Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1

# How to drug a cloud? Targeting intrinsically disordered proteins

2

**Vladimir N. Uversky** [1,*]

3

[1] *Department of Molecular Medicine and USF Health Byrd Alzheimer's Research Institute,*

4

*Morsani College of Medicine, University of South Florida, Tampa, FL 33612, USA*

5

6

7

Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

8 **Running Title:** Intrinsically disordered proteins as drug targets

9 **Corresponding author**: Vladimir N. Uversky, 120901 Bruce B. Downs Blvd., MDC07, Tampa,

10 Florida 33612, USA; phone: 1-813-974-5816; Fax: 1-813-974-7357; email: vuversky@usf.edu

11 **The number of text pages**: 131 (68 without references and figure legends)

12 **The number of tables**: 0

13 **The number figures**: 14

14 **The number of references**: 472

15 **The number of words in the *Abstract***: 230

16



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

17 **Abbreviations**:

| | | |
|---|---|---|
| 18 | Ab | antibody |
| 19 | AD | Alzheimer's disease |
| 20 | AI | artificial intelligence |
| 21 | ALS | amyotrophic lateral sclerosis |
| 22 | BC | biomolecular condensate |
| 23 | bHLHe39 | basic helix-loop-helix protein |
| 24 | bHLHZip | basic region/helix-loop-helix/leucine zipper |
| 25 | CB | Cajal body |
| 26 | CBP | CREB-binding protein |
| 27 | CDR | complementarity determining region |
| 28 | CP | chimeric polypeptide |
| 29 | CPP | cell penetrating peptide |
| 30 | cpSG | chloroplast stress granule |
| 31 | DBD | DNA-binding domain |
| 32 | Dox-CP | doxorubicin-conjugated chimeric polypeptide |
| 33 | ELP | elastin-like polypeptide |
| 34 | ESFT | Ewing's sarcoma family of tumors |
| 35 | EWS | Ewing's sarcoma |
| 36 | EWSR1 | EWS breakpoint region 1 |
| 37 | HCV | hepatitis C virus |
| 38 | HIV-1 | human immunodeficiency virus-1 |
| 39 | HLB | histone locus body |
| 40 | HMGB1 | high mobility group protein B1 |
| 41 | HRMT1L2 | protein arginine N-methyltransferase 1 |
| 42 | HSV | herpes simplex viruse |
| 43 | HTS | high-throughput screening |
| 44 | IDR | intrinsically disordered region |



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

45 IDP intrinsically disordered protein
46 IFN interferon
47 ITF inducible transcription factor
48 LBD ligand binding domain
49 LLPS liquid-liquid phase separation
50 LlMT metallothionein from *Littorina littorea*
51 mAb monoclonal antibody
52 Max Myc-associated factor X
53 ML machine learning
54 MLO membrane-less organelle
55 MoRF molecular recognition feature
56 MT metallothionein
57 NAFLD nonalcoholic fatty liver disease
58 nSB nuclear stress body
59 OPT Oct1/PTF/transcription
60 PcG polycomb group
61 PD Parkinson's disease
62 PDB protein data bank
63 PML progressive multifocal leukoencephalopathy
64 PNC perinucleolar compartment
65 POD PML oncogenic domains,
66 PPI protein-protein interaction
67 PTM posttranslational modification
68 RHA RNA helicase A
69 RNP ribonucleoprotein
70 SG stress granule
71 SNB Sam68 nuclear body
72 SNP single nucleotide polymorphism



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

| | | |
|---|---|---|
| 73 | SSD | signal sensing domain |
| 74 | TAD | transactivation domain |
| 75 | TF | transcription factor |
| 76 | TPPP/p25 | tubulin polymerization promoting protein |
| 77 | | |



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

78 **Abstract**

79 Biologically active proteins/regions without stable structure (i.e., intrinsically disordered proteins
80 and regions (IDPs and IDRs)) are commonly found in all proteomes. They have a unique
81 functional repertoire that complements the functionalities of ordered proteins and domains.
82 IDPs/IDRs are multifunctional promiscuous binders capable of folding at interaction with
83 specific binding partners on a template- or context-dependent manner, many of which undergo
84 liquid-liquid phase separation, leading to the formation of membrane-less organelles and
85 biomolecular condensates. Many of them are frequently related to the pathogenesis of various
86 human diseases. All this defines IDPs/IDRs as attractive targets for the development of novel
87 drugs. However, their lack of unique structures, multifunctionality, binding promiscuity, and
88 involvement in unusual modes of action preclude direct use of traditional structure-based drug
89 design approaches for targeting IDPs/IDRs, and make disorder-based drug discovery for these
90 "protein clouds" challenging. Despite all these complexities there is continuing progress in the
91 design of small molecules affecting IDPs/IDRs. This article describes the major structural
92 features of IDPs/IDRs and the peculiarities of the disorder-based functionality. It also discusses
93 the roles of IDPs/IDRs in various pathologies, and shows why the approaches elaborated for
94 finding drugs targeting ordered proteins cannot be directly used for the intrinsic disorder-based
95 drug design, and introduces some novel methodologies suitable for these purposes. Finally, it
96 emphasizes that regardless of their multifunctionality, binding promiscuity, lack of unique
97 structures, and highly dynamic nature, "protein clouds" are principally druggable.

98

99 **Significance Statement**



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

100 Intrinsically disordered proteins and regions are highly abundant in nature, have multiple
101 important biological functions, are commonly involved in the pathogenesis of a multitude of
102 human diseases, and are therefore considered as very attractive drug targets. Although dealing
103 with these unstructured multifunctional protein/regions is a challenging task, multiple innovative
104 approaches have been designed to target them by small molecules.

105



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

106 **Table of Contents**

107 I. Introduction dancing protein clouds:

108 I.A. Functions beyond the classic “lock-and-key”

109 I.B. Spatio-temporal heterogeneity of IDPs: Structural dissection of dancing protein clouds

110 I.C. Intrinsic disorder and structure-function continuum

111 I.D. Intrinsic disorder and liquid-liquid phase separation

112 II. Diseases and disorder-based protein complexity: Too many ways to get something wrong

113 III. How to target a dancing protein cloud

114 III.A. Why one would want to target an IDP?

115 III.B. Some general strategies for targeting IDPs

116 III.C. Targeting transcription factors

117 III.C.1. General considerations

118 III.C.2. Crucial hybrids: Order and intrinsic disorder in transcription factors

119 III.C.3. Transcription factors as potential and actual drug targets

120 III.C.4. Targeting ordered domains of transcription factors

121 III.C.5. Disorder-based rational drug design: the p53-Mdm2 story

122 III.C.6. cMyc-Max journey

123 III.C.7. Targeting EWS-FLI1 and other oncogenic translocation-generated fusion proteins



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

124 III.C.8. Androgen receptor

125 III.D. Irrational drug design: Targeting functionally misfolded IDPs/IDRs

126 III.E. Targeting aggregating IDPs/IDRs

127 III.F. Disorder-based development of consensus interferons

128 III.G. Unstructural vaccinology: Fighting fire with fire

129 III.H. Targeting liquid-liquid phase separation

130 III.I. Self-assembling “smart” IDP-based containers for targeted drug delivery

131 III.J. Pharmacological chaperones

132 IV. Concluding remarks: Clouds in clouds

133

134

135

136

137



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

138 # I. Introduction

139 ***I.A. Introducing dancing protein clouds: Functions beyond the classic "lock-and-key"***

140 Most biochemistry textbooks use the "lock-and-key" model to describe the molecular principles
141 of protein functionality, which depicts an enzyme as a lock, its active site as a key-hole, and a
142 substrate as a key, and which claims that only the correctly shaped/sized substrate (key) could fit
143 into the key-hole (active site) of the particular lock (enzyme). This theory was proposed in 1894
144 by German chemist Hermann Emil Louis Fischer (1852-1919) to explain why in the enzymatic
145 hydrolysis of glucoside multimers, one enzyme could hydrolyze α- but not β-glycosidic bonds,
146 whereas a similar enzyme could hydrolyze β- but not α-glycosidic bonds (Fischer, 1894;
147 Lemieux and Spohr, 1994). This highly mechanistic model was exceptionally fruitful and
148 actually served as a "big-bang" moment of the creation of the universe of modern protein
149 science, since most of the knowledge generated in the field by the end of the last century was in
150 one way or another rooted in the lock-and-key principle and the associated sequence-structure-
151 function paradigm, according to which a unique amino acid sequence of a protein defines its
152 unique 3D structure, which is crucial for unique protein function (Uversky, 2002a). Furthermore,
153 the ever-increasing content of the protein data bank (PDB), where, as of June 08, 2024, unique
154 3D structures determined by X-ray diffraction are reported for over 185,000 proteins, provides
155 compelling support for the static view of a functional protein structure.

156 However, the situation changed drastically at the turn of the century, when it was recognized that
157 not all biological functions of proteins require stable well-defined structure in solution, with
158 many functions originating from the lack of ordered structure in a protein molecule (Dunker et
159 al., 2001; Dunker and Obradovic, 2001; Dunker et al., 2008a; Dunker et al., 2008b; Dyson and



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

160 Wright, 2005; Tompa, 2002b; Uversky, 2002a; b; Uversky and Dunker, 2010b; Uversky et al.,
161 2000a; Wright and Dyson, 1999b). These intrinsically disordered proteins and regions (IDPs and
162 IDRs, respectively) exist as structural ensembles either at the secondary or at the tertiary
163 structure level. Curiously, although the origin of "unfoldomics" (Dunker et al., 2008a; Uversky,
164 2024) is attributed to a handful of papers published in the early 2000s (Dunker et al., 2001;
165 Dunker and Obradovic, 2001; Tompa, 2002b; Uversky, 2002a; b; Uversky et al., 2000a; Wright
166 and Dyson, 1999b), these biologically active proteins without unique structures were actually
167 discovered and rediscovered multiple times. This lengthy prehistory generated a wide trail of
168 terms used for the description of these proteins, which were depicted in literature as floppy,
169 pliable, rheomorphic (Holt and Sawyer, 1993), flexible (Pullen et al., 1975), mobile (Cary et al.,
170 1978), partially folded (Linderstrom-Lang and Schellman, 1959), natively denatured (Schweers
171 et al., 1994), natively unfolded (Uversky et al., 2000b; Weinreb et al., 1996), natively disordered
172 (Daughdrill et al., 2005), intrinsically unstructured (Tompa, 2002a; Wright and Dyson, 1999a),
173 intrinsically denatured, (Schweers et al., 1994) intrinsically unfolded (Weinreb et al., 1996),
174 intrinsically disordered (Dunker et al., 2001), vulnerable (Chen et al., 2008), chameleon
175 (Uversky, 2003a), malleable (Fuxreiter et al., 2008), 4D proteins (Tsvetkov et al., 2008), protein
176 clouds (Dunker and Uversky, 2010), dancing proteins (Livesay), proteins waiting for partners
177 (Janin and Sternberg, 2013), and several other names often representing different combinations
178 of 'natively, naturally, inherently, and intrinsically' with 'unfolded, unstructured, disordered, and
179 denatured' among several other terms (Dunker et al., 2013). Earlier, the "dancing protein cloud"
180 term was introduced by superimposing the aforementioned "dancing proteins" and "protein
181 clouds" descriptors (Uversky, 2016a). It was also emphasized: "Although the phrase "dancing
182 protein clouds" sounds like a parody, the term actually has deep meanings. The presence of a



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

183 unique structure in a given protein means that when one would look at the sample containing this
184 protein, s/he would find that all protein molecules are alike, that the structure of an individual
185 molecule barely changes over time, and that the ensemble-averaged (or time-averaged) structure
186 is identical, or at least very similar, to the structures of all individual protein molecules in that
187 sample. In other words, if one would overlay all those individual structures, a crisp and clear
188 image would be generated, similar to those found in the PDB, and this ensemble-averaged
189 structure would not change much over time. On the other hand, the lack of a unique structure in a
190 given protein would create a highly dynamic ensemble, members of which would possess very
191 different structures at any given moment, and the structure of any given molecule would change
192 dramatically over time (therefore the "dancing protein" analogy). If one would try to overlay all
193 those structures, all those dancing protein molecules, a cloud-like, fuzzy entity would be
194 generated, and the shape of this cloud would not be static, dramatically changing with time and
195 in response to subtle environmental perturbations" (Uversky, 2016a). This idea is illustrated by
196 **Figure 1** showing the NMR solution structure of one of the IDPs, barnacle (*Megabalanus rosa*)
197 cement protein MrCP20 (PDB ID: 6LEK; (Mohanram et al., 2019)), where 10 models are
198 overlaid, generating a complex conformational ensemble that clearly resembles a fuzzy cloud.

199 Although originally considered as being rather rare exceptions, IDPs/IDRs, which fail to form
200 unique 3D-structures under physiological conditions, are highly abundant in different proteomes
201 (Dunker et al., 2000; Tokuriki et al., 2009; Uversky, 2010a; Ward et al., 2004; Xue et al., 2012;
202 Xue et al., 2010), and are especially prevalent in eukaryotes (Dunker et al., 2000; Oldfield et al.,
203 2005a; Uversky, 2010a; Ward et al., 2004; Xue et al., 2012). The natural abundance of intrinsic
204 disorder can be understood via a unique usefulness of this feature for some proteins functions
205 (Daughdrill et al., 2005; Dunker et al., 2002a; Dunker et al., 2002b; Dunker et al., 2005; Dunker



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

206 et al., 1998; Dunker et al., 2001; Dunker and Obradovic, 2001; Dyson and Wright, 2005; Tompa,
207 2002b; 2005; Tompa and Csermely, 2004; Uversky, 2002a; b; 2003b; Uversky and Dunker,
208 2010b; Uversky et al., 2000a; Uversky et al., 2005; Vucetic et al., 2007; Wright and Dyson,
209 1999b; Xie et al., 2007). Since the disorder-based functionality cannot be described within the
210 frame of the traditional structure-function paradigm, a more comprehensive view of the protein
211 structure/function relationships is needed, where the disorder-based functionality complements
212 the functions of ordered proteins (Vucetic et al., 2007; Xie et al., 2007). In fact, the majority of
213 signaling proteins contain significant levels of intrinsic disorder (Dunker et al., 2002a), and
214 IDPs/IDRs, being capable of interactions with multiple partners and being involved in high-
215 specificity/low-affinity interactions, often play central roles in regulation and control of
216 numerous cellular signaling pathways and in promoting the assembly of supra-molecular
217 complexes (Dunker et al., 2005; Uversky et al., 2005). IDPs are multifunctional proteins capable
218 of promiscuous interactivity with numerous and often unrelated partners. This makes them
219 crucial organizers and controllers of complex protein-protein interaction (PPI) networks, in
220 which IDPs/IDRs often act as hubs (Daughdrill et al., 2005; Dosztanyi et al., 2006; Dunker et al.,
221 2005; Ekman et al., 2006; Haynes et al., 2006; Oldfield et al., 2008; Patil and Nakamura, 2006;
222 Singh et al., 2007; Uversky, 2011b).

223 Below is a list of some of the most important functional advantages of intrinsic disorder (Brown
224 et al., 2002; Cortese et al., 2008; Daughdrill et al., 2005; Dunker et al., 2002a; Dunker et al.,
225 2002b; Dunker et al., 2005; Dunker et al., 1998; Dunker et al., 2001; Dunker and Obradovic,
226 2001; Dunker et al., 1997; Dyson and Wright, 2002a; 2005; Iakoucheva et al., 2002; Mollica et
227 al., 2016; Plaxco and Gross, 1997; Pontius, 1993; Romero et al., 2001; Schulz, 1979; Uversky,
228 2013a; Uversky et al., 2005; Wright and Dyson, 1999b):



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

229 1) Most IDPs/IDRs are characterized by multifunctionality, which is based on their mosaic
230 architecture, where multiple relatively short and differently folded or disordered
231 functional elements are spread within the amino acid sequences (DeForte and Uversky,
232 2016; Uversky, 2013a; d; 2016a; 2019).

233 2) Multifunctionality of 'moonlighting' proteins (i.e., proteins that perform multiple
234 unrelated functions, each being independently regulated) relies on the presence of IDRs
235 (Tompa et al., 2005).

236 3) Biological activities and functions of IDPs/IDRs (due to their high accessibility) are
237 easily modulated by various enzymatically catalyzed posttranslational modifications
238 (PTMs), such as acetylation, glycosylation, phosphorylation, sumoylation, and
239 ubiquitination. (Darling and Uversky, 2018; Iakoucheva et al., 2004; Pejaver et al., 2014).

240 4) IDPs/IDRs are efficiently regulated via rapid proteolytic degradation.

241 5) IDPs/IDRs are commonly subjected to alternative splicing, which results in the
242 production of diverse forms and thereby represents an important means for regulation and
243 redirection.

244 6) IDPs/IDRs can undergo disorder-to-order transitions under a variety of conditions
245 (Dunker et al., 2002a; Dunker et al., 2005; Dunker et al., 2001; Dyson and Wright,
246 2002a; 2005; Fink, 2005; Iakoucheva et al., 2002; Oldfield et al., 2005b; Oldfield et al.,
247 2008; Tompa, 2002b; Uversky, 2002a; b; Uversky et al., 2000a; Uversky et al., 2005;
248 Wright and Dyson, 1999b). Besides changes in the protein environment, such folding can
249 also be promoted by interaction with binding partners, such as other proteins, nucleic



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

250 acids, membranes, or small molecules (Daughdrill et al., 2005; Dunker et al., 2002a;
251 Dunker et al., 2002b; Dunker et al., 2005; Dunker et al., 2001; Dunker and Obradovic,
252 2001; Dyson and Wright, 2002a; 2005; Iakoucheva et al., 2002; Plaxco and Gross, 1997;
253 Pontius, 1993; Schulz, 1979; Spolar and Record, 1994; Uversky et al., 2005; Wright and
254 Dyson, 1999b). The protein regions capable of such binding-induced folding are known
255 as molecular recognition features (MoRFs) (Cheng et al., 2007; Mohan et al., 2006;
256 Oldfield et al., 2005b; Vacic et al., 2007a). They are known to play crucial roles in
257 various biological processes, such as cell signaling, recognition, and regulation (Disfani
258 et al., 2012).

259 7) Many IDRs can act as morphing MoRFs, which fold differently at interaction with
260 different partners (Hsu et al., 2012; Oldfield et al., 2008), and many IDPs/IDRs can
261 readily bind to multiple partners by changing shape (Dunker et al., 2001; Karush, 1950;
262 Kriwacki et al., 1996). All this defines the unique disorder-based binding plasticity,
263 where, depending on the template provided by binding partners, IDPs/IDRs can mold into
264 specific bound conformations (which can be very different).

265 8) IDPs/IDRs commonly contain short linear motifs (SLiMs) and eukaryotic linear motifs
266 (ELMs) with a typical length between 5 and 25 residues. These linear motifs are
267 characterized by noticeable conservation (the presence of a short consensus pattern, with
268 conserved residues that are interspersed with rather freely exchangeable, variable
269 positions). Being dynamic switch-like elements, which are frequently generated and
270 erased in evolution, these short linear motifs can often be involved in mediating specific
271 protein-protein interactions (Fuxreiter et al., 2007b; Linding et al., 2003; Neduva and
272 Russell, 2005; Puntervoll et al., 2003).



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

273 9) Many IDPs and IDRs contain transient elements of secondary structure, pre-structured
274 motifs (PreSMos) (Chi et al., 2007; Lee et al., 2012), which were shown to pre-exist (Lee
275 et al., 2000) or be pre-formed prior to their binding to targets (Zitzewitz et al., 2000).
276 Therefore, PreSMos, which are also known by other names, such as pre-organized
277 (Bienkiewicz et al., 2002), pre-ordered (Sayers et al., 2000), or local structural elements
278 (Lee et al., 2000), transient structure (Ramelot et al., 2000), preformed structural element
279 (PSE) (Fuxreiter et al., 2004), primary contact site (PCS) (Csizmok et al., 2005), and
280 linear motifs (Fuxreiter et al., 2007b), act as active sites of IDPs/IDRs that mediate target
281 protein binding in many, if not all, extended IDPs/IDRs (Lee et al., 2012).

282 10) The bound forms of IDPs could be either relatively non-compact (i.e., remain
283 substantially disordered or fuzzy) (Dosztanyi and Tompa, 2008; Fuxreiter and Tompa,
284 2012; Uversky, 2011b; 2013b) or be tightly folded (Tompa and Fuxreiter, 2008; Uversky,
285 2002b; 2011b).

286 11) To accommodate the peculiarities of interaction with various partners, IDPs/IDRs employ
287 binding fuzziness based on the utilization of different binding mechanisms, such as
288 stabilization of the binding-competent secondary structure elements within the contact
289 region, establishing long-range electrostatic interactions, involvement in the transient
290 physical contacts with the partner, or formation of highly disordered complexes without
291 any apparent ordering.

292 12) IDPs/IDRs can be involved in cascade interactions (or "binding chain reactions:), where
293 binding to a first partner induces partial folding of an IDP/IDR, causing the formation of



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

294 a new binding site suitable for interaction with a second partner(Fuxreiter et al., 2014;
295 Uversky, 2013c).

296 13) The presence of intrinsic disorder allows decoupling specificity and affinity of binding,
297 which is central for the formation of specific but weak complexes involved in reversible
298 signaling processes, where turning a signal off is as important as turning it on (Dunker et
299 al., 2001; Schulz, 1979).

300 14) IDPs/IDRs, being extended and flexible, are characterized by a greater capture radius,
301 which allows them to have faster kinetics of interaction and spatially search through a
302 wider interaction space. Due to their conformational pliability, they are also able to
303 overcome steric restrictions.

304 15) Due to their extended linear conformations, IDPs/IDRs are capable of holding
305 overlapping binding sites.

306 16) IDPs/IDRs also have increased interaction (surface) area per residue that broadens their
307 binding mechanisms and enables larger interaction surfaces in protein-protein and
308 protein-ligand complexes than those obtained between rigid partners.

309 17) The large interaction surfaces allow some IDPs/IDRs to form very stable intertwined
310 complexes.

311 18) IDPs/IDRs can form transient encounter complexes, which makes spatial orientation
312 requirements less stringent.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

313 19) The structural flexibility and conformational dynamics of IDPs/IDRs provides important
314 means for masking (or not) of interaction sites and allows interaction between bound
315 partners.

316 20) IDPs/IDRs can be engaged in one-to-many binding, where a single IDP/IDR is capable of
317 binding to several structurally diverse partners.

318 21) IDPs/IDRs also participate in many-to-one binding, where many different IDPs/IDRs
319 interact with a single ordered protein.

320 22) IDPs/IDRs are characterized by a broad variability of their evolutionary conservation,
321 with some of them being highly conserved and others possessing very high evolutionary
322 rates and rapidly evolving into sophisticated and complex interaction centers (scaffolds)
323 that can be easily tailored to the needs of different organisms.

324 23) Some IDPs/IDRs have functions that are entropic in nature that not only do not require
325 any folding (Dunker et al., 2002a; Dunker et al., 2002b; Dunker et al., 2001; Tompa,
326 2002b; 2005; Uversky, 2002a; b; Uversky and Dunker, 2010b) but actually originate
327 from the lack of stable well-defined structure in a protein molecule or a protein region
328 (Dunker et al., 2001; Dunker and Obradovic, 2001; Dunker et al., 2008a; Dunker et al.,
329 2008b; Dyson and Wright, 2005; Tompa, 2002b; Uversky, 2002a; b; Uversky and
330 Dunker, 2010b; Uversky et al., 2000a; Wright and Dyson, 1999b).

331 In relation to the disorder-based signaling and regulatory interactions (as well as to the related
332 roles of IDPs/IDRs in the pathogenesis of different diseases), one of the major functional
333 advantages of IDPs/IDRs is their ability to undergo disorder-to-order transitions upon binding



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

334 that gives them a possibility, during the formation of "signaling" complexes characterized by
335 high specificity and low affinity, to decouple the specificity and affinity of binding (Dunker et
336 al., 2001; Schulz, 1979). Also, many IDPs/IDRs can act as shape-shifters capable of interaction
337 with partners by changing shape or undergoing partner-specific folding (Dunker et al., 2001; Hsu
338 et al., 2012; Hsu et al., 2013; Karush, 1950; Kriwacki et al., 1996; Oldfield et al., 2008; Uversky,
339 2013b; d). Numerous other advantages of intrinsic disorder for signaling, regulation, and control-
340 related protein functions have been systemized in several dedicated studies (Brown et al., 2002;
341 Cortese et al., 2008; Dunker et al., 1998; Dunker et al., 2001; Dunker et al., 1997; Romero et al.,
342 2001; Uversky, 2013a; Wright and Dyson, 1999b) and are schematically summarized in **Figure**
343 **2**.

344

345 ***I.B. Spatio-temporal heterogeneity of IDPs: Structural dissection of dancing protein clouds***

346 The characteristic feature of IDPs/IDRs is their inability to spontaneously fold under conditions
347 which trigger folding of ordered proteins/domains. The ability or inability of a protein to
348 spontaneously fold under physiological conditions is defined by the peculiarities of its amino
349 acid sequence. Therefore, based on the sequence-structure relationships, the universe of protein
350 amino acid sequences can be divided into at least two very different categories: sequences that
351 naturally fold into ordered proteins or domains, and sequences that yield IDPs/IDRs (Uversky,
352 2013a; Wathen and Jia, 2009). In fact, multiple features can differentiate amino acid sequences
353 coding for IDPs/IDRs from sequences encoding ordered globular proteins/domains. These
354 features include, but are not limited to, the amino acid composition, charge, evolutionary
355 conservation, flexibility, hydrophobicity, and sequence complexity. For example, ordered and



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

356 disordered proteins and regions can be differentiated based on the peculiarities of their amino
357 acid compositions, as IDPs/IDRs are enriched in so-called disorder-promoting residues A, G, D,
358 M, K, R, S, Q, P, and E, and are depleted in order-promoting amino acids C, W, Y, I, F, V, L, H,
359 T, and N, many of which would commonly be found in the hydrophobic cores of folded globular
360 proteins (Dunker et al., 2001; Radivojac et al., 2007; Vacic et al., 2007b; Williams et al., 2001)
361 (see **Figure 3A**). Various disorder predictors utilize these and other sequence differences. Due to
362 the complexity of the protein intrinsic disorder phenomenon and because of the multiple features
363 that are used by different tools to differentiate ordered and disordered sequences, a more
364 complete representation of the disorder status of an individual protein of interest or of a protein
365 dataset can be achieved by comparing and combining several predictors (Bourhis et al., 2007;
366 Dosztanyi et al., 2007; Dosztanyi and Tompa, 2008; Esnouf et al., 2006; Ferron et al., 2006; He
367 et al., 2009; Huang et al., 2012; Oldfield et al., 2005a; Uversky, 2017a).

368 It was also pointed out that the inability of IDPs/IDRs to spontaneously fold and the requirement
369 for some of them to bind to specific partners in order to gain more ordered structures indicated
370 that their amino acid sequences do not contain an entire "folding code" defining the ability of
371 foldable proteins to spontaneously gain their unique biologically active structures. Since many
372 IDPs/IDRs can fold at interaction with specific partners, it seems that these binding partner(s)
373 can supplement at least part of the missing portion of their "folding code". Importantly, this
374 feature also defines an important difference between ordered and disordered proteins in the
375 molecular principles of their functions, where ordered proteins first undergo spontaneous folding
376 and then bind their partners, whereas IDPs/IDRs remain disordered until they find and bind to
377 their partners (Uversky, 2013a; d; Uversky and Dunker, 2013). Furthermore, many disorder-
378 based functions do not require any folding (Dunker et al., 2002a; Dunker et al., 2002b; Dunker et



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

379 al., 2001; Tompa, 2002b; 2005; Uversky, 2002a; b; Uversky and Dunker, 2010b), and some
380 IDPs/IDRs preserve significant amount of disorder in their bound states, forming so-called fuzzy
381 complexes (Fuxreiter and Tompa, 2012; Tompa and Fuxreiter, 2008). On the other hand, the
382 removal of the restrictions posed by the need to spontaneously fold into ordered structure
383 dramatically increases the sequence space available to IDPs/IDRs (at least those which either do
384 not fold at all, or do not completely fold at binding) in comparison with the sequence space
385 available to foldable proteins and domains (Uversky, 2013a; d). These considerations are
386 illustrated by **Figure 3B** showing the difference in the dimensions of the sequence spaces
387 encoding for ordered/compact and extended intrinsically disordered proteins within the charge-
388 hydropathy phase space.

389 Importantly, the structural heterogeneity of IDPs/IDRs is not limited by the aforementioned
390 peculiarities of their amino acid sequences, being further extended due to the capability of
391 different parts of a protein to be (dis)ordered to different degrees, and the overall ability of
392 IDPs/IDRs to have different levels and depth of disorder (Uversky et al., 2005). In fact, even at
393 the whole molecule level, intrinsic disorder can be found in three different forms, as IDPs can
394 exist in the collapsed (molten globule-like), or extended (coil- or pre-molten globule-like) forms
395 (Daughdrill et al., 2005; Dunker et al., 2001; Turoverov et al., 2010; Uversky, 2002a; b; 2003b;
396 Uversky and Dunker, 2010b; Uversky et al., 2000a; Williams et al., 2001). Furthermore, the
397 structural heterogeneity does not stop there, as not only the entire protein molecule but its
398 different parts can be (dis)ordered to different degrees as well (Uversky, 2013a; d), indicating
399 that foldability (or structure-coding potential) is non-homogeneously distributed within the
400 amino acid sequences of IDPs/IDRs. As a result, different parts of a molecule are ordered or
401 disordered to different degrees, and a typical IDP/IDR normally contains potentially foldable,



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

402 partially foldable, differently foldable, or not foldable at all protein segments (Uversky, 2013a;
403 d). Therefore, IDPs/IDRs can be more or less compact and possess smaller or larger amounts of
404 flexible secondary/tertiary structure (Dunker et al., 2001; Dunker and Obradovic, 2001; Uversky,
405 2002a; b; Uversky and Dunker, 2010b; Uversky et al., 2000a).

406 Furthermore, this distribution of differently (dis)ordered regions is constantly changing in time,
407 and a given segment of a protein molecule can potentially show different structures or lack of
408 structure at different time points. As a result, at any given moment, an IDP/IDR has a structure
409 different from what was seen at another moment (Uversky, 2013a; d). Clearly, this consideration
410 represents another way to end up with the "dancing protein cloud" view of a dynamic protein
411 structure. One should also keep in mind that many proteins exist as complex structural hybrids
412 possessing ordered and differently disordered domains, thereby defining another level of
413 structural heterogeneity crucial for their functions (Dunker et al., 2013).

414

415 ***I.C. Intrinsic disorder and structure-function continuum***

416 The previous section draws a complex picture of a highly dynamic and very heterogeneous
417 protein structure, where only the entire protein molecules are expected to be disordered to
418 different degrees, but various protein segments (even rather short ones) can be differently
419 disordered as well (DeForte and Uversky, 2016; Uversky, 2013a; d; 2016a; e; 2019). The
420 resulting mosaic architecture of a protein molecule includes foldons (regions capable of
421 spontaneous folding), non-foldons (segment that do not fold), semi-foldons (regions that are
422 always in a semi-folded state), inducible foldons (segments that can gain structure (at least
423 partially fold) at interaction with binding partners), inducible morphing foldons (regions capable



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

424 of folding to the different structures at interaction with different binding partners), and unfoldons
425 (important less stable parts of ordered proteins that must unfold (or undergo order-disorder
426 transition, at least partially) in order to make protein active) (DeForte and Uversky, 2016;
427 Uversky, 2013a; d; 2016a; e; 2019). Again, the entire protein has a highly dynamic and
428 morphing structure, which is not rigid or crystal-like, and where at different time moments,
429 different parts of a molecule can be (dis)ordered to different degrees (DeForte and Uversky,
430 2016; Uversky, 2013a; d; 2016a; 2019). Furthermore, it is also very clear that the classification
431 of proteins as ordered and disordered is an obvious oversimplification, as the structure-disorder
432 space of a protein represents a continuum, with no obvious boundary between ordered and
433 disordered proteins (DeForte and Uversky, 2016; Uversky, 2013a). It is also important to
434 emphasize here that a protein molecule is an inherently flexible entity, and even the most ordered
435 proteins do not resemble crystals or "solid rocks", possessing instead some degree of flexibility,
436 which is crucial for their biological activities (Ma et al., 1999).

437 This complex mosaic-like structural organization of proteins, where different regions are
438 dynamically (dis)ordered to different degrees and constantly undergo conformational exchange,
439 not only defines their highly flexible and heterogeneous "anatomy" but serves as a very
440 important foundation of the unique disorder-based "physiology". This idea is illustrated by
441 **Figure 4** showing that all the aforementioned differently (dis)ordered structural segments of
442 proteins might have very different functions. Therefore, although based on the classical
443 structure-function paradigm, where a unique protein function is determined by a unique protein
444 structure, the structural "floppiness" (or the presence of intrinsic disorder) would be
445 incompatible with protein functionality, and in reality many protein functions originate from the
446 lack of ordered structure in a protein molecule. Furthermore, since all those foldons, semi-



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

447 foldons, non-foldons, inducible foldons, inducible morphing foldons, and unfoldons can be
448 found within one protein molecules, it is clear that a protein with such heterogeneous structure is
449 inherently multifunctional. In other words, protein structural continuum defines protein
450 multifunctionality. This indicates that at the global level, protein functionality depends on
451 comprehensive structure/function relationships and cannot be described within the frames of the
452 traditional structure-function paradigm. In fact, the protein sequence-function relationships are
453 best described using a two-pathway approximation, where the traditional sequence-to-structure-
454 to-function pathway is used to describe the functionality of enzymes and membrane transport
455 proteins, whereas a new sequence-to-disordered ensemble-to-function pathway should be added
456 for proteins and protein regions involved in signaling, regulation, and control (Dunker et al.,
457 2002a; Dunker et al., 2002b; Dunker et al., 2001; Dunker and Obradovic, 2001; Uversky, 2002a;
458 b).

459 Furthermore, the biological complexity is directly linked to the size of a functional proteome and
460 not to the size of the corresponding genome (Schluter et al., 2009). Originally, this increase in
461 the functional proteome was ascribed to the allelic variations (i.e., single or multiple point
462 mutations, indels, single nucleotide polymorphisms (SNPs)), different pre-translational
463 mechanisms affecting mRNA (e.g., production of numerous mRNA variants by the alternative
464 splicing and mRNA editing), and changes induced in proteins by various posttranslational
465 modifications (PTMs) (Farrah et al., 2013; Farrah et al., 2014; Kim et al., 2014; Reddy et al.,
466 2015; Smith et al., 2013; Uhlen et al., 2005), which represent specific means by which a single
467 gene can encode not a single protein but multiple proteoforms, which are different protein
468 products of a single gene (Smith et al., 2013). Later, additional means, such as intrinsic disorder,



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

469 structural flexibility, and functioning were included in the list of factors contributing to the
470 increase in protein structural and functional diversity (Uversky, 2016d; 2019).

471 All these considerations indicate that the relationships between genes and proteins are better
472 described by an "one-gene – many-proteins – many-functions" model (Uversky, 2016c; d)
473 rather than by a classic "one gene – one enzyme" hypothesis, according to which each gene
474 is responsible for producing a single enzyme that in turn affects a single step in a metabolic
475 pathway (Beadle and Tatum, 1941). Furthermore, a "protein structure-function continuum"
476 model, where a functional protein exists as a dynamic conformational ensemble containing
477 multiple proteoforms of different origin characterized by a broad spectrum of structural
478 features and possessing different functionalities, provides a global link between protein
479 structure and function (Uversky, 2015).

480

481 ***I.D. Intrinsic disorder and liquid-liquid phase separation***

482 Recent years have witnessed a dramatic increase in the interest of researchers in the
483 intriguing intracellular entities known as membrane-less organelles (MLOs) or biomolecular
484 condensates (BCs) (Brangwynne, 2013; Brangwynne et al., 2009; Brangwynne et al., 2015; Dundr
485 and Misteli, 2010; Feric et al., 2016; Mitrea and Kriwacki, 2016; Uversky, 2017b; c; Uversky et al.,
486 2015; Zhu and Brangwynne, 2015). These cellular foci or dots are known by different names,
487 such as ribonucleoprotein (RNP) bodies, or RNP droplets, or RNP granules. They extend the
488 realm of the membrane-encapsulated eukaryotic organelles, such as chloroplasts,
489 endoplasmic reticulum, Golgi apparatus, lysosomes, mitochondria, nucleus, and vacuoles.
490 Although for the first time the nucleolus, which is the best known and the most studied



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

491 MLO, was described about 200 years ago (reviewed in (Pederson, 2011)), and although other
492 MLOs were regularly found in eukaryotic cells, these cellular bodies were mostly underappreciated
493 by the scientific community, being often considered as obscure objects participating in completely
494 different cellular processes. Furthermore, nobody even supposed that these highly diversified
495 organelles could have common mechanical properties and share common mechanisms of formation
496 (Antifeeva et al., 2022; Fonin et al., 2022).

497 More than a hundred different MLOs were eventually found in the cytoplasm, nucleus,
498 mitochondria (and chloroplasts) of eukaryotic cells, as well as in the cytoplasm of bacteria
499 and archaea. The incomplete list of these biomolecular condensates in eukaryotic cells
500 include cytoplasmic centrosomes (Decker et al., 2011), germline P-granules (germ cell
501 granules or nuage) (Brangwynne et al., 2009; Chuma et al., 2009), neuronal RNA granules
502 (Kiebler and Bassell, 2006), processing or P-bodies (Decker et al., 2007), stress granules
503 (SGs) (Wippich et al., 2013), as well as chloroplast stress granules (cpSGs) (Uniacke and
504 Zerges, 2008) and mitochondrial RNA granules (Antonicka and Shoubridge, 2015). In the
505 nucleous, one can find Cajal bodies (CBs) (Strzelecka et al., 2010), chromatin (Li et al.,
506 2007), cleavage bodies (Li et al., 2006), histone locus bodies (HLBs) (Nizami et al., 2010),
507 nuclear gems or Gemini or coiled bodies (Gubitz et al., 2004; Matera and Frey, 1998),
508 nuclear speckles or interchromatin granule clusters (Lamond and Spector, 2003), nuclear
509 pores (Grossman et al., 2012), nuclear stress bodies (nSBs) (Biamonti, 2004; Biamonti and
510 Vourc'h, 2010), nucleolus (Shav-Tal et al., 2005), Oct1/PTF/ transcription (OPT) domains
511 (Harrigan et al., 2011), perinucleolar compartment (PNC) (Huang, 2000), paraspeckles (Fox
512 et al., 2002), PML-bodies (PML oncogenic domains, PODs) (Maul et al., 2000), polycomb
513 bodies (PcG bodies) (Pirrotta and Li, 2012), and Sam68 nuclear bodies (SNBs) (Huang,



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

514 2000).

515 MLOs are spherical micron-sized droplets (Nott et al., 2015) that have unique morphologies
516 and specific distribution patterns. They typically contain both RNA and proteins, and
517 structurally can include layers (i.e., might themseves represent phases in phases), which
518 might have different biophysical properties and functions (Hirose et al., 2023). The
519 importance of MLOs is defined by the fact that they represent "an intricate solution of the
520 cellular need to facilitate and regulate molecular interactions by physically isolating target
521 molecules in specialized compartments in a reversible and controllable way" (Uversky,
522 2017c).

523 Despite their localization within the different cellular compartments, and desipte their very
524 different functions, such as as microreactors-fermenters, network hubs controlling various
525 cellular signaling pathways or as storages of target molecules, the biogenesis of MLOs/BCs
526 is driven by a unique process of liquid-liquid phase separation (LLPS). Here, under the
527 approproate condition (typically above a specific critical concentration of the molecules
528 capable of LLPS) a homogeneous solution spontaneously separates into two distinct
529 immiscible liquids, or "phases": a dense phase, and a dilute phase, both characterized by high
530 water content and not separated by the membranes. Among the fundamental principles
531 defining MLO formation is the critical involvement of the polyvalent stochastic protein-
532 protein interactions typcal for IDPs/IDRs (Uversky, 2016b; 2017c; Uversky et al., 2015).
533 Therefore, it is not surprising that although MLOs differ from each other by specific sets of
534 their resident proteins, it seems that they always contain IDPs (Uversky, 2017c). In other
535 words, MLOs/BCs often represent a way of the intracellular compartmentalization of
536 IDPs/IDRs (Darling et al., 2018; Meng et al., 2015; Uversky, 2017b; c; d).



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

537 The characteristic features of MLOs are their cell size–dependent dimensions (Brangwynne,
538 2013) and liquid-like behavior (Brangwynne et al., 2009; Brangwynne et al., 2011; Feric and
539 Brangwynne, 2013; Wippich et al., 2013), with MLOs typically being just slightly denser
540 than the rest of the cytoplasm or nucleoplasm (Handwerger et al., 2005; Updike et al., 2011).
541 MLOs are mesoscopic cellular compartments with highly dynamic nature, components of
542 which are not protected from the environment, being in direct contact with the surrounding
543 cytoplasm, nucleoplasm, mitochondrial matrix, or chloroplast stroma (Pederson, 2001; Phair
544 and Misteli, 2000). The lack of encapsulating membranes also indicates that the structural
545 integrity and biogenesis of MLOs are entirely controlled and regulated by protein–protein,
546 protein–nucleic acid (Dundr and Misteli, 2010), and protein-membrane interactions
547 (Nesterov et al., 2021).

548 Being classified as liquid-droplet phases or different liquid state of the nucleoplasm,
549 cytoplasm, matrix, or stroma, MLOs possess a high level of internal dynamics (Aggarwal et
550 al., 2013; Brangwynne, 2013; Brangwynne et al., 2009; Brangwynne et al., 2011; Feric and
551 Brangwynne, 2013; Li et al., 2012; Wippich et al., 2013), clearly representing characteristic
552 examples of fluid, disorder-based ensembles. Depending on the place of their origin, MLOs
553 are classified as 1D, 2D, or 3D assemblages, where 1D MLOs are found as loqiod droplets
554 on liner cellular sturcutres, such as chromatin and cytoskeleton, 2D MLOs originate as a
555 result of the phase separation in/on the membranes, and 3D MLOs are formed via the LLPS
556 in the bulk of the nucleoplasm/cytoplasm/matrix/stroma (Nesterov et al., 2021). The resulting
557 1D, 2D, and 3D MLOs can influence each other (Nesterov et al., 2021). It was also
558 hypothesized that the LLPS, being triggered by environmental changes, can initiate
559 propagation of 1D, 2D, and 3D MLOs within the cell interior or can be transmitted in a form



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

560 of an autowave, with both processes being characteried by low energy demand representing
561 an efficent way of the adjustustement of the cell signaling and metabolic systems to new
562 demands (Nesterov et al., 2021). Such interconnection of phase separation at different
563 dimensions might serve as an important means of intracellular communication and regulation
564 (Nesterov et al., 2021).

565 Since the formation of many (if not all) MLOs is diven by LLPS of specific IDPs (Csizmok
566 et al., 2016; Elbaum-Garfinkle et al., 2015; Lin et al., 2015; Meng et al., 2015; Mitrea et al.,
567 2016; Nott et al., 2015; Toretsky and Wright, 2014; Uversky, 2017c; Uversky et al., 2015), it
568 is clear that the protein intrinsic disorder, biological phase separation, and MLO phenomena
569 are interlinked (Antifeeva et al., 2022; Fonin et al., 2022; Nesterov et al., 2021; Turoverov et
570 al., 2019; Uversky, 2017c). This interconnection redefines the organizational principles of
571 living matter from a rather mechanistic model, where functions of proteins are determined by
572 their rigid globular structures and where intracellular processes occur within the rigid
573 membrane-encapsulated organelles, to a new model, where highly dynamic "biological soft
574 matter" (IDPs and MLOs) positioned at the “edge of chaos” represents a critical foundation
575 of life and defines the complexity and evolution of living things (Turoverov et al., 2019).

576

## 577 II. Diseases and disorder-based protein complexity: Too many ways to get 578 something wrong

579 The facts assembled in previous sections indicate that the high abundance and enormous
580 complexity of IDPs, combined with their crucial roles in various regulatory and signaling
581 processes, have unique functional advantages (otherwise IDPs would not be commonly present



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

582 in all living things). However, the very same facts clearly suggest that this disorder-based
583 complexity must be tightly controlled. Otherwise it might backfire, as within a complex and
584 highly interconnected system, there are too many ways of getting something wrong. In fact,
585 structural plasticity and binding promiscuity are crucial for protein multifunctionality. However,
586 being taken out of control, they can easily promote a functional disaster (such as what would
587 happen if a multifunctional IDP would exercise its promiscuous binding by interacting with the
588 wrong partners at a wrong time in a wrong place). Similarly, uncontrolled assembly of
589 promiscuous binders in a limited volume within a cell might create ideal conditions for
590 pathological protein aggregation. Therefore, these different forms of deregulation of IDPs
591 represent an important addition to the known arsenal of means associated with protein
592 dysfunctions causing development of many pathological conditions.

593 In fact, the failure of a specific peptide or protein to adopt its functional conformational
594 state accompanied by protein misfolding, loss of normal function, gain of toxic function, and/or
595 protein aggregation are linked to a broad range of human diseases (Uversky, 2009a; 2010b).
596 Often, diseases are associated with dysfunction of a particular protein (or a set of proteins), and
597 dysfunctions of different proteins might have different triggers. In fact, for some disease-related
598 proteins, the formation of pathologic conformation(s) represents an intrinsic propensity. For other
599 proteins, conformational changes leading to the increased propensity to misfold are triggered by
600 mutations, or improper posttranslational modifications (acetylation, advanced glycation (i.e., the
601 end-stage of the non-enzymatic glycosylation), deamidation, glycosylation, methylation,
602 phosphorylation, or racemization), or impaired interactions with chaperones, or interactions with
603 various intracellular or extracellular factors, such as other proteins, nucleic acids, and other
604 endogenous factors or lost binding partners (including other proteins, small molecules, nucleic



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

605 acids, etc.). For still other proteins, misfolding and dysfunction are associated with exposure to
606 internal or external toxins or are linked to oxidative damage. Furthermore, abnormal alternative
607 splicing that commonly affects IDPs has been associated with numerous human diseases, such as
608 Alzheimer's disease (Lovestone et al., 1994), azoospermia (Ma et al., 1993), cancers (Geng et
609 al., 2024; Tao et al., 2024; Venables, 2004; Wojtys and Oron, 2023), cardiometabolic disorders
610 (Miyazaki, 2023), genetic disorders (Shi et al., 2024), inflammatory bowel disease (Zou et al.,
611 2023), inherited retinal diseases (Sundaresan et al., 2023), myotonic dystrophy (Roberts et al.,
612 1997), and Parkinson's disease (Beyer et al., 2008a; Beyer et al., 2008b), to name a few. All
613 these misfolding- and dysfunction-promoting factors can act additively, independently, or
614 synergistically.

615 Altogether, there are hundreds of different maladies associated with protein dysfunction.
616 These conformational diseases or proteinopathies (Walker and LeVine, 2000) can affect a single
617 organ, or be spread through multiple tissues, and can be hereditary or sporadic. In various forms
618 of amyloidosis, there are more than 50 different proteins, which prior to fibrillation have
619 different structures (β-sheets, α-helices, or contain both α-helices and β-sheets). Such pathology-
620 associated proteins can be globular or intrinsically disordered. The broad involvement of
621 IDPs/IDRs in the pathology of different diseases is typically associated with their deregulated
622 involvement in recognition, regulation, and cell signaling and is reflected in the "disorder in
623 disorders" or $D^2$ concept (Uversky et al., 2008). In other words, proteinopathies may originate
624 from the misfolding, misidentification, misregulation, and missignaling of causative IDPs/IDRs
625 (Midic et al., 2009; Uversky, 2009a; 2010b; Uversky et al., 2008; Uversky et al., 2009). Despite
626 the fact that IDPs/IDRs are commonly associated with various diseases, the IDRs themselves
627 might not be directly responsible for increased disease risk (Pajkos et al., 2012; Tompa, 2009).



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

628 Although the countless roles of IDPs in the pathogenesis of various diseases undoubtedly
629 represent an exciting subject, a detailed consideration of this phenomenon is outside the scope of
630 this review, and interested readers are encouraged to look for recent studies focused on this
631 subject (e.g., see (Alberti and Hyman, 2016; Ambadipudi and Zweckstetter, 2016; Choudhary et
632 al., 2022; Darling and Uversky, 2017; 2018; Hivare et al., 2023; Pancsa et al., 2019; Popelka and
633 Uversky, 2022; Raasakka and Kursula, 2020; Santofimia-Castano et al., 2020; Tsang et al., 2020;
634 Uversky, 2013b; 2018)).

635

## 636 III. How to target a dancing protein cloud

### 637 *III.A. Why one would want to target an IDP?*

638 Should we consider IDPs/IDRs as potential drug targets? The answer is undoubtedly yes. In fact,
639 there are multiple reasons to very seriously consider targeting IDPs and IDRs. The arguments
640 supporting this statement were already emphasized in the previous sections of this review. How
641 can one ignore proteins that constitute a very significant part of a human proteome, have
642 numerous crucial functions, act as multifunctional promiscuous binders capable of binding and
643 folding in a template-dependent manner, and are tightly involved in the pathogenesis of various
644 human diseases (Cheng et al., 2006; Dunker and Uversky, 2010; Metallo, 2010; Uversky, 2012)?
645 Therefore, it is absolutely clear that IDPs should be targeted (or at least, serious attempts should
646 be made to develop drugs specifically targeting IDPs/IDRs). On the other hand, it is also
647 absolutely clear that these proteins and protein regions cannot be subjected to the rational,
648 structure-based drug design (which became an essential tool for fast and cost-efficient lead
649 discovery for ordered proteins, and where prior knowledge of a target protein structure represents



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

650 the most important prerequisite for the successful development of a new drug), as they do not
651 have stable structures. Therefore, the druggability of "protein clouds" that do not possess ordered
652 structure and exist as highly flexible and extremely dynamic conformational ensembles is
653 questionable (if not impossible) from the viewpoint of the traditional structure-based rational
654 drug design approaches (Joshi and Vendruscolo, 2015). In fact, it seems that most of the prior
655 knowledge of what should be done to find a small molecule capable of high affinity and
656 selectivity binding to a specific "grove" or "cavity" on the surface of a "rock" would not work
657 for a fuzzy cloud, and therefore, in the intrinsic disorder-based drug design, new approaches
658 based on outside-the-box thinking are required to find drugs targeting IDPs/IDRs, affecting their
659 interactions, and modulating their (multi)functionality (Uversky, 2012).

660

661 ***III.B. Some general strategies for targeting IDPs***

662 In addition to the obvious complication of the lack of unique 3D structure, which makes
663 IDPs/IDRs mostly unsuitable for rational drug design, where prior knowledge of a target protein
664 structure represents the most important prerequisite for the successful development of a new
665 drug, the use of IDPs/IDRs as drug targets is further complicated by another crucial hurdle,
666 namely their binding promiscuity. In fact, depending on their interactions with specific partners,
667 IDPs/IDRs, being neomorphic moonlighting proteins, can form both physiological and
668 pathological complexes defining their ability to have physiological or pathological functions
669 (Szenasi et al., 2017). This need to find a drug lead that would be capable of selective targeting
670 of the pathological and not the physiological complexes of a given IDP represents a new type of
671 challenge in drug development. A convincing illustration of this complexity is given by the



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

672 analysis of two IDPs involved in the pathogenesis of Parkinson's disease and other
673 synucleinopathies, tubulin polymerization promoting protein (TPPP/p25) and α-synuclein
674 (Szenasi et al., 2017). These two proteins are known to be co-enriched and co-localized in
675 pathological brain inclusions (Szenasi et al., 2017). These highly disordered proteins are engaged
676 in the broad spectrum of protein-protein interactions, both forming tightly connected PPI
677 networks (see **Figure 5**). Consideration of all these PPIs to find which of them would lead to the
678 formation of physiological or pathological complexes is a daunting task. However, the available
679 information for TPPP/p25 indicated that this protein is able to form physiological (TPPP/p25-
680 tubulin) and pathological (TPPP/p25-α-synuclein) complexes. Analysis of these systems
681 revealed that formation of these complexes relies on different parts of the TPPP/p25, a fact that
682 should simplify the search for potential interaction inhibitors. However, TPPP/p25 showed
683 exceptional functional resilience, where, due to its high conformational plasticity and chameleon
684 nature, the removed binding segments were functionally replaced by other segments (Szenasi et
685 al., 2017). Despite these challenges, this study concluded that the disorder-based interfaces can
686 be accurately identified, and finding such interfaces represents a promising approach for
687 targeting promiscuous IDPs/IDRs (Szenasi et al., 2017).

688 Therefore, although the development of drugs targeting IDPs and IDRs represent a challenging
689 task, the novel field of finding small molecules capable of modulation of the functionality of
690 IDPs/IDRs is rapidly progressing, and several innovative strategies for the effective discovery
691 and design of the drugs affecting the disorder-based interactions were successfully elaborated
692 (Dunker and Uversky, 2010). Some of the currently available approaches for targeting
693 IDPS/IDRS and disorder-based interactions are briefly outlined below, along with illustrative
694 examples.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

695 An obvious way to deal with IDPs is to use their structural heterogeneity, as many IDPs
696 represent complex hybrid systems containing both ordered domains and IDRs. Here, ordered
697 domains can be subjected to the traditional routine of drug discovery elaborated for ordered
698 proteins, where the drugs blocking ligand binding sites in such ordered domains are found using
699 either random screening of possible compounds or rational drug design based on the prior
700 knowledge of the domain's 3D structure (Dunker and Uversky, 2010; Uversky, 2012). Drugs
701 inhibiting interaction of IDPs/IDRs with ordered partners can also be identified by utilizing the
702 available structural information for these ordered partners. If an IDR can fold at binding to its
703 partner (i.e., if it operates via the coupled binding and folding mechanism) and if the resulting
704 interaction sites are relatively small and compact, then they can be easily mimicked by small
705 molecules. These approaches indicate that small molecules directly targeting IDP/IDR can be
706 found using strategies that look similar to traditional drug discovery approaches. However, these
707 forms of rational drug design are still disorder-based and therefore will clearly benefit from the
708 prior knowledge of the intrinsically disordered nature of the binding partners (Dunker and
709 Uversky, 2010; Uversky, 2012).

710 It is known that for interaction with their partners, many IDPs/IDPRs use relatively short
711 contiguous regions that become ordered upon binding (Garner et al., 1999; Oldfield et al.,
712 2005b). Furthermore, IDPs/IDRs are capable of context- or template-dependent folding, forming
713 very different structures in their bound forms complexed with different partners (Dunker et al.,
714 2001; Hsu et al., 2012; Karush, 1950; Kriwacki et al., 1996; Oldfield et al., 2008). In such cases,
715 successful inhibition of the disorder-based PPI interactions can be achieved via misfolding of the
716 foldable binding sites into structures, which are unlike their structures in the bound states. In
717 other words, small molecules would force folding-upon-binding IDRs to form ordered structures



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

718 incompatible with binding to a partner. Therefore, in these cases, the disorder-based functions
719 and formation of biologically important protein-protein complexes are prevented by small
720 molecules capable of inducing (wrong) order in the targeted IDPs/IDRs (Dunker and Uversky,
721 2010; Hammoudeh et al., 2009; Metallo, 2010; Uversky, 2012; Wang et al., 2007; Yin et al.,
722 2003).

723 One of the ways for an IDP/IDR to escape unwanted interaction is to spontaneously form a non-
724 interactive cage sequestering interaction-prone preformed fragments; i.e., to functionally misfold
725 (Uversky, 2011a). This mechanism can be utilized to find small molecules capable of
726 stabilization of the different members of such functionally misfolded conformational ensembles,
727 and thereby precluding the targeted protein from biological interactions (Uversky, 2012).

728

729 ***III.C. Targeting transcription factors***

730 *III.C.1. General considerations*

731 Both the control of the gene activity in response to the specific stimuli and the expression of
732 specific gene are crucially dependent on the regulation of gene transcription (Latchman, 1997).
733 Important players in the related processes are transcription factors (TFs). These sequence-
734 specific DNA-binding factors have several important functions, such as recognition and binding
735 of specific DNA sequences, recruitment of the RNA polymerase to specific genes, control of the
736 genetic information transfer from DNA to mRNA, and acting as specific activators or repressors
737 of transcription via positive or negative regulation of gene transcription. To conduct these
738 numerous functions, TFs can either act alone or in a complex with other proteins that can
739 activate or deactivate the TF action (Latchman, 1997). Depending on what they do and how they



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

740 are controlled, TFs are classified as general or basal TFs that form a pre-initiation complex and
741 are needed for the transcription by RNA polymerase II, upstream TFs that stimulate or repress
742 transcription by binding upstream of the initiation site, and inducible TFs (ITFs), which are
743 products of the immediate-early genes capable of activation or repression of the transcription of
744 the late-response genes via specific binding to the regulatory DNA sequences upstream of the
745 initiation site. All ITFs and many other TFs can be rapidly induced in cells in response to
746 activating stimuli (Ziólkowska and Przewlocki, 2002).

747

748 *III.C.2. Crucial hybrids: Order and intrinsic disorder in transcription factors*

749 TFs are characterized by functional modularity for which they contain one or more DNA-binding
750 domains (DBDs), at least one transactivation domain, and signal sensing domain(s) (SSDs, such
751 as ligand binding domains, LBDs) capable of recognition of the external signals and transmission
752 them to the rest of the transcription complex, thereby leading to the up- or down-regulation of
753 gene expression. Often, DBDs and SSDs reside on separate proteins that interact within the
754 transcription complex to regulate gene expression.

755 The aforementioned multifunctionality and functional modularity of TFs also suggest that these
756 important proteins might represent structural hybrids containing ordered domains and functional
757 IDRs. In line with this hypothesis, the Protein Data Bank does not have 3-D structures of the
758 whole eukaryotic TFs, but contains abundant structural information on the TF modules (e.g.
759 DBDs and LBDs) (Minezaki et al., 2006). Furthermore, crucial roles of IDRs in TF functionality
760 was demonstrated for some specific TFs (Dyson and Wright, 2002b; 2005), and, based on a
761 comprehensive bioinformatics analysis, it was conceded that most TFs (82-94%) contain long



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

762 IDRs, with the eukaryotic TFs being significantly more disordered than the prokaryotic TFs (Liu
763 et al., 2006a; Minezaki et al., 2006). For example, in human TFs, almost 50% of the amino acid
764 sequences are occupied by IDRs, with more than half of the human TFs containing small DBDs
765 and long IDRs (Minezaki et al., 2006). Intrinsic disorder is heterogeneously distributed within
766 TFs, as their activation regions are typically essentially more disordered than the DBDs (Liu et
767 al., 2006a). Furthermore, in addition to the ordered DBDs that are well-structured in the non-
768 bound state, some DBDs can be considered as inducible foldons, as they are highly unstructured
769 in the unbound form and undergo a disorder-to-order transition upon DNA binding (Liu et al.,
770 2006a). Importantly, it was pointed out that TFs commonly contain inducible foldons (Liu et al.,
771 2006a), also known as molecular recognition features, MoRFs (Oldfield et al., 2005b), and even
772 inducible morphing foldons that can adopt different conformations when binding to different
773 partners (Oldfield et al., 2008). Because of the abundant presence of functional disorder in TFs,
774 it is not surprising to see that their functionality is regulated by alternative splicing (Hofstetter et
775 al., 2010) and posttranslational modification (Avalos et al., 2002).

776

777 *III.C.3. Transcription factors as potential and actual drug targets*

778 Because of their crucial roles in a wide spectrum of physiological and pathological processes,
779 TFs are commonly considered as promising drug targets (Latchman, 2000), and ~ 10% of
780 currently prescribed drugs directly target TFs from the nuclear receptor family (Gronemeyer et
781 al., 2004; Overington et al., 2006). Furthermore, it is clear that the functionality of TFs can be
782 modulated indirectly via numerous signaling cascades. Being multifunctional structural hybrids,
783 TFs represent important illustrative examples of the utilization of principally different molecular



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

784 mechanisms for (dis)order-based drug discovery. In fact, small molecules can be found that
785 either block the ligand binding sites in ordered TF modules or inhibit interaction of the
786 intrinsically disordered TF with the ordered TF partner by blocking the binding site of the
787 ordered partner, or directly target IDRs in TFs.

788

789 *III.C.4. Targeting ordered domains of transcription factors*

790 DBDs or LBDs, which are often well-structured TF modules (e.g., in nuclear receptors),
791 commonly serve as targets for the small molecules capable of recognition and blocking their
792 specific DNA- or ligand-binding sites. For example, the LBDs of all nuclear receptors studied so
793 far have similar 3-D structure, where a compact structure with a ligand-binding pocket is formed
794 by 11 helices, and where the mobile twelfth helix acts as a movable lid over the ligand-binding
795 pocket and controls the entrance to this pocket (Gronemeyer et al., 2004; Wurtz et al., 1996), the
796 size of which varies between family members (Wurtz et al., 1996). Furthermore, the orientation
797 of the twelfth helix relative to the reminder of an LBD is crucial for the LBD action as a signal
798 transducer, where in the absence of ligands, LBDs bind to a set of transcriptional co-repressors,
799 whereas in the ligand-bound form, LBDs specifically interact with transcriptional co-activators
800 (Gronemeyer et al., 2004). The available structural information was used to develop small
801 molecule pharmaceuticals with a large spectrum of agonist or antagonist activities, as well as a
802 set of selective modulators of the nuclear receptors which were capable, in a targeted cell-type-
803 selective manner, to modulate and control a subset of the functions of these important proteins
804 (Gronemeyer et al., 2004).

805



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

806 *III.C.5. Disorder-based rational drug design: the p53-Mdm2 story*

807 One of the three potential targets of structure-aided drug design are ordered proteins interacting
808 with IDRs of TFs. This type of interaction is commonly based on the insertion of a relatively
809 short fragment of IDR to a groove or cavity on the surface of an ordered partner, which can be
810 targeted by small molecules reasonably well (Cheng et al., 2006). Obviously, for the ordered
811 proteins with known 3D structures, the use of special computational approaches from the realm
812 of *in silico* screening tools can aid in finding the potential interaction surfaces that can be
813 targeted by small molecules (Liang et al., 1998) capable of inhibition of protein-protein
814 interactions (Joce et al., 2010). One of the most convincing examples of this approach for
815 finding/designing drugs capable of inhibition of a disorder-based binding of a TF to an ordered
816 partner is given by the successful development of the inhibitors of the interaction of the
817 disordered fragment of the cellular tumor antigen p53 (residues 15-29) with the ordered domain
818 of E3 ubiquitin-protein ligase Mdm2 (residues 17-125).

819 With more than 120,000 papers in PubMed talking about it, transcription regulator p53 does not
820 require a lengthy introduction. Activated p53 accumulates in the nucleus, where it causes
821 induction or inhibition of over 150 genes via binding to specific DNA sequences (Balint and
822 Vousden, 2001; Zhao et al., 2000). Therefore, it is not surprising that p53 is known as a
823 caretaker, keeping other genes healthy (i.e. suppressing mutation) and helping the genome
824 recover from damaging mutations, and also as a gatekeeper, controlling or inhibiting cell growth
825 (Deininger, 1999). This protein, being a crucial tumor suppressor, also acts as a major
826 contributor to the progression of various cancers, as it is mutated in over 50% of all human
827 tumors (Deininger, 1999). Similar to other TFs, p53 has a modular structure possessing an N-
828 terminal intrinsically disordered transactivation domain (TAD, which includes an



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

829 aforementioned Mdm2 binding region), the centrally-located mostly ordered DBD, and the C-
830 terminal intrinsically disordered tetramerization and regulatory domain. Besides Mdm2, the N-
831 terminal TAD of p53 can bind CBP/p300, CSN5/Jab1, RPA, TFIID, TFIIH, among many other
832 proteins (Anderson and Appella, 2004). Similarly, the C-terminal regulatory domain is also a
833 promiscuous binder interacting with 14-3-3, hGcn5, GSK3β, PARP-1, TAF1, TRRAP, and
834 S100B(ββ) and many other proteins (Anderson and Appella, 2004). The intrinsically disordered
835 p53 TAD binding to Mdm2 is accompanied by the disorder-to-order transition in the p53 TAD
836 (Cheng et al., 2006) and has several crucial outputs, such as direct inhibition of the p53
837 functioning as an activator or inhibitor of other genes, proteasomal degradation of the p53 after
838 the Mdm2-driven ubiquitination, and transport p53 out of the nucleus due to the presence of a
839 nuclear export signal in Mdm2 (Anderson and Appella, 2004). Since all these outputs cause the
840 shutting down of p53, an inhibition of the p53-Mdm2 interaction by small drug-like molecules
841 capable of blocking the p53 binding site of Mdm2 represents a promising approach for restoring
842 the crucial cellular p53 functions (Chene, 2004; Klein and Vassilev, 2004; Vassilev, 2004;
843 Vassilev et al., 2004). Several such small molecules were found, and one of them, Nutlin-2, was
844 shown to efficiently mimic the p53 residues crucial for the Mdm2 binding (Klein and Vassilev,
845 2004; Vassilev, 2004; Vassilev et al., 2004). **Figure 6** shows p53 and Mdm2 from an interesting
846 angle, revealing that both proteins are expected to contain high levels of intrinsic disorder. In
847 fact, predicted disordered residues constitute more than 65% of the sequences of these proteins.
848 **Figure 6** also shows that the p53-Mdm2 complex originates from the interaction of an inducible
849 foldon of p53 (residues 15-29) with the ordered N-terminal domain of Mdm2 (residues 17-125,
850 SWIB/MDM2). Based on the UniProt annotations, the intrinsically disordered N-terminal TAD
851 of p53, besides binding to Mdm2, can interact with Mdm2, histone acetyltransferase p300,



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

852 CREB-binding protein (CBP), high mobility group protein B1 (HMGB1), and protein arginine
853 N-methyltransferase 1 (HRMT1L2) whereas SWIB/MDM2 domain binds p53, p73, and E2F1.
854 This binding promiscuity of both partners clearly represents an additional level of complexity of
855 the development of drugs targeting this complex.

856 The molecular principles of the disorder-to-order transition-based interactions in one of the
857 binding partners are understood rather well (Cheng et al., 2006; Oldfield et al., 2005b). In fact,
858 the IDRs capable of undergoing disorder-to-order transitions at interaction with their binding
859 partners are potentially predictable (Cheng et al., 2006; Dosztanyi et al., 2009; Fuxreiter et al.,
860 2004; Fuxreiter et al., 2007a; Meszaros et al., 2009; Oldfield et al., 2005b). These bioinformatics
861 tools developed for predicting foldable binding sites in disordered regions (Oldfield et al.,
862 2005b) can be used to find potentially druggable short disordered binding regions (Cheng et al.,
863 2006). Since as a result of the coupled binding and folding processes, these short disorder-based
864 binding motifs fold into small and compact structures, they can be potentially mimicked by small
865 molecules capable of inhibiting such interactions (Cheng et al., 2006). Therefore, in these cases,
866 the small molecules mimic a critical motif of the disordered partner, which folds upon binding
867 into a specific structural element, and competes with this region for its binding site on the
868 structured partner.

869

870 *III.C.6. cMyc-Max journey*

871 The interaction between two intrinsically disordered TFs c-Myc and Max serves as a very
872 important illustration of a danger of using prior knowledge accumulated while working on one
873 set of systems on a system operating on different principles. The attractiveness of targeting this



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

874 complex stems from the fact that the proto-oncogene c-Myc (also known as Myc proto-oncogene
875 protein, c-Myc, class E basic helix-loop-helix protein 39 (bHLHe39), and transcription factor
876 p64), being involved in regulation of multiple genes controlling a wide spectrum of cellular
877 processes ranging from apoptosis to cell growth, differentiation, and metabolism, is
878 overexpressed in most human cancers (Dang, 1999) and that the c-Myc activation is driven by
879 the heredimerization of this protein with its partner, Myc-associated factor X (Max, also known
880 as class D basic helix-loop-helix protein 4 (bHLHd4)). The unbound forms of both Max and c-
881 Myc are mostly disordered (see **Figure 7**), whereas interaction of their basic region/helix-loop-
882 helix/leucine zipper (bHLHZip) domains is accompanied by their mutual coupled binding and
883 folding, leading to the formation of a helical coiled coil complex (see **Figure 7**) (Hammoudeh et
884 al., 2009; Metallo, 2010). Therefore, disruption of this heterodimetic complex represents a
885 reasonable strategy for c-Myc inhibition. However, using known structures of triple c-Myc-Max-
886 DNA complexes in rational structure-based drug design was unsuccessful, as the small
887 molecules identified by this approach failed to produce inhibitory effect. The mystery was
888 resolved when several specific inhibitors of the c-Myc-Max interactions were identified using the
889 traditional high throughput screening (Wang et al., 2007; Yin et al., 2003). Using solution NMR
890 analysis of the monomeric c-Myc it was established that the identified inhibitors bind to one of
891 three discrete sites within its bHLHZip domain (residues 366−375, 375−385, and 402−409).
892 Importantly, all these binding sites were located within the C-terminal region of c-Myc that was
893 disordered when not bound to Max. Interaction of these small molecules with the c-Myc
894 bHLHZip domain induced local folding of binding regions into structures incompatible with the
895 formation of the Myc–Max dimer; i.e., it promoted local misfolding that made the protein
896 dysfunctional (Hammoudeh et al., 2009; Metallo, 2010). Therefore, this case revealed that the



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

897 locally induced misfolding might represent a principally new way of inhibition of the disorder-
898 based interaction by small molecules.

899 It is important to emphasize here that although structure-based drug design is difficult for the
900 Myc-Max system and IDPs generally, pharmacophore models have proven possible. For
901 example, structural diversity of Myc-Max inhibitors was successfully extended using a
902 pharmacophore-based *in silico* screen of a large compound library linked to a rapid *in vivo*
903 screen (Han et al., 2019). Here, a pharmacophore model, which is a molecular framework
904 describing the essential features of a compound that allow it to bind to a biological target, was
905 created. To find compounds potentially targeting Myc-Max system, the model included 5 point
906 pharmacophoric features, such as one aromatic hydrophobic, two hydrogen bond donors, one
907 hydrogen bond acceptor, and one hydrophobic feature. This pharmacophore model was used to
908 screen a 16 million compound library, and 61 potential hits were identified and tested for their
909 possibility to affect Myc activity (Myc-Max-DNA complex formation, Myc transcriptional
910 activity, and Myc-Max-dependent cell viability). As a result, a set of small molecule acting as
911 Myc inhibitors was developed. The compounds were capable to engage Myc inside cells, disrupt
912 Myc/Max dimers, and impair Myc-driven gene expression, indicating that the proposed approach
913 of sampling a larger chemical space coupled with the rapid screening of candidates in mice
914 provides a promising way for the discovery of small molecules affecting IDP functions (Han et
915 al., 2019).

916

917 *III.C.7. Targeting EWS-FLI1 and other oncogenic translocation-generated fusion proteins*



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

918 Many types of cancer originate from the oncogenic chromosomal translocations, which is
919 described as the exchange of genetic components between two non-homologous chromosomes
920 (Nussenzweig and Nussenzweig, 2010), and if it happens across the coding regions of two genes,
921 it generates a chimeric gene which can be translated into a chimeric protein. One of the
922 illustrative examples of this type of cancer is given by a family of highly malignant tumors of
923 bone and soft tissue known as the Ewing's sarcoma family of tumors (ESFT), the second-most
924 common primary bone malignancy in children, adolescents, and young adults (Delattre et al.,
925 1992; Erkizan et al., 2010). ESFT is believed to originate from the chromosomal translocation
926 events, where the *EWS breakpoint region 1* (*EWSR1*, or *EWS*) gene on the chromosome 22 is
927 fused to one of the five *ETS* gene family members, *FLI1*, *ERG*, *ETV1/ER81*, *E1AF/PEA3*, and
928 *FEV*, generating a set of the EWS-ETS chimeric proteins, 85% of which is EWS-FLI1
929 (Lawrence et al., 2014). The only genetic alteration in these pediatric tumors is the appearance of
930 the EWS/ETS oncogenic fusion proteins, which function as aberrant transcription factors
931 (Crompton et al., 2014; Tirode et al., 2014), and whose persistent expression is absolutely
932 required for transformed phenotype maintenance (Kovar et al., 1996).

933 As a result of the chromosomal translocation, the RNA-binding domain of EWS is replaced by
934 the DNA-binding domain of FLI1 (Delattre et al., 1992), and the resulting EWS−FLI1 chimeric
935 protein acts as a transcription factor modulating a diverse group of target genes (Uren et al.,
936 2004). An IDR of EWS-FLI1 is required for maximal transactivation of transcription (Ng et al.,
937 2007; Uren et al., 2004). Structurally, EWS−FLI1 can be divided to two domains, a highly
938 disordered N-terminal domain of the EWS origin and a structured DBD of the FLI1 origin (see
939 **Figure 8**). EWS-FLI1 binds to DNA as a part of ternary protein complexes, with the adjacent
940 DNA sequences dictating the choice of the binding partners (Watson et al., 1997). Most of the



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

941 EWS−FLI1 protein-protein interactions happen within the disordered N-terminal domain, with
942 some well-established protein partners of EWS-FLI1 being BARD1, CREB-binding protein,
943 FOS-JUN dimers, hRPB3 subunit of RNA polymerase II, RNA helicase A (RHA), and TFIID
944 (Erkizan et al., 2010). This binding promiscuity of EWS-FLI1 is responsible for the involvement
945 of this protein in the formation of various nuclear complexes which are facilitated by the EWS-
946 FLI1 IDR and lead to oncogenesis (Erkizan et al., 2010). However, the C-terminally located IDR
947 of EWS-FLI1 was shown to drive some of the critical interaction of this TF with other
948 transcriptional regulators and govern the binding of other proteins to the adjacent DNA
949 regulatory elements (Erkizan et al., 2010). The oncogenic function and the neoplastic
950 transformation potential of the fusion EWS-FLI1 protein is driven by interaction of this hybrid
951 protein with RHA (Erkizan et al., 2009; Toretsky et al., 2006). Therefore, the finding of the
952 inhibitors of this EWS-FLI1-RHA interaction represents a promising way of the anti-ESFT drug
953 development. However, it is also recognized that the intrinsically disordered nature of the
954 oncogenic EWS-ETS proteins and the lack of the small molecule recognizable pockets make
955 these hybrid proteins (as well as TFs in general (Koehler, 2010)) difficult subjects for direct
956 targeting [38], indicating that to mitigate the oncogenic functions of the oncogenic EWS-ETS
957 proteins one should also identify and target functionally relevant EWS/ETS-associated factors
958 (Gollavilli et al., 2018). Despite these concerns, a small molecule YK-4-279 capable of
959 inhibiting the EWS-FLI1 interaction with RHA was found using a screen of a library of 3,000
960 compounds (Erkizan et al., 2009). This observation indicated that, likely similar to the
961 aforementioned c-Myc-Max case, the found lead compound was able to bind to the intrinsically
962 disordered protein–protein interaction site of EWS-FLI1 (Erkizan et al., 2009). An YK-4-279
963 derivative, TK-216, with demonstrated *in vitro* and *in vivo* antitumor activity in ESFT models



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

964 was developed for clinical trials (Federman et al., 2017; Selvanathan et al., 2017; Spriano et al.,
965 2019).

966

967 *III.C.8. Androgen receptor*

968 An important example of drug development to target a complex, modular transcription factor is
969 given by a set of small molecules interacting with an intrinsically disordered transactivation
970 domain of the androgen receptor (AR) (Obst et al., 2024). AR is a modular, ligand-activated
971 transcription factor from the super family of nuclear steroid receptors. Being activated by
972 androgens, AR is involved in regulation of cardiovascular, musculoskeletal, and hematopoietic
973 systems, whereas its misbehavior is associated with various pathologies including prostate
974 cancer, where AR is commonly overexpressed (Davey and Grossmann, 2016). In AR, one can
975 find three functional domains, the N-terminal intrinsically disordered transactivation domain
976 (TAD, residues 1-557), the DNA-binding domain (DBD, residues 560-660) that contains two
977 zinc-finger motifs (residues 560-580 and 596-620), and the C-terminal ligand-binding domain
978 (LBD, residues 669-920). TAD, which is a primary driver of the AR transcriptional activity, is
979 intrinsically disordered (Lavery and McEwan, 2005; McEwan et al., 2007; Tan et al., 2015).

980 The intrinsically disordered nature of TAD defines the multifunctionality and binding
981 promiscuity of AR, which is known to have almost 300 binding partners (Dai et al., 2023; Dai et
982 al., 2017; Heemers and Tindall, 2007; Obst et al., 2024). It was also indicated that the ability of
983 TAD to adopt numerous unique conformations enabling rapid and transient protein–protein
984 interactions with many structurally diverse binding partners is defined by the conformational
985 plasticity of its intrinsically disordered structure (Obst et al., 2024). This idea is illustrated by



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

986 **Figure 9A** representing the functional disorder profile generated for human AR by the $D^2P^2$
987 platform (Oates et al., 2013) and showing that the N-terminally-located TAD of this protein
988 contains 11 MoRFs, as well as by **Figure 9B** representing the 3D model generated by AlphaFold
989 (Jumper et al., 2021) and showing high overall prevalence of disorder in AR.

990 It is therefore not surprising that AR transactivation domain inhibitors (ARTADIs) have attracted
991 the attention of researchers, as these compounds can block AR activity potentially without the
992 adverse effects associated with androgen deprivation therapy (ADT) (Ban et al., 2021; Banuelos
993 et al., 2016; Obst et al., 2024; Sadar et al., 2008; Yi et al., 2024; Yi et al., 2023). It was found
994 that ARTADIs can exclusively target intrinsically disordered TAD of AR and do not prevent
995 ligands from binding to the AR-LBD. In particular, these compounds were effective in
996 modulating AR activity when the structured portion of the receptor had been circumvented (e.g.,
997 in castration-resistant prostate cancer, CRPC) leaving the disordered TAD as the viable
998 remaining target (Vaishampayan et al., 2017). The molecular mechanism of the ARTADI action
999 was revealed by the all-atom molecular dynamics computer simulations, which showed that
1000 these compounds can bind at the interface of two transiently helical regions and induce the
1001 formation of partially folded collapsed helical states (Zhu et al., 2022). It was also shown that
1002 TAD is crucial for the AR activity as transcription factor, allowing AR translocation to the
1003 nucleus upon activation by androgens, where it undergoes LLPS and forms transcriptional
1004 condensates, mesoscale nuclear "speckles", associated with the key AR functions (Basu et al.,
1005 2023). Such function-related phase separation of AR was modulated by short transient helices
1006 found in the regions of a TAD sequence that are rich in aromatic residues. Importantly,
1007 ARTADIs were shown to partition into AR condensates, where they interacted with aromatic
1008 residues in the AR TAD, eventually trapping this domain in a conformation that disfavors



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1009 interactions with effector partners. These observations were then used to improve the ARTADI
1010 potency (Basu et al., 2023). As such, AR represents a noteworthy example of the importance of
1011 having means for targeting the IDRs of hybrid proteins containing ordered and intrinsically
1012 disordered domains.

1013

1014 ***III.D. Irrational drug design: Targeting functionally misfolded IDPs/IDRs***

1015 IDPs/IDRs, being characterized by high conformational dynamics and flexibility and the
1016 presence of highly interaction-prone binding elements, can escape unwanted interactions with
1017 non-native partners via the functional misfolding mechanism, where the interaction-prone
1018 elements are sequestered inside the non-interactive or less-interactive cage (Uversky, 2011a). In
1019 line with this hypothesis, it is known that, despite the absence of persistent structural elements in
1020 many IDPs/IDRs, their conformational space is not completely random but contains a rather
1021 limited set of preferential conformations, which can be structurally characterized by combining
1022 NMR spectroscopy with advanced structure determination/modeling techniques of computational
1023 biology (Allison et al., 2009; Bernado et al., 2005; Bertoncini et al., 2005; Dedmon et al., 2005;
1024 Jensen et al., 2009; Salmon et al., 2010; Sung and Eliezer, 2007; Vendruscolo, 2007; Wu et al.,
1025 2008; Wu et al., 2009). For example, in the case of a canonical IDP α-synuclein it was shown
1026 that the fluctuating structural ensemble of this protein in solution is characterized by the presence
1027 of a wide range of conformations, including relatively compact transient states originating from
1028 long-range contacts between the temporarily populated elements of the residual local structure
1029 (Bertoncini et al., 2005; Dedmon et al., 2005). These relatively compact transient conformations
1030 represent an illustrative example of the aforementioned functionally misfolded state of an IDP.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1031 It seems that this functionally misfolded conformational ensemble represents an attractive drug
1032 target, assuming that small molecules can be found that would be capable of stabilization of
1033 different members of this ensemble, thereby precluding the targeted protein from making
1034 biological interactions. It is important to emphasize here that this approach is principally
1035 different from the aforementioned direct targeting of short IDRs, as here, a small molecule is
1036 expected to bind to a highly dynamic surface generated by the transient contacts between the
1037 interaction-prone elements. Within the context of the functionally misfolded conformational
1038 ensemble model, a search for such small molecules is more reminiscent of the well-established
1039 structure-based rational drug design approach elaborated for ordered proteins, assuming that the
1040 structures of the individual members of the ensemble can be guessed. In this case, tools
1041 originally developed for rational structure-based drug design for ordered proteins (e.g., *in silico*
1042 screening methods based on the high-throughput docking of large small molecule libraries to
1043 structured proteins (Lavecchia and Di Giovanni, 2013; McInnes, 2007)) can be utilized to find
1044 small molecules that are potentially able to interact with these guessed structures, thereby
1045 stabilizing them and locking the cage.

1046 The idea that the proposed approach might actually work was presented in a study by Toth et al.,
1047 who used a complex combinatorial approach comprising a set of computational and
1048 experimental techniques to find a drug-like phenyl-sulfonamide compound (ELN484228)
1049 capable of targeting α-synuclein, and showing substantial biological activity in the cellular
1050 models of α-synuclein-mediated dysfunction (Toth et al., 2014). In their search for small
1051 molecules targeting intrinsically disordered α-synuclein, the authors first randomly selected 100
1052 structures from the conformational ensemble containing 40,000 α-synuclein structural models
1053 derived from comprehensive NMR analysis. Then, they selected 22 more compact structures and



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

1054 used them as targets in the computational fragment probe mapping (Kortvelyesi et al., 2003) to
1055 identify potentially druggable hot spots (Toth et al., 2014). Based on this analysis, eight potential
1056 binding pockets, which were typically formed through relatively long-range tertiary contacts,
1057 were identified. Each of these pockets was located in a different member of the α-synuclein
1058 ensemble. In the next step, each of these binding pockets was used as a target for computational
1059 docking of a library of ~ 33,000 fragment-like compounds with molecular masses under 325 Da,
1060 leading to the identification of 89 potential ligands for these binding pockets (Toth et al., 2014).
1061 At the final stage, one of the identified *in silico* hits, ELN484228, was used in α-synuclein
1062 aggregation assays and in cellular models of α-synuclein malfunction (Toth et al., 2014). This
1063 analysis revealed the protective role of ELN484228 in cellular models of α-synuclein-mediated
1064 vesicular dysfunction (Toth et al., 2014). The importance of this study cannot be overestimated,
1065 as it serves as a solid proof of the idea that the dynamic and highly heterogeneous
1066 conformational ensembles of IDPs contain specific functionally misfolded members, which are
1067 relatively compact, contain small molecule binding pockets, and therefore can be targeted by
1068 drugs. These findings open intriguing and very promising possibilities in the field of drug
1069 discovery for pathology-related IDPs/IDRs.

1070

1071 ***III.E. Targeting aggregating IDPs/IDRs***

1072 Since protein misfolding diseases, including numerous neurodegenerative disorders, are
1073 characterized by the pathological aggregation of misfolded species that initiates an avalanche of
1074 events leading to neurodegeneration and death (Uversky, 2009a; 2010b), overpowering the
1075 pathological protein aggregation by small molecules is considered as a very promising research



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1076 area. However, protein aggregation involves multiple parallel assembly pathways and the
1077 simultaneous existence of various metastable structures, making this research a daunting task
1078 (Liu and Bitan, 2012). Furthermore, many conformational diseases are driven by the misbehavior
1079 of IDPs/IDRs (Gadhave et al., 2020; Uversky, 2009a; 2012; 2014; Wang et al., 2011). To deal
1080 with these challenges, several strategies were proposed to find small molecules that are able to
1081 (Uversky, 2012; 2014; Wang et al., 2011): (*i*) directly bind to IDRs and block their aggregation
1082 by keeping them in the interaction-incompetent conformation; or (*ii*) interact with IDP/IDR and
1083 promote formation and stabilization of non-toxic and non-amyloidogenic oligometric species; or
1084 (*iii*) interact with amyloidogenic protein and dramatically accelerate its aggregation to minimize
1085 the duration of the toxic oligomer formation stage. One of the ways to perturb the
1086 oligomerization and aggregation processes is the use of small molecules known as "molecular
1087 tweezers" (Fokkens et al., 2005; Prabhudesai et al., 2012; Sinha et al., 2011) capable of caging
1088 the reactive residues (lysines) of the aggregating proteins (such as amyloid β-protein (Aβ), tau,
1089 islet amyloid polypeptide, and α-synuclein) inside the electron-rich torus-shaped cavity
1090 decorated with two peripheral anionic phosphonate groups (Prabhudesai et al., 2012; Sinha et al.,
1091 2011).

1092 Formation of stable, nontoxic oligomers located off the fibrillation pathway was efficiently
1093 induced in an important IDP involved in the pathogenesis of Parkinson's disease, α-synuclein, by
1094 different various small molecules (including several polyphenols) (Ehrnhoefer et al., 2008;
1095 Uversky, 2007; 2010b; Uversky and Eliezer, 2009). The small molecule-induced formation of
1096 such oligomers was shown to inhibit fibrillation of α-synuclein (Masuda et al., 2006; Uversky,
1097 2007; 2010b; Uversky and Eliezer, 2009; Yamaguchi et al., 2010). Similarly, a set of lead-like
1098 compounds and drug-like molecules was identified in a high-throughput chemical microarray



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1099 surface plasmon resonance imaging-based search for finding small molecules capable of binding
1100 to a monomeric tau protein, which is an important IDP involved in the pathogenesis of various
1101 neurodegenerative diseases collectively known as tauopathies (Pickhardt et al., 2015). Based on
1102 the premise that the mature amyloid fibrils are typically less toxic than some of the soluble
1103 oligomeric species transiently accumulating during the protein fibrillation process, it was
1104 hypothesized that the acceleration of the transition from oligomers to fibrils might represent an
1105 important, albeit an absolutely counter-intuitive, therapeutic strategy (Ross and Poirier, 2005).
1106 Systematic analyses of the consequences of the drug-accelerated aggregation of several proteins
1107 provided strong support to this hypothesis (Liu and Bitan, 2012).

1108 Finally, information on the small molecules known to inhibit aggregation of IDPs related to the
1109 pathogenesis of neurodegenerative diseases was used to generate fragment-based libraries of
1110 IDP-specific drugs by screening the existing large generic libraries of small molecules (Joshi et
1111 al., 2016). The usefulness of this approach providing a way to generate molecular scaffolds of
1112 drugs potentially targeting neurodegeneration-related IDPs was illustrated by creating IDP-
1113 specific libraries of small molecules capable of targeting the IDPs related to the pathogenesis of
1114 the Alzheimer's and Parkinson's diseases, Aβ, tau, and α-synuclein (Joshi et al., 2016).

1115

1116 ***III.F. Disorder-based development of consensus interferons***

1117 Interferons (IFNs) are a group of signaling proteins produced and released by the host cells in
1118 response to the presence of different pathogens, such as bacteria, parasites, tumor cells, viruses,
1119 and their diverse products (De Andrea et al., 2002). Since the release of IFNs by the infected
1120 cells represents a unique signal to the neighboring cells aiming at triggering the protective



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1121 mechanisms used by the immune system to eradicate pathogens, IFNs serve as cytokines for
1122 inter-cell communication (Parkin and Cohen, 2001). There are three major types of human INFs
1123 that are grouped based on the receptors they use to trigger the subsequent signals. Type I
1124 represents the largest group of IFNs that includes a set of IFNs-α encoded by at least 23 genes
1125 with no intron sequences (Pestka et al., 1987), IFN-β, IFN-δ, IFN-ε, IFN-κ, IFN-τ, and IFN-ϖ
1126 (Liu, 2005; Platanias, 2005). Type I IFNs bind to a specific multichain IFN-α/β receptor IFNAR,
1127 which is a complex containing at least two subunits, IFNAR1 and IFNAR2 (de Weerd et al.,
1128 2007). The only type II IFN in humans is IFN-γ, which binds to the oligomeric IFN-γ receptor
1129 (IFNGR) consisting of IFNGR1 and IFNGR2 chains (Parkin and Cohen, 2001). Finally, for
1130 transduction of their signals, the type III IFNs use a receptor complex that comprises IL10R2 and
1131 IFNLR1 chains (Kalliolias and Ivashkiv, 2010).

1132 IFN-α, being discovered more than 50 years ago by Isaacs and Lindenmann (Isaacs and
1133 Lindenmann, 1957) as an anti-viral agent, is secreted by nearly all cell types following the
1134 stimulation by viruses, bacteria, nucleic acids, and protozoa (Sen and Lengyel, 1992). Therefore,
1135 it is not surprising that IFNs-α, which can be further subdivided into several subtypes (e.g., IFN-
1136 α1, -α2, -α4, -α5, -α6, -α7, -α8, -α10, -α13, -α14, -α16, -α17, and -α21), are among the best
1137 studied IFNs. Different IFNs-α show high levels of sequence similarity, and sequences of human
1138 IFNs-α share between 70 and 80% homology, and about 35% identity with human IFN-β (El-
1139 Baky and Redwan, 2015; Genin et al., 2009). In line with this sequence conservation, structural
1140 analysis revealed a high level of structural similarity between these proteins, where IFNs-α
1141 (IFN-α1/13 and IFN-α2) and IFN-β are characterized by a compact structure composed of five
1142 major α-helices (named helix A to E) connected by a long loop and three shorter loops
1143 (Radhakrishnan et al., 1996). However, although the global α-helical fold of IFNs is conserved,



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1144 the arrangements of their loops show some noticeable differences (see **Figures 10A**, **10B**, and
1145 **10C**) (El-Baky et al., 2015b). Furthermore, analysis of the reported structures revealed the
1146 presence of noticeable structural flexibility in IFN-α proteins, suggesting that some of their
1147 regions can be intrinsically disordered (El-Baky et al., 2015b). This idea was further supported
1148 by the analysis of the per-residue intrinsic disorder propensity of 17 human IFNs-α, which
1149 clearly revealed the presence of several flexible or disordered regions, mostly within the N-
1150 terminal regions of these proteins (see **Figure 10D**) (El-Baky et al., 2015b).

1151 Recombinant IFN-α2 is the only interferon that is currently used for treatment of chronic
1152 hepatitis B and C, and leukemia (Borden et al., 2007; Gutterman, 1994). Furthermore, to improve
1153 the anti-viral efficiency of natural recombinant IFNs-α, a synthetic IFN-α, the consensus
1154 interferon cIFN-α, was created two decades ago (Blatt et al., 1996; Fish et al., 2008). This
1155 consensus interferon, being an artificial recombinant second-generation type I interferon,
1156 developed based on the sequence conservation among the fourteen natural human IFNs-α
1157 subtypes (Klein et al., 1988), was shown to possess 10- to 100-fold higher anti-viral and anti-
1158 proliferative activities than the naturally occurring α-interferon subtypes in humans (Blatt et al.,
1159 1996; Keeffe et al., 1997; Ozes et al., 1992; Sjogren et al., 2005; Tong et al., 1997). This higher
1160 efficiency of the cIFN-α was linked to the higher affinity of this protein to the type I IFN
1161 receptors than the naturally occurring IFN-α (Blatt et al., 1996).

1162 Comparison of the disorder profiles generated for two consensus IFNs-α with the mean disorder
1163 profile calculated as averaged disorder predispositions of 17 human IFNs-α revealed a
1164 remarkable similarity between these three disorder profiles (El-Baky et al., 2015b) (see **Figure**
1165 **11**). Furthermore, these three disorder profiles were closer to each other than the disorder profiles
1166 of most of the individual IFNs-α (cf. **Figure 10D**). Based on these observations it was



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1167 hypothesized that "if the peculiarities of disorder distribution within the protein sequence are
1168 related to protein functionality, then comparison of the disorder profiles of artificial proteins
1169 (query profiles) with the averaged disorder predisposition profile of human IFNs-α (target
1170 profile) can be used in the design of novel cIFNs. The goal here would be to achieve a close
1171 similarity between the query and target profiles by manipulating the cIFN sequence" (El-Baky et
1172 al., 2015b).

1173

1174 ***III.G. Unstructural vaccinology: Fighting fire with fire***

1175 Despite intensive research spanning several decades, there has been no advancement in finding
1176 efficient vaccines against hepatitis C virus (HCV), human immunodeficiency virus-1 (HIV-1),
1177 and herpes simplex viruses (HSVs). In addition to the accepted objective reasons for such failure,
1178 such as the capability of HSVs to establish latency (Stanfield and Kousoulas, 2015), the high
1179 mutation rates of the HCV and HIV-1 RNA viruses (Goh et al., 2019; Goh, 2017b; Ward, 2007)
1180 leading to the presence of a complex mixture of heterogeneous viral strains, "quasispecies" in the
1181 HIV-1 infected persons (Meyerhans et al., 1989) or multiple HCV genotypes (Palladino et al.,
1182 2018; Smith et al., 2014), the presence of neotopes (Khattar et al., 2013), and the highly
1183 glycosylated nature of proteins used as primary vaccine targets against these viruses (see, e.g.,
1184 (Chakraborty et al., 2020; Leonard et al., 1990; Vigerust and Shepherd, 2007)), an important but
1185 mostly overlooked factor is the presence of highly flexible regions and IDRs in human antibodies
1186 (Abs) and in the major HIV-1, HCV, and HSV immunogens (Uversky, 2022). Such structural
1187 flexibility prevents the utilization of structure-based reverse vaccinology and calls for utilization
1188 of tools designed for the analysis of disordered and flexible proteins, while looking at the
1189 intrinsically disordered viral antigens and their interactions with intrinsically disordered/flexible



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1190 Abs, i.e., unstructural vaccinology. In fact, the foundation of structure-based reverse
1191 vaccinology, which was successfully utilized for the identification of novel vaccine antigens and
1192 the improvement of the safety and immunogenicity of vaccine antigens (Burton, 2002; 2010;
1193 Masignani et al., 2002; Rappuoli, 2001; Rinaudo et al., 2009), is the use of information from the
1194 crystallographic structure of a complex between a neutralizing monoclonal antibody (mAb) and a
1195 complementary epitope to rationally design better antigens capable of acting as vaccine
1196 immunogens (Masignani et al., 2002; Rappuoli, 2001). However, considering antigen-antibody
1197 complexes as rigid, motionless structures with steric “lock-and-key”-type complementarity to
1198 each other is a clear oversimplification, as biological molecules are not crystals but represent
1199 dynamic conformational ensembles characterized by structural fluctuations of different
1200 amplitude happening at multiple time-scales. Furthermore, the binding sites of all the partners
1201 involved in the complex formation are not fixed in space and time, being instead relational
1202 entities engaged in mutual tuning, the scale of which ranges from rather minimal structural
1203 adjustments to global binding-induced folding (Uversky, 2022). Therefore, the “flexible keys and
1204 adjustable locks” model could provide a better description of the protein-based interactions that
1205 rely on the mutual adjustments of the partners via coordinated induced complementarity and fit
1206 (Edmundson et al., 1987). In line with these considerations, intrinsic disorder or structural
1207 flexibility is abundantly present on both sides of the Abs-antigen system, as antigen-binding sites
1208 of Abs are flexible/disordered, and many antigens contain noticeable levels of intrinsic disorder,
1209 or are at least characterized by high conformational plasticity (Uversky, 2022).

1210 For example, being one of the major HIV-1 antigens, gp120 contains multiple IDRs coinciding
1211 with, or located in close proximity to, the functional regions of this protein, such as highly
1212 genetically diverse variable regions, CD4-binding loop, and all N-linked glycosylation sites



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1213 (Uversky, 2022). Furthermore, although "crystallizable" variants of the HIV-1 glycoprotein
1214 gp120 (gp120 cores) were generated by removal of some disordered/flexible regions (see **Figure**
1215 **12**), the resulting truncated forms were still characterized by noticeable structural variability, as
1216 between 20% and 30% of each structurally characterized gp120 core contained some unique
1217 structural features, not seen in other forms (Uversky, 2021). Similarly, a hypervariable region of
1218 the HCV envelope glycoprotein E2 (residues 1-27), which shields the more conserved epitopes
1219 of this protein from neutralizing antibodies (The Lancet Gastroenterology, 2021), and the N-
1220 linked glycosylation sites of this protein were predicted to be disordered (Uversky, 2022). In the
1221 HSVs, all 12 surface glycoproteins were predicted to contain high levels of intrinsic disorder,
1222 with many important functional features of HSV glycoproteins and their N-glycosylation sites
1223 being overlapped or located within, or in close proximity to, the IDRs (Uversky, 2022). Taken
1224 together, these data for three viruses indicated the importance of intrinsic disorder in establishing
1225 a flexible glycan-IDR shield protecting potential epitopes from neutralizing antibodies (Uversky,
1226 2022).

1227 Let's now look at another side of the dynamic antigen-antibody complex and briefly consider
1228 some of the key features of the highly flexible structure of Abs. For a detailed analysis of this
1229 structural flexibility, interested readers are encouraged to check reference (Uversky, 2021). In
1230 fact, structures of Abs are highly dynamic at multiple levels. For example, in the Y-shaped IgG,
1231 an obvious illustration of structural pliability is given by the flexible linkers/hinges between the
1232 antigen-binding fragments $F_{ab}$ (arms) and the constant fragment $F_c$ (stem). As a results of this
1233 high conformational flexibility of hinges, Abs exist as highly dynamic conformational
1234 ensembles, and the known crystal Ab structures represent "snapshots" of these ensembles
1235 (Saphire et al., 2001). Furthermore, the discontinuous "active sites" of Abs, which are formed by



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1236 the 50-70 hypervariable residues containing paratopes (binding sub-sites chemically and
1237 structurally complementary to the epitopes), are characterized by high conformational dynamics
1238 as well. These antigen-binding sites of anti-protein Abs represent the short segments located
1239 within the six complementarity determining regions (CDRs), known as L1, L2, L3 on the light
1240 chain and H1, H2, and H3 on the heavy chain (Goldsby et al., 2003), and which are typically
1241 relatively flat. However, it was pointed out that human Abs are able to embed into the canyons
1242 and clefts on the antigen surface (Saphire et al., 2001; Smith et al., 1996) as their H3 loops
1243 located in the center of the binding sites are often extended (Barbas et al., 1993; Kunert et al.,
1244 1998; Sanna et al., 1995; Saphire et al., 2001), being characterized by a very broad spectrum of
1245 distinctive structural features containing on average 10 times more unique conformations than
1246 the other loops (Regep et al., 2017). The intrinsic disorder status of this loop in both heavy and
1247 light chains of a typical IgG was confirmed by a bioinformatics analysis (Uversky, 2022). The
1248 idea of high structural flexibility of the H3 loop was further supported by a comparative analysis
1249 of several anti-HIV Abs of human or bovine origin (Uversky, 2021), which revealed that the
1250 length of the H3 loop can vary in a very broad range, from 4 residues in a non-neutralizing HIV
1251 antibody 13H11 (PDB ID: 3MO1) to 16 residues in a broadly reactive and potent HIV-1
1252 neutralizing human antibody PG9 (PDB ID: 3U1S; (McLellan et al., 2011)), and to 60 residues
1253 in a potent HIV-1 bNAb NC-Cow1 (PDB ID: 6OO0; (Stanfield et al., 2020)), which is folded
1254 into a mini domain (knob) on an extended stalk structure (Stanfield et al., 2020) (see **Figure 13**).

1255 The presence of the "knob on a stalk" structure within the exceptionally long CDR H3 of the
1256 NC-Cow1 bNAb (which protrude up to 40 Å above the tips of the other CDR loops) defines its
1257 ability to efficiently bind to the HIV Env trimer BG505 SOSIP, as the "knob on the stalk" can
1258 navigate "through the dense glycan shield on Env to target a small footprint on the gp120 CD4



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1259 receptor binding site with no contact of the other CDRs to the rest of the Env trimer" (Stanfield
1260 et al., 2020). In other words, the "knob on an extended stalk" structure makes an extra-long CDR
1261 H3 a perfect "penetrator" capable of traversing through the glycan shield on Env. It was also
1262 pointed out that the neutralization potential of a given Ab correlates with the length of its CDR
1263 H3 loop, and bNAbs, which are typically characterized by high neutralization potential, tend to
1264 have long CDR H3 loops (Purtscher et al., 1994; Zhou and Xu, 2018), which allow them to
1265 breach the dense glycan shield of HIV Env, thereby ensuring access to the protein surface of this
1266 viral glycoprotein (Kong et al., 2013; Zhou and Xu, 2018). One should also remember that the
1267 CDR H3 loops of different Abs were all predicted to be flexible or disordered, with the disorder
1268 degree being proportional to the length of the H3 loop (Uversky, 2021; Uversky, 2022). These
1269 observations not only indicate that the neutralizing efficiency of bNAbs is driven by intrinsic
1270 disorder, but also suggest that the structures reported for this region in different Abs are likely to
1271 represent snapshots of highly dynamic conformational ensembles, with some of these structures
1272 being induced by the crystal lattice or by the interactions of Abs with the antigens (Uversky,
1273 2022).

1274 Taken together, these and many related observations suggest that intrinsic disorder and structural
1275 flexibility play crucial roles in immune response, where intrinsic disorder of viral antigens helps
1276 them evade at least some of the protective mechanisms of the hosts, and where, by having
1277 flexible/disordered antigen-binding sites of Abs, the "immune system follows the "if you can't
1278 fight them join them" principle and "fights fire with fire" by utilizing intrinsic disorder/structural
1279 flexibility of Abs to overcome intrinsic disorder-based "invisibility" of viral antigens" (Uversky,
1280 2022). This also generates serious doubts about the overall applicability of the computational
1281 tools elaborated to be used in the rational structure-based reversed vaccinology. Since these



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1282 important considerations cannot be ignored, it was claimed: “We should stop playing with
1283 motionless toys, as the reality is much more complex than the static picture drawn by the classic
1284 “lock-and-key” model. In fact, it is even more complex than the complex interplay of “flexible
1285 keys and adjustable locks”. It is time for the emergence of unstructural vaccinology, where the
1286 phenomenon of intrinsic disorder is taken into account while thinking about novel approaches for
1287 designing vaccines against “flexible” viruses that act as dynamic “shape-shifters” (Goh, 2017a).
1288 In other words, we need to start using experimental and computational tools designed for the
1289 analysis of disordered and flexible proteins, while looking at intrinsically disordered viral
1290 antigens and their interactions with intrinsically disordered/flexible Abs” (Uversky, 2022).

1291

1292 ***III.H. Targeting liquid-liquid phase separation***

1293 In addition to a broad spectrum of crucial roles in cellular organization, signaling, and regulation
1294 of various cellular processes, LLPS is involved in the pathogenesis of numerous diseases,
1295 thereby serving as an important novel subject of the research on disease biology and
1296 development of therapeutics (Alberti and Dormann, 2019; Wang et al., 2021; Zbinden et al.,
1297 2020). In fact, in neurodegenerative disorders, such as Alzheimer’s disease (AD), Parkinson’s
1298 disease (PD), and amyotrophic lateral sclerosis (ALS) associated with the accumulation of
1299 neuropathological protein aggregates in the brain (Arai et al., 2006; Kosik et al., 1986; Masters et
1300 al., 1985; Spillantini et al., 1998), LLPS is recognized as the stage preceding aggregation that
1301 plays a number of significant roles in the pathogenesis of these diseases (Ambadipudi et al.,
1302 2017; Carey and Guo, 2022; Ray et al., 2020). Phase separation has been linked to cancer as
1303 well, with many oncogenic proteins undergoing LLPS and driving formations of MLOs and BCs
1304 involved in the promotion of tumor growth, survival, and metastasis (Kamagata et al., 2020;



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1305 Mehta and Zhang, 2022; Ren et al., 2022; Shi et al., 2021; Tong et al., 2022; Xie et al., 2023;
1306 Zamudio et al., 2019; Zhang et al., 2020). The aforementioned hybrid oncoprotein EWS-FLI1
1307 contributes to the altered gene expression patterns resulting in oncogenic cell transformation by
1308 undergoing LLPS and forming nuclear condensates (Ahmed et al., 2021; Boulay et al., 2017).
1309 Furthermore, oncogenesis is linked to dysregulation (El-Naggar and Sorensen, 2018; Song and
1310 Grabocka, 2023) and abnormal dynamics of SGs (Grabocka and Bar-Sagi, 2016; Gupta et al.,
1311 2017; Li et al., 2021; Somasekharan et al., 2015). Finally, pathogenesis of several metabolic
1312 diseases, such as obesity, diabetes, and nonalcoholic fatty liver disease (NAFLD) is linked to
1313 protein LLPS (Fonteneau et al., 2022; Gao et al., 2022; Pytowski et al., 2020). Consequently,
1314 targeting phase-separating proteins and resulting MLOs/BCs might have great potential in the
1315 therapeutic interventions of various diseases, and therefore emerged as a promising strategy for
1316 drug discovery (Mitrea et al., 2022; Vendruscolo and Fuxreiter, 2022). Among the approaches
1317 that can be utilized in the discovery of drug targeting phase-separated protein states or proteins
1318 undergoing LLPS are the use of small molecules as:

1319 a) Inhibitors capable of modulating the LLPS of proteins involved in specific diseases
1320 (Babinchak et al., 2020; Dai et al., 2021; Fang et al., 2019; Girdhar et al., 2020; Oka et
1321 al., 2016; Pradhan et al., 2021; Ramesh et al., 2023; Richard et al., 2019; Sawner et al.,
1322 2021; Song et al., 2016; Takada and Makishima, 2020; Wang et al., 2022; Xu et al.,
1323 2022a; Xu et al., 2022b), or
1324 b) A means to modulate the protein-protein interactions in condensates (Liu et al., 2023;
1325 White et al., 2019), or
1326 c) Specific IDR binders capable of altering the conformation or aggregation propensity of
1327 IDRs (Fang et al., 2019), or



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1328 d) A means to induce changes in the IDR energy landscape (Heller et al., 2018) or affecting
1329 interactions of IDRs with other proteins or cellular components involved in LLPS, or
1330 e) Modulators of the physicochemical properties of the cellular environment leading to the
1331 disruption of the formation or stability of liquid-like condensates (Patel et al., 2022), or
1332 f) Modulators of the cellular proteostasis and protein quality control systems affecting the
1333 functionality of chaperones (Baughman et al., 2018; Bruinsma et al., 2011; Wilhelmus et
1334 al., 2006; Zourlidou et al., 2004) and autophagy (Ma et al., 2022).

1335 Ever-increasing understanding of the phase-separated protein states leads to the development of
1336 new approaches for their utilization in drug discovery (Mitrea et al., 2022; Richard et al., 2019;
1337 Vendruscolo and Fuxreiter, 2022). Small molecules or compounds capable of LLPS modulation
1338 can be identified through the development of assays that capture the dynamics and properties of
1339 phase-separated condensates (Zhou et al., 2020). The discovery of novel therapeutics that
1340 effectively modulate phase-separated protein states and provide new opportunities for treating
1341 LLPS-associated diseases are linked to emerging technologies, such as high-throughput
1342 screening (HTS) techniques, development of biomimetic systems that aim to recreate cellular
1343 environments and conditions in a controlled laboratory environment (Bina et al., 2022;
1344 Jobdeedamrong et al., 2023; Razzak and De Brabander, 2011), and the integration of artificial
1345 intelligence (AI) and machine learning (ML) to accelerate the identification and optimization of
1346 potential drug candidates (Dara et al., 2022; Jobdeedamrong et al., 2023; Paul et al., 2021; Zhou
1347 et al., 2020) and streamlining the drug discovery process by predicting the potential off-target or
1348 side effects and toxicity profiles (Dara et al., 2022; Paul et al., 2021).

1349

1350 ***III.I. Self-assembling "smart" IDP-based containers for targeted drug delivery***



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1351 Importantly, IDPs have found specific applications in the form of self-assembling “smart”
1352 containers for targeted drug delivery. These new IDP-based materials utilize an intriguing
1353 possibility of extended IDPs to show a “turned out” response to heat reflected in the temperature-
1354 induced formation of some residual secondary structure (Kim et al., 2000; Permyakov et al.,
1355 2003; Timm et al., 1992; Uversky et al., 2001; Uversky et al., 2002). In this context, the term
1356 “turned out” response is used to emphasize the specific response of extended IDPs (native coils
1357 and native pre-molten globules) to changes in their environment, which is different from that of
1358 ordered proteins. In fact, although extreme temperatures or pH act as denaturing factors leading
1359 to the disruption of ordered protein structures (i.e., their (at least partial) destructuration), these
1360 same conditions (elevated temperature or extremely acidic and/or alkaline conditions) would
1361 cause the appearance of more structure in IDPs, i.e., their partial folding (Uversky, 2009b;
1362 2013d). Among important members of such proteins with the “turned out” response are elastin-
1363 like polypeptides (ELPs) consisting of repeats of the VPGXG pentapeptide with the "guest
1364 residue" X being any amino acid with the exception of proline. Deep understanding of the
1365 specific features determining the capability of ELP to undergo a reversible phase transition from
1366 the disordered, highly solvated conformation below the inverse transition temperature ($T_t$) to a
1367 new condensed phase comprising desolvated and aggregated polypeptides when the temperature
1368 is raised above $T_t$ (Meyer and Chilkoti, 1999; Urry, 1988; 1992; 1997) resulted in the
1369 development of a unified model for accurate prediction of the $T_t$ values for different ELPs based
1370 on their composition, chain length, and concentration (McDaniel et al., 2013b). The $T_t$ of an ELP
1371 is unique to each polymer and inversely related to the polypeptide concentration, number of
1372 monomer repeats, and hydrophobicity of the fourth or variable X residue of the pentapeptide
1373 repeat (Chilkoti et al., 2002; Meyer and Chilkoti, 2004; Urry, 1992; Urry et al., 1991).



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1374 Depending on the peculiarities of the ELP sequence, the resulting condensed phase can be either
1375 nanoscale particles (micelles), or micron-scale coacervates, or viscous gels (Hassouneh et al.,
1376 2012). Therefore, ELPs represent a unique system with a controllable separation to the
1377 mechanically different phases. Since ELPs are non-toxic, biodegradable biopolymers displaying
1378 good pharmacokinetics (Janib et al., 2013; Liu et al., 2006b; Paiva and Martins, 2011), they have
1379 found multiple uses in the development of various self-assembling drug-loaded nanoparticles.
1380 For example, fusing ELP to a cysteine-rich short peptide generated a chimeric polypeptide (CP)
1381 capable of covalent binding of various hydrophobic molecules including chemotherapeutics and
1382 undergoing a conjugation-induced spontaneous self-assembly into nanoparticles (MacKay et al.,
1383 2009; McDaniel et al., 2013a), which were able to effectively treat solid tumors upon intra-
1384 venous injection, as illustrated by the doxorubicin-conjugated chimeric polypeptide (Dox-CP)
1385 (MacKay et al., 2009). In another application of this technology, ELP-based “heat seeking”
1386 polypeptide nanoparticles loaded with anticancer drugs were designed to thermally target solid
1387 tumors (McDaniel et al., 2014). Here, the CP possessing thermal sensitivity within the clinically
1388 relevant temperature interval was designed that formed CP-Dox nanoparticles at 40–45°C
1389 (McDaniel et al., 2014). It was also shown that the nanoparticles were able to reversibly
1390 aggregate in tumors in a temperature-dependent manner and that the thermal cycling of the
1391 tumors between 37 and 42°C represented an effective way of targeting the nanoparticles to
1392 tumors (McDaniel et al., 2014). Fusing specific cell penetrating peptides (CPPs) to ELPs allowed
1393 for controllable and rapid delivery of the cargo molecule to the target cells (Bidwell et al., 2009;
1394 Bidwell et al., 2007; Bidwell and Raucher, 2005; 2010; Massodi et al., 2005; Massodi et al.,
1395 2009), and, being combined with local hyperthermia, such peptides showed enhanced
1396 permeability and high retention effects (Ryu and Raucher, 2014). A logical extension of this idea



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1397 was design of thermally responsive delivery systems, where fusion of a selected bioactive
1398 peptide or protein to a peptide delivery vector containing CPP and ELP enhanced cellular uptake
1399 and resulted in a noticeable increase in the activity of this protein/peptide by the hyperthermia
1400 treatment (Bidwell and Raucher, 2005; Massodi et al., 2005; Massodi et al., 2010; Massodi and
1401 Raucher, 2007; Mikecin et al., 2014; Shamji et al., 2008).

1402

1403 ***III.J. Pharmacological chaperones***

1404 Pharmacological chaperones are small molecules capable of stabilization of a native protein
1405 conformation or native protein complex. The idea is based on the discovery and successful
1406 utilization of tafamidis, a small molecule capable of stabilizing the native homotetrameric form
1407 of transthyretin and preventing dissociation, misfolding, aggregation, and amyloid fibril
1408 formation of this protein related to the pathogenesis of familial amyloid polyneuropathy, as well
1409 as familial and sporadic amyloid cardiomyopathy (Bulawa et al., 2012; Miroy et al., 1996). This
1410 concept of pharmacological chaperones has gained the attention of the researchers (Chiti and
1411 Kelly, 2022; Ringe and Petsko, 2009; Vendruscolo, 2023), and several drugs acting via this
1412 mechanism have been approved by the FDA, and many other compounds with a similar
1413 mechanism of action are under development (see (Vendruscolo, 2023) for review). Although the
1414 idea of pharmacological chaperones was originally proposed as a means for stabilization of the
1415 native structure of ordered proteins, it was recognized that this principle can be used to target
1416 IDPs/IDRs as well (Biesaga et al., 2021; Follis et al., 2008; Heller et al., 2015; Lohr et al., 2022;
1417 Robustelli et al., 2022; Ruan et al., 2019; Vendruscolo, 2023). Despite the fact that IDPs/IDRs
1418 cannot form stable interactions with small molecules due to the lack of specific binding pockets,



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1419 several small molecules were shown to interact with IDPs/IDRs (Ban et al., 2017; Follis et al.,
1420 2008; Ruan et al., 2020; Toth et al., 2014; Zhu et al., 2022). The proposed mechanisms of action
1421 of pharmacological chaperones on IDPs/IDRs include induction of a population shift in the
1422 conformational landscape of an IDP/IDR (Ban et al., 2017), promotion of IDP folding reflected
1423 in the formation of a collapsed state (Zhu et al., 2022) or even formation of a well-defined
1424 binding pocket (Follis et al., 2008; Toth et al., 2014; Vendruscolo, 2023; Zhu et al., 2013).
1425 Pharmacological chaperones have also been reported that utilize entropy and not enthalpy and
1426 work via the "disordered binding mechanism" (Heller et al., 2020; Heller et al., 2015; Lohr et al.,
1427 2022). According to this mechanism, the complex between an IDP/IDR and a small molecule is
1428 stabilized by an increase in entropy. This is possible when the small molecule competes with the
1429 transient intramolecular interactions of the protein and makes it even more disordered. In other
1430 words, the binding of small molecules by IDPs/IDRs can affect their conformational space by
1431 creating an entropic expansion, where more protein conformations become populated, thereby
1432 emphasizing that the entropic contributions can act as the main driving force of protein–ligand
1433 interaction (Heller et al., 2015).

1434 Recently, it was demonstrated that the concept of pharmacological chaperones can also be
1435 applied to the discovery of a small molecule that modulates the LLPS driven by the pathology-
1436 related IDPs (Dada et al., 2024). Here, the idea was that pharmacological interventions can be
1437 utilized to inhibit aggregation of a target protein (α-synuclein) within the liquid-like condensates
1438 into solid-like amyloid fibrils. Experiments conducted *in vitro* and in a *Caenorhabditis elegans*
1439 model of Parkinson's disease revealed that a small molecule spermine-containing aminosterol
1440 claramine (which is structurally similar to two natural products isolated from the liver of dogfish
1441 sharkstrodusquemine and squalamine) can act as a pharmacological chaperone modulating the



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1442 aggregation behavior of α-synuclein within the condensed state (Dada et al., 2024). Analysis
1443 revealed that the aminosterol claramine modulate the α-synuclein phase separation by stabilizing
1444 α-synuclein condensates and inhibiting fibrillation of this protein (Dada et al., 2024). Based on
1445 these exciting observations, the authors concluded that the inhibition of protein aggregation
1446 within condensates represents a possible therapeutic route that can be relevant to the treatment of
1447 many neurodegenerative diseases, where aggregation of disease causing proteins is accelerated
1448 within the liquid condensates formed through phase separation (Dada et al., 2024).

1449

1450 ## IV. Concluding remarks: Clouds in clouds

1451 Biologically active proteins without unique structures represent a new reality of molecular
1452 biology. There are multiple important reasons why IDPs/IDRs cannot be ignored anymore. First
1453 of all, they are vastly abundant in the protein universe (Dunker et al., 2000; Tokuriki et al., 2009;
1454 Uversky, 2010a; Ward et al., 2004; Xue et al., 2012; Xue et al., 2010), with highly disordered proteins
1455 (i.e., proteins, which are, based on the results of PONDR® VSL2 analysis, contain at least 50%
1456 the disordered residues and are characterized by mean disorder scores exceeding the 0.5
1457 threshold) accounting for 36.3% of human proteome (Mohammed and Uversky, 2022). This high
1458 prevalence of IDPs/IDRs in the human proteome and the fact that disorder is evolutionary
1459 conserved (IDPs/IDRs are abundantly found in all the proteomes from all the kingdoms of life
1460 analyzed so far) embrace a very important message: disorder is important. This conjecture is
1461 supported by the fact that, being a highly conservative lady, nature would not waste time, energy,
1462 and resources, which are typically very scarce, to generate something useless. In line with these
1463 considerations, vast evidence is accumulated to show that IDPs/IDRs are biologically important,



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1464 possessing crucial cellular functions complementing the functional repertoire of ordered proteins.
1465 Furthermore, intrinsic disorder represents a means for the almost unlimited extension of the
1466 modes of protein action, control, and regulation. Among numerous ways of how disorder is used
1467 by proteins in their various biological activities, its roles in protein multifunctionality and
1468 protein-protein interactions are unsurpassed. If one could think of any very unusual and never-
1469 seen-before way of connecting proteins to their partners (other proteins, nucleic acids, other
1470 biopolymers, membranes, or small molecules), with a very high probability, such an interaction
1471 mode would be based on intrinsic disorder. Being "edge of chaos" systems (Uversky,
1472 2013d)(Turoverov et al., 2019), IDPs/IDRs are extremely sensitive to their environment and can
1473 undergo fast conformational switching in response to subtle environmental changes, with the
1474 scale and direction of such conformational changes being dependent on the nature of the
1475 environmental stimuli. These high sensitivity and responsiveness define IDPs/IDRs as crucial
1476 cellular controllers, but also indicate that these controllers must be tightly controlled themselves.
1477 In fact, as was already emphasized, within a complex and highly interconnected system there are
1478 too many ways of getting something wrong. Therefore, it is not surprising that
1479 misregulation/dysregulation, deregulation, miscommunication, misfunction, and malfunction of
1480 IDPs/IDRs are commonly disastrous and linked to numerous human diseases. All this defines an
1481 ever-increasing desire of researchers to better understand these highly abundant multifunctional
1482 promiscuous binders in order to use IDPs/IDRs and disorder-based functionality as novel drug
1483 targets.

1484 However, all this also indicates that IDPs/IDRs and disorder-based functions are difficult drug
1485 targets, as well-established protocols of structure-based rational drug design cannot be directly
1486 used to find drugs targeting structure-less and highly dynamic systems. As a result, a



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1487 comprehensive computational analysis of the current druggable human proteome, which includes
1488 1027 human proteins known to be targeted by the existing drugs, revealed a low abundance of
1489 drugs targeting IDPs, with the druggable human proteome being heavily biased towards ordered
1490 proteins (Hu et al., 2016). Again, this bias is defined by a set of drug discovery techniques that
1491 were originally elaborated to find small molecules capable of modulating the functions of
1492 ordered proteins from limited set of functional families, such as cell surface receptors, enzymes,
1493 ion channels, nuclear hormone receptors, and transporters. Regardless of differences in the
1494 biological functions of these targets, the basic principles of their molecular mechanisms of action
1495 are similar – they all have structurally defined binding sites that specifically interact with the
1496 endogenous ligands or substrates. Therefore, the molecular mechanisms of action of small-
1497 molecule drugs affecting these differently functioning ordered targets are rather similar, as drugs,
1498 being used as antagonists and inhibitors, achieve their effects by over competing those
1499 endogenous ligands or substrates. Consequently, traditional, structure-based rational drug design
1500 is deeply rooted in prior knowledge of the structural organization of a targeted protein and a deep
1501 understanding of the molecular mechanisms underlying its biological activity, as the goal here is
1502 to find a small molecule that is precisely positioned within the uniquely organized binding site of
1503 a targeted protein.

1504 Because of the lack of ordered structures in their unbound states, the ability to fold in a context-
1505 dependent manner and preserve noticeable disorder and structural flexibility outside their
1506 binding site, IDPs/IDRs are considered as impossible targets for structure-based rational drug
1507 design. Further complication is given by the multitudes of ways by which small molecules can
1508 interact with IDPs/IDRs, where several chemically dissimilar small molecules can interact with
1509 one IDR binding site, and several different small molecules can bind to independent interaction



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1510 sites in one IDP, where the ability of IDPs/IDRs to interact with multiple structurally different
1511 proteins is paralleled by their ability to bind a variety of chemically different small molecules
1512 (Metallo, 2010), where the point-like, local folding can be induced in an IDP by binding of a
1513 small molecule with the remaining protein preserving disorder, and where the conformational
1514 plasticity and flexibility of the recognition/binding elements of IDPs/IDRs define the their ability
1515 to bind structurally different molecules with similar affinity (Metallo, 2010).

1516 To further illustrate complications associated with intrinsic disorder, the "cloud in cloud" or
1517 "ligand cloud around the protein cloud" binding mechanism is briefly outlined below. In the first
1518 case, the overall flexibility of the polypeptide chain containing well-defined binding sites defines
1519 a model that can be described as "fixed ligand cloud within protein cloud". **Figure 14A** shows
1520 the solution NMR structure of the metallothionein (MT) from the snail *Littorina littorea* (LlMT)
1521 in the complex with $Cd^{2+}$ (Baumann et al., 2017). The conformational ensemble representing the
1522 solution structure of LlMT includes 20 structurally different models of a polypeptide chain
1523 coordinating 9 metal ions (thus, the entire ensemble includes 20 polypeptide structures and 180
1524 $Cd^{2+}$ ions). LlMT contains three individual domains, each comprising a single structurally well-
1525 defined three-metal cluster, where three $Cd^{2+}$ ions are present in the (S-thiolate)$_4$-coordinated
1526 form (there are 27 Cys residues in LlMT capable of coordinating 9 metal ions) (Baumann et al.,
1527 2017). Although individual domains are well-folded (Baumann et al., 2017), they undergo rather
1528 substantial mutual movements, resulting in the overall structure of LlMT resembling a cloud.
1529 Since each structure included in the LlMT conformational ensemble contains 9 well-coordinated
1530 $Cd^{2+}$ ions, we are dealing with the "fixed ligand cloud within protein cloud" model here.
1531 Importantly, MTs represent a class of cysteine-rich polypeptides that contain little secondary
1532 structure but are capable of binding a large number of metal ions, such as $Zn^{2+}$, $Cd^{2+}$, and $Cu^{+}$



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1533 (Blindauer and Leszczyszyn, 2010; Henkel and Krebs, 2004; Kagi and Schaffer, 1988; Romero-
1534 Isart et al., 2010). It was also emphasized that the apo-forms of MTs are intrinsically disordered
1535 and undergo binding-induced folding at interaction with metal ions, such as $Cd^{2+}$, $Cu^{+}$, $Hg^{+}$, and
1536 $Zn^{2+}$ (Baumann et al., 2017). In line with these observations, **Figure 14B** represents a 3D
1537 structural model of LlMT generated by AlphaFold showing that this protein is expected to be
1538 mostly disordered. Further support of the highly disordered nature of LlMT is given by **Figure**
1539 **14C** representing its intrinsic disorder profile. Therefore, the aforementioned "fixed ligand cloud
1540 within protein cloud" model originates from the local multi-site metal-ion-binding-induced
1541 folding of an IDP combined with the preservation of global structural flexibility.

1542 To illustrate another level of complexity associated with utilization of IDPs/IDRs as potential
1543 drug targets, **Figure 15** represents the outputs of the comprehensive computational analysis of
1544 interaction between the c-$Myc_{370–409}$ (a ligand-binding element of the aforementioned
1545 intrinsically disordered proto-oncogene c-Myc) and its 10074-A4 ligand (Jin et al., 2013). This
1546 analysis revealed that the "protein cloud"-like structure of c-$Myc_{370–409}$ represents a complex
1547 conformational ensemble, in which one can find several structurally different clusters, members
1548 of which can efficiently bind ligands. Furthermore, **Figure 15** shows that, contrary to the
1549 traditional binding of small molecules to ordered proteins, where a dominant binding structure is
1550 formed, the binding of the ligand to a given member of the c-$Myc_{370–409}$ conformational
1551 ensemble occurred at a broad set of sites, generating a "ligand cloud around the protein cloud"
1552 binding mode (Jin et al., 2013). It is clear that reality can be even more complex. In fact, for long
1553 IDPs, instead of being around a protein cloud, the ligand cloud can be located within a protein
1554 cloud as illustrated by **Figure 14**. Therefore, the rarity of drugs targeting IDPs/IDRs can be
1555 explained by global challenges associated with dealing with such "cloud-around-cloud" or



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1556 "cloud-in-cloud" binding scenarios. This also indicates that some new tricks should be developed
1557 to find a way to drug IDPs/IDRs.

1558 To overcome at least some of these difficulties and challenges, several approaches were
1559 elaborated for disorder-based drug discovery (Ambadipudi and Zweckstetter, 2016; Arai et al.,
1560 2024; Chen and Tou, 2013; Cheng et al., 2006; Choudhary et al., 2022; Dunker and Uversky,
1561 2010; Joshi and Vendruscolo, 2015; Metallo, 2010; Ruan et al., 2019; Ruan et al., 2020; Saikia
1562 and Baruah, 2024; Saurabh et al., 2023; Tsafou et al., 2018; Uversky, 2012; Uversky and
1563 Dunker, 2010a; Wang et al., 2023; Yu et al., 2016; Yu et al., 2024). Drug molecules can be
1564 designed that mimic a critical foldable region (inducible foldon) of the disordered partner that
1565 undergoes a binding-induced folding and successfully compete with this inducible foldon for its
1566 binding site on the ordered partner (Cheng et al., 2006; Kim et al., 2019). Other small molecules
1567 can induces local misfolding of IDR and make the corresponding region incapable of the protein-
1568 protein interaction (Dunker and Uversky, 2010; Metallo, 2010; Tsafou et al., 2018). Other small
1569 molecules can stabilize different members of the functionally misfolded ensemble (Uversky,
1570 2011a), thereby preventing targeted protein from functional interactions (Uversky, 2012). Such
1571 small molecules acting as stabilizers of the non-functional misfolded members of the disordered
1572 conformational ensemble using an *in silico* structure-based computational docking screen, where
1573 the members of the conformational ensemble are treated as ordered proteins and are used in a
1574 virtual screening to identify potential ligands (Toth et al., 2014). Furthermore, small molecules
1575 can inhibit or accelerate protein aggregation and modulate the LLPS potential of targeted
1576 IDPs/IDRs. Finally, IDPs can be utilized in the development of novel means for drug delivery.

1577 Concluding, although many approaches are currently used to discover small molecules affecting
1578 various disorder-based function of proteins, it is clear that innovative techniques are required to



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

1579 find and design drugs for successful modulation of the (multi)functions and dysfunctions of
1580 IDPs/IDRs. In fact, one should also keep in mind that most of the techniques currently used for
1581 discovering and designing drugs for IDPs/IDRs are, in one way or another, still rooted in the
1582 approaches originally elaborated for rational structure-based drug design. However, the
1583 molecular mechanisms of many disorder-based functions are unique to IDPs/IDRs and seem to
1584 be impossible from the viewpoint of order-based functions, indicating that finding novel ways to
1585 modulate such "impossible" functions requires outside the box thinking. Despite these
1586 shortcomings, it is clear that the intrinsic disorder-based functions are principally druggable and
1587 can be modulated by small molecules. Therefore, the future of this field is exciting, bright, and
1588 promising. It is expected that better understanding of the molecular mechanisms underlying
1589 multifunctionality of IDPs/IDRs, their binding promiscuity, and unusual binding modes will lead
1590 to the identification of unique targets and elaboration of novel approaches to design drugs
1591 affecting disorder-based functionalities.

1592

1593 **Acknowledgments**

1594 Over the years, I have collaborated with more than 12,500 colleagues from more than 2,750
1595 research organizations in 89 countries/territories. I extend my sincere gratitude to all of them, as
1596 the presented research would be impossible without them.

1597

1598 **Funding**

1599 This work received no external funding.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1600

1601 **Conflict-of-interest statement**

1602 The author has no an actual or perceived conflict of interest with the contents of this article.

1603

1604 **Data Availability Statement**

1605 The author declares that all the data supporting the findings of this study are contained within the
1606 paper.

1607

1608 **Authorship Contributions**

1609 Participated in research design, conducted research, performed literature analysis, wrote the
1610 manuscript: Uversky, V.N.

1611

1612 **References**

1613 Aggarwal S, Snaidero N, Pahler G, Frey S, Sanchez P, Zweckstetter M, Janshoff A, Schneider A, Weil
1614 MT, Schaap IA, Gorlich D and Simons M (2013) Myelin membrane assembly is driven by a
1615 phase transition of myelin basic proteins into a cohesive protein meshwork. *PLoS biology*
1616 **11**:e1001577.

1617 Ahmed NS, Harrell LM, Wieland DR, Lay MA, Thompson VF and Schwartz JC (2021) Fusion protein
1618 EWS-FLI1 is incorporated into a protein granule in cells. *Rna* **27**:920-932.

1619 Alberti S and Dormann D (2019) Liquid-Liquid Phase Separation in Disease. *Annu Rev Genet* **53**:171-
1620 194.

1621 Alberti S and Hyman AA (2016) Are aberrant phase transitions a driver of cellular aging? *Bioessays*
1622 **38**:959-968.

1623 Allison JR, Varnai P, Dobson CM and Vendruscolo M (2009) Determination of the free energy landscape
1624 of alpha-synuclein using spin label nuclear magnetic resonance measurements. *J Am Chem Soc*
1625 **131**:18314-18326.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1626 Ambadipudi S, Biernat J, Riedel D, Mandelkow E and Zweckstetter M (2017) Liquid–liquid phase
1627 separation of the microtubule-binding repeats of the Alzheimer-related protein Tau. *Nature*
1628 *Communications* **8**:275.

1629 Ambadipudi S and Zweckstetter M (2016) Targeting intrinsically disordered proteins in rational drug
1630 discovery. *Expert Opin Drug Discov* **11**:65-77.

1631 Anderson CW and Appella E (2004) Signaling to the p53 tumor suppressor through pathways activated
1632 by genotoxic and nongenotoxic stress, in *Handbook of Cell Signaling* (Bradshaw RA and Dennis
1633 EA eds) pp 237-247, Academic Press, New York.

1634 Antifeeva IA, Fonin AV, Fefilova AS, Stepanenko OV, Povarova OI, Silonov SA, Kuznetsova IM,
1635 Uversky VN and Turoverov KK (2022) Liquid-liquid phase separation as an organizing principle
1636 of intracellular space: overview of the evolution of the cell compartmentalization concept. *Cell*
1637 *Mol Life Sci* **79**:251.

1638 Antonicka H and Shoubridge EA (2015) Mitochondrial RNA Granules Are Centers for
1639 Posttranscriptional RNA Processing and Ribosome Biogenesis. *Cell Rep*.

1640 Arai M, Suetaka S and Ooka K (2024) Dynamics and interactions of intrinsically disordered proteins.
1641 *Curr Opin Struct Biol* **84**:102734.

1642 Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, Mann D, Tsuchiya K, Yoshida M,
1643 Hashizume Y and Oda T (2006) TDP-43 is a component of ubiquitin-positive tau-negative
1644 inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem*
1645 *Biophys Res Commun* **351**:602-611.

1646 Avalos JL, Celic I, Muhammad S, Cosgrove MS, Boeke JD and Wolberger C (2002) Structure of a Sir2
1647 enzyme bound to an acetylated p53 peptide. *Mol Cell* **10**:523-535.

1648 Babinchak WM, Dumm BK, Venus S, Boyko S, Putnam AA, Jankowsky E and Surewicz WK (2020)
1649 Small molecules as potent biphasic modulators of protein liquid-liquid phase separation. *Nature*
1650 *Communications* **11**:5574.

1651 Balint EE and Vousden KH (2001) Activation and activities of the p53 tumour suppressor protein. *Br J*
1652 *Cancer* **85**:1813-1823.

1653 Ban D, Iconaru LI, Ramanathan A, Zuo J and Kriwacki RW (2017) A Small Molecule Causes a
1654 Population Shift in the Conformational Landscape of an Intrinsically Disordered Protein. *J Am*
1655 *Chem Soc* **139**:13692-13700.

1656 Ban F, Leblanc E, Cavga AD, Huang CF, Flory MR, Zhang F, Chang MEK, Morin H, Lallous N, Singh
1657 K, Gleave ME, Mohammed H, Rennie PS, Lack NA and Cherkasov A (2021) Development of an
1658 Androgen Receptor Inhibitor Targeting the N-Terminal Domain of Androgen Receptor for
1659 Treatment of Castration Resistant Prostate Cancer. *Cancers (Basel)* **13**.

1660 Banuelos CA, Tavakoli I, Tien AH, Caley DP, Mawji NR, Li Z, Wang J, Yang YC, Imamura Y, Yan L,
1661 Wen JG, Andersen RJ and Sadar MD (2016) Sintokamide A Is a Novel Antagonist of Androgen
1662 Receptor That Uniquely Binds Activation Function-1 in Its Amino-terminal Domain. *J Biol Chem*
1663 **291**:22231-22243.

1664 Barbas CF, 3rd, Collet TA, Amberg W, Roben P, Binley JM, Hoekstra D, Cababa D, Jones TM,
1665 Williamson RA, Pilkington GR and et al. (1993) Molecular profile of an antibody response to
1666 HIV-1 as probed by combinatorial libraries. *J Mol Biol* **230**:812-823.

1667 Basu S, Martinez-Cristobal P, Frigole-Vivas M, Pesarrodona M, Lewis M, Szulc E, Banuelos CA,
1668 Sanchez-Zarzalejo C, Bielskute S, Zhu J, Pombo-Garcia K, Garcia-Cabau C, Zodi L, Dockx H,
1669 Smak J, Kaur H, Batlle C, Mateos B, Biesaga M, Escobedo A, Bardia L, Verdaguer X, Ruffoni A,



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1670 Mawji NR, Wang J, Obst JK, Tam T, Brun-Heath I, Ventura S, Meierhofer D, Garcia J,
1671 Robustelli P, Stracker TH, Sadar MD, Riera A, Hnisz D and Salvatella X (2023) Rational
1672 optimization of a transcription factor activation domain inhibitor. *Nature structural & molecular*
1673 *biology* **30**:1958-1969.

1674 Baughman HER, Clouser AF, Klevit RE and Nath A (2018) HspB1 and Hsc70 chaperones engage
1675 distinct tau species and have different inhibitory effects on amyloid formation. *J Biol Chem*
1676 **293**:2687-2700.

1677 Baumann C, Beil A, Jurt S, Niederwanger M, Palacios O, Capdevila M, Atrian S, Dallinger R and Zerbe
1678 O (2017) Structural Adaptation of a Protein to Increased Metal Stress: NMR Structure of a
1679 Marine Snail Metallothionein with an Additional Domain. *Angewandte Chemie* **56**:4617-4622.

1680 Beadle GW and Tatum EL (1941) Genetic Control of Biochemical Reactions in Neurospora. *Proc Natl*
1681 *Acad Sci U S A* **27**:499-506.

1682 Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN and Bourne PE
1683 (2000) The Protein Data Bank. *Nucleic Acids Res* **28**:235-242.

1684 Bernado P, Bertoncini CW, Griesinger C, Zweckstetter M and Blackledge M (2005) Defining long-range
1685 order and local disorder in native alpha-synuclein using residual dipolar couplings. *J Am Chem*
1686 *Soc* **127**:17968-17969.

1687 Bertoncini CW, Jung YS, Fernandez CO, Hoyer W, Griesinger C, Jovin TM and Zweckstetter M (2005)
1688 Release of long-range tertiary interactions potentiates aggregation of natively unstructured alpha-
1689 synuclein. *Proc Natl Acad Sci U S A* **102**:1430-1435.

1690 Beyer K, Domingo-Sabat M, Humbert J, Carrato C, Ferrer I and Ariza A (2008a) Differential expression
1691 of alpha-synuclein, parkin, and synphilin-1 isoforms in Lewy body disease. *Neurogenetics* **9**:163-
1692 172.

1693 Beyer K, Domingo-Sabat M, Lao JI, Carrato C, Ferrer I and Ariza A (2008b) Identification and
1694 characterization of a new alpha-synuclein isoform and its role in Lewy body diseases.
1695 *Neurogenetics* **9**:15-23.

1696 Biamonti G (2004) Nuclear stress bodies: a heterochromatin affair? *Nat Rev Mol Cell Biol* **5**:493-498.

1697 Biamonti G and Vourc'h C (2010) Nuclear stress bodies. *Cold Spring Harb Perspect Biol* **2**:a000695.

1698 Bidwell GL, 3rd, Davis AN and Raucher D (2009) Targeting a c-Myc inhibitory polypeptide to specific
1699 intracellular compartments using cell penetrating peptides. *J Control Release* **135**:2-10.

1700 Bidwell GL, 3rd, Fokt I, Priebe W and Raucher D (2007) Development of elastin-like polypeptide for
1701 thermally targeted delivery of doxorubicin. *Biochem Pharmacol* **73**:620-631.

1702 Bidwell GL, 3rd and Raucher D (2005) Application of thermally responsive polypeptides directed against
1703 c-Myc transcriptional function for cancer therapy. *Mol Cancer Ther* **4**:1076-1085.

1704 Bidwell GL, 3rd and Raucher D (2010) Cell penetrating elastin-like polypeptides for therapeutic peptide
1705 delivery. *Adv Drug Deliv Rev* **62**:1486-1496.

1706 Bienkiewicz EA, Adkins JN and Lumb KJ (2002) Functional consequences of preorganized helical
1707 structure in the intrinsically disordered cell-cycle inhibitor p27(Kip1). *Biochemistry* **41**:752-759.

1708 Biesaga M, Frigole-Vivas M and Salvatella X (2021) Intrinsically disordered proteins and biomolecular
1709 condensates as drug targets. *Curr Opin Chem Biol* **62**:90-100.

1710 Bina M, Sciortino F and Mahrir AN (2022) Phase separation in polymer-based biomimetic structures
1711 containing planar membranes. *Biointerphases* **17**.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1712 Blatt LM, Davis JM, Klein SB and Taylor MW (1996) The biologic activity and molecular
1713 characterization of a novel synthetic interferon-alpha species, consensus interferon. *Journal of*
1714 *interferon & cytokine research : the official journal of the International Society for Interferon*
1715 *and Cytokine Research* **16**:489-499.

1716 Blindauer CA and Leszczyszyn OI (2010) Metallothioneins: unparalleled diversity in structures and
1717 functions for metal ion homeostasis and more. *Nat Prod Rep* **27**:720-741.

1718 Borden EC, Sen GC, Uze G, Silverman RH, Ransohoff RM, Foster GR and Stark GR (2007) Interferons
1719 at age 50: past, current and future impact on biomedicine. *Nature Reviews Drug Discovery* **6**:975-
1720 990.

1721 Boulay G, Sandoval GJ, Riggi N, Iyer S, Buisson R, Naigles B, Awad ME, Rengarajan S, Volorio A,
1722 McBride MJ, Broye LC, Zou L, Stamenkovic I, Kadoch C and Rivera MN (2017) Cancer-
1723 Specific Retargeting of BAF Complexes by a Prion-like Domain. *Cell* **171**:163-178.e119.

1724 Bourhis JM, Canard B and Longhi S (2007) Predicting protein disorder and induced folding: from
1725 theoretical principles to practical applications. *Curr Protein Pept Sci* **8**:135-149.

1726 Brangwynne CP (2013) Phase transitions and size scaling of membrane-less organelles. *The Journal of*
1727 *cell biology* **203**:875-881.

1728 Brangwynne CP, Eckmann CR, Courson DS, Rybarska A, Hoege C, Gharakhani J, Julicher F and Hyman
1729 AA (2009) Germline P granules are liquid droplets that localize by controlled
1730 dissolution/condensation. *Science* **324**:1729-1732.

1731 Brangwynne CP, Mitchison TJ and Hyman AA (2011) Active liquid-like behavior of nucleoli determines
1732 their size and shape in Xenopus laevis oocytes. *Proc Natl Acad Sci U S A* **108**:4334-4339.

1733 Brangwynne CP, Tompa P and Pappu RV (2015) Polymer physics of intracellular phase transitions. *Nat*
1734 *Physics* **11**:899–904.

1735 Brown CJ, Takayama S, Campen AM, Vise P, Marshall TW, Oldfield CJ, Williams CJ and Dunker AK
1736 (2002) Evolutionary rate heterogeneity in proteins with long disordered regions. *J Mol Evol*
1737 **55**:104-110.

1738 Bruinsma IB, Bruggink KA, Kinast K, Versleijen AA, Segers-Nolten IM, Subramaniam V, Kuiperij HB,
1739 Boelens W, de Waal RM and Verbeek MM (2011) Inhibition of α-synuclein aggregation by small
1740 heat shock proteins. *Proteins* **79**:2956-2967.

1741 Bulawa CE, Connelly S, Devit M, Wang L, Weigel C, Fleming JA, Packman J, Powers ET, Wiseman RL,
1742 Foss TR, Wilson IA, Kelly JW and Labaudiniere R (2012) Tafamidis, a potent and selective
1743 transthyretin kinetic stabilizer that inhibits the amyloid cascade. *Proc Natl Acad Sci U S A*
1744 **109**:9629-9634.

1745 Burton DR (2002) Antibodies, viruses and vaccines. *Nat Rev Immunol* **2**:706-713.

1746 Burton DR (2010) Scaffolding to build a rational vaccine design strategy. *Proc Natl Acad Sci U S A*
1747 **107**:17859-17860.

1748 Carey JL and Guo L (2022) Liquid-Liquid Phase Separation of TDP-43 and FUS in Physiology and
1749 Pathology of Neurodegenerative Diseases. *Front Mol Biosci* **9**:826719.

1750 Cary PD, Moss T and Bradbury EM (1978) High-resolution proton-magnetic-resonance studies of
1751 chromatin core particles. *Eur J Biochem* **89**:475-482.

1752 Chakraborty S, Berndsen ZT, Hengartner NW, Korber BT, Ward AB and Gnanakaran S (2020)
1753 Quantification of the Resilience and Vulnerability of HIV-1 Native Glycan Shield at Atomistic
1754 Detail. *iScience* **23**:101836.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1755 Chen CY and Tou WI (2013) How to design a drug for the disordered proteins? *Drug Discov Today*
1756 **18**:910-915.

1757 Chen J, Liang H and Fernandez A (2008) Protein structure protection commits gene expression patterns.
1758 *Genome Biol* **9**:R107.

1759 Chene P (2004) Inhibition of the p53-MDM2 interaction: targeting a protein-protein interface. *Mol*
1760 *Cancer Res* **2**:20-28.

1761 Cheng Y, LeGall T, Oldfield CJ, Mueller JP, Van YY, Romero P, Cortese MS, Uversky VN and Dunker
1762 AK (2006) Rational drug design via intrinsically disordered protein. *Trends Biotechnol* **24**:435-
1763 442.

1764 Cheng Y, Oldfield CJ, Meng J, Romero P, Uversky VN and Dunker AK (2007) Mining alpha-helix-
1765 forming molecular recognition features with cross species sequence alignments. *Biochemistry*
1766 **46**:13468-13477.

1767 Chi SW, Kim DH, Lee SH, Chang I and Han KH (2007) Pre-structured motifs in the natively
1768 unstructured preS1 surface antigen of hepatitis B virus. *Protein Sci* **16**:2108-2117.

1769 Chilkoti A, Dreher MR and Meyer DE (2002) Design of thermally responsive, recombinant polypeptide
1770 carriers for targeted drug delivery. *Advanced Drug Delivery Reviews* **54**:1093-1111.

1771 Chiti F and Kelly JW (2022) Small molecule protein binding to correct cellular folding or stabilize the
1772 native state against misfolding and aggregation. *Curr Opin Struct Biol* **72**:267-278.

1773 Choudhary S, Lopus M and Hosur RV (2022) Targeting disorders in unstructured and structured proteins
1774 in various diseases. *Biophys Chem* **281**:106742.

1775 Chuma S, Hosokawa M, Tanaka T and Nakatsuji N (2009) Ultrastructural characterization of
1776 spermatogenesis and its evolutionary conservation in the germline: germinal granules in
1777 mammals. *Molecular and cellular endocrinology* **306**:17-23.

1778 Cortese MS, Uversky VN and Dunker AK (2008) Intrinsic disorder in scaffold proteins: getting more
1779 from less. *Prog Biophys Mol Biol* **98**:85-106.

1780 Crompton BD, Stewart C, Taylor-Weiner A, Alexe G, Kurek KC, Calicchio ML, Kiezun A, Carter SL,
1781 Shukla SA, Mehta SS, Thorner AR, de Torres C, Lavarino C, Sunol M, McKenna A, Sivachenko
1782 A, Cibulskis K, Lawrence MS, Stojanov P, Rosenberg M, Ambrogio L, Auclair D, Seepo S,
1783 Blumenstiel B, DeFelice M, Imaz-Rosshandler I, Schwarz-Cruz YCA, Rivera MN, Rodriguez-
1784 Galindo C, Fleming MD, Golub TR, Getz G, Mora J and Stegmaier K (2014) The genomic
1785 landscape of pediatric Ewing sarcoma. *Cancer Discov* **4**:1326-1341.

1786 Csizmok V, Bokor M, Banki P, Klement É, Medzihradszky KF, Friedrich P, Tompa K and Tompa P
1787 (2005) Primary contact sites in intrinsically unstructured proteins: the case of calpastatin and
1788 microtubule-associated protein 2. *Biochemistry* **44**:3955-3964.

1789 Csizmok V, Follis AV, Kriwacki RW and Forman-Kay JD (2016) Dynamic Protein Interaction Networks
1790 and New Structural Paradigms in Signaling. *Chem Rev*.

1791 Dada ST, Toprakcioglu Z, Cali MP, Rontgen A, Hardenberg MC, Morris OM, Mrugalla LK, Knowles
1792 TPJ and Vendruscolo M (2024) Pharmacological inhibition of alpha-synuclein aggregation within
1793 liquid condensates. *Nat Commun* **15**:3835.

1794 Dai B, Zhong T, Chen ZX, Chen W, Zhang N, Liu XL, Wang LQ, Chen J and Liang Y (2021) Myricetin
1795 slows liquid-liquid phase separation of Tau and activates ATG5-dependent autophagy to suppress
1796 Tau toxicity. *J Biol Chem* **297**:101222.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1797 Dai C, Dehm SM and Sharifi N (2023) Targeting the Androgen Signaling Axis in Prostate Cancer. *J Clin*
1798 *Oncol* **41**:4267-4278.

1799 Dai C, Heemers H and Sharifi N (2017) Androgen Signaling in Prostate Cancer. *Cold Spring Harb*
1800 *Perspect Med* **7**.

1801 Dang CV (1999) c-Myc target genes involved in cell growth, apoptosis, and metabolism. *Mol Cell Biol*
1802 **19**:1-11.

1803 Dara S, Dhamercherla S, Jadav SS, Babu CM and Ahsan MJ (2022) Machine Learning in Drug
1804 Discovery: A Review. *Artif Intell Rev* **55**:1947-1999.

1805 Darling AL, Liu Y, Oldfield CJ and Uversky VN (2018) Intrinsically Disordered Proteome of Human
1806 Membrane-Less Organelles. *Proteomics* **18**:e1700193 (1700112 pages).

1807 Darling AL and Uversky VN (2017) Intrinsic Disorder in Proteins with Pathogenic Repeat Expansions.
1808 *Molecules* **22**.

1809 Darling AL and Uversky VN (2018) Intrinsic Disorder and Posttranslational Modifications: The Darker
1810 Side of the Biological Dark Matter. *Front Genet* **9**:158.

1811 Daughdrill GW, Pielak GJ, Uversky VN, Cortese MS and Dunker AK (2005) Natively disordered
1812 proteins, in *Handbook of Protein Folding* (Buchner J and Kiefhaber T eds) pp 271-353, Wiley-
1813 VCH, Verlag GmbH & Co. KGaA, Weinheim, Germany.

1814 Davey RA and Grossmann M (2016) Androgen Receptor Structure, Function and Biology: From Bench
1815 to Bedside. *Clin Biochem Rev* **37**:3-15.

1816 Dayhoff GW, 2nd and Uversky VN (2022) Rapid prediction and analysis of protein intrinsic disorder.
1817 *Protein Sci* **31**:e4496.

1818 De Andrea M, Ravera R, Gioia D, Gariglio M and Landolfo S (2002) The interferon system: an overview.
1819 *Eur J Paediatr Neurol* **6 Suppl A**:A41-46; discussion A55-48.

1820 de Weerd NA, Samarajiwa SA and Hertzog PJ (2007) Type I interferon receptors: biochemistry and
1821 biological functions. *J Biol Chem* **282**:20053-20057.

1822 Decker CJ, Teixeira D and Parker R (2007) Edc3p and a glutamine/asparagine-rich domain of Lsm4p
1823 function in processing body assembly in Saccharomyces cerevisiae. *The Journal of cell biology*
1824 **179**:437-449.

1825 Decker M, Jaensch S, Pozniakovsky A, Zinke A, O'Connell KF, Zachariae W, Myers E and Hyman AA
1826 (2011) Limiting amounts of centrosome material set centrosome size in C. elegans embryos.
1827 *Current biology : CB* **21**:1259-1267.

1828 Dedmon MM, Lindorff-Larsen K, Christodoulou J, Vendruscolo M and Dobson CM (2005) Mapping
1829 long-range interactions in alpha-synuclein using spin-label NMR and ensemble molecular
1830 dynamics simulations. *J Am Chem Soc* **127**:476-477.

1831 DeForte S and Uversky VN (2016) Order, Disorder, and Everything in Between. *Molecules* **21**.

1832 Deininger P (1999) Genetic instability in cancer: caretaker and gatekeeper genes. *Ochsner J* **1**:206-209.

1833 Delattre O, Zucman J, Plougastel B, Desmaze C, Melot T, Peter M, Kovar H, Joubert I, de Jong P,
1834 Rouleau G and et al. (1992) Gene fusion with an ETS DNA-binding domain caused by
1835 chromosome translocation in human tumours. *Nature* **359**:162-165.

1836 Disfani FM, Hsu WL, Mizianty MJ, Oldfield CJ, Xue B, Dunker AK, Uversky VN and Kurgan L (2012)
1837 MoRFpred, a computational tool for sequence-based prediction and characterization of short
1838 disorder-to-order transitioning binding regions in proteins. *Bioinformatics* **28**:i75-83.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1839 Dosztanyi Z, Chen J, Dunker AK, Simon I and Tompa P (2006) Disorder and sequence repeats in hub
1840 proteins and their implications for network evolution. *J Proteome Res* **5**:2985-2995.

1841 Dosztanyi Z, Meszaros B and Simon I (2009) ANCHOR: web server for predicting protein binding
1842 regions in disordered proteins. *Bioinformatics* **25**:2745-2746.

1843 Dosztanyi Z, Sandor M, Tompa P and Simon I (2007) Prediction of protein disorder at the domain level.
1844 *Curr Protein Pept Sci* **8**:161-171.

1845 Dosztanyi Z and Tompa P (2008) Prediction of protein disorder. *Methods Mol Biol* **426**:103-115.

1846 Dundr M and Misteli T (2010) Biogenesis of nuclear bodies. *Cold Spring Harb Perspect Biol* **2**:a000711.

1847 Dunker AK, Babu MM, Barbar E, Blackledge M, Bondos SE, Dosztányi Z, Dyson HJ, Forman-Kay J,
1848 Fuxreiter M, Gsponer J, Han K-H, Jones DT, Longhi S, Metallo SJ, Nishikawa K, Nussinov R,
1849 Obradovic Z, Pappu R, Rost B, Selenko P, Subramaniam V, Sussman JL, Tompa P and Uversky
1850 VN (2013) What's in a name? Why these proteins are intrinsically disordered. *Intrinsically*
1851 *Disordered Proteins* **1**:e24157.

1852 Dunker AK, Brown CJ, Lawson JD, Iakoucheva LM and Obradovic Z (2002a) Intrinsic disorder and
1853 protein function. *Biochemistry* **41**:6573-6582.

1854 Dunker AK, Brown CJ and Obradovic Z (2002b) Identification and functions of usefully disordered
1855 proteins. *Adv Protein Chem* **62**:25-49.

1856 Dunker AK, Cortese MS, Romero P, Iakoucheva LM and Uversky VN (2005) Flexible nets. The roles of
1857 intrinsic disorder in protein interaction networks. *Febs J* **272**:5129-5148.

1858 Dunker AK, Garner E, Guilliot S, Romero P, Albrecht K, Hart J, Obradovic Z, Kissinger C and
1859 Villafranca JE (1998) Protein disorder and the evolution of molecular recognition: theory,
1860 predictions and observations. *Pac Symp Biocomput*:473-484.

1861 Dunker AK, Lawson JD, Brown CJ, Williams RM, Romero P, Oh JS, Oldfield CJ, Campen AM, Ratliff
1862 CM, Hipps KW, Ausio J, Nissen MS, Reeves R, Kang C, Kissinger CR, Bailey RW, Griswold
1863 MD, Chiu W, Garner EC and Obradovic Z (2001) Intrinsically disordered protein. *J Mol Graph*
1864 *Model* **19**:26-59.

1865 Dunker AK and Obradovic Z (2001) The protein trinity--linking function and disorder. *Nat Biotechnol*
1866 **19**:805-806.

1867 Dunker AK, Obradovic Z, Romero P, Garner EC and Brown CJ (2000) Intrinsic protein disorder in
1868 complete genomes. *Genome Inform Ser Workshop Genome Inform* **11**:161-171.

1869 Dunker AK, Obradovic Z, Romero P, Kissinger C and Villafranca E (1997) On the importance of being
1870 disordered. *PDB Newsletter* **81**:3-5.

1871 Dunker AK, Oldfield CJ, Meng J, Romero P, Yang JY, Chen JW, Vacic V, Obradovic Z and Uversky VN
1872 (2008a) The unfoldomics decade: an update on intrinsically disordered proteins. *BMC Genomics*
1873 **9 Suppl 2**:S1.

1874 Dunker AK, Silman I, Uversky VN and Sussman JL (2008b) Function and structure of inherently
1875 disordered proteins. *Curr Opin Struct Biol* **18**:756-764.

1876 Dunker AK and Uversky VN (2010) Drugs for 'protein clouds': targeting intrinsically disordered
1877 transcription factors. *Curr Opin Pharmacol* **10**:782-788.

1878 Duret L, Gasteiger E and Perriere G (1996) LALNVIEW: a graphical viewer for pairwise sequence
1879 alignments. *Comput Appl Biosci* **12**:507-510.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1880 Dyson HJ and Wright PE (2002a) Coupling of folding and binding for unstructured proteins. *Curr Opin*
1881 *Struct Biol* **12**:54-60.

1882 Dyson HJ and Wright PE (2002b) Coupling of folding and binding for unstructured proteins. *Curr Opin*
1883 *Struct Biol* **12**:54-60.

1884 Dyson HJ and Wright PE (2005) Intrinsically unstructured proteins and their functions. *Nat Rev Mol Cell*
1885 *Biol* **6**:197-208.

1886 Edmundson AB, Ely KR, Herron JN and Cheson BD (1987) The binding of opioid peptides to the Mcg
1887 light chain dimer: flexible keys and adjustable locks. *Mol Immunol* **24**:915-935.

1888 Ehrnhoefer DE, Bieschke J, Boeddrich A, Herbst M, Masino L, Lurz R, Engemann S, Pastore A and
1889 Wanker EE (2008) EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway
1890 oligomers. *Nature structural & molecular biology* **15**:558-566.

1891 Ekman D, Light S, Bjorklund AK and Elofsson A (2006) What properties characterize the hub proteins of
1892 the protein-protein interaction network of Saccharomyces cerevisiae? *Genome Biol* **7**:R45.

1893 El-Baky NA, Linjawi MH and Redwan EM (2015a) Auto-induction expression of human consensus
1894 interferon-alpha in Escherichia coli. *BMC biotechnology* **15**:14.

1895 El-Baky NA and Redwan EM (2015) Therapeutic alpha-interferons protein: structure, production, and
1896 biosimilar. *Prep Biochem Biotechnol* **45**:109-127.

1897 El-Baky NA, Uversky VN and Redwan EM (2015b) Human consensus interferons: Bridging the natural
1898 and artificial cytokines with intrinsic disorder. *Cytokine Growth Factor Rev* **26**:637-645.

1899 El-Naggar AM and Sorensen PH (2018) Translational control of aberrant stress responses as a hallmark
1900 of cancer. *J Pathol* **244**:650-666.

1901 Elbaum-Garfinkle S, Kim Y, Szczepaniak K, Chen CC, Eckmann CR, Myong S and Brangwynne CP
1902 (2015) The disordered P granule protein LAF-1 drives phase separation into droplets with tunable
1903 viscosity and dynamics. *Proc Natl Acad Sci U S A* **112**:7189-7194.

1904 Erkizan HV, Kong Y, Merchant M, Schlottmann S, Barber-Rotenberg JS, Yuan L, Abaan OD, Chou TH,
1905 Dakshanamurthy S, Brown ML, Uren A and Toretsky JA (2009) A small molecule blocking
1906 oncogenic protein EWS-FLI1 interaction with RNA helicase A inhibits growth of Ewing's
1907 sarcoma. *Nat Med* **15**:750-756.

1908 Erkizan HV, Uversky VN and Toretsky JA (2010) Oncogenic partnerships: EWS-FLI1 protein
1909 interactions initiate key pathways of Ewing's sarcoma. *Clin Cancer Res* **16**:4077-4083.

1910 Esnouf RM, Hamer R, Sussman JL, Silman I, Trudgian D, Yang ZR and Prilusky J (2006) Honing the in
1911 silico toolkit for detecting protein disorder. *Acta Crystallogr D Biol Crystallogr* **62**:1260-1266.

1912 Fan X and Kurgan L (2014) Accurate prediction of disorder in protein chains with a comprehensive and
1913 empirically designed consensus. *J Biomol Struct Dyn* **32**:448-464.

1914 Fang MY, Markmiller S, Vu AQ, Javaherian A, Dowdle WE, Jolivet P, Bushway PJ, Castello NA, Baral
1915 A, Chan MY, Linsley JW, Linsley D, Mercola M, Finkbeiner S, Lecuyer E, Lewcock JW and
1916 Yeo GW (2019) Small-Molecule Modulation of TDP-43 Recruitment to Stress Granules Prevents
1917 Persistent TDP-43 Accumulation in ALS/FTD. *Neuron* **103**:802-819.e811.

1918 Farrah T, Deutsch EW, Hoopmann MR, Hallows JL, Sun Z, Huang CY and Moritz RL (2013) The State
1919 of the Human Proteome in 2012 as Viewed through PeptideAtlas. *Journal of Proteome Research*
1920 **12**:162-171.

1921 Farrah T, Deutsch EW, Omenn GS, Sun Z, Watts JD, Yamamoto T, Shteynberg D, Harris MM and
1922 Moritz RL (2014) State of the Human Proteome in 2013 as Viewed through PeptideAtlas:



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1923 Comparing the Kidney, Urine, and Plasma Proteomes for the Biology- and Disease-Driven
1924 Human Proteome Project. *Journal of Proteome Research* **13**:60-75.

1925 Federman N, Meyers PA, Daw NC, Toretsky J, Breitmeyer JB, Singh AS, Miller LL, Oltersdorf T, Jezior
1926 D and Jessen KA (2017) A phase I, first-in-human, dose escalation study of intravenous TK216
1927 in patients with relapsed or refractory Ewing sarcoma, American Society of Clinical Oncology.

1928 Feric M and Brangwynne CP (2013) A nuclear F-actin scaffold stabilizes ribonucleoprotein droplets
1929 against gravity in large cells. *Nature cell biology* **15**:1253-1259.

1930 Feric M, Vaidya N, Harmon TS, Mitrea DM, Zhu L, Richardson TM, Kriwacki RW, Pappu RV and
1931 Brangwynne CP (2016) Coexisting Liquid Phases Underlie Nucleolar Subcompartments. *Cell*
1932 **165**:1686-1697.

1933 Ferron F, Longhi S, Canard B and Karlin D (2006) A practical overview of protein disorder prediction
1934 methods. *Proteins* **65**:1-14.

1935 Fink AL (2005) Natively unfolded proteins. *Curr Opin Struct Biol* **15**:35-41.

1936 Fischer E (1894) Einfluss der configuration auf die wirkung der enzyme. *Ber Dt Chem Ges* **27**:2985-
1937 2993.

1938 Fish EN, Harrison SA and Hassanein T (2008) The role of consensus interferon in the current treatment of
1939 chronic hepatitis C viral infection. *Gastroenterol Hepatol (N Y)* **4**:1-12.

1940 Fokkens M, Schrader T and Klarner FG (2005) A molecular tweezer for lysine and arginine. *J Am Chem*
1941 *Soc* **127**:14415-14421.

1942 Follis AV, Hammoudeh DI, Wang H, Prochownik EV and Metallo SJ (2008) Structural rationale for the
1943 coupled binding and unfolding of the c-Myc oncoprotein by small molecules. *Chem Biol*
1944 **15**:1149-1155.

1945 Fonin AV, Antifeeva IA, Kuznetsova IM, Turoverov KK, Zaslavsky BY, Kulkarni P and Uversky VN
1946 (2022) Biological soft matter: intrinsically disordered proteins in liquid-liquid phase separation
1947 and biomolecular condensates. *Essays Biochem* **66**:831-847.

1948 Fonteneau G, Redding A, Hoag-Lee H, Sim ES, Heinrich S, Gaida MM and Grabocka E (2022) Stress
1949 Granules Determine the Development of Obesity-Associated Pancreatic Cancer. *Cancer Discov*
1950 **12**:1984-2005.

1951 Fox AH, Lam YW, Leung AK, Lyon CE, Andersen J, Mann M and Lamond AI (2002) Paraspeckles: a
1952 novel nuclear domain. *Current biology : CB* **12**:13-25.

1953 Fuxreiter M, Simon I, Friedrich P and Tompa P (2004) Preformed structural elements feature in partner
1954 recognition by intrinsically unstructured proteins. *J Mol Biol* **338**:1015-1026.

1955 Fuxreiter M and Tompa P (2012) Fuzzy complexes: a more stochastic view of protein function. *Adv Exp*
1956 *Med Biol* **725**:1-14.

1957 Fuxreiter M, Tompa P and Simon I (2007a) Local structural disorder imparts plasticity on linear motifs.
1958 *Bioinformatics* **23**:950-956.

1959 Fuxreiter M, Tompa P and Simon I (2007b) Structural disorder imparts plasticity on linear motifs.
1960 *Bioinformatics* **23**:950-956.

1961 Fuxreiter M, Tompa P, Simon I, Uversky VN, Hansen JC and Asturias FJ (2008) Malleable machines
1962 take shape in eukaryotic transcriptional regulation. *Nat Chem Biol* **4**:728-737.

1963 Fuxreiter M, Toth-Petroczy A, Kraut DA, Matouschek A, Lim RY, Xue B, Kurgan L and Uversky VN
1964 (2014) Disordered proteinaceous machines. *Chem Rev* **114**:6806-6843.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

1965 Gadhave K, Gehi BR, Kumar P, Xue B, Uversky VN and Giri R (2020) The dark side of Alzheimer's
1966 disease: unstructured biology of proteins from the amyloid cascade signaling pathway. *Cell Mol*
1967 *Life Sci* **77**:4163-4208.

1968 Gao XK, Rao XS, Cong XX, Sheng ZK, Sun YT, Xu SB, Wang JF, Liang YH, Lu LR, Ouyang H, Ge H,
1969 Guo J-s, Wu H-j, Sun QM, Wu H-b, Bao Z, Zheng LL and Zhou YT (2022) Phase separation of
1970 insulin receptor substrate 1 drives the formation of insulin/IGF-1 signalosomes. *Cell Discovery*
1971 **8**:60.

1972 Garner E, Romero P, Dunker AK, Brown C and Obradovic Z (1999) Predicting binding regions within
1973 disordered proteins. *Genome Informatics* **10**:41-50.

1974 Geng DY, Chen QS, Chen WX, Zhou LS, Han XS, Xie QH, Guo GH, Chen XF, Chen JS and Zhong XP
1975 (2024) Molecular targets and mechanisms of different aberrant alternative splicing in metastatic
1976 liver cancer. *World J Clin Oncol* **15**:531-539.

1977 Genin P, Vaccaro A and Civas A (2009) The role of differential expression of human interferon--a genes
1978 in antiviral immunity. *Cytokine Growth Factor Rev* **20**:283-295.

1979 Girdhar A, Bharathi V, Tiwari VR, Abhishek S, Deeksha W, Mahawar US, Raju G, Singh SK,
1980 Prabusankar G, Rajakumara E and Patel BK (2020) Computational insights into mechanism of
1981 AIM4-mediated inhibition of aggregation of TDP-43 protein implicated in ALS and evidence for
1982 in vitro inhibition of liquid-liquid phase separation (LLPS) of TDP-432C-A315T by AIM4.
1983 *International Journal of Biological Macromolecules* **147**:117-130.

1984 Goh GK (2017a) *Viral Shapeshifters: Strange Behaviors of HIV and Other Viruses*, Simplicity Research
1985 Institute.

1986 Goh GK, Dunker AK, Foster JA and Uversky VN (2019) HIV Vaccine Mystery and Viral Shell Disorder.
1987 *Biomolecules* **9**:178.

1988 Goh GKM (2017b) *Viral Shapeshifters: Strange Behavoirs of HIV and Other Viruses*, Simplicity
1989 Research Institute, Singapore.

1990 Goldsby R, Kindt TJ, Osborne BA and Kuby J (2003) Antigens in *Immunology* (Goldsby R, Osborne BA,
1991 Kuby J, Kindt TJ, Marcus DA and Punt J eds) pp 57-75, W. H. Freeman and Company, New
1992 York.

1993 Gollavilli PN, Pawar A, Wilder-Romans K, Natesan R, Engelke CG, Dommeti VL, Krishnamurthy PM,
1994 Nallasivam A, Apel IJ, Xu T, Qin ZS, Feng FY and Asangani IA (2018) EWS/ETS-Driven
1995 Ewing Sarcoma Requires BET Bromodomain Proteins. *Cancer research* **78**:4760-4773.

1996 Grabocka E and Bar-Sagi D (2016) Mutant KRAS Enhances Tumor Cell Fitness by Upregulating Stress
1997 Granules. *Cell* **167**:1803-1813.e1812.

1998 Grasberger BL, Lu T, Schubert C, Parks DJ, Carver TE, Koblish HK, Cummings MD, LaFrance LV,
1999 Milkiewicz KL, Calvo RR, Maguire D, Lattanze J, Franks CF, Zhao S, Ramachandren K, Bylebyl
2000 GR, Zhang M, Manthey CL, Petrella EC, Pantoliano MW, Deckman IC, Spurlino JC, Maroney
2001 AC, Tomczuk BE, Molloy CJ and Bone RF (2005) Discovery and cocrystal structure of
2002 benzodiazepinedione HDM2 antagonists that activate p53 in cells. *J Med Chem* **48**:909-912.

2003 Gronemeyer H, Gustafsson JA and Laudet V (2004) Principles for modulation of the nuclear receptor
2004 superfamily. *Nat Rev Drug Discov* **3**:950-964.

2005 Grossman E, Medalia O and Zwerger M (2012) Functional architecture of the nuclear pore complex.
2006 *Annu Rev Biophys* **41**:557-584.

2007 Gubitz AK, Feng W and Dreyfuss G (2004) The SMN complex. *Exp Cell Res* **296**:51-56.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2008 Gupta N, Badeaux M, Liu Y, Naxerova K, Sgroi D, Munn LL, Jain RK and Garkavtsev I (2017) Stress
2009 granule-associated protein G3BP2 regulates breast tumor initiation. *Proceedings of the National*
2010 *Academy of Sciences* **114**:1033-1038.

2011 Gutterman JU (1994) Cytokine Therapeutics - Lessons from Interferon-Alpha. *Proceedings of the*
2012 *National Academy of Sciences of the United States of America* **91**:1198-1205.

2013 Hammoudeh DI, Follis AV, Prochownik EV and Metallo SJ (2009) Multiple independent binding sites
2014 for small-molecule inhibitors on the oncoprotein c-Myc. *J Am Chem Soc* **131**:7390-7401.

2015 Han H, Jain AD, Truica MI, Izquierdo-Ferrer J, Anker JF, Lysy B, Sagar V, Luan Y, Chalmers ZR, Unno
2016 K, Mok H, Vatapalli R, Yoo YA, Rodriguez Y, Kandela I, Parker JB, Chakravarti D, Mishra RK,
2017 Schiltz GE and Abdulkadir SA (2019) Small-Molecule MYC Inhibitors Suppress Tumor Growth
2018 and Enhance Immunotherapy. *Cancer Cell* **36**:483-497 e415.

2019 Handwerger KE, Cordero JA and Gall JG (2005) Cajal bodies, nucleoli, and speckles in the Xenopus
2020 oocyte nucleus have a low-density, sponge-like structure. *Mol Biol Cell* **16**:202-211.

2021 Harrigan JA, Belotserkovskaya R, Coates J, Dimitrova DS, Polo SE, Bradshaw CR, Fraser P and Jackson
2022 SP (2011) Replication stress induces 53BP1-containing OPT domains in G1 cells. *The Journal of*
2023 *cell biology* **193**:97-108.

2024 Hassouneh W, MacEwan SR and Chilkoti A (2012) Fusions of elastin-like polypeptides to
2025 pharmaceutical proteins. *Methods in enzymology* **502**:215-237.

2026 Haynes C, Oldfield CJ, Ji F, Klitgord N, Cusick ME, Radivojac P, Uversky VN, Vidal M and Iakoucheva
2027 LM (2006) Intrinsic disorder is a common feature of hub proteins from four eukaryotic
2028 interactomes. *PLoS Comput Biol* **2**:e100.

2029 He B, Wang K, Liu Y, Xue B, Uversky VN and Dunker AK (2009) Predicting intrinsic disorder in
2030 proteins: an overview. *Cell Res* **19**:929-949.

2031 Heemers HV and Tindall DJ (2007) Androgen receptor (AR) coregulators: a diversity of functions
2032 converging on and regulating the AR transcriptional complex. *Endocr Rev* **28**:778-808.

2033 Heller GT, Aprile FA, Michaels TC, Limbocker R, Perni M, Ruggeri FS, Mannini B, Löhr T, Bonomi M
2034 and Camilloni C (2020) Small-molecule sequestration of amyloid-β as a drug discovery strategy
2035 for Alzheimer's disease. *Science advances* **6**:eabb5924.

2036 Heller GT, Bonomi M and Vendruscolo M (2018) Structural Ensemble Modulation upon Small-Molecule
2037 Binding to Disordered Proteins. *J Mol Biol* **430**:2288-2292.

2038 Heller GT, Sormanni P and Vendruscolo M (2015) Targeting disordered proteins with small molecules
2039 using entropy. *Trends Biochem Sci* **40**:491-496.

2040 Henkel G and Krebs B (2004) Metallothioneins: zinc, cadmium, mercury, and copper thiolates and
2041 selenolates mimicking protein active site features--structural aspects and biological implications.
2042 *Chem Rev* **104**:801-824.

2043 Hirose T, Ninomiya K, Nakagawa S and Yamazaki T (2023) A guide to membraneless organelles and
2044 their various roles in gene regulation. *Nat Rev Mol Cell Biol* **24**:288-304.

2045 Hivare P, Mujmer K, Swarup G, Gupta S and Bhatia D (2023) Endocytic pathways of pathogenic protein
2046 aggregates in neurodegenerative diseases. *Traffic* **24**:434-452.

2047 Hofstetter G, Berger A, Fiegl H, Slade N, Zoric A, Holzer B, Schuster E, Mobus VJ, Reimer D,
2048 Daxenbichler G, Marth C, Zeimet AG, Concin N and Zeillinger R (2010) Alternative splicing of
2049 p53 and p73: the novel p53 splice variant p53delta is an independent prognostic marker in
2050 ovarian cancer. *Oncogene* **29**:1997-2004.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2051 Holt C and Sawyer L (1993) Caseins as rheomorphic proteins: interpretation of primary and secondary
2052 structures of the as1-, b-, and k-caseins. *J Chem Soc Faraday Trans* **89**:2683-2692.

2053 Hsu WL, Oldfield C, Meng J, Huang F, Xue B, Uversky VN, Romero P and Dunker AK (2012) Intrinsic
2054 protein disorder and protein-protein interactions. *Pac Symp Biocomput*:116-127.

2055 Hsu WL, Oldfield CJ, Xue B, Meng J, Huang F, Romero P, Uversky VN and Dunker AK (2013)
2056 Exploring the binding diversity of intrinsically disordered proteins involved in one-to-many
2057 binding. *Protein Sci* **22**:258-273.

2058 Hu G, Wu Z, Wang K, Uversky VN and Kurgan L (2016) Untapped Potential of Disordered Proteins in
2059 Current Druggable Human Proteome. *Curr Drug Targets* **17**:1198-1205.

2060 Huang F, Oldfield C, Meng J, Hsu WL, Xue B, Uversky VN, Romero P and Dunker AK (2012)
2061 Subclassifying disordered proteins by the ch-cdf plot method. *Pac Symp Biocomput*:128-139.

2062 Huang S (2000) Review: perinucleolar structures. *Journal of structural biology* **129**:233-240.

2063 Huang X and Miller W (1991) A time-efficient, linear-space local similarity algorithm. *Advances in*
2064 *applied mathematics* **12**:337-357.

2065 Iakoucheva LM, Brown CJ, Lawson JD, Obradovic Z and Dunker AK (2002) Intrinsic disorder in cell-
2066 signaling and cancer-associated proteins. *J Mol Biol* **323**:573-584.

2067 Iakoucheva LM, Radivojac P, Brown CJ, O'Connor TR, Sikes JG, Obradovic Z and Dunker AK (2004)
2068 The importance of intrinsic disorder for protein phosphorylation. *Nucleic Acids Research*
2069 **32**:1037-1049.

2070 Isaacs A and Lindenmann J (1957) Virus interference. I. The interferon. *Proc R Soc Lond B Biol Sci*
2071 **147**:258-267.

2072 Janib SM, Liu S, Park R, Pastuszka MK, Shi P, Moses AS, Orosco MM, Lin YA, Cui H, Conti PS, Li Z
2073 and MacKay JA (2013) Kinetic quantification of protein polymer nanoparticles using non-
2074 invasive imaging. *Integr Biol (Camb)* **5**:183-194.

2075 Janin J and Sternberg MJE (2013) Protein flexibility, not disorder, is intrinsic to molecular recognition.
2076 *F1000 Biol Rep* **5**:2.

2077 Jensen MR, Markwick PR, Meier S, Griesinger C, Zweckstetter M, Grzesiek S, Bernado P and
2078 Blackledge M (2009) Quantitative determination of the conformational properties of partially
2079 folded and intrinsically disordered proteins using NMR dipolar couplings. *Structure* **17**:1169-
2080 1185.

2081 Jin F, Yu C, Lai L and Liu Z (2013) Ligand clouds around protein clouds: a scenario of ligand binding
2082 with intrinsically disordered proteins. *PLoS Comput Biol* **9**:e1003249.

2083 Jobdeedamrong A, Cao S, Harley I, Crespy D, Landfester K and Caire da Silva L (2023) Assembly of
2084 biomimetic microreactors using caged-coacervate droplets. *Nanoscale* **15**:2561-2566.

2085 Joce C, Stahl JA, Shridhar M, Hutchinson MR, Watkins LR, Fedichev PO and Yin H (2010) Application
2086 of a novel in silico high-throughput screen to identify selective inhibitors for protein-protein
2087 interactions. *Bioorg Med Chem Lett* **20**:5411-5413.

2088 Joshi P, Chia S, Habchi J, Knowles TP, Dobson CM and Vendruscolo M (2016) A Fragment-Based
2089 Method of Creating Small-Molecule Libraries to Target the Aggregation of Intrinsically
2090 Disordered Proteins. *ACS Comb Sci* **18**:144-153.

2091 Joshi P and Vendruscolo M (2015) Druggability of Intrinsically Disordered Proteins. *Adv Exp Med Biol*
2092 **870**:383-400.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2093 Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Zidek
2094 A, Potapenko A, Bridgland A, Meyer C, Kohl SAA, Ballard AJ, Cowie A, Romera-Paredes B,
2095 Nikolov S, Jain R, Adler J, Back T, Petersen S, Reiman D, Clancy E, Zielinski M, Steinegger M,
2096 Pacholska M, Berghammer T, Bodenstein S, Silver D, Vinyals O, Senior AW, Kavukcuoglu K,
2097 Kohli P and Hassabis D (2021) Highly accurate protein structure prediction with AlphaFold.
2098 *Nature* **596**:583-589.

2099 Kagi JH and Schaffer A (1988) Biochemistry of metallothionein. *Biochemistry* **27**:8509-8515.

2100 Kalliolias GD and Ivashkiv LB (2010) Overview of the biology of type I interferons. *Arthritis Res Ther*
2101 **12 Suppl 1**:S1.

2102 Kamagata K, Kanbayashi S, Honda M, Itoh Y, Takahashi H, Kameda T, Nagatsugi F and Takahashi S
2103 (2020) Liquid-like droplet formation by tumor suppressor p53 induced by multivalent
2104 electrostatic interactions between two disordered domains. *Sci Rep* **10**:580.

2105 Karush F (1950) Heterogeneity of the binding sites of bovine serum albumin. *J Am Chem Soc* **72**:2705-
2106 2713.

2107 Keeffe EB, Hollinger FB, Bailey R, Bain VG, Bala K, Balart L, Black M, Bonkovsky H, Cassidy W,
2108 Craig JR, Donovan J, Dusheiko GM, Ehrinpreis M, Everson G, Feinman S, Foust RT, Fromm H,
2109 Hauser S, Heathcote EJ, Hoefs JC, Hunter E, James S, Jensen DM, Killenberg P, Krawitt EL, Lee
2110 S, Lee WM, vanLeeuwen DJ, Lesesne H, Lieberman H, Lissoos T, Lumeng L, Lyche K,
2111 McHutchison JG, Minuk G, Mullen K, Payne KM, Pimstone N, Pockros PJ, Poterucha J, Reddy
2112 KR, Rosenblate H, Shafritz D, Smith C, Tong MJ, White H and Willems B (1997) Therapy of
2113 hepatitis C: Consensus interferon trials. *Hepatology* **26**:S101-S107.

2114 Khattar SK, Samal S, LaBranche CC, Montefiori DC, Collins PL and Samal SK (2013) Comparative
2115 immunogenicity of HIV-1 gp160, gp140 and gp120 expressed by live attenuated newcastle
2116 disease virus vector. *PLoS One* **8**:e78521.

2117 Kiebler MA and Bassell GJ (2006) Neuronal RNA granules: movers and makers. *Neuron* **51**:685-690.

2118 Kim MS, Pinto SM, Getnet D, Nirujogi RS, Manda SS, Chaerkady R, Madugundu AK, Kelkar DS,
2119 Isserlin R, Jain S, Thomas JK, Muthusamy B, Leal-Rojas P, Kumar P, Sahasrabuddhe NA,
2120 Balakrishnan L, Advani J, George B, Renuse S, Selvan LDN, Patil AH, Nanjappa V,
2121 Radhakrishnan A, Prasad S, Subbannayya T, Raju R, Kumar M, Sreenivasamurthy SK,
2122 Marimuthu A, Sathe GJ, Chavan S, Datta KK, Subbannayya Y, Sahu A, Yelamanchi SD,
2123 Jayaram S, Rajagopalan P, Sharma J, Murthy KR, Syed N, Goel R, Khan AA, Ahmad S, Dey G,
2124 Mudgal K, Chatterjee A, Huang TC, Zhong J, Wu XY, Shaw PG, Freed D, Zahari MS,
2125 Mukherjee KK, Shankar S, Mahadevan A, Lam H, Mitchell CJ, Shankar SK, Satishchandra P,
2126 Schroeder JT, Sirdeshmukh R, Maitra A, Leach SD, Drake CG, Halushka MK, Prasad TSK,
2127 Hruban RH, Kerr CL, Bader GD, Iacobuzio-Donahue CA, Gowda H and Pandey A (2014) A
2128 draft map of the human proteome. *Nature* **509**:575-581.

2129 Kim MY, Na I, Kim JS, Son SH, Choi S, Lee SE, Kim JH, Jang K, Alterovitz G, Chen Y, van der Vaart
2130 A, Won HS, Uversky VN and Kim CG (2019) Rational discovery of antimetastatic agents
2131 targeting the intrinsically disordered region of MBD2. *Sci Adv* **5**:eaav9810.

2132 Kim TD, Ryu HJ, Cho HI, Yang CH and Kim J (2000) Thermal behavior of proteins: heat-resistant
2133 proteins and their heat-induced secondary structural changes. *Biochemistry* **39**:14839--14846.

2134 Klein C and Vassilev LT (2004) Targeting the p53-MDM2 interaction to treat cancer. *Br J Cancer*
2135 **91**:1415-1419.

2136 Klein ML, Bartley TD, Lai PH and Lu HS (1988) Structural Characterization of Recombinant Consensus
2137 Interferon-Alpha. *Journal of Chromatography* **454**:205-215.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2138 Koehler AN (2010) A complex task? Direct modulation of transcription factors with small molecules.
2139 *Curr Opin Chem Biol* **14**:331-340.

2140 Kong L, Lee JH, Doores KJ, Murin CD, Julien JP, McBride R, Liu Y, Marozsan A, Cupo A, Klasse PJ,
2141 Hoffenberg S, Caulfield M, King CR, Hua Y, Le KM, Khayat R, Deller MC, Clayton T, Tien H,
2142 Feizi T, Sanders RW, Paulson JC, Moore JP, Stanfield RL, Burton DR, Ward AB and Wilson IA
2143 (2013) Supersite of immune vulnerability on the glycosylated face of HIV-1 envelope
2144 glycoprotein gp120. *Nature structural & molecular biology* **20**:796-803.

2145 Kortvelyesi T, Dennis S, Silberstein M, Brown L, 3rd and Vajda S (2003) Algorithms for computational
2146 solvent mapping of proteins. *Proteins* **51**:340-351.

2147 Kosik KS, Joachim CL and Selkoe DJ (1986) Microtubule-associated protein tau (tau) is a major
2148 antigenic component of paired helical filaments in Alzheimer disease. *Proc Natl Acad Sci U S A*
2149 **83**:4044-4048.

2150 Kovar H, Aryee DN, Jug G, Henockl C, Schemper M, Delattre O, Thomas G and Gadner H (1996)
2151 EWS/FLI-1 antagonists induce growth inhibition of Ewing tumor cells in vitro. *Cell Growth*
2152 *Differ* **7**:429-437.

2153 Kriwacki RW, Hengst L, Tennant L, Reed SI and Wright PE (1996) Structural studies of $p21^{Waf1/Cip1/Sdi1}$ in
2154 the free and Cdk2-bound state: conformational disorder mediates binding diversity. *Proc Natl*
2155 *Acad Sci U S A* **93**:11504-11509.

2156 Kunert R, Ruker F and Katinger H (1998) Molecular characterization of five neutralizing anti-HIV type 1
2157 antibodies: identification of nonconventional D segments in the human monoclonal antibodies
2158 2G12 and 2F5. *AIDS Res Hum Retroviruses* **14**:1115-1128.

2159 Kyte J and Doolittle RF (1982) A simple method for displaying the hydropathic character of a protein. *J*
2160 *Mol Biol* **157**:105-132.

2161 Lamond AI and Spector DL (2003) Nuclear speckles: a model for nuclear organelles. *Nat Rev Mol Cell*
2162 *Biol* **4**:605-612.

2163 Latchman DS (1997) Transcription factors: an overview. *Int J Biochem Cell Biol* **29**:1305-1312.

2164 Latchman DS (2000) Transcription factors as potential targets for therapeutic drugs. *Curr Pharm*
2165 *Biotechnol* **1**:57-61.

2166 Lavecchia A and Di Giovanni C (2013) Virtual screening strategies in drug discovery: a critical review.
2167 *Curr Med Chem* **20**:2839-2860.

2168 Lavery DN and McEwan IJ (2005) Structure and function of steroid receptor AF1 transactivation
2169 domains: induction of active conformations. *Biochem J* **391**:449-464.

2170 Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, Meyerson M, Gabriel
2171 SB, Lander ES and Getz G (2014) Discovery and saturation analysis of cancer genes across 21
2172 tumour types. *Nature* **505**:495-501.

2173 Lee H, Mok KH, Muhandiram R, Park KH, Suk JE, Kim DH, Chang J, Sung YC, Choi KY and Han KH
2174 (2000) Local structural elements in the mostly unstructured transcriptional activation domain of
2175 human p53. *J Biol Chem* **275**:29426-29432.

2176 Lee SH, Kim DH, Han JJ, Cha EJ, Lim JE, Cho YJ, Lee C and Han KH (2012) Understanding pre-
2177 structured motifs (PreSMos) in intrinsically unfolded proteins. *Curr Protein Pept Sci* **13**:34-54.

2178 Lemieux UR and Spohr U (1994) How Emil Fischer was led to the lock and key concept for enzyme
2179 specificity. *Adv Carbohydrate Chem Biochem* **50**:1-20.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2180 Leonard CK, Spellman MW, Riddle L, Harris RJ, Thomas JN and Gregory TJ (1990) Assignment of
2181 intrachain disulfide bonds and characterization of potential glycosylation sites of the type 1
2182 recombinant human immunodeficiency virus envelope glycoprotein (gp120) expressed in Chinese
2183 hamster ovary cells. *J Biol Chem* **265**:10373-10382.

2184 Li B, Carey M and Workman JL (2007) The role of chromatin during transcription. *Cell* **128**:707-719.

2185 Li H, Lin PH, Gupta P, Li X, Zhao SL, Zhou X, Li Z, Wei S, Xu L, Han R, Lu J, Tan T, Yang DH, Chen
2186 ZS, Pawlik TM, Merritt RE and Ma J (2021) MG53 suppresses tumor progression and stress
2187 granule formation by modulating G3BP2 activity in non-small cell lung cancer. *Mol Cancer*
2188 **20**:118.

2189 Li L, Roy K, Katyal S, Sun X, Bleoo S and Godbout R (2006) Dynamic nature of cleavage bodies and
2190 their spatial relationship to DDX1 bodies, Cajal bodies, and gems. *Mol Biol Cell* **17**:1126-1140.

2191 Li P, Banjade S, Cheng HC, Kim S, Chen B, Guo L, Llaguno M, Hollingsworth JV, King DS, Banani SF,
2192 Russo PS, Jiang QX, Nixon BT and Rosen MK (2012) Phase transitions in the assembly of
2193 multivalent signalling proteins. *Nature* **483**:336-340.

2194 Liang J, Edelsbrunner H and Woodward C (1998) Anatomy of protein pockets and cavities: measurement
2195 of binding site geometry and implications for ligand design. *Protein Sci* **7**:1884-1897.

2196 Lin Y, Protter DS, Rosen MK and Parker R (2015) Formation and Maturation of Phase-Separated Liquid
2197 Droplets by RNA-Binding Proteins. *Mol Cell* **60**:208-219.

2198 Linderstrom-Lang K and Schellman JA (1959) Protein structure and enzyme activity, in *The Enzymes*
2199 (Boyer PD, Lardy H and Myrback K eds) pp 443-510, Academic Press, New York.

2200 Linding R, Russell RB, Neduva V and Gibson TJ (2003) GlobPlot: Exploring protein sequences for
2201 globularity and disorder. *Nucleic Acids Res* **31**:3701-3708.

2202 Liu J, Perumal NB, Oldfield CJ, Su EW, Uversky VN and Dunker AK (2006a) Intrinsic disorder in
2203 transcription factors. *Biochemistry* **45**:6873-6888.

2204 Liu M, Wu C, Wang R, Qiu J, She Z, Qu J and Xia J (2023) Modulating Liquid–Liquid Phase Separation
2205 of Nck Adaptor Protein against Enteropathogenic Escherichia coli Infection. *ACS Central Science*
2206 **9**:2358-2368.

2207 Liu T and Bitan G (2012) Modulating self-assembly of amyloidogenic proteins as a therapeutic approach
2208 for neurodegenerative diseases: strategies and mechanisms. *ChemMedChem* **7**:359-374.

2209 Liu W, Dreher MR, Furgeson DY, Peixoto KV, Yuan H, Zalutsky MR and Chilkoti A (2006b) Tumor
2210 accumulation, degradation and pharmacokinetics of elastin-like polypeptides in nude mice. *J*
2211 *Control Release* **116**:170-178.

2212 Liu YJ (2005) IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell
2213 precursors. *Annu Rev Immunol* **23**:275-306.

2214 Livesay DR (2010) Protein dynamics: dancing on an ever-changing free energy stage. *Curr Opin*
2215 *Pharmacol* **10**:706-708.

2216 Lohr T, Kohlhoff K, Heller GT, Camilloni C and Vendruscolo M (2022) A Small Molecule Stabilizes the
2217 Disordered Native State of the Alzheimer's Abeta Peptide. *ACS Chem Neurosci* **13**:1738-1745.

2218 Lovestone S, Reynolds CH, Latimer D, Davis DR, Anderton BH, Gallo JM, Hanger D, Mulot S,
2219 Marquardt B, Stabel S and et al. (1994) Alzheimer's disease-like phosphorylation of the
2220 microtubule-associated protein tau by glycogen synthase kinase-3 in transfected mammalian
2221 cells. *Current biology : CB* **4**:1077-1086.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2222 Ma B, Kumar S, Tsai CJ and Nussinov R (1999) Folding funnels and binding mechanisms. *Protein Eng*
2223 **12**:713-720.

2224 Ma K, Inglis JD, Sharkey A, Bickmore WA, Hill RE, Prosser EJ, Speed RM, Thomson EJ, Jobling M,
2225 Taylor K and et al. (1993) A Y chromosome gene family with RNA-binding protein homology:
2226 candidates for the azoospermia factor AZF controlling human spermatogenesis. *Cell* **75**:1287-
2227 1295.

2228 Ma X, Lu C, Chen Y, Li S, Ma N, Tao X, Li Y, Wang J, Zhou M, Yan Y-B, Li P, Heydari K, Deng H,
2229 Zhang M, Yi C and Ge L (2022) CCT2 is an aggrephagy receptor for clearance of solid protein
2230 aggregates. *Cell* **185**:1325-1345.e1322.

2231 MacKay JA, Chen M, McDaniel JR, Liu W, Simnick AJ and Chilkoti A (2009) Self-assembling chimeric
2232 polypeptide-doxorubicin conjugate nanoparticles that abolish tumours after a single injection.
2233 *Nature materials* **8**:993-999.

2234 Masignani V, Rappuoli R and Pizza M (2002) Reverse vaccinology: a genome-based approach for
2235 vaccine development. *Expert Opin Biol Ther* **2**:895-905.

2236 Massodi I, Bidwell GL, 3rd and Raucher D (2005) Evaluation of cell penetrating peptides fused to elastin-
2237 like polypeptide for drug delivery. *J Control Release* **108**:396-408.

2238 Massodi I, Moktan S, Rawat A, Bidwell GL, 3rd and Raucher D (2010) Inhibition of ovarian cancer cell
2239 proliferation by a cell cycle inhibitory peptide fused to a thermally responsive polypeptide carrier.
2240 *Int J Cancer* **126**:533-544.

2241 Massodi I and Raucher D (2007) A thermally responsive Tat-elastin-like polypeptide fusion protein
2242 induces membrane leakage, apoptosis, and cell death in human breast cancer cells. *J Drug Target*
2243 **15**:611-622.

2244 Massodi I, Thomas E and Raucher D (2009) Application of thermally responsive elastin-like polypeptide
2245 fused to a lactoferrin-derived peptide for treatment of pancreatic cancer. *Molecules* **14**:1999-
2246 2015.

2247 Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL and Beyreuther K (1985) Amyloid
2248 plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci U S A*
2249 **82**:4245-4249.

2250 Masuda M, Suzuki N, Taniguchi S, Oikawa T, Nonaka T, Iwatsubo T, Hisanaga S, Goedert M and
2251 Hasegawa M (2006) Small molecule inhibitors of alpha-synuclein filament assembly.
2252 *Biochemistry* **45**:6085-6094.

2253 Matera AG and Frey MR (1998) Coiled bodies and gems: Janus or gemini? *Am J Hum Genet* **63**:317-321.

2254 Maul GG, Negorev D, Bell P and Ishov AM (2000) Review: properties and assembly mechanisms of
2255 ND10, PML bodies, or PODs. *Journal of structural biology* **129**:278-287.

2256 McDaniel JR, Bhattacharyya J, Vargo KB, Hassouneh W, Hammer DA and Chilkoti A (2013a) Self-
2257 assembly of thermally responsive nanoparticles of a genetically encoded peptide polymer by drug
2258 conjugation. *Angewandte Chemie* **52**:1683-1687.

2259 McDaniel JR, MacEwan SR, Li X, Radford DC, Landon CD, Dewhirst M and Chilkoti A (2014) Rational
2260 design of "heat seeking" drug loaded polypeptide nanoparticles that thermally target solid tumors.
2261 *Nano Lett* **14**:2890-2895.

2262 McDaniel JR, Radford DC and Chilkoti A (2013b) A unified model for de novo design of elastin-like
2263 polypeptides with tunable inverse transition temperatures. *Biomacromolecules* **14**:2866-2872.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2264 McEwan IJ, Lavery D, Fischer K and Watt K (2007) Natural disordered sequences in the amino terminal
2265 domain of nuclear receptors: lessons from the androgen and glucocorticoid receptors. *Nucl Recept*
2266 *Signal* **5**:e001.

2267 McInnes C (2007) Virtual screening strategies in drug discovery. *Curr Opin Chem Biol* **11**:494-502.

2268 McLellan JS, Pancera M, Carrico C, Gorman J, Julien JP, Khayat R, Louder R, Pejchal R, Sastry M, Dai
2269 K, O'Dell S, Patel N, Shahzad-ul-Hussan S, Yang Y, Zhang B, Zhou T, Zhu J, Boyington JC,
2270 Chuang GY, Diwanji D, Georgiev I, Kwon YD, Lee D, Louder MK, Moquin S, Schmidt SD,
2271 Yang ZY, Bonsignori M, Crump JA, Kapiga SH, Sam NE, Haynes BF, Burton DR, Koff WC,
2272 Walker LM, Phogat S, Wyatt R, Orwenyo J, Wang LX, Arthos J, Bewley CA, Mascola JR, Nabel
2273 GJ, Schief WR, Ward AB, Wilson IA and Kwong PD (2011) Structure of HIV-1 gp120 V1/V2
2274 domain with broadly neutralizing antibody PG9. *Nature* **480**:336-343.

2275 Mehta S and Zhang J (2022) Liquid–liquid phase separation drives cellular function and dysfunction in
2276 cancer. *Nature Reviews Cancer* **22**:239-252.

2277 Meng F, Na I, Kurgan L and Uversky VN (2015) Compartmentalization and Functionality of Nuclear
2278 Disorder: Intrinsic Disorder and Protein-Protein Interactions in Intra-Nuclear Compartments. *Int J*
2279 *Mol Sci* **17**.

2280 Meszaros B, Simon I and Dosztanyi Z (2009) Prediction of protein binding regions in disordered proteins.
2281 *PLoS Comput Biol* **5**:e1000376.

2282 Metallo SJ (2010) Intrinsically disordered proteins are potential drug targets. *Curr Opin Chem Biol*
2283 **14**:481-488.

2284 Meyer DE and Chilkoti A (1999) Purification of recombinant proteins by fusion with thermally-
2285 responsive polypeptides. *Nature Biotechnology* **17**:1112-1115.

2286 Meyer DE and Chilkoti A (2004) Quantification of the effects of chain length and concentration on the
2287 thermal behavior of elastin-like polypeptides. *Biomacromolecules* **5**:846-851.

2288 Meyerhans A, Cheynier R, Albert J, Seth M, Kwok S, Sninsky J, Morfeldt-Manson L, Asjo B and Wain-
2289 Hobson S (1989) Temporal fluctuations in HIV quasispecies in vivo are not reflected by
2290 sequential HIV isolations. *Cell* **58**:901-910.

2291 Midic U, Oldfield CJ, Dunker AK, Obradovic Z and Uversky VN (2009) Unfoldomics of human genetic
2292 diseases: illustrative examples of ordered and intrinsically disordered members of the human
2293 diseasome. *Protein Pept Lett* **16**:1533-1547.

2294 Mikecin AM, Walker LR, Kuna M and Raucher D (2014) Thermally targeted p21 peptide enhances
2295 bortezomib cytotoxicity in androgen-independent prostate cancer cell lines. *Anticancer Drugs*
2296 **25**:189-199.

2297 Minezaki Y, Homma K, Kinjo AR and Nishikawa K (2006) Human transcription factors contain a high
2298 fraction of intrinsically disordered regions essential for transcriptional regulation. *J Mol Biol*
2299 **359**:1137-1149.

2300 Miroy GJ, Lai Z, Lashuel HA, Peterson SA, Strang C and Kelly JW (1996) Inhibiting transthyretin
2301 amyloid fibril formation via protein stabilization. *Proc Natl Acad Sci U S A* **93**:15051-15056.

2302 Mitrea DM, Cika JA, Guy CS, Ban D, Banerjee PR, Stanley CB, Nourse A, Deniz AA and Kriwacki RW
2303 (2016) Nucleophosmin integrates within the nucleolus via multi-modal interactions with proteins
2304 displaying R-rich linear motifs and rRNA. *Elife* **5**.

2305 Mitrea DM and Kriwacki RW (2016) Phase separation in biology; functional organization of a higher
2306 order. *Cell Commun Signal* **14**:1.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2307 Mitrea DM, Mittasch M, Gomes BF, Klein IA and Murcko MA (2022) Modulating biomolecular
2308 condensates: a novel approach to drug discovery. *Nature Reviews Drug Discovery* **21**:841-862.

2309 Miyazaki T (2023) Calpain and Cardiometabolic Diseases. *Int J Mol Sci* **24**.

2310 Mohammed AS and Uversky VN (2022) Intrinsic Disorder as a Natural Preservative: High Levels of
2311 Intrinsic Disorder in Proteins Found in the 2600-Year-Old Human Brain. *Biology (Basel)* **11**.

2312 Mohan A, Oldfield CJ, Radivojac P, Vacic V, Cortese MS, Dunker AK and Uversky VN (2006) Analysis
2313 of molecular recognition features (MoRFs). *J Mol Biol* **362**:1043-1059.

2314 Mohanram H, Kumar A, Verma CS, Pervushin K and Miserez A (2019) Three-dimensional structure of
2315 Megabalanus rosa Cement Protein 20 revealed by multi-dimensional NMR and molecular
2316 dynamics simulations. *Philos Trans R Soc Lond B Biol Sci* **374**:20190198.

2317 Mollica L, Bessa LM, Hanoulle X, Jensen MR, Blackledge M and Schneider R (2016) Binding
2318 Mechanisms of Intrinsically Disordered Proteins: Theory, Simulation, and Experiment. *Front Mol*
2319 *Biosci* **3**:52.

2320 Nair SK and Burley SK (2003) X-ray structures of Myc-Max and Mad-Max recognizing DNA. Molecular
2321 bases of regulation by proto-oncogenic transcription factors. *Cell* **112**:193-205.

2322 Neduva V and Russell RB (2005) Linear motifs: evolutionary interaction switches. *FEBS Lett* **579**:3342-
2323 3345.

2324 Nesterov SV, Ilyinsky NS and Uversky VN (2021) Liquid-liquid phase separation as a common
2325 organizing principle of intracellular space and biomembranes providing dynamic adaptive
2326 responses. *Biochim Biophys Acta Mol Cell Res* **1868**:119102.

2327 Ng KP, Potikyan G, Savene RO, Denny CT, Uversky VN and Lee KA (2007) Multiple aromatic side
2328 chains within a disordered structure are critical for transcription and transforming activity of
2329 EWS family oncoproteins. *Proc Natl Acad Sci U S A* **104**:479-484.

2330 Nizami Z, Deryusheva S and Gall JG (2010) The Cajal body and histone locus body. *Cold Spring Harb*
2331 *Perspect Biol* **2**:a000653.

2332 Nott TJ, Petsalaki E, Farber P, Jervis D, Fussner E, Plochowietz A, Craggs TD, Bazett-Jones DP, Pawson
2333 T, Forman-Kay JD and Baldwin AJ (2015) Phase transition of a disordered nuage protein
2334 generates environmentally responsive membraneless organelles. *Mol Cell* **57**:936-947.

2335 Nussenzweig A and Nussenzweig MC (2010) Origin of chromosomal translocations in lymphoid cancer.
2336 *Cell* **141**:27-38.

2337 Oates ME, Romero P, Ishida T, Ghalwash M, Mizianty MJ, Xue B, Dosztanyi Z, Uversky VN, Obradovic
2338 Z, Kurgan L, Dunker AK and Gough J (2013) D(2)P(2): database of disordered protein
2339 predictions. *Nucleic Acids Res* **41**:D508-516.

2340 Obst JK, Tien AH, Setiawan JC, Deneault LF and Sadar MD (2024) Inhibitors of the transactivation
2341 domain of androgen receptor as a therapy for prostate cancer. *Steroids* **210**:109482.

2342 Oka M, Mura S, Yamada K, Sangel P, Hirata S, Maehara K, Kawakami K, Tachibana T, Ohkawa Y,
2343 Kimura H and Yoneda Y (2016) Chromatin-prebound Crm1 recruits Nup98-HoxA9 fusion to
2344 induce aberrant expression of Hox cluster genes. *Elife* **5**:e09540.

2345 Oldfield CJ, Cheng Y, Cortese MS, Brown CJ, Uversky VN and Dunker AK (2005a) Comparing and
2346 combining predictors of mostly disordered proteins. *Biochemistry* **44**:1989-2000.

2347 Oldfield CJ, Cheng Y, Cortese MS, Romero P, Uversky VN and Dunker AK (2005b) Coupled folding
2348 and binding with alpha-helix-forming molecular recognition elements. *Biochemistry* **44**:12454-
2349 12470.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2350 Oldfield CJ, Meng J, Yang JY, Yang MQ, Uversky VN and Dunker AK (2008) Flexible nets: disorder
2351 and induced fit in the associations of p53 and 14-3-3 with their partners. *BMC Genomics* **9 Suppl**
2352 **1**:S1.

2353 Overington JP, Al-Lazikani B and Hopkins AL (2006) How many drug targets are there? *Nat Rev Drug*
2354 *Discov* **5**:993-996.

2355 Ozes ON, Reiter Z, Klein S, Blatt LM and Taylor MW (1992) A comparison of interferon-Con1 with
2356 natural recombinant interferons-alpha: antiviral, antiproliferative, and natural killer-inducing
2357 activities. *Journal of interferon research* **12**:55-59.

2358 Paiva LR and Martins ML (2011) A multiscale model to evaluate the efficacy of anticancer therapies
2359 based on chimeric polypeptide nanoparticles. *Applied Physics Letters* **98**.

2360 Pajkos M, Meszaros B, Simon I and Dosztanyi Z (2012) Is there a biological cost of protein disorder?
2361 Analysis of cancer-associated mutations. *Mol Biosyst* **8**:296-307.

2362 Palladino C, Ezeonwumelu IJ, Marcelino R, Briz V, Moranguinho I, Serejo F, Velosa JF, Marinho RT,
2363 Borrego P and Taveira N (2018) Epidemic history of hepatitis C virus genotypes and subtypes in
2364 Portugal. *Sci Rep* **8**:12266.

2365 Pancsa R, Schad E, Tantos A and Tompa P (2019) Emergent functions of proteins in non-stoichiometric
2366 supramolecular assemblies. *Biochim Biophys Acta Proteins Proteom* **1867**:970-979.

2367 Parkin J and Cohen B (2001) An overview of the immune system. *Lancet* **357**:1777-1789.

2368 Patel A, Mitrea D, Namasivayam V, Murcko MA, Wagner M and Klein IA (2022) Principles and
2369 functions of condensate modifying drugs. *Front Mol Biosci* **9**:1007744.

2370 Patil A and Nakamura H (2006) Disordered domains and high surface charge confer hubs with the ability
2371 to interact with multiple proteins in interaction networks. *FEBS Lett* **580**:2041-2045.

2372 Paul D, Sanap G, Shenoy S, Kalyane D, Kalia K and Tekade RK (2021) Artificial intelligence in drug
2373 discovery and development. *Drug Discov Today* **26**:80-93.

2374 Peciak K, Tommasi R, Choi JW, Brocchini S and Laurine E (2014) Expression of soluble and active
2375 interferon consensus in SUMO fusion expression system in E. coli. *Protein expression and*
2376 *purification* **99**:18-26.

2377 Pederson T (2001) Protein mobility within the nucleus--what are the right moves? *Cell* **104**:635-638.

2378 Pederson T (2011) The nucleoulus. *Cold Spring Harbor perspectives in biology* **3**:a000638.

2379 Pejaver V, Hsu WL, Xin F, Dunker AK, Uversky VN and Radivojac P (2014) The structural and
2380 functional signatures of proteins that undergo multiple events of post-translational modification.
2381 *Protein Sci* **23**:1077-1093.

2382 Peng K, Vucetic S, Radivojac P, Brown CJ, Dunker AK and Obradovic Z (2005) Optimizing long
2383 intrinsic disorder predictors with protein evolutionary information. *J Bioinform Comput Biol*
2384 **3**:35-60.

2385 Peng ZL and Kurgan L (2012) Comprehensive comparative assessment of in-silico predictors of
2386 disordered regions. *Curr Protein Pept Sci* **13**:6-18.

2387 Permyakov SE, Millett IS, Doniach S, Permyakov EA and Uversky VN (2003) Natively unfolded C-
2388 terminal domain of caldesmon remains substantially unstructured after the effective binding to
2389 calmodulin. *Proteins* **53**:855--862.

2390 Pestka S, Langer JA, Zoon KC and Samuel CE (1987) Interferons and their actions. *Annual review of*
2391 *biochemistry* **56**:727-777.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2392 Phair RD and Misteli T (2000) High mobility of proteins in the mammalian cell nucleus. *Nature* **404**:604-
2393 609.

2394 Pickhardt M, Neumann T, Schwizer D, Callaway K, Vendruscolo M, Schenk D, St George-Hyslop P,
2395 Mandelkow EM, Dobson CM, McConlogue L, Mandelkow E and Toth G (2015) Identification of
2396 Small Molecule Inhibitors of Tau Aggregation by Targeting Monomeric Tau As a Potential
2397 Therapeutic Approach for Tauopathies. *Curr Alzheimer Res* **12**:814-828.

2398 Pirrotta V and Li HB (2012) A view of nuclear Polycomb bodies. *Curr Opin Genet Dev* **22**:101-109.

2399 Platanias LC (2005) Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol*
2400 **5**:375-386.

2401 Plaxco KW and Gross M (1997) Cell biology. The importance of being unfolded. *Nature* **386**:657, 659.

2402 Pontius BW (1993) Close encounters: why unstructured, polymeric domains can increase rates of specific
2403 macromolecular association. *Trends Biochem Sci* **18**:181-186.

2404 Popelka H and Uversky VN (2022) Theater in the Self-Cleaning Cell: Intrinsically Disordered Proteins or
2405 Protein Regions Acting with Membranes in Autophagy. *Membranes (Basel)* **12**.

2406 Prabhudesai S, Sinha S, Attar A, Kotagiri A, Fitzmaurice AG, Lakshmanan R, Ivanova MI, Loo JA,
2407 Klarner FG, Schrader T, Stahl M, Bitan G and Bronstein JM (2012) A novel "molecular tweezer"
2408 inhibitor of alpha-synuclein neurotoxicity in vitro and in vivo. *Neurotherapeutics* **9**: 464-476.

2409 Pradhan A, Mishra S, Surolia A and Panda D (2021) C1 Inhibits Liquid–Liquid Phase Separation and
2410 Oligomerization of Tau and Protects Neuroblastoma Cells against Toxic Tau Oligomers. *ACS*
2411 *Chemical Neuroscience* **12**:1989-2002.

2412 Pullen RA, Jenkins JA, Tickle IJ, Wood SP and Blundell TL (1975) The relation of polypeptide hormone
2413 structure and flexibility to receptor binding: the relevance of X-ray studies on insulins, glucagon
2414 and human placental lactogen. *Mol Cell Biochem* **8**:5-20.

2415 Puntervoll P, Linding R, Gemund C, Chabanis-Davidson S, Mattingsdal M, Cameron S, Martin DM,
2416 Ausiello G, Brannetti B, Costantini A, Ferre F, Maselli V, Via A, Cesareni G, Diella F, Superti-
2417 Furga G, Wyrwicz L, Ramu C, McGuigan C, Gudavalli R, Letunic I, Bork P, Rychlewski L,
2418 Kuster B, Helmer-Citterich M, Hunter WN, Aasland R and Gibson TJ (2003) ELM server: A new
2419 resource for investigating short functional sites in modular eukaryotic proteins. *Nucleic Acids Res*
2420 **31**:3625-3630.

2421 Purtscher M, Trkola A, Gruber G, Buchacher A, Predl R, Steindl F, Tauer C, Berger R, Barrett N,
2422 Jungbauer A and et al. (1994) A broadly neutralizing human monoclonal antibody against gp41
2423 of human immunodeficiency virus type 1. *AIDS Res Hum Retroviruses* **10**:1651-1658.

2424 Pytowski L, Lee CF, Foley AC, Vaux DJ and Jean L (2020) Liquid–liquid phase separation of type II
2425 diabetes-associated IAPP initiates hydrogelation and aggregation. *Proceedings of the National*
2426 *Academy of Sciences* **117**:12050-12061.

2427 Raasakka A and Kursula P (2020) Flexible Players within the Sheaths: The Intrinsically Disordered
2428 Proteins of Myelin in Health and Disease. *Cells* **9**.

2429 Radhakrishnan R, Walter LJ, Hruza A, Reichert P, Trotta PP, Nagabhushan TL and Walter MR (1996)
2430 Zinc mediated dimer of human interferon-alpha(2b) revealed by X-ray crystallography. *Structure*
2431 **4**:1453-1463.

2432 Radivojac P, Iakoucheva LM, Oldfield CJ, Obradovic Z, Uversky VN and Dunker AK (2007) Intrinsic
2433 disorder and functional proteomics. *Biophys J* **92**:1439-1456.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2434 Ramelot TA, Gentile LN and Nicholson LK (2000) Transient structure of the amyloid precursor protein
2435 cytoplasmic tail indicates preordering of structure for binding to cytosolic factors. *Biochemistry*
2436 **39**:2714-2725.

2437 Ramesh M, Balachandra C, Baruah P and Govindaraju T (2023) Cyclic dipeptide-based small molecules
2438 modulate zinc-mediated liquid-liquid phase separation of tau. *J Pept Sci* **29**:e3465.

2439 Rao JN, Jao CC, Hegde BG, Langen R and Ulmer TS (2010) A combinatorial NMR and EPR approach
2440 for evaluating the structural ensemble of partially folded proteins. *J Am Chem Soc* **132**:8657-
2441 8668.

2442 Rappuoli R (2001) Reverse vaccinology, a genome-based approach to vaccine development. *Vaccine*
2443 **19**:2688-2691.

2444 Ray S, Singh N, Kumar R, Patel K, Pandey S, Datta D, Mahato J, Panigrahi R, Navalkar A, Mehra S,
2445 Gadhe L, Chatterjee D, Sawner AS, Maiti S, Bhatia S, Gerez JA, Chowdhury A, Kumar A,
2446 Padinhateeri R, Riek R, Krishnamoorthy G and Maji SK (2020) α-Synuclein aggregation
2447 nucleates through liquid–liquid phase separation. *Nature Chemistry* **12**:705-716.

2448 Razzak M and De Brabander JK (2011) Lessons and revelations from biomimetic syntheses. *Nature*
2449 *Chemical Biology* **7**:865-875.

2450 Reddy PJ, Ray S and Srivastava S (2015) The Quest of the Human Proteome and the Missing Proteins:
2451 Digging Deeper. *Omics-a Journal of Integrative Biology* **19**:276-282.

2452 Regep C, Georges G, Shi J, Popovic B and Deane CM (2017) The H3 loop of antibodies shows unique
2453 structural characteristics. *Proteins* **85**:1311-1318.

2454 Ren J, Zhang Z, Zong Z, Zhang L and Zhou F (2022) Emerging Implications of Phase Separation in
2455 Cancer. *Advanced Science* **9**:2202855.

2456 Richard JW, Hyun OL, Ina P, Arun P, Thom D, Satoshi K, Sukhleen K, Eric Nathaniel A, Lara M,
2457 Anastasia CM, Marcus J, Xiaojie Z, Edgar B, Anatol F, Nicolas LF, Jared S, Udai P, Della CD,
2458 Benjamin GD, Andrew JB, Andreas H, Marc B, Simon A and Anthony AH (2019) Small
2459 molecules for modulating protein driven liquid-liquid phase separation in treating
2460 neurodegenerative disease. *bioRxiv*:721001.

2461 Rinaudo CD, Telford JL, Rappuoli R and Seib KL (2009) Vaccinology in the genome era. *J Clin Invest*
2462 **119**:2515-2525.

2463 Ringe D and Petsko GA (2009) Q&A: What are pharmacological chaperones and why are they
2464 interesting? *Journal of biology* **8**:1-4.

2465 Roberts R, Timchenko NA, Miller JW, Reddy S, Caskey CT, Swanson MS and Timchenko LT (1997)
2466 Altered phosphorylation and intracellular distribution of a (CUG)n triplet repeat RNA-binding
2467 protein in patients with myotonic dystrophy and in myotonin protein kinase knockout mice. *Proc*
2468 *Natl Acad Sci U S A* **94**:13221-13226.

2469 Robustelli P, Ibanez-de-Opakua A, Campbell-Bezat C, Giordanetto F, Becker S, Zweckstetter M, Pan AC
2470 and Shaw DE (2022) Molecular Basis of Small-Molecule Binding to alpha-Synuclein. *J Am*
2471 *Chem Soc* **144**:2501-2510.

2472 Romero-Isart N, Oliva B and Vašák M (2010) Influence of bonding interactions on the structure and
2473 dynamics of metallothioneins. *Journal of molecular modeling* **16**:387-394.

2474 Romero P, Obradovic Z, Li X, Garner EC, Brown CJ and Dunker AK (2001) Sequence complexity of
2475 disordered protein. *Proteins* **42**:38-48.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2476 Ross CA and Poirier MA (2005) Opinion: What is the role of protein aggregation in neurodegeneration?
2477 *Nat Rev Mol Cell Biol* **6**:891-898.

2478 Ruan H, Sun Q, Zhang W, Liu Y and Lai L (2019) Targeting intrinsically disordered proteins at the edge
2479 of chaos. *Drug Discov Today* **24**:217-227.

2480 Ruan H, Yu C, Niu X, Zhang W, Liu H, Chen L, Xiong R, Sun Q, Jin C, Liu Y and Lai L (2020)
2481 Computational strategy for intrinsically disordered protein ligand design leads to the discovery of
2482 p53 transactivation domain I binding compounds that activate the p53 pathway. *Chem Sci*
2483 **12**:3004-3016.

2484 Ryu JS and Raucher D (2014) Elastin-like polypeptides: The influence of its molecular weight on local
2485 hyperthermia-induced tumor accumulation. *Eur J Pharm Biopharm*.

2486 Sadar MD, Williams DE, Mawji NR, Patrick BO, Wikanta T, Chasanah E, Irianto HE, Soest RV and
2487 Andersen RJ (2008) Sintokamides A to E, chlorinated peptides from the sponge Dysidea sp. that
2488 inhibit transactivation of the N-terminus of the androgen receptor in prostate cancer cells. *Org*
2489 *Lett* **10**:4947-4950.

2490 Saikia B and Baruah A (2024) Recent advances in de novo computational design and redesign of
2491 intrinsically disordered proteins and intrinsically disordered protein regions. *Arch Biochem*
2492 *Biophys* **752**:109857.

2493 Salmon L, Nodet G, Ozenne V, Yin G, Jensen MR, Zweckstetter M and Blackledge M (2010) NMR
2494 characterization of long-range order in intrinsically disordered proteins. *J Am Chem Soc*
2495 **132**:8407-8418.

2496 Sanna PP, Williamson RA, De Logu A, Bloom FE and Burton DR (1995) Directed selection of
2497 recombinant human monoclonal antibodies to herpes simplex virus glycoproteins from phage
2498 display libraries. *Proc Natl Acad Sci U S A* **92**:6439-6443.

2499 Santofimia-Castano P, Rizzuti B, Xia Y, Abian O, Peng L, Velazquez-Campoy A, Neira JL and Iovanna J
2500 (2020) Targeting intrinsically disordered proteins involved in cancer. *Cell Mol Life Sci* **77**:1695-
2501 1707.

2502 Saphire EO, Parren PW, Pantophlet R, Zwick MB, Morris GM, Rudd PM, Dwek RA, Stanfield RL,
2503 Burton DR and Wilson IA (2001) Crystal structure of a neutralizing human IGG against HIV-1: a
2504 template for vaccine design. *Science* **293**:1155-1159.

2505 Saurabh S, Nadendla K, Purohit SS, Sivakumar PM and Cetinel S (2023) Fuzzy Drug Targets: Disordered
2506 Proteins in the Drug-Discovery Realm. *ACS Omega* **8**:9729-9747.

2507 Sawner AS, Ray S, Yadav P, Mukherjee S, Panigrahi R, Poudyal M, Patel K, Ghosh D, Kummerant E,
2508 Kumar A, Riek R and Maji SK (2021) Modulating α-Synuclein Liquid-Liquid Phase Separation.
2509 *Biochemistry* **60**:3676-3696.

2510 Sayers EW, Gerstner RB, Draper DE and Torchia DA (2000) Structural preordering in the N-terminal
2511 region of ribosomal protein S4 revealed by heteronuclear NMR spectroscopy. *Biochemistry*
2512 **39**:13602-13613.

2513 Schluter H, Apweiler R, Holzhutter HG and Jungblut PR (2009) Finding one's way in proteomics: a
2514 protein species nomenclature. *Chem Cent J* **3**:11.

2515 Schulz GE (1979) Nucleotide Binding Proteins, in *Molecular Mechanism of Biological Recognition*
2516 (Balaban M ed) pp 79-94, Elsevier/North-Holland Biomedical Press, New York.

2517 Schweers O, Schonbrunn-Hanebeck E, Marx A and Mandelkow E (1994) Structural studies of tau protein
2518 and Alzheimer paired helical filaments show no evidence for beta-structure. *J Biol Chem*
2519 **269**:24290-24297.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2520 Selvanathan SP, Moseley E, Graham GT, Jessen K, Lannutti B, Üren A and Toretsky JA (2017) TK-216:
2521 A novel, first-in-class, small molecule inhibitor of EWS-FLI1 in early clinical development, for
2522 the treatment of Ewing Sarcoma. *Cancer research* **77**:694-694.

2523 Sen GC and Lengyel P (1992) The interferon system. A bird's eye view of its biochemistry. *J Biol Chem*
2524 **267**:5017-5020.

2525 Shamji MF, Chen J, Friedman AH, Richardson WJ, Chilkoti A and Setton LA (2008) Synthesis and
2526 characterization of a thermally-responsive tumor necrosis factor antagonist. *J Control Release*
2527 **129**:179-186.

2528 Shav-Tal Y, Blechman J, Darzacq X, Montagna C, Dye BT, Patton JG, Singer RH and Zipori D (2005)
2529 Dynamic sorting of nuclear components into distinct nucleolar caps during transcriptional
2530 inhibition. *Mol Biol Cell* **16**:2395-2413.

2531 Shi Q, Kang K and Chen YG (2021) Liquid-liquid phase separation drives the β-catenin destruction
2532 complex formation. *Bioessays* **43**:e2100138.

2533 Shi W, Tang J and Xiang J (2024) Therapeutic strategies for aberrant splicing in cancer and genetic
2534 disorders. *Clin Genet* **105**:345-354.

2535 Sickmeier M, Hamilton JA, LeGall T, Vacic V, Cortese MS, Tantos A, Szabo B, Tompa P, Chen J,
2536 Uversky VN, Obradovic Z and Dunker AK (2007) DisProt: the Database of Disordered Proteins.
2537 *Nucleic Acids Res* **35**:D786-793.

2538 Singh GP, Ganapathi M and Dash D (2007) Role of intrinsic disorder in transient interactions of hub
2539 proteins. *Proteins* **66**:761-765.

2540 Sinha S, Lopes DH, Du Z, Pang ES, Shanmugam A, Lomakin A, Talbiersky P, Tennstaedt A, McDaniel
2541 K, Bakshi R, Kuo PY, Ehrmann M, Benedek GB, Loo JA, Klarner FG, Schrader T, Wang C and
2542 Bitan G (2011) Lysine-specific molecular tweezers are broad-spectrum inhibitors of assembly
2543 and toxicity of amyloid proteins. *J Am Chem Soc* **133**:16958-16969.

2544 Sjogren MH, Sjogren R, Holtzmuller K, Winston B, Butterfield B, Drake S, Watts A, Howard R and
2545 Smith M (2005) Interferon alfacon-1 and ribavirin versus interferon alpha-2b and ribavirin in the
2546 treatment of chronic hepatitis C. *Digestive Diseases and Sciences* **50**:727-732.

2547 Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT and Simmonds P (2014) Expanded
2548 classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype
2549 assignment web resource. *Hepatology* **59**:318-327.

2550 Smith LM, Kelleher NL and Consortium for Top Down P (2013) Proteoform: a single term describing
2551 protein complexity. *Nat Methods* **10**:186-187.

2552 Smith TJ, Chase ES, Schmidt TJ, Olson NH and Baker TS (1996) Neutralizing antibody to human
2553 rhinovirus 14 penetrates the receptor-binding canyon. *Nature* **383**:350-354.

2554 Somasekharan SP, El-Naggar A, Leprivier G, Cheng H, Hajee S, Grunewald TG, Zhang F, Ng T, Delattre
2555 O, Evdokimova V, Wang Y, Gleave M and Sorensen PH (2015) YB-1 regulates stress granule
2556 formation and tumor progression by translationally activating G3BP1. *The Journal of cell biology*
2557 **208**:913-929.

2558 Song GJ, Nam Y, Jo M, Jung M, Koo JY, Cho W, Koh M, Park SB and Suk K (2016) A novel small-
2559 molecule agonist of PPAR-γ potentiates an anti-inflammatory M2 glial phenotype.
2560 *Neuropharmacology* **109**:159-169.

2561 Song MS and Grabocka E (2023) Stress Granules in Cancer. *Rev Physiol Biochem Pharmacol* **185**:25-52.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2562 Spillantini MG, Crowther RA, Jakes R, Hasegawa M and Goedert M (1998) alpha-Synuclein in
2563 filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies.
2564 *Proc Natl Acad Sci U S A* **95**:6469-6473.

2565 Spolar RS and Record MT, Jr. (1994) Coupling of local folding to site-specific binding of proteins to
2566 DNA. *Science* **263**:777-784.

2567 Spriano F, Chung EYL, Gaudio E, Tarantelli C, Cascione L, Napoli S, Jessen K, Carrassa L, Priebe V,
2568 Sartori G, Graham G, Selvanathan SP, Cavalli A, Rinaldi A, Kwee I, Testoni M, Genini D, Ye
2569 BH, Zucca E, Stathis A, Lannutti B, Toretsky JA and Bertoni F (2019) The ETS Inhibitors YK-4-
2570 279 and TK-216 Are Novel Antilymphoma Agents. *Clin Cancer Res* **25**:5167-5176.

2571 Stanfield B and Kousoulas KG (2015) Herpes Simplex Vaccines: Prospects of Live-attenuated HSV
2572 Vaccines to Combat Genital and Ocular infections. *Curr Clin Microbiol Rep* **2**:125-136.

2573 Stanfield RL, Berndsen ZT, Huang R, Sok D, Warner G, Torres JL, Burton DR, Ward AB, Wilson IA and
2574 Smider VV (2020) Structural basis of broad HIV neutralization by a vaccine-induced cow
2575 antibody. *Sci Adv* **6**:eaba0468.

2576 Strzelecka M, Trowitzsch S, Weber G, Luhrmann R, Oates AC and Neugebauer KM (2010) Coilin-
2577 dependent snRNP assembly is essential for zebrafish embryogenesis. *Nature structural &*
2578 *molecular biology* **17**:403-409.

2579 Sundaresan Y, Banin E and Sharon D (2023) Exonic Variants that Affect Splicing - An Opportunity for
2580 "Hidden" Mutations Causing Inherited Retinal Diseases. *Adv Exp Med Biol* **1415**:183-187.

2581 Sung YH and Eliezer D (2007) Residual structure, backbone dynamics, and interactions within the
2582 synuclein family. *J Mol Biol* **372**:689-707.

2583 Szenasi T, Olah J, Szabo A, Szunyogh S, Lang A, Perczel A, Lehotzky A, Uversky VN and Ovadi J
2584 (2017) Challenging drug target for Parkinson's disease: Pathological complex of the chameleon
2585 TPPP/p25 and alpha-synuclein proteins. *Biochim Biophys Acta* **1863**:310-323.

2586 Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT,
2587 Morris JH, Bork P, Jensen LJ and Mering CV (2019) STRING v11: protein-protein association
2588 networks with increased coverage, supporting functional discovery in genome-wide experimental
2589 datasets. *Nucleic Acids Res* **47**:D607-d613.

2590 Takada I and Makishima M (2020) Peroxisome proliferator-activated receptor agonists and antagonists: a
2591 patent review (2014-present). *Expert Opin Ther Pat* **30**:1-13.

2592 Tan MH, Li J, Xu HE, Melcher K and Yong EL (2015) Androgen receptor: structure, role in prostate
2593 cancer and drug discovery. *Acta Pharmacol Sin* **36**:3-23.

2594 Tao Y, Zhang Q, Wang H, Yang X and Mu H (2024) Alternative splicing and related RNA binding
2595 proteins in human health and disease. *Signal Transduct Target Ther* **9**:26.

2596 The Lancet Gastroenterology H (2021) The hunt for a vaccine for hepatitis C virus continues. *Lancet*
2597 *Gastroenterol Hepatol* **6**:253.

2598 Timm DE, Vissavajjhala P, Ross AH and Neet KE (1992) Spectroscopic and chemical studies of the
2599 interaction between nerve growth factor (NGF) and the extracellular domain of the low affinity
2600 NGF receptor. *Protein Sci* **1**:1023--1031.

2601 Tirode F, Surdez D, Ma X, Parker M, Le Deley MC, Bahrami A, Zhang Z, Lapouble E, Grossetete-
2602 Lalami S, Rusch M, Reynaud S, Rio-Frio T, Hedlund E, Wu G, Chen X, Pierron G, Oberlin O,
2603 Zaidi S, Lemmon G, Gupta P, Vadodaria B, Easton J, Gut M, Ding L, Mardis ER, Wilson RK,
2604 Shurtleff S, Laurence V, Michon J, Marec-Berard P, Gut I, Downing J, Dyer M, Zhang J, Delattre
2605 O, St. Jude Children's Research Hospital-Washington University Pediatric Cancer Genome P and



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2606 the International Cancer Genome C (2014) Genomic landscape of Ewing sarcoma defines an
2607 aggressive subtype with co-association of STAG2 and TP53 mutations. *Cancer Discov* **4**:1342-
2608 1353.

2609 Tokuriki N, Oldfield CJ, Uversky VN, Berezovsky IN and Tawfik DS (2009) Do viral proteins possess
2610 unique biophysical features? *Trends Biochem Sci* **34**:53-59.

2611 Tompa P (2002a) Intrinsically unstructured proteins. *Trends Biochem Sci* **27**:527-533.

2612 Tompa P (2002b) Intrinsically unstructured proteins. *Trends Biochem Sci* **27**:527-533.

2613 Tompa P (2005) The interplay between structure and function in intrinsically unstructured proteins. *FEBS*
2614 *Lett* **579**:3346-3354.

2615 Tompa P (2009) Structural disorder in amyloid fibrils: its implication in dynamic interactions of proteins.
2616 *Febs J* **276**:5406-5415.

2617 Tompa P and Csermely P (2004) The role of structural disorder in the function of RNA and protein
2618 chaperones. *FASEB J* **18**:1169-1175.

2619 Tompa P and Fuxreiter M (2008) Fuzzy complexes: polymorphism and structural disorder in protein-
2620 protein interactions. *Trends Biochem Sci* **33**:2-8.

2621 Tompa P, Szasz C and Buday L (2005) Structural disorder throws new light on moonlighting. *Trends*
2622 *Biochem Sci* **30**:484-489.

2623 Tong MJ, Reddy KR, Lee WM, Pockros PJ, Hoefs JC, Keeffe EB, Hollinger FB, Hathcote EJ, White H,
2624 Foust RT, Jensen DM, Krawitt EL, Fromm H, Black M, Blatt LM, Klein M, Lubina J, Bailey R,
2625 Bala K, Balart L, Bonkovsky H, Cassidy W, Craig JR, Donovan J, Dusheiko GM, Ehrinpreis M,
2626 Everson G, Feinman S, Hauser S, Hunter E, James S, Killenberg P, Lee S, vanLeeuwen DJ,
2627 Lesesne H, Lieberman H, Lissoos T, Lumeng L, Lyche K, Minuk G, Mullen K, Payne KM,
2628 Pimstone N, Poterucha J, Rosenblate H, Shafritz D, Smith C and Willems B (1997) Treatment of
2629 chronic hepatitis C with consensus interferon: A multicenter, randomized, controlled trial.
2630 *Hepatology* **26**:747-754.

2631 Tong X, Tang R, Xu J, Wang W, Zhao Y, Yu X and Shi S (2022) Liquid–liquid phase separation in tumor
2632 biology. *Signal Transduction and Targeted Therapy* **7**:221.

2633 Toretsky JA, Erkizan V, Levenson A, Abaan OD, Parvin JD, Cripe TP, Rice AM, Lee SB and Uren A
2634 (2006) Oncoprotein EWS-FLI1 activity is enhanced by RNA helicase A. *Cancer research*
2635 **66**:5574-5581.

2636 Toretsky JA and Wright PE (2014) Assemblages: functional units formed by cellular phase separation.
2637 *The Journal of cell biology* **206**:579-588.

2638 Toth G, Gardai SJ, Zago W, Bertoncini CW, Cremades N, Roy SL, Tambe MA, Rochet JC, Galvagnion
2639 C, Skibinski G, Finkbeiner S, Bova M, Regnstrom K, Chiou SS, Johnston J, Callaway K,
2640 Anderson JP, Jobling MF, Buell AK, Yednock TA, Knowles TP, Vendruscolo M, Christodoulou
2641 J, Dobson CM, Schenk D and McConlogue L (2014) Targeting the Intrinsically Disordered
2642 Structural Ensemble of alpha-Synuclein by Small Molecules as a Potential Therapeutic Strategy
2643 for Parkinson's Disease. *PLoS One* **9**:e87133.

2644 Tsafou K, Tiwari PB, Forman-Kay JD, Metallo SJ and Toretsky JA (2018) Targeting Intrinsically
2645 Disordered Transcription Factors: Changing the Paradigm. *J Mol Biol* **430**:2321-2341.

2646 Tsang B, Pritisanac I, Scherer SW, Moses AM and Forman-Kay JD (2020) Phase Separation as a Missing
2647 Mechanism for Interpretation of Disease Mutations. *Cell* **183**:1742-1756.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2648 Tsvetkov P, Asher G, Paz A, Reuven N, Sussman JL, Silman I and Shaul Y (2008) Operational definition
2649 of intrinsically unstructured protein sequences based on susceptibility to the 20S proteasome.
2650 *Proteins* **70**:1357-1366.

2651 Turoverov KK, Kuznetsova IM, Fonin AV, Darling AL, Zaslavsky BY and Uversky VN (2019)
2652 Stochasticity of Biological Soft Matter: Emerging Concepts in Intrinsically Disordered Proteins
2653 and Biological Phase Separation. *Trends Biochem Sci* **44**:716-728.

2654 Turoverov KK, Kuznetsova IM and Uversky VN (2010) The protein kingdom extended: ordered and
2655 intrinsically disordered proteins, their folding, supramolecular complex formation, and
2656 aggregation. *Prog Biophys Mol Biol* **102**:73-84.

2657 Uhlen M, Bjorling E, Agaton C, Szigyarto CA, Amini B, Andersen E, Andersson AC, Angelidou P,
2658 Asplund A, Asplund C, Berglund L, Bergstrom K, Brumer H, Cerjan D, Ekstrom M, Elobeid A,
2659 Eriksson C, Fagerberg L, Falk R, Fall J, Forsberg M, Bjorklund MG, Gumbel K, Halimi A,
2660 Hallin I, Hamsten C, Hansson M, Hedhammar M, Hercules G, Kampf C, Larsson K, Linskog M,
2661 Lodewyckx W, Lund J, Lundeberg J, Magnusson K, Malm E, Nilsson P, Odling J, Oksvold P,
2662 Olsson I, Oster E, Ottosson J, Paavilainen L, Persson A, Rimini R, Rockberg J, Runeson M,
2663 Sivertsson A, Skollermo A, Steen J, Stenvall M, Sterky F, Stromberg S, Sundberg M, Tegel H,
2664 Tourle S, Wahlund E, Walden A, Wan JH, Wernerus H, Westberg J, Wester K, Wrethagen U, Xu
2665 LL, Hober S and Ponten F (2005) A human protein atlas for normal and cancer tissues based on
2666 antibody proteomics. *Molecular & Cellular Proteomics* **4**:1920-1932.

2667 Uniacke J and Zerges W (2008) Stress induces the assembly of RNA granules in the chloroplast of
2668 Chlamydomonas reinhardtii. *The Journal of cell biology* **182**:641-646.

2669 Updike DL, Hachey SJ, Kreher J and Strome S (2011) P granules extend the nuclear pore complex
2670 environment in the C. elegans germ line. *The Journal of cell biology* **192**:939-948.

2671 Uren A, Tcherkasskaya O and Toretsky JA (2004) Recombinant EWS-FLI1 oncoprotein activates
2672 transcription. *Biochemistry* **43**:13579-13589.

2673 Urry DW (1988) Entropic elastic processes in protein mechanisms. I. Elastic structure due to an inverse
2674 temperature transition and elasticity due to internal chain dynamics. *J Protein Chem* **7**:1-34.

2675 Urry DW (1992) Free energy transduction in polypeptides and proteins based on inverse temperature
2676 transitions. *Prog Biophys Mol Biol* **57**:23-57.

2677 Urry DW (1997) Physical chemistry of biological free energy transduction as demonstrated by elastic
2678 protein-based polymers. *Journal of Physical Chemistry B* **101**:11007-11028.

2679 Urry DW, Luan CH, Parker TM, Gowda DC, Prasad KU, Reid MC and Safavy A (1991) Temperature of
2680 Polypeptide Inverse Temperature Transition Depends on Mean Residue Hydrophobicity. *Journal*
2681 *of the American Chemical Society* **113**:4346-4348.

2682 Uversky VN (2002a) Natively unfolded proteins: a point where biology waits for physics. *Protein Sci*
2683 **11**:739-756.

2684 Uversky VN (2002b) What does it mean to be natively unfolded? *Eur J Biochem* **269**:2-12.

2685 Uversky VN (2003a) A protein-chameleon: conformational plasticity of alpha-synuclein, a disordered
2686 protein involved in neurodegenerative disorders. *J Biomol Struct Dyn* **21**:211-234.

2687 Uversky VN (2003b) Protein folding revisited. A polypeptide chain at the folding-misfolding-nonfolding
2688 cross-roads: which way to go? *Cell Mol Life Sci* **60**:1852-1871.

2689 Uversky VN (2007) Neuropathology, biochemistry, and biophysics of alpha-synuclein aggregation. *J*
2690 *Neurochem* **103**:17-37.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2691 Uversky VN (2009a) Intrinsic disorder in proteins associated with neurodegenerative diseases. *Front*
2692 *Biosci* **14**:5188-5238.

2693 Uversky VN (2009b) Intrinsically disordered proteins and their environment: effects of strong
2694 denaturants, temperature, pH, counter ions, membranes, binding partners, osmolytes, and
2695 macromolecular crowding. *Protein J* **28**:305-325.

2696 Uversky VN (2010a) The mysterious unfoldome: structureless, underappreciated, yet vital part of any
2697 given proteome. *J Biomed Biotechnol* **2010**:568068.

2698 Uversky VN (2010b) Targeting intrinsically disordered proteins in neurodegenerative and protein
2699 dysfunction diseases: another illustration of the D(2) concept. *Expert Rev Proteomics* **7**:543-564.

2700 Uversky VN (2011a) Intrinsically disordered proteins may escape unwanted interactions via functional
2701 misfolding. *Biochim Biophys Acta* **1814**:693-712.

2702 Uversky VN (2011b) Multitude of binding modes attainable by intrinsically disordered proteins: a portrait
2703 gallery of disorder-based complexes. *Chem Soc Rev* **40**:1623-1634.

2704 Uversky VN (2012) Intrinsically disordered proteins and novel strategies for drug discovery. *Expert Opin*
2705 *Drug Discov* **7**:475-488.

2706 Uversky VN (2013a) A decade and a half of protein intrinsic disorder: biology still waits for physics.
2707 *Protein Sci* **22**:693-724.

2708 Uversky VN (2013b) Intrinsic disorder-based protein interactions and their modulators. *Curr Pharm Des*
2709 **19** 4191-4213.

2710 Uversky VN (2013c) Intrinsic disorder-based protein interactions and their modulators. *Curr Pharm Des*
2711 **19**:4191-4213.

2712 Uversky VN (2013d) Unusual biophysics of intrinsically disordered proteins. *Biochim Biophys Acta* **1834**
2713 932-951.

2714 Uversky VN (2014) The triple power of D(3): protein intrinsic disorder in degenerative diseases. *Front*
2715 *Biosci (Landmark Ed)* **19**:181-258.

2716 Uversky VN (2015) Functional roles of transiently and intrinsically disordered regions within proteins.
2717 *FEBS J* **282**:1182-1189.

2718 Uversky VN (2016a) Dancing Protein Clouds: The Strange Biology and Chaotic Physics of Intrinsically
2719 Disordered Proteins. *J Biol Chem* **291**:6681-6688.

2720 Uversky VN (2016b) Intrinsically disordered proteins in overcrowded milieu: Membrane-less organelles,
2721 phase separation, and intrinsic disorder. *Curr Opin Struct Biol* **44**:18-30.

2722 Uversky VN (2016c) (Intrinsically disordered) splice variants in the proteome: implications for novel
2723 drug discovery. *Genes Genom* **38**:577-594.

2724 Uversky VN (2016d) p53 Proteoforms and Intrinsic Disorder: An Illustration of the Protein Structure-
2725 Function Continuum Concept. *Int J Mol Sci* **17**:1874.

2726 Uversky VN (2016e) Paradoxes and wonders of intrinsic disorder: Complexity of simplicity. *Intrinsically*
2727 *Disord Proteins* **4**:e1135015.

2728 Uversky VN (2017a) How to Predict Disorder in a Protein of Interest. *Methods Mol Biol* **1484**:137-158.

2729 Uversky VN (2017b) Intrinsically disordered proteins in overcrowded milieu: Membrane-less organelles,
2730 phase separation, and intrinsic disorder. *Curr Opin Struct Biol* **44**:18-30.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2731 Uversky VN (2017c) Protein intrinsic disorder-based liquid-liquid phase transitions in biological systems:
2732 Complex coacervates and membrane-less organelles. *Adv Colloid Interface Sci* **239**:97-114.

2733 Uversky VN (2017d) The roles of intrinsic disorder-based liquid-liquid phase transitions in the "Dr.
2734 Jekyll-Mr. Hyde" behavior of proteins involved in amyotrophic lateral sclerosis and
2735 frontotemporal lobar degeneration. *Autophagy* **13**:2115-2162.

2736 Uversky VN (2018) Intrinsic Disorder, Protein-Protein Interactions, and Disease. *Adv Protein Chem*
2737 *Struct Biol* **110**:85-121.

2738 Uversky VN (2019) Protein intrinsic disorder and structure-function continuum. *Prog Mol Biol Transl Sci*
2739 **166**:1-17.

2740 Uversky VN (2021) On the irrationality of rational design of an HIV vaccine in light of protein intrinsic
2741 disorder. *Arch Virol* **166**:1283-1296.

2742 Uversky VN (2022) Fight fire with fire: the need for a vaccine based on intrinsic disorder and structural
2743 flexibility. *Exploration of Immunology* **2**:731-748.

2744 Uversky VN (2024) Functional unfoldomics: Roles of intrinsic disorder in protein (multi)functionality.
2745 *Adv Protein Chem Struct Biol* **138**:179-210.

2746 Uversky VN and Dunker AK (2010a) Understanding protein non-folding. *Biochim Biophys Acta*
2747 **1804**:1231-1264.

2748 Uversky VN and Dunker AK (2010b) Understanding protein non-folding. *Biochim Biophys Acta*
2749 **1804**:1231-1264.

2750 Uversky VN and Dunker AK (2013) The case for intrinsically disordered proteins playing contributory
2751 roles in molecular recognition without a stable 3D structure. *F1000 Biol Rep* **5**:1.

2752 Uversky VN and Eliezer D (2009) Biophysics of Parkinson's disease: structure and aggregation of alpha-
2753 synuclein. *Curr Protein Pept Sci* **10**:483-499.

2754 Uversky VN, Gillespie JR and Fink AL (2000a) Why are "natively unfolded" proteins unstructured under
2755 physiologic conditions? *Proteins* **41**:415-427.

2756 Uversky VN, Gillespie JR and Fink AL (2000b) Why are "natively unfolded" proteins unstructured under
2757 physiologic conditions? *Proteins* **41**:415-427.

2758 Uversky VN, Kuznetsova IM, Turoverov KK and Zaslavsky B (2015) Intrinsically disordered proteins as
2759 crucial constituents of cellular aqueous two phase systems and coacervates. *FEBS Lett* **589**:15-22.

2760 Uversky VN, Li J and Fink AL (2001) Evidence for a partially folded intermediate in alpha-synuclein
2761 fibril formation. *J Biol Chem* **276**:10737--10744.

2762 Uversky VN, Oldfield CJ and Dunker AK (2005) Showing your ID: intrinsic disorder as an ID for
2763 recognition, regulation and cell signaling. *J Mol Recognit* **18**:343-384.

2764 Uversky VN, Oldfield CJ and Dunker AK (2008) Intrinsically disordered proteins in human diseases:
2765 introducing the D2 concept. *Annu Rev Biophys* **37**:215-246.

2766 Uversky VN, Oldfield CJ, Midic U, Xie H, Xue B, Vucetic S, Iakoucheva LM, Obradovic Z and Dunker
2767 AK (2009) Unfoldomics of human diseases: linking protein intrinsic disorder with diseases. *BMC*
2768 *Genomics* **10 Suppl 1**:S7.

2769 Uversky VN, Permyakov SE, Zagranichny VE, Rodionov IL, Fink AL, Cherskaya AM, Wasserman LA
2770 and Permyakov EA (2002) Effect of zinc and temperature on the conformation of the gamma
2771 subunit of retinal phosphodiesterase: a natively unfolded protein. *J Proteome Res* **1**:149--159.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2772 Vacic V, Oldfield CJ, Mohan A, Radivojac P, Cortese MS, Uversky VN and Dunker AK (2007a)
2773 Characterization of molecular recognition features, MoRFs, and their binding partners. *J*
2774 *Proteome Res* **6**:2351-2366.

2775 Vacic V, Uversky VN, Dunker AK and Lonardi S (2007b) Composition Profiler: a tool for discovery and
2776 visualization of amino acid composition differences. *BMC Bioinformatics* **8**:211.

2777 Vaishampayan U, Montgomery R, Gordon M, Smith D, Barber K, de Haas-Amatsaleh A, Thapar N,
2778 Chandhasin C, Perabo F and Chi K (2017) EPI-506 (ralaniten acetate), a novel androgen receptor
2779 (AR) N-terminal domain (NTD) inhibitor, in men with metastatic castration-resistant prostate
2780 cancer (mCRPC): Phase 1 update on safety, tolerability, pharmacokinetics and efficacy. *Annals of*
2781 *Oncology* **28**:v274.

2782 Vassilev LT (2004) Small-molecule antagonists of p53-MDM2 binding: research tools and potential
2783 therapeutics. *Cell Cycle* **3**:419-421.

2784 Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C,
2785 Klein C, Fotouhi N and Liu EA (2004) In vivo activation of the p53 pathway by small-molecule
2786 antagonists of MDM2. *Science* **303**:844-848.

2787 Venables JP (2004) Aberrant and alternative splicing in cancer. *Cancer research* **64**:7647-7654.

2788 Vendruscolo M (2007) Determination of conformationally heterogeneous states of proteins. *Curr Opin*
2789 *Struct Biol* **17**:15-20.

2790 Vendruscolo M (2023) Thermodynamic and kinetic approaches for drug discovery to target protein
2791 misfolding and aggregation. *Expert Opin Drug Discov* **18**:881-891.

2792 Vendruscolo M and Fuxreiter M (2022) Protein condensation diseases: therapeutic opportunities. *Nature*
2793 *Communications* **13**:5550.

2794 Vigerust DJ and Shepherd VL (2007) Virus glycosylation: role in virulence and immune interactions.
2795 *Trends Microbiol* **15**:211-218.

2796 Vucetic S, Xie H, Iakoucheva LM, Oldfield CJ, Dunker AK, Obradovic Z and Uversky VN (2007)
2797 Functional anthology of intrinsic disorder. 2. Cellular components, domains, technical terms,
2798 developmental processes, and coding sequence diversities correlated with long disordered
2799 regions. *J Proteome Res* **6**:1899-1916.

2800 Walker LC and LeVine H, 3rd (2000) The cerebral proteopathies. *Neurobiol Aging* **21**:559-561.

2801 Wang B, Zhang L, Dai T, Qin Z, Lu H, Zhang L and Zhou F (2021) Liquid–liquid phase separation in
2802 human health and diseases. *Signal Transduction and Targeted Therapy* **6**:290.

2803 Wang C, Lu H, Liu X, Gao X, Tian W, Chen H, Xue Y and Zhou Q (2022) A natural product targets
2804 BRD4 to inhibit phase separation and gene transcription. *iScience* **25**:103719.

2805 Wang H, Hammoudeh DI, Follis AV, Reese BE, Lazo JS, Metallo SJ and Prochownik EV (2007)
2806 Improved low molecular weight Myc-Max inhibitors. *Mol Cancer Ther* **6**:2399-2408.

2807 Wang H, Xiong R and Lai L (2023) Rational drug design targeting intrinsically disordered proteins. *Wiley*
2808 *Interdisciplinary Reviews: Computational Molecular Science* **13**:e1685.

2809 Wang J, Cao Z, Zhao L and Li S (2011) Novel strategies for drug discovery based on Intrinsically
2810 Disordered Proteins (IDPs). *Int J Mol Sci* **12**:3205-3219.

2811 Ward B (2007) Antiviral Vaccines, in *Fundamentals of Molecular Virology* (Acheson NH ed) pp 428-
2812 455, Wiley, Hoboken, NJ, USA.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2813 Ward JJ, Sodhi JS, McGuffin LJ, Buxton BF and Jones DT (2004) Prediction and functional analysis of
2814 native disorder in proteins from the three kingdoms of life. *J Mol Biol* **337**:635-645.

2815 Wathen B and Jia Z (2009) Folding by numbers: primary sequence statistics and their use in studying
2816 protein folding. *Int J Mol Sci* **10**:1567-1589.

2817 Watson DK, Robinson L, Hodge DR, Kola I, Papas TS and Seth A (1997) FLI1 and EWS-FLI1 function
2818 as ternary complex factors and ELK1 and SAP1a function as ternary and quaternary complex
2819 factors on the Egr1 promoter serum response elements. *Oncogene* **14**:213-221.

2820 Weinreb PH, Zhen W, Poon AW, Conway KA and Lansbury PT, Jr. (1996) NACP, a protein implicated
2821 in Alzheimer's disease and learning, is natively unfolded. *Biochemistry* **35**:13709-13715.

2822 White MR, Mitrea DM, Zhang P, Stanley CB, Cassidy DE, Nourse A, Phillips AH, Tolbert M, Taylor JP
2823 and Kriwacki RW (2019) C9orf72 Poly(PR) Dipeptide Repeats Disturb Biomolecular Phase
2824 Separation and Disrupt Nucleolar Function. *Mol Cell* **74**:713-728.e716.

2825 Wilhelmus MM, Otte-Höller I, Wesseling P, de Waal RM, Boelens WC and Verbeek MM (2006)
2826 Specific association of small heat shock proteins with the pathological hallmarks of Alzheimer's
2827 disease brains. *Neuropathol Appl Neurobiol* **32**:119-130.

2828 Williams RM, Obradovic Z, Mathura V, Braun W, Garner EC, Young J, Takayama S, Brown CJ and
2829 Dunker AK (2001) The protein non-folding problem: amino acid determinants of intrinsic order
2830 and disorder. *Pac Symp Biocomput*:89-100.

2831 Wippich F, Bodenmiller B, Trajkovska MG, Wanka S, Aebersold R and Pelkmans L (2013) Dual
2832 specificity kinase DYRK3 couples stress granule condensation/dissolution to mTORC1 signaling.
2833 *Cell* **152**:791-805.

2834 Wojtys W and Oron M (2023) How Driver Oncogenes Shape and Are Shaped by Alternative Splicing
2835 Mechanisms in Tumors. *Cancers (Basel)* **15**.

2836 Wright PE and Dyson HJ (1999a) Intrinsically unstructured proteins: re-assessing the protein structure-
2837 function paradigm. *J Mol Biol* **293**:321-331.

2838 Wright PE and Dyson HJ (1999b) Intrinsically unstructured proteins: re-assessing the protein structure-
2839 function paradigm. *J Mol Biol* **293**:321-331.

2840 Wu KP, Kim S, Fela DA and Baum J (2008) Characterization of conformational and dynamic properties
2841 of natively unfolded human and mouse alpha-synuclein ensembles by NMR: implication for
2842 aggregation. *J Mol Biol* **378**:1104-1115.

2843 Wu KP, Weinstock DS, Narayanan C, Levy RM and Baum J (2009) Structural reorganization of alpha-
2844 synuclein at low pH observed by NMR and REMD simulations. *J Mol Biol* **391**:784-796.

2845 Wurtz JM, Bourguet W, Renaud JP, Vivat V, Chambon P, Moras D and Gronemeyer H (1996) A
2846 canonical structure for the ligand-binding domain of nuclear receptors. *Nat Struct Biol* **3**:87-94.

2847 Xie H, Vucetic S, Iakoucheva LM, Oldfield CJ, Dunker AK, Uversky VN and Obradovic Z (2007)
2848 Functional anthology of intrinsic disorder. 1. Biological processes and functions of proteins with
2849 long disordered regions. *J Proteome Res* **6**:1882-1898.

2850 Xie Q, Cheng J, Mei W, Yang D, Zhang P and Zeng C (2023) Phase separation in cancer at a glance.
2851 *Journal of Translational Medicine* **21**:237.

2852 Xu B, Chen J and Liu Y (2022a) Curcumin Interacts with α-Synuclein Condensates To Inhibit Amyloid
2853 Aggregation under Phase Separation. *ACS Omega* **7**:30281-30290.

2854 Xu B, Mo X, Chen J, Yu H and Liu Y (2022b) Myricetin Inhibits α-Synuclein Amyloid Aggregation by
2855 Delaying the Liquid-to-Solid Phase Transition. *Chembiochem* **23**:e202200216.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2856 Xue B, Dunker AK and Uversky VN (2012) Orderly order in protein intrinsic disorder distribution:
2857 Disorder in thirty five hundred proteomes from viruses and the three domains of life. *Journal of*
2858 *Biomolecular Structure and Dynamics* **30**:137-149.

2859 Xue B, Williams RW, Oldfield CJ, Dunker AK and Uversky VN (2010) Archaic chaos: intrinsically
2860 disordered proteins in Archaea. *BMC Syst Biol* **4 Suppl 1**:S1.

2861 Yamaguchi Y, Masuda M, Sasakawa H, Nonaka T, Hanashima S, Hisanaga S, Kato K and Hasegawa M
2862 (2010) Characterization of inhibitor-bound alpha-synuclein dimer: role of alpha-synuclein N-
2863 terminal region in dimerization and inhibitor binding. *J Mol Biol* **395**:445-456.

2864 Yi Q, Han X, Yu HG, Chen HY, Qiu D, Su J, Lin R, Batist G and Wu JH (2024) SC912 inhibits AR-V7
2865 activity in castration-resistant prostate cancer by targeting the androgen receptor N-terminal
2866 domain. *Oncogene* **43**:1522-1533.

2867 Yi Q, Liu W, Seo JH, Su J, Alaoui-Jamali MA, Luo J, Lin R and Wu JH (2023) Discovery of a Small-
2868 Molecule Inhibitor Targeting the Androgen Receptor N-Terminal Domain for Castration-
2869 Resistant Prostate Cancer. *Mol Cancer Ther* **22**:570-582.

2870 Yin X, Giap C, Lazo JS and Prochownik EV (2003) Low molecular weight inhibitors of Myc-Max
2871 interaction and function. *Oncogene* **22**:6151-6159.

2872 Yu C, Niu X, Jin F, Liu Z, Jin C and Lai L (2016) Structure-based Inhibitor Design for the Intrinsically
2873 Disordered Protein c-Myc. *Sci Rep* **6**:22298.

2874 Yu Q, Wang Z, Tu Y, Cao Y, Zhu H, Shao J, Zhuang R, Zhou Y and Zhang J (2024) Proteasome
2875 activation: A novel strategy for targeting undruggable intrinsically disordered proteins. *Bioorg*
2876 *Chem* **145**:107217.

2877 Zamudio AV, Dall'Agnese A, Henninger JE, Manteiga JC, Afeyan LK, Hannett NM, Coffey EL, Li CH,
2878 Oksuz O, Sabari BR, Boija A, Klein IA, Hawken SW, Spille JH, Decker TM, Cisse, II, Abraham
2879 BJ, Lee TI, Taatjes DJ, Schuijers J and Young RA (2019) Mediator Condensates Localize
2880 Signaling Factors to Key Cell Identity Genes. *Mol Cell* **76**:753-766.e756.

2881 Zbinden A, Pérez-Berlanga M, De Rossi P and Polymenidou M (2020) Phase Separation and
2882 Neurodegenerative Diseases: A Disturbance in the Force. *Developmental Cell* **55**:45-68.

2883 Zhang JZ, Lu TW, Stolerman LM, Tenner B, Yang JR, Zhang JF, Falcke M, Rangamani P, Taylor SS,
2884 Mehta S and Zhang J (2020) Phase Separation of a PKA Regulatory Subunit Controls cAMP
2885 Compartmentation and Oncogenic Signaling. *Cell* **182**:1531-1544.e1515.

2886 Zhao R, Gish K, Murphy M, Yin Y, Notterman D, Hoffman WH, Tom E, Mack DH and Levine AJ
2887 (2000) Analysis of p53-regulated gene expression patterns using oligonucleotide arrays. *Genes*
2888 *Dev* **14**:981-993.

2889 Zhou M, Li W, Li J, Xie L, Wu R, Wang L, Fu S, Su W, Hu J, Wang J and Li P (2020) Phase-separated
2890 condensate-aided enrichment of biomolecular interactions for high-throughput drug screening in
2891 test tubes. *J Biol Chem* **295**:11420-11434.

2892 Zhou T and Xu K (2018) Structural Features of Broadly Neutralizing Antibodies and Rational Design of
2893 Vaccine. *Adv Exp Med Biol* **1075**:73-95.

2894 Zhu J, Salvatella X and Robustelli P (2022) Small molecules targeting the disordered transactivation
2895 domain of the androgen receptor induce the formation of collapsed helical states. *Nat Commun*
2896 **13**:6390.

2897 Zhu L and Brangwynne CP (2015) Nuclear bodies: the emerging biophysics of nucleoplasmic phases.
2898 *Curr Opin Cell Biol* **34**:23-30.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2899 Zhu M, De Simone A, Schenk D, Toth G, Dobson CM and Vendruscolo M (2013) Identification of small-
2900 molecule binding pockets in the soluble monomeric form of the Abeta42 peptide. *J Chem Phys*
2901 **139**:035101.

2902 Ziólkowska B and Przewlocki R (2002) Chapter I Methods used in inducible transcription factor studies:
2903 focus on mRNA, in *Handbook of Chemical Neuroanatomy* (Kaczmarek L and Robertson HA eds)
2904 pp 1-38, Elsevier.

2905 Zitzewitz JA, Ibarra-Molero B, Fishel DR, Terry KL and Matthews CR (2000) Preformed secondary
2906 structure drives the association reaction of GCN4-p1, a model coiled-coil system. *J Mol Biol*
2907 **296**:1105-1116.

2908 Zou C, Zan X, Jia Z, Zheng L, Gu Y, Liu F, Han Y, Xu C, Wu A and Zhi Q (2023) Crosstalk between
2909 alternative splicing and inflammatory bowel disease: Basic mechanisms, biotechnological
2910 progresses and future perspectives. *Clin Transl Med* **13**:e1479.

2911 Zourlidou A, Payne Smith MD and Latchman DS (2004) HSP27 but not HSP70 has a potent protective
2912 effect against alpha-synuclein-induced cell death in mammalian neuronal cells. *J Neurochem*
2913 **88**:1439-1448.

2914

2915



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2916 **Figure legends**

2917 **Figure 1**. NMR solution structure of barnacle (*Megabalanus rosa*) cement protein MrCP20
2918 (PDB ID: 6LEK; (Mohanram et al., 2019)), where 10 models are overlaid generating a complex
2919 conformational ensemble that clearly resembles a fuzzy cloud.

2920 **Figure 2**. Schematic representation of major functional advantages of IDPs/IDRs over ordered
2921 proteins and domains. Modified from (Uversky, 2018).

2922 **Figure 3**. Peculiarities of the amino acid sequences of intrinsically disordered proteins. **A**.
2923 Amino acid determinants defining structural and functional differences between the ordered and
2924 intrinsically disordered proteins. Fractional difference in the amino acid composition
2925 (compositional profile) between the typical IDPs from the DisProt database (Sickmeier et al.,
2926 2007) and a set of completely ordered proteins (Berman et al., 2000) calculated for each amino
2927 acid residue. The fractional difference was evaluated as $(C_{DisProt}-C_{PDB})/C_{PDB}$, where $C_{DisProt}$ is the
2928 content of a given amino acid in a DisProt database (Sickmeier et al., 2007), and $C_{PDB}$ is the
2929 corresponding content in the dataset of fully ordered proteins from PDB select 25 (Berman et al.,
2930 2000). Positive bars colored in red and pink correspond to residues found more abundantly in
2931 IDPs, whereas negative bars colored in blue and cyan show residues, in which IDPs are depleted.
2932 Amino acid types are ranked according to their increasing disorder-promoting potential
2933 (Radivojac et al., 2007). Fractional differences are shown for proteins in DisProt 3.4 (cyan and
2934 pink bars) and DisProt release 2023_12 (blue and red bars). **B**. Evaluation of the charge-
2935 hydropathy space available for mouse proteins. The space accessible to the sequences encoding
2936 compact proteins is shown as a light cyan area, whereas the space accessible to sequences
2937 encoding IDPs is depicted as light red area. These two areas are defined by four boundaries, (i)
2938 the known boundary separating compact proteins and extended IDPs ($\langle R \rangle = 2.785 \langle H \rangle - 1.151$,



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

where $\langle R \rangle$ and $\langle H \rangle$ correspond to the absolute mean charge and mean hydropathy, respectively (Uversky et al., 2000b)); (ii) the mirror image of this boundary ($\langle R \rangle = -2.785 \langle H \rangle + 1.151$, where $\langle R \rangle$ and $\langle H \rangle$ correspond to the mean charge and mean hydropathy, respectively); (iii) two boundaries showing logical limits of the CH-space ($\langle R \rangle = -1.125 + 1.125 \langle H \rangle$ and $\langle R \rangle = 1.00 - \langle H \rangle$), evaluated for a series of hypothetical polypeptides containing different proportions of Ile (which is, according to the Kyte and Doolittle scale, is the most hydrophobic residue with the normalized hydropathy of 1 (Kyte and Doolittle, 1982)) and a negatively charged Asp (which is characterized by the normalized Kyte and Doolittle hydropathy of 0.1111 (Kyte and Doolittle, 1982)) or a positively charged Arg (which is characterized by the normalized Kyte and Doolittle hydropathy of 0.0 (Kyte and Doolittle, 1982)). Vertical dashed line represents a hypothetical boundary separating soluble compact proteins and membrane proteins.

**Figure 4**. Schematic representation of the mosaic nature of the protein structure-function space. Differently (dis)ordered segments (foldons, semi-foldons, inducible foldons, inducible morphing foldons, non-foldons, and unfoldons) might have different functions. Being found within one protein, these pieces of structural mosaic define protein multifunctionality.

**Figure 5**. Structural and functional characterization of TPPP/p25 (A, B) and α-synuclein (C, D) known to be involved in the pathogenesis of Parkinson's disease and other synucleinopathies. **A**. 3D model generated for human TPPP/p25 by AlphaFold (Jumper et al., 2021). Structure is colored based on the AlphaFold-generated per-residue confidence score (pLDDT) that between 0 and 100, where regions with very high confidence score (pLDDT > 90) are shown by blue color, confidently predicted regions (90 > pLDDT > 70) are shown by cyan color, whereas regions predicted with low (70 > pLDDT > 50) and very low confidence (pLDDT < 50) are shown by



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2962 yellow and orange color, respectively. Some regions with low pLDDT may be unstructured in
2963 isolation. **B**. Protein-protein interaction network centered at human TPPP/p25 (UniProt: O94811)
2964 generated by the Search Tool for the Retrieval of Interacting Genes (STRING, which a quality-
2965 controlled database that uses experimentally and computationally derived data to detail
2966 functional interactions between proteins (Szklarczyk et al., 2019)) using a default medium
2967 confidence of 0.4 for the minimum required interaction score. This network includes 41 nodes
2968 connected by 139 edges. It is characterized by the average node degree of 6.78 and the average
2969 local clustering coefficient of 0.839. Since the expected number of edges for the random set of
2970 proteins of the same size and degree distribution drawn from the genome is 57, this network has
2971 significantly more interactions than expected and is characterized by the PPI enrichment p-value
2972 $< 10^{-16}$. **C**. NMR solution structure of the SLAS-micelle bound human α-synuclein (PDB ID:
2973 2KKW, (Rao et al., 2010)) showing an octopus-like appearance of this IDP. **D**. STRING-
2974 generated PPI network of human α-synuclein (UniProt: O94811) with a high confidence of 0.7
2975 for the minimum required interaction score. Network includes 122 nodes connected by 768
2976 edges, which is significantly higher than the expected number of edges (259), indicating that this
2977 network has significantly more interactions than expected and is characterized by the PPI
2978 enrichment p-value $< 10^{-16}$. With the average node degree of 12.6 and the average local
2979 clustering coefficient of 0.69, this α-synuclein-centered network is highly connected.

2980 **Figure 6**. Intrinsic disorder and binding-induced folding of human p53 (UniProt ID: P04637)
2981 and Mdm2 (UniProt ID: Q00987). **A**. AlphaFold-generated model of the 3D structure of human
2982 p53. Note that this static structure represents a snapshot of a highly dynamic conformational
2983 ensemble, as structures of regions shown by orange and yellow colors are predicted with low and
2984 very low confidence and therefore are disordered. **B**. Intrinsic disorder profile of human p53



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

2985 generated by the RIDAO platform (Dayhoff and Uversky, 2022). Here, residues with the
2986 disorder scores above the threshold of 0.5 (this threshold is shown by thin black solid line) are
2987 considered disordered, whereas residues with disorder scores between 0.15 (this threshold is
2988 shown by thin dashed black line) and 0.5 are considered flexible. **C**. AlphaFold-generated model
2989 of the 3D structure of human Mdm2. **D**. RIDAO-generated intrinsic disorder profile of human
2990 Mdm2. Note the extremely high level of intrinsic disorder in this protein. **E**. Crystal structure of
2991 the human Mdm2 (blue structure) in a complex with a p53 peptide (red structure) (PDB ID:
2992 1T4F; (Grasberger et al., 2005)). Positions of the regions of these proteins used in the
2993 crystallization experiments within their amino acid sequences are shown in plot **D**.

2994 **Figure 7**. Intrinsic disorder and binding-induced folding of human c-Myc (UniProt ID: P01106)
2995 and Max (UniProt ID: P61244). **A**. AlphaFold-generated model of the 3D structure of human C-
2996 Myc. Note that long α-helices that correspond to the basic region/helix-loop-helix/leucine zipper
2997 (bHLHZip) domain cannot exist in solution as they are not involved in formation of any
2998 hydrophobic core, and therefore they represent regions with very strong helical propensity and
2999 high binding potential. Such long standing alone α-helical regions predicted by AlphFold
3000 typically correspond to coiled-coil domains of proteins. **B**. Intrinsic disorder profile of human c-
3001 Muc generated by the RIDAO platform (Dayhoff and Uversky, 2022). **C**. AlphaFold-generated
3002 model of the 3D structure of human Max. **D**. RIDAO-generated intrinsic disorder profile of
3003 human Max. Note that this protein is also predicted to have two long α-helices corresponding to
3004 its coiled-coil domain. **E**. Crystal structure of the Myc-Max recognizing DNA (PDB ID: 1NKP;
3005 (Nair and Burley, 2003)). Structures of C-Myc and Max are shown by orange and red colors,
3006 whereas DNA is shown in blue and light-blue. Positions of the regions of these proteins used in
3007 the crystallization experiments within their amino acid sequences are shown in plot **D**.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

3008 **Figure 8**. Intrinsic disorder profiles of human EWS (top plot), FLI1 (bottom plot), and the EWS-
3009 FLI1 chimeric protein (middle plot) generated by the RIDAO platform (Dayhoff and Uversky,
3010 2022). Regions of the EWS and FLI1 incorporated to the EWS-FLI1 chimera are shown by gray
3011 and cyan shades, respectively.

3012 **Figure 9**. Modular structure and functional intrinsic disorder of human androgen receptor
3013 (UniProt ID: P10275). **A**. Functional disorder profile generated by the $D^2P^2$ platform (Oates et
3014 al., 2013) showing the outputs of several disorder predictors, such as VLXT, VSL2b, PrDOS,
3015 IUPred and Espritz. Position of conserved functional domains are shown below the bars with the
3016 intrinsic disorder prediction outputs. The colored bar containing blue and green shades represents
3017 the consensus disorder prediction, whereas yellow zigzagged bars show positions of MoRFs. **B**.
3018 3D model generated by AlphaFold (Jumper et al., 2021).

3019 **Figure 10**. Structural alignment of human IFN-α1/13 (PDB ID: 3ux9, yellow ribbon), IFN-α2
3020 (PDB IDs: 3s9D, chain A, gray ribbon; 3s9D, chain C, orange ribbon; and 1itf, red ribbon) and
3021 human IFN-β (PDB ID: 1au1, blue ribbon). Images A, B, and C correspond to the different
3022 views (left-side, central and right-side) of the aligned structures. D. Evaluation of intrinsic
3023 disorder propensities of various subtypes of human IFN-α by the PONDR® VSL2 algorithm
3024 (Peng et al., 2005), which, based on the comprehensive assessment of *in silico* predictors of
3025 intrinsic disorder, is one of the more accurate stand-alone disorder predictors (Fan and Kurgan,
3026 2014; Peng and Kurgan, 2012). Data for different subtypes are represented by curves of different
3027 color (see the corresponding keys on the plot). Dashed, thick, dark pink line represents the
3028 consensus disorder plot, where the disorder propensity was calculated by averaging the disorder
3029 propensity data for the individual IFNs-α. Position of known α-helices and regions of missing
3030 electron density are also indicated. Shaded light pink area shows the position of the signal



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

3031 peptide (residues 1-23). Residues with the PONDR® VSL2 values above the threshold of 0.5
3032 (this threshold is shown by thin black solid line) are considered disordered, whereas residues
3033 with disorder scores between 0.15 (this threshold is shown by thin dashed black line) and 0.5 are
3034 considered flexible.

3035 **Figure 11**. Comparison of the intrinsic disorder propensities of consensus interferons and the
3036 mean disorder propensity evaluated for 17 human IFN-α proteins. Plot represents disorder
3037 profiles calculated for two consensus interferons, cIFN-α2 (El-Baky et al., 2015a) and IFN-con
3038 (Peciak et al., 2014) and the mean disorder profile based on the data shown in **Figure 10**. Shaded
3039 gray area shows the position of the signal peptide (residues 1-23).

3040 **Figure 12**. Comparison of the HIV-1 glycoprotein gp120 (residues 33-516 of the UniProt ID:
3041 P03377) and a gp120 core (UniProt ID: A0A0M3KKW8). **A**. Pairwise sequence alignment
3042 conducted by an alignment tool for protein sequences, SIM (https://web.expasy.org/sim/) (Huang
3043 and Miller, 1991). Results of the alignment were visualized using LALNVIEW, a graphical
3044 viewer program for pairwise alignments (Duret et al., 1996)Intrinsic disorder profiles of gp120
3045 (**B**) and gp120 core (**C**) generated by the RIDAO platform (Dayhoff and Uversky, 2022).

3046 **Figure 13**. Multiple structure alignment of the heavy chains of $F_{ab}$ of the anti-HIV Abs of human
3047 or bovine origin: Non-neutralizing HIV antibody 13H11 (PDB ID: 3MO1; orange structure);
3048 broadly reactive and potent HIV-1 neutralizing human antibody PG9 (PDB ID: 3U1S; blue
3049 structure) (McLellan et al., 2011); potent HIV-1 broadly neutralizing antibody NC-Cow1 (PDB
3050 ID: 6OO0; green structure) (Stanfield et al., 2020).

3051 **Figure 14**. Structural characterization of the metallothionein from the snail *Littorina littorea*
3052 (LlMT). **A**. Illustration of the “clouds in cloud” model. NMR solution structure of the LlMT



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

3053 (gray structures) in complex with $Cd^{2+}$ (small pink spheres) (PDB ID: 5ML1; (Baumann et al.,
3054 2017)). This conformational ensemble includes 20 structural models, with each chain binding
3055 nine metal ions. **B**. Alpha-Fold generated 3D structural model of LlMT. **C**. Intrinsic disorder
3056 profile of LlMT generated by several commonly used disorder predictors.

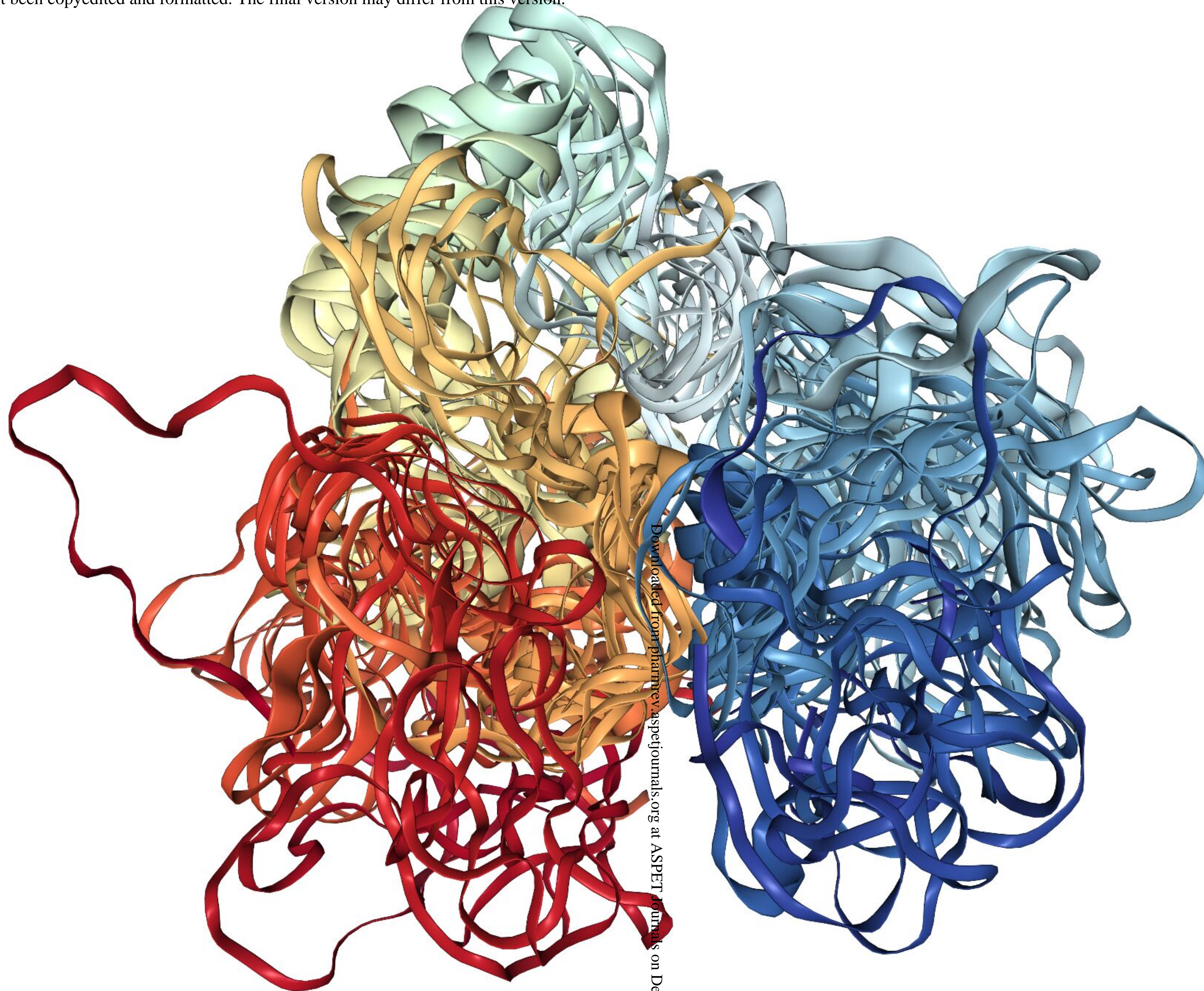
3057 **Figure 15**. Illustration of the ligand cloud concept. Figure represent the first eight clustering
3058 groups of the ligand-bound conformations of c-$Myc_{370–409}$ fragment from the explicit solvent
3059 simulations together with the clouds of bound 10074-A4 ligand. In each cluster, the N- and C-
3060 termini of the c-$Myc_{370–409}$ fragment are shown by blue and red colors, whereas the structures of
3061 10074-A4 ligand in the each group are depicted as green dots positioned at the centers of mass.
3062 The fractional cluster populations are: **A**: 14.3%, **B**: 13.9%, **C**: 13.7%, **D**: 10.4%, **E**: 7.5%, **F**:
3063 6.9%, **G**: 5.4%, and **H**: 5.2%. Figure is reproduced with permission from Jin F., Yu C., Lai L.,
3064 Liu Z. (2013) Ligand clouds around protein clouds: A scenario of ligand binding with
3065 intrinsically disordered proteins. PLoS Comput Biol. 9 (10): e1003249. doi:
3066 10.1371/journal.pcbi.1003249 (57).

3067



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.



Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Figure 1

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

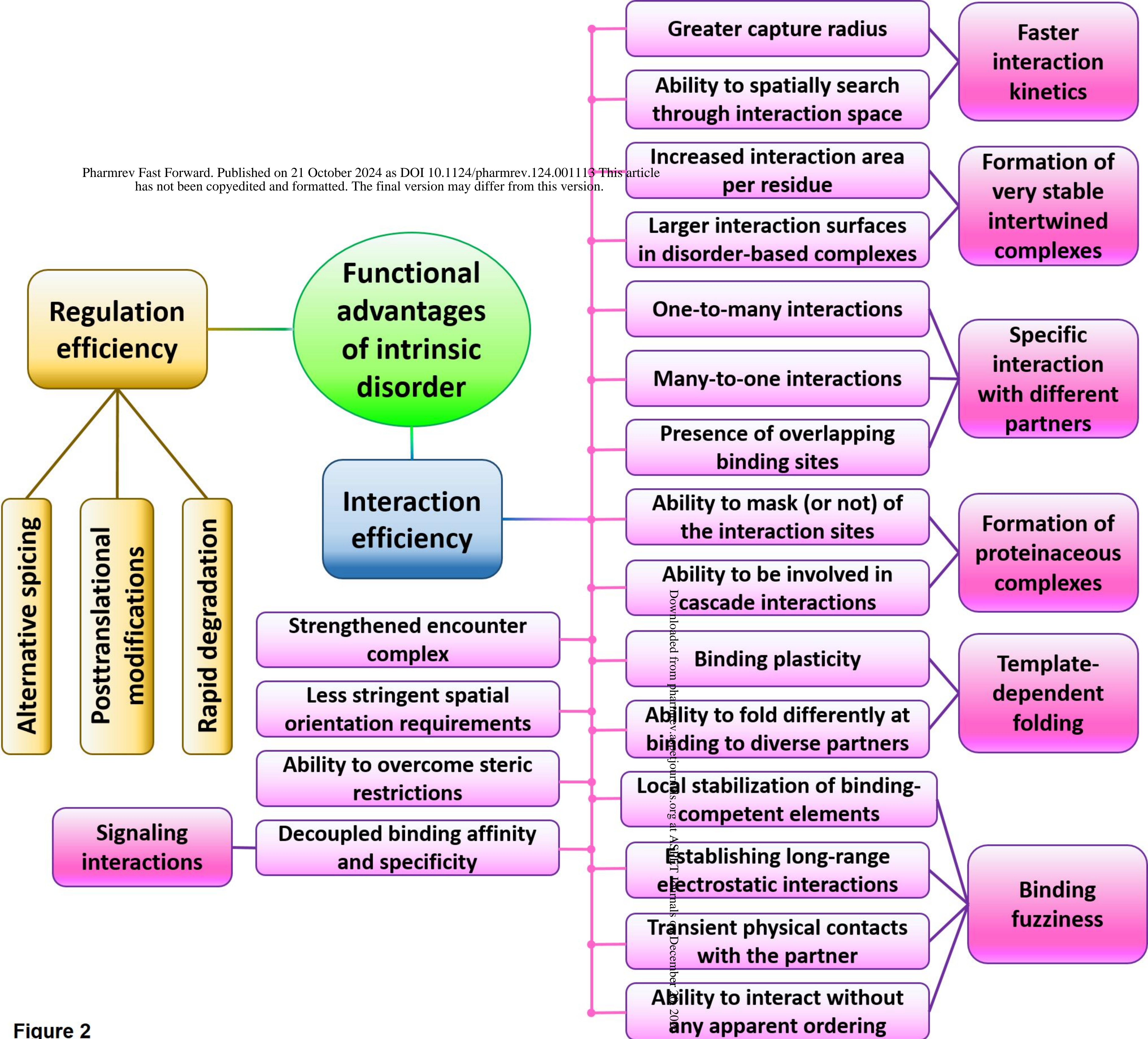



Figure 2

Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

A

(DisProt - PDB Select 25) / PDB Select 25

0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6

W C Y I F V L H M N A T R G K D Q E S P

DisProt release 2023_12
DisProt release 2023_12
DisProt 3.4
DisProt 3.4

B

Mean net charge

1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00

0.00 0.25 0.50 0.75 1.00

Mean scaled hydropathy

Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

**Figure 3**

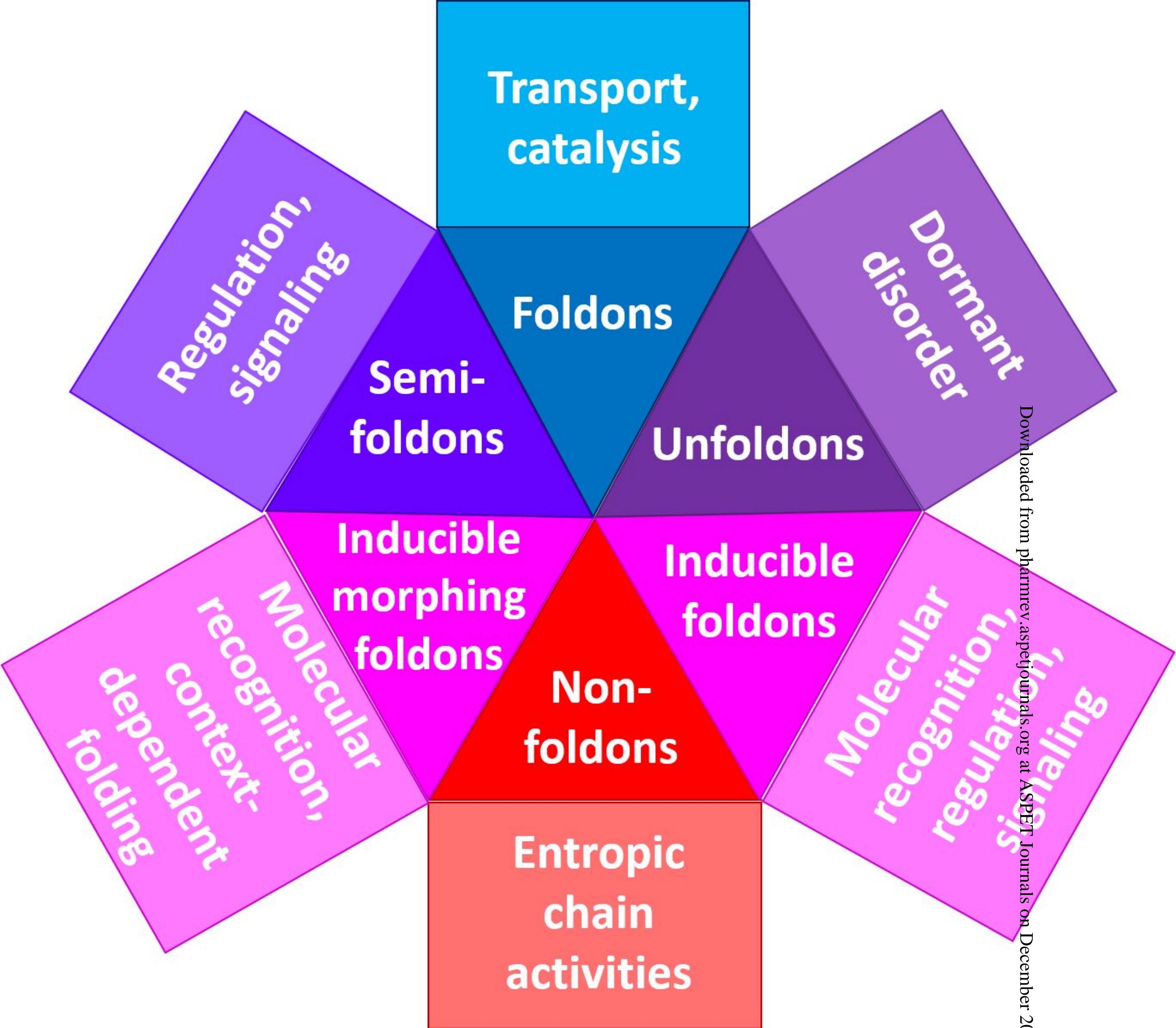



Figure 4

Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

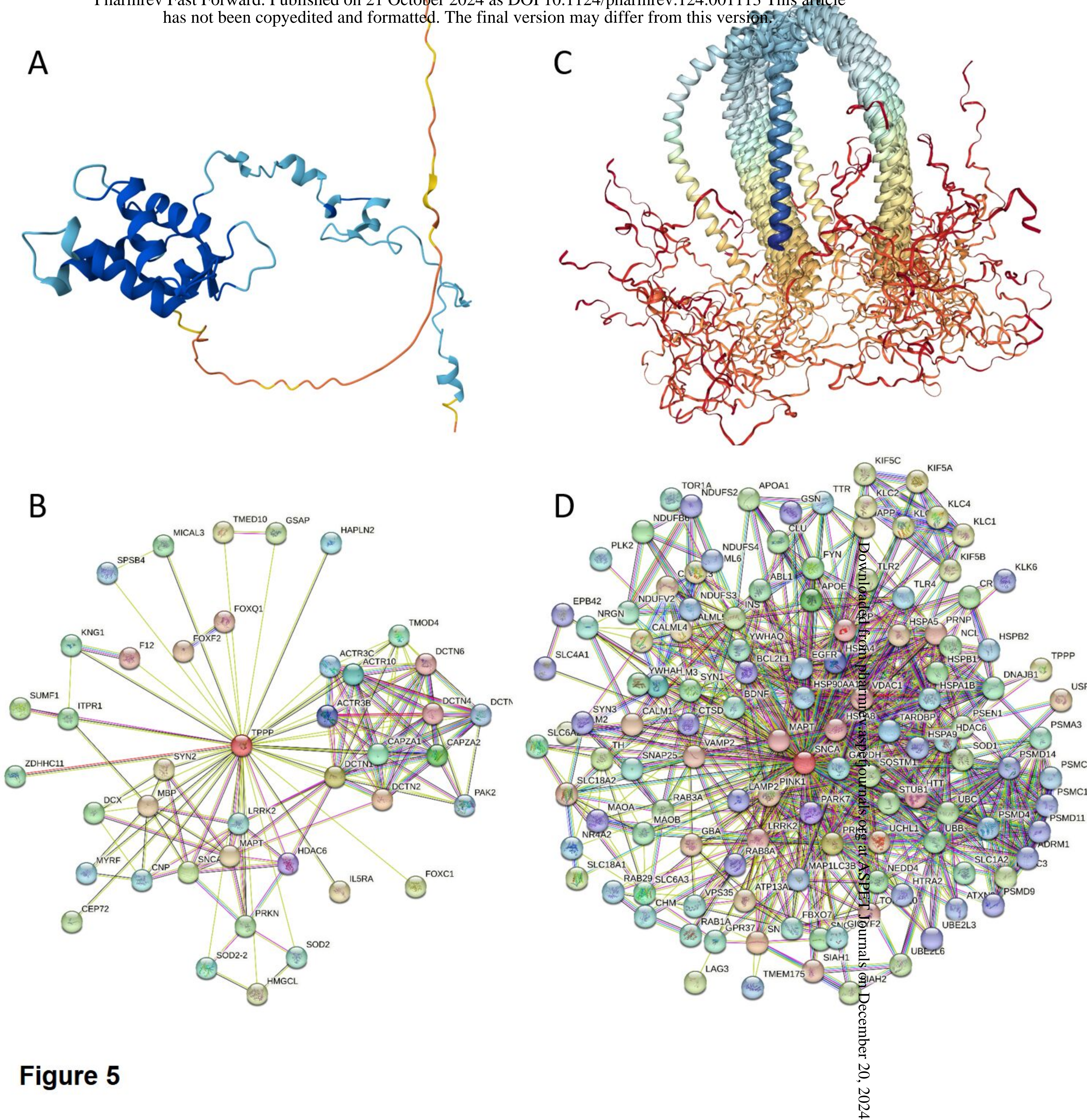
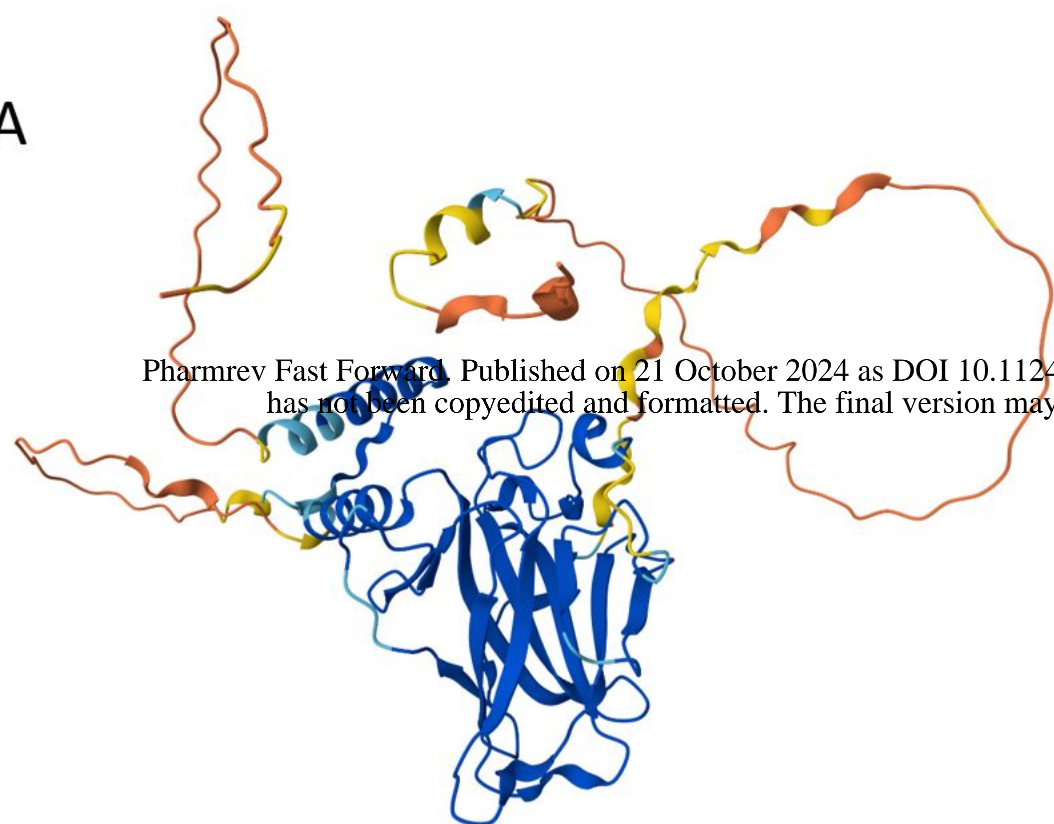
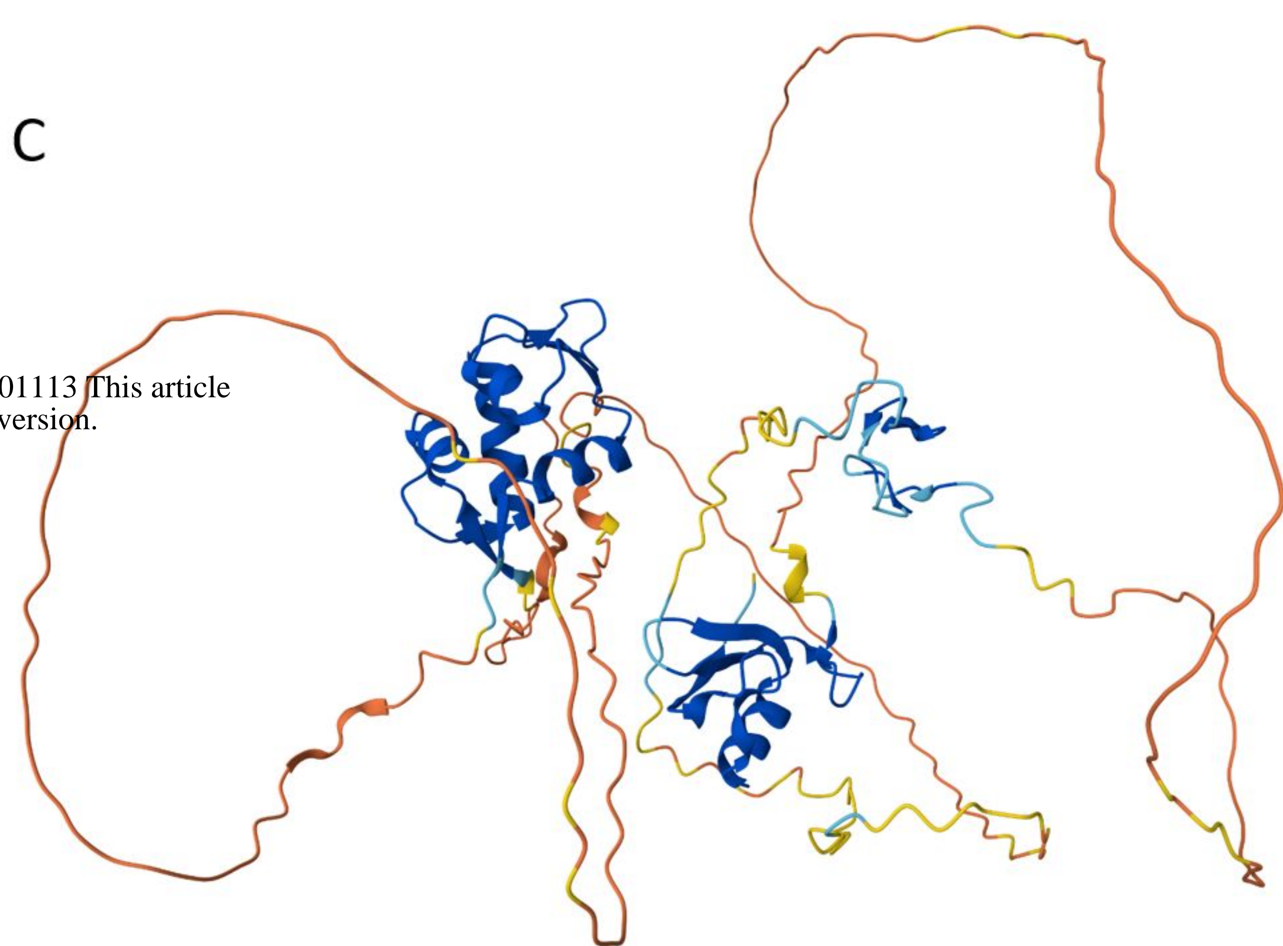


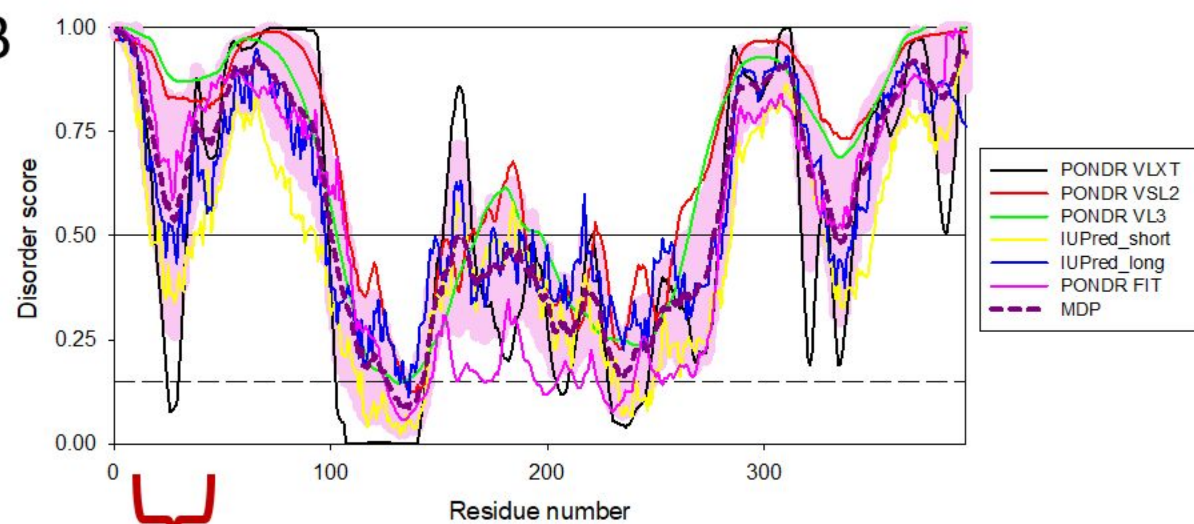

Figure 5

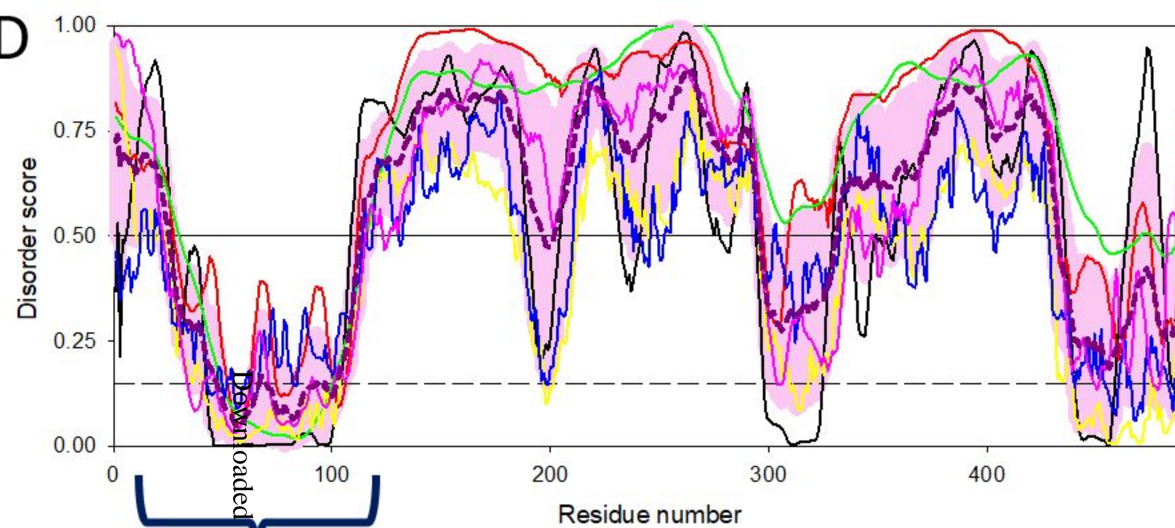
Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

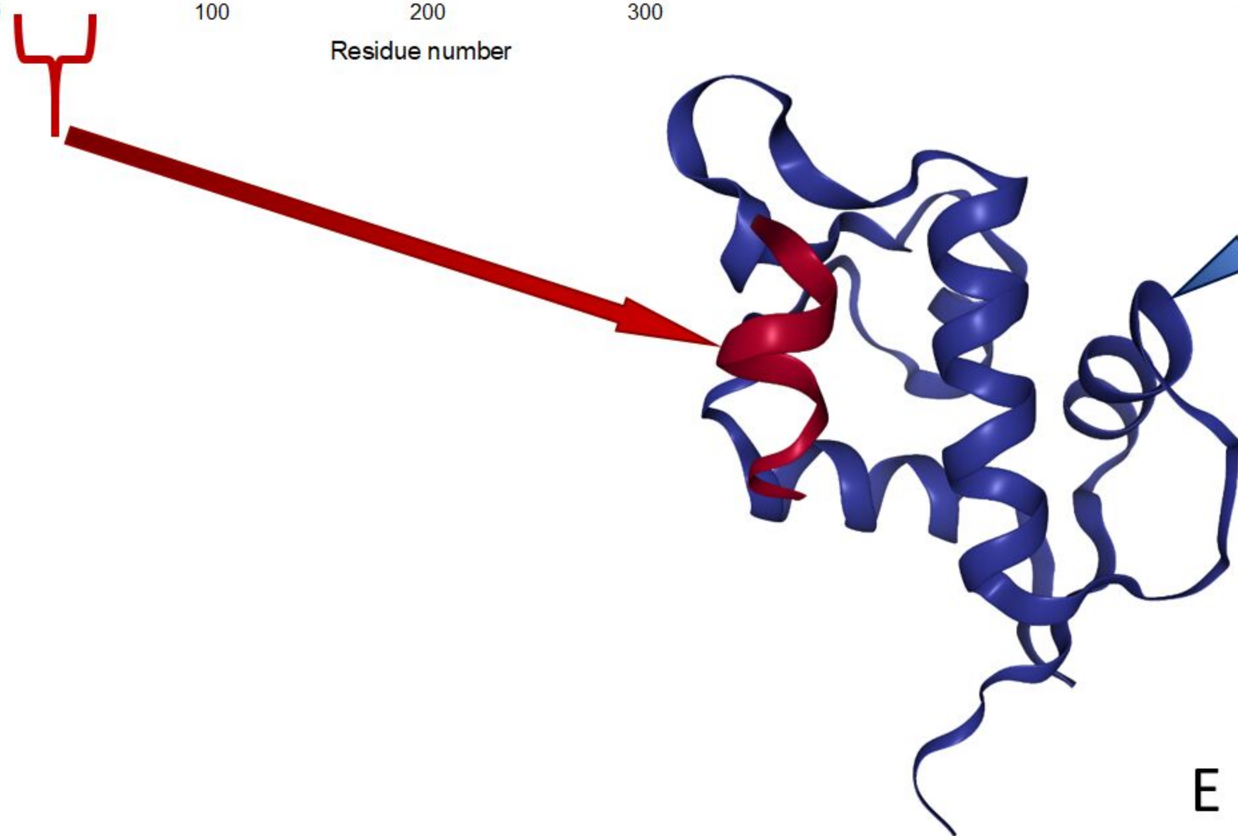



Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

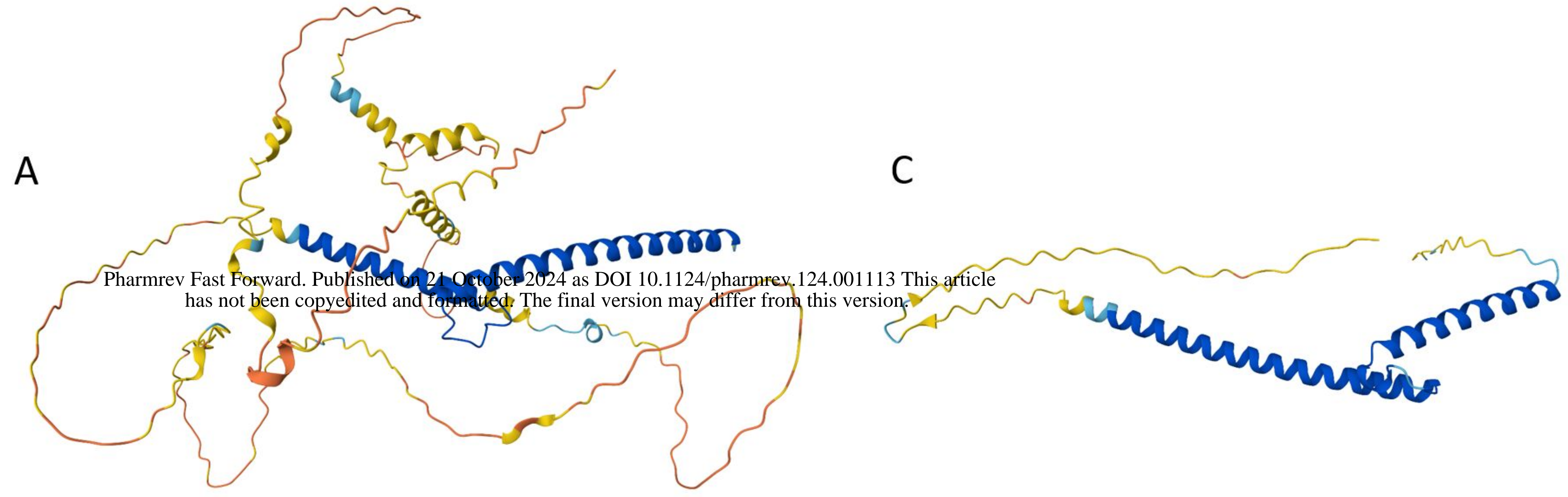


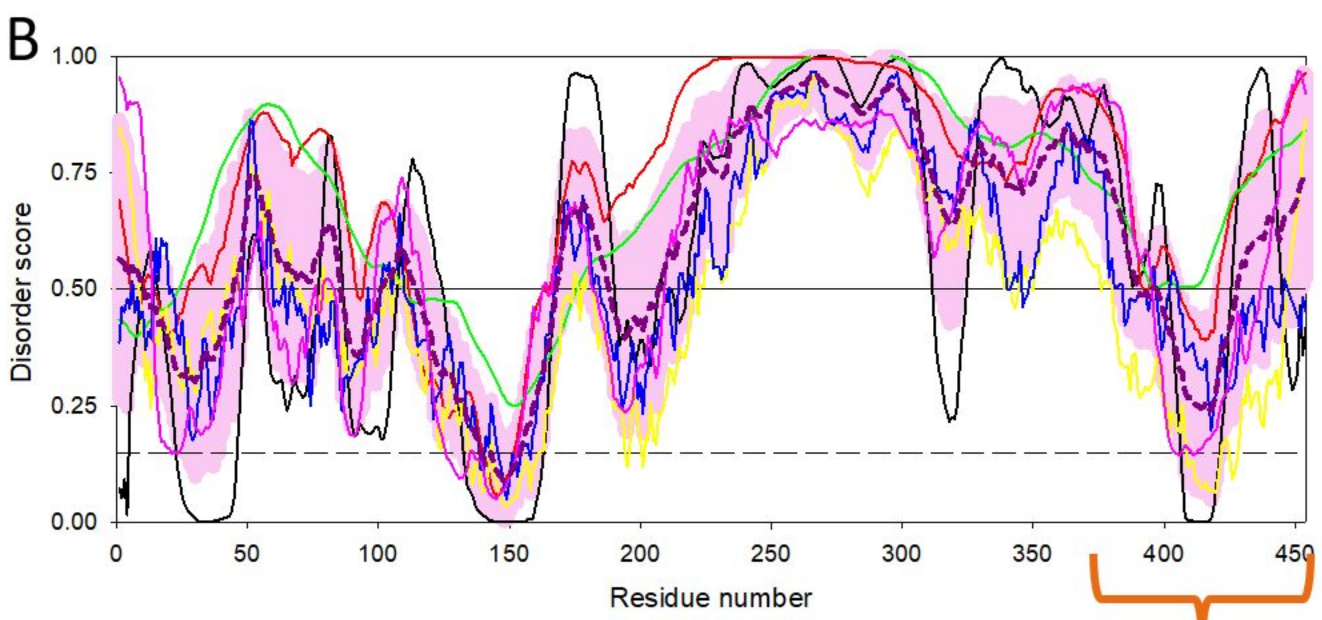



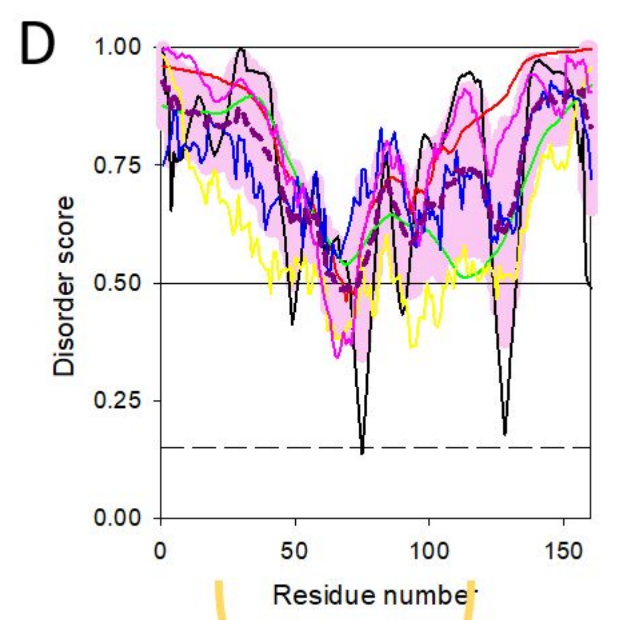

Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

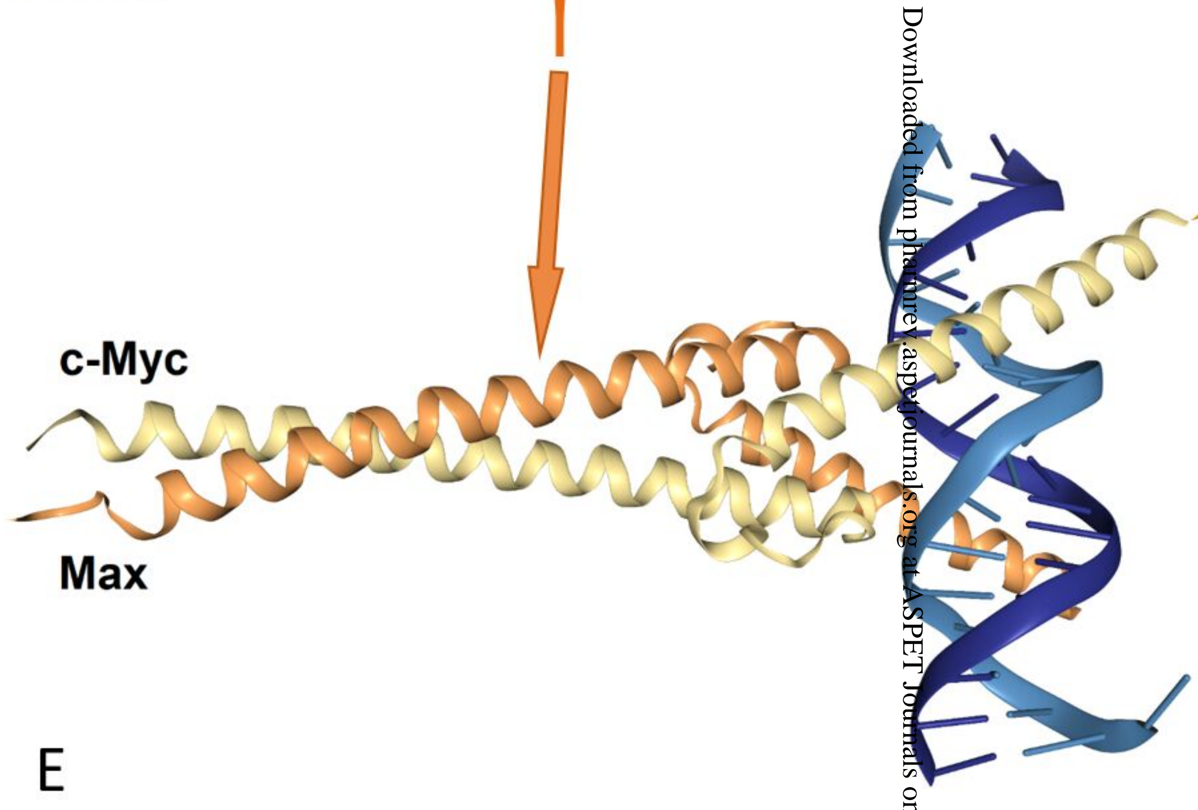
**Figure 6**




Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.




Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Figure 7

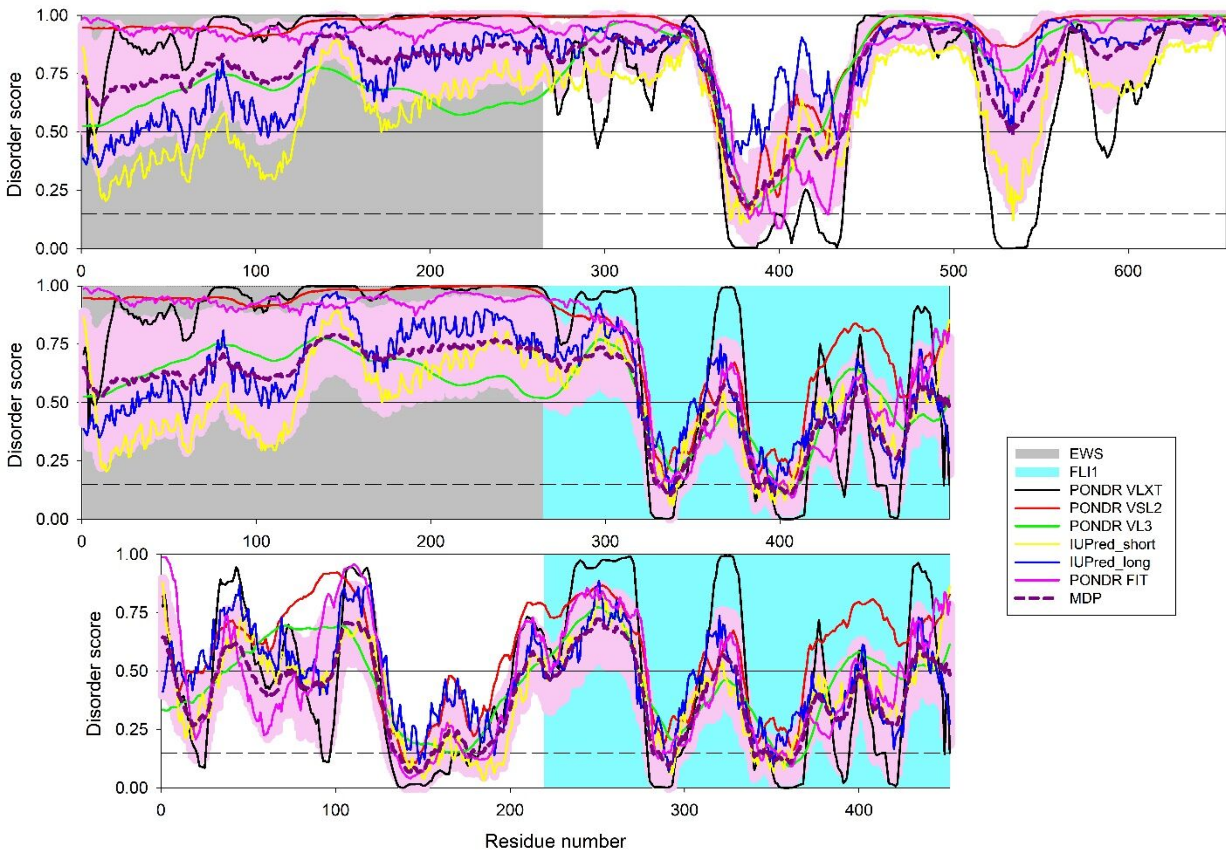



**Figure 8**

Predicted Disorder Agreement

0 50 100 150 200 250 300 350 400 450 500 550 600 650

700 750 800 850 900

Esprittz-D
Espritz-X
Espritz-N
IUPred-L
IUPred-S
PV2
PrDOS
VSL2b
VLXT

Predicted Domains

MoRF Regions

PTM Sites

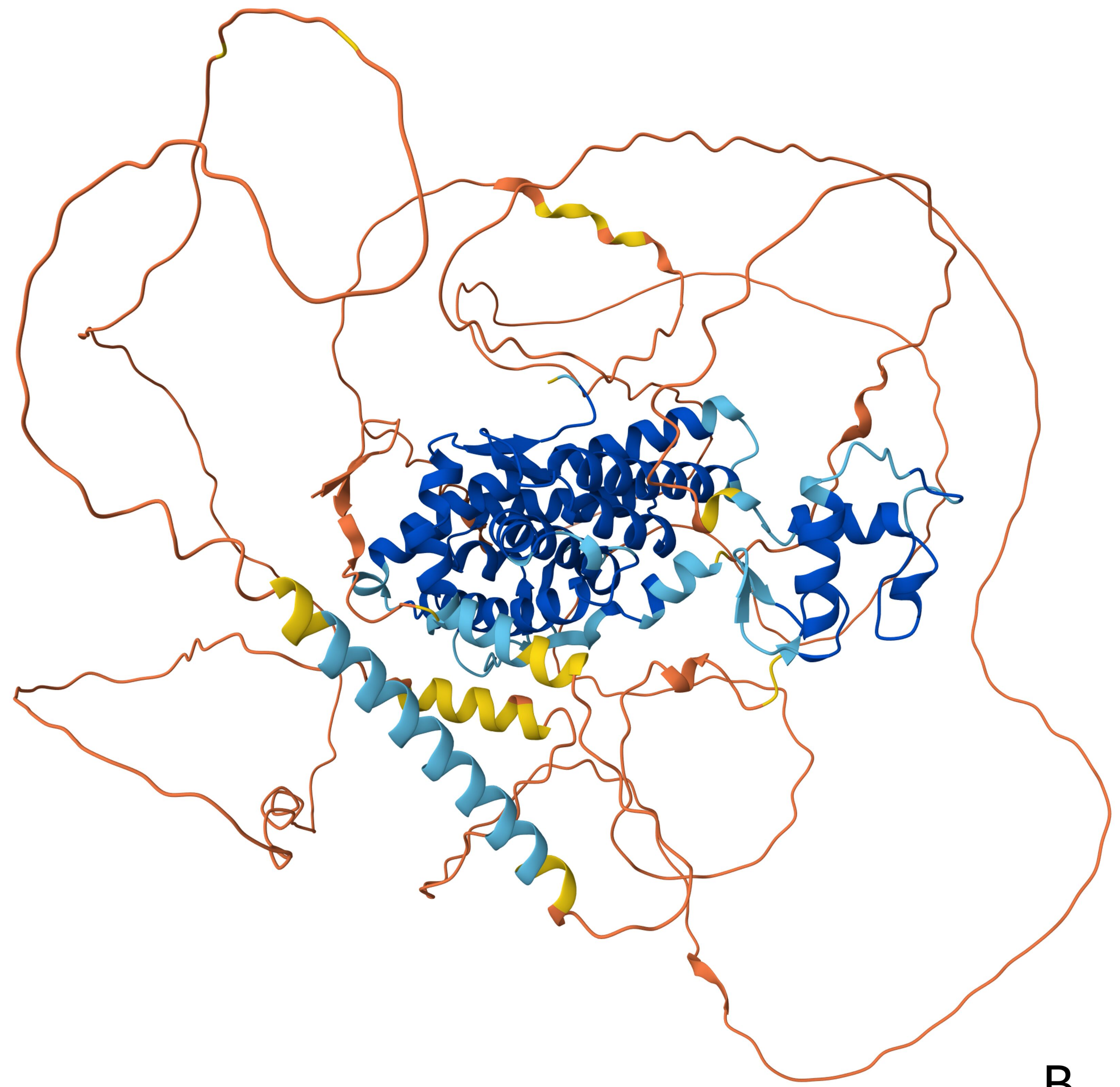
Key:
Predicted SCOP Structure
Weaker Support
Pfam Conserved Domain
Predicted Disorder
Predicted MoRFs
Curated PTM Site

Disorder:
Espritz-D
Espritz-X
Espritz-N
IUPred-L
IUPred-S
PV2
PrDOS
VSL2b
VLXT

Superfamilies:
[1] Nuclear receptor ligand-binding domain
[2] Glucocorticoid receptor-like (DNA-binding domain)

Pfams:
[3] PF02166.11 (Family)
[4] Ligand-binding domain of nuclear hormone receptor
[5] Zinc finger, C4 type (two domains)
[6] PB010071 (Pfam-B)
[7] PB008983 (Pfam-B)
[8] PB008001 (Pfam-B)
[9] PB010624 (Pfam-B)

A



B

**Figure 9**

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

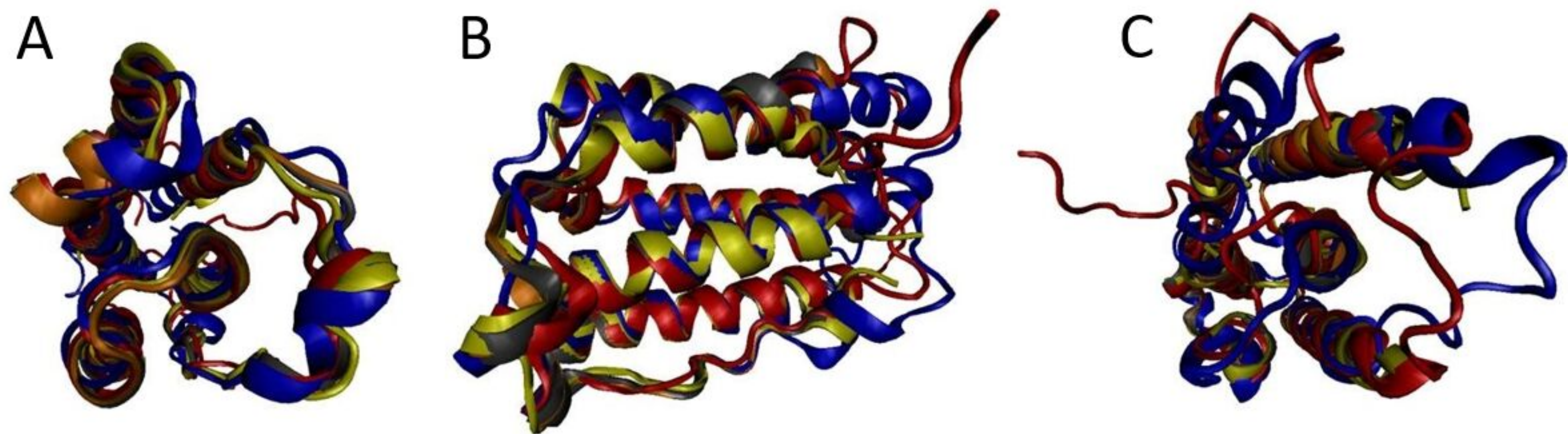
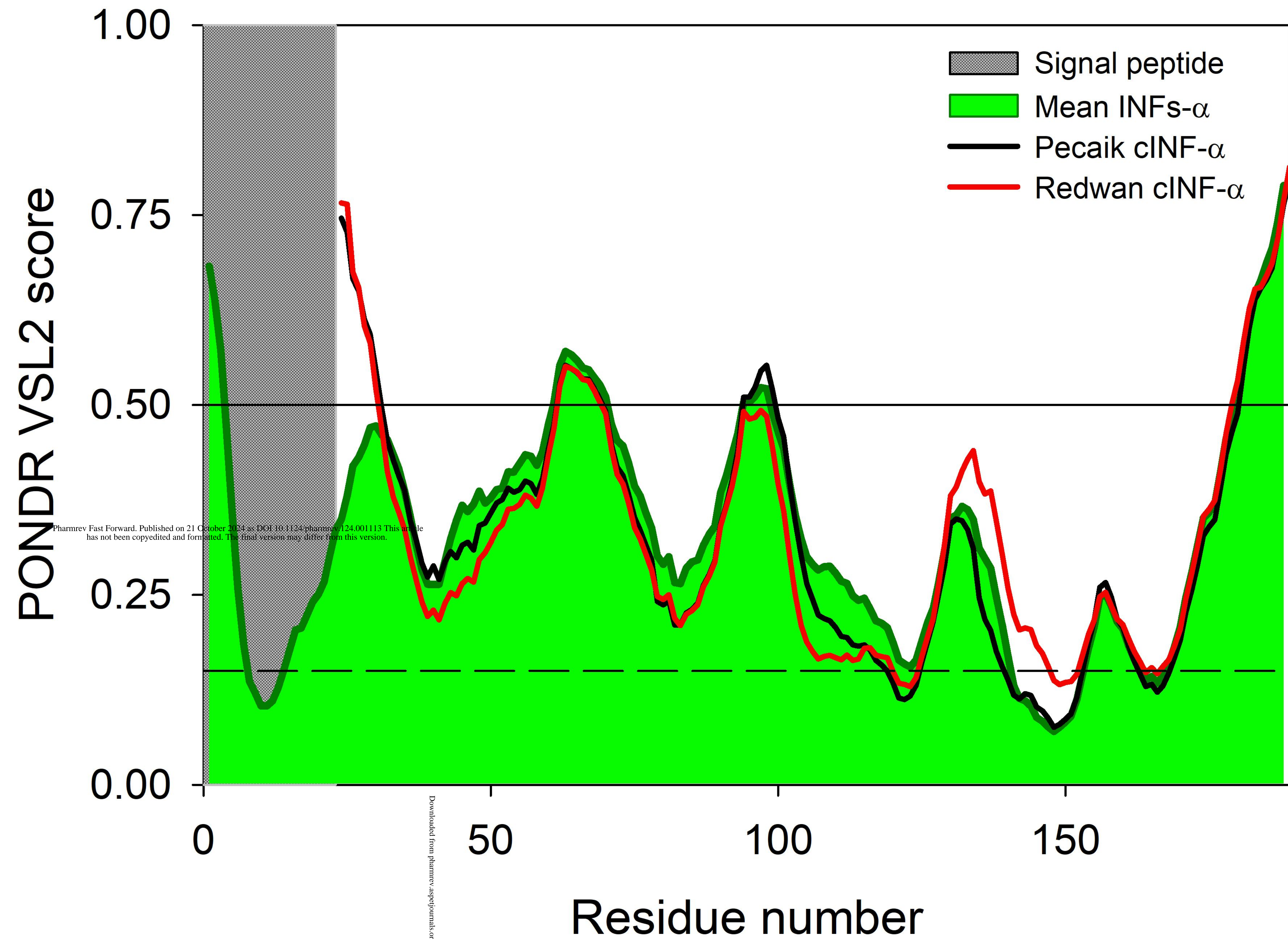



**Figure 10**

Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024




Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

**Figure 11**

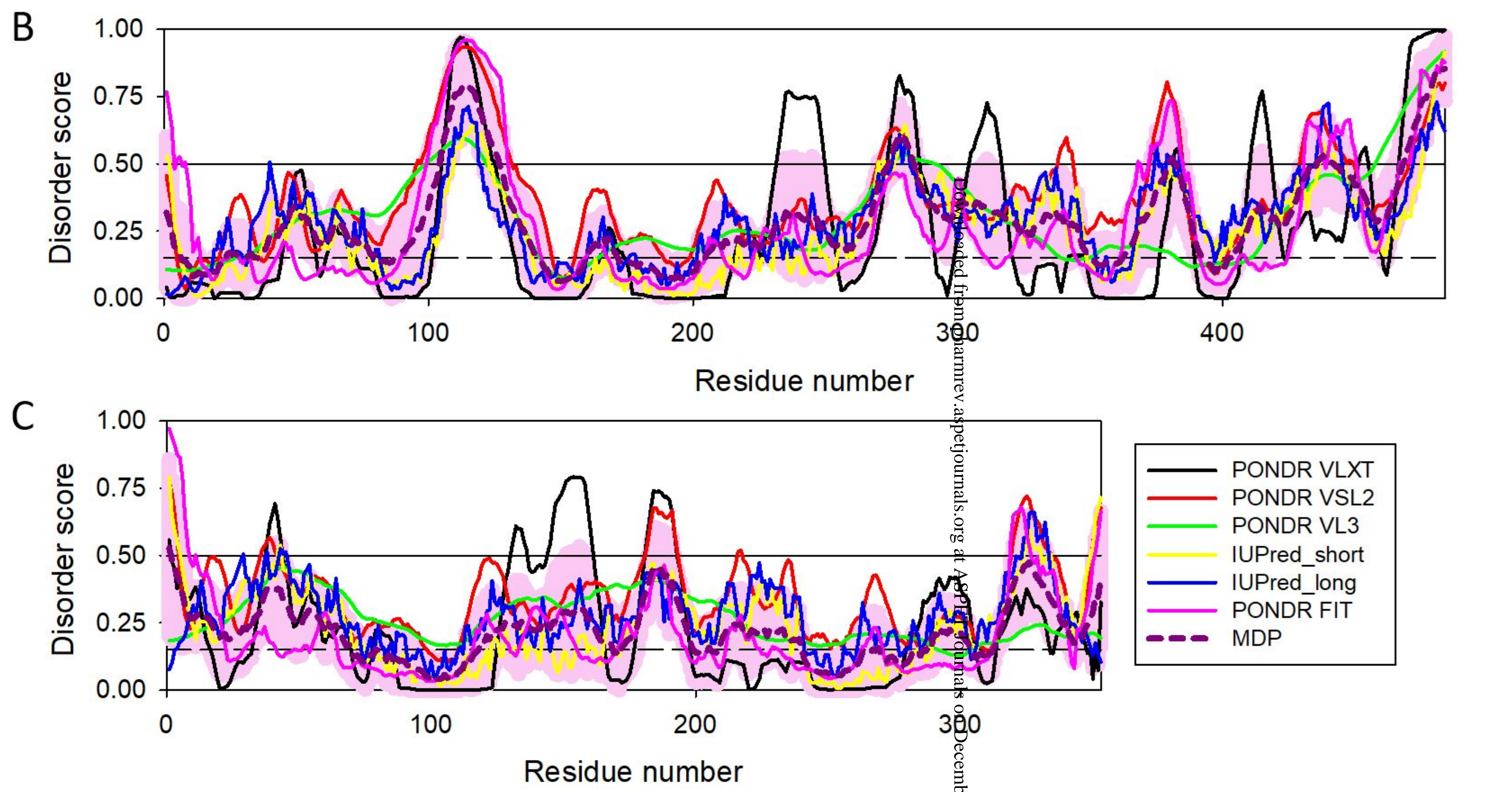
```
P03377|33-516    12 VWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEVVLVNVTENFNMWKNDMVEQMHEDIISLWDQSLKPCVKLTPLCVSLKCTDLGNATNTNSSNTNSSSGEMMMEKGEIKNCS
A0A0M3KKW8        1 VWKDADTTLFCASDAKAHETEVHNVWATHACVPTDPNPQEIHLENVTENFNMWKNNMVEQMQEDVISLWDQSLQPCVKLT----------------------------------------
                    *** * ***********   ********************   * *********** ***** ** ******** ******

P03377|33-516   132 FNISTSIRGKVQKEYAFFYKLDIIPIDNDTTSYTLTSCNTSVITQACPKVSFEPIPIHYCAPAGFAILKCNNKTFNGTGPCTNVSTVQCTHGIRPVVSTQLLLNGSLAEEEVVIRSANFT
A0A0M3KKW8       81 --------------------------------------GGSVIKQACPKISFDPIPIHYCTPAGYVILKCNDKNFNGTGPCKNVSSVQCTHGIKPVVSTQLLLNGSLAEEEIIIRSENLT
                                                          *** ***** ** ******* ***  ***** * ******* *** ******* *****************  *** * *

P03377|33-516   252 DNAKTIIVQLNQSVEINCTRPNNNTRKSIRIQRGPGRAFVTIGKIGNMRQAHCNISRAKWNATLKQIASKLREQFGNNKTIIFKQSSGGDPEIVTHSFNCGGEFFYCNSTQLFNSTWFNS
A0A0M3KKW8      163 NNAKTIIVHLNKSVEINCTRPSNGGS----------------GSGGDIRKAYCEINGTKWNKVLKQVTEKLKEHF-NNKTIIFQPPSGGDLEITMHHFNCRGEFFYCNTTQLFNNTCIGN
                    ******* ** ********* *                   *  *  * * * *   ****  ***   ** * * *******   **** **  * *** ******* ***** *

P03377|33-516   372 TWSTEGSNNTEGSDTITLPCRIKQFINMWQEVGKAMYAPPISGQIRCSSNITGLLLTRDGGNNNNGSEIFRPGGGDMRDNWRSELYKYKVVKIE
A0A0M3KKW8      266 E-TMKGCNGT-----ITLPCKIKQIINMWQGTGQAMYAPPIDGKINCVSNITGILLTRDGGANNTSNETFRPGGGNIKDNWRSELYKYKVVQIE
                         * * *     ***** *** *****  * ******* * * * ***** ******* **   * ******   ************* **
```

A




Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Figure 12

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This article has not been copyedited and formatted. The final version may differ from this version.

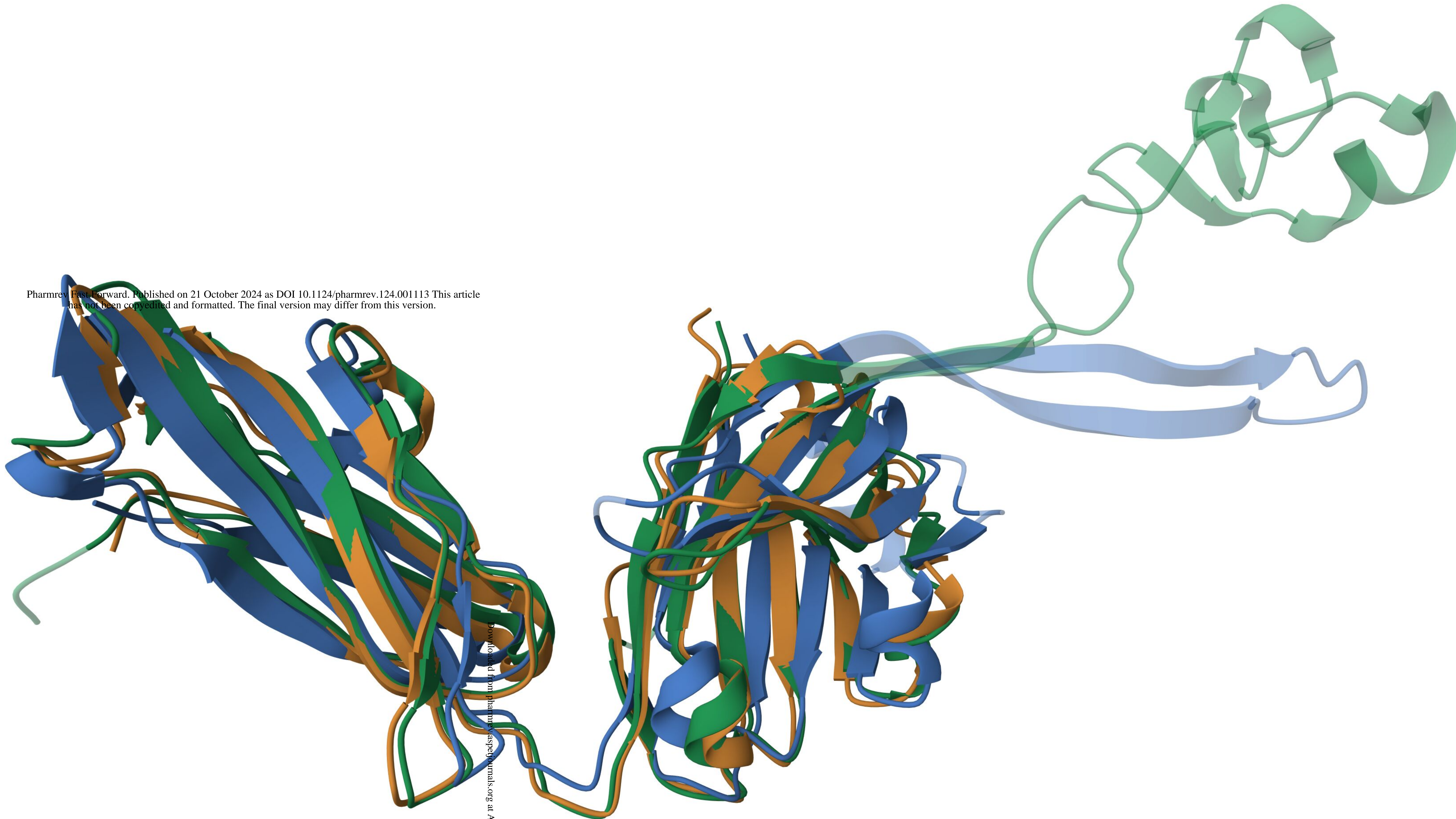
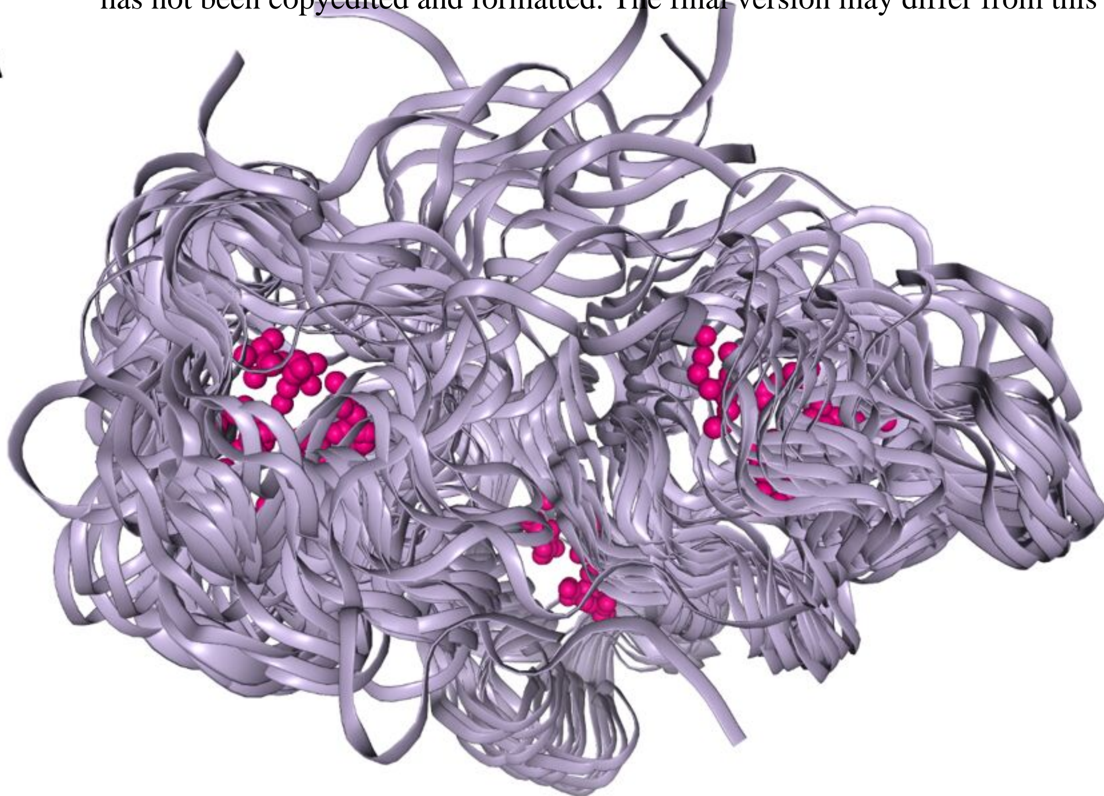
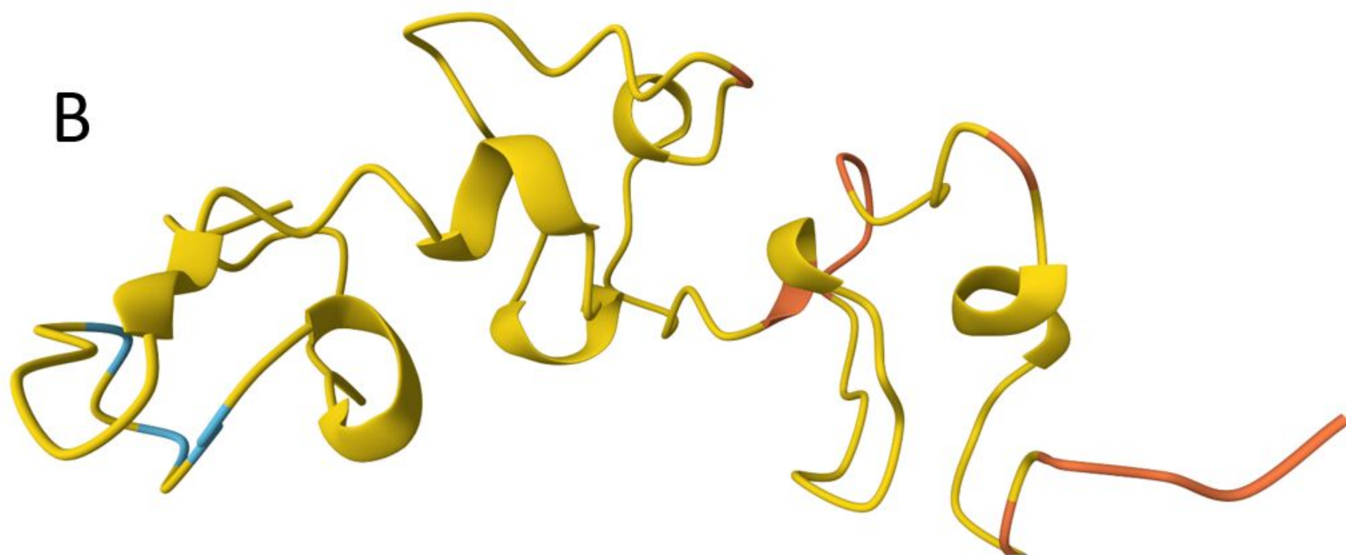


Figure 13

Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

Pharmrev Fast Forward. Published on 21 October 2024 as DOI 10.1124/pharmrev.124.001113 This ar
has not been copyedited and formatted. The final version may differ from this version.



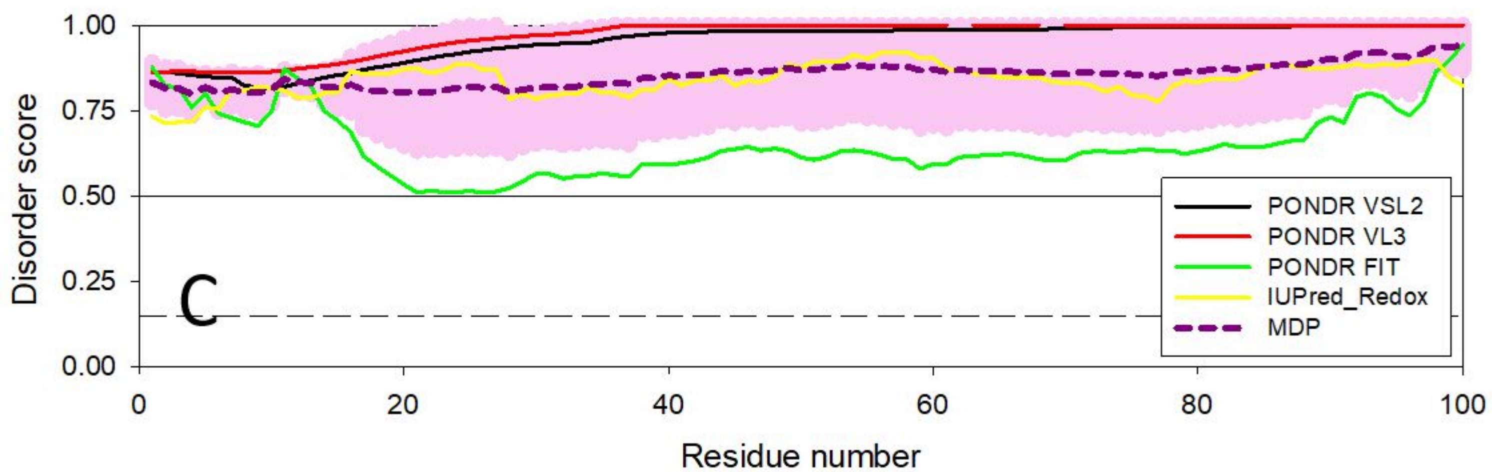




Figure 14

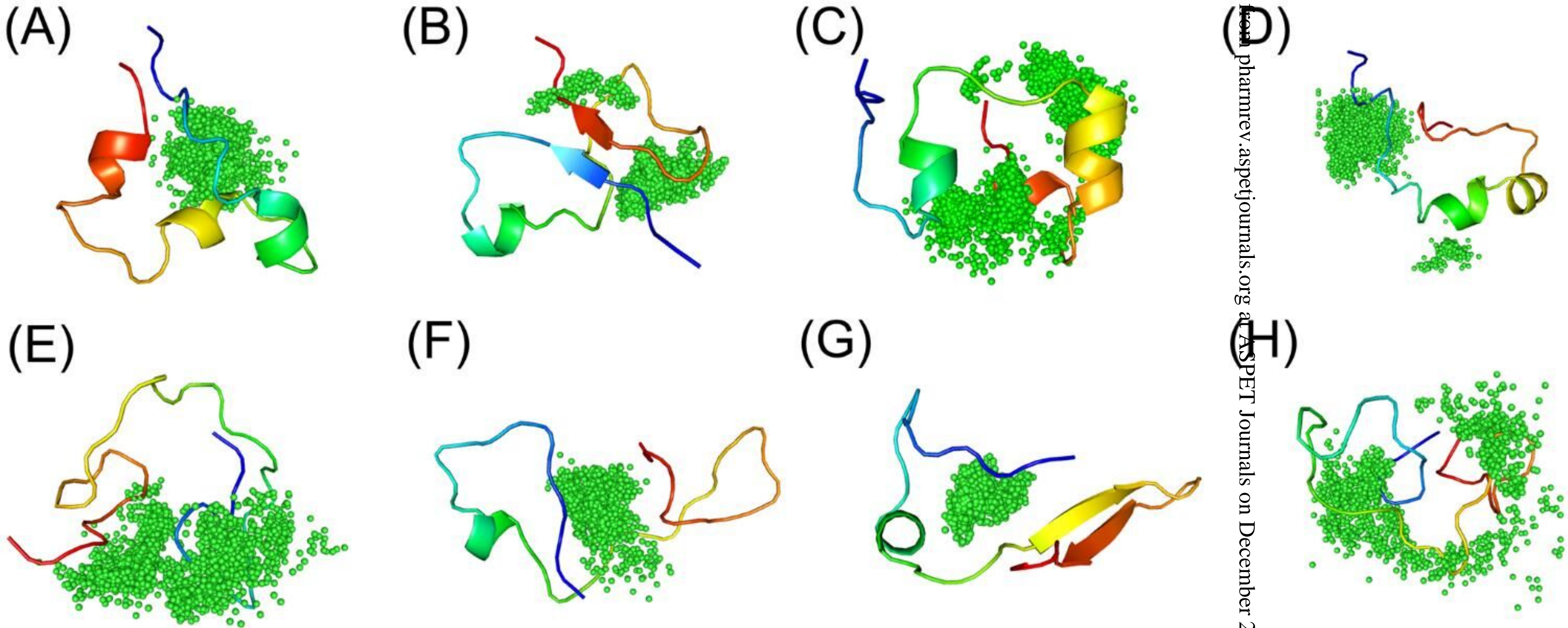



Figure 15

Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024