

1 **How to drug a cloud? Targeting intrinsically disordered proteins**

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

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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 virus
43	HTS	high-throughput screening
44	IDR	intrinsically disordered region

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	LIMT	metallothionein from <i>Littorina littorea</i>
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

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

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**

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.

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## 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  $\alpha$ - but not  $\beta$ -glycosidic bonds,  
146 whereas a similar enzyme could hydrolyze  $\beta$ - but not  $\alpha$ -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

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

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

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):

- 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

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).

- 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



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.

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

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

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

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 hydrophathy 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,

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

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-

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,



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; Dunder  
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

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,

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 themselves 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 despite 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 appropriate 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 typical 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).

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 liquid droplets  
554 on linear cellular structures, 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

560 of an autowave, with both processes being characterized by low energy demand representing  
561 an efficient way of the adjustment 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 driven by LLPS of specific IDPs (Csizmek  
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; Toretzky 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

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

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 ( $\beta$ -sheets,  $\alpha$ -helices, or contain both  $\alpha$ -helices and  $\beta$ -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).

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



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

672 analysis of two IDPs involved in the pathogenesis of Parkinson's disease and other  
673 synucleinopathies, tubulin polymerization promoting protein (TPPP/p25) and  $\alpha$ -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- $\alpha$ -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.

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

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

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 (Ziółkowska 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

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

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 remainder 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

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



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, TFIIF, 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, GSK3 $\beta$ , PARP-1, TAF1, TRRAP, and  
834 S100B( $\beta\beta$ ) 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,

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

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

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*

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

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

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

986 **Figure 9A** representing the functional disorder profile generated for human AR by the D<sup>2</sup>P<sup>2</sup>  
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



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  $\alpha$ -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.

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  $\alpha$ -synuclein, and showing substantial biological activity in the cellular  
1050 models of  $\alpha$ -synuclein-mediated dysfunction (Toth et al., 2014). In their search for small  
1051 molecules targeting intrinsically disordered  $\alpha$ -synuclein, the authors first randomly selected 100  
1052 structures from the conformational ensemble containing 40,000  $\alpha$ -synuclein structural models  
1053 derived from comprehensive NMR analysis. Then, they selected 22 more compact structures and

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  $\alpha$ -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  $\alpha$ -synuclein  
1062 aggregation assays and in cellular models of  $\alpha$ -synuclein malfunction (Toth et al., 2014). This  
1063 analysis revealed the protective role of ELN484228 in cellular models of  $\alpha$ -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

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 (Gadhav 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  $\beta$ -protein (A $\beta$ ), tau,  
1089 islet amyloid polypeptide, and  $\alpha$ -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,  $\alpha$ -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  $\alpha$ -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

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, A $\beta$ , tau, and  $\alpha$ -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

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 IFNs  
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- $\alpha$  encoded by at least 23 genes  
1125 with no intron sequences (Pestka et al., 1987), IFN- $\beta$ , IFN- $\delta$ , IFN- $\epsilon$ , IFN- $\kappa$ , IFN- $\tau$ , and IFN- $\omega$   
1126 (Liu, 2005; Plataniias, 2005). Type I IFNs bind to a specific multichain IFN- $\alpha/\beta$  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- $\gamma$ , which binds to the oligomeric IFN- $\gamma$  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 (Kallioliias and Ivashkiv, 2010).

1132 IFN- $\alpha$ , 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- $\alpha$ , which can be further subdivided into several subtypes (e.g., IFN-  
1136  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 4$ ,  $\alpha 5$ ,  $\alpha 6$ ,  $\alpha 7$ ,  $\alpha 8$ ,  $\alpha 10$ ,  $\alpha 13$ ,  $\alpha 14$ ,  $\alpha 16$ ,  $\alpha 17$ , and  $\alpha 21$ ), are among the best  
1137 studied IFNs. Different IFNs- $\alpha$  show high levels of sequence similarity, and sequences of human  
1138 IFNs- $\alpha$  share between 70 and 80% homology, and about 35% identity with human IFN- $\beta$  (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- $\alpha$   
1141 (IFN- $\alpha 1/13$  and IFN- $\alpha 2$ ) and IFN- $\beta$  are characterized by a compact structure composed of five  
1142 major  $\alpha$ -helices (named helix A to E) connected by a long loop and three shorter loops  
1143 (Radhakrishnan et al., 1996). However, although the global  $\alpha$ -helical fold of IFNs is conserved,

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- $\alpha$  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- $\alpha$ , 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- $\alpha$ 2 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- $\alpha$ , a synthetic IFN- $\alpha$ , the consensus  
1154 interferon cIFN- $\alpha$ , 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- $\alpha$   
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  $\alpha$ -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- $\alpha$  was linked to the higher affinity of this protein to the type I IFN  
1161 receptors than the naturally occurring IFN- $\alpha$  (Blatt et al., 1996).

1162 Comparison of the disorder profiles generated for two consensus IFNs- $\alpha$  with the mean disorder  
1163 profile calculated as averaged disorder predispositions of 17 human IFNs- $\alpha$  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- $\alpha$  (cf. **Figure 10D**). Based on these observations it was

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- $\alpha$  (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



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 Massignani 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 (Massignani 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

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

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: 6O00; (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

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

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;

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

- 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***

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) deconstruction), 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 ( $T_i$ ) to a  
1367 new condensed phase comprising desolvated and aggregated polypeptides when the temperature  
1368 is raised above  $T_i$  (Meyer and Chilkoti, 1999; Urry, 1988; 1992; 1997) resulted in the  
1369 development of a unified model for accurate prediction of the  $T_i$  values for different ELPs based  
1370 on their composition, chain length, and concentration (McDaniel et al., 2013b). The  $T_i$  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).



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

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,

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 ( $\alpha$ -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

1442 aggregation behavior of  $\alpha$ -synuclein within the condensed state (Dada et al., 2024). Analysis  
1443 revealed that the aminosterol claramine modulate the  $\alpha$ -synuclein phase separation by stabilizing  
1444  $\alpha$ -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<sup>®</sup> 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,

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, malfunction, 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

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

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 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* (LIMT)  
1521 in the complex with  $\text{Cd}^{2+}$  (Baumann et al., 2017). The conformational ensemble representing the  
1522 solution structure of LIMT 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  $\text{Cd}^{2+}$  ions). LIMT contains three individual domains, each comprising a single structurally well-  
1525 defined three-metal cluster, where three  $\text{Cd}^{2+}$  ions are present in the (S-thiolate)<sub>4</sub>-coordinated  
1526 form (there are 27 Cys residues in LIMT 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 LIMT resembling a cloud.  
1529 Since each structure included in the LIMT conformational ensemble contains 9 well-coordinated  
1530  $\text{Cd}^{2+}$  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  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ , and  $\text{Cu}^+$

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  $\text{Cd}^{2+}$ ,  $\text{Cu}^+$ ,  $\text{Hg}^+$ , and  
1536  $\text{Zn}^{2+}$  (Baumann et al., 2017). In line with these observations, **Figure 14B** represents a 3D  
1537 structural model of LIMT generated by AlphaFold showing that this protein is expected to be  
1538 mostly disordered. Further support of the highly disordered nature of LIMT 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<sub>370-409</sub> (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<sub>370-409</sub> 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<sub>370-409</sub> 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



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 induce 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

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

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1603

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1605 The author declares that all the data supporting the findings of this study are contained within the  
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1607

1608 **Authorship Contributions**

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

1611

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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  $(C_{\text{DisProt}} - C_{\text{PDB}}) / C_{\text{PDB}}$ , where  $C_{\text{DisProt}}$  is the  
2928 content of a given amino acid in a DisProt database (Sickmeier et al., 2007), and  $C_{\text{PDB}}$  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 hydrophathy 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 ( $\langle R \rangle = 2.785 \langle H \rangle - 1.151$ ,

2939 where  $\langle R \rangle$  and  $\langle H \rangle$  correspond to the absolute mean charge and mean hydrophathy, respectively  
2940 (Uversky et al., 2000b)); (ii) the mirror image of this boundary ( $\langle R \rangle = -2.785 \langle H \rangle + 1.151$ ,  
2941 where  $\langle R \rangle$  and  $\langle H \rangle$  correspond to the mean charge and mean hydrophathy, respectively); (iii)  
2942 two boundaries showing logical limits of the CH-space ( $\langle R \rangle = -1.125 + 1.125 \langle H \rangle$  and  $\langle R \rangle =$   
2943  $1.00 - \langle H \rangle$ ), evaluated for a series of hypothetical polypeptides containing different  
2944 proportions of Ile (which is, according to the Kyte and Doolittle scale, is the most hydrophobic  
2945 residue with the normalized hydrophathy of 1 (Kyte and Doolittle, 1982)) and a negatively  
2946 charged Asp (which is characterized by the normalized Kyte and Doolittle hydrophathy of 0.1111  
2947 (Kyte and Doolittle, 1982)) or a positively charged Arg (which is characterized by the  
2948 normalized Kyte and Doolittle hydrophathy of 0.0 (Kyte and Doolittle, 1982)). Vertical dashed  
2949 line represents a hypothetical boundary separating soluble compact proteins and membrane  
2950 proteins.

2951 **Figure 4.** Schematic representation of the mosaic nature of the protein structure-function space.  
2952 Differently (dis)ordered segments (foldons, semi-foldons, inducible foldons, inducible morphing  
2953 foldons, non-foldons, and unfoldons) might have different functions. Being found within one  
2954 protein, these pieces of structural mosaic define protein multifunctionality.

2955 **Figure 5.** Structural and functional characterization of TPPP/p25 (A, B) and  $\alpha$ -synuclein (C, D)  
2956 known to be involved in the pathogenesis of Parkinson's disease and other synucleinopathies. **A.**  
2957 3D model generated for human TPPP/p25 by AlphaFold (Jumper et al., 2021). Structure is  
2958 colored based on the AlphaFold-generated per-residue confidence score (pLDDT) that between 0  
2959 and 100, where regions with very high confidence score (pLDDT > 90) are shown by blue color,  
2960 confidently predicted regions (90 > pLDDT > 70) are shown by cyan color, whereas regions  
2961 predicted with low (70 > pLDDT > 50) and very low confidence (pLDDT < 50) are shown by

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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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

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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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**.

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<sup>2</sup>P<sup>2</sup> 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- $\alpha$ 1/13 (PDB ID: 3ux9, yellow ribbon), IFN- $\alpha$ 2  
3020 (PDB IDs: 3s9D, chain A, gray ribbon; 3s9D, chain C, orange ribbon; and 1itf, red ribbon) and  
3021 human IFN- $\beta$  (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- $\alpha$  by the PONDR<sup>®</sup> 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- $\alpha$ . Position of known  $\alpha$ -helices and regions of missing  
3030 electron density are also indicated. Shaded light pink area shows the position of the signal

3031 peptide (residues 1-23). Residues with the PONDR<sup>®</sup> 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- $\alpha$  proteins. Plot represents disorder  
3037 profiles calculated for two consensus interferons, cIFN- $\alpha$ 2 (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<sub>ab</sub> 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: 6O00; green structure) (Stanfield et al., 2020).

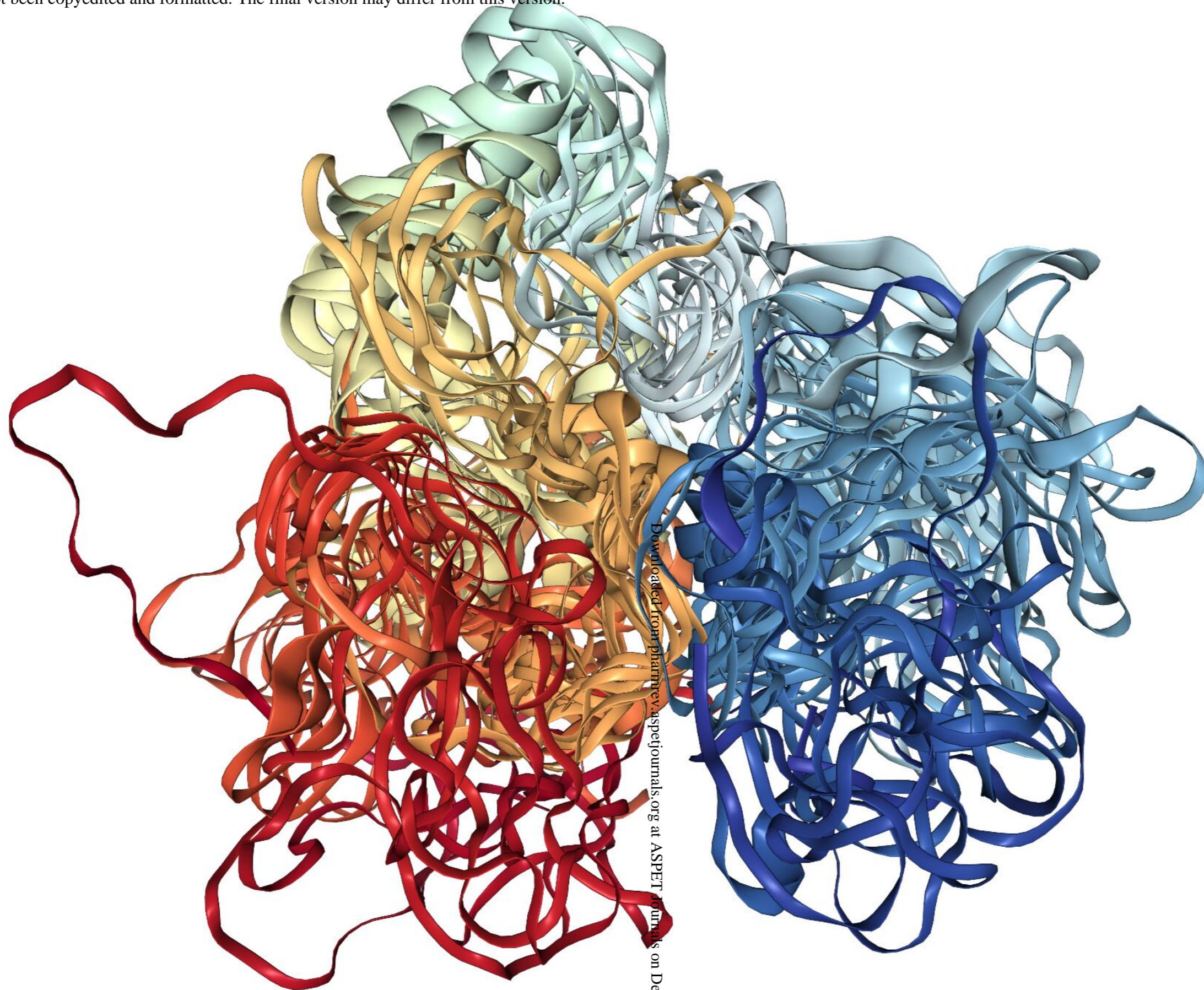
3051 **Figure 14.** Structural characterization of the metallothionein from the snail *Littorina littorea*  
3052 (LIMT). **A.** Illustration of the “clouds in cloud” model. NMR solution structure of the LIMT



3053 (gray structures) in complex with  $\text{Cd}^{2+}$  (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 LIMT. **C.** Intrinsic disorder  
3056 profile of LIMT generated by several commonly used disorder predictors.

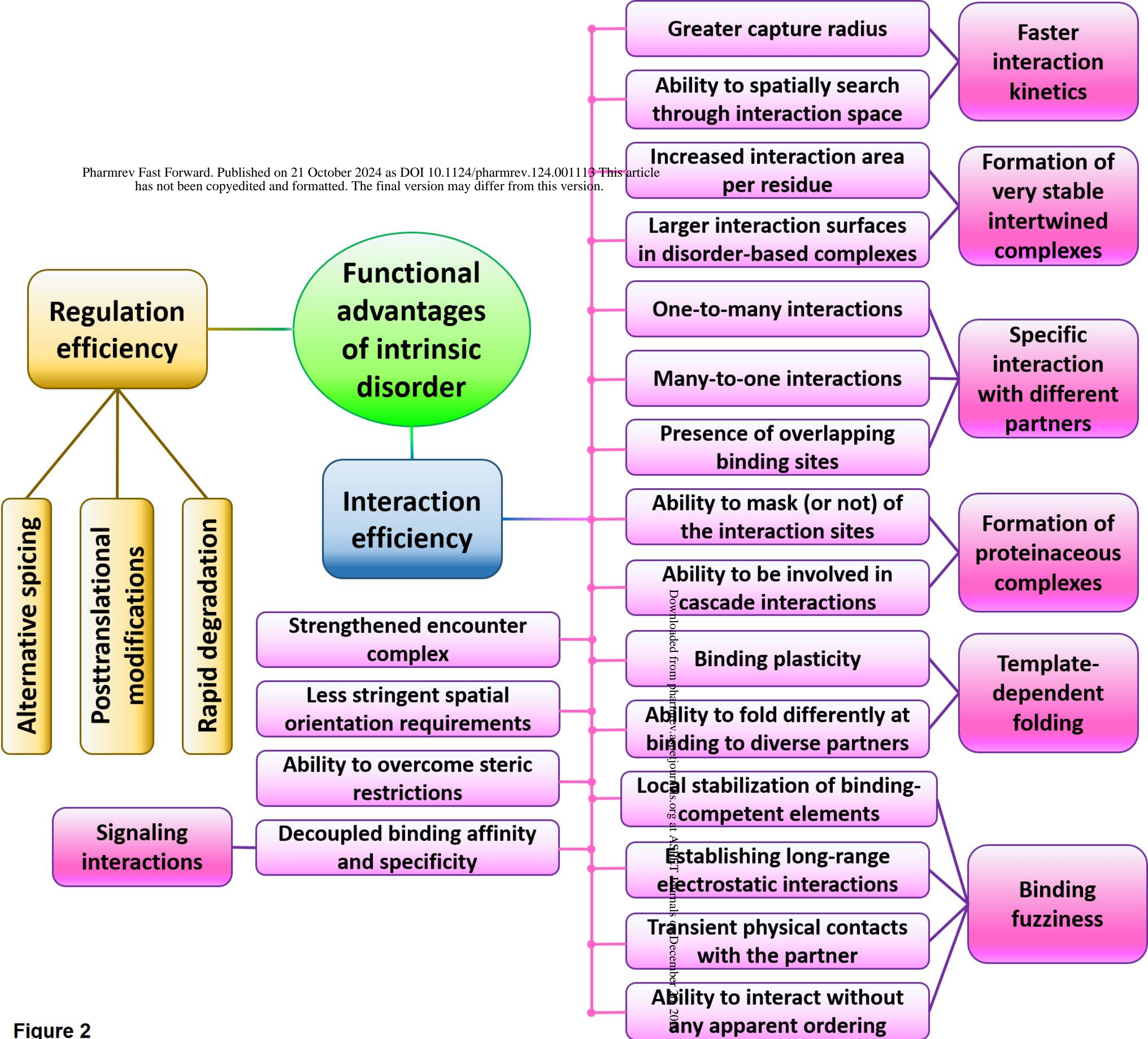
3057 **Figure 15.** Illustration of the ligand cloud concept. Figure represent the first eight clustering  
3058 groups of the ligand-bound conformations of c-Myc<sub>370-409</sub> fragment from the explicit solvent  
3059 simulations together with the clouds of bound 10074-A4 ligand. In each cluster, the N- and C-  
3060 termini of the c-Myc<sub>370-409</sub> 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).

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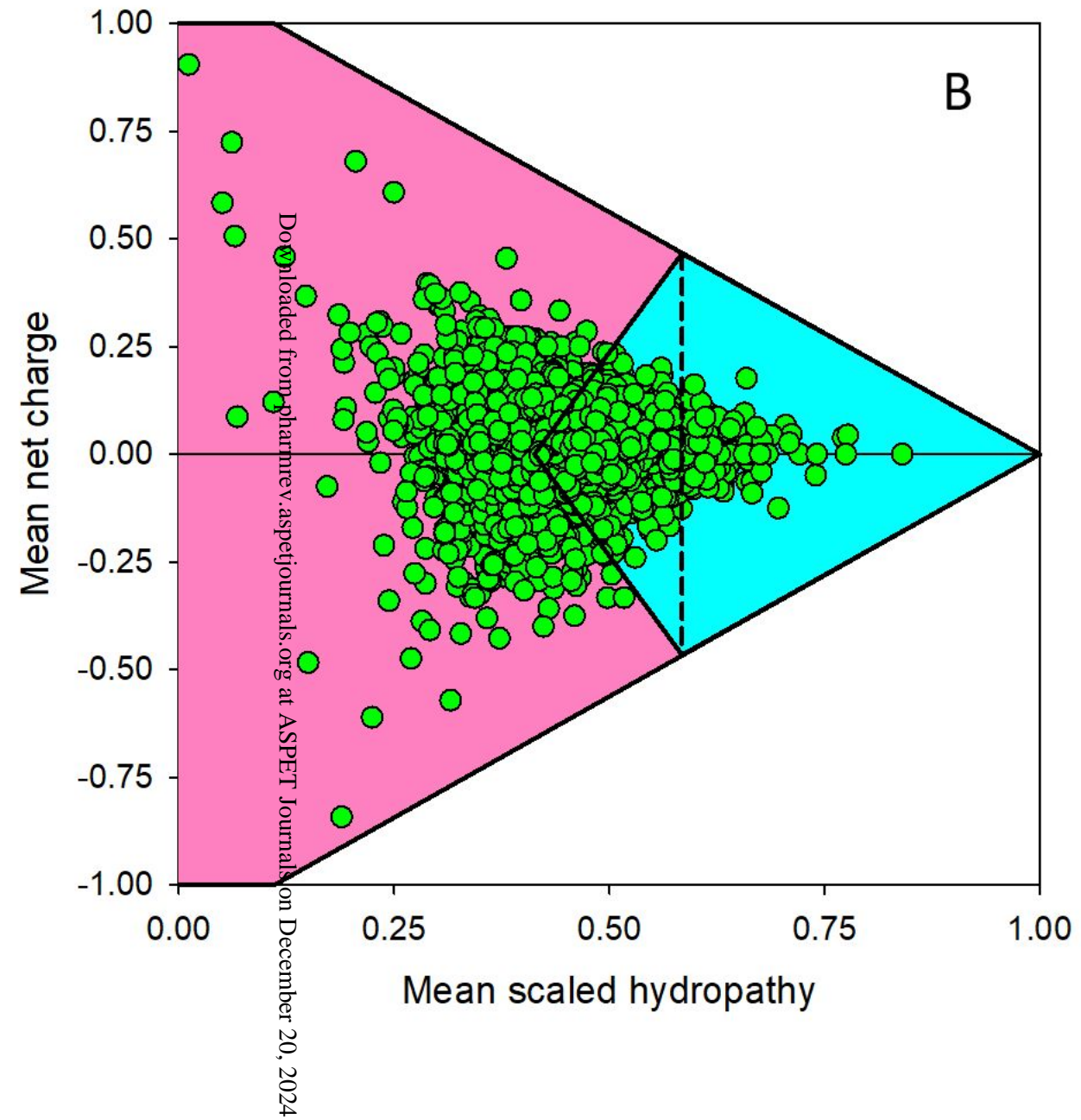
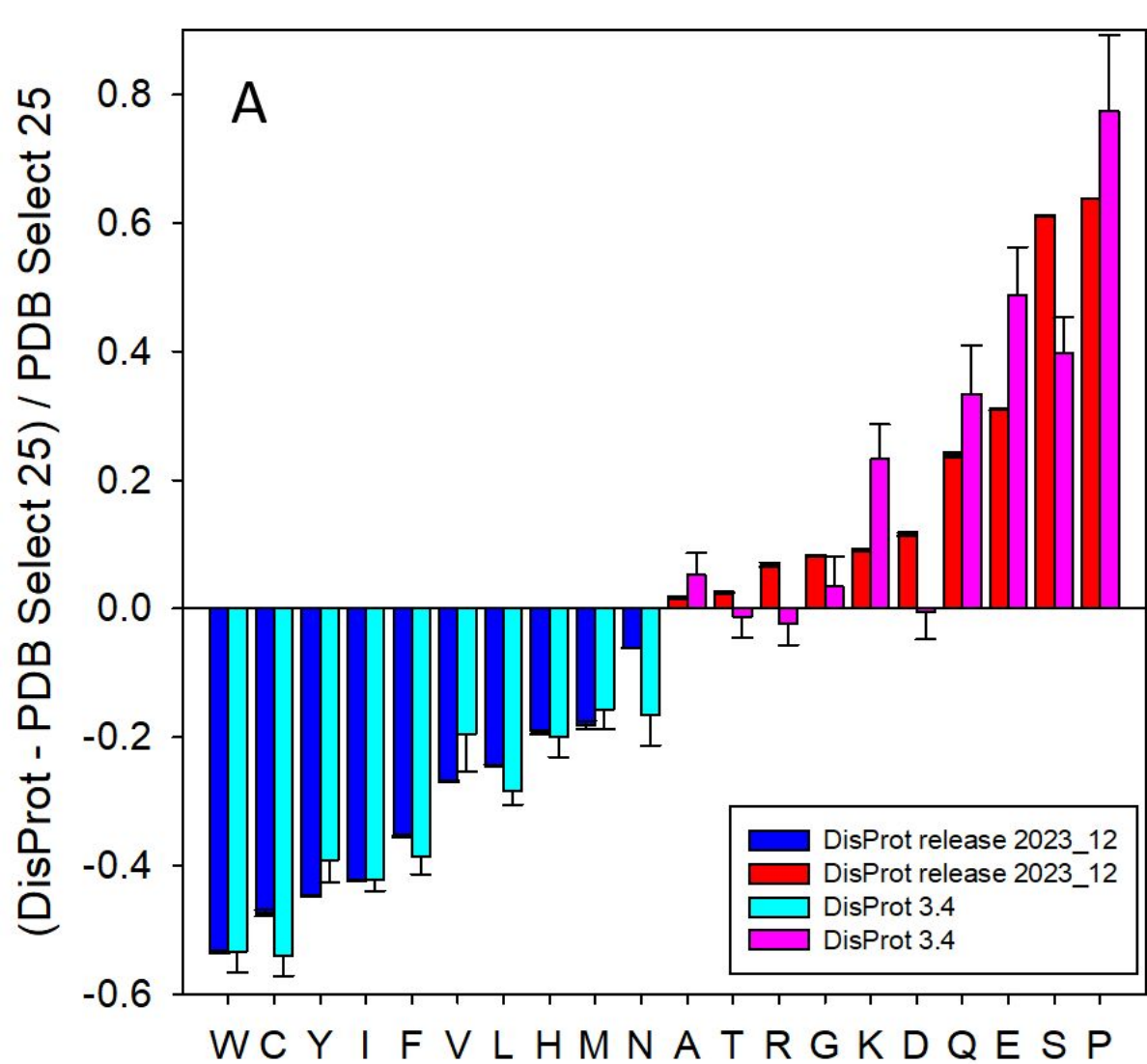
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Figure 1

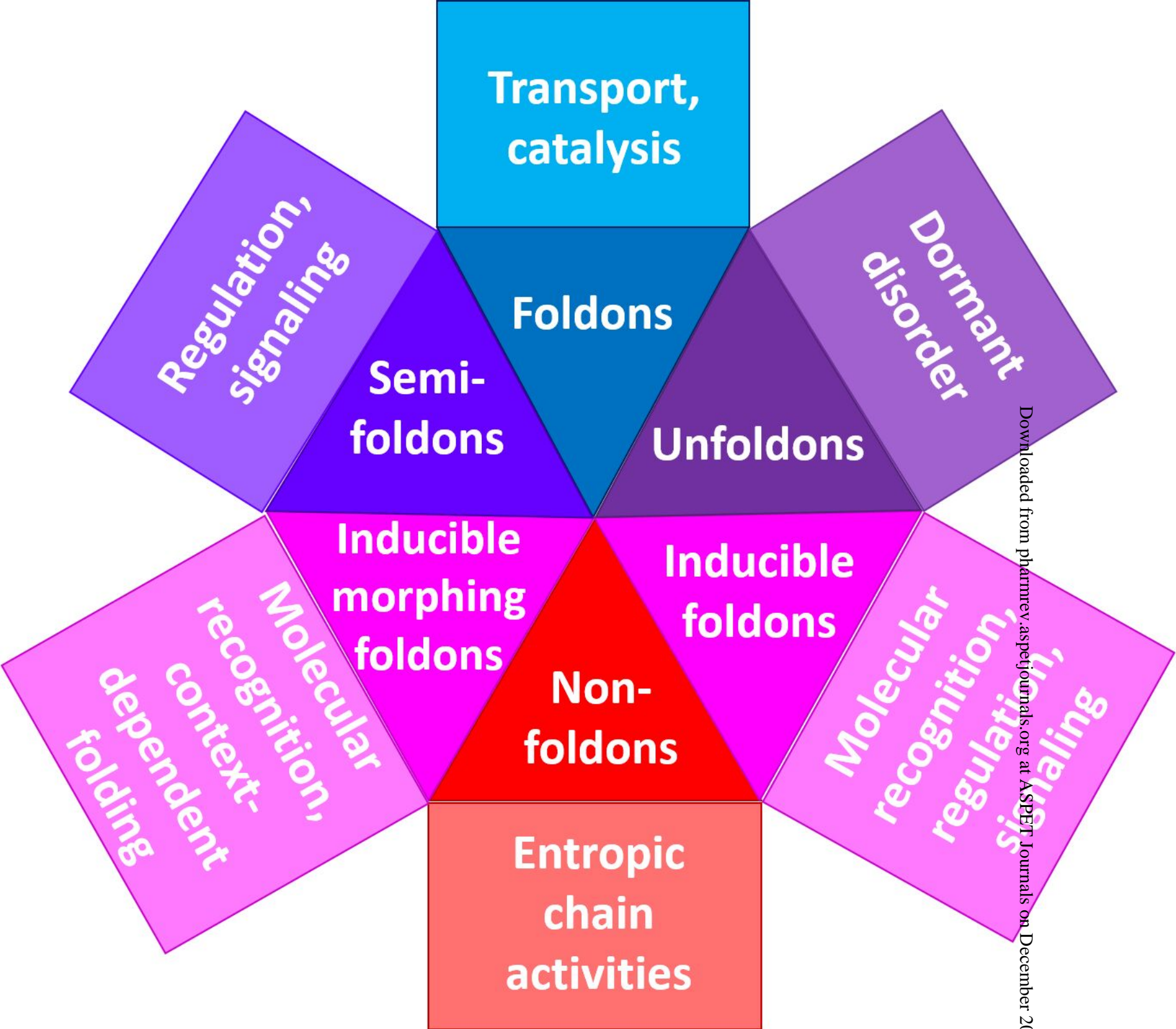


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Figure 2

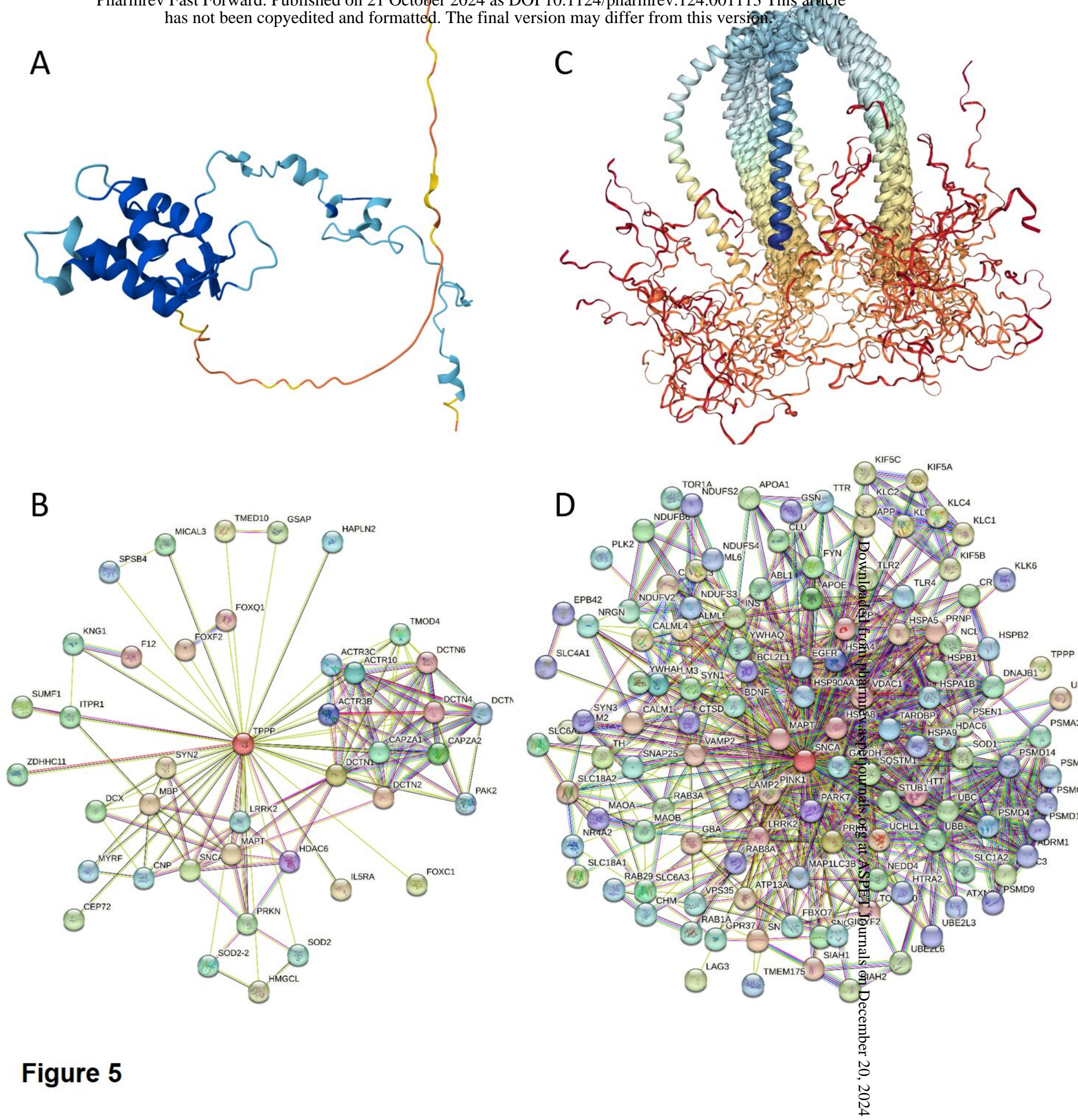


**Figure 3**



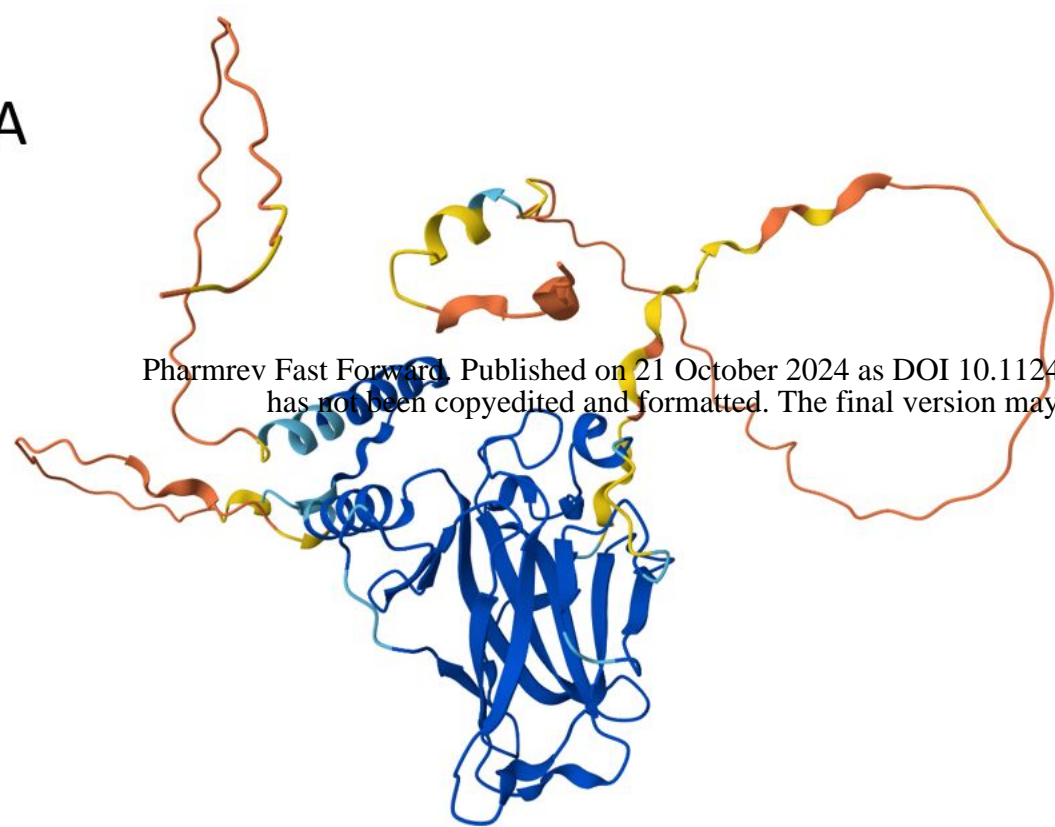
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**Figure 4**

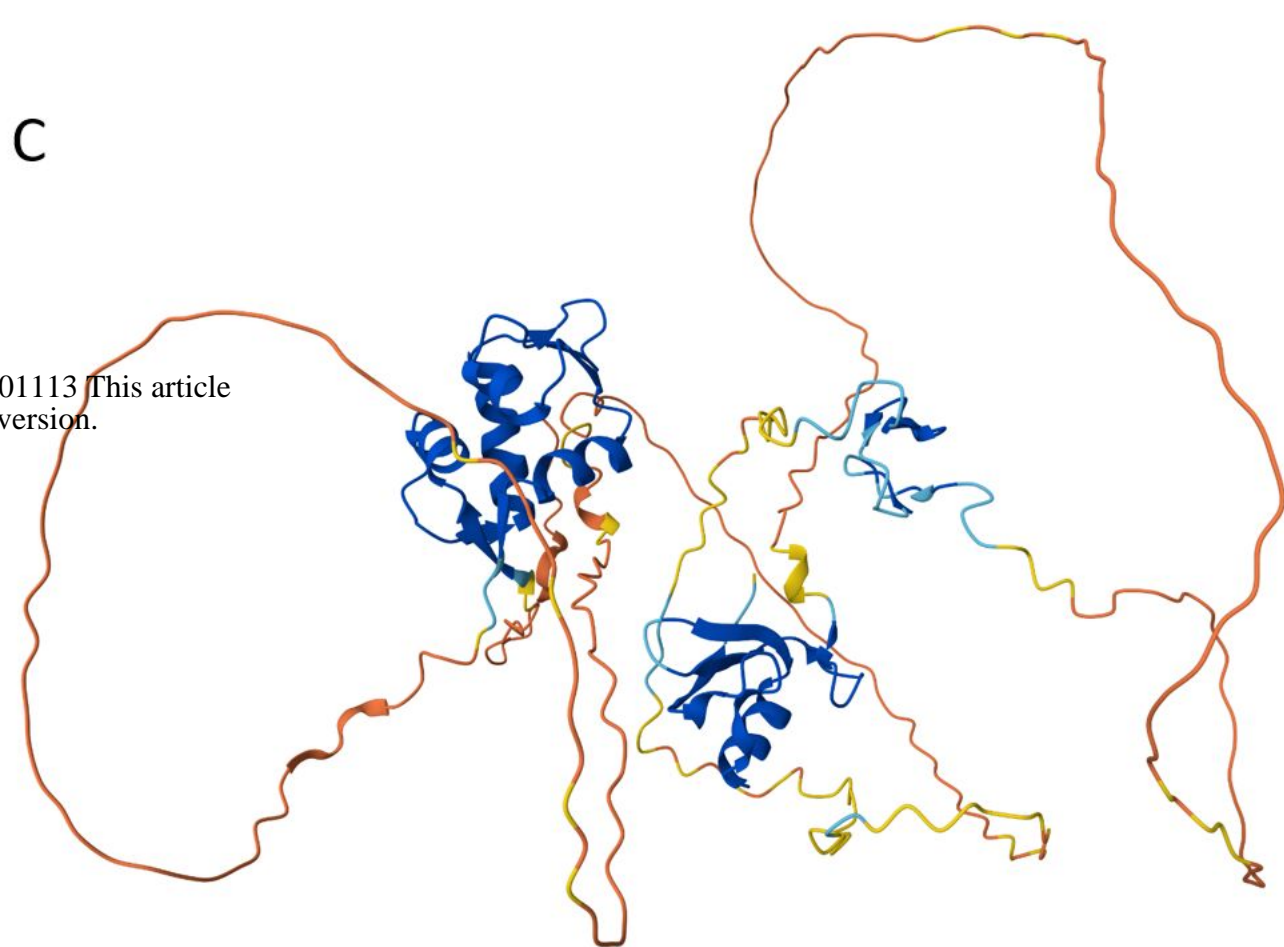


**Figure 5**

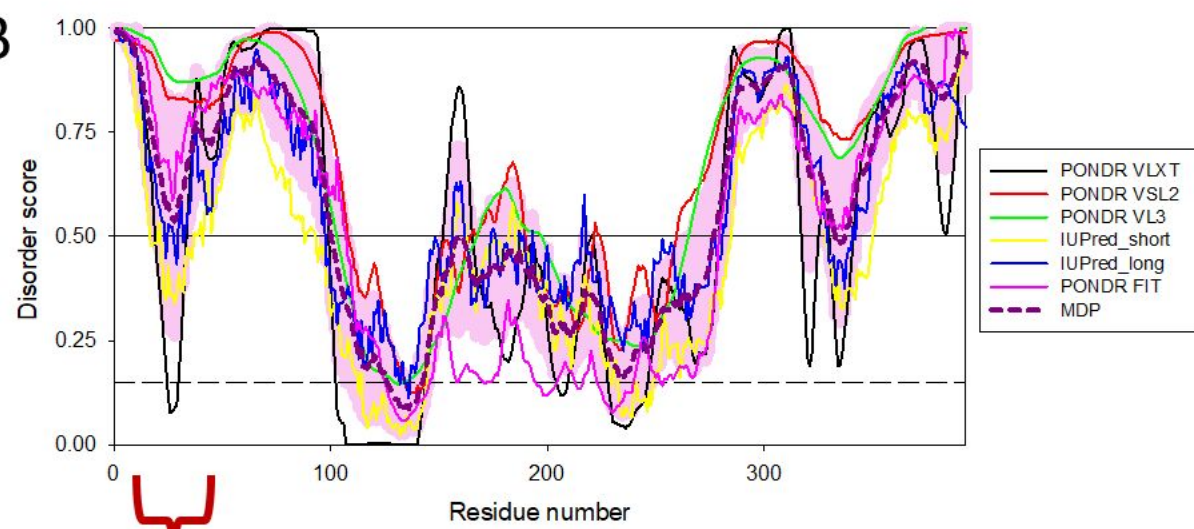
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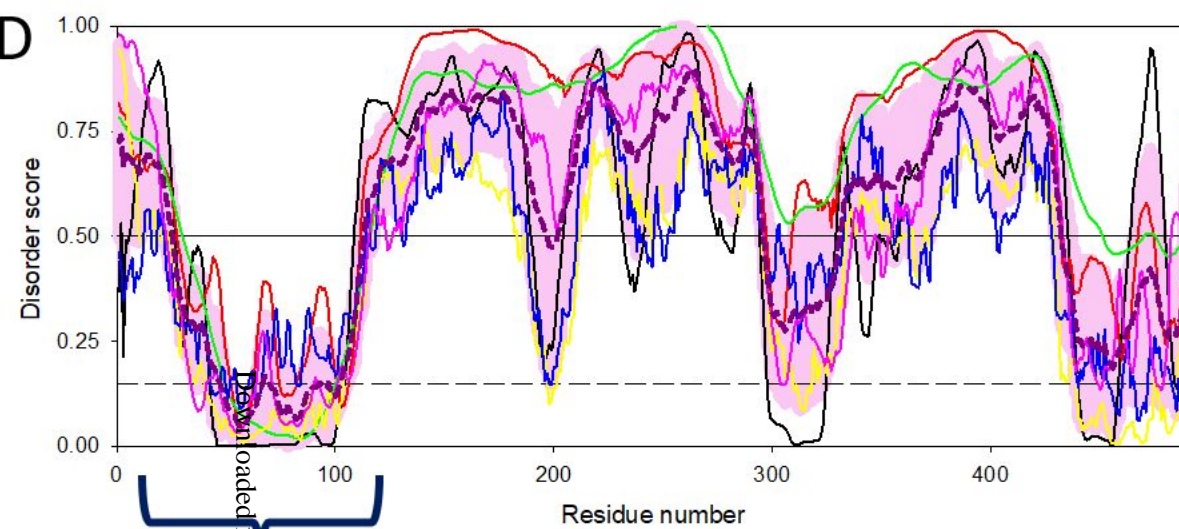
C



B



D



E

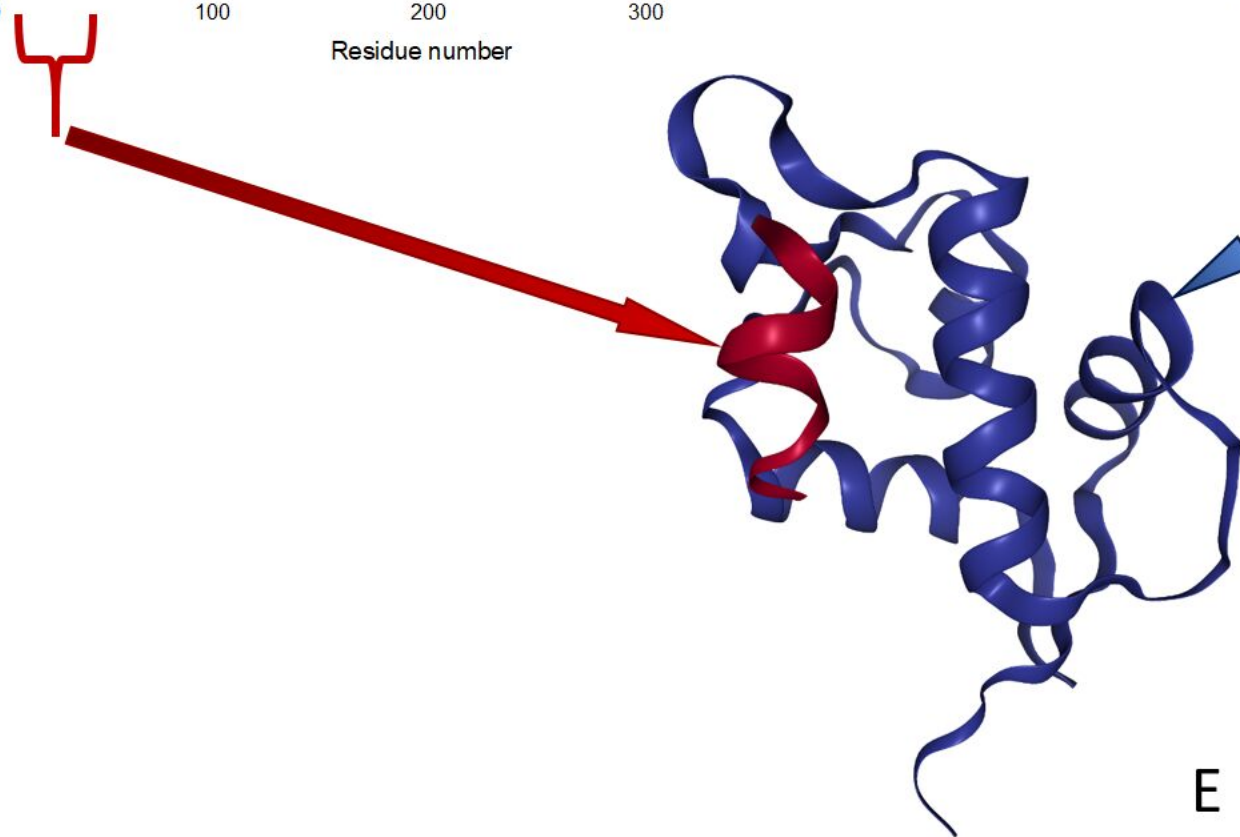
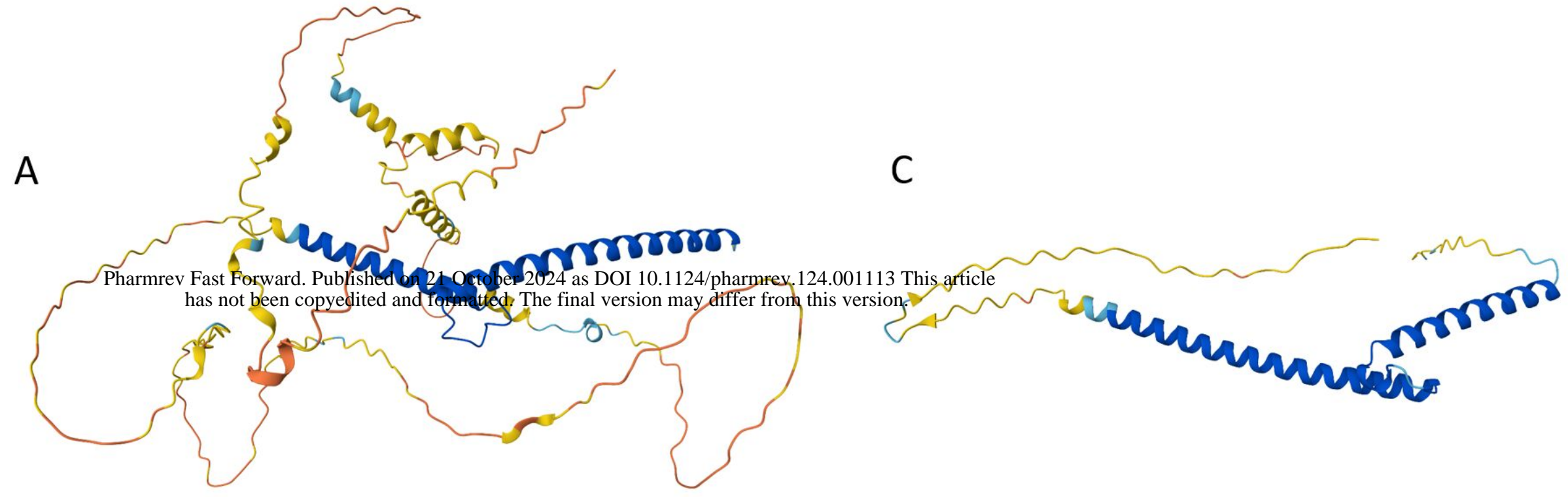
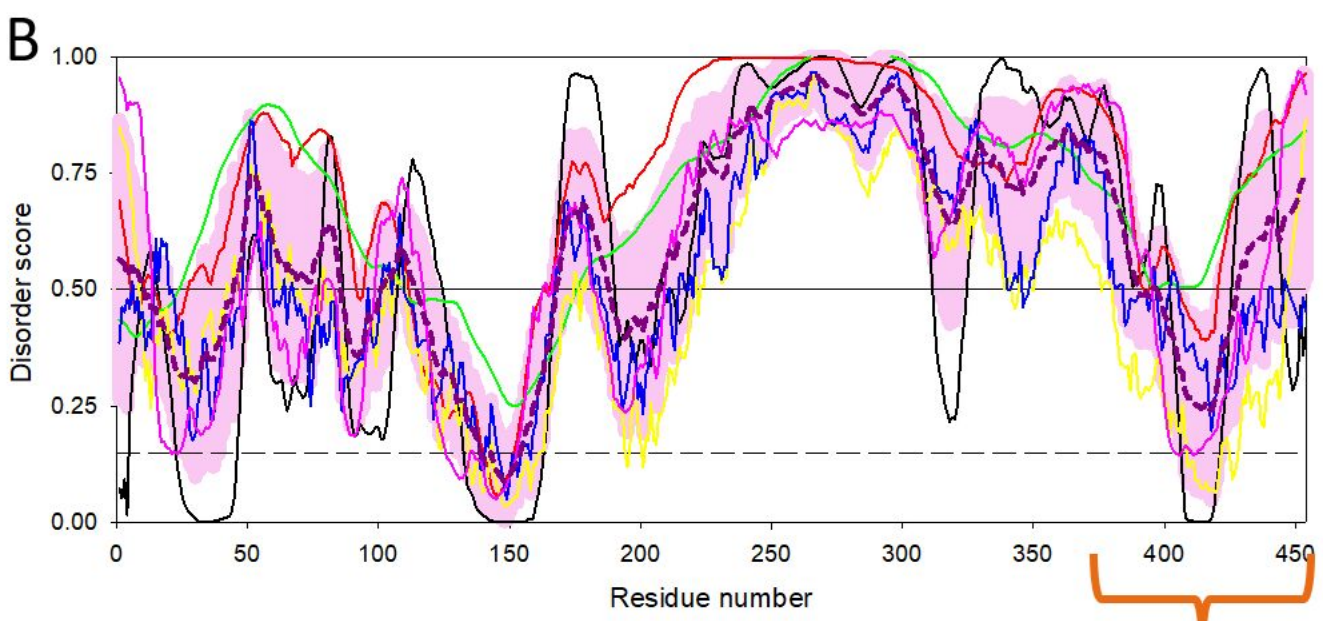


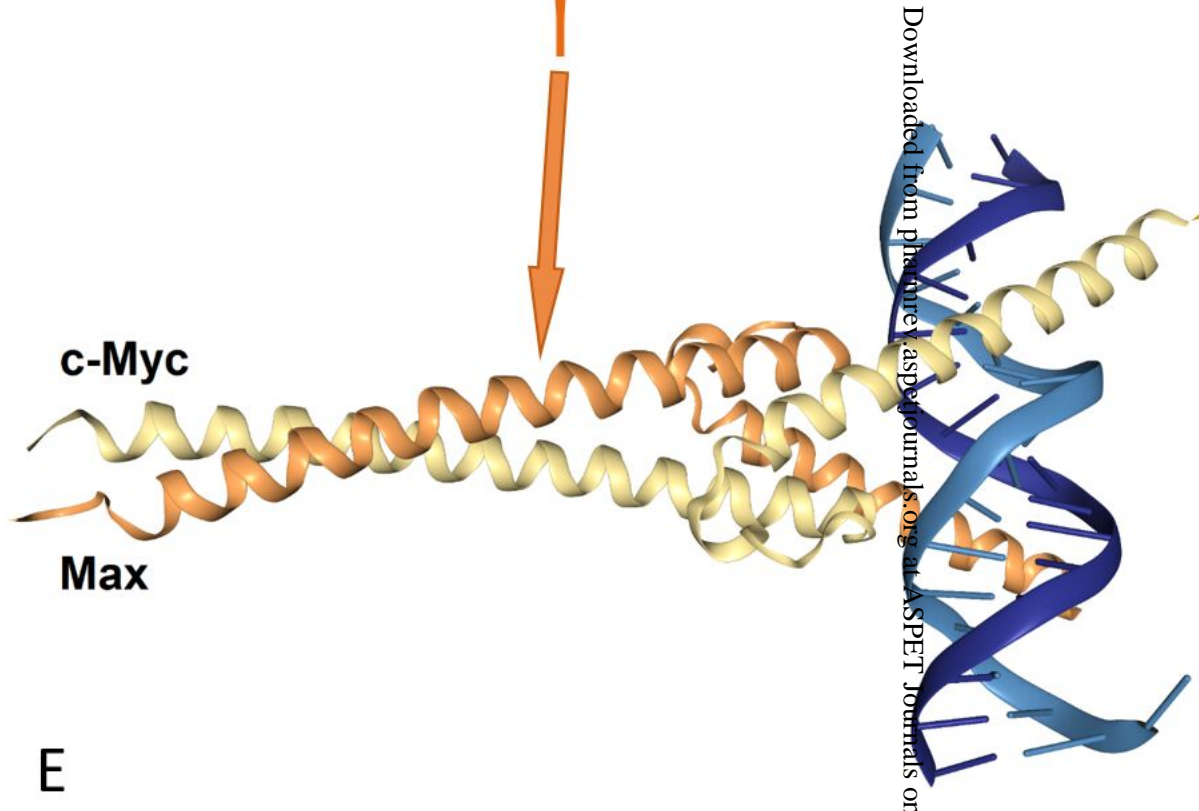
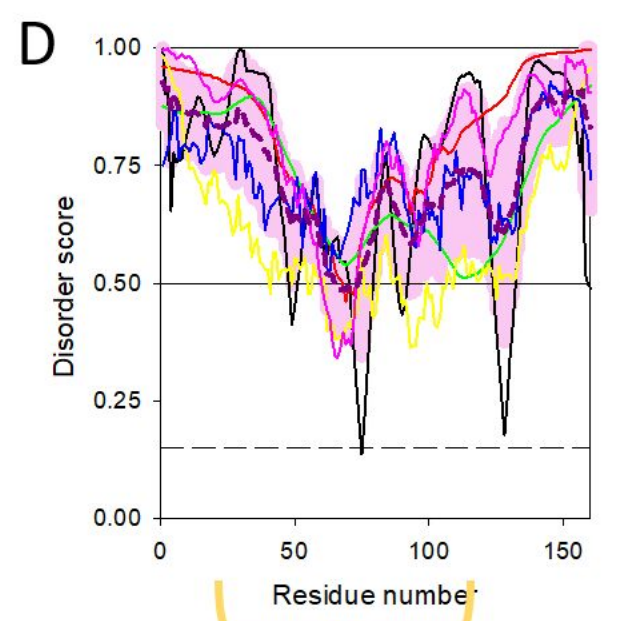
Figure 6



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- PONDRL VLXT
- PONDRL VSL2
- PONDRL VL3
- IUPred\_short
- IUPred\_long
- PONDRL FIT
- MDP



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**Figure 7**



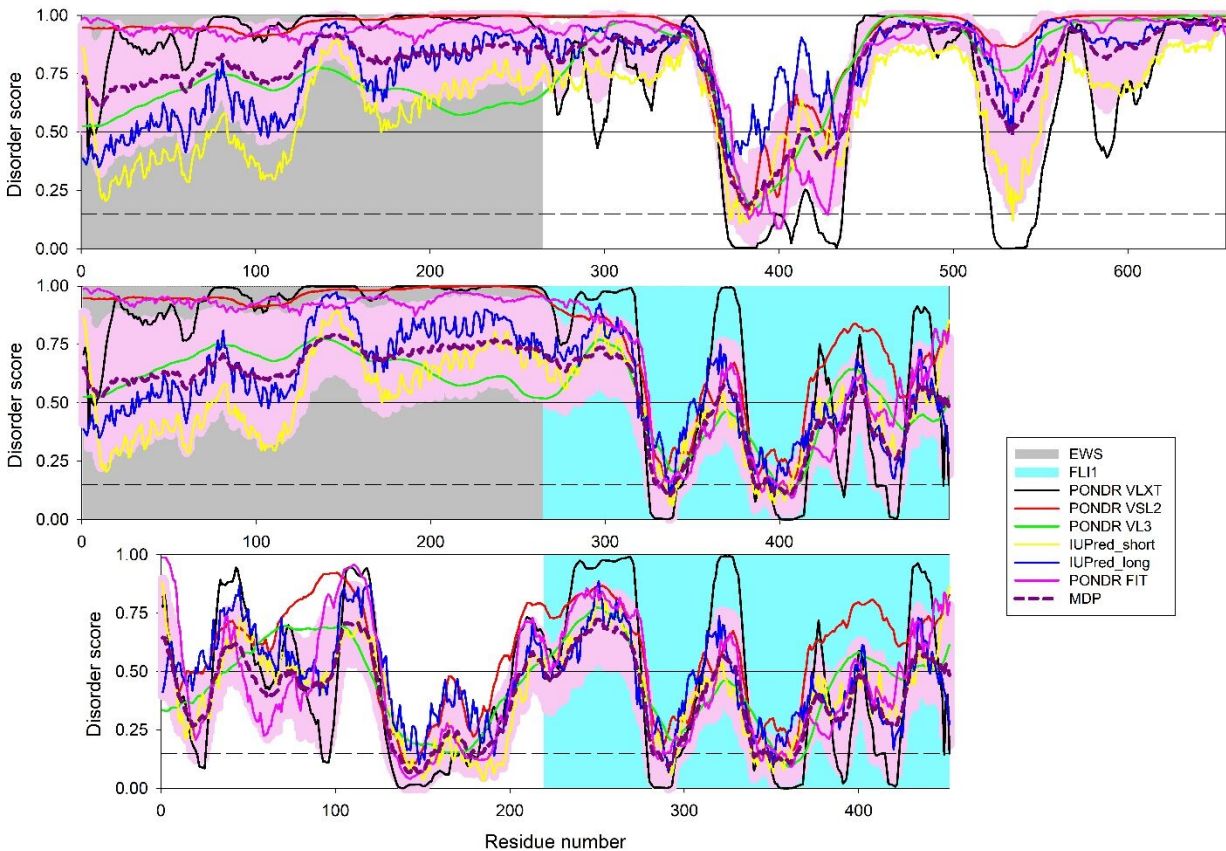
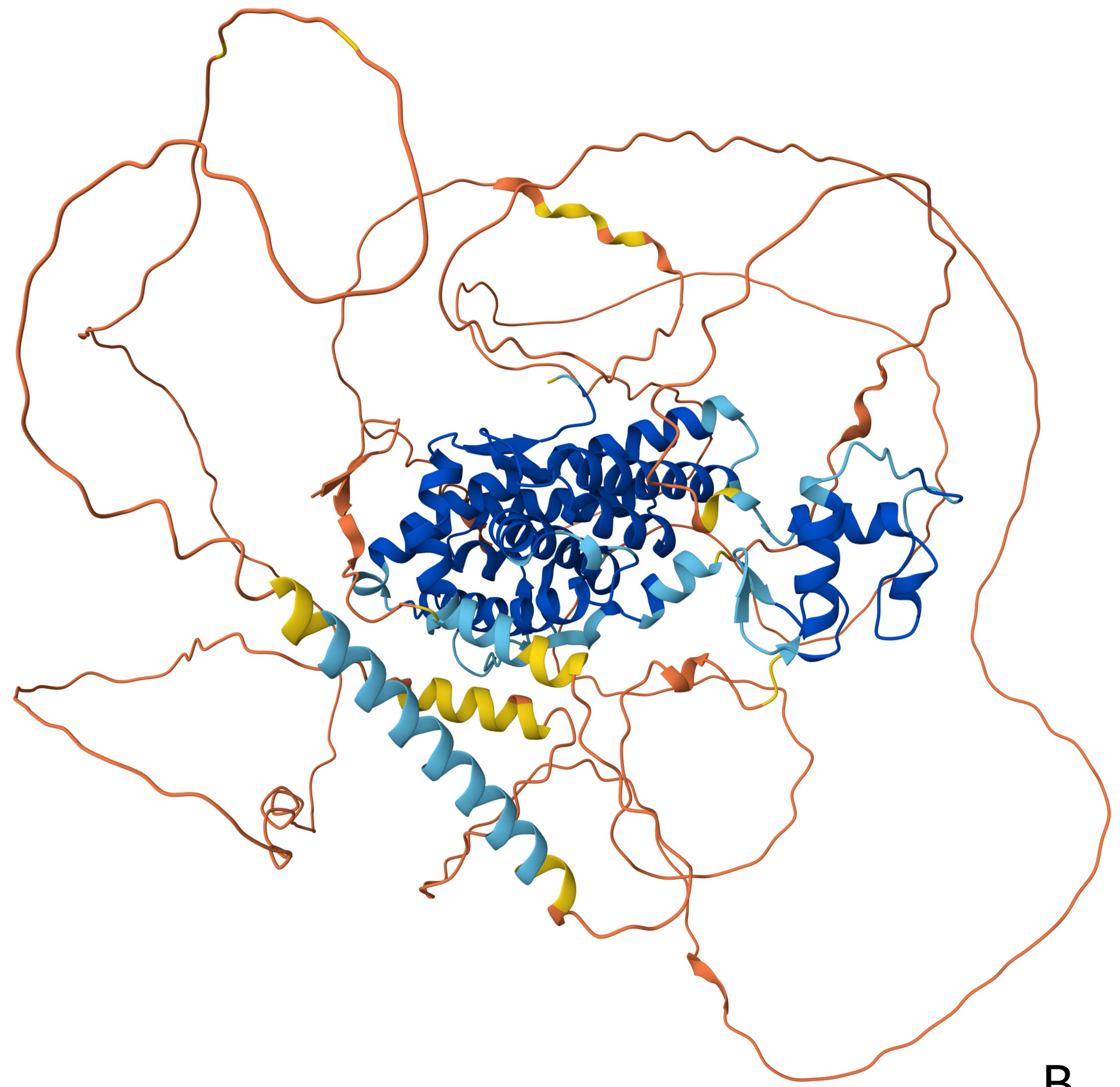
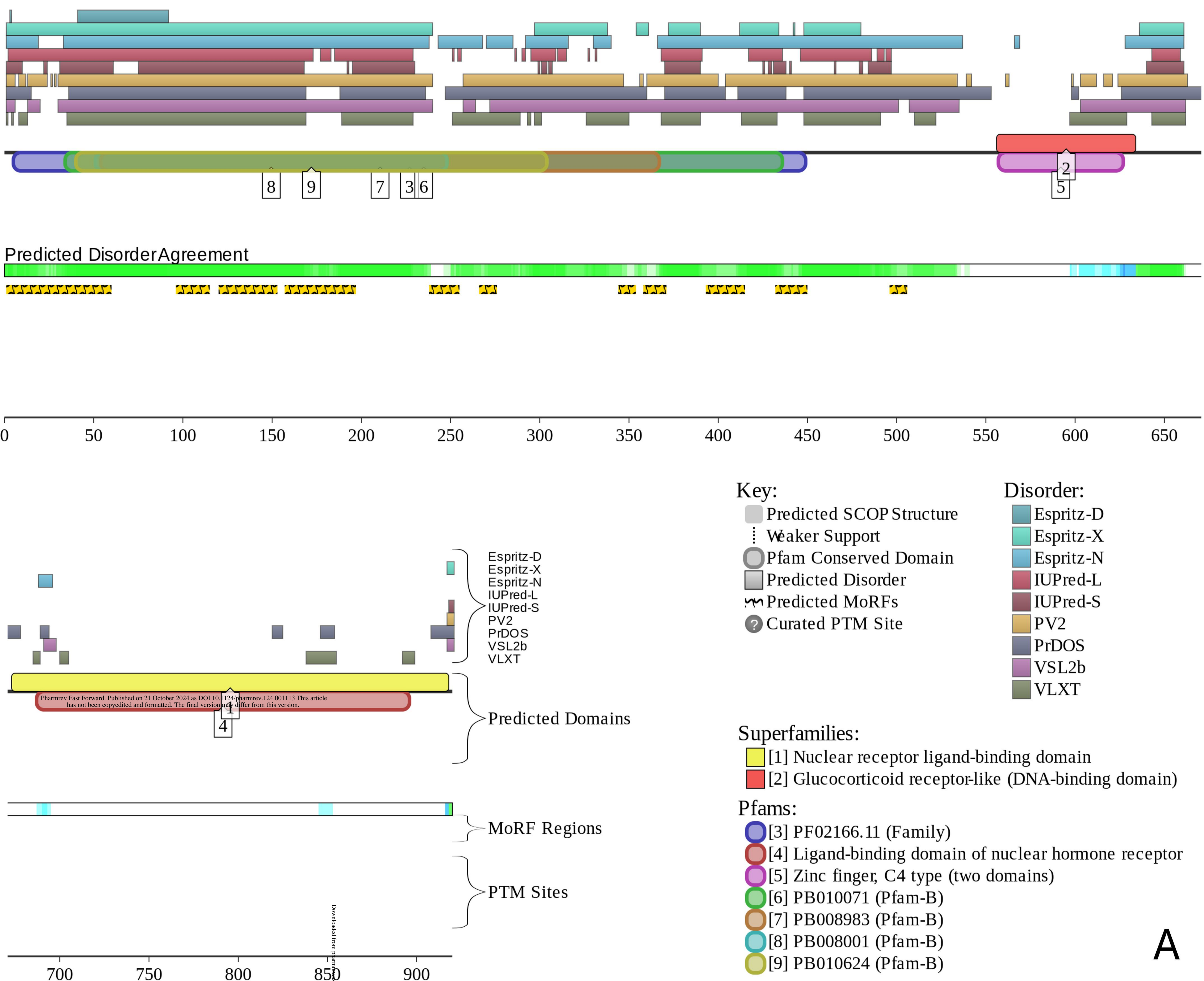


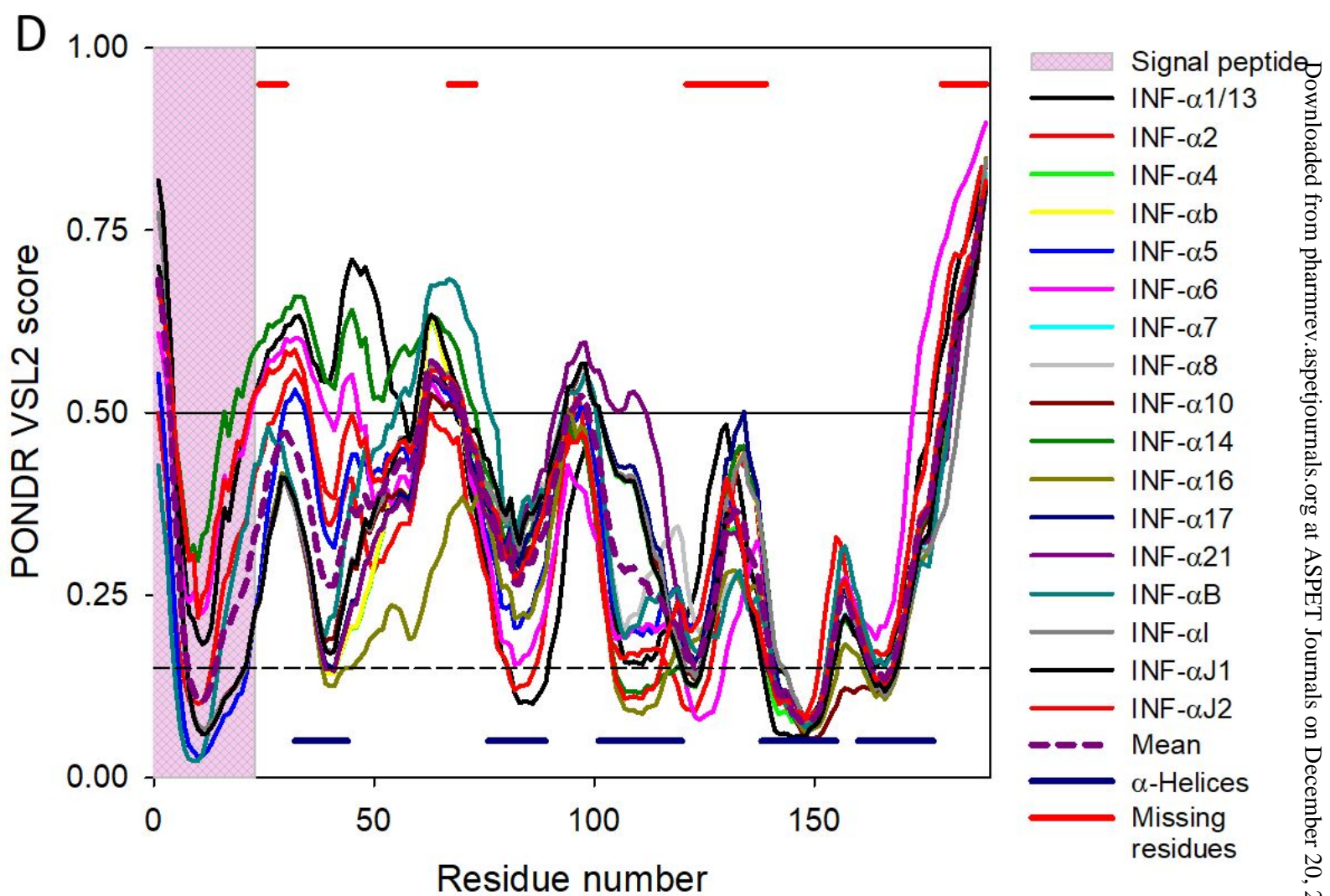
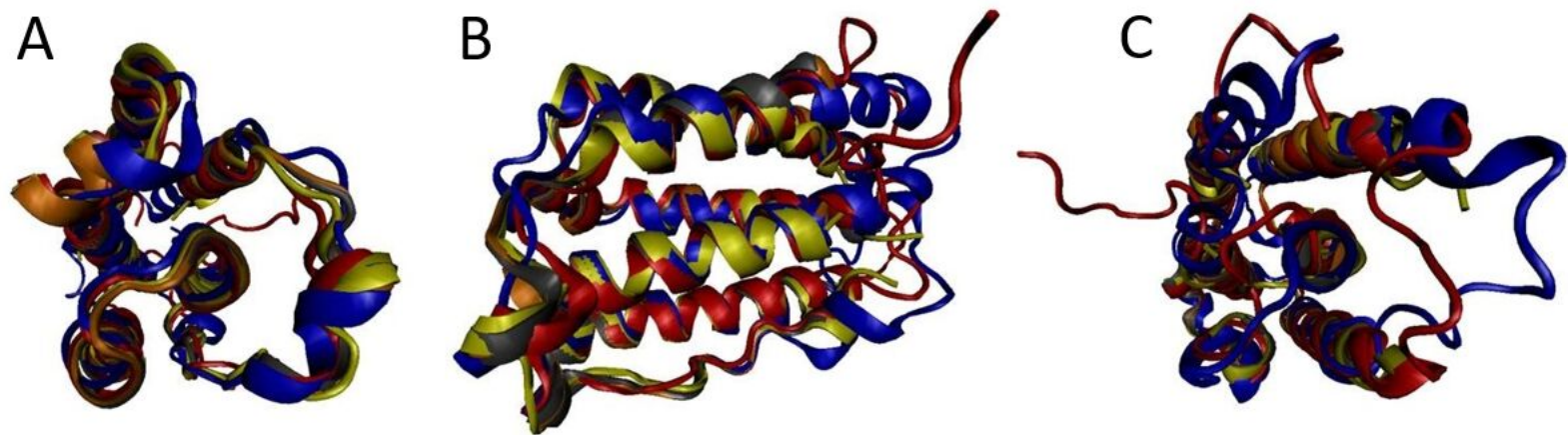
Figure 8



A

B

Figure 9



**Figure 10**

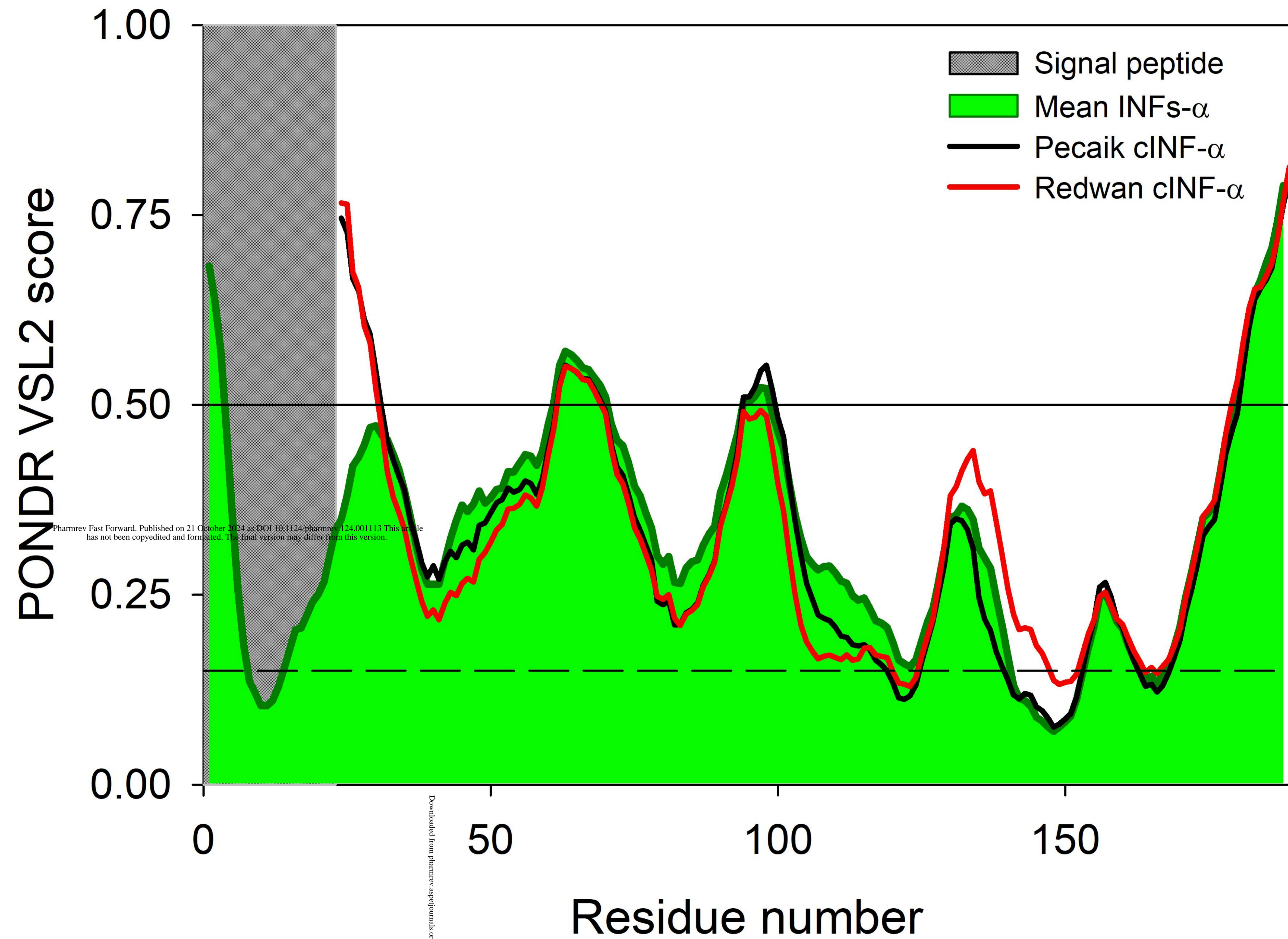


Figure 11

P03377|33-516  
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1 VWKDADTTLFCASDAKAHETEVHNWVWATHACVPTD PNPQEIHLNVTENFNMWKNNMVEQMVEDVISLWDQSLQPCVKLT-----  
\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\*

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132 FNI STSIRGKVQKEYAFFYKLDI IPIDNDTTSYTLTSCNTSVITQACPVSFEPIPIHYCAPAGFAILKCNKTFNGTGPC TNVSTVQC THGIRPVVSTQ LLLNGSLAE EEEVIR SANFT  
81 -----GGSVIKQACPKISFDPIPIHYCTPAGYVILKCNKDFNGTGPCKNVSSVQC THGIKPVVSTQ LLLNGSLAE EEEI IIRSENLT  
\*\*\* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\*

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252 DNAKTII VQLNQSVEINCTRPNNNTRKSI RIQRGPGRAFVTIGKIGNMRQAHCNISRAKWNATLKQIASKLREQFGNNKTIIFKQSSGGDPEIVTHSFNCGGEFFYCNSTQLFNSTWENS  
163 NNAKTII VHLNKSVEINCTRPSNGGS-----GSGGDIRKAYCEINGTKWNKVLKQVTEKLKEHF-NNKTIIFQPPSGGDLEITMHHFNCRGEFFYCNSTQLFNNTCIGN  
\*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\*

P03377|33-516  
A0A0M3KKW8

372 TWSTEGSNNTEGSDTITLPCR I KQFINMWQEVGKAMYAPPISGQIRCSSNITGLLLTRDGGNNNNGSEIFRPGGGMRDNWRSELYKYKVVKIE  
266 E-TMKGCNGT-----ITLPCKIKQIINMWQGTGQAMYAPPIDGKINCVSNITGILLTRDGGANNTSNETFRPGGGNIKDNWRSELYKYKVVQIE  
\* \* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\* \* \*\*\*\*\*

A

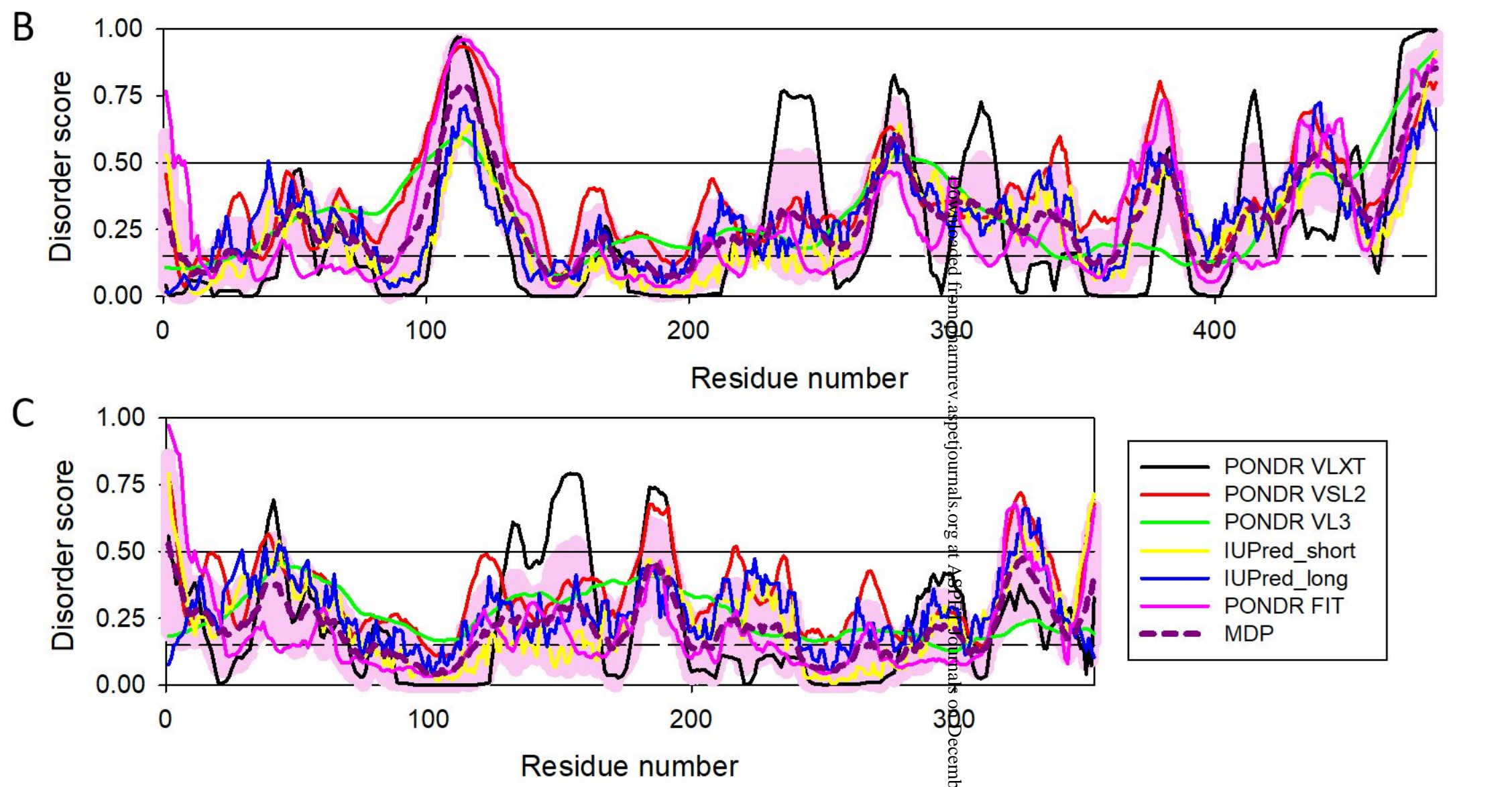
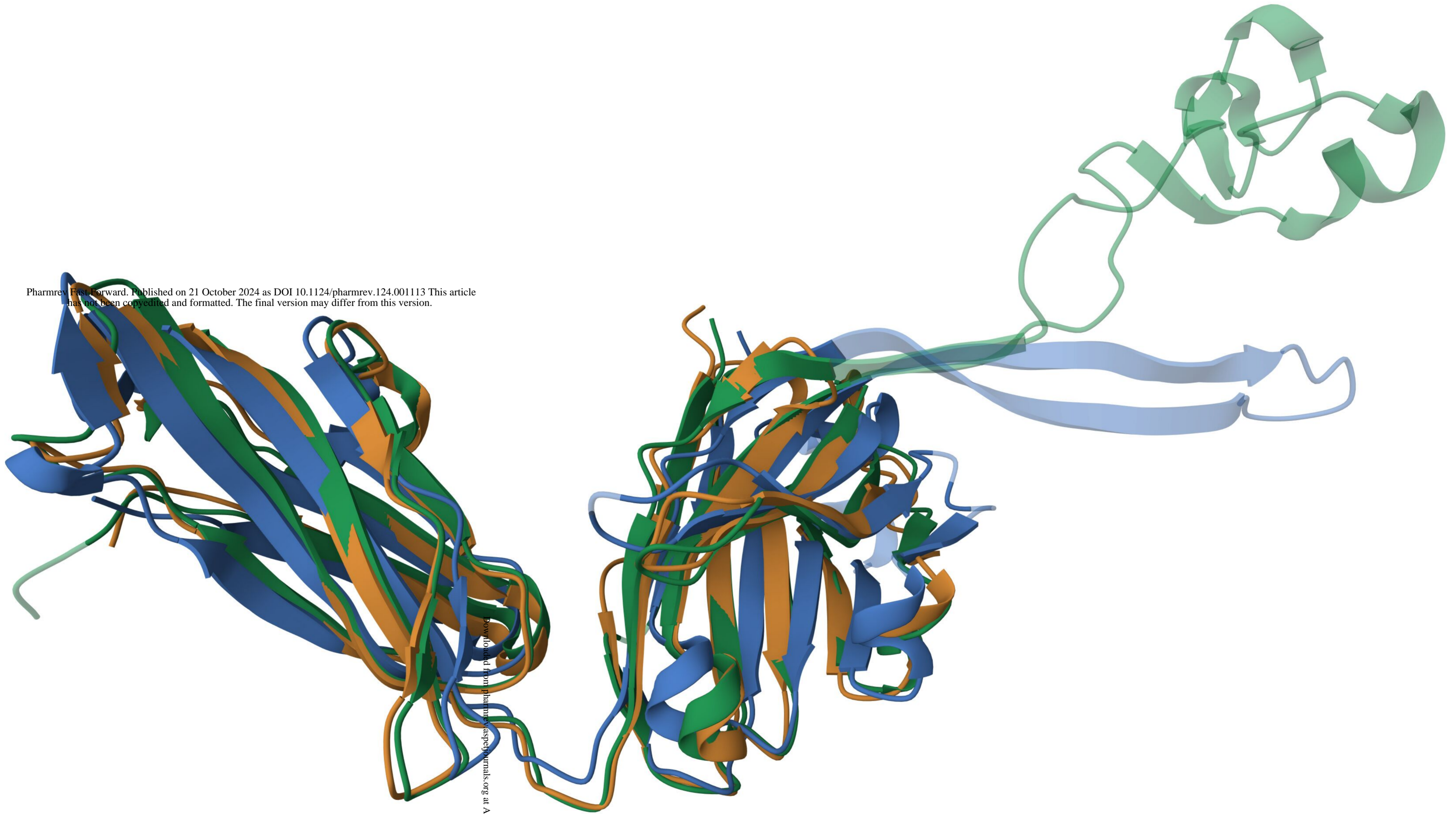


Figure 12

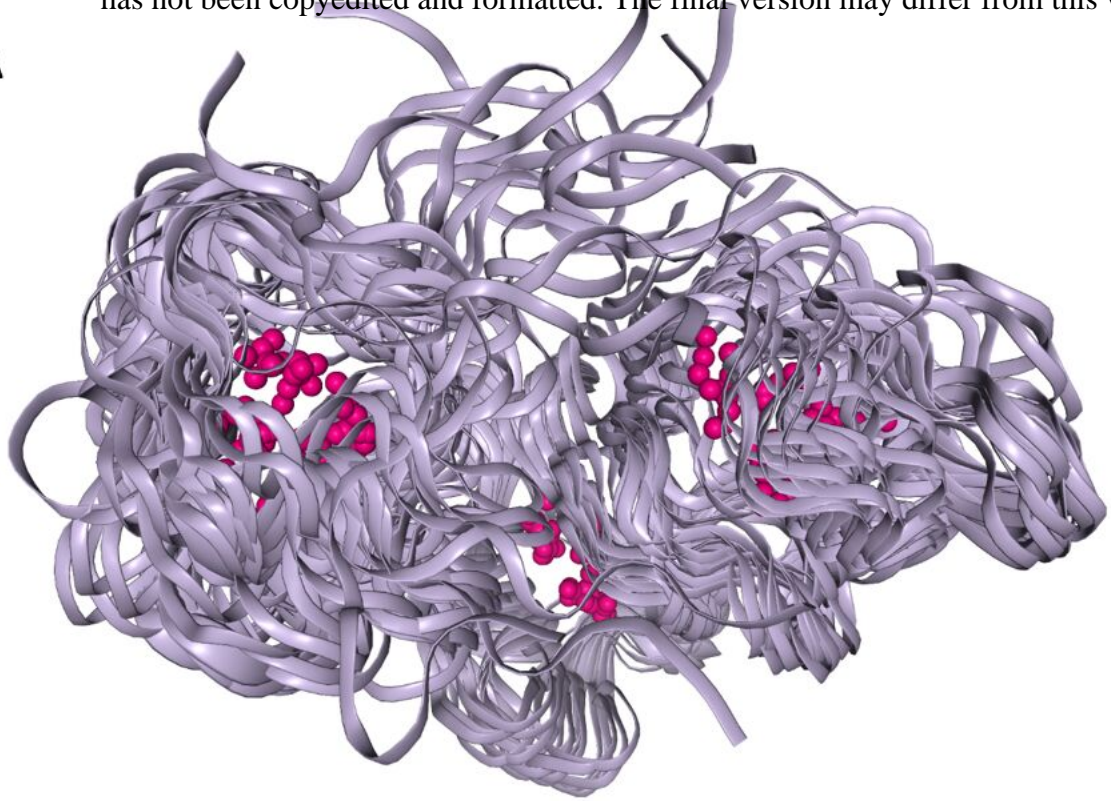
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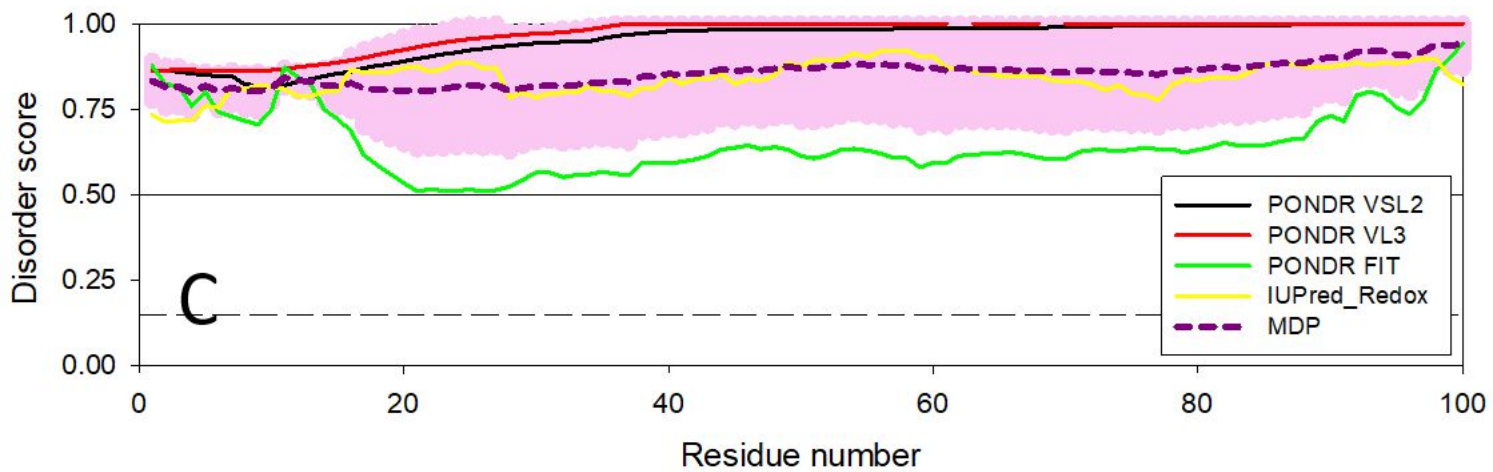
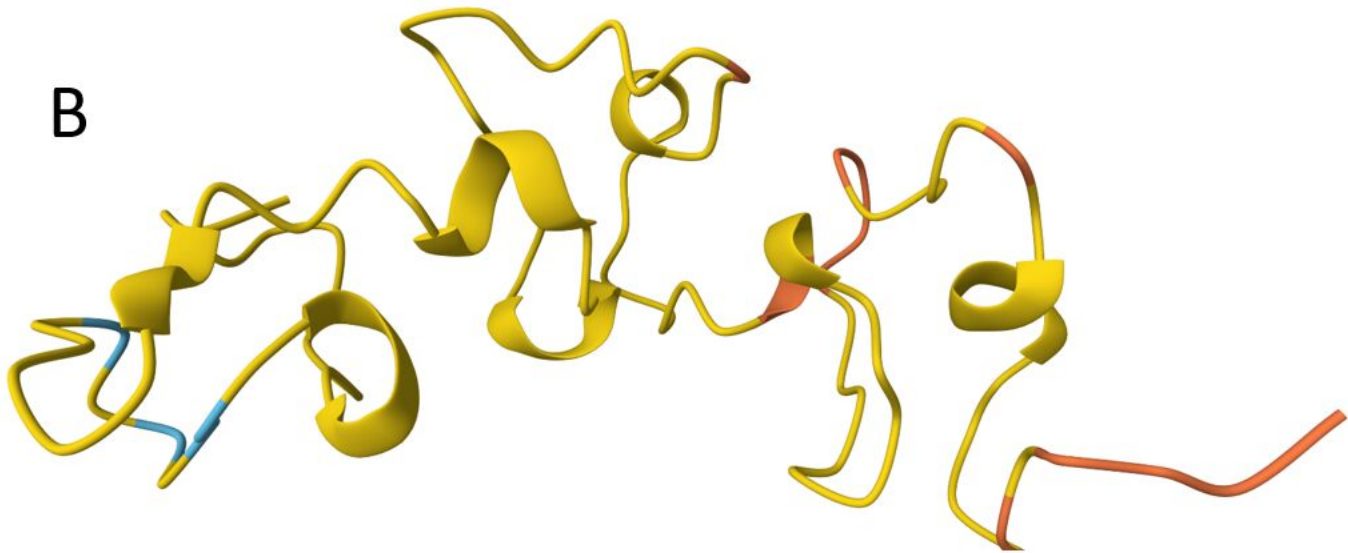
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**Figure 13**

**A**



**B**



**C**

**Figure 14**

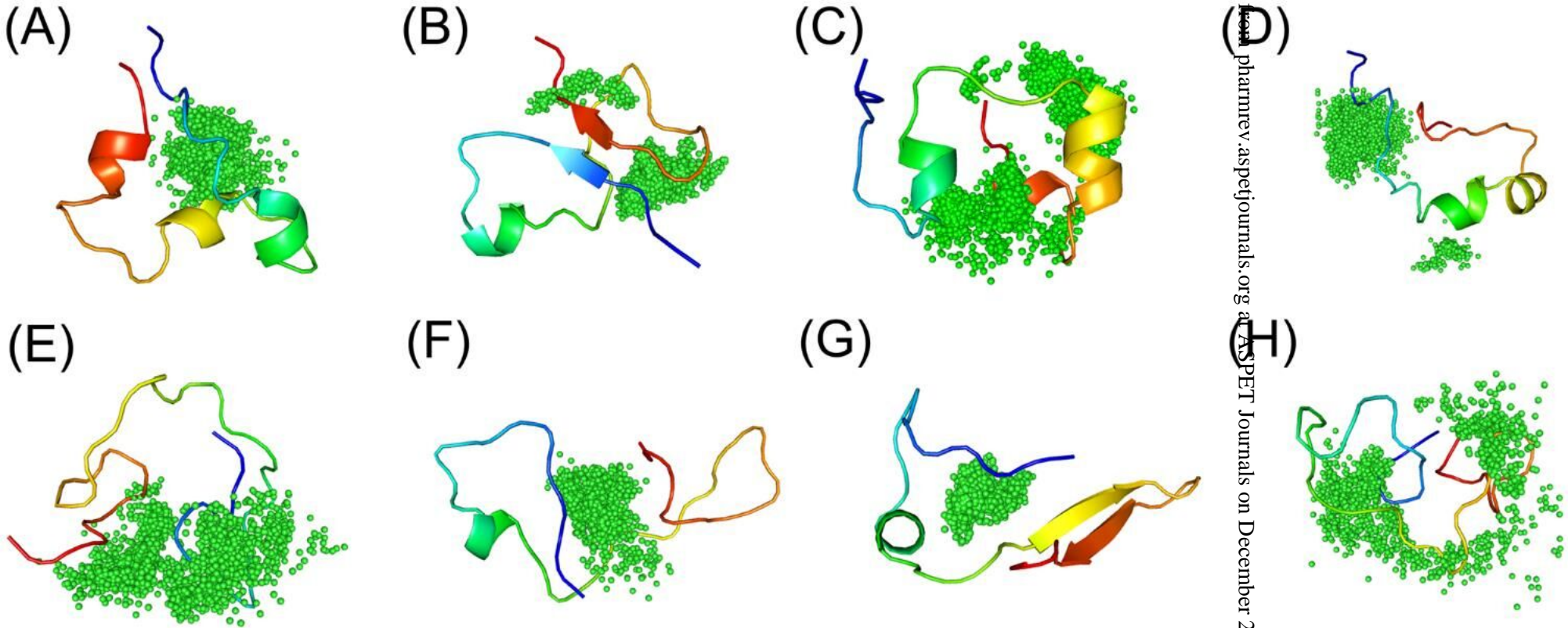


Figure 15