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Note

Letter to the Editor: No folding upon binding of intrinsically disordered proteins: Still interesting but not unique and novel. A commentary on "A novel mode of interaction between intrinsically disordered proteins. by Hibino, E. and Hoshino, M., Biophysics and Physicobiology 17, 86–93 (2020). DOI: 10.2142/biophysico.BSJ-2020012"

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The emergence of intrinsically disordered proteins (IDPs) and intrinsically disordered regions (IDRs) has introduced a new paradigm of coupled binding and folding that suggests that IDPs and IDRs fold upon binding to their partners [1]. However, in 2004, a novel and previously unrecognized no folding upon binding mechanism has been first reported for several IDPs—cytoplasmic domains of signaling subunits from different cell receptors: ζ_{cyt}, CD3δ_{cyt}, CD3ε_{cyt}, CD3γ_{cyt}, FcRγ_{cyt}, Igα_{cyt} and Igβ_{cyt} [2]. The IDPs studied in this work were all found to form specific homodimers under physiological conditions without folding upon dimerization [2]. This unusual phenomenon is distinct from non-specific aggregation behavior seen in many systems (e.g., elastin [3]). Later, in 2007, the no folding upon binding mechanism has been demonstrated for the heterodimeric complex of the well-folded simian immunodeficiency virus (SIV) Nef protein and intrinsically disordered ζ_{cyt} where ζ_{cyt} remains disordered upon binding to Nef [4]. Graphically, these two groundbreaking findings are illustrated in Figure 1A. Since then, other examples of homo-and heterodimeric complexes of IDPs/IDRs with their disordered or well-folded protein partners where IDP/IDR remain largely disordered upon binding have been reported and reviewed in detail elsewhere [5–10]. Table 1 summarizes some of the advancements in this field, focusing on IDP/IDP (IDR/IDR) homo- and heterodimers [2,4,11–18].

In the recently published study by Hibino and Hoshino entitled "A novel mode of interaction between intrinsically disordered proteins" [19] in Biophysics and Physicobiology, the eukaryotic transcription factors Sp1 and TAF4 were reported to have long IDRs. The authors found that "One of the IDRs in Sp1 exhibited homo-oligomer formation. In addition, the same region was used for the interaction with another IDR found in the TAF4 molecule. In both cases, we have not detected any significant conformational change in that region, ..." (Abstract [19]). The authors further concluded that these findings suggest "... a prominent and novel binding mode for IDPs/IDRs, which are not categorized by the well-accepted concept of the coupled folding and binding mechanism" (Abstract [19]). This conclusion has been illustrated by the authors in Graphical Abstract (Fig. 1B) where they compared the

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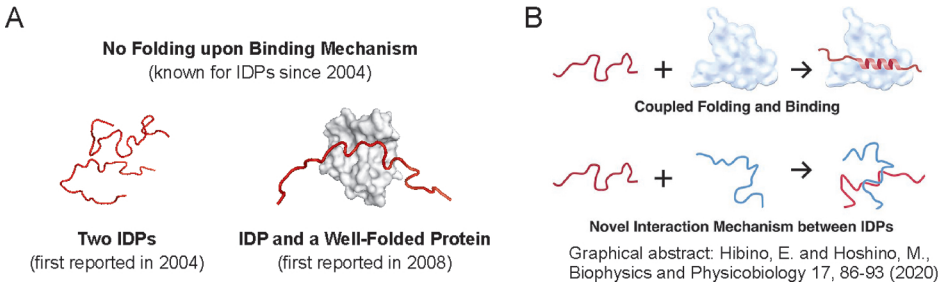

Figure 1 Graphical representation of the "uncoupled binding and folding" and "coupled binding and folding" interactions of IDPs/IDRs. (A) The no folding upon binding mechanism of interactions between two identical IDP/IDR (homodimers) (first reported in 2004 [2]) and between IDP/IDR and a well-folded protein (first reported in 2008 [4]). (B) This figure was taken from the graphical abstract by Hibino, E. and Hoshino, M. (2020) A novel mode of interaction between intrinsically disordered proteins. Biophys. Physicobiol., 17, 86–93, licensed under CC-BY-NC-SA 4.0. Source: https://doi.org/10.2142/biophysico.BSJ-2020012. Top panel. The coupled binding and folding mechanism of interactions between IDP/IDR and a well-folded protein. Bottom panel. The no folding upon binding mechanism of interaction between two different IDPs/IDRs first reported in 2016 [16] and called by the authors [16,19,22] as a "novel interaction mechanism between IDPs".

Table 1 Examples of homo- and heterodimeric complexes where IDP/IDR have been demonstrated to remain largely disordered suggesting the no folding upon binding mechanism*

Homodimer				Homodimer			
IDP/IDR-1	IDP/IDR-2	Year	Ref.	IDP/IDR-1	IDP/IDR-2	Year	Ref.
ζ_{cyt}	ζ_{cyt}	2004	[2]	UmuD2	UmuD2	2007	[11]
$CD3\delta_{cyt}$	$CD3\delta_{cyt}$			C-TRPV1	C-TRPV1	2012	[12]
$CD3\varepsilon_{cyt}$	$CD3\varepsilon_{cyt}$			Sp1	Sp1	2012	[13]
$CD3\gamma_{cyt}$	$CD3\gamma_{cyt}$			aaUsp-NTD	aaUsp-NTD	2014	[14]
$FcR\gamma_{cyt}$	$FcR\gamma_{cyt}$			HMGA2	HMGA2	2015	[15]
$Ig\beta_{cyt}$	$Ig\beta_{cyt}$						
$Ig\alpha_{cyt}$	$Ig\alpha_{cyt}$						
Heterodimer				Heterodimer			
IDP/IDR	Folded protein	Year	Ref.	IDP/IDR-1	IDP/IDR-2	Year	Ref.
ζ_{cyt}	SIV Nef	2008	[4]	Sp1	TAF4	2016	[16]
				4.1G CTD	NuMA	2017	[17]
				H1	ProTα	2018	[18]

* Abbreviations: 4.1G CTD, C-terminal domain of protein 4.1G; NuMA, nuclear mitotic apparatus protein; H1, linker histone H1.0; ProTα, nuclear protein prothymosin-α, HMGA2, high mobility group protein AT-hook 2; recombinant N-terminal domain of the Usp isoform B from *A. aegypti*; C-TRPV1, C-terminal domain of transient receptor potential protein thermal-sensitive non-selective ion channel; UmuD2, product of the umuD gene in *Escherichia coli*; Sp1, eukaryotic transcription factor Sp1, TAF4, eukaryotic transcription factor TAF4.

coupled folding and binding mechanism with the no folding upon binding mechanism, calling the latter as a "novel interaction mechanism between IDPs". The title and abstract of the paper [19] both seriously mislead the Reader, making an impression that the authors are the first who reported this mechanism.

In this regard, we thought it proper to remind the readership of Biophysics and Physicobiology of our pioneering study of 2004 that revealed for the first time the no folding upon binding mechanism of interaction between two IDPs (Fig. 1A, Table 1) [2], which mechanistically is the same to that reported by Hibino and Hoshino (Fig. 1B) [19].

It should be highlighted that despite the growing evidence that IDPs can dimerize but not necessarily fold upon dimerization (Fig. 1A, Table 1), the existence of this unusual propensity of IDPs is still questioned and debated today not only in reviews [10,20] but also in structural studies of IDPs [21]. Thus, another example of IDPs/IDRs capable of remaining largely disordered upon dimerization first reported in 2016 [16] and further detailed by Hibino and Hoshino in 2020 [19] is interesting and important. However, while referring to our previous work [2,4] as "similar examples" [19], the authors do not comparatively analyze their findings in light of these and other studies in the field (Table 1) [19]. Instead, the authors compare their results with the "coupled folding and binding" concept and call the no folding upon binding mechanism known since

2004 (Table 1) as "...a prominent and novel binding mode for IDPs/IDRs" in their studies [16,19,22]. This not only does not correspond to reality, but it also does not properly acknowledge the contribution of other researchers in the field.

In summary, we believe that it is important that since 2004 [2], the no folding upon binding mechanism for IDPs/IDRs attracts more and more attention from the scientific community. However, we also strongly believe that proper citation and discussion of previous work in the field, while avoiding the use of misleading statements and conclusions is critical to provide our further progress in science.

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