Protein Disorder and Multivalency in Regulation of Molecular Machines: Focus on Dynein, DNA Repair, and SARS-CoV2 Nucleocapsid

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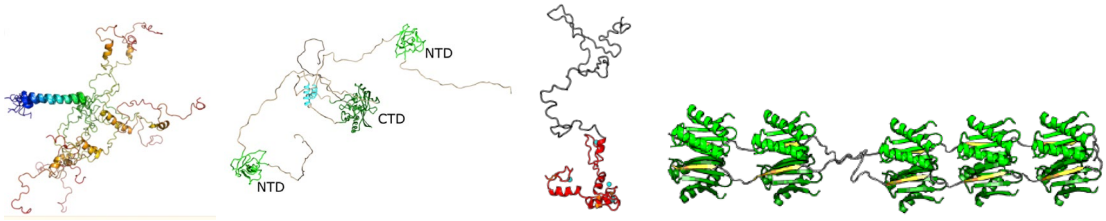
Multiple essential processes, including those associated with signaling, cell division, and cellular differentiation, involve proteins with high prevalence of intrinsically disordered regions (IDRs). It is now well established that IDRs can regulate protein function through various mechanisms including posttranslational modification and intra and intermolecular protein interactions that either promote or inhibit complex assembly. Elucidating the impact of multiple conformational equilibria on specific binding in vitro and functional role in vivo, especially those involving IDRs, however, have been challenging. Here we show the interplay of phosphorylation, linker lengths, and autoinhibition on regulation of protein interactions in dynamic assemblies that require multivalent interactions for their activation.

The cargo attachment complex of dynein has high level of disorder that has hindered cryo-electron microscopy and X-ray crystallography resolution of its structure and interactions. Using proteins from *Chaetomium thermophilum* (CT), we identify long range intramolecular interactions between the N-terminal single α-helix of intermediate chain IC which is the binding region for $p150^{Glued}$ and NudE, and an alpha helix corresponding to LC7 binding site, closer to the C-terminal of the disordered domain, thus causing autoinhibition manifested by reduced binding to non-dynein proteins $p150^{Glued}$ and NudE. We demonstrate that this autoinhibition is relieved by assembly with the light chains at multivalent sites (Jara et al. Elife 2022. 11:e80217)

Other dynamic complex assemblies that require binding to other proteins for their activation include the DNA repair protein 53BP1 that is regulated by its oligomerization domain (OD) and binding to dimeric hub protein LC8. We show that 53BP1 OD is a trimer that forms a heterogeneous mixture of complexes when bound to dimeric LC8 with the largest mass corresponding to a dimer-of-trimer bridged by 3 LC8 dimers. The stability of the bridged complex is tuned by multivalency, the sequence of the second LC8 site, and the length of the linker separating the LC8 binding domain and OD. 53BP1 mutants deficient in forming bridged species fail to impact 53BP1 focus formation in human cell culture studies, suggesting LC8 promotes recruitment of 53BP1 at sites of DNA damage.

Another system is the nucleocapsid protein N from the SARS-CoV2. We demonstrate how the nucleocapsid functions are regulated by specific phosphorylation in the intrinsically disordered SR region that links the RNA binding domain to the dimerization domain (Estelle et al., PNAS Nexus 2023, Stuwe et al. J Biol Chem 2024).



1. Phosphorylation in the Ser/Arg-rich region of the nucleocapsid of SARS-CoV-2 regulates phase separation by inhibiting self-association of a distant helix.
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2. RNA structure and multiple weak interactions balance the interplay between RNA binding and phase separation of SARS-CoV-2 nucleocapsid.
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3. Quantifying cooperative multisite binding in the hub protein LC8 through Bayesian inference.
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4. Linker Length Drives Heterogeneity of Multivalent Complexes of Hub Protein LC8 and Transcription Factor ASCIZ.
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5 NMR Analysis of the Interactions and Conformational Plasticity of Dynein Intermediate Chain.
   Jara KA, **Barbar EJ.** Methods Mol Biol. 2023;2623:241-256. doi: 10.1007/978-1-0716-2958-1_15.PMID: 36602690

6. Multivalency, autoinhibition, and protein disorder in the regulation of interactions of dynein intermediate chain with dynactin and the nuclear distribution protein.
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7. Multivalent binding of the hub protein LC8 at a newly discovered site in 53BP1.
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