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Review

Polybivalency and disordered proteins in ordering macromolecular assemblies

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ABSTRACT

Intrinsically disordered proteins (IDPs) are prevalent in macromolecular assemblies and are thought to mediate protein recognition in complex regulatory processes and signaling pathways. The formation of a polybivalent scaffold is a key process by which IDPs drive early steps in macromolecular assemblies. Three intrinsically disordered proteins, IC, Swallow and Nup159, are core components, respectively, of cytoplasmic dynein, bicoid mRNA localization apparatus, and nuclear pore complexes. In all three systems, the hub protein LC8 recognizes on the IDP, short linear motifs that are fully disordered in the apo form, but adopt a β-strand when bound to LC8. The IDP/LC8 complex forms a bivalent scaffold primed to bind additional bivalent ligands. Scaffold formation also promotes self-association and/or higher order organization of the IDP components at a site distant from LC8 binding. Rigorous thermodynamic analyses imply that association of additional bivalent ligands is driven by entropic effects where the first binding event is weak but subsequent binding of additional ligands occurs with higher affinity. Here, we review specific examples of macromolecular assemblies in which polybivalency of aligned IDP duplexes not only enhances binding affinity and results in formation of a stable complex but also compensates unfavorable steric and enthalpic interactions. We propose that polybivalent scaffold assembly involving IDPs and LC8-like proteins is a general process in the cell biology of a class of multi-protein structures that are stable yet fine-tuned for diverse cellular requirements.

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1. Introduction

Multivalent interactions involving the linked associations of ligands binding to multiple sites on a receptor confer increased affinity compared to binding to monovalent receptors. Multivalent interactions function in diverse biological systems, including antibody antigen associations, interactions of multisubunit toxins such as cholera toxin and botulinum neurotoxin with their target cells, lectin–carbohydrate and protein–glycan interactions. Bivalent interactions are a subset of multivalent interactions involving associations of bivalent ligands with a receptor having two binding sites for that ligand.

While multivalent interactions are common and well described for ordered receptors and small ligands [1–3] their role in intrinsically disordered proteins (IDPs) is only recently appreciated in supramolecular protein assembly [4,5], and in polyphosphorylation [6]. A key feature of bivalent interactions of disordered proteins in macromolecular assembly is that, in the process, two monovalent IDP chains are incorporated into a bivalent macromolecular receptor or scaffold with each chain carrying one binding site. The

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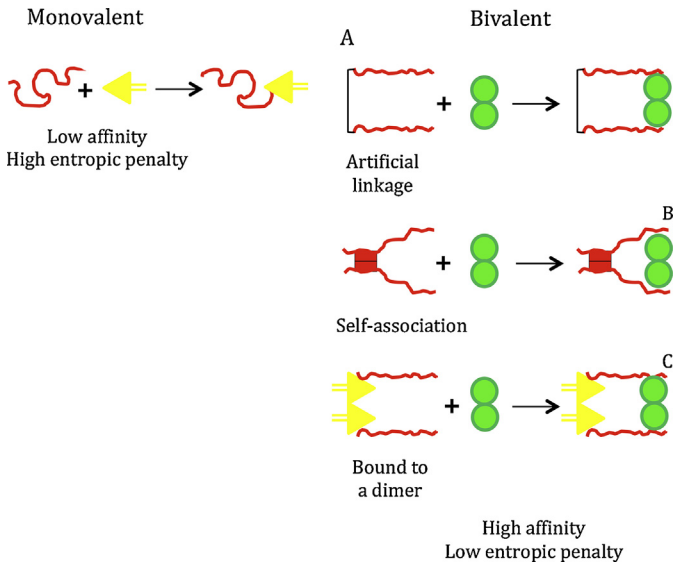

Fig. 1. Scheme illustrating bivalent interactions in disordered proteins. A high entropic penalty is associated with monovalent interactions. Aligning two chains of a disordered protein by an artificial linker, black line (A), through inter-chain (self-association) interactions, red bars (B), or by their interactions with a dimeric protein, yellow triangles (C) reduces the entropic penalty of a second binding event, green spheres.

two IDP chains may be linked by binding a dimeric protein with two symmetrical binding sites, by self-association, or by a synthetic linkage (Fig. 1). For such a disordered protein system with multisite recognition motifs for different bivalent ligands, alignment of two chains, either by binding a bivalent ligand, or by self-association, results in a duplex with multiple additional bivalent sites. We refer to this duplex as a polybivalent scaffold. For all cases (bivalent or polybivalent), the outcome is the same: the first binding event pays the entropic cost so that a subsequent event occurs with lower entropic penalty when compared to a monovalent ligand. In summary, bivalent events organize two disordered chains into a bivalent, partially disordered system that has higher binding affinity for other protein(s) at other recognition motif(s). The partial disorder resides in the incorporated IDP chains, which retain disordered regions.

Binding enhancement arising from bivalency/polybivalency has been demonstrated in assembly of Swallow/LC8 complex [7,8], the nuclear pore protein Nup159/LC8 complex [9] and the dynein intermediate chain/light chains complex [4,5,10,11]. A common feature among the three systems is that a segment of their disordered regions has one or more recognition motifs for the hub protein LC8 [12]. On Swallow, one LC8 dimer binds a recognition sequence C-terminal to a weak coiled-coil region and promotes stable coiled-coil self-association. On nucleoporin Nup159, five LC8 dimers bind at positions N-terminal to a predicted coiled-coil and form a rigid beads-on-a-string rod, possibly with a kink. On dynein intermediate chain, IC, binding of one LC8 dimer creates a bivalent IC duplex with enhanced affinity to another LC8-like dimer, Tctex1, and promotes self-association of IC at a distant domain. In these examples, bivalency involves not only interactions between intrinsically disordered chains and bivalent protein dimers but also self-association interactions.

Nup159 and IC are canonical examples of polybivalent scaffolds because Nup159 binds multiple LC8 dimers, and IC binds several bivalent ligands. The multiple bivalent sites provide the potential for mutual enhancement of affinity for an additional ligand(s), as well as for coiled-coil interchain interactions. Initial binding to these multiple bivalent sites is associated with a large entropic penalty. By pre-paying this entropic cost, bivalency either

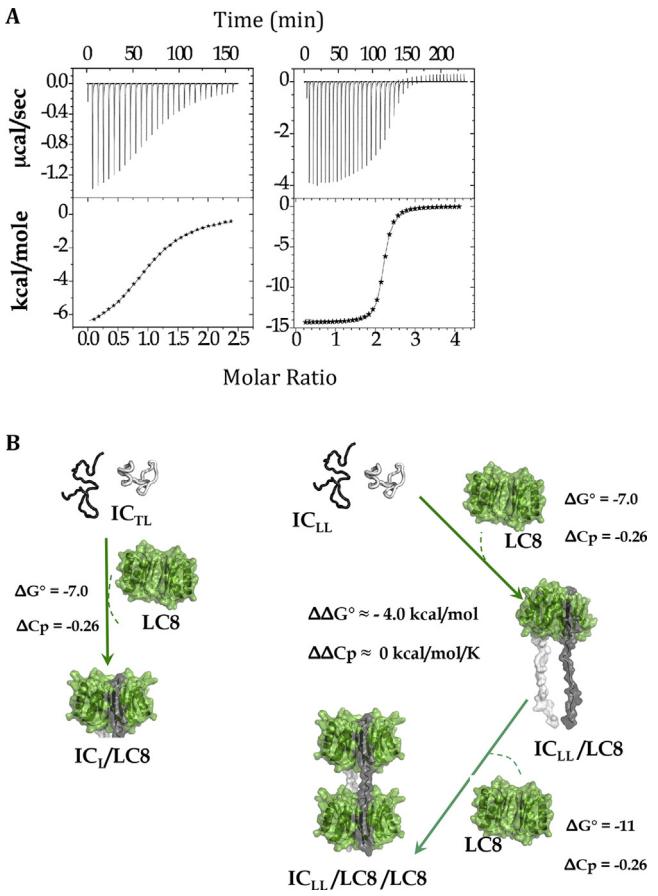

Fig. 2. Thermodynamics of LC8 binding to monovalent and bivalent IC. (A) Representative ITC thermograms (top panels) and binding isotherms (bottom panels) for titration of IC_{TL} (left) and IC_{LL} (right) with LC8. (B) Binding free energies ΔG° (kcal/mol), and ΔCp (kcal/mol/K) are given for each step. The difference in free energy and heat capacity changes between the second and first binding events of LC8 to IC_{LL} (right) are expressed as $\Delta\Delta G^\circ$ and $\Delta\Delta Cp$, respectively, and are shown in the center. Binding parameters for the first IC_{LL}/LC8 binding event are assumed to be similar to those to IC_L (left). Binding parameters for the second IC_{LL}/LC8 binding event are inferred from average values determined from direct measurements. Figures adapted from [5].

significantly enhances binding affinity of subsequent bivalent ligands, or energetically compensates unfavorable interactions associated with complex formation. Each of these scenarios is described below.

2. Bivalency enhances binding affinity and results in formation of a stable complex

To evaluate the thermodynamic parameters of binding enhancement of the IC component of the dynein cargo complex, we used a simplified IC segment, IC_{LL}, that has two recognition motifs for LC8 separated by a 3–5 residues linker. IC_{LL} is a variant of wild type IC with the Tctex1 recognition sequence replaced by the LC8 recognition sequence; the resulting disordered IC_{LL} has two identical LC8 recognition motifs. IC_{LL} is a suitable model because Tctex1 and LC8 are structural homologs with similar tertiary and quaternary structures, and similar binding affinity to IC.

Isothermal titration calorimetry, ITC, does not resolve individual binding events for IC_{LL} binding to two LC8 dimers. Instead, the output is a macroscopic K_d value of 0.5 μM and a stoichiometry of two IC_{LL} chains per two LC8 dimers (Fig. 2) with an average value for ΔG° of −9.0 kcal/mol, and a ΔCp of −0.26 kcal/mol/K. Distinct thermodynamic parameters for each binding are inferred using the