Canadian Association of Physicists
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Contribution ID: **3365** Type: **Oral Competition (Graduate Student) / Compétition orale (Étudiant(e) du 2e ou 3e cycle)**

(G*) Force Without Form: Delineating a Disordered Protein Complex with Single-Molecule Fluorescence Spectroscopy

Tuesday, 7 June 2022 09:00 (15 minutes)

Intrinsically disordered proteins (IDPs) play critical roles in regulatory protein interactions, but detailed structural/dynamics characterization of their ensembles remain challenging, both in isolation and they form dynamic 'fuzzy'complexes. Such is the case for mRNA cap-dependent translation initiation, which is regulated by the interaction of the predominantly folded eukaryotic initiation factor 4E (eIF4E) with the intrinsically disordered eIF4E binding proteins (4E-BPs) in a phosphorylation-dependent manner. Fluorescence spectroscopy provides crucial insights into the dimensions and dynamics of IDPs which inform the molecular basis of their function. Single-molecule Förster resonance energy transfer showed that the conformational changes of 4E-BP2 induced by binding to eIF4E are non-uniform along the sequence; while a central region containing both motifs that bind to eIF4E expands and becomes stiffer, the C-terminal region is less affected. Fluorescence anisotropy decay revealed a nonuniform segmental flexibility at different sites along the chain. Dynamic quenching of these fluorescent probes by intrinsic aromatic residues measured via fluorescence correlation spectroscopy report on transient intra- and inter-molecular contacts on ns-μs timescales. The chain rigidity around sites in the C-terminal region far away from the two binding motifs significantly increased upon binding to eIF4E, suggesting that this region is also involved in the highly dynamic 4E-BP2:eIF4E complex. Our time-resolved fluorescence data paint a sequence-level rigidity map of three states of 4E-BP2 differing in phosphorylation or binding status and distinguish regions that form contacts with eIF4E. We are now conducting single-molecule experiments aimed at resolving site-specific interactions and kinetics of the eIF4E:4E-BP2 complex. Our results constitute an important step towards a mechanistic understanding of the biological function of IDPs via integrative modelling.

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