Decoding Molecular Plasticity in the Dark Proteome

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The mechanisms by which intrinsically disordered proteins (IDPs) engage in rapid and highly selective binding is a subject of considerable interest and represents a central paradigm to nuclear pore complex (NPC) function. Nuclear transport receptors (NTRs) can move through the central channel of the NPC which is filled with hundreds of phenylalanine-glycine-rich nucleoporins (FG-Nups) reaching millimolar concentrations with elusive conformational plasticity. Since site-specific labeling of proteins with small but highly photostable fluorescent dyes inside cells remains the major bottleneck for directly studying protein dynamics in the cellular interior, we have now developed a semi-synthetic strategy based on novel artificial amino acids that are easily and site-specifically introduced into any protein by the natural machinery of the living cell via a newly developed thin-film synthetic organelle that equips the living cell with up to three genetic codes. This allowed us to develop an experimental approach combining site-specific fluorescent labeling of IDPs in non-fixed cells with fluorescent lifetime imaging microscopy (FLIM) to directly decipher the plasticity of FG-Nups via FRET. Our study enabled a conformational look on the condensated IDPs in the sub-resolution (roughly $(50\ nm)^3$ small cavity) cavity of the NPC. By measuring the end-to-end distances of different segments of the labeled FG-Nups using time resolved scanning FRET and anisotropy spectroscopy, we can extract the scaling exponent and dynamics, which directly describes the conformations of FG-Nups at their functional status as well as the solvent quality in the cellular and even inner NPC environment.

Yu M, Heidari M, Mikhaleva S, Tan PS, Mingu S, Ruan H, Reinkermeier CD, Obarska-Kosinska A, Siggel M, Beck M, Hummer G, Lemke EA. Deciphering the conformations and dynamics of FG-nucleoporins in situ. Nature. 2023 May;617(7959):162-169