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Small Molecules, Big Impact: Quantifying the Influence of Molecular Influencers on Protein Interactions

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Motivation

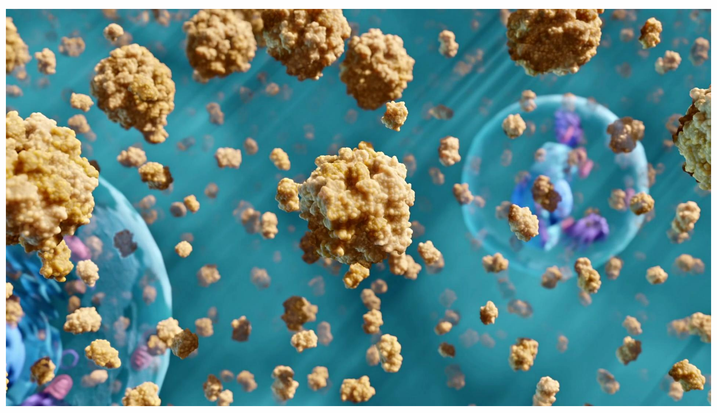
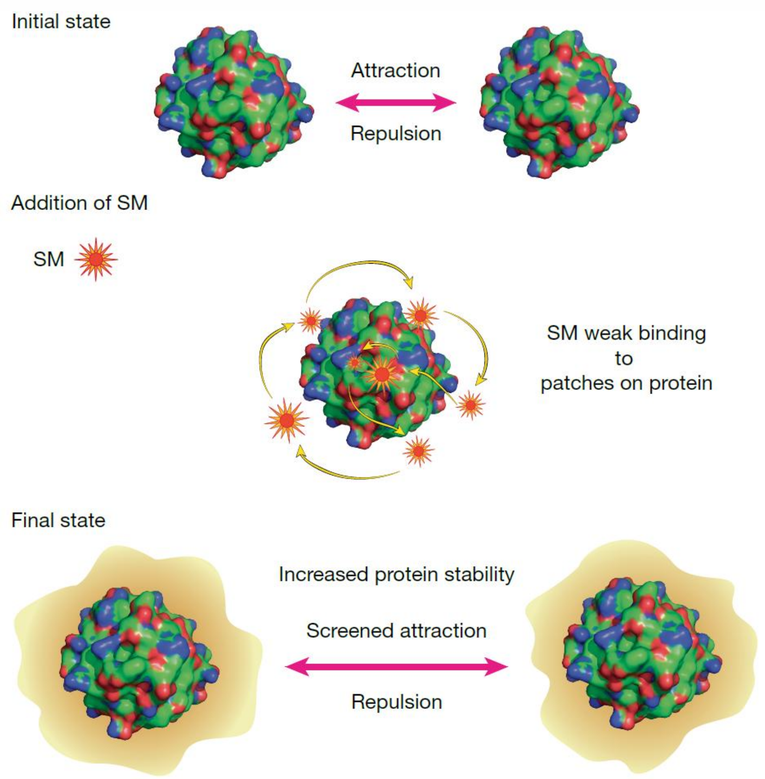


Illustration of protein interactions and aggregation (beige) in solution against the backdrop of living cells.

Protein dynamics determine biological function and understanding how protein interactions are influenced by other proteins and small molecules, such as amino acids, is key to connecting dynamics with function. There is growing evidence that the dynamics of proteins are a function of the cellular environment.

Amino acids stabilize protein dispersions.

To precisely measure the stability in solution and the interactions of proteins, we measured the second osmotic virial coefficient (B_{22}) and the binding affinity (K_D), both proxies for interaction strength by applying self-interaction chromatography and surface plasmon resonance studies. We have developed a theory that explains all the experimental observations and agrees quantitatively with the measurements we performed. It postulates that the **effect is due to weak amino acid-protein screening interactions that in turn modulate the protein self- and cross-interactions** (*1,2*).



Schematic of the proposed theoretical framework for colloidal stability caused by small molecules *(1)*.

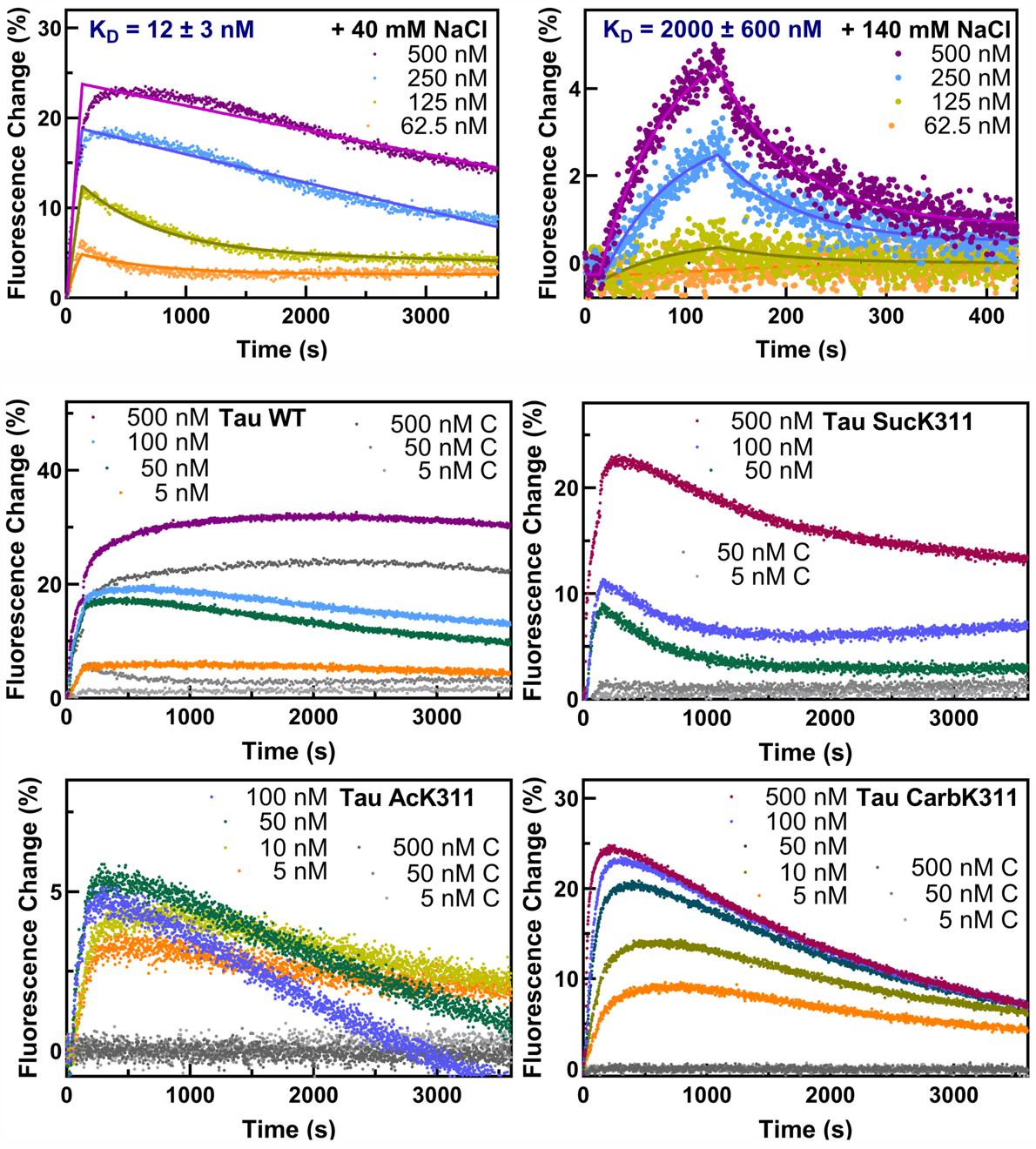
How do molecular influencers shape protein interaction dynamics?

For protein cross-interactions between lysozyme and whey protein isolates, we showed that millimolar concentrations of glutamine, proline, and arginine can stabilize interactions and shift binding affinity by orders of magnitude *(2)*. This has physiological relevance, as intracellular free amino acid concentrations range from a few to ~100 mM *(3)*. We propose that amino acids represent one manifestation of a broader principle: **the molecular environment regulates protein interaction dynamics.** This local cellular environment is **defined by ionic composition, small molecules, post-translational modifications (PTMs), molecular crowding, and condensates**—collectively termed **molecular influencers**.

We recently found that **changing NaCl concentration from 40 to 140 mM** in phosphate buffer shifts the K_D of tau by two orders of magnitude. These preliminary results suggest that **salt ions can substantially alter the molecular environment of intrinsically disordered proteins** such as tau, markedly more strongly than in proteins with defined structures.

PTMs alter protein charge distribution. Preliminary results with **tau variants carrying a single PTM** at K311—succinylation (SucK311), acetylation (AcK311), or carbamylation (CarbK311)—**show distinct binding kinetics** with wildtype (WT) tau and **altered interactions with polyanions e.g. DNA** (grey curves).

We hypothesize that **neighboring amino acid residues in tau compete for interaction partners**—including salt ions, polyanions, metabolites, and other tau monomers—as a function of their concentration and protein charge distribution modulated by PTMs. **Understanding these dynamics requires deciphering how molecular "influencers" act across spatial and temporal scales in model and native living systems**. The currently existing large gap between *in vitro* and *in vivo* results reflects differences in molecular conditions, forces, and drivers, preventing quantitative and predictive models of protein interactions in cells. **Correlative multidisciplinary approaches are key to advance on bridging this gap**.



(1) T. Mao, X. Xu, P.M. Winkler *et al.*, *Nature*. (2025). (2) P. M. Winkler *et al.*, *J. Phys. Chem. B*. (2024).
(3) R. Rollin, J.-F. Joanny, P. Sens, *eLife*. (2023).