Modulating Protein-Protein Interactions Using Osmolytes: Implications for Protein Stability and Aggregation

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Organisms such as *Escherichia coli* initially respond to osmotic stress by taking up inorganic ions, which helps partially restore cytoplasmic water concentration and growth rates. However, long-term survival requires a more effective strategy for re-establishing homeostasis. Nature offers a simple solution: the accumulation of small organic molecules known as osmolytes. Classical models often assume osmolytes are preferentially excluded from protein surfaces, overlooking their interactions with surface charges and their influence on protein-protein interactions (PPIs).
We used a two-dimensional microfluidic assay and vapor pressure osmometry to investigate this question. Specifically, we attached a negatively charged protein to two-dimensionally fluid phospholipid membranes mainly made of phosphatidylcholine lipids. Protein and lipid molecules were labeled with dyes and studied within polydimethylsiloxane (PDMS) microfluidic devices on glass substrates. Our research showed that at least three main factors influence how osmolytes modify PPIs. Although trimethylamine N-oxide (TMAO) is known to counteract urea's denaturing effects, we found that the urea-to-TMAO ratio needed for this effect is not the same in all cases. These results challenge traditional views of osmolyte exclusion and reveal a complex, interaction-based mechanism. This work offers new insights into how excipients regulate PPIs and has direct implications for improving protein formulations, stability in crowded environments, and rational biotherapeutic design.

Keywords:
Protein-Protein Interactions, protein stability, osmolytes, TMAO, urea

References:

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