École polytechnique fédérale de Lausanne

Institute of Materials - Institute of Bioengineering

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Supramolecular NanoMaterials and Interfaces Laboratory

Amino Acids Effect on Protein-Protein Interactions

SPS Annual Meeting 2024

Pamina M. Winkler

September 12th, 2024

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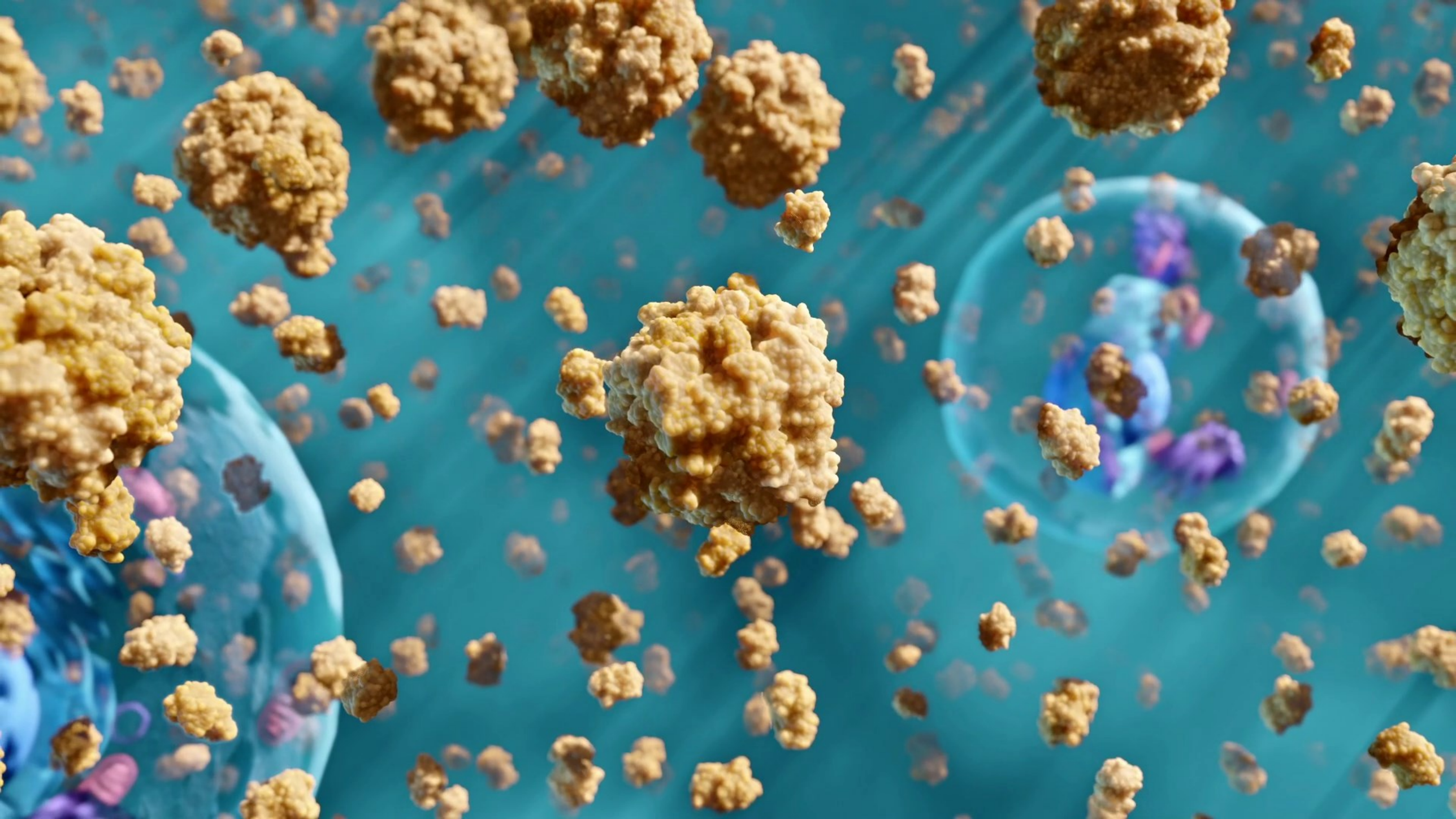
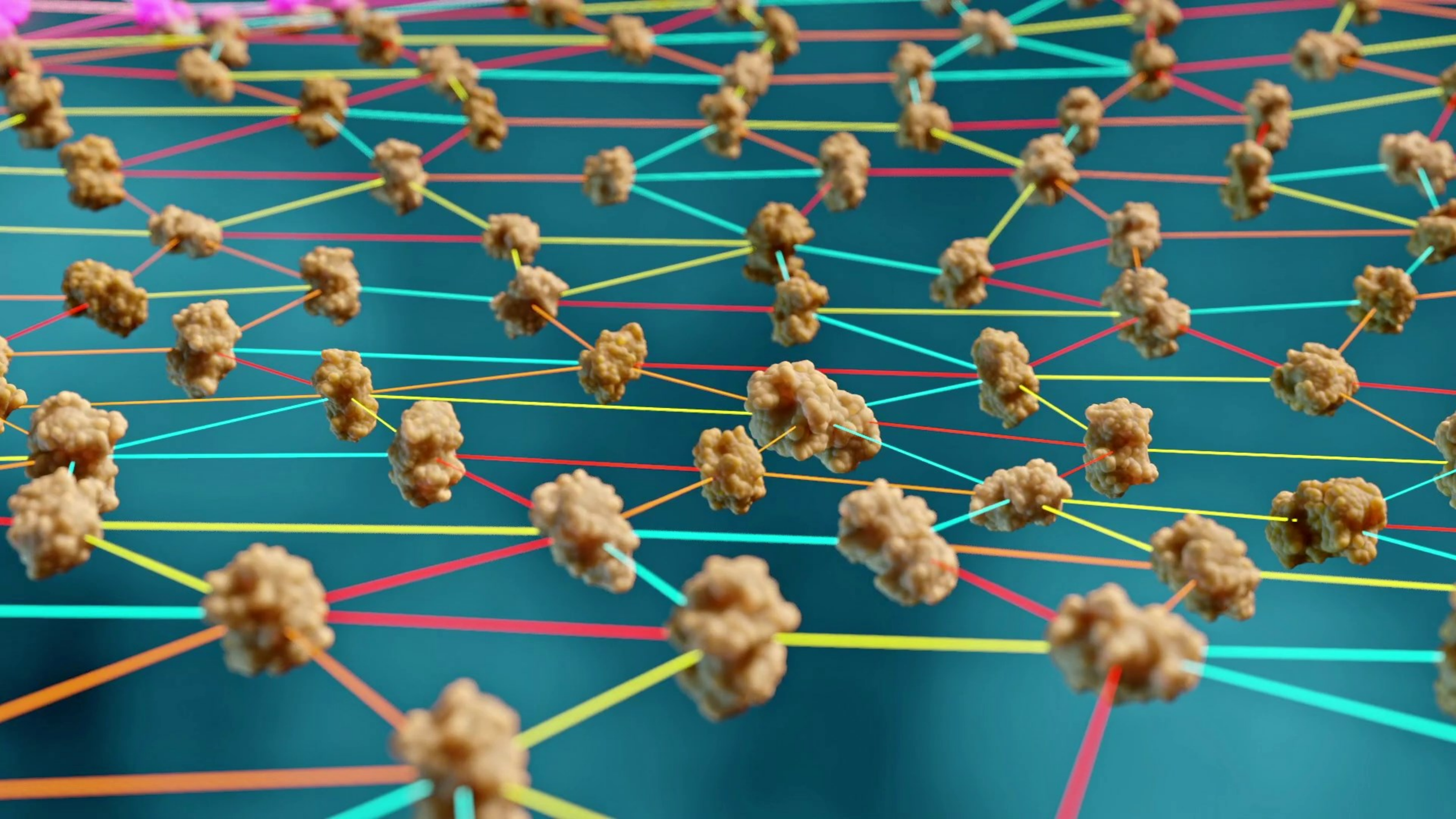


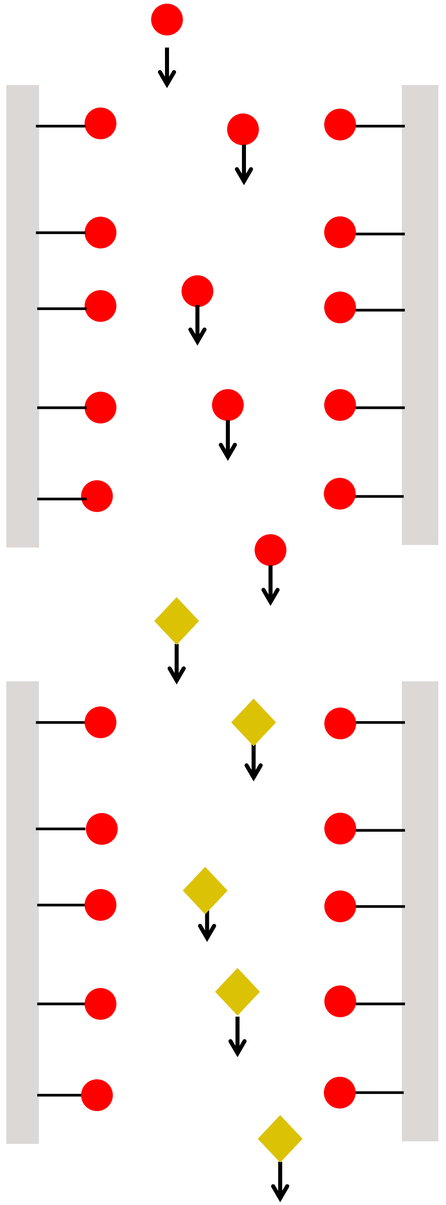


Self- and Cross-Interaction Chromatography (SIC/CIC)

Self-Interaction Chromatography (SIC)
→ Extension Cross-Interaction Chromatography (CIC)

- The protein of interest is covalently immobilized on the resin.
- The resin is packed into a small chromatography column.
- The protein of interest is injected and passed through the column.
- The elution profile is monitored which reflects the interaction of the immobilized protein with the protein free in solution.

- From the elution profile the retention volume is obtained which is a measure of the ensemble average strength of protein–protein interaction energy of the two proteins under the solution conditions used. → B_2



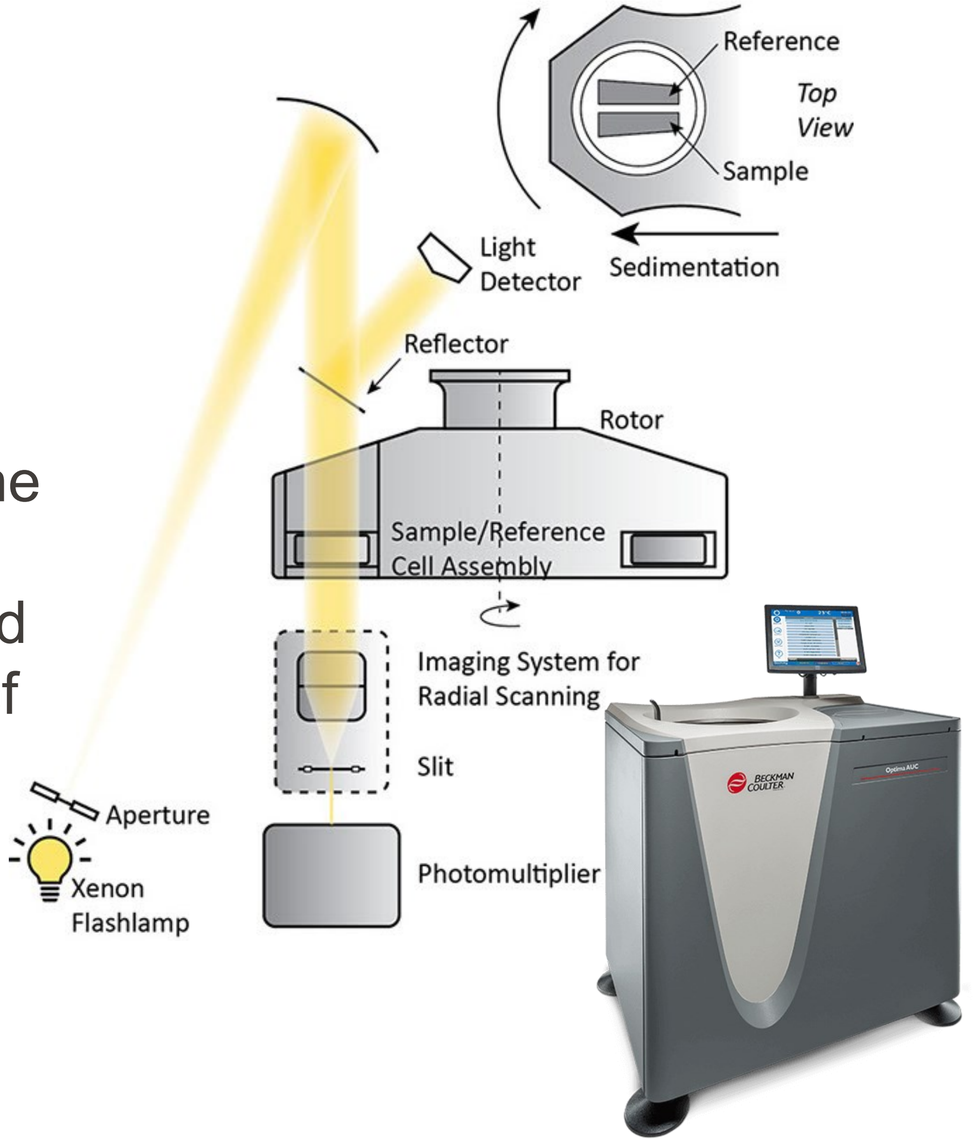
Analytical ultracentrifugation (AUC)

Mass will redistribute in a gravitational field until the **gravitational potential energy exactly balances the chemical potential energy at each radial position**.

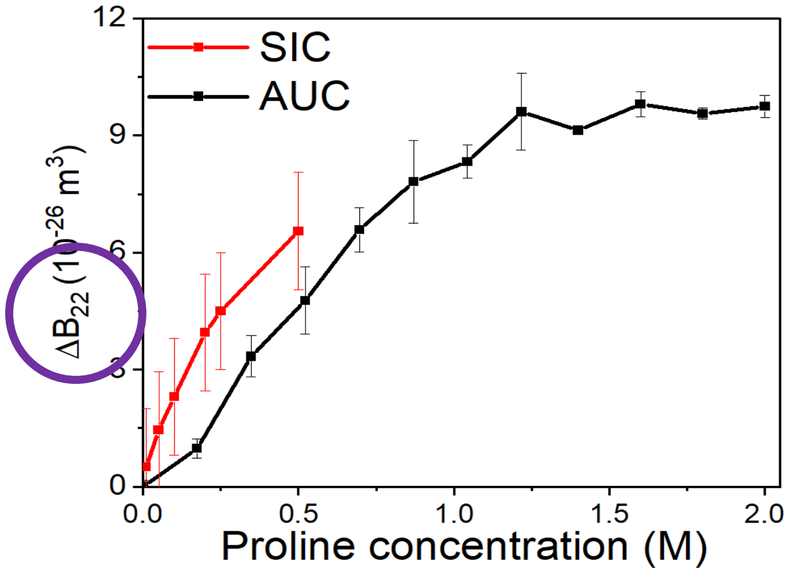
Two experimental modes of AUC:

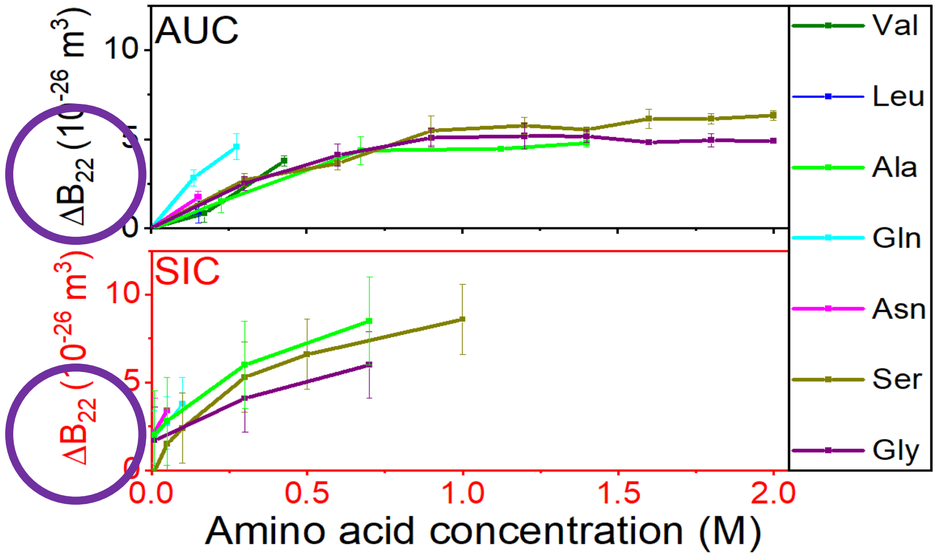
1. **sedimentation velocity (SV) :** allows to determine the presence of possible aggregation.
2. **sedimentation equilibrium (SE):** is currently the gold standard method for determining molecular weights of macromolecules in their native buffer solution.

SE-AUC allows for a direct comparation of the second osmotic coefficient (B_2) with SIC and SAXS.



Amino acids stabilize protein dispersions





free energy/site from a mean field approach

$$\frac{\Delta G}{kT} = \phi ln\,\phi + (1-\phi) ln\,(1-\phi) + \chi\,\phi\,(1-\phi)$$

interaction → incompatibility parameter χ

$$\chi = \frac{z}{kT}\left(\epsilon_{cs} - \frac{\epsilon_{ss} + \epsilon_{cc}}{2}\right)$$

interaction strength → second virial coefficient B_{22}

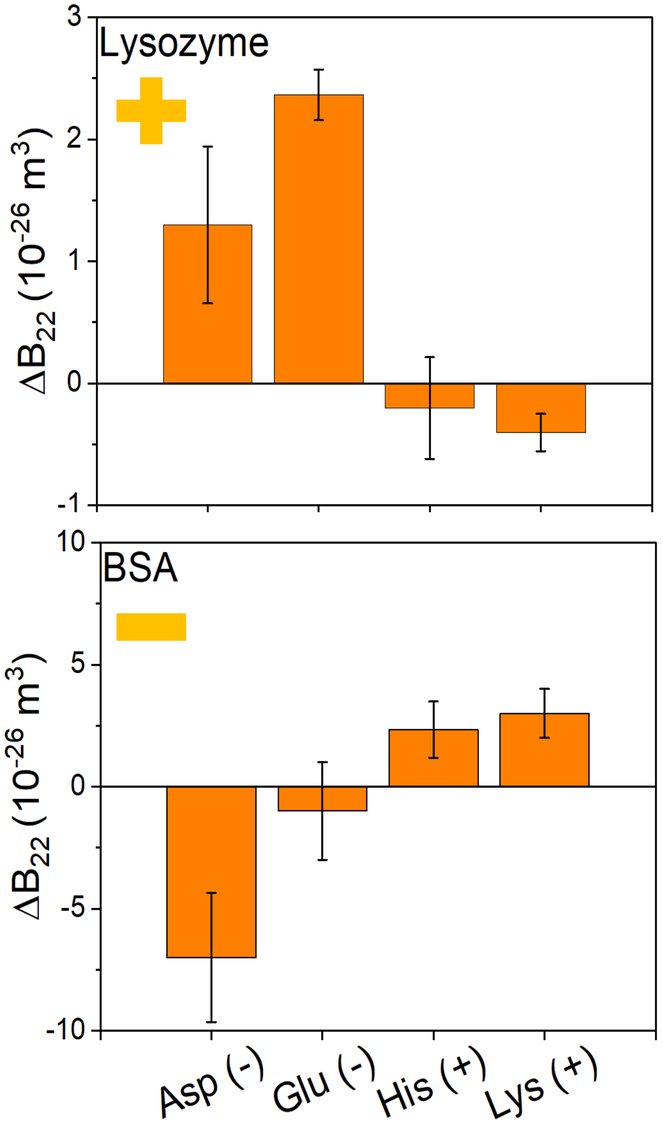
$$B_{22} = a^3(1-2\chi)$$

...

$$\Delta B_{22} = (a^3 - {B_{22}}^o)\frac{Kc}{1+Kc}$$

T. Mao*, X. Xu*, P.M. Winkler*, Cécilia Siri… F. Stellacci, submitted/arXiv:2404.11574 (2024).

Amino acids stabilize protein : theory vs experiment?



Testing consequences of the proposed theory:

1. AAs with charged side chains will have a stabilizing effect only on proteins of the opposite charge.

2. For peptides formed by three or four prolines, ΔB_{22} are basically the same ones as generated by proline itself.

3. The theory does not imply any special property for AAs. It only requires molecules to have weak interactions with the colloids. → 1,1,1-Tris(hydroxymethyl)ethane (TME) that is highly water-soluble but has an exposed methyl group.

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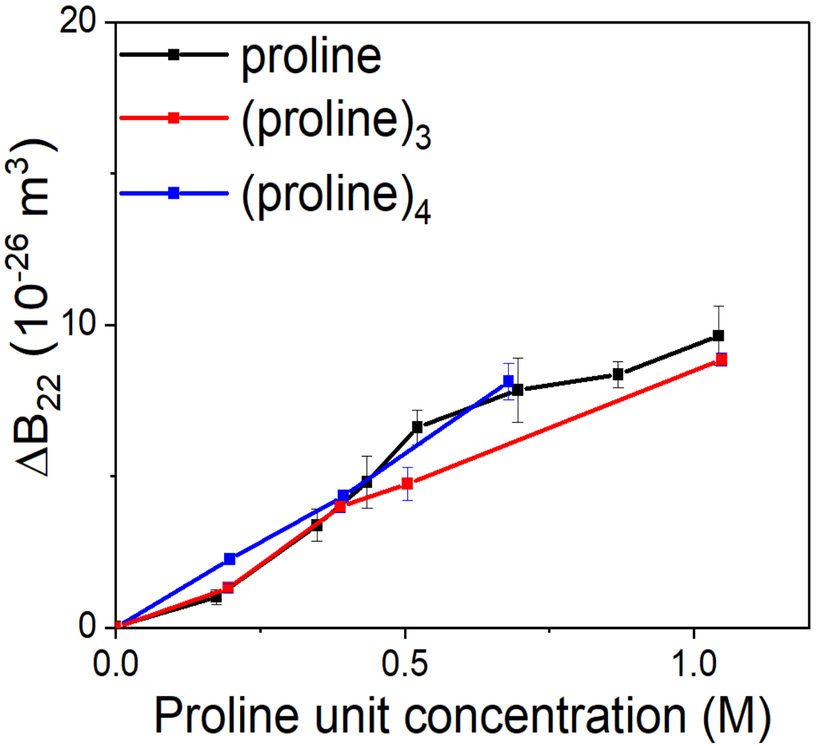

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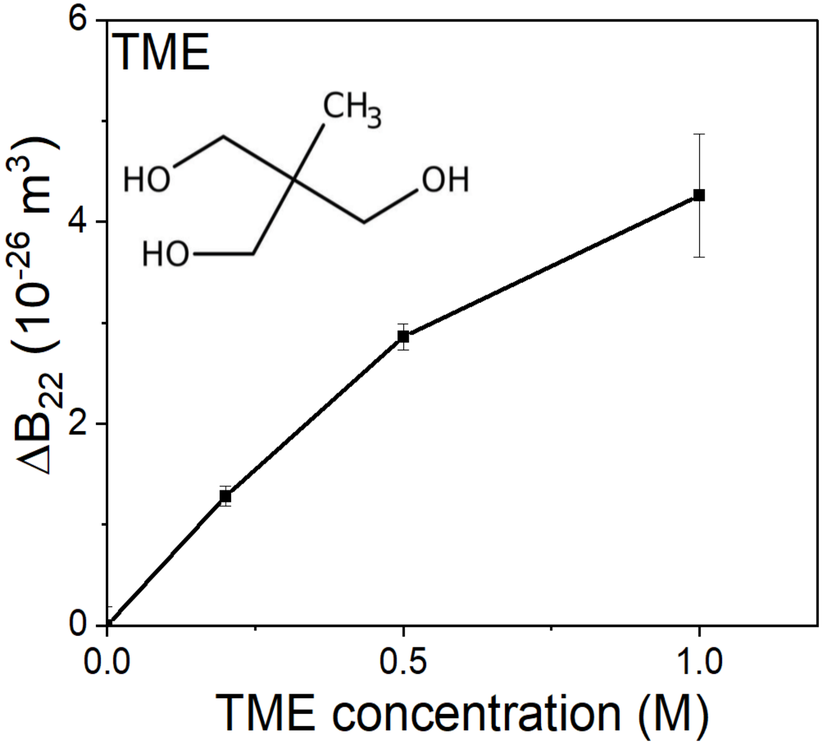

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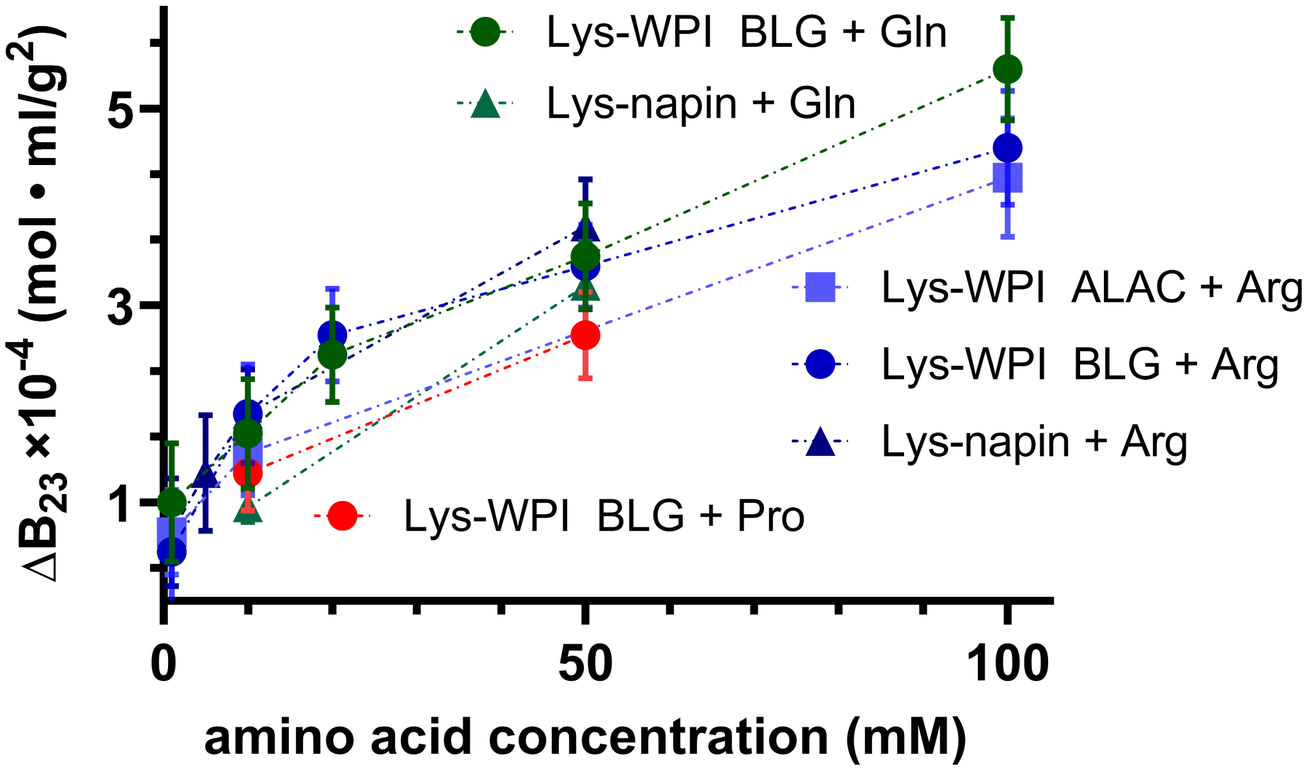
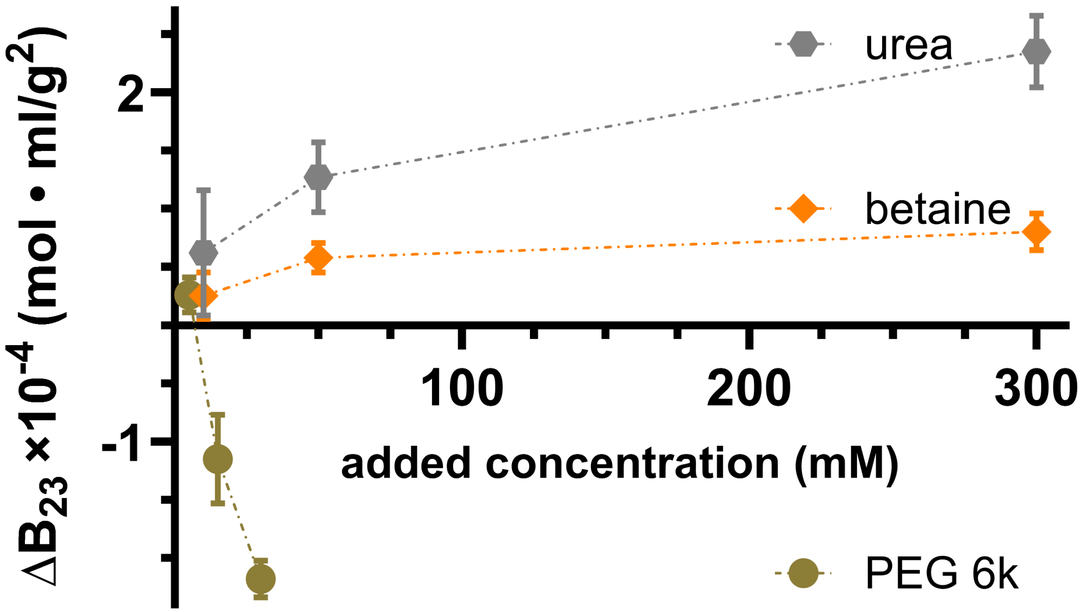
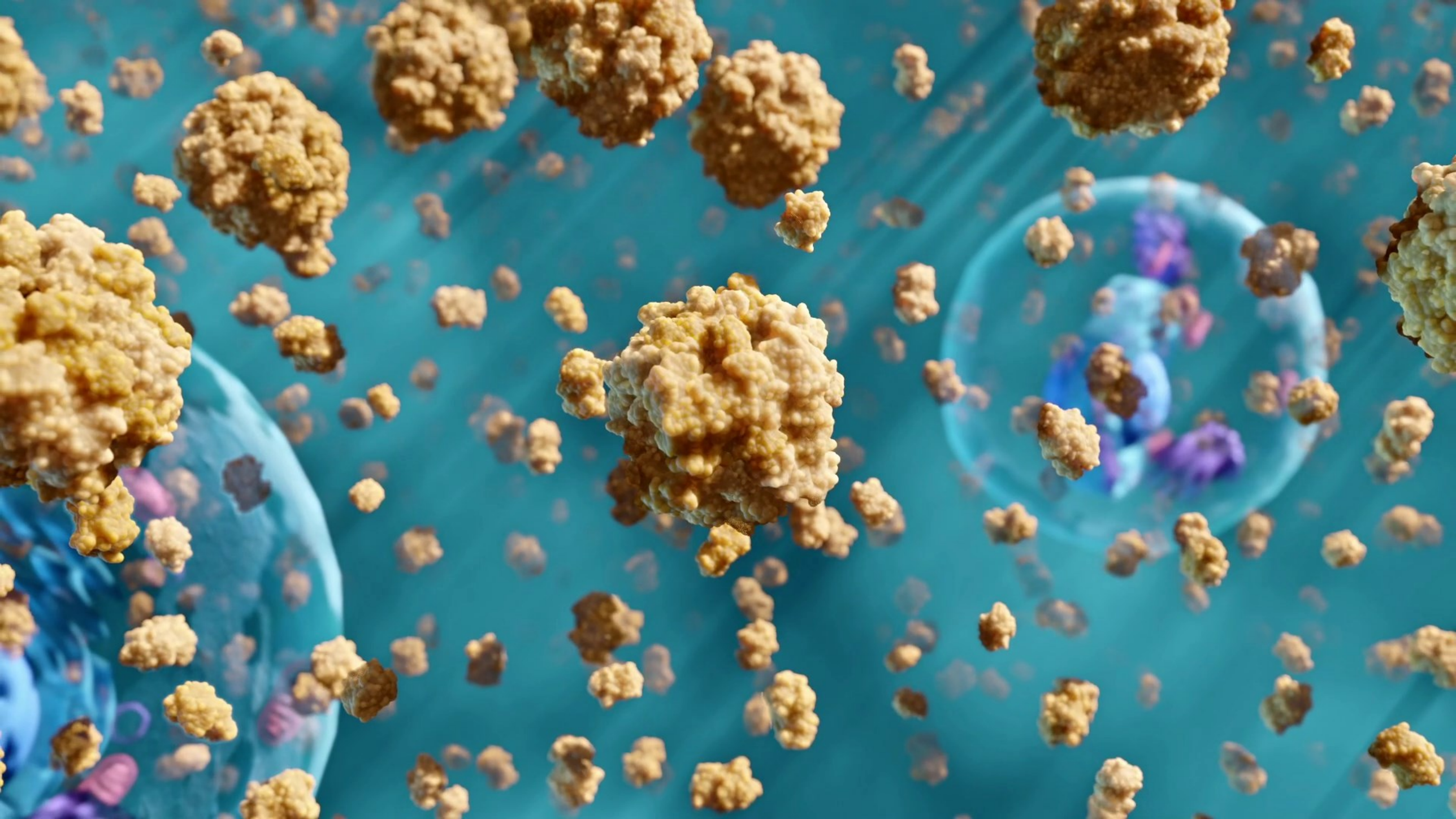
Protein cross-interactions in presence of small molecules





P.M. Winkler, …,F. Stellacci, J. Phys. Chem. B (2024).



Outlook

We have shown that **weak interactions between small molecules and proteins provide sizeable effects on the stability and their interactions.**

Amino acids interacting with proteins **at (milli)molar concentrations** lead to **strong changes in the interaction strength** (second osmotic virial coefficient).

The effects shown exist for amino acids as well as short peptides, both are abundant in cell cytosols.

Cells under osmotic pressure produce more amino acids, but they also produce more peptides and amino acids when they degrade intracellular proteins.

Our data shows that the increase in such intracellular concentration of AAs and peptides need to be considered as an important class of small molecules to influence protein-protein interactions.

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Thank you for your attention!

Special thanks *to Francesco Stellacci, Cécilia Siri and the chairs and organizers.*

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