Contribution ID: **44** Type: **Plakat // Poster**

PEG and Ficoll modulation of NS3/4A protease kinetics and structure

Monday, 8 September 2025 17:00 (2 hours)

The total concentration of macromolecules in biological environments ranges from approximately 80 g/L in blood plasma to 300 g/L in bacterial cytoplasm. This crowding reduces available volume and introduces non-specific interactions that can alter solute properties such as diffusion, protein folding, and substrate binding. Despite these effects, most in vitro studies of therapeutic targets are performed under dilute conditions that do not reflect the intracellular environment.
We investigated how crowding affects the activity and structure of the hepatitis C virus NS3/4A protease, a key enzyme in viral replication. To mimic intracellular conditions, we used synthetic crowding agents: linear polyethylene glycol (PEG 2,000 and 4,000) and branched polysaccharide Ficoll 70. Proteolytic activity was determined at 50-200 g/L crowder concentrations using a fluorogenic peptide substrate. NS3/4A structural changes were monitored via Trp-induced intrinsic fluorescence.
PEG reduced NS3/4A activity in a concentration- and size-dependent manner, with PEG 2,000 showing a stronger inhibitory effect than PEG 4,000. At 100 g/L, PEG 2,000 impaired substrate binding, while PEG 4,000 improved it, but both reduced turnover. At 200 g/L, catalytic efficiency decreased by ~30% for both PEGs. In contrast, Ficoll enhanced NS3/4A activity despite impaired substrate binding at 100 and 200 g/L. Turnover increased with concentration, and at 200 g/L, catalytic efficiency nearly doubled.
Both PEG and Ficoll promoted NS3/4A folding, but to different extents. PEG induced folding into a more compact structure with Trp residues buried in the protein hydrophobic core, with PEG 2,000 showing a stronger effect than PEG 4,000. Such folding may restrict enzyme flexibility, thus reducing activity. Ficoll 70 induced only partial folding with moderate reduction in Trp-solvent exposure. This conformation may favor activity relative to the unordered structure in dilute buffer.
Overall, we demonstrated that crowding modulates NS3/4A activity and structure in a crowder-specific manner. Folding changes partly explain activity shifts, but additional effects such as altered diffusion and substrate interactions also contribute. Therefore, incorporating crowding agents into in vitro studies can increase their physiological relevance, supporting the investigation of therapeutic targets and drug development.

Acknowledgements:
We acknowledge support from the National Science Centre, Poland (2022/47/O/NZ1/01933).

References:

1. Minton, A.P. *Biopolymers* **20**, 2093-2120 (1981).
2. Taliani, M. et al. *Anal Biochem* **240**, 60-67 (1996).
3. Popielec, A. et al. *Biochimie* **176**, 169-180 (2020).
4. Ostrowska, N. et al. *PLoS Comput Biol* **19**, e1011054 (2023).

Primary authors: Ms LOBKA, Małgorzata (University of Warsaw, Centre of New Technologies, S. Banacha 2c, 02-097, Warsaw, Poland); Prof. TRYLSKA, Joanna (University of Warsaw, Centre of New Technologies, S. Banacha 2c, 02-097, Warsaw, Poland)

Presenter: Ms LOBKA, Małgorzata (University of Warsaw, Centre of New Technologies, S. Banacha 2c, 02-097, Warsaw, Poland)

Session Classification: Sesja plakatowa

Track Classification: Biofizyka // Biophysics