Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Institute for Molecular Physical Science (IMPS)

Einladung zu einem Kolloquium

Datum/Zeit:	**Dienstag, 16.04.2024, 16.45 Uhr**
Referent:	**Prof. Enrica Bordignon** Université de Genève, Genève Switzerland
Titel:	*Using EPR in an integrative approach to unveil properties of macromolecular complexes and molecular aggregates*
Ort:	**HCI G7**

Electron Paramagnetic Resonance (EPR) is used in conjunction with site-directed spin labeling methods for the analysis of structure and dynamics of proteins in vitro and in cellular milieu. Here we explore advantages and challenges of the method to extract kinetic information on the conformational changes of large macromolecular complexes (Tc toxins) and to monitor the rotational dynamics of proteins undergoing liquid-liquid phase separation (γD-crystallin).

Tc toxins are virulence factors of many insects and human pathogenic bacteria. They attach as soluble prepores to receptors on host cells and following acidification in the late endosome, perforate the cell membrane like a syringe to translocate toxic enzymes through their pore-forming channel. In vitro and in the absence of receptors, however, the prepore-to-pore transition is initiated by high pH. Although this complex transformation has been structurally well studied, the functional aspects of this large-scale rearrangement, such as the reaction pathway with possible intermediate states and the resulting temporal evolution, have remained elusive. Here, we used an integrated biophysical approach comprising EPR, single-molecule fluorescence spectroscopy and cryo-EM to monitor the multi state kinetics of the prepore-to-pore transition.[1]

Liquid-liquid phase separation (LLPS) is a commonly observed phenomenon for intrinsically unfolded and globular water-soluble proteins, which is finely tuned by protein concentration, temperature, pressure, crowders and co-solutes. Here, using molecular dynamics simulations[2] and EPR we investigate the change in the rotational diffusion of a globular protein, γD-crystallin, undergoing LLPS in vitro in aqueous solutions containing different co-solutes. The spin-labeled crystallin proteins were used as viscosity nanoprobes, enabling a direct correlation between effects induced by bulk viscosity on isolated proteins and by molecular crowding in the condensed phase. This study further validates the predictive power of MD simulations and highlights the relevance of using a sensitive nanoprobes to extract the viscosity of condensates.

1) P. Njenga Ng'ang'a, J. Folz, S. Kucher, D. Roderer, Y. Xu, O. Sitsel, A. Belyy, D. Prumbaum, R. Kühnemuth, T. E. Assafa, M. Dong, C. A. M. Seidel, E. Bordignon, S. Raunser. Multi-state kinetics of the syringe-like injection mechanism of Tc toxins. BioRxiv, 2024, https://doi.org/10.1101/2024.01.16.575634

2) S. Mukherjee, S. Ramos, S. Pezzotti, A. Kalarikkal, T. Prass, L. Galazzo, D. Gendreizig, N. Barbosa, E. Bordignon, M. Havenith, L.V. Schäfer. Entropy Tug-of-War Determines Solvent Effects in the Liquid-Liquid Phase Separation of a Globular Protein. J. Phys. Chem. Lett., 2024, accepted.

Gäste sind willkommen