Contribution ID: **1588** Type: **Poster**

Structural studies and crystallization of ferritin-based protein complex

Monday 28 October 2024 18:50 (20 minutes)

Ferritin is a protein complex responsible for storing iron in living systems. One of its most remarkable properties is an ability to spontaneously form a spherical hollow protein globule consisting of 24 subunits, this process known as self-assembly [1]. This unique characteristic, combined with ferritin's exceptional stability across a wide range of temperatures and pH levels [2], make it a promising tool in numerous biotechnological applications, including drug delivery, nanotechnology, and vaccine development [3].

Crystallization plays a crucial role in the structural characterization of proteins. Through the formation of ordered structures, the atomic details of proteins can be investigated using X-ray diffraction and other techniques. Understanding the high-resolution structure of ferritin-based complexes can be beneficial for their use in biotechnology, particularly for drug delivery.

In previous work, we developed a model system based on ferritin derived from *Helicobacter pylori* [4]. In this construct (FerSUMO), ferritin was genetically fused at the N-terminal region with both a His-tag and SMT3 protein, a homolog of the human Small Ubiquitin-like Modifier (SUMO-tag). Small-Angle X-ray Scattering (SAXS) experiments confirmed that under specific expression conditions, the FerSUMO protein assembles into globules containing 24 subunits [4].

In this study, we further investigated the FerSUMO complex. We performed Blue Native PAGE (BN-PAGE), Size-Exclusion Chromatography (SEC), and Negative Stain Transmission Electron Microscopy (NS-TEM) to provide additional information about its structural organization. We also explored the crystallization behavior of the FerSUMO complex using the vapor diffusion method. An extensive screening of crystallization conditions was conducted, followed by an optimization phase. The optimal crystallization conditions for the FerSUMO complex were determined to involve a buffer system containing 100 mM Tris base and BICINE at pH 8.5, along with high-molecular-weight polyethylene glycol (PEG 4000 and PEG 8000). Under these conditions, rhombic crystals with a diameter of about 50 μm were obtained. These crystals require further investigation through X-ray diffraction to resolve the structural details of the protein complex.

This work was supported by the Ministry of Science and Higher Education of the Russian Federation under agreement 075-03-2024-117, project FSMG-2021-0002.

Literature list

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Primary author: GETTE, Margarita (Moscow Institute of Physics and Technology (MIPT))

Co-authors: KUKLIN, Alexander (JINR); VLASOV, Alexey (MIPT); Dr MANUKHOV, Ilya (Moscow Institute of Physics and Technology (MIPT)); OKHRIMENKO, Ivan (MIPT); TILINOVA, Oksana (Moscow Institute of Physics and Technology); Dr BAZHENOV, Sergey (Moscow Institute of Physics and Technology (MIPT)); Dr KURKIN, Tikhon (Moscow Institute of Physics and Technology (MIPT)); SUDAREV, Vsevolod; Dr ZAGRYADSKAYA, Yuliya (Moscow Institute of Physics and Technology (MIPT)); RYZHYKAU, Yury (MIPT, JINR); Mr SEMENOV, Yury (Moscow Institute of Physics and Technology (MIPT))

Presenter: GETTE, Margarita (Moscow Institute of Physics and Technology (MIPT))

Session Classification: Poster session & Welcome drinks

Track Classification: Life Science