

# **Title: Kinetics of the interaction among proteins involved in the biogenesis of iron-sulfur clusters**

**Marcin Jeleń (marcin.jelen@phdstud.ug.edu.pl)**

## **Abstract:**

Biogenesis of mitochondrial iron-sulfur (FeS) cluster proteins requires the interaction of multiple proteins with the highly conserved 14-kDa scaffold protein Isu1, on which clusters are built prior to their transfer to recipient proteins. The assembly process of FeS on Isu involves interaction of molecular scaffold with both Nfs1, the cysteine desulfurase serving as a sulfur donor, and the yeast frataxin homolog (Yfh1) serving as a regulator of desulfurase activity and/or iron donor. Besides proteins forming the core assembly complex (Isu1-Nfs1(Isd11)-Yfh1), additional factors are required for *de novo* FeS cluster synthesis. Namely, mitochondrial ferredoxin reductase Arh1 and ferredoxin Yah1 were reported to form an electron transfer chain that supplies electrons for the reduction of the persulfide sulfur ( $S^0$ ) to the sulfide ( $S^{2-}$ ) present in the FeS cluster.

After the initial phase of FeS cluster synthesis on Isu, the cluster is released from the scaffold, transferred to target apoproteins and inserted into the polypeptide chain. The release and transfer of the Isu1-bound FeS cluster is executed by the Hsp70 chaperone system. A mitochondrial Hsp70 molecular chaperone system is central to the transfer process of FeS cluster from Isu to the recipient proteins. In *Saccharomyces cerevisiae* it is composed of the Hsp70 Ssq1 and its J protein co-chaperone Jac1, as well as the nucleotide release factor Mge1. Isu1 is well defined client protein for the Jac1/Ssq1 pair

Main aim of my project is kinetic analysis of Isu1 interactions with proteins of assembly and transfer complexes using Bio-layer interferometry technique. Using this technique I've determined the interaction of the apo form of Isu1 with components of assembly complex core, Nfs1(Isd11) and Yfh1 and the interaction with Hsp70 chaperone transfer system. Obtained results confirmed that Isu1 binds strongly to Nfs1 (Isd11) while Yfh1 has strong affinity only to Isu1:Nfs1 (Isd11) preformed double complex but doesn't bind to those components alone. Efficient binding of Ssq1 to Isu1 requires the cooperation with J-protein, Jac1, in the presence of ATP, Isu1 doesn't bind to Ssq1 alone. Future plans include analogous analysis using holo form of Isu1 under anaerobic conditions.