Many proteins require for their function the presence of iron and sulfur complexes termed iron-sulfur (FeS) clusters. Biological utility of the clusters relies on their unique ability to bind and release electrons. FeS proteins function in key metabolic processes: electron transfer chains, nitrogen assimilation, cofactor biosyntheses, biogenesis of ribosomes, DNA replication and repair. Despite a relatively simple chemical structure for most FeS clusters, their biogenesis is a complex and not well-understood process involving dedicated proteins forming multi-protein complexes. Biological activity of these complexes depends on protein-protein interactions among their components. So far, our studies of FeS cluster biogenesis have focused on identification of those interactions. In this proposal, we aim to dissect those interactions functionally, by testing which of them are critical for FeS cluster synthesis *de novo* and which are important for FeS cluster transfer onto recipient proteins. To reach this goal, we plan to reconstitute the key steps of FeS cluster biogenesis in a test tube by utilizing purified proteins. Next, we will test the importance of their individual protein-protein interactions for FeS cluster synthesis and transfer. To this end, we will replace in our reconstituted system, one by one, fully active proteins by their variants defective in interaction with binding partners. A novel aspect of our proposal is to use in biochemical experiments proteins of the thermophile fungus *Cheatomium thermophilum*. This recently developed model system has proved to be a good source of proteins that are usually more stable than their counterparts obtained from standard model organisms, such as baker yeast. The significance of our proposal is two-fold. Firstly, we will uncover molecular mechanisms behind a very important, but poorly understood, metabolic process. Secondly, as disruption of this process in humans, e.g. by mutations in genes encoding FeS biogenesis proteins leads to many, often fatal, pathologies, our results will provide an understanding of the molecular basis of these diseases and in the long run may allow development of knowledge based therapies.