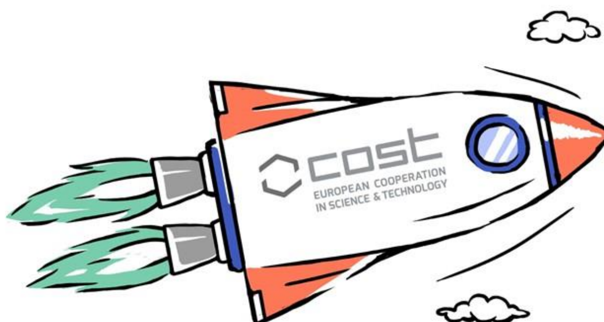

COST Action CA21115

Report date: November 2024

WG5. FeS Clusters and Proteins as Drug Targets

General description: This report describes the protocols developed and used by Action members for investigating FeS clusters and proteins as drug targets for making new drugs

Period: Nov 2022 – Nov 2024

Section 1. Computational methods to study FeS proteins

Broken-Symmetry Density Functional Theory (BS-DFT) is a specialized approach within Density Functional Theory (DFT) that is used to handle systems with unpaired electrons or magnetic properties, particularly those involving transition metals or open-shell systems like certain metal clusters, metal centers in proteins, or organic radicals. It is especially useful in studying molecules with complex spin configurations, such as in in FeS clusters showing a strong antiferromagnetic coupling or high-spin states. This advance computational approach was employed to predict and optimize the structure of the [4Fe–4S]- cluster-containing HypCD complex and to investigate its electronic and magnetic properties.

Paper 1:

Kwiatkowksi, A., Caserta, G. , Schulz, AC, Frielingsdorf, S., Pelmenschikov, V., Weisser, K., Belsom, A., Rappsilber, J., Sergueev,I., Limberg, C., Mroginski, MA, Zebger, I. and O Lenz, O. ATP-triggered Fe(CN)2CO synthon transfer from the maturase HypCD to the active site of apo-[NiFe]-hydrogenase. **Manuscript under revision in JACS.** (https://chemrxiv.org/engage/api-gateway/chemrxiv/assets/orp/resource/item/667d4fcc01103d79c5447e12/original/atp-triggered-fe-cn-2co-synthon-transfer-from-the-maturase-hyp-cd-to-the-active-site-of-apo-ni-fe-hydrogenase.pdf)

Hybrid Quantum Mechanics/Molecular Mechanics (QM/MM) calculations were carried out to elucidate the electronic structure of an unusual double cubane cluster (DCC) cofactor in the DCCP protein and the FeS cluster of the DCCP-R reductase partner. Similar to nitrogenases, this protein can catalyze the reduction of small molecules. Within the QM/MM framework, the FeS clusters and

adjacent side chains are modeled using Broken-Symmetry Density Functional Theory (BS-DFT), as described earlier, while the surrounding protein matrix is treated using a more computationally efficient classical molecular mechanics approach. Understanding the electronic structure of a system enables precise predictions of chemical reactivity, redox potentials, substrate affinity, spectroscopic signatures, and other key physicochemical properties of FeS moieties in proteins. This cutting-edge multiscale technique is currently being applied to other FeS-containing proteins such as MitoNEET.

Paper 2:

Zitarre, U., Katz, S., Pelmenschikov, V., Elghobashi-Meinhardt, N., Jeoung, JH., Dobbek, H., Mroginski, M.A., Zebger, I., The Double-cubane cluster protein reductase: an atypical archerase that displays a non-canonical activation mechanism. **Manuscript to be submitted.**

Commented [VP1]: Perhaps, the title can have a more specific message.

Protein-protein docking algorithms are computational methods used to predict the interaction between two or more proteins. These algorithms aim to determine how proteins bind together by modeling the spatial arrangement of their surfaces and identifying the most favorable binding orientations. Some wide-used protein-protein docking algorithms are HADDOCK, ClusPro , AlphaFold2 and ZDock among others. We have used this technique to predict the binding pose of FeS proteins, such as the human radical S-adenosylmethionine-dependent nucleotide dehydratase (SAND), with their potential electron transfer partner. The predictability of these well-established algorithms was improved by combining them with **Coarse Grained (CG) – and All Atoms (AA) – classical molecular dynamics (MD)simulations**. These additional MD simulations allow us to explore the conformational space of the protein complex, enabling the identification of other thermodynamically stable states or even transient complexes. Classical MD simulations can be also used to predict and characterize substrate channeling pathways in proteins as recently done on the mitochondrial outer membrane protein mitoNEET, an FeS protein involved in immunometabolism.

Paper 3:

Thao Nghi Hoang, Meritxell Wu Lu, Alberto Collauto, Peter-Leon Hagedoorn, Maria Andrea Mroginski, Maxie M. Roessler, Kourosh H. Ebrahimi," O_2 level modulates MitoNEET gasotransmitter sensory function", **Manuscript under Revision in COMMSCHEM**

Protein-ligand docking is a computational technique used to predict the preferred orientation of a ligand when bound to a protein target, often with the goal of drug discovery. It simulates how a small molecule (ligand) fits into a binding site on a protein. This technique can also be used to insert cofactors in incomplete protein structural models. Within this COST- Action we have mainly employed the AI-based Alphafill tool as well as a flexible docking algorithm implemented in the Autodock Vina program to generate holistic structural models of the human radical S-adenosylmethionine-dependent nucleotide dehydratase (SAND) harboring the radical S-adenosylmethionine (SAM) moiety and the nucleoside CTP ligand. Protein-ligand docking algorithms can be integrated with classical molecular dynamics (MD) simulations to refine structural models, enhancing the accuracy of predicted binding poses by accounting for dynamic interactions and conformational flexibility.

Paper 4

Nghi Thao Hoang, Deborah Grifagni, Meritxell Wu Lu, Yujie Sheng, Theo Situmorang, Pei-Hsin Tai, Astrid Maluta, Mohammed Hakil, Yvain Nicolet, Shahram Kordasti, Peter-Leon Hagedoorn, Maria-Andrea Mroginski, Simone Ciofi-Baffoni, Kourosh H. Ebrahimi . Discovery of the Electron Transfer Partner of the innate immune antiviral radical-SAM enzyme**. Manuscript in preparation**

Section 2. FeS proteins as potential targets for discovering new antibiotics

Amalia STEFANIU, as drug-designer, and Misu Moscovici, for preparative research

Collaboration with the Clinical Hospital for Infectious and Tropical Diseases "Dr. Victor Babes", Bucharest România. We selected antibiotic multiple resistant microorganisms and started FeS proteins stimulated biosynthesis, their isolation by cell lysis, and purification as native structures from the wild strains, adapting usual purification techniques, including ion-exchange and affinity chromatography.

Obviously, we have no yet leading molecules, but a lot of **computational screening was made and it is still in progress on potential ligands for such proteins, using DFT algorithms (B3LYP with SPARTAN Software) and molecular docking simulations (with CLC Drug Discovery Workbench).**

Section 3. Targeting mitochondrial iron metabolism for developing new therapeutics

R. Sutak

We don't really have any particular method or protocol to mention, but we have started a new collaboration within the WG:

"A collaboration between the laboratory of R. Sutak in Prague and the laboratory of S. Besteiro in Montpellier has started on the project of **mitochondrially targeted iron chelators**. R. Sutak is working on the use of these compounds as potent antiparasitic agents in the framework of the COST action and the collaboration with S. Besteiro has allowed the inclusion of his main model Toxoplasma gondii among the target pathogens. His research has shown promising effects against this parasite and a joint publication is planned for next year."

Section 4. Investigating FDX2 as a potential drug target using NMR spectroscopy

S. Ciofi, F. Cantini (Florence) and I. Turel (Ljubljana)

We have investigated the **interaction of FDX2 with selected ruthenium complexes** (some were not enough soluble to run NMR experiments).

The interaction studies between [2Fe-2S] FDX2 and Ru complexes were investigated mapping chemical shift changes on the **1H-15N HSQC spectra** acquired upon step-wise additions of the ligand. As a result, we detected the interaction of one compound. Further we wanted to perform study of this protein and ruthenium complex with ESI MS but experiments were not successful due to instability.

Section 5. Discovery of FeS enzymes producing antiviral lead molecules

A novel assay was developed by Ebrahimi lab at King's College London. The assay named VITAS (Viral polymerase-Inhibition Toxin-Associated Selection). The VITAS assay is based on the hypothesis that an ANA is formed due to the expression and activity of an enzyme in E. coli. The ANA inhibits viral T7 RNA polymerase (Pol)-mediated expression of a toxin protein allowing cell growth. The VITAS assay fundamentally differs from the commonly used live/dead assays in drug discovery. Traditional assays rely on chemical or biological labelling of cells during/after treatment with known purified lead molecules synthesised either chemically or using a purified enzyme. Moreover, purifying oxygen-sensitive radical-SAM enzymes is not straightforward, limiting the use of in vitro assays. These commonly used assays cannot easily be adopted for protein engineering to rapidly screen the activity of enzyme variants in a large library. In sharp contrast, the VITAS assay eliminates the need to purify enzymes and the NPs. Therefore, it enables the screening of enzymes' activity, facilitating the mining of the repository of natural enzymes and protein engineering to discover new ANAs. The VITAS assay is sensitive to the SANDs' activity, unlike the previously reported fluorescence-based assay showing similar activity for human and microbial SANDs.

Paper

Alharbi, A. F., Kim, H., Chumroo, D., Ji. Y., Hakil, M., Ebrahimi, K. H., VITAS, a sensitive in vivo selection assay to discover enzymes producing antiviral natural products, CHEMICAL COMMUNICATIONS 2023,59, 5419-5422

Section 6. Engineering iron-storage proteins for making new drug delivery systems and therapeutics.

Two members of the COST Action, Ebrahimi and Nicolet, worked on engineering iron-storage protein, an essential component of iron homeostasis and vital for FeS biogenesis. They report a new method to control ferritin nanocage assembly and show its application in drug delivery and the development of new therapeutics.

Naturally occurring protein nanocages like ferritin are self-assembled from multiple subunits. Because of their unique cage-like structure and biocompatibility, there is a growing interest in their biomedical use. A multipurpose and straightforward engineering approach does not exist for using nanocages to make drug-delivery systems by encapsulating hydrophilic or hydrophobic drugs and developing

vaccines by surface functionalization with a protein like an antigen. Here, a versatile engineering approach is described by mimicking the HIV-1 Gap polyprotein precursor. Various PREcursors of nanoCages (PREC) are designed and created by linking two ferritin subunits via a flexible linker peptide containing a protease cleavage site. These precursors can have additional proteins at their N-terminus, and their protease cleavage generates ferritin-like nanocages named protease-induced nanocages (PINCs). It is demonstrated that PINC formation allows concurrent surface decoration with a protein and hydrophilic or hydrophobic drug encapsulation up to fourfold more than the amount achieved using other methods. The PINCs/Drug complex is stable and efficiently kills cancer cells. This work provides insight into the precursors' design rules and the mechanism of PINCs formation. The engineering approach and mechanistic insight described here will facilitate nanocages' applications in drug delivery or as a platform for making multifunctional therapeutics like mosaic vaccines.

Paper

Sheng, Y., Chen, Z., Cherrier, M. V., Martin, L, Bui, T. T. T., Lynham, S., Nicolet, Y., Ebrahimi, K. H., A Versatile Virus-Mimetic Engineering Approach for Concurrent Protein Nanocage Surface-Functionalization and Cargo Encapsulation, SMALL 20, 2024, 2310913