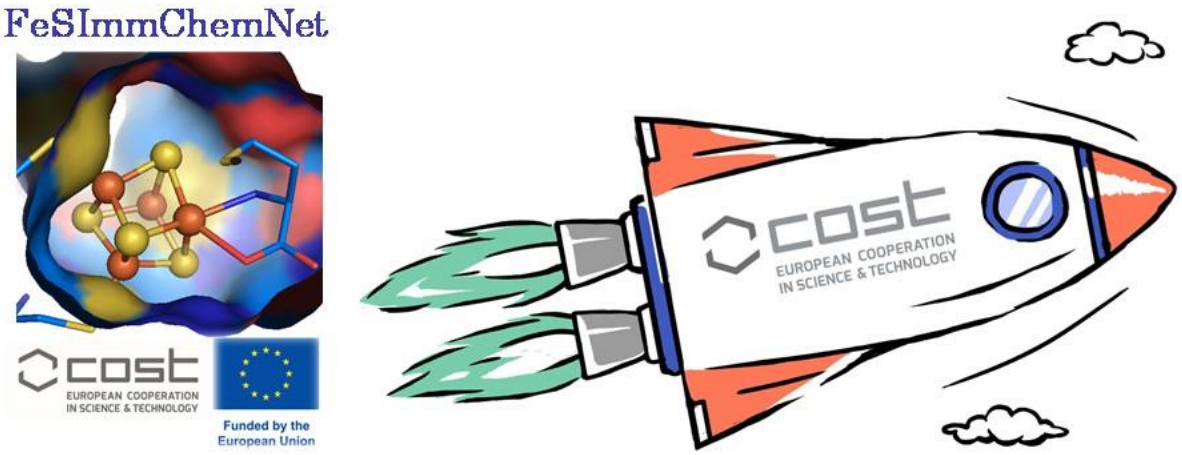

COST Action CA21115

Report date: November 2024

WG2. FeS Clusters in Viral Replication

General description: This report describes the protocols developed and used by Action members to study and work with FeS proteins involved in viral replication

Period: Nov 2022 – Nov 2024

Section 1. Computational methods to study human FeS protein SAND involved in restricting viral replication

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 1

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. Discovery of FeS enzymes producing antiviral lead molecules inhibiting viral replication

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