Title

Computational Approaches to Structural and Functional Characterization of Hypothetical Proteins Associated with Pyrite-Dependent Growth in *Allochromatium vinosum*

Authors

Yoshitha Evuri[1], Jonathon E Mohl[1,2]

Bioinformatics Program, The University of Texas at El Paso, El Paso, TX

Abstract

Pyrite (FeS_2) is an iron sulfide mineral that can serve as an electron source for certain microorganisms. *Allochromatium vinosum*, a photosynthetic purple sulfur bacterium, can grow under pyrite-supported conditions, although the molecular mechanisms involved remain incompletely understood. This study investigated five hypothetical proteins (Alvin0016, Alvin0022, Alvin0929, Alvin1150, and Alvin1152) whose biological functions have not been experimentally characterized. These proteins were selected as an initial representative subset to establish a computational framework for structural and functional characterization, with the long-term objective of extending the analysis to the broader set of uncharacterized proteins potentially involved in pyrite-dependent growth. Understanding these proteins may provide insights into microbial electron transfer and metabolic adaptation. Three-dimensional protein structures were predicted using AlphaFold2, a deep learning-based structure prediction tool. I-TASSER was used to predict potential protein functions and ligand-binding sites through structural comparisons, while AutoDock Vina evaluated protein–ligand interactions by estimating docking affinities.

Alvin0022 showed the strongest predicted interaction with heme (−7.6 kcal/mol), an iron-containing molecule involved in biological electron transfer, suggesting a possible role in redox metabolism. Alvin0016 exhibited predicted ATP binding (−5.5 kcal/mol), while Alvin1150 interacted with bacteriochlorophyll A (−5.6 kcal/mol), a photosynthetic pigment. Alvin0929 interacted with bedaquiline (−5.2 kcal/mol), a synthetic antibiotic, whereas Alvin1152 showed weak binding to beryllium difluoride (BeF_2; −2.6 kcal/mol), an experimental phosphate analog. These ligands were selected from I-TASSER predictions to investigate possible binding-site characteristics rather than confirm physiological interactions. Overall, these findings generate preliminary functional hypotheses, establish a computational approach for systematic characterization of additional hypothetical proteins, and identify candidate proteins for future experimental investigation of pyrite-dependent growth.