BioFluCas: Designing multi-molecularly crowded Biomimetic neoteric Fluids to enhance In vitro biological Cascade reactions

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INTRODUCTION

In plants and several other organisms, carboxysomes are specialized structures that house enzymes such as carbonic anhydrase (CA) and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) which works in cascade, wherein CA helps to Rubisco by increase CO_2 concentration near Rubisco for the transformation of CO_2 and ribulose 1,5-biphosphate into 3-phosphoglycerate, an intermediate needed for glucose biosynthesis. Inspired by this, BioFluCas project aims to design an effective CA-Rubisco based enzymatic cascade reaction (ECR) for artificial synthesis of glucose precursor using CO_2. However, in nature, biological ECRs take place in a confined cellular space filled with various biomolecules including polysaccharides, protein, nucleic acids, and several other small molecules. Such molecularly crowded environment governed the stability and activity of enzymes inside cellular environment. Traditional approaches, which use single molecular crowding agents and standard buffer systems, fall short in accurately replicating the complexity of in vivo conditions when studying ECRs and protein stability, which often results unfolding of enzyme under extreme conditions. Neoteric solvents such as ionic liquid (ILs) and deep eutectic solvent (DESs) offer unique properties including the ability to dissolve a wide range of biomacromolecules and protein-friendly nature. As a result, they offer suitable characteristics for mimicking a biological cell environment. The BioFluCas project proposes that combining ILs or DESs with macro- and small-molecular crowding agents will more effectively replicate the complex cellular environment to improve ECRs. Such multi-molecularly crowded biomimetic neoteric fluids (MCBNFs) would outperform traditional single-crowder systems, particularly in improving the efficiency of ECRs such as HRP-GOx and CA-Rubisco cascades (**Fig. 1**).

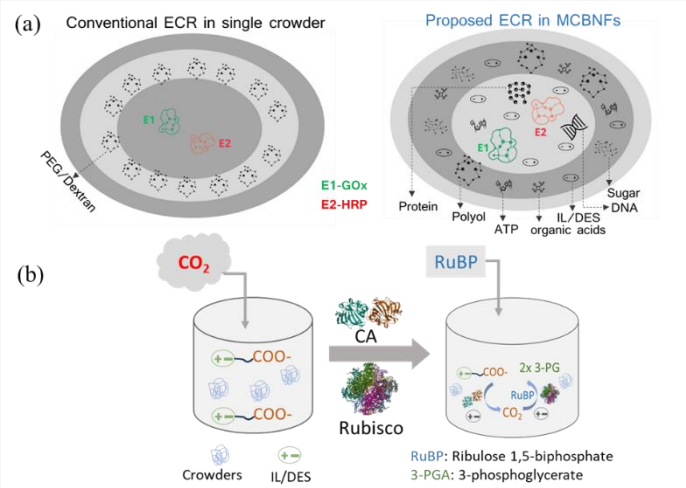

Fig. 1: (a) Traditional single crowder-based *Vs* proposed ECR in MCBNFs. (b) Atmospheric CO_2 to glucose precursor synthesis in MCBNFs

AIM OF THE PROJECT AND APPROACH

MCBNFs will be created by dissolving various crowding agents in ILs or DESs. The effects of MCBNFs on the structural stability of enzymes like HRP, GOx, CA, and Rubisco will be studied using spectroscopic, microscopic, and computational methods. Kinetic assays of these enzymes will be performed, with a focus on the HRP-GOx system to explore proximity, substrate channeling, and protein-protein interactions. Additionally, a proof-of-concept study will be conducted to demonstrate the enhancement of in vitro photosynthetic CO_2 fixation using MCBNF. To achieve the general goal of BioFluCas project the detailed research approach is divided into five research tasks (T). T-1: Develop and characterize biomimetic multi-molecularly crowded fluids using ILs and DESs; T-2: Investigate the stability and kinetics of enzymes (HRP, GOx, CA, and Rubisco) in presence of MCBNFs; T-3: Investigate how MCBNFs affect substrate channeling, kinetic efficiency, and stability under harsh conditions in the GOx-HRP cascade reaction; T-4: Study protein-protein interactions and enzyme stability in MCBNF using molecular dynamics simulations; T-5: Explore in vitro CO_2 fixation in the presence of MCBNFs by modulating CA-Rubisco cascade efficiency.

SIGNIFICANCE OF THE PROJECT RESULTS

The engineered MCBNFs will unveil a better model for investigating in vitro biological cascade reactions which are important in metabolic pathways understanding. The project results would also make a significant contribution to the green solvents research field in that they reveal a more detailed knowledge on how ILs and DESs replicate natural cells using multi-molecular crowding agents. The results of multienzyme reactions such as GOx-HRP and CA-Rubisco cascades in MCBNFs will promote industrial biocatalysis and carbon capture technologies hence contribute to sustainable development goals.