Tracking Photosynthetic Metabolite Transport Through Bacterial Microcompartments Using Enhanced Sampling Simulations

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Bacterial Microcompartments (BMCs) are proteinaceous organelles that sequester key metabolic reactions to increase enzymatic efficiency and prevent the loss of volatile or cytotoxic intermediates. Among them, carboxysomes (CBs) are of particular interest, as they sequester ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) to enhance carbon fixation by elevating the local CO_2 concentration and thereby improving rubisco's catalytic efficiency. The BMC shell functions as a selective, semi-permeable barrier, and its permeability is central to maintaining metabolite gradients between the organelle interior and the cytoplasm. While prior studies have demonstrated selective transport of Calvin cycle metabolites into CB hexameric pores, the precise mechanisms governing molecular influx and efflux through both the pores remain unclear. Here, we employed enhanced sampling molecular dynamics simulations to quantify the permeability coefficients of key metabolites—HCO_3^-, CO_2, O_2, ribulose bisphosphate (RuBP), and 3-phosphoglycerate (3-PGA)—through the hexameric and trimeric pores of the BMC shell. Our results reveal that the pores preferentially facilitate the passage of HCO_3^-, 3-PGA, and RuBP, while providing lower permeability for CO_2 and O_2. These findings offer mechanistic insight into metabolite transport across BMC shells and establish a predictive framework for tuning shell permeability. This approach enables the rational engineering of BMC-based metabolic modules, opening new opportunities in synthetic biology for enhancing carbon fixation, bioenergy production, and sustainable biodesign.