Construction of DNA-based artificial compartments for enzyme cascade reactions

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In cells, enzymes are spatially organized to perform specific sequential reactions within the compartments such as membrane-bound or membraneless organelles.[1] Artificial compartments have been constructed using liposomes, proteins, or polymers, but the applications of these carriers face the challenges of low enzyme loading yields and the difficulty in controlling the location and number of enzymes. With the advantages of precise addressability, DNA scaffolds provide the ideal platforms for enzyme assembly.[2] In this study, a series of DNA hexagonal prisms with different dimensions were prepared to construct the artificial compartments for enzyme reactions.

A series of scaffold systems were developed for the cascade reactions of xylose reductase (XR) and xylitol dehydrogenase (XDH) from the D-xylose metabolic pathway. The DNA scaffolds of 3D hexagonal prism (HP), medium HP (MHP), and shallow HP (SHP) were prepared by the DNA origami method.[3] XR and XDH were specifically located to the scaffold in the open state by the modular adaptor method,[4] followed by the closing process of the scaffold induced by the closing keys (short DNAs).[5] XR and XDH were encapsulated in the closed states of HP, MHP, and SHP with an estimated interenzyme distance of ~18 nm, ~10 nm, and ~4 nm, respectively. Alditol oxidase (AldO) was used as a competing enzyme for XDH to evaluate the free diffusion of intermediates (Figure 1).

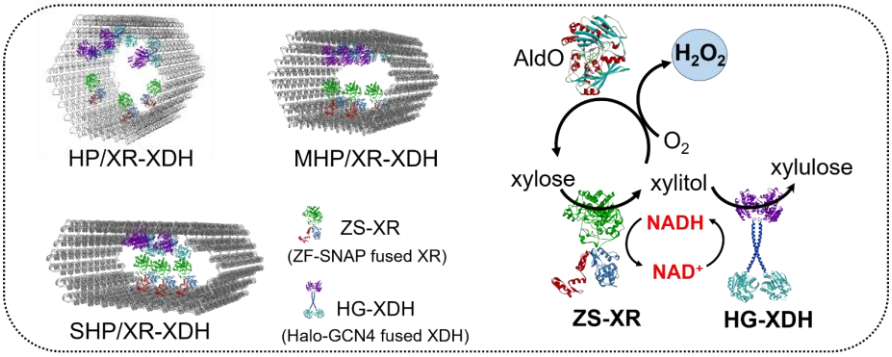

Figure 1. Cascade enzyme reactions of XR and XDH on the DNA scaffolds.

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