Proteins associate with each other to form complex collaborative network in order to perform certain functions, such as multi-step reactions in metabolic process, receiving and transferring signals in signaling pathways, and host cell infection. Scaffolds have evolved in nature to organize proteins to enhance collaborative efficiency. Inspired by features and advantages of natural scaffolds, synthetic scaffolds are developed for customized applications by organizing proteins into functional modules.

In the first objective of this thesis, DNA was used as a scaffold to organize 5 proteins involved in converting cellulose to H2O2 for application in cellulosic fuel cell. DNA scaffolds feature simple hybridization rules and precise programming of structures. These features allow precise control of order and distance of the 5 proteins, enabling investigation on substrate channeling effect of adjacent enzymes and synergistic effect of enzymes in close proximity. The organization of proteins is based on specific hybridization of DNA linkers with DNA template. Chemical conjugation or HaloTag conjugation was adopted to modify the 5 proteins with DNA linkers. Although DNA can be programmed into structures in 1 to 3-dimensional, the heavy programming load and cost of the 5 enzyme cascade prevented the use of DNA as 3-dimensional scaffolds, leading to the exploration of native 3-dimensional scaffolds.

In the second objective, functionalization of protein nanoparticles was investigated to develop 3-dimensional scaffolds. 3-dimensional scaffolds play an important role in signal amplification of biosensors, drug delivery and biocatalysis. Native protein nanoparticles can self-
assemble into stable monodisperse structures and are biocompatible. However, previous functionalization methods can lead to incorrect folding of nanoparticle structures or loss of control over specific modifications that limit and complicate the application of protein nanoparticles. To develop a simple and modular functionalization method, an enzyme-mediated ligation strategy was investigated; sortase-mediated ligation enabled specific conjugation of functional proteins, such as elastin-like polypeptide, monomeric and tetrameric enzymes onto E2 protein nanoparticles from Bacillus stearothermophilus, a model 3-dimesnional scaffold.

Finally, to further simplify the use of native 3-dimensional scaffolds, one-pot synthesis of protein scaffolds that are ready to use after production were developed. It is inspired by the biogenesis of outer membrane vesicles (OMVs), which naturally formed into 20-250 nm proteoliposomes during growth cycle of bacteria. They feature the simultaneous formation of nanostructures and displaying of functional proteins. Membrane proteins were investigated as anchors for displaying heterologous proteins to the exterior and interior membrane surfaces, which allowed simultaneous display of proteins of different functionalities, such as detection and reporter moieties. One-pot synthesized sensors were generated based on OMVs and successful antigen detection by ELISA and cell imaging were demonstrated.

In summary, three different types of synthetic scaffolds were engineered for customized applications and novel functionalization strategies have been developed for 3-dimensional scaffolds. The findings discussed in this thesis can contribute to expanding the applications of synthetic scaffolds.