

# **Construction of functional protein hydrogels utilizing the interaction between subunits of oligomeric proteins**

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Association-controllable hemoprotein assemblies and hydrogels were constructed from a fusion protein (FP) with two c-type cytochrome units utilizing 3D domain swapping (3D-DS), where the same structural region is exchanged three-dimensionally between molecules of the same protein. FP was expressed with *E. coli* and purified by ion exchange and size exclusion chromatography. The dimer and trimer of FP, oligomerized by 3D-DS, were prepared by the procedure of ethanol addition, lyophilization, and redissolution of the residual. The obtained 3D-DS FP dimer constructed hemoprotein assembly exhibiting a dynamic structural change between ring and linear forms, regulated by CO and imidazole binding. The oligomerization of the FP 3D-DS dimer depended on the temperature and protein concentration. The structural change between ring and linear structure of the dimer of FP 3D-DS dimer was directly observed by the high-speed atomic force microscopy in solution. Highly concentrated 3D-DS FP trimer formed protein hydrogels, which decomposed by CO and imidazole binding. In this study, protein nanostructures and protein hydrogels were constructed through the interaction between proteins. These results contribute as the basis of future functional supramolecular protein assemblies and drug delivery systems using protein-based structures.