Reversible and mutli-cyclic protein-protein interaction in bacterial cellulosome-mimic system

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The type II cohesin domain and type II dockerin of bacterial cellulosome were cloned from *Clostridium thermocellum* and expressed in *Escherichia coli* with the fusion of tobacco mosaic virus coat protein (TMVcp) and enhanced green fluorescent protein (EGFP), respectively. The TMVcp-cohesin fusion protein was assembled to the stable and rod-shaped nanostructure (TMVcp-Coh rod) under a particular buffer condition, where many active cohesin proteins are biologically and densely displayed around the 3-dimensional surface of TMVcp-Coh rod. We confirmed that the Ca^{+2}-dependent binding and dissociation between native cohesin and dockerin were reproduced with the two recombinant fusion proteins, TMVcp-cohesin and EGFP-dockerin, using EGFP-dockerin as a fluorescent reporter. The multi-cyclic binding-dissociation operation of TMVcp-Coh rod and EGFP-dockerin was successfully performed with maintaining the reversible cohesin-dockerin interaction in every cycle. In this study, EGFP that was fused to dockerin as a proof-of-concept can be switched to other functional proteins/peptides that need to be used in multi-cyclic operation.