13:00 – 13:45

Self-assembling complexes based on protein-protein interactions

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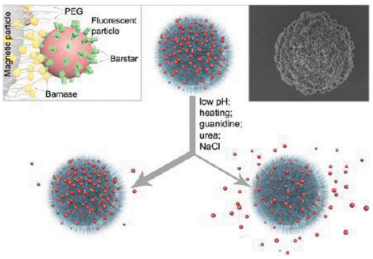

Figure Concept of multipoint contacts between the components of the colloidal assembly (left), SEM image of the barnase-barstar system assembled structure (right) and general schematic view of the self-assembly system behavior upon disassembly of the preassembled structures in protein-denaturing conditions (center).

Numerous self-assembly systems have been designed that allow for control of their behavior in terms of assembly-disassembly in different conditions by triggering these transitions with relatively gentle stimuli of various nature. For example, in nucleic acid-based systems, disassembly may be triggered upon temperature denaturation of DNA duplexes holding particles within the assembly; other examples include disassembly induced by competing oligonucleotides, lowering of pH, and divalent cations of transition metals, etc. These approaches to achieve controllability of the assemblies using various stimuli have a great potential in the construction of "smart" materials for a number of applications (drug delivery, biosensors, etc.). In this work, we address the question of stability of the barnase-barstar system (BBS) -"glued" assemblies subject to destruction. To this end, we test their behavior under severe protein denaturing conditions such as high temperature and low pH as well as high salt and chaotropic agent (urea and guanidinium hydrochloride) concentrations.

Yet in other situations it is desirable to design materials that would not readily disassemble under appropriate conditions. For example, self-assembled multifunctional theranostic agents are expected to demonstrate significant stability to ensure retention of all functional modules within a single entity

to be able to perform all programmed functions (e.g., imaging, drug delivery, stimulus-responsiveness) with equal efficiency. We studied protein-assisted nanoparticle self-assembly systems (namely, those based on barnase-barstar, streptavidin-biotin, antibody-antigen, and protein A-immunoglobulin interactions) that exhibit unexpected robustness in denaturing conditions intolerable for most proteins. Use of proteinaceous "molecular glues" for nanoparticle self-assembly purposes is of interest due to the advantages of introducing new functionalities to the self-assembled structures via additional protein modules fused to initial molecules mediating assembly. Among them, we find the barnase-barstar pair particularly noteworthy due to benefits offered by genetic engineering of this entirely protein based system and ease of heterologous prokaryotic expression of the proteins in ample amounts. The barnase-barstar system has found applications in bioengineering and design of a number of fusion proteins and supramolecular constructs. To the benefit of barnase and barstar genetic engineering, N- and C-termini of both proteins are not involved in the molecular interface of the proteins within the complex, so they are available for fusions such as those with antibodies, fluorescent proteins, and bacterial toxins, which can be used as additional functional modules of the hybrid protein-particle constructions. That distinguishes the BBS from the other above-mentioned protein-based self-assembly approaches, which are generally used per se.

Experiments show that the obtained constructs possess unusual stability and tolerate conditions far beyond physiological ones. The BBS was also compared to other widely used self-assembly systems mentioned above, in terms of resistance of the preassembled structures to the extreme conditions as well as with respect to their ability to mediate assembly of the initial conjugates involving the components of these systems. Unexpectedly, whereas in the former case the tested systems demonstrate a relatively similar behavior, their performance in the latter differs substantially.

The results on applications of barnase-barstar platform with important types of the nanoparticles, including quantum dots, luminescent nanodiamonds, colloidal gold, magnetic NPs, luminescent upconversion NPs as well as delivery of pseudomonas exotoxin A and radioisotope to the HER2/neu overexpressing human adenocarcinoma cells also are reviewed.