

“Smart Polymer-Smart Protein Bioconjugates”

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Over the past twenty years, we have been combining “smart” polymer molecules with different biomolecules for many unique applications in medicine and biotechnology. “Smart” polymers are sharply responsive to small physical stimuli. (1) For example, a small temperature or pH change, or exposure to specific wavelengths of light, can cause the smart polymer in an aqueous solution to convert from a hydrated coil to a collapsed, hydrophobic globule, leading to phase separation. Reversal of the stimulus will cause the polymer chain to rehydrate and redissolve.

Our early work on these interesting hybrid materials was mostly focused on the random conjugation of a smart polymer to a protein, usually effected through reaction of an activated group on the polymer with a protein lysine amine group. The bioconjugate may be precipitated from solution by stimulating the smart polymer to phase separate. Such phase separation has been used for separation (and recycle) of an enzyme from its product in a bioprocess solution, for separation of an affinity protein with its bound ligand from a complex solution, for recovery, removal or assay of the ligand (this is similar to affinity chromatography done in solution), and for separation of an immune complex sandwich of an antibody/antigen/second labeled antibody from a diagnostic sample, for assay of the antigen (this is similar to ELISA done in solution). (1) This technology has limitations: one such limitation is that it requires a reactive lysine amine group on the protein, and not all proteins have accessible lysine groups, and another limitation is that the conjugation site is not controlled, and the polymer may be conjugated to a lysine that is intrinsic to the activity of the protein, thus interfering with the activity of the protein. Fortunately, the sequences and crystal structures of many proteins are now available, permitting us to clone specific mutants for such site-specific conjugations. (2) We have focused our efforts on the conjugation of a smart polymer to a cysteine –SH group, which has been selectively cloned into the protein at a specific site. By conjugating the polymer near the binding site of the protein, we have been able to control the ligand binding activity of the protein (2), and we have also effected the release of a bound ligand when the smart polymer is cycled through its phase separation transition. (3) We have extended these studies to pH-sensitive polymers (4) and also to thermally-induced, size-controlled binding of biotinylated proteins to streptavidin. (5) We recently synthesized photo-sensitive polymers and conjugated them to an enzyme for photo-induced “on-off” control of the enzyme-substrate reactions. (6,7) This talk will highlight our latest results with these interesting conjugates, including our recent applications to microfluidic systems.