Design of Smart Polymer Constructs for Use in Microfluidic Diagnostic Assays

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Introduction

In this talk, I will describe the design, synthesis and properties of (a) random conjugates of smart polymers with proteins, (b) site-specific conjugates of smart polymers and proteins, (c) dual-responsive random and block copolymers and their bioconjugates, and (d), conjugates of nano-beads with smart polymers and biomolecules, and their applications in microfluidic devices.

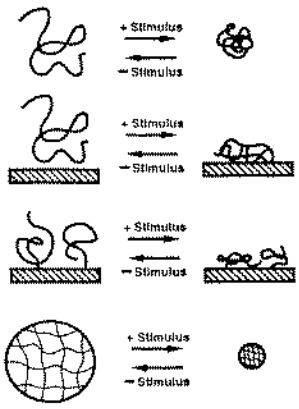

Fig. 1. Smart polymers include (a) polymers in solution that either phase separate or form gels upon stimulation, (b) polymers that adsorb and desorb reversibly from surfaces upon stimulation, (c) grafted polymers that change the nature of the surface wettability upon stimulation, and (d) hydrogels that collapse or reswell sharply upon stimulation. All these phenomena are reversible.

Random Smart Polymer-Protein Bioconjugates

We began to work with smart polymers about 25 years ago, in the early 1980s. We began by randomly conjugating a smart polymer, poly(N-isopropyl acrylamide) or PNIPAAm, to lysine amino sites on proteins to yield temperature-responsive bioconjugates that phase-separated out of aqueous solutions *sharply* above a critical temperature, called the LCST. (Indeed, this *sharp phase separation* is the reason such a polymer is called a "smart" polymer). We found that we could heat an aqueous solution of the PNIPAAm-protein bioconjugate to cause the smart polymer to sharply phase separate around 28°C in buffer, and that led to phase separation of the bioconjugate. We noticed that free PNIPAAm enhanced the precipitation process. We were also able to reverse this process by cooling. (eg, see Hoffman, 2000 and Stayton, 2005)

Among the proteins we conjugated with PNIPAAm were enzymes, affinity proteins such as Protein A and Streptavidin, and antibodies. We applied these bioconjugates for uses in a variety of processes, including enzyme recovery and recycle in an enzyme bioprocess, affinity separation and recovery of a specific product in a recombinant cell culture process, and isolation and quantification of a specific molecule such as in an immunoassay. (eg, see Hoffman, 2000 and Stayton, 2005)

Conjugates of nano-beads with smart polymers and biomolecules, and their applications in microfluidic devices

In the last 6 years we have end-linked PNIPAAm to aminated nano-beads, and found that the beads could be aggregated by heating above the LCST of PNIPAAm. We also linked biotin-PEG to the beads and that allowed us to bind Streptavidin to the beads. The Streptavidin was then used as a linker to bind biotinylated antibodies, ssDNA or enzymes to the smart beads. We are using these "doubly-smart" nano-beads in microfluidic devices, for a variety of affinity separations, diagnostic assays and lab-on-a-chip enzyme bioprocesses. (Malmstadt, 2003, 2004 and Ebara, 2006)

Table 1: Important Characteristics of Microfluidic Devices
- **Many complex, sequential procedures can be done in a single device**
- **Small dimensions = rapid diffusion and efficient separations**
- **Streamline flow eliminates undesirable mixing**
- **Relatively inexpensive to fabricate (esp. PDMS)**
- **Small sample volumes and reagent volumes**
- **Therefore, more convenient and less waste**
- **Easy to take anywhere**
- **Very suitable for "Point of Care" diagnostics**

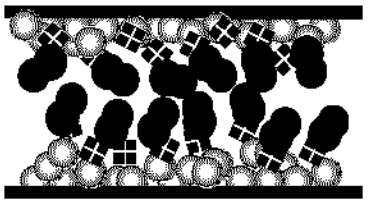

Fig. 2. Schematic of microfluidic channel showing smart nanobeads aggregated in a heated region, with Streptavidin (SA) (red squares) bound to a biotin-PEG spacer on the bead surfaces, biotinylated capture biomolecules (blue circles) bound to the SA, and target molecules (red circles) bound to the capture biomolecules.

Site-specific conjugates of smart polymers and proteins

Beginning in the early 1990s, we conjugated temperature-, pH- and light-responsive polymers at specific sites on recognition proteins; most often the site we select is near the active site of the protein. In order to do this, we need to know the crystal structure and amino acid sequence of the protein. This allows us to visualize the three dimensional composition and conformation of the active site; this information enables us to select a specific amino acid for mutation, which we need to do in order to create a special reactive site that is different from lysine amino groups for conjugation of the smart polymer. We then clone a mutant of the protein with a cysteine inserted in a prominent position near the active site. This provides us a reactive thiol group near the active site, and then we conjugate the smart polymer to that site, using chemistry that is specific to the thiol group. When the polymer is stimulated to collapse by changing temperature, pH or light, it will block the active site, turning "off" the ability of that site to recognize and react; the reverse of the stimulus restores the polymer solubility, and this opens up the active site and turns the protein activity back "on". In this way, we are able to "switch" protein recognition processes *on* and *off* reversibly by blocking and unblocking protein recognition sites caused by thermal-, pH- or light-driven changes in the smart polymer solubility. We are applying this principle to affinity separation and enzyme processes. (Shimoboji, 2001 and 2002)

Dual-responsive random and block copolymers and their bioconjugates

We have also copolymerized pairs of monomers that have different responsivities to different stimuli, leading to formation of dual-responsive copolymers that are responsive to two specific stimuli. We have used normal, random free radical copolymerization techniques for this purpose. More recently, we are using living free radical polymerizations such as RAFT or ATRP to prepare block copolymers with dual sensitivities. (Kulkarni,2006) In this way, we have synthesized combined pH-and temperature-responsive copolymers and also temperature- and light-responsive copolymers. We have conjugated these interesting smart polymers to proteins, and this talk will also include descriptions of the properties and applications of such doubly smart, dual responsive bioconjugates

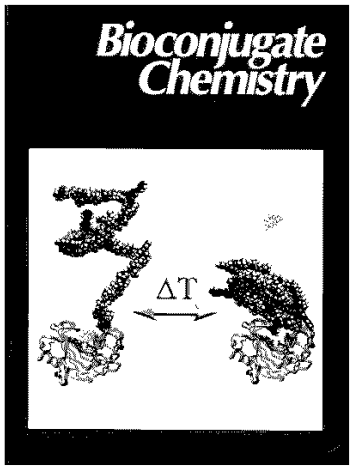

Fig. 3: Cover of Bioconjuate Chemistry, May/June 2003 issue showing a dual sensitive smart polymer, responsive to both light and temperature, conjugated to the enzyme endogluconase. (Shimoboji, 2001 and 2002)

Acknowledgements

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