Really smart bioconjugates of smart polymers and receptor proteins

Allan S. Hoffman,1* Patrick S. Stayton,1 Volga Bulmus,1 Guohua Chen,1 Jingping Chen,1 Chuck Cheung,1 Ashutosh Chilkoti,1 Zhongli Ding,1 Liangchang Dong,1 Robin Fong,1 Chantal A. Lackey,1 Cynthia J. Long,1 Morikazu Miura,2 John E. Morris,3 Niren Murthy,1 Yoshikuni Nabeshima,1 Tae Gwan Park,1 Ollie W. Press,1 Tsuyoshi Shimoboji,1 Sara Shoemaker,1 Heung Joon Yang,1 Nobuo Monji,2 Robert C. Nowinski,2 Carole Ann Cole,2 John H. Priest,2 J. Milton Harris,3 Katsuhiko Nakamae,4 Takashi Nishino,4 Takashi Miyata4

1University of Washington, Seattle, Washington
2Genetic Systems, Inc., Seattle, Washington
3Shearwater Polymers, Inc., Huntsville, Alabama
4Kobe University, Kobe, Japan

Received 12 June 2000; accepted 13 June 2000

Abstract: Over the past 18 years we have been deeply involved with the synthesis and applications of stimuli-responsive polymer systems, especially polymer-biomolecule conjugates. This article summarizes our work with one of these conjugate systems, specifically polymer-protein conjugates. We include conjugates prepared by random polymer conjugation to lysine amino groups, and also those prepared by site-specific conjugation of the polymer to specific amino acid sites that are genetically engineered into the known amino acid sequence of the protein. We describe the preparation and properties of thermally sensitive random conjugates to enzymes and several affinity recognition proteins. We have also prepared site-specific conjugates to streptavidin with temperature-sensitive polymers, pH-sensitive polymers, and light-sensitive polymers. The preparation of these conjugates and their many fascinating applications are reviewed in this article. © 2000 John Wiley & Sons, Inc. J Biomed Mater Res, 52, 577–586, 2000.

Key words: smart polymers; stimuli-responsive polymers; intelligent polymers; bioconjugates; polymer conjugation; recognition proteins; site-specific conjugation; affinity separation; immunoassay

INTRODUCTION

Smart polymers and their bioconjugates

Stimuli-responsive, “intelligent” polymers are polymers that respond with large property changes to small physical or chemical stimuli. They are also known as “smart,” “stimuli-responsive,” or “environmentally-sensitive” polymers. These polymers can take many forms; they may be dissolved in aqueous solution, adsorbed or grafted on aqueous-solid interfaces, or crosslinked in the form of hydrogels (Fig. 1).1–3 Many different stimuli have been investigated, and they are listed in Table I. Typically, when the polymer’s critical response is stimulated, the smart polymer in solution will show a sudden onset of turbidity as it phase separates, and the surface-adsorbed or grafted smart polymer will collapse, converting the interface from hydrophilic to hydrophobic. The smart polymer crosslinked in the form of a hydrogel will exhibit a sharp collapse, and release much of its swelling solution. These phenomena are reversed when the stimulus is reversed, although the rate of reversion is often slower when the polymer has to redissolve or the gel has to reswell in aqueous media.

Smart polymers may be physically mixed with or chemically conjugated to biomolecules to yield a large family of polymer-biomolecule systems that can respond to biological as well as to physical and chemical stimuli. Biomolecules that may be polymer-conjugated include proteins and oligopeptides, sugars and polysaccharides, single and double-stranded oligonucleotides and DNA plasmids, simple lipids and phospholipids, and a wide spectrum of recognition proteins. We have also prepared site-specific conjugates to streptavidin with temperature-sensitive polymers, pH-sensitive polymers, and light-sensitive polymers. The preparation of these conjugates and their many fascinating applications are reviewed in this article. © 2000 John Wiley & Sons, Inc. J Biomed Mater Res, 52, 577–586, 2000.

Key words: smart polymers; stimuli-responsive polymers; intelligent polymers; bioconjugates; polymer conjugation; recognition proteins; site-specific conjugation; affinity separation; immunoassay
ligands and synthetic drug molecules. In addition, polyethylene glycol (PEG) may also be conjugated to the smart polymer backbone to provide it with "stealth" properties (Fig. 2). The conjugate of a synthetic polymer and a biomolecule produces a new, hybrid type of molecule that can synergistically combine the individual properties of the two components to yield new and unusual properties. One could say that these bioconjugates are "doubly smart." Among the most important polymer-biomolecule conjugates are polymer-drug conjugates\(^4\)–\(^7\) and polymer-protein conjugates.\(^8\) There have been a number of successful applications in medicine and biotechnology for such smart polymer-biomolecule systems, and as such they represent an important extension of polymeric biomaterials beyond their well known uses in implants and medical devices.

We have been combining smart polymers with a wide variety of biomolecules over the last fifteen years.\(^9\)–\(^27\) Our major activity in this area has been to conjugate temperature-sensitive polymers to proteins, both randomly\(^10\)–\(^20\) and at specific sites,\(^21\)–\(^27\) and this work will be the focus of this review article. There are also many others who have randomly conjugated smart polymers to proteins, especially for affinity separations\(^28\)–\(^33\) and enzyme recovery,\(^34\)–\(^40\) but to our knowledge, we are the only ones who have synthesized and investigated site-specific smart polymer bioconjugates.

There are many polymers that exhibit thermally induced precipitation (Table II), but the polymer we have studied most extensively is poly(N-isopropylacrylamide), or PNIPAAm. This polymer is soluble in water below 32°C, and it precipitates sharply as temperature is raised above 32°C. The precipitation temperature is called the lower critical solution temperature, or LCST. If NIPAAm monomer is copolymerized with more hydrophilic monomers such as acrylamide, the LCST decreases (Fig. 3).

<table>
<thead>
<tr>
<th>Physical</th>
<th>Chemical</th>
<th>Biochemical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>pH</td>
<td>Enzyme substrates</td>
</tr>
<tr>
<td>Ionic strength</td>
<td>Specific ions</td>
<td>Affinity ligands</td>
</tr>
<tr>
<td>Solvents</td>
<td>Chemical agents</td>
<td>Other biochemical agents</td>
</tr>
<tr>
<td>E.M. radiation (UV, visible)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric fields</td>
<td>Mechanical stress, strain</td>
<td></td>
</tr>
<tr>
<td>Sonic radiation</td>
<td>Magnetic fields</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Schematic figure of the different types of responses of "intelligent" polymer systems to environmental stimuli.\(^3\)

Figure 2. Schematic illustration of the variety of natural or synthetic biomolecules which may be conjugated to a smart polymer. In some cases, only one molecule may be conjugated, such as a recognition protein, which may be linked to the protein at a reactive terminal group of the polymer, or it may be linked at a reactive pendant group along the polymer backbone. In other cases more than one molecule may be conjugated along the polymer backbone, such as a targeting ligand along with many drug molecules.\(^5\)
Synthesis of polymer-biomolecule conjugates

Random conjugation

Proteins may be conjugated randomly to one end of a polymer, or to pendant groups along the polymer backbone (Fig. 4). We have utilized chain transfer free radical polymerization to synthesize oligomers with one functional end group, which can then be derivatized to form a reactive group that can be conjugated to the protein. We have also copolymerized NIPAAm with reactive comonomers to yield a random copolymer with pendant reactive groups, which have then been conjugated to the protein.24

Normally, the lysine amino groups are the reactive sites for random polymer conjugation to proteins, and N-succinimide attachment chemistry is most often utilized. Other possible sites include —COOH groups of aspartic or glutamic acid, —OH groups of serine or tyrosine, and -SH groups of cysteine residues. The most likely attachment site will be determined by the reactive group on the polymer and the reaction conditions, especially the pH. Because these conjugations are generally carried out in a nonspecific way, they can interfere sterically with the protein’s active site, or modify its microenvironment, and the bioactivity of the protein is then usually reduced (see Ding et al.18 for an exception to this).

Site-specific conjugation

We have also been able to conjugate the smart polymer to a specific site on some proteins by inserting a specific reactive amino acid at a selected site, such as a cysteine with its reactive —SH thiol group (Fig. 5). This is done by genetically-engineering a site-specific mutation into the DNA sequence of the protein, and then cloning the mutant in cell culture. Thus, this method is applicable only to proteins whose complete peptide sequence is known. The preparation of the reactive smart polymer is similar to the method described above, but now the reactive end or pendant groups and the reaction conditions are specifically designed to favor conjugation to —SH groups rather than to —NH₂ groups. Typical mercaptyl-reactive

![Figure 3.](image)

**Figure 3.** Copolymerization of a thermally sensitive polymer, PNIPAAm, with a more hydrophilic comonomer, AAm, raises the LCST of the copolymer, while copolymerization with a more hydrophobic comonomer, N-test butyl-acrylamide (N-tBAAm), lowers the LCST.

![Figure 4.](image)

**Figure 4.** Various types of random and site-specific smart polymer-protein conjugates. In the latter case, conjugation near the active site of the protein is intended to provide stimulus control of the recognition process of the protein for its ligand, while conjugation far away from the active site should avoid any interference of the polymer with the protein’s natural activity.

### Table II

<table>
<thead>
<tr>
<th>Ether Groups</th>
<th>Alcohol Groups</th>
<th>Substituted Amide Groups</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(ethylene oxide) (PEO)</td>
<td>Hydroxypropyl acrylate</td>
<td>Poly(N-substituted acrylamides)</td>
<td>Poly(methacrylic acid)</td>
</tr>
<tr>
<td>Poly(EO/PO) random copolymers</td>
<td>Hydroxypropyl methycellulose</td>
<td>Poly(N-acryloyl pyrrolidine)</td>
<td></td>
</tr>
<tr>
<td>PEO-PPO-PEO triblock surfactants</td>
<td>Hydroxypropyl cellulose</td>
<td>Poly(N-acryloyl piperidene)</td>
<td></td>
</tr>
<tr>
<td>Alkyl-PEO block surfactants</td>
<td>Methylcellulose</td>
<td>Poly(acryl-L-amino acid amides)</td>
<td></td>
</tr>
<tr>
<td>Poly(vinyl methyl ether)</td>
<td>Poly(vinyl alcohol) derivatives</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
polymer groups include maleimide and vinyl sulfone groups. For “site-specific” conjugation to —NH₂ groups, it should be noted that control of the reaction pH can provide more favorable conjugation to the terminal amine group vs. pendant lysine amine groups. Furthermore, concerning the latter, some lysine amine groups will be more accessible, and therefore more reactive than others.

The specific site for polymer conjugation can be located far away from the active site (in order to avoid interference with the biological functioning of the protein), or nearby, or even within the active site, in order to control the ligand-protein recognition process and the biological activity of the protein (Fig. 4).²¹,²²

Properties and applications of random, smart polymer-protein bioconjugates

Phase separations for recovery

The thermally induced precipitation of a PNIPAAm-protein bioconjugate from a complex solution will simultaneously and selectively remove only the protein that is conjugated to the PNIPAAm from the solution. We have used this phenomenon for the separation of an enzyme from its reaction solution, in order to enable both recovery of the product from the supernatant and the recycling of the enzyme (Fig. 6).¹⁶–¹⁸

If the conjugated protein forms a complex with another biomolecule, for example by affinity recognition, then the complex will also be selectively precipitated from solution (Fig. 7). We have used this phenomenon to selectively remove IgG from solution as a PNIPAAm-protein A/IgG complex, in a fashion similar to affinity chromatography, but in this case it is carried out by reversible phase separation from a solution, instead of flowing through and eluting from a packed column.¹⁵

This thermally induced affinity precipitation process may be extended to stimuli-induced phase separation of a biotinylated target molecule which is complexed to avidin or streptavidin. In this case, a biotinylated target molecule is first complexed with an excess of avidin or streptavidin molecules in solution, such that at least one of the four (on average) biotin binding sites remains free. Then, an end-linked biotin-smart polymer conjugate is permitted to bind to the free site on the avidin or streptavidin molecules. Following this, the bioconjugate/affinity complex can be phase-separated by raising the temperature above the LCST of the smart polymer (or by changing the pH if...
it is a pH-sensitive smart polymer), which selectively removes the biotinylated target molecule from solution.32

Phase separation immunoassays

We have extended the affinity phase separation concept to the selective isolation and assay of an analyte from a complex mixture such as a serum sample. This is done by conjugating a first antibody to the polymer, complexing the analyte by affinity to the first antibody, and then introducing a second, labeled antibody, which then binds to the analyte by affinity to a similar or a different site (epitope) on the analyte. This yields a temperature-sensitive polymer conjugated to an immune complex sandwich, which can then be selectively removed by thermally-induced precipitation. This is an especially important separation step, because an excess of the labeled, second antibody is usually added to the sample. Washing and dissolution in cold buffer permits easy assay of the analyte (Fig. 8a).10–14 This immunoassay resembles an enzyme-linked immunosorbant assay (ELISA) done in solution. This concept has been extended to assay of two different analytes in the same test sample. If NIPAAm is copolymerized with a more hydrophilic or a more hydrophobic comonomer, then copolymers with higher and lower LCSTs can be obtained. If one of each of these two different LCST copolymers is conjugated to a different antibody, then two different analytes may be assayed in the same serum sample by sequentially raising the temperature of the system, to sequentially phase-separate the two different poly-

---

**Figure 8.** (a) Schematic illustration of the stimuli-induced phase-separation of a random conjugate of a smart polymer and a recognition protein coupled to a specific analyte which is coupled to a second, labeled recognition protein, to form a smart polymer-immune complex sandwich conjugate. This process is essentially an immunoassay such as ELISA (enzyme-linked immunosorbant assay) carried out in solution.1–3 (b) Schematic illustration of the same process carried out with one test sample, in order to assay two separate analytes in the same sample by using two different smart polymers having different LCSTs (e.g., see Fig. 3). This two step process may use two temperature-sensitive polymers, two pH-sensitive polymers, or one of each.
mer-conjugated immune complex sandwiches (Fig. 8b). One could also carry out such a dual affinity separation or dual immunoassay using combinations of two different pH-sensitive smart polymers, or one temperature- and one pH-sensitive smart polymer.

Properties and applications of site-specific, smart polymer-protein bioconjugates

Site-specific cytochrome-b5 conjugates located away from the active site

We first created a mutant cytochrome-b5 with a cysteine located far from the active binding site, and conjugated PNIPAAm to the thiol group using maleimide chemistry. We showed that the cytochrome-b5 was reversibly precipitated and redissolved by thermal cycling through the LCST, and also that the conjugation did not significantly reduce the activity of the protein. This is an extension of the randomly conjugated enzyme phase-separation process discussed above, as shown in Figure 6. The site-specific conjugation is designed to assure a minimal loss in activity of the protein after conjugation with the smart polymer.

Site-specific conjugates located near the active site

Conjugation of a responsive polymer to a specific site near the ligand-binding pocket of a genetically engineered protein is a powerful new concept. The site-specific placement of a smart polymer near the active site of a protein can permit sensitive environmental control of the ligand/protein receptor recognition process, which controls all living systems. Then, small changes in environmental conditions can cause large changes in the polymer conformation, leading to reversible “blocking” or “unblocking” of the protein’s active site, and such changes can also lead to triggered release of a bound ligand from the protein binding site (Fig. 9).

Site-specific streptavidin (SA) conjugates that control biotin-SA binding

We then selected streptavidin (SA) to study. SA has become one of the most widely used proteins in affinity separations, laboratory assays, and clinical diagnostics because of its very high binding affinity for biotin. There are also several interesting in vivo applications under development for the use of SA with biotinylated drugs or imaging agents. In order to attempt to control the biotin binding process, we decided to conjugate PNIPAAm near or within the active site of SA. We initially selected the N49 site, which is located on the loop above the biotin-binding pocket of SA, and genetically engineered a unique cysteine thiol group at that site, called the N49C mutant (native SA has no cysteine residues). After that we conjugated a vinyl sulfone-terminated PNIPAAm to the thiol group at that site. We found that the thermally induced collapse of the polymer acted as a “molecular gate” to control the association of biotin with SA, which had been immobilized on a solid surface to avoid its precipitation from solution (Fig. 9). Using another SA mutant, E116C, we found that the molecular weight of the polymer had an important influence on the gating process, which was only efficient above PNIPAAm MW of ca. 7,000, supporting the concept of steric interference of the biotin at the binding site.

Triggered release of bound biotin from site-specific SA conjugates

When biotin is bound to a PNIPAAm-E116C SA conjugate at temperatures below the LCST, we found that raising the temperature to thermally induce the collapse of the polymer “triggers” the release of some of the bound biotin molecules (Fig. 9). By cycling the temperature through the LCST several times, we were able to release all of the bound biotin. Such triggered release of bound ligands could be used to release therapeutics, such as for topical drug delivery to the skin or mucosal surfaces of the body, and also for localized delivery of drugs within the body by stimulated release at pretargeted sites using...
noninvasive, focused stimuli, or delivery of stimuli from catheters. Triggered release could also be used to release and recover affinity bound ligands from chromatographic and other supports in eluate-free conditions, including the capture and release of specific cell populations to be used in stem cell and bone marrow transplantation. These processes could involve two different stimuli-responsive polymers with sensitivities to the same or different stimuli. For delicate target ligands such as peptides and proteins, recovery could be effected without the need for time-consuming and harsh elution conditions. Triggered release could also be used to remove inhibitors, toxins, or fouling agents from the recognition sites of immobilized or free enzymes and affinity molecules, such as those used in biosensors, diagnostic assays, or affinity separations. This could be used to “regenerate” such recognition proteins for extended process use.

Site-specific conjugates of PNIPAAm-SA linked by hybridized DNA sequences to control the distance of the PNIPAAm from the SA binding site

We recently applied an interesting construct to control the distance of the PNIPAAm from the active site. For this purpose, we conjugated one sequence of complementary nucleotides to the E116C site near the binding pocket of SA, and a second sequence to the end of a PNIPAAm chain. Then, by controlling the location and length of the complementary sequence, the self-assembly via hybridization of the two single chain DNA sequences was used to control the distance of the polymer from the SA binding site. This is illustrated in Figure 10.27

Size-selective blocking of biotinylated proteins by site-specific PNIPAAm-SA conjugates

We have most recently investigated the gating of biotinylated proteins by a site-specific poly(N-N,N-diethylacrylamide) or (PDEAAm)-SA conjugate, and discovered that the gating process is sensitive to the size of the protein. For this study, we cloned a double lysine SA mutant, E51K/N118K, that was designed to localize two reactive lysine amino groups near the biotin binding pocket, and thus to enhance the conjugation efficiency for amino-reactive succinimidyl groups on PDEAAm. Computer modeling has suggested that the two engineered lysine residues are more solvent-accessible than the native SA lysines or the N-terminal amine. Previous characterization of the existing amine reactivity of native SA showed that its few native lysine amino groups are relatively unreactive. Thus, we were able to preferentially conjugate one of the genetically engineered lysines at the E51K/N118K sites with amine-reactive PDEAAm. (It is unlikely that both lysine groups could be simultaneously conjugated with high efficiency).

We found that the largest biotinylated protein we studied, IgG (MW 150 kD), was unable to bind to the SA conjugate whether the polymer was above or below its LCST, while the smallest biotinylated protein we studied, Protein G (MW 6.2 kD), was able to bind whether the polymer was hydrated or collapsed. An intermediate size biotinylated protein, bovine serum albumin, or BSA (MW 67 kD), exhibited increased binding as temperature was raised through the LCST of the PDEAAm (ca. 23°C in PBS). This surprising result seemed to contradict our observations of biotin binding by itself, which was blocked from binding above the LCST of PNIPAAm (coincidentally, the LCSTs of both polymers in water are ca. 32°C; addition of buffer and salt will lower their LCSTs). We have concluded that the result with biotinylated BSA is related to the tetrameric structure of SA, which has four binding sites, two each on opposite faces. Our data reveal that we have, at best, only conjugated one polymer molecule on each face, probably due to steric considerations. Thus, the increase in binding of biotinylated BSA as temperature is increased through the LCST reflects the increasing exposure of the adjacent SA binding site, which is on the same face of SA as the conjugated site. This result is not related to binding at the conjugated site itself, which probably blocks binding of any size biotinylated protein, whether the polymer is above or below its LCST. Thus, below the LCST, the hydrated polymer sterically interferes with the access of the biotinylated BSA at the adjacent binding site, while above the LCST, its collapse is enough to sterically expose that adjacent site, permitting the biotinylated BSA to bind.

Site-specific conjugation of a pH- and temperature-sensitive polymer to SA

We have observed similar results to those seen with the PNIPAAm-E116C SA conjugates when we pre-
pared an E116C SA conjugate with a PNIPAAm-acrylic acid (AAc) copolymer that exhibits combined temperature- and pH-sensitivity. By incorporation of as little as 5.5 mol % of AAc in a random copolymer with NIPAAm, we obtained a copolymer that is completely soluble at 37°C and pH 7.4, and insoluble at 37°C and pH 4.0. When this copolymer is conjugated to the E116C site of SA, biotin binding at 37°C is significantly reduced at pH 4.0, compared to pH 7.4. This is most likely due to the more compact copolymer coil at pH 4.0 and 37°C, compared to that at pH 7.4 and 37°C. After we allowed biotin to bind at 37°C and pH 7.4, we were able to trigger the release of some of the bound biotin by changing the conditions to pH 4.0.

Site-specific conjugates of temperature- and light-sensitive polymers to SA

In order to create photo control of enzyme activity we have synthesized two different copolymers that exhibit combined temperature- and photo-sensitivity. The copolymers were prepared by copolymerizing N,N-dimethyl acrylamide (DMA) with two different light-sensitive comonomers, 4-phenylazophenyl acrylate (AZAA) and N-4-phenylazophenyl acrylamide (AZAAm) (Fig. 11). The copolymers were identified as DMAA and DMAAm, respectively. DMAAm and DMAA showed ca. 8–10°C differences in their LCSTs when irradiated with UV and visible (VIS) light. Surprisingly, they showed the opposite photocoupling responses from each other using the same photo-stimuli. Under the isothermal conditions at their photo-responsive temperatures, DMAAm precipitated under UV light irradiation (350 nm), while DMAA dissolved under the same UV light irradiation. This difference is related to the difference of the linkage between the azobenzene groups and the polymer backbone, which are either amide or ester bonds. We derivatized the copolymers with vinyl sulfone end groups for specific conjugation to the E116C SA thiol group.

One of these copolymer-E116C SA conjugates exhibited blocking of free biotin and triggered release of bound biotin under VIS irradiation, while the other demonstrated the same phenomena under UV irradiation. These opposite photo-responsive biotin blocking or releasing responses corresponded to the original photo-induced phase transition properties of the copolymers. The vinyl sulfone-terminated DMAAm and DMAA copolymers are also being utilized for site-specific conjugation to a mutant enzyme. That site-specific enzyme conjugate is currently in preparation for use as a photo-switchable enzyme. Such light-controlled binding and release of site-specific protein conjugates may be utilized as a molecular switch for various applications in biotechnology, medicine, and bioelectronics.

CONCLUSIONS

We have conjugated stimuli-responsive polymers to random and specific sites on a variety of useful and important proteins, and in the latter case, we demonstrated the ability of the polymer to control the protein-ligand recognition and binding process. Table 3 summarizes the different possible molecular actions of these “really smart” bioconjugates. Site-specific conjugation combines genetic engineering techniques that allow us to place the mutant residue in one (or more) selected location(s), with polymerization techniques that permit us to vary the molecular weight (thus the polymer coil size) and the composition of the stimuli-responsive polymer (thus the type and magnitude of the stimulus to be applied). Therefore, each polymer-protein conjugate can be molecularly engineered for each particular application. It should be emphasized that the site-specific placement of responsive polymers near the binding sites of recognition proteins can provide very sensitive and precise environmental control of the receptor protein-ligand recognition process, which is the process controlling all living systems. Furthermore, triggered-release of a bound ligand may have many interesting applications in drug delivery, delivery of chemical or biochemical reagents in a process reaction, and signal generation in general.

Table III
Molecular Actions of “Smart” Polymer-Engineered Protein Conjugates

<table>
<thead>
<tr>
<th>Action Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Control of enzyme-substrate reaction rates</td>
</tr>
<tr>
<td>* Control of affinity ligand binding rates and selectivities</td>
</tr>
<tr>
<td>* Eluate-free recovery of affinity ligands</td>
</tr>
<tr>
<td>* Size-dependent enzyme-substrate reactions and affinity separations</td>
</tr>
<tr>
<td>* Capture and recovery of specific cells</td>
</tr>
<tr>
<td>* Response to and generation of optical or electrical signals</td>
</tr>
<tr>
<td>* Ability to switch protein recognition processes and products “on” and “off”</td>
</tr>
</tbody>
</table>
Table IV

<table>
<thead>
<tr>
<th>Applications of “Smart” Polymer-Engineered Protein Conjugates</th>
</tr>
</thead>
<tbody>
<tr>
<td>· Affinity separations of molecules and cells</td>
</tr>
<tr>
<td>· Clinical diagnostics and immunoassays</td>
</tr>
<tr>
<td>· Environmental sensors and biosensors</td>
</tr>
<tr>
<td>· Regeneration of “fouled” sensors</td>
</tr>
<tr>
<td>· Cell culture (fermentation) processes</td>
</tr>
<tr>
<td>· Triggered release (removal) of enzyme-bound inhibitors, products, toxins and fouling agents</td>
</tr>
<tr>
<td>· Triggered release of drugs</td>
</tr>
<tr>
<td>· Drug “discovery”</td>
</tr>
<tr>
<td>· Information storage and retrieval</td>
</tr>
</tbody>
</table>

There have been a number of successful uses and there are many more potential applications for these exciting “doubly smart” polymer-protein conjugates in medicine and biotechnology (Table 4), and as such they represent an important extension of polymeric biomaterials beyond their well known uses in implants and medical devices.

The main author of this article (ASH) would like to express his great gratitude for the many creative ideas of his students and colleagues and their productive collaborations over the past 18 years in the area of smart polymer-protein bioconjugates. We all share the 2000 Founders’ Award of the Society for Biomaterials.

References