Design and Applications of Responsive Polymers in Diagnostics, Separations, Bioprocesses, and Drug Delivery

Allan S. Hoffman, Patrick S. Stayton
plus many students, postdocs and collaborators,
Bioengineering Department, University of Washington, Box 355061, Seattle, WA 98195
hoffman@u.washington.edu

Random conjugates of smart polymers and proteins: We have been designing and synthesizing a wide variety of stimuli-responsive polymers (which are sometimes called "smart" polymers) for many different applications in biotechnology and medicine. We have conjugated or complexed the responsive polymers to (a) proteins such as streptavidin, antibodies and enzymes, (b) nucleic acids and (c) a variety of drugs. Random conjugation of the smart polymer to a protein is usually carried out by reaction of a terminal or pendant group of the polymer to a protein's lysine amine group. Such a conjugate of a smart polymer and an affinity protein (called the capture molecule) may recognize and bind to the affinity binding partner of the protein (called the target molecule), and then this polymer-protein conjugate may be phase-separated from solution by a specific stimulus, selectively removing the target molecule from the complex mixture. In this way, an affinity separation process may be carried out in solution, rather than in a packed chromatographic column. Such a process might be used to recover a product from a process stream, or to remove a toxin from a fluid mixture. If a second, labeled affinity protein for the target molecule is added to the complex mixture, it will bind to the target molecule and then the three, affinity-linked proteins (eg, immune complex "sandwich") may be removed by stimulating the smart polymer to phase separate. The signal from the labeled protein will then be an indication of the concentration of the target molecule in the test solution. This is like an immunoassay carried out in solution rather than in a well on a plate reader (ELISA)

Site-specific smart polymer-protein conjugates: We have extended this technology to site-specific protein-polymer conjugates, where the conjugation site is near the protein's active site. To do this we use site-specific mutagenesis to form mutant proteins with cysteine -SH groups near the active site, to which we conjugate our smart polymer. This permits us to block and unblock the active, recognition site of the protein by different stimuli, and sometimes to do this without phase separation of the conjugate.

Dual-responsive smart copolymers: We have synthesized polymers and copolymers with dual responsivities to combinations of temperature, pH and light (visible-UV) stimuli. The polymer structures may be random, block or graft copolymers. Most recently we have used living free radical polymerization techniques such as RAFT to form block copolymers with two different sensitivity blocks. We have demonstrated control of an enzyme bioprocess using both light- and temperature-sensitive copolymer-enzyme conjugates.

Microfluidic applications: We have similarly bound the smart polymer and capture biomolecule (affinity protein or nucleic acid) to nanobeads and utilized the beads for applications in microfluidic devices for point-of-care diagnostics, which operate with affinity capture of target molecules as a first step followed by stimulation to effect physical separation as a second step. The responsive polymers may be stimulated to phase separate and this causes the protein conjugate to bind by hydrophobic interactions to the channel wall of the device. Site-specific conjugation of dual-sensitivity random, block or graft polymers to selected proteins may be especially useful for these purposes.

Intracellular drug delivery applications: The endosome of a cell is acidic and fusogenic peptide sequences in viral protein coats respond to the lowered pH to disrupt the endosomal membranes, releasing their genomic cargo into the cytosol. We are taking advantage of this mechanism by designing synthetic, biomimetic polymers that are pH-responsive, and act similarly to the pH-responsive viral peptides. We have

applied pH-sensitive and combined pH- and temperature-sensitive polymers and copolymers to enhance intracellular drug delivery, especially to facilitate endosomal escape to the cytosol of protein drugs or nucleic acid drugs such as plasmid DNA, antisense ODNs and siRNA.

Selected references: A) Smart Polymer Conjugates and Hydrogels

T.G. Park and A.S. Hoffman, "Effect of Temperature Cycling on the Activity and Productivity of Immobilized b-Galactosidase in a Thermally Reversible Hydrogel Bead Reactor", *Appl. Biochem. and Biotech.*, 19, 1-9 (1988)

L.C. Dong and A.S. Hoffman, "A Novel Approach for Preparation of pH- and Temperature-Sensitive Hydrogels for Enteric Drug Delivery", *J. Contr. Release*, 15, 141-152 (1991)

P.S. Stayton, et al., "Control of protein-ligand recognition using a stimuli-responsive polymer", *Nature,* 378, 472-474 (1995)

A. S. Hoffman, et al., "Really Smart Bioconjugates of Smart Polymers and Receptor Proteins", *J. Biomed. Mater. Res.,* 52, 577-586 (2000)

Z. Ding, et al., "A Smart Polymer Shield that Controls the Binding of Different Size Biotinylated Proteins to Streptavidin", *Nature*, 411, 59 - 62 (2001)

T. Shimoboji, et al., "Mechanistic Investigation of Smart Polymer-Protein Conjugates", *Bioconj. Chem.*, 12, 314-319 (2001)

T. Shimoboji, et al., "Photoresponsive polymer-enzyme switches", *PNAS*, 99, 16592-6 (2002)

N. Malmstadt, et al., "A Smart Microfluidic Affinity Chromatography Matrix Composed of Poly (N-isopropylacrylamide)-Coated Beads",*Anal Chem,* 75, 2943-2949 (2003)

N. Malmstadt, et al, "Smart Mobile Affinity Matrix for Microfluidic Immunoassays", *Lab Chip*, 4, 412-415 (2004)

P.S. Stayton, et al., "Smart polymer-streptavidin conjugates", *Methods Mol Biol.* 283: 37-44 (2004)

S. Kulkarni, et al., "Meso-scale Smart Polymer-Protein Particles that Form Reversibly and with Controlled Sizes", *Bioconj Chem.*, 15, 747-53 (2004)

P.S. Stayton, et al., "Intelligent Biohybrid Materials for Therapeutic and Imaging Agent Delivery", *Proc. IEEE*, 93, 726-736 (2005)

S. Kulkarni, et al., "Controlling the Activity and Aggregation of Conjugates of Streptavidin with Smart Block Copolymers Prepared via the RAFT Copolymerization Technique", *Biomacromol,* (2006)

X. Yin, et al., "Temperature- and pH-Responsiveness of Poly(*N*-Isopropylacrylamide-*co*-Propylacrylic acid) Copolymers Prepared by RAFT Polymerization" *Biomacromol*, (2006)

M. Ebara, et al., "Switchable surface traps for injectable bead-based chromatography in PDMS microfluidic channels", *Lab Chip*, (2006)

Selected references: B) Smart Polymers in Intracellular Drug Delivery

C.A. Lackey, et al., "A Biomimetic pH-Responsive Polymer Directs Endosomal Release and Intracelllar Delivery of an Endocytosed Antibody Complex", *Bioconj. Chem.* 13, 996-1001 (2002)

N. Murthy, et al., "Bioinspired polymeric carriers that enhance intracellular delivery of biomolecular therapeutics" *Bioconj. Chem.* 14, 412-419 (2003)

N. Murthy, et al., "Design and synthesis of pH-Responsive Polymeric Carriers That Target Uptake and Enhance The Intracellular Delivery of Oligonucleotides to Hepatocytes " *J. Cont. Rel*., 89(3); 365-374 (2003)

V. Bulmus, et al., "A new pH-responsive and glutathione-reactive, membrane-disruptive polymeric carrier for intracellular delivery of biomolecular drugs", *J. Contr. Rel* , 93 105– 120 (2003)

C.Y. Cheung, et al., "Poly(propylacrylic acid) reduces the Inactivation of Polyplexes by Serum", *J. Biomater. Sci, Pol. Ed.*, 16, 163-180 (2005)

T. Kiang, et al., "Formulation of Chitosan/DNA Nanoparticles with Poly(propylacrylic acid) Enhances Gene Expression", *J. Biomater. Sci, Pol. Ed*,, 15, 1405-1422 (2005)

D. Pack, et al., “Design and Development of Polymers for Gene Delivery”, *Nature Reviews, Drug Discovery,* 4, 581-593 (2005)

G. Tae, et al., “PEG-Crosslinked Heparin is an Affinity Hydrogel for Sustained Release of VEGF”, *J. Biomater. Sci, Pol. Ed.,* 17, 187-197 (2006)

S.M. Henry, et al., “pH-Responsive Poly(styrene-alt-maleic anhydride) Copolymers for Intracellular Drug Delivery”, *Biomacromol,* (2006)