Smart Polymers, Then and Now

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

Introduction: This talk is mainly intended to give the early history of my involvement with smart polymers, since it was in that same period of time that I developed my friendship and respect for Stuart Cooper. Stuart has made immense contributions to our field in basic research, in teaching, in our published literature, and in academic administration. Any one of these would have been sufficient for us to salute Stuart on his 65th birthday, but taken together he stands alone in our field as the truly Complete Biomaterials Man of the 20th and 21st Centuries! Happy birthday, Stu, and many more!!

In the beginning of this talk I will describe when and why I first thought about using smart polymers in biotechnology. The story begins in 1982 when I met Bob Nowinski, MD, PhD. We were put together by a mutual friend who knew about Nowinski's needs, and who also knew about my work focused on using polymers in new ways as biomaterials. Nowinski had recently founded a new company in Seattle called Genetic Systems Corporation (GSC). GSC had licensed many monoclonal antibodies (MoAb) from the Fred Hutchinson Cancer Research Center, and they wanted to develop both therapeutic and diagnostic products based on those antibodies. In particular, Nowinski realized that there was a growing market for immunoassays used to screen blood in blood banks for previous exposure to various viruses such as HIV and Hepatitis B. Abbott was already selling their ELISA kits to blood banks for this use, and to encourage the banks to use their kits, Abbott was giving away the plate readers that were needed to run the assay. Nowinski wanted to use GSC's antibodies in a new immunoassay that would compete with ELISA and Abbott for the blood bank screening business. He asked me if I could selectively separate certain proteins out of blood serum "using polymers". So I began to try to solve this problem as a consultant to GSC in 1982.

First I looked into ELISA immunoassays, and realized that the target wasn't simply one protein, but it was an "immune complex sandwich" formed by a capture MoAb complexed to the target protein analyte—probably another MoAb that had formed as an immune response to a viral protein coat—and complexed once more to another, labeled antibody to the target analyte. This second MoAb to the target analyte could be labeled with a radiolabel or a fluorescent label. The three proteins together formed an "immune complex sandwich", which was the actual "analyte" being assayed in the ELISA plate reader. So I began to think about how to combine a polymer and the immune complex sandwich, and then how to cause the polymer-protein complex to separate out of solution, either by phase separation or by physical adsorption to a surface.

My first idea was based on an earlier observation that my then postdoc, Buddy Ratner, and I had made while we were working on the radiation grafting of hydroxyethyl methacrylate (HEMA) to silicone rubber in the early 1970s. Although the monomer HEMA was water- soluble, PolyHEMA was not water soluble, and the polymer phase separated as it formed. So I thought we could conjugate vinyl groups to a protein and then copolymerize the monomer-derivatized protein with HEMA, causing it to phase separate as a polymer-protein conjugate. The protein would be a model antibody such as IgG, and represent the first, capture MoAb in the immunoassay. Later we would try the whole immune complex sandwich. We monomer-conjugated the protein, and used an aqueous redox initiator to copolymerize it with HEMA, and the protein phase-separated with the polyHEMA. It also worked with the immune complex sandwich, and with blood samples. GSC was excited by this technology, filed patents, and put a team of scientists together to try to make it work as an immunoassay. They envisaged a simpler, scaled-down version of the fluorescence-activated cell sorter (FACS) as their assay method, counting the small labeled particles as they formed. They described it in their 1983 annual report as "The technology combines recent advances

in the fields of polymer chemistry, monoclonal antibodies and DNA probes". In 1984 they established a 22.5 million dollar "diagnostic partnership" that involved a number of partners, including Institute Pasteur in Paris with their AIDS diagnostic IP, and Applied Biosystems, a California company that had recently successfully developed a family of protein and DNA sequencers and synthesizers. GSC called it a "polymerization-induced phase separation immunoassay" or PIPSIA.

Meanwhile, I was concerned with two possible problems with this assay: the first was the need to exclude oxygen and the second was the potential presence of polymerization inhibitors in serum. So I began to look for other ways to separate such protein complexes with polymers. In 1983 the ACS had their annual meeting in Seattle, and I ran into an old friend, Jim Guillet, a chemistry professor from the University of Toronto. I asked Jim if he knew about polymers that phase separated from aqueous solutions, and he immediately told me about poly(N-isopropyl acrylamide) or PNIPAAm, which he had worked with in the late 1960s. *This was the moment that I began my involvement with "smart" polymers.*

I told GSC about this second possibility for phase-separating the immune complex sandwich, and they put three chemists on this project. It soon became apparent that the thermally-induced phase separation with PNIPAAm was a much more efficient method than the polymerization assay, and results showed sensitivities down to ng/ml concentrations of the target antigen. This was as good or better that the current ELISA sensitivity. Thus, in 1984-5 GSC filed patents and began to develop the "thermally-induced phase separation immunoassay" or TIPSIA. However, now heating and cooling were required in the modified FACS test equipment being developed. This slowed the commercial development of the assay. (PCR had not been invented and there were no simple heating and cooling machines on the market. The lack of heating and cooling in the FACS machines available at that time significantly slowed the development of the assay).

Meanwhile, Abbott continued to sell their ELISA kits to blood banks and to give away the ELISA readers. Nowinski decided that GSC should get into the immunoassay business sooner rather than later, and GSC began to develop and market their own ELISA kits, using their proprietary antibodies. Thus, some time in the mid 1980s, GSC decided to stop development of this new immunoassay. Late in the 1980s GSC was sold to Bristol Myers, and Nowinski became a vice president of BM. Subsequently the diagnostics IP (many patents had issued) was spun off to a new company called Sanofi-Pasteur Laboratories Diagnostics. Ultimately that company was sold to Bio-Rad, who now own all of the phase separation immunoassay patents. Nowinski later left BM to co-found ICOS in Seattle with two others.

My attention turned to work on hydrogels of PNIPAAm, sometimes including other comonomers such as acrylic acid (AAc). This and subsequent research on smart polymer systems was carried out at the University of Washington. We incorporated drugs in the smart gels and showed that we could release a non-steroidal anti-inflammatory drug gradually and linearly with time from a NIPAAm-AAc copolymer gel at enteric pHs, while avoiding release at gastric pHs. (The NIPAAm component resisted swelling at 37°C, while the AAc component only induced swelling when it became ionized at physiologic pH). We also incorporated enzymes and cells in the PNIPAAm gels and showed that we could turn them on and off, and on again, by cycling temperature around the phase separation temperature of PNIPAAm.

In 1992 Pat Stayton arrived at the University of Washington, and my attention turned back to PNIPAAm-protein conjugates. He suggested that we could conjugate PNIPAAm to specific sites cloned into streptavidin (SA) by site-specific mutagenesis, especially sites near the biotin binding sites of SA. We did that, and then we cycled temperature to thermally-induce the collapse or rehydration of the conjugated PNIPAAm chain. In this way, we were able to control the binding of biotin and biotinylated proteins, depending on the size of the latter.

I will finish my presentation with a few brief comments about our latest work with smart polymers. Once again, Happy Birthday Stuart!

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