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by Robert Stevenson

20th International Light Scattering Symposium

The interaction of light with matter is a complicated and useful phenomenon. Chemists are quite comfortable with simple absorbance of light by atoms and small molecules. Beer's law describes the phenomenon and, as a result, photometry is very useful, especially for small molecules. However, as the analyte gets much larger, significant light scattering occurs. The physics quickly gets much more complicated. But modern instrumentation, including software, can still factor out these effects and deliver useful results in our computers.

The 20th International Light Scattering Colloquium (ILSS) attracted about 80 scientists to the beautiful and historic Four Seasons Biltmore Hotel in Santa Barbara, CA, October 19 and 20, 2009. One cannot imagine a more stimulating setting, with the gentle Pacific Ocean surf 50 feet from the lectern. The setting complemented the two-day program, which covered advances in light scattering instrumentation and enabled applications. The atmosphere was relaxing, which set the mind free to concentrate on the science. It must be working for **Wyatt Technology Corp.** (Santa Barbara, CA), since it was named one of the top 10 best places to work for a second consecutive year by *The Scientist*.

Dr. James Burns, Senior VP of Drug and Biomaterials R&D for **Genzyme** (Cambridge, MA) opened the meeting with a plenary report on the use of light scattering in product development. He began by describing the use of hyaluronan (HA) in ocular surgery. HA prevents adhesion, thus making lens replacement practical. The trick was to develop a synthetic route that provided HA with physical properties similar to the natural product isolated originally from chicken and rooster combs. **Genzyme** used steric exclusion chromatography with multiangle light scattering (SEC-MALS) to optimize the analytical process. Along the way, researchers found evidence of distinct free and entangled regions for the polymer that also correlated with molecular weight and performance.

A second report studied the role of HA in cartilage, where it holds proteoglycans together and also imbibes water to act as a shock absorber, much like Silly Putty® (Crayola, Easton, PA). It was found that with low-frequency movement, the energy is dissipated with viscous flow, but with high-frequency impact, the entangled molecular network resists deformation, acting as a shock absorber. This led to a product that can be injected into a knee joint, reducing pain and restoring normal movement for up to six months. Competitive products use smaller HA molecules that lack the entanglement and associated impact cushioning, plus they are readily cleared from the body.

Saccharides, including starch

Prof. Bruce Hamaker (Purdue University, Lafayette, IN) introduced the topic of polysaccharides for food, including starches and how they change during processing, cooking, and digestion. In general, the molecules are very large, and can be part of even larger structures. He reported that asymmetric flow field-flow fractionation (AF4) combined with MALS detection is the best technology for characterizing starch, since it provides molecular weight (MW) distribution as well as the RMS radius, Rg. The two most abundant components of starch are amylopectin (AMP, 10^7–10^9 Da) and amylose (AML, ~10^5–10^6 Da). A typical starch granule contains about five 10^7 molecules of AMP. Origin and prior treatment of the starch affect the structure significantly. The nutritional value of starch is highly dependent on AMP, since the texture and preparation are tied to glucose through AMP.

Typically, dimethylsulfoxide (DMSO) is used to dissolve the sample prior to assay by SEC-MALS. Linear velocity in the column needs to be kept low to prevent degradation by shear forces. One example used this technology to study the starch content of nine varieties of rice grown in regions of the U.S.A. The MW and Rg distributions were noticeably different. For amylopectin, the degree of branching correlated with growing at lower temperature (from Missouri), producing the highest percentage of short chains. Another study focused on the shortened bowl life of corn flakes with some sources of corn, but not others. The rapid loss of crispness is due to fragmentation of the amylopectin during processing, which affects the thickness of the flake. Prof. Hamaker was very optimistic that AF4 with MALS-RI (refractive index) will improve and facilitate measurement of the tertiary and quaternary structure of amylopectin, leading to new properties and products. For example, if the structure is stabilized, the rate of glucose release can be reduced. Perhaps this can diminish the urge to snack. Two similar reports using SEC-MALS for characterization of starch were included in the poster session.

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Proteins

Almost half of the program focused on proteins as the analyte class. Prof. Andreas Pluckthun (Universitat Zürich, Switzerland) presented a thoughtful, critical review on the development of protein therapeutics. Protein therapeutics work by interacting with other proteins in vivo. This immediately leads to more questions about the stoichiometry, location, and stability of the interaction. Depending on the molecular weight, he uses either SEC or AF4 for the characterization of the association reaction.

However, the candidate therapeutic usually suffers from side reactions such as agglomeration, oxidation, and unstable tertiary structure. Prof. Pluckthun sees these as opportunities to redesign the protein. For example, cysteines can be removed to eliminate oxidation susceptibility. Dimerization can be eliminated by changing the association site.

Prof. Pluckthun selected ankyrin repeat proteins as an attractive scaffold since it has several helices that provide astounding theoretical diversity of 7.2×10^7 in each helix. These variants can be directly programmable from the DNA or randomly generated by ribosome display. He calls these designed ankyrin repeat proteins (DARPs). He contrasted DARPs with antibody-based technology: "There are lots of proteins, with lots of different important epitopes in lots of different organisms. Is there really no better idea around than to make monoclonal antibodies to all of them? Do you really want to handle a million cell lines?" He clearly thinks that DARPs offer a superior alternative. He went on to show that for one cell marker antibodies and DARPs bonded to different epitopes. Perhaps diversity may be useful.

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Detergent selection for membrane proteins

Dr. David Veesler of the Université Aix-Marseille I & II (Marseille, France) compared solubilization of membrane proteins with various detergents. This is an important topic, since membrane proteins constitute a third of the open-reading frames of all genomes. The protein detergent complexes (PDCs) can be separated with SEC, but the calibration curve is seldom accurate since the complex is the item being separated. He reported a method for characterizing PDCs with SEC-MALS, which gives the molecular weight from light scattering. He compared the solubilization of Methanosarcina mazei CorA transporter with detergents *N*-dodecyl-*N*,*N*-dimethylamine-*N*-oxide (LDAO) and dodecyl maltoside (DDM). He found that DDM solubilized the transport active pentamer of CorA, while the LDAO disrupted the pentamer to the non-native monomer. Detergent selection is an important issue. The value of this approach will probably rest on how well it works for the majority of membrane proteins.

Light scattering in protein therapeutics

A lecture by Dr. Reb Russel of **Bristol-Myers Squibb** (**BMS**, Doylestown, PA) showed that SEC with dynamic light scattering (DLS) detection is much more robust and powerful than DLS alone. Some examples that he discussed included: 1) Characterizing the molecular weight distribution of polyethylene glycol (PEG), which is used in a variety of products at **BMS**. Neither MS nor sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) provides results that are as reliable. 2) Characterizing the PEGylation of a protein. Results showed five peaks corresponding in molecular weight to multimers, atmospheric pressure ionization (API), and fragments. 3) Conformational heterogeneity of a protein product was revealed by SEC-MALS. MALS showed that the front of the peak had a molecular weight that was about 15 kDa lower than the material in the last half of the peak. 4) Samples taken in support of a comparability study clearly showed that some were not comparable, but others were indistinguishable based upon this test. 5) A comparison of SEC-MALS with analytical ultracentrifugation (AUC) for analysis of low- and high-molecular-weight impurities showed similar results, but the SEC had a much shorter turnaround time and was also less labor intensive.

Virus-like particles (VLPs)

The light scattering meeting was followed by a one-day users' meeting focused on AF4-MALS. This did not stop Prof. Anton Middelburg (University of Queensland, Australia) from describing the use of AF4-MALS for developing vaccines consisting of virus-like particles self-assembled from large proteins, in this case glutathione S-transferase (GST). He found that AF4-MALS provided the unique ability to differentiate between correctly assembled VLPs and closely related structural contaminants. While the initial results showed formation of some VLPs, the yield was low. The assembly process was then optimized by controlling the second virial coefficient to promote assembly.

Nuclear magnetic resonance (NMR) complements light scattering

Light scattering provides information about the size and motion of large particles, but Angela M. Gronenborn of the University of Pittsburgh Medical School (PA) showed that NMR can provide a much more detailed structure of large, complicated molecules. She said that it is so easy now to determine the primary and secondary solution structure that high school students can do it. NMR reveals amazing differences in protein association that change with substitution of a few or even one amino acid. In NMR studies, domain swapping between two identical proteins is the most common mechanism. The rate of reaction differs greatly from faster than NMR to separable by gel filtration, ion exchange, etc.

Gold nanoparticles

Dr. Anil K. Patri, Deputy Director of the National Characterization Laboratory (Frederick, MD), reported on the characterization of nanoparticles, particularly gold. Gold nanoparticles (GNPs) are a candidate for therapeutics. The activity depends on GNP size. With AF4, it is possible to separate a 10-nm GNP ladder into discrete bands. A high-ionic-strength mobile phase favors resolution, but additives can create adducts. In one case, PEGylation of GNP was followed by both batch DLS and AF4-DLS. For 20-nm GNPs, PEGylation increased the diameter to 49.4 nm (batch DLS) compared to 48.1 nm for AF4-MALS. At the conclusion, he invited the public to contact the National Characterization Laboratory for help in characterizing potential therapeutics (visit http://ncl.cancer.gov/assay_cascade.asp).

Polymers

Synthesis, followed by characterization of very large organic polymers, is an important commercial topic and an intellectual challenge. This was demonstrated by Prof. and Nobel Laureate Robert H. Grubbs of the California Institute of Technology (Pasadena). He received the award for his work on olefin metathesis using ruthenium complexes. His lecture touched on

many facets of organic synthesis, but the topic of very large cyclic polymers really caught my eye. He used a catalytic ring expansion metathesis reaction to make very long (megadalton) secondary amines (polyamines). Most are cyclic, but a few are not. These are large, so how to assay for cyclics? He took advantage of the natural avidity that crown ethers have for amines. He added 24 crown 8 to the solution. The cyclics remained unchanged, but the long, open-chain polymers threaded themselves down the barrel of the crowns in a matter of minutes. The cyclic polymers have low solubility in methylene glycol diethyl ether, but the crown–linear complex is very soluble. So precipitation works, as does liquid chromatography. He also made a stationary phase with 24 crown 8 as a bonded stationary phase. The cyclics come through in the void volume and the linear polyamines are retained by threading.

This may seem esoteric, but think of the possible products. Normally, oils age and wear by fission to smaller molecules, which lowers viscosity and lubricity. However, cyclic polymers are less viscous than the corresponding linear chain. So it might be possible to tune a mixture of linear and cyclic polymers to have constant viscosity as they wear. The loss of viscosity from degradation of the linear polymer could be offset by opening the cyclic polymer to a long, viscous linear polymer. This work required light scattering to follow the reactions and characterize the products.

Second virial coefficient

Just two years ago, **Wyatt** introduced the Calypso, an automated system for characterizing protein–protein interactions with static light scattering. Using three syringe pumps, the Calypso provides the concentration gradients in reagents that control the interactions. This flows through a DAWN MALS detector (**Wyatt**) plus a concentration-sensitive detector such as UV absorbance or RI. The base of applications is growing nicely. These include determination of the second virial coefficient (A_2) of proteins. This property is gaining recognition as an important parameter in controlling self- and heteroassociation of large molecules. It is important in crystallization and prediction of solubility. Several examples of protein–protein binding were presented. Calypso technology is gaining acceptance over more established techniques such as surface plasmon resonance (SPR) and AUC, primarily due to experimental throughput and the ability to handle and elucidate the stoichiometry of a reaction.

Summary and outlook

One of the key measures of the degree of technology adoption is use in process control. Usually a technology will first appear in process development laboratories, and later in process control and product release. Light scattering is clearly a recognized technology practiced by leading firms and also well accepted by the regulators. As the frontiers of research impinge on the new nanoworld, we will need techniques such as light scattering to guide our way and map our progress.

Wyatt deserves special recognition for organizing and hosting a first-class technical meeting focused on a technology niche. By mixing application reports with theory, the meeting achieved a very good balance with something for everybody. Equally important was the organization of the creature comforts. Attendees were provided a comfortable atmosphere for both novices and experts to talk shop. Personally, I was amazed at the warm hospitality exhibited by the entire **Wyatt** team during the meeting. About 20% of the firm was in attendance, so there was always an expert available. All in all, the 20th International Light Scattering Colloquium was a class act.

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