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If It's Spring, There's Chemical Biology in New Haven

Meeting Review

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The annual Yale Chemical Biology Symposium took place on May 12 and sparkled with exciting talks from scientists representing the breadth of chemical biology research. The program featured Drs. Laura Kiessling, Ann Valentine, Chad Mirkin, Ronald Raines, and Benjamin Turk, with Dr. Christopher Walsh presenting the keynote address. This year's talks touched on a variety of topics including stem cells, nanoscience, and the biosynthesis of natural products. The importance of bioinorganic chemistry and the essential contributions of metalloenzymes interlaced the talks throughout the day.

On Friday, May 12, members of the Yale and northeastern U.S. scientific communities braved torrential rain to participate in the annual Yale Chemical Biology Symposium. Although the event did not have a stated theme, this year's talks held a distinct bioinorganic flavor while showcasing a broad spectrum of chemical biology questions.

The day began with a talk from Dr. Laura Kiessling from the University of Wisconsin at Madison who described the initial stages of her work designing chemical approaches to controlling cell-fate decisions in embryonic stem (ES) cells. Differentiation relies on signaling, which is often mediated through clustered receptors, a process promoted by multivalent ligands. Biological roles for multivalency have been a long-standing interest of the Kiessling lab. Dr. Kiessling described her collaboration with Dr. Jamie Thompson to create synthetic versions of the niches, which are local environments where stem cells interact with morphogens, other cells, and the extracellular matrix and that determine how a stem cell will differentiate. Kiessling's synthetic niches are based on alkane thiol self-assembled monolayers and small-molecule or peptide-based morphogens. In a pilot assay, members of her group screened for peptides derived from laminin (a major component of Mattrigel, a mixture of mouse extracellular matrix proteins currently essential for culturing ES cells) that could induce ES cell proliferation on the synthetic surface. A population of self-renewing ES cells could create a pool on which to subsequently study differentiation without the need for harvesting primary cells from tissue. They were able to identify specific morphological changes in the treated ES cells in response to treatment with different peptides. Moreover, by looking at two different protein markers for differentiation, they could identify cells undergoing changes to their cellular program on the molecular level. To launch this approach, the Kiessling group developed array-patterning technology based on cytophobic fluorinated alkane thiols and applied a recently identified chemically defined culture medium to create fully controllable growth conditions. With an eye to the future, Dr. Kiessling discussed the potential for using this system to uncover specific differentiation signals and their relevant cellular receptors.

Dr. Ann Valentine of Yale University discussed work from her lab on titanium bioactivity. Eliciting periodic chuckles from the audience, she wittily placed her work in context by describing the challenges of securing funding to investigate metal-based therapeutics, the checkered history of titanium-based therapeutics like titanocene dichloride, and the success of titanium-based implants. A significant consequence of putting titanium into the human body is that it winds up distributed throughout and associates with both chromatin and specific proteins via an unknown mechanism. Dr. Valentine opined that understanding the basic chemistry of titanium complexes in aqueous solutions may provide insight into these observations. The major mechanistic concern in cells is that the titanium ligands hydrolyze, making the bioavailable species dramatically different from the starting compound. For example, titanocene's two chloride ligands are known to exchange with water within minutes of exposure to an aqueous environment. Dr. Valentine described one of her lab's goals: the development of stable, water-soluble titanium complexes. She went on to present results characterizing the stability of titanium citrate and ascorbate complexes. Such complexes may allow more specific targeting of titanium to cancer cells, for example, which could be cytotoxic. Moving to a more complicated system, Dr. Valentine discussed the role of transferrin as a cellular metal-ion shuttle. Although principally responsible for transporting iron into cells, transferrin binds to ~40 metal ions, including titanium and the bismuth found in PeptoBismol. Many tumors are known to require excess iron, and consequently, have increased expression of transferrin on their cells' surfaces. Using an inorganic model compound of transferrin and the protein itself, work from her lab has defined the thermodynamic parameters for titanium binding. From this and the work of others, she suggested that transferrin plays a key role in the activity of titanium in vivo.

The symposium turned to nanoscience as Dr. Chad Mirkin of Northwestern discussed challenges for the future at the interface of biology, engineering, chemistry, and materials science. He began by describing a remarkable advance in Dip-Pen lithography to expand the scale of the procedure. By increasing the number of pens acting in parallel and aligned by gravity, workers in his lab have been able to create an apparatus capable of creating in excess of 50,000 copies of a single entity (he showed a 2005 nickel at 80 nm resolution). Snazzy tricks aside, Dr. Mirkin described many areas where such technology could make a serious impact, including X-ray crystallography, catalyst-development, and whole-cell analyses. As one example, he presented a study of a nanoarray of immobilized viral particles, which retained full infectivity and, on the basis of a variety of genetic markers, were identical to unrestrained particles. Such arrays might facilitate the study of viral

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cooperativity. Switching gears, Dr. Mirkin introduced DNA nanoparticle conjugates (100–200 copies of a DNA on a 13 nm particle) that retain the recognition properties of the constituent DNA sequences and their development into molecular diagnostics. He began by laying out an approach to achieve 10^{-18}-fold sensitivity in single-mismatch detection and then moved on to explain how the technology could serve as the underpinning for "biological barcode" detection approach. His goal is to create an ultrasensitive diagnostic system with the ability to detect both nucleic acids and proteins. The approach combines a magnetic bead decorated with a specific antibody and the DNA-conjugated nanoparticle, magnetic separation of antibody-bound protein (or nucleic acid) from a patient sample, subsequent release of the "barcode DNA," and detection of the DNA sequence (in amounts proportional to the isolated macromolecule) with exquisite specificity. As an example, he discussed the merits of identifying changes in prostate-specific antigen (PSA) levels in postoperative men where levels are currently below detection, or the need for rigorous results to address the question of whether PSA is a marker for breast cancer. Dr. Mirkin concluded by highlighting the opportunities to develop the barcode approach for identifying other biomarkers, as well as small molecules and even metal ions.

After a break for lunch and a poster session, the symposium continued with a talk by Dr. Ronald Raines from the University of Wisconsin at Madison. He began by reacquainting the audience with the triple helix found in collagen, formed from repeats of a triplet of amino acids, and left the audience members looking at their arms as he cited the statistic that collagen provides three-quarters of the dry weight of skin. More surprisingly, 28% of the collagen repeats contain proline and 38% hydroxyproline, an amino acid created via posttranslational modification by α-ketoglutarate-dependent prolyl 4-hydroxylase, an iron-dependent enzyme. Prolyl hydroxylase activity is essential for *C. elegans* survival, and in humans, loss of prolyl 4-hydroxylase activity leads to scurvy. Conversely, overexpression of the enzyme produces fibrosis. Dr. Raines described the synthesis of α-ketoglutarate analogs as inhibitors, and experiments in worms to confirm their efficacy. Turning to the modification itself, he described efforts from his lab to investigate the functional role for the hydroxyl group. To test an existing mechanistic mode that proposes localization of two water molecules within each collagen repeat via hydrogen bonding with the hydroxyls, he and his colleagues synthesized a 4-fluoroproline analog and characterized the biophysical properties of fluorine-modified triple helices. Fluorinated helices had higher melting temperatures than either prolyl- or hydroxyprolyl-containing ones. Stereochemically, fluorination in the native *R* configuration was critical to stability. Dr. Raines concluded by describing evidence based on studies of the fluoro analog as well as other proline derivatives for a significant stereoelectronic contribution, fostered by hydroxyproline in the native polymer, to the organization of the collagen-peptide backbone, in contrast to the water-based helical stabilization model.

Yale University's Benjamin Turk described the efforts of his laboratory to develop a peptide-based inhibitor of anthrax lethal toxin. Lethal toxin, which persists after antibiotic treatment of the infection, kills cellular macrophages via necrosis. It comprises two components, the protective antigen involved in pore formation and lethal factor (LF), a zinc-dependent metalloprotease. All six known cellular targets for LF are MAP-kinase kinases (MKKs). These kinases are the central enzymes in the three-component phosphorylation cascades that transduce a stimulus through a MKK-kinase, to the MKK, and onto the MAP kinase (MAPK) that phosphorylates multiple substrates, leading to a cellular response. LF cleaves six of the seven MKKs, which removes the MAPK docking sites and turns signaling off. Dr. Turk's lab used a screening approach to uncover the substrate sequence preferences of the LF protease. They utilized three screens to look for sequence determinants upstream and downstream of the cleavage site. Experimental analyses revealed some selectivity for a hydrophobic amino acid and a proline in positions P1′ and P2′, respectively (the first and second positions following the cleavage site), and a preference for tyrosine at P2 and one for basic residues at positions P4–P6 upstream of the cleavage site. Subsequent studies showed that the consensus peptide derived from the screens was a good substrate for LF, and it is now used in small-molecule screens. Subsequently, the Turk group tested a related substrate-based inhibitor bearing a metal chelator at its C terminus and compared it to a known, structurally similar, matrix metalloprotease (MMP) inhibitor (also bearing a chelating group). The MMP inhibitor was efficacious in an in vivo assay of lethal toxin activity. Turk also commented on collaborative structural analyses of LF bound separately to the peptide substrate and to the MMP inhibitor, which revealed that the tyrosine favored at P1′ fit into a deep hydrophobic pocket with the rest of the peptide stretched across the protein in an extended conformation. He concluded by highlighting the lab's ongoing work to determine which of the MKKs is the critical target for LF.

Christopher Walsh of Harvard University Medical School delivered the keynote address, closing the symposium. Walsh began by introducing the audience to the astonishing array of halogenated compounds produced by natural biosynthetic pathways. The talk focused on two enzyme classes that catalyze halogenation—flavin-dependent enzymes and non-heme Fe(II) α-ketoglutarate-dependent enzymes. Mechanistically, the flavin-dependent enzymes utilize $FADH_2$ and oxygen to transfer chlorine to electron-rich aromatic rings. As an example, Walsh discussed studies of the overexpression of two enzymes from the rebeccamycin gene cluster, RebH and RebF, the former a flavin-dependent halogenase and the latter a flavin reductase. In tandem, the expressed proteins catalyze the chlorination of tryptophan. Detailed mechanistic studies from his laboratory suggested that the active chlorinating species is HOCl or a chemical equivalent. Turning to halogenation by non-heme Fe(II) α-ketoglutarate-dependent enzymes, Walsh described two enzymes involved in syringomycin synthesis, SyrB1 and SyrB2. SyrB1 is a nonribosomal peptide synthase didomain protein containing A and T domains. SyrB2 is a non-heme Fe(II) α-ketoglutarate-dependent halogenase. Following anaerobic purification, members of the Walsh group demonstrated that

SyrB2 bound with α-ketoglutarate binds nearly a full equivalent of iron. For halogenation of a SyrB1 bound substrate, the enzyme is dependent on the α-ketoglutarate cofactor, oxygen, and iron. The Walsh and Drennan labs (MIT) were able to show, using X-ray crystallography, that the bound halogen (bromine was used in the structure determination) replaced an expected carboxylate ligand, derived from an Asp residue, in the iron coordination sphere. Using the characteristic replacement of an Asp conserved in non-heme Fe(II) α-ketoglutarate-dependent oxygenases with Ala, the Walsh group identified other such halogenases. Dr. Walsh finished the halogenation story with the discussion of other examples of biological halogenation, including the barbamide synthetic pathway and the mechanism for coronatine biosynthesis.