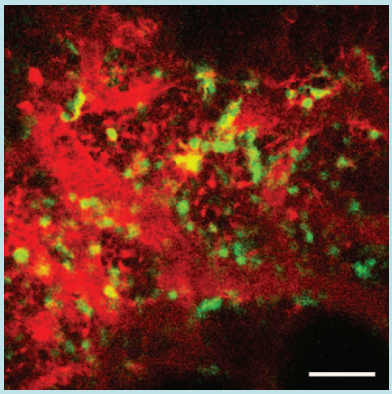
Spotlight

No Bones About It



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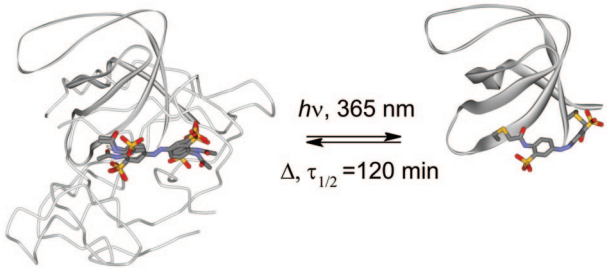
Osteoclasts are a type of bone cell responsible for bone resorption, the breaking down of bone tissue in normal bone remodeling processes. However, osteoclast dysfunction contributes to disorders such as osteoporosis and rheumatoid arthritis, and so understanding the molecular basis for osteoclast differentiation and regulation has important therapeutic implications. Osteoclast precursors migrate to and also from the bone surface where they eventually complete the differentiation process to become mature osteoclasts. Now, Ishii *et al.* (*Nature*, published online Feb 8, 2009; DOI: 10.1038/nature07713) report that the lipid mediator sphingosine-1-phosphate (S1P) is a key regulator of osteoclast precursor migration.

S1P is known to mediate cell migration in other tissues. Examination of S1P receptor mRNA and protein expression in osteoclast precursor cells revealed a decrease in receptor levels after stimulation with cytokines that promote osteoclast differentiation. The osteoclast precursors exhibited positive chemotaxis along an S1P gradient *in vitro*, which also decreased significantly upon differentiation. To explore the relevance of these findings *in vivo*, the migration of osteoclast precursors in the bone marrow spaces of mice was explored. Intravital two-photon imaging, a technique that facilitates visualization of cells located in deep tissue environments, revealed that exposure to a selective S1P receptor agonist induced the migration of osteoclast precursors from the bone marrow into the circulation. In additional experiments, it was observed that mice deficient in an S1P receptor suffered from increased osteoporosis. The authors proposed that loss of S1P receptor function resulted in reduced levels of osteoclast precursor recirculation with increased levels in the bone marrow. The potential therapeutic applications of targeting this pathway were illustrated when mice subjected to ovariectomy, which leads to osteoporosis, exhibited significant reduction in bone density loss upon treatment with an S1P receptor agonist. This study illuminates an important regulatory pathway for osteoclastogenesis and a potential therapeutic strategy for treatment of bone destructive disorders. **Eva J. Gordon, Ph.D.**

Photoswitchable Folding

Numerous diverse processes, including vision and photosynthesis, are mediated by proteins whose structure and function are sensitive to light. Use of protein engineering technology offers a systematic approach to the design of proteins whose activity can be controlled with light, perhaps limiting the ability to create photocontrollable proteins only to the imagination. Now, Zhang *et al.* (*J. Am. Chem. Soc.* 2009, *131*, 2283−2289) describe their use of photoswitchable intramolecular cross-linkers to control the folding of the SH3 domain from the tyrosine kinase Fyn (referred to as FynSH3).

FynSH3 is an excellent model for protein folding studies, since the stability and folding energetics of the protein and several mutants have been extensively characterized. BSBCA, a cysteine-reactive, azobenzene-derived cross-linker, was chosen to bestow photocontrol into FynSH3 folding, since it undergoes light-controlled *cis*−*trans* isomerization about the N═N double bond, which results in a substantial distance differential. Molecular modeling studies were employed for the selection of possible sites for cross-linking in which the distance between the cysteine sulfur atoms was well matched with the *cis* form of the cross-linker but not the *trans*. Three mutant proteins were generated, in each of which two native residues were replaced with cysteine residues for reaction with the cross-linker. The mutants were screened for photocontrol of conformation using circular dichroism and further characterized by UV−vis and NMR spectroscopy. Indeed, in one of the mutants, introduction of the *trans* form of the cross-linker led to significant de-



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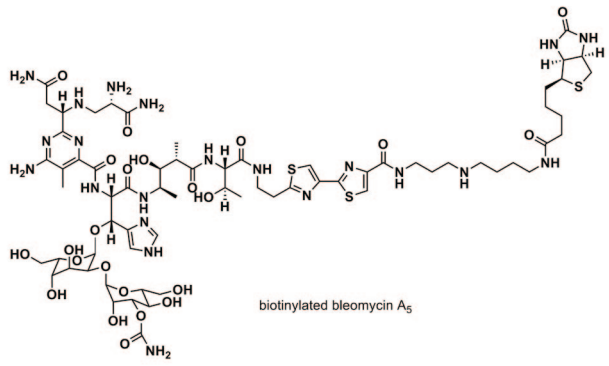
stabilization and unfolding of the protein. However, upon photoisomerization to the *cis* form, the folded, active state of the protein was restored. The demonstration of photocontrol of protein folding presented here can be extended to other globular proteins, offering an exciting new strategy for controlling protein folding. **Eva J. Gordon, Ph.D.**

Bubbles of Bleomycin

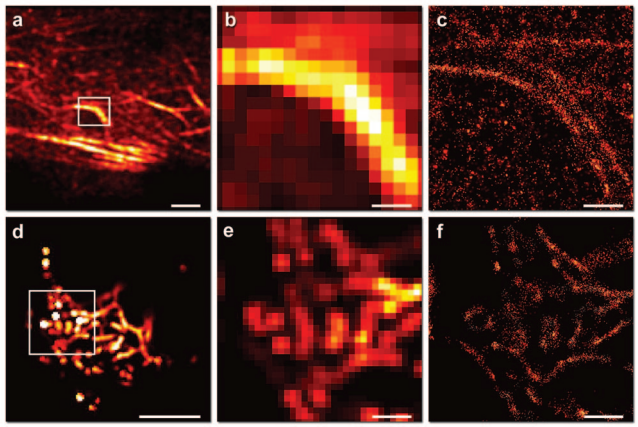
Bleomycins, glycosylated natural products used clinically in the treatment of certain cancers, execute their anticancer activity through the selective oxidative cleavage of DNA and, perhaps, RNA. But how does bleomycin selectively target cancer cells while sparing their normal cell counterparts? Chapuis *et al.* (*J. Am. Chem. Soc.* 2009, *131*, 2438−2439) use an innovative approach employing microbubbles to explore the structural features of bleomycin that contribute to its tumor-specific behavior.



Microbubbles are widely used in ultrasound-based diagnostic imaging techniques as contrast agents and intravascular blood flow agents. Comprising an outer shell of albumin, carbohydrates, or lipids enclosing a core of either air, nitrogen, or a perfluorocarbon, microbubbles are small enough to flow freely though the circulation. The authors hypothesized that appropriately derivatized microbubbles could serve as a useful vehicle for monitoring how molecules such as the bleomycins interact with tumor cells. To this end, a biotin moiety was synthetically attached to bleomycin A_5, and the conjugate was mixed with microbubbles derivatized with streptavidin. The resulting bleomycin-conjugated microbubbles were introduced into a flow chamber containing cultured human breast cancer cells. Using an inverted microscope fitted with a camera, the bleomycin-modified microbubbles could readily be visualized adhering to the cells. In contrast, neither did microbubbles lacking bleomycin adhere to the cells nor did bleomycin-conjugated microbubbles adhere to a normal breast cell line. To explore the structural features of bleomycin that might contribute to this selectivity, a biotinylated bleomycin derivative lacking the disaccharide unit was prepared and evaluated in a similar manner. Notably, this conjugate did not interact with either the normal or cancerous breast cells, highlighting a key role for the carbohydrate in cancer cell targeting. This clever approach offers a general and effective method for exploration of the selective cancer cell targeting of bleomycin and other tumor targeting compounds. **Eva J. Gordon, Ph.D.**

Photoconvertible and Photostable

Photoconvertible fluorescent proteins, such as the green-to-red photoconverting protein EosFP, are powerful tools for exploring dynamic cellular processes and other high resolution microscopy applications. However, EosFP exists in solution as a tetramer, which interferes with its utility as a fusion partner with proteins of interest. Tandem dimer (tdEos) and monomeric (mEos) EosFP variants developed to alleviate this issue suffer from inaccurate localization in fusions and improper maturation, respectively. Now, McKinney *et al.* (*Nat. Methods* 2009, *6*, 131−133) report the generation of a new EosFP variant, mEos2, that exhibits superior localization properties and photostability in a variety of high resolution imaging applications.

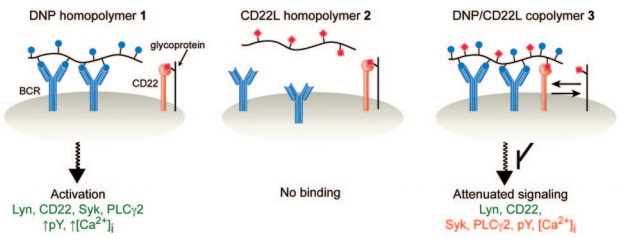


Four key mutations to mEos led to the generation of mEos2. Characterization of mEos2 revealed similar spectral, brightness, pK_a, photoconversion, contrast, and maturation properties as previous EosFP variants, though some dimeric character was present. However, live-cell and photoactivated localization microscopy (PALM) imaging experiments indicated that mEos2 exhibited superior behavior in time-lapse imaging of numerous processes including mitosis, cell division, mitochondrial fusion, and gap junction plaque behavior. Moreover, fusions of mEos2 with signal peptides and targeting proteins yielded expected localization patterns, in contrast to the localization defects and aggregation observed with earlier variants. Finally, total internal reflection fluorescence imaging and PALM analysis revealed that the localization precision of mEos2

was comparable or superior to that achieved with earlier versions. Thus, mEos2 is a valuable addition to the toolkit of imaging agents for live-cell and super-resolution imaging applications, as it exhibits improved photostability, targeting, and localization precision characteristics. **Eva J. Gordon, Ph.D.**

Antigenic Suppression

B cells are key players in the innate and acquired immune response, but they can also be key culprits in the progression of autoimmune diseases. Deciphering the intricate pathways that regulate B cell activation might facilitate development of methods to control B cell behavior, for example, by stimulating activation in response to an infection or dampening it to prevent autoimmune activity. CD22, a coreceptor that resides on the B cell surface, attenuates signaling through the B cell receptor. Ligands for CD22 include sialylated glycoproteins that also reside on the B cell surface and interact with CD22 *in cis*. However, it is unclear if other sialylated ligands influence CD22 activity. Now, Courtney *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2009, *106*, 2500−2505) present the use of synthetic, sialylated glycopolymers to explore whether *trans* interactions between CD22 and multivalent sialylated ligands affect B cell activation.

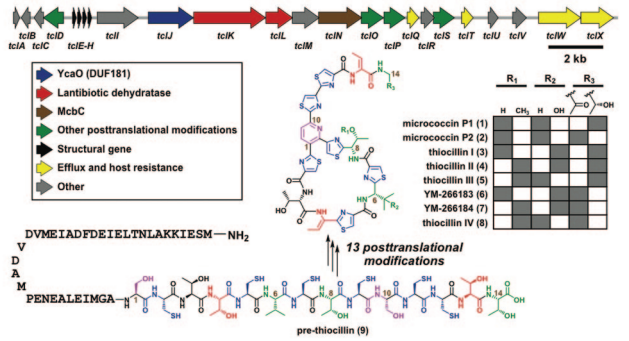


Courtney, A. H., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, *106*, 2500−2505. Copyright 2009 National Academy of Sciences, U.S.A.

Three synthetic polymers were meticulously designed to address the complex relationship between CD22 and the B cell activity. One polymer displayed only a 2,4-dinitrophenyl (DNP) group, which activates B cells directly through a DNP-specific B cell receptor. The second polymer displayed a sialic acid terminated trisaccharide known to bind to cell surface CD22. The third polymer displayed both the DNP group and the trisaccharide and thus was capable of engaging both the B cell receptor and CD22. Several physiological events that occur upon B cell activation, including the transient influx of extracellular calcium into the cytosol and the phosphorylation of various signaling molecules, were examined upon exposure to the polymers. The results indicated that antigens that engage both CD22 and the B cell receptor have an inhibitory effect on signaling through the B cell receptor. This suggests that antigens displaying CD22 ligands can suppress B cell activation. The intriguing clues into the interplay between CD22, sialylated antigens, and the B cell receptor uncovered in this study will facilitate development of novel molecular tools and therapeutic agents that function by modulating B cell activity. **Eva J. Gordon, Ph.D.**

A Thirteen-Step Program

Thiazolylpeptides are a class of peptidic antibiotics that inhibit protein synthesis. These antibiotics are characterized by sprinklings of oxazole, thiazole and pyridine, piperidine, or dehydropiperidine rings. Evidence suggests that the biosynthesis of these natural products begins with ribosomal peptide synthesis, followed by posttranslational modifications to generate the nonpeptide heterocycles. Wieland Brown *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2009, *106*, 2549−2553) use bioinformatic, genetic, and chemical methods to investigate the mechanism of thiazolylpeptide biosynthesis, revealing an impressive assortment of 13 posttranslational modifications that contribute to the creation of these important antibiotics.



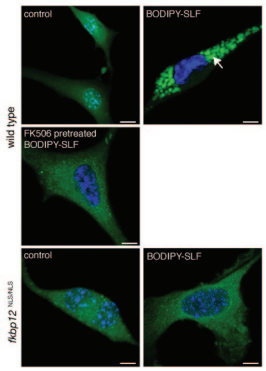
Wieland Brown, L. C., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, *106*, 2549−2553. Copyright 2009 National Academy of Sciences, U.S.A.

A bioinformatics search based on a peptide sequence thought to encode several thiazolylpeptides pointed to a region at the 5′ end of a cluster of genes, termed *tcl*, in a strain of the bacteria *Bacillus cereus*. Disruption of the *tcl* gene cluster by insertional mutagenesis prevented thiazolylpeptide production, providing genetic evidence that this cluster was indeed responsible for thiazolylpeptide biosynthesis. Further bioinformatic analysis of the *tcl* cluster revealed a striking grouping of enzymes homologous to those that convert amino acid residues into various heterocycles in the biosynthesis of other peptidic antibiotic families. Detailed examination of the likely biosynthetic mechanism of the thiazolylpeptide thiocillin indicated that 13 of the 14 amino acids undergo one of six different posttranslational modifications to achieve the final product. In addition, a remarkable 10 of the 14 residues are predicted to undergo heterocyclization or dehydration, and other modifications include hydroxylation, *O*-methylation, and decarboxylation. Notably, further bioinformatic characterization of the *tcl* gene cluster led to the identification of several additional gene clusters likely to produce novel thiazolylpeptides, suggesting that this class of antibiotics may be much larger than the ∼50 members identified to date. **Eva J. Gordon, Ph.D.**

Spotlight

Pharmacologically Stabilized

Despite extraordinary progress over the past two decades, drugs developed to treat AIDS are often plagued by poor pharmacological properties. As a result, treatment with these therapeutics requires high doses and/or coadministration with metabolic enzyme inhibitors. This not only increases treatment costs but can lead to adverse side effects and poor patient compliance. While the use of prodrugs is a common strategy for overcoming some of the pharmacological issues surrounding anti-HIV therapeutics, such compounds require enzymatic processing for activity. Marinec *et al.* (*Proc. Natl. Acad. Sci.* 2009, *106*, 1336−1341) stray from this conventional approach, presenting an innovative strategy for creating a "pharmacologically stabilized" derivative of the HIV protease inhibitor amprenavir.



Marinec, P. S., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, *106*, 1336−1341. Copyright 2009 National Academy of Sciences, U.S.A.

The strategy draws upon on the unique properties of the natural product FK506. FK506 is a macrolide that binds with high affinity to FKBP, a protein that resides in the cytoplasm of blood cells. The authors hypothesized that by creating a bifunctional molecule composed of an FK506-like component covalently linked to an amprenavir-like component, the HIV protease activity associated with amprenavir could be recruited to the cytoplasm of blood cells and away from key metabolic enzymes that can only access free drug in the plasma. To this end, a bifunctional inhibitor termed SLFavir was synthesized, and *in vitro* experiments confirmed that the compound retained antiprotease activity. To explore the metabolic stability of SLFavir, its localization in mouse whole blood samples and in live mice was determined. Indeed, whereas amprenavir divides its time relatively equally between blood cells and plasma, SLFavir clearly prefers to reside in the cells. Moreover, it was demonstrated that SLFavir retains a dramatically increased half-life in both blood samples and in mice when compared with amprenavir. Hinting at the promising therapeutic potential of this strategy, an SLFavir derivative exhibited activity significantly higher than that of amprenavir when tested for activity against live HIV in cultured cells. Notably, this novel approach for improving the pharmacological stability of HIV inhibitors might be extended to other drug classes as well. **Eva J. Gordon, Ph.D.**

An RNA Enzyme Reinvents Itself

The RNA world hypothesis proposes that the first biological molecules on Earth were RNA because it is a biopolymer that can both encode genetic information and perform catalytic functions. Among a prebiotic soup, increasingly complex reactions were catalyzed by RNA, and these laid the stepping stones toward self-sufficient organisms. As with any biological system, replication is a key hallmark. To simulate the situation in an RNA world, several laboratories have used *in vitro* evolution to select RNAs that can perform ligation or polymerization reactions that mimic an ancient replication machinery. One such selection uncovered an RNA enzyme, R3C, that could ligate together two RNA oligomers. However, unlike the enzymes found in the protein world, this RNA enzyme had a hard time efficiently utilizing the substrates it was provided. Now, with a further evolved version of R3C, Lincoln and Joyce (*Science* 2009, *323*, 1229−1232) show that not only is efficient catalysis possible, but sustained replication can be achieved as well.

In a clever twist, the base pairing of the RNA enzyme with its two oligomer substrates was arranged such that the reaction product was another RNA ligase molecule. By serial transfers of exhausted reactions into new vessels containing more substrate oligomers, this cross-replication reaction could continue indefinitely and with an overall doubling time of approximately 1 h. A 200 year-old Charles Darwin would be proud to see that the authors even went on to introduce selection and competition into the mix. Twelve pairs of self-replicating enzymes were synthesized with different substrate binding arms and similar but distinct catalytic cores. Feeding these enzymes a variety of sequences for the 4-oligomer substrates meant that 132 new recombinant RNA enzymes could arise. After letting these reactions proceed over the course of 100 h with many serial dilutions into fresh substrate, recombinants dominated and the original ligase molecules fell to the minority. This report shows the power of evolution and replication in a test tube and points toward interesting future directions, while keeping a firm eye on the prebiotic past. **Jason G. Underwood, Ph.D.**