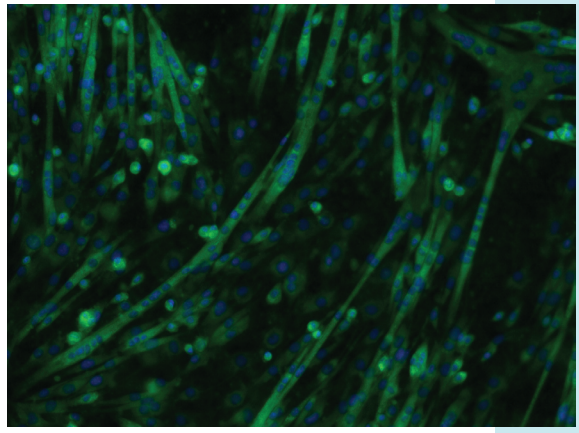
Spotlight

Screening for Screening's Sake

High-throughput screening continues to be an invaluable tool for the discovery of new biological probes and potential drugs. However, a screen is only as good as the reagents it employs. Less than ideal detection reagents, for example, can significantly hamper the efficiency and usefulness of a screen. Wagner *et al.* (*J. Am. Chem. Soc.* 2008; DOI: 10.1021/ja077656d) now report a strategy for discovering effective new detection reagents that simplifies high-throughput cell-based screening for small molecules that affect muscle cell differentiation.



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Small molecules that prevent or promote muscle cell differentiation can help us understand muscle development as well as have important applications in regenerative medicine. Immature muscle cells, or myoblasts, can be distinguished from their differentiated counterparts, myotubes, using the somewhat cumbersome process of immunofluorescence of myotube-specific proteins. In the search for a small fluorescent molecule capable of distinguishing between myoblasts and myotubes directly, 1606 fluorescent compounds were added to both myoblasts and myotubes. Of these, six were identified that fluoresced in myotubes but not myoblasts. One of these compounds, called E26, was tested as a myogenesis probe in a screen in which 84 kinase inhibitors, including some known myogenesis inhibitors, were added to myoblasts in differentiation media. By monitoring the fluorescent signal from E26, the authors found that 17 of the kinase inhibitors prevented myoblast differentiation. Importantly, when the signal from E26 was compared with that using immunofluorescence in cells treated with the known myogenesis inhibitor rapamycin, a comparable decrease was observed, an indication that E26 is a valid detection reagent for cell-based screens exploring myogenesis. This clever approach for improving the efficiency and simplicity of high-throughput screens can be more broadly applied toward the discovery of fluorophores capable of distinguishing other cellular states as well. **Eva J. Gordon, Ph.D.**

Shedding Skin with miRNAs

During development, pluripotent stem cells give rise to many different cell types, and these transitions are marked by profound changes in gene expression. The recent flurry of microRNA (miRNA) research is proving that non-coding RNAs can make important contributions to proper regulation as cells differentiate. Switching on one miRNA gene can mean the down-regulation of numerous proteins by virtue of its complementarity to cognate mRNAs. Armed with this hypothesis, Yi *et al.* (*Nature* 2008, *452*, 225–229) look skin-deep for a candidate miRNA that could play a role in the specification of mature skin cells during fate commitment.

In the skin, the underlying proliferative stem cells in the basal layer give rise to the suprabasal cells, which are less proliferative and are programmed for terminal differentiation. By profiling mouse embryonic stages where skin is either single layered or stratified, the authors discovered that miR-203 was highly enriched upon stratification. But, what role might this miRNA play in these cells? To pursue this question, an impressive set of experiments addressed both loss of function and gain of function of this miRNA in the skin. They began by testing the consequence of overexpressing miR-203 in the skin, but then went on to inhibit miR-203 with a complementary oligonucleotide, and by ablating the miRNA in the skin with a conditional knockout of a key miRNA processing enzyme, Dicer. Expressing too much miR-203 made the epidermis too thin, and far lower numbers of the basal precursor cells were viable. Inhibiting the miRNA's function by the antisense approach or Dicer knockout had the opposite effect. The epidermis showed dramatic proliferation, and a key marker of the basal stem layer, p63, was misexpressed in suprabasal cells. The p63 protein was previously known to play a role in keeping the basal cells in their proliferative stem cell state, so a unique connection between these two stories emerged when the 3′ untranslated region of p63 displayed binding sites for miR-203. These sites are phylogenetically conserved in vertebrates back to zebrafish, an indication that miR-203 may have orchestrated the formation of the skin for >350 million years. **Jason G. Underwood, Ph.D.**

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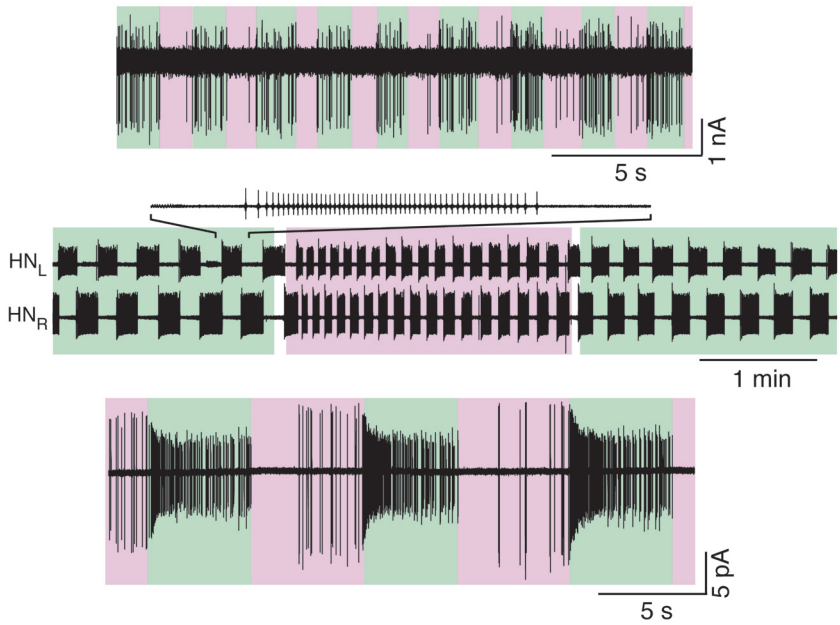
Eukaryotic Ion Channels See the Light

Light provides an attractive means for eliciting physiological responses from excitable cells because it is noninvasive and can be implemented with exquisite spatial and temporal control. Unfortunately, eukaryotic cells do not have ion channels that are directly activated by light. Although caged neurotransmitters that are released upon exposure to light and ion channels engineered to be light sensitive have been developed, these methods have inherent disadvantages that can limit precise control over neurotransmitter or ion channel activity. Fortin *et al.* (*Nat. Methods*, published online March 2, 2008; DOI: 10.1038/nmeth.1187) now report the development of small-molecule photoswitchable affinity labels (PALs) that confer light sensitivity to endogenous ion channels.

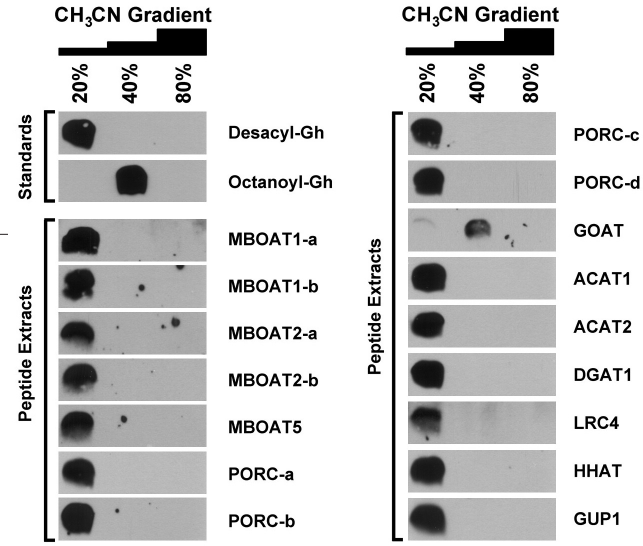
The PALs are designed to target voltage-gated potassium (K^+) channels and possess three key elements: a protein-binding ligand, a photoisomerizable tether, and an electrophilic group. A quaternary ammonium group was chosen as the protein-binding ligand to confer specificity to K^+ channels. An azobenzene group was selected as the photoisomerizable tether because its configuration is light sensitive: exposure to 360–400 nm light causes a trans to cis isomerization. The PAL molecule can only reach the pore, and thus block ion conduction, when the azobenzene is in the extended trans configuration; when it is in the bent cis configuration, the channel is active. Finally, PALs with three distinct electrophilic groups (acrylamide, chloroacetamide, and epoxide) were created, enabling covalent attachment to the channel *via* nucleophilic side chains near the pore.



The authors demonstrate that PAL treatment can impart light sensitivity to endogenous K^+ channels in various cell types and tissues, including human kidney cells, rat neurons, rat cerebellar slices, heart interneuron cells in a medicinal leech, and rat retinal ganglion cells. This promising approach illuminates a new path toward controlling the many K^+-channel-dependent physiological responses, such as neuronal communication and cardiac signaling. Notably, this approach could be extended to manipulate other ion channels by modification of the protein-binding ligand element of the PAL. **Eva J. Gordon, Ph.D.**

Spotlight

Fighting Obesity: Food for Thought

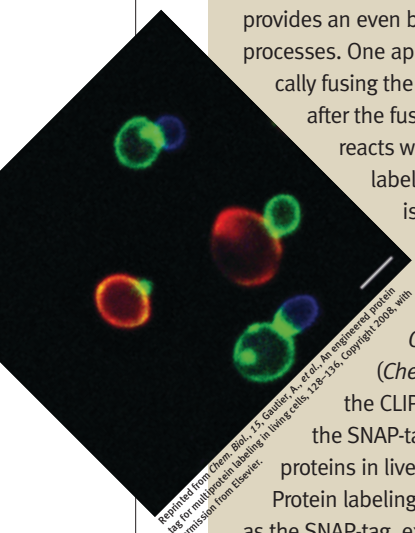


Reprinted from *Cell*, *132*, Yang, J., *et al.*, Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone, 387–396, Copyright 2008, with permission from Elsevier.

Ghrelin, a 28 amino acid peptide hormone secreted by cells of the "hungry" stomach, was discovered nearly a decade ago. Levels of this hormone have been shown to rise just before meals. In addition, numerous studies have found ghrelin guilty by association in obesity. Not surprisingly, the idea of targeting ghrelin to combat the obesity epidemic has received considerable attention in recent years. It has been shown that ghrelin is cleaved from a precursor peptide, and a highly conserved serine of this hormone is modified with an octanoyl group. Octanoylated ghrelin is the target of choice because it possesses growth-hormone-releasing activity and because the octanoyl modification has not been found in other peptides. Until now, the enzyme that catalyzes this modification had not been found. Now, Yang *et al.* (*Cell* 2008, *132,* 387–396, published on Feb 8, 2008; DOI 10.1016/j.cell.2008.01.017) discover ghrelin *O*-acyltransferase (GOAT), which catalyzes the octanoylation of ghrelin.

The authors used a bioinformatic approach to identify a putative enzyme, GOAT, thought to catalyze the octanoylation of ghrelin and developed an assay to separate processed ghrelin from precursors. Using this assay, they showed that that GOAT mediates the vital octanoylation reaction. Further, the authors identified residues essential for catalysis and also showed that GOAT mRNA is found in highest abundance in the stomach. As discussed by the authors, it seems only a matter of time before small molecules are designed that fight fat by inhibiting GOAT. **Anirban Mahapatra, Ph.D.**

A SNAP and a CLIP



Reprinted from *Chem. Biol.*, *15*, Gautier, A., *et al.*, An engineered protein tag for multiprotein labeling in living cells, 128–136, Copyright 2008, with permission from Elsevier.

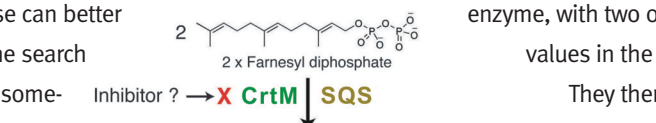
Visualization of a protein enables a unique perspective on a biological event of interest; visualization of two distinct proteins *simultaneously* in live cells, though technically challenging, provides an even bigger window into the complex world of cellular processes. One approach for visualizing proteins involves genetically fusing the protein of interest to a molecular tag that, after the fusion protein is expressed in cells, covalently reacts with a small molecule containing a fluorescent label. For example, fusion of the SNAP-tag, which is derived from the human DNA repair protein O^6-alkylguanine-DNA alkyltransferase, to a protein of interest enables reaction of the tagged protein with a variety of fluorescent O^6-benzylguanine derivatives. Gautier *et al.* (*Chem. Biol.* 2008, *15*, 128–136) now introduce the CLIP-tag that, when used in conjunction with the SNAP-tag, enables the simultaneous labeling of two proteins in live cells.

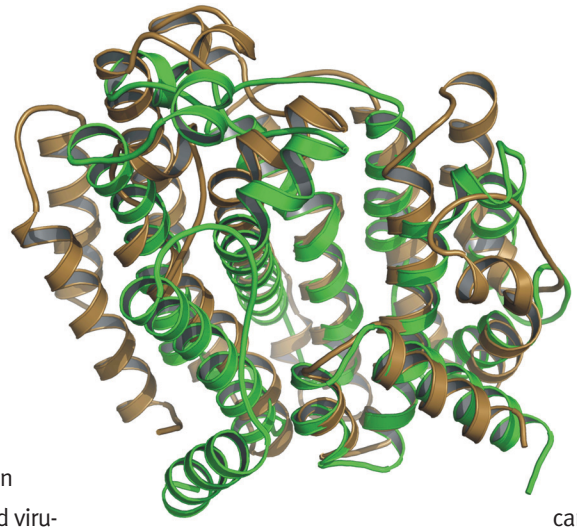
Protein labeling with the CLIP-tag employs a similar strategy as the SNAP-tag, except that the CLIP-tag has been engineered to react selectively with O^2-benzylcytosine rather than O^6-benzylguanine. After determining that SNAP and CLIP fusion proteins could be labeled simultaneously with different fluorescent tags both *in vitro* and in living cells, the authors demonstrated two applications of the technology. First, two proteins residing in different subcellular locations were simultaneously labeled and detected, an indication that double labeling can be used for parallel determination of protein localization. Next, double pulse-chase labeling experiments were conducted in which incorporation of the yeast cell adhesion protein Aga2p into the cell wall of budding yeast was monitored. Cells expressing either Aga2p-CLIP or Aga2p-SNAP fusions were mixed and labeled with orthogonal tags, allowed to grow for 1 h, and then labeled again with a different set of tags. It was clearly shown that cell wall growth and incorporation of a new protein is directed toward the bud in yeast cells, signifying the value of this technology for exploration of dynamic cellular processes. Development of the CLIP-tag offers an exciting expansion to the scope of investigations possible with protein visualization methods. **Eva J. Gordon, Ph.D.**

New Tricks to Terminate Your Staph

The emergence of nastier antibiotic-resistant bacterial strains has raised the ante in the search for new ways to combat infections. One new-school idea for therapy does not involve killing the bacterial threat but rather crippling the bug with a drug so that the body's natural immune response can better fight off the infection. The search for these compounds is sometimes performed with small-molecule libraries, but a recent study shows that rational design of an antibacterial drug can be inspired by the human proteome.

In a new paper, Liu *et al.* (*Science* 2008, *319*, 1391–1394) study the virulence factor, staphyloxanthin, from *Staphylococcus aureus*, that causes deadly antibiotic-resistant Staph infections.





From Liu C.-I, *et al.*, *Science*, March 7, 2008, DOI: 10.1126/science.1153018. Reprinted with permission from AAAS; Epub Feb 14, 2008.

The authors determined the structure of a key enzyme in the synthesis of this pigmented virulence factor, dehydrosqualene synthase (CrtM). The enzyme fold showed remarkable similarity to the human enzyme squalene synthase (SQS), an important enzyme in cholesterol biosynthesis. The proteins displayed only moderate homology by primary structure, but the three-dimensional structures displayed a very similar shape, probably due to the similar reactions that Staph CrtM and the human SQS catalyze. Inhibiting cholesterol synthesis has stood out as the pharmaceutical industry's favorite pastime of late, so the authors took advantage of human SQS inhibitors as putative anti-virulence candidates. They tested eight compounds for CrtM inhibition and found that three phosphonosulfonate compounds did inhibit the enzyme, with two of these three showing K_i values in the low nanometer range.

They then went back and crystallized three inhibitors bound to the CrtM enzyme by X-ray crystallography. This analysis showed that the compounds bind specifically and form numerous electrostatic and hydrophobic interactions with side chains on the bacterial enzyme. The study even goes on to try one of the inhibitors, BPH-652, *in vivo* by challenging mice with *S. aureus* in the presence and absence of the candidate drug. Remarkably, after 3 days, the treated mice showed bacterial counts 98% lower than the control group. This study showcases the power of structural biology combined with drug screening and proves that crippling a bacterium can be nearly as effective as killing it. It also reproves Sir Alexander Fleming's 1928 result; just as on his moldy Staphylococcus plate, sometimes a drug can come from where you'd least expect it. **Jason G. Underwood, Ph.D.**