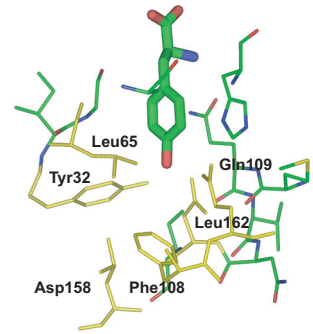
acs chemical biology

In this ISSUE



Phosphotyrosine Fake Out

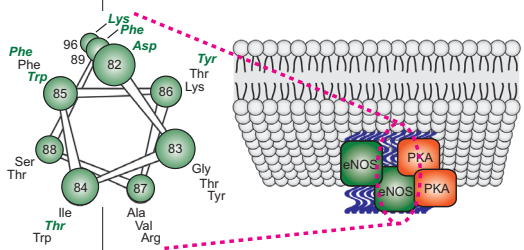
Cells rely on the phosphorylation of proteins, generally on serine, threonine, or tyrosine residues, to trigger signaling events. However, the dynamic nature of protein phosphorylation, coupled with the fact that many proteins can be phosphorylated at multiple locations, complicates the meticulous investigation of any one phosphorylation event. Xie *et al.* (p 474 and Point of View p 454) "fake out" the system by instilling site-specific, non-hydrolyzable phosphotyrosine mimics into proteins of interest.

The phosphotyrosine mimic *para*-carboxymethyl-phenylalanine (*p*CMF) was synthesized in four steps. Incorporation of *p*CMF into proteins was accomplished with an orthogonal amber suppressor transfer RNA (tRNA)/aminoacyl-tRNA synthetase pair evolved from a library of active-site mutants of the tyrosyl tRNA synthetase from the archaea *Methanococcus jannaschii*. Five rounds of positive and negative selection, followed by DNA sequencing of the mutants and SDS-PAGE and mass spectrometry analysis of a test protein, confirmed the creation of an efficient and specific tRNA/aminoacyl-tRNA synthetase pair for *p*CMF incorporation into proteins. Next, *p*CMF was incorporated into a fragment of the protein signal transducer and activator of transcription 1 (STAT1), in place of Tyr701. Electrophoretic mobility shift assays demonstrated that the *p*CMF-containing STAT1 can engage in biological interactions similar to those of the wild-type protein, because it underwent homodimerization and bound to a DNA duplex containing an M67 site. Thus, this bit of trickery enables the creation of stable mimics of phosphorylated proteins, providing a novel tool for investigating the contribution of individual phosphotyrosine residues.

A Double Barrel Shotgun Approach

Proteins are promiscuous. A single protein can interact with hundreds of different partners. In many cases, the factors governing these interactions are murky at best, especially for proteins whose structures are inherently difficult to study, such as membrane proteins. Levin *et al.* (p 493) attack this problem full blast by using what they term "double barrel" shotgun scanning to investigate the binding of the transmembrane protein caveolin-1 with two of its binding partners, endothelial nitric oxide synthase (eNOS) and protein kinase A (PKA).

Traditional shotgun scanning approaches employ combinatorial libraries to substitute specific amino acid residues with a 1:1 ratio of either alanine or an amino acid homologous to the wild-type residue; then, phage display assays are used to assess the effects on binding to a single partner. Here, a double barrel shotgun scan of caveolin-1 scaffolding domain (CSD) binding to both eNOS and PKA was undertaken. Binding interactions were evaluated with an oligomer complementation approach, in which synthetic CSD is added to phage-displayed CSD before the interacting protein, and by dynamic light scattering. Both of these experiments provided compelling evidence that CSD forms large oligomers and, along with studies with deletion mutants, enabled the authors to propose an intriguing model for how CSD mediates binding to and inhibition of its partners.



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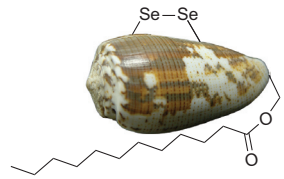
A Snail Tale

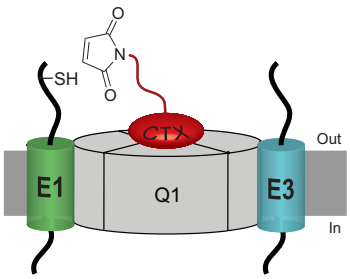
The venom of marine cone snails is extraordinarily potent and causes rapid immobilization or paralysis prior to the sad, inevitable fate of the snail's prey. The venom is composed of small, disulfide-rich peptides called conotoxins. Voltage- and ligand-gated ion channels have been identified as the primary targets of the conotoxin peptides, so these compounds and derivatives thereof are potential drug leads for various neurological disorders. Because conotoxins are fairly easily synthesized, incorporation of structural modifications for structure–activity analysis and improved pharmacological properties is relatively straightforward. Craik and Adams (p 457) review recent approaches for altering the structures of these remarkable peptides in pursuit of new medicines and valuable biological tools.



Several strategies for altering the properties of conotoxins have been attempted. Various amino acid substitutions, such as the replacement of naturally occurring L-amino acids with D-amino acids and the incorporation of post-translational modifications such as sulfation, hydroxylation, or the addition of lipid tags or non-natural backbone spacers, have yielded compounds with enhanced stability or activity. In addition, capping of the ends of the peptides, which occurs naturally in many conotoxins, can lead to reduced susceptibility to proteolytic degradation. Disulfide bond engineering, such as switching the connectivity of the disulfide bonds or replacing the cysteine residues with selenocysteine, is a third approach that has led to peptides with improved characteristics. Finally, cyclization of the peptides is another well-validated approach that has led to conotoxin derivatives with enhanced pharmacological properties. Exploration of the various structural modifications possible with this class of peptides is still in its infancy, but it is ripe with therapeutic potential.

Bring on the Scorpions!

KCNQ1 (Q1) K^+ channels are prominent in heart-muscle tissue and inner-ear neurons, among other tissues, and improper functioning of these chan-



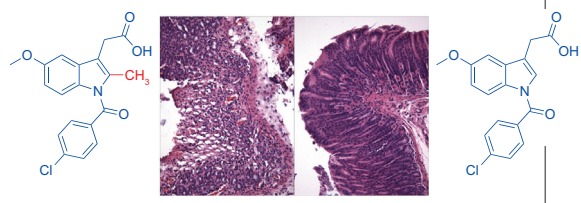
nels is associated with certain cardiac and auditory disorders. Q1 channels associate with the KCNE family of β-subunit transmembrane peptides, which help modulate the various K^+ currents needed for proper channel function. The recent discovery of all five KCNE peptides within single tissues suggested that heteromeric Q1–KCNE complexes composed of two or more different KCNE peptides complexed to the same Q1 channel might exist. Morin and Kobertz (p 469 and Point of View p 451) cleverly design an irreversible Q1 channel inhibitor to further explore the nature of Q1–KCNE complexes.

The researchers exploited the Q1 inhibitory properties of charybdotoxin (CTX), a scorpion toxin, by creating a cysteine-reactive CTX derivative. Strategic replacement of specific N-terminal amino acids on the KCNE peptides E1 and E4 with cysteine led to the generation of two irreversible Q1 inhibitors. These inhibitors were used to probe for the presence of hybrid complexes. Indeed, the authors demonstrated that heteromeric complexes exist and, furthermore, that certain KCNE peptides possess greater control over opening and closing the gate than others. This innovative strategy could be applied to the investigation of other ion channel complexes as well.

Exploring COX Tox

Cyclooxygenase (COX) inhibitors have been on quite the roller-coaster ride over the last decade or so. First heralded as remarkable new drugs that were easier on the stomach than other nonsteroidal, anti-inflammatory agents, most were dramatically taken off the market a few years ago when an increased risk of heart attack became apparent. Despite all the hype, much remains to be deciphered regarding COX biology and the off-target effects of known COX inhibitors. To this end, Felts *et al.* (p 479) examine the off-target activities of two COX inhibitor analogues that exhibit drastically reduced COX inhibitory activity.

On the basis of evidence that a specific methyl group present in the COX inhibitors indomethacin and suldinac sulfide is critical for their interaction with COX, the researchers generated the desmethyl versions of the drugs. Various assays demonstrated that the compounds exhibited off-target activities similar to those of the COX inhibitors, including the ability to activate the nuclear transcription factor peroxisome proliferator-activated receptor γ, trigger apoptosis, and induce adipocyte differentiation. Remarkably, however, the desmethyl indomethacin derivative had no apparent adverse effect on the gastrointestinal tract, an indication that the gastrointestinal toxicity of indomethacin is directly correlated to its inhibition of COX. These compounds are incredibly useful tools for deciphering COX biology, and their "off-target" activities may be their ticket to help fight other diseases, such as cancer and diabetes.



Sex Hormones Go Both Ways

Interaction of sex hormones such as estrogen and androgen can lead to two dramatically different general cellular responses, depending, in part, on where the interactions take place. If the interaction is at the nuclear membrane, gene transcription results. However, if the interaction takes place in the cytosol, sex hormone receptors can interact with the protein kinase Src, triggering a signaling cascade. Kim *et al.* (p 484) have developed an assay that exploits the interaction of the androgen receptor (AR) with Src to help characterize agonist and antagonist induction of this pathway.

The researchers employ a protein-fragment complementation assay by using the AR, Src, and firefly luciferase in their assay. The interaction between AR and Src in response to ligand enables a proximity-induced recovery of luciferase activity and a consequent increase in luminescence. AR agonists known to activate gene transcription were evaluated for their ability to promote binding of AR to Src. It is interesting that certain compounds were effective inducers of gene transcription but not signaling, and *vice versa*. In addition, evaluation of AR antagonists revealed that these compounds could in fact inhibit association of AR with Src. This assay provides a new method for evaluating the effects of various sex hormone receptor ligands on cell signaling pathways and distinguishing their actions from gene transcription events. This approach could help decipher the role of these hormones in certain cancers and other diseases.