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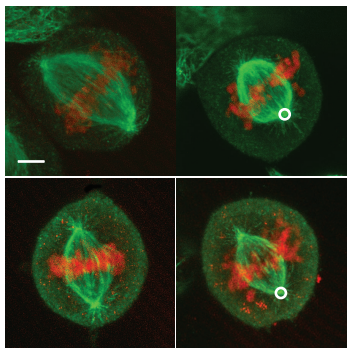
Protein Inactivation in a SNAP

Observation of protein activity in real time offers a unique and powerful perspective into cellular processes. Chromophore-assisted laser inactivation (CALI), a technique in which a protein labeled with a photosensitizer is inactivated upon exposure to light, enables analysis of protein function in live cells with high spatial and temporal resolution. However, limited methods for targeting photosensitizer dyes to proteins have hampered development of this promising protein analysis strategy. Now, Keppler and Ellenberg (DOI 10.1021/cb800298u) report the use of the SNAP-tag labeling approach to efficiently and selectively label proteins with a photosensitizer for CALI applications.

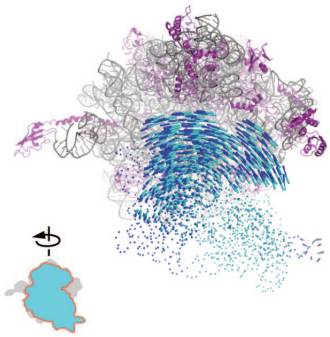
SNAP-tag labeling exploits the reactivity of human O^6-alkylguanine-DNA alkyltransferase (AGT), which can be reacted selectively with fluorescent O^6-benzylguanine derivatives. Two members of the tubulin family, α- and γ-tubulin, were generated as AGT, or SNAP-tag, fusion proteins and labeled with a benzylguanine fluorescein derivative. Using confocal imaging, the dramatic effect of inactivation of each protein on cell division was easily monitored, demonstrating the effectiveness of this approach.



Translocation: Last but Not Least

The translation of messenger RNA (mRNA) into proteins is an extraordinary, fundamental biological process directed by large protein−RNA complexes called ribosomes. The vital function of ribosomes also makes them compelling drug targets, and indeed various antibiotics are known to interfere with ribosomal activity. Thus, knowledge of the molecular mechanisms underlying ribosomal function benefits both basic research and medicinal purposes. Shoji *et al.* (DOI 10.1021/cb8002946) hone in on the final step of protein translation on the ribosome, translocation, reviewing its structural and dynamic aspects, as well as the mechanisms behind the antibiotics that target it.

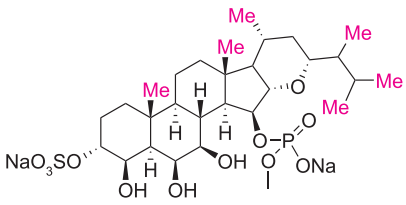
Ribosomal translocation encompasses the delivery of transfer RNAs (tRNAs) to appropriate adjacent sites on the ribosome in preparation for addition of the next amino acid to the growing polypeptide chain. This fascinating process ultimately requires significant movement of the tRNAs along with dramatic conformational changes in the ribosome. Equally as fascinating are the intricate schemes employed by antibiotics to interfere with this ribosomal movement that is so critical to the translocation process.



Double-Difference Makes a Difference

As far back as 3000 B.C., plants containing mind-altering cannabinoids, such as that found in marijuana, have been used for therapeutic purposes. However, inherent challenges in characterizing the membrane-bound cannabinoid receptors, coupled with the high water insolubility of cannabinoids, has hampered our ability to study these fascinating interactions. Using cell-based screening and a technique called saturation transfer double-difference (STDD) NMR spectroscopy, Pereira *et al.* (DOI 10.1021/cb800264k) report the discovery of a new cannabinoid receptor agonist and explore its interactions with two human cannabinoid receptors, CB1 and CB2.

Screening of a marine natural product extract library for binding to CB1 and CB2 expressed in insect cells led to the identification of the phosphorylated sterol sulfate haplosamate A as a selective cannabinoid receptor agonist. STDD NMR, which can provide atomic level detail of protein−ligand interactions for which crystallographic data is not easily obtained, confirmed the selective interaction between haplosamate A and the cannabinoid receptor and enabled additional structure−activity characterization of this novel interaction.



Haplosamate A

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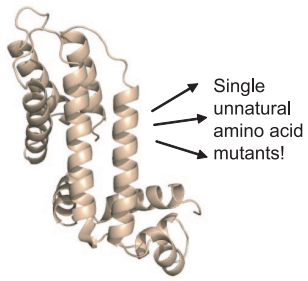
Efficient Mutagenesis

The many innovative methods developed to create mutant proteins have contributed significantly to our understanding of protein structure and function. However, for certain applications, such as generation of a large number of defined, single amino acid mutants, even the most sophisticated approaches still entail a tedious, perhaps even impractical process. Daggett *et al.* (DOI 10.1021/cb800271f) now present an efficient, convergent method for creating a collection of mutants in which every amino acid in a given protein can be independently and efficiently replaced.

The method exploits a transposon reaction to facilitate systematic replacement of each codon present within a given gene. As proof of principle, a library of glutathione-*S*-transferase mutants in which each amino acid was individually replaced with the unnatural, photoreactive amino acid *p*-benzoylphenylalanine, was created. This approach for creating "rationally diversified" protein sequences can be expanded to generate various other protein libraries, such as those containing different amino acid derivatives or proteins with not just one but multiple mutations.



Stopping the Bleeding

Platelets are critical players in the formation of blood clots and are thus welcome visitors in response to certain injuries. However, unwanted blood clotting can cause serious conditions such as heart attacks and strokes. Though several drugs exist to combat overzealous platelet activity, such medications often come with the unfortunate side effect of excessive bleeding, which has proven as deadly as the heart attacks that the drugs were developed to prevent. In the search for safer antiplatelet agents, Singh *et al.* (DOI 10.1021/cb8002094) now report the development of small molecule antagonists of a novel antiplatelet target, the platelet EP_3 receptor for prostaglandin E_2 (PGE_2).

Meticulous examination of the binding elements inherent in PGE_2 led to the development of a bicyclic acylsulfonamide, termed DG-041, that acted as a selective EP_3 antagonist. *In vitro* and *in vivo* studies demonstrated the efficacy of DG-041 in preventing platelet aggregation. Importantly, treatment with DG-041 did not appear to effect bleeding times, suggesting that this compound has exciting potential as a safer alternative to current antiplatelet therapeutics.