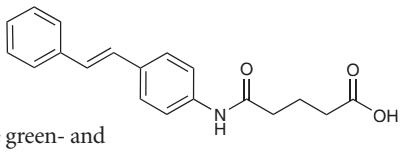
Native ion channels lighten up

Ion channels mediate action potentials in excitatory neurons in response to stimuli such as ligand-receptor binding and changes in membrane potential. Recently, investigators have engineered ion channels to be gated by light, which provides an orthogonal tool for probing mechanisms of neuronal excitability. To date, the success of light activation approaches has depended on engineered ion channels that must be expressed in neurons. To generalize the approach, Fortin *et al.* devised a method that uses molecules designed to function as photoswitchable affinity labels (PALs) to endow endogenous potassium channels with light-activated gating properties. The authors created a series of PALs that contained a known K^+ channel blocker (a quaternary ammonium group) tethered to an electrophilic tag through an azobenzene bridge. Ammonium group binding led to light-dependent tethering of the PAL to K^+ channels, which caused the channels to become responsive to light pulses: longer-wavelength light (500 nm) blocks the channel by favoring the *trans* isomer of azobenzene, and shorter-wavelength light (380 nm) isomerizes azobenzene to the *cis* form and opens the channel. The PALs showed no toxic effects and targeted many voltage-dependent K^+ (Kv) channels, but did not affect Na^+ or Ca^{2+} channels. Tuning the wavelength of the input light modulated the firing frequency and threshold potential of hippocampal neurons, both in culture and in neural tissue such as rat cerebellar slices and heart central pattern generator interneurons of the leech *Hirudo medicinalis*. Retinal ganglion cell neurons from rats were sensitive to PAL treatment, which opens up the possibility that ion channel regulation by light could be developed to restore visual transmission in animals. (*Nat. Methods*, published online 2 March 2008, doi:10.1038/nmeth.1187) *TLS*

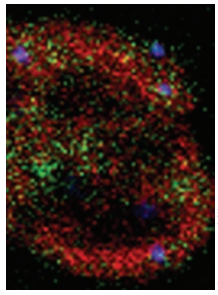
Antibody luminescence



Antibodies that emit green, purple or blue light when in complex with stilbene have proven to be useful biosensors. For the green- and purple-emitting complexes, antibody binding induces an increase in fluorescence by preventing photoinduced *cis-trans* isomerization of stilbene. In contrast, the blue antibody–stilbene complex produces bright and long-lasting luminescence upon exposure to UV light. To investigate the mechanism of luminescence emission, Debler *et al.* mutated each of the seven antibody residues in the van der Waals contact with stilbene, including a tryptophan found at the base of the deep stilbene binding site that forms a π-stacking interaction with the terminal stilbene phenyl ring. When this tryptophan was mutated to phenylalanine, the intensity and duration of the luminescence were dramatically reduced. The authors solved the structures of the green and purple antibody–stilbene complexes and found substantially shallower stilbene binding pockets that did not reach this tryptophan, suggesting that the stillbene-tryptophan stacking is critical for luminescence. Based on these results, the authors propose a mechanism in which photon absorption generates an electron-deficient singlet excited state of stillbene. An electron is then rapidly transferred from tryptophan to the excited stilbene molecule to form a charge-transfer excited complex. Because of the close stacking between the two aromatic rings, strong blue luminescence is favored over fluorescence. It is likely that the newly developed understanding of the photophysics underlying the bright blue luminescence of the antibody-small molecule complex will aid in the design of highly selective biosensors. (*Science* **319**, 1232–1235, 2008) *JK*

Written by Mirella Bucci, Joanne Kotz & Terry L Sheppard

The eye of a proteolytic storm


Anna von Mikecz

Many neurodegenerative diseases are characterized by the formation of insoluble protein inclusions. For instance, in Huntington's disease, an expansion of a polyglutamine (polyQ) region of the huntingtin protein causes its aggregation into β-sheet–containing amyloid fibrils. The biological consequences of polyQ-containing aggregates are not completely known, but it has been suggested that nuclear-localized aggregates causes global impairment of the ubiquitin-proteasome (UPS) degradation system. Indeed, nuclear inclusions (NIs) of polyQ contain ubiquitin and proteasome components, implying that they are storage sites for damaged or misplaced proteins. However, evidence exists that NIs have proteolytic activity. Chen *et al.* used a previously described silica nanoparticles (silica-NPs) strategy to induce intranuclear protein aggregates. The aggregates form exclusively within the nucleoplasm of treated cells but are not cytotoxic like NIs formed by conventional methods. These NIs form amyloid-like structures and recapitulate the protein composition and solubility of polyQ-induced nuclear protein aggregates precisely. The authors used inhibitors and activators of the UPS to show that proteasomal activity of the NIs correlates with protein aggregation and that there is a sustainable induction of nuclear proteasomal activity that corresponds to NI formation. Also, using a microinjection-based method that allowed them to pinpoint the location of proteolysis of a fluorescent substrate, they showed that a significant subpopulation of silica-NP–induced NIs are proteolytically active. These findings support the idea that NIs represent sites of proteasomal protein degradation. (*J. Cell Biol.* 180, 697–704, 2008) *MB*

Metal ambivalence

Carbonic anhydrase (CA) catalyzes the interconversion of carbon dioxide and bicarbonate. Although CAs generally use Zn^{2+} as an essential cofactor, CAs from marine diatoms use Cd^{2+} for catalysis. Xu *et al.* now report the crystal structures of the Cd^{2+}, Zn^{2+} and apo forms of cadmium CA (CDCA). Although the two proteins have no amino acid sequence or global structural similarity, critical CDCA active-site residues are placed with striking similarity to those in the active site of the beta CAs of plants, suggesting structural mimicry between the two enzymes. Biochemical studies revealed that both Cd^{2+} and Zn^{2+} can facilitate high CDCA activity and that one metal can readily be replaced by the other. The apo form of CDCA is in a more open conformation than the metal-bound form, which may decrease the energetic cost of metal release and, therefore, facilitate metal exchange. Most metalloenzyme active sites are finely tuned for ensuring metal specificity, and non-native metals typically support little or no catalytic activity. The ability of CDCA to function efficiently with Zn^{2+} and Cd^{2+} seems to be a unique adaptation to the metal-deficient conditions of the ocean surface. CA is a critical enzyme in the transfer of carbon from the atmosphere to the ocean, and cadmium is depleted at the ocean surface by phytoplankton uptake and regenerated through remineralization at the ocean depths. Therefore, CDCA provides an intriguing link between global carbon and cadmium cycles. (*Nature* **452**, 56–61, 2008). *JK*