SENSORS AND PROBES

Led to the near infrared

Two groups describe near-infrared fluorophores that can be used in applications ranging from cellular to clinical imaging.

Fluorescence imaging is shifting to the red, and for good reason. Near-infrared (NIR) probes are ideal for biological imaging because few endogenous molecules in organisms absorb or emit in the NIR region: there is little background autofluorescence to contend with. Furthermore, NIR light is less damaging to delicate cells and is safe even for clinical applications.

Two recent reports add new NIR small-molecule fluorophores to a growing toolbox of red-shifted probes. Kai Johnsson of the École Polytechnique Fédérale de Lausanne and his colleagues report an NIR fluorophore for imaging proteins in cells using super-resolution microscopy (Lukinavičius *et al.*, 2013). John Frangioni of the Beth Israel Deaconess Medical Center in Boston and his group report a promising NIR fluorophore for image-guided surgery (Choi *et al.*, 2013).

Although an assortment of NIR small-molecule probes exist, most cannot cross cell membranes; invasive techniques are needed to get them into cells. Johnsson's team turned to a class of silicon-containing rhodamine (SiR) derivatives with excellent imaging properties and membrane permeability, described by Tetsuo Nagano's group at the University of Tokyo. The team reasoned that they could make benzylguanine derivatives of these SiR probes to enable covalent labeling of SNAP-tag fusion proteins. Finding that nonspecific binding was unacceptably high, they modified the SiR probe structure to reduce its hydrophobicity. As it turned out, this not only reduced nonspecific probe binding but also increased its membrane permeability.

Johnsson's team went on to create SiR derivatives to also label CLIP- and Halo-tagged fusion proteins. The SiR probes breezed through internal membranes in human cells, allowing fusion proteins expressed in various organelles to be labeled. The researchers have used the probes to label proteins in cortical neurons in rat brain sections and to perform super-resolution microscopy in live cells to localize structures with high precision.

SiR probes are particularly well suited for live-cell imaging because they covalently bind their specific targets, which likely allows the fluorophores to maintain a zwitterionic form—that is, positive and negative charges decorate the probe at various locations, but the overall charge is neutral—whereas unreacted probes aggregate or bind nonspecifically to other molecules and assume a nonfluorescent form.

Frangioni's group, on the other hand, has been interested in developing an NIR fluorophore for *in vivo* and clinical applications, particularly for image-guided surgery. Existing NIR probes tend to bind and stick to lots of things, creating a high background signal. In previous work, Frangioni's team reported a zwitterionic heptamethine indocyanine NIR fluorophore, dubbed ZW800-1, with reduced nonspecific binding and reduced background signal. This probe contains a reactive carboxyl handle allowing simple, one-step coupling reactions to amine-containing targeting ligands.

In their most recent work, Frangioni's team coupled ZW800-1 to ligands or antibodies such that the probe would light up cells expressing the target of interest, suggesting utility for applications including immunocytometry and histopathology. ZW800-1–conjugated ligands could be targeted to tumors in mice, allowing image-guided surgery. They also conjugated the probe to fibrinogen to image blood clots in a rat model of internal bleeding. In all of these applications, ZW800-1 showed much better performance than two of the best commercially available NIR dyes, exhibiting almost no background signal.

ZW800-1 was processed by the kidneys within 4 hours of intravenous injection into an animal. This rapid clearance is likely due to the unique zwitterionic structure of the probe. Fast probe elimination by the renal system is important for potential clinical applications; indeed, the first human clinical trials are under way.

As new probes such as these useful tools emerge, we are sure to see a greater movement of imaging into the near infrared.

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RESEARCH PAPERS

Choi, H.S. *et al.* Targeted zwitterionic near-infrared fluorophores for improved optical imaging. *Nat. Biotechnol.* **31**, 148–153 (2013).

Lukinavičius, G. *et al.* A near-infrared fluorophore for live-cell super-resolution microscopy of cellular proteins. *Nat. Chem.* **5**, 132–139 (2013).