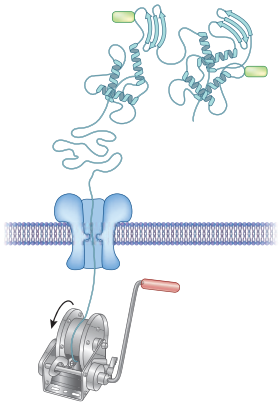
Nanopores for proteins

Nanopores hold promise for single-protein characterization.

The ability to sequence DNA with nanopores has been a technical coup, but as polymers go, DNA is pretty tame: besides having a relatively uniform secondary structure and charge, it is made up of only four bases. Compare this to proteins and their 20 amino acids, variable charge and hydrophobicity, and considerable secondary and tertiary structure. Despite this complexity, nanopores may soon be useful for assessing protein structural domains, modifications and interactions.

Nanopore sensors measure the disruption of ionic current as a single molecule transits the pore; the level of disruption depends on the molecule's sequence and structural features. The same voltage that generates current across the pore causes negatively charged DNA to enter it. To coax weakly charged proteins into the pore, researchers have had to add a string of negatively charged amino acids (*Nat. Biotechnol.* **31**, 247–250, 2013) or a short stretch of DNA (*Nat. Nanotechnol.* **8**, 288–295, 2013) to one end. Optimizing the efficiency of adding the leader will be important for generalizing and scaling up assays.



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Pulling proteins through nanopores can provide information on structure and modifications.

Threading a leader through the pore causes some proteins to continue unwinding, but others need more force to denature and pull them through. Adding a recognition site for the unfoldase ClpX to the leader made it possible to ratchet target protein through a pore in a sequence-independent fashion (*Nat. Biotechnol.* **31**, 247–250, 2013). For tightly folded proteins, denaturants may need to be tested.

Nanopores have been used to characterize functional aspects of unfolding proteins during translocation. One study used them to discriminate site-specific phosphorylation states of thioredoxin (*Nat. Biotechnol.* **32**, 179–181, 2014). Engineering larger protein pores (*J. Am. Chem. Soc.* **135**, 13456–13463, 2013) may also allow for folded domains or proteins to enter the pore in order to assay protein interactions with other proteins, drugs or substrates.

Ultimately, designing motors that move protein through the pore one amino acid at a time will be a critical step toward single-protein sequencing. It may be impossible to resolve the signature of amino acids individually or in each of their bewildering number of combinations. But recognizing domains and coarse features may be sufficient to identify proteins, characterize unfolding or protein states, or assess interactions. We look forward to seeing nanopores become established tools for studying proteins at the single-molecule level. **Tal Nawy**

Imaging at depth

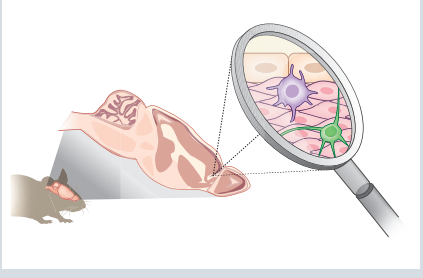
A closer look into the depths of organs such as the brain is within reach.

The spatial organization and functional properties of organs or tissues are preferably studied in the intact state. Several emerging techniques achieve this goal either in fixed or in living samples.

The opacity of biological material complicates imaging deep within a specimen. 'Clearing' the tissue can overcome this hurdle in fixed samples with protocols such as CUBIC, iDISCO or PACT, all of which preserve fluorescent immunolabeling signals. These techniques were established recently and await application to a variety of different questions.

Clearing techniques are not available for live specimens. Alternative approaches can reduce light scattering and improve transparency in these circumstances: for example, by employing nonlinear excitation such as with two-photon microscopy, which does not excite molecules along the path of incident light, or by imaging with near-infrared illumination, which is poorly scattered by biological material. The advent of genetically encoded near-infrared probes and nanoparticles has been particularly useful for non-invasive cancer studies or cell tracking *in vivo*. At the same time, progress has been made in shifting the imaging window to longer wavelengths, allowing for even deeper tissue penetration.

Another strategy for dealing with light scattering is to forgo imaging with light. In photoacoustic or optoacoustic imaging, incident light is absorbed within a tissue and converted into ultrasound via thermoelastic expansion. Ultrasound is less sensitive to scattering than light, enabling imaging at greater depths than with conventional microscopy while maintaining good resolution. The different absorption properties of biological materials make label-free imaging possible. In addition, absorption differences between saturated and unsaturated hemoglobin allow for functional imaging in the brain.



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Imaging deep within intact tissue samples or animals.

Finally, the characteristics of different biological materials introduce imaging aberrations, a problem that worsens with depth. Adaptive optics is used in astronomy to correct for optical aberrations, and it has recently been used to image transparent biological specimens such as the zebrafish embryo. Efforts in this direction may soon allow for use of adaptive optics in less transparent organisms.

Researchers now have an unprecedented choice of techniques for imaging tissue deep within a specimen. We hope that further developments in this area will spur the analysis of cells and tissues in their native context. **Nina Vogt**