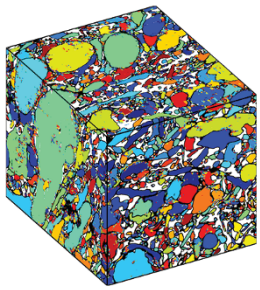
IMAGING

Simple tools for difficult imaging

Small chemical tweaks during tissue preparation make a big difference for imaging thick, complex biological samples.

Understanding biological complexity often requires that cellular populations be analyzed within tissues. But imaging in thick tissue samples is notoriously difficult. Two recent papers show how seemingly minor modifications in sample preparation can have big effects.



Modified from Pallotto *et al.* (2015) and provided by K. Briggman

Segmented cells in tissue with preserved extracellular spaces.

The work of Kwanghun Chung and colleagues at MIT shows that tissue samples up to a millimeter thick that have been fixed with paraformaldehyde (PFA) can be treated with glutaraldehyde (GA) to form a stable 'tissue gel' that can be subjected to multiple rounds of antibody stripping and probing without undue loss of structural integrity or antigenicity. The researchers successfully labeled nine antigens in the same human brain sample and demonstrated that 12 rounds (at minimum) of labeling produce no image distortion. The constructs can also withstand high temperatures (80 °C), so that tissue can be optically cleared rapidly, within days.

A general problem in working with thick samples is that any agent used to modify the tissue—whether a fixative or a labeling antibody—needs to diffuse into its interior. In addition, superficial layers can act as a 'sink' for reagents that interact with their targets; typically, superficial layers are modified much more readily in a given time than deeper ones. Chung and colleagues demonstrate an approach to tackle this problem.

Making use of the fact that GA does not cross-link protein under acidic conditions, they essentially render the fixation conditional. After GA has been allowed to penetrate the tissue at pH 3, the buffer is returned to neutral, enabling uniform fixation throughout the sample. Remarkably, this principle can also be applied to other agents, such as molecular labels; all one

BIOPHYSICS

WATCHING PROTEINS FOLD ON THE RIBOSOME

Researchers monitor cotranslational protein folding in real time.

The mechanism of protein folding has been the subject of intense research over the past several decades. Most studies are carried out on full-length proteins in solution. Although these studies are informative, they do not give a complete picture of how proteins fold in cells as they are synthesized by the ribosome. Such cotranslational folding is poorly understood, as is the potential role of the ribosome in directing proper folding of proteins.

"It has been long suggested that cotranslational folding differs from the spontaneous folding-unfolding of proteins in solution, because the narrow polypeptide exit tunnel of the ribosome restricts the choice of potential conformations for folding," notes Marina Rodnina of the Max Planck Institute for Biophysical Chemistry. To better understand these differences, she and lab members Wolf Holtkamp, a postdoctoral fellow, and Goran Kokic, an undergraduate student, developed methods to monitor a protein's structure as it is synthesized by the ribosome.

For their experiments, the team used a purified *Escherichia coli in vitro* translation system to translate a 112–amino acid protein consisting of an α-helical domain and linker. They then studied the folding of the emerging, or nascent, protein using fluorescence resonance energy transfer (FRET), which can measure the distance between two fluorophores. They generated synchronized initiated translating complexes in which the first amino acid was labeled with a fluorophore. They then allowed translation to proceed by adding in the necessary factors. For site-specific labeling of an internal position in the nascent protein, they included a lysine tRNA charged with a fluorescently labeled lysine derivative.

The team then obtained FRET measurements to examine the effects of the extent of translation on the structure of the protein. The team expected that folding would be hindered in short versions of the protein because of confinement by the polypeptide exit

needs is to identify the right chemical on-off switch. For instance, antibody binding to antigen and dye labeling of lipid can be inhibited by SDS and therefore rendered conditional by this additive (of course, samples must already be fixed for this to work).

Powerful though the GA-based fixation approach may be, it does still require prior fixation with PFA as well as long incubations under harsh conditions. Although Chung and colleagues found that antigenicity and sample structure were well preserved, this could vary depending on the parameters being studied. It also remains a fact that fixation of any kind is likely to cause some perturbation to the sample. Indeed, a second paper from Kevin Briggman and colleagues at the US National Institutes of Health revisits a known artifact of aldehyde-based fixation: extracellular space is lost in aldehyde-fixed tissue, the researchers remind us, in contrast to rapidly frozen tissue. Briggman and colleagues sought to determine whether they could manipulate this artifact, to wit, increase the extracellular space and thus improve automated cell segmentation in electron micrographs for the reconstruction of neuronal circuits.

In a spirit similar to that of the MIT group, Briggman and colleagues modified the buffer in which they fixed mouse neural tissue, increasing the osmolarity of the solution to prevent cell swelling and loss of extracellular space. They observed that this increases the accuracy of cell segmentation in 2D and 3D electron micrographs, does not cause macroscopic changes to the sample, can preserve ultrastructure, and makes it easier to identify gap junctions than in conventionally prepared samples. In addition, perhaps not surprisingly, antibodies penetrate better into samples in which extracellular space has been preserved.

It is an agreeable irony that our increasingly sophisticated ability to image complex, multidimensional biological samples seems to benefit in no small way from an understanding of fundamental, low-tech methods of tissue preparation.

Natalie de Souza

RESEARCH PAPERS

Murray, E. *et al.* Simple, scalable proteomic imaging for high-dimensional profiling of intact systems. *Cell* **163**, 1500–1514 (2015).

Pallotto, M. *et al.* Extracellular space preservation aids the connectomic analysis of neural circuits. *eLife* doi:10.7554/eLife.08206 (9 December 2015).

channel, and as expected, when only 41 amino acids of the protein were translated, there was low FRET signal, demonstrating that the protein was held in an extended form by the exit channel. Conversely, when the full-length protein was synthesized such that the entire α-helical domain was free from the exit channel, a FRET signal consistent with the folded protein was observed. Interestingly, for intermediate lengths of the protein, a high FRET signal was measured that was distinct from the signals of both the folded protein and the extended form, indicating that the nascent peptide formed a unique compact structure.

These results were corroborated by further experiments using mutants of the protein with disrupted folding and by studies using photoinduced electron transfer between the first labeled amino acid and engineered tryptophan residues, a technique that is very useful for probing structures at short length scales. These experiments provided evidence complementary to that from the team's FRET studies and confirmed that the protein folded into a compact structure distinct from the final folded structure after leaving the exit channel, a result that Rodnina recalls was "most surprising."

These experiments provide important evidence that the ribosome can affect the structure of translated proteins prior to final folding. The ribosome could have the potential to prevent misfolding or even direct proper folding of proteins in the complex cellular milieu by inducing or stabilizing folding intermediates, although future work will be necessary to further elucidate its role. Overall, the future is bright for these complementary approaches, and Rodnina hopes to use them to study how other protein domains fold, as well as to examine how other factors, such as chaperones, affect protein folding on the ribosome.

Rita Strack

RESEARCH PAPERS

Holtkamp, W. *et al*. Cotranslational protein folding on the ribosome monitored in real time. *Science* **350**, 1104–1107 (2015).