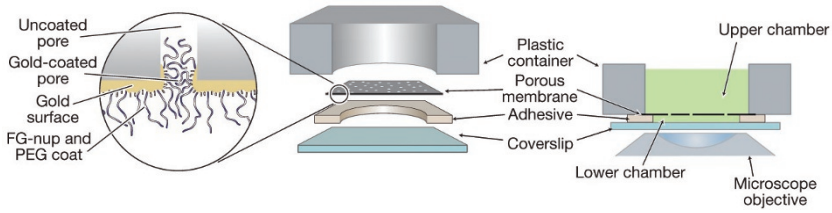
CELL BIOLOGY

Mimicking a pore

A functionalized polycarbonate nanosorter mimics fundamental properties of the nuclear pore complex.

The nuclear pore complex (NPC) is a heterogeneous scaffold of proteins that selectively filters biomolecules moving between the nucleus and the cytoplasm. The NPC is an ancient yet highly conserved gate decorated with phenylalanine-glycine (FG)-nucleoporins, natively disordered protein domains that interact directly with the transporters that ferry biological cargo through the pore.

In an effort to determine whether an artificial pore lined with FG-nucleoporins is sufficient to recapitulate NPC selectivity, Brian Chait and Mike Rout, in conjunction with their colleagues at Rockefeller University, engineered a nanosorter that mimics the NPC. "We worked for many years on a detailed description of the nuclear pore complex," says Chait, and "what we didn't find was any sort of motor molecules or ATPases that would make this thing move in a concerted way." Rout adds, "We knew that the NPC was a gate, but when we think of a gate, we think of something that opens and shuts." The absence of conventional molecular motors and moving parts means that the NPC is an unusual gate. Chait says, "At that point, we realized that this machine would have to work by diffusion."



Schematic of the NPC mimic, including a single pore, the device that supports the membrane and the experimental setup on a microscope. FG-nup, FG-nucleoporin. Reprinted from *Nature*.

The initial impression that the NPC was a hole surrounded by FG-nucleoporins did not change as more details emerged. "This is a virtual gating machine; it is always open to the right kind of things, but always closed to the wrong things," says Chait. To verify this hypothesis, they decided to try and build one. They engineered a porous polycarbonate membrane that was functionalized with FG-repeat domains conjugated to the membrane through a thin layer of gold. The NPC mimic, or nanosorter, incorporated several key features of the NPC, among them that the nanopores had appropriate diameters, FG-nucleoporins were present in appropriate orientations and densities, and the business end of the membrane itself was thin. The researchers tested their NPC

IMAGING AND VISUALIZATION

THE MANY AGES OF A PROTEIN

Monomeric fluorescent timers determine protein age within the cell.

Proteins have a finite lifetime in cells. They are synthesized, perform their functions and are eventually degraded. A general method to measure the 'age' of proteins at different spatial locations would yield insight into the timing of intracellular events and pathways of cellular organization.

In recently published work, Vladislav Verkhusha and colleagues at the Albert Einstein College of Medicine in New York present monomeric fluorescent timers designed for this purpose.

Several years ago, in studies on the maturation of red fluorescent proteins, Verkhusha and colleagues realized that red chromophore maturation goes through a blue, protonated GFP-like form. This observation was critical to the present work. As Verkhusha explains, "Knowing the mechanism of maturation immediately suggested many applications. One of them was to slow the maturation from the blue to the red form, and this has led to our fluorescent timers."

The researchers used both site-directed and random mutagenesis on the monomeric variant of DsRed, mCherry, to generate blue-to-red fluorescent timers that mature at slow, medium or fast rates. By measuring the ratio of red to blue fluorescence at a particular location in the cell, they could determine the 'age' of the protein at that location.

Another timer protein, DsRed-Timer, was previously reported, but it is tetrameric and thus prone to causing aggregation of fusion partners. "DsRed-Timer could be used to trace the timing of a gene promoter, but not of the gene product," Verkhusha says.

Applying their medium-rate fluorescent timer (Medium-FT), Verkhusha and colleagues examined the traffic of the LAMP-2A protein in HeLa cells. Previous studies have suggested that the lysosomal LAMP proteins can reach their destination either through direct trafficking from the Golgi to the late endosome, or through an indirect pathway via endocytosis from the plasma membrane.

The researchers expressed a pulse of the LAMP-2A–Medium-FT fusion and measured the red-to-blue ratio at different times and intracellular locations. They observed that the measured 'age' of the detected proteins increased from Golgi to plasma membrane to endocytic vesicles and lysosomes, supporting a predominantly indirect route of LAMP-2A targeting to the lysosome.

Although it may be possible to carry out similar experiments using photoactivatable proteins, Verkhusha points out that fluorescent timers have some advantages. "Typically, photoactivation leads to a rather limited pool of fluorescent protein. Then this pool goes all over the cell and at some point we reach the detection limit," he says. "Also, photoactivatable

mimic by measuring the flux of fluorescently labeled proteins from one chamber to another on the opposite side of the membrane. Amazingly, their relatively simple nanosorter recapitulated many fundamental properties of the NPC, including selectivity.

Looking forward, this nanosorter should prove highly useful to scientists interested either in understanding how the NPC works or in its practical applications as a selective filter. In this first application of the nanosorter, an unanticipated role emerged for transporters that carry cargo through the pores. As Rout explains, "Rather than just being carriers of cargo back and forth across the NPC, [the transporters] seem to help exclude things that are not supposed to be there." Thus, the transporters are an unexpected but possibly major component of the gate between nuclear and cytoplasmic compartments. Further experiments of this nature, with more sophisticated nanosorters, should provide greater mechanistic insight into NPC selectivity and function.

Another application that Chait and Rout envision for improved versions of this nanosorter is molecular sorting. According to Rout, "Currently, molecular sorting is based on chromatography, and that's great, but it has its limitations. It would be nice to have alternative techniques in your pocket. Biological membranes are selective filters. Once we fully understand the principles of how they work, we could design our own." Chait adds, "Ultimately, we may be able to sort all kinds of different molecules from very messy milieus."

Amy Donner

RESEARCH PAPERS

Javanovic-Talisman, T. *et al.* Artificial nanopores that mimic the transport selectivity of the nuclear pore complex. *Nature* advance online publication, 21 December 2008.

proteins are useful for fast events, perhaps over tens of minutes, but timers, because their maturation takes hours, allows us to use them for much slower processes."

The researchers also used kinetic models of timer maturation to estimate the absolute age of the protein at different cellular locations. The timers are not limited to use with heterologous promoters but may be fused to proteins expressed under their endogenous promoters as well. It may be particularly interesting to use them to study proteins with complex subcellular localization patterns or that undergo spatially localized post-translational modifications.

Potential users of these mCherry-based fluorescent timers, Verkhusha emphasizes, should keep in mind that several of the caveats that apply to all red fluorescent protein fusions also apply here. Potential mistargeting of timer fusions and pH sensitivity of the timer must be taken into account. More specifically, because DsRed-derived proteins can undergo light-induced maturation when illuminated with high-intensity violet light, it may be preferable for images to be collected in the red channel prior to the blue channel.

With sufficient attention, however, these monomeric fluorescent timers will be useful tools for tracing individual proteins in space and time.

Natalie de Souza

RESEARCH PAPERS

Subach, F.V. *et al.* Monomeric fluorescent timers that change color from blue to red report on cellular trafficking. *Nat. Chem. Biol.* **5**, 118–126 (2009).

NEWS IN BRIEF

GENOMICS

Finding noisy promoters

Genetically identical populations of unicellular organisms often show a surprising amount of phenotypic variation. Freed *et al.* developed a fluorescence-activated cell sorting method to identify 'noisy' promoters in *Salmonella enterica* ssp. I serovar *Typhimurium*. They created a GFP-tagged genomic plasmid library and subjected populations to fluctuating selection for GFP expression, thus enriching for promoters exhibiting high noise; the noisiest promoters were involved in flagella synthesis.

Freed, N.E. *et al. PLoS Genet.* **4**, e1000307 (2008).

MOLECULAR LIBRARIES

Libraries against libraries

The selection of antigen-specific antibodies is slow because antibodies are typically selected against one antigen at a time. Bowley *et al.* now present a co-selection method for identifying antibody-antigen pairs from libraries displayed in distinct platforms: yeast and phage. This selection method, in tandem with high-throughput antibody-antigen pair validation, should allow simultaneous identification of all antibody-antigen pairs in the mix and, theoretically, should saturate the proteome.

Bowley, D.R. *et al. Proc. Natl. Acad. Sci. USA* **106**, 1380–1385 (2009).

PROTEOMICS

Predicting high-responding peptides

In targeted proteomics and biomarker discovery applications, it is most effective to set the mass spectrometer to selectively detect specific peptides in multiple reaction monitoring (MRM) mode. Fusaro *et al.* present a computational predictor to help identify signature peptides that are unique to one protein (proteotypic) as well as most likely to produce a high ion current response in the mass spectrometer, based on their physicochemical properties.

Fusaro, V.A. *et al. Nat. Biotechnol.* **27**, 190–198 (2009).

BIOPHYSICS

Single-molecule filament disassembly

RAD51 plays a central role in homologous recombination. The protein polymerizes around a single-stranded DNA to form a duplex-invading nucleoprotein filament, which then disassembles after strand exchange. van Mameren *et al.* used a powerful single-molecule approach, combining optical tweezers with fluorescence microscopy and microfluidics, to investigate the molecular mechanism of nucleoprotein filament disassembly.

van Mameren, J. *et al. Nature* **457**, 745–748 (2009).

STEM CELLS

Distinguishing human embryonic stem cells

Aggressive cancers express human embryonic stem (hES) cell–associated genes, suggesting that hES cells are vulnerable to transformation. Partially transformed cells with cancer cell characteristics should be avoided in clinical applications. Towards developing an approach to identifying such cells, Werbowetski-Ogilvie *et al.* characterized two hES cell lines expressing pluripotency markers at high levels and show that although they are not malignant, they show signs of neoplastic progression.

Werbowetski-Ogilvie, T.E. *et al. Nat. Biotechnol.* **27**, 91–97 (2009).