CHEMICAL BIOLOGY

Smaller can be better

By splitting the FlAsH-ReAsH tetracysteine recognition sequence into two pieces, researchers show that these small-molecule fluorophores can report protein folding or protein-protein interactions.

Fluorescence is not only beautiful, but also useful. Fluorescence imaging tools have become indispensable for cell biology. An entire rainbow of fluorescent proteins is now available to researchers for a multitude of applications. Small-molecule fluorophores, which lack the advantage of genetic encodability but have the advantage of petiteness, are also beneficial in many situations.

Alanna Schepartz of Yale University and her colleagues report the design of a new concept for fluorescent reporting based on the binding of FlAsH or ReAsH biarsenical reagents to a split tetracysteine motif, which they call "bipartite tetracysteine display." These fluorophores had been shown several years ago by Roger Tsien and coworkers to specifically label proteins tagged with a tetracysteine (CCPGCC) sequence and only become fluorescent upon forming a biarsenical tetracysteine complex. The bipartite tetracysteine display method now uses these popular fluorophores to report on protein folding or protein-protein interactions. By placing two cysteine-cysteine pairs such that they are far apart in primary sequence—but such that they come into close proximity when the protein is folded (or when two proteins interact)—the fluorescent biarsenical complex can be reconstituted.

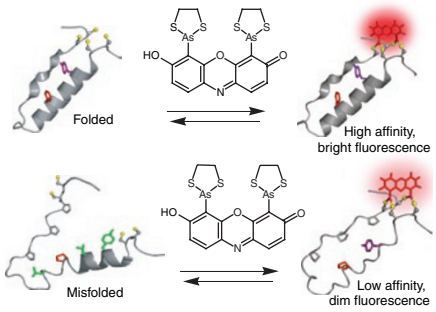

Figure 1 | Bipartite tetracysteine display can distinguish between folded and misfolded proteins. The folded protein (top) has a high affinity for ReAsH and forms a brightly fluorescent complex. The misfolded protein (bottom) has a much lower affinity for ReAsH and forms a dim complex. Reprinted from *Nature Chemical Biology*.

The idea came to Schepartz while she was preparing a lecture about FlAsH labeling for her chemical biology class. She was chatting with her postdoc Nathan Luedtke (now a faculty member at the University of Zurich) about her lecture and mentioned that she could not believe that FlAsH (and ReAsH) would only form complexes with the specific CCPGCC sequence. "And Nathan, who's exceptionally quick on the uptake, realized immediately that it could be a really interesting project," recounts Schepartz.

FRET, fluorescence resonance energy transfer, is used to monitor protein folding and protein-protein interactions. Bipartite tetracysteine display offers an alternative that does not require two bulky fluorescent proteins, which can interfere with protein folding and function. "In addition," says Schepartz, "the fluorescent changes induced by the binding of a biarsenical should be higher than the changes in fluorescence due to FRET."

By engineering dicysteine motifs into a set

GENE TRANSFER

THERE IS A VIRUS GOING AROUND

Researchers can manipulate gene expression in tumors *in vivo* by specifically making them susceptible to viral infection.

Human cancers progress in fits and starts, when a few cells acquire spontaneous mutations that change gene expression patterns and sometimes allow those cells to escape tumor-suppression pathways. Any given protein may have several roles in these processes, but what role that protein has when is difficult to uncover. As Memorial Sloan-Kettering Cancer Center researcher Yi-Chieh Nancy Du describes, "many somatic gene mutations and the altered expression of many genes have been discovered in cancers, but it is not easy to distinguish genes important for tumorigenesis from 'passenger' events." To answer those questions, researchers need to target established tumor cells at different stages. Now, Du and her colleagues in Harold Varmus's laboratory describe a system that makes tumor cells susceptible to infection by an avian retrovirus, allowing researchers to manipulate gene expression in the tumor at defined time points.

There are several ways to induce transgene expression, including by Cre-*lox*–based recombination and under tetracycline- or estrogen-dependent regulation. These techniques, however, require the genomic insertion and genetic transmission of the construct, and each new transgenic line is costly in terms of money and time. Additionally, these methods affect most cells in the induced tumor and may not accurately emulate human cancer progression.

When looking for other approaches, Du and colleagues proposed to introduce the genes of interest into the developing tumors. Previous work on the avian leukosis virus had shown that ectopic expression of the TVA receptor makes a mammalian cell susceptible to avian viral infection. If a tumor cell expresses this receptor, infection with the avian retrovirus can introduce new DNA into the cell, allowing researchers to manipulate gene expression *in vivo*. As a beneficial side-effect of low infection rates, few cells would receive the gene of interest, which more closely mimics the spontaneous mutations that cause the progression of human cancers.

To develop this method, the researchers turned to a well-known tumor model in which SV40 T antigen is expressed specifically in pancreatic β cells. T antigen blocks two tumor suppressor pathways, causing a reproducible tumor progression in pancreatic islets that mimics that of human cancers. In this model, they could co-express the T antigen with the TVA receptor for the avian viral vector, inducing virus-susceptible tumors. Researchers then have a choice of two ways to analyze phenotypes: *in vivo* infection of tumors for physiological studies or *in vitro* cell lines from these tumors for molecular and biochemical studies.

of model protein sequences, the researchers tested whether bipartite tetracysteine display could be used to monitor intramolecular protein folding and intermolecular protein-protein dimerization *in vitro*. In testing a wide variety of different contexts, they found that "in some cases the proteins [modified for bipartite tetracysteine display] are better behaved than in other cases, but in no case yet have we flat-out failed," says Schepartz, suggesting that the steric requirements for FlAsH or ReAsH binding to the bipartite tetracysteine motif are not quite as stringent as one might think.

They also showed that they could distinguish well-folded and misfolded proteins. Proteins with destabilizing point mutations still formed complexes with FlAsH or ReAsH, but the fluorescence was much dimmer in comparison to well-folded proteins (**Fig. 1**). They have not observed the formation of nonspecific fluorescent complexes of proteins that would otherwise not interact, though Schepartz does clarify that they have not extensively tested for this. Notably, the researchers also showed that their method can be used to detect protein folding and protein-protein interactions in live mammalian cells.

Schepartz and her colleagues believe that bipartite tetracysteine display offers a useful small-molecule alternative to FRET for designing new post-translational modification or protein-protein interaction sensors. The method should also be compatible with other techniques such as electron microscopy and could be used in high-throughput screening applications, for example, to identify small molecules that either stabilize or disrupt protein dimerization.

The future for this method indeed looks very 'bright'.

Allison Doerr

RESEARCH PAPERS

Luedtke, N.W. *et al.* Surveying polypeptide and protein domain conformation and association with FlAsH and ReAsH. *Nat. Chem. Biol.* **3**, 779–784 (2007).

Du and colleagues found that, although infection with a control virus had no effect on tumor progression, genes could be transmitted to the tumor cells and dramatically influenced tumor behavior. For instance, expression of a dominant-negative E-cadherin (a cell adhesion molecule) in these tumors caused similar phenotypes as coexpression of T antigen and dominant-negative E-cadherin in these pancreatic islet tumors. Opportunely, the researchers used this system to characterize a previously unknown role of the anti-apoptotic protein Bcl-xL in the cytoskeletal rearrangements that affect metastasis.

This system works particularly well for tumors that have stereotyped progression. As Du explains, "knowing the timing of development of hyperplastic lesions is important for introducing these avian viruses" at the desired stage of tumorigenesis. Once these variables are known, however, "this method has the flexibility to deliver a combination of the avian viruses encoding different genes simultaneously or sequentially to study their interactions in tumorigenesis," she says. So using viruses to deliver genes may not just save money and time over traditional transgenic techniques, but also make it possible to manipulate tumors *in vivo* in unprecedented ways.

Katherine Stevens

RESEARCH PAPERS

Du, Y.-C.N. *et al.* Assessing tumor progression factors by somatic gene transfer into a mouse model: Bcl-xL promotes islet tumor cell invasion. *PLoS Biol.* **5**, e276 (2007).

NEWS IN BRIEF

GENE TRANSFER

Human *ROSA26* locus

The *ROSA26* locus in the genome of mouse embryonic stem cells is easy to target and expresses transgenes well. Irion *et al.* identified the human equivalent of the *ROSA26* locus on chromosome 3. They integrated various genes into that locus and followed the multilineage differentiation of the targeted cells. Their locus provides a safe landing spot for transgenes and makes worries about gene silencing or disruption of endogenous genes because of random integration a thing of the past.

Irion, S. *et al. Nat. Biotechnol.* **25**, 1477–1482 (2007).

IMMUNOCHEMISTRY

High-throughput antibodies

Antibodies are key reagents for the study of protein function. Schofield *et al.* now screen a phage display library to identify human monoclonal antibodies on an unprecedented scale. They identified antibodies to 72% of 404 antigen targets, with an average of 25 specific clones to each. As with all high-throughput efforts, quality control and validation are key elements of the work.

Schofield, D.J. *et al. Genome Biol.* **8**, R254 (2007).

STEM CELLS

Fingerprinting stem cells

Haematopoetic stem cells differentiate to give rise to all the cells in the blood. By performing global gene expression analysis on these cells and on their differentiated progeny, Chambers *et al.* identified molecular fingerprints for specific cell types and cell lineages in the blood. This resource will be useful for developing markers and for identifying regulators of differentiation and cell fate specification in haematopoesis.

Chambers, S.M. *et al. Cell Stem Cell* **1**, 578–591 (2007).

CHEMISTRY

Synthetic lectins

Lectins are highly specific carbohydrate receptors that are being increasingly applied as tools for carbohydrate detection. Ferrand *et al.* designed a synthetic lectin analog that can recognize the disaccharide cellobiose with very high affinity and specificity. As the analog is much smaller than natural lectins, this represents notable progress toward the design of synthetic carbohydrate receptors for practical use as sensors.

Ferrand, Y. *et al. Science* **318**, 619–622 (2007).

IMAGING AND VISUALIZATION

Single-molecule tracking by fours

The need to track single fluorescently labeled proteins in living cells has created a desire for three-dimensional single-particle tracking methods using low-level illumination. Lessard *et al.* show that they can track single quantum dots using only 10 μW of energy by using four optical fibers coupled to individual detectors to effectively create four confocal pinholes that provide location information for feedback control–based tracking.

Lessard, G.A. *et al. Appl. Phys. Lett.* **91**, 224106 (2007).