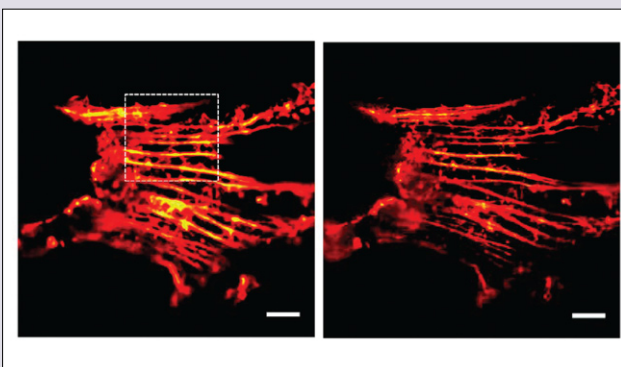


Citations

STEDy Improvement

Just as nanofluidics has stolen some of the limelight from microfluidics, advances in nanoscopy are beginning to make standard microscopy seem a bit passe. One of the first techniques described for optical imaging at the nanoscale is stimulated emission depletion microscopy, or STED, which couples excitation light with a quenching beam in a surrounding ring. The result is a smaller fluorescing spot, leading to so-called super-resolution optical microscopy. While the technology is impressive, the tools leave a bit to be desired. Unlike the array of fluorophores available for traditional confocal microscopy, the options for STED microscopy have generally been limited to far-red organic dyes. Recently, a HaloTag-based labeling approach has been described that is compatible with STED microscopy, but the fluorescent ligand is cell impermeant and thus restricted in its potential uses. Another class of fluorophore-binding factors are the fluorogen activating proteins (FAPs), which bind (and thereby render fluorescent) the far-red dye malachite green. In an article in *Bioconjugate Chemistry*, Fitzpatrick et al. show that an engineered variant of this system is ideally suited to live-cell imaging using STED microscopy. Resolution is similar to that obtained when using organic dyes with STED, but the genetically encoded FAP enables the types of protein-labeling studies commonly performed in traditional



Actin as visualized by confocal microscopy (left) and STED nanoscopy (right). Reprinted with permission © 2009 ACS.

fluorescence microscopy. Together with a new far-red variant of DsRed, which is described in a recently published *Biochemistry* article, the FAP–malachite green labeling method should accelerate the use of STED nanoscopy for dynamic imaging of subcellular components in living cells.

Fitzpatrick et al. 2009. STED nanoscopy in living cells using fluorogen activating proteins. *Bioconjugate Chem.* 20:1843-1847.

Diversity Matters

There is seemingly endless variety in protein detection platforms these days, but the options contract sharply when it comes to acceptable sample types. Biomolecules that autofluoresce or absorb at inconvenient wavelengths aren't welcome in many ELISAs or protein microarrays. By the same token, if a sample strays outside narrow pH, ionic strength, or temperature constraints, electrochemical or microcantilever-based techniques are off limits. In a technical report appearing in *Nature Medicine*, Gaster et al. describe a detection scheme intended to be as open as possible to diverse sample conditions. The assay mirrors a sandwich ELISA, but instead of a colorimetric or other matrix-sensitive detection method, readout is by magnetoresistive sensors. The components of the assay include a capture antibody immobilized on a magneto-nano sensor chip, a biotinylated detection antibody, and a streptavidin-conjugated magnetic nanoparticle tag. Magnetic fields induced in the latter can be detected in real time at the sensor surface. Because biological materials do not generate detectable magnetic fields, signal generation and capture are insensitive to matrix-mediated interference. In addition, the sensor does not waver even in the face of vast pH (4–10) and temperature shifts (–20–48°C),

and the optical properties of the sample have no effect on detection. Side-by-side comparison with traditional ELISA showed a compelling advantage in linear dynamic range (6 versus 2 orders of magnitude) and a 1000-fold boost in sensitivity. The lack of matrix interference was demonstrated by equal detection sensitivity whether test biomarkers were spiked into simple buffers or complex solutions such as serum and urine. Signals in saliva were noticeably lower, which may be a consequence of protease degradation or sample viscosity. Data from ongoing monitoring of serum samples for tumor markers in a mouse model of colorectal cancer are shown as one application, but the technology is as appropriate for basic research as for clinical diagnostics.

Gaster et al. 2009. Matrix-insensitive protein assays push the limits of biosensors in medicine. *Nat Med.* 15:1327-1332.

Expressing Our Synbodies

With the growing interest in the field of proteomics, there is a need to generate more protein affinity reagents than is possible using standard antibody approaches, which tend to be costly and time-consuming due in large measure to the need for animal immunization.

While alternative binders based on engineered immunoglobulin domains, protein scaffolds, and aptamers can be easier to obtain, they still require multiple rounds of time- and labor-intensive *in vitro* selection and amplification, steps that are not amenable to high-throughput methodologies. In addition, because alternative binding reagents based on small molecule ligands are commonly selected from combinatorial libraries, they typically have only moderate binding affinities for a target protein when compared to antibodies. Increased binding affinity can be achieved by attaching two or more ligands to a tethering molecule to create a higher affinity, multivalent binding reagent. In a recent issue of the *Journal of the American Chemical Society*, Williams et al. describe a novel strategy for the scalable production of such synthetic antibodies—which they term synbodies—by tethering two peptide ligands into a single high-affinity bivalent protein binding reagent using DNA. The authors first used peptide microarrays to isolate moderate affinity peptide binders to a specific target protein. Two peptides that bind to different sites on the target protein were then tethered to a short DNA oligonucleotide by amine coupling to modified bases at various positions, with each peptide on a separate strand. The optimal distance and orientation of the two peptides for generating the strongest

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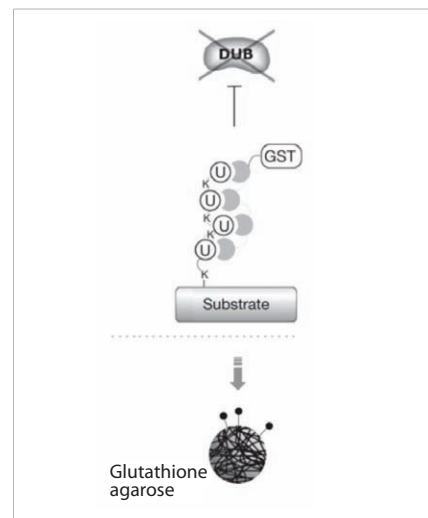
binding synbody was determined using surface plasmon resonance (SPR) analysis where all possible pairs (both hetero- and homo-) of peptides attached to the DNA at a variety of locations were screened on a chip. Using the yeast Gal80 regulatory protein and human transferrin as targets, pairs of peptides with individual binding affinities in the low micromolar range were transformed into synbodies with ~1000-fold higher binding affinities, levels of affinity comparable to monoclonal antibodies. These synbodies were shown to work in ELISA and protein pull-down assays, further demonstrating their effectiveness as substitutes for antibodies.

Williams et al. Creating protein affinity reagents by combining peptide ligands on synthetic DNA scaffolds. *J. Am Chem Soc.* 2009 Nov 6. [Epub ahead of print; DOI: 10.1021/ja9051735]

Together 4-Ever

To keep ubiquitylated proteins from falling apart during purification, try giving them the protective embrace of a tetrameric ubiquitin-binding protein. Tandem-repeated ubiquitin

binding entities (TUBEs), described in *EMBO Reports* by Hjerpe et al., are designed to shield modified proteins against de-ubiquitylating enzymes and proteasomal degradation. In TUBEs, the 4 ubiquitin-binding domains are combined in a construct that also houses 3 tags (GST, His, and SV5). Using TUBEs for GST pull-downs in cell lysates efficiently captures polyubiquitylated proteins and protects them even during extended (16 h) incubation. The technique avoids the artifacts possible when overexpressing tagged ubiquitin to explore the “ubiquitome,” and does not require cysteine protease inhibitors, which are known to form undesirable protein adducts. Moreover, TUBEs capture more target protein than ubiquitin monoclonal antibodies, and are compatible with secondary purification steps, as shown by immunoprecipitation of ubiquitylated p53 after TUBE pull-down. These properties should make TUBEs equally valuable for characterization of purified ubiquitylated proteins as for monitoring ubiquitylation responses in cultured cells, and will speed additional insights into this important class of post-translational modifications.



Tandem-repeated ubiquitin-binding entities (TUBEs; gray semi-circles) protect ubiquitylated proteins from de-ubiquitylating enzyme (DUB) and allow for GST pull-down. Reprinted with permission © 2009 NPG.

Hjerpe et al. 2009. Efficient protection and isolation of ubiquitylated proteins using tandem ubiquitin-binding entities. *EMBO Rep.* 10:1250-1258.

miRNA Quantification: Microarrays or Sequencing?

Microarrays have been the preferred method for gene expression analysis for the past decade. But recent advances in sequencing technology, along with the idea that sequence data provides more quantitative measurements, have challenged that position. While past comparison studies have shown that gene expression ratios from microarray and sequencing data from biological samples correlate, a new study by Willenbrock et al. published in *RNA* has, for the first time, directly compared data generated from microarray and next-generation sequencing using samples with defined RNA content. The authors compared Exiqon's locked nucleic acid-based microarrays with sequence data acquired using the Illumina GA-II next-generation sequencing platform. They addressed the question of relative and absolute RNA abundance as well as the sensitivity and reproducibility of each method by challenging both platforms to analyze a library of 744 synthetic RNA sequences corresponding to 708 human mature miRNAs (miRNAs) and 36 miRNA sequences with potential for cross-hybridization. This experimental design allowed the authors to directly compare the quantities determined by each technique to known RNA concentrations and sequences, without interference from unspecified RNA components. They found that microarray data values correlated better with the known RNA concentration than sequence data, indicating that microarray data may more accurately portray levels of absolute RNA abundance. The authors surmise that this may be the result of continuing microarray probe

optimization over several array generations, limiting the variation in hybridization efficiency. For both platforms, only moderate correlations were detected in absolute expression measurements, but quantification ratios of one sample compared to another correlated very well. Although both platforms generated highly reproducible data, microarrays were found to be more sensitive, especially at lower concentrations. Sequence read variation may account for this reduced sensitivity observed using next-generation sequencing. More than 130,000 unique sequences were produced, although only 744 synthetic RNAs were present in the original samples. Many of the sequences generated matched RNA reference sequences but were truncated or elongated, while others did not resemble any of the synthetic RNAs and were potentially introduced during the PCR-based amplification step necessary for sample preparation. The authors concluded that while sequencing offers advantages such as the potential to discover new sequence variants and deliver highly reproducible expression data, microarray still surpasses next-generation sequencing for absolute RNA quantification.

Willenbrock et al. 2009. Quantitative miRNA expression analysis: comparing microarrays with next-generation sequencing. *RNA.* 15: 2028-2034.

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