Spotlight

Entrained to the Membrane

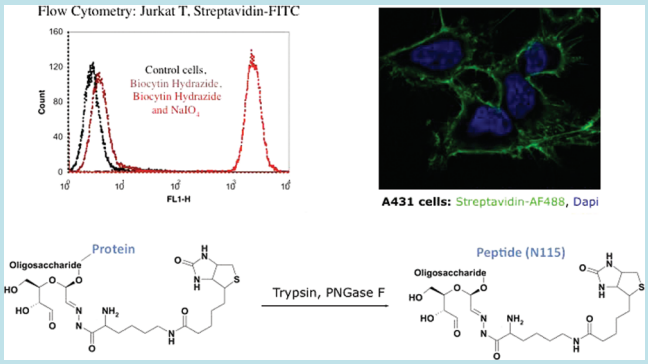

Image courtesy of Bernd Wollscheid.

The purification and characterization of specific cell types has historically involved antibody labeling and cell sorting. Cells of the immune system in particular are often classified by what proteins reside on the outside of the cell. More recently, the surface proteome is emerging as a key obstacle for stem cell biologists wishing to define the various lineages that arise during cell differentiation. Now, a new proteomics method takes a crack at the surface topology problem without the aid of antibodies. Wollscheid *et al.* (*Nat. Biotechnol.* 2009, *27*, 378−386) took advantage of the reactivity of a post-translational modification commonly found on membrane proteins, N-linked glycosylation.

In a technique termed cell surface capture, a biotin linker was reacted with oxidized N-linked sugars before cell disruption and proteolysis. This clever trick added an affinity purification handle to extracellular peptides for subsequent capture and enrichment over the more abundant intracellular peptides. Then, using a sensitive mass spectrometry method, the peptides were mapped back to the genome to generate an extracellular inventory. By comparing coupling reactions performed with isotopically light or heavy reagents, they could even quantitatively compare two samples. In proof-of-principle experiments, the authors honed the method and showed its application to cultured cells, as well as cells from primary tissues and organs. They went on to ask several novel biological questions with this elegant new tool. What proteins are turned on during T and B cell activation, and further, what proteins change upon differentiation of pluripotent stem cells into neural precursor cells? The results not only confirmed several known changes in extracellular topology but also uncovered dozens of new proteins that change during these cellular transitions. This technique could be used in reverse for finding new targets for antibody-based enrichment strategies or could stand alone as a new tool to characterize and quantify those proteins poking out of the cell. Jason G. Underwood, Ph.D.

Expansion Diseases Meet a New Nemesis

Numerous genetic diseases are caused by the expansion of trinucleotide repeat sequences within important genes. Some expansions cause RNA processing issues, while others add a problematic homopolymeric peptide within the encoded protein. Now that mapping of many disease expansions has honed in on specific mRNAs, an increased effort to selectively target causal mRNAs or their trinucleotide repeats is underway.

Two debilitating disorders of the nervous system, Machado−Joseph disease and Huntington's disease, get their phenotypes from expanded CAG repeats in the mRNA of Ataxin-3 and Huntingtin genes, respectively. In both of these cases, the genes have natural CAG repeats present in healthy humans, but disease states see one allele expanding beyond 40 repeats with the severity correlating with the number. Since both Ataxin-3 and Huntingtin proteins are important for cellular functions, targeting the bad allele while leaving the good one intact is critical to any gene therapy strategies.

A new antisense approach takes aim at the problem spot in these mRNAs, the CAG repeats, and with impressively selective results. Hu *et al.* (*Nat. Biotechnol.* 2009, *27*, 478−484) tried several flavors of antisense oligonucleotides to target the repeats in these mRNAs or the sequences just adjacent to the repeats. Through various iterations, they modified the backbone to peptide nucleic acid or locked nucleic acid and also tried terminal modifications with positively charged amino acids to increase cell permeability. Using cell lines with a wild-type and disease allele of Ataxin-3 or Huntingtin, they convincingly showed that gene expression from the expansion allele is far more inhibited by the antisense compounds. This inhibition does not correlate with loss of transcription, so the compounds may block translation of the expansion mRNA. With optimized modifications and concentrations, the antisense compounds showed very low cell toxicity, so this new brand of expansion targeting may represent a novel therapeutic. **Jason G. Underwood, Ph.D.**

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Spotlight

RNAs Wears a New Cap

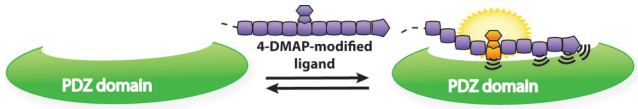
During RNA transcription, a special modification is placed on the 5′ end of eukaryotic mRNAs. This methylated guanosine cap structure plays key roles in the cell, most notably in RNA stability and translational efficiency. When making RNA for *in vitro* studies of RNA splicing or translation, scientists often use a dinucleotide analogue of the cap that acts as a initiation substrate for phage polymerases. The resulting transcript is more stable and shows higher translation efficiency in cell extracts or when it is introduced into living cells. However, this dinucleotide trick results in half of the caps incorporating in the wrong orientation with the methylated base in the body of the transcript rather than on the cap structure. This situation does not provide the same advantage during translation. Now, a bioorganic group has synthesized a new answer to this dilemma.

Kore *et al.* (*J. Am. Chem. Soc.* 2009, *131*, 6364−6365) used a locked nucleic acid (LNA) methyl-guanosine to substitute for one of the bases in the dinucleotide. This sugar modification links up the 2′ and 4′ carbons of the ribose ring *via* a methylene bridge. The LNA architecture conformationally constrains the sugar, resulting in higher stability and increased melting temperatures when annealed to a complementary sequence. After demonstrating that this new LNA cap could be incorporated by a phage polymerase in a single orientation, the authors went on to show its advantages in cellular assays. A luciferase mRNA capped with the LNA cap showed 50% higher stability when introduced into cultured human cells compared to the conventional cap. In addition, the translation of luciferase protein increased about 3-fold. While further studies in other assays will be necessary, this synthesis and early characterization indicates that LNA may be the best cap for an RNA to wear for *in vitro* studies. **Jason G. Underwood, Ph.D.**

Probing Dynamic Domains

PDZ domains are one of the most abundant protein interaction modules mediating cellular signaling in eukaryotic cells. These compact modules typically assemble macromolecular complexes by binding to the C-terminal regions of interacting proteins. PDZ domains bind transiently to multiple proteins with relatively weak (low micromolar) affinity and thus are key elements in the plasticity of these protein assemblies. This promiscuity, together with structural features of the domains themselves, makes it difficult to develop binding probes based on a residue replacement strategy, where a conserved ligand residue is replaced by a reporter group with similar properties. However, Sainlos *et al.* (*J. Am. Chem. Soc.* 2009, *131*, 6680−6682) now report a clever method for developing fluorogenic peptides to probe the dynamics of PDZ-domain interactions.

The design of the probes was based on peptide sequences from natural ligands of PDZ domains, with the addition of a fluorophore

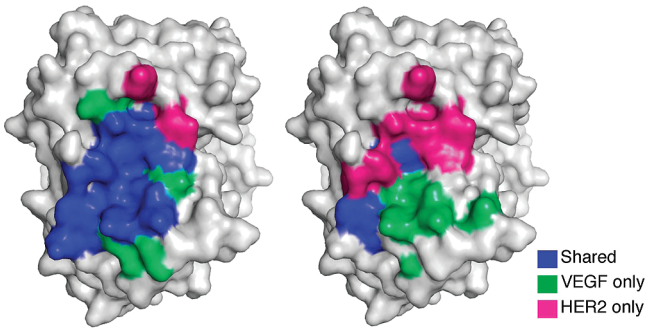


from the dimethylaminophthalimide family that served as an environmental sensor. In an initial step, a peptide library based on the C-terminal binding motif of the PDZ-binding protein Stargazin was used to determine the optimal location for the fluorophore and the ideal linker length between the peptide backbone and the fluorophore. Afterward, a new library of probes based on three different PDZ domain-binding sequences were then synthesized to afford probes with unique specificity for the partner PDZ domains. In each case, the increases in fluorescence (up to 265-fold) correlated with the binding affinity of the probes for their target PDZ domains. Fluorescence titrations and isothermal titration calorimetry studies indicated that the fluorescent peptides bound more tightly to their target PDZ domains than unlabeled peptides. Initial structural studies did not show an obvious origin for this enhanced binding and fluorescence emission, which underscored how this general approach could prove useful for designing probes for other similar domains when the rational replacement strategy falls short. **Sarah A. Webb, Ph.D.**

Antibody "Keys" That Fit Multiple Locks

Many targeted therapies, such as Herceptin for certain types of breast tumors, are based on antibodies that bind to a single antigen, following the paradigm of a "lock-and-key" mechanism. If an antibody with a single specificity works, could antibodies with specificity for two or more proteins prove even more effective? Bostrom *et al.* (*Science* 2009, *323*, 1610−1614) set out to engineer variants of the antibody Herceptin that maintain their original binding specificity but could simultaneously interact with another target protein. An efficient natural immune response might involve multispecific antibodies, but previously no one had isolated an antibody that could recognize two unrelated proteins.

The antibody Herceptin recognizes human epidermal growth factor receptor (HER2) primarily through interactions in the heavy chain complementarity determining regions. Therefore, a library of new variants were generated through mutations in the light chain. Selecting this library against vascular endothelial growth factor (VEGF), death receptor 5, and the complement binding fragment of immunoglobulin G allowed the researchers to fish out the antibody, bH1, which had the highest affinity for both HER2 and VEGF. Although structures of bH1 bound to HER2 and to VEGF showed significant overlap in the two binding sites, studies of the energetic contributions of the individual mutations in bH1 indicated that the heavy chain residues primarily mediate HER2 binding while interactions



From Bostrom *et al.*, *Science*, 2009, DOI: 10.1126/science.1165480. Reprinted with permission from AAAS.

in light chain contribute to VEGF binding. *In vivo* studies suggested that the bH1 variant bH1−44 has the same pharmacological activity as either Herceptin or an anti-VEGF antibody. However, it is not yet clear if bH1 would be as effective as a two-antibody regimen, either mixed or given separately. In addition to describing the first fully functional dual-specific antibodies, this study provides insights into how natural antibodies may develop their diverse repertoire.

Sarah A. Webb, Ph.D.