Citations

Buckle Up

In recent years, keeping up with the developments in fluorescence-based nucleic acid detection platforms has made for a dizzying ride. Some early strategies used dual labeling of a probe oligonucleotide with a quencher dye and a fluorescent reporter. The probe would self-quench its own signal until it hybridized with a target DNA strand, a step that would cause the quencher to separate in space from the fluorophore, thereby recovering the fluorescent signal. The disadvantage of this approach is the requirement for a label at each end of the oligonucleotide probe. Thereafter, it became apparent that nanostructures such as gold nanoparticles or carbon nanotubes could quench fluorescently labeled oligonucleotide probes, sidestepping the need for an attached quencher dye. This single-label strategy rests on the tendency of ssDNA to associate with such nanostructures and dissociate upon hybridizing with a complementary-stranded target. Now, in an article newly published in *Nucleic Acids Research*, Wang et al. introduce a new quenching nanostructure—the nanobelt. The appeal of the nanobelt is its extremely easy synthesis, which involves adding ammonium persulfate to an aqueous solution of *p*-phenylenediamine monomers at room temperature and collecting the precipitate. As the “nanobelt” name implies, the product of this reaction is a long, thin structure several hundred nanometers in

breadth and tens of micrometers long. This platform adsorbs fluorescently labeled ssDNA probes through stacking interactions involving the bases and quenches the fluorescent signal. When target DNA is added, the nucleobases of the probe now hybridize with the complementary sequence, liberating the probe from the surface and restoring fluorescence. Testing showed that the intensity of fluorescence recovery was dependent on the amount of target DNA and was sensitive to single-base mismatches. The kinetics of probe adsorption and release were faster than the times required for these steps when using single-walled carbon nanotubes, but substantially slower than graphene-based nanostructures. However, given the heavy time and labor demands of synthesizing the latter, nanobelts still come out looking as the more convenient and efficient option. In addition, the nanobelt quenching/recovery paradigm is compatible with three different fluorophores, allowing multiplexing for simultaneous detection of multiple targets.

Wang et al. Conjugation polymer nanobelts: a novel fluorescent sensing platform for nucleic acid detection. *Nucleic Acids Res.* [Epub ahead of print, December 22, 2010; doi:10.1093/nar/gkq1294].

SUMO Match

One of the most studied posttranslational modifications, phosphorylation, is believed to involve the actions of several hundred kinases and perhaps 150 phosphatases. The regulation and consequences of these enzymes' activities have in many cases been well elucidated. By contrast, the triggers and pathways involved in the posttranslational modification known as SUMOylation—the addition of a small ubiquitin-like modifier (SUMO) to a target lysine residue—remain less understood. Of particular interest is the significance of poly-SUMO chains, which have been implicated in meiosis and stress responses. Establishing a comprehensive list of those proteins that become poly-SUMO conjugates demands an easy method for purifying these targets, a challenge Bruderer et al. met in a recent issue of *EMBO Reports*. The authors took the four SUMO interaction motifs of the RING-finger 4 (RNF4) protein (which is known to direct ubiquitination in a poly-SUMO-specific manner) and coupled this subdomain to beads to create a poly-SUMO affinity matrix. Significantly, this bait has low affinity for single SUMO groups, enabling a stoichiometric selectivity not possible in previous SUMO conjugate detection strategies,

Views of the binding between SUMO and a SUMO-interacting domain, the basis for purification of poly-SUMO conjugates. Reproduced by permission © 2011 the RCSB PDB.

where single SUMO groups were picked up as efficiently as poly-SUMO chains. In addition, the method showed commendable depth (an estimated 80% of poly-SUMO chains were recovered) and excellent stringency, as assessed by comparison with pull-downs using bait with mutated interaction motifs. Since previous work had shown enhanced poly-SUMOylation after heat shock, the authors used nuclear and cytoplasmic extracts prepared from HeLa cells exposed to 43°C for 30 min. To focus on poly-SUMO conjugates themselves instead of factors pulled down by indirect associations, the authors sliced the gels into nine molecular weight regions before

mass spectrometric protein identification, and compared apparent with predicted molecular weight. This enabled the results to be filtered for proteins with a molecular weight discrepancy consistent with modification by two or more SUMOs. All told, over 300 putative poly-SUMO conjugates were detected, with enrichment in factors involved in DNA damage checkpoint response and DNA repair. These factors should provide fertile ground for teasing out the complex interactions involved in this signaling pathway, and, given the high degree of sequence conservation in SUMO interaction motifs, this capture strategy should be effective in metazoans and yeast alike.

Bruderer et al. 2011. Purification and identification of endogenous polySUMO conjugates. *EMBO Rep.* 12:142-148.

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