Citations

On Demand

Some factories concentrate on churning out as much product as can be sold, while others are demand-driven, responding to specific customer orders. The second approach, which offers opportunities for rapid, customized response, is the biological philosophy adopted by Schroeder et al., who describe a new remotely triggered nanofactory for protein production. As the authors explain in *Nano Letters*, a major goal in nanotechnology is developing materials that can respond to stimuli. In particular, Schroeder et al. were interested in temporal and spatial control of protein synthesis. Their nanofactory consists of a self-assembled lipid bilayer, a minimal *E. coli* S30 extract, and plasmid DNA. The phospholipids form a vesicle-like enclosure, the bacterial extract supplies the raw materials and enzymes, and the plasmid is the source of a cDNA template. Before analyzing the potential for on-demand manufacturing, the authors needed to confirm that the factories worked at all. Imaging of vesicles supplied with a GFP-expressing plasmid showed green fluorescence, confirming appropriate folding of this marker protein. Likewise, production of luciferase yielded the expected luminescence when substrate was added to lysed vesicles. Interestingly, the productivity of the nanoscale factories showed size dependence. As vesicle diameter

was reduced from 400 to 170 nm, protein production tended to increase, likely reflecting more efficient interactions between the crammed-together reagents. When the diameter dipped to 100 nm, however, there was no detectable production. The impediment proved to be the plasmid template, which apparently could not supercoil efficiently enough to fit within the nanosized factory. This consideration aside, and having established the production capabilities of their nanoparticles, Schroeder et al. set about programming in stimulus responsiveness. To this end, they conjugated a photolabile protective group to the DNA, thereby blocking transcription. In a key test, the authors injected the vesicular nanofactories into mice, then irradiated the injection site to uncage the template. Luciferase signal was easily detected in a UV-dependent manner and persisted for at least 24 hours. This proof-of-principle demonstration of on-demand protein synthesis from an artificial nanoscale production system should stimulate efforts to realize localized delivery of therapeutics in vivo.

A. Schroeder, et al. Remotely activated protein-producing nanoparticles. *Nano Lett.* [Epub ahead of print, May 8, 2012; doi:10.1021/nl2036047].

Western Aid

Perhaps half of the proteins encoded by the human genome are phosphorylated, and this post-translational modification helps regulate vital processes such as signal transduction and the cell cycle. However, monitoring phosphorylation by Western blotting can be frustrating. Although anti-phosphotyrosine antibodies are reliable, antibodies against phosphoserine and phosphothreonine tend to be sequence dependent. A number of non-antibody-based dyes have been introduced, but high backgrounds and poor sensitivities limit their popularity. ³²P-labeling is a context-independent alternative to Westerns, but its toxicity can perturb cells, and few relish the hassle of working with this beta emitter. Another option, mass spectrometry (MS), suffers from low ionization and fragmentation of phosphopeptides, necessitating sophisticated instrumentation not usually available for routine use. Undaunted by this litany of disappointment in phosphoprotein detection, Iliuk et al. describe sensitive phosphorylation analysis via a modified Western blotting method. The technique, published in *Molecular and Cellular Proteomics*, uses a soluble hyperbranched nanopolymer called pIMAGO. The reagent is functionalized with titanium ions, which have affinity and selectivity for phosphate groups. The authors previously used this property for enriching phosphopeptides before MS, and

Titanium- and biotin-functionalization of the pIMAGO nanopolymer for phosphate detection and chemiluminescent readout.
Source: *Molecular and Cellular Proteomics*

showed that pIMAGO could be deployed in phosphoprotein detection via ELISA. For use in Westerns, the pIMAGO dendrimer was decorated with biotin so that post-blotting, it could be detected either by avidin-HRP (for traditional chemiluminescence) or a streptavidin-conjugated fluorophore (for infrared imaging). After pIMAGO acted pilot tests involving purified proteins and simple mixtures, Iliuk et al. blotted HeLa extract that had been immunoprecipitated with an anti-phosphotyrosine antibody and showed pIMAGO-probed Westerns were just as specific for phosphoproteins as those using anti-phosphotyrosine antibody. pIMAGO also performed equivalently to established anti-phosphotyrosine antibodies in *in vitro* kinase assays. To compare with MS analysis, the authors immunoprecipitated wild-type or mutant Polo-like kinase 1 (Plk1) from transfected cells. Some of the sample was Western blotted using pIMAGO with either chemi-

luminescent or fluorescent detection and the remainder was analyzed through a MS-based quantitative phosphoproteomic workflow. Using the more quantifiable fluorescence detection results, Iliuk et al. uncovered a 40% increase in phosphorylation of immunoprecipitated proteins from the mutant Plk1 sample, a finding that was confirmed by MS-based measurements. These tests establish pIMAGO as a robust probe of phosphorylation, and although phosphosite-specific antibodies will still have a place for detailed characterization, pIMAGO should appeal to researchers looking to quickly determine whether a protein of interest undergoes phosphorylation changes in response to particular stimuli.

A. Iliuk, et al. Chemical visualization of phosphoproteomes on membrane. *Mol Cell Proteomics.* [Epub ahead of print, May 16, 2012; doi:10.1074/mcp.O112.018010].

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