The unnatural amino acid azidohomoalanine (AHA) is an analog of methionine, which the cell's translational machinery will insert in place of methionine if methionine is left out of the mix. The side chain of AHA contains an azide functional group which is not found in nature, but spontaneously reacts with an alkyne (another functional group not found in nature) to form a covalent bond. To pull out the newly synthesized azide-tagged proteins from the total proteomic pool, Schuman and her colleagues developed an alkyne-based affinity reagent containing a Flag antibody epitope and biotin, which forms an extremely tight noncovalent association with the protein avidin. They entitled their labeling strategy 'BONCAT', or 'bioorthogonal noncanonical amino acid tagging'.

As proof of principle, the researchers exposed human embryonic kidney 293 cells to AHA for 2 hours. They used western blotting to verify that AHA was incorporated into a wide range of proteins. They incubated cell lysates with the biotin–Flag–alkyne affinity reagent and used avidin resin to isolate the newly synthesized AHA-tagged proteins. They digested the immobilized proteins on the resin, and subjected the fragments to shotgun mass-spectrometry analysis and database searching (**Fig. 1**). They successfully identified 1,028 nonredundant peptides, which made up 195 different newly synthesized proteins with a diverse range of functions and biochemistries.

Schuman and her colleagues believe their method provides a unique and simple route to obtaining temporal 'snapshots' of diverse mammalian cellular proteomes. They suggest that BONCAT may be particularly useful for metabolic labeling in post-mitotic cell cultures, in which stable isotope labeling with heavy and light amino acid variants has been challenging.

Allison Doerr

RESEARCH PAPERS
Dieterich, D.C. *et al.* Selective identification of newly synthesized proteins in mammalian cells using bioorthogonal noncanonical amino acid tagging (BONCAT). *Proc. Natl. Acad. Sci. USA* **103**, 9482–9487 (2006).

Their theory allows you to find the experimental signal, defined as a 'generalized coordinate', which will provide the optimal resolution possible within your experimental design parameters such as bead size and trap stiffness that may be constrained for various reasons. This promises to provide researchers with a simple way of getting the best resolution they can at lower spring tensions, which are less likely to negatively affect the system under study.

Coauthor Yann Chemla cautions, "It is important to emphasize that environmental and instrumental sources of noise are still important, and our theory isn't a prescription for getting you high resolution all the time. You really have to build the instrument very carefully and you have to worry about the temperature stability of your room, the mechanical stability of the instrument and even its acoustic isolation."

Now that many labs are considering dual-trap machines for their own experiments, these findings should be a great aid to researchers who want to probe the workings of enzymes and motors at angstrom-level resolution.

Daniel Evanko

RESEARCH PAPERS
Moffitt, J.R. *et al.* Differential detection of dual traps improves the spatial resolution of optical tweezers. *Proc. Natl. Acad. Sci. USA* **103**, 9006–9011 (2006).

NEWS IN BRIEF

CHEMICAL BIOLOGY

Controlling protein activity with a small molecule–dependent intein

A primary goal of chemical genetics is to discover a small-molecule partner for every protein, capable of modulating its activity. As an alternative to extensive small-molecule synthesis and screening, Yuen *et al.* developed a genetic strategy to control protein function using a 4-hydroxytamoxifen–dependent, intein-based molecular switch. This molecular switch is particularly attractive for investigating cell-signaling pathways.

Yuen, C.M. *et al. J. Am. Chem. Soc.* **128**, 8939–8946 (2006).

CHROMATIN TECHNIQUES

Measuring accessibility of chromosomal DNA on a second time scale

The mechanism by which proteins find access to DNA packaged in chromatin is a long standing question in the field. Bucceri *et al.* now have harnessed the light-dependent DNA repair enzyme, photolyase, to monitor the accessibility of specific DNA loci in live cells and at high resolution.

Bucceri, A. *et al. EMBO J.*; published online 15 June 2006.

IMAGING AND VISUALIZATION

A fluorescent nitric-oxide sensor for imaging in live cells

Nitric oxide (NO) is an important cell signaling messenger, but its direct detection *in vivo* has been extremely difficult. Lim *et al.* now present a sensor consisting of a copper and fluorescein complex, which upon reaction with NO results in fluorescence, making possible the direct imaging of NO in live cells.

Lim, M.H. *et al. Nat. Chem. Biol.* **2**, 375-380 (2006).

CELL BIOLOGY

Tumor expression profiling with immuno-LCM

Buckanovich *et al.* present a method combining immunohistochemistry and laser-capture microdissection (immuno-LCM) to purify distinct cell populations from complex tissue samples. They used this tool to carefully isolate specific cell populations from tumor microenvironments to facilitate analysis of the underlying molecular events by RNA expression profiling.

Buckanovich, R.J. *et al. Cancer Biol. Ther.*; published online 9 June 2006.

MICROSCOPY

Probing cell-surface glycans with atomic force microscopy

There is great general interest from the glycobiology community in new methods for investigating glycan modifications on cell-surface proteins. Using lectins as probes, Lekka *et al.* used atomic force microscopy to interrogate both the identity of the oligosaccharides as well as their relative densities on the surfaces of living cells.

Lekka, M. *et al. Chem. Biol.* **13**, 505–512 (2006).