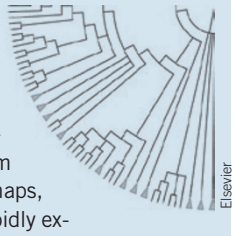
Casting a kinase net



Kinases and their substrates form complex dynamic interaction networks, with numerous kinases phosphorylating the same substrate and individual kinases interacting with various substrates. A better understanding of this complicated system will come from the completion of phosphorylation network maps, which are highly fragmentary. In hopes of rapidly expanding the phosphorylation network and of mapping phosphorylation sites to specific kinases, Linding *et al*. developed a computational method that combines existing methods of motif-based predictions with contextual information such as colocalization and co-occurrence in the genome and literature. Unlike motif-based prediction, which only predicts kinase families, the integrated strategy can predict specific kinases responsible for phosphorylation events. After finding that they could increase their prediction accuracy by 2.5-fold over the motif-based method alone by incorporating contextual information to analyze known *in vivo* phosphorylation sites, the authors analyzed a complete human phosphoproteome containing 7,207 sites on 2,540 proteins. The large resulting network revealed several new biological insights. For instance it predicted several new proteins to be phosphorylated by the kinase ATM, which regulates the cellular response to DNA double-strand breaks. These findings were verified using biochemistry and with a mass-spectrometry technique called multiple reaction monitoring, which accelerates the validation of individual kinase-substrate relationships *in vivo*. The ability to assign new functional roles to kinases demonstrates the power of the integrated computational approach in revealing complex interaction networks. (*Cell*, published online 29 June 2007, doi:10.1016/j.cell.2007.05.052) *MB*

Plus 19 makes Tre

Despite considerable progress in treating HIV infection, current approaches have focused on targeting viral enzymes or inhibiting viral-host cell fusion with small molecules. Although effective, current therapies do not reverse chronic infection, as HIV genetic sequences remain integrated into the host genome. An alternative strategy to treating retroviral infection involves the selective removal of HIV genetic sequences from host chromosomes. A major first step toward this goal has been achieved by Sarkar *et al.*, who now report that an evolved Cre recombinase variant selectively excises HIV proviral DNA from host DNA. The authors sought to identify recombinase enzymes that would recognize and cleave asymmetric DNA sequences within the long terminal repeat (LTR) of HIV proviral DNA. Using their previously developed approach of 'substrate-linked protein evolution', the authors screened a library of Cre variants to identify recombinases that would operate on a set of HIV LTR target sites (LoxLTRs). After 126 rounds of the evolutionary protocol, they identified 50 Cre variants that recombined LoxLTRs. The most active variant, Tre, which diverged from Cre by 19 amino acid changes, acted on a low level on *loxP*, the original Cre DNA substrate, but showed efficient recombination of LoxLTR sequences in mammalian cells, which resulted in reduced levels of *Tat* gene activation using an appropriate reporter construct. Further, Tre treatment was shown to excise HIV proviral DNA from HIV-infected cells with no effect on overall cell viability. Though the application of Tre in a therapeutic setting remains a distant goal, the current study demonstrates the possibility of proviral excision as a viable option and the generality of directed protein-evolution approaches. (*Science* **316**, 1912–1915, 2007) *TLS*

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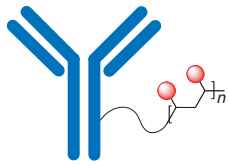
Elemental, my dear



The simultaneous and sensitive measurement of a variety of biological parameters is inherently challenging owing to the complexity of biological systems and the limitations of current instrumentation. Lou *et al.* now make significant strides in overcoming these problems by developing antibodies tagged with different elements for analysis by inductively coupled plasma mass spectrometry (ICP-MS). This technique allows the deconvolution of many inputs and features high sensitivity, allowing biomolecules with copy numbers as low as 100 molecules per cell to be observed. To put ICP-MS into service, the authors first created a polymer incorporating approximately 30 repeats of DOTA, a metal-chelating ligand with high-affinity for lanthanide ions. After selective covalent attachment of the polymer to five antibodies and treating each with a different metal ion, they were able to prepare conjugates that could individually report on the presence of the corresponding antigen; the multiplexed detection of these five cell surface markers demonstrated good agreement with known expression levels in two different cell types. Further, the matching responses obtained using individual versus mixed pools of antibodies indicated that the metals are indeed tightly coupled to their original antibody, confirming that the ICP-MS measurement properly reflects on the antigen of interest. In addition to exploring the makeup of the cell surface, this technique provides new opportunities in cell fingerprinting with applications in diagnostics. (*Angew. Chem.* published online 29 May 2007, doi:10.1002/anie.200700796) *CG*

The specifics of cold

Enzymes from thermophilic organisms are useful biocatalysts because of their obvious utility at high temperatures, and have also served as instructive examples for the investigation of basic principles in catalysis. In particular, the decline of enzyme activity concomitant with decreasing temperature has demonstrated the importance of protein dynamics in reaction mechanisms. But although protein structure is clearly affected by temperature, it is not obvious whether there should be any temperature dependence of substrate specificity. Lutz *et al.* explore this issue in the context of the *Thermotoga maritima* thymidine kinase (TK), an enzyme that transfers a phosphate from ATP to thymidine to generate ADP and TMP. They observed that unlike at high temperatures, where TK is highly specific, the enzyme at low temperatures was more tolerant of unnatural substrates. Investigating this phenomenon, the authors observed a discontinuity in the reaction rates at 70 °C and a change in emission wavelength of a bound fluorescent nucleotide analogue at the same temperature. Together, the data suggest that at low temperatures, TK is not optimally structured for catalysis, and that the subsequent reorganization serves as a kinetically slow step which makes the apparent rate of substrate binding effectively the same regardless of substrate. It remains to be seen whether this kinetic mechanism extends to other thermophilic enzymes; in the meantime, this finding has significant potential for extending biocatalytic possibilities. (*J. Am. Chem. Soc.* published online 26 June 2007, doi:10.1021/ja0734391) *CG*