Biotechnology
Paper alert

A selection of interesting papers that were published in the two months before our press date in major journals most likely to report significant results in biotechnology.

- • of special interest
- •• of outstanding interest

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Contents (chosen by)

Analytical biotechnology

Selected by Catherine Halliwell and Tony Cass
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• High-throughput screens for postgenomics: studies of protein crystallization using microsystems technology. Juárez-Martinez G, Steinmann P, Roszak AW, Isaacs NW, Cooper JM: *Anal Chem* 2002, 74:3505-3510.
Significance: A major focus of structural genomics is the isolation, characterisation and structure determination of the many thousands of putative proteins revealed by the human genome project. A limiting factor to this is the crystallisation of individual proteins, which is labour-intensive and not readily amenable to high-throughput methods for combinatorial screening of crystallisation conditions.
Findings: The authors describe the fabrication of a micro-machined minaturised array of crystallisation chambers, and its evaluation using hen egg white lysozyme as a model protein. A simple method for generating a thermal gradient across the array was included, to permit the screening of temperature as well as chemical variables in protein crystallisation. Protein crystals of sufficient quality for diffraction studies were produced in a number of chambers in the centre of the array.

• Avidin: a natural bridge for quantum dot–antibody conjugates. Goldman ER, Balighian ED, Mattoussi H, Kuno MK, Mauro JM, Tran PT, Anderson GP: *J Am Chem Soc* 2002, 124:6378-6382.
Significance: Fluorescent labelling of biomolecules is of great importance in diagnostic applications, such as DNA and protein arrays, and biological imaging. In this study, quantum dots, nanocrystalline fluorescent CdSe–ZnS core particles, are conjugated to antibodies via avidin–biotin chemistries.
Findings: Avidin, a highly positively charged protein, was found to adsorb to the negatively charged surface of quantum dots. Biotinylated antibodies could then be conjugated to the quantum dots with high affinity and subsequently used in fluoroimmunoassays. The sensitivity of the assay was comparable to existing methods, but has the advantages associated with quantum dots, namely, size-dependent tunable fluorescence emission wavelengths, photostability and high quantum yields.

• Sorting fluorescent nanocrystals with DNA. Gerion D, Parak WJ, Williams SC, Zanchet D, Micheel CM, Alivisatos AP: *J Am Chem Soc* 2002, 124:7070-7074.
Significance: Fluorescence labelling of target DNA with organic fluorescent dyes is common to many DNA arrays, however, organic fluorophores require different excitation sources and have broad, overlapping emissions that limit their use in multiplex labelling and analysis. Fluorescent CdSe–ZnS nanocrystals are an alternative to organic dyes in DNA labelling and offer the advantages of broad absorption spectrums, narrow emission spectrums and photostability.
Findings: Gold substrates were covalently modified with thiol-terminated oligonucleotides, which then hybridised directly with oligonucleotides covalently attached via a heterobifunctional cross-linking agent to thiol-silane-modified CdSe-ZnS nanocrystals. Multicolour microarray studies were conducted by sorting DNA–nanocrystal conjugates of different emission colours using the selective hybridisation of their DNAs to specific locations on the gold substrates.

• β-Lactamase protein fragment complementation assays as ***in vivo*** and ***in vitro*** sensors of protein–protein interactions. Galarneau A, Primeau M, Trudeau LE, Michnick SW: *Nat Biotechnol* 2002, 20:619-622.
Significance: Protein arrays as tools for investigating protein–protein interactions require a strategy for detecting these poorly understood interactions, which can be used in a high-throughput format. The authors demonstrate a strategy for detecting protein–protein interactions, based on a protein fragment complementation assay (PCA) of TEM-1 β-lactamase.
Findings: Interactions between two proteins genetically fused to fragments of β-lactamase brought the fragments into proximity to form a constitutively active β-lactamase that could be detected by colorimetric and fluorescence assays. A number of protein pairs were studied using *in vitro* assays, including apoptotic proteins Bcl2 and Bad. The homodimerisation of Smad 3 was also investigated. The fluorescence assays could also be performed *in vivo*, using HEK293 cells transfected with the fusion constructs.

Plant biotechnology

Selected by Jim Dunwell
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• Successful boosting of a DNA measles immunization with an oral plant-derived measles virus vaccine. Webster DE, Cooney ML, Huang Z, Drew DR, Ramshaw IA, Dry IB, Strugnell RA, Martin JL, Wesselingh SL: *J Virol* 2002, 76:7910-7912.
Significance: There have been several reports on the potential value of edible vaccines, but the best means of utilising such

vaccines remains to be determined. This study represents the first demonstration of an enhanced immune response to a prime-boost vaccination strategy combining a DNA vaccine with edible plant technology.
Findings: The authors investigated the value of edible vaccine technology for immunisation against measles, which despite eradication attempts remains a global health concern. They demonstrate that a single-dose DNA immunization followed by multiple boosters, delivered orally as a plant-derived vaccine, can induce significantly greater quantities of measles-virus-neutralizing antibodies than immunization with either DNA or plant-derived vaccines alone.

• Increasing plant susceptibility to ***Agrobacterium*** infection by overexpression of the ***Arabidopsis*** nuclear protein VIP1. Tzfira T, Vaidya M, Citovsky V: *Proc Natl Acad Sci USA* 99:10435-10440.
Significance: Agrobacterium is a unique model system and a major biotechnological tool for the genetic manipulation of plant cells. It is still unknown, however, whether host cellular factors exist that are limiting for infection, and whether their over-expression in plant cells can increase the efficiency of the infection. The results reported here may lead to improved methods for *Agrobacterium*-mediated transformation.
Findings: The authors examined the effect of overexpression in tobacco plants of an *Arabidopsis* gene, VIP1, which encodes a recently discovered cellular protein required for *Agrobacterium* infection. The results indicated that elevated intracellular levels of VIP1 render the host plants significantly more susceptible to transient and stable genetic transformation by *Agrobacterium*, probably because of the increased nuclear import of the transferred DNA.

• A set of modular plant transformation vectors allowing flexible insertion of up to six expression units. Goderis IJ, De Bolle MF, Francois IE, Wouters PF, Broekaert WF, Cammue BP: *Plant Mol Biol* 2002 50:17-27.
Significance: There is increasing interest in the development of methods that will allow the introduction of multiple gene combinations into transgenic plants. The vectors described here may have particular value for that purpose.
Findings: The authors constructed an *Agrobacterium* binary vector that has a multiple cloning site consisting of 13 hexanucleotide restriction sites, six octanucleotide restriction sites and five homing endonuclease sites. This vector set is ideally suited for the construction of transformation vectors containing multiple expression cassettes and/or other elements such as matrix attachment regions. With this modular vector system, six different expression units were constructed in as many auxiliary vectors and assembled together in one plant transformation vector. The transgenic nature of *Arabidopsis thaliana* plants transformed with this vector was assessed, and the expression of each of the six genes was demonstrated.

Environmental biotechnology

Selected by Lawrence P Wackett
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•• Genome shuffling of ***Lactobacillus*** for improved acid tolerance. Patnaik R, Louie S, Gavrilovic V, Perry K, Stemmer WP, Ryan CM, Del Cardayre S: *Nat Biotechnol* 2002, 20:707-712.
Significance: Lactic acid is produced from renewable resources via fermentation using *Lactobacillus* strains. The significant industrial demand for lactic acid production from sugars is expected to increase with expansion in the use of polylactide polymers. The fermentation process could be improved for increased product yield and ease of product recovery by enhancing the acid tolerance of *Lactobacillus*.
Findings: Because increased acid tolerance is a complex phenotype, different techniques were used consecutively to improve this property. Classical chemical mutagenesis and selection was used to generate a pool of mildly more acid-tolerant strains. The different strains were then subjected to whole genome DNA shuffling via protoplast fusion techniques and the recombinants were screened. Clones were identified that grow at substantially lower pH than the starting strain. Moreover, strains were obtained that produce three times more lactic acid at pH 4.0 than the starting strain.

•• Biodegradation of 1,2,3-trichloropropane through directed evolution and heterologous expression of a haloalkane dehalogenase gene. Bosma T, Damborsky J, Stucki G, Janssen DB: *Appl Environ Microbiol* 2002, 68:3582-3587.
Significance: 1,2,3-Trichloropropane is a side-product from epichlorohydrin manufacture. The development of biological methods to transform this compound into useful compounds will generate a more efficient and environmentally friendly manufacturing process. Efforts to isolate bacteria that grow on 1,2,3-trichloropropane have been unsuccessful. In this study, a bacterium was engineered to grow on this substrate. This could be used as a vehicle to select for improved enzymes and organisms that are able to biotransform 1,2,3-trichloropropane.
Findings: DNA shuffling methods were used to generate haloalkane dehalogenase mutants which were screened for halide release from 1,2,3-trichloropropane. The most catalytically efficient enzyme contained two amino acid substitutions, which were modeled using X-ray structural data for haloalkane dehalogenase. The k_{cat}/K_m for 1,2,3-trichloropropane was improved eightfold by the mutations. A strain was constructed that contained the mutant haloalkane dehalogenase and enzymes to metabolize 2,3-dichloro-1-propanol. The strain was shown to both grow on, and to metabolize, 1,2,3-trichloropropanol.

•• Role of the cytochrome P450 NocL in nocardicin A biosynthesis. Kelly WL, Townsend CA: *J Am Chem Soc* 2002, 124:8186-8187.
Significance: Certain actinomycetes of the genus *Nocardia* produce novel β-lactam antibiotics, the most potent example being nocardicin A. Nocardicin A contains a novel oxime functional group, the origin of which has been obscure. Here, it is shown that a cytochrome P450 monooxygenase is involved in the last step of nocardicin A biosynthesis.
Findings: The cytochrome P450 *NocL* gene was cloned and expressed in *Escherichia coli* and the recombinant cytochrome P450 purified to homogeneity. The cytochrome P450 was shown to oxidize the amino group of a precursor compound to generate nocardicin A.

•• Crystal structure of D-hydantoinase from ***Bacillus stearothermophilus***: insight into the stereochemistry of enantioselectivity. Cheon YH, Kim HS, Han KH, Abendroth J, Niefind K, Schomburg D, Wang J, Kim Y: *Biochemistry* 2002, 41:9410-9417.

Significance: Biotechnological production of enantiopure D-*p*-hydroxyphenylglycine is required for the manufacture of major antibiotics such as penicillins and cephalosporins. Microbial D-specific hydantoinases are used to generate D-*p*-hydroxyphenylglycine and other important D-amino acids. Stereocontrol of the hydantoin hydrolysis reaction is crucial to generate high optical purity in the products. Knowledge of the three-dimensional structure of a hydantoinase would allow opportunities for rational engineering of the active site to better control the stereochemical course of the hydrolysis reaction with important industrial substrates.
Findings: The crystal structure of the *Bacillus stearothermophilus* hydantoinase was solved at 3.0 Å resolution. The enzyme was shown to have a classic TIM barrel fold and resembled the recently solved structure of dihydroorotase. The enzyme binds two zinc atoms per dimer, which are bridged by a carbamylated lysine residue. Regions were identified that may control enantioselectivity, and were designated stereochemistry gate loops. Sequences were compared amongst microbial hydantoinases of known enantioselectivity to further characterize the stereochemistry gate loops.

•• Heterogeneous structure of silk fibers from ***Bombyx mori*** resolved by solid-state NMR spectroscopy. Asakura T, Yao J, Yamane T, Umemura K, Ulrich AS: *J Am Chem Soc* 2002, 124:8794-8795.
Significance: Silk is currently used in fabrics, but its use would be expanded if recombinant silk proteins could be mass-produced; however, the silk protein forms different structures when spun by the silkworm or made in recombinant form. The current study uses solid-state NMR to learn more about the structure of silk proteins, to help bridge the gap between native and mass-produced silk.
Findings: Labelling techniques, coupled to ^{13}C solid-state NMR, were used to gain insight into the structural features of silk I and II fibers. This approach allowed an estimation of the percentage of the protein in distorted β-turns, β-sheet with parallel alanine residues, and β-sheet with alternating alanine residues. Thus, different forms of silk were compared.

•• Biomimetic synthesis and optimization of cyclic peptide antibiotics. Kohli RM, Walsh CT, Burkart MD: *Nature* 2002, 418:658-661.
Significance: Ring-closing reactions are very important in generating a wide range of bioactive products. Currently, most cyclic compounds are generated by whole cells that produce a limited variety of structures of a given structural type. The current paper demonstrates that synthesis might be done very efficiently *in vitro*, with the potential to generate large libraries of chemical structures that can be screened for novel biological activity.
Findings: TycC, the C-terminal thioesterase domain that catalyzes ring-closure reactions, has a very broad substrate specificity. TycC was shown to catalyze release and cyclization of intermediates tethered to a solid-phase amine-terminal polyethylene glycol amide resin. Analogs of tyrocidin A with improved biological activity were synthesized in this manner.

•• Global analysis of the ***Deinococcus radiodurans*** proteome by using accurate mass tags. Lipton MS, Pasa-Tolic L, Anderson GA, Anderson DJ, Auberry DL, Battista JR, Daly MJ, Fredrickson J, Hixson KK, Kostandarithes H *et al.*: *Proc Natl Acad Sci USA* 2002, 99:11049-11054.
Significance: *Deinococcus radiodurans* is being broadly studied because of its ability to survive high flux ionizing radiation at levels that kill almost all other organisms. Moreover, the organism is being used as a platform for engineering biodegradative activities that will work effectively in radioactive-organic mixed waste. The complete genome of *D. radiodurans* has been completed, but this only provides limited insights into the organism's physiological capabilities. In this study, more than half of the proteome of *D. radiodurans* was studied, with the goal of providing insights into the organism's physiological responses to stress. Moreover, this work represents the broadest single coverage of the proteome for any organism studied to date and pushes the boundaries for mass spectrometric analysis.
Findings: Proteins were extracted from cells grown under different culturing conditions and digested with trypsin to yield a complex peptide mixture. The peptides were subjected to tandem mass spectrometry and peptide masses were identified using sequence information derived from the genome sequencing project. The proteins identified were organized into functional categories. The expression levels for different proteins were compared under different growth conditions. New proteins related to stress responses were identified in this way.

Pharmaceutical biotechnology

Selected by Steven Projan
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• Vancomycin-resistant enterococci (VRE) in broiler flocks 5 years after the avoparcin ban. Heuer OE, Pedersen K, Andersen JS, Madsen M: *Microbial Drug Resistance* 2002, 8:133-138.
Significance: Vancomycin-resistant strains of enterococci are a continuing, if not growing, clinical problem in hospitals. Although the relative virulence of the enterococci, especially *Enterococcus faecium*, is hotly debated it is clear that there is increased morbidity and mortality among patients infected with those bacteria. Vancomycin-resistant enterococci (VRE) are particularly difficult to treat, owing at least in part to their multidrug-resistant phenotype. Avoparcin is a glycopeptide antibiotic that has been widely used as a growth promoter in animals for meat production, mainly in Europe. As avoparcin and vancomycin are similar structurally and share the same mechanism of action in bacterial killing, it is not surprising that vancomycin-resistant strains are cross-resistant to avoparcin. Furthermore it has been assumed that avoparcin use selected for vancomycin-resistant isolates that have made their way into human patients. Indeed, the use of avoparcin has been banned throughout Europe since 1997 and in Denmark since 1995. In this context, the authors sought to determine if there has been a reduction in VRE strains colonizing broiler chickens. One important point concerning the emergence and spread of VRE, which is often lost in the debate over the use of antimicrobial agents as growth promoters, is that the VRE problem was, and is, more widespread in the United States where neither avoparcin nor vancomycin was used as a growth promoter. The simple explanation for this paradox is that vancomycin is more widely used therapeutically in the US (mainly for the treatment of methicillin-resistant *Staphylococcus aureus*), thereby exerting the selective pressure.
Findings: The authors found that, among flocks on Danish farms that had previously used avoparcin, VRE was isolated

from 74.3% (104 of 140) of the broiler flocks. On farms that do not use avoparcin, only 9.1% (2 of 22) of flocks had VRE. The authors noted that this result differs from one published in 2000 in a study of farms in the Netherlands (van der Bogaard *et al. J Antimicrob Chemother* 46:146). However, rather than account for this as a valid difference between Dutch and Danish farms, the authors accounted for this disparity by differences in isolation methodology. In the present study, the authors directly selected on medium with vancomycin, when they attempted the same isolation protocol on medium without vancomycin the percentage of flocks with VRE isolates dropped to 12.1% for farms with previous avoparcin use. Clearly, there are two lessons from this study: one is the importance of the method of isolation and the other is the persistence of resistant strains. This persistence is all the more remarkable if one considers that flocks of broiler chickens turn over at high frequency and probably also reflects negatively on the diet of the broiler chicken.

•• From genetic footprinting to antimicrobial drug targets: examples in cofactor biosynthetic pathways. Gerdes SY, Scholle MD, D'Souza M, Bernal A, Baev MV, Farrell M, Kurnasov OV, Daugherty MD, Mseeh F, Polanuyer BM *et al.*: *J Bacteriol* 2002, 184:4555-4572.
Significance: Over the past decade there have been many approaches employed to identify 'novel' bacterial targets. The authors of this study focus on the identification and prioritization of targets that have the promise of delivering broad-spectrum agents. This particular study clearly has benefited from previous work both in the development of novel genetic approaches towards identifying 'essential' genes as well as from the large and increasing amount of genome sequence data of diverse, pathogenic bacteria. In this case the authors employed a 'genetic footprinting' method using *Escherichia coli* to identify genes that cannot be disrupted by transposon insertion. This method involves saturation of the target chromosome in a large number of cells followed by passaging the cells and mapping of the insertions by PCR. After identifying the 'non-disruptable' gene set the authors then focused on three specific adenylate cofactor biosynthetic pathways (for the production of NAD(P), CoA and FAD) to prioritize specific targets with known functions within those pathways.
Findings: The authors compared their footprinting results from the first 50 minutes of the *E. coli* chromosome with essential data gleaned from the literature by the Genetic Resource Committee of Japan. Of the 81 'known essential' genes the authors found no insertions in 70 of them (86%). For the 111 'non-essential' genes 98 (or 88%) were found to be disrupted. In this instance essentiality is defined purely in genetic terms (i.e. failure to obtain a gene knockout is taken as the *sine qua non* of essentiality). Indeed, this need not be the case (some excellent target genes can readily be disrupted when the gene is effectively duplicated and some other genes cannot be disrupted yet do not represent valid targets). That being stated, this analysis provides an excellent insight as to how effective the method is in identifying genes that can be disrupted. Equally interesting was the bioinformatic analyses of the three cofactor pathways. For example, a comparison of the three (distantly related) adenylyl transferases from the three cofactor pathways revealed that *ribF* (encoding riboflavin kinase) was conserved and probably essential in all bacterial species analyzed. By contrast, *nadD* (NaMN adenylyltransferase) was not found in the fastidious bacterium *Haemophilus influenzae,* despite the fact that *nadD* was found to be essential in both *E. coli* and *Staphylococcus aureus*. Likewise, *coaD* (pantetheine-phosphate adenylyltransferase) was not present on the chromosomes of either *Mycoplasma genitalium* and *H. influenzae*.

Food biotechnology

Selected by Jeroen Hugenholtz and Michiel Kleerebezem
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• ***Listeria monocytogenes*** bile salt hydrolase is a PrfA-regulated virulence factor involved in the intestinal and hepatic phases of listeriosis. Dussurget O, Cabanes D, Dehoux P, Lecuit M, the European Listeria genome Consortium, Buchrieser C, Glaser P, Cossart P: *Mol Microbiol* 2002, 45:1095-1106.
Significance: The authors identify a functional bile salt hydrolase (BSH) in *Listeria monocytogenes* and demonstrate that this enzyme is an important virulence factor involved in listeriosis.
Findings: Through comparative genomics, the authors have identified a putative *bsh* gene in *Listeria monocytogenes*. It was subsequently shown that the gene codes for a functional enzyme that confers bile salt resistance. This activity was increased at low oxygen concentration. Involvement of the *bsh* gene in virulence was indicated by the positive regulation through PrfA, the transcriptional regulator of known virulence genes. Further evidence for involvement of BSH in listeriosis was derived from colonisation and infection studies with mutants lacking the *bsh* gene.

•• Heterologous expression of the ***Lactococcus lactis*** bacteriocin, nisin, in a dairy ***Enterococcus*** strain. Li H, O'Sullivan DJ: *Appl Environ Microbiol* 2002, 68:3392-3400.
Significance: Nisin is one of the few bacteriocins that is applied, in practice, for 'natural' food preservation. This paper describes the successful incorporation of nisin production in another lactic acid bacterium, potentially providing a more broad application of this bacteriocin in (fermented) food.
Findings: The lactococcal transposon Tn*5307*, coding for nisin biosynthesis and nisin immunity, was previously incorporated in *Enterococcus*. The resulting transconjugant did exhibit immunity to nisin, but was not able to produce it. In this paper it was confirmed that the whole nisin transposon, including the structural gene for nisin (*nisA*), was present in the transconjugant. Analysis of mRNA showed that *nisA* was not transcribed at all and that nisin immunity was much lower than observed for a lactococcal nisin-producer. These data suggest poor expression of the nisin operon. Transcription of the *nisA* gene, resulting in nisin production, could be induced in *Enterococcus* by the addition of (high concentrations of) external nisin. Apparently, the autoinduction by nisin works much less efficiently in this new host than in *Lactococcus lactis*.

Chemical biotechnology

Selected by Nicholas J Turner* and Robert E Speight†
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• An enzyme library approach to biocatalysis: development of nitrilases for enantioselective production of carboxylic acid derivatives. DeSantis G, Zhu Z, Greenberg WA, Wong K, Chaplin J, Hanson SR, Farwell B, Nicholson LW, Rand CL, Weiner DP *et al.*: *J Am Chem Soc* 2002, 124:9024-9025.

Significance: A large (>200) and diverse collection of nitrilase enzymes has been generated by expression of genomic DNA libraries and subsequent screening to identify enzymes with desired characteristics. In particular, nitrilases have been identified that have high enantioselectivity and activity towards substrates that are of importance as pharmaceutical intermediates.
Findings: Nitrilase enzymes have attracted considerable interest as biocatalysts for the conversion of organonitriles to the corresponding carboxylic acids. The reactions typically proceed under mild conditions (neutral pH, room temperature) compared with their non-enzymatic counterparts. Moreover, they are generally stereoselective and chemoselective, thereby providing access to single enantiomer products that are useful intermediates for agrochemicals and pharmaceuticals. A limitation of this technology has been the relatively few (~15) microbial nitrilases that have been documented, which restricts the range of different compounds that can be processed. By exploiting the diversity found in nature, the authors of this paper have generated a library of new nitrilase enzymes by cloning and expressing genomic DNA derived from environmental sources. All of the nitrilases were shown to be unique at the sequence level and also possessed the conserved catalytic triad Glu-Lys-Cys, which is characteristic for this enzyme class. The nitrilases were initially screened against mandelic acid and its derivatives, resulting in the identification of one enzyme (nitrilase-I) that yielded (*R*)-mandelic acid in quantitative yield and 98% enantiomeric excess. Of particular interest was the desymmetrisation of 3-hydroxyglutaronitrile, a reaction that has proved difficult to accomplish in the past with existing nitrilases. In the present work a second enzyme (nitrilase-III) was identified that yielded the mono-carboxylic acid of (*R*)-configuration in 98% yield and 95% enantiomeric excess. Esterification of the product to the corresponding ethyl ester provided a key intermediate used in the manufacture of the cholesterol lowering agent Lipitor.

• Evolution of an antibiotic resistance enzyme constrained by stability and activity trade-offs. Wang X, Minasov G, Shoichet BK: *J Mol Biol* 2002, 320:85-95.
Significance: Protein evolution is the key to changing substrate specificity, both in nature and in directed evolution experiments, but substituting amino acids during activity evolution could also have a negative effect on protein stability. This concern was investigated in detail here.
Findings: In this paper, the authors investigated protein destabilisation effects that accompany evolution-linked amino acid substitutions. This approach involved cloning and purifying a number of variants of the TEM-1 β-lactamase protein, which had evolved resistance against cephalosporin antibiotics in the clinic. As well as losing activity against the original substrate penicillin, these proteins also exhibited reduced thermodynamic stability. The structures of three of these enzymes were determined and showed that both the change in specificity and reduced stability were due to an enlarged active site. However, several variants also possessed a Met182Thr substitution that was far from the active site and restored the lost stability without affecting the new activity. This suggests that in both nature and directed evolution experiments, unexpected substitutions far from the active site may be particularly important for protein stabilisation.

• A bacterial small-molecule three-hybrid system. Althoff EA, Cornsih VW: *Angew Chem Int Ed* 2002, 41:2327-2330.
Significance: Although small-molecule three-hybrid systems exist in yeast, a bacterial version would have a significant effect on the possible library sizes and number of potential applications.
Findings: A methodology is presented for the identification of protein–small-molecule interactions using a bacterial version of the yeast three-hybrid assay. In these proof-of-concept experiments a small molecule was used to facilitate the interaction of the λ repressor (λcI) with the N-terminal domain of the α-subunit of RNA polymerase (αNTD), which is necessary for transcription of a reporter gene. This interaction was achieved via fusions of FKBP12 to λcI and dihydrofolate reductase (DHFR) to αNTD. These fusion proteins could be dimerized by a molecule consisting of a fusion of methotrexate and a synthetic analogue of FK506, which interact with DHFR and FKBP12, respectively. Expression of the two fusion proteins coupled with addition of the molecule resulted in specific expression of the β-galactosidase reporter protein, demonstrating the potential utility of this technology. Replacement of DHFR with a protein library or methotrexate with a small-molecule library could allow the rapid screening of a large number of small-molecule–protein interactions.