

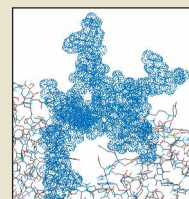
Focus on biomanufacturing and bioprocessing



Twenty-one years after the first biopharmaceutical, insulin, entered commercial manufacture, reliable large-scale production of high-quality recombinant protein remains a significant scientific and engineering challenge. Failure to consider downstream capacity can have catastrophic business consequences, as was illustrated by the Enbrel debacle of 2000, when Immunex (Seattle, WA, USA) failed to meet market demand for its drug and was ultimately swallowed up by Amgen (Thousand Oaks, CA, USA). The influence of manufacturing on a protein's clinical activity is assuming even greater significance as first-generation biopharmaceuticals, such as human growth hormone and insulin, lose patent protection in Europe and generic versions become a possibility [News Feature, p. 1343]. This is posing problems for regulators attempting to assess the comparability of different versions of a protein produced by two manufacturing processes [Perspective, p. 1383], and though the European Union has pioneered legislation in this regard [Profile, p. 1341], Schellekens highlights several unresolved technical issues that may reduce incentives for generic manufacturers to enter the market [Commentary,

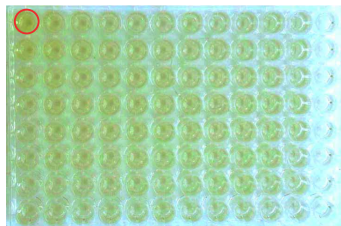


p. 1357]. Noronha Pissarra also notes that a shortage of expertise and resources may hinder development of the biogenerics sector [Commentary, p. 1355]. What is clear is that traditional expression systems (mammalian cells and bacterial cells) still hold sway [Feature, p. 1365]. Transgenic animals and plants, on the other hand, are likely to play niche roles, for example, producing proteins that require complex post-translational modification but are refractory to synthesis in animal cell culture. In the latter system, production capacity has surged in the past few decades, but transformation remains largely hit or miss and instability of transformants is still problematic [Reviews, p. 1393]. In addition, advances in our understanding of protein folding and aggregation in bacteria may make expression systems, such as *Escherichia coli*, more competitive for complex human proteins [Reviews, p. 1399]. Efforts to humanize glycosylation pathways in fungi could also offer cost savings and increased ease of genetic manipulation [Reviews, p. 1409]. Plant cell culture systems, although useful for producing small molecules and secondary metabolites, such as paclitaxel (Taxol), still face significant challenges to compete with mammalian systems [Reviews, p. 1415].



NC, LD, GTO & AM

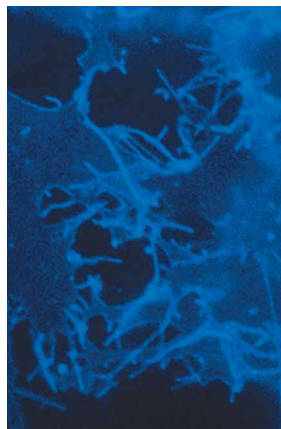
Gassy expressions



Fussenegger and colleagues infuse a breath of (not so) fresh air into the toolbox available for manipulating mammalian expression systems. Their contribution consists of engineering an acetaldehyde-inducible promoter that controls

transcription in a gas concentration-dependent manner and is thus potentially more precisely tunable than existing expression systems. The authors demonstrate the potential of their approach by engineering Chinese hamster ovary cells that express interferon- β and secreted alkaline phosphatase, a human glycoprotein. The main advantages of this expression system include tight regulation capability, rapid induction kinetics and compatibility with most mammalian cell systems. [Letter, p. 1440] GTO

Protein highlighter



Lukyanov and colleagues describe a new fluorescent protein, photo-switchable cyan fluorescent protein (PS-CFP), that changes color from cyan to green in response to irradiation at 405 nm. PS-CFP has the highest contrast of any known monomeric photoactivatable protein, with a 1,500-fold change in the green-to-cyan fluorescence ratio after activation. PS-CFP fluorescence is stable over a pH range of 4.8–9, allowing targeting to acidic organelles. Several examples illustrate how PS-CFP is used to track protein movements: a fusion

of PS-CFP and a gene of interest is expressed throughout a cell, fusion proteins in a small region of the cell are photoactivated, and changes in both cyan and green fluorescence are monitored. Of particular interest is an experiment in which the exchange of cargo proteins between two endosomes is visualized for the first time. [Letters, p. 1435, News and Views p. 1374] KA

In This Issue written by Kathy Aschheim, Nadia Cervoni, Laura DeFrancesco, Michael Francisco, Andrew Marshall and Gaspar Taroncher-Oldenburg.

Patent roundup

- Will the patent expiry of several first generation protein drugs create opportunities for generic manufacturers? [News Feature, p. 1343] *AM*
- Teitelbaum and Cohen report on a recent US Federal Circuit decision that expands the legal definition of a publication, with important implications for what is considered novel and thus patentable. [Patent Article, p. 1449] *MF*
- Recently published patent applications in antiviral vaccines. [New Patents, p. 1451] *MF*

Quantifying drug-protein interactions

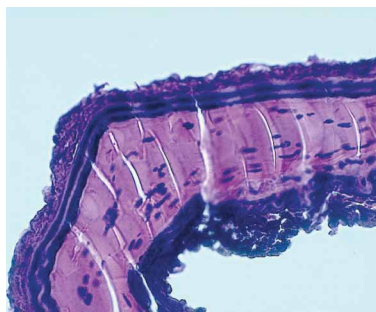
Hage and colleagues have developed an approach to quantitatively characterize allosteric interactions between two drugs (ibuprofen/*S*-lorazepam acetate, *S*-oxazepam hemisuccinate/*R*-oxazepam hemisuccinate and *L*-tryptophan/phenytoin) while binding to human serum albumin (HSA). The technique involves immobilizing HSA on a liquid chromatography column followed by the addition of a known concentration of one drug, while injecting small amounts of a second drug. By measuring the change in the amount of time it takes for one drug to traverse the column in the presence of the second drug, the authors can quantitatively determine the allosteric effect that each drug has on the other, when binding to HSA. The method can be used to ascertain both positive and negative allosteric interactions as well as to measure direct competition. Moreover, this approach is not limited to the study of drug interactions with HSA, but can potentially be used to study the interaction between substances with other biopolymers or receptors. [Letters, p. 1445, News and Views, p. 1376] *NC*

Next month in

**nature
biotechnology**

- Dairy genome
- HIV-inducible gene silencing
- Improved fluorescent proteins
- Dendritic cells with enhanced immune capacity

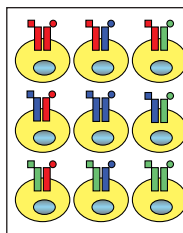
Dynamic duo for clots



The high incidence of cardiovascular disease has led to the extensive use of anticoagulant drugs. Lack of control of anticoagulant activity can lead to serious bleeding associated with increased morbidity and mortality rates for cardiovascular disease patients undergoing surgery.

Bruce Sullenger and colleagues now demonstrate the application of an anticoagulant aptamer drug in animals, whose activity can be reversed by the binding of an antidote oligonucleotide. Systemic administration of the antidote oligonucleotide neutralizes anticoagulation activity of the aptamer in clinically predictive models of cardiovascular disease in both pigs and mice. In addition, the antidote prevents hemorrhage in the clipped tails of mice that receive the anticoagulant aptamer, demonstrating the efficacy of the antidote in a surgical trauma model. [Letters, p. 1423; News and Views, p. 1373] *NC*

Four by four



Tetramers of major histocompatibility complex (MHC) class I-peptide complexes are widely used to detect antigen-specific T-cell responses to viral infection, cancer, transplants and other conditions. As tetramers, they compensate for the relatively weak binding between MHC-peptide complexes and T-cell receptors (TCRs). Kuroda and colleagues have approached this interaction

from the other direction, generating tetramers of TCRs for specific recognition of MHC-peptide complexes. They isolate an array of TCR α and β chains from peptide-specific oligoclonal T-cell populations and screen all possible combinations of these chains to identify $\alpha\beta$ pairs that bind with high affinity. The screening is carried out in fly cells in the absence of accessory molecules that can stabilize the MHC-TCR interaction. The authors apply TCR tetramers to rank the affinities of several peptides for an MHC class I molecule (Mamu-A*01) and to identify macaques expressing this allele. [Letters, p. 1429] *KA*