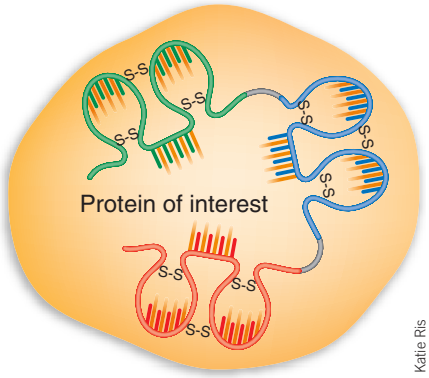
PROTEIN BIOCHEMISTRY

Strength in numbers

A promising new class of proteins for therapeutic purposes makes an entrance; small in size, with multiple low-affinity binding sites for their targets and many disulfide bonds, they are easy to produce and almost completely nonimmunogenic and ready for a clinical trial.

It started about 20 years ago, with the idea that antibodies would make good therapeutics; by targeting a protein on a diseased cell they should swiftly and specifically bring about the destruction of this cell. A compelling idea indeed, but according to Willem Stemmer—an antibody engineer—not a hugely successful one because of problems in antibody production and stability. Stemmer and his colleagues from the biotech company Avidia have now taken an approach—recently described in *Nature Biotechnology*—that deviates from classical antibody engineering. Instead of using large antibody scaffolds with one high-affinity domain for the target, they linked small protein domains, each with relatively low target affinity, resulting in a cumulative effect of strong binding to the target.

Their starting point were A domains, repetitive stretches of 35 amino acids, mainly found on the extracellular portions of human receptor proteins, which bind to different epitopes on the same target. Stemmer explains nature's ingenuity in this approach: "Combinatorial methods for creating binding proteins are the most efficient. Each domain by itself has a small affinity for the target but in combination you get strong binding through an avidity effect." His group developed phage display libraries that started with the human repertoire of A domains and created a highly diverse pool of monomers by synthetic recombination. They then screened the monomers against a target protein; once candidates were found, they added another monomer and screened the new library of dimers against the target. After iteration, Stemmer obtained a trimer with very high binding affinity for its target protein, the cytokine interleukin 6 (**Fig. 1**). They appropriately named these molecules avimers, for avidity multimer, and showed that their anti–interleukin 6 avimer binds with high affinity and inhibits the proliferation of leukemia cells by stimulation with interleukin 6.



Katie Ris

Figure 1 | Avimers are made up of multiple small protein domains, each with many disulfide bridges and unique binding regions for the target protein.

Stemmer argues that the combinatorial nature of avimers gives them unique possibilities as therapeutics, for example, in cancer

GENE REGULATION

CONTROLLING GENE EXPRESSION IN TIME AND SPACE

An inducible expression system allowing temporal and dosage control of genes offers an elegant tool for studying how gene expression gradients influence embryonic development.

The importance of gene dosage is well established, especially in developmental processes such as body patterning and limb growth, which rely on the exquisite, coordinated control of the activity of multiple genes. Many such genes act along gradients in which gene activity peaks in cells from certain embryonic regions and then tapers off in more distally situated cells as mRNAs and proteins diffuse outward. A cell's position within a gradient is often reflected in the extent of the cell's response to the gradient factor, but this isn't always the case. For example, in *Drosophila* larvae, the cells responsible for wing formation—the wing imaginal disc—grow in response to the morphogen Decapentaplegic (DPP), but even though DPP levels form a sharp gradient, cell growth is even throughout the disc, seemingly independent of DPP levels.

Until recently, nobody really knew why. "There were various different explanations that people suggested, and there wasn't really very good support for any of them," explains Kenneth Irvine of Rutgers University. "It was sort of a dirty little secret in the field—we'd all just say that DPP patterns influence growth, but we sort of glossed over the fact that we really didn't know how this worked!" Hoping to solve that riddle, Irvine and graduate student Dragana Ragulja developed a gene control system that would allow them to finely manipulate the timing and extent of DPP response at a level that was not previously possible.

They generated transgenes for a constitutively activated form of the DPP receptor with an upstream activating element that responds to the binding of a transactivator fused to a progesterone receptor domain, expressed from a second transgene. In the presence of an activator compound—the progesterone analog RU486—the activated receptor is rapidly produced, simulating DPP activation. According to Irvine, timing is everything in developmental studies. "Most signaling pathways have some kind of feedback," he says, "and so responses are dampened over time. And technically, because of the way these genetic experiments are done, people have only looked at

therapy. "You can treat a disease at multiple different levels," he says. "You can have domains that inhibit proteins necessary for extravasation and domains that induce killing of the tumor cell." Although the cure for cancer with avimer therapy is not yet around the corner, their application to the clinic is not far off, and Stemmer is confident that clinical trials will begin next year on the use of their interleukin-6 avimer to treat autoimmune diseases.

One requirement for the clinical application of a molecule is low immunogenicity, a requirement the avimers fulfill, partly owing to their small size and partly because of a large number of disulfides. Work with other high-disulfide density proteins has shown that such proteins yield low immune responses, most likely because the presence of disulfide bonds makes the protein hard to cleave by intracellular proteases in antigen presenting cells. This cleavage is a prerequisite to the presentation of the peptides on the surface of these cells, which in turn triggers an immune response.

Another requirement for clinical application is the ability to manufacture the drug. Stemmer presents data showing the efficient production of avimers in bacteria, which should greatly reduce the cost of manufacturing relative to that for antibodies. In addition, the multiple disulfide bonds contribute to a high-temperature and stress stability, which may lead to longer drug shelf lives, and simplify the shipping and storage processes.

With their high target affinity, specificity and low immunogenicity, avimers appear to be well positioned for their first appearance in the clinic.

Nicole Rusk

RESEARCH PAPERS

Silverman, J. *et al.* Multivalent avimer proteins evolved by exon shuffling of a family of human receptor domains. *Nat. Biotechnol.* **23**, 1556–1561 (2005).

long-term responses, where long-term is like two days after you turn on the pathway." This system, on the other hand, allows rapid and dosage-dependent gene activation, allowing studies with far shorter time scales. Working with chimeric animals—with genetically modified cells positioned at different locations within the wing disc—Irvine and Ragulja were able to manipulate DPP-response gradients and immediately observe the impact on cell growth in different regions. The data suggested that the essential factor for cell growth is not the concentration of DPP, but rather the slope of the gradient, with growth resulting where neighboring cells show markedly different levels of DPP activity.

Irvine's group is now applying the same system to other gradient-dependent molecules, but Irvine adds that there has also been considerable interest from other groups looking to achieve equally sensitive modulation for their own genes of interest. "There's a number of different systems where it's nice to add this element of temporal control," he says, "and this is a pretty simple way to do it."

Michael Eisenstein

RESEARCH PAPERS

Rogulja, D. & Irvine, K.D. Regulation of cell proliferation by a morphogen gradient. *Cell* **123**, 449–461 (2005).

NEWS IN BRIEF

GENE REGULATION

Artificial cell-cell communication in yeast *Saccharomyces cerevisiae* using signaling elements from *Arabidopsis thaliana*

After constructing two hybrid strains of yeast—a 'sender' strain that expresses and secretes a plant-derived hormone, and a 'receiver' strain that expresses a hybrid signal transduction pathway capable of responding to the hormone—Chen & Weiss demonstrate the engineering of simple, synthetic networks that can model a variety of cell-cell communication processes.

Chen, M.-T. & Weiss, R. *Nat. Biotechnol.* **23**, 1551–1555 (2005).

PROTEOMICS

Quantitative analysis of protein phosphorylation in mouse brain by hypothesis-driven multistage mass spectrometry

The sensitive detection of site-specific *in vivo* phosphorylation changes for a given target protein can pose a complex problem. Jin *et al.* offer a promising new solution, in which protein samples from drug-treated animals are subjected to a mass spectrometry strategy that allows simultaneous measurement of phosphorylation at many different residues, even at low levels.

Jin, M. *et al. Anal. Chem.*; published online 9 November 2005.

CELL BIOLOGY

Unmodified cadmium telluride quantum dots induce reactive oxygen species formation leading to multiple organelle damage and cell death

It has been known for some time that quantum dots alone are cytototoxic and require modification for *in vivo* use. Lovrić *et al.* find that cells treated with 'naked' quantum dots undergo nonclassical apoptosis, exhibiting organelle damage and elevated generation of reactive oxygen species, and they suggest that the gradual loss of surface modifications may lead to *in vivo* toxicity.

Lovrić, J. *et al. Chem. Biol.* **12**, 1227–1234 (2005).

BIOINFORMATICS

Kernel-based machine learning protocol for predicting DNA-binding proteins

Bhardwaj *et al.* describe a support vector machine (SVM)-based computational method for the characterization of DNA-binding proteins. After training their system for the analysis of features that distinguish DNA-binding from non–DNA-binding proteins, they are capable of achieving prediction with 85–90% accuracy without relying on homology or motif information.

Bhardwaj, N. *et al. Nucleic Acids Res.* **33**, 6486–6493 (2005).

IMAGING AND VISUALIZATION

Light-switching excimer probes for rapid protein monitoring in complex biological fluids

Background signal can be an issue when using fluorescence for quantitative protein analysis. Yang *et al.* describe a modified aptamer whereby binding of target protein puts the molecule into an 'excimer' conformation that brings two pyrene molecules into close proximity, resulting in a fluorescence shift that is quantitative and can be easily distinguished from background.

Yang, C.J. *et al. Proc. Natl. Acad. Sci. USA* **102**, 17278–17283 (2005).