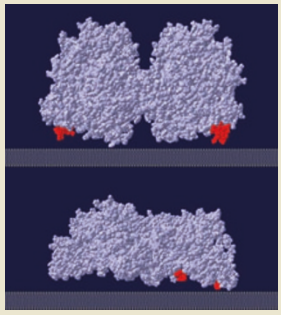
Orientation tips for proteins



Predicting how a specific protein interacts with an artificial surface is a key problem when creating protein-based biosensors, protein microarrays and other applications involving surface-immobilized proteins. Currently, no approach is available that can rapidly predict the most likely orientation in which a protein will bind to a surface. Now Talasaz *et al.* propose an approach to predicting the most optimal alignment of a protein on a surface by modeling the underlying electrostatic interactions. Specifically, they develop an algorithm for determining the orientation (out of all possible orientations) of a protein on a surface with the minimum electrostatic free energy. When the algorithm was applied to two vertebrate proteins (a mitochondrial creatine kinase and a type I hexokinase) whose orientations in the cellular membrane had already been characterized in detail, the predicted orientations were the same as those experimentally determined (see picture). One advantage of the algorithm is that it can enable alignment of a protein on a surface without resorting to complex structural studies or time-consuming molecular dynamic simulations. More importantly, it opens up the possibility of rationally engineering a protein (or surface) of interest so that orientation of the active site is optimally accessible to a particular analyte or substrate of interest, all without resorting to traditional mutagenesis. (*Proc. Natl. Acad. Sci. USA* **103**, 14773–14778, 2006) *GTO*

Staunching blood flow

Methods used to control bleeding—cauterization, ligation or vasoconstriction—can be difficult to control and can cause tissue damage. Now, Ellis-Behnke and coworkers describe a peptide that does the trick in just seconds. In previous work, the authors used two different peptide motifs to make self-assembling nanofiber scaffolds that provided a matrix for regrowing neurons in rodent brain. During the course of these studies, one of the scaffolds, comprising four arginine, alanine, aspartate and alanine repeats (RADA-16), was observed to stop bleeding. In the current work, they applied RADA-16 to a variety of lesions and found in all cases that bleeding was stopped in less than 15 seconds, whereas control animals bled for minutes. Because RADA-16 is made up of alternating positively and negatively charged amino acids, the protein self-assembles in physiological conditions to form interwoven nanofibers (10–20 nm in length). The scaffolds have several advantages over other polymeric biomaterials. They form fibers on the same scale as the extracellular matrix, they break down into amino acids, they are free of animal or chemical contaminants and they appear to be immunologically inert. The mechanism is unknown and the authors saw no evidence of clotting in electromicrographs of healed lesions. Given that roughly half of surgical time is spent managing bleeding, self-assembling peptide scaffolds could greatly reduce the time patients spend in surgery. (*Nanomed. Nanotechnol. Biol. Med.* published online 13 October 2006, doi:10.1016/j.nano.2006.08.001) *LD*

Research Highlights written by Laura DeFrancesco, Peter Hare, Teresa Moogan, Gaspar Taroncher-Oldenburg & Jan-Willem Theunissen

Targeting IL-10 receptors for antivirals

Persistent infections with HIV, or hepatitis B or C viruses are accompanied by inactivation of virus-specific T-cells. To determine how viruses trigger immunosuppression, Brooks *et al.* show that a persistent infection by lymphocytic choriomeningitis virus (LCMV) of mice results in upregulated interleukin-10 (IL-10)—a cytokine with immunosuppressive effects—expression in dendritic cells and fewer functionally active $CD4^+$ and $CD8^+$ T cells. In IL-10–deficient mice, however, an increased frequency of virus-specific T-cells against several LCMV immunodominant and subdominant epitopes effectively eliminates the virus. In addition, resistance to re-infection indicated development of memory T-cells. The authors show that *in vivo* treatment with an antibody against the IL-10 receptor increases T-cell function, effectively eradicates the virus shortly after viral exposure and reduces viral titers during persistent infection. Future studies are required to determine whether inhibition of IL-10 signaling results in control or elimination of viral infections in human patients. (*Nat. Med.* **12,** 1301–1309, 2006) *JWT*

How IFN potentiates bacterial infection

Although it has been observed for years that viral infection makes people susceptible to severe bacterial infection, the mechanism underlying this phenomenon remained unknown. Zinkernagel and colleagues now show that increased levels of type I interferon (IFN I) may be at the root of the problem. In model studies where mice were infected first with lymphocytic choriomeningitis virus (LCMV), which induces a strong IFN I response, and then with *Listeria monocytogenes*, the animals exhibited bacterial titers 1,000-fold higher than mice infected with bacteria only. In a different experiment, when the authors used an anti-granulocyte antibody (GR1) to deplete animals of granulocytes (which are crucial for *L. monocytogenes* clearance from viral-infected mice), these animals also became more susceptible to bacterial infection. Finally, apoptotic granulocytes were observed in bone marrow cell suspensions soon after LCMV infection in normal mice, but not in knockout mice lacking IFN I receptors. The results suggest that treatment with anti-IFN I compounds or a granulocyte-stimulating factor during early viral infection may reduce the incidence of bacterial superinfections and sepsis, a life-threatening condition that strikes up to 200,000 people in the United States alone each year. (*Proc. Natl. Acad. Sci. USA* **103**, 15535–15539, 2006) *TM*

Top-down mass spectrometry

In contrast to bottom-up mass spectrometry (MS), top-down approaches involve transferring an undigested protein to the gas phase before dissociation. This allows the mass of an intact protein to be used to identify all splicing and post-translational modifications, with each assignable to disparate regions on the polypeptide chain from their corresponding fragment mass values. Until now, however, the tight three-dimensional folding of large molecules when ionized and transferred into the gas phase prevented sufficient fragmentation, restricting application of the approach to proteins <50 kDa in size. Now, McLafferty and colleagues optimize the MS protocol, by modifications that include addition of ammonium salts of citrate, succinate and tartrate to the electrospray ionization solution, to enable fragmentation of proteins four times as large (>2,000 amino acids). Although the approach is only suitable for purified proteins and requires improvement before translation to large-scale analyses on a proteomic scale, it opens up a much larger universe of proteins to direct MS analysis without prior digestion. (*Science* **314**, 109–112, 2006) *PH*