Metal in restrained means

Andy Morby

Precious, profitable, and poisonous: Metals and their derivatives are these and more. The properties of metals, from bronze and iron to present-day alloys, have inevitably led to increasing demand and the corresponding development of elegant and efficient methods for their isolation. Unfortunately, this has also resulted in the mobilization of toxic species, such as Hg(II), Cd(II) and Pb(II), into the environment. In this context, mechanisms for the removal of metal ions from solution are of use for recovery and environmental protection. On pages 1017–1020 of this issue, Sousa et al.[1] detail a novel method for the accumulation of metal ions from solution using a genetically modified strain of *Escherichia coli* that carries a metal-binding peptide on its outer membrane.

The recovery of metals from their ores (biomining) and the removal of toxic metal species from polluted sites or industrial waste (bioremediation) are two areas in which the ability of microorganisms to accumulate metal species from their surroundings may be exploited. Diverse physicochemical mechanisms underlie microbial accumulation of metal ions, some of which are dependent on active metabolism. The simplest mechanisms use microbial biomass, which is rich in oxygen, nitrogen, and sulfur groups to "mop up" free metal ions[2].

The first commercial metal immobilization system was dependent on active metabolism and was based on the precipitation of metal ions by microbial sulfide[3]; this process, although efficient, lacked specificity. Recent advances have been made using microbially enhanced chemisorption of Ni(II); in this method, microbes create a uranyl phosphate-based matrix on the cell surface, which can then be used to chelate Ni(II) and Neptunium species[4,5].

A well-documented phenomenon is the affinity of Ni(II) for histidine; indeed, the addition of a polyhistidine tag to a protein allows one-step purification using a matrix containing immobilized Ni(II)[6]. Increased intracellular synthesis of histidine has also been shown to increase tolerance to, and accumulation of, Ni(II) in certain plant species[7]. Sousa et al. have expanded on these observations and introduced either one or two hexahistidine peptides into the outer membrane protein LamB[8]. These groups are solvent-accessible and lie on the surface of *E. coli*; the modified cells bind up to 10-fold more Cd(II) than control cells. This dramatically increased ability to bind Cd(II) exceeded the expectations of the researchers, who believe that these histidine-rich regions in *E. coli* not only bind Cd(II) directly, but also in some way stimulate the intrinsic cellular metal-binding capacity. This last point is of particular interest and perhaps suggests that even cells that already accumulate large amounts of metal ions can be made more efficient. In addition to binding Cd(II), the modified cells also bind to a nickel resin, a property with a range of possible uses, including rapid recovery of cell mass along with its associated metal load.

The majority of microorganisms studied and ultimately used in biosorption applications are isolated from the environment and not engineered to accomplish the task. The work of Sousa et al. demonstrates the potential of the genetic approach and will generate enthusiasm for a mixing of minds and methodologies.

Andy Morby is an NERC research fellow at the school of molecular and medical biosciences, University of Wales, College of Cardiff, Museum Avenue, P.O. Box 911, Cardiff CF1 3US, UK (morby@cardiff.ac.uk).

1. Sousa, C., Cebolla, A., and de Lorenzo, V. 1996. *Nature Biotechnology* **14**:1017–1020.
2. White, C., Wilkinson, S.C., and Gadd, G.M. 1995. *Intl. Biodeterior. Biodeg.* **35**:17–40.
3. Barnes, L.J. 1990. European Patent Application 90203480.0.
4. Bonthrone, K.M., Basnakova, G., Lin, F., and Macaskie, L.E. 1996. *Nat. Biotechnol.* **14**:635–638.
5. Basnakova, G. and Macaskie, L.E. 1996. *Biotechnol. Letts.* **18**:257–262.
6. Hochuli, E., Dhebeli, H., and Schacher, A.J. 1987. *Chromatography* **411**:177–184.
7. Kramer, U., Cotter-Howells, J.D., Charnock, J.M., Baker, A.J.M., and Smith, J.A.C. 1996. *Nature* **379**:635–638.
8. Hofnung, M. 1991. *Methods Cell Biol* **4**:77–105

Taking DNA probes into a protein world

Sanjay Tyagi

The burgeoning field of DNA-based diagnostics exploits the capacity of nucleic acid strands to anneal specifically to complementary molecules. In this issue of *Nature Biotechnology* (pp. 1021–1025), Drolet et al.[1] describe an assay that uses DNA probes in a novel fashion. Their oligonucleotide probe does not hybridize to a complementary nucleic acid, but binds to a protein target. This oligonucleotide probe assumes a conformation that allows it to fit within a pocket of its protein target. Using this mode of molecular recognition, the authors have developed an ELISA-type assay for a growth factor. This approach expands the utility of nucleic acids beyond their traditional role as hybridization probes.

The oligonucleotide probes of Drolet et al. are created and used in a manner analogous to antibodies. From the vast field of possible shapes and structures inherent in a partially or fully randomized collection of oligonucleotides, it is possible to isolate an oligonucleotide that can bind to any target of interest. A library of all possible sequences is created on a DNA synthesizer and an in vitro selection scheme is used for the isolation of the desired molecule. Methods of in vitro evolution and selection that allow the isolation of a particular sequence from hundreds of billions of other possibilities were first introduced in 1967[2]. These methods have recently received a new lease on life with the introduction of PCR. Using a scheme termed SELEX[3] (systematic evolution of ligands by exponential enrichment), it has been possible to find nucleic acid ligands for a wide variety of molecules, including proteins and organic dyes[4]. In this scheme, a library of oligonucleotides is panned for molecules that exhibit an affinity for a desired target. Those that are capable of binding to the target are exponentially amplified. This process is repeated until a molecule of desired specificity and affinity is found.

Drolet et al. have used SELEX to find a DNA ligand for vascular endothelial growth factor (VEGF), a protein involved in angiogenesis[5]. Using this DNA ligand as a detector probe and an antibody as a capture probe, they developed a sandwich-ELISA type assay for VEGF. The DNA ligand was tagged with a fluorescein moiety in order to produce a chemiluminescent signal via an antifluorescein Fab and alkaline phosphatase detection system. Their assay exhibits a specificity equivalent to a common sandwich-ELISA.

One advantage of nucleic acid ligands over antibodies is that animals are not

Sanjay Tyagi is an associate at the Public Health Research Institute, 455 First Avenue, New York, NY 10016 (sanjay@phri.nyu.edu).