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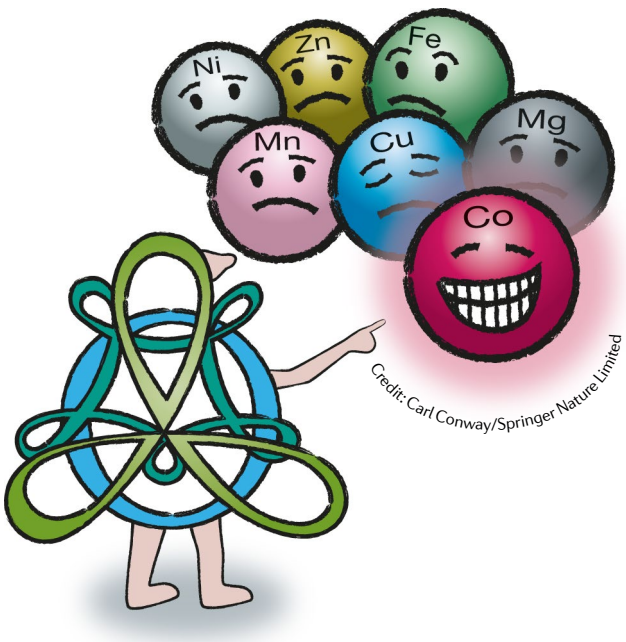
METALLOPROTEINS

Finding the right match

> such measurements offer the promise of making in vivo metalation open to manipulation

Metalloenzymes need metal ions, and not just any ion will do — a redox-active Fe^{II} enzyme will be ineffective if it acquires a Zn^{II} that was earmarked for a hydrolytic enzyme. Mismetalation, which occurs when one element is in short supply or is out-competed for binding, sometimes affords active enzymes but more often than not leads to problems. So, how much of each metal is available? We can easily measure the total metal content in a cell, but just how much is available to proteins is less obvious. Bacterial metal-sensing proteins ensure that metal ions are present in the correct concentration and, as a team led by Nigel Robinson and Peter Chivers now describes in *Nature Chemical Biology*, define free energies for correct metalation.

The Irving–Williams series ranks the propensity of aquated bivalent metal ions to undergo substitution with other ligands. This increases from left to right, often rationalized in terms of decreasing ionic radii, and peaks at Cu for reasons of ligand field stabilization. There are more influences on metal–ligand bonding, but this 'natural' stability order is remarkably general. It is thought that when a cell needs to metalate one apo-enzyme with an early metal ion and another apo-enzyme with a later metal, it has the early metal at a higher available concentration than the later metal. This compensates for the lower affinity earlier metal ions have for proteic ligands, and this trick has been proved by exploiting the cells own metal detectors. Cells measure availability of each metal using a separate transcriptional regulator — a sensor protein that, like apo-enzymes, cannot selectively bind its cognate metal but can selectively detect it if its allosteric mechanism is tuned to the correct buffered metal concentration.



Credit: Carl Conway/Springer Nature Limited

How metal sensing can lead to homeostasis and correct metalation was a question that prompted Chivers, Robinson and colleagues to identify and overexpress sensors in *Salmonella*. The team then used spectrophotometry and fluorescence anisotropy to measure equilibrium constants for metal + protein ⇌ metal·protein, protein + DNA ⇌ protein·DNA and metal + protein + DNA ⇌ metal·protein·DNA. "Extensively calibrated multiple-reaction monitoring mass spectrometry was used to establish the numbers of molecules of each sensor per cell," says Deenah Osman, co-first author of the study, "after which, equations were developed to describe the equilibria and reveal the previously unknown metal availabilities at which each sensor triggers a response." Metal binding influences protein–DNA interactions, and this positive or negative allostery can be quantified in terms of free energy. For example, Co^{II} has a negative allosteric effect on the interaction its sensor protein RcnR has with DNA, such that when Co^{II} is scarce one has more RcnR·DNA, a complex that represses the expression of a metal efflux protein. If Co^{II} and other ions are at appropriate availabilities, then Co^{II} can find its way into CbiK (a donor to vitamin B_{12}) even in the presence of Cu^{I}, a demonstrably stronger binder.

Osman and colleagues found that bacterial sensors of earlier, weakly binding metals only alter transcription at high concentrations of the cognate metal. The behaviours of Mg and Mn–Zn differ so vastly that their available cytosolic concentrations span the range from ~10^{-18} M (for Cu^{I}) to ~10^{-3} M (for Mg^{II}) despite total concentrations of each metal being within two orders of magnitude. The present approach for quantifying metal availability relies on thermodynamic equilibria, with complications arising if a metal is kinetically trapped in an inert folded protein. Nevertheless, Robinson notes "such measurements offer the promise of making in vivo metalation open to manipulation for industrial biotechnology, to develop antimicrobials that promote mis-metalation and to understand mis-metalation in disease."

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ORIGINAL ARTICLE Osman, D. et al. Bacterial sensors define intracellular free energies for correct enzyme metalation. *Nat. Chem. Biol.* **15**, 241–249 (2019)