

especially as the fully reduced enzyme is out of electrostatic balance².

The partial reversibility of the catalytic cycle by an imposed electric field led to the postulate that the transitions between the 'peroxy' (P) and 'oxoferryl' (F) intermediates, and between the F and 'oxidized' (O) intermediates, are coupled to the pumping of two protons each². O~ is now⁴ postulated to be the next intermediate after F and to have the energy to pump two protons, but to decay to the de-energized O. With the existence of O~, a reversal of O to F and then to P with equal energy inputs for pumping two protons each is impossible, as the reversal of O to F would now require the input of sufficient energy to translocate four charges. This is in contrast to the experiment represented in Fig. 3 of ref. 2, which shows that 2.2 charges are transported for the reversal from O to F.

Starting from fully reduced cytochrome *c* oxidase, a catalytic cycle in which only three protons are pumped (two during oxidation and one during re-reduction)⁷ fits the data of Fig. 3 in ref. 4 as well as a two-plus-two cycle, fits the data of Fig. 2 in ref. 4 better than a two-plus-two cycle, and avoids any need for the energy-rich state O~.

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Verkhovskiy et al. reply — Ten years ago, one of us showed that all proton translocation by cytochrome *c* oxidase seems to be coupled to the enzyme's oxidative catalytic half-cycle¹. This led to the assumption that all proton translocation takes place during this half of the cycle. Our more recent data² contradict that idea, as well as Michel's hypothesis³, but our original findings¹ still stand.

The main reason for our proposal that proton translocation during the reductive half of the cycle may still be energetically coupled to the preceding oxidative phase was our observation that, after the energy-rich O~ state had relaxed to O, there was no proton translocation associated with reduction². Michel does not mention this, although it contradicts his model³.

Michel is right that our² Fig. 2a shows the release of about 1.2 H⁺, but this was in a single experiment. In the text² we reported the release of 1–2 H⁺. This variation is due to the difficulty of having

all the enzyme molecules reduced by exactly four electrons. When more than four electrons are available (Fig. 2b of ref. 2), there is no such difficulty and the enzyme is always fully reduced. The result in Fig. 2b therefore shows much less variation, and consistently yielded four released H⁺. In the single trace of Fig. 2a, all enzyme molecules had not been fully reduced by four electrons. This can be seen from the small, slow tail in the corresponding absorbance trace, resulting from the slow decay of oxygen intermediates that arise when the fraction of enzyme with less than four electrons reacts with oxygen.

All the enzyme relevant to these experiments is correctly orientated relative to the membrane. We ascertained that incorrectly orientated enzyme is not reduced under our experimental conditions by the charged reductants ferrocytochrome *c* and hexammine ruthenium [II].

It has been shown⁴ that, in conditions similar to those used for our Fig. 2b (ref. 2), any additional turnovers resulting from an excess of oxygen over the enzyme would be very slow (roughly 15 seconds), as such turnovers depend on slow intermolecular reactions. Because the reaction in Fig. 2b is much faster, Michel's claim that the result is due to multiple turnovers cannot be correct. Michel's conclusion that the O₂ concentration was superstoichiometric in this experiment is also flawed, because we ascertained the stoichiometry of O₂ versus enzyme by titrating the reduced enzyme spectroscopically with known amounts of O₂. Thus, both the enzyme concentration and the number of protons released were determined on the basis of the concentration of O₂ added.

Michel's comments about extinction coefficients are therefore not relevant, and they are also incorrect. He concludes that the path length was ~1 cm in our experiments², but we stated⁵ that the path length varies from experiment to experiment depending on the optical geometry, and that it must be determined separately each time. A calculation based on Fig. 2 of ref. 5 would have shown this.

Finally, assuming there would have been both an excess of O₂ and an excess of reductant (cytochrome *c*) in the experiment² shown in our Fig. 2b, and, as claimed by Michel, the first turnover would pump three protons and the subsequent turnovers four protons each, we can calculate that nine turnovers would be needed to yield 3.9 H⁺ per O₂ (note that H⁺ per O₂ is the primary quantity measured). Michel calculates 1.8 turnovers, which would give only 3.4 protons, far below the number observed. Obtaining these extra turnovers would require not only an excess of O₂, but also an excess of ferrocytochrome *c*. Our experiment had neither of these but, even if

it had both, our result could still not be explained by multiple turnovers.

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Microporous materials

Electrochemically grown photonic crystals

There is a particular group of microporous materials (dielectric structures that exhibit periodicity in three dimensions, called photonic crystals) that are of special interest because of their unique optical properties¹: they can manipulate light in much the same way that a superconductor manipulates electrons. Here we use a technique involving electrochemical deposition within a colloidal template to create porous materials that are stronger and have a higher refractive index than has previously been possible.

In the quest to increase refractive-index contrast in microporous materials, attempts have been made to use colloidal assemblies as templates in a replication process^{2–5}. The feasibility of this approach is supported by calculations predicting that a matrix having a high index of refraction and containing a face-centred cubic lattice of spheres of low refractive index, notably air, can give rise to a complete gap in the photonic band structure^{6,7}. Although such structures can be built up layer by layer through conventional lithography, processing difficulties limit the formation of more than a few layers^{8,9}.

Structures that have substantial three-dimensional periodicity are required for many photonic applications, but so far it has proved difficult to fully mineralize the interstitial space of a colloidal assembly and generate such a three-dimensional structure. In principle, this involves only two steps: the mineralization of the interstitial space of a colloidal assembly, and the subsequent removal of the initial colloid template (and possibly some by-products of chemical reactions). Previous promising attempts have produced replicas with only moderate density and thus an effective index of refraction that is smaller than that of the bulk material. They have also been mechanically fragile^{2–5,10}.

Colloidal assemblies are attractive because of their size tuneability and three-

dimensional structure. A critical issue in creating photonic materials is how to fill the interstitial space completely with a component that has a high refractive index. We therefore electrodeposited group II–VI semiconductors into colloidal sediments, filling the interstitial space with materials of high refractive index (Fig. 1). The electrodeposited materials still allow the colloidal template to be removed, which is necessary to maximize the refractive-index contrast.

Electrodeposition should be an ideal way to fill topologically complex structures because it starts from deep within the structure and then grows out towards the exposed surfaces. We obtained microporous cadmium–selenium (CdSe) structures by potentiostatic¹¹ deposition in the interstitial space of a polystyrene colloidal template (Fig. 1a,b). Cadmium sulphide (CdS) was also grown by galvanostatic¹² (Fig. 1c) and potentiostatic (results not shown) deposition in colloidal assemblies, again yielding three-dimensionally periodic structures after the template is removed.

Because both CdS and CdSe have high refractive indices (2.5 at 600 nm and 2.75 at 750 nm, respectively), these structures can have deep gaps in their photonic band structure after the template is removed. CdS and CdSe are promising materials for photonic applications because, unlike most other high-refractive-index materials, they are optically transparent in the visible and near-infrared region of the spectrum.

One of the critical requirements for the existence of a true omnidirectional photonic band gap is three-dimensional periodicity and uniformity. Our template-directed electrodeposition of semiconductors results in three-dimensional structures (Fig. 1b,c), as indicated by the clearly visible holes into the layer below in all the systems. These holes form at contact points between the spheres of the template, and their arrangement on a triangular lattice is evidence that the hollow voids are on a hexagonal close-packed array.

As well as determining the three-dimensional structure, the holes between the voids allow for the complete removal of the template by solvent or acid, or through burnout. Our electrodeposited materials appear to be dense, as observed in high-magnification scanning electron micrographs of other template-directed electrodepositions¹¹. As a result, the microporous structures shrink by only a tiny amount (<2%) when the template is removed.

Template-directed electrodeposition is well suited to creating three-dimensional microstructures: many materials of high refractive index can be electrodeposited, including semiconductors of groups II–VI, III–V and IV, all of which are very reluctant to form three-dimensional microstructures by traditional techniques. These materials, which have many desirable optical and

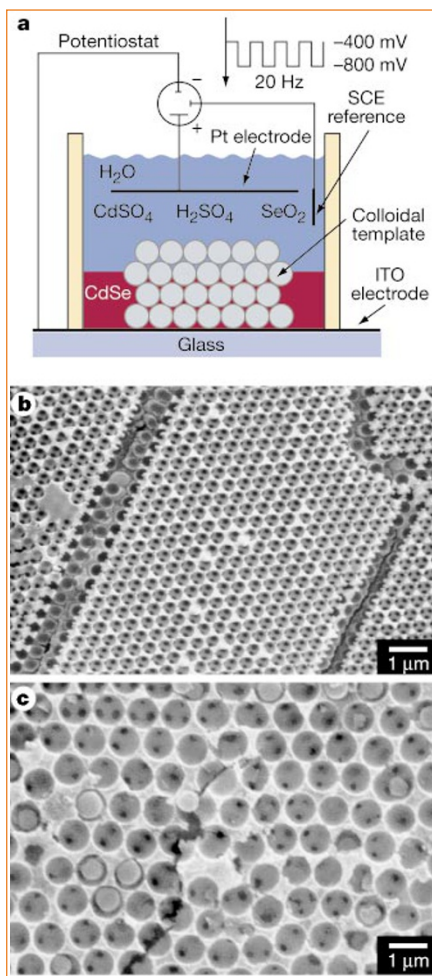


Figure 1 Electrodeposition in colloidal assemblies. **a**, Schematic representation of the experimental set-up. **b, c**, Scanning electron micrographs of the product. **b**, CdSe potentiostatically deposited in the interstitial space of a polystyrene colloidal assembly formed from polystyrene spheres 0.466 μm in diameter. After the electrodeposition, the polystyrene template was fully dissolved with toluene. SCE, standard calomel electrode; ITO, indium tin oxide. **c**, CdS galvanostatically grown around a silica colloidal template formed from silica spheres 1 μm in diameter. The template was partly dissolved after electrodeposition by dipping the sample into an aqueous 4.8% hydrogen fluoride solution for 10 min. This did not completely dissolve the template, allowing the relation between the colloidal template and the resulting three-dimensional structure to be seen directly.

electronic properties, are commonly used in the semiconductor industry. The potential offered by integrating photonic band-gap structures with semiconductor devices could revolutionize optoelectronics and optical computing. It will be straightforward to extend our methods to metallic materials, which can readily be electrodeposited, and these three-dimensionally microperiodic metallic structures could well have unusual mechanical or thermal properties.

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addendum

Colour categories in a stone-age tribe

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The colour-naming pattern of Berinmo speakers may appear consistent with that obtaining in tritanopia¹, a colour-vision disorder that has an increased frequency in other tropical areas² and can arise from chronic exposure to short-wavelength light³. Furthermore, it could be argued that an isolated tribe may have become tritanopic through a shared genetic defect. However, our Berinmo speakers were not tritanopic. They were tested with the City University Colour Vision Test⁴ that specifically assesses tritanopia. The test consists of ten plates, and all speakers who failed any of the plates were eliminated from our study. The failure rate was 7 out of 83 speakers tested during the course of the study.

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corrections

Magnet levitation at your fingertips

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We inadvertently omitted a second reference to the work of I. A. H. Boerdijk (*Phillips Res. Rep.* **11**, 45–56; 1956), where the first demonstration, to our knowledge, of levitation of a magnet using non-superconducting diamagnetic materials as stabilizers was reported. The more stable two-sided configuration was first described by Ponisovskii (*Prib. Tekh. Eksp.* **24**, 7–14; 1981). We were unaware of the earlier work until our experiments were completed.

Tropical tree gene flow and seed dispersal

M. B. Hamilton
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Two numbers were transposed in the last column of Table 1: for site 7, the values for populations 41-2 and 3209 should have been 1.0 and 0.0, respectively.