

New mechanism for modulating colour vision

Single cones start making a different opsin as young salmon move to deeper waters.

Each cone photoreceptor in the retina responds to light in a limited range of wavelengths, giving it a spectral phenotype. This phenotype is determined by the most prevalent of the photoreceptor's visual-pigment proteins (opsins) and is assumed to remain unchanged during an animal's lifetime. Here we show that in the Pacific pink salmon, *Oncorhynchus gorbuscha*, single cones can switch their spectral phenotype from ultraviolet to blue by regulating the production of the appropriate opsins as the fish grow older. This photoreceptor plasticity may operate to modulate colour vision as the salmon's lifestyle changes.

The vertebrate retina has several spectral types of cone photoreceptor. Each photoreceptor contains a visual pigment that is maximally sensitive to ultraviolet (UV), blue, green or red light¹ and consists of a protein (opsin) attached to a chromophore (a derivative of vitamin A₁ or vitamin A₂)¹. It is the combined input from cones containing different visual pigments, each with a different spectral phenotype, that allows an animal to perceive colour.

Changes in colour sensitivity in the outer retina arise from altering the density of different cone types² or by switching chromophores (vitamin A₂-based visual pigments absorb light of longer wavelengths than their vitamin A₁ counterparts)³. Another way to modulate colour vision would be to switch between different opsins, a mechanism that we investigate here in the cone photoreceptors of the Pacific pink salmon.

To determine whether it is possible for such a phenotypic transformation to occur: we measured the absorbance of visual pigment along the outer segment of individual cones by using microspectrophotometry³ (the outer segment of a cone consists of stacked lipid bilayers that contain the visual pigment)¹; we also labelled opsin messenger RNAs by using *in situ* hybridization⁴ with salmon-specific molecular probes.

We found that all the single cones in recently hatched fish (weight about 0.4 g) contain a visual pigment with maximum absorbance in the ultraviolet (λ_{\max} of the visual pigment: 365–375 nm); however, as the fish grew (weight exceeding 0.8 g), the single cones stopped producing UV-opsin and started to produce blue-opsin (λ_{\max} of the visual pigment: 425–435 nm) in a transformation event that proceeds from the ventral to the dorsal retina.

When absorbance is measured from the tip to the base of a cone outer segment undergoing such a transformation, the absorbance is ultraviolet-dominated at the

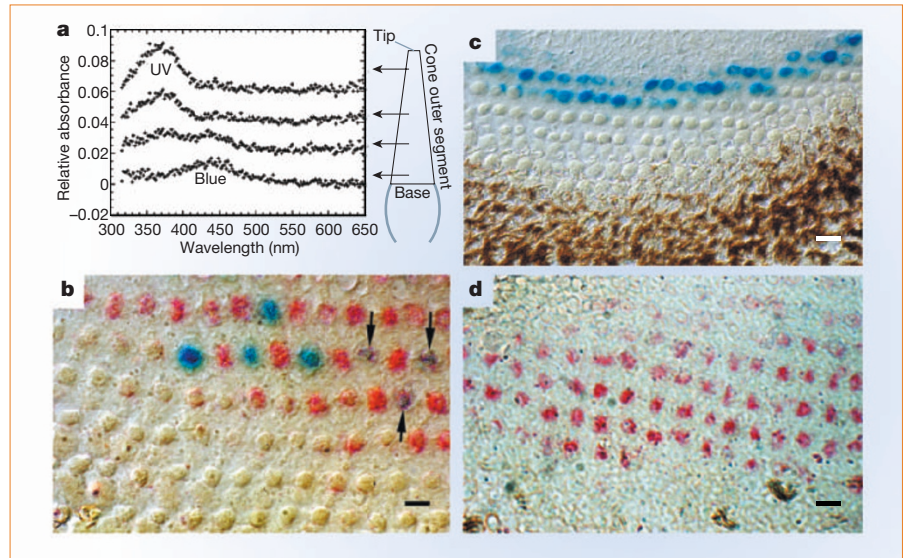


Figure 1 Absorbance measurements and double *in situ* labelling of single cones in Pacific pink salmon with ultraviolet- and blue-opsin riboprobes. **a**, Cones show increased absorbance in the ultraviolet at the tip of the outer segment (right) and increased absorbance of blue light at the base, with intermediate absorbances in between (traces are composite averages from seven cones). **b**, Tangential retinal section showing single cones labelled by *in situ* hybridization with probes specific for mRNAs encoding UV-opsin (shown in blue) or blue-opsin (in red); cones coexpressing both mRNAs appear purple (arrows). **c,d**, Single cones are labelled with only the UV-opsin riboprobe before the switch in opsin expression (**c**), and with only the blue-opsin riboprobe afterwards (**d**). Scale bars: **b**, 5.5 μm ; **c**, 7.8 μm ; and **d**, 13 μm .

tip, becoming blue-dominated at the base (Fig. 1a). As the outer-segment bilayers are produced at the base and removed from the tip⁵, these results show that the UV-opsin is being replaced by blue-opsin during the transformation.

In agreement with this finding, fish in the process of transformation have a mixture of ultraviolet- and blue-sensitive cones, with some cones expressing both opsins together (Fig. 1b). Before the transformation, single cones express only UV-opsin (Fig. 1c); afterwards, they express only blue-opsin (Fig. 1d). Salmonid fish also lose some single (corner) cones following this transformation², further reducing their ultraviolet/blue sensitivity.

This opsin switch in the single cones of pink salmon represents a previously undiscovered way to modulate colour vision. The transformation is linked to a progressive change in the lifestyle of the salmon⁶, which starts as a planktivore in surface waters, where ultraviolet light is abundant, and becomes a fish-eating predator in deeper waters, where blue-green light prevails. As several other vertebrates are known to co-express different opsins in a cone^{7–10}, such a mechanism for temporal modulation of colour vision may be widespread.

Christiana L. Cheng,
Iñigo Novales Flamarique

Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

e-mail: inigo@sfu.ca

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Buckminsterfullerenes

A non-metal system for nitrogen fixation

In all nitrogen-fixation processes known so far — including the industrial Haber–Bosch process¹, biological fixation by nitrogenase enzymes² and previously described homogeneous synthetic systems^{3–5} — the direct transformation of the stable, inert dinitrogen molecule (N₂) into ammonia (NH₃) relies on the powerful redox properties of metals. Here we show that nitrogen fixation can also be achieved by using a non-metallic buckminsterfullerene (C₆₀) molecule, in the

form of a water-soluble C_{60} : γ -cyclodextrin (1:2) complex⁶, and light under nitrogen at atmospheric pressure. This metal-free system efficiently fixes nitrogen under mild conditions by making use of the redox properties of the fullerene derivative.

Treatment of a γ -cyclodextrin-bicapped C_{60} complex⁶ (**1** in Fig. 1) with 100 equivalents of sodium bisulphite ($Na_2S_2O_4$) as a reducing reagent in water under 1 atmosphere of nitrogen and with visible light at 60 °C for 1 hour gave ammonia in 33% yield (based on the amount of C_{60} in the reaction); prolonging the reaction time to 24 hours did not increase the yield. The combination of $Na_2S_2O_4$, **1** and N_2 is essential for the reaction, as control reactions lacking any one of these reactants produced no ammonia. Replacing **1** by γ -cyclodextrin, C_{60} or a simple mixture of C_{60} and γ -cyclodextrin, or performing the reaction at 25 °C, failed to produce ammonia.

The pH of the reaction must be kept neutral, as yields of ammonia were lower under acidic or basic conditions. When the reaction was carried out under an atmospheric pressure of $^{15}N_2$, the formation of $^{15}NH_4^+$ ($^{15}ND_4^+$; -363 p.p.m. in D_2O) was confirmed by ^{15}N NMR spectroscopy after acidification of the crude product. This result confirms that molecular nitrogen undergoes conversion into ammonia in this system.

Visible light was also essential to the reaction as no ammonia was formed in the absence of light. An increased yield of ammonia (45%) was obtained when the reaction mixture was irradiated with a high-pressure mercury lamp (450 W) without a slit. Strong absorption in the ultraviolet region, with weaker but significant bands in the visible region, was seen in the spectrum of **1** in water: characteristic features of the C_{60} moiety were observed at wavelengths of 212 (strong), 259 (strong), 333 (strong), 408 (medium), 536 (weak) and 601 (weak) nanometres (results not shown).

These results indicate that charge transfer between nitrogen and the reduced and electronically excited **1** (exciplex formation) is probably involved in the reduction of N_2 , given that C_{60} and its derivatives are easily excited by low-energy light and that their excited states readily promote electron and energy transfer compared with the ground-state species⁷. Light may therefore assist electron and energy transfer from C_{60} in **1** to N_2 (probably coordinated to **1**). A similar effect of light has been described for a transition-metal complex⁸.

We consider that all of the electrons required for the formation of ammonia are supplied by the reduced C_{60} in **1**, because C_{60} in the complex is readily reduced to the corresponding C_{60}^- and C_{60}^{2-} species by using $Na_2S_2O_4$ (ref. 9). The cyclic voltammogram of **1** (in water under both argon and nitrogen atmospheres) reveals two waves: a reversible

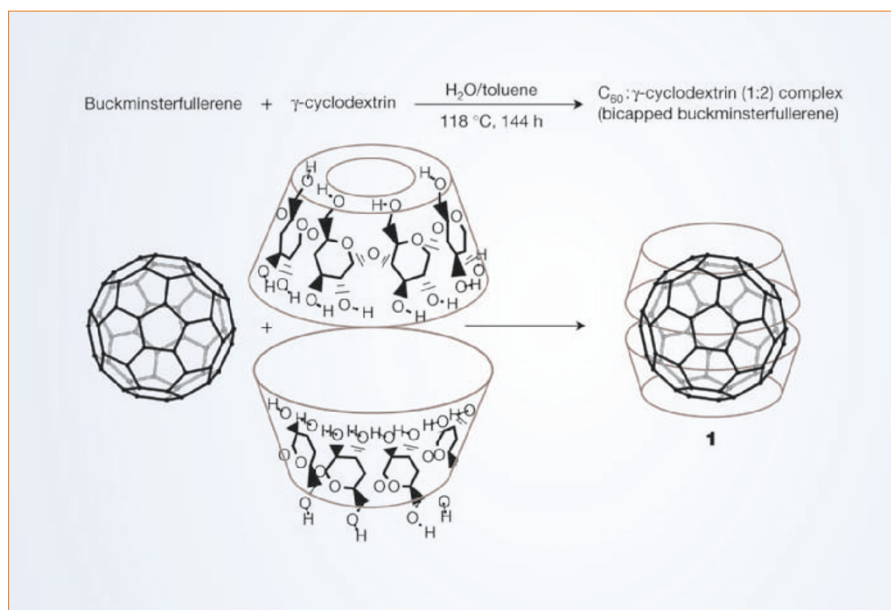


Figure 1 Preparation of γ -cyclodextrin-bicapped C_{60} complex (**1**), which is used in the fixation of nitrogen to ammonia. A mixture of C_{60} (0.400 g; 0.555 mmol) and γ -cyclodextrin (1.200 g; 0.925 mmol) was stirred in a water (160 ml)/toluene (60 ml) mixture at 118 °C for 48 h and then γ -cyclodextrin (0.600 g; 0.463 mmol) was added twice more every 48 h; complex **1** was produced in 70% yield (1.464 g; 0.391 mmol) as a purple solid, with each molecule being coordinated with 24 H_2O molecules (stoichiometry from thermogravimetric and differential thermal analysis). In the fixation reaction, a suspension of **1** (37.4 mg; 0.010 mmol) under 1 atmosphere of nitrogen with $Na_2S_2O_4$ (174 mg; 1.00 mmol) in water (10 ml) was magnetically stirred at 60 °C for 1 h under visible light from a fluorescent lamp. The yield of ammonia was quantified by using indophenol reagent.

wave at -0.61 V (the half-wave electric potential) assignable to the one-electron redox process C_{60}/C_{60}^- ; and an irreversible wave at -1.09 V (the cathodic peak potential) assignable to the one-electron redox process C_{60}^-/C_{60}^{2-} . A saturated calomel electrode was used as the reference electrode. The results indicate that the reduced complexes of **1** have sufficiently low reduction potentials⁵ to reduce the coordinated N_2 molecule.

Some molybdenum and tungsten complexes with nitrogen can be protonated by sulphuric acid to produce ammonia in high yields as a result of the presence of the metal atoms³. We therefore investigated whether protonation occurs in C_{60} -coordinated N_2 . The reaction of **1** with an excess of dilute sulphuric acid under 1 atmosphere of nitrogen at 60 °C for 1 hour produced ammonia in 15% yield (based on C_{60}). Control experiments run for over 1 hour gave no ammonia at 25 °C, or when **1**, H_2SO_4 or N_2 were absent. The yield of ammonia was lower than that obtained in the reaction using $Na_2S_2O_4$. Visible light was again essential for reaction, with the yield of ammonia being increased to 25% under a high-pressure mercury lamp (as specified above).

The infrared and Raman spectra of **1** unfortunately showed no clear-cut absorption that could be assigned to the $\nu_{N=N}$ band of coordinated N_2 on **1**. No peaks other than that due to $^{15}N_2$ (-75 p.p.m. in D_2O) were observed by ^{15}N NMR on treatment of **1** in D_2O under one atmospheric pressure of $^{15}N_2$. These results indicate that complex formation between N_2 and **1** is not

sufficient to be detected by infrared, Raman and NMR spectroscopy. However, it is possible that molecular N_2 might coordinate to the surface of the C_{60} molecule in **1** in an end-on mode.

Yoshiaki Nishibayashi*, **Makoto Saito***,
Sakae Uemura*, **Shin-ichi Takekuma†**,
Hideo Takekuma†, **Zen-ichi Yoshida†**

*Department of Energy and Hydrocarbon Chemistry,
Graduate School of Engineering, Kyoto University,
Yoshida, Sakyo-ku, Kyoto 606-8501, Japan
e-mail: uemura@scl.kyoto-u.ac.jp

†Department of Applied Chemistry, Faculty of
Science and Engineering, Kinki University, 3-4-1
Kowakae, Higashi Osaka, Osaka 577-8502, Japan

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Earth science: An alternative origin for the 'Silverpit crater'

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