# Protein function below 220 K

SIR — Rasmussen et al. report[1] that crystalline ribonuclease A loses its catalytic function below 220 K, because its structure becomes too rigid to undergo the small adjustments in atomic positions needed to bind substrates productively. In 1978, we found[2] a similar transition in the magnetic behaviour of azidemethaemoglobin in which the haem iron is in a thermal spin equilibrium between two spin states.

Most ferric iron complexes remain in the same spin state over a wide temperature range and thus obey the Curie–Weiss law, $\chi = 1/(T-\theta)$, where χ is the magnetic susceptibility, T the absolute temperature and θ a constant; but in some complexes the separation in energy between the low ($^2T_2$) and high ($^6A_1$) spin states is so small that it approaches the thermal energy. Such compounds exhibit a temperature-dependent spin equilibrium. If the low-spin state is the ground state, they show a region at low temperature where they are pure low spin ($S = \frac{1}{2}$) and obey the Curie–Weiss law; then a transitional region where their paramagnetic susceptibility rises with rising temperature until the high- ($S = \frac{5}{2}$) and low-spin states are equally populated; and finally, a region of mixed spin where the Curie–Weiss law is obeyed again[3].

The spin transition is coupled to stereochemical changes. For example, in
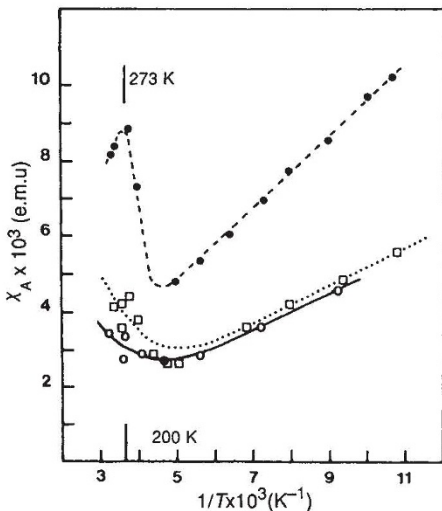
Temperature dependence of paramagnetic susceptibilities of carp azidemethaemoglobin in the R-structure without the allosteric effector inositolhexaphosphate (open circles and full line); in the T-structure with inositolhexaphosphate (solid circles and broken line); and azidemetmyoglobin (squares and dotted line). The susceptibility χ is related to the effective magnetic moment μ_e by the equation $\mu_e = 2.828\sqrt{\chi T}$.

high-spin tetraphenylporphyrin $Fe^{3+}N_3^-$, $Fe-N_{porph} = 2.067$ Å, the iron is displaced by 0.45 Å from the plane of the porphyrin and has a magnetic moment near 5.92 Bohr magnetons at 20 °C. In the low-spin pyridine complex of the same compound $Fe-N_{porph} = 1.99$ Å, the iron lies in the porphyrin plane, and it has a spin-only magnetic moment of 1.73 Bohr magnetons at 20 °C, which is raised somewhat by orbital contributions. When a thermal spin equilibrium exists, as in azidemethaemoglobin, the iron oscillates between the two positions and spin states.

Addition of certain allosteric effectors has been found to raise the spin equilibrium of azidemethaemoglobin to higher spin by switching the quaternary structure from the R (relaxed) to the T (tense) state, because the latter stretches the Fe–N bonds (see figure). In both states the paramagnetic susceptibility falls on going from room temperature to about 220 K, where it begins to follow the Curie–Weiss law, rising linearly with $1/T$. In the R-structure the magnetic

moment goes down to 2.2 Bohr magnetons before it rises again; in the T-structure the rise begins at 2.8 Bohr magnetons. In either structure the rise starts at 220 K because the globin has become too rigid for the iron atom to oscillate between two alternative positions; whatever spin equilibrium has been reached at 220 K becomes frozen in.

The loss of mobility observed by different methods in at least three proteins — ribonuclease (crystalline and suspended in supercooled liquid), haemoglobin and myoglobin[4] in frozen solutions — suggests that 'freezing' at about 220 K may be a general property of protein structures.

M. F. Perutz
*MRC Laboratory of Molecular Biology,
Hills Road, Cambridge CB2 2QH, UK*

1. Rasmussen, B. F., Stock, A. M., Ringe, D. & Petsko, G. A. *Nature* **357**, 423–424 (1992).
2. Messana, C. *et al. Biochemistry* **17**, 3652–3662 (1978).
3. Perutz, M. F. *A. Rev. Biochem.* **48**, 327–386 (1979).
4. Frauenfelder, H., Sligar, S. G. & Wolynes, P. G. *Science* **254**, 1598 (1991).

Kiwi's egg size and moa

SIR — Morgan's novel speculation[1] on why kiwi have a relatively enormous egg is that kiwi became nest parasitic on moa to avoid having their ground-laid eggs gobbled by these large omnivorous ratites. Natural selection (Morgan argues) then favoured kiwi with larger eggs, as these were less easily detected in the moa's communal nest. Later, when the moa vanished and the rat arrived, kiwi survived by (presumably) learning to incubate their own eggs again, but this time in holes in the ground — the implication being that these holes afforded some protection against egg predation by these mammals.

Morgan may have sought to challenge Gould's views[2] on how natural selection might account for the oversized kiwi egg, but she presents an argument that runs counter to the existing data on the biology of both moa and kiwi. First, kiwi eggs have been reported in none of the more than 40 subfossil moa nests that have been found[3,4]. Second, the suggestion that moa were omnivorous is pure speculation. Present evidence points to browse as the preferred diet of moa, along with some fruit, seeds and grass[5]. Third, moa nests contained only one, or occasionally two eggs[3,4]. This is inconsistent with Morgan's assertion that moa had communal nests. Fourth, why kiwi should flee underground when rats arrived is bewildering. Rats and other mammalian predators are a major cause of egg loss in most New Zealand birds, but not in kiwi. In fact, the main predator of kiwi nests is the weka (*Gallirallus australis*), a native rail. Nesting in a

burrow may give some protection against this avian predator, but little if any against rats.

Asking why kiwi eggs are so large in relation to the size of the bird may obscure a likely explanation by the nature of the question. Most ratites are large, and have eggs that are allometrically in proportion to their body size. If the ancestral ratites were also large (which seems a reasonable supposition), the question that should really be asked is what were the selective pressures that may have operated on kiwi to favour a reduction in body size? The availability of a niche for a forest-dwelling insectivore is one possibility.

Finally, several compelling benefits of the large, yolky eggs to kiwi hatchlings have been suggested[6,7], challenging Morgan's claim that the kiwi's relatively enormous egg is of no obvious advantage to the chick.

Murray Potter
*Department of Ecology,
Massey University, Palmerston North,
New Zealand*
John McLennan
*DSIR Land Resources,
Goddard Lane, Havelock North,
New Zealand*

1. Morgan, E. *Nature* **355**, 401 (1992).
2. Gould, S. J. *Bully for Brontosaurus* (Hutchinson Radius, 1991).
3. Hartree, W. H. *New Zealand Archaeol. Ass. Newslett.* **3**, 28 (1960).
4. Anderson, A. *Prodigious Birds* (Cambridge University Press, 1989).
5. Burrows, C. J. *et al. Rec. Canterbury Mus.* **9**, 309–336 (1981).
6. Calder, W. A. III *BioScience* **29**, 461–467 (1979).
7. Gould, S. J. *Nat. Hist.* **11**, 20–29 (1986).