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Proteins as nanomagnets and magnetoreceptors

It is an appealing notion that a protein molecule could act as a nanomagnet. A genetically encodable biomolecule with a permanent magnetic moment at room temperature could have a range of applications: a magnetogenetic actuator, a magnetic tag for purifying and immobilizing enzymes, a contrast agent for magnetic resonance imaging, and a basis for a biomimetic magnetic sensing device, to name just a few. A magnetic protein could perhaps also function as the sensor in the magnetic compass that enables small songbirds to navigate the huge distances between their breeding and wintering grounds. Attractive though such possibilities may be, how realistic are they?

Iron-containing nanoparticles, for example the magnetosome structures that allow magnetotactic bacteria to orient in the Earth's magnetic field, have intrinsic magnetism because they contain hundreds of thousands of iron (Fe) atoms which interact strongly with their immediate neighbours leading to a macroscopic alignment of their electron spins and hence a permanent magnetic dipole moment. Many proteins bind Fe atoms, often in the form of iron-sulphur (Fe-S) clusters, but these groupings are usually few and far between, such that the strong spin-spin coupling required for permanent magnetism would seem to be out of the question.

There was therefore some excitement in 2016 when Can Xie and his colleagues reported the discovery of a magnetic iron-containing protein complex (Qin et al., 2016). Modelling suggested a cylindrical oligomeric structure, 24 nm long and 15 nm wide, composed of 20 molecules of a homologue of the bacterial Fe-S cluster assembly protein, IscA1, adorned with 10 molecules of the flavoprotein, cryptochrome (Cry). Containing 40 Fe atoms, with the closest Fe-S centres ~1 nm apart, this ~900 kDa complex was reported to align spontaneously, like a nanoscopic compass needle, in the Earth's magnetic field. The authors suggested that the avian analogue of IscA1, dubbed MagR, in association with avian cryptochrome-4 (Cry4), could be the detector in the magnetic compass sense of migratory songbirds and other animals. Xie's proposal combines aspects of two leading hypotheses for the biophysical mechanism of animal magnetoreception. One – the radical pair mechanism – for which there is currently the most convincing evidence, is based on magnetically sensitive, light-induced electron transfer reactions in Cry4 (Hore & Mouritsen, 2016). The other relies on biogenic superparamagnetic or ferrimagnetic Fe-containing nanoparticles (e.g., magnetite, Fe_3O_4) coupled to mechanoreceptors or ion channels in nerve cells (Nordmann et al., 2017).

The claim that supramolecular MagR/Cry4 complexes could have an intrinsic magnetic moment sufficient to align them in a magnetic field as weak as the Earth's (25-65 μT) was initially met with blunt disbelief. In an article on the broader area of magnetogenetics, Meister estimated that one would need to cram ~10^6 closely packed Fe atoms into each complex before there would be any possibility of a permanent magnetic moment (Meister, 2016). Separately, Winklhofer and Mouritsen calculated that the magnetic moment would have to be 10^7 times larger than the value Xie had measured for MagR/Cry4 to account for the reported orientation in the Earth's magnetic field (Winklhofer & Mouritsen, 2016). Nevertheless, and despite a few negative replication attempts, there have been a number of independent studies over the last few years that seem to confirm some of the properties claimed for MagR/Cry4. Three are summarized here.

First, at least three independent laboratories have had success using Fe_3O_4-SiO_2 magnetic beads to purify and immobilise catalytically active enzymes when expressed as MagR fusion proteins (Jiang et al., 2017; Kang et al., 2021; Liu et al., 2022; Wang et al., 2019). In no case was an attempt made to co-express Cry4 suggesting, perhaps, that it is not a prerequisite for MagR magnetization. However, Pekarsky et al. found that *E. coli* cells overexpressing fruit-fly MagR had no measurable permanent magnetization at low temperature and attributed their limited success at protein purification to ionic rather than magnetic interactions (Pekarsky et al., 2021).

Second, the fabrication of electrochemically-based magnetic sensors has been reported by two independent research groups (Cheng et al., 2023, 2024; Xue et al., 2020). The output current of these devices, containing a layer of pigeon MagR/Cry4 immobilised on an electrode, was found to be sensitive to applied magnetic fields down to ~1 mT. The effect is thought to be due to a magnetically-induced alignment of the adsorbed MagR/Cry4 molecules which reduces the scattering of charge carriers. One of the two laboratories found that neither MagR nor Cry4 on its own showed similar effects, that the magnetic responses of the devices required light, and that MagR/Cry4 had no measurable permanent magnetic moment (Xue et al., 2020).

Third, transfection of pigeon MagR/Cry4 genes into *E. coli* cells and eukaryotic mesenchymal stem cells cultured with exogenous iron resulted in enhanced T_2 contrast in magnetic resonance images (MRI) of the cells (Li et al., 2023a, 2023b). The effect was not found for transfection with the MagR gene alone or in the absence of exogenous Fe^{3+}. It seems that MagR/Cry4 promotes the formation of intracellular paramagnetic iron oxide granules which accelerate the transverse relaxation observed by MRI. Given the failure of

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several laboratories to confirm that MagR/Cry4 has a permanent magnetic moment, one wonders whether some of the other magnetic effects claimed for MagR/Cry4 could be due to the biosynthesis of (or contamination by) magnetic Fe_3O_4 or Fe_2O_3 nanoparticles.

What to make of all these articles? Either all of them, along with others not mentioned here, unwittingly report experimental artefacts or, unlikely as it may seem, MagR/Cry4 really does have unprecedented magnetic properties. The few theories have been put forward to account for these observations shed little light. Nor is it at all clear what role Cry4 might play in MagR/Cry4. Xie suggests that movement of electrons between MagR and Cry4 could be important (Qin et al., 2016; Xie, 2022). He proposes a structural model in which a central cylindrical stack of five disk-shaped MagR tetramers is surrounded by a sheath of Cry4 molecules. Within each tetramer is a ring (diameter ~1 nm) of four Fe-S clusters (one per monomer) and a concentric, perpendicular ring (diameter ~2 nm) of 16 aromatic amino acid residues (one tyrosine and three phenylalanines per monomer). It is imagined that this arrangement would provide a ~5 nm electron transfer pathway between the Cry4 chromophores (flavin adenine dinucleotide) in the periphery and the Fe-S loops in the core, via a chain of four tryptophans in Cry4, a bridge comprising two tyrosines separated by ~1 nm, and the Tyr-Phe ring in MagR within which electrons are supposed to circulate (Xie, 2022). It will be interesting to see whether this speculation is confirmed by experiment.

Clearly, more needs to be known about iron-binding in MagR/Cry4. To this end, Xie and colleagues have now completed a detailed study, published in four recent articles in *Zoological Research*, the results of which can be summarized as follows. (1) Pigeon MagR binds iron in the form of Fe^{3+} and as Fe-S centres; the former requires the presence of a conserved tyrosine residue (Zhou et al., 2023). (2) The 25 N-terminal amino acid residues in pigeon MagR are essential for the stability of the MagR/Cry4 complex and enhance the Fe-binding efficiency of eukaryotic MagR relative to prokaryotic IscA (Zhang et al., 2024b). (3) There are only three sequence variations between the MagR proteins from (non-migratory) pigeon and (migratory) European robin. Two of them account for the higher Fe^{3+} and Fe-S cluster affinity of the robin protein and for the different oligomeric states of pigeon (tetrameric) and robin (dimeric) MagR. Both proteins are diamagnetic at 300 K (Wang et al., 2024). (4) The stability of MagR and its affinity for Fe^{3+} and Fe-S clusters have increased during the course of evolution from prokaryotes, molluscs, arthropods, bony fishes, reptiles, and birds to mammals (Zhang et al., 2024a).

Despite these new insights, fundamental questions still surround MagR/Cry4. What is the origin of any unusual intrinsic magnetism? Is the Cry4 component essential and, if so, what is its function? Does the MagR/Cry4 protein complex exist *in vivo*? And, most intriguingly of all in my view, could it be an indispensable component of the compass magnetoreceptor in birds or other animals? Whatever the answers turn out to be, they are likely to be interesting.

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