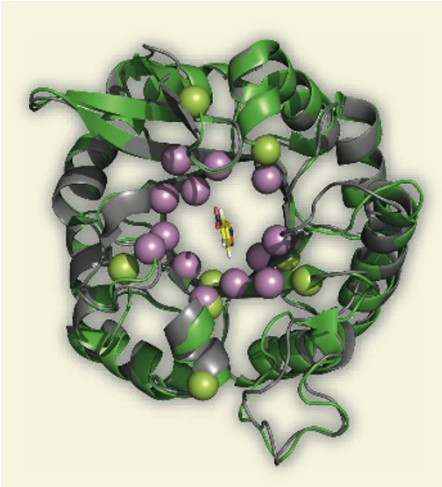

Figure 1 | Enzymes by design. Röthlisberger *et al.*[1] have computationally designed and prepared the first enzyme capable of catalysing a non-biological reaction. Here, the computational model (grey) is overlaid with the crystal structure of the actual protein (green); the two overlap almost perfectly. The substrate is shown at the centre of the structure. The design process involved modifying the amino-acid sequence of a naturally occurring protein. Residues selected computationally to form the active site are shown as purple spheres. Additional mutations that were introduced *in vitro* to optimize the enzyme's performance are shown as green spheres.

But some reports of catalysis by designed enzymes have fared rather better — especially those that are based on sound crystallographic evidence[6–8]. An essential step in demonstrating the success of a designer enzyme, therefore, is the determination of a high-resolution crystal structure for the protein, to verify that the designed catalytic features are present. The results of Röthlisberger *et al.*[1] and Jiang *et al.*[2] are remarkable in the spectacular agreement between their computationally predicted enzyme models and the experimentally determined structures (Fig. 1).

Röthlisberger *et al.*[1] made an enzyme that catalyses the Kemp elimination reaction (see Fig. 1a on page 190 for a reaction scheme). The Kemp elimination is initiated by the removal of a hydrogen ion from a carbon–hydrogen bond in the substrate; the minimum requirement for catalysis of the reaction is the presence of a base to perform this step. The authors therefore identified two amino acids — aspartic acid and histidine — that have side chains that can act as bases under physiological conditions, and used these as the starting points of their putative active sites. They decorated models of the proposed active sites with other chemical groups found in proteins, choosing those that could interact favourably with groups in the substrate. They then used state-of-the-art quantum-mechanical methods to precisely place all the groups in the models to maximize stabilization of the transition state of the substrate. The authors thus obtained a large ensemble of designs for catalytic sites in enzymes.

Next, Röthlisberger *et al.* selected about 100 proteins that could be used as scaffolds for their proposed active sites. The criteria for selection were the availability of high-resolution crystal structures and the presence of pre-organized cavities, with a preference for proteins that behave well in experiments (that is, those that have good solubility, are expressed easily in cells, and so on). The authors then used computational methods to search each of the proteins for specific regions that could accommodate the sites, narrowing down the vast number of possibilities to about 100,000 promising leads. These were whittled down further using an automated modelling technique to find the optimal amino-acid sequence in defined shells around the active site, selecting sequences that maintained protein stability and integrity.

This computational screening method picked out 59 candidate enzymes, which the authors expressed in cells and evaluated for their ability to catalyse the target reaction. Only eight of the proteins had measurable catalytic activity. The team then used *in vitro* evolution to further optimize one of their successful leads (designated KE07), mutating the amino-acid sequence in both random and directed locations. After several rounds of mutation and screening, Röthlisberger *et al.* obtained improved enzymes that were up to 200 times more active than KE07. The best two of these mutants accelerate the rate of the Kemp elimination reaction to about a million times that of the uncatalysed version.

The strategy used by Röthlisberger *et al.*[1] promises to be general, as the same group[2] has successfully applied the procedure to another chemical transformation known as the retro-aldol reaction, which is very different from the Kemp elimination. The complexity of the design procedure is underlined by the number of interdisciplinary groups involved in the work, and by the huge amount of computational power required to solve the problem — donated from hundreds of thousands of idling computers around the world as part of a project known as Rosetta@home[9].

Those in the know might say that the performance of the designed enzymes is far from impressive — the reaction-rate enhancements for typical, naturally occurring enzymes are anywhere between 10,000 and 1 billion times higher than those of the artificial enzymes described in these papers[1,2]. Furthermore, the chosen reactions are relatively easy targets. The Kemp elimination is accelerated by several catalysts, including various synthetic compounds, catalytic antibodies and even serum albumin. Similarly, the retro-aldol reaction is catalysed by antibodies[10] and by various peptides[11,12]. Indeed, the rate enhancements reported by Röthlisberger *et al.*[1] are equivalent to those of only the most sophisticated catalytic antibodies[13,14]; the enhancements obtained by Jiang *et al.*[2] for the retro-aldol reaction are even more modest.

Another limitation of the design process is that, although naturally occurring enzymes

50 YEARS AGO

A Hundred Years of Evolution. By Dr. G. S. Carter — It is fundamental to the neo-Darwinian theory that Weismann's concept of the inviolability of germ plasm by soma is correct, and that mutational changes in the gene complex arise solely at random; Dr. Carter (p. 87) accepts Weismann's doctrine as "undeniable when once pointed out". It is arguable, however, that the "separateness of the gonad from the rest of the soma" is a philosophical concept of the same order as that of the soul and the body. As such it may have been valid in the state of biological knowledge in Weismann's time, but it has to-day become undermined to the point of collapse ...That mutation is random is purely theoretical, depending in the first place on the validity of the divorce between germ plasm and soma, and in the second upon the absence of evidence to the contrary.

From *Nature* 10 May 1958.

100 YEARS AGO

To be told that life exists on Mars tells us but little of its nature ... Perhaps on Mars there is only one living being, a gigantic vegetable the branches or pseudopodia of which embrace the planet like the arms of an octopus, suck water from the melting polar snows, carry it to other parts of the planet, and are visible to us as the Martian canals. Lowell adduces the straightness of the canals as a proof that they are artificial products of intelligent beings. But they are certainly no straighter than the somewhat similarly interlaced pseudopodia seen in certain Heliozoa, Foraminifera, and Radiolaria ... My position is that one may admit that Prof. Lowell's brilliant researches prove the existence of life on Mars, and still ask from him further evidence before we are convinced that that life is intelligent.

From *Nature* 7 May 1908.

50 & 100 YEARS AGO