Expanding the universe of protein structures through *de novo* protein design

Supervisory team:

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Project description:

The number of natural protein structures is limited, estimated at 2,000 – 10,000 different folds. These are used by biology in many different contexts, being adapted through natural selection to give an array of functions. By contrast, even combining just two types of secondary structure—alpha-helices and beta-strands—the number of possible structures is effectively infinite. This difference between the known protein structures and those that are possible has been termed the *dark matter of protein space*.

Delving into these *dark protein structures* is interesting for a number of reasons: first, are the currently known natural proteins somehow special, and others simply not accessible or stable? Second, if we could engineer completely new protein structures, what might we be able to do with them?

The question is: how do we get into the *dark space*? For some time, protein designers have been able to mimic natural protein structures, because we can learn so-called *sequence-to-structure* relationships from known examples. For completely new structures, however, there are no such direct relationships to guide the designs. Thus, we need entirely new approaches to *completely de novo protein design.*

We have started to do this for one class of dark protein structure known as the *alpha-helical barrels*. These are related to natural alpha-helical bundles and coiled-coil proteins. In the natural cases, 2 – 4 helices come together to make bundles with solid hydrophobic cores. In the alpha-helical barrels however, 5 or more helices assemble, and rather than having solid cores they have central channels, **Figure**. Moreover, the diameters of the channel scale linearly with the number of helices in the barrel—a 5-helix bundle has a ≈5 Å channel and so on—making them appealing targets for downstream functional designs. As there are few or no naturally occurring examples for most of the barrel sizes, we have developed computational methods to allow the design of entirely new alpha-helical barrels from scratch.

The proposed PhD project will develop this programme further by creating single-channel alpha-helical barrels that will be produced recombinantly in *E. coli* ready for structural characterisation by solution-phase biophysics and X-ray crystallography. Successful designs will be used as scaffolds to introduce functions such as small-molecule binding, enzyme-like catalysis and membrane-spanning pores.

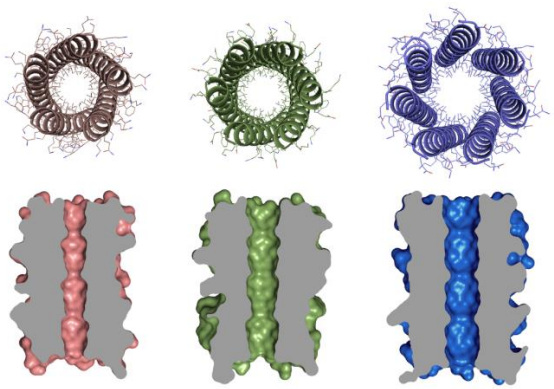

Figure: Computationally design alpha-helical barrels. From left to right these have 5, 6 and 7 helices and central channels of diameters ≈5, 6 and 7 Å. Thomson *et al.* ***Science*** **346** 485-488 (2014).