Peptide design and assembly: from test tubes to cells

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Protein design—*i.e.*, the construction of entirely new protein sequences that fold into prescribed structures—has come of age: it is possible now to generate a wide variety stable protein folds from scratch using rational and/or computational approaches. A challenge for the field is to move from what have been largely *in vitro* exercises to protein design in living cells and, in so doing, to augment biology. This talk will illustrate what is currently possible in this nascent field using *de novo* α–helical coiled-coil peptides as building blocks.[1]

Coiled coils are bundles of 2 or more α helices that wrap around each other to form rope-like structures. They are one of the dominant structures that direct natural protein-protein interactions. Our understanding of coiled coils provides a strong basis for building new proteins from the bottom up. The first part of this talk will survey this understanding,[1] our design methods,[2,3] and our current "toolkit" of *de novo* coiled coils.[4-6]

Next, I will describe how the toolkit can be used to direct protein-protein interactions and build complex protein assemblies in bacterial cells. First, in collaboration with the Savery lab (Bristol), we have used homo- and hetero-oligomeric coiled coils as modules in engineered and *de novo* transcriptional activators and repressors.[7] Secondly, with the Warren (Kent) and the Verkade (Bristol) labs, we have engineered hybrids of a *de novo* heterodimer and a natural component of bacterial microcompartments to form a "cytoscaffold" that permeates *E. coli* cells (Figure).[8] This can be used to support the co-localisation of functional enzymes.

1. Coiled-coil design: updated and upgraded.
DN Woolfson. ***Subcellular Biochemistry 82***, 35-61 (2017)
2. CCBuilder: an interactive web-based tool for building, designing and assessing coiled-coil-protein assemblies.
CW Wood *et al.* ***Bioinformatics 30***, 3029-3035 (2014)
3. ISAMBARD: an open-source computational environment for biomolecular analysis, modelling and design
CW Wood *et al.* ***Bioinformatics 33***, 3043–3050 (2017)
4. A basis set of *de novo* coiled-coil peptide oligomers for rational protein design and synthetic biology
JM Fletcher *et al.* ***ACS Synth Biol 1***, 240-250 (2012).
5. A set of *de novo* designed parallel heterodimeric coiled coils with quantified dissociation constants in the micromolar to sub-nanomolar regime
F Thomas *et al.* ***J Am Chem Soc 135***, 5161-5166 (2013).
6. Computational design of water-soluble □□helical barrels.
AR Thomson *et al.*, ***Science 346***, 485-488 (2014)
7. Guiding biomolecular interactions in cells using *de novo* protein-protein interfaces
AJ Smith *et al.* bioRxiv 486902; doi**:** https://doi.org/10.1101/486902
8. Engineered synthetic scaffolds for organizing proteins within the bacterial cytoplasm.
MJ Lee *et al.*, ***Nature Chem Biol 14***, 142-147 (2018)