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Sculpting cavities in alpha-helical bundles to accommodate ligands of different size and shapes

Katarzyna Ozga[a], Rokas Petrenas[a], Joel J. Chubb[a], and Derek N. Woolfson[a,b,c,d]

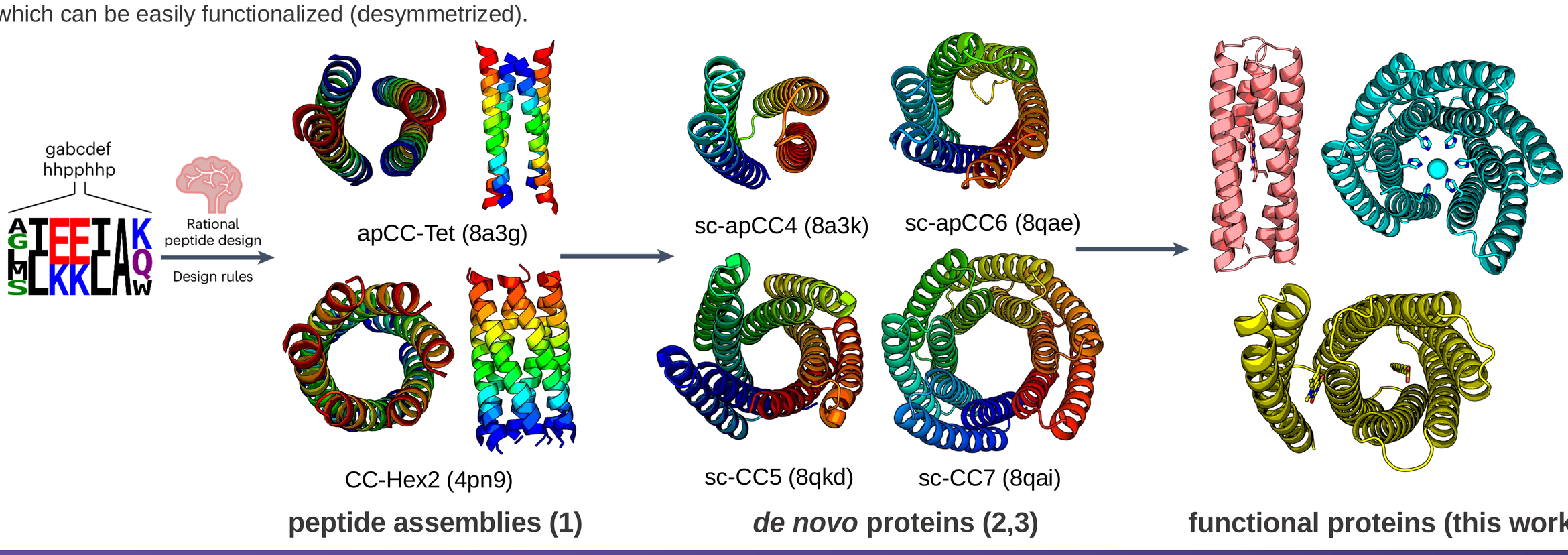
[a] School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK ,
[b]Max Planck-Bristol Centre for Minimal Biology, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK ,
[c]School of Biochemistry, Biomedical Sciences Building, University of Bristol, University Walk, Bristol BS8 1TD, UK,
[d]BrisEngBio, School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK

Abstract

The advancement of AI methods, including RoseTTAFold and AlphaFold (structure prediction from sequence), RFdiffusion (backbone design), ProteinMPNN (sequence design), and more comprehensive tools like Chroma (backbone and sequence design), has made protein structure and function design highly accessible (e.g. through platforms like Colab notebooks) and remarkably successful. However, **rational design** still offers two significant advantages: it minimizes the need for extensive sequence sampling and enhances understanding. In the **Woolfson lab**, we employ a hybrid approach that combines rational and computational methods to design functional and complex coiled-coil proteins, beginning with a deep understanding of the **sequence design rules** for coiled-coil peptide assemblies.

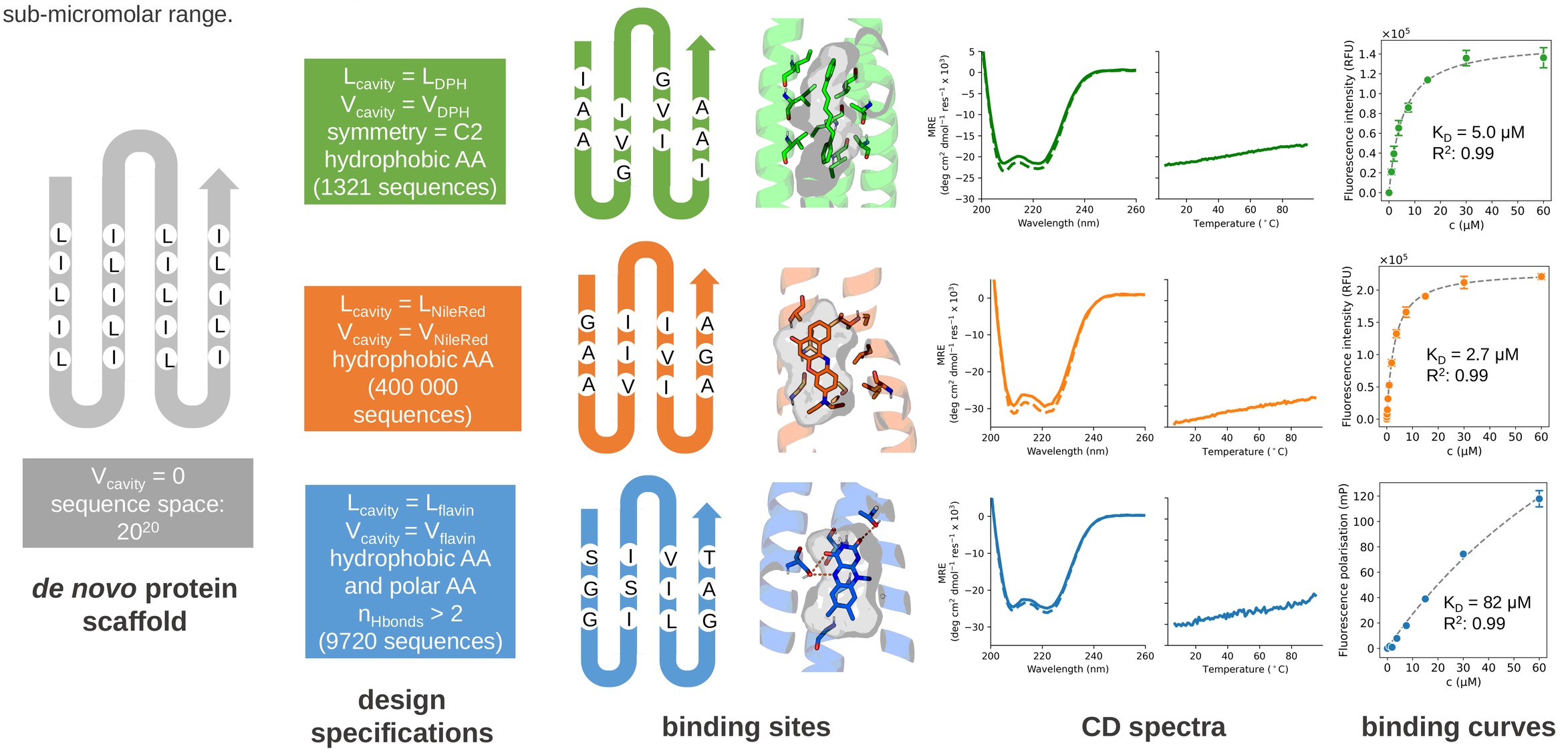
Coiled-coil protein design

The relationship between sequence and structure for coiled-coil peptides was uncovered through bioinformatic analysis of natural coiled coils, complemented by extensive biochemical studies (1). This understanding paved the way for the ***de novo*** design of larger, more complex assemblies (**barrels**), which are rare in nature. In recent work, these coiled-coil peptide assemblies have been further utilized as seeds for template-based loop design using MASTER and ProteinMPNN (2). This approach enables the creation of robust, highly thermostable proteins with varying pore sizes and chemistries, which can be easily functionalized (desymmetrized).



RASSCoL: introducing binding sites within coiled coils

In this work, we introduce the **R**apid **A**ssessment of **S**ize, **S**hape, and **C**omplementarity of **L**igand (**RASSCoL**) as an **ultra-fast protocol** for designing binding sites for various small molecules, including metals, cofactors, and transition states, within coiled-coil proteins. The process begins with a significant reduction of the sequence space by applying **design specifications** that can be quickly assessed at the **sequence level**, e.g. rough estimation of whether the introduced side chains volumes will cumulatively sum up to the target volume of ligand. Next, the filtered sequences are packed onto a scaffold using FASPR and pre-scored with AutoDock Vina, achieving a computing time of just **0.3 seconds per sequence** on a single CPU. The top 100 sequences are then selected for a series of short molecular dynamics (MD) simulations with the ligand to more accurately calculate binding affinity and predict the stability of the mutants. The two best designs for each ligand were subsequently tested experimentally, demonstrating specificity and binding affinities in the low to sub-micromolar range.



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