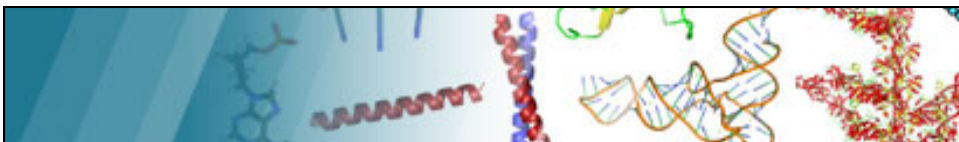




Generating, using and expanding a toolkit of peptide components for protein design & synthetic biology

Dek Woolfson, Chemistry and Biochemistry



Synthetic Components Network - SCN: Synthetic Biology from the bottom up

www.bristol.ac.uk/scn

Next meeting: Bristol, December 5 & 6

SCN member institutions



University of
BRISTOL



The
University
Of
Sheffield.



University of the
West of England

Scientific aims of the SCN

- A biomolecular design and engineering approach to synthetic biology via:
 - “instructing” biomolecules to fold and assemble;
 - “creating” a toolkit of biomolecular components for self-assembly;
 - “engineering” functional biomolecular systems;
 - “guiding” self-organisation and complexity (emergent properties).

✦ About this talk

- (1) One view on synthetic biology
- (2) Peptide components for synthetic biology
- (3) Applying the components in chemical biology/materials science
- (4) Using the components to make peptide-based vesicles
- (5) A new scaffold for protein engineering and systems chemistry

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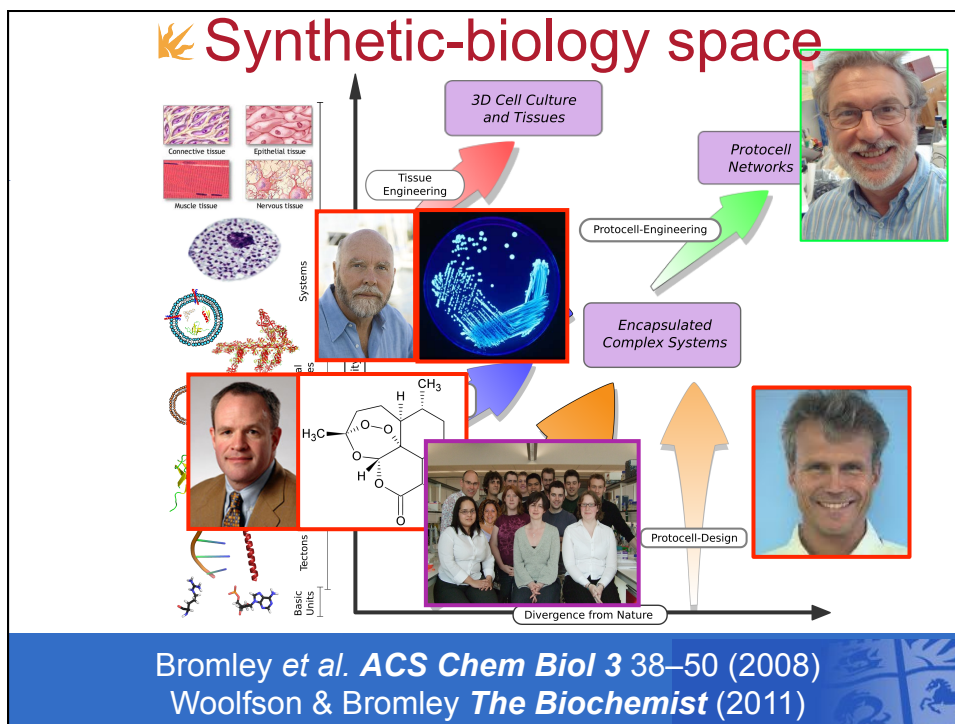
✦ Synthetic biology

*"Synthetic Biology is
(A) the design and construction of new
biological parts, devices, and systems, and
(B) the re-design of existing, natural
biological systems for useful purposes."*

<http://syntheticbiology.org/>

*How can we make the engineering of
biological systems easier and more reliable?*

*Make general rather than "bespoke"
designed peptide components.*



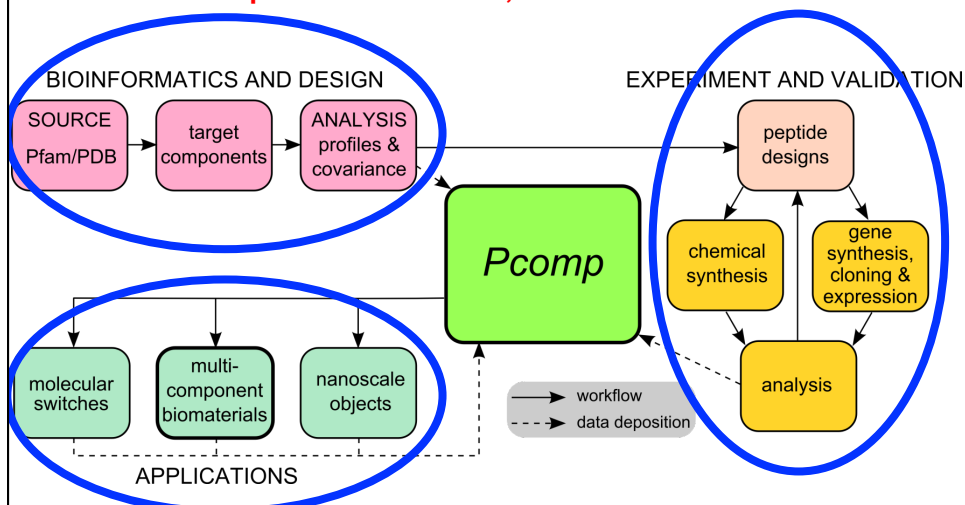
🔥 About this talk

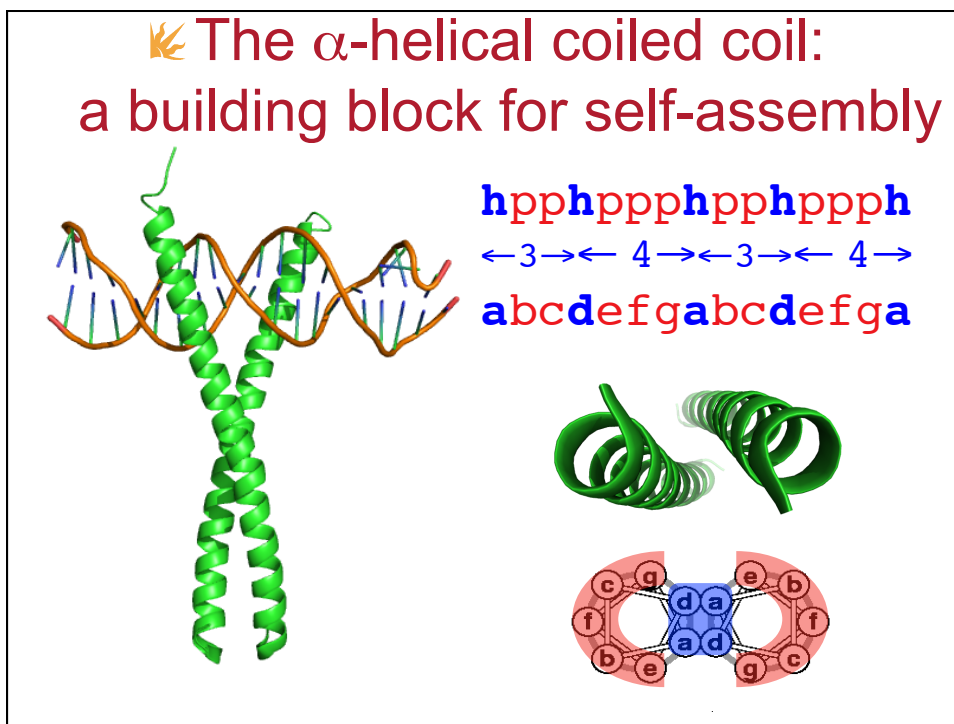
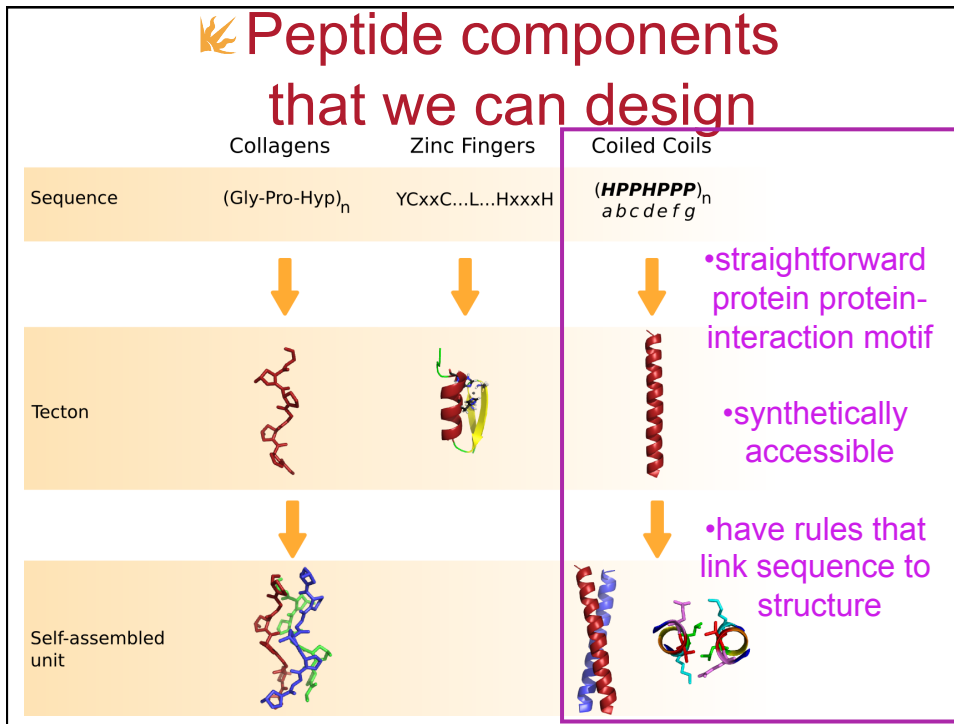
- (1) One view on synthetic biology
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🔥 A toolkit of peptide components: *Pcomp*

**Small; simple/simplified; independently folded; orthogonal;
predictable/reliable; well-characterised.**





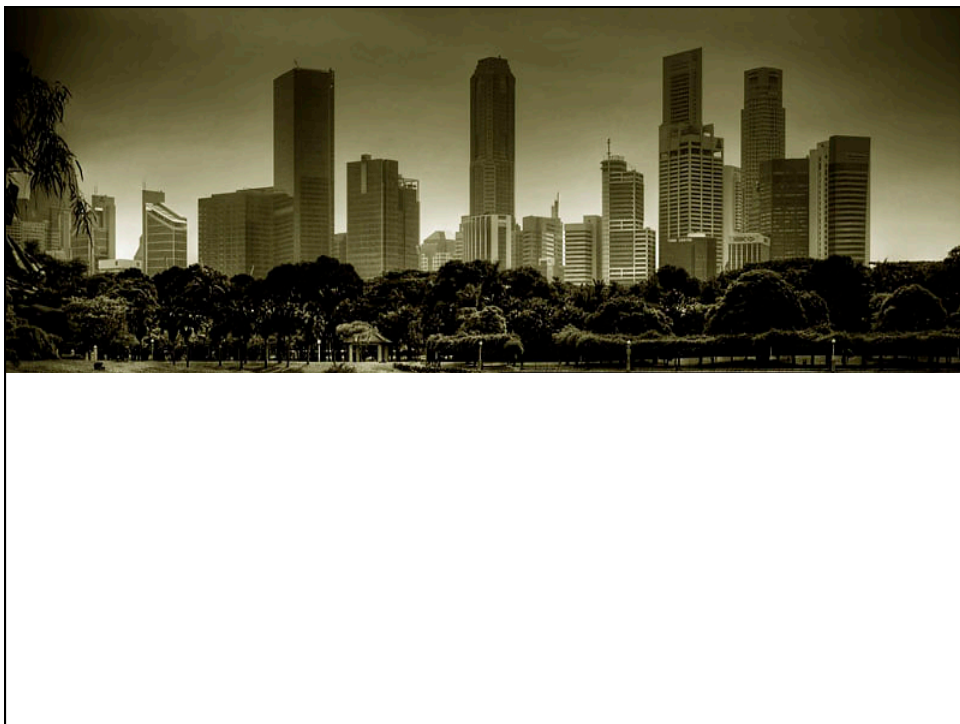
Yes, but

90%

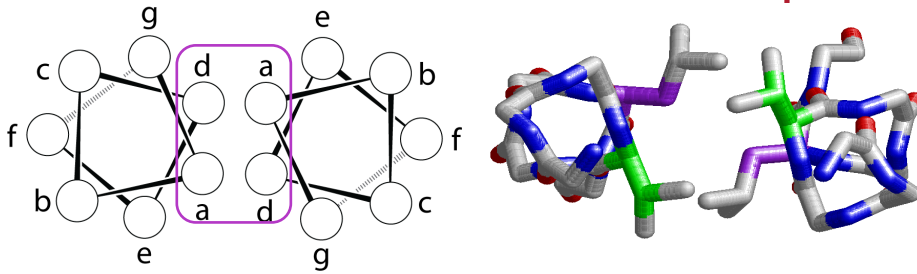
Can we start *Pcomp* with a designed Basis Set of these coiled coils and then build with them?

www.coiledcoils.chm.bris.ac.uk

Oli Testa *et al.* *Nucleic Acids Res* 37, 315 (2009)
 Fay Moutevelis & Woolfson *J Mol Biol* 385, 726 (2009)

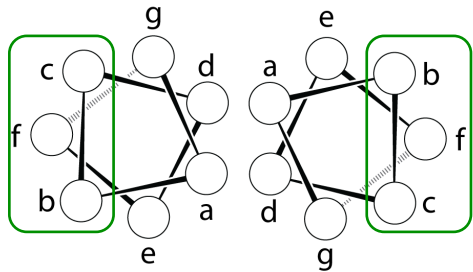


🔥 Coiled-coil Basis Set: Step 1



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🔥 Coiled-coil Basis Set: Step 3



- Vanilla
- Helix favouring
- Polar
- 1 x Trp, Tyr, or IodoPhe

gabcdef gabcdef gabcdef gabcdef

BS2 **G**E**I**A**A**L**K**Q **E**I**A**A**L**K**K** **E**I**A**A**L**K**W** **E**I**A**A**L**K**Q**G

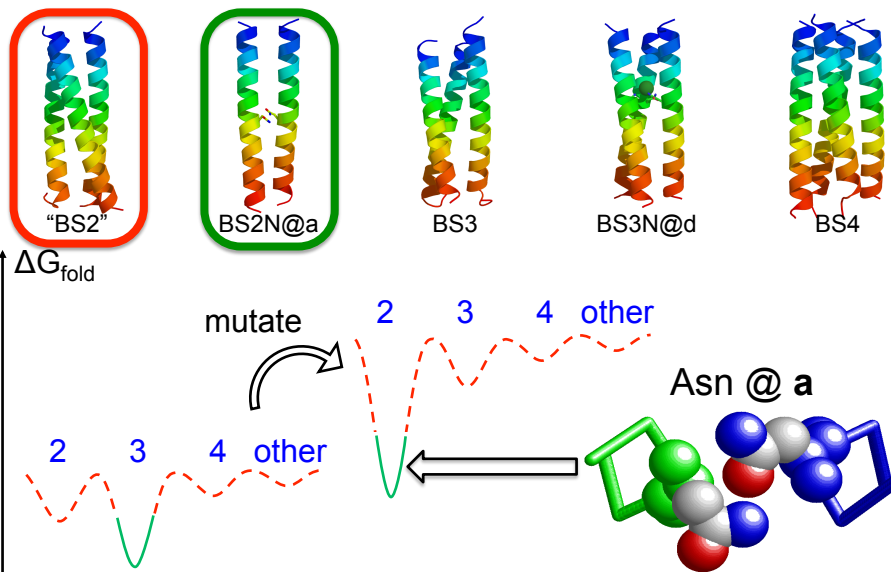
BS3 **G**E**I**A**A**I**K**Q **E**I**A**A**I**K**K** **E**I**A**A**I**K**W** **E**I**A**A**I**K**Q**G

BS4 **G**E**L**A**A**I**K**Q **E**L**A**A**I**K**K** **E**L**A**A**I**K**W** **E**L**A**A**I**K**Q**G

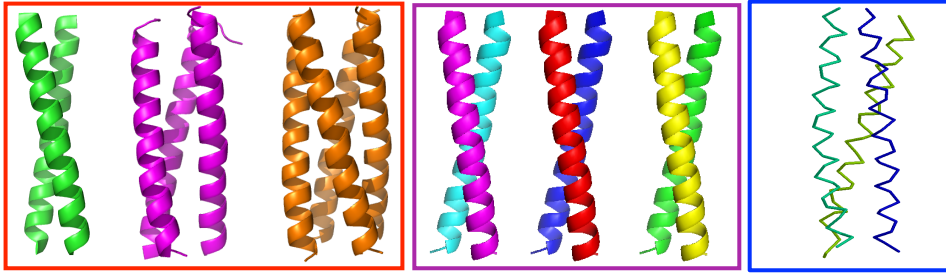
Add terminal linkers, Cys, mass tags as necessary.

🔥 BS2, or "the dimer that's a trimer"

helicity, oligomerisation state, T_M , K_D , X-ray crystal structures



✦ Our designed coiled-coil tectons



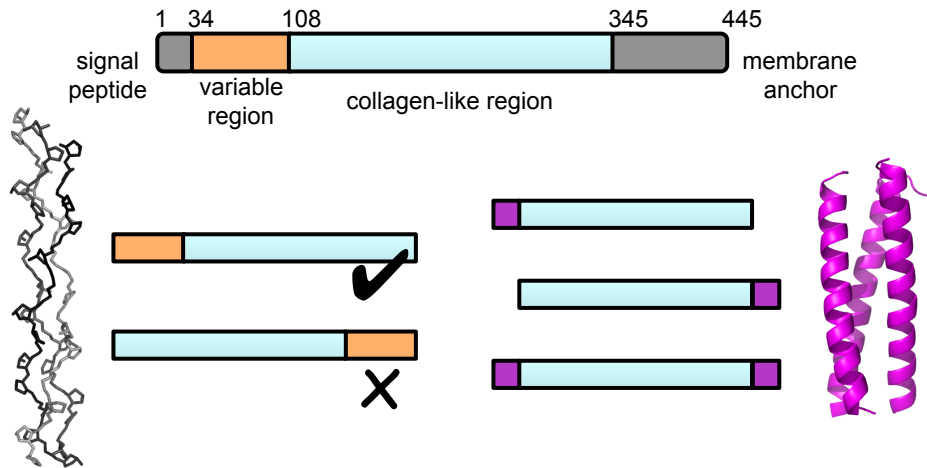
Bromley *et al.* *J. Am. Chem. Soc.* **131**, 928 (2009)
Nautiyal *et al.* *Biochemistry* **34** 11645 (1995)

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BS3 nucleating a bacterial collagen: *Streptococcal* collagen-like protein 2

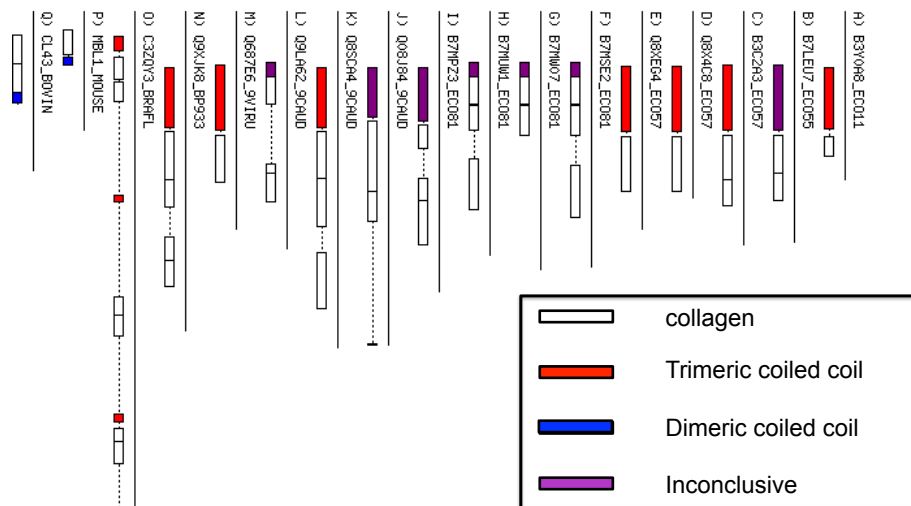


Barbara Brodsky & Ayumi Yoshizumi; Jordan Fletcher
J. Biol. Chem. 280, 1751 (2011)

Unfolding & refolding experiments



Protein families with CCs & CLs



Gail Bartlett and Tom Vincent

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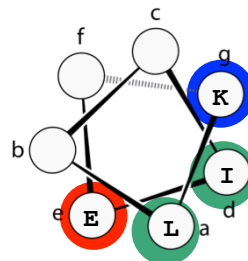
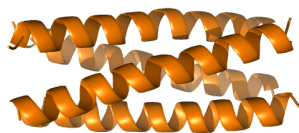
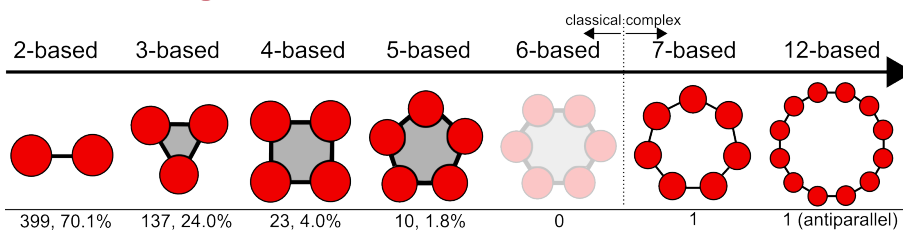
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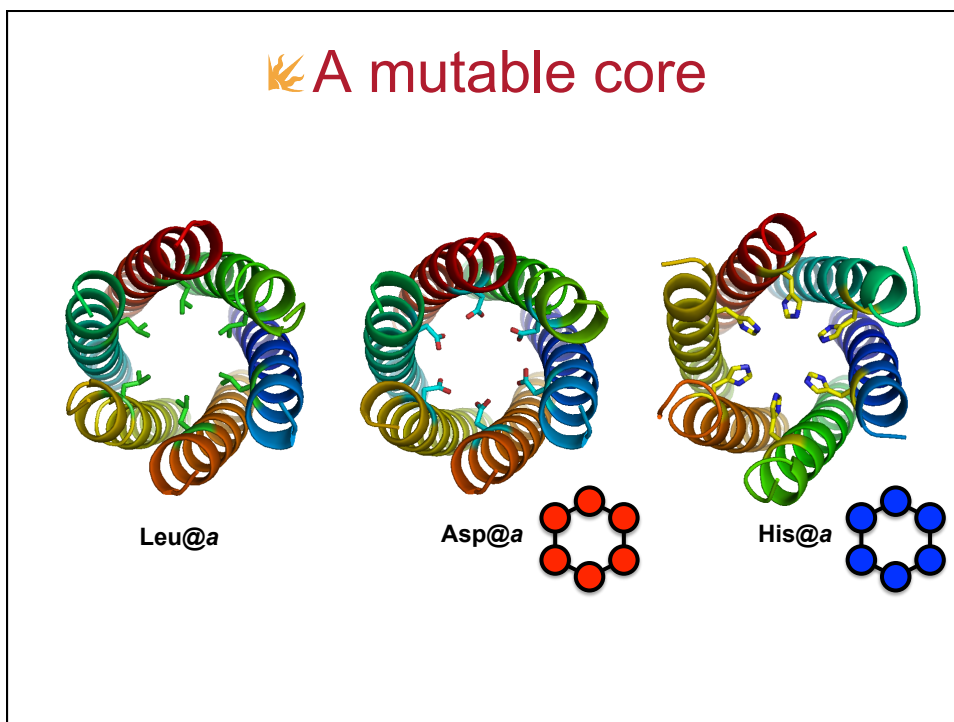
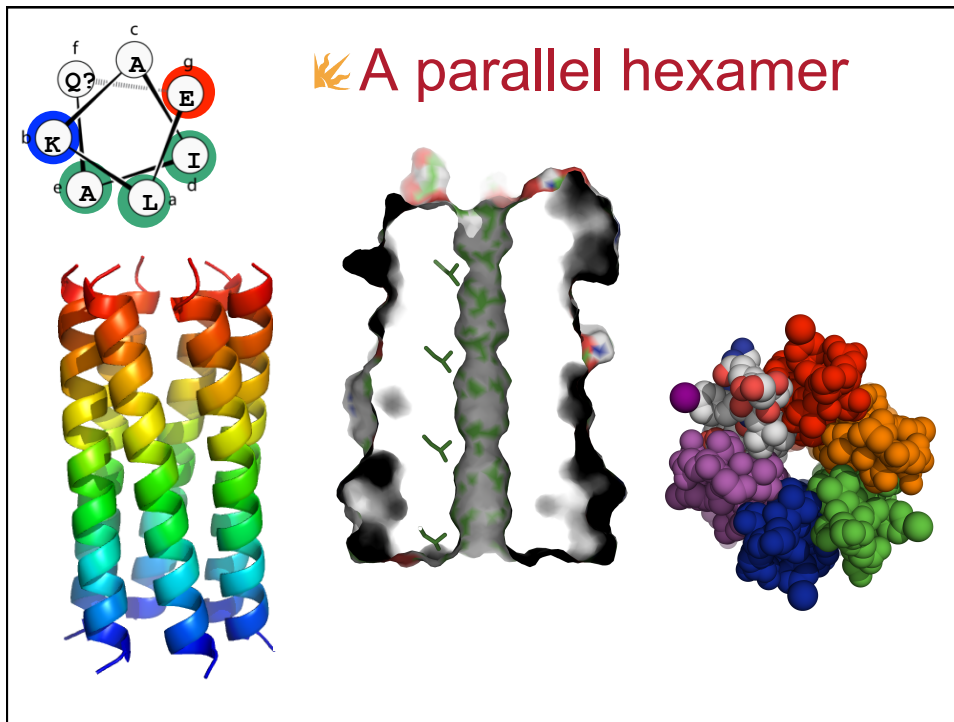
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✦ Using BS4 to expand the toolkit

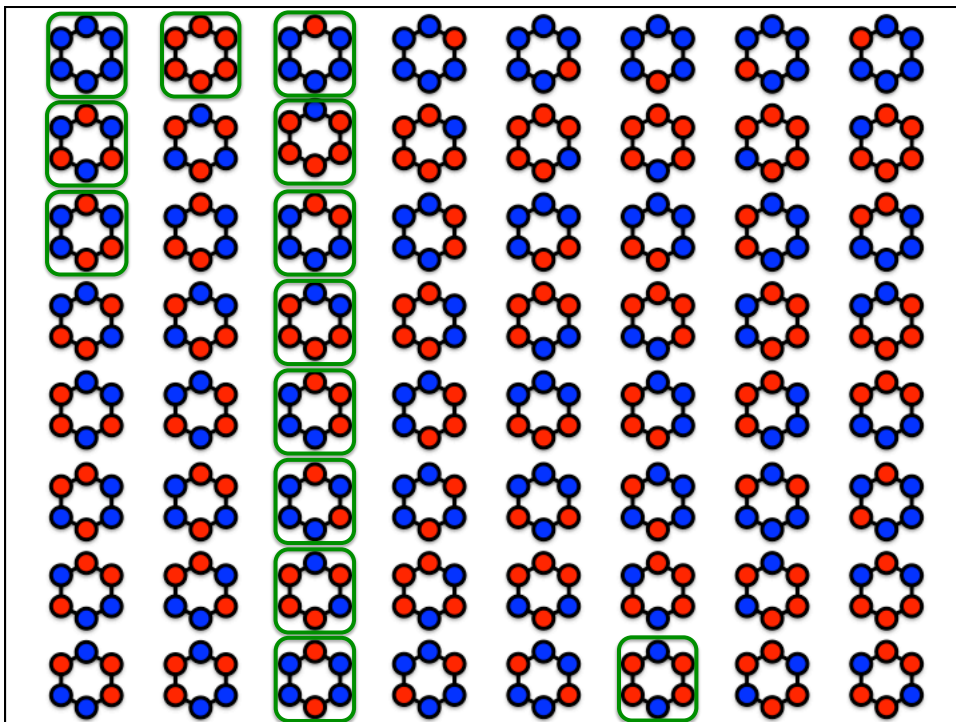
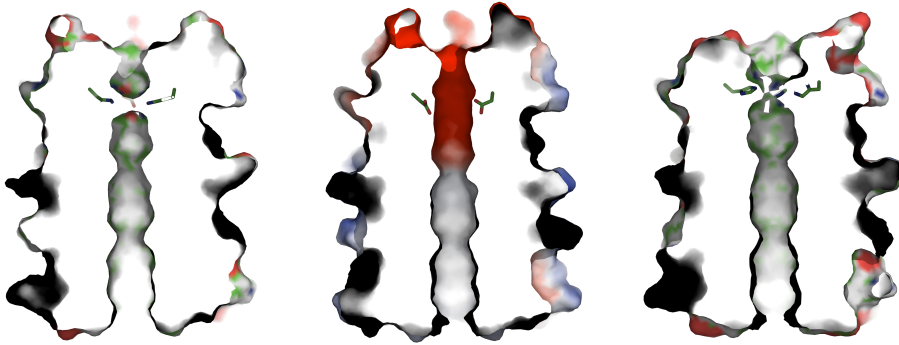
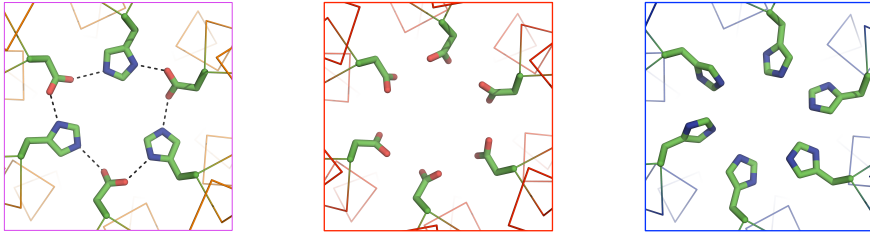


Moutevelis & Woolfson *J Mol Biol* 385, 726 (2009)

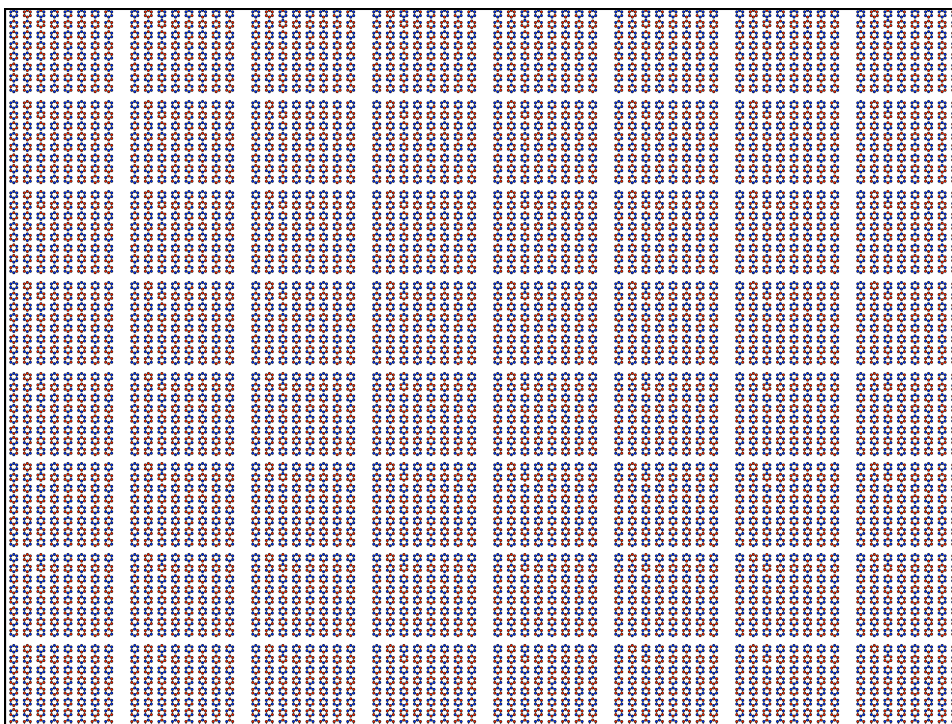
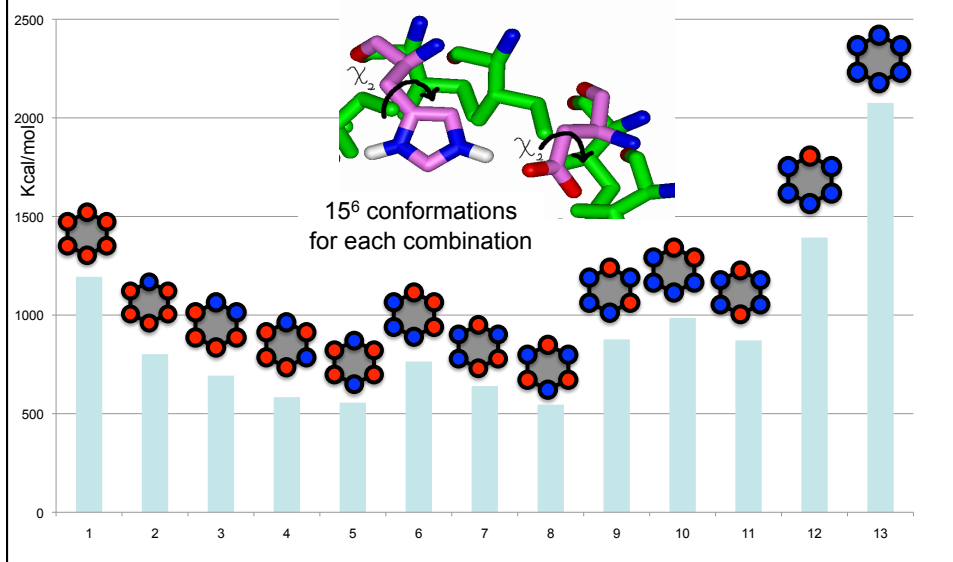
After Min Lu, Cornell



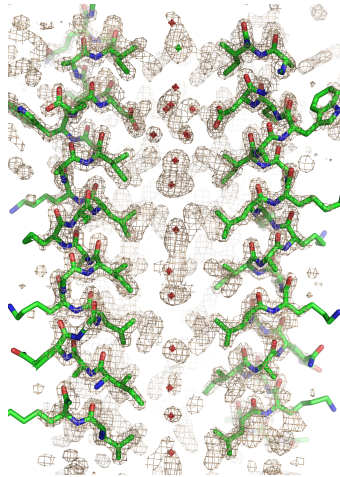
✦ The experiment & comparison



MM Calculations for the Electrostatic & van der Waals' Energy



✦ Towards functional components by design: ion-channel proteins



1.75 Å resolution

✦ Summary

- Begun to populate *Pcomp*, a toolkit for peptide design and assembly. We would welcome other contributors.
- We've had to revise some of our rules and understanding of coiled-coil folding and assembly.
- Begun to use the *CC Basis set* to apply it in chemical biology, and to build new materials.
- Discovered a new coiled-coil structure, a robust parallel hexamer, which has an exciting future ahead of it.

Synthetic biology: design and engineering through understanding
22—26 August 2011
Keele University, UK

www.biochemistry.org/Harden.aspx



Designers

Craig Armstrong
Gail Bartlett
Tom Vincent

Aimee Boyle
Marc Bruning
Bertie Chi
Jordan Fletcher
Thom Sharp
John Simms
Drew Thomson
Nathan Zaccal

Collaborators

Paula Booth
Leo Brady
Noah Linden
Richard Sessions
Paul Verkade

Barbara Brodsky
Andrei Lupas

Funders

BBSRC, EPSRC, HFSP, MRC, Unilever, UofB, UofS & Wolfson-Royal Society