



Generating and applying a toolkit of *de novo* peptides for synthetic biology

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BBSRC

🔥 Aim: a toolkit for peptide assembly

Small; straightforward/simplified; independently folded; orthogonal; predictable/reliable; well-characterised; **proteinogenic & vanilla.**

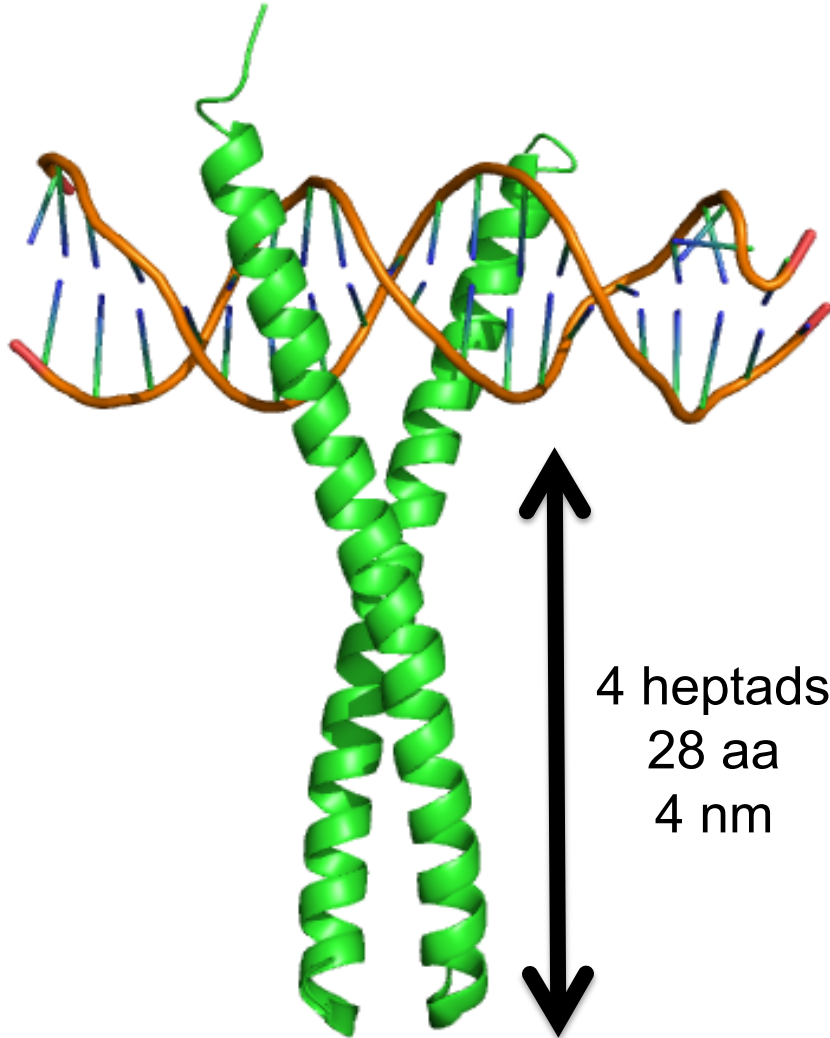
Microwave-assisted Fmoc SPPS



General rather than “bespoke” solutions to the protein-design problem.

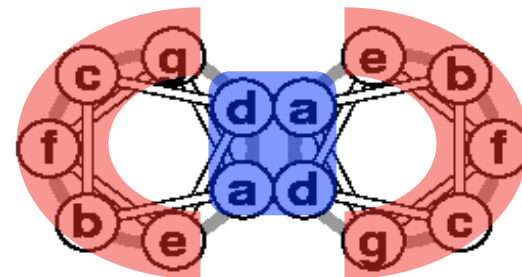
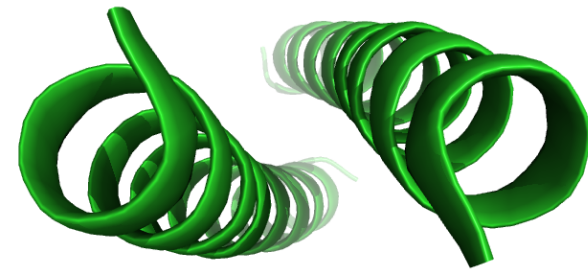
Jordan Fletcher *et al.* *ACS Synth. Biol.* 1, 240-250 (2012)

🦋 The α -helical coiled coil: a starting point for self-assembly

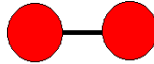
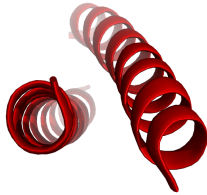
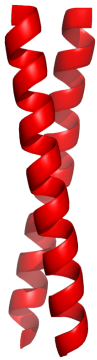


4 heptads
28 aa
4 nm

h p p h p p p h p p h p p p h
← 3 → ← 4 → ← 3 → ← 4 →
a b c d e f g a b c d e f g a

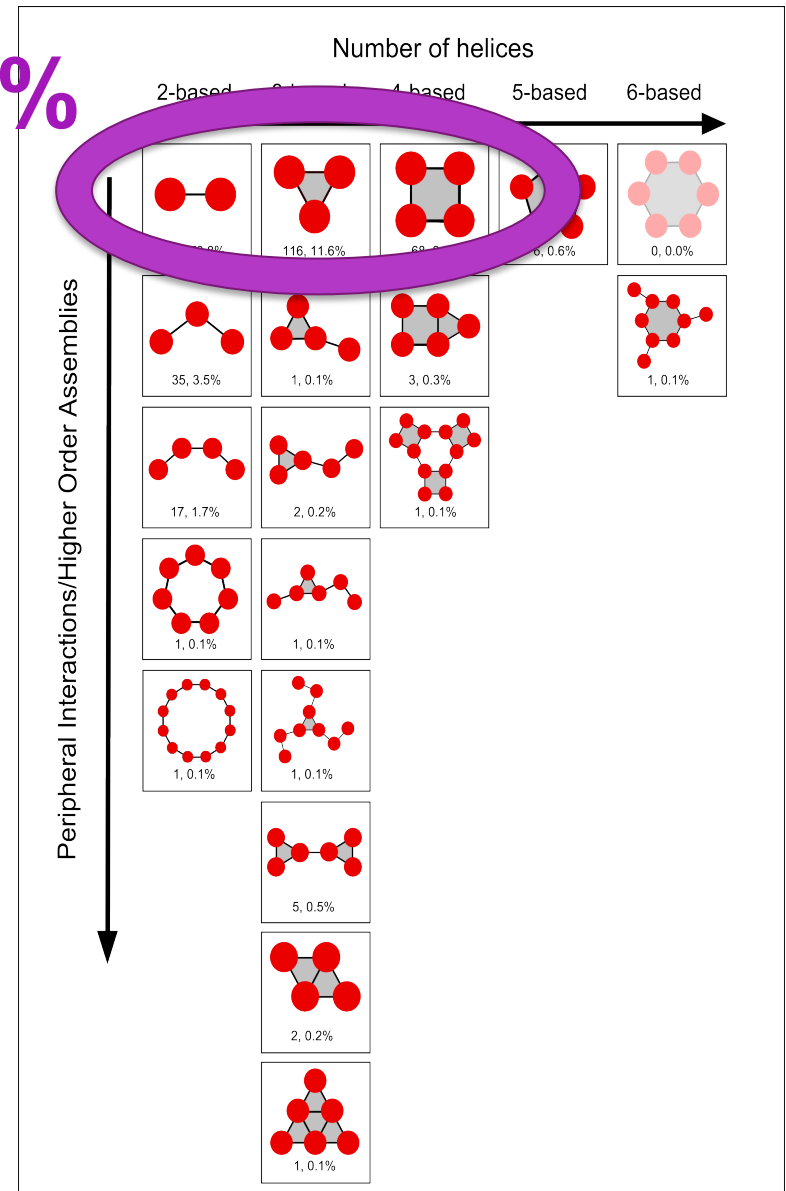


But it's more complicated



www.coiledcoils.chm.bris.ac.uk

90%

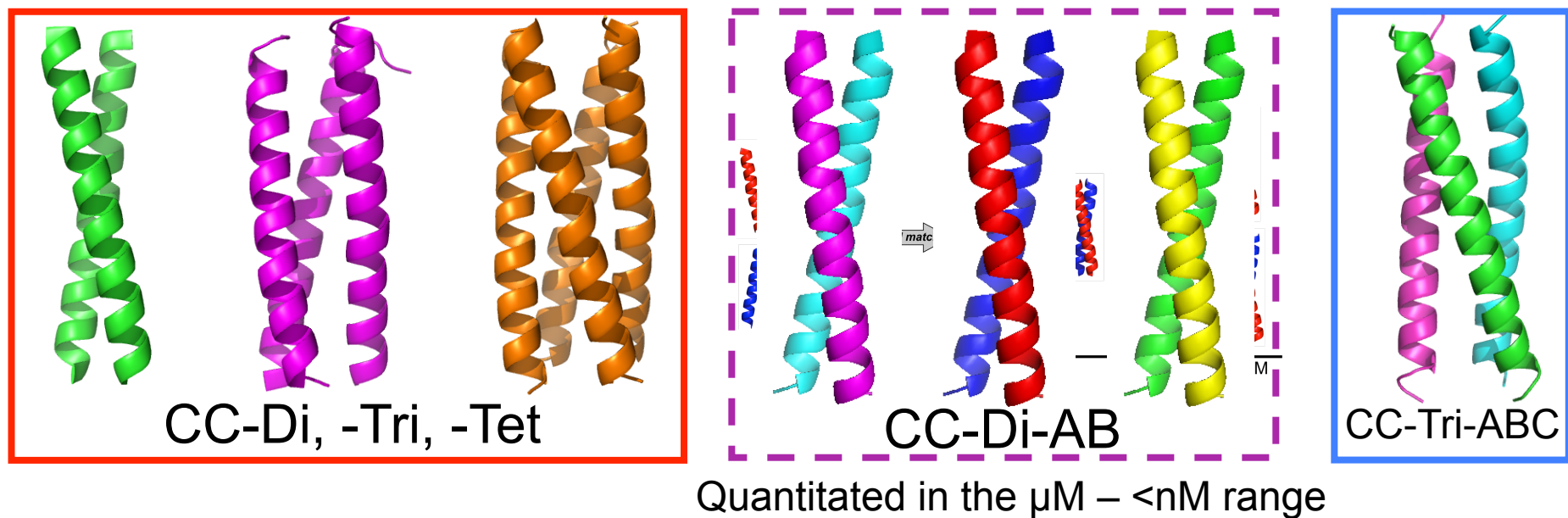


John Walshaw & Woolfson, *J Mol Biol* 307, 1427-1450 (2001)

Oli Testa *et al.* *Nucleic Acids Res* 37, 37, D315-D322 (2009)

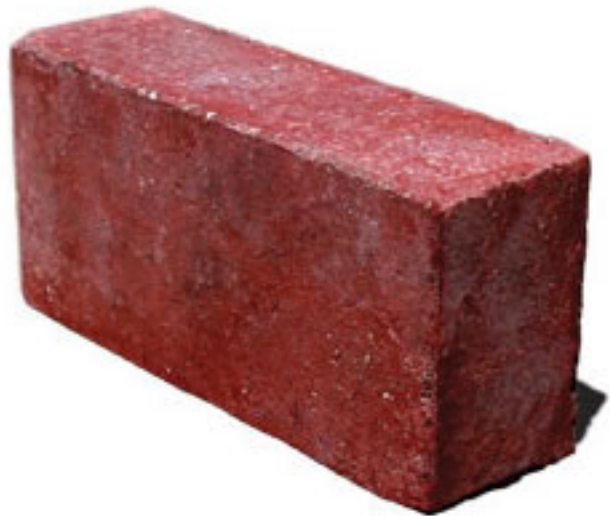
Fay Moutevelis & Woolfson *J Mol Biol* 385, 385, 726-732 (2009)

🌿 Our toolkit so far: protein assemblies without *biobaggage*

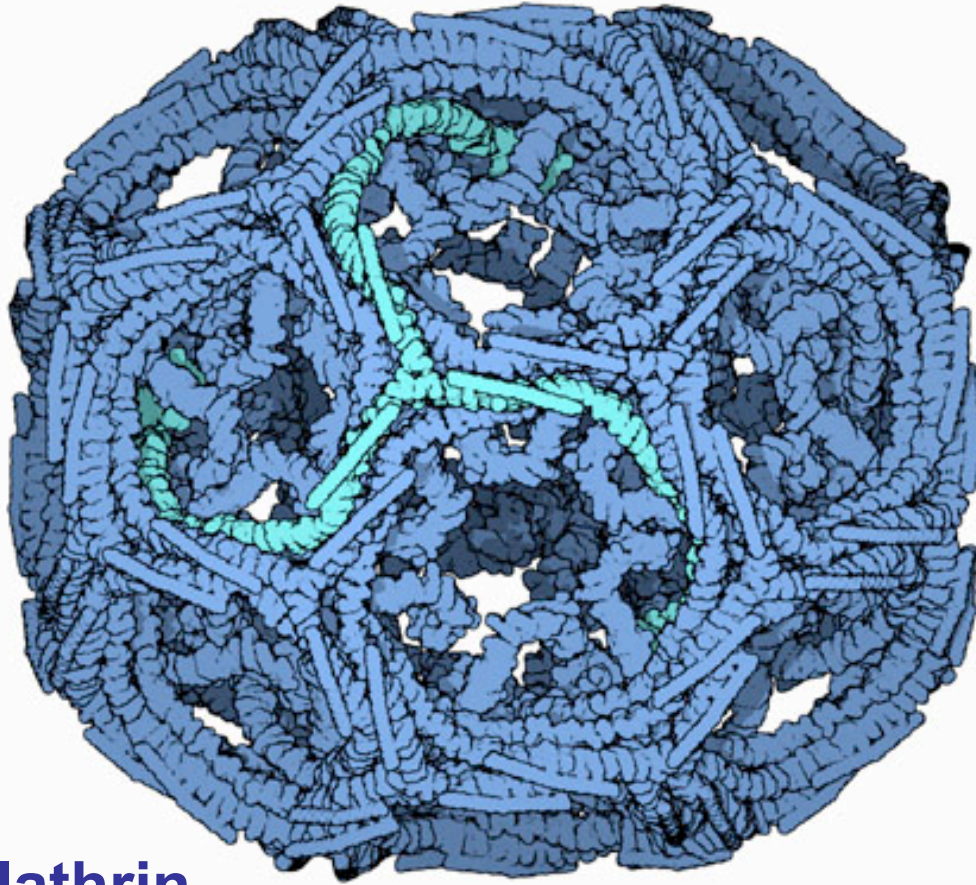


Jordan Fletcher *et al.* *ACS Synth Biol* (2012);
Beth Bromley *et al.* *J. Am. Chem. Soc.* **131**, 928 (2009);
Franziska Thomas *et al.*, *J. Am. Chem. Soc.* **135**, 5161 (2013).

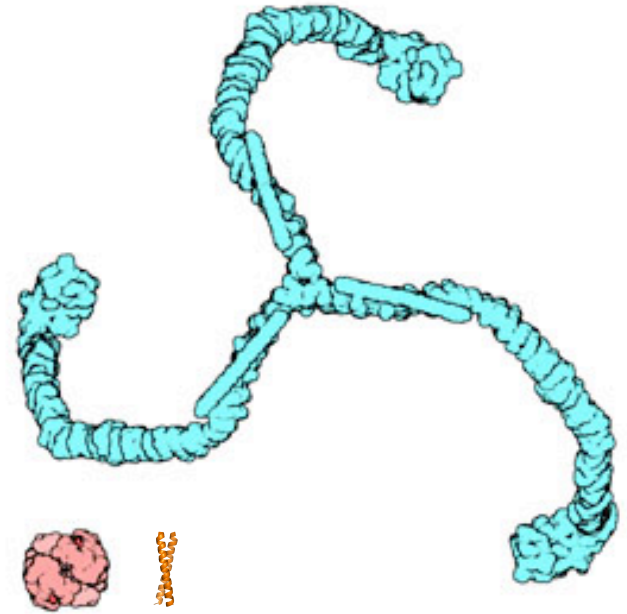
🔥 Building with the toolkit



🔥 Combining components: self-assembled peptide cages



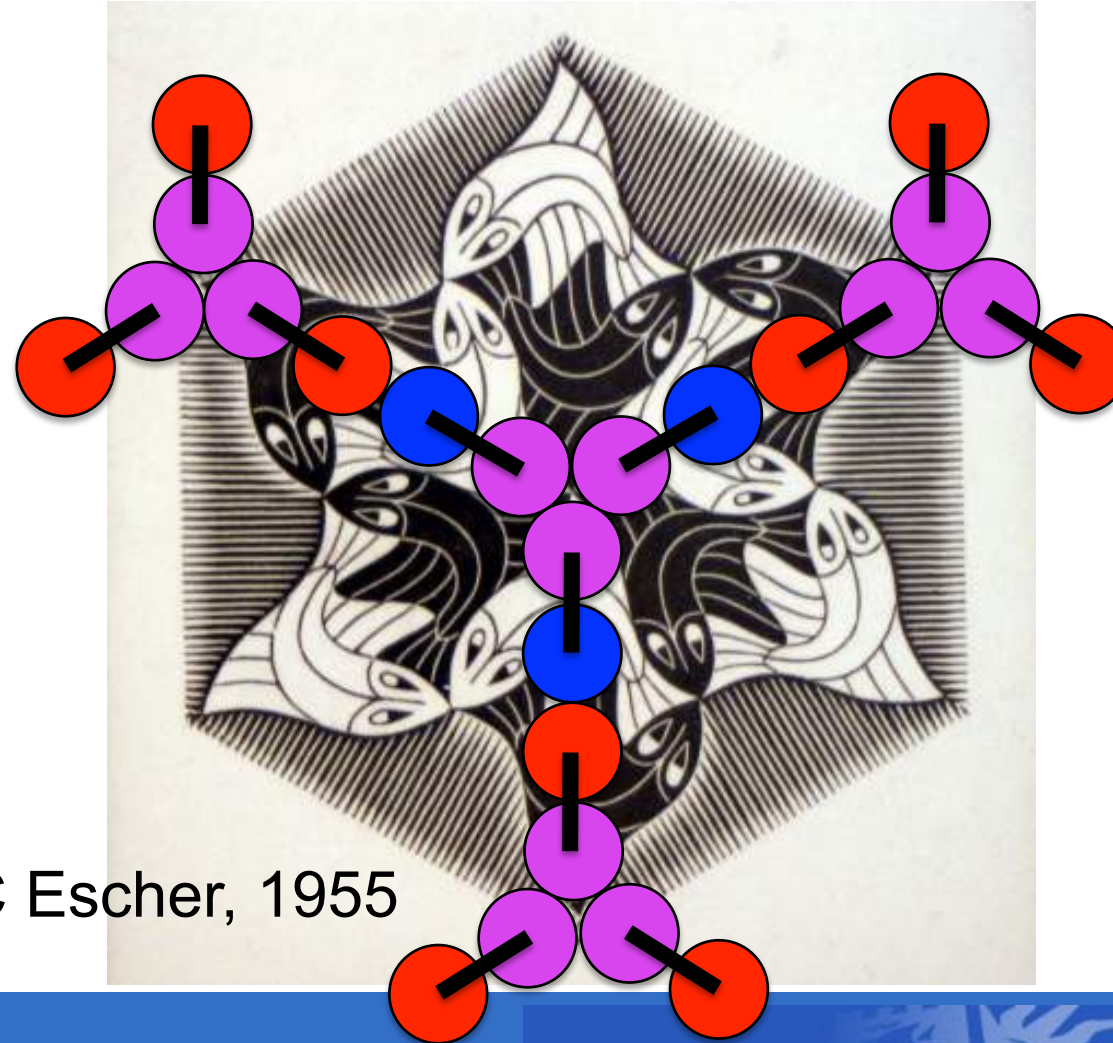
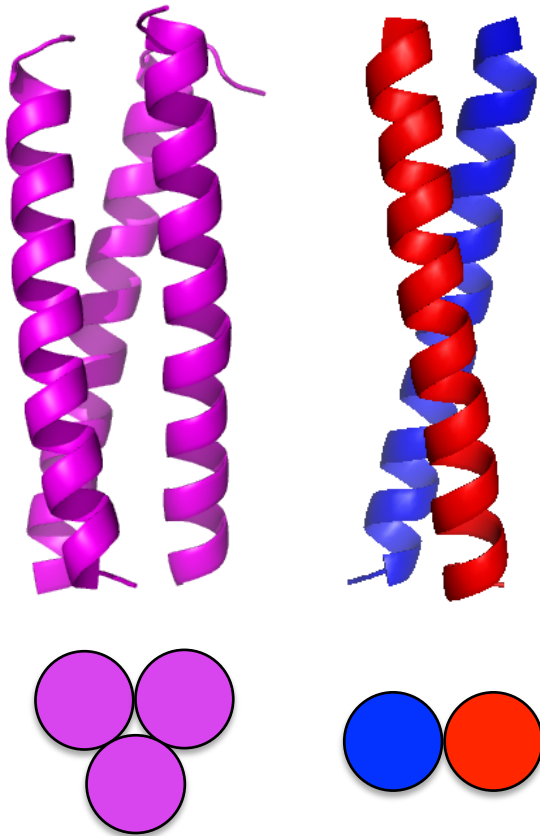
clathrin



haemoglobin

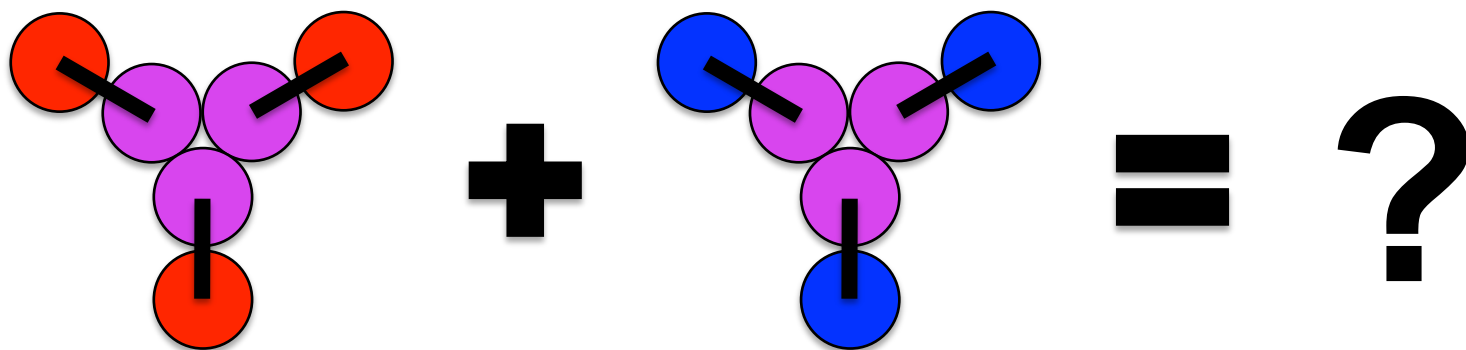
Graham T. Johnson and David S. Goodsell (Scripps)

Combining CC-Tri & CC-Di-*AB*; towards peptide-based vesicles

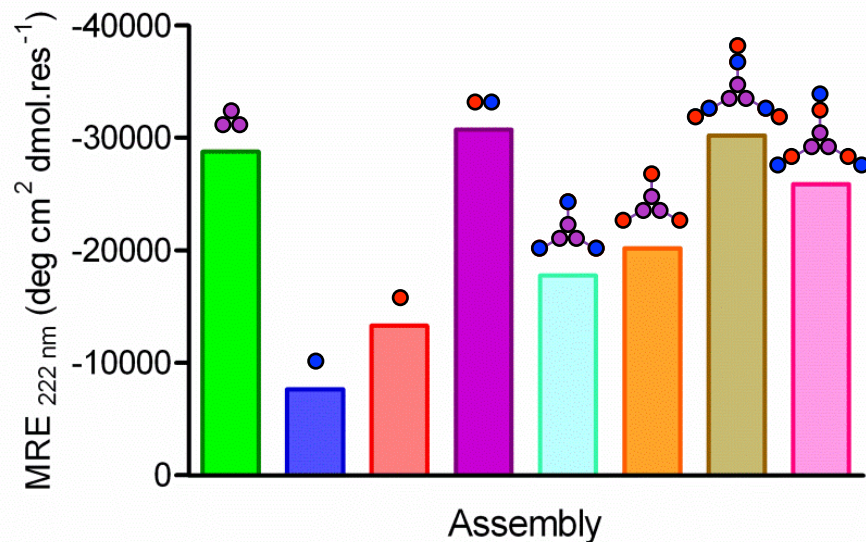


Jordan Fletcher *et al.* *Science* 340: 595-599 (2013)

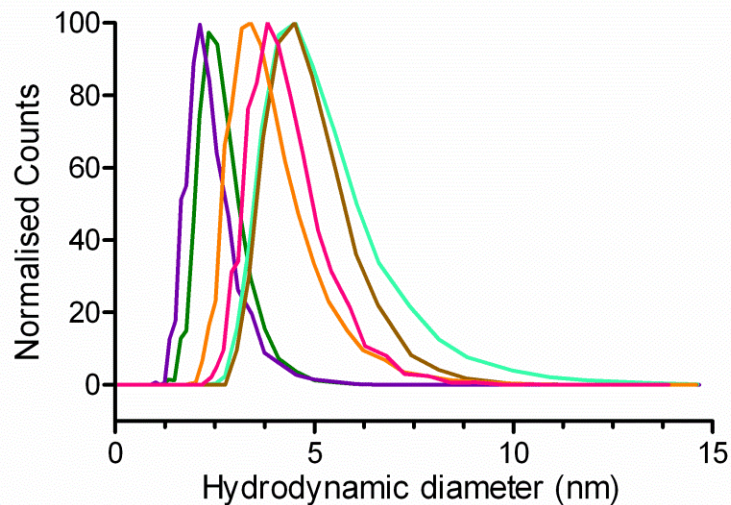
🌿 Making and characterizing the hubs; confirming modularity



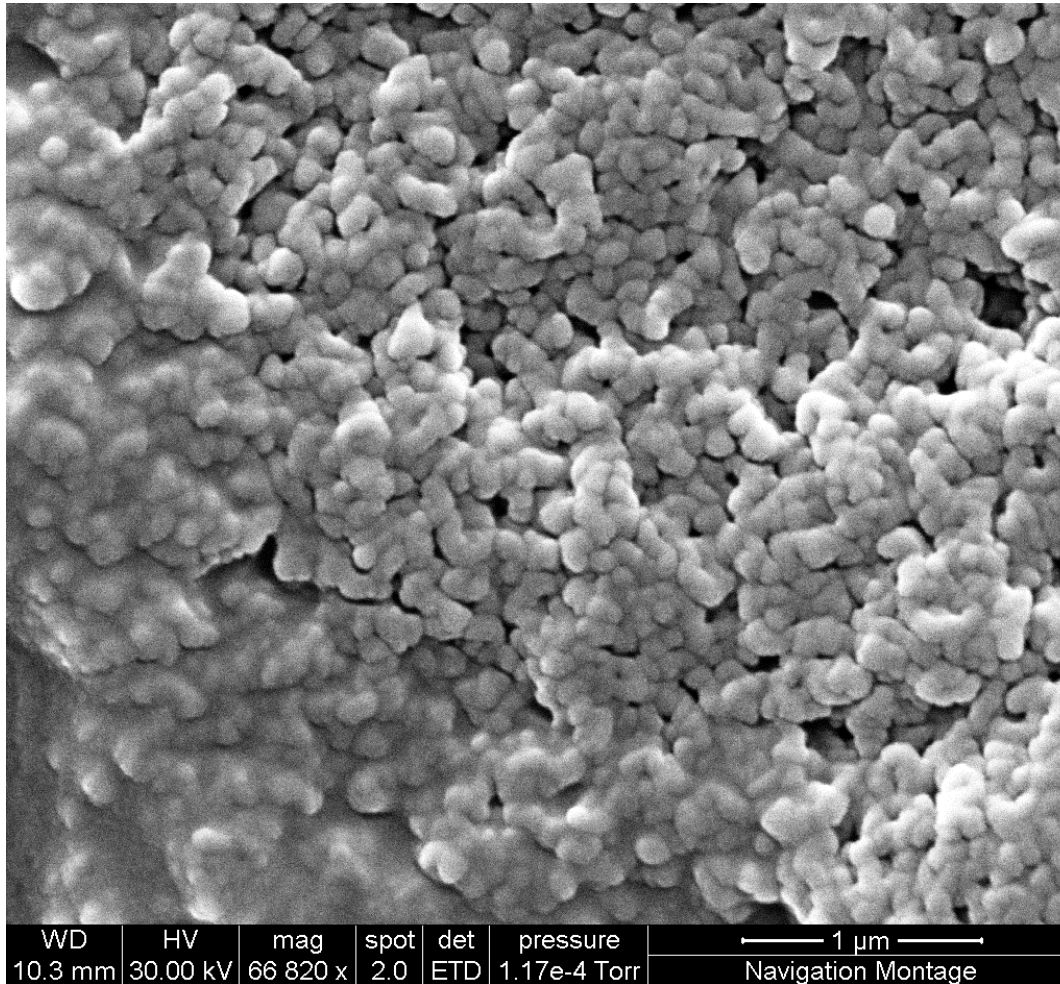
CD spectroscopy



Dynamic Light Scattering

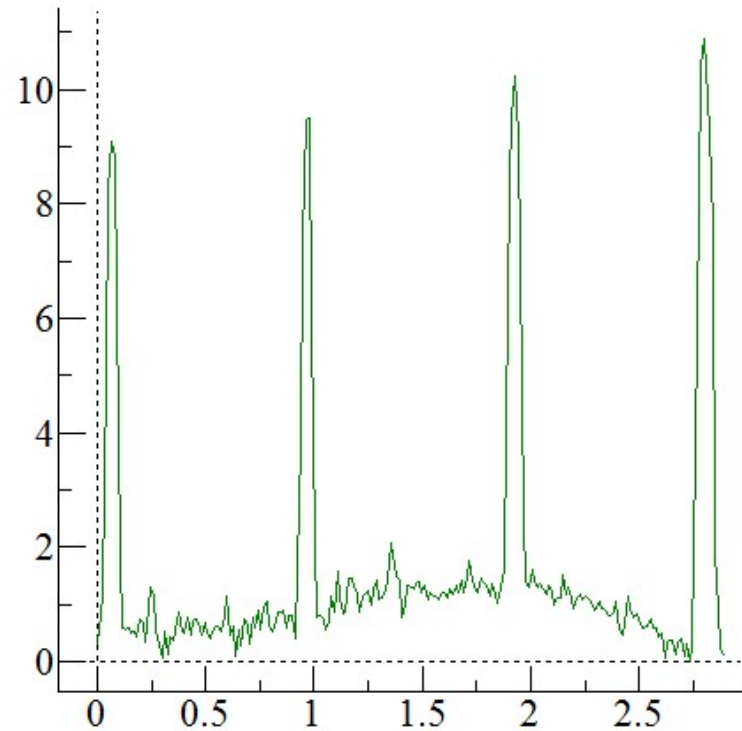
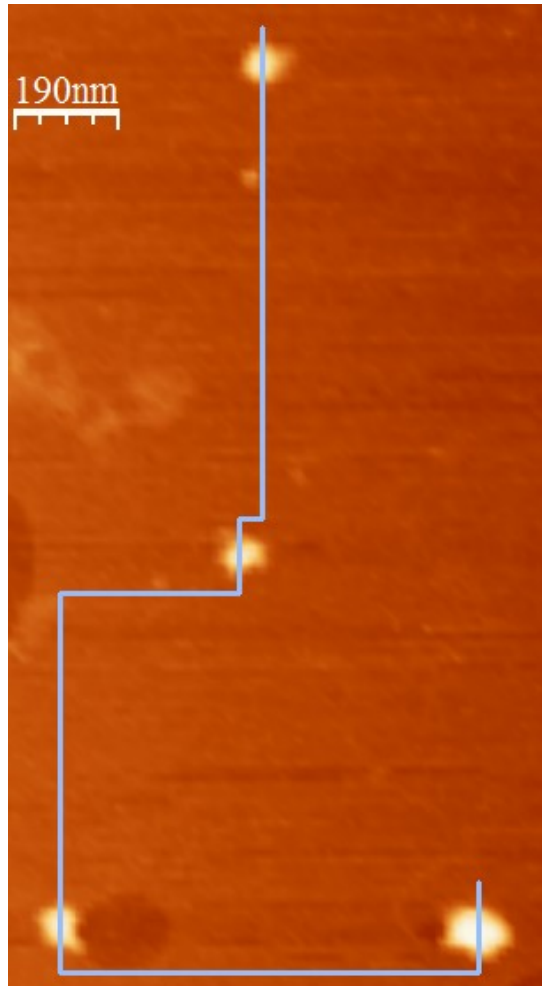


Scanning Electron Microscopy; mixing the hubs gives spheres



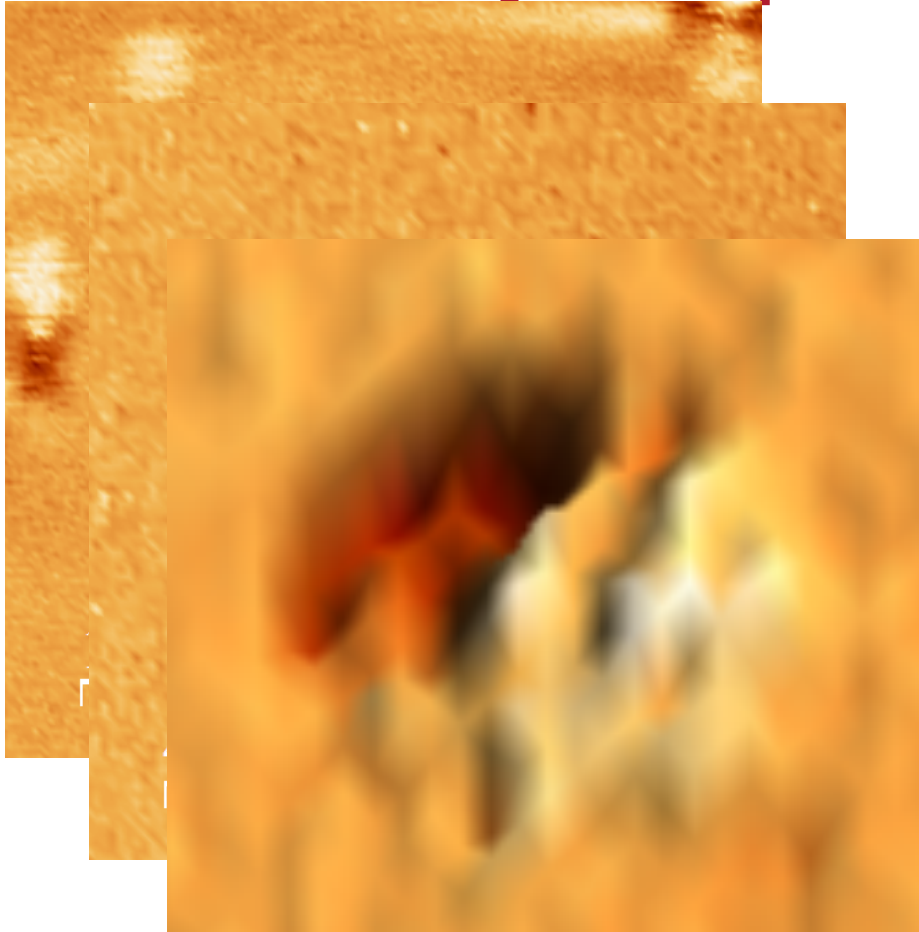
Thom Sharp, Judith Mantell and Jordan Fletcher

🌿 “Dry” Atomic Force Microscopy; unilamellar vesicles



Rob Harniman, Andy Collins and Jordan Fletcher

🌿 “Wet” Atomic Force Microscopy; not just spheres, but cages



- Instrument built in-house at NSQI
- Non-contact imaging with lateral molecular force microscopy
- Coupled to a scattered evanescent wave detection system

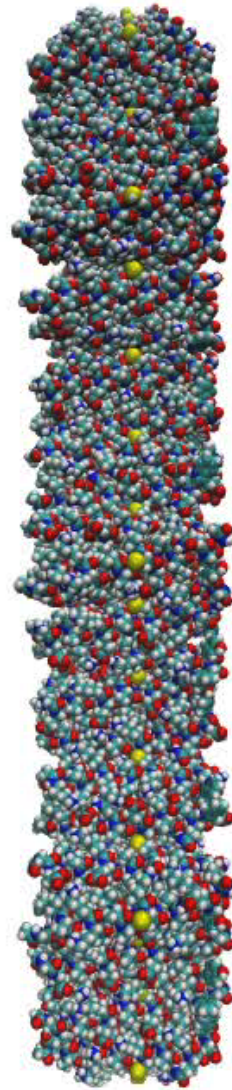
Why spheres?

Models and Molecular Dynamics

Richard Sessions, Noah Linden & DNW



19-Hexagon Model: Before MD



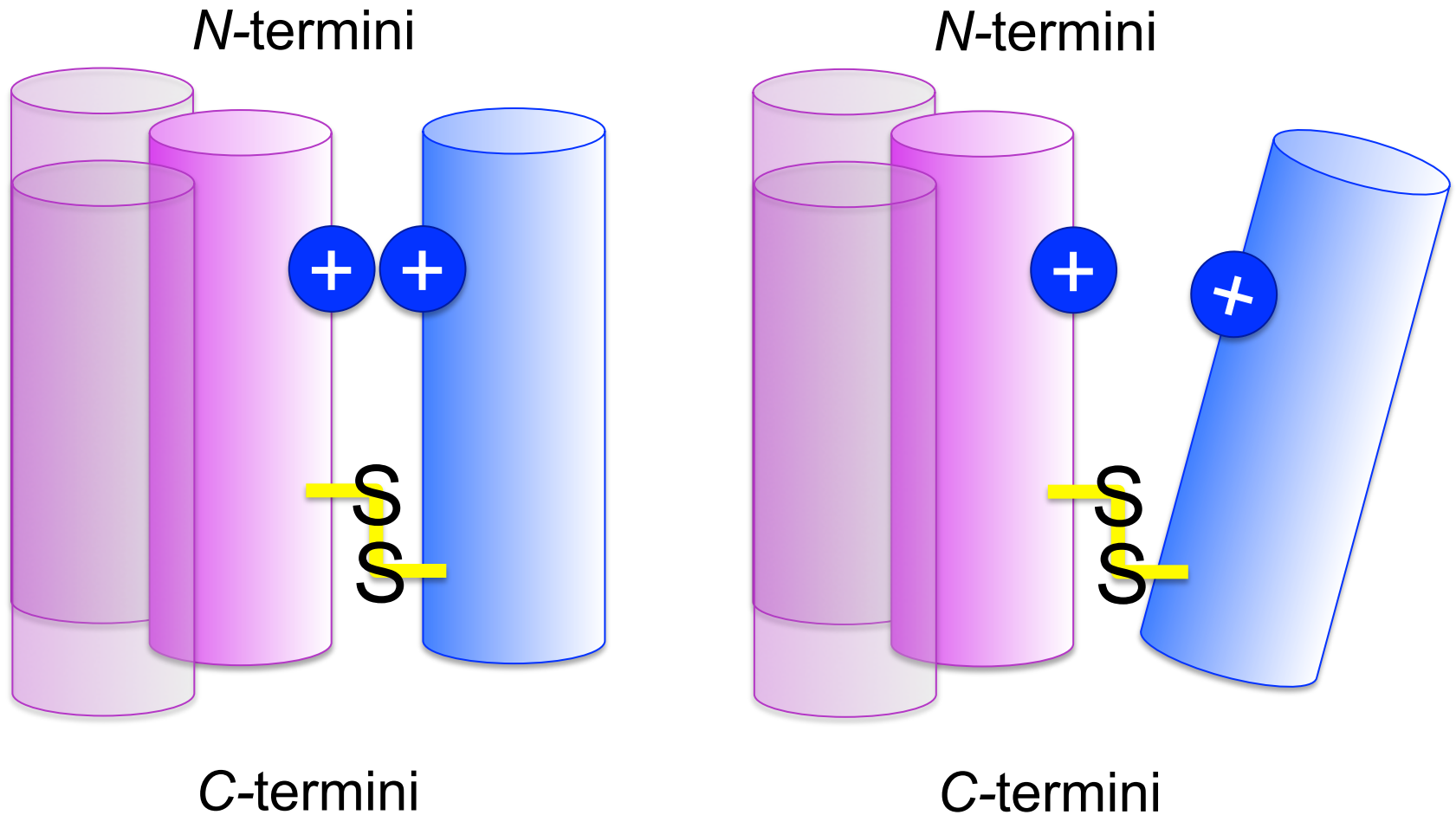
N-terminus (left)

C-terminus (right)

306 peptide chains; 2,440,958 atoms; water not shown

🔥 Why spheres?

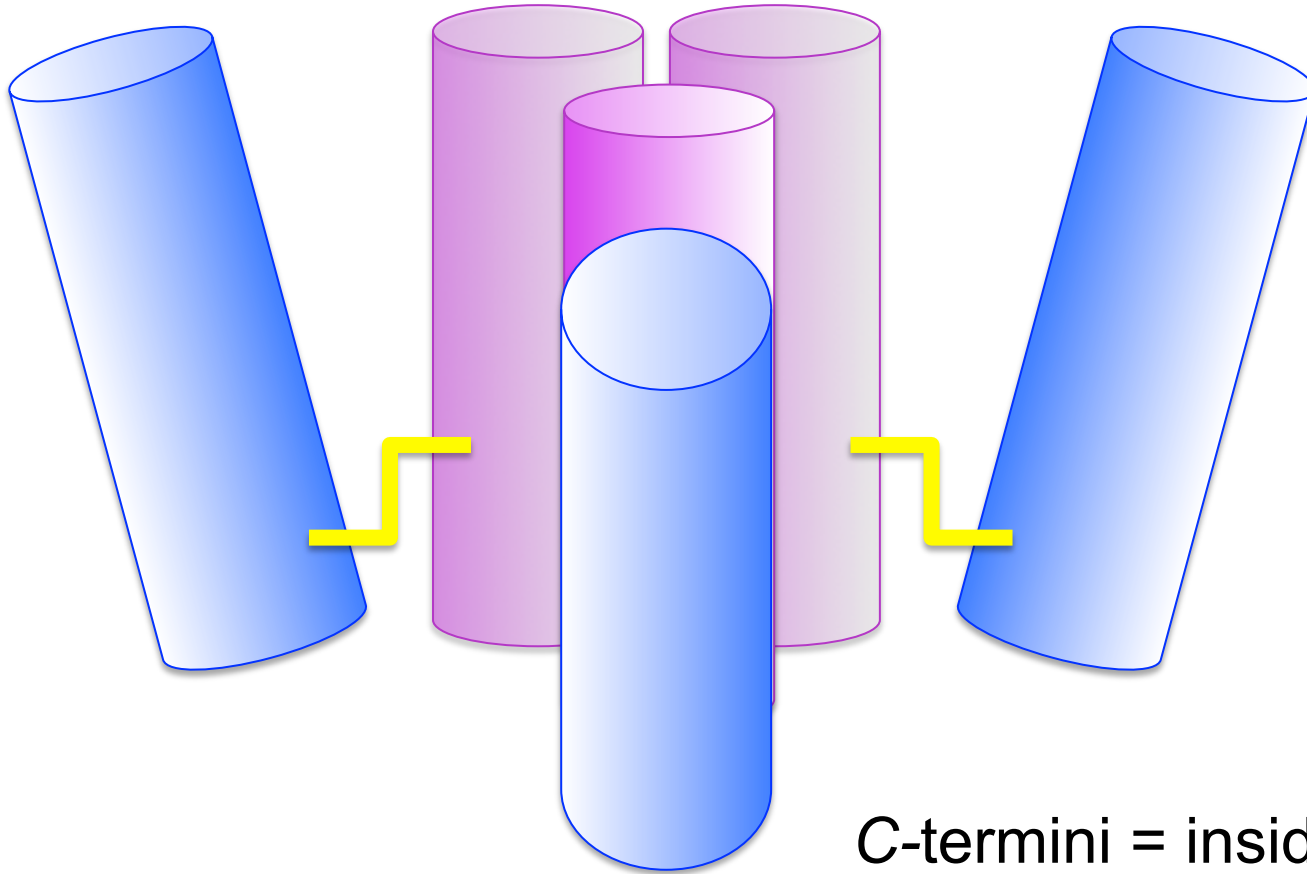
Inspecting the MD



🔥 Why spheres?

Inspecting the MD

N-termini = outside



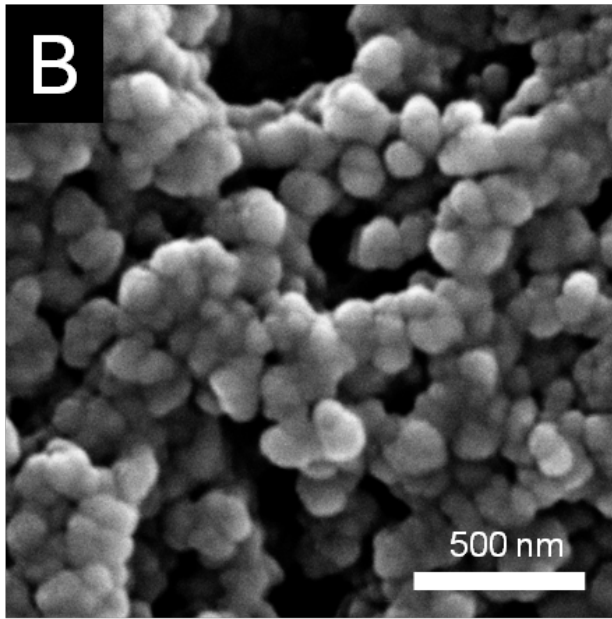
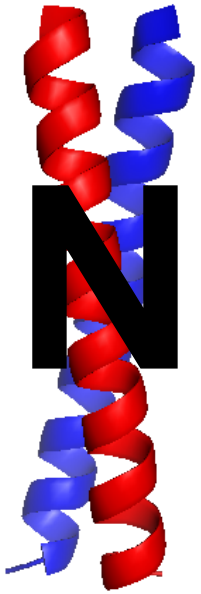
C-termini = inside

🌿 Why monodisperse spheres?

?

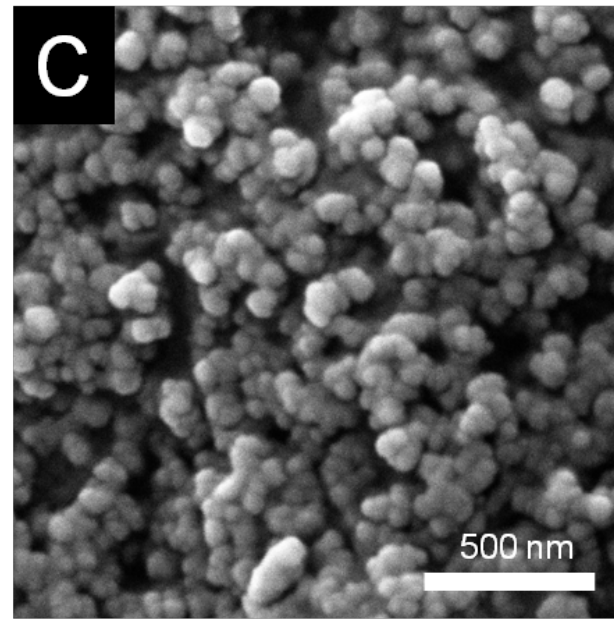


🔥 Rational peptide redesign; changing modules to control size

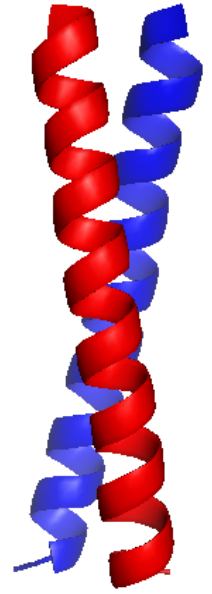


$K_D \sim \mu\text{M}$

$97 \pm 19 \text{ nm}$



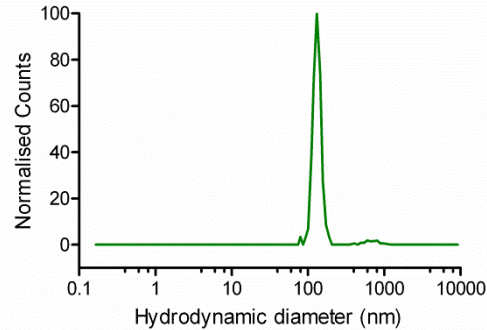
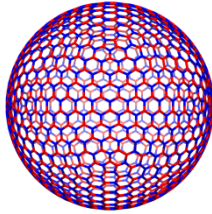
$68 \pm 12 \text{ nm}$



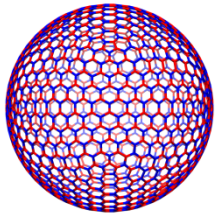
$K_D \sim \text{nM}$



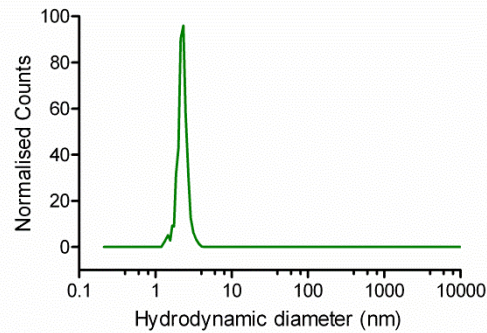
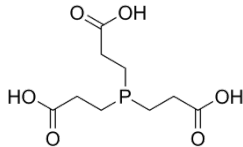
A disappearing act



132 ± 42 nm



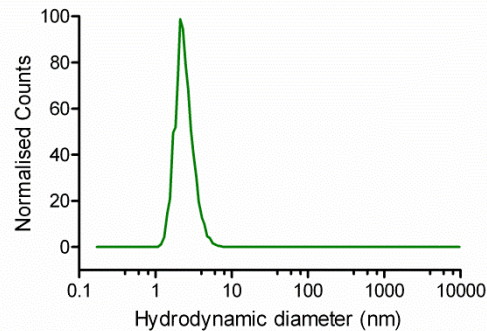
+



2.3 ± 0.9 nm

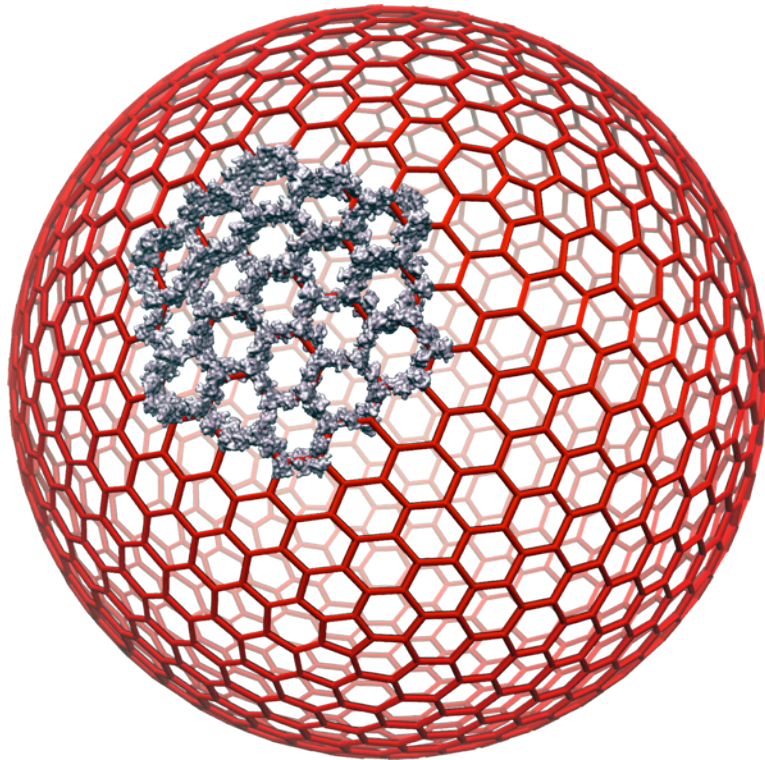


+



2.5 ± 0.6 nm

🔥 Where next for the SAGEs?



- Virus-like particles
– vaccine development
- Targeted delivery of bioactive molecules to cells
- Enzyme encapsulations and enzyme factories

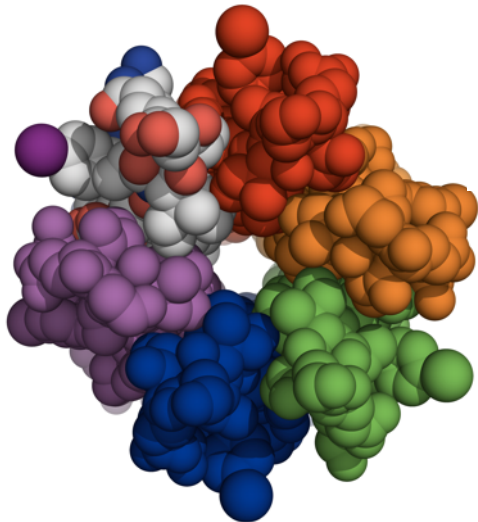
Summary



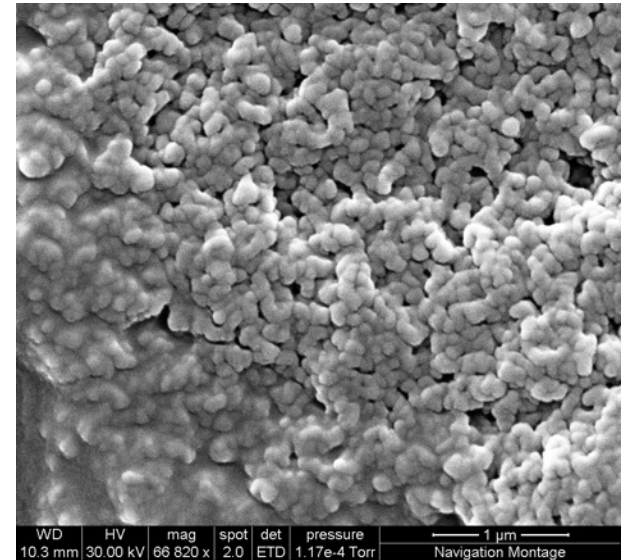
Collagen nucleation and tissue engineering materials
J Biol Chem 2011



A toolkit
ACS Synth Biol 2012



Unseen protein structures
Nature Chem Biol 2011



Encapsulation materials
Science 2013



The Woolfson Lab: Gail Bartlett



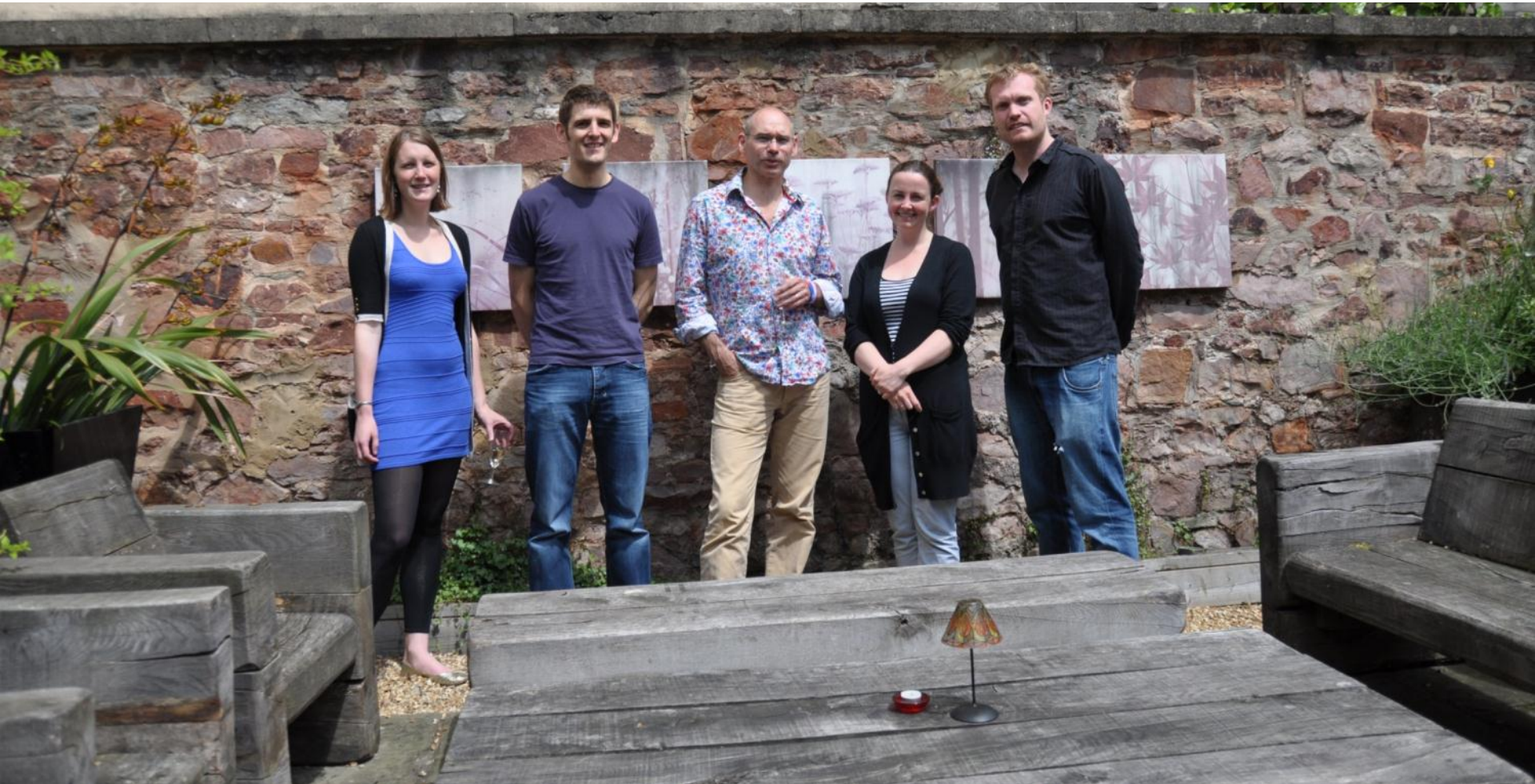
The Woolfson Lab: Drew Thomson



The Woolfson Lab: Jordan Fletcher



The Woolfson Lab: Aimee Boyle



The Woolfson Lab: Marc Bruning



BBSRC, EPSRC, HFSP, Leverhulme, RS-Wolfson

Massimo Antognozzi, Paula Booth, Leo Brady,
Noah Linden, Merv Miles, Richard Sessions & Paul Verkade



Craig Armstrong, Bertie Chi, Thom Sharp, Tom Vincent & Freddy Barnes, **Andy Collins, Rob Harniman, Judith Mantell, Nathan Zaccai**