National
Institute of Chemistry
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Design of topological proteins based on concatenated coiled-coil modules

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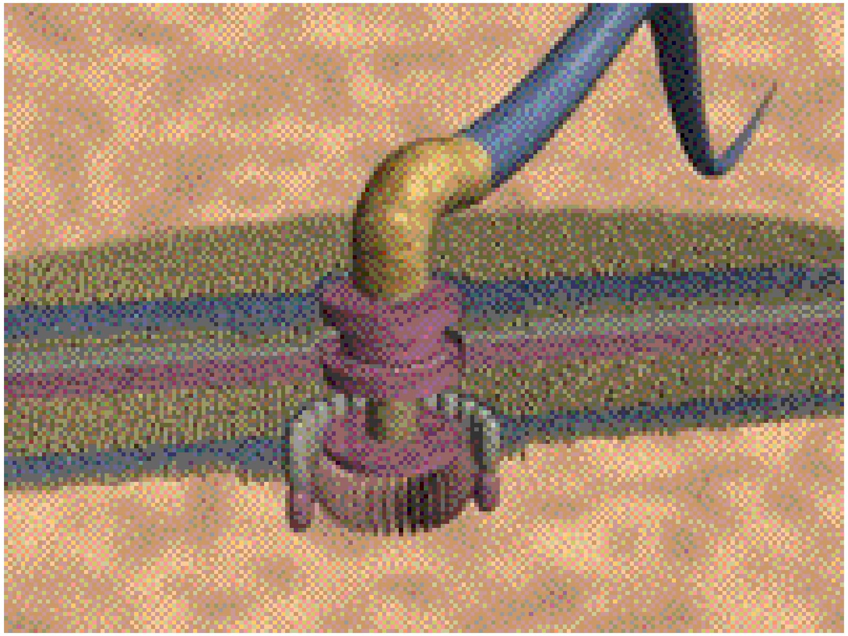
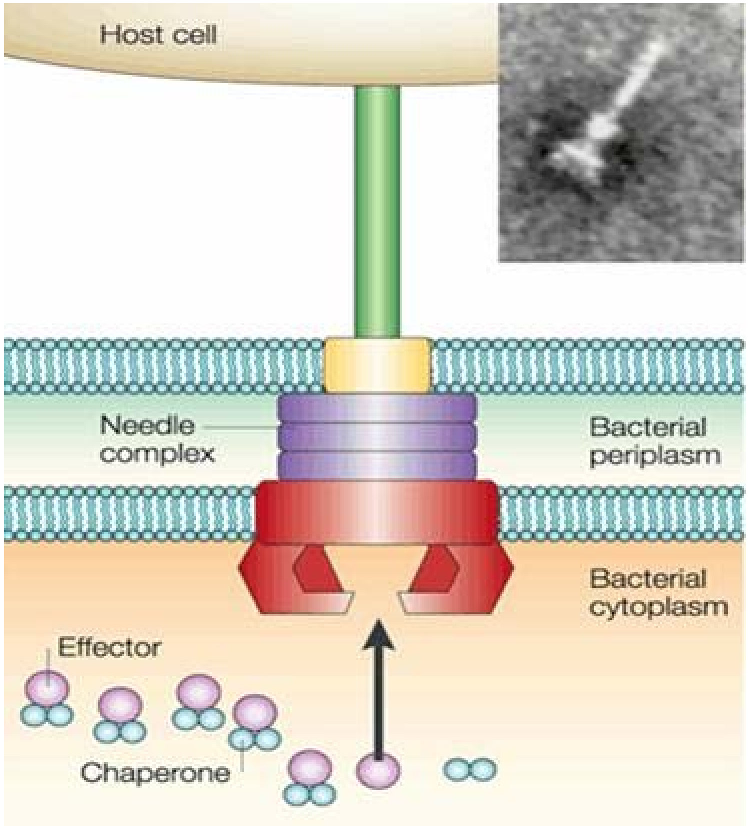
Topofolds

- Concept of modular topological polypeptide folds
- Designed single polypeptide chain tetrahedon
- DNA as the prototyping material to design the folding pathway

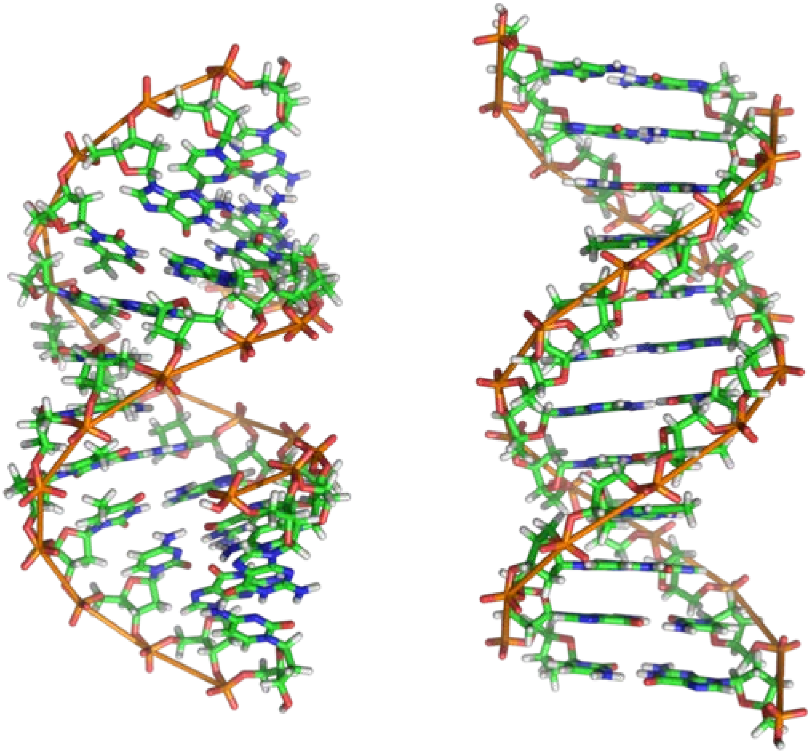
Natural molecular machines





http://www.arn.org/mm/mm.htm

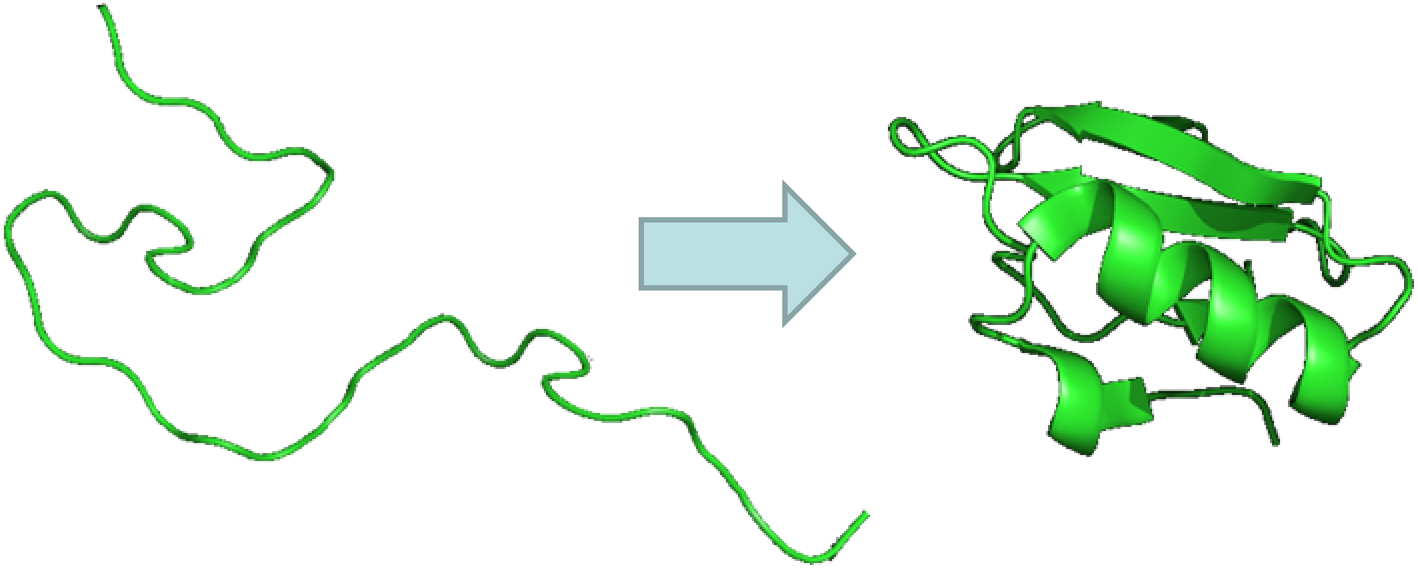
Natural biopolymers with designable structure



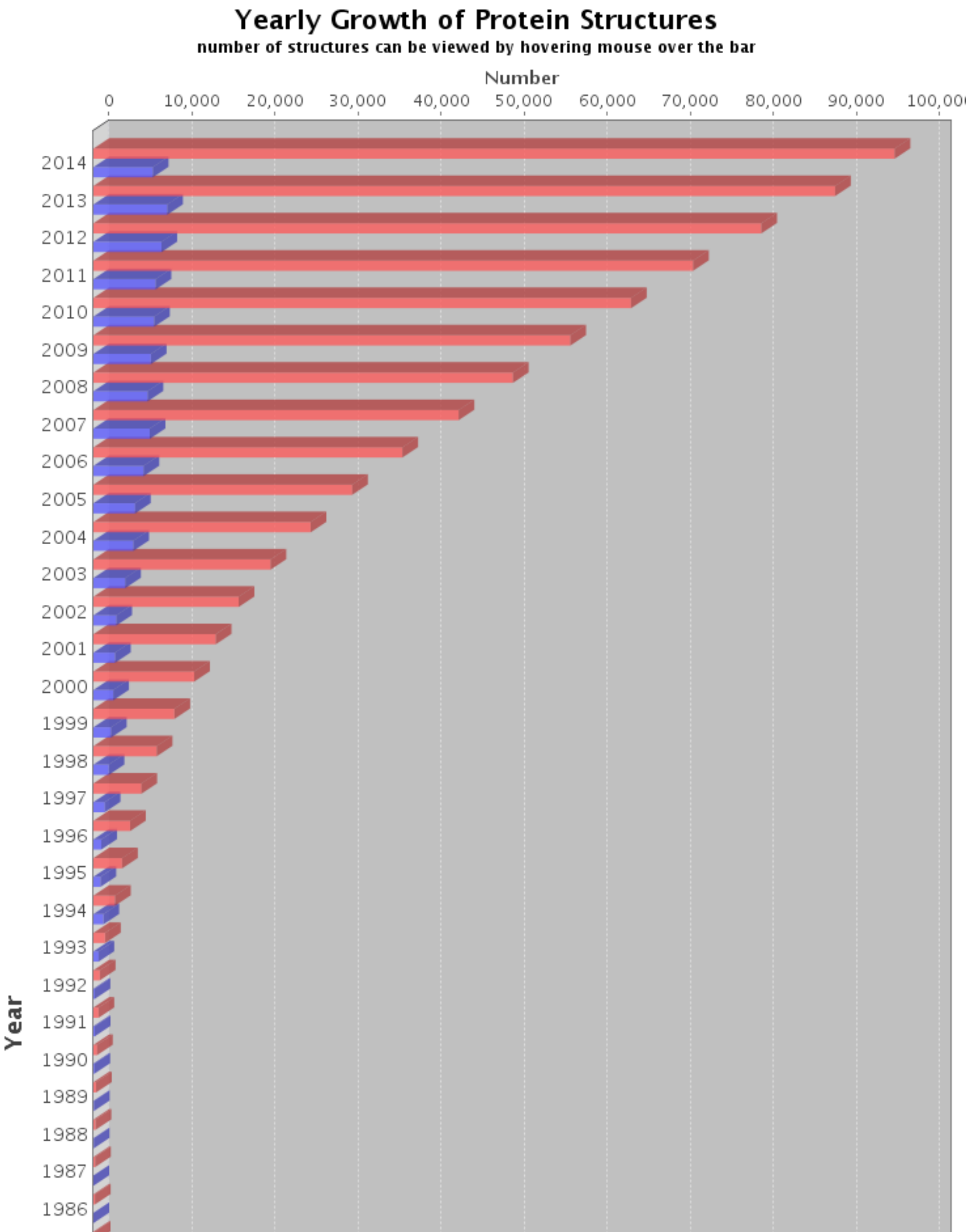
„To a first approximation
All nucleic acids are the same and
All proteins are diferent“.

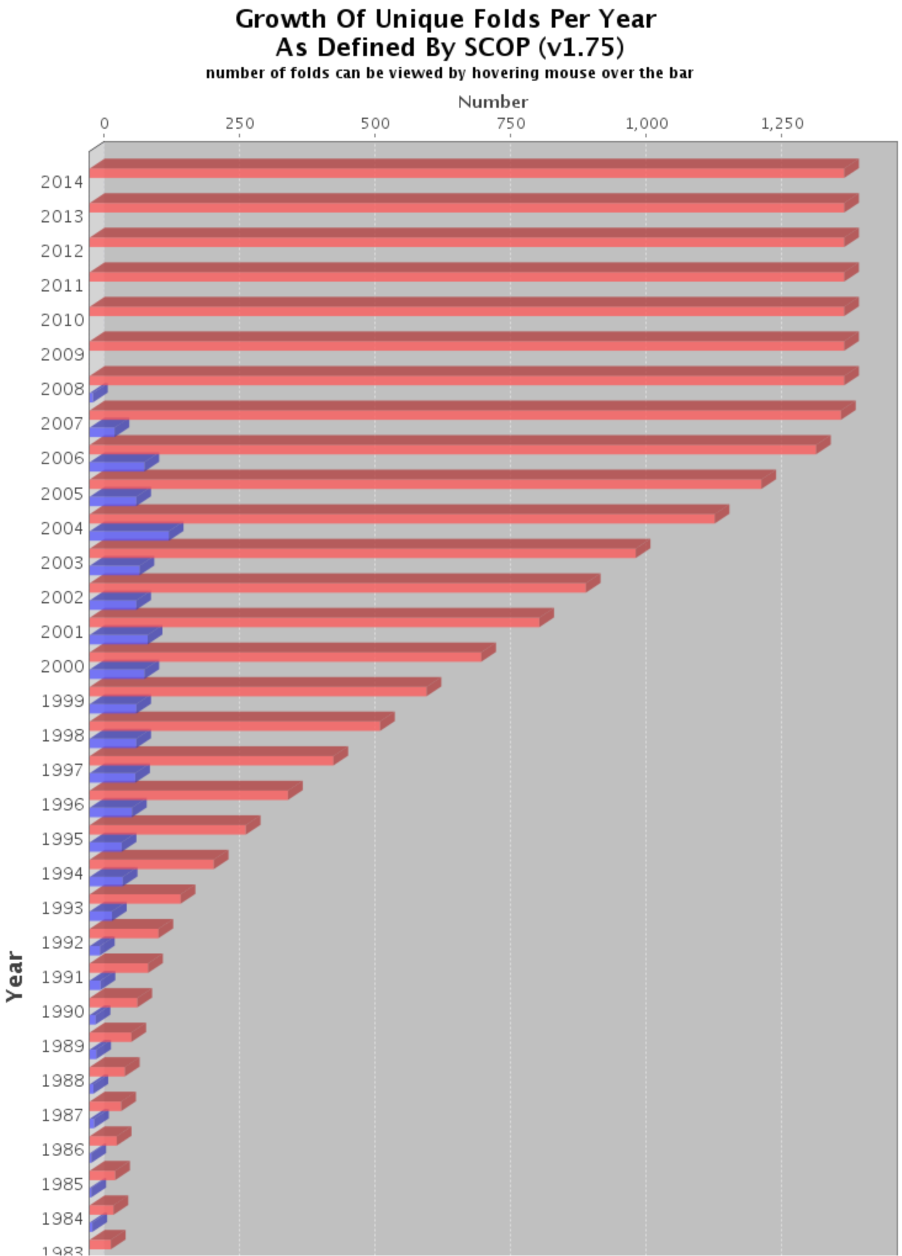
Biochemistry 101

Natural protein origami

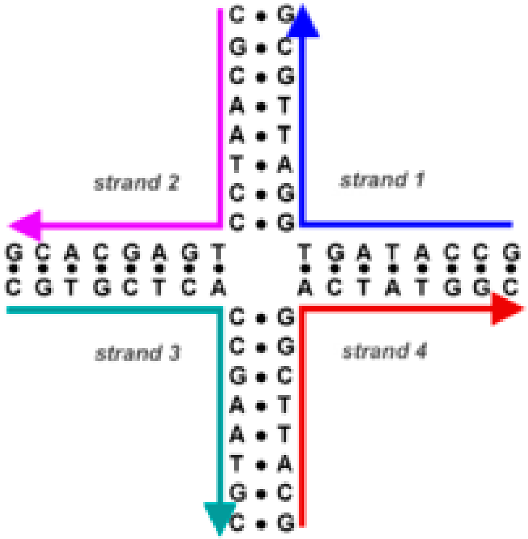


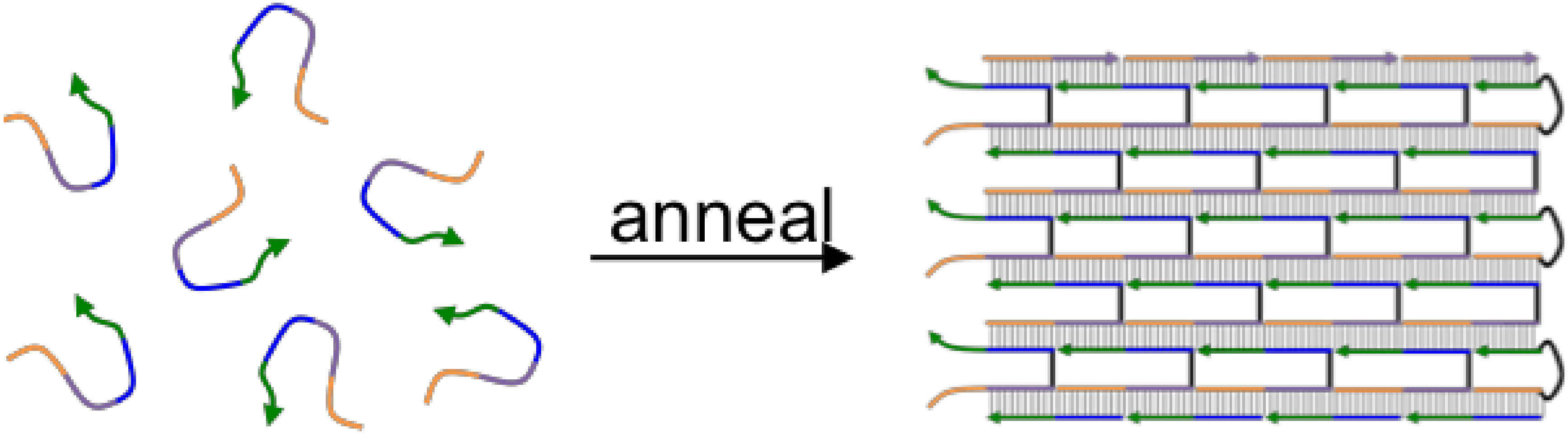
Natural protein folds



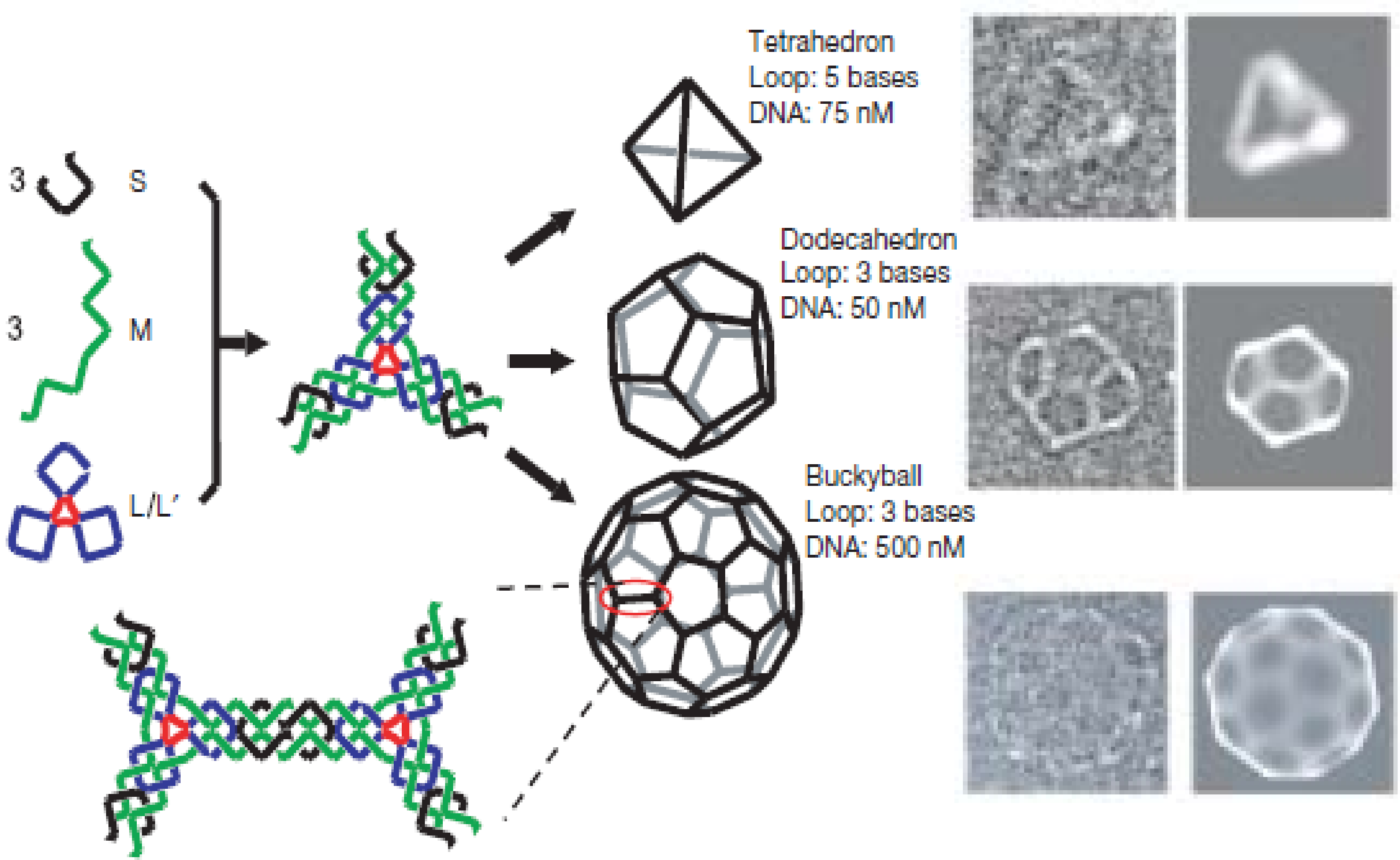


Long range modular interactions in designed DNA nanostructures





Designed DNA nanostructures



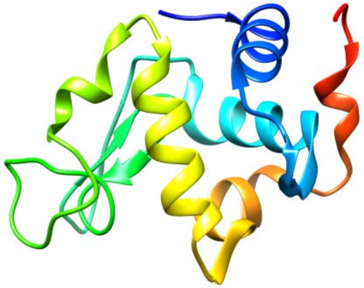
He et al., Nature, 2008

Evolved and designed bionanostructures

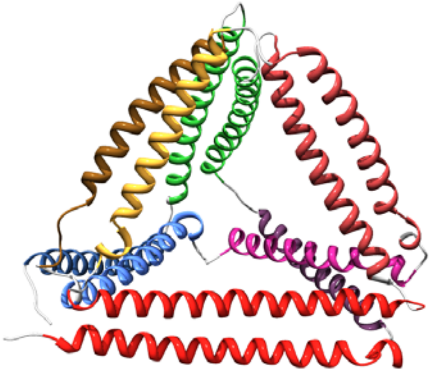
DNA

Protein

Evolved compact fold



Modular fold



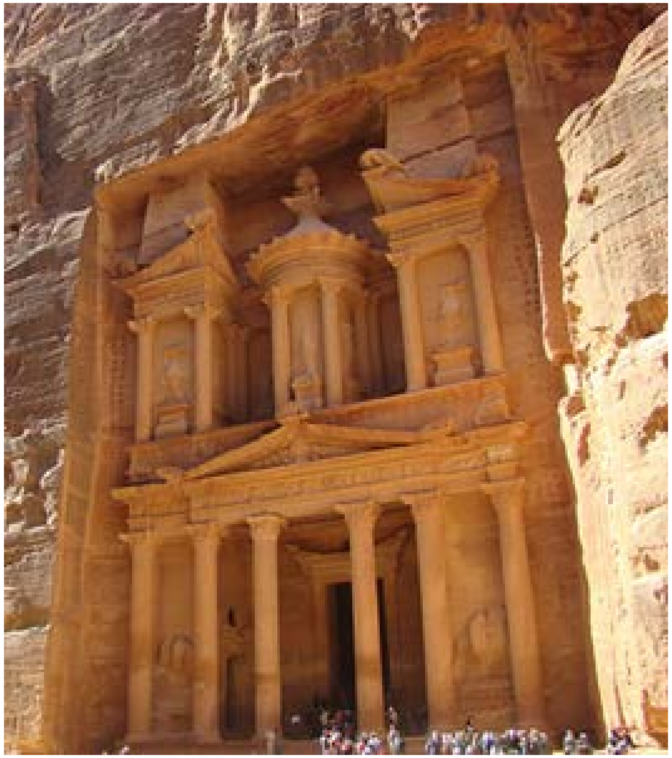
Nucleic acids and polypeptides as building blocks

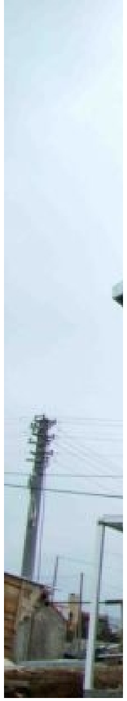
DNA	**proteins**
contains 4 nucleotides with similar properties -	contains 20 AA with different chemical properties +
can fold into defined 3D structures +/-	can fold into defined 3D structures +
used to store information in nature -	builds structures and functional devices in nature +
prepared mainly by synthesis +/-	produced by cell factories at low cost +
easy to program (W-C base pairs) +	structure-encoding information is complex -

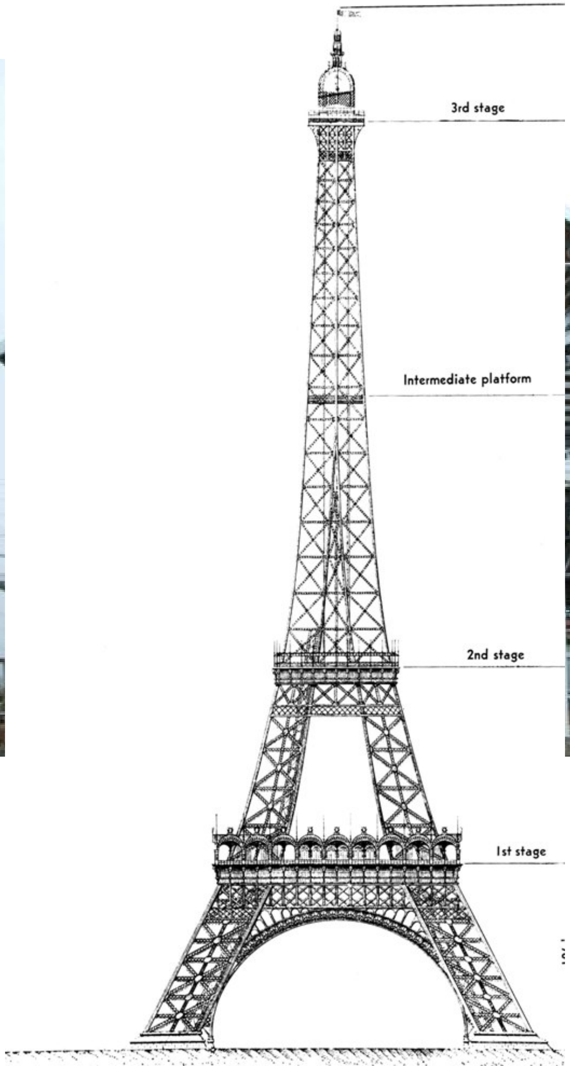
Construction of buildings

Artisanal – ad hoc, unique

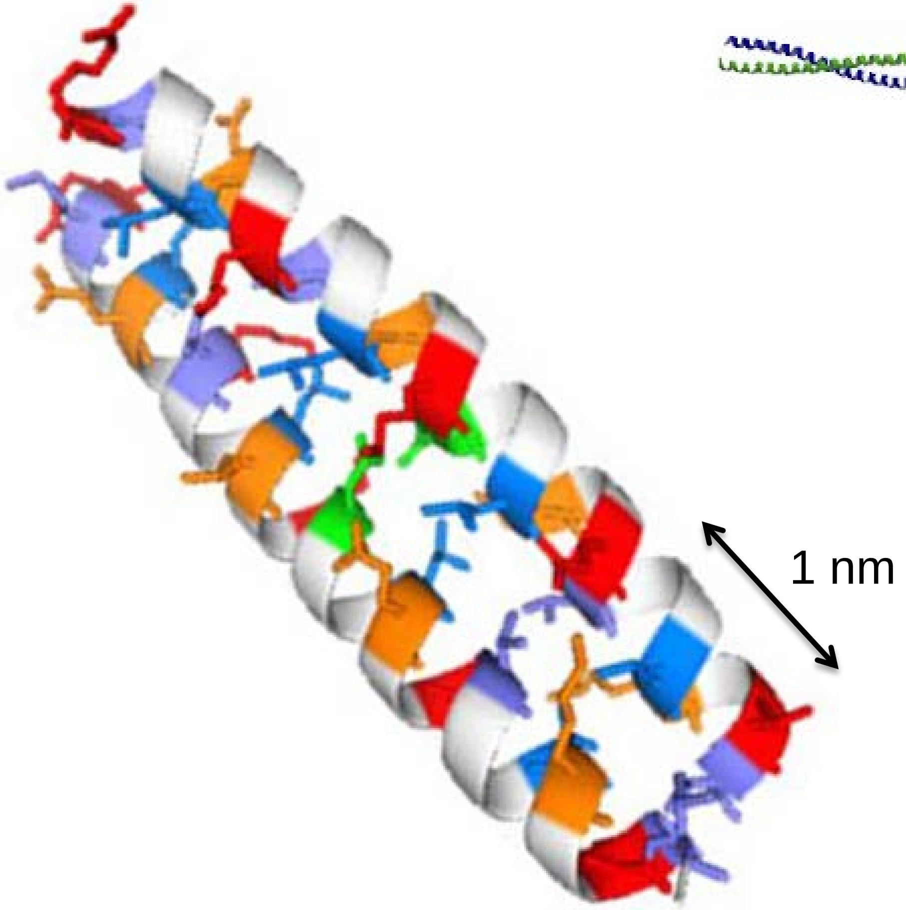
Engineered - modular



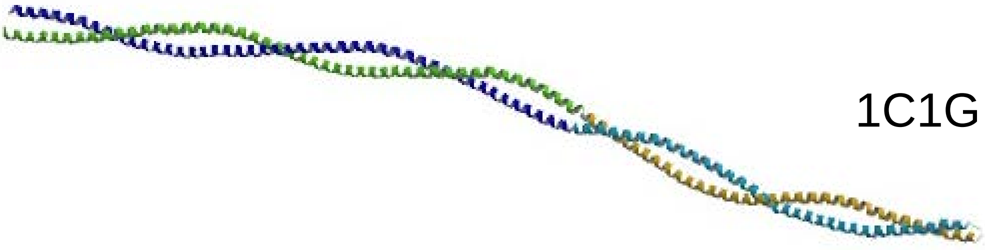




Coiled-coils as building blocks



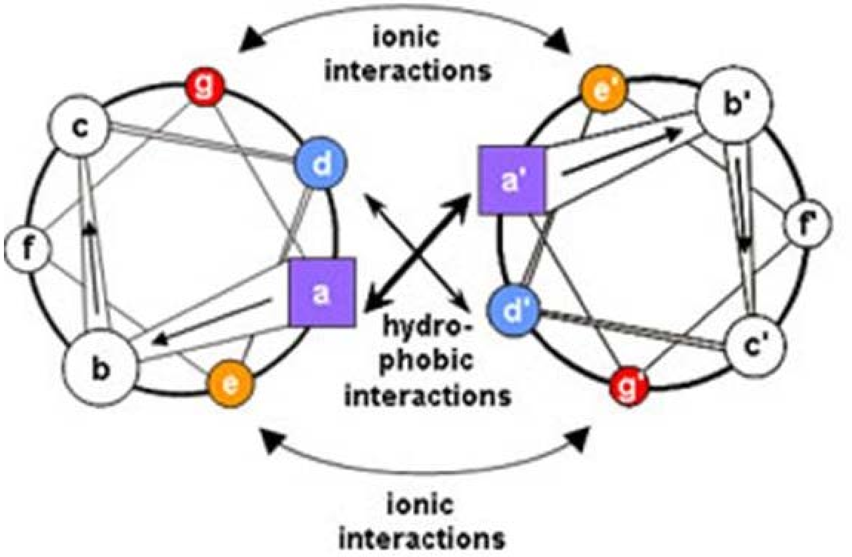
Length: 3 - > 50 nm (hundreds of residues)



Coiled-coil design rules

➢ We used the principles governing the selectivity and stability of CC segments to **design and experimentally test** a set of peptides

Heptad repeat-specific pattern



Stabilization

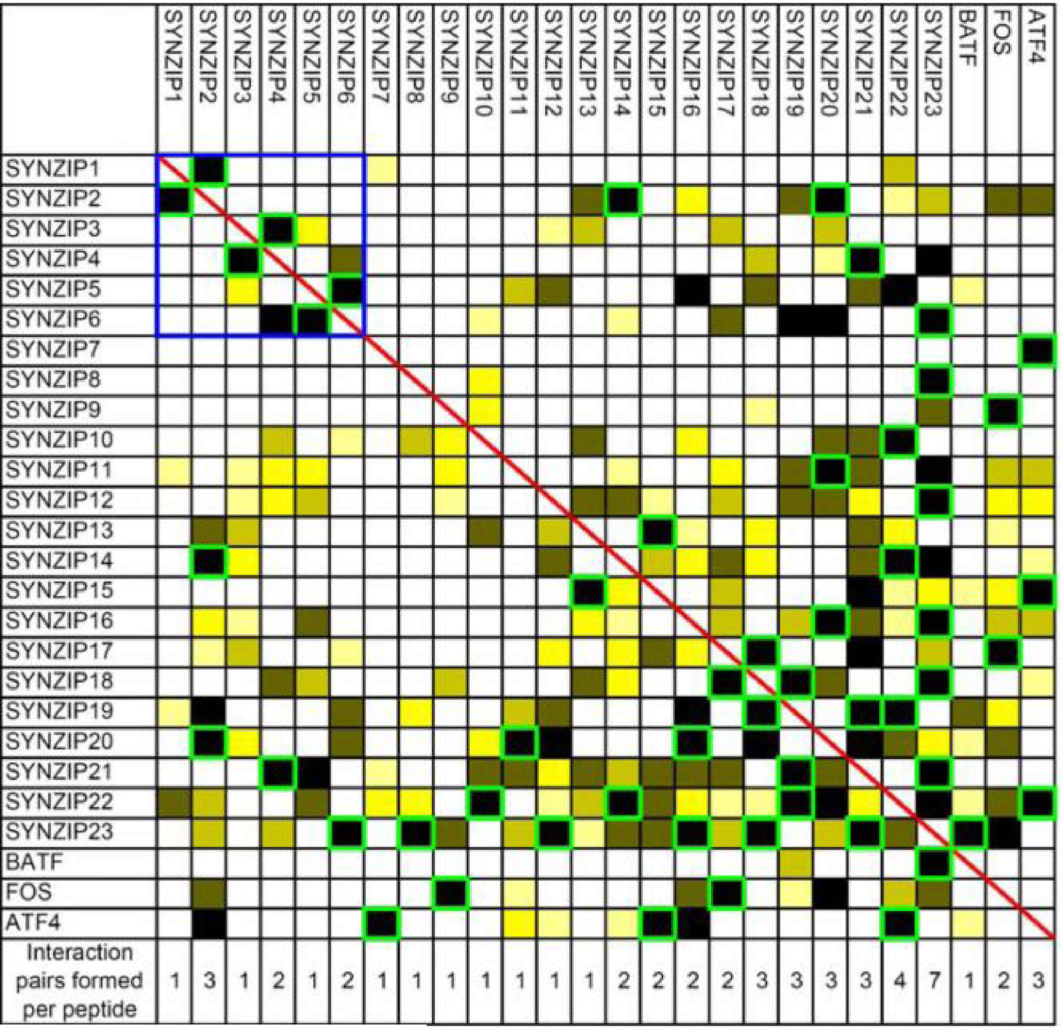
- hydrophobic residues at positions ***a*** and ***d***
- opposite charged residues at positions ***e*** and ***g***

Destabilization

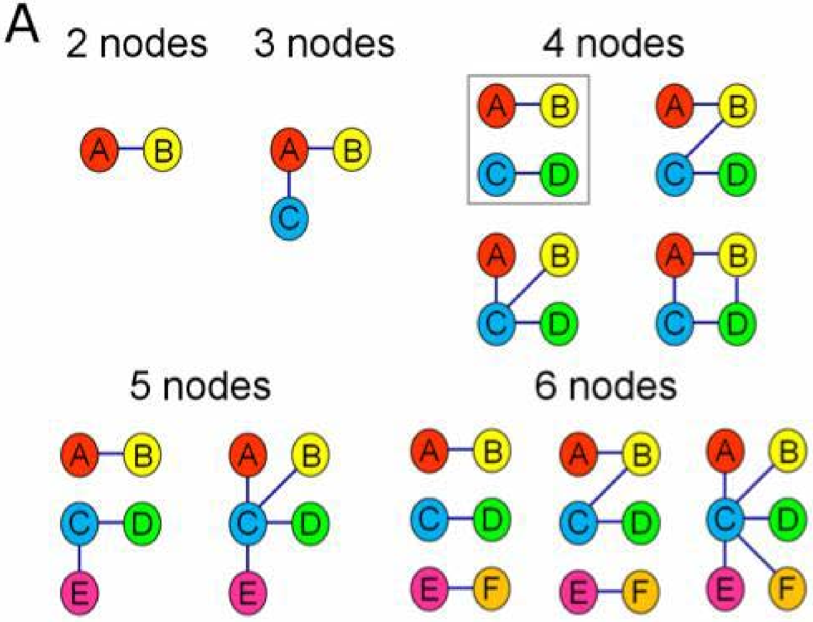
- Negative design motif based on burial of polar Asn residues
- maximize the difference between designed (target) and unwanted combinations of residues

- positions ***b*, *c*** and ***f*** can be chemically modified to introduce desired function into the coiled-coil assembly

Orthogonality of the native coiled-coil dimers

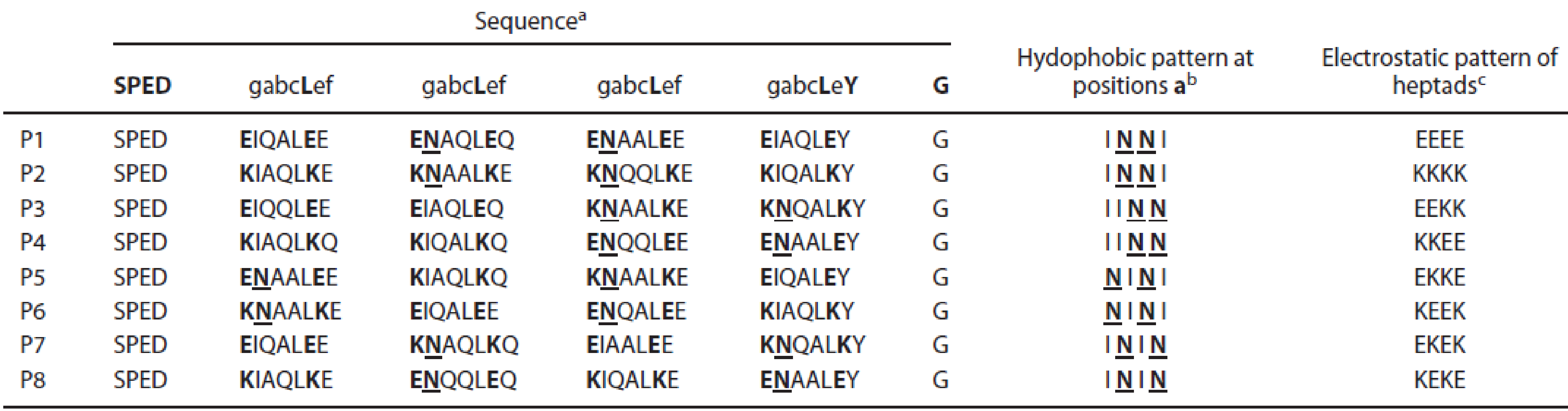


Analysis of pairwise interactions between 55 natural bZIP CC segments

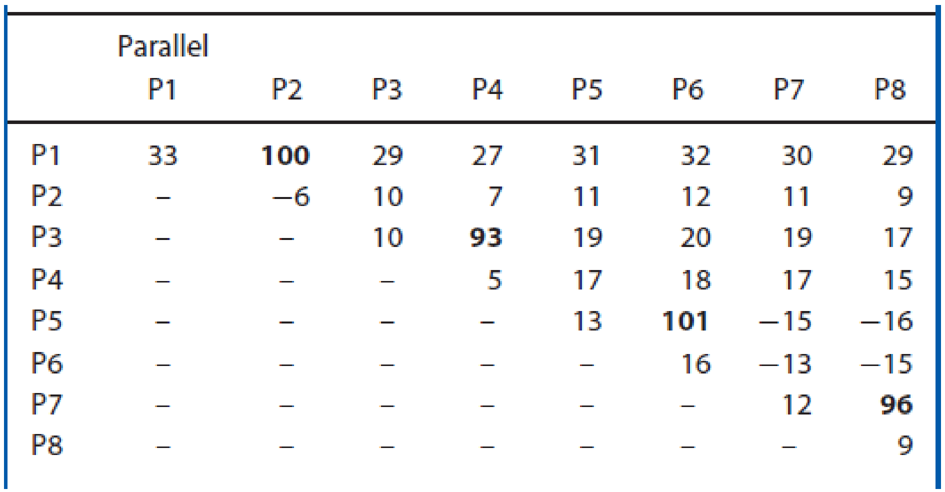


Reinke et al. (Keating lab), JACS 2011

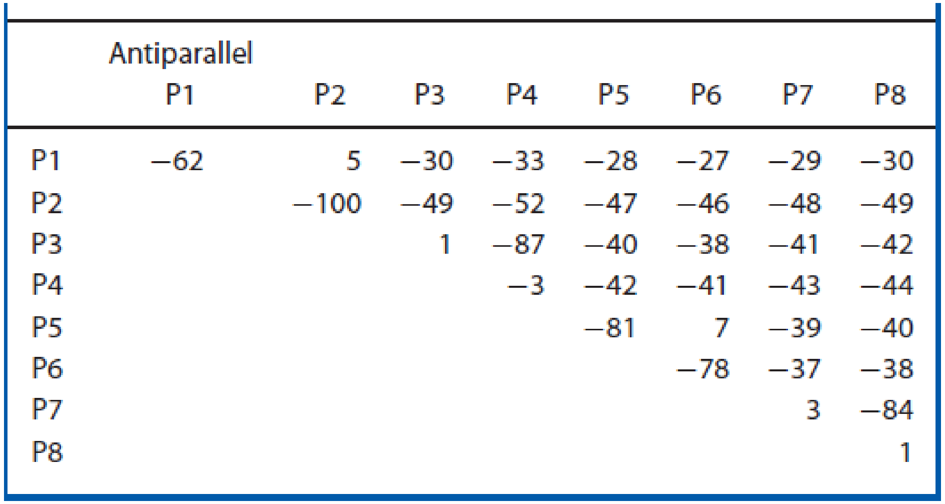
Design of orthogonal coiled-coil dimers



	Sequence[a]						Hydophobic pattern at positions **a**[b]	Electrostatic pattern of heptads[c]
	SPED	gabc**L**ef	gabc**L**ef	gabc**L**ef	gabc**L**e**Y**	**G**		
P1	SPED	**E**IQAL**E**E	**EN**AQL**E**Q	**EN**AAL**E**E	**E**IAQL**E**Y	G	I **N** **N** I	EEEE
P2	SPED	**K**IAQL**K**E	**KN**AAL**K**E	**KN**QQL**K**E	**K**IQAL**K**Y	G	I **N** **N** I	KKKK
P3	SPED	**E**IQQL**E**E	**E**IAQL**E**Q	**KN**AAL**K**E	**KN**QAL**K**Y	G	I I **N** **N**	EEKK
P4	SPED	**K**IAQL**K**Q	**K**IQAL**K**Q	**EN**QQL**E**E	**EN**AAL**E**Y	G	I I **N** **N**	KKEE
P5	SPED	**EN**AAL**E**E	**K**IAQL**K**Q	**KN**AAL**K**E	**E**IQAL**E**Y	G	**N** I **N** I	EKKE
P6	SPED	**KN**AAL**K**E	**E**IQAL**E**E	**EN**QAL**E**E	**K**IAQL**K**Y	G	**N** I **N** I	KEEK
P7	SPED	**E**IQAL**E**E	**KN**AQL**K**Q	**E**IAAL**E**E	**KN**QAL**K**Y	G	I **N** I **N**	EKEK
P8	SPED	**K**IAQL**K**E	**EN**QQL**E**Q	**K**IQAL**K**E	**EN**AAL**E**Y	G	I **N** I **N**	KEKE



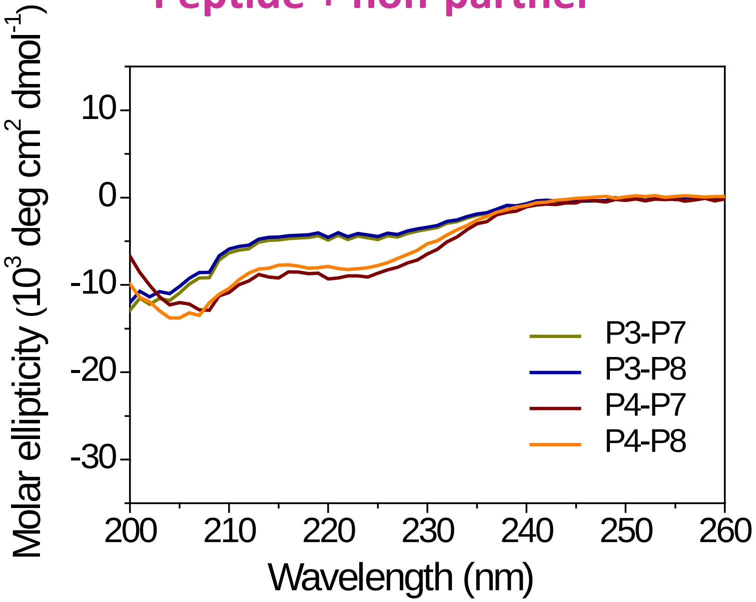
	Parallel							
	P1	P2	P3	P4	P5	P6	P7	P8
P1	33	**100**	29	27	31	32	30	29
P2	–	−6	10	7	11	12	11	9
P3	–	–	10	**93**	19	20	19	17
P4	–	–	–	5	17	18	17	15
P5	–	–	–	–	13	**101**	−15	−16
P6	–	–	–	–	–	16	−13	−15
P7	–	–	–	–	–	–	12	**96**
P8	–	–	–	–	–	–	–	9



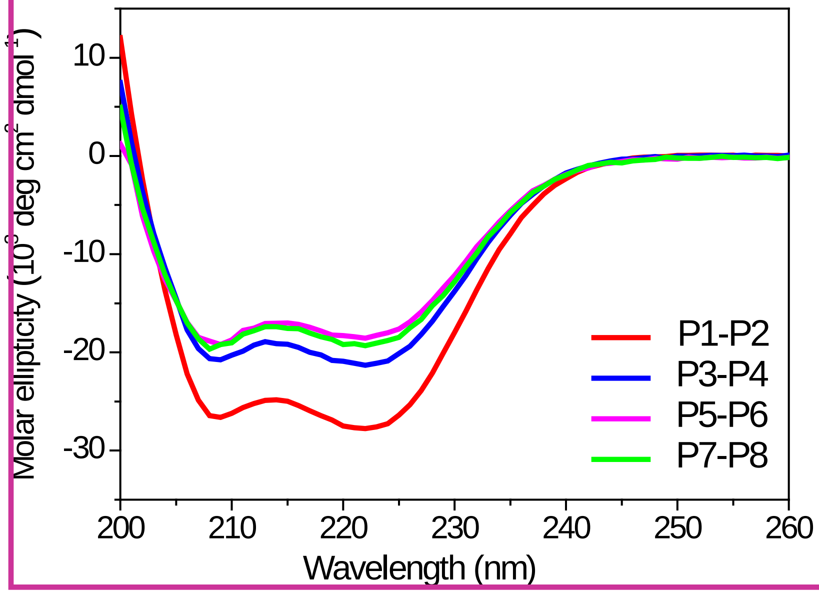
	Antiparallel							
	P1	P2	P3	P4	P5	P6	P7	P8
P1	−62	5	−30	−33	−28	−27	−29	−30
P2		−100	−49	−52	−47	−46	−48	−49
P3			1	−87	−40	−38	−41	−42
P4				−3	−42	−41	−43	−44
P5					−81	7	−39	−40
P6						−78	−37	−38
P7							3	−84
P8								1

Orthogonality of designed coiled-coil peptides

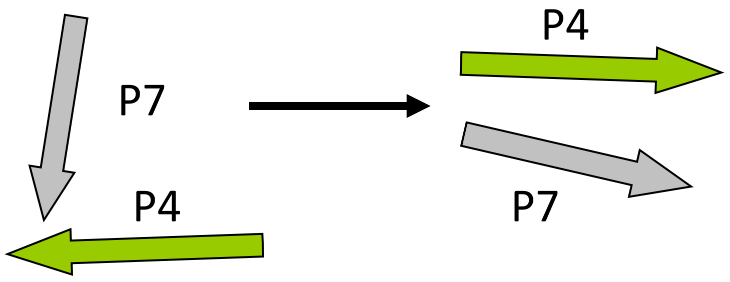
Peptide + non-partner



Peptide + designed partner



Peptide + non-partner

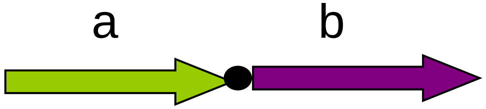


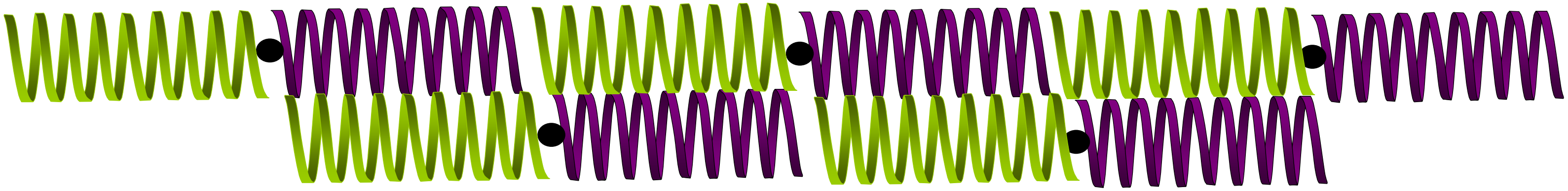
Peptide + its partner

Gradišar and Jerala, J.Pept.Sc., 2011

Linking of coiled-coil forming segments

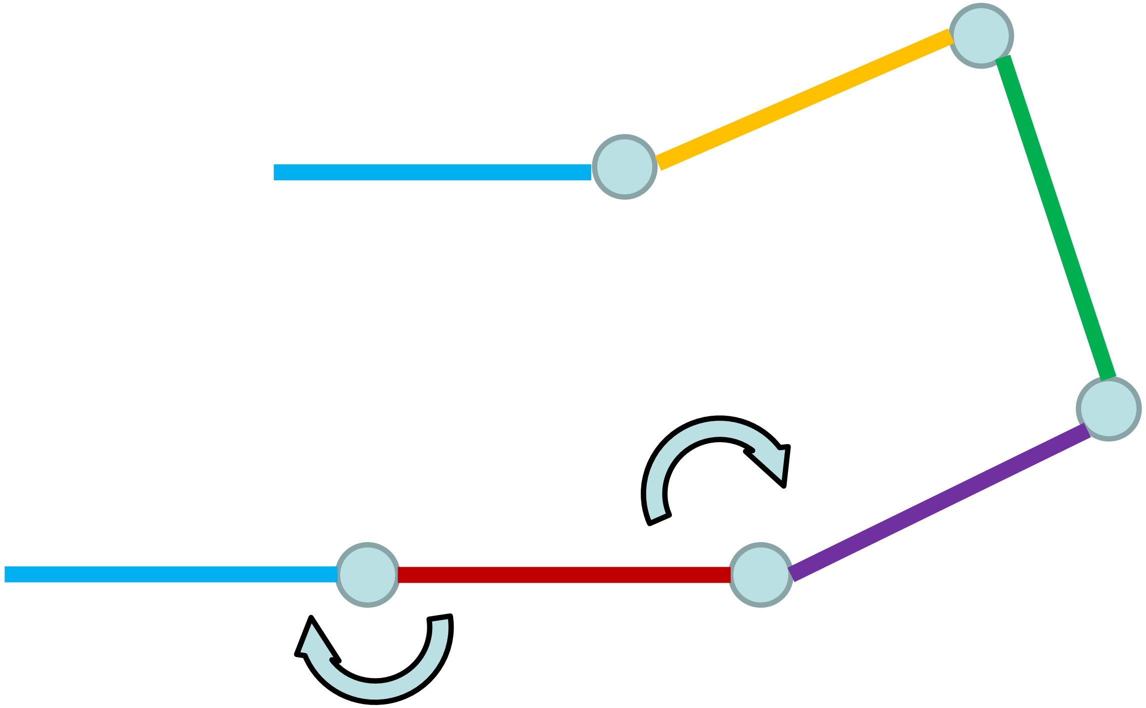
Building block = **2** coiled-coil-forming segments



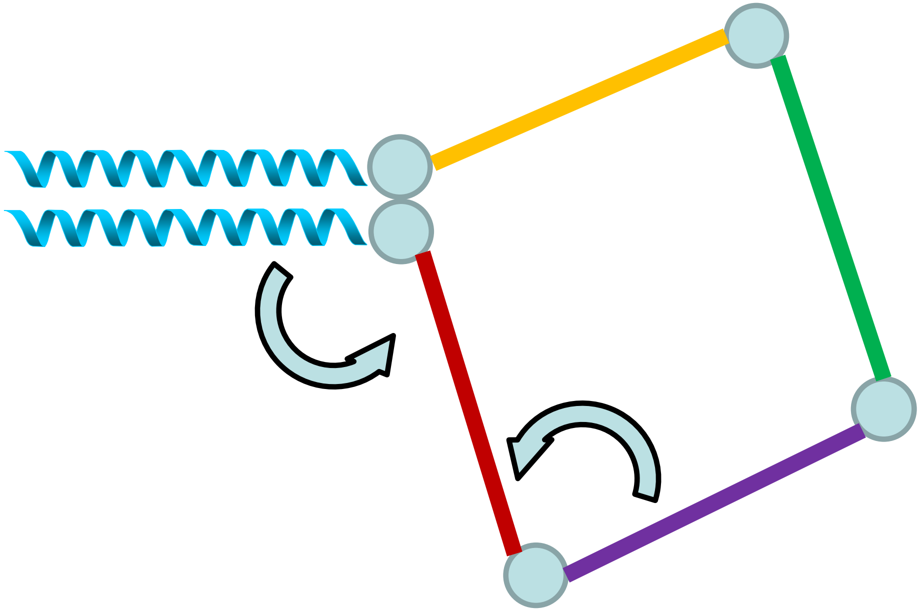


Two linked coiled-coil-forming segments can only form **fibrils** (nearest neighbor interactions)

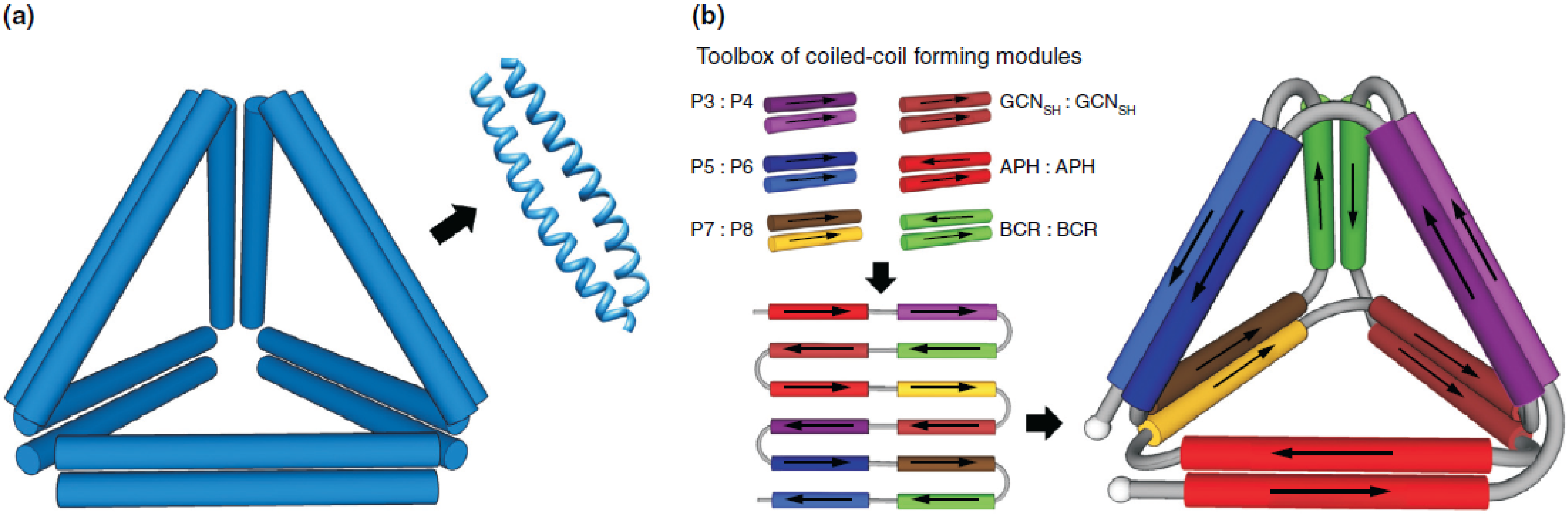
Flexible linker connecting interacting elements



Flexible linker connecting interacting elements



Deconstructing shape into modules



Toolbox of coiled-coil forming modules

Deconstruction of a polyhedron into rigid building blocks

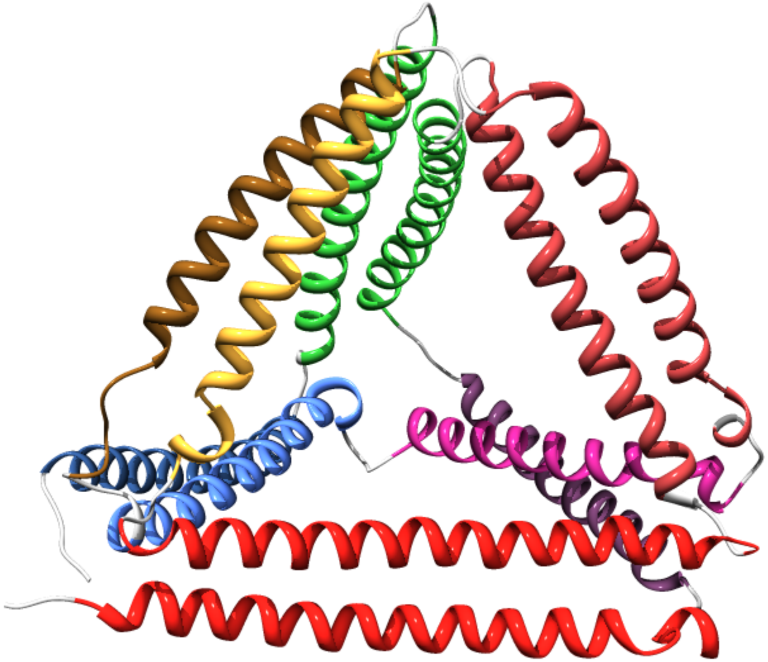
Sequential order of concatenated coiled-coil forming modules

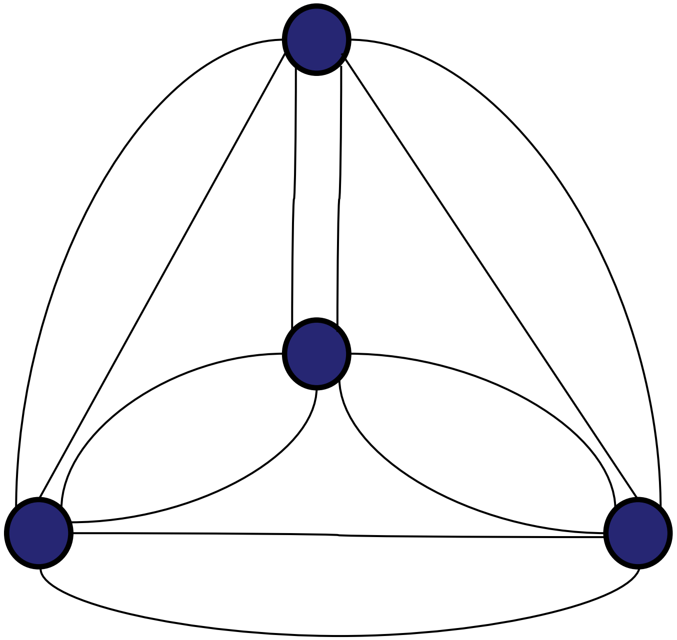
Self-assembled tetrahedron

Current Opinion in Chemical Biology

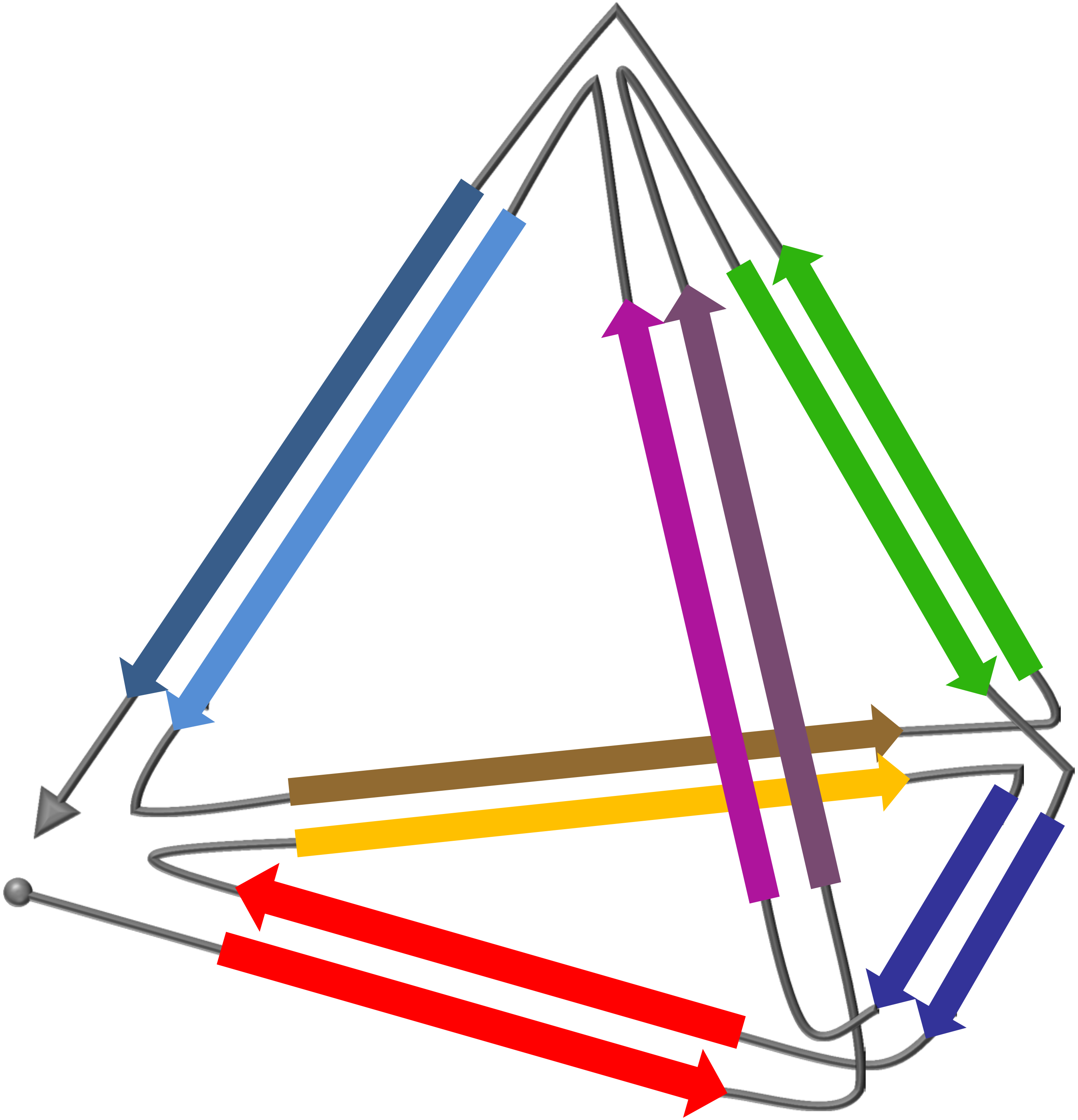
Božič-Abram et al., Cur.Op.Chem.Biol. 2013

Construction of the tetrahedron

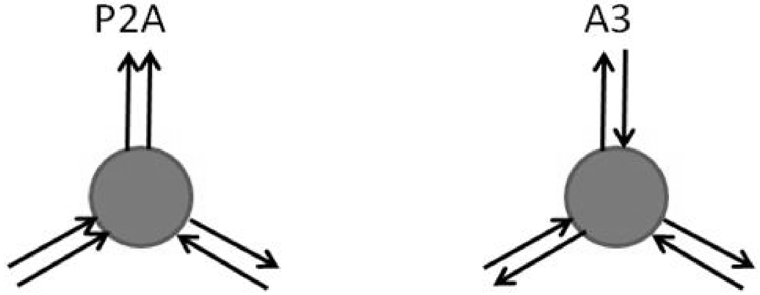




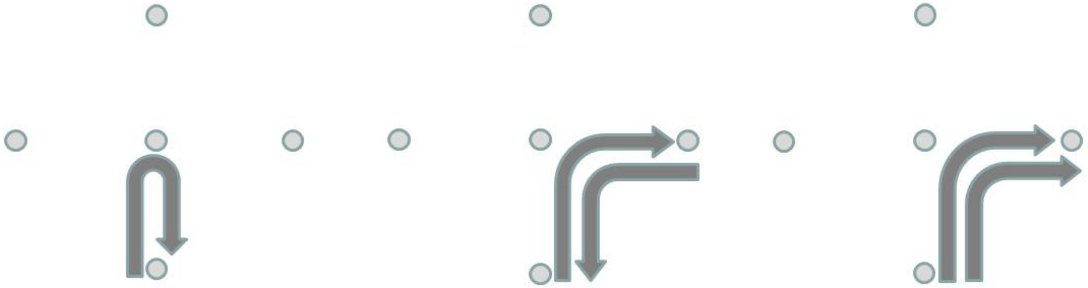
Can the tetrahedral edges be traversed exactly twice, forming coiled-coils at each edge ?



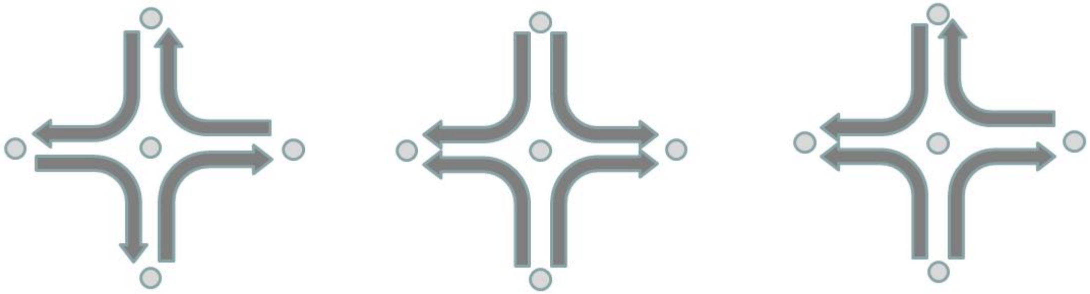
Topological solutions for a tetrahedron



Two types of vertices of the degree of 3 (6).

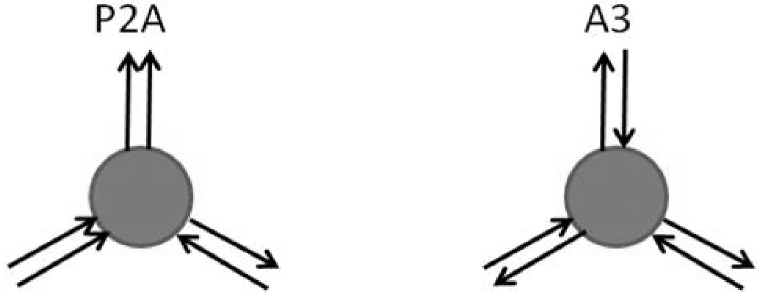


Disallowed paths

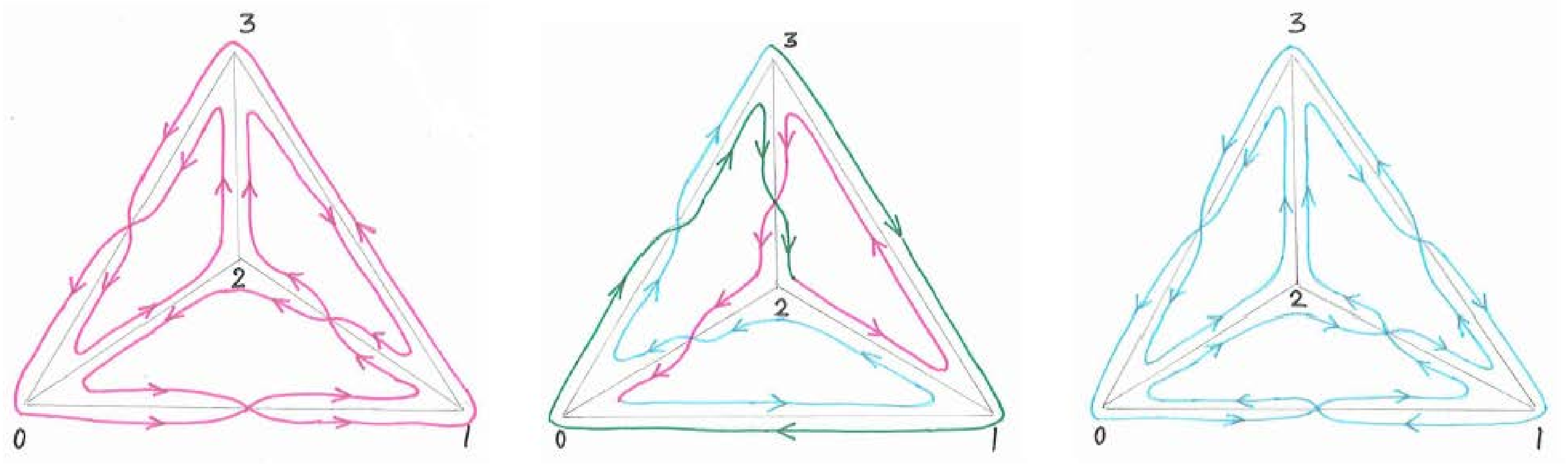


Allowed paths

Topological solutions for a tetrahedron

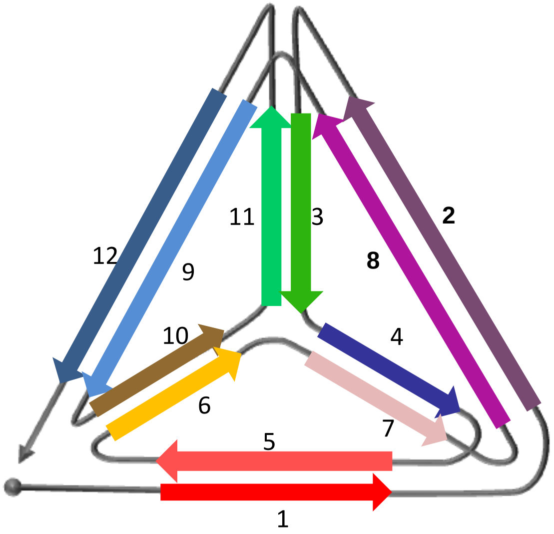


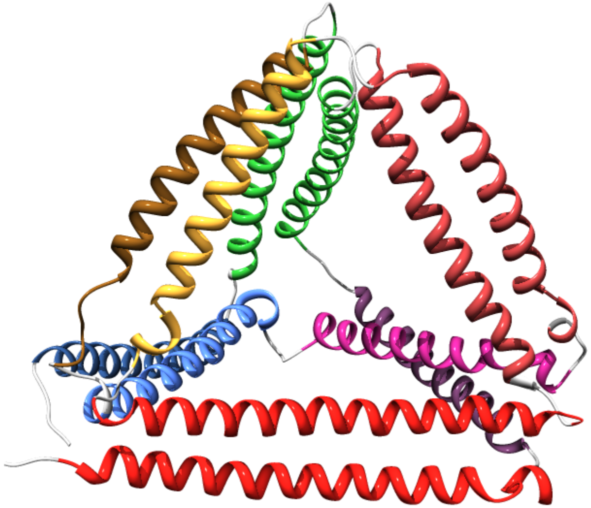
Two possible types of vertices of the degree of 3 (6).

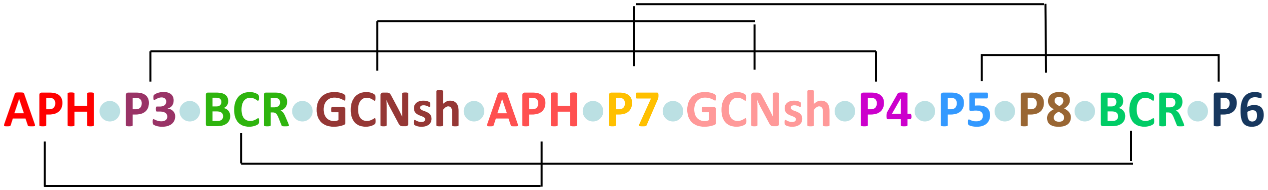


Three possible topologies to construct a tetrahedron but could be realized by 28 different combinations of segments.

Design of a tetrahedron-forming polypeptide



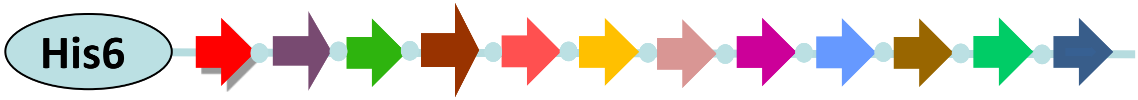



APH•P3•BCR•GCNsh•APH•P7•GCNsh•P4•P5•P8•BCR•P6

4 parallel dimers

2 antiparallel dimers

TET12

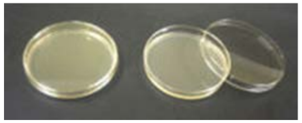


- flexible tetrapeptide linker

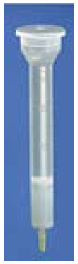
SGPG

Polypeptide production, isolation and self-assembly

Production in *E.coli*

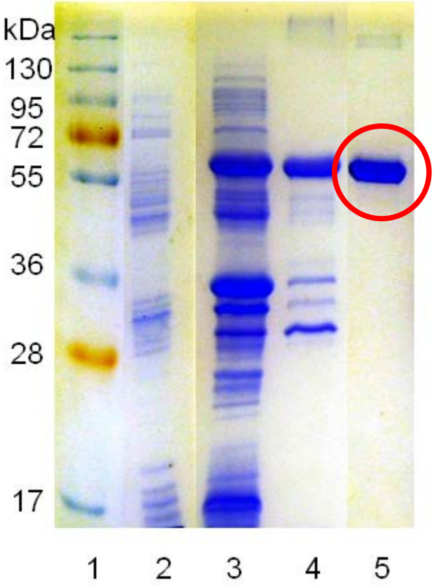


Protein purification



Affinity chromatography
HPLC-RP

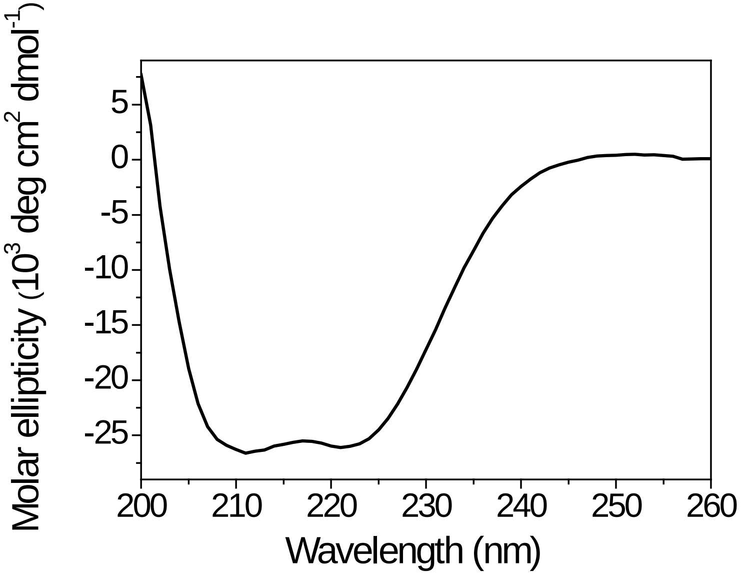
SDS-PAGE



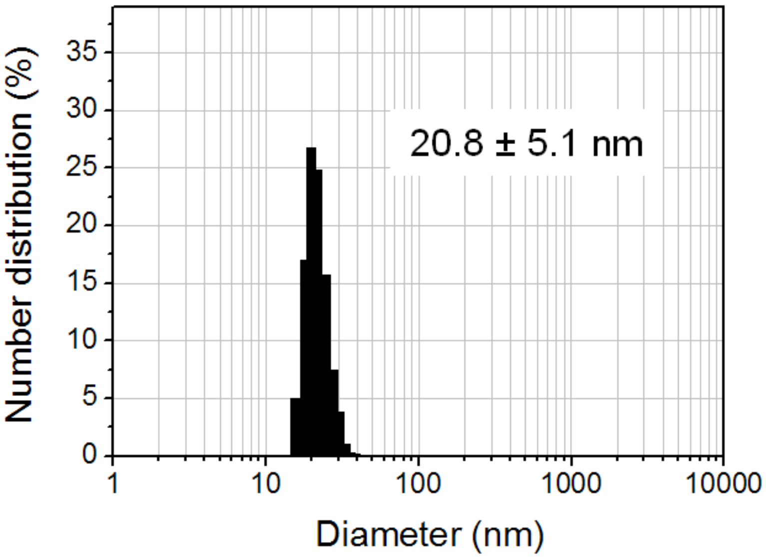
1. Standard
2. Supernatant of cell lysate
3. Inclusion bodies
4. TET12 purified on NiNTA
5. TET12 purified on RP-HPLC

In vitro self-assembly

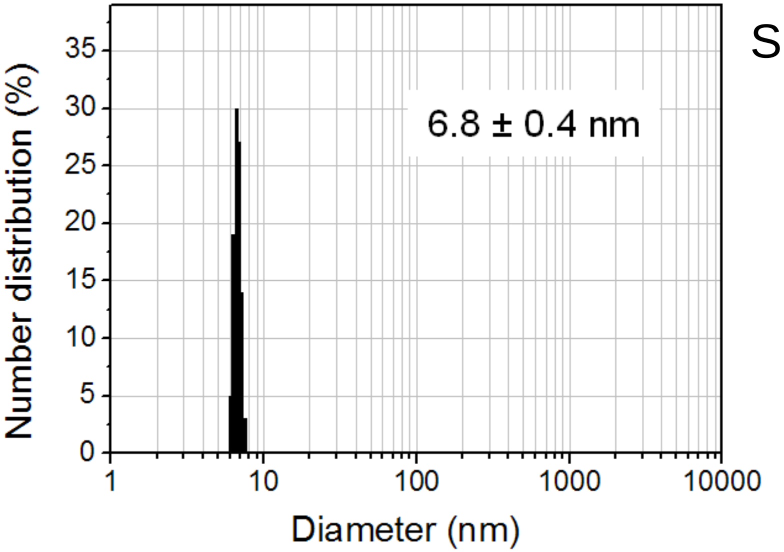
- **Dialysis** at low polypeptide concentration



Characterization of hydrodynamic size by DLS

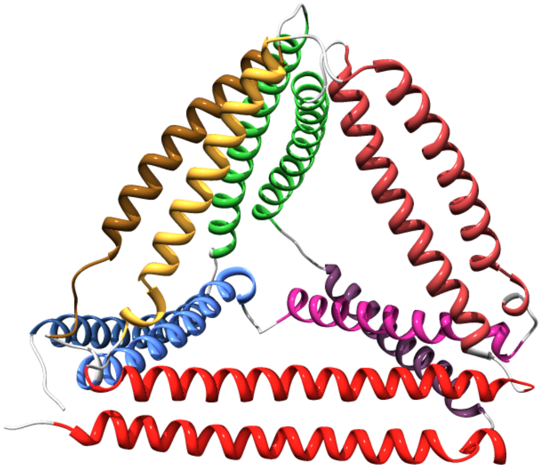


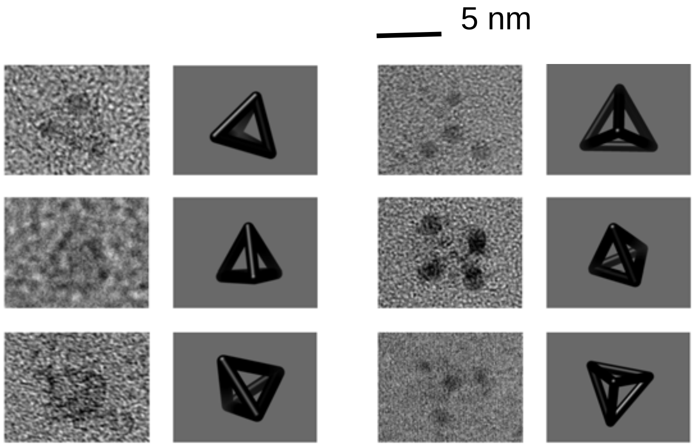
Denatured TET12

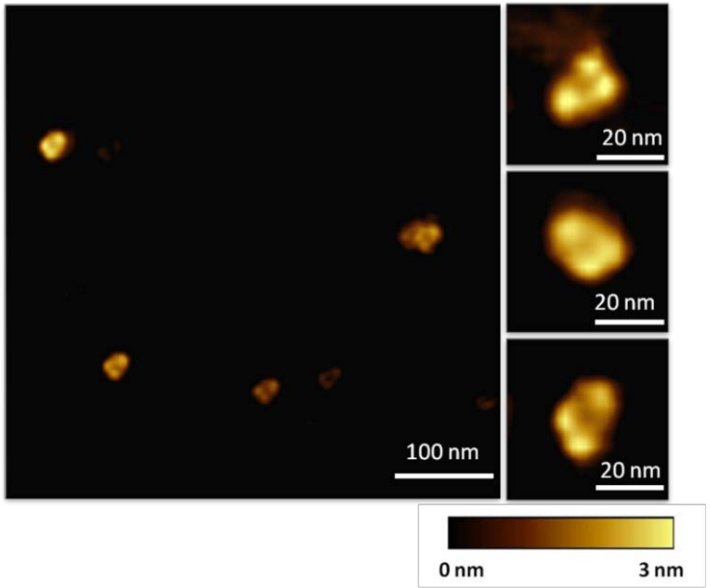


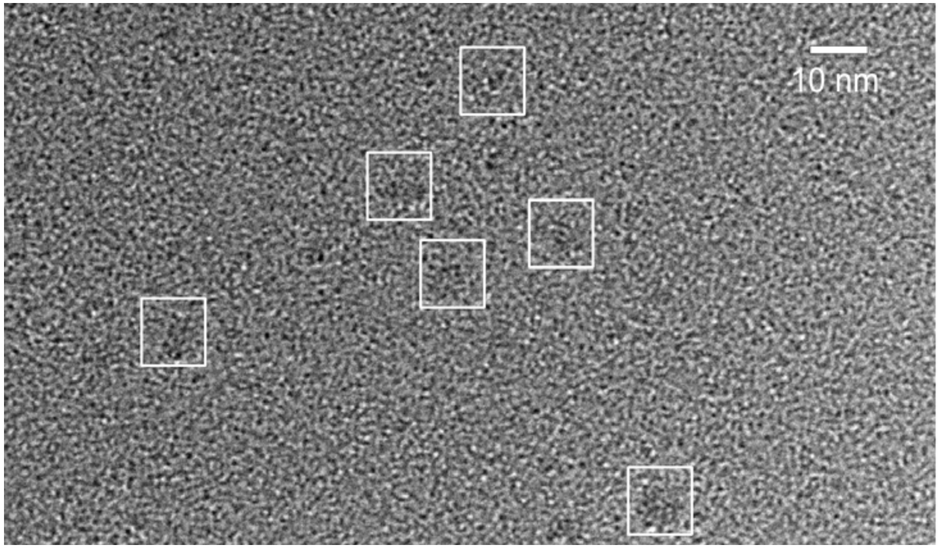
Self-assembled TET12

TEM and AFM imaging



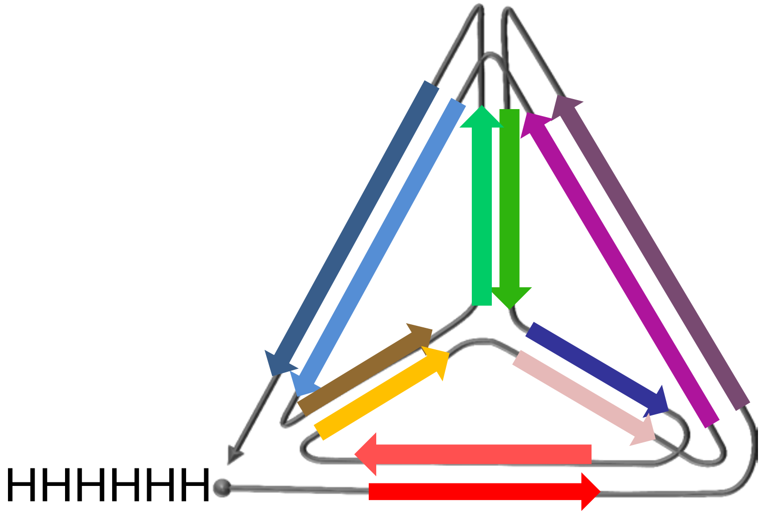


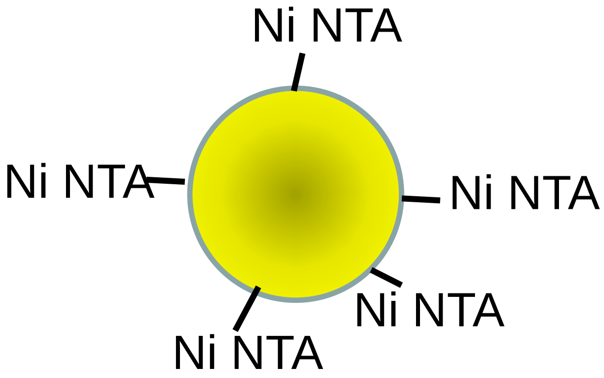


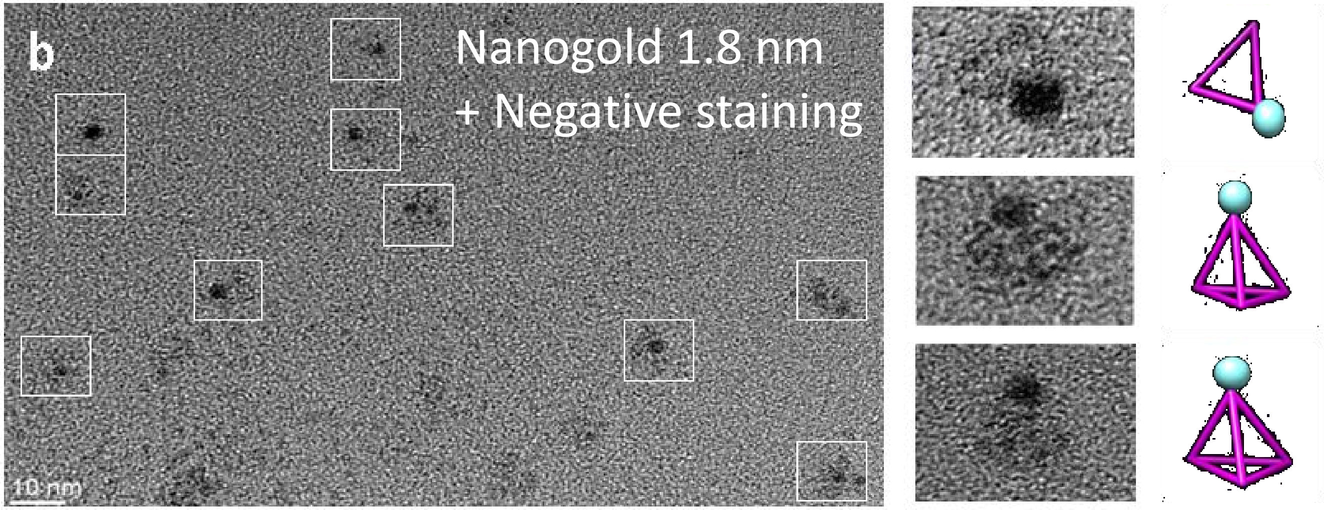


Gradišar et al., Nature Chem. Biol. 2013

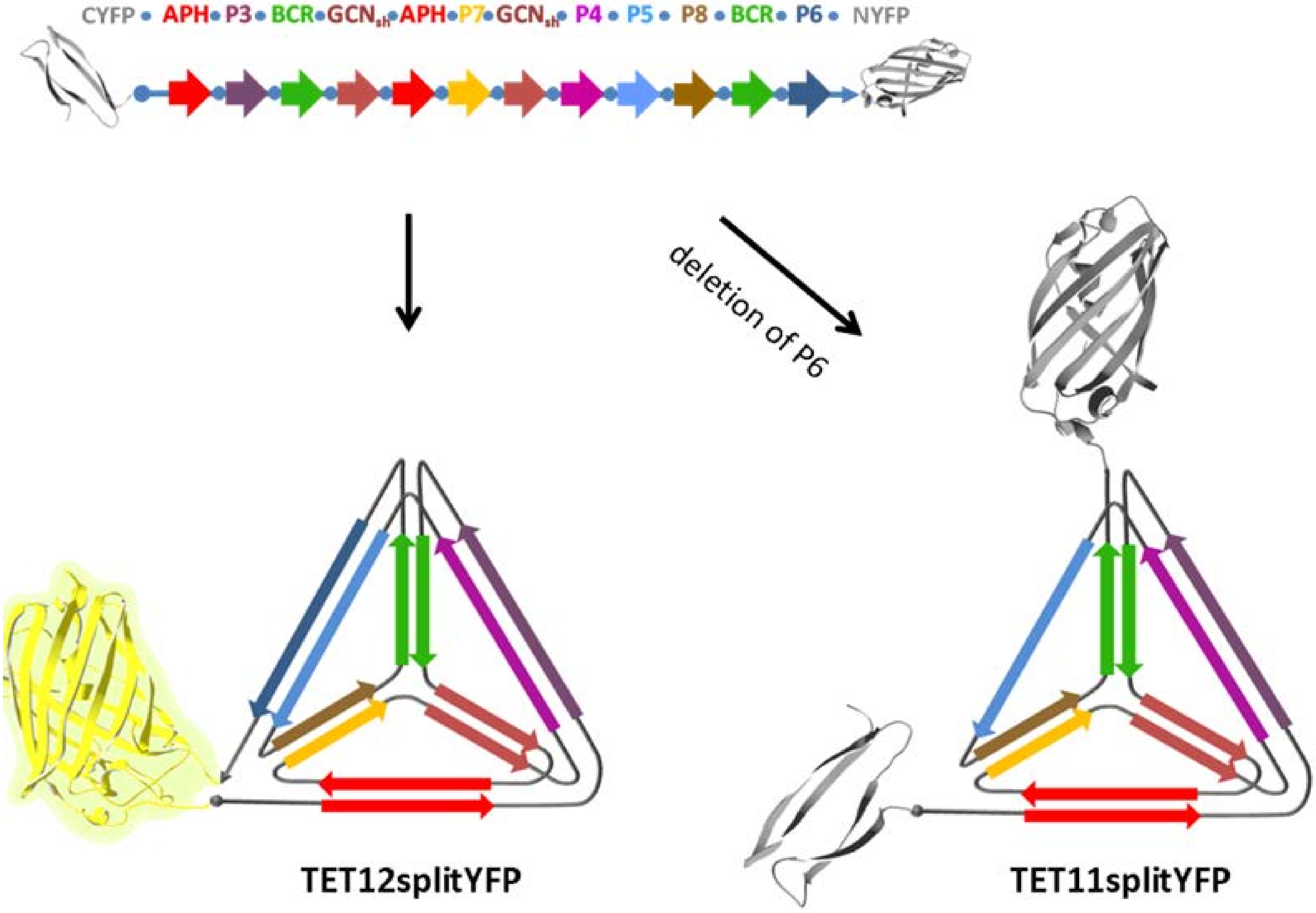
Detection of the N-terminal end of TET12



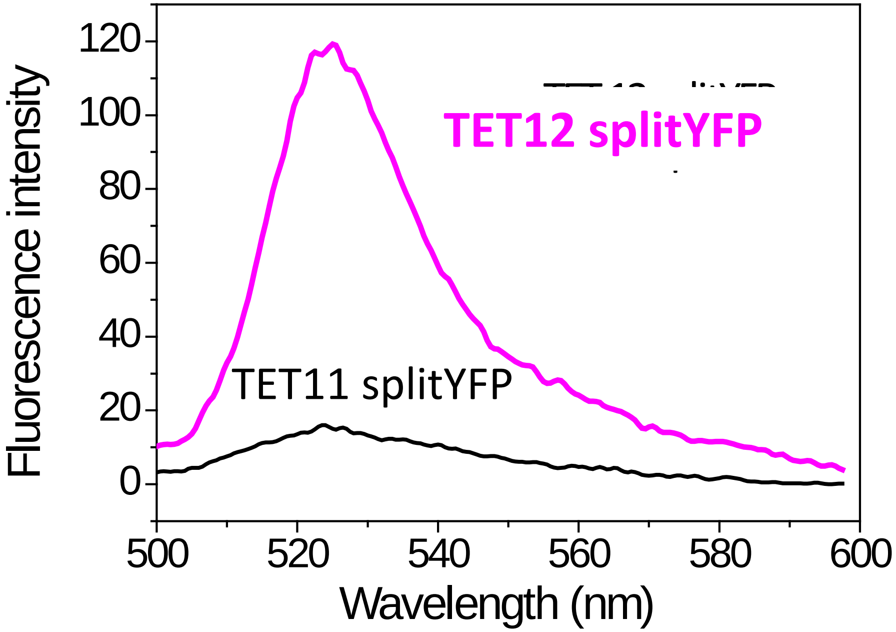




Termini of the tetrahedral path coincide



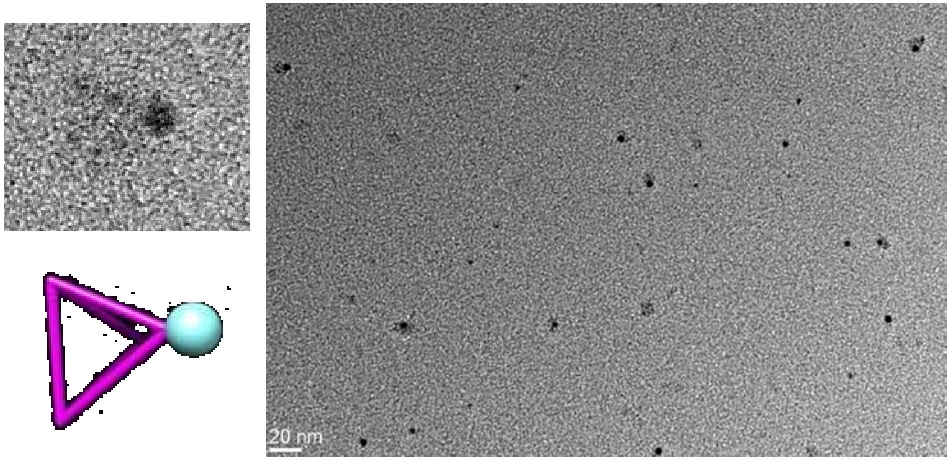
Coincidence of termini by YFP reconstitution



In vitro reconstitution

No fluorescence in producing bacteria

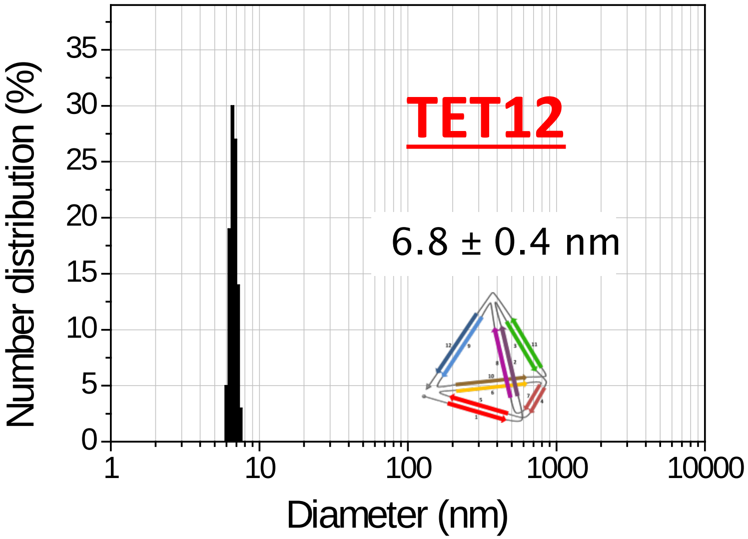
TET12 splitYFP

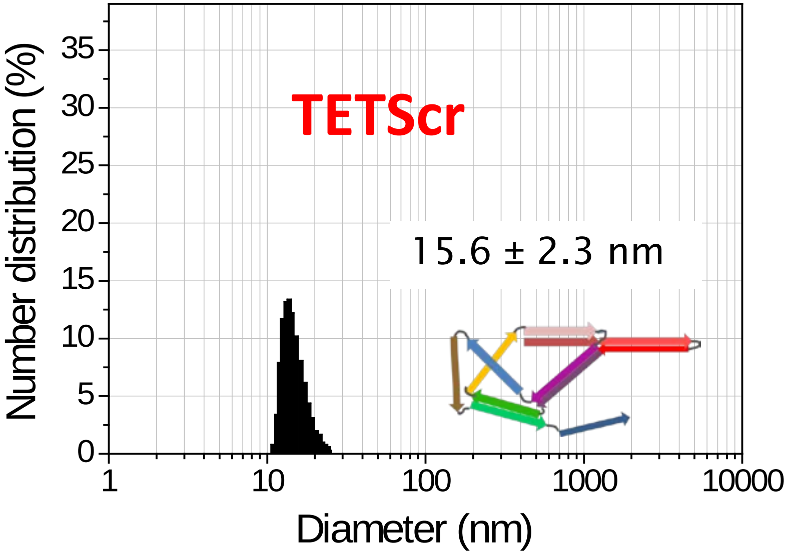


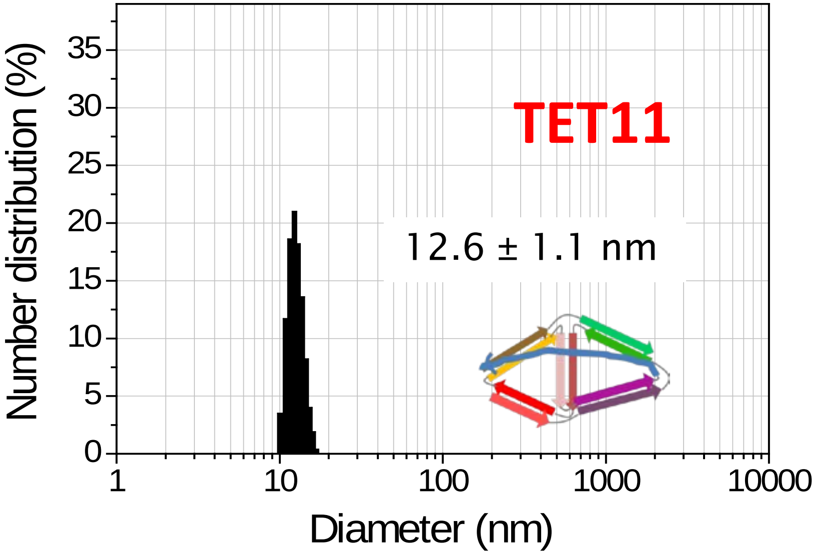
- ➢Fluorescence is reconstituted only in TET12 splitYFP but not for TET11 splitYFP

Gradišar et al., Nature Chem. Biol. 2013

Correct order of segments defines the structure

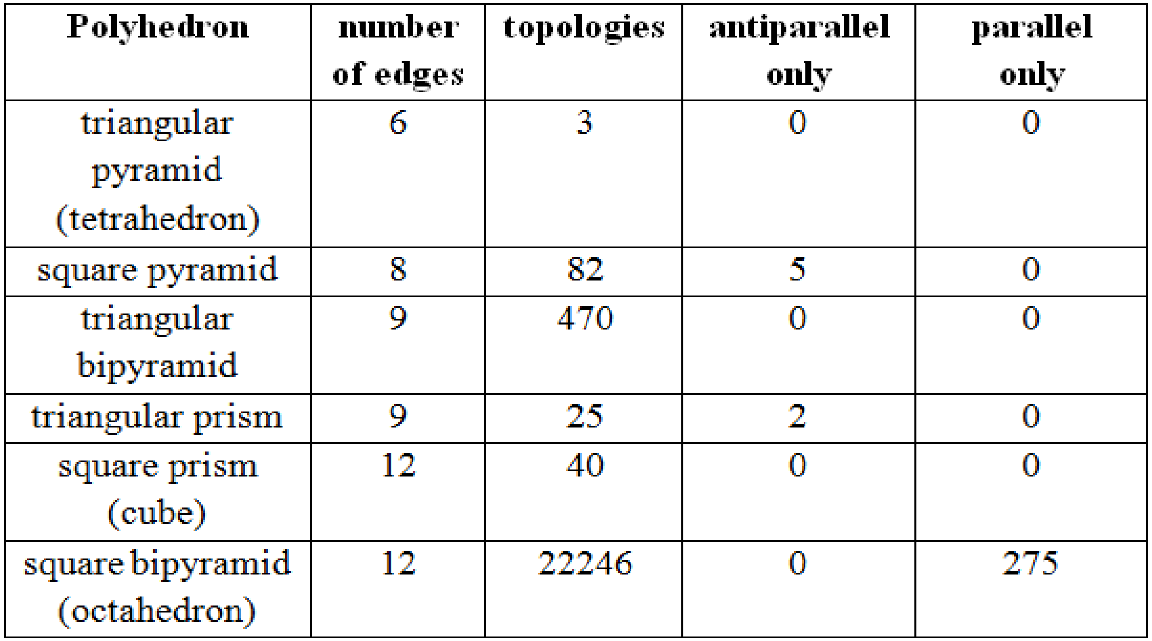




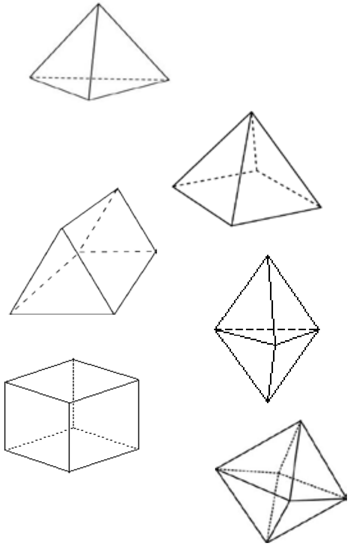


Next steps

- **Increasing the complexity of topological folds**



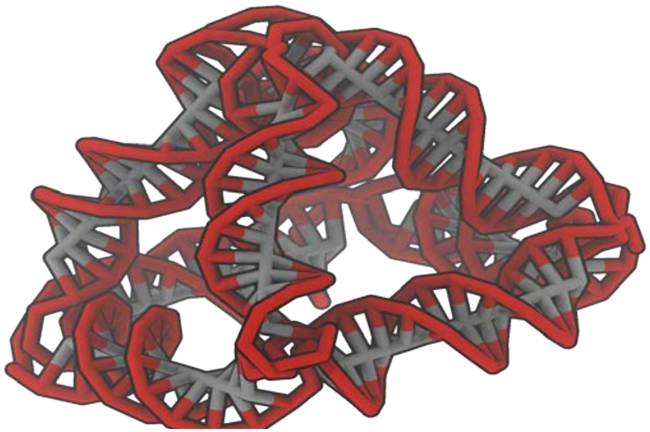
Polyhedron	number of edges	topologies	antiparallel only	parallel only
triangular pyramid (tetrahedron)	6	3	0	0
square pyramid	8	82	5	0
triangular bipyramid	9	470	0	0
triangular prism	9	25	2	0
square prism (cube)	12	40	0	0
square bipyramid (octahedron)	12	22246	0	275

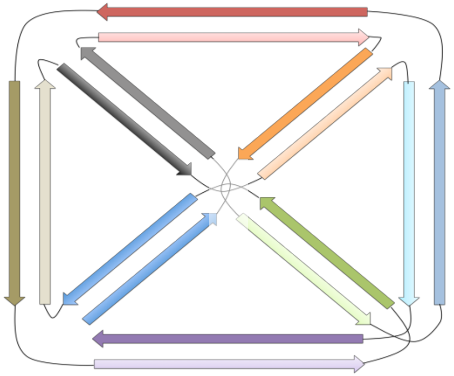


- **Establishing the foundations**:
  - Expand the toolbox of building blocks
  - Selection of loop sequences
  - *In vivo* folding ?

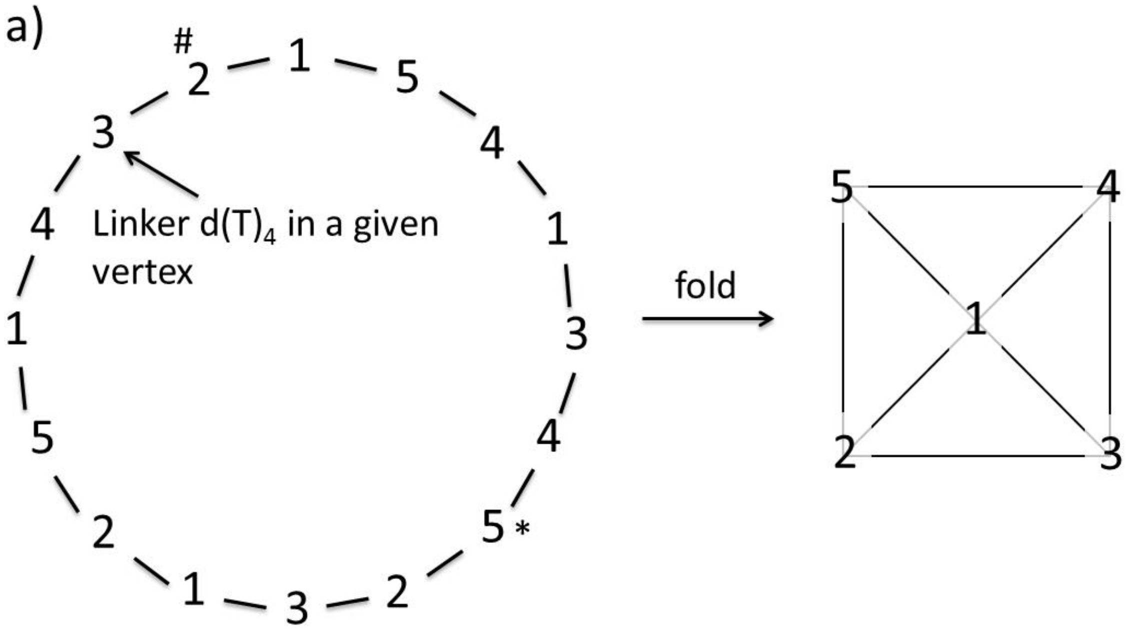
DNA as the prototyping material

Square pyramid from a single DNA chain

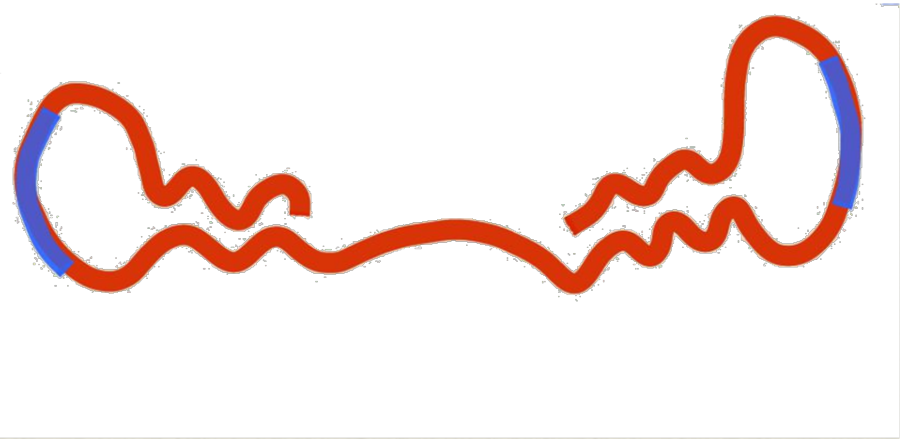




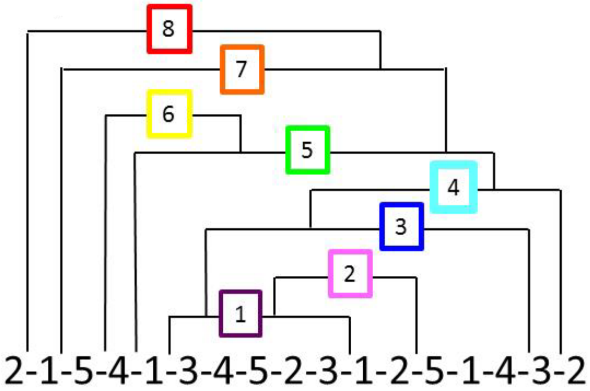
Only 2 circular paths to fold single chain antiparallel square pyramid



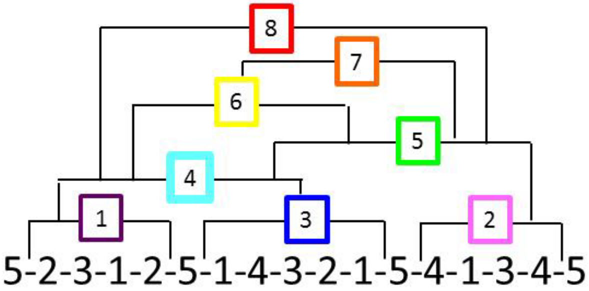
Selection of the folding pathway



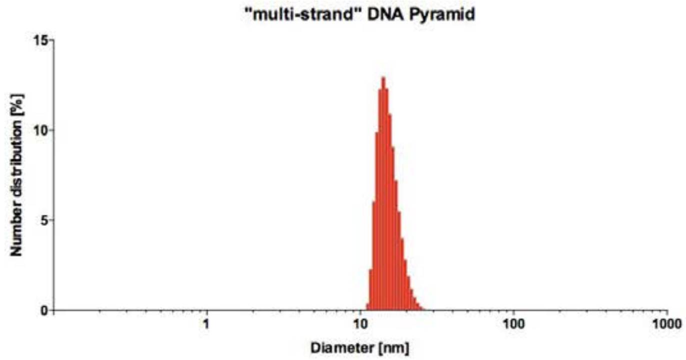
“Optimal“ arrangement

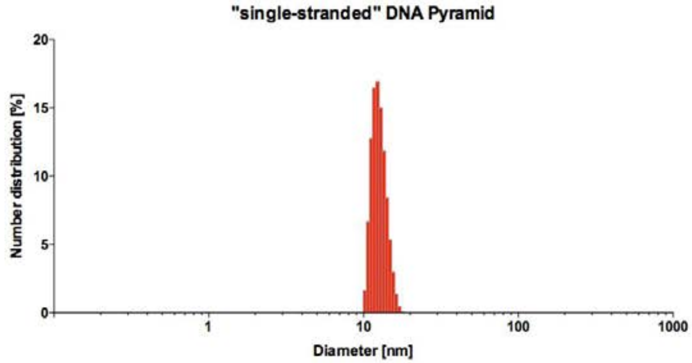


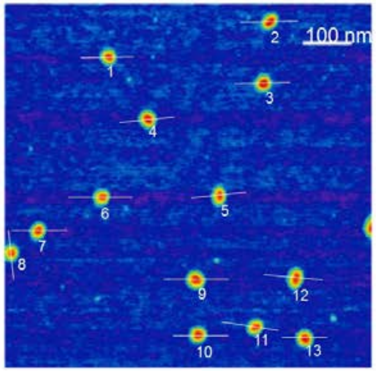
“Kineticaly hindered“ arrangement

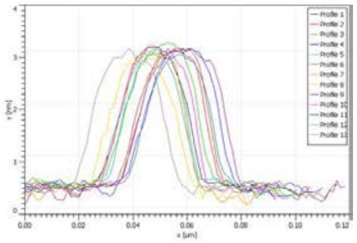


Single chain DNA pyramid



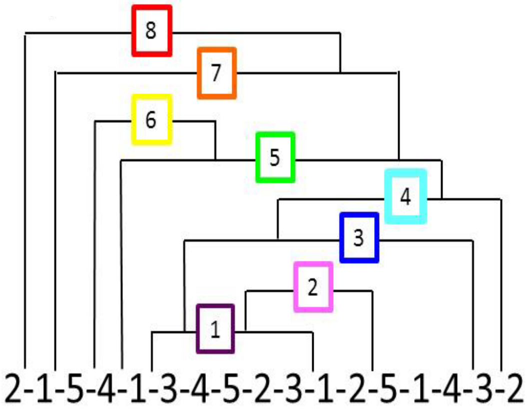






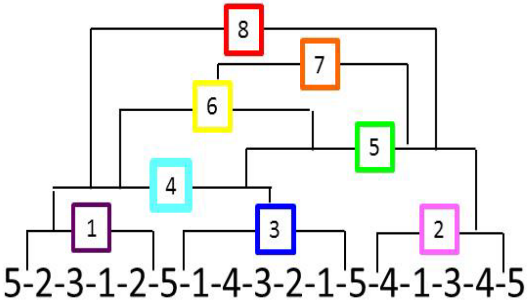
Kinetics of folding depending on the topology

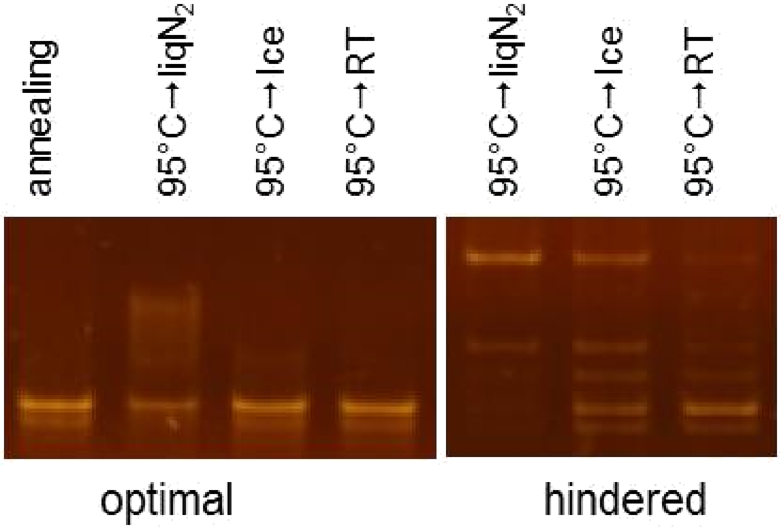
a) favorable



b) unfavorable

- folding topology



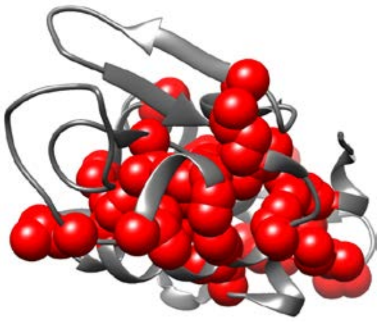


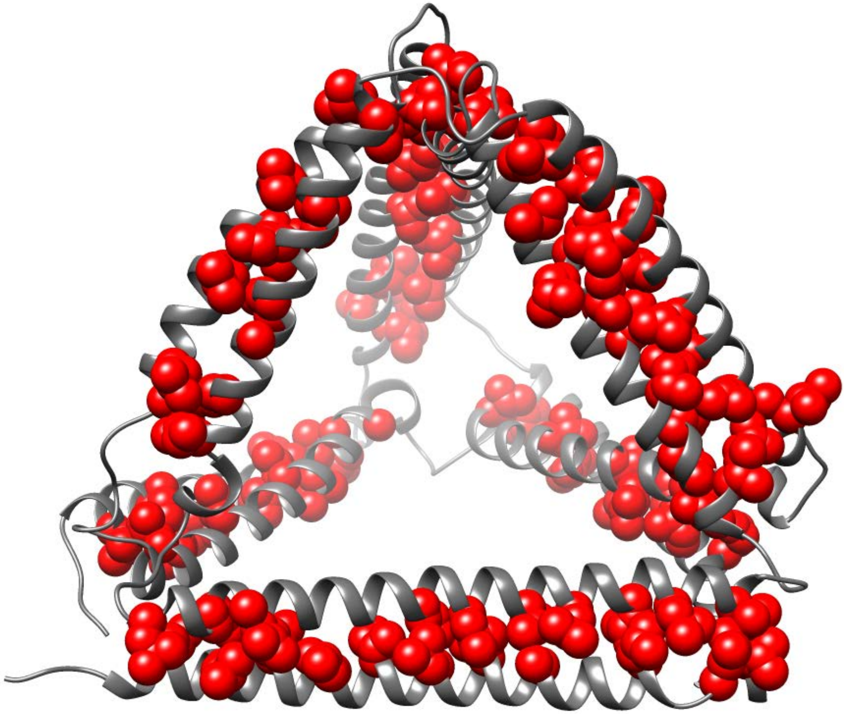
DNA pyramid

Natural and topological protein fold

NATIVE PROTEIN FOLD

TOPOLOGICAL PROTEIN FOLD



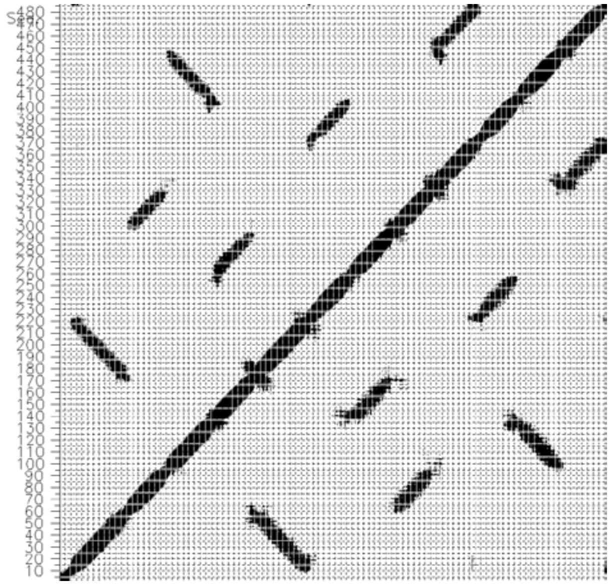


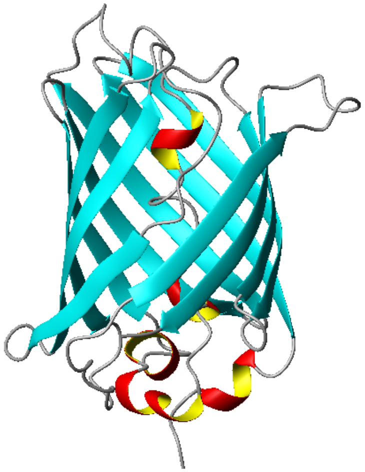
Compact and continuous hydrophobic core joining secondary structure elements

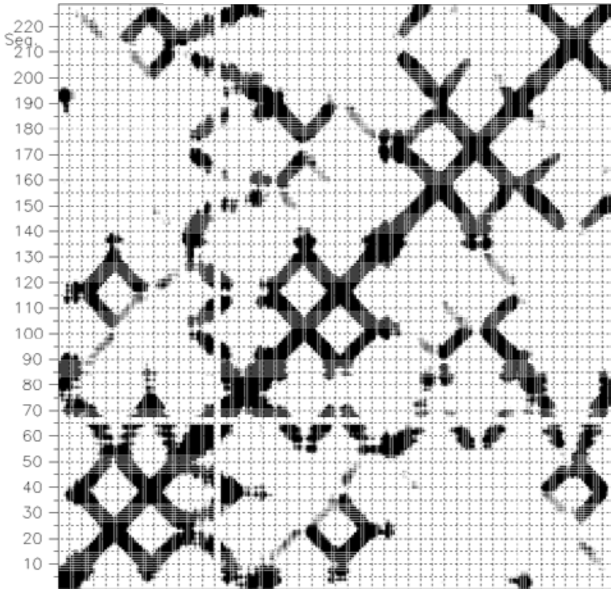
Hydrophobic core limited to within each building block

Topology defines the fold !

Fold definition by long-range interactions

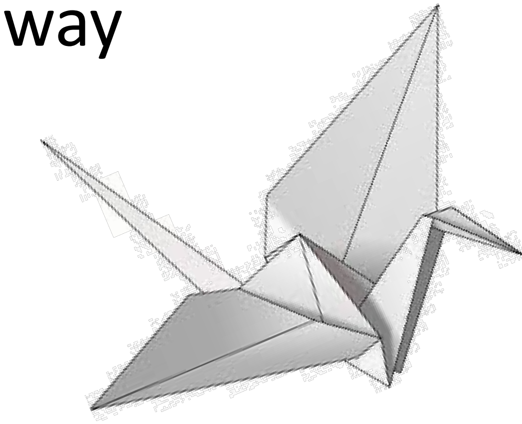






Summary

- Concatenated coiled-coil-based modules can be used to design new type of a **topological protein fold** based on similar principles as DNA nanostructures
- Tetrahedral fold not been found in nature has been designed and succesfully folded
- The arrangement of interacting segments according to stability allows define teh folding pathway



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