Nanosciences

Third Year PhD Exam

Elisa Martino

Dr. Gianpiero Garau

Dr. Stefano Luin

Pisa, 20/10/2020

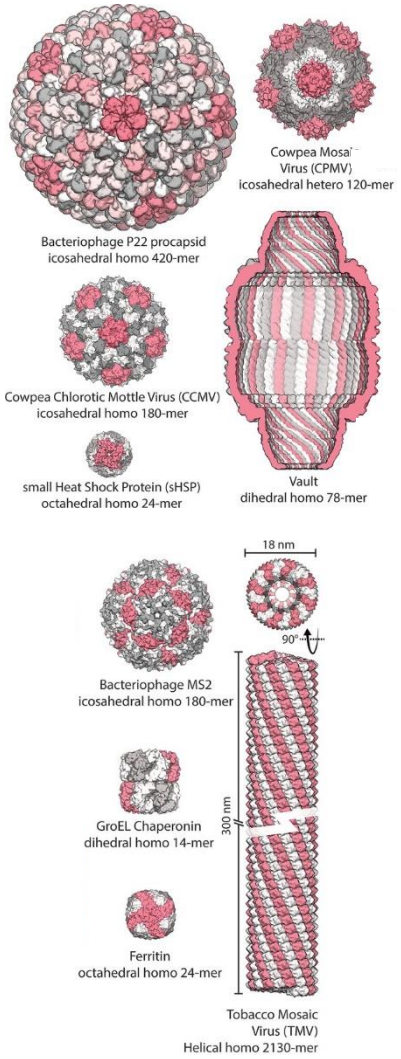
National Enterprise for nanoScience and nanoTechnology

NEST

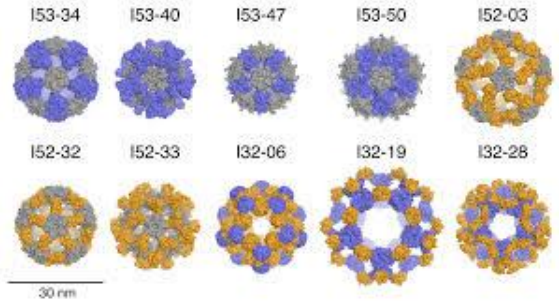
PhD Project:

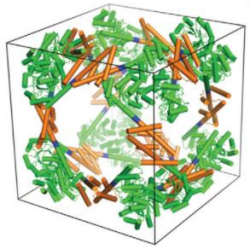
3D Nano-bioarchitectures

Natural protein cages



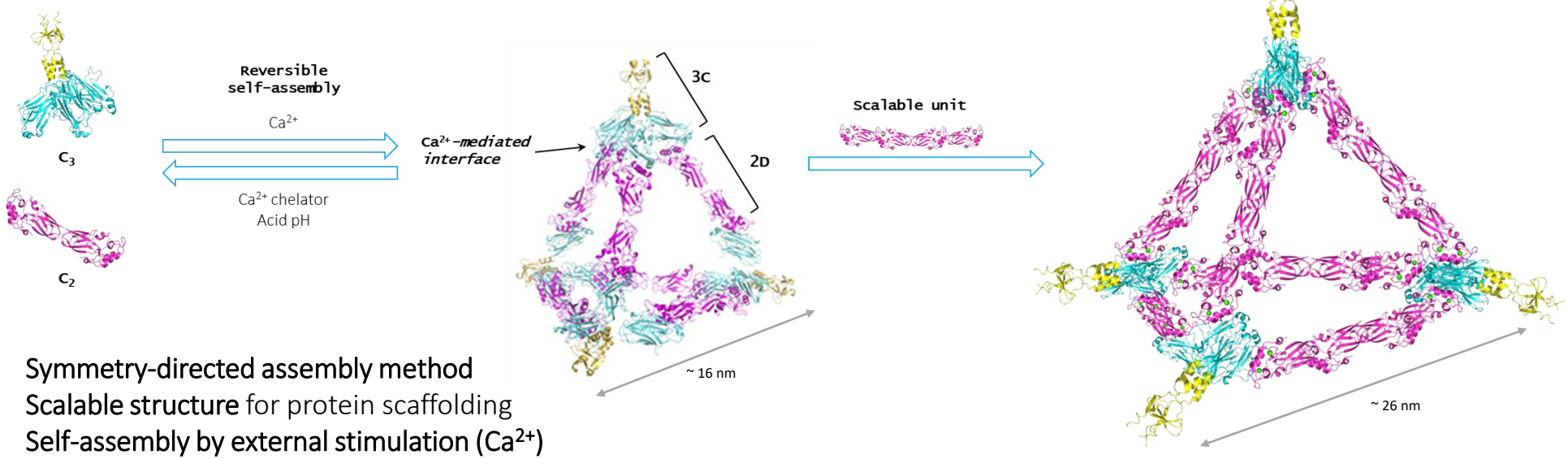
Artificial protein cages





Objective: development of novel protein-cage nanostructures through symmetry-directed assembly method

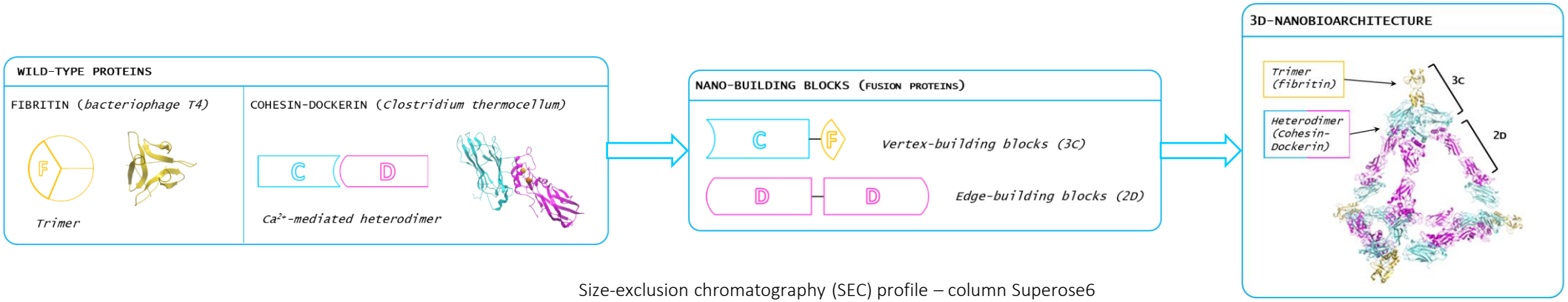
TETRAHEDRAL MODULAR 3D-NANOBIOARCHITECTURE



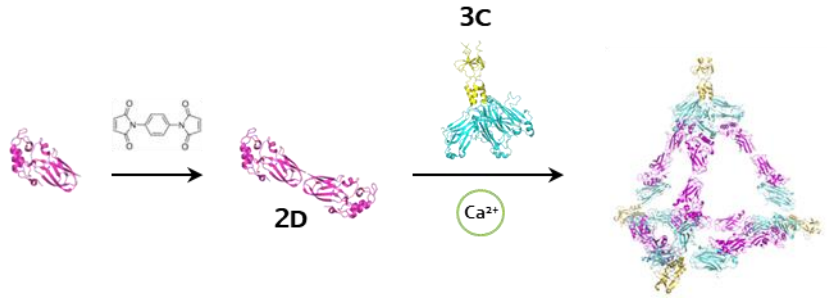
- Symmetry-directed assembly method
- Scalable structure for protein scaffolding
- Self-assembly by external stimulation (Ca^{2+})

PhD Project:

Original design and assembly

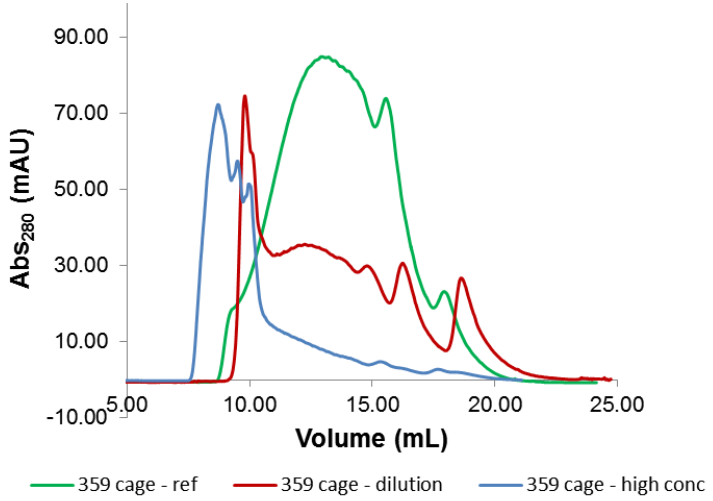


Heterodimer-driven assembly



1) chemical crosslinking on monomeric dockerin
2) calcium addition → heterodimer with 3C

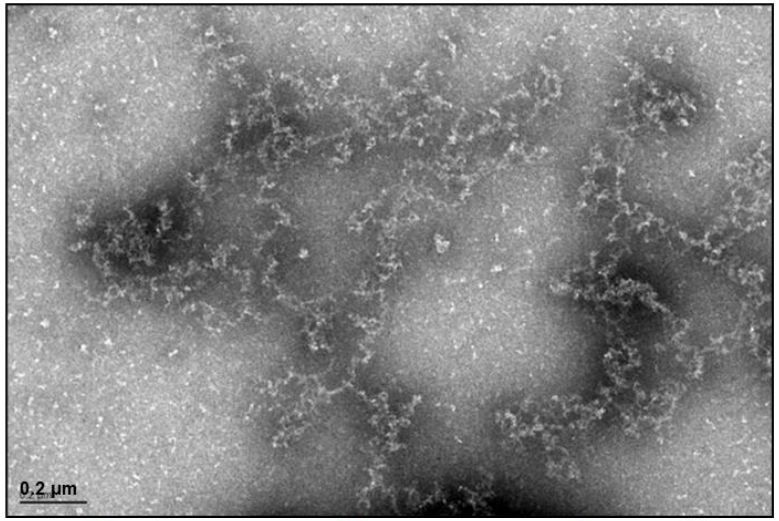
Size-exclusion chromatography (SEC) profile – column Superose6



359 - ref: 3C 26,5 µM, 2D 35,5 µM;
359 - dilution: 3C 10 µM, 2D 15 µM
359 – high conc: 3C 60 µM, 2D 90 µM

3C-bb: 6H-Cohesin-[2aa linker]-Fibritin (CF3)
2D-bb: Cross-linked XDoc59SH (59)

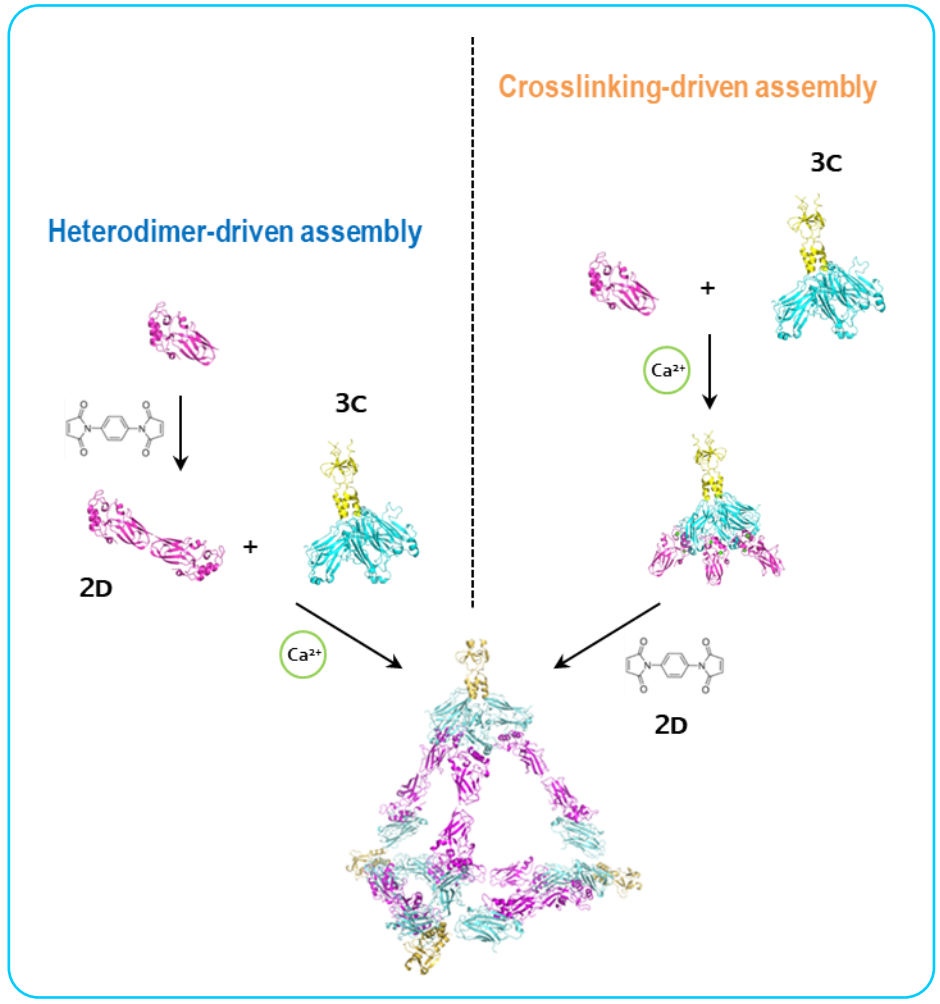
Negative staining on selected fraction



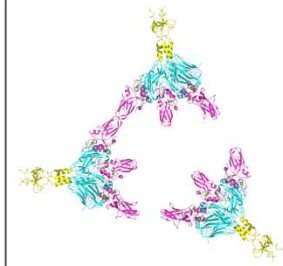
3C-bb: 6H-Cohesin-1[2aa linker]-Fibritin (CF1)
2D-bb: Cross-linked XDoc59SH (59)

New crosslinking assembly strategy:

Crosslinking-driven assembly



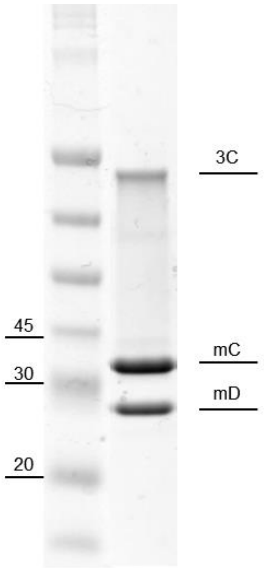
- Heterodimer formation and crosslinking dimerization order is inverted:
  1) Calcium addition → heterodimer between monomeric Doc and 3C
  2) chemical crosslinking on intermediate
- Intramolecular-like mechanism).



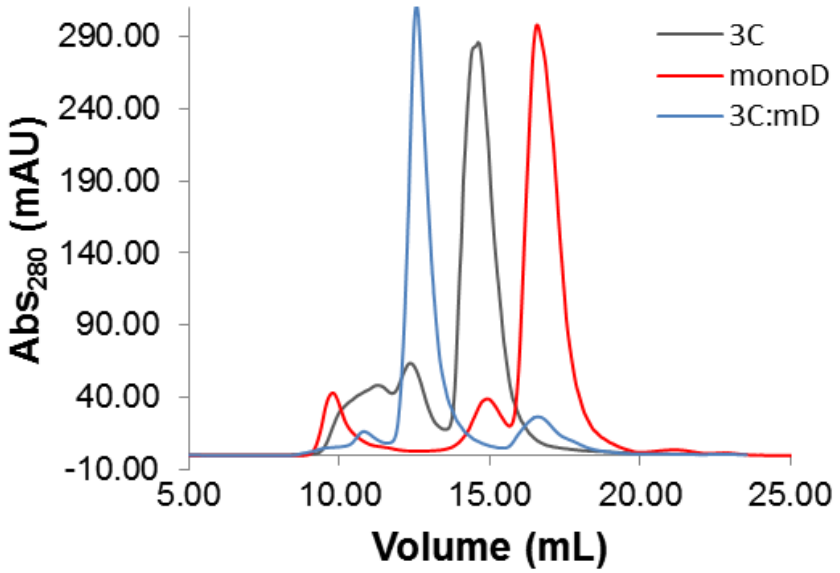
Intramolecular-like mechanism

Synthesis of the pre-assembled intermediate (3C:monoD)

SDS-PAGE

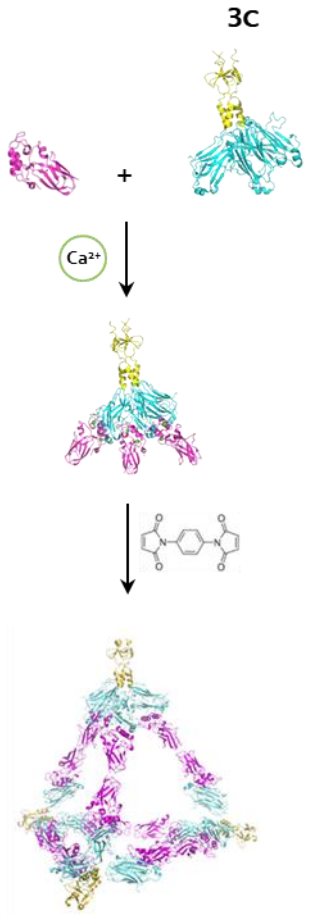


SEC profile – column Superdex200



New crosslinking assembly strategy:

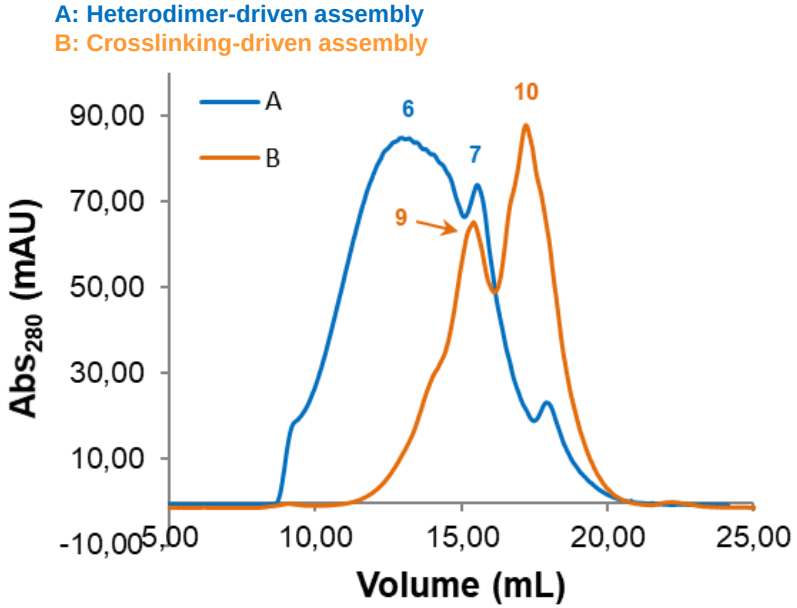
Crosslinking-driven assembly



Crosslinking-driven assembly

- High molecular structures suppressed
- Still no formation of predominant species
- Incomplete reaction (some starting material present)

SEC profile – column Superose6



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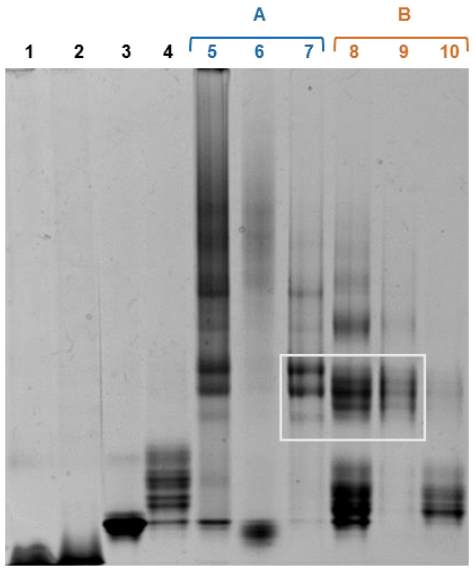
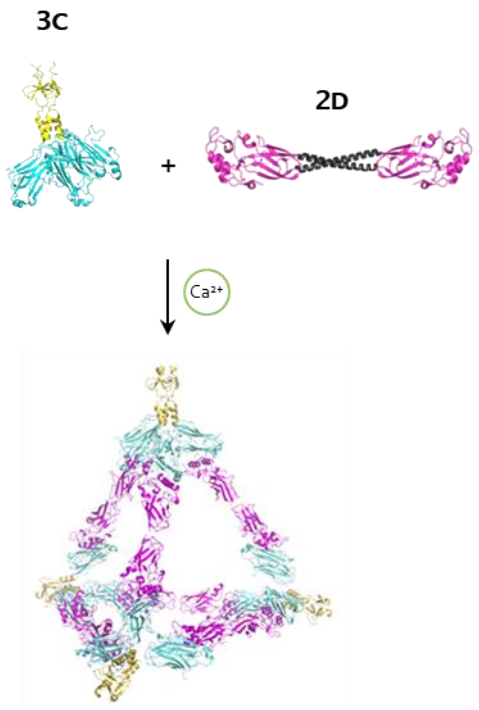


1: XDoc59SH
2: crosslinked-XD59
3: CF3 (2aa linker)
4: CF3:59SH complex Ca
5: 359 A pre-SEC
6: 359 A – peak 1
7: 359 A – peak 2
8: 359 B pre-SEC
9: 359 B – peak 1
10: 359 B – peak 2

- Both previous approaches did not lead to the desired structure
- **Structural characterization of pre-assembled complex** underway (crystals obtained) → possible re-design of crosslinking
- Further optimization of the crosslinking-driven assembly

Assembly strategy without crosslinking:

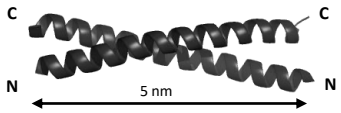
«Biomimetic» assembly



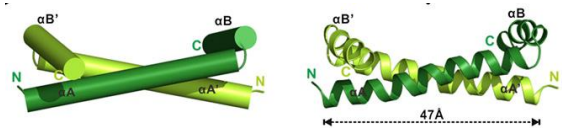
Biomimetic assembly

- No crosslinking reaction for 2D synthesis
- Dimerizing domain (coiled-coil) for 2D auto-assembly
- Antiparallelel orientation of coiled-coil
- Heterodimer-driven assembly

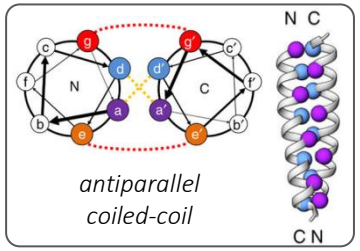
Antiparallel coiled-coils domains selected:



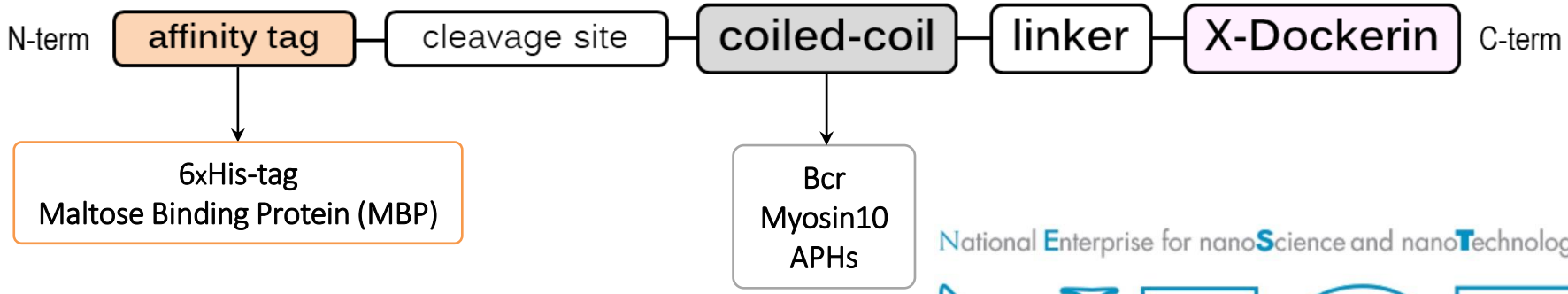
1) Bcr: oligomerization domain of Bcr-Ap1 oncoprotein, aa 28-68



2) Myosin10 aa 883-934, K_D = 0.6 µM. aa 883-910 → αA, minimal for dimer



3) APHs: artificial coiled-coils sequences. Computationally-designed.



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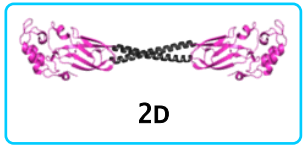
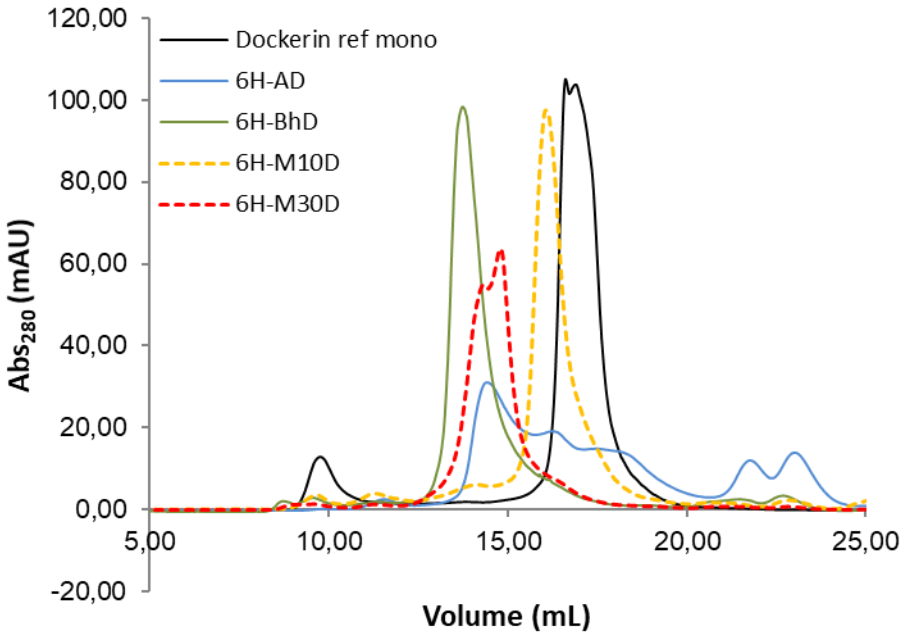
«Biomimetic» assembly

Synthesis of 2D-bb with coiled coil domains



Construct	Affinity tag	Coiled-Coil	Linker	Solubility
6H-BD	6xHis	**Bcr**	none (direct fusion)	totally insoluble
MBP-BD	MBP	**Bcr**	none (direct fusion)	after removal of MBP solubility drops
6H-BhD	6xHis	**Bcr**	[EAAAK] - helical	soluble
MBP-BhD	MBP	**Bcr**	[EAAAK] - helical	after removal of MBP solubility drops
6H-BgsD	6xHis	**Bcr**	[GS] – BamHI restriction site	soluble
6H-BeD	6xHis	**Bcr**	[E] casual mutation	To test
6H-M10D	6xHis	**Myo10**	none (direct fusion with XDoc)	soluble
MBP-M10D	MBP	**Myo10**	none (direct fusion with XDoc)	after removal of MBP solubility drops
6H-M30D	6xHis	**Myo10**	20 AA (truncation at aa 930)	soluble
MBP-M30D	MBP	**Myo10**	20 AA (truncation at aa 930)	soluble after MBP removal – MBP association
6H-M20D	6xHis	**Myo10**	10 AA (truncation at aa 920)	To test
6H-AD	6xHis	**APH**	none (direct fusion)	low solubility
MBP-AD	MBP	**APH**	none (direct fusion)	after removal of MBP protein precipitation

SEC profile – column Superdex200



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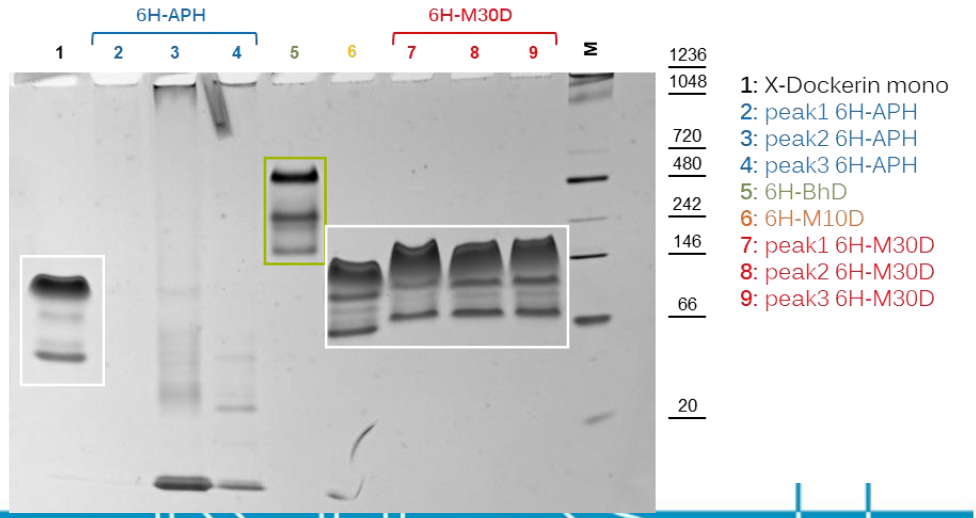


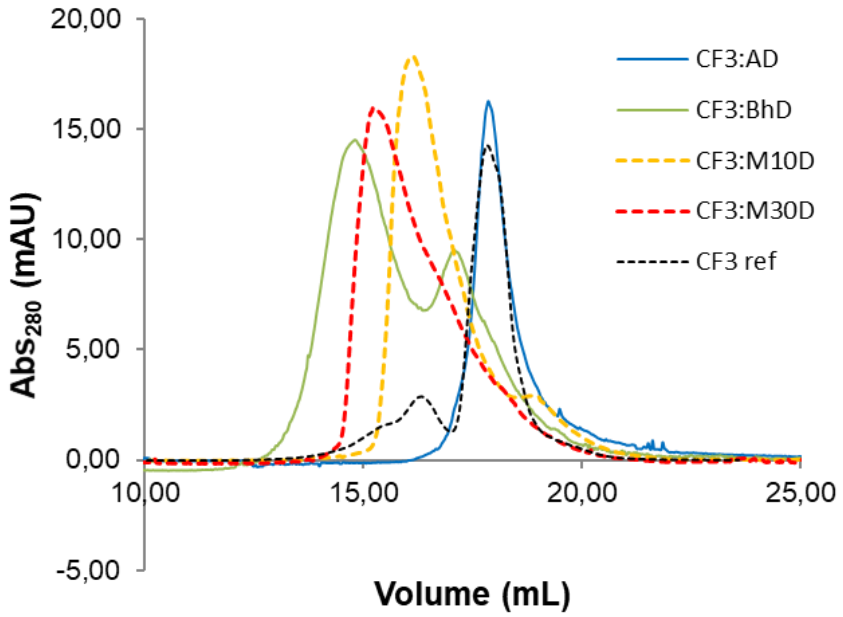
«Biomimetic» assembly

Preliminary assembly tests

- Less equilibrium forms in solution

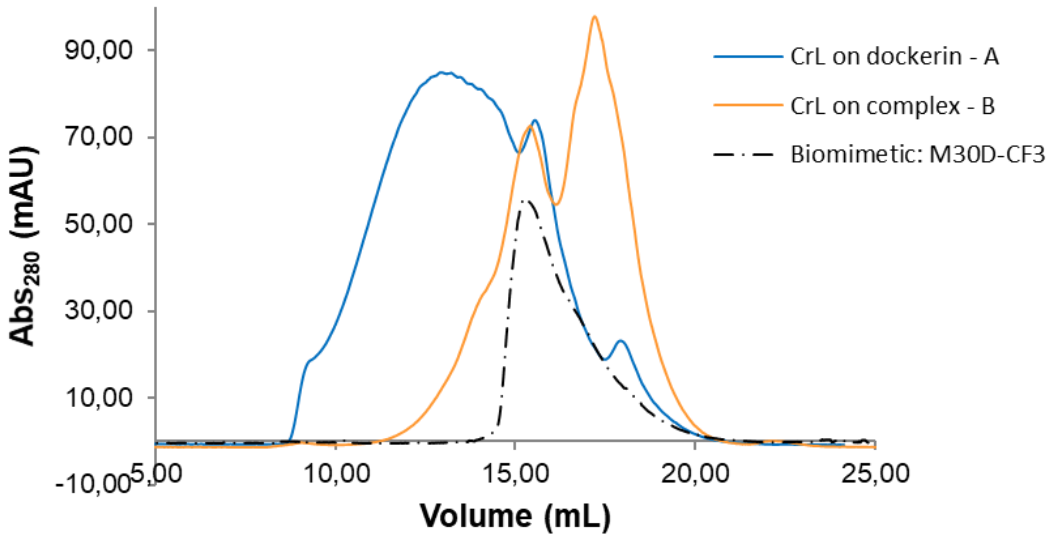
«Biomimetic» assembly: first attempts

SEC profile – column Superose6



Comparison between assembly methods

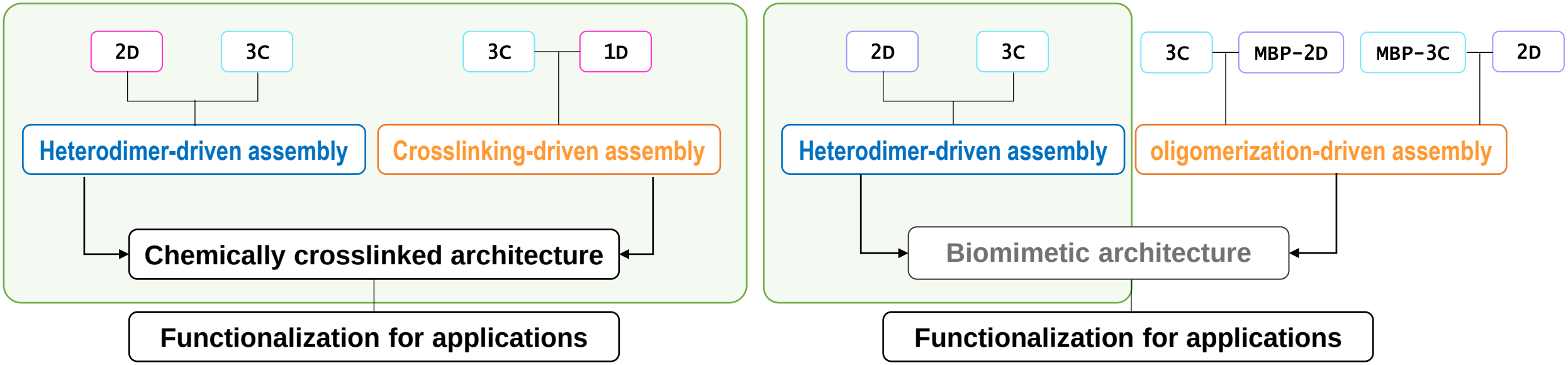
SEC profile – column Superose6



- Investigate the structures → negative staining
- Test different assembly conditions (pH, buffer, concentration)

Conclusions and Outlook

- **Chemically crosslinked architectures:** much more difficult to obtain than expected. Still some possible optimization of crosslinking-driven assembly methodolgy.
- Expansion of assembly strategies: **biomimetic architecture.** Many different assembly strategies still to investigate.
- Possible functionalization: fluorophores/fluorescent proteins as tool for differences among the assemblies.



Seminars: 6 hours
22-25/09/2020: GeCry School - From Gene to Protein Crystal Structure

Review on "Artificial self-assembling protein-cages" *in preparation*

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