Nanosciences

Second Year PhD Exam

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National Enterprise for nanoScience and nanoTechnology

NEST

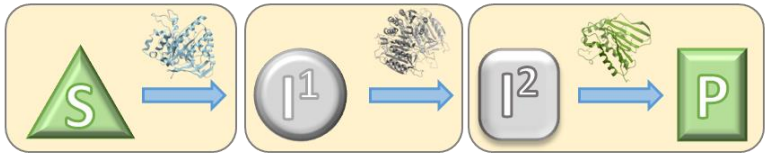
PhD Project:

3D Nano-bioarchitectures for biocatalytic cascades

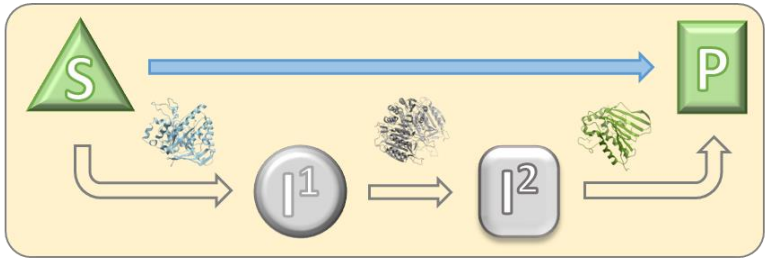
Biocatalytic cascades: multi-enzyme one-pot reactions

- ✓ Improved process → no isolation of intermediates
- ✗ Co-immobilization of multiple enzymes needed

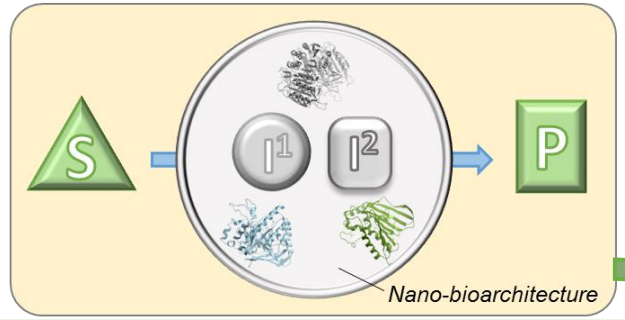
Sequential biocatalytic reaction

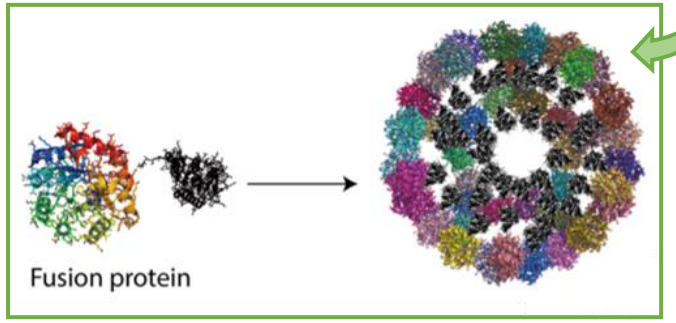


Multi-enzymatic cascade



Multi-enzymatic cascade in nanobioreactor





Nano-bioarchitectures as scaffolds

- ✓ Protein-based scaffold → simultaneous production of catalyst and support
- ✓ Compartimentalization and spatial organization of enzymes → high local concentrations of substrates

Objective of the project

1. Development of a novel protein-scaffold → 3D nano-bioarchitecture
2. Application to organic synthesis → biocatalytic cascades

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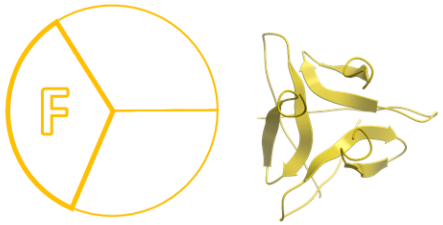
1. Nano-bioarchitecture

Design of the protein cage

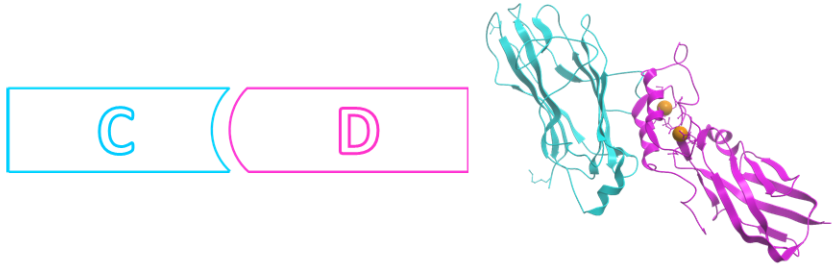
WILD-TYPE PROTEINS

FIBRITIN



Trimer

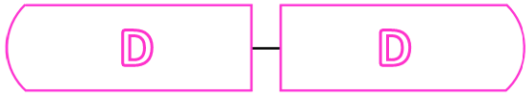
COHESIN-DOCKERIN



Ca^{2+}-mediated dimer

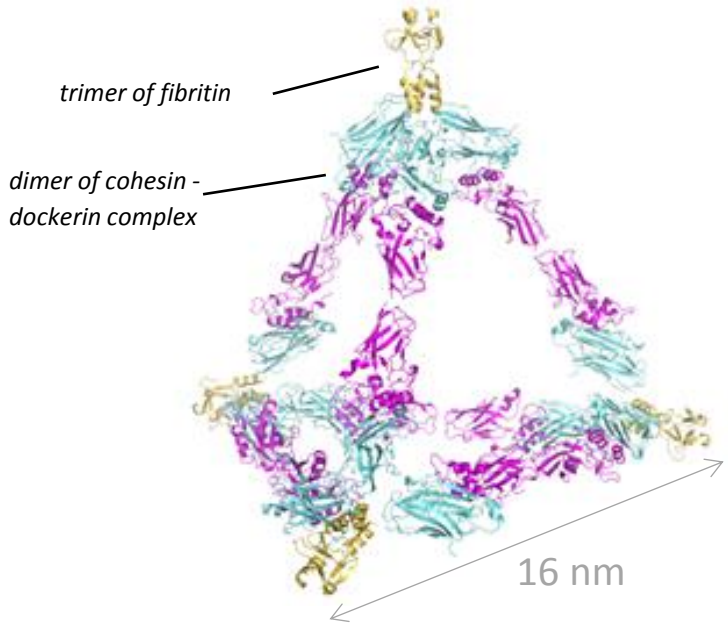
NANO-BUILDING BLOCKS (FUSION PROTEINS)

Vertex-building blocks



Edge-building blocks

3D-NANOBIOARCHITECTURE



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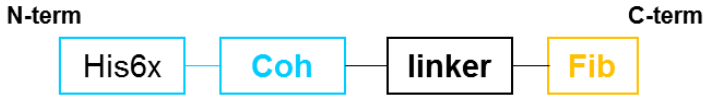
1. Nano-bioarchitecture

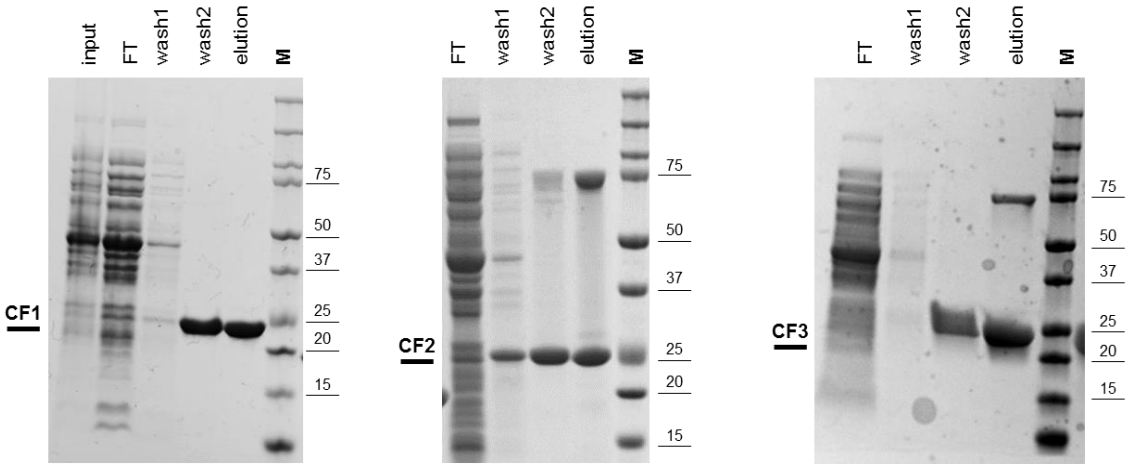
Synthesis of the nano-building blocks

Vertex BBs



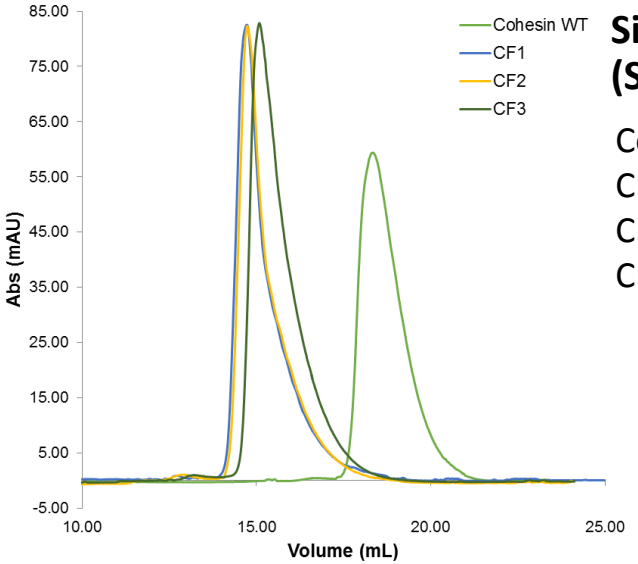
CF1: linker 12 aa
CF2: linker 9 aa
CF3: linker 2 aa

Purification of vertex BBs



✓ High yield 30-50 mg/L of colture
✓ Very pure protein after first purification

Trimer formation

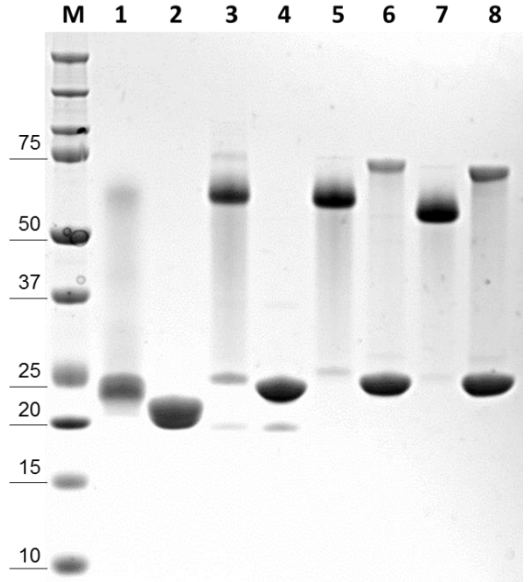


Size Exclusion Chromatography (SEC) on:

Cohesin WT: 20.52 kDa
CF1: 24.69 kDa (trimer: 74.07 kDa)
CF2: 24.36 kDa (trimer: 73.08 kDa)
CF3: 23.65 kDa (trimer: 70.95 kDa)

SDS PAGE

1: Cohesin WT (nonboiled)
2: Cohesin WT (boiled)
3: CF1 (nonboiled)
4: CF1 (boiled)
5: CF2 (nonboiled)
6: CF2 (boiled)
7: CF3 (nonboiled)
8: CF3 (boiled)

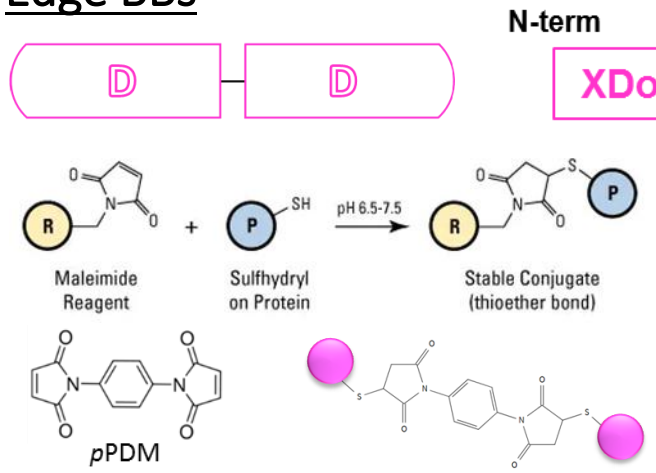


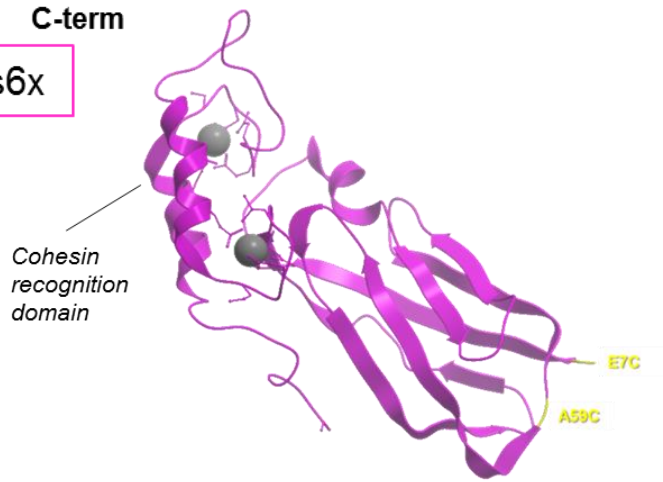
✓ Formation of highly stable trimer

1. Nano-bioarchitecture

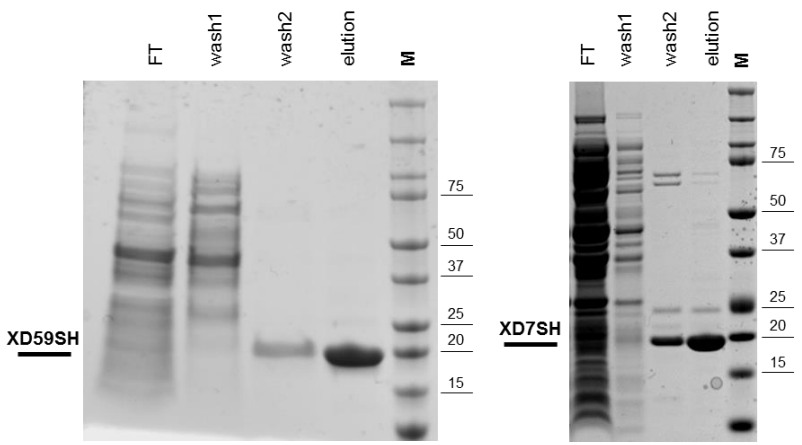
Synthesis of the nano-building blocks

Edge BBs

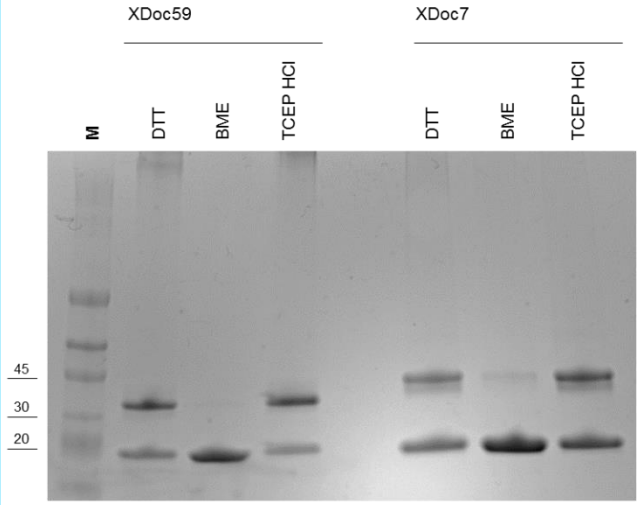




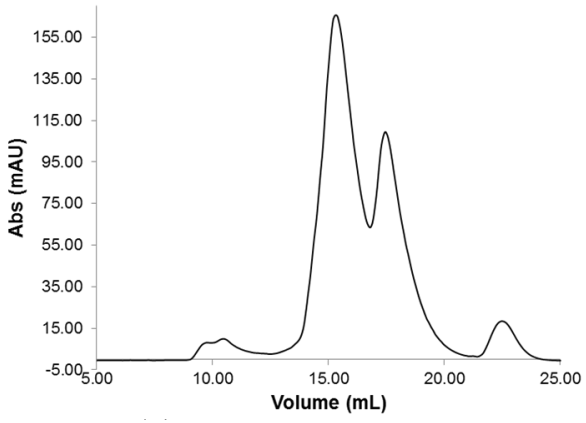
Purification of XDoc59SH and XDoc7SH



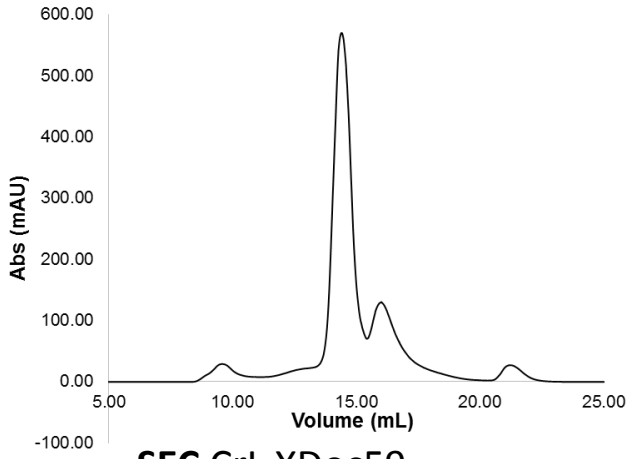
Cross-linking reaction to form bis-XDockerins



MW: XDoc59SH 18.83 kDa; BD59 37,66 kDa; XDoc7SH 18,77 kDa; BD/ 37,54 kDa



SEC CrL XDoc7



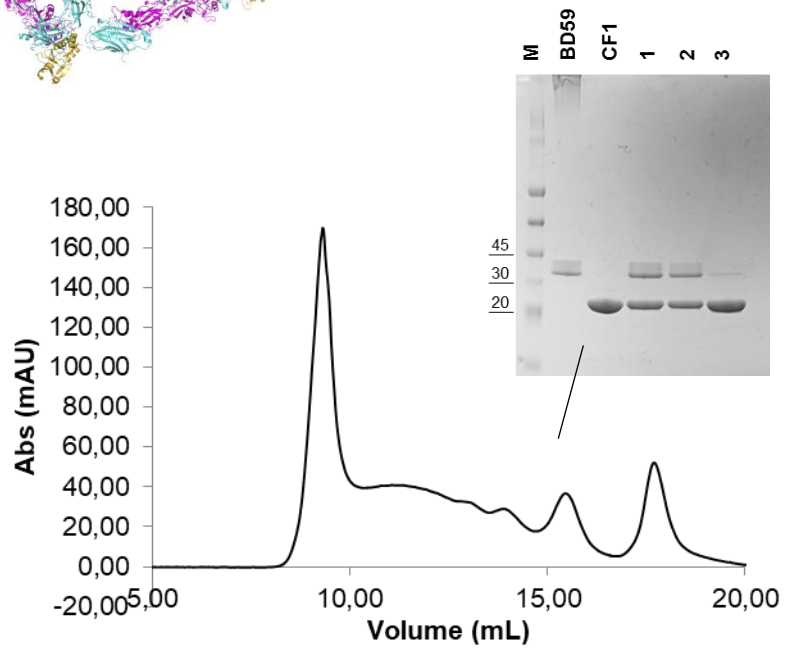
SEC CrL XDoc59

- Many cross-linking conditions tested: pH, T, buffer, additives
- Critical parameter: reducing agent
- Different reactivity of the two thiols
- ✓ XDoc59SH good cross-linking

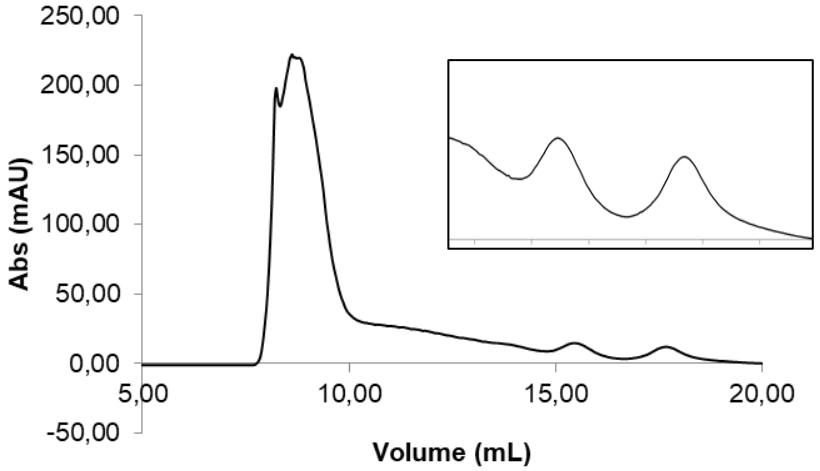
1. Nano-bioarchitecture

Assembly protein cage

- **Equilibrium forms** orbserved
- Many assembly conditions tested: pH, T, buffer, additives, concentration, addition order, different nano-BB
  → no significant variability
- Critical parameter: low concentration



SEC Low concentration



SEC High concentration

Characterization of the observed forms is underway through:

- Negative staining (Cryo-EM)
- ✓ Crystallization → crystals obtained

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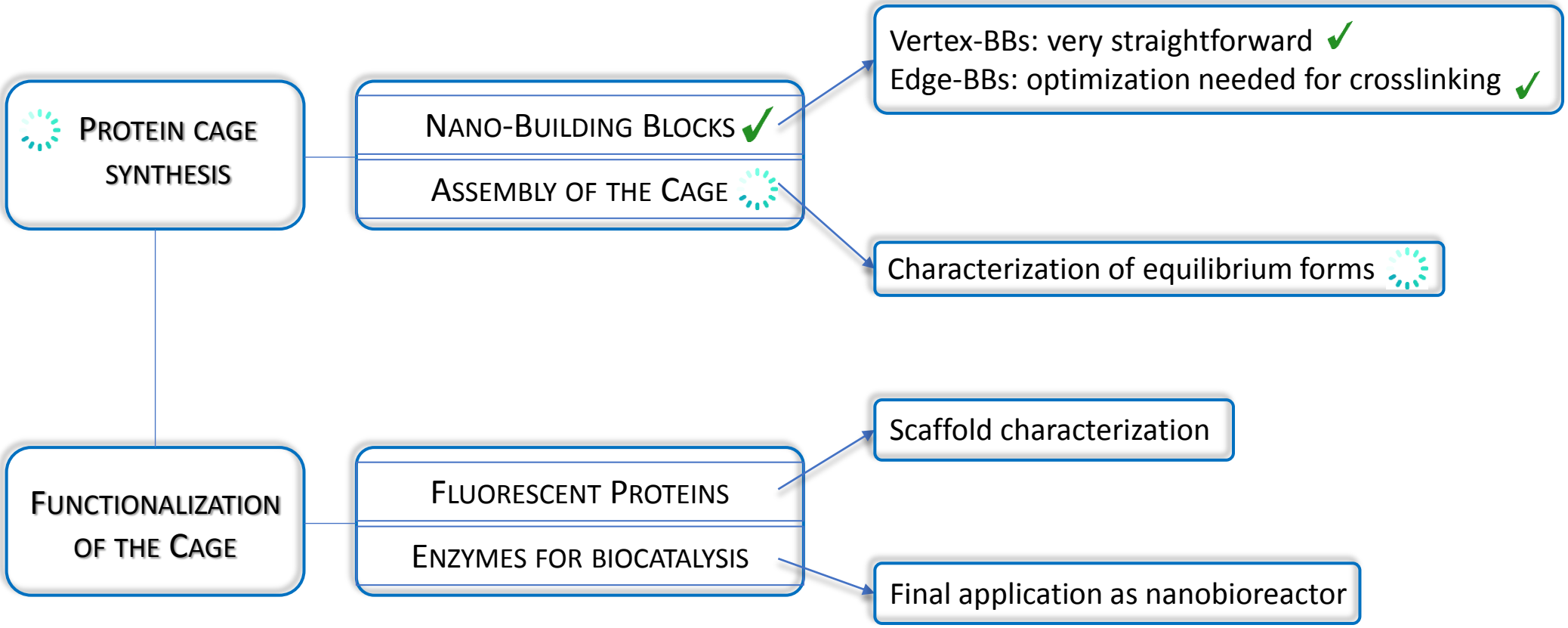
Conclusions and Outlook



Courses and Conferences

Attended courses	Hours
Ciclo di seminari – Biophysical Sciences (Exam on 24/10/19)	45
Topics in Structural Biology	20

Attended Seminars and Workshops	Hours
Seminars	12
Workshop (ERC Starting Grant SLaMM Workshop) “Advanced theranostic nanomedicine in oncology”	10
Advanced Course (Rome) “Trends in Enzymology and Biocatalysis”	26
Congress (NEST meeting) “Highlights in Nanoscience”	16

Thanks for your attention

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