Binary Protein Crystals for the Assembly of Inorganic Nanoparticle Superlattices

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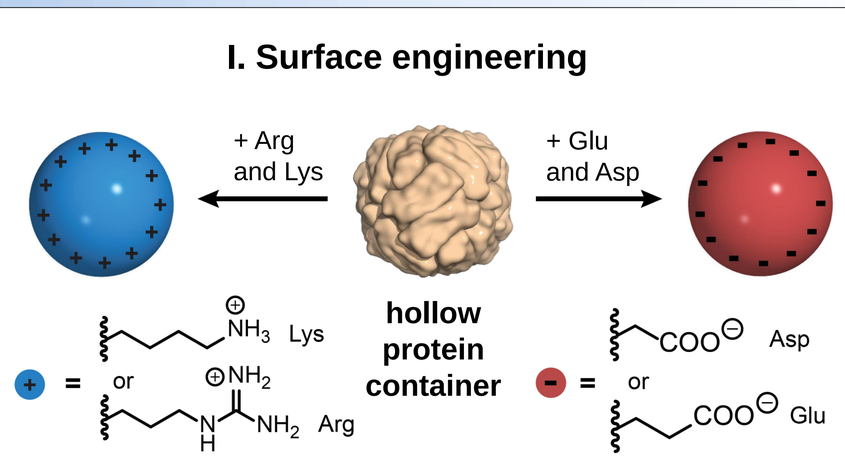
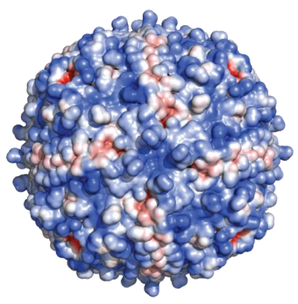
General strategy

- protein containers as templates for the organization of nanoparticles
- generate binary structures with crystalline order
- future application in catalysis, sensing, optoelectronics



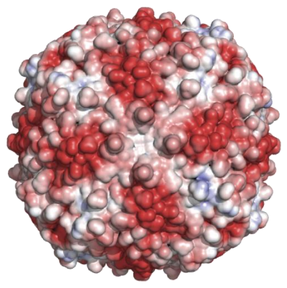
Surface engineering of human ferritin



Ftn(pos)

9 mutations per subunit

24 subunits Ø ~12 nm



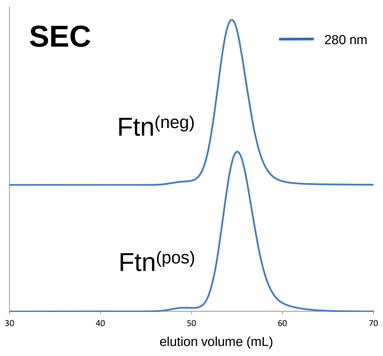
Ftn(neg)

4 mutations per subunit

- ***in silico* protein design** with the Rosetta fixbb module
- overexpression in *E. coli*
- elaborate purification protocol

Characterization:

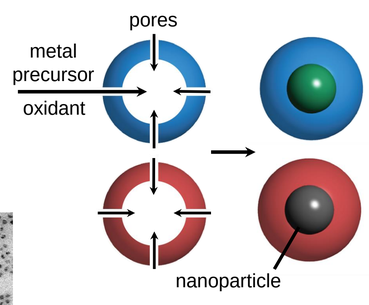
- mass spectrometry
- SDS/native PAGE
- circular dichroism spectroscopy
- TEM/DLS



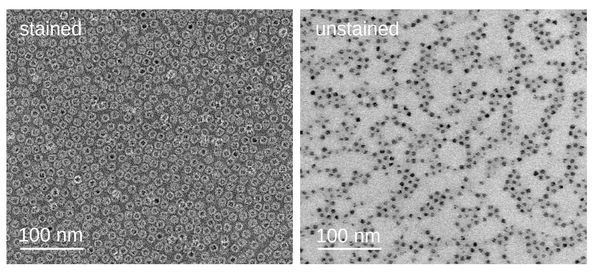
In situ nanoparticle synthesis

a) $2\ Ce^{3+} + H_2O_2 + 2\ H_2O \xrightarrow{65\ ^\circ C} 2\ CeO_2 + 6\ H^+$

b) $3\ Co^{2+} + H_2O_2 + 2\ H_2O \xrightarrow{65\ ^\circ C} Co_3O_4 + 6\ H^+$



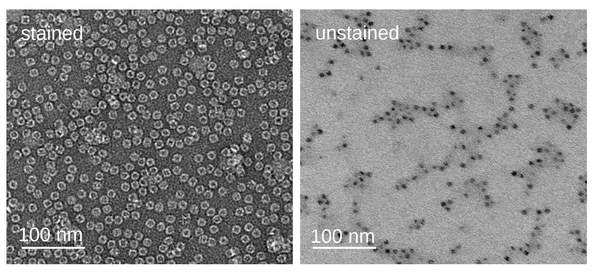
CeO_2 nanoparticles in $Ftn^{(pos)}$



TEM

Protein shell size	NP core size
12.2 ± 0.7 nm	5.8 ± 0.9 nm

Co_3O_4 nanoparticles in $Ftn^{(neg)}$



Protein shell size	NP core size
12.3 ± 0.7 nm	5.6 ± 1.1 nm

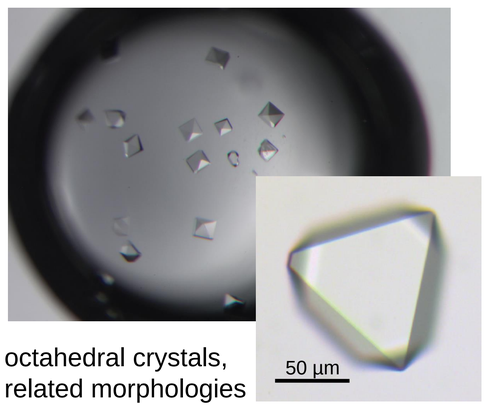
Size-constrained synthesis

Conclusion

In summary, we produced binary superlattices of inorganic nanoparticles by exploiting electrostatic interactions between engineered protein containers. Importantly, the protein shell determines the structure of the assembly whereas functionality can be readily imparted by the choice of cargo, e.g., nanoparticles, enzymes, small molecules, or a combination of these. By varying the lattice parameters with different crystallization conditions, multifunctional biohybrid materials with tunable structures could be accessible.

Crystallization trials and structure determination

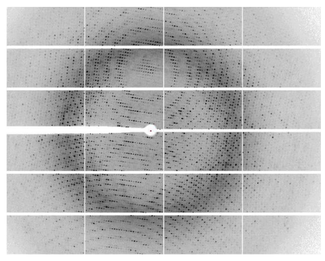
- protein crystallization screening with **empty** ferritin
- crystal optimization in manual plate setup



octahedral crystals, related morphologies

Single crystal X-ray diffraction

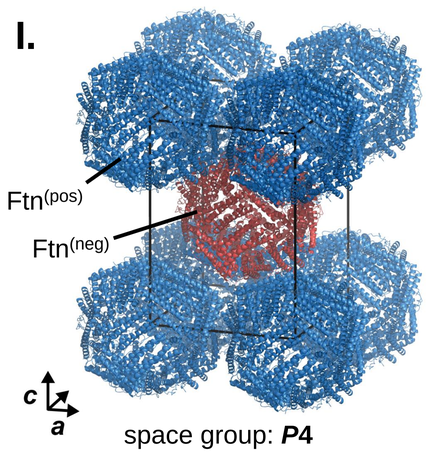
resolution 1.8 - 1.9 Å



PX-I and PX-III SLS, Villigen, CH

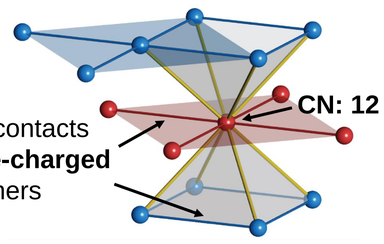
Lattice parameters controlled by crystallization conditions

I.



space group: ***P4***

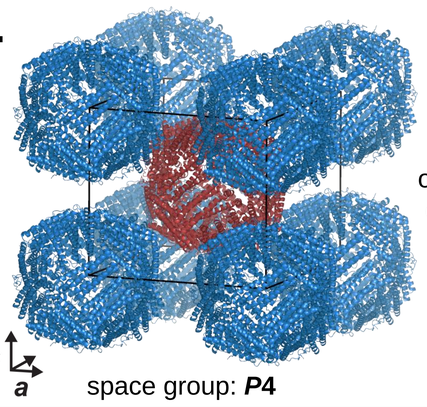
Tetragonal binary protein structure



additional contacts between **like-charged** containers

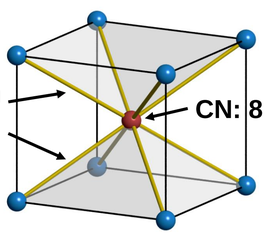
cell parameters: *a* = 126.6 Å, *c* = 174.9 Å

II.



space group: ***P4***

Same space group, different coordination



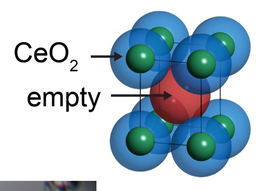
only contacts between **oppositely-charged** containers

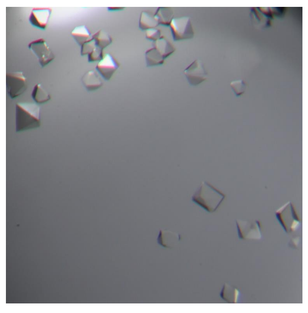
cell parameters: *a* = 153.3 Å, *c* = 135.8 Å

Protein-nanoparticle assembly and characterization

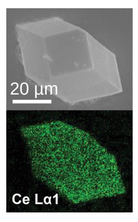
Unitary nanoparticle lattice

(A)

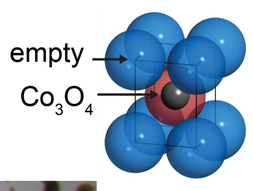


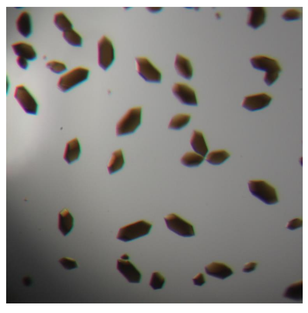


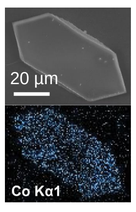
SEM/EDX



(B)

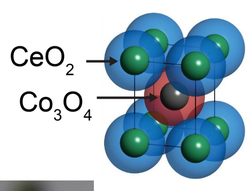


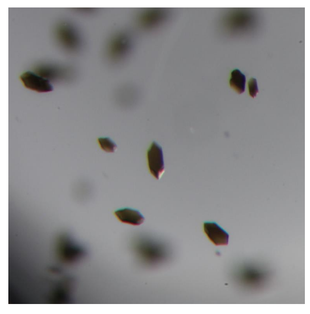


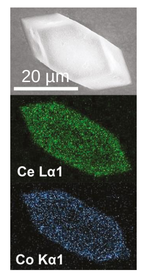


Binary nanoparticle lattice

(C)







Small-angle X-ray scattering (SAXS)

Large domain sizes, crystal structure independent from nanoparticle cargo

References

- Künzle, M., Eckert, T. & Beck, T.: 'Binary Protein Crystals for the Assembly of Inorganic Nanoparticle Superlattices' *J. Am. Chem. Soc.* **2016**, *138*, 12731-12734.
- Beck, T., Tetter, S., Künzle, M. & Hilvert, D.: 'Construction of Matryoshka-Type Structures from Supercharged Protein Nanocages' *Angew. Chem. Int. Ed.* **2015**, *54*, 937-940.

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