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Terminal supraparticle assemblies from similarly charged protein molecules and nanoparticles

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1. Introduction

- Supraparticles (SPs) is a type of protein-nanoparticles (NPs) hybrid structures represents a case of stable self-limited terminal assemblies.
- Be a potential analytical[1] and drug delivery tool[2].
- Integrate biological functions of proteins with optical / electrical properties of metallic / semiconducting materials.
- Geometrical motifs for assemblies between NPs and biomacromolecules were limited because size and shape of SPs assemblies could not be controlled.
- Terminal assemblies, which could only be formed with inherent size restriction, were not known for hybrid NP–biomacromolecule systems because of small number of particles, bad uniformity or stability.

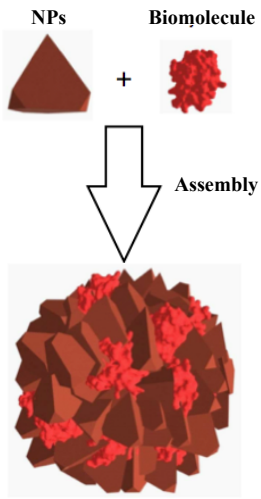


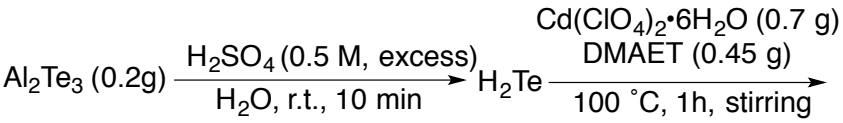
Fig.1 Terminal assembly of SPs.

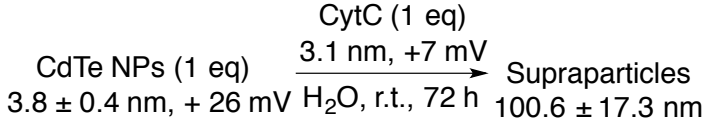
This work:

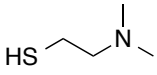
- Preparation of a new type of protein–NP hybrid structures, SPs, by the balance of attractive and repulsive forces between the building blocks. It spontaneously assembles from CdTe NPs and cytochrome C (CytC).

2. Results and Discussion

Preparation of SPs:





DMETA: 2-(dimethylamino) ethanethiol 

2.1 Self-assembly of CdTe NPs and CytC

- Uniformly sized, spherical SPs with a TEM diameter of 94 ± 5.6 nm when the ratio of CdTe and CytC is 1:1 (*Fig. 2*).
- When CdTe NPs or CytC is excess, SPs aggregate.

➢ A specific tendency of CdTe-CytC pairs to form spheres

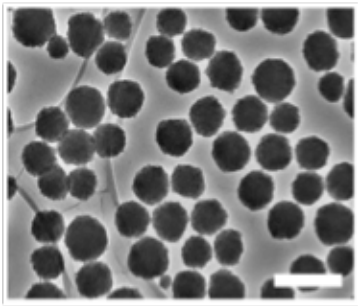


Fig. 2 TEM image of CdTe/CytC SP. Scale bar: 200 nm.

2.2 Supraparticle characterization

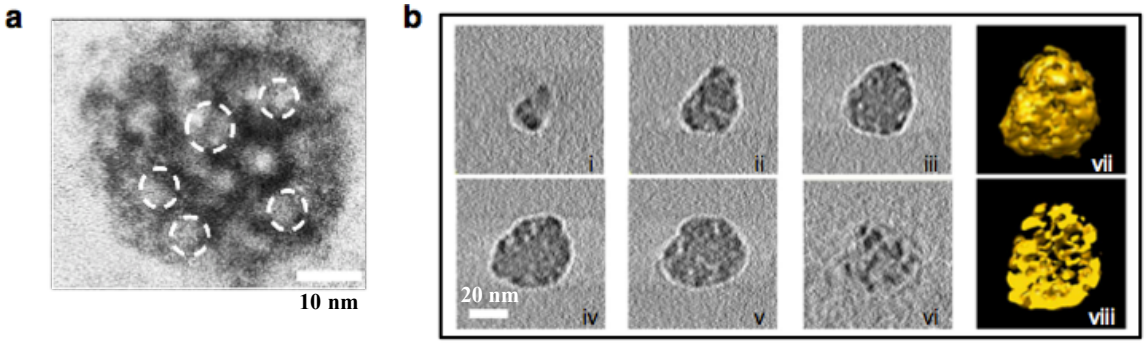


Fig. 3 (a) High-resolution TEM (HR-TEM) image of the SP. (b) TEM tomographic reconstructions of CdTe / CytC SP: X-Y slices (i-vi) of the SP, shown in every 4.8 nm through the volume. 3D surface rendering(vii) and cross-section (viii) of the SP.

- Consists of reticulated electron-transparent and electron-dense areas (*Fig. 3a*). These areas interpenetrated each other to form a network of tightly interconnected NP-CytC network (*Fig. 3b*).
- Peak shifted from 409 nm to 415 nm in UV (*Fig. 4*) and it indicated a change in the oxidation state of the haem group in the protein from Fe^{3+} to Fe^{2+} upon SPs assembly.

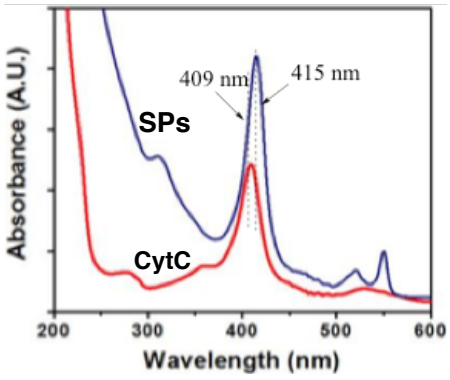


Fig. 4 UV-Vis spectra of CytC and SPs.

- After assembly with NPs, the proteins remained in a folded state otherwise the soret band around 400 nm would have blue shift or there would be the disappearance of the peaks in 500-600 nm region.

2.3 Assembly mechanism

2.3.1 Time dependend observation

- Wider size distribution in the early stages (24 h) (*Fig. 5 and 6b*) indicates the gradual emergence of SP with an equilibrium diameter.
- The initial increase and then decrease of DLS values (*Fig. 5*) show that large aggregates with a broad size distribution form quickly, and subsequently condense and stabilize in size by 72h.

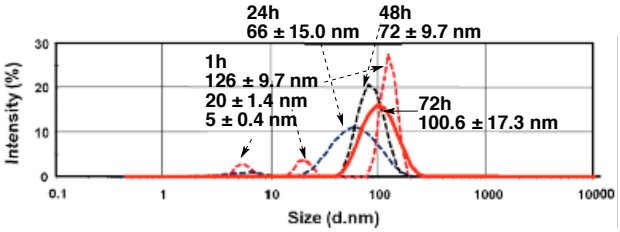


Fig. 5 DLS curves for particle size distribution for different times of self-assembly process between CdTe and CytC.

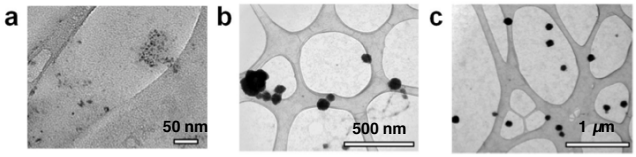


Fig. 6 CdTe/CytC SPs in the course of the assembly 3 hrs (a), 24 hrs (b), and 72 hrs (c).

2.3.2 Simulation

- The effective non-covalent interactions between NPs and CytC are described by the empirical 12-6 Lennard-Jones potential (van der Walls interaction).
- When the ratio of NPs / CytC was 1:1, the simulation result matched well with the experiment data (*Fig. 8a*).
- When the inter-SP charge–charge repulsion was not considered the simulation model, the results are in disagreement with the experiments (*Fig. 8b*).
- When the SPs reach their terminal size they become spherical in shape.
- ➢ The assembly mechanism results from the balance between the net attractive forces between the NPs and CytC and their electrostatic repulsion.[3]

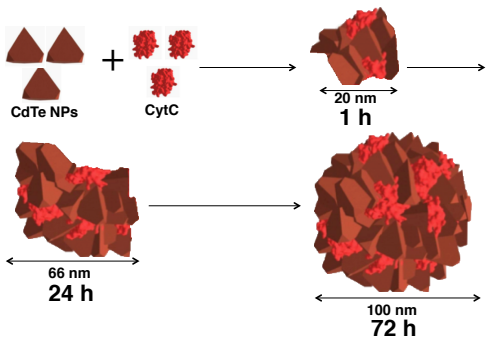


Fig. 7 Formation process of SPs.

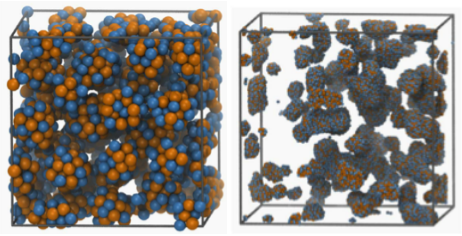


Fig. 8 (a) Spherical assemblies formed by a mixture of 2000 units; (b) Exactly the same to (a), but without considering the inter-SP charge-charge repulsion.

2.4 Photoenzymatic activity

$$NO_3^- + NADPH \xrightarrow[SPs + NRed,\ h\gamma]{} NO_2^-$$

NADPH: nicotinamide adenine dinucleotide phosphate; NRed: nitrate reductase.

- In the absence of light, the presence of the SPs has virtually no effect on the activity of the enzyme. (*Fig*. 9a, 1, 2 and 3)
- None of the control experiments shows enzyme activity as high as for the illuminated SP-NRed in the presence of NADPH. (*Fig*. 9a, 4 and 5)

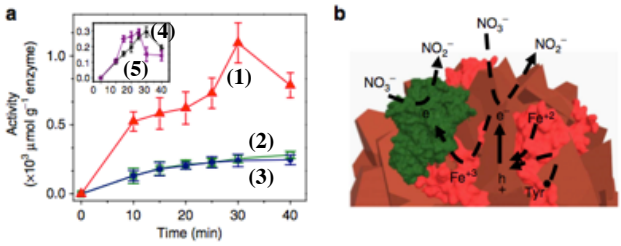


Fig. 9 (a) Formation of nitrite for SP-NADPH-NRed(1) excited at 470 nm and for NADPH-NRed(2) and SP-NADPH-NRed in dark(3). Inset: formation of nitrite for NADPH-NRed being excited at 470 nm in presence of only CdTe NPs(4) or CytC(5). (b) Schematics of the reactions on the photoexcitation of SP-NADPH-NRed.

- The SPs are essential for the photoenzymatic NO_3^- reduction because of electron transfer from NADPH-CdTe-CytC-NRed-NO_3^-. (*Fig*. *9b*)
- The assembled SP remains intact for 20 min of photoreaction. After 30 min, SP decomposes (confirmed by TEM) and activity decreases.

3. Conclusion

- Positively charged CdTe NPs and proteins self-organize into self-limiting SPs, following a pattern previously unseen for the individual components.
- It is the competition between electrostatic repulsion and non-colvalent attractive interactions (mainly affected by van-der-Walls interaction) that make the self-assemble occurred.
- It might open the door to a new diverse family of colloids and uncover unknown biological effects of NPs present in the environment.

References

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