論文の内容の要旨

Organofullerene Self-assembly for Electron Microscopy and Tomography
（有機フラーレン集合体形成と電子顕微鏡法および電子線トモグラフィー応用）

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1. Introduction

The structural analyses of nanomaterials are an essential process for developing their functionalization and application in various fields. Among various microscopic methods, transmission electron microscopy (TEM) is a powerful and versatile analytical tool for imaging specimens with high spatial resolution in three-dimensional spaces[1]. To display their inherent structures in isolated states without deformation nor degradation, the sample preparations should be specially cared[2]. To date, various methods such as drop cast and embedment in resin or vitrified water (cf. cryo-EM) are widely developed and applied to accomplish the above requirements. However, there is a lack of application of molecular assembly systems, which have an essential advantage on fine-tunability of nanoscale environments for specimen observation, due to their fragilities under high vacuum and electron beam irradiation. In my doctoral course study, various molecular assemble architectures are constructed with penta-substituted[60]fullerenes[3] having their tolerance against to electron beam, and their structural and chemical tunability were utilized to display target specimens in ideal isolated states and orientations for efficient structural analyses.

2. Self-assembly of organofullerene nanosphere incorporating specimens for electron tomography analysis

Electron tomography (ET) is a powerful method to reconstruct 3-D data of target nanomaterials without averaging the numerous numbers of specimens and be suitable for capturing the inherent structure. However, the image acquisition processes at various tilt angles in its procedure often suffer from decreasing the specimen contrast due to the increase of optical thickness of supporting materials. To tackle the issue, I presented the application of spherical supporting materials prepared from molecular assembly of organofullerenes and succeeded to collect specimen images in a constant contrast.

The method is simple that only requires the mixing of nanoparticle dispersions and organofullerene solutions in THF and DMF to prepare multiple-component nanospheres (Figure 1). Therefore, it is applicable for analysis of various specimens from inorganic nanoparticles to biomolecules including the virus, while typical preparation procedure of core-shell particles based on polymers required multiple steps and limit the applicable substrates to be incorporated. The key idea is an introduction of a rate control mechanism of nucleation and growth steps of molecular assembly. In this research, an equilibrium between neutral fullerene (**C7H**) and its potassium salt (**C7K**) in water was utilized and enable the formation of fullerene assemblies incorporating specimens from water/buffer.

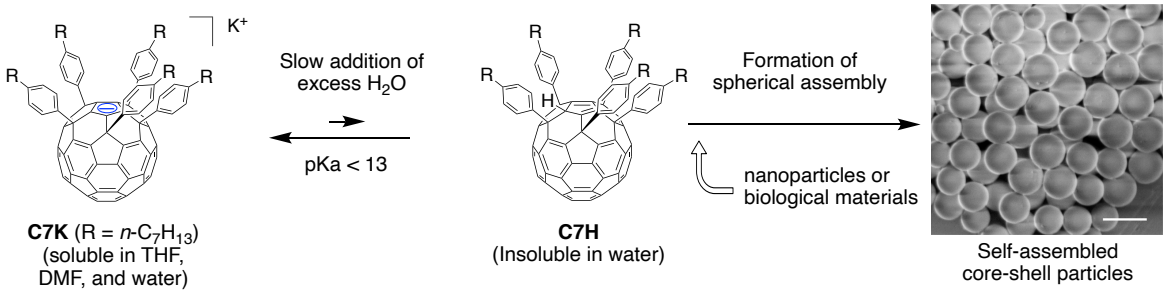

Figure 1. Self-assembled core-shell particle formation by sequestering **C7H** from unfavorable equilibrium with **C7K** in water. Scale bar: 1 μm.

A fullerene spherical assembly (fullersphere, FS) as a shell part of the multiple-component spheres was prepared through the investigation of five fullerene compounds (**CnH**, n = 0, 1, 7, and 13). While all suspensions prepared by water injection to their potassium salt (**CnK**, Figure 2a) in organic solvent indicate the existence of molecular assembly (Figure 2b), electron microscopic characterization revealed that the substrate introduced medium alkyl chains (**C7H**) as the best component to form FS (Figure 2e). The diameter of **C7H**-FS can be controlled from 30 nm to 2.5 μm by the selection of solvents and injection rate of water (Figure 2g).

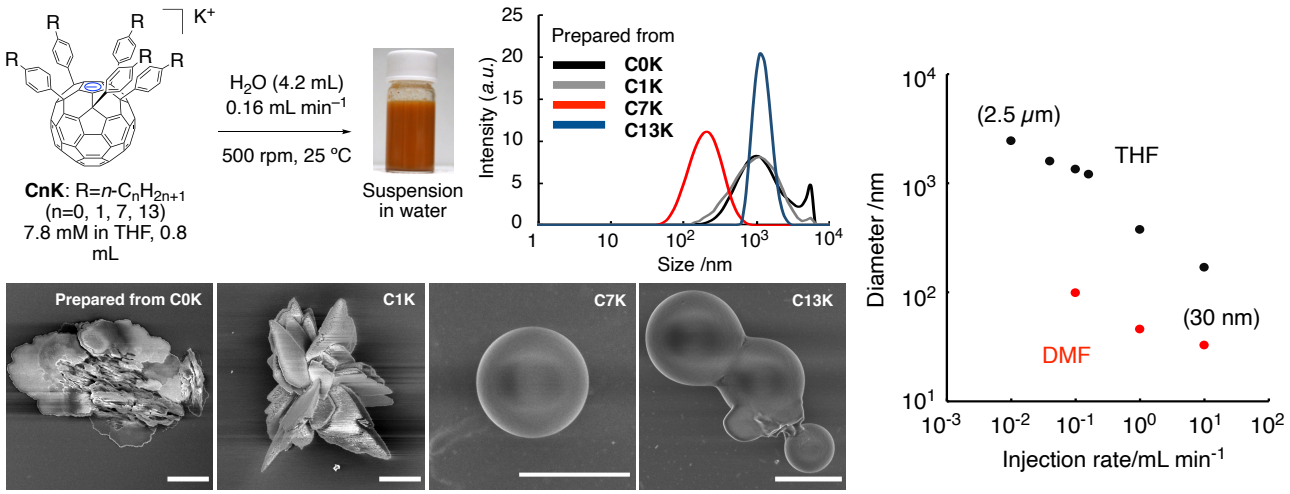

Figure 2. Formation of micro-assembly prepared from **CnK**. (a) A typical procedure to obtain a suspension of micro-assembly. (b) DLS data for the suspensions prepared from **CnK** are shown as intensity-weighted size distribution.

(c-f) STEM images of fullerene assemblies prepared from **CnK**. Scale bars: 1 μm. (g) A plot of average diameter of **C7H** -FS against injection rate of water.

The specimen having its denisty larger than that of FS (d = 1.25 g mL^{-1}) can be visualized by TEM (Figure 3a). Thus, the method is applicable for imaging various specimens from inorganic nanoparticles to biomolecules including the virus. To give an example of incormoration of gold nanoparticle (AuNP, 5 nm), the embedding ratio of the nanoparticles in the **C7H**-FSs were calculated to be 8-12% independent on their ligands charge when dispersion of the concentration of 5.0 x10^{16} particles/L were used. The individual particles were observed with TEM at a beam acceleration voltage of 80-200 kV. TEM images of 5.0 nm AuNPs show lattice fringes (2.4 Å) assignable to interplane distance of Au crystals with fcc structure (Figures 3b). Similarly, TEM images of 6 nm CdSe/ZnS quantum dots (QDs) also show lattice fringes (3.7 Å) assignable to interplane distance of CdSe as a QD core (Figure 3c). Note that the **C7H**-FS underwent no structural damage induced by electron beam irradiation without any conductive coatings or staining. During the above observations, the structure of **C7H**-FS did not collapse nor deform with total electron dose of 5 x 10^{7} e^{-} nm^{-2} that supply enough contrast for atomic-resolution images of lattice structures of the inorganic nanoparticles.

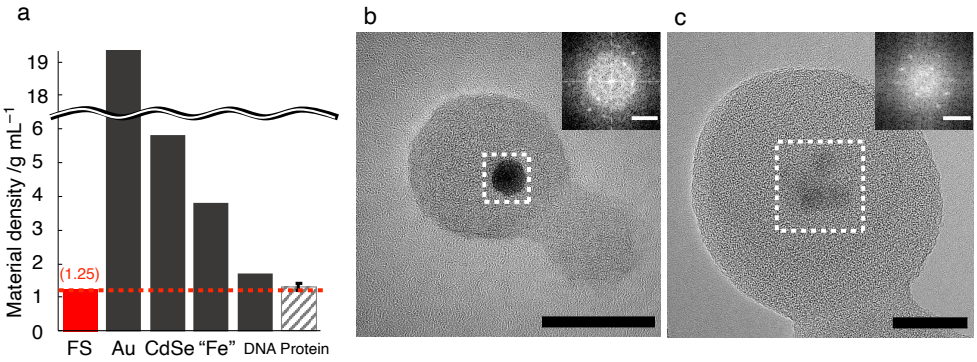

Figure 3. TEM images of NPs in C7H-FS. (a) The density of various materials compared with that of **C7H**-FS. "Fe" denotes ferrihydrite in ferritin. (b) TEM image of a single AuNP (5 nm,) in **C7H**-FS. (c) TEM image of three CdSe/ZnS QDs (6 nm) in **C7H**-FS. Insets indicate the FFT image corresponding to the nanoparticles. Scale bars: 20 nm for b and c, and 0.2 nm^{-1} for the insets.

Finally, we applied the multiple-component **C7H**-FS for ET of incorporated specimens by suspending individual spheres on the edge of holey carbon grids to obtain high tilt angle images without background contrast increase Upon deposition of **C7H**-FSs incorporating 5 nm AuNPs dispersed in water to a holey carbon grid, many spherical particles are found on the side edges of the holes. We chose a sphere which is located on the cross point of the rotating axis and the hole edge (Figure 4a) and collected a series of images with continuous rotation to ±60°. Figure 4b shows a high-tilt image of the **C7H**-FS with retaining its grey contrast, while the grid edge becomes dark with increasing tilt angles. A determination of 3-D coordinates of four AuNPs in this experiment (Figure 4c) ensuress the applicability of **C7H**-FS system for the structural analysis of more complexed structures in the future.

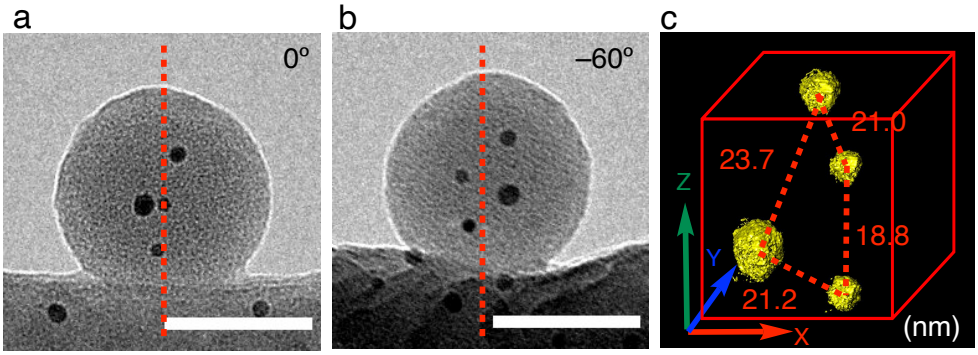

Figure 4. Electron tomography of AuNPs incorporated in **C7H**-FSs on the edge of holey carbon grids. (a) TEM image of four AuNPs@FS on an edge of a holey carbon film with an in-column omega energy filter. Red lines indicate tilt axis. Scale bar: 50 nm. (b) A –60° tilted TEM image of the specimen in a. (c) Reconstructed image of AuNPs@FS shown in a and b.

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4. 第 4 章

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5. 第 5 章

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6. Conclusion

During my doctoral course study, I prepared and applied molecular assemblies made of penta-substituted [60]fullerenes as platforms for observation of various nanomaterials. The achievement including the following: (1) Observation of nanoparticles in atomic resolution without agglomeration by fixing their isolated structures through embedment into fullerene amorphous particles. (2-4) Undisclosed. These results will lead new practical applications of molecular assembly systems into electron microscopic research. The author anticipates that further chemical derivatization of organofullerenes and development of the hierarchical molecular assembly systems based on this work would contribute the structural analyses of wider specimens in EM research. Besides, some concepts of supra molecular chemistry described in this thesis would be applicable beyond the organofullerene skeleton, and the present study is expected to become a milestone of opening the new application field of molecular assembly systems.

7. Reference

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3) Homma, T. *et al. J. Am. Chem. Soc.* **2011**, *133*, 6364-6370.