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Dynamic Process of Gold Nanoparticle Assembly using Fluorinated Surface Ligands in Solutions

(溶液中におけるフッ素化リガンド分子修飾金ナノ粒子の動的な自己集合プロセスに関する研究)

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Graduate School of Chemical Sciences and Engineering,
Hokkaido University

2016
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Chapter 1

Introduction
1.1 Background

Self-assembly is the spontaneous organization of the separated or linked components into ordered ensambles without human’s intervenion.\textsuperscript{1,2} Molecular self-assembly was extensively investigated in the past several decades. Molecules can spontaneously form 1D nanowire,\textsuperscript{3} 2D planer structure\textsuperscript{4} and 3D nanovesicles.\textsuperscript{5-7} For example, Kunitake \textit{et al.} firstly reported the formation of bio-membrane-like bilayer structure from a small organic molecule (didodecyldimethylammonium bromide).\textsuperscript{5} The building block of small molecule can be further extended to block copolymer and protein for the fabrication of nanovesicles.\textsuperscript{6,7} Molecular self-assembly provides a promising strategy to create a large variety of nanomaterials. The mechanism for molecular self-assembly generally includes two pathways (Scheme 1-1).\textsuperscript{8} Molecules can spontaneously form thermodynamically stable equilibrium structure via a reversible process. On the other hand, molecules can initially form kinetically trapped structure, and further transform into a thermodynamically stable structure via dynamic process of self-assembly utilizing the noncovalent interactions, including electrostatic interaction, van der Waals force, π effect and hydrophobic effect.\textsuperscript{9}

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\textit{Scheme 1-1. General pathways for molecular self-assembly. Reproduced from Whitelam \textsuperscript{8} with permission from American Physical Society.}
In recent years, self-assembly of metal nanoparticles (MNPs) based on the interactions, which are involved in the biological or molecular systems, has become a hot topic in the fundamental research.\textsuperscript{10,11} Because the size of MNPs is similar to that of the biomolecules. The surface of MNPs can be facially functionalized to be biocompatible. Therefore, MNPs are generally regarded as biomolecule-like NPs or function-like biomolecules.\textsuperscript{12} Especially, the biophysical interaction study between NPs and biomolecules, cell and organism has been widely explored recently.\textsuperscript{13} Aida \textit{et al.} found that the chaperonin proteins can enfold cadmium selenide (CdSe) NPs. The NPs in the cavity of protein complexes were released under the action of ATP.\textsuperscript{14} Wang \textit{et al.} reviewed the recent advances related to NPs with a biocompatible surface functionalization acting as enzyme-like activity, which were expected to be the next generation of artificial enzymes.\textsuperscript{15} Further, surface ligand coated NPs could self-assemble into multi-complex but highly ordered nano-architectures acting as a similar fashion to the self-assembly of molecules in some perspectives.\textsuperscript{16} So the fundamental understanding of the interactions involved in the self-assembly of MNPs can further reflect some perspectives of the biological self-assembly process in nature.\textsuperscript{11}

The ability to control dynamic process of self-assembly of MNPs into 1D, 2D and 3D assemblies depends on the understanding of the interactions between the MNPs (Figure 1-1).\textsuperscript{17,18} The attractive and repulsive interaction, namely competing interactions, are crucial for the controlled self-assembly of NPs.\textsuperscript{10,18-22} The attractive interaction is generally regarded as short range while the repulsive interaction is long range.\textsuperscript{17} Further, the anisotropic and isotropic characters of the competing interactions are also quite important for the understanding on the formation mechanism of the assemblies.\textsuperscript{11,19,21,23}
Figure 1-1. Surface functionalization and self-assembly of MNPs into 1D, 2D or 3D NP assembly.

Wang et al. found that isotropic surface functionalization of NPs also generated the anisotropic 1D NP chain assembly due to a profound anisotropic character of electrostatic repulsion together with a short range anisotropic dipolar interactions.\(^{19}\) Tang et al. found anisotropic character of electrostatic interactions and a directional hydrophobic attractions directed the self-assembly of CdTe nanocrystals into 2D free-floating sheets in solution.\(^{23}\) Cao et al. reported the formation of colloidal supraparticles (SPs) using the anisotropic interactions of CdSe-CdS nanorod under a thermodynamic equilibrium.\(^{24}\) Kotov et al. reported the formation of monodispersed SPs with narrow size distribution from poly-dispersed NPs with wide size distribution through a self-limited self-assembly process (Figure 1-2a).\(^{21}\) Van der Waals attraction drove the formation of initial
aggregation. Due to the negative charge provided by citrate anions on the surface of NPs, the dynamic renormalization of electrostatic repulsion of NPs or SPs finally balanced with van der Waals attraction. Finally, terminal assembly with certain size was formed.\textsuperscript{21,25} Further, both positively charged cytochrome C (Cyt C) protein and cadmium telluride nanoparticles (CdTe NPs) were reported to form terminal SPs by a counterbalancing electrostatic repulsion and the intermolecular attraction (Figure 1-2b).\textsuperscript{26} Kraus \textit{et al.} reported citrate coated gold nanoparticles (GNPs) with negative charge in the presence of hemoglobin (Hb) with positive charge produced stable hybrid cluster due to a multilayer coating of Hb on the surface of agglomerates (Figure 1-2c).\textsuperscript{27} Kumacheva \textit{et al.} reported the structure transitions of 3D globules or linear chains by tuning the delicate balance between the competing nanoscale interactions, including attractive hydrophobic and repulsive electrostatic interactions of positive charged GNPs factionalized with thiol-terminated polystyrene.\textsuperscript{22}

Figure 1-2. Formation of (a, b) SPs and (c) clusters by the-self-assembly of (a) CdSe NPs with negative charge. Reproduced from Kotov \textit{et al.}\textsuperscript{21} with permission from the Nature Publishing Group; (b) CdTe NPs with positive charge and Cyt C with positive charge. Reproduced from Kotov \textit{et al.}\textsuperscript{26} with permission from the Nature Publishing Group; (c) gold nanoparticles (AuNPs) with negative charge and hemoglobin (Hb). Reproduced from Kraus \textit{et al.}\textsuperscript{27} with permission from the American Chemical Society.
Compared with SPs, metal nanoparticle vesicle (MNV) with a hollow interior exhibited uniquely collective chemical and physical properties.\textsuperscript{28-45} Therefore, MNVs have attracted particular attention for their potential applications in imaging\textsuperscript{28}, catalysis,\textsuperscript{34} photothermal therapy\textsuperscript{42}, theranostics\textsuperscript{46} and drug delivery carrier.\textsuperscript{43,45,47} Fabrication of MNVs based on self-assembly of multi-complex polymer or small surface ligand coated NPs were developed respectively. Compared with multi-complex polymer, small surface ligand with a short length has the potential advantage over polymers to reduce the interparticle distance, thus inducing stronger coupling between NPs. Further, MNVs made by small surface ligand can potentially enlarge the interior space to increase the payload capacity. But the design of small surface ligand with variable interactions to self-assemble with NPs into MNVs still remains a challenging topic.

1.2 Objective

Our group firstly reported the formation of gold nanoparticle vesicles (GNVs) by the self-assembly of semi-fluorinated-oligo (ethylene glycol) ligand (SFL) tethered GNPs\textsuperscript{33,47}. But SFL can’t self-assemble with GNPs diameter larger than 20 nm or binary mixtures of GNPs to create multi-complex nanostructures for the extension of plasmonic applications. Therefore, my objectives in this thesis include:

1) creation of GNVs composed of single-sized GNPs with diameter larger than 20 nm in solution and investigation on their plasmonic coupling;
2) investigation on the dynamic process of GNVs for the study of formation mechanism of GNVs;
3) fabrication of hierarchical assemblies composed of GNPs with two different sizes.

To achieve these objectives, sugar-terminated fluorinated-oligo (ethylene glycol)
ligand (SUFL) with a neutral head, carboxylic acid-terminated fluorinated-oligo (ethylene glycol) ligand (CFL) with a weakly negative charge and sodium carboxylate-terminated fluorinated-oligo (ethylene glycol) ligand (SCFL) with a negative charge were designed and synthesized respectively based on our previously reported SFL. Glucose-terminated fluorinated-oligo (ethylene glycol) (GFL) was chosen as a representative SUFL for investigation. In chapter 2, the formation of GNVs composed of single-sized GNPs (15 or 30 nm in a diameter) in solution using GFL was demonstrated (Scheme 1-2). In chapter 3, formation of GNV composed of single-sized GNPs (15 or 30 nm in a diameter) using CFL in solution (Scheme 1-2), which was induced by electrostatic repulsive interaction, was disclosed. So the unique design of CFL or SCFL is based on the incorporation of a pair of competing interaction (attractive interaction and electrostatic repulsive interaction) into one single small molecule. In chapter 4, size-segregation of binary mixtures of GNPs in the presence of GFL or CFL into yolk/shell assembly in solution was discussed (Scheme 1-2). In chapter 5, the main conclusions in this thesis are included. Another important feature of the research in this thesis is that I am particularly interested in the self-assembly process of GNPs in solution and the formation of assemblies in solution.
Scheme 1-2. Chemical structure of the surface ligands and self-assembly with single-sized GNPs or binary mixtures of GNPs described in this thesis.
1.3 References


(38) Hickey, R. J.; Haynes, A. S.; Kikkawa, J. M.; Park, S.-J. Controlling the self-


(46) Liu, Y.; Yin, J.; Nie, Z. Harnessing the collective properties of nanoparticle

Chapter 2

Formation of gold nanoparticle vesicles composed of single-sized gold nanoparticles in solution
2.1 Introduction

Gold nanoparticle vesicle (GNV) with a hollow interior, which was widely applied to encapsulate or release the substances in a controllable manner,\textsuperscript{1-4} has attracted particular attention for the potential applications in theranostics,\textsuperscript{5} imaging\textsuperscript{6} and biomedical area.\textsuperscript{4,7,8,9} Duan \textit{et al.} developed plasmonic GNVs, which were assembled from amphiphilic biocompatible polymer coated gold nanoparticles (GNPs), for encapsulating anti-cancer drugs, targeting cancer cell and controlled releasing drugs in acidic endocytic organelles under external stimulus, such as light\textsuperscript{2} or pH\textsuperscript{3}. Nie \textit{et al.} reported the formation of giant GNVs, which hydrodynamically drove the self-assembly of amphiphilic polymer tethered gold nanorod using a microfluidic device. The controlled release of the payload was achieved using irradiation of near-infrared (NIR) light (700 to 1000 nm).\textsuperscript{1} Recently, they reported that the extinction spectra of GNVs made by block copolymer tethered 40 nm GNPs gave two plasmonic peaks, resulting in a strong absorbance (743 nm) in the NIR range due to the occurrence of `plasmon hybridization’, which was used for the photothermal therapy for cancer treatment.\textsuperscript{7} Even though the fabrication and plasmonic application of GNVs based on the multi-complex polymers coated GNPs were reported, the dynamic process of self-assembly of GNPs or the formation of GNVs in solution was still limitedly understood. The fundamental understanding on the formation mechanism of GNVs in solution can enhance our ability to manipulate and extend their potential applications.

Our group reported that the small surface ligand semi-fluorinated oligo(ethylene glycol) (OEG) ligand (SFL) coated GNPs could spontaneously generate GNVs composed of single-sized GNPs without template,\textsuperscript{10} which could be further functionalized as drug delivery carrier.\textsuperscript{4} But SFL can’t self-assemble with GNPs larger than 20 nm for extending
their plasmonic applications. In this chapter, sugar-terminated fluorinated-OEG ligand (SUFL) was designed. Because sugar can provide stronger inter- and intra-molecular hydrogen bonding, thus providing stronger interactions than OEG. Glucose-terminated fluorinated-OEG ligand (GFL) was chosen as a representative SUFL for the discussion. GFL can not only self-assemble with 15 nm GNPs in to GNVs, but also can spontaneously self-assemble with 30 nm GNPs into GNVs. The UV-vis absorption spectra of GNVs composed of 30 nm GNPs gave two plasmonic peaks, which were located at 550 and 700 nm respectively, resulting in a strong absorption in NIR region. This collective plasmon coupling between 30 nm GNPs on the surface of GNV was revealed by a finite-difference time-domain (FDTD) simulation. Importantly, the size of GNV could be controlled by varying the concentration of 30 nm GNPs, indicating that both kinetics and thermodynamics were involved in the formation of GNV. Time-dependent study of UV-vis, DLS and STEM measurements showed that quick aggregation and slow self-assembly process were involved in the formation of GNV in solution. A snap shot obtained from X-ray laser diffraction imaging clearly indicated that GNV composed of 30 nm GNPs was truly formed in solution rather than a drying process.

## 2.2 Experimental

### General information

All commercially available reagents were used without further purification. All air and moisture sensitive reactions were carried out in N₂-flushed round bottom flask sealed with rubber septa, and the reagents were injected with a syringe. Thin-layer chromatography (TLC) was performed on glass backed pre-coated silica gel plate (60F254, Merck & Co., Inc., USA). The reaction was monitored with TLC using Cerium molybdate (10% Cerium
(IV) sulfate, 15% aqueous sulfuric acid solution). Products were isolated by a column chromatography on silica-gel (Kanto Chemical, 60N, spherical, neutral, 40 - 50 μm). NMR spectra were acquired on JEOL spectrometer with 400 MHz for $^1$H NMR, 372 MHz for $^{19}$F NMR and 100 MHz for $^{13}$C NMR. Matrix-assisted laser desorption/ionization-time of flight mass spectra (MALDI-TOF MS) were measured using Voyager-DE STR-H (Applied Bio Systems) or Ultraflex-S (Bruker Daltonics) with 2,5-dihydroxybenzonic acid as matrix. High resolution electrospray ionization mass spectra (HR-ESI MS) were collected with an Exactive LCMS Mass Spectrometer (Thermo Fisher Scientific Inc., Japan) by Instrumental Analysis Division, Equipment Management Center, Creative Research Institution of Hokkaido University.

GNPs coated with citric acid in aqueous solution (5, 10, 15 or 30 nm in a diameter) were purchased from British Biocell International (BBI), Ltd. (Britain) and concentrated using a centrifuge CF-16RX (Hitachi-Koki, Ltd., Japan). Ultraviolet-visible (UV-vis) absorption spectra were measured with UV-2600/2700 (Shimadzu Corporation, Japan). Dynamic light scattering (DLS) analysis were measured with Delsa Nano HC (Beckman Coulter, Inc., Japan). The surface of NP assemblies was imaged based on the secondary electron mode of scanning transmission electron microscopy (SE-SEM) with an accelerating voltage of 200 kV or field-emission scanning electron microscopy (FE-SEM) with an accelerating voltage of 5 kV. The interior of NP assemblies was acquired using transmission electron mode of STEM (TE-STEM) with an accelerating voltage of 200 kV. Transmission electron microtomography was carried out using an electron microscopy (JEM-2200FS, JEOL Co., Ltd., Japan) with an accelerating voltage of 200 kV. Pulsed coherent X-ray solution scattering (PCXSS) experiment was performed using the Spring-8 angstrom compact free-electron laser (SACLA).
Self-assembly of GFL and GNPs

The aqueous dispersions of citric acid-coated GNPs (500 μL, 30 nm in diameter) were concentrated by centrifugation (8,600 rcf, 6 min). After removing the supernatant, concentrated GNPs (50 μL) was then added into dioxane solution of GFL (450 μL, 0.22 mM). The mixture was gently stirred (200 rpm) for 1 h. Samples for STEM measurement were prepared directly casting 3 μL assembly solution without purification onto TEM grid with high resolution carbon supporting membrane and dried at room temperature under N₂ for overnight.

Calculation of GFL-surface coverage on GNPs by inductively coupled plasma optical emission spectroscopy (ICP-OES)

A water-containing dioxane solution of GFLs-GNPs (30 nm in a diameter) was prepared at the same condition for GNV. The solvent was changed to water by repeated centrifugation of the GNPs to remove the free GFLs. The surface GFL coverage per one single GNP was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES). The surface number of Au atoms for each GNP was set according to the reference.¹¹

General procedure for microtome experiment

EPON 812 (4.6 mL), dodecenyl succinic anhydride (DDSA) (2.0 mL) and methyl nadic anhydride (MNA) (3.5 mL) were transferred to a glass bottle with flat bottom using a plastic syringe. The mixture was stirred at room temperature for 15 min. Subsequently, 2,4,6-tri(dimethylamino methyl) phenol (DMP) (200 μL) was added into the above solution and stirred for 30 min. The above mixture (100 ~ 200 μL) was homogeneously mixed with freshly prepared sample of GFL-Au-30 NP assemblies (see experimental) in
10% H$_2$O in dioxane (200 μL). The solvent was removed under vacuum at room temperature and continued to keep in vacuum condition for 1 ~ 2 h to remove the air as much as possible. The GFL-Au-30 NP assemblies could be successfully immobilized in epoxy resin at 60 ºC for 1 ~ 2 days. The sample after polymerization was sliced using a Leica EM UC7 ultramicrotome and glass knife. To avoid the deformation of GFL-Au-30 assemblies as much as possible during the slice process, the slice speed was set to 0.05 mm/s. After transferring the slice sections from the surface of water to TEM grid, the sample was dried under N$_2$ in the glove box for overnight. The surface of the slice sample was coated with carbon with a thickness of 5 nm and observed using scanning transmission electron microscope (STEM).

**Electron microtomography**

Transmission electron microtomography (TEMT) was performed using an electron microscopy (JEM-2200FS, JEOL Co., Ltd., Japan) with an accelerating voltage of 200 kV. TEMT experiment of a GFL-Au-15 NP assembly was carried out tilting the sample from -76° to 73° at an angular interval of 1°. The tilt series of projections were aligned using the Au-5 NPs as fiducial markers. After the alignment, 3D images were reconstructed using the filtered back projection algorithm. The 3D reconstructed image of a GNV composed of Au-15 NPs was reconstructed using Image J software.
Synthesis of glucose-terminated fluorinated-oligo(ethylene glycol) ligand 6

Scheme 2-1. Synthetic route of glucose-terminated fluorinated-oligo(ethylene glycol) ligand 6.

10-Undecene-1-tosylate, 1

Compound 1 was synthesized according to our previous report.\textsuperscript{10} \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): $\delta$ / ppm = 1.22 – 1.37 (m, 13H), 1.59 – 1.66 (m, 2H), 2.00 – 2.05 (m, 2H), 2.45 (s, 3H), 4.01 (t, 2H, $J$ = 6.4 Hz), 4.91 – 5.02 (m, 2H), 5.76 – 5.86 (m, 1H), 7.34 (d, 2H, $J$ = 8.1 Hz), 7.79 (d, 2H, $J$ = 8.1 Hz).

2,2,4,4,5,5,7,7,8,8,10,10-dodecafluoro-3,6,9,12-tetraoxatricos-22-en-1-ol, 2

Compound 2 was synthesized according to our previous report.\textsuperscript{10} \textsuperscript{1}H NMR (400 MHz,
CDCl$_3$): $\delta$ / ppm = 1.26 – 1.40 (m, 13H), 1.56 – 1.60 (m, 3H), 2.01 – 2.03 (m, 2H), 2.31 (t, 1H, $J = 5.0$ Hz), 3.59 (t, 2H, $J = 4.6$ Hz), 3.79 (t, 2H, $J = 6.0$ Hz), 3.90 – 3.93 (m, 2H), 4.91 – 4.93 (m, 2H), 5.77 – 5.81 (m, 1H). $^{19}$F NMR (372 MHz, CDCl$_3$): $\delta$ / ppm = -77.48, -77.83, -80.31, -88.44, -88.63.

(2R,3R,4S,5R,6R)-6-(acetoxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl tetraacetate, 3

Acetic anhydride (60 mL) was added to a solution of D-(+)-glucose (5.4 g, 0.03 mol) in pyridine (60 mL). The mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, and the residue was purified using column chromatography (CHCl$_3$/ethyl acetate, 4/1, v/v) to give compound 3 (10.63 g, 90.9%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ / ppm = 2.00 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 2.08 (s, 3H), 2.17 (s, 3H), 4.06 – 4.11 (m, 2H), 4.25 (dd, 1H, $J = 4.1$ Hz), 5.02 – 5.16 (m, 2H), 5.46 (t, 1H, $J = 10$ Hz), 6.29 (d, 1H, $J = 8.7$ Hz). MALDI-TOF Mass (m/z): [M+Na] calcd for C$_{33}$H$_{44}$F$_{12}$NaO$_{14}$, 413.33; found, 414.08, [M+Na]; [M+K] calcd for C$_{33}$H$_{44}$F$_{12}$KO$_{14}$, 429.33; found, 430.07.

(2R,3R,4S,5R)-2-(acetoxymethyl)-6-((2,2,4,4,5,5,7,7,8,8,10,10-dodecafluoro-3,6,9,12-tetraoxatricos-22-en-1-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate, 4

Compound 2 (1.12 g, 1.99 mmol) and compound 3 (0.94 g, 2.40 mmol) were dissolved in 10 mL dehydrated CH$_2$Cl$_2$. The mixture was stirred at room temperature under N$_2$ protection. Boron trifluoride-ethyl ether complex (BF$_3$·Et$_2$O) (125 µL, 2.40 mmol) was quickly injected into the reaction system using a syringe and then left stirring for overnight at room temperature. Crushed ice was added to deactivate the unreacted BF$_3$, and concentrated under vacuum. The residue was purified using column chromatography (Ethyl acetate/hexane, from 1/5 to 1/4, v/v) to give compound 4 (0.15 g, 8.38%). $^1$H NMR
(400 MHz, CDCl$_3$): $\delta$ / ppm = 1.23 – 1.36 (m, 14H), 1.53 – 1.63 (m, 3H), 1.99 (s, 3H), 2.00 – 2.04 (m, 8H), 2.07 (s, 3H), 3.58 (t, 2H, $J$ = 6.6 Hz), 3.68 – 3.72 (m, 1H), 3.79 (t, 2H, $J$ = 10.0 Hz), 3.91 – 3.99 (m, 1H), 4.05 – 4.15 (m, 2H), 4.25 (dd, 1H, $J$ = 4.6 Hz), 4.62 (d, 1H, $J$ = 8.0 Hz), 4.89 – 4.92 (m, 1H), 4.93 – 5.00 (m, 1H), 5.05 (d, 2H, $J$ = 8.2 Hz), 5.08 (t, 1H, $J$ = 9.8 Hz), 5.19 (t, 1H, $J$ = 9.4 Hz), 5.74 – 5.82 (m, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ / ppm = 170.68, 170.23, 169.43, 169.32, 139.30, 125.48, 124.67, 122.65, 121.86, 119.84, 119.06, 114. 68, 114.13, 100.69, 73.18, 72.46, 72.23, 70.77, 70.01, 69.71, 69.40, 68.11, 67.22, 66.90, 66.58, 61.66, 34.00, 29.57, 29.53, 29.46, 29.37, 29.16, 28.97, 25.81, 20.73, 20.64, 20.27. $^{19}$F NMR (372 MHz, CDCl$_3$): $\delta$ / ppm = -77.52, -77.83, -88.41, -88.47, -88.66. MALDI-TOF Mass (m/z): [M+Na] calcd for C$_{16}$H$_{22}$NaO$_{11}$, 915.665; found, 916.150; [M+K] calcd for C$_{33}$H$_{44}$F$_{12}$KO$_{14}$, 931.773; found, 932.198.

(2R,3R,4S,5R)-2-(acetoxyethyl)-6-((2,2,4,4,5,5,7,7,8,8,10,10-dodecafluoro-25-oxo-3,6,9,12-tetraoxa-24-thiahexacosyloxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate, 5

Compound 4 (150 mg, 0.17 mmol), thiolacetic acid (64 mg, 0.84 mmol) and azobisisobutyronitrile (AIBN) (27 mg, 0.17 mmol) were dissolved in 2 mL dehydrated THF without stabilizer. The mixture was stirred under refluxing with a N$_2$ balloon for 4 h. After cooling to room temperature, the solution was concentrated under vacuum. The residue was purified by column chromatography (Ethyl acetate/hexane, 1/4, v/v) to give compound 5 (110 mg, 67.5%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ / ppm = 1.22 – 1.33 (m, 16H), 1.53 – 1.60 (m, 5H), 1.99 – 2.03 (m, 10H), 2.08 (s, 3H), 2.30 (s, 3H), 2.84 (t, 2H, $J$ = 7.5 Hz), 3.58 (t, 2H, $J$ = 6.6 Hz), 3.68 – 3.71 (m, 1H), 3.79 (t, 2H, $J$ = 9.8 Hz), 3.92 – 3.99 (m, 1H), 4.05 – 4.15 (m, 3H), 4.25 (dd, 1H, $J$ = 4.6 Hz), 4.63 (d, 1H, $J$ = 8.0 Hz), 5.02 (t, 1H, $J$ = 8.0 Hz), 5.09 (t, 1H, $J$ = 9.8 Hz), 5.19 (t, 1H, $J$ = 9.6 Hz). $^{13}$C NMR (100
MHz, CDCl$_3$): $\delta$ / ppm = 196.15, 170.65, 170.20, 169.42, 169.29, 139.26, 125.47, 124.67, 122.66, 121.86, 119.83, 119.05, 114.63, 114.50, 114.16, 100.68, 73.16, 72.45, 72.21, 70.76, 69.99, 69.69, 69.38, 68.10, 67.21, 66.89, 66.57, 61.64, 30.67, 29.55, 29.49, 29.36, 29.19, 29.14, 28.86, 25.80, 20.70, 20.61, 20.25. $^{19}$F NMR (372 MHz, CDCl$_3$): $\delta$ / ppm = -77.48, -77.86, -88.35, -88.44, -88.66. MALDI-TOF Mass (m/z): [M+Na] calcd for C$_{35}$H$_{48}$F$_{12}$NaO$_{15}$S, 991.782; found, 991.959, [M+K] calcd for C$_{35}$H$_{48}$F$_{12}$KO$_{15}$S, 1007.891; found, 1007.965.

(3R,4S,5S,6R)-2-((2,2,4,4,5,5,7,7,8,8,10,10-dodecafluoro-23-mercapto-3,6,9,12-tetraoxatricosyl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol, 6

A mixture of compound 5 (110 mg, 0.11 mmol), 28% sodium methoxide in methanol (30 µL, 0.58 mmol) were dissolved in 500 µL dehydrated MeOH. After the mixture were stirred overnight at room temperature, Dowex (50WX8-200) resin was then added for neutralization. After filtration, the resulting solution was evaporated under vacuum to yield compound 6 (74 mg, 86.0%). $^1$H NMR (400 MHz, d$_3$-MeOH): $\delta$ / ppm = 1.25 – 1.40 (m, 14H), 1.54 – 1.80 (m, 4H), 2.46 (t, 1H, $J = 7.3$ Hz), 2.66 (t, 1H, $J = 7.3$ Hz), 3.18 – 3.38 (m, 6H), 3.57 (t, 2H, $J = 6.4$ Hz), 3.61 – 3.68 (m, 1H), 3.88 (t, 3H, $J = 9.8$ Hz), 4.06 – 4.14 (m, 1H), 4.23 – 4.32 (m, 1H), 4.36 (d, 1H, $J = 7.8$ Hz). $^{13}$C NMR (100 MHz, d$_3$-MeOH): $\delta$ / ppm = 125.86, 125.42, 123.06, 122.62, 120.25, 119.81, 114.70, 114.13, 113.75, 113.72, 112.30, 104.17, 78.23, 77.88, 74.74, 73.82, 71.39, 70.66, 70.36, 70.05, 68.17, 67.86, 67.56, 62.66, 39.77, 35.23, 34.87, 30.60, 30.42, 30.29, 30.19, 29.40, 26.91, 24.94. $^{19}$F NMR (372 MHz, d$_3$-MeOH): $\delta$ / ppm = -78.76, -89.47, -89.68, -89.90, -90.06. MALDI-TOF Mass (m/z): [M+Na] calcd for C$_{25}$H$_{38}$F$_{12}$NaO$_{10}$S, 781.599; found, 781.875, [2M+Na] calcd for C$_{50}$H$_{74}$F$_{24}$NaO$_{20}$S$_2$, 1538.192; found, 1538.914. HR-ESI Mass (m/z):
[M+Na] calcd for $\text{C}_{25}\text{H}_{38}\text{O}_{10}\text{F}_{12}\text{NaS}$, 781.18863; found, 781.18791, [2M+Na] calcd for $\text{C}_{50}\text{H}_{74}\text{O}_{20}\text{F}_{24}\text{NaS}_2$, 1537.37238; found, 1537.36975.
Synthesis of maltose-terminated fluorinated-oligo(ethylene glycol) ligand 10

![Scheme 2-2. Synthetic route of maltose-terminated fluorinated-oligo(ethylene glycol) ligand 10.](image)

Compound 7 was synthesized according to the experimental procedure of compound 3. Compound 7 (6.50 g, 48.2%) was obtained from maltose (7.2 g, 20 mmol), acetic anhydride (45 mL) and pyridine (45 mL) through a column chromatography (CHCl₃/ethyl acetate, 1:1, v/v). ¹H NMR (600 MHz, CDCl₃): δ / ppm = 1.96 – 2.00 (m, 12H, CH₃), 2.02 (s, 3H, CH₃), 2.07 (s, 6H, CH₃), 2.11 (s, 3H, CH₃), 3.75 – 3.83 (m, 1H), 3.86 – 3.94 (m, 1H), 3.96 – 4.06 (m, 2H), 4.14 – 4.26 (m, 2H), 4.41(dd, 1H, J = 2.5 Hz), 4.83 (dd, 1H, J = 4.1 Hz), 4.95 (t, 1H, J = 9.2 Hz), 5.04 (t, 1H, J = 9.8 Hz), 5.24 (t, 1H, J = 9.4 Hz), 5.33 (t, 1H, J = 10 Hz), 5.38 (d, 1H, J = 3.9 Hz), 5.71 (d, 1H, J = 8.2 Hz). MALDI-TOF
(m/z): [M+Na] calcd for C\textsubscript{28}H\textsubscript{38}NaO\textsubscript{19}, 701.58; found, 703.28, [M+K] calcd for C\textsubscript{28}H\textsubscript{38}KO\textsubscript{19}, 717.69; found, 719.99.

\((2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-(((2R,3R,5R,6R)-4,5-diacetoxy-2-(acetoxymethyl)-6-((2,2,4,4,5,5,7,7,8,8,10,10-dodecafluoro-3,6,9,12-tetraoxatricos-22-en-1-yl)oxy)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate, 8\)

Compound 8 was synthesized according to the experimental procedure of compound 4. Compound 8 (1.7 g, 72%) was obtained from compound 7 (1.63 g, 2.4 mmol), compound 2 (1.12 g, 2.0 mmol), BF\textsubscript{3}Et\textsubscript{2}O (theoretical: 113 µL, 2.2 mmol, experimental: 125 µL), dehydrated CH\textsubscript{2}Cl\textsubscript{2} (10 mL) through a column chromatography (Ethyl acetate/hexane, 1:2, v/v). \(^1\)H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta / \text{ppm} = 1.21 – 1.36 \text{ (m, 12H, C}_2\text{H}_2\), 1.50 – 1.63 \text{ (m, 2H, C}_2\text{H}_2\), 1.95 – 1.96 \text{ (m, 9H, CH}_3\), 1.98 \text{ (s, 3H, CH}_3\), 2.00 \text{ (s, 3H, CH}_3\), 2.06 \text{ (s, 3H, CH}_3\), 2.10 \text{ (s, 3H, CH}_3\), 3.55 \text{ (t, 2H, J = 6.4 Hz, OCH}_2\), 3.62 – 3.70 \text{ (m, 1H), 3.76 \text{ (t, 2H, J = 9.6 Hz, OCH}_2\), 3.88 – 4.08 \text{ (m, 3H), 4.16 – 4.22 \text{ (m, 2H), 4.46 \text{(dd, 1H, J = 2.8 Hz), 4.62 \text{(d, 1H, J = 7.8 Hz), 4.78 – 4.96 \text{ (m, 4H), 4.01 \text{(t, 1H, J = 9.6 Hz), 5.20 \text{(t, 1H, J = 9.2 Hz), 5.31 \text{(t, 1H, J = 10.0 Hz), 5.37 \text{(d, 1H, J = 4.1 Hz), 5.74 – 5.82 \text{ (m, 1H).}}\)

\(^{13}\)C NMR (100 MHz, CDCl\textsubscript{3}): \(\delta / \text{ppm} = 170.58, 170.56, 170.43, 170.16, 170.00, 169.57, 169.46, 139.24, 132.00, 131.80, 130.08, 125.60, 124.60, 123.65, 122.64, 121.81, 119.89, 119.00, 114.15, 100.14, 95.65, 74.97, 73.13, 72.47, 72.42, 71.63, 70.02, 69.97, 69.66, 69.30, 68.59, 68.01, 67.27, 66.95, 66.64, 62.48, 61.49, 33.84, 29.53, 29.49, 29.42, 29.34, 29.13, 28.94, 25.77, 20.88, 20.77, 20.70, 20.61, 20.58, 20.22. \(^{19}\)F NMR (372 MHz, CDCl\textsubscript{3}): \(\delta / \text{ppm} = -77.52, -77.86, -88.44, -88.66.\) MALDI-TOF MS (m/z): [M+Na] calcd for C\textsubscript{45}H\textsubscript{60}F\textsubscript{12}NaO\textsubscript{22}, 1203.915; found, 1204.469, [M+K] calcd for C\textsubscript{45}H\textsubscript{60}F\textsubscript{12}KO\textsubscript{22}, 1220.024; found, 1220.670.
(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-(((2R,3R,5R,6R)-4,5-diacetoxy-2-(acetoxymethyl)-6-((2,2,4,4,5,5,7,7,8,8,10,10-dodecafluoro-25-oxo-3,6,9,12-tetraoxa-24-thiahexacosyl)oxy)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triy triacetate, 9

Compound 9 was synthesized according to the experimental procedure of compound 5. Compound 9 (1.52 g, 89.0%) was obtained from compound 8 (1.60 g, 1.36 mmol), thiolacetic acid (0.52 g, 6.80 mmol), AIBN (220 mg, 1.36 mmol), THF (Super dehydrated, with stabilizer) (16 mL) through a column chromatography (Ethyl acetate/hexane, 1:2, v/v). $^1$H NMR (400 MHz, CDCl$_3$): δ / ppm = 1.20 – 1.38 (m, 16H, CH$_2$), 1.50 – 1.60 (m, 4H, CH$_2$, containing water), 1.98 – 2.03 (m, 15H, 3CH$_3$), 2.09 (s, 3H, CH$_3$), 2.13 (s, 3H, CH$_3$), 2.30 (s, 3H, CH$_3$), 2.84 (t, 2H, $J = 7.3$ Hz, SCH$_2$), 3.58 (t, 2H, $J = 6.6$ Hz, OCH$_2$), 3.68 – 3.71 (m, 1H), 3.79 (t, 2H, $J = 10.0$ Hz, OCH$_2$), 3.92 – 4.10 (m, 6H), 4.19 – 4.26 (m, 2H), 4.49 (dd, 1H, $J = 2.5$ Hz), 4.65 (d, 1H, $J = 7.6$ Hz), 4.82 – 4.86 (m, 2H), 5.04 (t, 1H, $J = 9.8$ Hz), 5.23 (t, 1H, $J = 9.0$ Hz), 5.34 (t, 1H, $J = 10.0$ Hz), 5.40 (d, 1H, $J = 3.9$ Hz). $^{13}$C NMR (100 MHz, CDCl$_3$): δ / ppm = 196.20, 170.63, 170.20, 170.05, 169.63, 169.51, 122.64, 121.81, 100.18, 95.67, 77.31, 75.00, 73.19, 72.50, 72.42, 71.66, 70.05, 69.72, 69.34, 68.62, 68.03, 67.32, 67.01, 66.69, 62.50, 61.52, 30.72, 29.57, 29.51, 29.39, 29.22, 29.17, 28.89, 25.82, 20.94, 20.83, 20.77, 20.68, 20.64, 20.29. $^{19}$F NMR (372 MHz, CDCl$_3$): δ / ppm = -77.49, -77.83, -88.41, -88.66. MALDI-TOF MS (m/z): [M+Na] calcd for C$_{47}$H$_{64}$F$_{12}$NaO$_{23}$S, 1280.033; found, 1280.443. [M+K] calcd for C$_{47}$H$_{64}$F$_{12}$KO$_{23}$S, 1296.141; found, 1296.424.

(3R,4S,5S,6R)-2-((2,2,4,4,5,5,7,7,8,8,10,10-dodecafluoro-23-mercapto-3,6,9,12-tetraoxatricosyl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol, 10
Compound 10 was synthesized according to the experimental procedure of compound 6. Compound 10 (0.7 g, 64.8%) was obtained from compound 9 (1.48 g, 1.18 mmol), 28% sodium methaoxide in methanol (719 µL). $^1$H NMR (400 MHz, d$^3$-MeOH): δ / ppm = 1.25 – 1.40 (m, 14H, $CH_2$), 1.50 – 1.65 (m, 4H, $CH_2$), 2.64 (t, 1H, $J = 7.3$ Hz), 3.22 – 3.29 (m, 4H), 3.36 – 3.44 (m, 2H), 3.45 – 3.52 (m, 3H), 3.56 – 3.68 (m, 7H), 3.75 – 3.80 (m, 2H), 3.88 (t, 3H, $J = 10$ Hz ), 4.06 – 4.11 (m, 1H), 4.22 – 4.28 (m, 1H), 4.39 (d, 1H), 5.14 (d, 1H, $J = 3.9$ Hz ). $^{13}$C NMR (100 MHz, CDCl$_3$): δ / ppm = 125.87, 125.38, 123.06, 122.58, 120.24, 119.77, 114.70, 114.50, 114.12, 102.84,101.57, 101.36, 79.67, 76.32, 75.56, 73.72, 73.46, 73.00, 72.79, 72.53, 70.11, 69.37, 69.06, 68.75, 66.97, 66.65, 66.35, 61.39, 60.77, 38.48, 29.35, 29.30, 29.13, 28.99, 28.89, 28.12, 25.62. $^{19}$F NMR (372 MHz, CDCl$_3$): δ / ppm = -78.79, -89.47, -89.68, -89.90, -90.06. MALDI-TOF MS (m/z): [M+Na] calcd for C$_{31}$H$_{48}$F$_{12}$NaO$_{15}$S, 943.739; found, 944.063, [2M+Na] calcd for C$_{62}$H$_{94}$F$_{24}$NaO$_{30}$S$_2$, 1862.473; found, 1863.139, [2M+K]; calcd for C$_{62}$H$_{94}$F$_{24}$KO$_{30}$S$_2$, 1878.582; found, 1879.133. HR-ESI MS (m/z): [M+Na] calcd for C$_{31}$H$_{48}$F$_{12}$NaO$_{15}$S, 943.24145; found, 943.24049, [2M+Na] calcd for C$_{62}$H$_{94}$F$_{24}$NaO$_{30}$S$_2$, 1861.47803; found, 1861.47687.
Synthesis of maltotriose-terminated fluorinated-oligo(ethylene glycol) ligand 14


\[(3R,4R,5R,6R)\cdot 6\cdot (\text{acetoxymethyl})\cdot 5\cdot (\{(2R,3R,5R,6R)\cdot 3,4\cdot \text{diacetoxy}\cdot 6\cdot (\text{acetoxymethyl})\cdot 5\cdot (\{(2R,3R,4S,5R,6R)\cdot 3,4,5\cdot \text{triacetoxy}\cdot 6\cdot (\text{acetoxymethyl})\cdot \text{tetrahydro-2H-pyran-2-yl} \} \cdot \text{oxy}) \cdot \text{tetrahydro-2H-pyran-2,3,4-triyl triacetate, 11}\]

Compound 11 was synthesized according to the experimental procedure of compound 3. Compound 11 was obtained from maltotriose (5.57 g, 11 mmol), acetic anhydride (20 mL) and pyridine (20 mL). Column chromatography (CHCl3/ethyl acetate, 1:1, v/v) to
give (6.54 g, 61.7%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ / ppm = 1.97 – 2.05 (m, 112H), 2.08 – 2.10 (m, 26H), 2.14 – 2.16 (m, 30H), 2.22 (s, 6H), 3.85 – 3.87 (m, 3H), 3.90 – 3.95 (m, 15H), 3.98 – 4.04 (m, 10H), 4.08 – 4.11 (m, 4H), 4.12 – 4.16 (m, 6H), 4.22 – 4.26 (m, 6H), 4.27 – 4.30 (m, 4H), 4.42 – 4.47 (m, 10H), 4.71 – 4.75 (m, 5H), 4.83 (d, 2H, $J = 4.1$ Hz ), 4.84 (d, 2H, $J = 4.1$ Hz), 4.93 – 4.95 (m, 2H), 4.96 (d, 2H, $J = 8.9$ Hz), 5.04 – 5.05 (t, 3H, $J = 4.1$ Hz), 5.06 – 5.08 (t, 2H, $J = 3.8$ Hz), 5.25 (t, 4H, $J = 4.1$ Hz), 5.28 (t, 4H,), 5.31 – 5.46 (m, 15H, ), 5.50 (t, 2H, $J = 10$ Hz), 5.73 (d, 3H, $J = 8.2$ Hz) 6.22 (d, 2H, $J = 3.8$ Hz). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ / ppm = 14.24, 20.48, 20.64, 20.71, 20.88, 20.94, 20.97, 21.08, 60.05, 61.36, 61.40, 62.18, 62.27, 62.59, 62.69, 67.91, 68.52, 69.09, 69.38, 70.11, 70.16, 70.47, 70.99, 71.69, 71.79, 72.10, 72.30, 72.50, 72.70, 72.96, 73.20, 73.48, 75.17, 88.87, 91.27, 95.63, 95.69, 95.95, 96.00, 168.85, 169.00, 169.50, 169.69, 169.86, 169.90, 170.03, 170.39, 170.54, 170.58, 170.66, 171.17. MALDI-TOF (m/z): [M+Na] calcd for C$_{40}$H$_{54}$NaO$_{27}$, 989.83; found, 992.13, [M+K] calcd for C$_{40}$H$_{54}$KO$_{27}$, 1005.94; found, 1008.26.

(2R,3R,5R,6R)-2-(acetoxy methyl)-6-(((2R,3R,5R,6R)-4,5-diacetoxy-2-(acetoxy methyl)-6-(((2R,3R,4S,5R)-4,5-diacetoxy-2-(acetoxy methyl)-6-((2,2,4,4,5,5,7,7,8,8,10,10-dodecafluoro-3,6,9,12-tetraoxatricos-22-en-1-yl)oxy)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate, 12

Compound 12 was synthesized according to the experimental procedure of compound 4. Compound 12 (0.60 g, 41%) was obtained from compound 11 (1.16 g, 1.20 mmol), compound 2 (0.56 g, 1 mmol), BF$_3$Et$_2$O (66.8 µL, 1.3 mmol) through a column chromatography (Ethyl acetate/hexane, 1:2, v/v). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ / ppm = 1.12 – 1.35 (m, 13H, CH$_2$), 1.48 – 1.55 (m, 2H, CH$_2$), 1.89 – 2.03 (m, 24H, 8CH$_3$), 2.05
(s, 3H, CH₃), 2.10 – 2.16 (m, 6H, 2CH₃), 3.50 – 3.60 (m, 2H), 3.62 – 3.77 (m, 3H), 3.85 – 4.35 (m, 10H), 4.43 (t, 1H, J = 7.3 Hz), 4.60 (d, 1H, J = 7.8 Hz), 4.65 – 4.72 (m, 1H), 4.78 – 5.10 (m, 5H), 5.15 – 5.25 (m, 2H), 5.26 – 5.40 (m, 3H), 5.71 – 5.81 (m, 1H). ¹³C NMR (100 MHz, CDCl₃): δ / ppm = 170.63, 170.58, 170.48, 170.40, 170.07, 169.92, 169.76, 169.61, 169.49, 139.25, 125.60, 124.60, 122.64, 121.82, 119.89, 119.00, 114.15, 100.09, 95.86, 95.70, 74.89, 73.51, 73.12, 72.42, 71.74, 71.66, 70.47, 70.11, 69.97, 69.66, 69.37, 69.02, 68.56, 67.91, 67.25, 66.94, 66.63, 62.65, 62.33, 61.39, 33.84, 29.53, 29.49, 29.42, 29.34, 29.12, 28.94, 25.77, 20.94, 20.83, 20.71, 20.62, 20.58, 20.23. ¹⁹F NMR (372 MHz, CDCl₃): δ / ppm = -77.52, -77.89, -88.47, -88.69, -88.75. MALDI-TOF MS (m/z): [M+Na] calcd for C₅₇H₇₆F₁₂NaO₃₀, 1492.166; found, 1492.677, [M+K] calcd for C₅₇H₇₆F₁₂KO₃₀, 1508.074; found, 1508.838.


Compound 13 was synthesized according to the experimental procedure of compound 5. Compound 13 (0.39 g, 65%) was obtained from compound 12 (0.57 g, 0.39 mmol), thiolacetic acid (0.15 g, 1.96 mmol), AIBN (0.06 g, 0.39 mmol) through a column chromatography (Ethyl acetate/hexane, 1:1, v/v). ¹H NMR (400 MHz, CDCl₃): δ / ppm = 1.21 – 1.39 (m, 17H, CH₂), 1.50 – 1.60 (m, 4H, CH₂), 1.93 – 2.02 (m, 24H, 3CH₃), 2.06 (s, 3H, CH₃), 2.12 (s, 3H, CH₃), 2.14 (s, 3H, CH₃), 2.28 (s, 3H, CH₃), 2.83 (t, 2H, J = 7.3 Hz, SCH₂), 3.55 (t, 2H, J = 6.9 Hz, OCH₂), 3.69 – 3.73 (m, 1H), 3.77 (t, 2H, J = 10.0 Hz, OCH₂), 3.87 – 3.97 (m, 5H), 3.98 – 4.06 (m, 2H), 4.07 – 4.10 (m, 2H), 4.14 – 4.30 (m,
3H), 4.40 – 4.48 (m, 2H), 4.63 (d, 1H, $J = 7.8$ Hz), 4.69 (dd, 1H, $J = 4.2$ Hz), 4.79 – 4.84 (m, 2H), 5.04 (t, 1H, $J = 10.0$ Hz), 5.19 – 5.24 (m, 2H), 5.29 – 5.36 (m, 1H), 5.37 (d, 1H, $J = 4.1$ Hz). $^{13}$C NMR (100 MHz, CDCl$_3$): δ / ppm = 196.15, 170.67, 170.65, 170.61, 170.50, 170.41, 170.08, 169.94, 169.78, 169.64, 169.51, 125.45, 124.63, 122.64, 121.82, 119.82, 119.02, 114.58, 114.50, 114.45, 114.05, 113.64, 100.10, 95.86, 95.71, 77.33, 74.90, 73.52, 73.15, 72.43, 71.75, 71.68, 70.48, 70.12, 69.98, 69.68, 69.38, 69.03, 68.56, 67.92, 67.27, 66.96, 66.64, 30.67, 29.55, 29.52, 29.48, 29.35, 29.18, 29.13, 28.84, 21.09, 20.96, 20.85, 20.73, 20.65, 20.59, 20.26, 14.24, 14.17. $^{19}$F NMR (372 MHz, CDCl$_3$): δ / ppm = -77.58, -77.83, -88.41, -88.66, -88.78. MALDI-TOF MS (m/z): [M+Na] calcd for C$_{59}$H$_{80}$F$_{12}$NaO$_3$S, 1568.284; found, 1568.698, [M+K] calcd for C$_{59}$H$_{80}$F$_{12}$KO$_3$S, 1584.392; found, 1585.719.

(3R,4S,5S,6R)-2-((2,2,4,4,5,5,7,7,8,8,10,10-dodecafluoro-23-mercaptop-3,6,9,12-tetraoxatricosyl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol, **14**

Compound **14** was synthesized according to the experimental procedure of compound **6**. Compound **14** (196 mg, 71.8%) was obtained from compound **13** (0.39 g, 0.25 mmol), 28% sodium methaoxide in methanol (206 µL). $^1$H NMR (400 MHz, d$_3$-CH$_3$OH): δ / ppm = 1.25 – 1.40 (m, 15H, CH$_2$), 1.50 – 1.67 (m, 4H, CH$_2$), 2.66 (t, 1H, $J = 7.3$ Hz), 3.23 – 3.30 (m, 5H), 3.36 – 3.44 (m, 2H), 3.45 – 3.52 (m, 3H), 3.57 – 3.61 (m, 4H), 3.63 – 3.68 (m, 2H), 3.72 – 3.91 (m, 9H), 4.06 – 4.14 (m, 1H), 4.22 – 4.31 (m, 1H), 4.40 (d, 1H, $J = 7.8$ Hz), 5.12 – 5.14 (m, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$): δ / ppm = 126.83, 125.87, 123.07, 122.58, 121.10, 114.69, 114.52, 114.13, 102.82, 101.58, 101.36, 79.96, 79.70, 76.30, 75.55, 73.75, 73.58, 73.44, 73.00, 72.91, 72.53, 72.41, 72.03, 70.13, 69.36, 69.06, 68.74, 66.97, 66.66, 66.34, 61.39, 60.82, 38.47, 29.35, 29.32, 29.29, 29.12, 28.98, 28.88,
28.11, 25.62. $^{19}$F NMR (372 MHz, CDCl$_3$): $\delta$ / ppm = -78.76, -89.44, -89.68, -89.87, -90.03. MALDI-TOF MS (m/z): [M+Na] calcd for C$_{37}$H$_{58}$F$_{12}$NaO$_{20}$S, 1105.880; found, 1106.198, [2M+Na] calcd for C$_{74}$H$_{114}$F$_{24}$NaO$_{40}$S$_2$, 2186.754; found, 2187.417, [2M+K] calcd for C$_{74}$H$_{114}$F$_{24}$K$_{2}$O$_{40}$S$_2$, 2202.863; found, 2203.426. HR-ESI MS (m/z): [M+Na] calcd for C$_{37}$H$_{58}$F$_{12}$NaO$_{20}$S, 1105.29427; found, 1105.29285, [2M+Na] calcd for C$_{74}$H$_{114}$F$_{24}$NaO$_{40}$S$_2$, 2185.58368; found, 2185.58180.

2.3 Results and discussion

The chemical structures of the newly synthesized sugar-terminated fluorinated-oligo(ethylene glycol) ligands (SUFLs) were listed in Scheme 2-4. First, the formation of GNVs using small-sized GNPs in the presence of SUFL was examined. Citric acid-coated GNPs with a diameter of 15 nm (referred to as Au-15, 50 μL of H$_2$O) were added to SFL dissolved in dioxane (450 μL). The self-assembly was triggered directly adding GNPs (aq) to the dioxane solution of SUFL. The obtained assembly was denoted as XFL-Au-Y, where X stooded for the abbreviation of sugar head, while Y indicated the diameter of GNPs. Secondary electron of scanning transmission electron microscopy (SE-SEM) images (Figure 2-1a-c) showed that spherical assemblies with surface closely packed Au-15 NPs formed using GFL, MFL, and MAFL. Hydrodynamic diameter of spherical assemblies obtained from dynamic light scattering (DLS) (Figure 2-1d) increased with decreasing the number of glucose, indicating that the size of the assembly composed of Au-15 NPs could be controlled varying the ‘number of glucose’ in SUFLs. Transmission electron microscopy (TEM) images (Figures 2-2a) showed an obvious contrast between the edge and the center of the assemblies, indicating a hollow structure. Transmission electron microtomography (TEMT) was further carried out to confirm the inside of the
assemblies. The reconstructed images (Figure 2-2b and -2c) clearly demonstrated a spherical hollow structure with a monolayer of GNPs.

Scheme 2-4. (a) Chemical structures of fluorinated surface ligands in this chapter and (b) a presentative illustration of the formation of GNV composed of Au-15 NPs using GFL.
Figure 2-1.  (a-c) SE-SEM images and (d) hydrodynamic diameter of Au-15 NP assembly using (a) GFL; (b) MFL; (c) MAFL in 10% H₂O in dioxane. Scale bars: (a-c) 100 nm.

Figure 2-2.  (a) TEM, (b) a digitally sliced XY image at approximately in the middle of
the Au-15 NPs assembly. Data from reconstruction TEMT 3D image of (c). Note that the electrons were irradiated along Z direction (perpendicular to the image (b) in TEM). This indicates that the addition of GFLs to Au-15 NPs produce GNVs.

Figure 2-3. (a) SE-SEM image and (b-d) TE-STEM images of GFL-Au-30 assemblies (b), slice sections of GFL-Au-30 assemblies embedded in epoxy resin (c, d). Scale bars: (a) 100 nm; (b) 500 nm; (c, d) 200 nm.

Next, self-assembly of SUFL with GNPs of a diameter larger than 20 nm was investigated. Glucose-terminated fluorinated-oligo (ethylene glycol) (GFL) was chosen as a representative SUFL for the following discussion. SE-SEM image (Figure 2-3a) of a GFL-Au-30 NP assembly showed close packing between the neighboring Au-30 NPs.
Transmission electron mode of scanning transmission electron microscopy (TE-STEM) image (Figure 2-3b) showed assemblies composed of Au-30 NPs formed. Even though electron tomography was already applied to probe the inside of GFL-Au-30 assembly, which was prepared by directly casting the sample solution on a TEM grid, it’s still unclear that whether GFL-Au-30 assembly was composed of a monolayer of Au-30 NPs or not, due to a unclear deformation of the morphology of assembly. So I need to embed the GFL-Au-30 assemblies into epoxy resin. The GFL-Au-30 NP assemblies were successfully immobilized into epoxy resin utilizing a fast drying process to remove the solvent under vacuum condition. Microtome was further carried out to evaluate the inside of GFL-Au-30 NP assembly. TE-STEM images (Figure 2-3c and -3d) of a slice section of GFL-Au-30 NP assemblies showed a hollow interior, demonstrating that GNV composed of Au-30 NPs formed. While the expression of GNP cluster was defined as spherical assembly with a solid interior, in which was closely packed with GNPs.

The effect of number concentration of Au-30 NPs ($C_{\text{Na}_{30}}$) on the size of GFL-Au-30 assembly was further investigated. When $C_{\text{Na}_{30}} = 0.4 \times 10^{11}$ NPs/mL, clusters (Figure 2-4b) instead of GNVs with an average size of 100 nm obtained from DLS (Figure 2-4a) were formed. When $C_{\text{Na}_{30}} = 0.8 \times 10^{11}$ NPs/mL, GNVs were formed. The hydrodynamic diameter of GNVs increased and eventually reached a size-saturation with increasing $C_{\text{Na}_{30}}$. The size distribution of GNVs obtained from TE-STEM images matched well with hydrodynamic diameter from DLS, demonstrating that the size of GNVs could be controlled by changing $C_{\text{Na}_{30}}$. Accordingly, for the UV-vis absorption spectrum as shown in Figure 2-4d, there was almost no shift for the first plasmonic peak located at 550 nm, while a second plasmonic peak emerged and red-shifted to NIR region (700 nm) with
Figure 2-4. Effect of the concentration of Au-30 NPs on (a) hydrodynamic diameter of GFL-Au-30 assembly in 10% H$_2$O in dioxane and (b, c) SE-SEM images of GFL-Au-30 NP assemblies at $C_{\text{Au-30}} = 0.4 \times 10^{11}$ NPs/mL (b) and $2.8 \times 10^{11}$ NPs/mL. Scale bars: (b, c) 100 nm. (d) Normalized UV-vis extinction spectra of the assemblies of GFL-Au-30 NP in 10% H$_2$O in dioxane at $C_{\text{Au-30}} = 0.4 \times 10^{11}$ NPs/mL (red), $1.2 \times 10^{11}$ NPs/mL (green), $2.0 \times 10^{11}$ NPs/mL (blue), $2.8 \times 10^{11}$ NPs/mL (cyan), and monodispersed citric acid coated Au-30 NPs in H$_2$O (black). (e) Effect of hydrodynamic diameter on plasmonic peaks, which were obtained from (d). The hydrodynamic diameter in (e) was the average value of (a) at each corresponding concentration. The multi-plasmonic peaks in (e) are obtained via peak deconvolution of (d) using Origin 8.5 software. The interparticle distance for GFL-Au-30 NP assembly at each concentration is almost a constant (4 nm) according to the SE-SEM images, thus (d, e) shows the dependence of the size of GNVs on the absorption spectra and plasmonic peak in solution.
Figure 2-5. (a) Effect of ligand on normalized UV-vis absorption spectra of assemblies composed of Au-30 NPs or Au-15 NPs in water containing dioxane (90% dioxane, 10% H₂O) and (b, c) TE-STEM images of (b) GNV-like assemblies of GFL-Au-30 NPs and (c) aggregations of SFL-Au-30 NPs. Scale bars: (b) 300 nm, (c) 500 nm. This data indicated the two plasmonic peaks in the UV-vis absorption spectra were originated from GNV rather than aggregations. The absorption spectra of 200 nm GNVs composed of different-sized GNPs showed that Au-30 NPs can induce stronger plasmon coupling than Au-15 NPs.

increasing $C_{\text{NAu-30}}$. A correlation between the hydrodynamic diameter and plasmonic peaks of GNV in Figure 2-4e further clearly demonstrated that the size of GNV showed remarkable dependence on the plasmonic absorption. As a control, UV-vis absorption spectra (Figure 2-5a) of SFL-Au-30 NPs showed one broad plasmonic peak, while TE-STEM image (Figure 2-5c) gave aggregations. This strongly supported that two characteristic absorptions of GNV composed of Au-30 NPs were originated from the plasmon coupling between Au-30 NPs of a spherical GNV rather than aggregation. This
data also indicated that strong short range attractive interaction provided by the glucose head can induce the formation of GNV. While the GNV composed of Au-15 NPs with hydrodynamic diameter of 200 nm gave one single peak in the UV-vis absorption spectra (Figure 2-5a). The interparticle distance between GNP s on the surface of GNV was 4 nm, which was estimated through SE-SEM images. This indicated that the diameter of GNP s showed remarkable dependence on the UV-vis absorption spectra.

Finite-difference time-domain (FDTD) simulation was extensively applied to understand the surface plasmon resonance of metal NPs or the collective plasmon coupling between metal NPs. In this chapter, FDTD (Figure 2-6) was further carried out to disclose the plasmonic coupling of GNV. The 3D ideal model is constructed based on a single-layered GNV according to the experimental observations. The size of GNV is 200 nm. The surface interparticle distance of assemblies composed of only Au-30 or Au-15 NPs is 4 nm. The simulated extinction spectra, namely an overlay of scattering and absorption, of GNV composed of Au-15 or Au-30 NPs were in well accordance with the experimental data, supporting that the dependence of GNP s size on the plasmonic peaks of UV-vis absorption spectra (Figure 2-5a). Further, FDTD simulated extinction spectra of GNV composed of Au-30 NPs revealed that the plasmonic peak of 580 nm (experimental data: 550 nm) was originated from the plasmon resonance of the single Au-30 NP on the surface of GNV, while the simulated spectra of single Au-30 NP isolated from a dispersed condition showed one plasmonic peak of 540 nm. The difference of this plasmonic peak was probably due to the scattering, in which case the scattering from the single Au-30 NP, isolated from the mono-dispersed condition, was neglectable. While the scattering from GNVs was profound due to the larger size of GNVs than that of mono-dispersed Au-30 NPs according to our simulation result. The second plasmonic peak of
740 nm (Experimental data: 700 nm) was produced by a collective dipolar plasmon coupling between Au-30 NPs on the surface of GNVs, while 3D ideal model of a gold nanoshell with a thickness of 30 nm gave quadrupole resonance peaks rather than a simple dipolar plasmon coupling.\textsuperscript{13}

![Figure 2-6](image)

Figure 2-6. Simulated UV-vis extinction spectra of GNV of GFL-Au-15 (a), GFL-Au-30 (b) with a size of 200 nm and gold nano-shell with a thickness of 30 nm. This data indicates the FDTD simulation strongly supports the experimental observations.

![Figure 2-7](image)

Figure 2-7. Time dependent study of UV-vis absorption spectra (a) and hydrodynamic diameter (b) of self-assembly of GFL and Au-30 NPs in 10% H\textsubscript{2}O in dioxane.
Figure 2-8. Time dependent study of TE-STEM images (a-n) of self-assembly of GFL and Au-30 NPs in 10% H₂O in dioxane. Scale bars: (a-n) 300 nm.

The effect of Au-30 NP concentration revealed that both kinetics and thermodynamics were involved in GNV formation. The definition of kinetics for the formation of GNV in this thesis indicates an energetically unfavorable state or non-equilibrium fashion. An important feature of kinetics is that the size of assemblies is variable with increasing the incubation time due to a dynamic interplay of the attractive and repulsive interactions between functionalized NPs. So the kinetically trapped assemblies can be visualized through a direct observation in solution or a quenching approach utilizing drying process.
at different time intervals. While the definition of thermodynamics in this thesis showed an energetically favorable state or equilibrium fashion, resulting in a stable GNV with a relatively narrow size-distribution or a size-saturation state in solution.

To understand the formation mechanism of GNV, time course studies (Figure 2-7 and -8) of UV-vis, DLS and STEM measurements for the self-assembly of GFL and Au-30 NPs were carried out. UV-vis absorption spectrum (Figure 2-7a) gave one plasmonic peak with a red shift of 27 nm compared with monodispersed Au-30 NPs after homogeneous mixing Au-30 NPs and GFL for 1 min, indicating that aggregation of Au-30 NPs occurred. With increasing the incubation time, the UV-vis absorption spectrum was gradually separated into two plasmonic peaks located at 540 and 700 nm respectively. Time course study of DLS (Figure 2-7b) showed a gradual increase of the hydrodynamic diameter and finally reached a size-saturation in solution, supporting that both kinetics and thermodynamics were involved in the dynamic self-assembly process to produce thermodynamically stable GNP assemblies with a saturated size. Electron microscopy was used to observe kinetically trapped assemblies subjected to a drying process. TEM-STEM image (Figure 2-8) taken after incubation for 30 s showed that poly-dispersed 3D aggregations were formed, indicating that a quick aggregation occurred. With increasing the incubation time, the average size of assemblies became larger accompanying with disappearing of the small-sized aggregations through a slow self-assembly process. Eventually, a relatively monodispersed and well-defined GNV formed. This tendency is similar to the self-limiting behavior involved in the formation of supraparticles due to a balance between attractive interaction and repulsive interaction reported by Kotov et al.14
Table 2-1. GFL coverage per one single GNP obtained from ICP measurement

<table>
<thead>
<tr>
<th>Sample</th>
<th>Incubation time (min)</th>
<th>Ligand nm$^{2}$/GNP</th>
<th>Ligand coverage per one single GNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFL-Au-30 NPs</td>
<td>5</td>
<td>4.7</td>
<td>34.2%</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>5.6 ± 0.03</td>
<td>37.9%</td>
</tr>
</tbody>
</table>

Inductively coupled plasma optical emission spectroscopy (ICP-OES) was further carried out to investigate ligand exchange for self-assembly of GFL and Au-30 NPs at two different time points (5 min and 90 min). ICP-OES results showed that the surface GFL coverage per one single Au-30 NPs almost kept as a constant after mixing for 5 and 90 min (Table 2-1), indicating that ligand exchange completed within 5 min. While the formation of relatively mono-dispersed GNV took for 1h.

![Figure 2-9](image)

Figure 2-9. TE-STEM (a, b) and FE-SEM (c) images of influence of substrate on GFL-Au-30 assemblies during the drying process. Scale bars (a-c): 300 nm.

Generally, when NP assemblies formed in solution, the substrate should have no obvious influence on the structure or morphology of assemblies. Figure 2-9 showed that aggregations on elastic carbon coated TEM grid and GNVs on high resolution carbon supporting membrane coated TEM grid or on silicon wafer were observed (Figure 2-9). So a noticeable influence of the substrate on the structure of GFL-Au-30 NP assembly
was demonstrated. Therefore, it is necessary to prove whether GNV composed of Au-30 NPs was truly formed in solution or not. Pulsed coherent X-ray solution scattering (PCXSS) utilizing X-ray free electron laser, which has been proved as a powerful method to capture snapshots of substances “in solution”, such as NP assemblies\textsuperscript{15} and living bacteria,\textsuperscript{16} was applied to disclose whether or not the assemblies are truly formed in solution. PCXSS snapshots were captured after incubation of GFL with Au-30 NPs for 2 h. From Figure 2-7 and -8, incubation for 2 h was enough for the formation of GNV. Figure 2-10c, which was reconstructed from a representative single-shot coherent X-ray diffraction (CXD) pattern (Figure 2-10f) of an isolated assembly, indicated a two-dimensional projection of electron density distribution. The size of assembly from the reconstructed image (Figure 2-10c) was 150 nm, close to the average size obtained from electron microscopy and DLS measurement. To analyze the interior of the sample in Figure 2-10c, 3D ideal models of a mono-layered spherical GNV (150 nm) (Figure 2-10a) with a hollow interior and a spherical GNP clusters with solid interior composed of Au-30 NPs (Figure 2-10b) were constructed respectively. The corresponding CXD patterns of the simulated model were shown in Figure 2-10d and -10e respectively. The circular average of the experimental CXD pattern of GFL-Au-30 NP assembly (Figure 2-10g) matched well with that of the GNV model rather than GNP cluster model in the small angle area, strongly supporting the GNV composed of Au-30 NPs was truly formed in solution rather than a drying process.
Figure 2-10. Simulated 3D models (a and b) and the corresponding calculated CXD pattern (d and e); Reconstructed image (c) from the experimental CXD pattern (f); The circular average (g) of the simulated and experimental CXD pattern. Blue curve: experimental; Red curve: simulated GNP cluster; Green curve: simulated GNV.

2.4 Conclusion

GFL as a representative SUFL, can not only spontaneously self-assemble with Au-15 NPs into GNV, but also induce the GNV formation of Au-30 NPs in solution. The
absorption spectra of GNV of Au-30 NPs gave two plasmonic peaks in solution, which were located at 550 and 700 nm respectively, resulting in a strong absorption in NIR region, which was strongly supported by FDTD simulation. Importantly, GNV size could be controlled by the concentration of GNPs, indicating that both kinetics and thermodynamics were involved in the self-assembly process, which was strongly supported by time course studies of UV-vis, DLS and STEM measurements. PCXSS snapshot strongly supported that GNV composed of Au-30 NPs was truly formed in solution rather than during a drying process.
2.5 References


Chapter 3

Formation of gold nanoparticle vesicle-like assembly by a charged fluorinated ligand
Chapter 3 describes the formation of GNV-like assembly composed of 15 nm GNPs or 30 nm GNPs using a carboxylic acid-terminated fluorinated-OEG ligand (CFL) with weak negative charge and the formation mechanism study of GNV-like assembly. The concentration of Au-30 NPs showed independence on the size of the GNV-like assembly. Effect of NaCl on the self-assembly process of CFL and 30 nm GNPs revealed that an important influence of electrostatic repulsive interaction provided by the carboxylic acid head on the formation of GNV-like assembly. Time-dependent study of self-assembly process of CFL and Au-30 NPs using UV-vis, DLS and electron microscopy measurements supported that a kinetic process was involved in the formation of thermodynamically stable GNV-like assembly.
Chapter 4

Size-segregation of binary mixtures of gold nanoparticles into yolk/shell assembly in solution
4.1 Introduction

The controllable self-assembly of metal nanoparticles (NPs) has attracted a good deal of attention recently due to their wide-ranging potential applications.\textsuperscript{1-5} Various soft bottom-up approaches based on NP functionalization for their self-assembly have been explored.\textsuperscript{6-16} To achieve NP assemblies applicable to increasingly complex and novel functionalities, the self-assembly of NPs of different compositions or sizes is practically attractive. The assembly of NPs of different composites or sizes into a binary NP superlattice with a crystalline structure has been widely explored.\textsuperscript{17-20} However, the segregation in these studies was achieved on a solid substrate or liquid/air interface with external forces, such as a solvent evaporation or capillary force.\textsuperscript{21-23} Solution-based hierarchical assembly, which could simplify and extend the potential applications of functional NPs, still remains as an important theme.

In chapter 2 and chapter 3, the formation GNV composed of single-sized GNPs using GFL or CFL was introduced. The further question is that how about self-assembly of binary mixtures of GNPs in the presence of GFL or CFL in solution? In this chapter, I first proposed a two-step approach based on a sequential addition of citric acid coated GNPs to GFL in dioxane by varying the number ratio between binary mixtures of GNPs to investigate the self-assembly behavior. A rearrangement of small- and large-sized GNPs rather than a simple encapsulation process occurred in solution. Next, I found that binary mixtures of small and large GNPs (5/15, 5/30, 10/30, and 15/30 nm in diameter) using the number ratio obtained from the two-step protocol in the presence of GFL can spontaneously form size-segregated yolk/shell assembly. The outermost layer of the assembly is composed of a single layer of small-sized GNPs, while the larger-sized GNPs are located in the interior, forming what is referred to as a yolk/shell assembly. Time
course study revealed that small and large GNPs aggregated together, and these kinetically trapped aggregations were transformed into a size-segregated structure by repeating fusions. A yolk/shell structure was directly visualized in solution by X-ray laser diffraction imaging, indicating that the structure was truly formed in solution rather than a drying process. Further, a binary mixture of GNP pairs (5/30 or 10/30 in diameter) in the presence of CFL can also spontaneously self-assemble into a size-segregated yolk/shell assembly in solution.

4.2 Experimental

General information

Gold nanoparticles (GNPs) coated with citric acid in aqueous solution (5, 10, 15 or 30 nm in a diameter) were purchased from British Biocell International (BBI), Ltd. (Britain) and concentrated using a centrifuge CF-16RX (Hitachi-Koki, Ltd., Japan). Ultraviolet-visible (UV-vis) spectra were measured with UV-2600 (Shimadzu Corporation, Japan). Dynamic light scattering (DLS) analysis were measured with Delsa Nano HC (Beckman Coulter, Inc., Japan). The morphologies of GNP assembly were imaged using a scanning transmission electron microscope (STEM HD-2000) (Hitachi High-Tech Manufacturing & Service Co., Ltd, Japan) with an accelerating voltage of 200 kV. Scanning transmission electron microtomography was carried out using an electron microscopy (JEM-2200FS, JEOL Co., Ltd., Japan) with an accelerating voltage of 200 kV. The 3D images was reconstructed using Image J software and Amira 3D software. Pulsed coherent X-ray solution scattering (PCXSS) experiment was performed using the SPring-8 angstrom compact free-electron laser (SACLA).

Self-assembly and Characterization
Self-assembly of GFL and GNPs using two different sizes by two-step protocol

The commercially available 30 nm gold nanoparticles (Au-30 NPs) (500 μL, BBI solution) or Au-15 NPs (500 μL, BBI solution) coated with citric acid in H₂O were concentrated using centrifugation. The purification parameters were listed in Table 1. After removing the supernatant, the concentrated Au-30 NPs (26 μL) was then quickly added into GFL in dioxane (500 μL, 0.2 mM) with a H₂O content of 5%, and then vigorously stirred for 1 s under vortex to ensure the homogeneous mixing. The mixture was then gently shaken (200 rpm) for 30 min. The concentrated Au-15 NPs (29 μL) was then added into the above solution of GFL-Au-30 assembly with a final H₂O content of 10%. And the mixture was gently shaken (200 rpm) for 90 ~ 120 min.

Table 4-1. Experimental purification parameters for each diameter of GNPs

<table>
<thead>
<tr>
<th>GNP diameter (nm)</th>
<th>Centrifugation Speed (rcf)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>14,000</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>12,000</td>
<td>30</td>
</tr>
<tr>
<td>15</td>
<td>11,500</td>
<td>25</td>
</tr>
<tr>
<td>30</td>
<td>8,600</td>
<td>6</td>
</tr>
</tbody>
</table>

Control experiment 1

The second control experiment was based on a reversed addition of GNPs using two different sizes. The commercially available Au-30 NPs (500 μL, BBI solution) or Au-15 NPs (500 μL, BBI solution) coated with citric acid in H₂O were concentrated using centrifugation. The purification parameters were listed in Table 4-1. After removing the supernatant, the concentrated Au-15 NPs (26 μL) was then quickly added into GFL in dioxane (500 μL, 0.2 mM) with a H₂O content of 5%, and then vigorously stirred for 1 s under vortex to ensure the homogeneous mixing. The mixture was then gently shaken...
(200 rpm) for 30 min. The concentrated Au-30 NPs (29 μL) was then added into the above solution of GFL-Au-30 assembly with a final H₂O content of 10%. And the mixture was gently shaken (200 rpm) for 90 ~ 120 min.

**Self-assembly of GFL with binary mixtures of GNPs using one-step protocol**

The aqueous dispersions of citric acid coated Au-30 NPs (500 μL, number concentration of Au-30 NPs (C_{Na_{Au-30}}): 2.0×10^{11}/mL, N_{Au-30} = 10^{11}) and Au-15 NPs (500 μL, C_{Na_{Au-15}} = 1.4×10^{12}/mL, N_{Au-15} = 7×10^{11}) were purified using the above method respectively. After mixing with concentrated Au-15 and Au-30 NPs together to reach a final volume of 50 μL, which was then quickly added into GFL in dioxane (450 μL) with a final GFL concentration of 0.2 mM. Subsequently, the mixture was vigorously stirred for 1 s under vortex to ensure the homogeneous mixing, and then gently shaken (200 rpm) for 90 ~ 120 min.

**Self-assembly of CFL with binary mixtures of GNPs using one-step protocol**

The aqueous dispersions of citric acid coated Au-30 NPs (200 μL, C_{Na_{Au-30}} = 2.0×10^{11}/mL, N_{Au-30} = 0.4×10^{11}) and Au-5 NPs (80 μL, C_{Na_{Au-5}} = 5.0×10^{13}/mL, N_{Au-5} = 4.0×10^{12}) were purified using the above method respectively. After mixing with concentrated Au-5 and Au-30 NPs (N_{Au-5}/N_{Au-30} = 100:1) together to reach a final volume of 20 μL, which was then quickly added into CFL in dioxane (480 μL) with a final CFL concentration of 0.2 mM. Subsequently, the mixed solution was vigorously stirred for 1 s under vortex to ensure the homogeneous mixing. The mixture was then gently shaken (200 rpm) for 120 min.

To make a clear comparison of the experimental data, the samples for STEM measurement were prepared directly casting 3 μL solution without purification onto the
200 mesh copper TEM grid with elastic carbon coating or ultra-high resolution TEM grid and dried at room temperature under N\textsubscript{2} in glove box for overnight. So the STEM images in this chapter reflected a raw state of solution sample of the assembly using CFL during a drying process.

**Characterization**

**Electron microtomography**

Scanning transmission electron microtomography (SEMT) was performed using an electron microscopy (JEM-2200FS, JEOL Co., Ltd., Japan) with an accelerating voltage of 200 kV. SEMT experiment of GFL-Au-30&Au-5 NP assemblies was performed by tilting the samples from -62° to 64° at an angular interval of 1°. The tilt series of projections were aligned using the Au-5 NPs as fiducial markers. After the alignment, 3D images were reconstructed using the filtered back projection algorithm. Note that the 3D reconstructed image in this chapter was reconstructed using Image J and Amira software. As the Au-5 NPs on the surface and even in the interior were white color in the digitally sliced sections, segmentation was based on marking the background black and the Au-30 NPs white. After 3D reconstruction, transparency adjustment was applied to capture the inside of the GFL-Au-30&Au-5 NP assembly.

**Pulsed coherent X-ray solution scattering (PCXSS)**

A PCXSS experiment was performed using the SPring-8 angstrom compact free-electron laser (SACLA). A few micro litter of the suspension of the assemblies were enclosed in a micro liquid enclosure array (MLEA) three hours after mixing of GFL with the homogeneous mixtures of Au-15&Au-30 NPs. X-ray free electron laser (XFEL) pulses from SACLA was coherently focused, with a wavelength of 3.1 Å, down to a spot
size of 1.3 μm × 1.0 μm. The focused XFEL beam illuminated the samples in MLEA and we recorded single-shot coherent X-ray diffraction (CXD) patterns with a multiport charged-coupled device. Image reconstruction was performed for the CXD patterns after centrosymmetrization and 8 × 8 pixel binning. Relaxed averaged alternating reflections algorithm \(^{24}\) with shrink-wrap support \(^{25}\) (6000 steps) was used and subsequently the noise-tolerant hybrid input-output algorithm \(^{26}\) with fixed support (1000 steps). The hybrid input-output algorithm with iterative normalization \(^{27}\) was applied during the reconstruction calculations. In the shrink-wrap method, the support was updated every 50 iterations, and the kernel was initially set to 5 pixels and was gradually reduced down to 3 pixels. Using results with 100 different initial random seeds, correlation coefficients between all pairs of the results were calculated. Finally, 10 images with the highest similarity were selected and averaged.

**Calculation of GFL-surface coverage on GNPs by inductively coupled plasma optical emission spectroscopy (ICP-OES)**

A water-containing dioxane solution of GFLs-GNPs (Au-15 NPs) was prepared at the same condition for the size-segregated yolk/shell assembly. The solvent was changed to water by repeated centrifugation of the GNPs to remove the free GFLs. The surface GFL coverage per one single GNPs were determined by ICP-OES. We set the surface number of Au atoms of each GNPs according to the reference.\(^ {28}\)

**43 Results and discussion**

In chapter 2 and 3, the formation of GNV composed of small- or large- sized GNPs with narrow size distribution in the presence of GFL or CFL was demonstrated. The further question is that how about the self-assembly of binary mixtures (small and large
size) of GNPs with a wide size distribution in the presence of GFL or CFL in solution?

Scheme 4-1. Chemical structure of GFL used in this study, and self-assembly of GFL and GNPs with different sizes into a size-segregated assembly using a two-step protocol.

First, a two-step self-assembly approach (see experimental details) based on a sequential addition of GNPs into GFL in dioxane was proposed (Scheme 4-1). Au-30 NPs were chosen as large-sized GNPs, while Au-15 NPs were chosen as small-sized GNPs. The resulting assembly was denoted as GFL-Au-30-Au-15. The number concentration of Au-30 NPs ($C_{\text{Au-30}}$) was kept as a constant, $C_{\text{Au-30}} = 1.0 \times 10^{11}$ NPs/mL. $N_{\text{Au-15}}$ can be adjusted by varying the volume of Au-15 NPs. When $N_{\text{Au-15}}/N_{\text{Au-30}} = 1:1$, UV-vis absorption spectrum (Figure 4-1a) showed one broad peak and blue-shifted compared with the controlled experiment using only Au-30 NPs. While the hydrodynamic diameter of assemblies (Figure 4-1b) composed of Au-15 and Au-30 NPs under $N_{\text{Au-15}}/N_{\text{Au-30}} = 1:1$ (200 nm) was much larger than that of assembly composed of only Au-15 NPs (120 nm) under the same condition, but comparable to that of assembly composed of only Au-30 NPs. The surface of NP assemblies was imaged based on the secondary electron mode of scanning transmission electron microscopy (SE-SEM). The interior of NP assemblies was acquired using high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) or transmission electron mode of scanning transmission electron microscopy.
Figure 4-1. Effect of N_{Au-15}/N_{Au-30} on (a) normalized UV-vis absorption spectra (Red indicated GNV composed of only Au-30 NPs, black indicated GNV composed of only Au-15 NPs at Au-30 or Au-15 NP concentration of N_{Au-15}/N_{Au-30} = 7:1) and hydrodynamic diameter (b) for GFL-Au-30-Au-15 NP assemblies using a two-step protocol and the controlled experiment. Effect of N_{Au-15}/N_{Au-30} on SE-SEM images (c-e) and HAADF-STEM image (f) of GFL-Au-30-Au-15 assembly at N_{Au-15}/N_{Au-30} = 7:1. Scale bars: (c, d) 300 nm, (e) 100 nm and (f) 800 nm.

microscopy (TE-STEM). SE-SEM image (Figure 4-1c and -1d) showed that the surface of the assembly was packed with Au-30 and Au-15 NPs, which might influence the plasmonic coupling between Au-30, thus inducing the blue shift of the plasmonic peak in the UV-vis spectrum compared with assemblies composed of only Au-30 NPs. And with increasing N_{Au-15}/N_{Au-30}, a red shift of the plasmonic peak in the UV-vis absorption spectrum (Figure 4-1a) was observed. Accordingly, the hydrodynamic diameter increased and saturated at N_{Au-15}/N_{Au-30} = 7:1 (Figure 4-1b). SE-SEM image (Figure 4-1e) under
$N_{\text{Au-15}}/N_{\text{Au-30}} = 7:1$ clearly showed that the surface of the assemblies was covered by only Au-15 NPs, while HAADF-STEM image (Figure 4-1f) indicated that the assembly of Au-30 NPs was located in the interior. At this number ratio, the number of encapsulated Au-30 NPs might be different, which could be judged through HAADF-STEM image (Figure 4-1f), indicating a rearrangement of the Au-30 and Au-15 NPs occurred, rather than a simple encapsulation process.

To confirm whether the rearrangement of Au-30 and Au-15 NPs occurred using a simple sequential addition process, a reversed addition of Au-30 and Au-15 NPs, namely first fabrication of GNV composed of Au-15 NPs, subsequently added Au-30 NPs into the solution of GNV of Au-15 NPs. The resulting assembly for a reversed addition process was denoted as GFL-Au-15-Au-30. UV-vis absorption spectrum (Figure 4-2a) and DLS (Figure 4-2b) didn’t show obvious difference even the addition sequence was different. SE-SEM image (Figure 4-2c) showed that some Au-15 NPs were located on the surface of GFL-Au-30 assembly or even the surface was covered by Au-15 NPs, some Au-30 NPs in the inside of GFL-Au-15-Au-30 assembly can be observed through TE-STEM image (Figure 4-2d). These data clearly demonstrated the rearrangement of Au-15 and Au-30 NPs occurred. The optimized number ratio between small (5, 10 or 20 nm) and Au-30 NPs to form the size-segregated assembly based on a two-step sequential addition process was summarized in Table 4-2. The typical SE-SEM and HAADF-STEM images for the size-segregated assembly composed of two different size using a two-step protocol was summarized in Figure 4-3.
Figure 4-2. Effect of addition sequence on (a) UV-vis absorption spectra and (b) hydrodynamic diameter of GFL-Au-30-Au-15 from two-step protocol and GFL-Au-15-Au-30 assembly from a reversed two-step protocol. (c) SE-SEM and (d) TE-STEM of GFL-Au-15-Au-30 assembly. Scale bars: (c, d) 100 nm. These data indicated that a rearrangement of Au-15 and Au-30 NPs occurred.

Figure 4-3. SE-SEM (a-c) and HAADF-STEM images (d, e) of assemblies composed of binary mixtures of GNPs based on a two-step sequential addition process. (a) Au-
20/Au-30 ($N_{Au-20}/N_{Au-30} = 4:1$); (b, d) Au-10/Au-30 ($N_{Au-10}/N_{Au-30} = 10:1$); (c, e) Au-5/Au-30 ($N_{Au-5}/N_{Au-30} = 100:1$). Scale bars: (a) 600 nm; (b-e) 100 nm.

Table 4-2. Number ratio information of binary mixtures of GNPs

$C_{NAu-X}$ was refer to as the number concentration of GNPs. $N_{Au-X}$ indicated the number of GNPs. $V_{Au-X}$ indicated the volume of GNP solution. X indicated the diameter of GNPs.

<table>
<thead>
<tr>
<th>$N_{Au-X}/N_{Au-30}$</th>
<th>$N_{Au-30}$</th>
<th>$N_{Au-X}$</th>
<th>$V_{Au-X}$ (μL)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>X = 15</td>
<td>7:1</td>
<td>$7\times10^{11}$</td>
<td>500</td>
<td>$C_{NAu-15} = 1.4\times10^{12}$/mL</td>
</tr>
<tr>
<td>X = 10</td>
<td>10:1</td>
<td>$10^{11}$</td>
<td>175</td>
<td>$C_{NAu-10} = 5.7\times10^{12}$/mL</td>
</tr>
<tr>
<td>X = 5</td>
<td>100:1</td>
<td>$1\times10^{13}$</td>
<td>200</td>
<td>$C_{NAu-5} = 5.0\times10^{13}$/mL</td>
</tr>
</tbody>
</table>

Note: The number concentration was obtained from BBI solution following official website: http://www.bbisolutions.com/molar-concentration-of-nanoparticles/

The electron microscopy measurements were, however, made for dried samples dispersed on substrates in vacuum. It is necessary to prove that size-segregated assembly is spontaneously formed in solution, but not during the drying process. Pulsed coherent X-ray solution scattering (PCXSS) utilizing X-ray free electron lase was applied to disclose whether or not the assemblies are truly formed in solution. The image reconstructed from a single-shot coherent X-ray diffraction (CXD) pattern of an isolated assembly made by self-assembly of GFL with Au-30 and Au-15 NPs using a two-step protocol indicates the two-dimensional projection of electron density distribution. PCXSS experiment showed three different kinds of CXD pattern. The reconstructed images from CXD pattern (Figure 4-4) indicated spherical assemblies were truly formed in solution. Circular average of the CXD pattern (Figure 4-4) indicated that the size-segregated assembly of Au-30 and Au-15 NPs with different number of encapsulated Au-30 NPs and the assembly composed of only Au-15 NPs for two-step protocol was also observed.
Figure 4-4. Experimental CXD pattern (a-d) obtained from the assembly solution of GFL-Au-30-Au-15 NPs based on a sequential addition process as described in the experimental session (a-c), the controlled experiment of GNV made by only Au-15 NPs in solution (d) and the corresponding reconstructed image (e-h); (i) The circular average of experimental CXD pattern (a-c) of GFL-Au-30-Au-15 NP assembly and (d) of GFL-Au-15 assembly.
Circular average of the CXD pattern for an isolated assembly made by GFL and only Au-15 NPs under the same concentration as shown in the two-step protocol was in well accordance with the circular average of CXD pattern of Au-15 NPs assembly by the controlled experiment using two step protocol (Figure 4-4), strongly supporting that the formation of assembly composed of only Au-15 NPs in the two step protocol. These data were also in well accordance with observations using electron microscopy.

Scheme 4-2. Chemical structures of CFL and GFL used in this study, and self-assembly of the binary mixtures of GNPs with GFL or CFL into a yolk-shell assembly.

Next, self-assembly of homogeneous binary mixtures of small and Au-30 NPs in the presence of GFL was investigated. The number ratio between small and large size GNPs was obtained from the two-step protocol mentioned above. Mixtures of Au-30 and Au-15 NPs ($N_{Au-15}/N_{Au-30} = 7:1$, 50 µL) with GFL in dioxane (450 µL) produced spontaneous
Figure 4-5. (a-c) SE-SEM and (d-f) HAADF-STEM images of small and large NPs mixtures with GFL. (a, d) Au-30 & Au-15 (N_{Au-15}/N_{Au-30} = 7:1), (b, e) Au-30 & Au-10 (N_{Au-10}/N_{Au-30} = 10:1) and (c, f) Au-30 & Au-5 (N_{Au-5}/N_{Au-30} = 100:1) NPs. Scale bars: (a-f) 150 nm. These images indicate that small and large NPs form size segregated structures in which the surface is covered with a single layer of small GNP.

When using Au-5 or Au-10 as the smaller NPs,
Figure 4-6. The typical SE-SEM images (a, b) of a Au-30/Au-5 NP assembly and a Au-5 NP assembly. Scale bars: (a, b) 60 nm.

Figure 4-7. (a-e) HAADF-STEM images of GFL and (a) Au-30/Au-5 (N_{Au-5}/N_{Au-30} = 1:1), (b) Au-30/Au-10 (N_{Au-10}/N_{Au-30} = 1:1), (c) Au-15/Au-5 (N_{Au-5}/N_{Au-15} = 25:1), (d) Au-30/Au-15 (N_{Au-15}/N_{Au-30} = 1:1) and (e) GFL-Au-30/Au-15 (N_{Au-15}/N_{Au-30} = 10:1) NP assembly. Scale bars: (a-c) 100 nm, (d, e) 200 nm.
the encapsulated Au-30 NPs can be seen through the outer layer, even in SE-SEM images (Figure 4-5). The interparticle gap for small GNPs was clearly wider than that for the vesicle structures produced by small GNPs alone (Figure 4-6), which is probably due to the osmotic pressure. To confirm generality of size-segregated behaviors, Au-15 NPs was used as large particles, which produced vesicle structures as described in chapter 2. The binary mixture of GFL-Au-5/Au-15 (N_{Au-5}/N_{Au-15} = 25:1) also showed size segregation (Figure 4-7). This indicates that the size segregation phenomenon in this study is related to vesicle formation of the small NPs, but is independent of whether the large NPs alone form vesicles. STEM tomographic slice sections of the segregation of small (5 nm) and large (30 nm) NPs demonstrated that the assemblies are yolk/shell structure, in which a space exists between the interior assembly of Au-30 NPs and outermost layer assembly of Au-5 NPs (Figure 4-8).^30,31^ Figure 4-8 indicates a schematic presentation of Au-5 and Au-30 assembly obtained from TEMT, in which NP shapes were subjected to the spherical approximation. Sliced images of Figure 4-8d also clearly support the yolk/shell structure. When the proportionality of the small NPs (Au-5, -10, and -15 NPs) was reduced to N_{small-NP}:N_{Au-30} = 1:1, no obvious size segregation was observed (Figure 4-7). At this ratio, the total number of small NPs was insufficient to form Au-30-encapsulated size-segregated structures. In other words, the large excess number of small particles is required for the size segregation, supporting that the small GNPs formed vesicles by pushing large particles into the inner space due to entropy-driven depletion force.^^32^ To understand the dynamic size segregation phenomenon, a time-dependent study of the self-assembly of Au-30 and Au-15 NPs with GFL was carried out (Figures 4-9). Samples (3 μL) of the mixture were taken at the times shown in Figures 4-9, cast onto the TEM grid, and were subjected to drying. STEM images taken soon after mixing showed that the
Figure 4-8.  (a) A 3D reconstructed image of GFL-Au-5 & Au-30 assembly. Because of the insufficient tilt angular range in TEMT experiments, the 3D image appeared vague especially in Z direction. (b) A digitally sliced XY image from 3D reconstructed data. The Z position of the sliced image corresponds to the middle of the GFL-Au-5 & Au-30 assembly. (c) A schematic 3D representation of the GFL-Au-5 & Au-30 assembly. The center coordinates of all gold nanoparticles (both 5 and 30 nm particles) were determined form the experimentally obtained reconstructed 3D image (a), and virtual particles were generated from the center position. Thus, the 3D picture captures structural features but is not quantitative. (d) Schematic presentation of a GFL-Au-30 & Au-5 NP assembly in Figure 3c at different slice sections.

Au-30 and Au-15 NPs had already started to form small aggregations. After 5 min, the Au-15 NPs had started to form vesicles with the Au-30 NPs located inside, supporting the notion that the size segregation occurred at the early stage of assembly process. The aggregations were fused into size-segregated assemblies (5-30 min) and grown to spherical assemblies so that the surfaces were covered by Au-15 NPs, while the Au-30
NP assemblies were encapsulated. The image taken after 30 min clearly indicated that the yolk/shell structure was formed via a fusion of the outer layer of each assembly, similar in manner to the fusion of liposomes.

Figure 4-9. Time dependent study on UV-vis extinction spectra (a) and hydrodynamic diameter (b) of the assemblies of the mixture of Au-30 & Au-15 NPs (N_{Au-15}/N_{Au-30} = 7:1) in the presence of GFLs in 10% water-containing dioxane. (c-g) HAADF-STEM and (h-l) SE-STEM images of the assemblies at different time points. Scale bars: (c-l) 100 nm. (k) Scheme of the proposed self-assembly process of GFL and the mixture of Au-30 & Au-15 NPs into a size-segregated yolk/shell assembly.

I investigated the ligand exchange rate of GFLs for Au-15 or Au-30 NPs using
inductively coupled plasma optical emission spectroscopy (ICP-OES). GFL density on the surface of Au-15 or Au-30 NPs at 5 and 90 min remained almost constant, indicating that the ligand exchange for Au-15 or Au-30 NPs was completed within 5 min (Table 4-3 and Table 2-1 in chapter 2), while the size segregation took more than 90 min. Importantly, tetra(ethylene glycol)-terminated fluorinated OEG ligand, which lacked a glucose moiety, induced aggregations but not size-segregated structures (Figure 4-10).

Table 4-3. ICP result for the kinetic study of surface GFL coverage per one single Au-15 NP

<table>
<thead>
<tr>
<th>Sample</th>
<th>Incubation time (min)</th>
<th>Ligand nm² GNP</th>
<th>Ligand coverage per one single GNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFL-Au-15 NPs</td>
<td>5</td>
<td>5.4 ± 0.57</td>
<td>39.5%</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>5.6 ± 0.32</td>
<td>40.1%</td>
</tr>
</tbody>
</table>

Figure 4-10. TE-STEM (a) and SE-STEM image (b) of the control experiments for self-assembly with mixtures of Au-30&Au-15 NP pairs (N_{Au-15}/N_{Au-30} = 7:1) in the presence of a tetra (ethylene glycol)-terminated fluorinated-oligo (ethylene glycol) ligand in 10% H₂O in dioxane. Scale bars: (a, b) 200 nm.
Figure 4-11. Experimental CXD pattern (a,c) and reconstructed image (b,d) of GFL-Au-15/Au-30 NP assembly (a,b) and GFL-Au-15 NP assembly (c,d) in water-containing dioxane solution, measured by single-shot illumination by a femtosecond X-ray laser pulse. (e) Circular average of the CXD pattern of (a).
In some previous literature on supraparticle assemblies, surface ligands have been used as a stabilizer.\textsuperscript{33,34} GFL is a unique molecule that affords surface characteristics that enable the rearrangement of GNPs after initial aggregation, resulting in a thermodynamically stable yolk/shell structure. These data suggest that the fluorinated GFL surface promotes the rearrangement of GNPs after initial aggregation in solution, resulting in a thermodynamically stable yolk/shell structure. A time-course study using DLS measurements and UV-vis spectroscopy of these processes suggest that the self-assembly process took place in solution (Figure 4-9).

PCXSS utilizing X-ray free electron laser was applied to disclose whether or not the assemblies are truly formed in solution. The image reconstructed from a single-shot CXD pattern of an isolated assembly indicates the two-dimensional projection of electron density distribution (Figure 4-11). PCXSS snapshots were captured after the incubation of GFL with homogeneous mixture of Au-15 and Au-30 NPs for 3 h. Based on Figure 10, 3 h is long enough for the formation of the yolk/shell structure. The diameters of reconstructed image for Au-15/Au-30 mixture and Au-15 alone were approximately 200 and 150 nm, respectively, which were close to the average size obtained from electron microscopy and DLS measurements. The reconstructed images of the binary mixture of Au-15/Au-30 showed yolk/shell structure where Au-15 NPs form the outermost layer with Au-30 NPs located inside, while the images of the assembly made of Au-15 alone showed vesicles with a single layer. Figure 4-11e shows a circular average of the CXD pattern of Figure 4-11a. The circular average of the CXD pattern from the binary mixture of Au-15/Au-30 displays that the peaks due to structures with 15 nm periodicity, neighboring Au-15 particles, and 30 nm periodicity, neighboring Au-30 particles, were stronger than that with 23 nm periodicity, neighboring Au-15 and Au-30 particles. This
also strongly supports the segregation state of the binary mixture of Au-15&Au-30 NPs in solution.

Figure 4-12. UV-vis absorption spectra (a) and hydrodynamic diameter (b) of the assemblies of CFL and different NP pairs. (c, d) SE-SEM and (e, f) SE-STEM images of assembly of CFL and different NP pairs. (c, e) Au-30 & Au-5 ($N_{\text{Au-5}}/N_{\text{Au-30}} = 100:1$), (d, f) Au-30 & Au-10 ($N_{\text{Au-10}}/N_{\text{Au-30}} = 10:1$). Scale bars: (c, e) 100 nm, (d, f) 300 nm.
Further, self-assembly of CFL with binary mixtures of GNPs (Au-30&Au-5, N_{Au-5}/N_{Au-30} = 100:1; Au-30&Au-10, N_{Au-10}/N_{Au-30} = 10:1) was further investigated. UV-vis absorption spectra (Figure 4-12a) showed a red shift compared with that of monodispersed citric acid coated GNPs in water containing dioxane, indicating aggregation occurred in solution. DLS (Figure 4-12b) gave an average diameter of 150 nm for assembly of Au-5&Au-30 NP pairs or Au-10&Au-30 NP pairs. SE-SEM images (Figure 4-12c and -12d) clearly indicated that the surface of the assembly was covered by a single-layer of small-sized GNPs while Au-30 NPs can be observed even through the SE-SEM images. HAADF-STEM images (Figure 4-12e and -12f) indicated that assembly of Au-30 NPs were located inside of the assemblies. These results supported that a size-segregated yolk/shell assembly formed in solution.

4.4 Conclusion

The binary mixtures of GNPs coated with GFL could hierarchically assemble into a yolk/shell structure in solution. Time-course studies revealed that the size segregation occurred at the early stage of the self-assembly. The kinetically trapped aggregations transformed to energetically stable size-segregated yolk/shell structures. A snapshot obtained by PCXSS clearly indicates that the size-segregated assembly was formed in solution. The data indicate that the entropy-driven size segregation of fluorinated nanoparticles is powerful to make hierarchical assembly and this strategy has potential applications to the construction of new types of assembled structures with variable properties. Accordingly, CFL can also spontaneously self-assemble with a binary mixture of Au-5 and Au-30 NPs into a size-segregated yolk/shell assembly in solution.
4.5 References


Chapter 5

Conclusion
In this thesis, I described the design, synthesis of novel fluorinated surface ligands: glucose-terminated fluorinated-oligo(ethylene glycol) ligand (GFL) with a neutral head as a presentative sugar-terminated fluorinated-oligo (ethylene glycol), carboxylic acid-terminated fluorinated-OEG ligand (CFL) with a weak negative charge head and sodium carboxylate-terminated fluorinated-OEG ligand (SCFL) with a negative charge. GFL or CFL can not only self-assemble with single-sized gold nanoparticles (GNPs) into gold nanoparticle vesicles (GNVs), but also can induce the formation of size-segregated yolk/shell assembly using binary mixtures of GNPs. The formation of GNV and the dynamical process of self-assembly with GNPs using GFL or CFL in solution was extensively investigated.

**Summary of this thesis**

``Chapter 2 Formation of gold nanoparticle vesicles composed of single-sized gold nanoparticles in solution``

GFL with a neutral glucose head was designed based on the fact that the glucose head can provide stronger inter- and intra- hydrogen bonding, thus providing stronger short range attraction than oligo(ethylene glycol) head of semi-fluorinated-oligo(ethylene glycol) ligand (SFL). GFL can not only self-assemble with small-sized GNPs (less than 20 nm), but also self-assemble with Au-30 NPs into GNV in solution. The UV-vis absorption spectra of GFL-Au-30 assembly showed two separated peaks (550 and 700 nm), resulting in a strong absorption in the NIR region, which was strongly supported by a FDTD simulation. The size of GNV was controlled by the concentration of Au-30 NPs. Time dependent studies of UV-vis, DLS and STEM measurements showed that quick aggregation and slow fusion process were involved in the formation of GNV.
Chapter 3 Formation of gold nanoparticle vesicle-like assembly by a charged fluorinated ligand

CFLs can not only self-assemble with GNP size smaller than 20 nm, but also can induce the formation of GNV composed of Au-30 NPs in water containing dioxane. Importantly, the concentration of Au-30 NPs showed independence on the size of the GNVs, indicating thermodynamically stable GNVs formed in solution. Effect of salt on the self-assembly process demonstrated that the formation of GNV was prohibited even in the presence of a very low $C_{NaCl}$, indicating that electrostatic repulsive interaction was important for the formation of GNV. Time-dependent study of self-assembly of CFL and Au-30 NPs using UV-vis, DLS and STEM measurements indicated that both kinetics and thermodynamics were involved in the formation of thermodynamically stable GNV.

Chapter 4 Size-segregation of binary mixtures of gold nanoparticles into yolk/shell assembly in solution

Size-segregation of binary mixtures of GNPs in the presence of GFL or CFL into a yolk/shell structure in solution was demonstrated. Rearrangement between small and large size GNPs was involved in size-segregation. Time course studies revealed that the size segregation occurred at the early stage of the self-assembly. These kinetically trapped aggregations transformed into energetically stable size-segregated yolk/shell structure. A single snapshot obtained from PCXSS experiment clearly indicated that the size-segregated assembly was formed in solution. These data indicate that the entropy-driven size segregation of fluorinated nanoparticles is powerful to make hierarchical assembly and this strategy has potential applications to the construction of new types of assembled structures with variable properties. Accordingly, CFL can also spontaneously self-assemble with a pair of NPs (Au-5/Au-30 or Au-10/Au-30) into a size-segregated
yolk/shell assembly in solution.

**General conclusion**

In conclusion, I demonstrate that attractive interaction or repulsive interaction is important for the formation of GNV composed of single-sized GNPs using the newly designed fluorinated ligands. X-ray laser diffraction imaging clearly showed that the GNV formed in solution rather than a drying process. The extensive investigations on the dynamic process of self-assembly showed that quick aggregation and slow fusion process were involved in GNV formation in solution. A size-saturation state of GNVs was observed in solution with the dynamic interplay of attractive interaction and repulsive interaction. This size-limiting behavior and dynamic process of self-assembly in GNV formation in this thesis reveals some similarities to the self-assembly of globular proteins in nature.

The unique design of GFL or CFL also afforded novel self-assembly phenomenon in solution. Rearrangement of GNPs with two different sizes in the presence of the surface ligand induced the size-segregation, resulting in the formation of a novel yolk/shell assembly in solution. Three key processes, including aggregation, segregation and Ostwald ripening, were involved in the formation of energetically stable yolk/shell assembly. Depletion force for the size-segregation was further proved. This entropy-driven size-segregation of fluorinated nanoparticles is powerful to make hierarchical assemblies and this strategy has promising applications to construct new types of hybrid assembly structures with variable properties and develop new encapsulation process.

Importantly, a novel concept of surface ligand design based on the incorporation of a pair of competing interactions (attractive interaction and repulsive interaction) into one
single small molecule was firstly proposed and initially tested in this thesis. This concept can be further extended to the design of various small molecules and polymers for the surface functionalization and self-assembly of NPs in colloidal chemistry.