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**Orientation Control of Nanorods
with the Assistance of Polymer Brush**

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Doctor of Science

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Chapter 1

General Introduction

1.1 Why Is It Necessary to Control Anisotropic Nanoparticle Orientation?

A grand challenge in the fields of chemistry and materials science is to create materials and devices with new functionality that has a potential to bring about a revolution in our daily lifestyles. To tackle this challenge, organic and supramolecular chemists control molecular orientation by building sophisticated molecular architectures through the manipulation of the intermolecular non-covalent bonds such as hydrogen bonds, ionic bonds (electrostatic interactions), van der Waals interactions and hydrophobic interactions. Some example of the designed molecules includes self-assembled monolayers (SAMs), liquid crystal (LC) molecules, and lipid bilayers. SAMs of highly ordered and tightly packed organic molecules are powerful tools for controlling the physical and chemical properties on solid surfaces, which is of vital importance for applications including control of wetting and adhesion, chemical resistance and nano-fabrication. Optical properties of transmitted light through LC film are modulated depending on the structure and orientation of LC molecules, and thus LC molecules are used for applications in flat panel displays. In biological system, lipid bilayers, which consists of oriented amphiphilic phospholipids, serves as a selectively permeable membrane by providing a scaffold for enzymes and proteins that works to sustain life activities. These emergent properties, realized by the orientational control of functional molecules, has inspired colloidal chemists to develop methods for controlling the orientation of anisotropic mesoscale (midrange between nano and micro) materials such as nanorods, nanocarbon and protein-based nanotubes. This is because they are attractive materials with unique properties, and thus are considered promising for the construction of creating functional devices and materials. However, unlike molecular systems, where intermolecular interactions can relatively easily be designed, it is difficult to provide anisotropic mesoscale materials with directional information that enable their orientational control. Therefore, we colloidal chemists have pursued methods that allows to control orientation of anisotropic mesoscale objects over the past decade.

1.2 Current Strategies to Control over Anisotropic Nanoparticle Orientation

To date, a variety of strategies for orientation control of anisotropic nanoparticles have been developed¹⁻⁴. Here, we will overview the previous techniques for orientation control of anisotropic nanoparticles, and lastly, I will give my opinion on what directions are likely to encourage the most innovation and scientific progress in this field.

Drop casting. Drop casting is the most basic technique to assemble nanoparticles into ordered structures⁵⁻⁸. It requires just two steps: (i) drop casting of a small amount of nanoparticle dispersion on solid substrate and (ii) evaporation of the solvent. By exploring the optimal experimental conditions such as nanoparticle concentration, evaporation rate and solvent quality, among others, ordered structures from nanorods are formed during the drying process. For example, Ryan and co-workers achieve the formation of vertically oriented hexagonal two-dimensional arrays and multilayer three-dimensional superstructure arrays by optimizing the nanorod concentration⁵.

External field-directed assembly. Applying external forces on nanorods is also an effective way to achieve high levels of alignment of nanorods. Electric field⁹⁻¹¹ and magnetic field¹² are widely exploited to organize anisotropic nanoparticles into superlattices.

Template-assisted assembly. Templating is a superior way to direct assembly of nanorods on substrates¹³⁻²². The basic concept of the strategy is to arrange nanorods on chemically or geometrically patterned regions. In general, templates are fabricated through techniques such as optical and electron beam lithography and microcontact printing. This strategy takes advantage of both top-down and bottom-up approaches. Thus, it allows selective and precise placement of nanorods, providing ordered

arrays that are not attainable via other assembly methods. For example, flauraud and co-workers succeed in the arrangement of nanorods into complex motifs (*e.g.* the Latin alphabet and the Arabic numeral characters)²².

Liquid-liquid interfacial assembly. The change in interfacial energy for placing nanorods at liquid-liquid interfaces is described by several groups^{23–27}. Russel and coworkers demonstrate that individual nanorods align in parallel with the interfaces, which is consistent with their theoretical argument²³. On the other hands, Kim's group demonstrate that CTAB-coated nanorods align perpendicular to the plane of the oleic acid-water interface, which is consistent with their argument²⁷. By controlling this interfacial behavior of nanorods, high density assemblies of nanorods that are aligned in parallel with the interfaces could be produced.

Gas-liquid interfacial assembly. At a gas-liquid interface, Langmuir-Blodgett (LB) technique has been used for constructing horizontally oriented anisotropic nanoparticle arrays at the interface²⁸. The resulting assemblies can be transferred onto a solid substrate. A device-scale monolayer of vertically aligned and closely packed nanorods can be also produced using air-water interface²⁹.

It seems possible to produce the desired structures with the current self-assembly strategies. However, there are still some obstacles to shift orientation control technique for nanorods from a scientific curiosity to a practical methodology for fabrication of next generation materials and devices. Let me give you two examples of the obstacles.

First, let us assume the functional thin membrane that is used for applications such as selective separation and purification and controlled-release materials. The membrane thickness should be as thin as possible to achieve high throughput. It is desirable for the membrane to possess defined

pore size. Control over the pore orientation in the membrane is essential to achieve high performance. Ideal materials with a pore, such as carbon nanotube³⁰, protein-based nanoparticles³¹ and DNA pore^{32,33}, have already existed, so that self-assembly of orientation-controlled these materials into monolayer membrane considered to be a conceptually different approach to produce the membrane that fulfill the above requirements. However, the current self-assembly strategies for colloidal nanoparticles are difficult to be applicable to these materials.

Second, two dimensionally confined arrays of anisotropic nanoparticles prepared are static after being prepared, resulting in only a single set of properties. For example, the resultant structures formed after drying process are unsuitable for dynamic control of colloidal nanoparticle ordering because they are closely packed. Interfacial assembly would enable control over colloidal nanoparticle ordering with a certain spacing. However, the orientation of anisotropic nanoparticles is difficult to be altered dynamically because the orientation is thermodynamically stable at the interface. In other words, it remains challenging to achieve the dynamic alteration of properties of anisotropic nanoparticle assemblies confined in a 2D space.

1.3 End-Grafted Polymers

Grafting polymer molecules by one end to an interface is one of the effective methods to control over the polymer conformation because a grafted polymer experiences forces such as the osmotic pressure and excluded volume repulsions. The conformation of end grafted polymers can be divided into three classes: (i) pancake structure, (ii) mushroom structure and (iii) brush structure (**Figure 1. 1**)³⁴. When the density of the polymer molecules is very high, the polymer chains are in a stretched conformation (“brush-like”) which is far from the typical random coil conformation that polymer chains attain in solution. In contrast, at low grafting densities, the polymer chains adapt the mushroom conformation. However, if the polymer chains adsorbed onto the underlying substrate, the polymer chains obtain the

pancake conformation. In addition to grafting density, surrounding environmental conditions, for example, pH, temperature and ionic strength affects the conformation of end-grafted polymers. Inspired by knowledge on the phase behavior of end-grafted polymer chains from the macromolecular community, I hypothesized that the end-grafted polymers can be used for orientation control of anisotropic nanoparticle that can remove the obstacles.

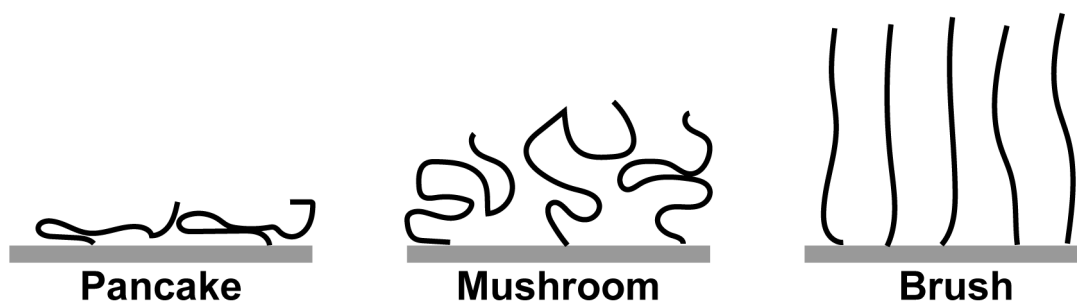


Figure 1. 1 Schematic illustration of three structures of end-grafted polymer chains.

1.4 DNA as a Unique Polyanion

DNA is an interesting biomolecule not only as a carrier of genetic information but also as a polyanion with unique physical property and excellent molecular recognition ability. The structure of DNA consists of the 5'-phosphate terminus, the 3' -OH terminus, the phosphate-sugar backbone, and the bases as side chain. The A-T and G-C bases between two antiparallel strands interact through hydrogen bonds and π - π stacking, to form a stable, double stranded (ds) DNA helix. The formation of base pairing affords a unique physical property and excellent molecular recognition ability to DNA. Furthermore, design of base sequence allows DNA to bind a variety of targets including small molecules, proteins, viruses, and cells^{35,36}. Thus, use of DNA as a component would broaden the potential of self-assembly strategy.

1.5 Gold Nanorods

Tremendous efforts have been devoted to the development of the shape-controlled nanocrystal over the several decades^{37,38,47–52,39–46}. Synthesis work have paved the way for the creation of nanocrystals, whose properties can be greatly expanded beyond their atomic or macroscopic forms. To bridge the gap between nanoscale materials and macroscopic applications, orientation control is of strategic importance. Although the range of accessible anisotropic nanoparticles drastically has increased^{30,38,39,41,46,53–55}, “rod-shaped gold nanoparticles (gold nanorods)” have several advantages in using them as model anisotropic nanoparticles: first, plasmonic properties of gold nanorods allows us to monitor and analyze their orientation in a simple way. Second, chemical synthetic methods for monodisperse gold nanorods are well-established. Third, surface properties of gold nanorods can be tuned. In the following subsections, these characteristics of gold nanorods rendering them a canonical example to develop the orientation control techniques will be described.

1.5.1 Plasmonic Properties of Gold Nanorods

When gold is divided into minuscule grains such as gold nanoparticles, interesting properties arise, which is referred to as localized surface plasmon resonance (LSPR)^{56,57}. LSPR is a phenomenon that occurs when light interacts with conductive particles that are smaller than the incident wavelength. When the nanoparticle is exposed to light of a specific wavelength, a motion of free electrons toward the nanoparticle surface is induced by the electromagnetic field of the light. Because of the restriction to the movement of these electrons within the nanoparticle, the pair of electric charges separated, namely a negative one in one side and positive charge in the opposite side, resulting in the formation of a dipole. Such a coherent displacement of electrons from the positively charged lattice exerts a restoring force on these driven electrons. As a result, the dipole can enter into resonance with the incident photon oscillations, leading to strong absorption of a specific wavelength. The energy

required to excite electrons in gold nanoparticles is affected by the dimensions of the gold nanoparticles and the arrangement of neighboring gold nanoparticles.

The plasmonic properties of the GNRs allows us to investigate their orientation and their assembly mode by simple spectroscopic analysis^{58,59}. GNRs exhibit two plasmonic modes: (i) transverse localized surface plasmon resonance (TLSPR), excited by the oscillation of electrons along the major axis of the GNRs and (ii) longitudinal localized plasmon resonance (LLSPR), excited by the oscillation of electrons along the minor axis of the GNRs (**Figure 1. 2a, b**). In the case of GNRs with the size of 34 nm × 10 nm, the TLSPR is located around 500 nm while the LLSPR is located around 800 nm. Furthermore, because of the optically anisotropic property, GNR change color depending on their orientation relative to the direction of the incident light⁶⁰. For example, when a GNRs orient perpendicular to the direction of the oscillating electric field, the GNR does not show a LLSPR band. However, the intensity peak for LLSPR band increases as the GNR orientation shifts from parallel direction to vertical direction with respect to the incident light. Furthermore, GNRs show polarization dependent optical response. Let us consider GNR monolayer arrays aligned in a single direction on a substrate (**Figure 1. 2c**). As the substrate is rotated with respect to the incident light, p-polarized light excites the LLSPR mode (**Figure 1. 2d**); however, s-polarized light does not excite LLSPR at any angle (**Figure 1. 2e**).

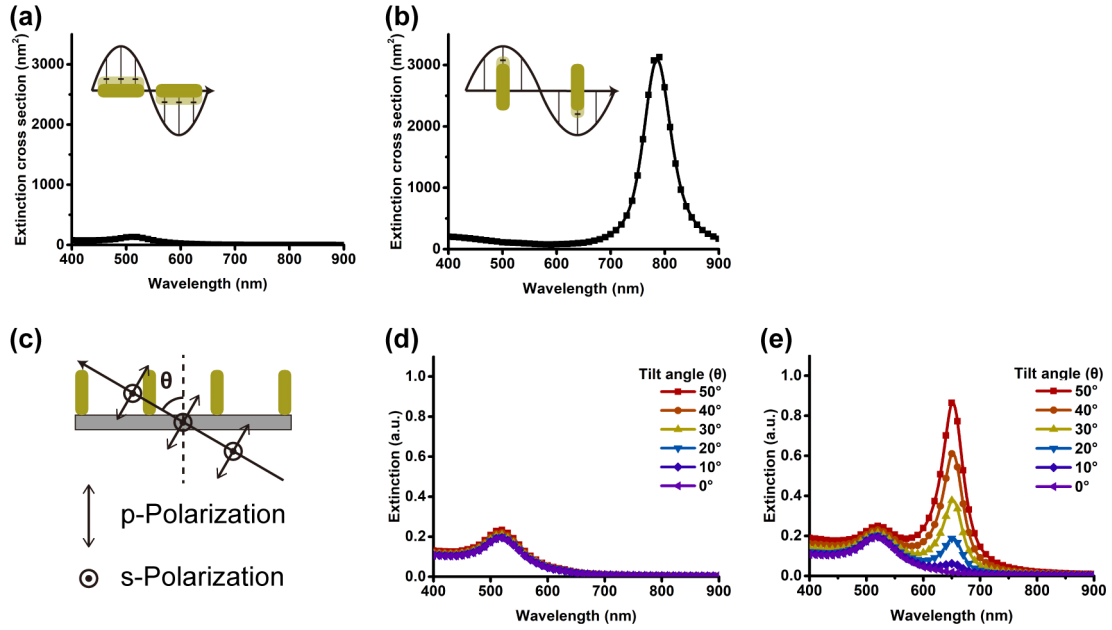


Figure 1. 2 FDTD-calculated GNR ($34 \text{ nm} \times 10 \text{ nm}$) optical properties. (a, b) Simulated extinction cross section of a gold nanorod with its longitudinal (a) and transverse (b) axis aligned to the polarization of the incoming light. (c) Scheme showing the extinction of GNRs under p- and s-polarized light. (d, e) Simulated extinction spectra of perfectly aligned GNR arrays under p-polarized (d) and s-polarized (e) light. The GNR arrays assumed to be arranged in a hexagonal pattern, and the center-to-center distance was 30 nm.

In addition to the GNR orientation, the GNR plasmonic properties provide information on how GNRs assemble. For example, theoretical work predicts that GNRs assembled in side-by-side mode show blue-shifting of the LSPR peak (**Figure 1. 3a**), whereas GNRs assembled in end-to-end mode show red-shifting of the LSPR peak (**Figure 1. 3b**)^{61,62}. Thus, we can examine the self-assembly process of gold nanorods in a simple manner.

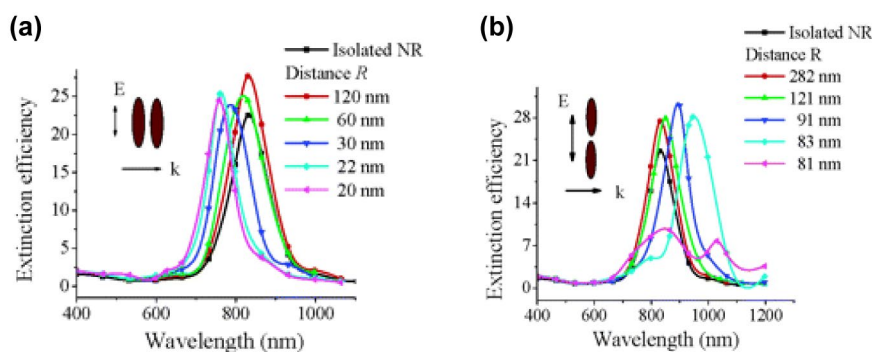


Figure 1. 3 Discrete dipole approximation (DDA) calculated GNR (80 nm $\times$ 20 nm) optical properties with their transverse axis aligned to the incoming light. (a) Simulated extinction cross section of GNRs assembled in a side-to-side orientation (b) Simulated extinction cross section of GNRs assembled in an end-to-end orientation. The values in the graphs are GNR center-to-center distance. Reprinted with permission from reference 62.

1.5.2 Synthesis of Gold Nanorods

In addition to the plasmonic properties of gold nanoparticles, outstanding progress in the chemical synthetic techniques for gold nanoparticle is also considered to be one of the causes that gold nanoparticles are focused on^{54,58,59,63,64}. The field of gold nanoparticle chemistry can be traced back to the pioneer work by Michael Faraday in 1851, in which the colloidal gold dispersions were synthesized by reduction of a gold salt with phosphorus dissolved in carbon disulfide⁶⁵. After several decades, Zsigmondy proposed a synthetic method combining his synthetic technique with Faraday's method⁶⁶. This method involves two step processes and is now termed as "seed-mediated" method. Another landmark study for the synthesis of gold nanoparticle using sodium citrate as a reducing reagent was reported by Turkevich et al. in 1951⁶⁷. In this study, they improved a previous preparation method reported by Hauser and Lynn. Until today, the sodium citrate method, in general referred to as "Turkevich method", has evolved through numerous improvements and then allowed for the large-scale synthesis of monodisperse spherical gold nanoparticles in a wide range of sizes⁶⁸.

Inspired by the advancement of the synthetic techniques for spherical nanoparticles, chemists have developed a wet chemical synthesis of metal nanorods about three decades ago. An early method to fabricate metal nanorods employed porous alumina^{69,70} or polycarbonate⁷¹ membrane templates to grow metal nanorods. A colloidal solution of nanorods can be obtained by releasing them from the template and disperse them into solvents, but the method is suffered from low yield. Around the beginning of the 20th century, novel method, which is “seed-mediated” method, allowing large-scale synthesis of gold nanorods was reported by Murphy et al. and El-Sayed et al^{54,72} (**Figure 1. 4**). The unique point is to use cationic surfactant hexadecyltrimethylammonium bromide (CTAB) as a surfactant to direct nanorod formation. Since then, several research groups have attempted to improve the yield of monodispersed gold nanorods^{73,74,83–85,75–82}, and thereby we can obtain monodispersed gold nanorods in high yield.

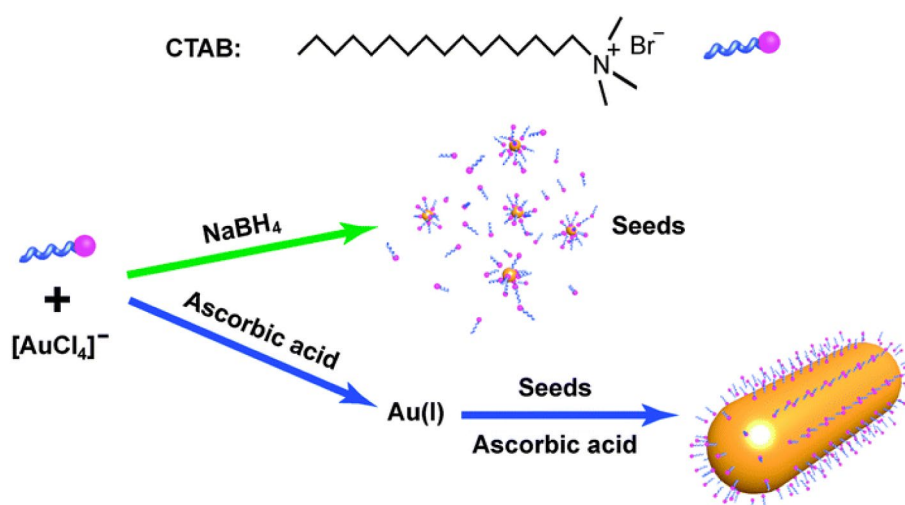


Figure 1. 4 Schematic representation of the “seed-mediated” method for the synthesis of gold nanorods. Reprinted with permission from ref. 59.

1.5.3 Modulation of Surface Properties of Gold Nanorods

The modulation of surface properties of gold can be achieved by the formation of self-assembled monolayers (SAMs)^{86,87}. SAMs are versatile molecular assemblies, highly ordered and oriented. A

representative SAM molecule consists of three units: a head group, a spacer and a functional group (Figure 1. 5). The head group is bound to a surface and the functional group determines the surface property. In case of gold surface, thiol ligands are the mostly used because the strength of the Au–S interactions provides basis to form robust self-assembled monolayers on gold surface. The spacer generally possesses alkyl chain, which facilitates packing arrangement of the molecules.

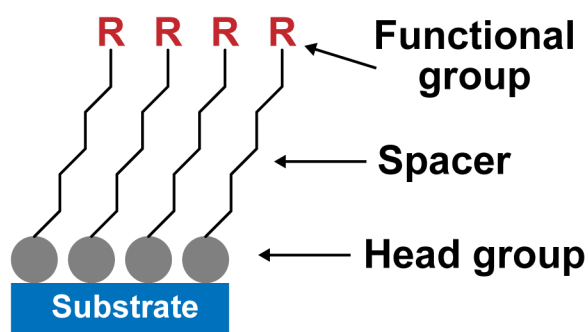


Figure 1. 5 Schematic diagram of a SAM monolayer.

SAM molecules are used for functionalization of gold nanorods to impart stability in the nanoparticle and modulate the interactions among neighboring nanoparticles. In general, as-prepared gold nanorods are protected with a capping reagent (CTAB) present in the synthetic reaction. Using various kinds of thiol ligands with terminal functional groups such as carboxylic ($-\text{COOH}$), amine ($-\text{NH}_2$) and hydroxy ($-\text{OH}$), hydrophobic, hydrophilic and charged gold nanorods can be prepared. Furthermore, surface properties of gold nanorods can be varied by mixing ligands containing different functional moieties together.

1.6 Aims of this project

In this project, I aim to develop new orientation control strategy that can produce ordered arrays or dynamically alterable structures from rod-shaped nanoparticles. More especially, I propose polymer brush directed self-assembly of rod-shaped nanoparticles utilizing electrostatic interactions. Electrostatic interactions can be adjusted through surface chemistry and by the ionic strength, and thus

can impart dynamic properties to the resulting structure. Schematic illustration of this work is given in **Figure 1. 6**.

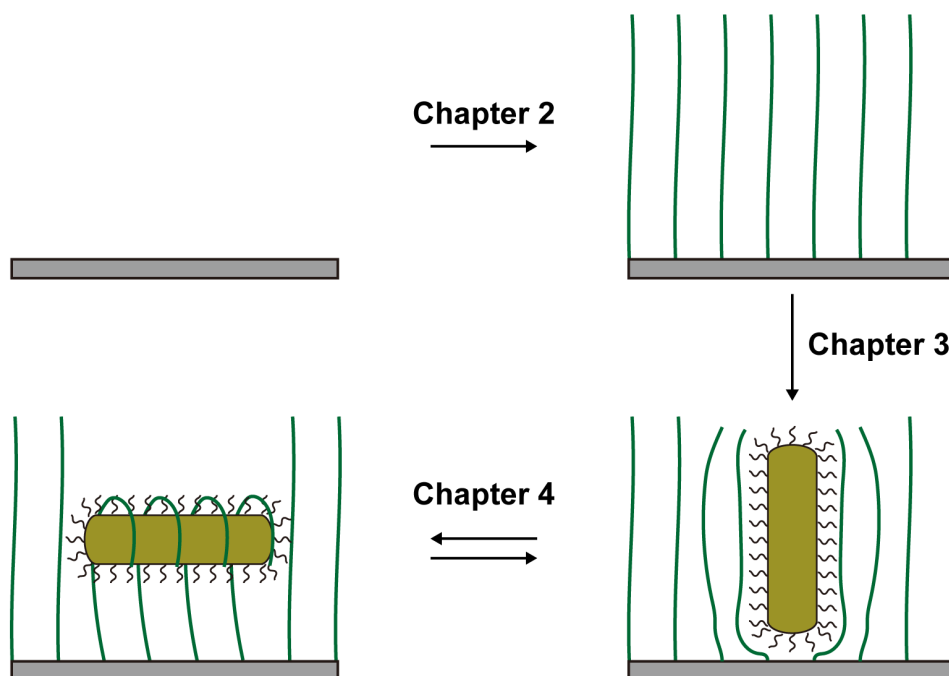


Figure 1. 6 Schematic illustration of this thesis contents. The concept of this study is to use end-grafted polymers as a matrix for orientation control of anisotropic nanoparticles.

In chapter 2, I will prepare DNA brushes in two ways: “grafting to” and “grafting from” techniques. Chapter 3 will describe a DNA brush-directed self-assembly method that produce vertically aligned gold nanorod arrays over large areas. Chapter 4 will study the salt-induced assembly behavior of gold nanorods electrostatically attached to substrate surfaces. Finally, in chapter 5, I will summarize this thesis.

Chapter 2

Preparation and Characterization of DNA Brush

2.1 Introduction

Major advances in the surface modification strategies – for example, layer-by-layer (LBL) assembly^{88–90}, self-assembled monolayer (SAM)^{86,87}, Langmuir-Blodgett (LB) technique⁹¹ and grafting method^{92,93} – have been made for both fundamental and applied scientific research. Especially, the dense grafting of polymer brush onto a solid surface through the one end has attracted much attention as a strategy for the development of functional materials. This is because grafted polymers can take on the stretched configurations and further change their conformation between collapsed and swelling states reversibly, which lead to adaptive and switchable surface^{94,95}. Synthesis of polymer brushes can be mainly divided into two methods: (i) “grafting to” method and (ii) “grafting from” method (**Figure 2. 1**). In the former method, polymer chains are immobilized directly onto a surface. In the latter method, initiators are anchored onto a surface followed by polymerization. Each strategy has pros and cons. For example, the “grafting to” method is experimentally simpler than the “grafting from” method. In contrast, the “grafting from” method can yield high-density long polymer brushes because the “grafting to” method can avoid the steric hindrance effect of pre-adsorbed polymer chains.

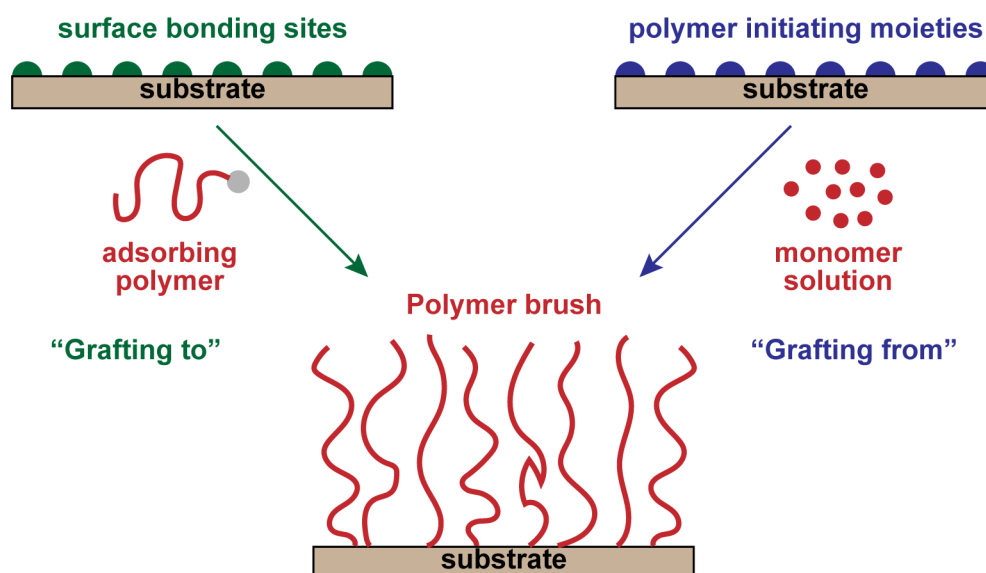


Figure 2. 1 Scheme depicting synthetic routes of polymer brushes: “grafting to” and “grafting to” strategies.

A DNA brush, a collection of DNA polymers end-grafted to a surface, has been used in the fields of biomedical applications, materials engineering research, and so on. These utilities largely rely on the molecular recognition of DNA molecules, which synthetic polymers do not exhibit. Most of the DNA molecular recognition comes from the formation of base pairing between A-T and G-C, resulting in the unique conformational varieties. Examples of the varieties include double stranded helix, G-quadruplex, C-rich i-motif and molecular beacon³⁵. These unique conformations strongly affect the physical properties of DNA. For example, while dsDNA behaves as a rigid polymer with a large persistence length (~ 50 nm), single-stranded (ss) DNA behaves as a flexible worm-like polymer with a short persistence length (~ 1 nm)⁹⁶. Although DNA is a unique polymer in terms of not only their chemical properties but also their physical properties, most of the previous studies on DNA brush has paid attention to only their chemical properties. This may be partly because there are only a few reports on the preparation of long dsDNA brushes. In addition, only a few works have been done on investigation of the crucial parameters governing the behavior of dsDNA brush such as grafting density and chain length⁹⁷.

In this chapter, DNA brushes are prepared by the “grafting to” and “grafting from” methods. For “grafting to” method, DNA polymers are synthesized using a standard polymerase chain reaction (PCR) and then immobilized onto a solid surface. For “grafting from” method, short oligonucleotides are immobilized onto a solid surface and subsequently polymerized using a DNA polymerase. The grafting density and chain length are investigated by Ultraviolet-visible (UV) spectroscopy and agarose gel electrophoresis. Furthermore, atomic force microscopy (AFM) is used to characterize the conformation of the DNA brush prepared via “grafting from” method both in aqueous solution and under ambient conditions.

2.2 Experimental

2.2.1 Materials

Oligonucleotides are purchased from Thermo Fisher Scientific, Inc. (USA) or Integrated DNA Technologies, Inc (USA). The sequences of the oligonucleotide and the chemical structure of the linker between DNA and modification site are shown in **Table 2. 1** and **Figure 2. 2**, respectively.

Table 2. 1 Oligonucleotides sequences used to synthesize DNA brushes.

Description	Sequence (5'→3')
Biotin-primer	BIOTIN -GTACGAACGCCATCGACTTACG
Alexa647-primer (148)	ALEXA647 -GAGCCCGTCGAGAATACTGGC
Biotin-T148	BIOTIN -T ₁₄₈
Biotin-dC ₁₅	BIOTIN -(GTCCT) ₂ CGA(GTCCT) ₂ C ₁₅
dG ₁₅	G ₁₅

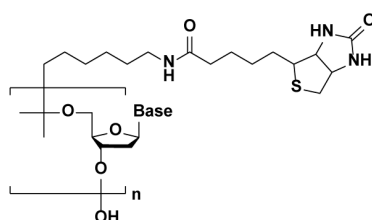


Figure 2. 2 Chemical structure of the linker between DNA and biotin-modification site. The aminohexyl (C6) linker is used to incorporate a biotin group onto the 5'-end of an oligonucleotide.

2-(carbomethoxy) ethyltrichlorosilane (CMETS) was purchased from Fluorochem, Ltd. (UK). Amine-PEG₂-Biotin was purchased from Thermo Fisher Scientific, Inc. (USA). H₂AuCl₄·3H₂O and NaBH₄ were purchased from Sigma-Aldrich, Inc. (USA). Ultrapure water (18.2 MΩcm⁻¹, Milli-Q, Millipore, USA) was used to make up all solutions. λ-DNA was purchased from Nippon Gene Co., Ltd. (Japan). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, hydrochloride (EDC) was purchased from Dojindo Laboratories Co., Ltd. (Japan). QIA quick PCR Purification Kit was purchased from Qiagen, Inc. (Germany). Quick Taq HS DyeMix was purchased from TOYOBO Co., Ltd. (Japan). All other salts and reagents, were purchased from Wako Pure Chemical Industries, Ltd. (Japan). Cuvettes were purchased from TOSOH Co., Ltd. (Japan). Glass substrates were purchased from Fujiwara

Scientific Company Co., Ltd. (Japan). X-ray photoelectron spectroscopy (XPS) analysis was performed with a JPS-9200 photoelectron spectrometer (JEOL). UV-vis absorption spectra were recorded with a UV-2600 (Shimadzu Corporation). Ultraviolet (UV) light was irradiated with a mercury/xenon lamp Lightningcure LC8 (Hamamatsu Photonics K.K.). Gel imaging was performed with a FLA-7000 (FUJIFILM). Atomic force microscopy analyses of 2D-patterned DNA brushes were performed with MFP-3D-BIO (Asylum Research) by using a cantilever of 0.09 N/m spring constant (BL-AC40TS-C2; Olympus).

2.2.2 Synthesis of DNA for “Grafting to” Method using PCR

Linear ds DNA was synthesized by a standard PCR procedure using λ DNA as a template and a pair of 5' modified primers, which have biotin and fluorescent marker respectively (**Figure 2. 3**). The biotin primer (10 μ M), Alexa647 primer (148) (10 μ M) and λ DNA (0.20 μ g/ μ L) were mixed at a ratio of 1:1:1. The primer-template mixed solution, MilliQ and Quick Taq HS DyeMix were mixed at a ratio of 3:22:25. Thermal cycling was carried out as follows: 2 min at 94°C, followed by 20 cycles of 20 sec at 94°C, 30 sec at 58°C and 1 min at 68°C. After the reaction was completed, the products were cooled to 4°C. Finally, a Qiagen PCR purification kit was used to remove the non-polymerized primers.

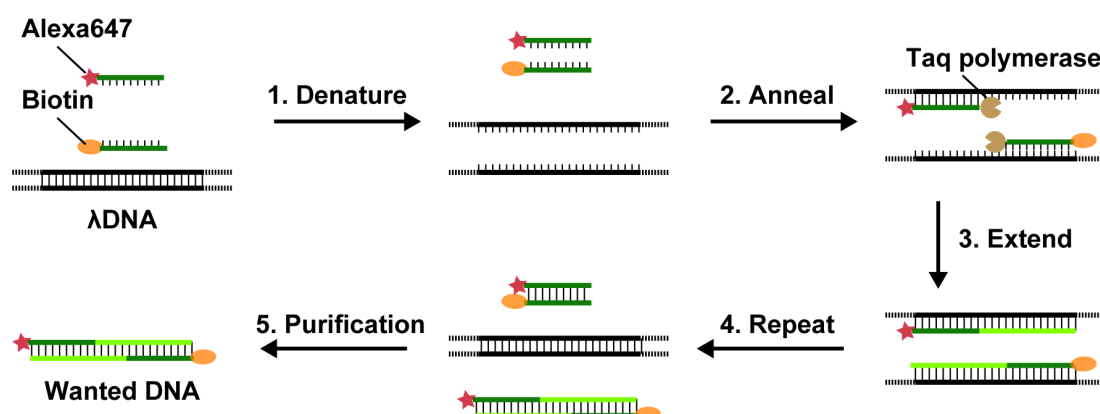


Figure 2. 3 Schematic depicting synthesis of biotin- and alexa647-modified dsDNA for “grafting to” method using a standard PCR procedure.

2.2.3 Preparation of Streptavidin Surface

Schematic of the preparation of streptavidin surface is shown in **Figure 2. 4**. A cuvette or substrate was cleaned with piranha solution (a 3:1 mixture of concentrated H_2SO_4 with H_2O_2) for 1 h, with subsequent rinsing in MilliQ, and dried in an incubator (60 °C). The cleaned cuvette or substrate was placed in a N_2 -filled glovebox below 20% relative humidity, and the inside surface of the cuvette or the substrate was immersed in 0.01% v/v superdehydrated toluene of CMETS. A silane coupling reaction was performed for 1 h, followed by rinsing with acetone, ethanol, and MilliQ. After the rinsing, the surface methoxy group was hydrolyzed by the treatment with concentrated HCl for 16 h. To immobilize the biotin, the hydrolyzed cuvette or substrate was soaked in a 10 mM HEPES-KOH buffer solution (pH = 7.4) containing EDC (50 mM) and amine-PEG₂-biotin (1 mM) for 1 h. Streptavidin was immobilized onto the cuvette or substrate via streptavidin–biotin interaction by incubating the biotinylated surface with a pH 7.7 Tris–HCl buffer containing streptavidin (100 µg/mL) for 1 h.

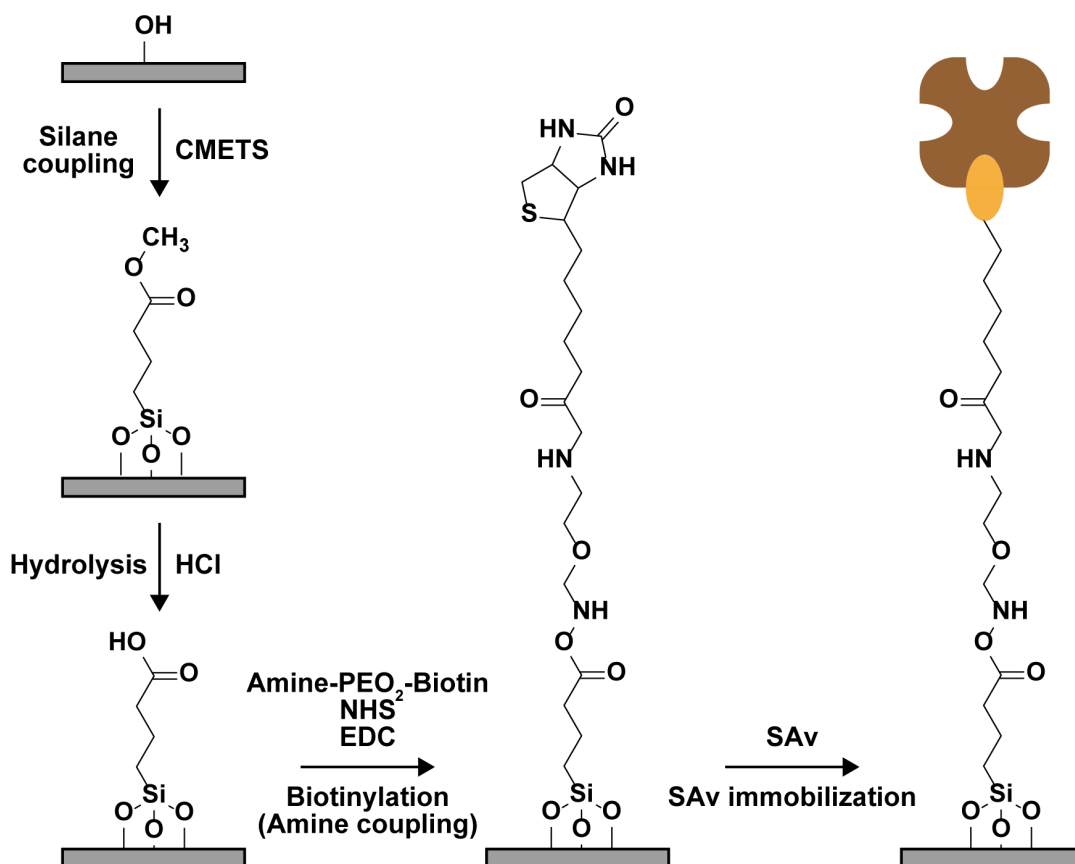


Figure 2. 4 Preparation scheme of streptavidin surface.

2.2.4 Preparation of DNA Brush via “Grafting to” Method

In “grafting to” method, synthesized DNA using PCR was immobilized through biotin-streptavidin interactions (Figure 2. 5). The biotinylated DNA was immobilized by soaking the streptavidin-immobilized cuvette or substrate in a pH 7.7 Tris-HCl buffer containing the biotinylated DNA and NaCl. The nongrafted DNA was removed by rinsing the inside surface of the cuvette or the substrate with pH 7.7 Tris-HCl buffer at least three times.

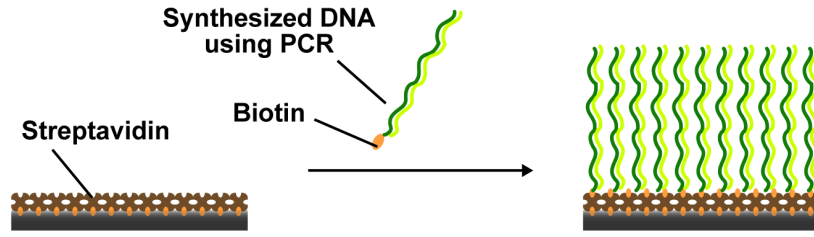


Figure 2. 5 Schematic illustration of DNA brush preparation via “grafting to” method.

2.2.5 Preparation of DNA Brush via “Grafting from” Method

In “grafting from” method, DNA slippage was used to produce DNA brush (**Figure 2. 6**). Biotin-dC₁₅ solution (1 μ M, in 10 mM Tris-HCl buffer, 200 mM NaCl, pH = 7.9) was applied onto the streptavidin surface. The complementary oligo-dG₁₅ solution was applied on the dC₁₅-coated surface to form hybridization complexes. The hybridized oligonucleotides, which have homo-sequence, were polymerized using Klenow fragment exonuclease minus (KF-) in 10 mM Tris-HCl buffer (pH = 7.9) with 0.25 units/ μ l KF-, 0.5 mM dCTP, 0.5 mM dGTP, 2 mM MgCl₂, 200 mM NaCl at 37 °C.

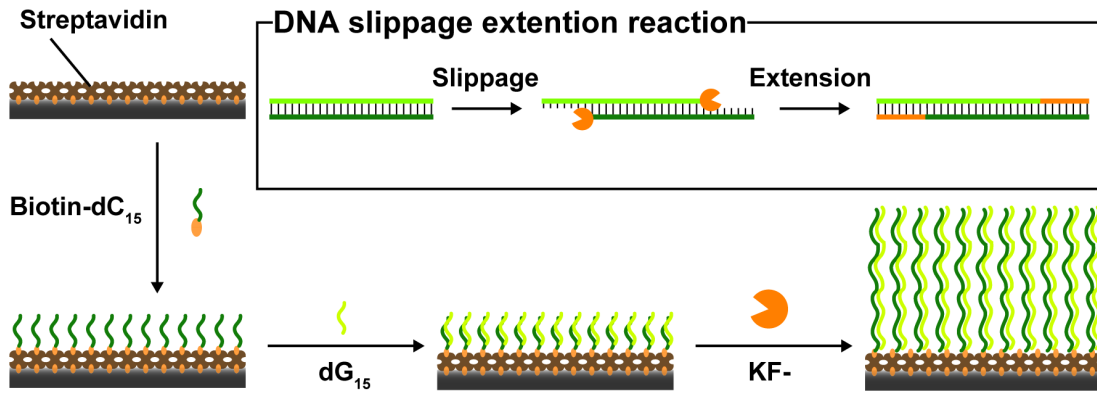


Figure 2. 6 Schematic illustration of DNA brush preparation via “grafting from” method.

2.2.6 Calculation of DNA Brush Density and Interchain Distance

For the “grafting to” approach, DNA brush density (σ) can be calculated as following:

$$\sigma = \frac{(Abs_{260} \text{ (before)} - Abs_{260} \text{ (after)}) \times V_{\text{DNA solution}} \times N_{\text{Avo}}}{l_o \times \varepsilon \times A_{\text{cuvette}}}$$

where Abs_{260} (before) and Abs_{260} (after) are the absorption values at 260 nm of DNA solution before and after DNA immobilization; l_o is the optical path length of the cuvette (in our experiment, $l_o = 0.1$ cm); ε is molar adsorption coefficient; $V_{DNA\ solution}$ is the volume of the added DNA solution (in our experiment, $V_{DNA\ solution} = 0.2$ mL); N_{Av0} is the Avogadro constant ($6.02 \times 10^{23} \text{ mol}^{-1}$); $A_{cuvette}$ is the area where DNA solution was immersed (in our experiment, $A_{cuvette} = 4.5 \times 10^8 \text{ }\mu\text{m}^2$). For DNA, ε is given by:

$$\varepsilon = \frac{Mw_{Ave.}}{1 \text{ } OD_{260} \text{ unit}}$$

where $Mw_{Ave.}$ is the average DNA molecular weight; $1 \text{ } OD_{260} \text{ unit}$ is absorbance units to nucleic acid concentration conversion at $l_o = 1$ cm. For dsDNA, $1 \text{ } OD_{260} \text{ unit}$ is 50 $\mu\text{g/ml}$. For ssDNA, $1 \text{ } OD_{260} \text{ unit}$ is 37 $\mu\text{g/ml}$.

The averaged DNA interchain distance (d) was estimated assuming that DNA chains arrange in a hexagonal pattern and are stretched to their counter length. Averaged DNA interchain distance (d) can be estimated as following:

$$d = \sqrt{\frac{2}{\sqrt{3} \times \sigma}}$$

This equation is also used for the averaged interparticle distance of particles.

For “grafting from” approach, the density of DNA was estimated as following equation assuming that dsDNA dispersed in an aqueous solution and dsDNA immobilized on substrate have the same absorption coefficient:

$$\sigma = \frac{Abs_{260} \times 1 OD_{260} \times 2^{-1}}{1 \text{ (cm)} \times Mw_{Ave.} \times N_b}$$

“2⁻¹” is placed in the equation as the cuvette has two surfaces where DNA was immobilized in the optical pathway.

2.3 Results and Discussion

2.3.1 Preparation of Streptavidin Surface

The water contact angles of the substrates were measured to characterize the surface alternation. Hydrophilic surface shows high contact angle and hydrophobic surface shows low contact angle. Thus, changes in contact angle provides evidence for surface alteration. Photos of a droplet on the substrates are shown in **Figure 2. 7a**. The contact angle values are plotted in **Figure 2. 7b**. Bare glass substrate treated with the piranha solution showed contact angle of 50° which is lower than that of the untreated bare glass substrate, which suggests that the piranha washing process cleaned organic residues off the bare glass surface. The silane coupling treatment increased contact angle of the glass substrate from 14° to 38° . Methoxy group is more hydrophobic than hydroxyl group, thus the increase in contact angle suggests that the cleaned substrate undergoes silane coupling reactions. A decrease in contact angle after HCl treatment is due to hydrolysis of the surface methoxy group. A treatment for biotin-immobilization caused a small increase in contact angle. Biotin is a hydrophobic molecule, and therefore the slight change in contact angle suggests biotin-immobilization. Streptavidin treatment increased slightly contact angle of the biotin-immobilized surface, which is against to my speculation that streptavidin immobilization results in hydrophilization of the biotin-immobilized surface since streptavidin is negatively charged. Considering contact angle measurement requires drying process, a possible reason is that streptavidin denatures, and as a result, expose its hydrophobic part to the air-water interface.

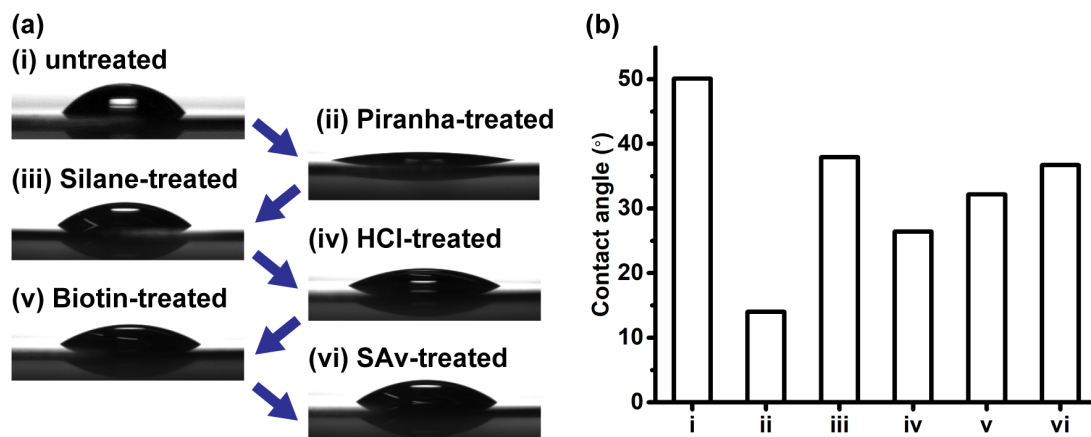


Figure 2. 7 Static contact angles of the substrates to water. (a) Images of water droplets on the substrates. (b) Contact angle values for (i) untreated, (ii) piranha-treated, (iii) silane-treated, (iv) HCl-treated, (v) biotin-treated, and (vi) streptavidin (SAv)- substrates.

Next, a chemical state of element present on the surfaces was analyzed by XPS technique to confirm the surface functionalization (**Figure 2. 8**). The signal intensity for C1s (ca. 410 eV) was enhanced by silane-coupling modification, and further enhanced by streptavidin immobilization, while the intensity for Si2s (ca. 160 eV) and Si 2p (ca. 110 eV) signals decreased. This changes in the XPS spectra indicate the accumulation of organic compounds. N1s signal (ca. 390 eV), derived from biotin-linker and streptavidin, was traced. The N1s signal intensity was increased by the biotinylation of the HCl treated surface, and was further increased by the streptavidin immobilization, indicating the successful immobilization of biotin and streptavidin. Thus, the XPS results indicate that bare glass undergoes the chemical surface functionalization in accordance with the scheme in **Figure 2. 4**.

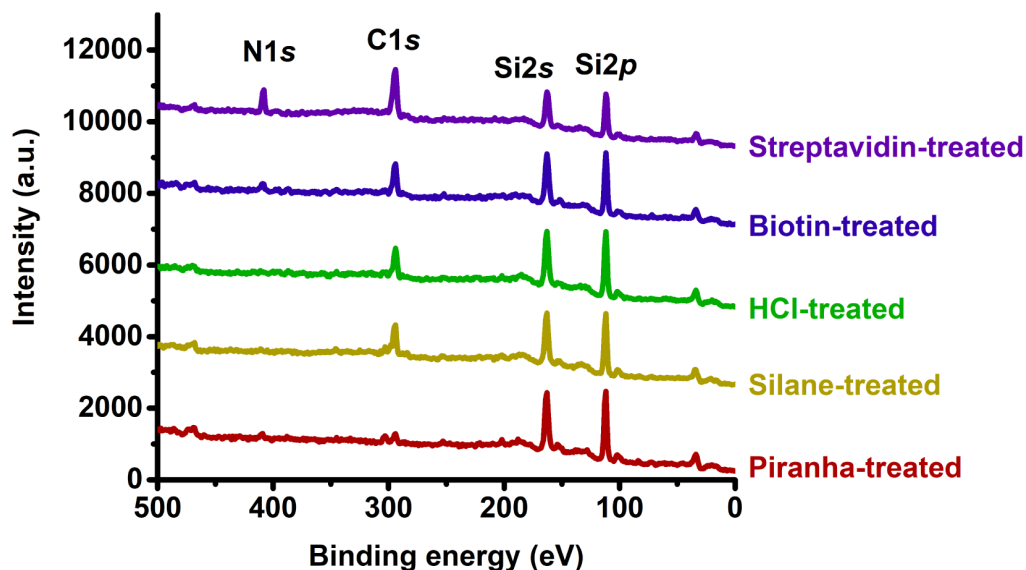


Figure 2. 8 XPS spectra of piranha-treated, silane-treated, HCl-treated, biotin-treated, and streptavidin-treated substrates.

Finally, AFM measurements of before and after the streptavidin immobilization were performed to directly visualize the immobilized streptavidin. AFM images of silane-treated substrate and streptavidin-treated substrate are shown in **Figure 2. 9**. Bumps of about 4-5 nm in height and about 20 nm in width were observed, while the bumps were not observed in AFM image before the streptavidin immobilization. Considering that streptavidin has a hydrodynamic diameter of 5 nm⁹⁸ and the width is larger owing to tip dilation, the bumps are streptavidin.

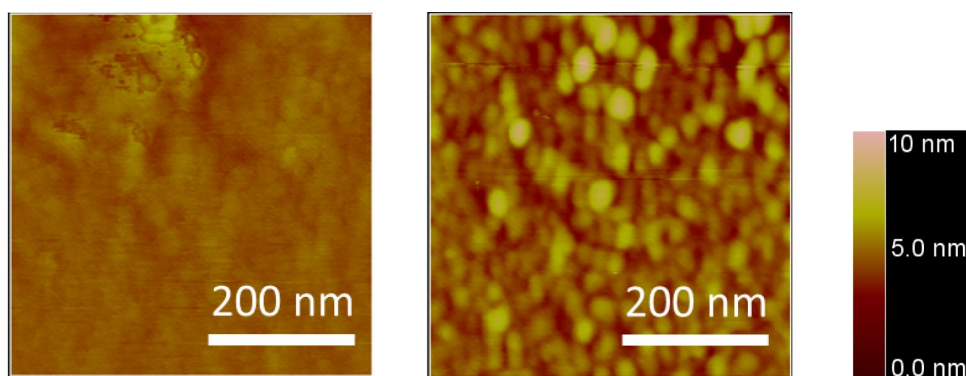


Figure 2. 9 AFM images of silane-treated (left) and streptavidin-coated substrates (right).

2.3.2 DNA Brush Prepared via “Grafting to” Method

148 bp dsDNA with biotin and alexa647 modifications at the 5' ends of the complementary strands was synthesized using biotin-primer and alexa647-primer (148). Assuming a standard B-form duplex of which helical pitch is 3.4 nm or 10 base pairs per turn, the length of the DNA is about 50 nm. Using the 148 bp dsDNA, dsDNA brushes were prepared.

First, I investigated with non-biotinylated 148 bp DNA as a negative control whether DNA polymers are immobilized via biotin-streptavidin interactions. Fluorescence signal intensity of the cuvettes were shown in **Table 2. 2**. For biotin modified DNA, strong fluorescence signal was detected. The fluorescence signal for non-biotin modified DNA was less than a hundredth of that for biotin modified DNA. Thus, most of the immobilized DNA found to be anchored via biotin-streptavidin interaction, rather than nonspecifically.

Table 2. 2 Fluorescent intensity per unit area of immobilized 148bp Alexa647-labeled dsDNA.

DNA	Fluorescence intensity (a.u.)
Biotinylated DNA	8.75×10^5
Non-biotinylated DNA	7.33×10^3

The absorbance at 260 nm of the DNA solution before (Abs_{260} (before)) and after (Abs_{260} (after)) the DNA immobilization was measured to obtain values of DNA brush density (σ) and mean DNA interchain distance (d). Values of Abs_{260} , the grafting densities and the mean DNA interchain distance are summarized in **Table 2. 3**. Streptavidin has about 5 nm hydrodynamic diameter and can bind up to four biotin molecules. Therefore, the smallest DNA interchain distance could be less than 5 nm. Considering the smallest DNA interchain distance was larger than 5 nm, DNA brush density was limited by steric and electrostatic barriers, rather than by shortage of surface binding sites.

The grafting density of 148 bp dsDNA obtained here were larger than critical values (σ_c) at which grafted polymer chains begin to overlap, and therefore it is inferred that the grafted DNA chains

repel each other due to steric repulsion or osmotic pressure which force them to stretch: σ_c is described as $\sigma_c \sim R^{-2} = l_a^{-2} N_a^{-1}$, where R is the end-to-end distance; l_a is the Kuhn length of the polymer; N_a is joint Kuhn segment of the polymer. Furthermore, a phase diagram of dsDNA brushes prepared by bar-ziv et al.⁹⁷ also supports that the dsDNA brush with the grafting densities obtained here could attain brush-like configuration.

Table 2. 3 Brush density and inter-chain distance of DNA brushes prepared via the “grafting to” approach.

Run	DNA	NaCl (mM)	DNA (nM)	Brush density (chains/ μm^2)	Mean Inter-chain distance (nm)
1	148 bp dsDNA	1000	200	24000	7.0
2	148 bp dsDNA	1000	100	17000	8.2
3	148 bp dsDNA	50	100	15000	8.8
4	148 bp dsDNA	10	100	8700	12
5	148 bp dsDNA	1	100	3800	17
6	148 base ssDNA	1000	200	18000	8.0

2.3.3 DNA Brushes Prepared via “Grafting from” Method

UV spectroscopic analysis was performed to quantify the enzymatic elongation of the dsDNA on the substrate. The absorbance at 260 nm showed that the amount of DNA on the substrates increased with polymerization time (**Figure 2. 10a**). The amounts of DNA on the substrate were calculated as 5.5×10^{-15} , 9.5×10^{-15} , 15×10^{-15} , 20×10^{-15} g/ μm^2 , respectively. To measure the elongated length of the dsDNA, the polymerized DNA from the substrates was harvested. To harvest the polymerized DNA from the substrate, desthiobiotin-dC₁₅ was used instead of biotin-dC₁₅. Desthiobiotin is a biotin analogue that binds less tightly to biotin-binding proteins and is easily displaced by biotin. The length of the harvested DNA was measured with gel electrophoresis (**Figure 2. 10b**). Two bands were mainly observed in each lane: one is a smearing band and the other is a relatively sharp band which corresponds to the lowest molecular weight. DNA molecules in clear bands grew in proportion to the

reaction time. The clear bands corresponded to 2300, 5700, 8200 and 9700 bp for the 15, 30 45- and 60-min extension reactions, respectively. The length of DNA chains in the smearing bands also increased with the polymerization time and was more than 2-times longer than that of DNA molecules in the clear band. According to a previous report, slippage extension reaction using KF- in solution produces a smearing band, which indicates the aggregation of the DNA molecules⁹⁹. The authors also report that the DNA grows in proportion to the time of the polymerization. Considering the previous literature on the slippage reaction using KF- in solution, it is inferred that the smearing bands observed here indicates the DNA entangled with each other, and the clear bands reflects the actual length of the polymerized DNA. Based on the estimation, the amount and length of the DNA polymerized on the surface are plotted as a function of polymerization time (**Figure 2. 10c**). The amount and length of the DNA polymerized on the surface were in proportion to the polymerization time. Next, the density of the polymerized DNA on the substrates was calculated. The density was approximately 1500 chains/ μm^2 regardless of DNA length (**Figure 2. 10d**). In general, density of polymer brush prepared via “grafting to” approach is significantly dependent on length of the dsDNA. As the length of the polymers become longer, the density of the polymer brush becomes low. Bar-ziv et al. have reported that the density of the immobilized DNA via streptavidin-biotin interactions decreased with increase in the length of the DNA⁹⁹. Thus, the “grafting from” method presented here can provide dense DNA brush with long DNA length.

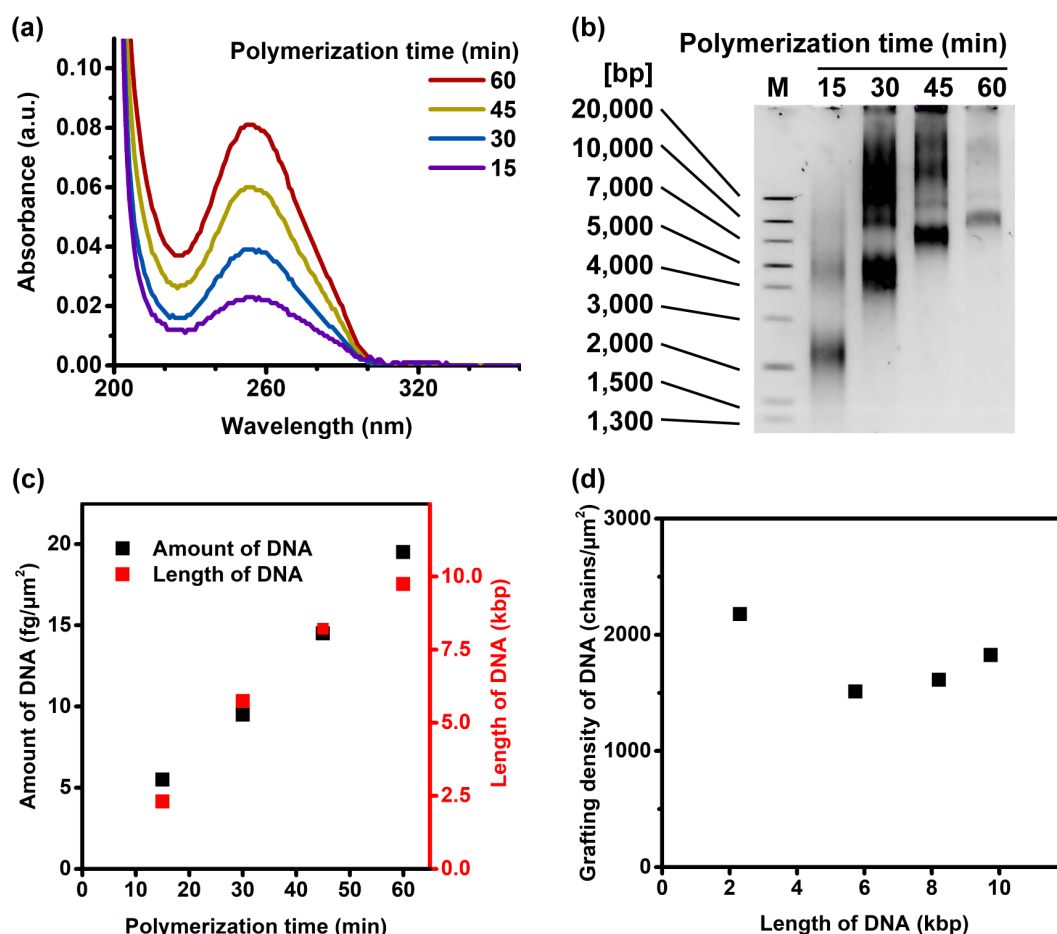


Figure 2. 10 (a) Absorption spectra of dsDNA brushes after 15, 30, 45- and 60-min polymerization. (b) Agarose gel electrophoresis of the harvested dsDNA polymerized on the surface. The DNA molecules were stained with SYBR green I. (c) Amount and length of DNA polymerized on the surface as a function of polymerization time. (d) Grafting density of DNA polymerized on the surface as a function of length of the DNA.

2.3.4 Characterization of Conformation of DNA Brush Prepared via “Grafting from”

Method

For the further characterization of the dsDNA brush, two dimensionally patterned dsDNA brushes were prepared. Patterned dsDNA brush was fabricated by UV-irradiating the streptavidin surface through a photomask prior to oligonucleotide immobilization in order to selectively inactivate the immobilized streptavidin (**Figure 2. 11a**). For the optimization of UV-irradiation time to fabricate

patterned streptavidin substrate, elongated DNA on selectively UV-irradiated streptavidin was stained with SYBR green I. The relative fluorescent intensity was plotted as a function of UV-irradiation time (**Figure 2. 11b**). After 2 min of UV irradiation, almost no fluorescent signal was detected. Based on this UV irradiation test, the streptavidin surface was irradiated with UV light for 2 min through the photomasks and then the initiator DNA was elongated on the substrate. As shown in **Figure 2. 11c**, 2D patterned dsDNA brush was produced with the UV-irradiation through the photomask.

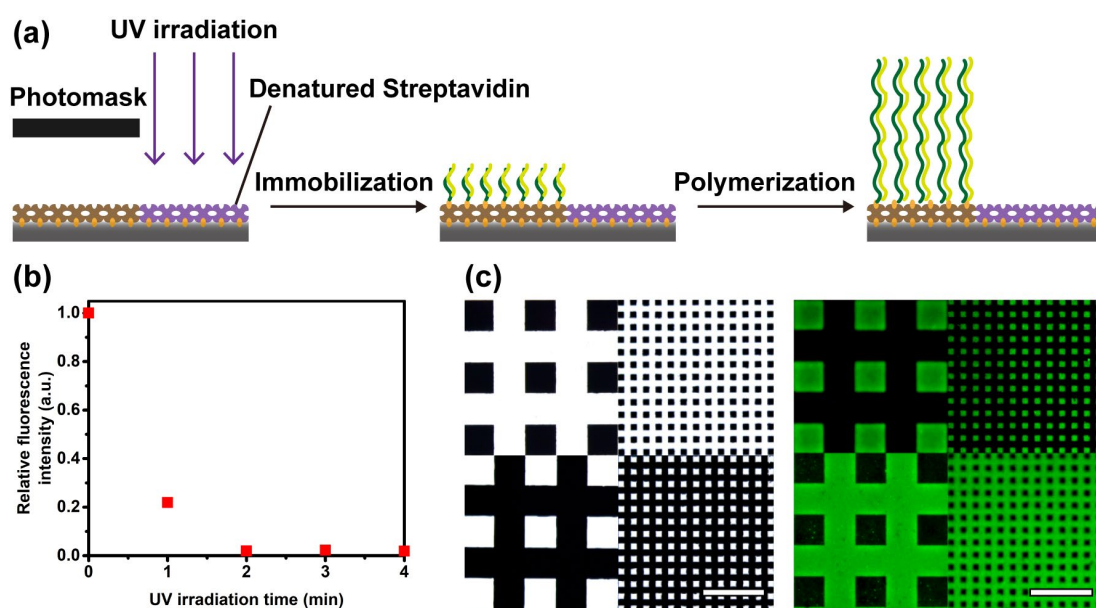


Figure 2. 11 (a) Schematic of DNA brush patterning by irradiating immobilized streptavidin with UV light. (b) Relative fluorescence intensity as a function of UV irradiation time. (c) Optical images of photomask (left images). Black regions do not allow light to pass through, while the white regions allow light to pass through. Fluorescent images of 2D-patterned dsDNA brush stained with SYBR green I (right images). The green represents stained regions with SYBR green I. The scale bars are 20 μm .

To characterize the conformation of the DNA brush prepared via “grafting from” method, the thickness of the DNA brush was measured by AFM. **Figure 2. 12a** shows AFM images of a region of the 2D patterned DNA brush array with the length of about 9200 bp and the grafting density of 1800 chains/ μm^2 . There was a clear contrast between the regions irradiated and not irradiated by UV light. Change in the measured height of the DNA brushes was observed depending on environmental

conditions. DNA brush heights are summarized in **Figure 2. 12b**. In MilliQ water, the measured height was about 150 nm. In NaCl aqueous solution, the measured heights were only one-third the height of that in MilliQ water. Under ambient condition, the measured height decreased to about 9.4 nm. Thus, the DNA brush attains swelling state in aqueous solution. However, these measured heights in aqueous solution are smaller than expected. For an ideal chain behavior of a long semiflexible polymer in solution (wormlike chain model), end-to-end distance (R) of 9200 bp dsDNA is estimated to be about 560 nm [$R = l_a N_a^{0.5} = 100 \text{ nm} \times (9200 \text{ bp} \times 0.34 \text{ nm/bp} \times (100 \text{ nm})^{-1})^{0.5} = 559 \text{ nm}$]. I speculated that AFM cantilever inserts into or compresses the DNA brush due to its low grafting density, and therefore the measured heights of the DNA brush in aqueous solution are underestimated compared to the real height. Further work and improvement will be necessary to measure the real height of the DNA brush in aqueous solution to discuss its conformation.

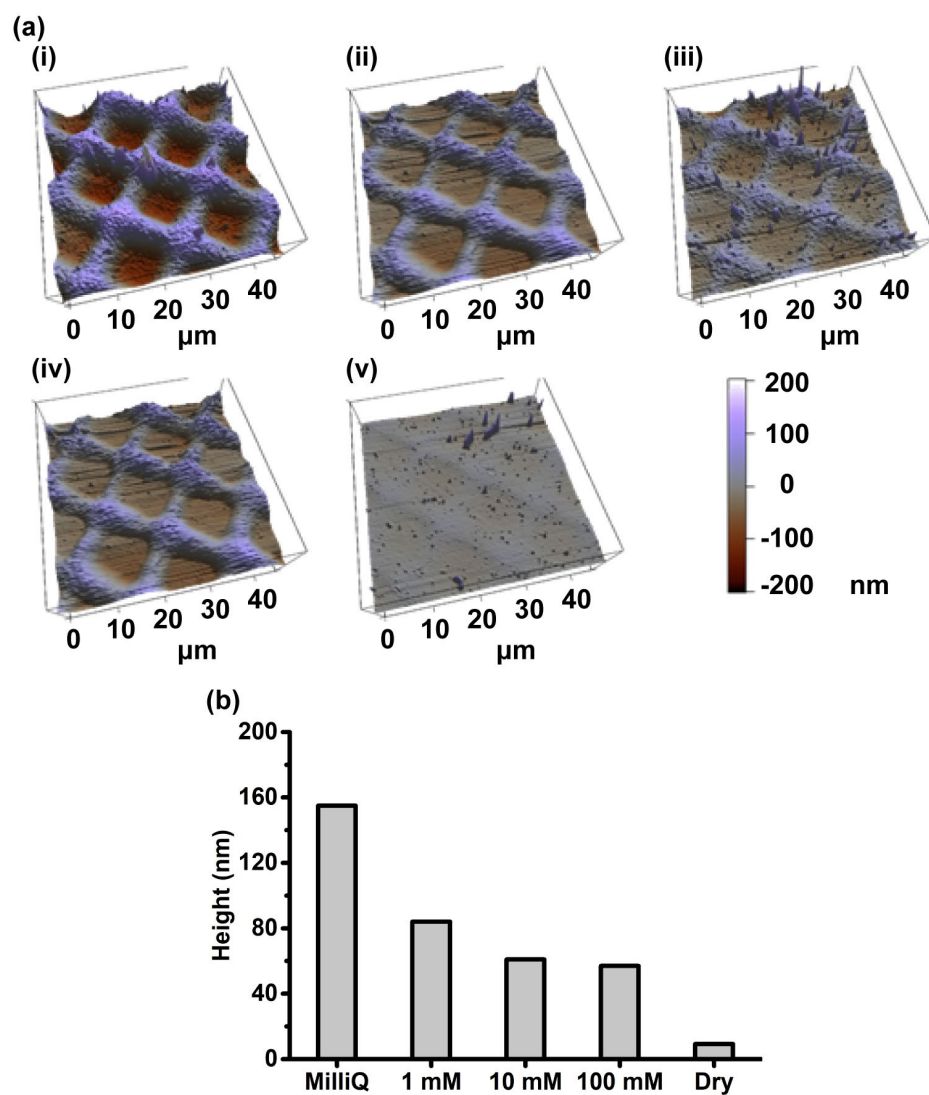


Figure 2. 12 (a) AFM images of a 2D-patterned DNA brush in MilliQ water (i), 1 mM NaCl (aq) (ii), 10 mM NaCl (aq) (iii), 100 mM NaCl (aq) (iv), and under dry condition (v). (b) The height of the dsDNA brush.

2.4 Conclusion

The author's purpose in this chapter is to prepare DNA brushes in two ways: "grafting to" and "grafting from" methods. For this purpose, first, streptavidin surface was prepared to graft DNA polymers through biotin-streptavidin interactions. Water contact angle measurement, XPS analysis and AFM observation confirmed the chemical surface alteration from bare glass to streptavidin. Fluorescence intensity measurement confirmed that biotinylated DNA polymers are adsorbed on a streptavidin surface by not non-specific binding but end attachment of their biotin end. It is found that the maximal density of DNA brush prepared via "grafting to" approach is limited by the interactions between DNA chains. Based on previous reports on polymer brush, it is inferred that grafted DNA chains stretch away from the surface. Finally, the preparation of DNA brushes via "grafting from" approach was shown. In this approach, hybridized oligonucleotides (dC₁₅-dG₁₅) were used as an initiator, and they were polymerized by a DNA polymerase (KF-). The DNA brushes prepared by this method have DNA chain density (1500 chains/ μm^2) independent of the length of the DNA. AFM analysis showed significant changes in their height under various salt concentrations or dried conditions. However, the real conformation of the DNA brushed remain unclear due to analytical limitations.

Chapter 3

Polymer Brush-Directed Vertical Alignment of Nanorod Arrays

3.1 Introduction

Self-assembly of nanoparticles (NPs) have attracted much attention as a powerful approach to produce NP arrays on a surface, which could lead to the development of next generation materials for various applications in the field of nanotechnology and nanoscience¹⁰⁰. The key parameters controlling the properties of NP arrays are the size, shape and composition of the NP as well as the packing order of them, and thus techniques to create NP arrays with desired structure have been pursued for several decades. Some examples of these approaches include layer-by-layer (LbL) assembly^{88,89}, drying-mediated self-assembly¹⁰¹ and interfacial assembly²⁴. Although these methods provide a facile way to create NP arrays, self-assembly technique capable of aligning nanorods (NRs) perpendicular to an interface with controlled inter-particle distance has been lacking. In the LbL assembly, charged NRs are deposited on an oppositely charged polymer layer in horizontal direction (in parallel with substrate)¹⁰². Drying-mediated self-assembly enables to produce perpendicularly oriented NR superlattices, but NR multilayers are formed simultaneously⁵. Interfacial assembly is a superior strategy to prepare an extensive NR monolayer where NRs are oriented vertically and tightly packed²⁹. However, in this approach, the NRs need to be modified with hydrophobic ligands which assemble them in a side-by-side configuration to regulate their interfacial behavior, and therefore the materials used as a building block is very limited.

A possible approach to remove limits to the area of vertically aligned NRs in theoretical terms could be use of a highly ordered structure of polymer oriented away from an interface, called polymer brush. Some theoretical studies on spatial arrangements of nanoparticles in polymer brush predict that cylinder-shaped nanoparticles in polymer brush prefers vertical orientation rather than horizontal one relative to the grafted polymer interface^{103,104}. This is because the free energy cost that a polymer brush pays to create a space for vertically oriented cylinders is less than for horizontally oriented ones (**Figure 3. 1**). However, to the best of my knowledge, no one has reported the vertical

alignment of NRs with the assistance of polymer brush.

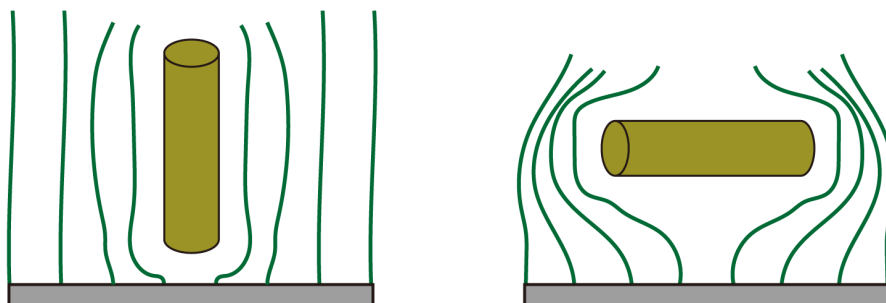


Figure 3. 1 Schematic illustration of vertically oriented (left) and horizontally oriented (right) nanoparticles placed in a polymer brush.

In this chapter, I demonstrate that polyelectrolyte brushes direct vertical alignment of extensive NR arrays. Specifically, I use DNA brushes, a collection of DNA attached by one end to an interface, as a scaffold to vertically align gold nanorods (GNRs). I hypothesized that positively charged GNRs attach to negatively charged DNA brushes along the extended chains (**Figure 3. 2**).

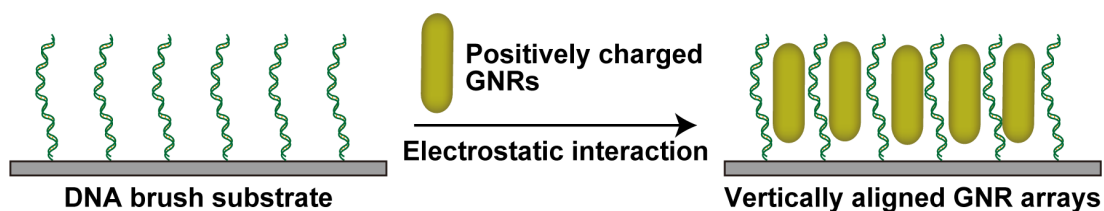


Figure 3. 2 Schematic illustration of vertical assembly of NRs with the assistance of a DNA brush.

3.2 Experimental

3.2.1 Materials

$\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, L-Ascorbic Acid and NaBH_4 were purchased from Sigma-Aldrich (USA). Epoxy resin (EPON812 RESIN), dodecenyl succinic anhydride (DDSA), methyl nadic anhydride (MNA) and 2, 4, 6-Tris(dimethylaminomethyl)phenol (DMP-30) were purchased from TAAB Laboratories Equipment,

Ltd. (UK). $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, L-Ascorbic Acid and NaBH_4 were purchased from Sigma-Aldrich (USA). 20-(11-Mercaptoundecanyloxy)-3, 6, 9, 12, 15, 18-hexaoxaicosane-1-amine ($\text{NH}_2\text{EG6C11}$) and 11-Mercaptoundecanol hexaethyleneglycol ether (EG6C11) were purchased from Dojindo Molecular technologies, Ltd. (Japan). All other salts and reagents were purchased from Wako Pure Chemical Industries, Ltd. (Japan). Scanning transmission electron microscopic (STEM) images were obtained using a STEM HD-2000 system (Hitachi High-Tech Manufacturing & Service Co., Ltd., Japan) with 200 kV acceleration voltage. Extinction spectra were recorded with a V-770 UV-vis spectrometer (JASCO Corporation, Japan). Dynamic light scattering (DLS) and zeta potential were recorded with a Zetasizer Nano ZS (Malvern Instruments Ltd., UK). Inductively coupled plasma atomic emission spectrometer (ICP-AES) analysis was performed with an ICPE-9000 (Shimadzu Corporation, Japan). Field emission scanning electron microscope (FE-SEM) images were obtained using a JSM-7800F (JASCO Corporation, Japan) with 200 kV acceleration voltage. Circular dichroism (CD) spectra were recorded with a J-820S (JASCO Corporation, Japan). Transmission electron microscopy (TEM) and transmission electron microtomography (TEMT) observations were carried out using a JEM-2200FS (JEOL, Ltd., Japan) with an accelerating voltage of 200kV.

3.2.2 Preparation of DNA Brushes

A 148 bp-DNA brush was prepared via “grafting to” approach. The preparation of DNA brushes is described in Chapter 2.

3.2.3 Synthesis of GNRs

Preparation of the seed solution. $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (250 μL , 0.01 M) was added to a CTAB aqueous solution (7.5 mL, 0.1 M) in a 50 mL plastic tube. An ice-cold NaBH_4 solution (600 μL , 0.01 M) was injected while the plastic tube was shaken gently. The solution was then left undisturbed for 2 h at

30 °C prior to use.

Growth reaction. $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (400 μL , 0.01 M), AgNO_3 (240 μL , 0.01 M), and ascorbic acid (64 μL , 0.1 M) were added to a CTAB aqueous solution (40 mL, 0.1 M) in a 100 mL plastic tube in the above order, with subsequent injection of the seed solution (96 μL). The growth solution was then shaken gently and left undisturbed for 3 h at 30 °C.

Centrifugal purification. The synthesized GNR solution (40 mL) was transferred to a 50 mL plastic tube. The GNR solution was concentrated 10-fold by centrifugation (15,000g, 20 min, 30 °C). The concentrated GNR solution (40 mL) was transferred to a new 50 mL plastic tube. Then, the GNR solution was centrifuged (15,000g, 20 min, 30 °C), and the supernatant (38.7 mL) was removed. The residue was diluted with MilliQ (38.7 mL). This purification procedure was performed twice.

3.2.4 Calculation of Size and Concentration of GNRs

The purified GNRs were characterized by STEM, UV-vis-NIR spectroscopy and ICP-AES. In the characterization, the GNRs are considered as right-circular cylinders with semispherical ends as shown in **Figure 3.3**.

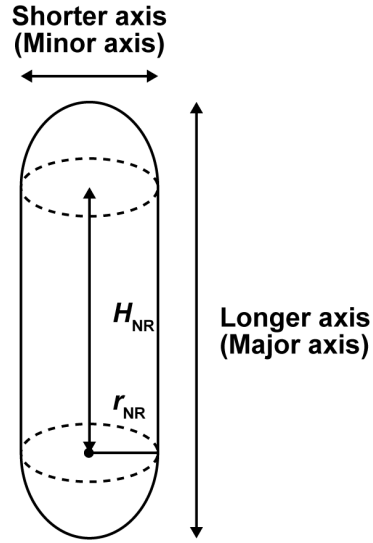


Figure 3. 3 Schematics of the GNR geometry. H_{NR} and r_{NR} are height and radius of the cylinder.

The concentration of GNRs per liter (C_{NR}) and the extinction coefficient for GNPs (ϵ_{NR}) were estimated using the following equations:

$$U_{NR} = \frac{4}{a^3} (\pi r^2 H_{NR} + \frac{4}{3} \pi r_{NR}^3)$$

$$C_{NR} = \frac{N_{gold}}{U_{NR} N_A}$$

$$\epsilon_{NR} = \frac{Abs_{LLSPR}}{L_w C_{NR}}$$

U_{NR} is the number of gold atoms fitting into each volume of the GNRs. The crystal structure of gold is face-centered cubic and has a lattice constant (a) of 4.0 Å; there are four gold atoms per unit cell.

H_{NR} and r_{NR} are height and radius of the GNRs. H_{NR} and r_{NR} were determined as the average of over 300 particles based on the STEM. N_{gold} is the measured number of gold atoms per liter. N_A is the Avogadro constant. Abs_{LLSPR} is absorbance at LLSPR of the GNRs. l_o is the optical path length of the cuvette. The purified GNRs were dissolved with aqua regia ($HNO_3/HCl = 1:3$). The amount of

gold contained in the aqua regia was quantitatively analyzed by ICP-AES.

3.2.5 Surface Modification of GNRs with PEG Alkanethiol Ligands

Different amounts of two kinds of alkanethiol ligands (the NH₂EG6C11 and EG6C11 ligand, see **Figure 3. 4**) were mixed in methanol. The percentages of the cationic ligands were 0%, 5%, 10%, 20%, 40%, 60%, 80% and 100%. For GNRs, the mixed alkanethiol ligand solution (50 μ L, 5 mM) was added to the purified GNR solution (1.45 μ L). The mixed solution was purified by two cycles of centrifugation.

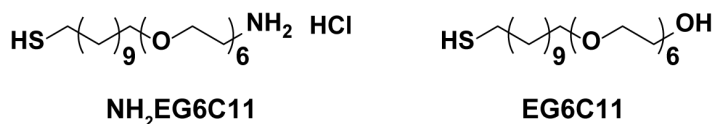


Figure 3. 4 Chemical structures of the PEG alkanethiol ligands (NH₂EG6C11 and EG6C11).

3.2.6 Adsorption of PEG Alkanethiol-capped GNRs onto DNA brush

The GNRs modified with the PEG Alkanethiol ligands were attached by soaking the inside surface of the DNA brush cuvettes in the GNR solution. The inside surface of the cuvette was washed with pH 7.7 Tris-HCl at least three times to remove the nonattached GNRs.

3.2.7 Quantitative measurement of the amount of adsorbed GNRs

The amount of the adsorbed GNPs was measured using UV-vis-NIR spectroscopy by subtracting the amount of the non-adsorbed GNRs from the added amount of the GNPs.

3.2.8 Transfer of Immobilized GNRs into the Solid State

EPON812 RESIN, DDSA, and MNA were mixed in a ratio of 4.6, 2.6, and 2.8, and subsequently stirred for 20 min. DMP-30 was then added into the mixed solution. After stirring for 60 min, the resin

solution was degassed under vacuum for 1 h. Then, the DNA brush/GNR substrates were rinsed 3 times with MilliQ, ethanol and the mixture solution. After the last rinse, the substrates were left in the mixture solution at 60 °C for 3 days for solidification. The solidified epoxy resin was decoupled from the substrates.

3.2.9 Preparation of Samples for Electron Microscopy Observations

For the FE-SEM observations, the solidified epoxy resin with GNR arrays was coated with carbon (10–20 nm). For the TEM and TEMT observations, thin slices (200–300 nm) which contains GNR arrays were produced using a microtome equipped with a diamond knife, placed on the TEM grid and then coated with osmium (5 nm). Prior to characterization by TEM and TEMT, the samples were irradiated with electron beam until the epoxy resin stopped shrinking.

3.2.10 Transmission Electron Microtomography

A series of TEM images were acquired at tilt angles of around $\pm 70^\circ$ at an angular interval of 1° . The tilt series of the TEM images were aligned and then reconstructed by filtered back projection.

3.2.11 Finite Difference Time Domain (FDTD) Calculation

The optical properties of the GNRs were estimated using an electromagnetic optical simulation based on the FDTD method. The FDTD method solves Maxwell's equations by discretizing the equations via central differences in time and space and then numerically solving these equations using software. The effect of the solvent has been accounted for by the refractive index of the ambient environment, that is, $n = 1.333$ for water. The FDTD calculation was performed by Dr. Masayuki Naya and Mr. Takeharu Tani (FUJIFILM Corporation).

3.3 Results and Discussion

3.3.1 Characterization of GNRs Before and After the Surface Modification with PEG

Alkanethiol Ligands

GNRs with mean diameter 10 nm and length 34 nm were synthesized based on a previously reported procedure by Murphy et al⁷⁸ (10 nm and 34 nm indicate the size of the metallic core). The STEM image of the GNRs were shown in **Figure 3. 5a**. The GNRs exhibited TLSPR around 510 nm and LLSPR around 794 nm (**Figure 3. 5b**). Spherical and cubic nanoparticles were obtained as byproducts. The yield of the byproducts was about 10%. The molar extinction coefficient was $\epsilon = 1.2 \times 10^9$ at 794 nm. The GNRs were modified with a mixture of cationic ligand (NH₂EG6C11) and a nonionic ligand (EG6C11) at a certain ratio. Hereafter, GNRs modified with a cationic ligand ratio of X% are denoted as X% cationic GNRs. The modification of GNRs by two kinds of alkanethiol ligands allows controlling the density of the positive charges on the surface of the GNRs. The ζ potential of the GNRs decreased with the ratio of the cationic ligands (**Table 3. 1**). The hydrodynamic diameter of the CTAB-stabilized and ligand-modified GNRs obtained by dynamic light scattering (DLS) show no evidence of aggregation (**Table 3. 1**). Extinction spectra of GNRs before and after surface modification did not show significant change in transverse and longitudinal plasmonic peak positions which supports the absence of aggregation (**Table 3. 1**). Ligand densities ranged from 4.6 to 6.1 molecules/nm², which are comparable to literature value¹⁰⁵ (**Table 3. 1**). Following the purification of alkanethiol ligand-modified GNRs by centrifugations, the GNRs were adsorbed onto the DNA brush.

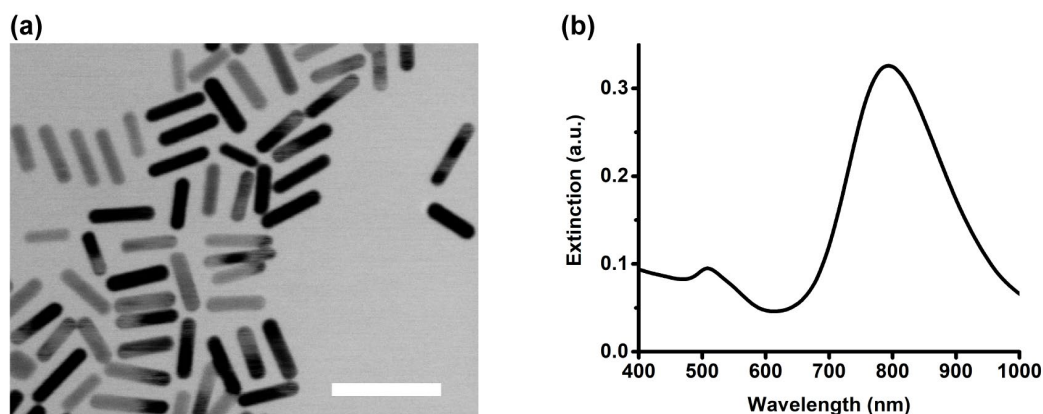


Figure 3. 5 (a) STEM image of the GNRs. The size was calculated as the average of over 300 particles based on STEM images. The scale bar is 60 nm. (b) Extinction spectrum of GNRs dispersed in aqueous solution.

Table 3. 1 Characterization of CTAB and alkanethiol ligands stabilized GNRs.

Run	GNRs	Zeta potential (mV)	Hydrodynamic diameter (nm)	Peak shift in LLSPR (nm)	Peak shift in TLSPR (nm)	Ligand density (molecules/nm ²)
1	CTAB	53.4	28.3			
2	100% cationic	65.3	34.9	+24	+2	4.6
3	80% cationic	63.9	33.3	+24	+2	5.3
4	60% cationic	59	31.8	+25	+2	5.4
5	40% cationic	53.7	31.8	+25	+2	5.8
6	20% cationic	43.1	30.6	+25	+2	5.6
7	10% cationic	39.1	30.1	+25	+2	6.1
8	5% cationic	33.4	29.7	+26	+2	5.8
9	0% cationic	13.1	28.6	+25	+2	5.3

3.3.2 Effect of GNR Surface Charge Density on Orientation of GNRs

First, 100% cationic GNRs were adsorbed onto the dsDNA brush with grafting density of 17000 chains/ μm^2 , and the extinction spectra were recorded with nonpolarized light (**Figure 3. 6**). The 100% cationic GNRs showed a strong peak for LLSPR at 920 nm, indicating that the GNR did not align vertically. However, the intensity of the peak for LLSPR decreased under a high salt concentration (2 M NaCl). Considering that electrostatic interaction is weakened in the presence of salt, this result

suggests that the intensity of the electrostatic attraction between the dsDNA brushes and the adsorbed GNRs influences the GNR orientation.

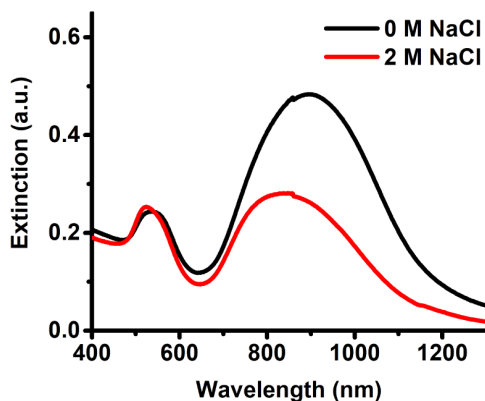


Figure 3. 6 Extinction spectra of the adsorbed GNRs modified with the cationic ligand on the DNA brush in 10 mM Tris-HCl (black line) and 10 mM Tris-HCl buffer containing 2M NaCl (red line). The GNR solution with concentration of 25 nM was used. The adsorption time was 60 min.

The effect of positive charge density on the surface of the GNRs on the orientation was investigated by tuning the mixing ligand ratio. As a result, different extinction spectra were obtained from the GNRs adsorbed on the dsDNA brush surfaces depending on the ligand ratio (**Figure 3. 7a**). Their particle densities were tuned to be the same as about 1800 particles/ μm^2 , which corresponding to about 25 nm in center-to-center GNR distance. The GNRs adsorbed on the inside surface of the cuvette showed purple (100% cationic GNRs) and pink (20% cationic GNRs), respectively. (**Figure 3. 7b**). As the proportion of the zeta potential of the GNRs decreased, the intensity of the peak for LLSR also decreased (**Figure 3. 7c**).

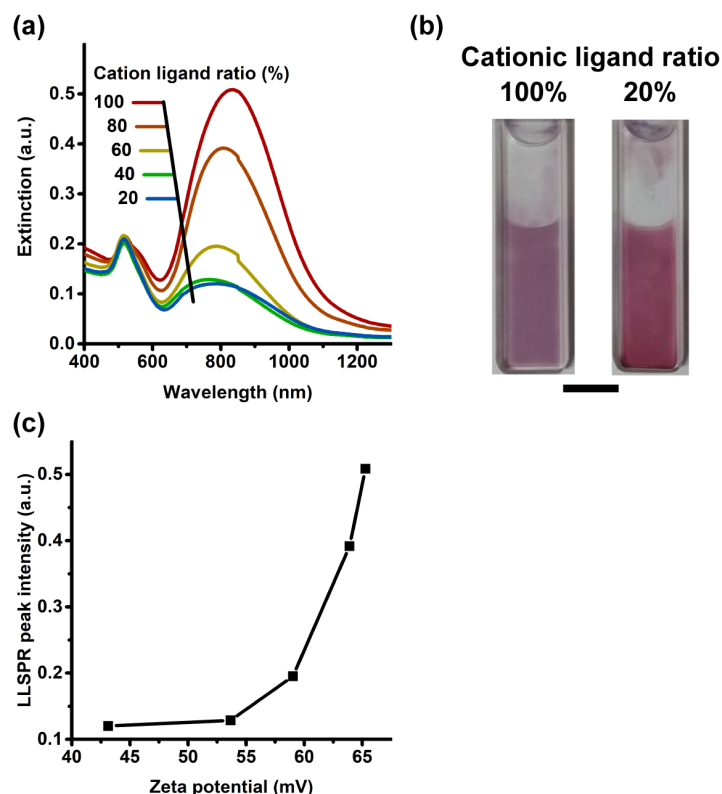


Figure 3. 7 (a) Extinction spectra of GNRs adsorbed on dsDNA brushes with its grafting density = 17000 chains/ μm^2 in 10 mM Tris-HCl (pH = 7.6). (b) Photos of the cuvette containing the adsorbed 100% and 20% cationic GNRs. The scale bar is 1 cm. (c) LLSPR peak intensity as a function of zeta potential of the GNRs. The GNR solution with concentration of 6.7 nM was used. The adsorption time was 12 h.

Next, optical analysis using polarized light was performed at various tilt angle. The photographs of the cuvette, wherein 20% cationic GNRs were adsorbed, through a polarizer clearly showed the polarization dependent optical properties (**Figure 3. 8a**). The cuvette turned from pink to purple under p-polarized illumination, but the color unchanged under s-polarized illumination (**Figure 3. 8b**). The extinction spectra of the GNRs with a low charge density (20% cationic) under polarized light is shown in **Figure 3. 8c, d**. When the cuvette was rotated, LLSPR mode around 661 nm that is differed from the LLSPR mode around 800 nm, was excited under p-polarized light (**Figure 3. 8c**) while no significant changes were observed under s-polarized light (**Figure 3. 8d**). These results indicate that the GNRs with a low positive charge density orients vertically on the dsDNA brush.

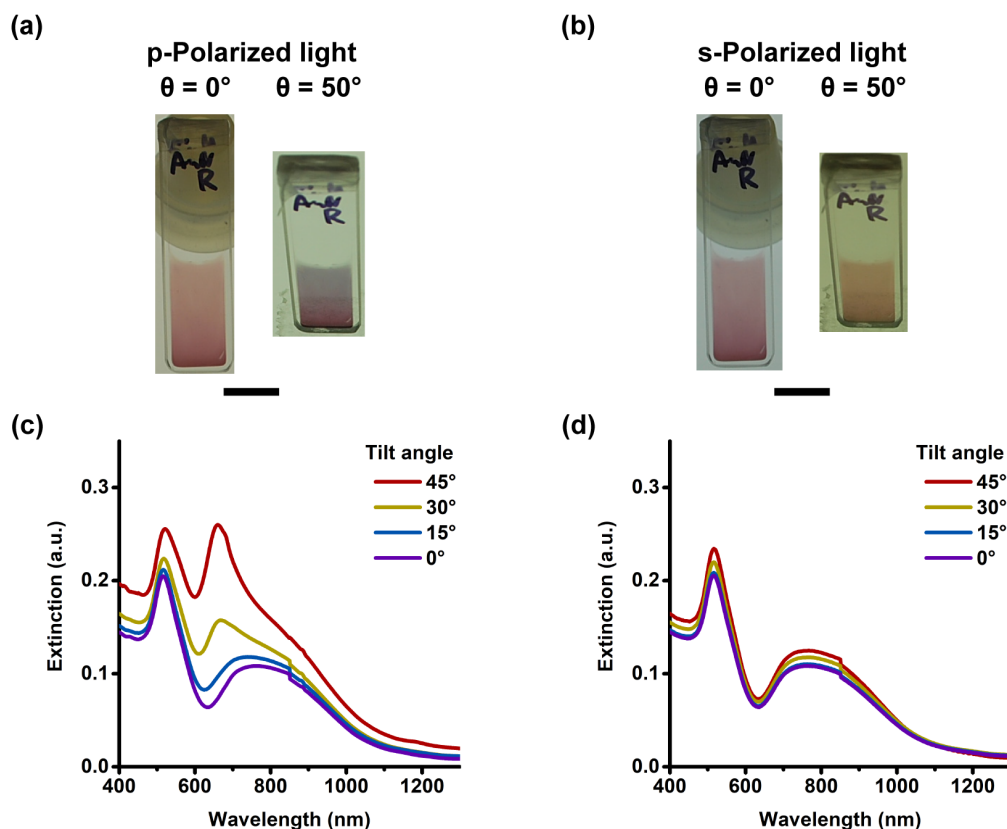


Figure 3. 8 (a, b) Photographs of the cuvette containing the adsorbed 20% cationic GNRs through a polarizer. (a) p-Polarized light. (b) s-Polarized light. The scale bars are 1 cm. Extinction spectra of GNRs using polarized light (both p- and s-polarized light at various tilt angle. These extinction spectra were recorded using the same sample as extinction spectrum (blue line) shown in Fig. 3. 7a. (c, d) Extinction spectra of 20% cationic GNRs adsorbed on the dsDNA brush under p-polarized (c) and s-polarized (d) light.

On the other hands, 100% cationic GNRs attached to the DNA brush showed the polarization dependent optical property which means horizontal alignment of the GNRs (**Figure 3. 9a, b**). In general, tilting the plane increases the measuring surface, which leads to increment of total extinction intensity. However, p-polarized light decreased slightly the intensity for the LLSRP peak with the increase in the tilt angle. Considering that horizontally oriented GNRs show such polarization dependent optical property, this result indicates that highly charged cationic GNRs are immobilized in not completely random but horizontal orientation.

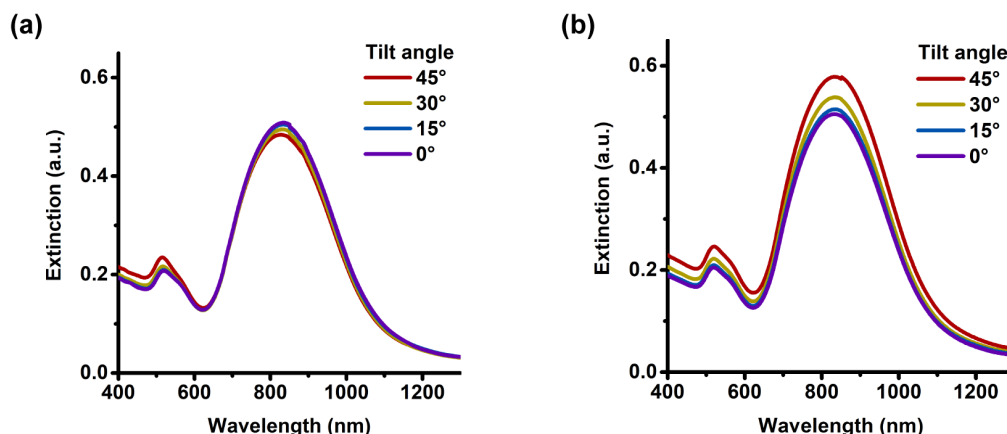


Figure 3. 9 Extinction spectra of 100% cationic GNRs adsorbed on the dsDNA brush under p-polarized (a) and s-polarized (b) light. These extinction spectra were recorded using the same sample as extinction spectrum (red line) shown in Fig. 3. 7a. The GNR solution with concentration of 6.7 nM was used. The adsorption time was 12h.

Next, field emission scanning electron microscopy (FE-SEM) was used to image the 20% cationic GNRs adsorbed onto the dsDNA brush. In my first attempt to image the immobilized GNRs, the sample was simply dried and then imaged. Before imaging, the extinction spectra of the GNRs before and after the drying process were recorded to confirm if the solution-phase structure was preserved. As shown in **Figure 3. 10a**, this approach revealed a strong peak for LLSPR, which means that the ordered structure was disturbed. The FE-SEM image also showed that the GNRs orient horizontally (**Figure 3. 10b**). This orientational change was thought to be caused by a lateral capillary force during evaporation of the solvent and suggested that enough free space existed around each GNRs for them lay flat. Therefore, to avoid any structural changes induced by the drying process, an epoxy resin embedding technique was employed to stabilize the GNRs. The GNRs were first embedded in the epoxy resin, which was subsequently decoupled from the glass surface after solidification, and then imaged by FE-SEM. As shown in **Figure 3. 10c**, epoxy resin embedding enabled to reduce the orientational change more than the simple dry method. Although the extinction spectra subtly changed before and after the epoxy resin embedding process, the GNRs were observed to be round (**Figure 3. 10d**). The diameter of the round GNRs was similar to that of their minor axis

(10 nm), which indicates the GNRs were generally aligned vertically.

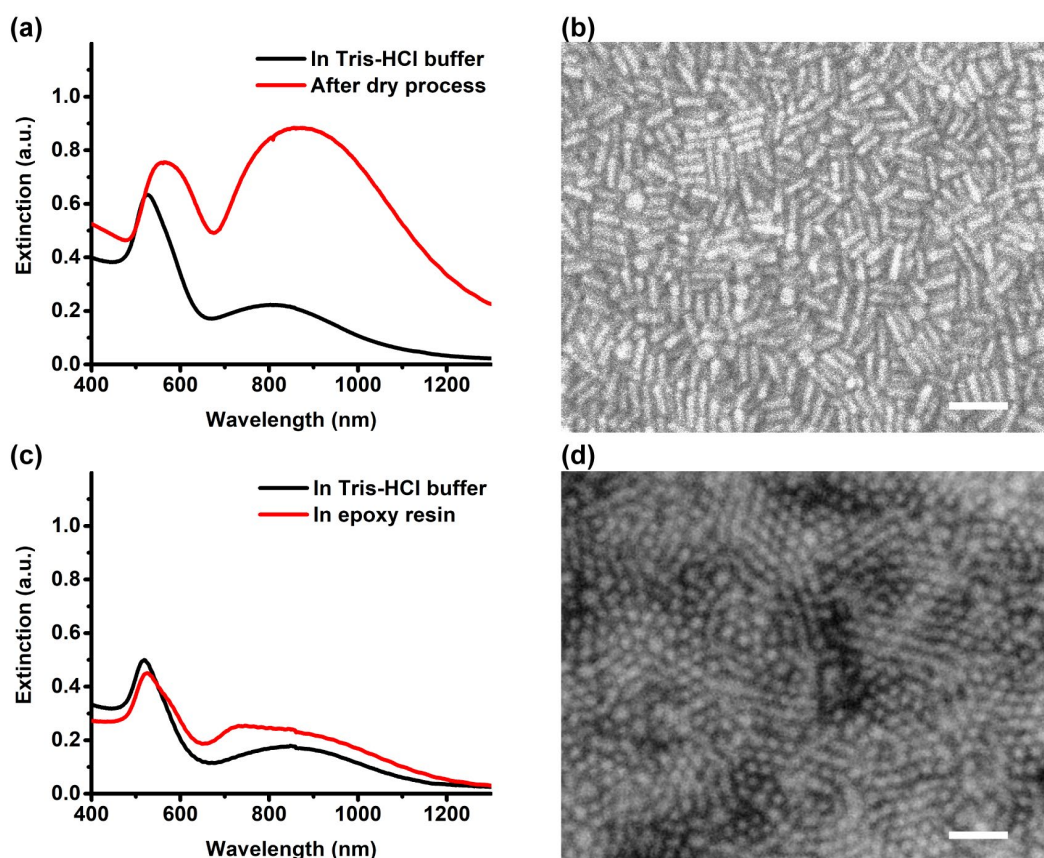


Figure 3. 10 (a) Extinction spectra of 20% cationic GNRs on the dsDNA brush in Tris-HCl buffer (pH=7.7) and after the drying process. (b) FE-SEM image of the adsorbed GNRs after the drying process. (c) Extinction spectra of the GNRs on the dsDNA brush before and after the epoxy resin embedding process. (d) FE-SEM image of the adsorbed GNRs in the epoxy resin. The scale bars are 60 nm. The GNR solution with concentration of 25 nM was used. The adsorption time was 60 min.

3.3.3 Effect of Interchain Distance of DNA brushes on Orientation of GNRs

The effect of average DNA interchain distance on the immobilized GNR orientation was investigated. dsDNA brushes with various interchain distances were prepared and 20% cationic GNRs were adsorbed onto them. The different extinction spectra were obtained from the GNRs adsorbed on the dsDNA brush surfaces depending on the DNA interchain distance (**Figure 3. 11a**). GNR extinction

intensity at the LLSPR wavelength was plotted as a function of average DNA interchain distance (**Figure 3. 11b**). The intensity for LLSPR peak increased with the decrease in DNA interchain distance, which means that a high surface coverage of the dsDNA is essential to align the GNRs vertically. Furthermore, 20% cationic GNRs onto the dsDNA brush with the low grafting density showed the polarization dependent optical property which means horizontal alignment of the GNRs (**Figure 3. 11d**). From these results, I deduced that these are because collective stretching effects are weakened as each tethered dsDNA chain become independent of its neighbors, as illustrated in **Figure 3. 11e**.

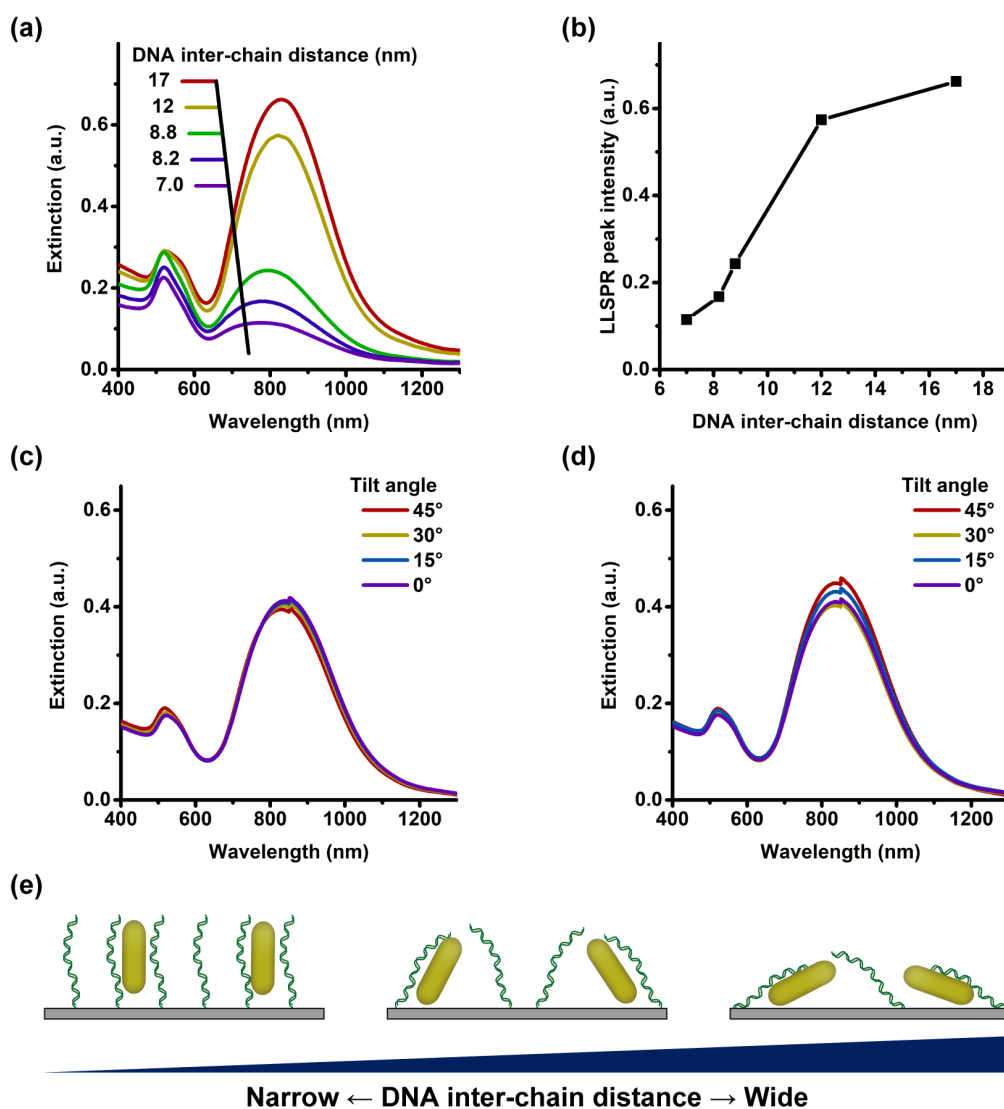


Figure 3. 11 (a) LLSPR extinction intensity of the adsorbed GNRs on dsDNA brushes at various interchain distances. Their particle densities were adjusted to be the same as about 1800 particles/ μm^2 . (b,c) Extinction spectra of 20% cationic GNRs adsorbed on the dsDNA brush with grafting density of 3800 chains/ μm^2 under p-polarized light (c) and s-polarized light (d). The GNR solution with concentration of 6.7 nM was used. The adsorption time was 12h. (e) Schematic illustration of DNA inter-chain distance dependency of the GNR orientation on the dsDNA brushes.

3.3.4 Why Surface Charge Density of GNRs Affects Orientation of GNRs

Based on the above results, I believed that the charge density on the surface of the GNRs affects how the tethered dsDNA chain associate with the GNRs: the tethered dsDNA chain wraps around positively charged GNRs or not. Yingling and coworkers recently simulated the conformational change of 30 bp dsDNA induced by interaction with gold spherical nanoparticles (GNPs) (a diameter of ~6 nm including a ligand layer)¹⁰⁶. The simulation predicts that the highly charged GNPs bends the dsDNA, and the GNPs with a low charge density allows the dsDNA to interact with the GNP without bending. Although the dsDNA length and nanoparticle size and shape in our experiment differs from those in the simulation, the above results can be similarly explained by the presence or absence of dsDNA chain bending (**Figure 3. 12**): the tethered dsDNA bends at the curved surface of the highly cationic GNRs (deformed duplex), leading to the random orientation as the dsDNA is no longer stretched vertically. In contrast, when the GNRs with a low charge density were used, the tethered dsDNA does not bend (B-form DNA) and the GNRs are oriented perpendicular to the substrate surface.

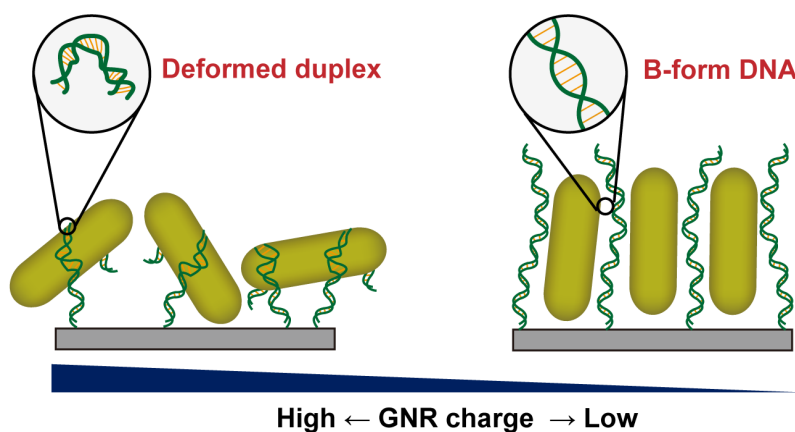


Figure 3. 12 Proposed mechanism of GNR charge dependency the GNR orientation on the dsDNA brushes.

To verify the proposed mechanism, the conformation of the tethered DNA chains upon association with the positively charged NPs was investigated by Circular dichroism (CD) spectroscopy. The CD spectra of the DNA dispersed in an aqueous solution showed a band of negative at 250 nm

and positive peaks at 280 nm, which are observed in the characteristic CD spectrum of DNA B-form duplex (**Figure 3. 13a**). Similarly, dsDNA brush alone showed the typical bands around 250 nm and 280 nm, indicating that grafted dsDNA takes on the B-form duplex (**blue line in Figure 3. 13b**). In contrast, DNA associated with highly charged GNRs (100% cationic) showed a spectrum shift from positive to negative values in the peak around 280 nm (**red line in Figure 3. 13b**). The spectrum shift indicates deformation of the B-form DNA. However, when the 20% cationic GNRs were adsorbed onto the dsDNA brush, the DNA showed the typical band of B-form duplex (**red line in Figure 3. 13b**)), indicating that the DNA brush associating with 20% cationic GNRs takes on B-form conformation. Therefore, the CD data support my hypothesis that adsorption of highly charged GNRs onto dsDNA brush induced the deformation of dsDNA by bending the DNA upon interaction with the curved cationic GNRs.

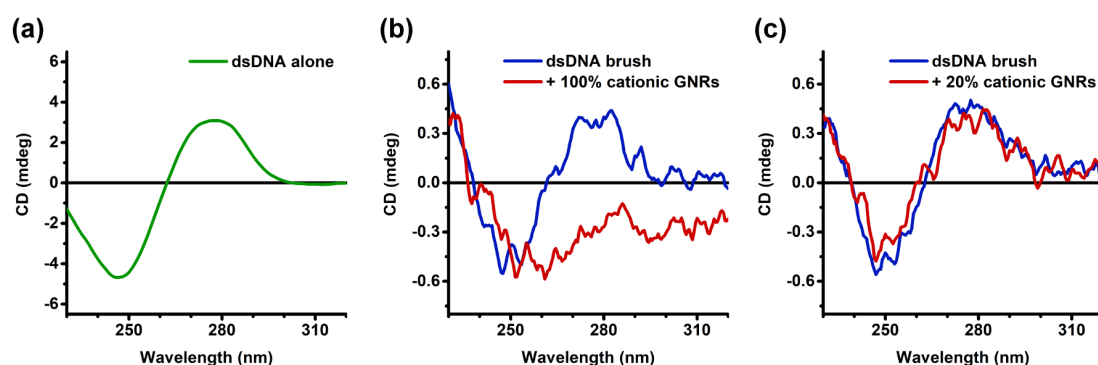


Figure 3. 13 CD spectra of the dsDNA. (a) CD spectrum of 148 bp dsDNA dispersed in 10 mM Tris-HCl buffer (pH = 7.6). (b, c) CD spectra of 148 bp dsDNA brushes in 10 mM Tris-HCl buffer (pH = 7.6). Blue line is the dsDNA brushes alone and red line is the dsDNA brushes associating with the cationic gold nanoparticles. (b) 20% cationic GNRs. (c) 100% cationic GNRs. The GNR solution with concentration of 6.7 nM was used. The adsorption time was 12h.

3.3.5 Effect of Persistent Length of the End-Grafted Polymer on Orientation of GNRs

The persistence is a critical parameter to control the elastic behavior of a charged polymer chain in an electrostatic interaction with an oppositely charged nanoparticle. The persistence length describes

stiffness of a polymer chain. The longer the persistence length is, the stiffer the polymer chain behaves. The elastic behavior is governed by a competition between the energy that must be paid to deform the polymer chain (E_p) and the energy that is gained due to the electrostatic interaction (E_g). In other words, at $E_p < E_g$ the polymer chain bends and wraps around the nanoparticle, and at $E_p > E_g$ the polymer chain binds to the nanoparticle without deformation. Most synthetic polymers are flexible polymers with a persistence length of the order 1 nm compared to dsDNA with persistence length of 50 nm. Such flexible polymers would be more bendable in an electrostatic interaction with a nanoparticle than dsDNA. Such flexible polymers would be more bendable in an electrostatic interaction with a nanoparticle than dsDNA, so that the persistence length of grafted polymers could be critical factor to achieve the orientation control of rod-shaped nanoparticles with the assistance of polymer brush.

Thus, the generality of these results was verified by using single stranded (ss)DNA instead of stiff dsDNA. First, a DNA brush composed of 148 base single stranded DNA (poly(dT)) was prepared and subsequently 20% cationic GNRs were adsorbed onto the ssDNA brush. The DNA brush density is 18000 chains/ μm^2 , which is equivalent to 8.0 nm in interchain distance. The GNR density is about 1800 particles/ μm^2 . **Figure 3. 14a, b** shows the extinction spectra of the 20% cationic GNRs adsorbed onto the ssDNA brush surface were recorded under polarized light. The optical property of the GNRs on the ssDNA brush surface depended on the polarization direction which means the GNRs oriented perpendicular to the surface. Therefore, it is found that there is no need to use rigid polymer like dsDNA in order to achieve the vertical alignment of rod-shaped nanoparticles. Furthermore, considering that ssDNA has a similar persistence length to that of synthetic polymers, these findings may apply to a broad range of synthetic polymers.

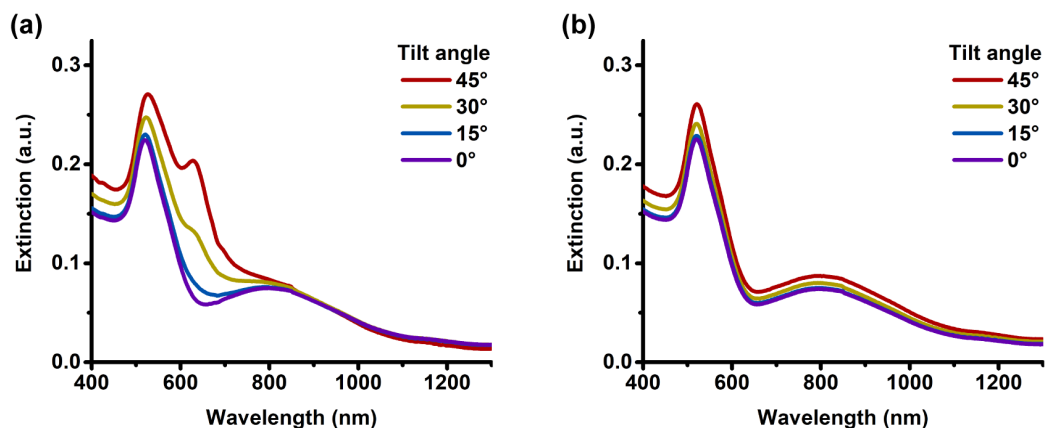


Figure 3. 14 Extinction spectra of 20% cationic GNRs adsorbed on the ssDNA brush under p-polarized (a) and s-polarized (b) light. The concentration of the GNR solution is 6.7 nM. The adsorption time was 12 h.

3.3.6 Plasmon Coupling Effect on GNR Interparticle Spacing

Next, to estimate the interparticle spacing of the aligned GNRs, the effect of plasmonic coupling neighboring GNRs was investigated. First, the extinction spectra of GNR arrays aligned perpendicular to a substrate with various center-to-center distance was simulated by FDTD calculation (**Figure 3. 15a**). To discuss the plasmonic coupling effect, incident light angle (θ) was fixed at 50° . From the extinction spectra, the simulated LLSPR peak positions were plotted as a function of the simulated GNR center-to-center distance (**Figure 3. 15b**). The peak position of the LLSPR excited by p-polarized light depends very sensitively on their interparticle spacing, i.e., as the interparticle spacing narrowed, the LLSPR excitation gradually blue-shifts due to plasmon coupling.

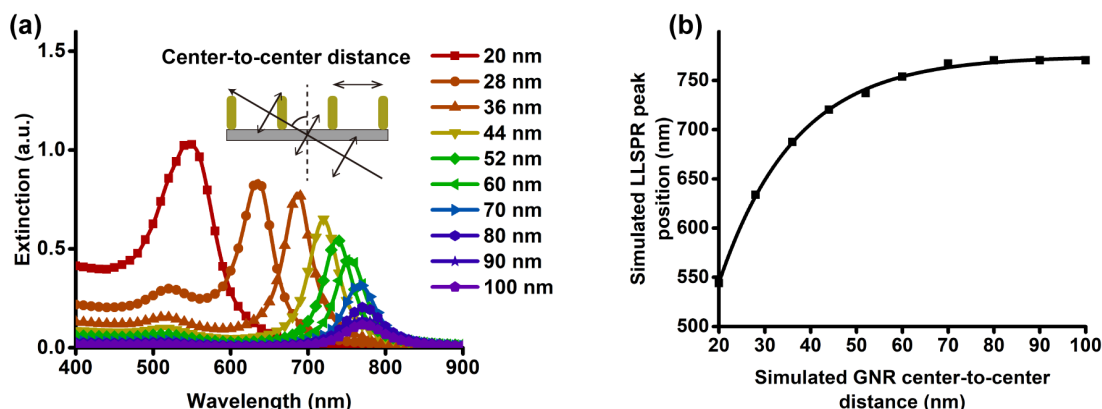


Figure 3. 15 FDTD-calculated optical properties of GNR arrays. (a) Simulated extinction spectra of perfectly aligned GNR arrays with various center-to-center distance under p-polarized light at $\theta = 50^\circ$. (b) Simulated LLSPR peak position on the various center-to-distances. Curve fitting was performed as an exponential curve ($y=y_0+A*\exp(B*x)$), where $y_0 = 774.43$, $A = -767.52$, and $B = -0.06028$. The data fit well ($R^2 = 0.999$) to the exponential formula.

This tendency was also observed in my experimental data. **Figure 3. 16** shows extinction spectra of 20% cationic GNRs on the dsDNA brush surface with different GNR densities under p-polarized light at $\theta = 0^\circ$ and 45° . The amount of adsorbed GNRs were 1300 and 680 particles/ μm^2 for adsorption times of 1 and 10 min. These values equate to the average center-to-center distances of the adsorbed GNRs of 30 nm and 41 nm. The experimentally observed LLSPR peak positions were 684 nm and 729 nm, and these positions corresponded to 36 and 46 nm, respectively, on the basis of the correlation curve calculated from the simulation. These values obtained from the extinction spectra were also consistent with the values obtained from quantitative measurement of the number of adsorbed GNR. Further, both the experimental results and simulation data showed that this vertical alignment was independent of the number of adsorbed GNRs.

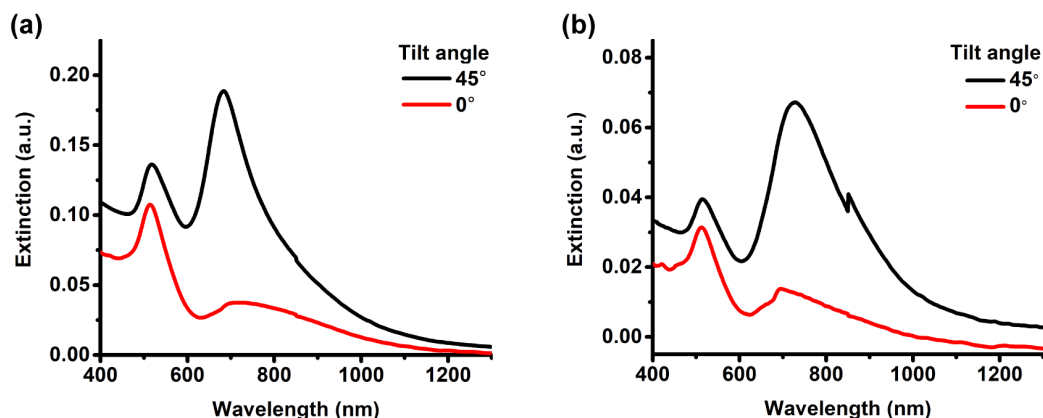


Figure 3. 16 Extinction spectra of 20% cationic GNRs on dsDNA brushes with different particle densities under p-polarized light at $\theta = 0^\circ$ (red line) and 45° (black line). (a) 1300 particles/ μm^2 . (b) 680 particles/ μm^2 . The concentration of the GNR solution is 6.7 nM. The adsorption time was 10 min (a) or 1 min (b).

3.3.7 Effect of Adsorbed Amount of GNRs on Orientation of GNRs

To fabricate vertically oriented and tightly packed NR monolayer arrays, I investigated the effect of adsorbed amount of GNRs on orientation of GNRs adsorbed onto DNA brushes. The extinction spectra of 10% cationic GNRs adsorbed onto the dsDNA brush as a function of particle density are shown in **Figure 3. 17a**. The maximum particle density was 29000 particles/ μm^2 at this experimental condition. In all the range of the particle density, the extinction spectra showed a weak peak for LLSPR. **Figure 3. 17b** shows the plot of the intensity ratio of LLSPR to TLSPR as a function of particle density. The intensity ratio increased with the particle density. As the GNR orientation changes from vertical direction to random orientation, the intensity ratio should increase, which means that the degree of anisotropy in GNR orientation decreased with particle density.

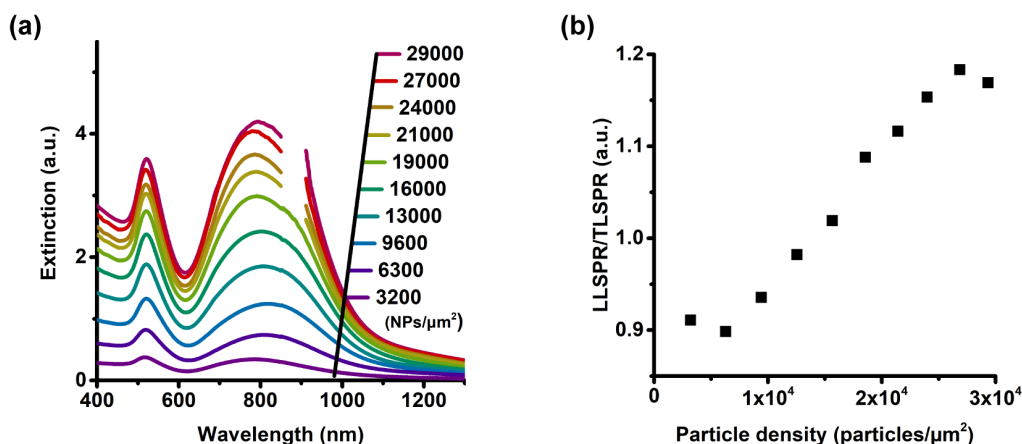


Figure 3. 17 (a) Extinction spectra of the GNRs immobilized on the 148 bp dsDNA brush, as a function of particle density [particles/ μm^2]. The grafting density of the dsDNA brush is 24000 chains/ μm^2 and 10% cationic GNRs were used. The extinction spectra were recorded in 10 mM Tris-HCl (pH = 7.6). The extinction values ranging from 910 nm to 850 nm was saturated due to an insufficient quantity of light falling onto the photodetector, so that these extinction values are not shown. (b) The intensity ratio of LLSPR to TLSPR of immobilized GNRs.

To reveal the cause of the averaged GNR orientation dependency on the particle density, 2D images of the GNRs on the dsDNA brush surface with the particle density of about 19000 particles/ μm^2 were obtained using STEM (**Figure 3. 18**). The dark and light regions were extensively observed, and most of the region was occupied by the dark regions (**Figure 3. 18a**). When zooming in the marked region, vertically standing GNRs and slightly tilting GNRs were observed in the light region (**Figure 3. 18b**). Thus, the light regions correspond to GNR monolayer arrays. On the other hands, the dark regions are multiple layers and GNR orientation was difficult to judge in the dark region. These 2D STEM images indicate that GNRs were deposited in multiple layers as the amount of the adsorbed GNRs increased.

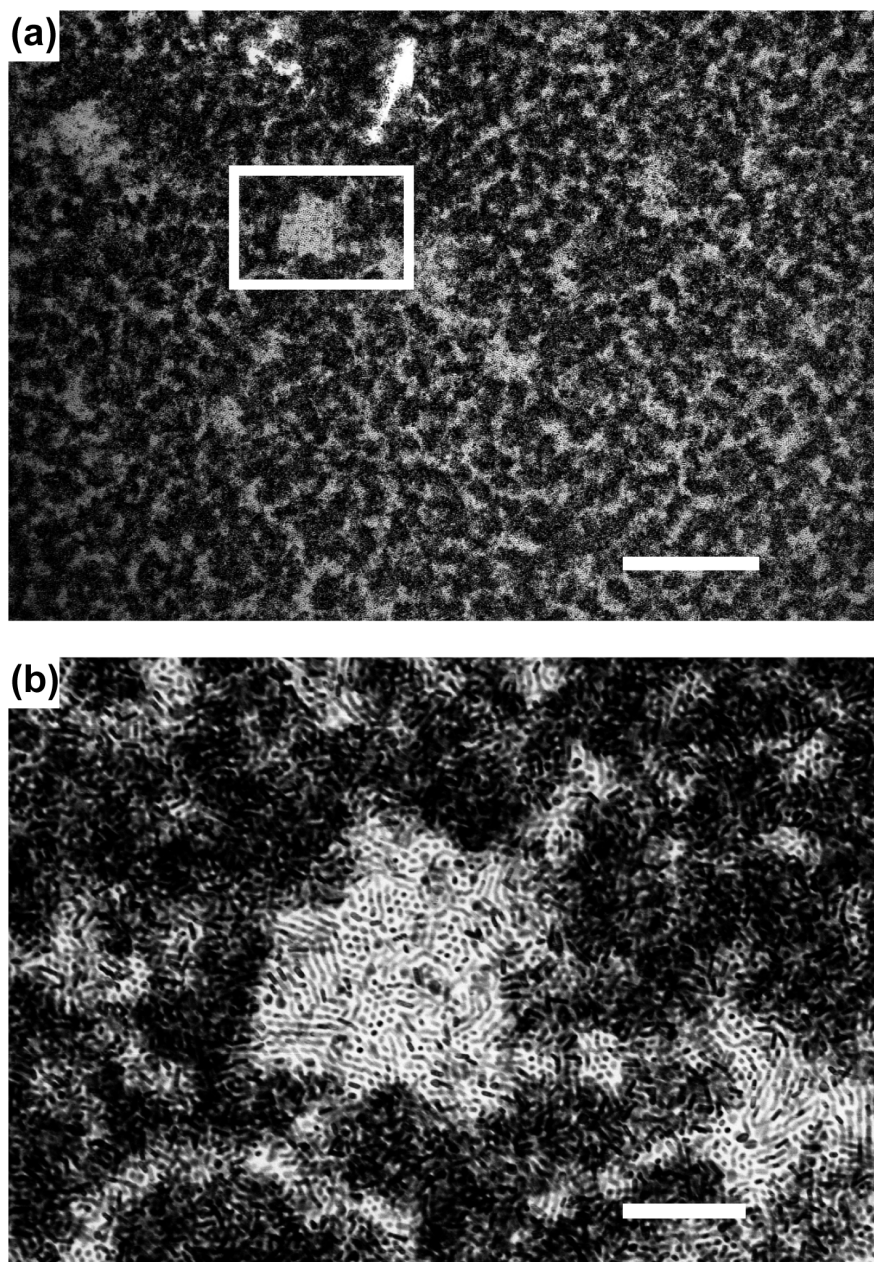


Figure 3. 18 (a) Large-area STEM image of the adsorbed GNRs on the 148 bp DNA brush. The scale bar represents 1 μm . (b) Zoomed image inside the white frame marked in (a). The scale bar represents 200 nm. Note that the adsorbed GNRs were embedded in epoxy resin before imaging to attempt to preserve the solution-phase structure in the solid state.

To elucidate the orientation of the deposited GNRs on the highly oriented GNR layer, 3D image of the same sample was obtained using transmission electron microtomography (TEMT) (Figure 3. 19). The GNR orientation in the bottom layer, which is the closest to the substrate, was

distinctively differed from that in the other layers. GNRs aligned vertically in the bottom layer (V-GNRs, colored in blue) and GNRs aligned horizontally in the other layers (H-GNRs, colored in green) (**Figure 3. 19a**). To quantitatively clarify the difference of the V- and H-GNR orientation, the angle distributions were plotted in the histograms (**Figure 3. 19b**). The angle median values of V- and H-GNRs were 67.9° and 13.7° , respectively. Thus, deposition of the GNRs on the vertically aligned GNR layer leads to the decrease in the degree of anisotropy in GNR, as illustrated in **Figure 3. 19c**.

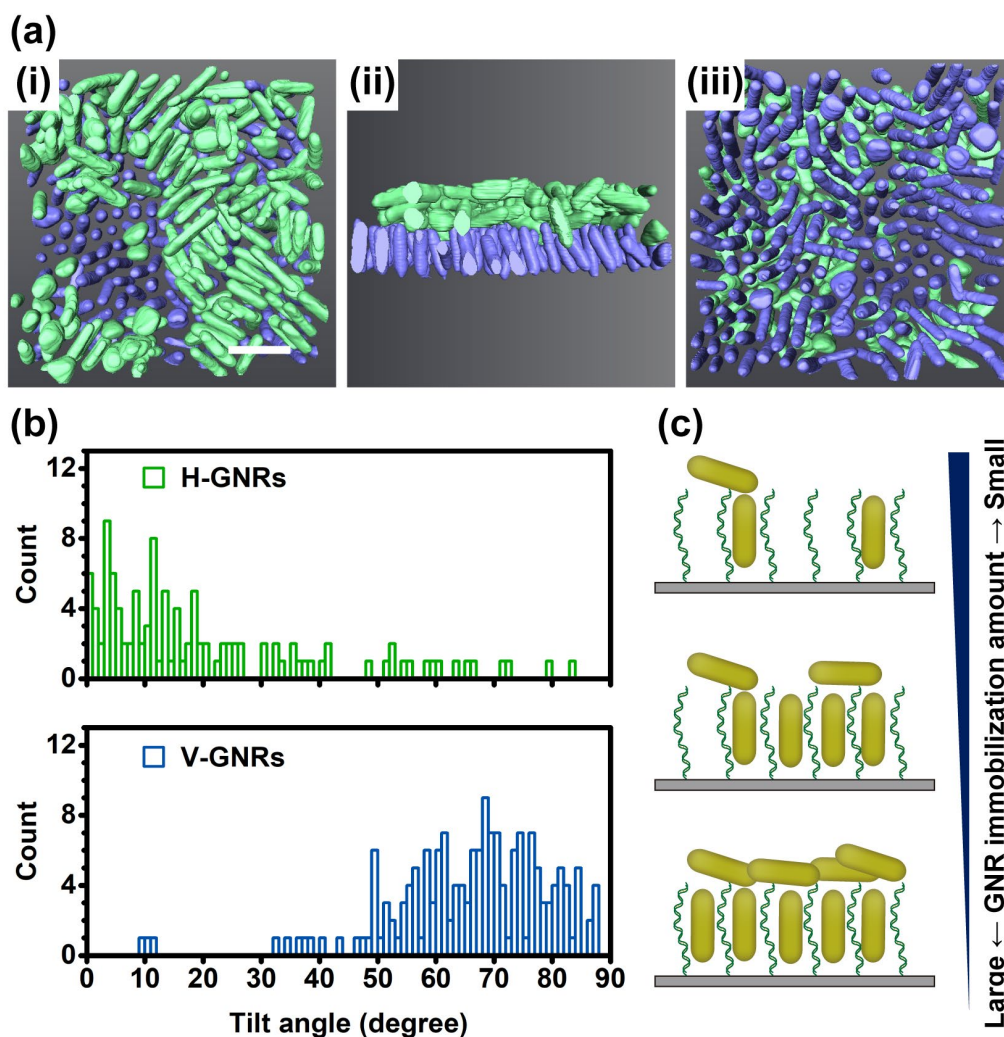


Figure 3. 19 (a) Electron tomography reconstruction images of the adsorbed GNRs with the particle density of about 19000 particles/ μm^2 on the 148 bp DNA brush. The scale bar represents 50 nm. (i) front view, (ii) cross sectional view, (iii) back view. (b) Histogram of tilt angle of the adsorbed GNRs. Tilt angle is the angle between the longer axis of the adsorbed GNRs and the substrate. The TEMT observation was performed using same sample in **Figure 3. 17**. Non-rod-like particles were not included in the histogram. (c) Schematic illustration of GNR immobilization amount dependency of the GNR orientation on the dsDNA brush.

3.3.8 Fabrication of Vertically Oriented and Tightly Packed GNR Monolayer Arrays

For further consideration to create the monolayer arrays, the possibility of selective detachment of the H-GNRs was examined by tuning ionic strength. Given that V-GNRs are surrounded by the stretched

dsDNA, V-GNRs formed more electrostatic interactions than H-GNRs do, and thus H-GNRs can be selectively removed by tuning ionic strength. To verify this hypothesis, the GNR arrays with the amount of saturated adsorption were prepared, and then immersed in buffer-solution with step-wise increase of NaCl concentration (C). **Figure 3. 20a** shows the extinction spectra depending on NaCl concentration. To roughly estimate the relative value of particle density at each NaCl concentration, the extinction (E_{NR}) intensity value at 400 nm wavelength ($E_{NR}@400$) was plotted as a function of NaCl concentration (**Figure 3. 20b**). When NaCl concentration was increased to 50 mM from 0 mM, extinction intensity value at 400 nm drastically decreased. The decline rate of the extinction intensity value was approximately 70%. Here, a salt concentration at which extinction intensity value at 400 nm markedly declined is defined as critical salt concentration ($C_{critical}$). For $C > C_{critical}$, a slow decline in extinction intensity at 400 nm was observed. These changes in extinction intensity value at 400 nm wavelength indicate that approximately 70% of the adsorbed GNRs were detached from the surface at $C = C_{critical}$ and the remained GNRs were removed gradually with the increase in C ($> C_{critical}$). Interestingly, at $C = 200$ mM, the GNRs remained on the surface showed only a sharp TLSPR peak (**Figure 3. 20c**). In other words, a LLSPR peak, which indicates deposition of H-GNRs, was not observed. Based on the above results, I estimated that most of the detached GNRs at $C_{critical}$ was H-GNRs and the remained V-GNRs were gradually dissociated from the surface.

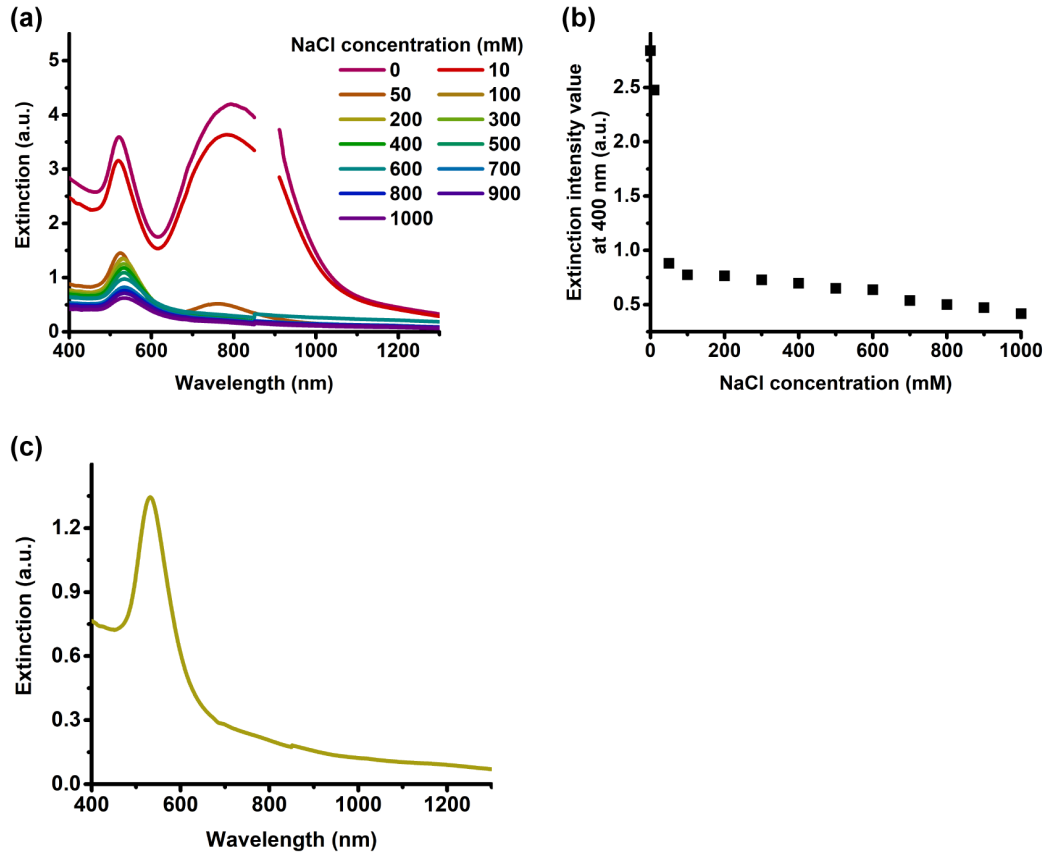


Figure 3. 20 (a) Extinction spectra of the 10% cationic GNRs immobilized on the 148 bp dsDNA brush. The extinction values ranging from 910 nm to 850 nm at NaCl concentration of 0 and 10 mM were not shown due to the saturation of the extinction values. (b) Extinction intensity value at 400 nm of the immobilized GNRs as a function of NaCl concentration. (c) Extinction spectrum of the immobilized GNRs at NaCl concentration = 200 mM.

To corroborate the estimation, the GNR arrays with saturated particle adsorption were immersed in NaCl solution ($C = C_{\text{critical}}$) and then embedded in epoxy resin to preserve their solution-phase orientations, followed by the observation of the GNRs by STEM and TEMT. **Figure 3. 21a** shows the electron microscopy image of the GNR arrays. In contrast to **Figure 3. 18a**, the area fraction of the dark regions was extremely low in comparison with that of the light regions. In the light regions, vertically aligned or slightly tilted GNRs were remained closely packing in a monolayer. The TEMT reconstruction image and histogram of the tilt angle distribution were shown in **Figure 3. 21b** and **Figure 3. 21c**. The angle median value was 67.7° , which is comparable to that obtained from V-GNRs

in **Figure 3. 19b**. The TEMT reconstruction image indicates the formation of vertically aligned GNR arrays at the monolayer level. These results indicate that H-GNRs deposited on a V-GNR layer were selectively removed by salt treatment while preserving the V-GNR layer, as illustrated in **Figure 3. 21e**.

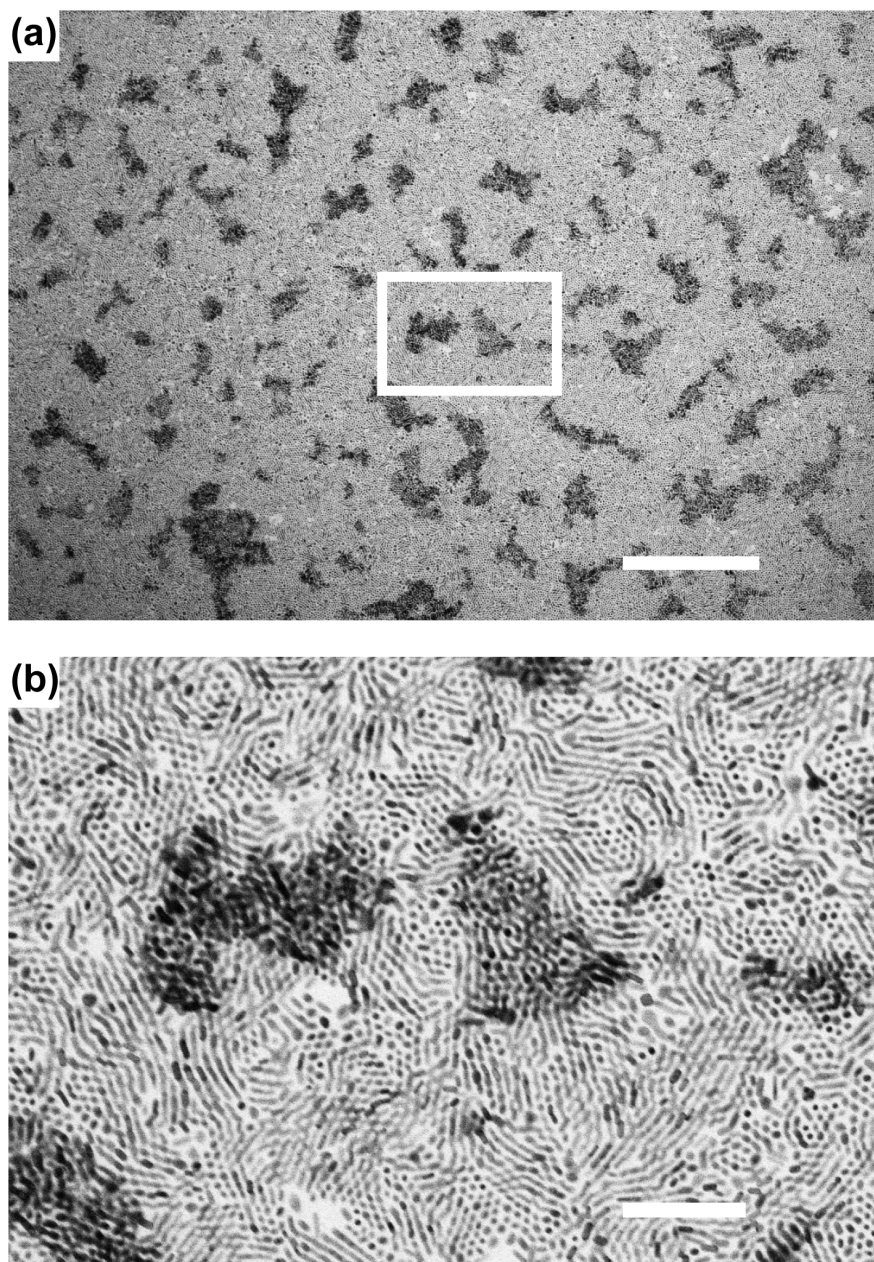
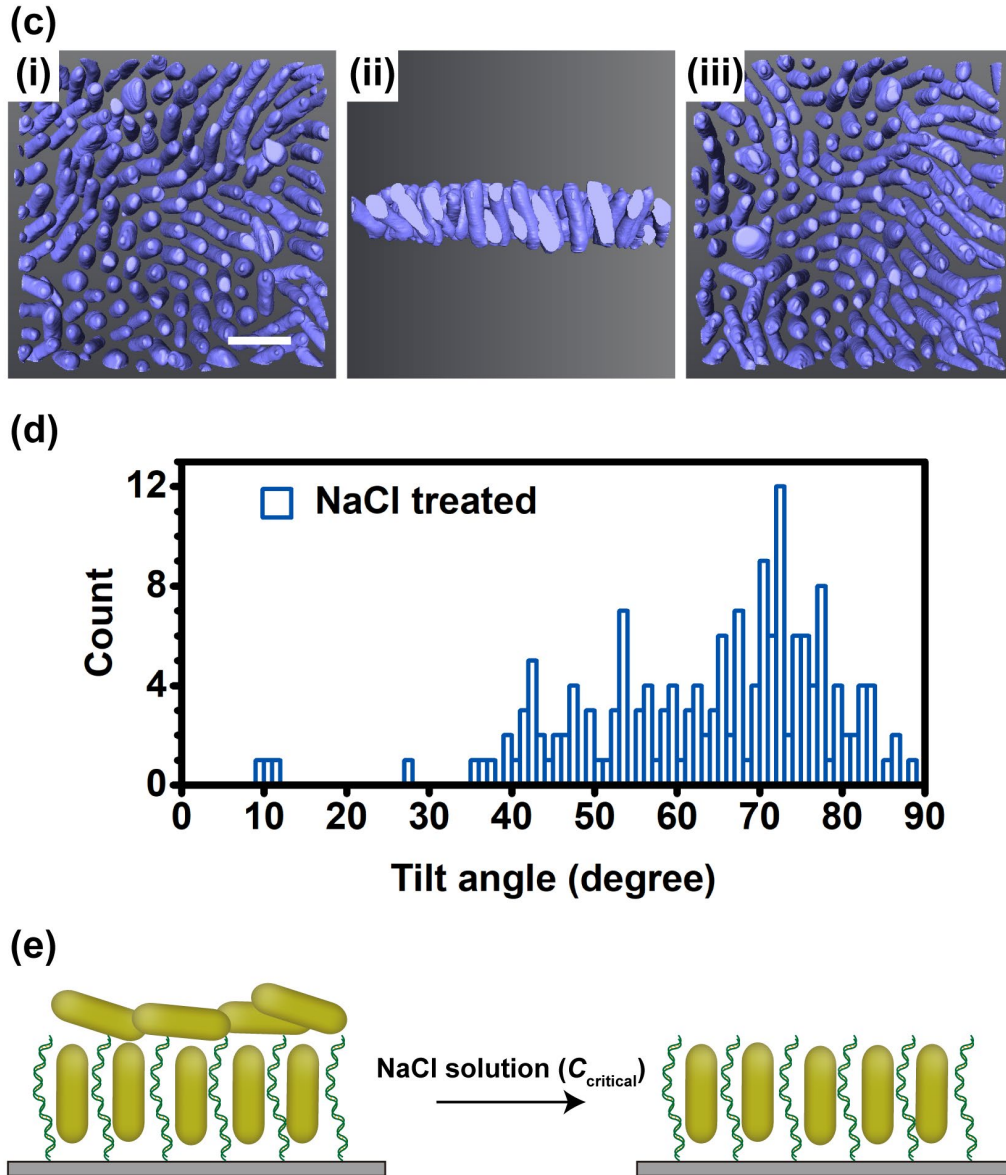


Figure 3. 21 (a) Large-area STEM image of NaCl-treated GNR arrays on the 148 bp DNA brush. The scale bar represents 1 μm . (b) Zoomed image inside the white frame marked in (a). The scale bar represents 200 nm. (c) Electron tomography reconstruction images of NaCl-treated GNR arrays on the 148 bp DNA brush. The scale bar represents 50 nm. (i) front view, (ii) cross sectional view, (iii) back view. (d) Histogram of tilt angle of the adsorbed GNRs. Tilt angle is the angle between the longer axis of the adsorbed GNRs and the substrate. Non-rod-like particles are not included in the histogram. Note that the adsorbed GNRs were embedded in epoxy resin before imaging to attempt to preserve the solution-phase structure in the solid state. (e) Schematic illustration of selective removal of H-GNR by immersing the substrate in NaCl solution.



To assess the quality of the GNR arrays of **Figure 3. 21c**, FDTD calculation was employed. **Figure 3. 21a** shows the theoretically calculated extinction spectra of a perfectly vertically standing GNR single layer with different center-to-center distance. As the center-to-center distance narrowed, a TLSPR peak position blue-shifted. To clarify the correlation, the center-to-center distance was plotted as a function of TLSPR peak position (**Figure 3. 21b**). The extinction spectrum in **Figure 3. 19c** showed a TLSPR peak at 532 nm, indicating that the averaged GNR center-to-center distance is estimated to be 18 nm. This value corresponds to 8 nm in particle-to-particle distance. Then, the two

normalized extinction spectra at LLSPR was plotted (**Figure 3. 21c**): (i) experimentally obtained extinction spectrum shown in **Figure 3. 20c**, and (ii) theoretically obtained extinction spectrum of vertically aligned GNR arrays with center-to-center distance of 18 nm. The experimentally obtained extinction spectrum was in excellent agreement with the calculated extinction spectrum. This agreement means that the orientation of GNRs adsorbed to the dsDNA brush is comparable to perfectly vertical one. Furthermore, the vertically oriented GNR arrays found to be tightly packed. The thiol ligand layer and dsDNA should present among nearest neighboring GNRs. The ligand layer thickness is 3.6 nm if the thiol ligands take on all-trans chain configuration. The diameter of dsDNA is 2 nm. Therefore, when the vertically oriented GNRs are closely packed, the particle-to-particle distance should be 9.6 nm, as described **Figure 3. 22d**. This value is higher than the value obtained from spectroscopic measurement (8 nm), which means that free space does not exist around each GNR.

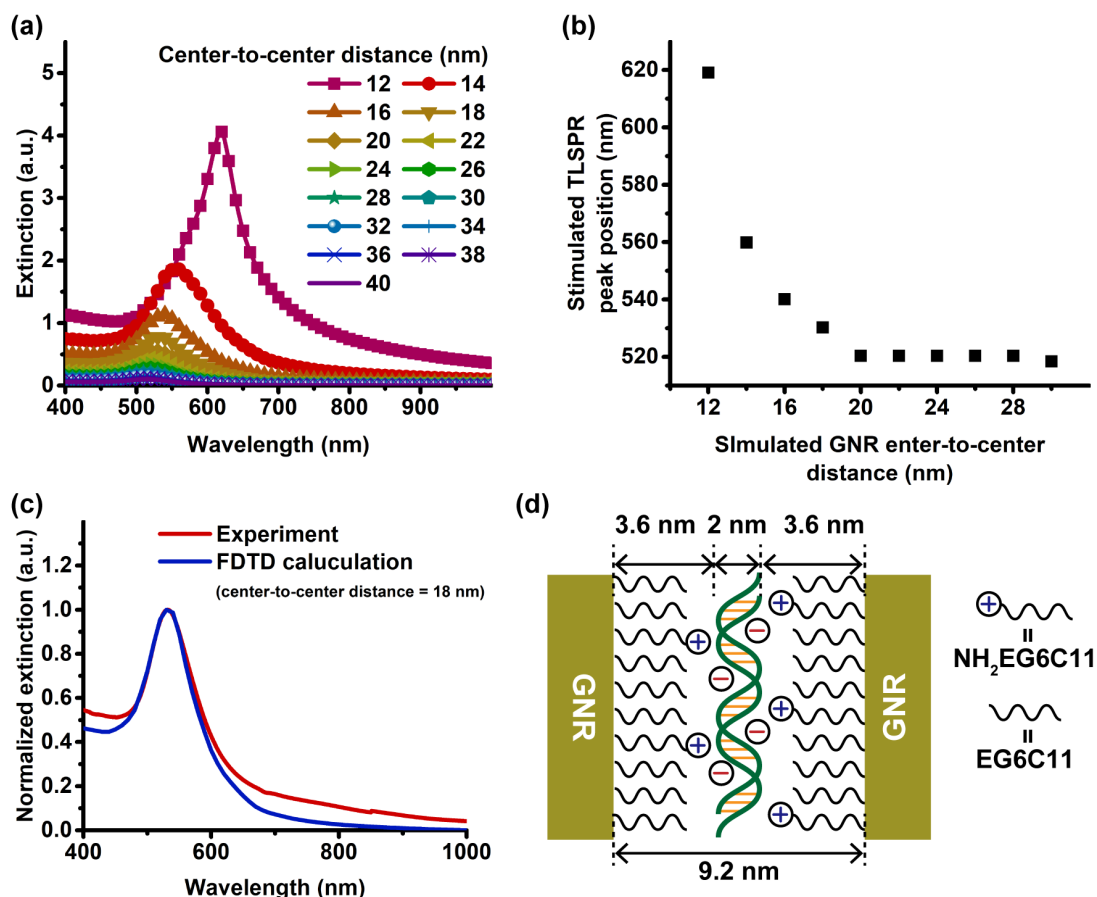


Figure 3. 22 (a) Simulated extinction spectra of vertically standing GNR monolayer with different center-to-center distance. (b) Stimulated LLSPR peak position on the different center-to-center distance. (c) Experimentally and theoretically obtained extinction spectra of GNRs. The experimental extinction spectrum is the same as **Figure 3. 20c**. Theoretical extinction spectrum of GNR monolayer with center-to-center distance of 18 nm is shown. The spectra are normalized by their TLSPR peak values. (d) Schematic diagram of the gap between nearest neighboring GNRs, wherein the alkanethiol ligand layer and dsDNA are present.

3.4 Conclusion

In this chapter, I proposed new technique for the self-assembly of GNRs into vertically aligned arrays with DNA brushes. With this technique, vertical alignment of GNR arrays can be achieved with controlled interparticle distance. There exist two important factors to achieve the assembly: (i) the moderate charge density on the surface of the nanorods and (ii) high grafting density of the DNA brush

enough to generate collective stretching effect. When these conditions are satisfied, our approach would be not limited to the self-assembly of GNRs, but a universal methodology for the assembly of one-dimensional nanoparticles. Furthermore, this technique provides many possibilities for control of nanorod orientation in more complex systems; for example, the inclusion of colloidal particles on a non-flat surface. I believe that our strategy serves as a promising general approach to the fabrication of a monolayer film composed of highly oriented rod-shaped nanoparticles, which leads to the development of next generation membrane materials for various applications such as selective separation and purification, sensing, catalysis and controlled-release materials.

Chapter 4

Salt-Induced Orientational Change of Gold Nanorods

Attached to a DNA Brush

4.1 Introduction

The study on the stimuli-induced assembly/disassembly behavior of nanoparticles is of great interest for both fundamental research and applications. To induce the assembly/disassembly of nanoparticles, a variety of stimuli and environmental changes has been used including changes in pH^{107,108,117,109–116} and temperature^{118–127}, and light^{128–131}. Most of these studies on the assembly/disassembly of the nanoparticles have been focused to “assembly/disassembly behavior of nanoparticles dispersed in solutions”. On the other hands, stimuli-induced assembly/disassembly of the nanoparticles attached to substrates has attracted much attention as an interesting phenomenon, which could be used in designing of systems for various applications. To name one example of the potential applications, confinement of the plasmonic nanoparticles in 2D space enables location-selective color change accompanied by the assembly/disassembly behavior. Therefore, the phenomenon could be used for development of plasmonic display. To date, chemists have developed methods to induce assembly/disassembly of nanoparticles attached to substrates^{132–134}. Especially, “polymer brush” surfaces have often been used as a matrix because assembly/disassembly of nanoparticles could be triggered by conformational transitions of polymer brushes in response to surrounding environments between swelling and collapse states. For example, Klitzing and colleagues report that thermally or salt-induced assembly/disassembly of spherical gold nanoparticles (GNPs) confined within poly(Nisopropylacrylamide) (PNIPAM)¹³³.

GNRs have an array of unique shape-dependent properties, which allows them to change their color depending on their orientation relative to incoming light. Furthermore, the dynamic modulation of the plasmonic response of GNRs can be achieved by tuning their size and aspect ratio. Thus, GNRs are not only anisotropic building blocks for self-assembly but also an attractive material for use to design systems that change color in response to external stimuli. Indeed, GNRs have already been used for the purpose. For example, Smalyukh and coworkers demonstrate that GNRs, which are

dispersed in surfactant-based lyotropic liquid crystals, change their color by control over their orientation through shearing¹³⁵ and electric fields¹³⁶. Yin and coworkers report that magnetic nanoparticle-attached GNRs, which are dispersed in an aqueous solution, change their color in response to magnetic fields¹³⁷.

In chapter 3, I obtained a result suggesting that a change in salt concentration induces orientational change of GNRs adsorbed onto a DNA brush. Herein, I study the orientational change behavior of GNRs on DNA brush surface triggered by increase in ionic strength. I believe that this phenomenon can give us a new insight into colloidal science, which leads to designing of novel plasmonic devices.

4.2 Experimental

4.2.1 Materials

All reagents and instruments used in this chapter are listed in chapter 3.

4.2.2 Preparation of DNA Brushes and Synthesis of MTAB Ligands

The preparation of DNA brush and the synthesis of GNRs are described in Chapter 2 and 3, respectively. The procedures of GNR adsorption onto DNA brushes and the quantitative measurement of the adsorbed GNR amount are described in Chapter 3. The techniques for the characterization including STEM imaging, DLS and zeta potential measurements, ICP-AES measurements, and extinction spectrometry are described in Chapter 3. (16-mercaptohexadecyl) tri-methylammonium bromide (MTAB) was synthesized by Dr. Kenya Kobayashi following a previous literature¹⁰⁵ (**Figure 4.1**).

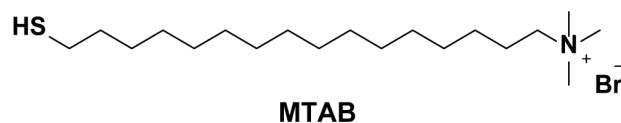


Figure 4. 1 Chemical structure of the cationic alkanethiol ligand (MTAB).

4.2.3 Surface Modification of GNRs with MTAB Ligands

A methanol solution containing MTAB ligands (1.26 mM, 71 μ L) was added to the GNR aqueous solution (4.6 nM, 1429 μ L). Centrifugal purification was performed twice to remove free MTAB ligands from the GNR solution, followed by a centrifugal concentration or a dilution with MilliQ to adjust the concentration of GNRs.

4.2.4 Adsorption of MTAB-capped GNPs onto DNA brushes

The GNRs modified with the MTAB ligands were attached by soaking the inside surface of the DNA brush cuvettes in the GNR solution. The inside surface of the cuvette was washed with pH 7.7 Tris-HCl at least three times to remove the nonattached GNRs.

4.2.5 Quantitative measurement of the amount of adsorbed GNPs

The amount of the adsorbed GNPs was measured using UV-vis-NIR spectroscopy by subtracting the amount of the non-adsorbed GNRs from the added amount of the GNPs.

4.3 Results and Discussion

4.3.1 Salt-Induced Assembly/disassembly of GNRs on DNA brushes

148 bp dsDNA brushes with grafting density of 16100 chains/ μ m² were prepared via the “grafting to” method. The average inter-chain distance of the dsDNA brush was about 8.5 nm. MTAB-capped GNRs were adsorbed onto the dsDNA brush. After removal of non-adsorbed GNRs, the extinction spectra of

the DNA brush/GNR hybrid were recorded in Tris-buffer solution, followed by in Tris-buffer solution containing 1000 mM NaCl. When NaCl concentration increases from 0 to 1000 mM, the color of DNA brush/GNR hybrid turned from red purple to blue purple (**Figure 4. 2**). At NaCl concentration of 0 mM, the GNR attached to the DNA brush exhibited a weak band for TLSPR and a strong band for LLSPR, meaning that the GNRs lie flat on the surface. In contrast, at NaCl concentration of 1000 mM, the TLSPR band slightly increased in intensity and the LLSPR band almost disappeared, suggesting that orientation of the GNRs changes from lateral to vertical direction. With the disappearance of LLSPR band, the extinction maximum of the TLSPR red-shifted from 525 nm to 537 nm.

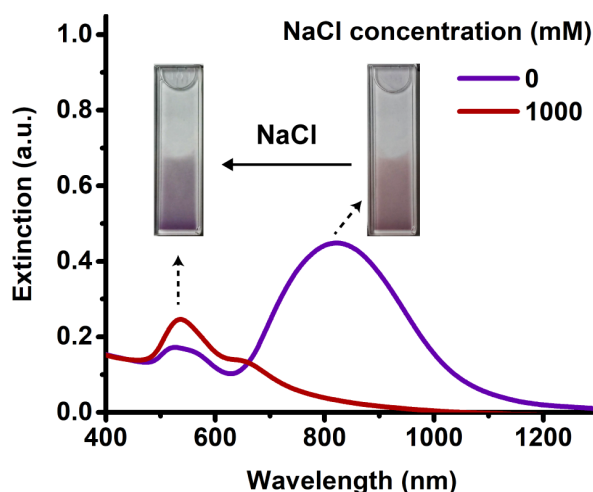


Figure 4. 2 Extinction spectra and photographs of the GNRs attached to the DNA brush. Purple line is the extinction spectrum in 10 mM Tris-HCl buffer. Red line is the extinction spectrum in 10 mM Tris-HCl containing 1000 mM NaCl.

Investigation of the spectrum changes with the stepwise increase in NaCl concentration revealed that orientational change of GNRs from lateral direction to vertical direction and side-by-side assembly occurs simultaneously. Based on the extinction spectra of GNRs when NaCl concentration is increased by 200 mM (**Figure 4. 3a**), I plotted the LLSPR peak position and the LLSPR peak intensity as a function of NaCl concentration (**Figure 4. 3b**). A blue-shift in the LLSPR peak and a decrease in the LLSPR peak occurs simultaneously. The extinction maximum of the LLSPR blue-

shifted from 816 nm to 649 nm with the increase in NaCl concentration from 0 mM to 1000 mM. Considering theoretical works predicting that side-by-side assembly leads to blue-shifting of the LLSPR, I believed that the addition of NaCl triggers the side-by-side assembly on the surface with orientational change of GNRs, as described in **Figure 4. 3c**.

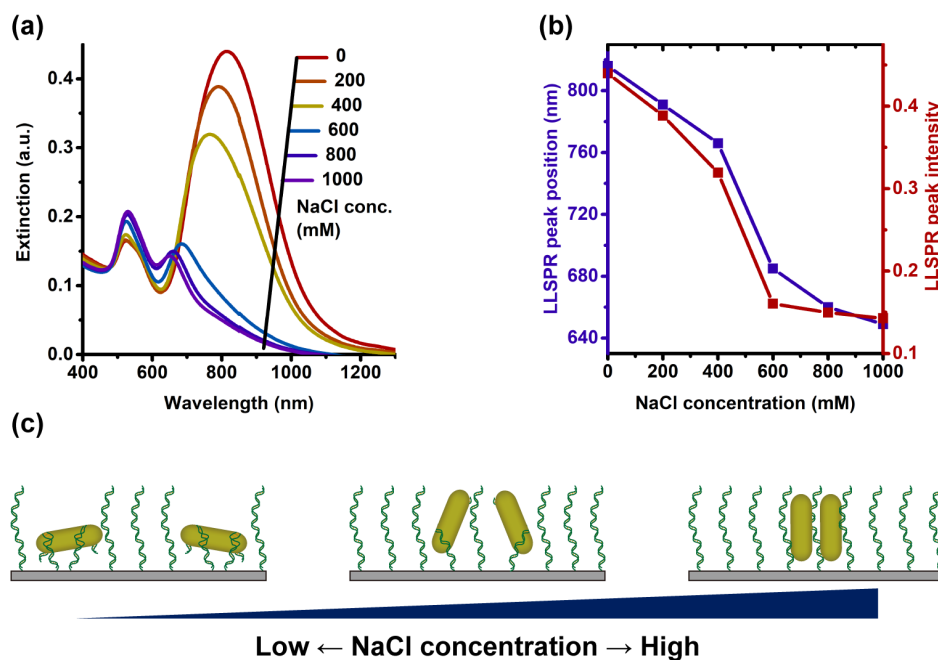


Figure 4. 3 (a) A series of extinction spectra that accompanied the increase in NaCl concentration. (b) Changes in LLSPR peak position (purple) and LLSPR peak intensity (red). (c) A sketch of the assembly process

The salt-induced color change of the GNRs attached to the DNA brush was reversible. I repeated changing the NaCl concentration from 0 mM to 1000 mM and back four times to test the reversibility of the color change. The extinction spectra of the GNR that accompanied by the change of the NaCl concentration are shown in **Figure 4. 4a**. At the initial state, the hybrid showed TLSPR peak at 525 nm and LLSPR peak at 822 nm. Plot of the GNR extinction at Ext@822 showed that the color change can be repeated (**Figure 4. 4b**). The reversibility of the color change means that the color change is not induced by the irreversible transformation of the GNRs. It is noted, however, that the salt-induced changes in LLSPR and TLSPR modes (in 1000 mM NaCl concentration) showed a

hysteresis that a larger initial decrease in LLSR intensity is followed by highly reproducible values. Thus, the orientational change behavior of the GNRs found to show a hysteresis, as described in **Figure 4. 4c**.

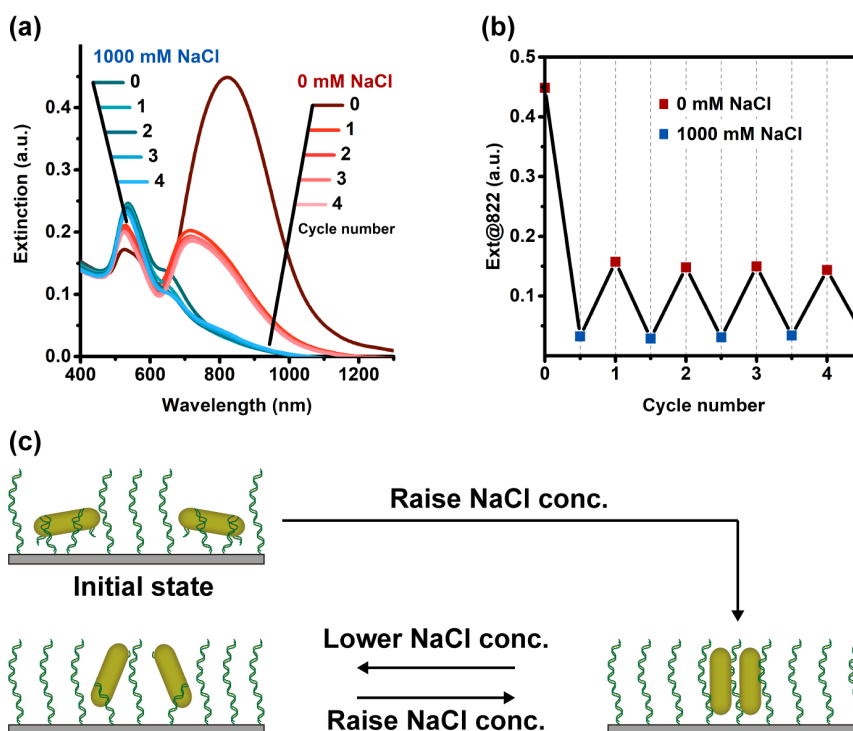


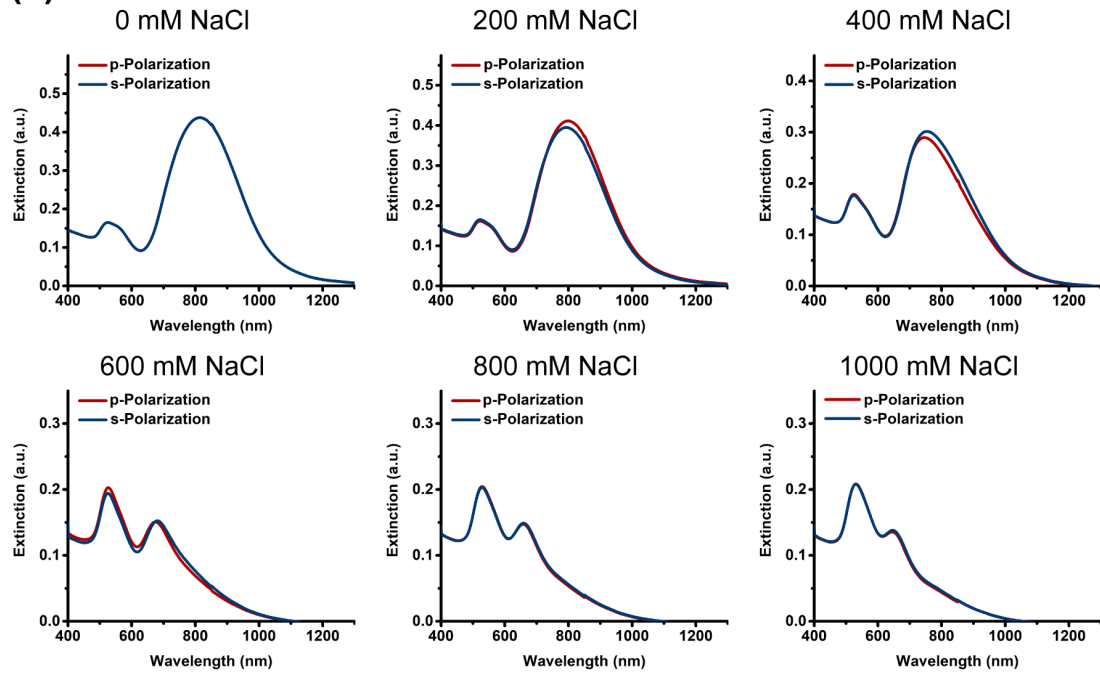
Figure 4. 4 (a) Extinction spectra of the DNA brush/GNRs hybrids for 4 cycles between 0 mM and 1000 mM NaCl in Tris-buffer. The numbers indicate the cycle number of NaCl concentration change. (b) Changes in the extinction at Ext@822. (c) A sketch of the reversible process.

Further investigations using polarized light (p-polarized and s-polarized light) corroborated that an increase in NaCl concentration triggers the orientational change of the GNRs attached to the DNA brush. **Figure 4. 5** shows the extinction spectra of the DNA brush/GNR hybrid at $\theta = 0^\circ$ (**Figure 4. 5a**) and 45° (**Figure 4. 5b**). Obviously, at $\theta = 0^\circ$, a polarization optical property was not observed (**Figure 4. 5a**). However, the DNA brush/GNR hybrid showed a polarization dependent optical property when the substrate is rotated (**Figure 4. 5b**). When NaCl concentration is 0 mM, 200 mM and 400 mM, the values of $Ext_s@LLSPR$ were higher than those of $Ext_p@LLSPR$. However, when NaCl concentration is 600 mM, 800 mM and 1000 mM, the values of $Ext_s@LLSPR$ were lower than

those of $\text{Ext}_p@LLSPR$. Plots of the ratios of LLSPR peak extinction for p-polarization ($\text{Ext}_p@LLSPR$) and LLSPR peak extinction for s-polarization ($\text{Ext}_s@LLSPR$) to NaCl concentration clarified that the hybrid displays the polarization-dependent optical property by rotating the substrate. $\text{Ext}_p@LLSPR$ exceeded $\text{Ext}_s@LLSPR$ with the increase in NaCl concentration (**Figure 4. 5c**), supporting that the orientation of the GNRs gradually changes from lateral to vertical direction with the increase in NaCl concentration.

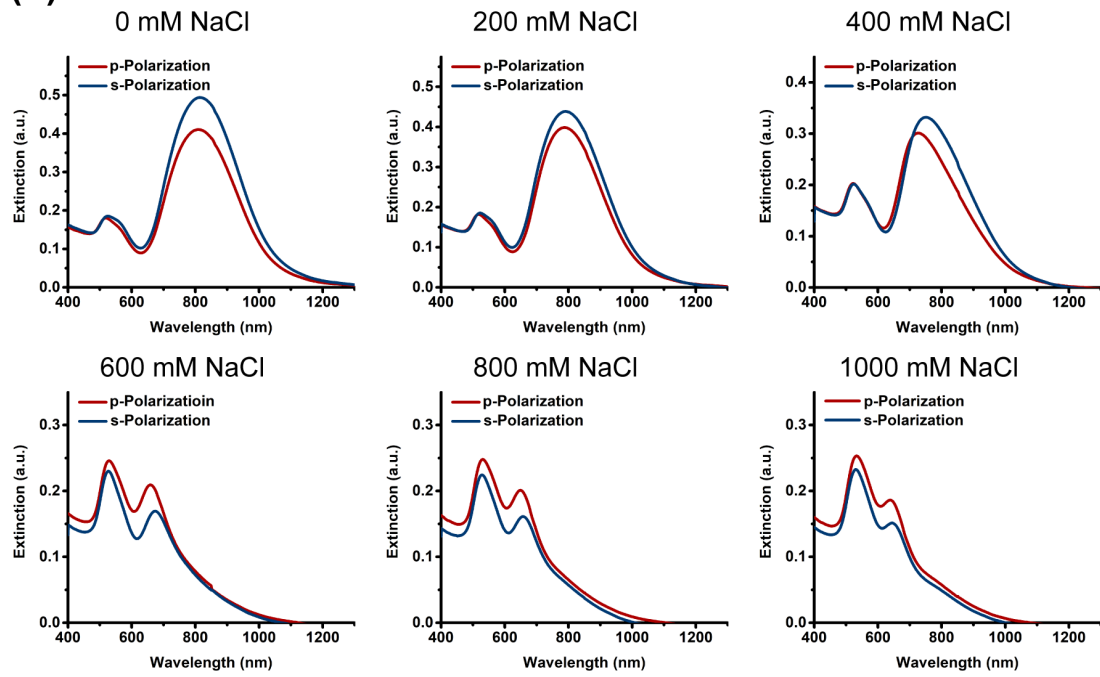
(a)

$\theta=0^\circ$



(b)

$\theta=45^\circ$



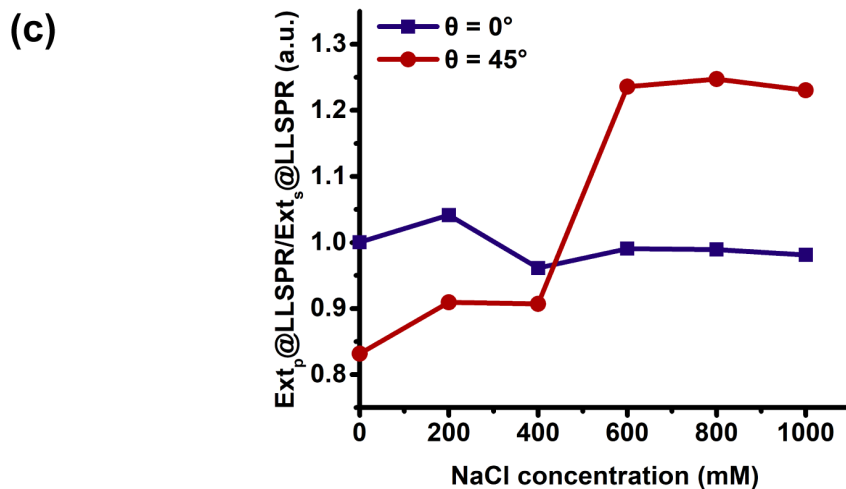


Figure 4. 5 Extinction spectra of the GNRs attached to the DNA brushes recorded through a polarizer at $\theta = 0^\circ$ (a) and 45° (b). (c) Plots of the ratios of LLSPR peak extinction for p-polarization (Ext_p@LLSPR) and LLSPR peak extinction for s-polarization (Ext_s@LLSPR)

4.4 Conclusion

In this chapter, I reported the salt-induced orientational change of GNRs attached to DNA brushes. Monitoring the process by optical spectroscopy revealed the two following. First, this phenomenon is reversible, but a hysteresis is observed at first cycle. Second, an increase in salt concentration induced side-by-side assembly of the GNRs with orientational change from lateral to vertical direction. Although further study of this phenomenon is still needed, I believe that screening of the stabilizing electrostatic repulsion among neighboring GNRs induces the on-surface assembly of GNRs. If so, design of surface ligands allows nanoparticles to assemble responding to chemical or physical stimuli such as pH, temperature and light, and thus these stimuli may be used to induce the on-surface aggregation. Therefore, I anticipate that the observed phenomena lead to development of novel plasmonic devices such as information storage, display and sensor.

Chapter 5

General Conclusion

The results presented in this thesis demonstrate that polymer brush-assisted self-assembly utilizing electrostatic interactions is a promising tool for the formation of ordered arrays of rod-shaped nanoparticles and provides a scaffold for their dynamic modulation. Interestingly, control over the electrostatic interactions among components such as grafted polyelectrolyte polymer and oppositely charged rod-shaped nanoparticles are important in the polymer brush-assisted self-assembly. Specifically, vertically aligned arrays of gold nanorods are achieved with the assistance of DNA brushes by adjusting the surface charge density. The dynamic control of the orientation and assembly state of gold nanorods is accomplished by tuning ionic strength of the system.

There are some future considerations to further optimize the ordered arrays or dynamically modulate them. Examples include surrounding environmental effects such as pH and temperature. In this thesis work, cationic ligand possesses a primary amine substitute and the surface charge density is influenced by change in pH. It is known that temperature affects electrostatic interactions. Thus, the orientation and assembly state of gold nanorods could respond to change in pH and temperature. In addition to surrounding environmental factors, molecular design of polymer chain and surface-ligand should also be considered to impart new functionality. For example, introduction of photo-responsive moiety to polymer brush or surface ligand in order to change conformation of polymer brush or surface-charge density could impart photo-responsiveness to the system.

I believe that the self-assembly strategy outlined in this thesis will hopefully contribute to the further development of self-assembly strategy for 1D building blocks, and thus ultimately lead to the creation of devices and materials that go beyond conventional ones.

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