Microfluidic Modeling of Cell Flow & Self-Assembly of Gold Nanorods with Different Lengths

by

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Abstract

The thesis is divided into two parts: (1) microfluidic modeling of blood cell flow in constricted microvasculature and (2) the kinetic study of self-assembly of Au nanorods with different lengths.

The passive mechanism of the flow of neutrophils was studied by using poly(dimethyl siloxane) microchannels with circular cross-sections as model blood vessels and agarose microgels as model cells. Their velocity and pressure profiles at various locations inside the microchannel with constrictions were studied as functions of (a) the initial velocity of the microgels, (b) the degree at which the channel-at-large tapered into the constriction, and (c) the size of microgels.

Previously, our group proposed that the kinetics of self-assembly of Au nanorods resembles that of the reaction-controlled step-growth polymerization. To investigate factors that affect the reactivity of functional groups, self-assembly experiments were performed for nanorods with different lengths and their kinetics was analyzed.
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Chapter 1
Introduction to Microfluidic Modeling of Blood Cell Flow in Circular Cross-Section Microchannels with Constriction

1.1 Microvascular system: flow of cells through constrained geometries

The study of the flow of blood cells in the cardiovascular system has great importance for understanding the response of the body to injuries and infections.\(^1\)–\(^5\) Cells encounter capillary segments with diameters smaller than the dimensions of cells during their transit through the pulmonary and systemic microcirculations. Blockage of capillaries may occur due to diseased red blood cells, e.g. in sickle cell anemia\(^6\) and thrombosis,\(^7\) or diseased capillaries, e.g. in atherosclerosis.\(^8\)

1.2 Studies of cell flow through pulmonary capillary network

Studies of the flow of blood cells through the pulmonary capillary network have attracted great attention. The pulmonary circulation is approximately a quarter of the volume of the systemic blood circulation and it is a low pressure system.\(^7\) Flow of neutrophils, the most abundant type of white blood cells, is of particular interest because they have an average diameter of 6-8 μm, which is larger than about 70% of the capillaries in the lungs.\(^9\) Therefore, they have to deform and change their rheological properties to flow in capillaries. Neutrophils are also significantly more resistant to deformation than the red blood cells and they also show adhesion to the endothelium.\(^10\) Both factors counteract neutrophil flow under confinement. Whereas red blood cells traverse the pulmonary circulation in a few seconds with little or no observable delay\(^6,11-16\)
at the entrance of a capillary segment, neutrophils stop for time intervals ranging from a few seconds to tens of minutes, in order to change their shape\textsuperscript{17} and they require a relatively long time to travel through the capillary.\textsuperscript{10} This behavior may lead to obstruction of capillary blood flow, also known as the leukocyte plugging phenomenon.\textsuperscript{10,18,19} Neutrophils’ long transit time also causes an increase in the concentration of neutrophils at discrete sites along the pulmonary circulation and an increase in pressure at the capillary segment entrance.\textsuperscript{19}

1.3 Passive and active mechanisms of neutrophil flow

The mechanism of the flow of neutrophils under constrained conditions can be passive or active, depending on the response of cells to the mechanical stimulation during their flow in narrow capillaries.\textsuperscript{20,21} Active behaviour originates from mechanosensing or signal transduction in the cell, which modulates the change in cell shape in response to the mechanical stimulus.\textsuperscript{22} The passive behaviour is governed by the pressure gradient across the capillary. In the absence of driving forces that activate cells, fluctuation in their viscoelastic continuum allows them to flow through constriction.\textsuperscript{23} Whereas for cellular systems it is difficult to disentangle these effects, the detailed fundamental study on model systems provides insights on the role of various characteristics on cell flow, e.g., the mechanical properties of cells, the degree of confinement, or cell-capillary interactions.

1.4 Microchannels as model blood vessels

Microfluidics provides a great tool in modeling the flow of cells through blood capillaries \textit{in vitro}.\textsuperscript{24} Blood capillaries have been mimicked by using microchannels fabricated in glass or
polymers. The flow of red blood cells\textsuperscript{6,11-16} and neutrophils\textsuperscript{21,24} has been studied in microchannels to relate cell type, shape, and mechanical properties to their flow behavior.\textsuperscript{25} Neutrophil activation upon its mechanical deformation under a threshold stimulus was observed when the cells passed through narrow channels fabricated in poly(dimethyl siloxane) (PDMS).\textsuperscript{21} In response to the changes in surrounding temperature and applied pressure, neutrophils changed their rheological properties by reorganizing their cytoskeleton and lowering their shear moduli.\textsuperscript{26} Under a sufficiently strong mechanical signal neutrophils formed pseudopods and crawled along the microchannels.\textsuperscript{21}

1.5 Microparticles as model blood cells
Fundamental studies of cell flow through constrained geometries are preferred on model cells, since living cells are dynamic systems with a large time-dependent variability in size, mechanical properties and adhesion or surface chemistry between cells of even the same line. Vesicles,\textsuperscript{27-29} polymer microbeads,\textsuperscript{30-36} microgels,\textsuperscript{37-39} or soft capsules\textsuperscript{40,41} have been used as model cells. Although the dimensions and the mechanical properties of these particles did not match the corresponding properties of blood cells, the use of model cells highlighted the importance of receptor-ligand and electrostatic interactions for cell adhesion,\textsuperscript{38,42,43} and allowed simulation of the renal filtration process\textsuperscript{5} and the passive mechanism of cell flow, e.g., the role of initial velocity under unbound conditions, pressure gradients, adhesion and friction.
1.6 Limitations of conventional microchannels with rectangular cross-sections

Currently, experimental studies of cell flow have been conducted in microchannels that had a rectangular cross-section, so that cells did not conformably plug the rectangular constrictions.\textsuperscript{21,39} Due to the limitation with the geometry of the channels, thorough examination of the effect of particular characteristics of cell flow, e.g, the degree of confinement or the mechanical properties of the cells could not be achieved. Attempts to fabricate microchannels with a circular cross-section were made by casting PDMS around glass capillaries\textsuperscript{44} and stainless steel rods.\textsuperscript{45-47} The main problem with these methods for studies of cell flow in constrained geometries was an insufficient ability to gradually change the diameter of the microchannel to create constrictions. Fabricating complex networks of microchannels was also difficult due to the rigid nature of the templates.\textsuperscript{48} The limitations of the above methods and microchannels with rectangular cross-sections counteract the development of the fundamental understanding of the role of passive mechanism on cellular flow under constrained conditions.

1.7 Modeling the passive mechanism of the flow of neutrophils in capillaries with constricted geometry

Here we present the results of the systematic studies of flow of the model cells in the constrained geometries of microchannels. We used agarose microgels flowing through the circular microchannels with a diameter smaller than that of the microgels. We systematically examined the role of the following variables on flow of microgels through the constriction: (i) the effect of varying initial microgel velocity; (ii) the effect of the varying entrance angle at the constriction,
(iii) the effect of the degree of confinement of microgels and (iv) the effect of the mechanical properties of microgels.
2.1 Materials

Sylgard 184 Silicone Elastomer Kit (PDMS) was purchased from Dow Corning Corp. (Midland, MI). SU-8 50 photoresist and SU-8 developer was purchased from MicroChem (USA). Silicon wafers were purchased from Wafer World, Inc. (West Palm Beach, FL). ACS grade acetone, methanol and isopropyl alcohol was purchased Sigma-Aldrich Canada. Ultra low gelling agarose was obtained from SeaPrep (Switzerland). All chemicals were used as received.

2.2 Fabrication of microfluidic devices

The microfluidic (MF) devices were fabricated using a soft lithography method\textsuperscript{30,49} in poly(dimethylsiloxane) (PDMS) (Sylgard 184 Silicone Elastomer Kit, Down Corning Corp., Midland, MI). Microchannel patterns were created as a positive relief of photoresist on a silicon wafer using SU-8 50 photoresist.\textsuperscript{50} A PDMS negative replica of the master was prepared from a mixture of the prepolymer PDMS with the crosslinking agent in ratio of 10:1. The mixture was degassed for 40 min under vacuum, poured on the master and cured over-night at 75 °C. After curing, holes were drilled in the inlets and outlet of the sheet. The patterned PDMS sheet was sealed to a planar PDMS sheet using plasma-induced bonding (550 mTorr in air, 90 s) in an oxygen plasma chamber (PDC-3XG, Harrick, USA).
The cross-sections of the MF microchannel were transformed into circular ones by polymerizing PDMS oligomer dissolved in hexane flowing through the microchannel around a \( \text{N}_2 \) stream introduced in the channel.\(^{48}\) Using a syringe with an 18 gauge needle and 17 gauge o.d. Teflon tubing (Small Parts, USA), the channel was manually filled with a solution of PDMS oligomer prepared by dissolving the oligomer and the initiator (in a 5 to 1 ratio) in hexane (Sigma-Aldrich Canada). Then a stream of \( \text{N}_2 \) from a tank was introduced in the channel via Teflon tubing at the pressure of 5 psi. The stream formed a gaseous thread with a circular cross-section. With the \( \text{N}_2 \) flowing into the microchannel, the device was placed for 10 min on a hot place at 100 °C. The PDMS oligomers polymerized around the gas stream and the hexane dissolving the PDMS evaporated. Once the oligomer was polymerized, the passage of \( \text{N}_2 \) was terminated and the microfluidic device was cooled to room temperature for use.\(^ {48} \) The diameters of the modified channel-at-large and of the constriction were 150 ± 5 μm and 50 ± 5 μm respectively. Finally, polyethylene tubing (Small Parts, USA) were placed in the drilled holes of the finished PDMS device and sealed with epoxy glue.

### 2.3 Preparation of microgels as model cells

Model cells were prepared from ultra-low gelling temperature (8 – 17 °C) agarose (Sigma Aldrich, Canada), using a MF method reported elsewhere.\(^ {51,52} \) Briefly, an agarose solution in the phosphate buffered saline (PBS) buffer (pH 7.4) was heated to 37 °C and emulsified in the MF droplet generator with a T- junction geometry. Mineral oil containing 3 wt.% of surfactant Span 80 was used as the continuous phase. The concentration of agarose, \( \text{C}_{\text{ag}} \), in the microgels was
0.75, 1, 1.5 and 2.0 wt.%). The suspension of microgels in mineral oil was collected in a centrifuge tube containing PBS at 2 °C. Following centrifugation at 1000 rpm, the mineral oil was removed, the microgels were transferred in a PBS buffer and stored for at least, 4 months without noticeable change in their dimensions or mechanical properties. The size of microgels was measured and analyzed using a light microscope (Olympus BX51) and Image Pro Plus Software (Media Cybernetics, Silver Spring, MD).

2.4 Setup of experiments of flow of microgels through constriction

Figure 1 (a) shows the optical transmission and fluorescence microscopy images, respectively, of the microfluidic (MF) channel used in the studies of the flow of model cells. Following the modification, the microchannel-at-large and the constriction had the diameters of 150 and 50 μm, respectively. The length of the constriction was 450 μm. The microchannels were fabricated to have different degrees of tapering between the channel-at-large and the constriction. In the present work, we used the MF devices with three values of entrance angles (defined as the angle θ between the horizontal axis along the channel and the converging wall into the constriction). The values of θ were 13°, 22°, and 35°.

The agarose microgels used in the present work were divided into four groups with a mean diameter, Dm, of 95, 105, 115 and 125 ± 5μm. Figure 1 (b) shows a typical optical microscopy image of the agarose microgels with a mean diameter of 105 ± 5 μm. The degree of confinement
was defined as $\alpha = D_m/D_c$, where $D_m$ was the diameter of microgels and $D_c$ was the diameter of the channel-at-large, varied from 1.9 to 2.5.

![Diagram](image)

Figure 1. (a) Optical fluorescence microscopy images of the microchannel with a circular cross-section and the orifice entrance angles of 13, 22, and 35° (top to bottom). The microchannel is filled with fluorescein solution. The scale bar is 150 μm. (b) Typical optical microscopy image of agarose microgels used in model cell studies. The scale bar is 100 μm.

Dispersion of microgels with a concentration of 100 000 microgels/mL was introduced in the microchannel via polyethylene tubing connected to a syringe reservoir on a syringe pump (Harvard Apparatus PHD 2000, Holliston, MA). This concentration of microgels ensured that the microgels did not interact with each other when they approached the constriction.

The range of initial velocities of the microgels in the channel-at-large varied from 1200 to 2300 μm/s. The lower end of the range was close to the mean resting capillary blood flow velocity of 650 – 950 μm/s.$^{53,54}$
2.5 Pressure measurements

In our experiments, microgels flowing through the microchannel exhibited distinct behaviour in various regions of the microchannel. In addition to constructing velocity profiles of microgels traversing the constriction, monitoring the pressure difference between upstream and downstream of the constriction was important. Figure 2 shows the experimental set-up of pressure measurement. The syringe containing the dispersion of microgels was mounted on a syringe pump and the syringe was connected to a pressure gauge (Media Gauge™, SSI Technologies, Inc., USA) measuring the pressure in the region from the inlet of the microchannel to the constriction. The outlet of the microchannel was connected to the second pressure gauge measuring the pressure downstream of the constriction region. A digital camera (Canon PowerShot A530) was used to video-record the readings of the two pressure gauges during the pressure measurement. In parallel, the motion of microgels through the constriction was imaged under 100 x magnification using an optical microscope (Olympus SZX12) and was video-recorded with a high-speed video camera (Casio EX-F1). We analyzed the two sets of videos and plotted the velocity of microgels and the corresponding pressure difference inside the microchannel against the position inside the microchannel.

Figure 2. The experimental set-up for measuring pressure upstream and downstream of the constriction region. (a) The pressure gauge on the left measures the pressure upstream of the constriction. The pressure gauge on the right measures the pressure downstream. (b) The pressure gauge connected to the syringe containing the dispersion of microgels enters through the inlet of the microchannel (left). The outlet of the microchannel (right) is connected to the pressure gauge measuring the downstream pressure.
2.6 Analysis of microgel velocity

The flow of microgels under constrained conditions was examined by analyzing their velocity profiles in the channel at large and the constriction. The motion of the microgels through the constriction was imaged under 100 x magnification using an optical microscope (Olympus SZX12) equipped with a high-speed camera (Casio EX-F1). The flow of microgels was video-recorded and the video clips were analyzed using Windows ® Movie Maker Version 2.6.4038.0 (Microsoft). To determine linear velocities of microgels, the Regions 1 to 6 of the microchannel with the total length of 2.5 mm was divided into 48-50 divisions with the width of ~51 μm. The velocity of microgels in each region was calculated and plotted as a function of the distance from the centre of the constriction. The velocity profiles of the microgels were normalized with respect to their initial velocity in the channel-at-large determined at 979 ± 61 μm to the left of the centre of the constriction. The normalized velocity profiles were averaged for at least 20 microgels and were plotted as normalized velocity vs. distance profiles.
Chapter 3
Microfluidic Modeling of Neutrophil Flow Using Agarose Microgels and Microchannels with Circular Cross-Sections

3.1 Velocity profiles of the continuous phase in circular cross-section microchannel

In addition to studying the flow behaviour of agarose microgels, the flow profile of the continuous phase was evaluated by flowing 10-µm polystyrene (PS) beads. Figure 3 shows the normalized velocity profile of an estimation of the fluid velocity as well as the profile of the PS beads in the modified circular cross-section microchannel. Estimation of the fluid velocity was based on Bernoulli’s equation, which explains that as the channel cross-sectional area decreases the flow rate of the fluid increases proportionally. The continuous phase velocity increases to 8 x the initial velocity using the estimated value, or 6 x the initial velocity using the values found with the PS beads. The difference between the two values is due to the increased drag on the PS beads. The PS beads are 10 µm in diameter, if we used smaller particles they would have less drag and the velocity in the orifice would be closer to our estimated value.
Figure 3. Normalized velocity profiles of the continuous phase in a circular cross-section microchannel. Red squares represent theoretical data and blue triangles represent the flow of 10-µm polystyrene beads in a microchannel with the constriction entrance angle of 13°. D represents the distance from the centre of the constriction.

3.2 Flow of microgel through a circular cross-section microchannel

Figure 4 (a) shows typical velocity profiles of the microgels flowing through microchannel illustrated in Figure 1 (a). A typical velocity profile (red circles) featured six characteristic regions corresponding to the flow of microgels through six regions of the channel (circled numbers).

In Region 1, a microgel moved with a constant velocity in the unconfined conditions in the channel-at-large. In our work, the velocity in Region 1 is referred to as the initial velocity, $v_{in}$. In the tapered zone, the decrease in the diameter of the channel led to the increase in the microgel velocity (Region 2). At the entrance of the constriction the microgels stalled (Region 3). As the pressure built-up, the microgel entered into the constriction (Region 4). In the constriction at
~125 μm from its entrance, the microgel velocity increased to approximately a constant value. Upon traversing the 450-μm long constriction, the microgel exited the constriction and entered the downstream channel-at-large with the mean diameter of 150 μm (Region 5). In this zone, the microgel had the largest velocity that was several times larger than the initial velocity in Region 1. This increase was the combination of the pressure build-up behind the microgel when it was flowing inside the constriction in Region 4 and the absence of the static and kinetic friction experienced when the microgel conformably plugged into the constriction and traversed with its surface area in contact with the wall of the microchannel. Region 6 starts ~1000 μm after the exit of the constriction and it is marked by decrease of the microgel velocity to the value close to the initial velocity. Figure 4 (b) shows snapshots of a microgel flowing in each of the 6 regions.

In the control experiment, we introduced microgels in the microchannel in the reversed direction, that is, from Region 6 to Region 1. The “backwards” velocity profile shown in blue squares in Figure 4 (a) is in close agreement to the “forwards” profile (red circles), indicating that the modification of the microchannel yield a geometry that was symmetric with respect to the vertical axis in the middle of the microchannel, producing no geometric obstructions to the flow of microgels in the constriction.
Figure 4. (a) Representative velocity profiles of the microgels traveling through the microchannel in a forward (from Region 1 to Region 6, red circles) and backward (from Region 6 to Region 1, blue squares) manner. The initial velocity of the microgel in the channel-at-large was 1200 µm s⁻¹. The vertical dashed lines (A1 – A7) represent lines dividing each region. (b) Optical microscopy images of a microgel flowing from Region 1 to Region 6. The channel entrance angle is 13 °. The scale bar is 150 µm.
3.3 Effect of initial velocity of microgels on their flow in constriction

The effect of initial velocity of microgels on their flow was studied by using the microchannel with the entrance angle of 13° and microchannels with the mean diameter of 100 μm. The initial velocities were $1200 \pm 100 \mu m/s$, $1800 \pm 100 \mu m/s$ and $2300 \pm 100 \mu m/s$. Figure 5 (a) and (b) shows the velocity profiles of the microgels supplied to the channel-at-large at different initial velocities.

In Region 2, microgels introduced at $2300 \pm 100 \mu m/s$ (green squares in Figure 5) showed the largest velocity increase before reaching the constriction entrance in Region 3. At the entrance of the constriction, the microgels with the initial velocity of $2300 \mu m/s$ had a finite, non-zero velocity at the constriction entrance, in contrast with microgels moving in the channel-at-large with the velocities of $1200 \pm 100 \mu m/s$ and $1800 \pm 100 \mu m/s$. We observed that as initial velocity of the microgels increased, the time for which the microgel was in Region 3 deforming itself into the constriction (residence time) decreased, from $1.64 \pm 0.77$ s, $1.09 \pm 0.40$ s, and $0.76 \pm 0.30$ s for initial microgel velocity values of $1200 \pm 100 \mu m/s$, $1800 \pm 100 \mu m/s$, and $2300 \pm 100 \mu m/s$, respectively. Our rationale was that the greater the microgel velocity, the greater the backpressure is when the microgel blocks the constriction entrance, decreasing the residence time of the microgel. Measurement of pressure to study the effect of initial velocity of microgels is currently under investigation. In Region 4, the stabilized velocity of the microgel was the highest for the microgel with the highest initial velocity. In Region 5, these microgels exited the constriction with the greatest velocity, which started decaying later than microgels with lower initial velocities.
Figure 5. (a) Velocity profiles of 100-µm microgels flowed through the microchannel with the entrance angle of 13° at different velocities. Blue triangles: 1200 ± 100 µm/s, red circles: 1800 ± 100 µm/s, and green squares: 2300 ± 100 µm/s. (b) Velocity profiles showing Regions 1 to 4.
To analyze the change in velocity of the microgels flowing at different initial velocities, we plotted the variation in normalized velocities against the length of the microchannel (Figure 6). The normalized velocity profiles of the microgels followed similar trends in Regions 1-3, as in Figure 5. In the constriction (Region 4), however, the microgels with a higher initial velocity moved through at the lowest normalized velocity, due to the high viscous friction they experience inside the constriction. When velocity increases the friction coefficient inside the constriction becomes higher and the microgels with the highest initial velocity traverse the constriction slower. The normalized velocity profile also showed that in Region 5, microgels with the highest velocity experienced the smallest increase in their velocity. Compared to the initial velocities, the highest velocities observed in Region 5 were 11 x, 5.7 x, and 2 x as high for the initial velocity values of 1200 ± 100 µm/s, 1800 ± 100 µm/s, and 2300 ± 100 µm/s, respectively. We speculate that the highest increase in the exit velocity for the microgels flowing at 1200 ± 100 µm/s occurred due to the accumulation of backpressure in the constriction. Their longer residence time led to the greater accumulation of pressure and their greater increase in velocity upon exiting the constriction.
Figure 6. (a) Normalized velocity profiles of microgels introduced in the channel-at-large with various initial velocities (blue triangles: 1200 ± 100 μm/s, red circles: 1800 ± 100 μm/s, green squares: 2300 ± 100 μm/s). (b) Close-up of the profiles in Regions 2 to 5 of the microchannel. The mean diameter of microgels is 100 μm and the entrance angle of the microchannel is 13°.
Measurement of pressure during the flow of microgels at various initial velocities is currently under investigation.

3.4 Effect of the entrance angle on microgel flow

To investigate the effect of varying the entrance angle on the flow of microgels, a dispersion of microgels with the mean diameter of 100 µm was introduced into microchannels with the entrance angle of 13°, 22°, or 35°. The initial velocity of microgels was 1200 ± 100 µm/s. Figure 7 (a) shows the velocity profiles of the microgels in the channels with different entrance angles. Figure 7 (b) shows the close-up of the velocity profiles in Regions 1 to 4.

The velocity increase in Region 2 occurred at different positions inside the microchannel; the maximum upstream velocity was measured at the position of -340 µm and -400 µm for the 35°- and 13°-channels, respectively (Figure 7 (b)). The rate of increase was abrupt for the 35° tapering, but small for the 13°-channel that tapered into the constriction more gradually. Microgels introduced through a 13°-channel started to deform earlier, due to the gradual tapering, which aided in their easier entrance in the constriction as well. The residence time or the time for which microgels were deforming themselves into the constriction was 1.09 ± 0.40, 2.42 ± 0.43, and 35.2 ± 18.6 s for the entrance angles of 13°, 22°, and 35°, respectively. The stall time of microgels at the orifice entrance was the shortest for the microchannel with the entrance angle of 13°. As the wall angle decreased, the magnitude of the contact force between the channel wall and the microgels increased and acted to deform the microgels before approaching the entrance of the constriction. We note that neutrophils exhibit a similar behaviours when they
traverse constriction with different entrance angles.\textsuperscript{56} It was also noticed that the larger the entrance angle, the smaller the microgel velocity in the constriction (Region 4) (Figure 7 (a)). Microgels experienced a shorter residence time in low-angle microchannels, which led to their higher normalized velocity.

The increase in velocity after the microgels exited the constriction was also affected by the degree of tapering. The microgels entering microchannels with the entrance angle of 13°, 22°, and 35° experienced the increase in normalized velocities to 6, 7.5, and 9, respectively. The buildup of backpressure and the residence time was the highest for the microgels in the 35°-channel, and the microgels exited the constriction with the highest velocity.
Figure 7. (a) Optical fluorescence microscopy images of the microchannels with entrance and exit angles of 13˚, 22˚, and 35˚. The channel is filled with an aqueous solution of 10-μm fluorescent silica beads. (b) Normalized velocity profiles of microgels traveling through an orifice with entrance angles (θ) of 35˚ (green curve), 22˚ (blue curve), and 13˚ (red curve). The scale bars are 150 μm. (c) Zoomed in velocity profiles of microgels before they reach the center of the constriction. The dispersion of agarose microgels with a mean diameter of 100 μm were introduced into the microchannel at an average initial velocity of 1200 μm.

3.5 Effect of confinement

To study the effect of confinement on the velocity profile of microgels, microgels with different diameters were introduced into the microchannel. The microgels were divided into 4 size groups: Group 1: 90 – 99 μm, Group 2: 110 – 119 μm, and Group 3: 120 – 129 μm. The flow rate of the syringe pump was fixed to yield the microgel velocity of 1200 ± 100 μm. The mean diameter of
microgels was 100 µm. The microchannel had the entrance angle of 13°. Approximately 20 microgels in each size group were analyzed to produce the velocity profile and the pressure profile.

Figure 8 shows the normalized velocity profile of the microgels in the four size groups. In Region 1, microgels experienced a slight difference in their initial velocity, but in the range of error. In Region 2, microgels with higher diameters started lowering their velocity earlier. The larger microgels were in contact with the tapering wall of the microchannel earlier than the smaller microgels and they stalled before reaching the entrance of the constriction. The stall time for the microgels were 1.21 ± 0.49 s, 1.91 ± 0.43 s and 2.30 ± 0.25 s for microgels of groups 1, 2, and 3, respectively. The exit velocity in Region 5 was the highest for microgels in Group 3. This is due to the backpressure they gained while they were in Region 3, slowly deforming their shapes to enter into Region 4. The onset of the relaxation of the exit velocity to values close to the initial velocities for microgels with different dimensions is currently under investigation.
Figure 8. Superimposed velocity profiles of all agarose microgels of different diameters (Group 1: 90 – 99 μm, red; group 2: 110 – 119 μm, green; and group 3: 120 – 129 μm, blue) flowing through a microchannel with constriction. (a) full flow profile. (b) A zoomed-in image of the entrance into the constriction. D stands for the distance from the centre of the constriction. The entrance angle of the microchannel is 13°.
Pressure measurements were performed for flow experiments of microgels with different sizes. Figure 9 shows the pressure profiles of microgels in Groups 1 (red circles) and 3 (blue triangles), as well as those with the diameter ranges of 80 – 89 µm (yellow squares), and 130 – 139 µm (purple diamonds). The notable trend is the large increase in pressure at the end of Region 4. As microgels started deforming their shapes to flow through the constriction, an increase in pressure was observed. The pressure rose continuously until the microgels came to the end of the constriction. As they exited, the pressure started decreasing and eventually decreased to the initial values after the microgels flowed through Region 6. The highest pressure values for microgels in Group 1 and 3 were 0.042 psi and 0.062 psi, respectively. The pressure profiles confirmed that larger microgels experienced larger pressure buildup. This feature coincided with the high exit velocity of the large microgels in Region 5.

Figure 9. Superimposed pressure profiles of all agarose microgels of different diameters (80 – 89 µm, yellow squares; Group 1: 90 – 99 µm, red circles; Group 3: 120 – 129 µm, blue triangles; and 130 – 139 µm, purple diamonds) flowing through a microchannel with the entrance angle of 13°.
3.6 Conclusion

We performed microfluidic flow experiments to study the passive mechanisms of the flow of neutrophils in blood capillaries with constriction. Agarose microgels were synthesized using a T-junction device and were used as model neutrophils. Microchannels with constriction were modified to have circular cross-sections to better replicate the geometry of capillaries. Dispersions of microgels were flowed into the microchannels at flow rates that are close to physiological conditions. Velocity profiles were constructed to study the flow behaviour of microgels at different locations inside the microchannel. The flows were studied as functions of the (a) initial velocity of microgels, (b) angle at which the channel-at-large tapers into the constriction, and (c) size of microgels. We found that the three variables affected the flow of microgels through constriction, as observed by the static time of microgels at the entrance of the constriction, velocity at different regions inside the channel, and the pressure observed as microgels flowed through. Instead of using complex biological systems to study the cell flow, our technique of using model cells and model capillaries made it possible to study the flow of cells by controlling one variable at a time.

Future studies will involve extensive study of pressure as functions of the variables stated above. Friction forces microgels experience as they flow through constriction will be investigated as well. The role and impact of active mechanisms on the flow of neutrophils will also be studied by flowing real neutrophils into our microchannels and comparing their behaviour with our model system.
Chapter 4
Introduction to Step-Growth Polymerization of Au Nanorods with Different Lengths

4.1 Introduction

The focus of nanoscience is shifting from syntheses of nanomaterials with different compositions and shapes to hierarchical organization of individual nanoparticles (NP) into larger nanostructures. Ensembles of NPs show new optical, electronic and magnetic properties that are distinct from those of isolated NPs. Self-assembly of NPs has been recognized as an efficient strategy for producing nanostructures with complex, hierarchical architectures. The past decade has witnessed great progress in NP self-assembly, yet the quantitative prediction of the architecture of NP ensembles and of the kinetics of their formation remains a challenge. It has become increasingly more important to attain comprehensive understanding of mechanisms leading to the formation of NP ensembles and the ways to control their structural characteristics.

Recently, our group reported on the marked similarity between the self-assembly of metal NPs and reaction-controlled step-growth polymerization. The NPs act as multifunctional monomer units, which form reversible, noncovalent bonds at specific bond angles and organize themselves into a colloidal polymer due to the hydrophobic interactions between polystyrene molecules tethered to the ends of the nanorods (NRs). The theories of kinetics and statistics for step-growth polymerization enable a quantitative prediction of the architecture of linear, branched, and cyclic self-assembled nanostructures as well as their aggregation numbers, size distribution, and the formation of structural isomers.
4.2 Organization of individual nanoparticles into ensembles

Inorganic NPs have a range of shape- and size-dependent properties, such as absorption, photoluminescence, surface-enhanced Raman cross-sections and conductivity,\(^5\) which differ dramatically from those of their bulk materials, and atoms or molecules. The properties of NP ensembles can be altered due to strong interactions between their excitons, magnetic moments or surface plasmons.\(^5\) Much interest in creating higher-order structures has risen to exploit their interesting collective properties and the possibility of using these properties in functional devices.\(^5\)

The potential applications of the collective properties of discrete nanostructures are in optical and electronic devices,\(^6-7\) sensing and imaging,\(^68,69\) biodiagnostics,\(^70\) and drug and gene delivery.\(^71\) Self-assembled structures of NPs have been used as bio- and chemical sensors by monitoring the changes in their optical properties. For example, the surface plasmon coupling between Au NPs was utilized in a colourimetric sensor for the detection of oligonucleotides. Colour change occurred when DNA-conjugated NPs underwent self-assembly mediated by hybridization of DNA with target oligonucleotides. Detection of micromolar concentrations of cysteine or glutathione was achieved using the self-assembly of Au NRs end-terminated with the amino acid.\(^72\) At the pH of the Au NR solution \(\sim 5.6\) (the isoelectric point of cysteine is 5.02) the cysteine was present in its zwitterionic form in solution. The zwitterionic groups at the ends of the NRs led to a two-point electrostatic interaction in a cooperative fashion. Effective sensing of cysteine/glutathione was possible since the coupling of plasmon absorption of the Au NRs led to
red-shift of the longitudinal surface plasmon wavelength (LSPW) of NRs and other amino acids, such as tyrosine and leucine. Au NRs functionalized with tyrosine and leucine did not have zwitterionic functional groups at the pH of ~5.6, and could not lead to the end-to-end self-assembly of the Au NRs.  

4.3 Self-assembly of inorganic nanoparticles

Self-assembly is a simple and cost-efficient approach for producing arrays of NPs in a controllable and predictable fashion and controlling their collective properties and applications. It is a process by which NPs or other discrete components spontaneously organize themselves into higher-order structures directly through specific interactions or indirectly through their environment. Direct interactions include interparticle forces, such as electrostatic forces, hydrogen bonding and biospecific forces, and indirect interactions can be created by using a self-assembly template or an external field.

In particular, self-assembly of one-dimensional (1D) NPs shows potential applications in optoelectronics and sensing. Several research groups reported on the assembly of colloidal particles in 1D polymer-like structure in solution, at interfaces, and by external fields.

4.3.1 Nanorod self-assembly mediated by biological molecules

Biorecognition between bioorganic ligands attached to NR surfaces is a heavily researched area of self-assembly. Au NR chains with the aggregation number of ~20 have been assembled using
specific forces acting between biological linkers such as biotin and streptavidin,\textsuperscript{75,76} oligonucleotides,\textsuperscript{77,78} oligonucleotides-metal ions,\textsuperscript{79} avidin and biotin, polypeptides,\textsuperscript{80} antibody-antigen,\textsuperscript{81} and aptamer-protein.\textsuperscript{82} This is a promising strategy not only because it provides a high binding specificity between the NRs, but also because the resulting structures have potential applications in sensing applications and in medical diagnostics. Currently, the use of biological ligands is dominated by the use of SH-terminated molecules, since they form the strongest bond with the Au surface \textit{via} Au-S linkage.\textsuperscript{83} For example, Pan et al.\textsuperscript{84} exploited linear 1D assembly of Au NRs with mercaptoalkyloligonucleotide at the ends of NRs. The NRs carrying thiolated oligonucleotides at long sides formed 3D lattices in which the NRs were organized in a side-by-side manner. The assembly in the lattices was governed by DNA hybridization between the complementary strands.

4.3.2 Nanorod self-assembly mediated by chemical reactions

In chemically mediated self-assembly, regioselective ligands on NRs form covalent bonds. For example, \(\alpha,\omega\)-alkanedithiol attached to the ends of two neighboring Au NRs formed Au-S bonds and linked the NRs in the end-to-end fashion.\textsuperscript{83} The work showed that the rate of self-assembly increased with a reducing the length of carbon chains in the linkers.\textsuperscript{83} The large activation energy of NR dimerization suggested that the assembly process was reaction-controlled. Mokari et al. synthesized cadmium selenide (CdSe) semiconductor quantum rods end-terminated with Au NPs and their strong coupling was observed.\textsuperscript{85}
4.3.3 Nanorod self-assembly mediated by physical forces

Au NRs end-terminated with cysteine or glutathione underwent self-assembly triggered by electrostatic attraction.\textsuperscript{72} The thiol groups of cysteine or glutathione bound to the ends of Au NRs and electrostatic attraction between the $-\text{NH}_3^+$ and $-\text{COO}^-$ groups of cysteine or glutathione led to the association of NRs in the end-to-end fashion.\textsuperscript{72} End-termination of Au NRs by 11-mercaptoundecanoic acid or 3-mercaptophosphonic acid can also reveal end-to-end assembly through hydrogen bonding.\textsuperscript{86} Nie et al.\textsuperscript{87,88} and Liu et al.\textsuperscript{63} achieved self-assembly of Au NRs end-terminated with thiolated polystyrene (PS) (Figure 10). The NRs were coated with cetyl trimethylammonium bromide (CTAB) along their long faces. The NRs were dissolved in dimethylformamide (DMF), which was a good solvent for both PS and CTAB. The solubility of PS was reduced with addition of water. The reduced solubility of PS led to unfavourable interactions of PS with the water-DMF mixture, and triggered the end-to-end assembly of the NRs.

![Figure 10](image_url)

**Figure 10.** The monomer building block: is an amphiphilic Au NR carrying a double layer of CTAB along the longitudinal side and polystyrene molecules grafted to both ends. In DMF/water the individual NRs undergo self-assembly to form NR chains.

4.4 Resemblance of step-growth polymerization and self-assembly of inorganic nanoparticles

Prediction of the topology of nanostructures and the kinetics of their growth is important for achieving effective scale-up of NPs into chains and exploit NP self-assembly in technological
Currently, the lack of models describing the kinetics and statistics of the self-assembly of 1D arrays does not allow the quantitative prediction of their structural features, such as the length of NP chains, the degree of branching, or the coexistence of rings, linear chains, and branched structures. No quantitative approach has been successfully applied to the prediction of chain topology and the kinetics of chain growth. Recently, attempts have been made to quantify the kinetics and elucidate the mechanism of aggregation and crystal growth by oriented attachment (OA). In OA, NPs with common crystallographic orientations combine to form larger anisotropic structures. The formation of nanowires of SnO$_2$ and TiO$_2$ NPs was quantified by making an analogy between OA and reaction-controlled step-growth polymerization. Chains were formed from the hydrothermally treated NPs, which were viewed as monomers with two active sites. The analysis was erroneous in that the reaction rate constant $k$ of each addition step had to be corrected to account for the size of growing chains, while it had to be independent of the length of chains during the entire process of OA, based on Flory’s assumption. The size distribution (or the aggregation number) of the growing chains was compared to Flory’s most probable distribution, however, mistakenly by using weight-distribution function, instead of the number-distribution function.

Recently, Liu et al proposed that the kinetics of self-assembly of multifunctional Au NRs, in fact, resembles that of the step-growth polymerization. The authors hypothesized that NPs act as multifunctional monomer units and organize themselves into macromolecule-like assemblies. Self-assembly or polymerization between Au NRs terminated with PS occurred due to the formation of reversible, noncovalent bonds at specific bond angles, and it yielded a colloidal polymer. The growth of NR chains was described by the kinetics and statistics of step-growth
polymerization. As a result, this work allowed the prediction of aggregation number of the NR chains and the size distribution of NR ensembles at a particular time.

4.4.1 Step-growth polymerization

Step-growth polymerization of monomer molecules by the stepwise reaction between the functional groups of multifunctional reactants. The process proceeds from a monomer to a dimer, trimer, tetramer, pentamer, etc. The evolution and distribution of chain lengths are characterized by monitoring, as a function of time, the changes in their number-average degree of polymerization (\( \bar{X}_n \)), weight-average degree of polymerization (\( \bar{X}_w \)), and polydispersity index PDI

\[
\bar{X}_n = \frac{\sum n_x x}{\sum n_x} \quad \bar{X}_w = \frac{\sum n_x x^2}{\sum n_x x} \quad PDI = \frac{\bar{X}_w}{\bar{X}_n} \quad (1)
\]

where \( x \) is the number of NRs in the chain and \( n_x \) is the number of chains containing \( x \) number of NRs. The extent of reaction \( p \) (equation 2) also describes the conversion of individual NRs into NR chains.

\[
p = 1 - \frac{[M]}{[M]_0} \quad (2)
\]

High molecular-weight polymer chains are obtained only near >98% of \( p \) or conversion. The degree of polymerization of polymer chains increases at a relatively slow pace. The reaction scheme is shown in Scheme 1.
Scheme 1: Progression of step-growth polymerization

- Monomer + monomer $\rightarrow$ dimer
- Dimer + monomer $\rightarrow$ trimer
- Dimer + dimer $\rightarrow$ tetramer
- Trimer + monomer $\rightarrow$ tetramer
- Trimer + dimer $\rightarrow$ pentamer
- Trimer + trimer $\rightarrow$ hexamer
- etc.

\[ n\text{-mer} + m\text{-mer} \rightarrow (n+m)\text{-mer} \]

The analysis of polymerization kinetics becomes complicated when the number of reaction becomes infinite. To simplify the analysis, a number of assumptions have been made: (1) the reactivities of the two functional groups of a bifunctional monomer are the same; (2) the reactivity of one functional group of a bifunctional reactant does not depend on whether the other functional group has reacted; (3) and the size of the growing polymer chain does not affect the reactivity of a functional group.\(^9^3\)

Step-growth polymerization occurs between bifunctional and/or polyfunctional monomers. The prime principles behind the kinetics of step-growth polymerization is the equal reactivity
assumption proposed by Flory.\textsuperscript{93} The concept of equal reactivity of functional groups makes the kinetics of step-growth polymerization identical to those of the analogous small molecule reaction, e.g. the reaction of esterification (Scheme 2). Table 1 shows rate constants for esterification in homologous compounds. There is a decrease in reactivity of the acid and alcohol functional groups of the two reactants with increased molecular weight, but the effect becomes significant only for $x < 3$ where $x$ is the degree of polymerization.

\begin{center}
\textbf{Scheme 2: Esterification of a series of homologous carboxylic acids}
\end{center}
\[ H(CH_2)_xCO_2H + C_2H_5OH \xrightarrow{HCl} H(CH_2)_xCO_2C_2H_5 + H_2O \]

\begin{center}
\textbf{Table 1: Rate constants for esterification in homologous compounds (temperature = 25 °C)}\textsuperscript{93}
\end{center}

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<th>Degree of Polymerization (x)</th>
<th>$k \times 10^4$ for H(CH$_2$)$_x$CO$_2$H</th>
<th>$k \times 10^4$ for (CH$_2$)$_x$(CO$_2$H)$_2$</th>
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4.4.2 Kinetics of step-growth polymerization of inorganic nanoparticles

Previously our group reported that the self-assembly of Au NRs follows the kinetics of step-growth polymerization.⁶³ The relationship between $\bar{X}_n$ and self-assembly time was linear and the reaction rate was proportional to the initial concentration of NRs. These featured characteristics are of reaction-controlled step-growth polymerization, in which the reactivity of functional groups of the individual NRs is independent of the chain length [the Flory’s assumption⁹²]. The theories of kinetics and statistics for step-growth polymerization enable a quantitative prediction of their aggregation numbers, size distribution, and the formation of structural isomers. However, the reactivity of different types of monomers in this model is not predicted.

We propose that as long as they have the same functional groups, the factors affecting the reactivity of PS-tethered NRs are the length of an individual NR, the surface charge of the NR, and the relative volume of PS ligands. Studies of these factors are yet to be conducted. They are necessary to attain deeper understanding of the kinetics of step-growth polymerization of inorganic NPs. The length of a NR (the monomer) can affect the reactivity of the SH-PS functional group, because as the monomer becomes longer, its excluded volume increases. The excluded volume refers to the volume a part of a polymer molecule cannot occupy, due to the presence of another part of the polymer molecule already occupying the space.⁹⁴ Excluded volume is proportional to the square of the length of the molecule (in our system, it was the length of the NR). The surface charge density can also affect the rate of self-assembly. Electrostatic repulsion originates from interactions between CTAB-coated sides of NRs. The surface charge factor is also related to the length of a NR in that for the same charge density, longer NRs have a higher positive charge, leading to stronger repulsion between the side facets.
of NRs. Finally, the reactive volume $\phi_r$ defined as $\phi_r = V_{pol}/(V_{NR}+V_{pol})$, where $V_{pol}$ and $V_{NR}$ are the volumes of the SH-PS ligand on the ends of the NRs and the volume of individual NRs, respectively, is another factor affecting the rate of self-assembly. Changing the molecular weight of SH-PS on the ends of the NRs or $V_{pol}$ is expected to affect the probability of the NRs to self-assemble in an end-to-end fashion. Along with the surface charge aspect, varying the $V_{NR}$ can also be accomplished by varying the length of the NR.

Understanding how each of the factors (the surface charge, the reactive volume, and the excluded volume of the NR) affects the reactivity of the SH-PS functional group is crucial for future development of the self-assembly technique. Quantitative and systematic analyses of the factors can be applied to the prediction of chain topology and the kinetics of chain growth. In our work we focused on studies of the effect of the length of NR on the reactivity of the functional group (PS ligand). To keep the length of the NR as the only variable, the diameter of the NRs, molecular weight of SH-PS, and surface charge density of NRs were maintained constant. This investigation will provide insight for future studies involving (a) self-assembly of Au NRs in different length ranges, (b) self-assembly of NRs of different metals, and even (c) co-assembly of Au NRs with different lengths and/or other metals.
Chapter 5
Materials and Methods for Self-Assembly of Au Nanorods

5.1 Materials
Hexadecyltrimethylammonium bromide (99%) and benzyldimethylammonium chloride hydrate, gold (III) chloride trihydrate (HAuCl₄·3H₂O), sodium borohydride (98%), L-ascorbic acid (99%), and silver nitrate (99%) were purchased from Sigma-Aldrich. Thiol terminated polystyrene ($M_n = 12000, M_w/M_n = 1.09$) was from Polymer Source Inc. Deionized water (18.2 MΩ) was used in all the experiments. Imaging and characterization of Au NRs and their self-assembly were achieved by obtaining UV spectra and transmission electron microscopy (TEM) images. UV absorbance of etching and self-assembly was measured by a Varian Cary 5000 UV-VIS-NIR Spectrophotometer. The TEM images of Au NRs were recorded by Hitachi HD-2000 and H-7000 transmission electron microscopes. Dimensions of NRs were determined by measuring ~100 NRs per sample by Image J 1.43u (National Institutes of Health, USA).

5.2 Experimental procedure
The procedure used for the synthesis and self-assembly of NRs included the following steps: the synthesis of Au NRs, fractionation of the NRs by depletion flocculation, shortening of the NRs by selective etching, surface modification by exchanging the benzylidimethylhexadecylammoniumchloride (BDAC) ligand with CTAB coated NRs with CTAB, the attachment of thiolated PS to the ends of NRs, and the self-assembly of NRs.
5.3 Synthesis of Au nanorods

Au NRs with the mean length and width of 99 and 13 nm, respectively (aspect ratio of ~7) were synthesized by following the seed-mediated growth method developed by Nikoobakht and El-Sayed. The NRs were stabilized with a BDAC/CTAB binary surfactant system as a stabilizer. The synthesis was scaled up to obtain a 100 mL dispersion of the NRs. Seed Au NPs were prepared by mixing chloroauric acid (HAuCl₄) (0.12 mL, 15mM) with an aqueous solution of CTAB (2.5 mL, 0.20M), 1.0 mL of deionized water, and sodium borohydride (NaBH₄) (0.50 mL, 10mM) in ice-cold water. For the preparation of a growth solution, CTAB (2.0 g, 0.20 M) and BDAC (2.97 g, 0.25 M) were dissolved in 90 mL of DI water at 60 °C. After cooling down to room temperature, the solution of CTAB/BDAC was mixed with aqueous solutions of silver nitrate (AgNO₃) (5.0 mL, 4.0 mM) and HAuCl₄ (5.0 mL, 15mM). Following the addition of ascorbic acid (1.24 mL 0.788M), the dark-yellow solution turned colorless. Finally, 0.10 mL of a 60-min-aged seed solution was added to the growth solution and the mixture was incubated unstirred at 27.0 °C for over 20-24 hours.

5.4 Depletion-induced separation of Au nanorods

Synthesis of long Au NRs accompanies the presence of unwanted NPs with the diameter of ~20 nm. To separate these NPs from NRs, the solution of as-synthesized Au NRs with the NP concentration of 0.1 nM was warmed up to 60 °C and mixed with 1.65 g of CTAB and 2 g of BDAC to increase of the concentration of CTAB from 0.055 to 0.100 M and BDAC from 0.075 to 0.125 M, respectively. The total surfactant concentration equaled to the micelle molar concentration of ~3.6 mM, which is $10^7$ times higher than the concentration of the long NRs.
Upon dissolution of the surfactants in the NR solution, it was slowly cooled down to ~ 20 °C. Under cooling, the mixture became opaque due to the flocculation of NRs. Sedimentation of long NRs occurred at the bottom of the solution flask after 24 h. The supernatant consisting of NPs and short NRs was carefully removed from the flask using a glass pipette. Deionized water was added to the sediment to redisperse the NRs by lowering the surfactant concentration.

5.5 Etching of Au nanorods

The shortening of Au NRs was carried out by adding a solution of H$_2$O$_2$ (1M) into the Au NR solution with a volume ratio of H$_2$O$_2$ to the NR solution of 1:10. Etching was quenched by 3-fold dilution of the NR mixture with DI water. This step was followed by two rounds of centrifugation at 14000 rpm for 10 min and 10000 rpm for 20 min (Eppendorf Centrifuge 5430R). Shorter Au NRs were obtained at increased etching time. The resulting precipitates were redispersed in a large amount of DI water. Five different lengths of Au NRs were attained by time-dependent etching; the NRs had the average lengths of 95, 80, 65, 50, and 35 nm.

5.6 CTAB exchange

The long sides of etched Au NRs were coated with the CTAB/BDAC surfactant mixture. This mixture was replaced with CTAB. The etched NRs were centrifuged at 10000 rpm for 20 min and the supernatant was removed. The precipitated NRs were redispersed in AgNO$_3$ (50 µl, 4.0 mM), CTAB (1 mL, 0.1 M), and L-ascorbic acid (12.4 µl, 0.788 M) and incubated for more than 30 min. The procedure was repeated twice to ensure the exchange of BDAC with CTAB. The
resulting NRs were centrifuged at 10000 rpm for 20 min twice to remove excess CTAB from the system.

5.7 Attachment of polystyrene to both ends of nanorods

The solutions of NRs with various lengths were centrifuged twice at 10000 rpm for 20 min to remove the supernatant. The precipitated NRs were redispersed in DI water (30 µl). A tetrahydrofuran (THF) solution of SH-PS was prepared at the concentration of SH-PS 0.2 mg/mL. The NRs were rapidly injected into 3 mL of the SH-PS solution and sonicated for 30 min. After sonication, the solution of NRs was incubated for 24 h at room temperature. The excess SH-PS in the THF solution was removed by 8 – 9 times of centrifugation at 8500 rpm for 25 – 30 minutes.

5.8 Self-assembly of Au nanorods

The THF in the NR solutions was evaporated using air flow. The precipitated NRs were redispersed in 1.5 g of N-dimethylformamide (DMF). The NR concentrations were calculated with their LSPW absorbance values and their corresponding molar extinction coefficients. It was ensured that all the NR solutions had the same initial NR concentration, [NR]₀. The self-assembly process was triggered by slow, drop-wise addition of 1.5 g of water-DMF mixture with the water concentration of C_w = 30 wt. % to the NR solution in DMF. The final concentration of water in the reaction mixture was 15 wt. %. The time at which the last drop of water-DMF mixture was added was considered as the start time of the self-assembly (t = 0). The self-assembly process was monitored using their absorbance spectra as a function of time. In parallel,
samples for transmission electron microscopy (TEM) were prepared by applying a drop of the NR self-assembly solution onto a carbon-coated TEM grid, leaving it for 10 sec and quickly drying them with KimWipes.

5.9 Data collection and analysis

The self-assembly was characterized by the number average degree of polymerization ($\bar{X}_n$), weight average degree of polymerization ($\bar{X}_w$), polydispersity index (PDI), and the number fractions of linear x-mer chains for each sample. We examined a few randomly selected locations on the TEM grids. At these locations, the number of all chains and individual NRs were included in the analysis. The total number of NRs counted for each sample was ~1500.
Chapter 6
Step-Growth Polymerization of Au Nanorods with Different Lengths

6.1 Synthesis of high aspect-ratio Au nanorods

To synthesize longer NRs (with the aspect ratio in the range of 6 to 10), a binary-surfactant system consisting of benzyldimethylhexadecylammonium chloride (BDAC) and CTAB was used. The experimental details are given in Section 5.3. Figure 11 shows a typical TEM image of as-synthesized long NRs with the mean diameter and length of 13 ± 1 and 99 ± 14 nm, respectively. Undesired by-products in the form of short NRs and nanospheres (4 – 20 nm) were also observed at a concentration of ~10 %, which was unacceptable for the study of self-assembly of NRs.

Figure 11. Dark field TEM image of as-synthesized long NRs (diameter = 13 nm, and length = 99 nm) with nanosphere and nanocube byproducts. The scale bar is 200 nm.
6.2 Depletion-induced separation of long Au nanorods from impurities

Separation of unwanted byproducts (short NRs and spherical NPs) was crucial for the study of kinetics and statistics of NR self-assembly. Although several separation methods, such as capillary electrophoresis, column chromatography, and centrifugation, have been explored to separate the NPs with different size and shapes, we found that depletion-induced separation approach is the most efficient and facile way in our case. In our work, separation of long NRs from short NRs and nanospheres was accomplished through the formation of reversible flocculates of NRs by increasing the concentration of BDAC/CTAB surfactants in the NR solution.

Depletion interactions between NRs occurred when the separation distance between the NRs was on the order of the size of BDAC and CTAB micelles. The exclusion of the surfactant micelle from the space between the NRs resulted in a region of pure solvent between the NRs. An osmotic pressure then developed due to the concentration gradient and pushed the NRs toward each other through the depletion force. The potential energy rising from the depletion of micelles between two NRs at contact depended on the interparticle contact area of Au NRs. Therefore, NPs with the same mass, but different shapes could be separated effectively.

An effective attraction between NRs originated from the addition of BDAC/CTAB above their critical surfactant micelle molar concentration CMC (CMC_{BDAC} = 0.0005 M) and CTAB (CMC_{CTAB} = 0.001 M). At CMC the number of surfactant micelles was sufficient to induce an
attractive forces between the Au NRs and cause their flocculation. To induce depletion interaction, BDAC and CTAB were added to the NR solution at the surfactant concentration of 0.225 M (0.125 M of BDAC and 0.1 M of CTAB). This concentration was equivalent to the micelle concentration of ~3.6 mM, which was $10^7$ times greater than the concentration of NRs.

The short NRs and NPs remained in the solution. The flocculated NRs were separated and redispersed when the surfactant micelle concentration was decreased with the addition of deionized water. Figure 12 shows a TEM image of the long NRs after their fractionation using depletion forces. The NP impurities were removed very effectively and NRs with high monodispersity remained.

![TEM image of long NRs](image)

Figure 12. A TEM image of long NRs after depletion-induced separation from nanosphere and nanocage impurities. The scale bar is 100 nm.
6.3 Preparation of nanorods with different lengths by selective shortening of long Au nanorods

Synthesis of Au NRs of different aspect ratios has been achieved by tuning the concentration ratios of BDAC, CTAB, and AgNO$_3$. However, the resulting NRs differ in both their diameters and lengths simultaneously. In our work, to keep the length as the only variable, it was important to maintain the diameter of the NRs and the volume of SH-PS tethered to the ends of NRs. Using different recipes to synthesize NRs with different lengths and fractionating the desired NRs using the depletion forces could be very inefficient and time-consuming. Instead, we prepared Au NRs with different lengths but the same diameter by synthesizing long NRs and subsequently reducing their lengths. A few methods of selectively etching the ends of NRs have been reported. For example, Tsung et al. explored the oxidation of Au NR tips by dissolving oxygen in the aqueous solution of NRs with concentrated acid. Bubbling oxygen in the presence of CTAB surfactant was the major technical difficulty of the reaction since CTAB generated an immense amount of foam when agitated. Also, the system was under thermal treatment which could potentially lead to thermally induced reshaping of the NRs. Sreeprasad et al. reported a selective etching method using oxygen, Cu$^{2+}$, and ascorbic acid. Their process also took place at high temperatures. Zou et al. reported a room-temperature etching method of the NR tips in the presence of fine concentrations of FeCl$_3$. The halide ions were used to reduce the electron potential of the Au species, which enabled Fe$^{3+}$ ions to selectively etch the Au NR ends with less CTAB passivating the surface. The method did not require increase in temperature, however, it fell short due to the challenge in removing Fe$^{3+}$ ions and complexing Cl$^-$ ions in order to stop the etching.
In our work, we selected the method reported by Ni et al. Tailoring the lengths of Au NRs was accomplished by using H$_2$O$_2$ to selectively etch the ends of NRs. H$_2$O$_2$ was used as the oxidizing agent because it is relatively easy to handle and heating is not required. We monitored the reaction by following the blue-shift of longitudinal surface plasmon wavelength (LSPW) of the NRs using UV-vis spectroscopy. We observed a slow change in LSPW immediately after the addition of H$_2$O$_2$ because the ends of the NRs were coated with BDAC/CTAB surfactants initially. After ~20 nm of blue-shift in LSPW the etching rate became linear as a function of the oxidation time. We controlled the rate of NR etching by varying the concentration of H$_2$O$_2$ added. When the desired LSPW corresponding to a particular NR length was reached, the reaction was stopped by dilution of the NR solution with H$_2$O and centrifugation. In our system, the as-synthesized NRs with the length of 99 nm were etched by this method to produce 5 different lengths: 95 ± 9, 80 ± 6, 65 ± 6, 50 ± 9, and 35 ± 7 nm with the diameter of 13 ± 1 nm.

### 6.4 Exchange of the binary surfactant system with a single surfactant system

The etched NRs were stabilized with a mixture of BDAC/CTAB surfactants and we conducted ligand exchange to replace BDAC with CTAB. This step was intended to simplify the surfactant composition and to ensure the complete coverage of the sides of NRs with CTAB after etching. We used two routes (Route 1 and Route 2) of surfactant exchange. Figure 17 shows TEM images of the Au NRs after the ligand exchange steps.

In Route 1, the etched NRs coated with BDAC/CTAB in water were centrifuged to remove the supernatant and were subsequently dispersed in a solution of CTAB (0.1 M). The NRs in the
solution of CTAB were left for 2 hrs. Then centrifugation was carried out at 8500 rpm for 10 min, in order to remove the supernatant. In the following SH-PS attachment step in THF, the NRs aggregated, which indicated that they became colloidal unstable (Figure 13 (a)). In Route 2, the NRs were centrifuged to remove the supernatant and subsequently redispersed in the following solution in the following order: aqueous AgNO$_3$ (4 mM), CTAB (0.1 M), and L-ascorbic acid (0.0788 M). Our rationale was as follows. It has been stated by Jana et al. that Ag$^+$ is deposited on the {110} side facets of Au NRs in the form of AgBr (Br$^-$ ions introduced with CTAB) which restricts the growth of the {110} facets.$^{106-108}$ The side facets passivated with AgBr provide attachment points for CTAB. Elemental Ag is also known to form through underpotential deposition (UPD) and passivate the {110} facets of Au NRs.$^{106,109-112}$ UPD is the deposition of silver at a potential much less than what is needed for bulk deposition.$^{111,112}$ Ascorbic acid was added to the NR solution as a mild reducing agent for Ag$^+$ ions. Immediately following the addition of ascorbic acid, two cycles of centrifugation at 10 000 rpm for 20 min were performed to allow the surfactant exchange to occur and then the supernatant was removed. The NRs dispersed in THF after the SH-PS attachment showed very little aggregation of NRs (Figure 13 (b)).
6.5 Attachment of polystyrene to the ends of Au nanorods

Following the ligand-exchange step, CTAB-coated NRs with different lengths were dispersed in the THF containing SH-PS (M_w = 12 k) at the concentration of 0.2 mg/ml. Preferential binding of CTAB along the longitudinal sides (the \{110\} facets) of the NRs left the ends (the \{100\} facets) of the NRs deprived of CTAB and allowed for the binding of SH-PS to the NR ends\(^{113}\).

Figure 14 shows TEM images of the NRs end-terminated with SH-PS.
Figure 14. TEM images of Au NRs of different lengths. (a) As-synthesized long NRs with the length of 110 nm, (b) 95 nm (LSPW = 1185 nm), (c) 80 nm (LSPW = 1079 nm), (d) 65 nm (LSPW = 939 nm), (e) 50 nm (LSPW = 822 nm), and (f) 35 nm (LSPW = 685 nm). The scale bar is 150 nm.

Before commencing self-assembly, the initial molar concentration of the NRs, $[\text{NR}]_0$, was determined by measuring the intensity of extinction of the NRs at their surface plasmon wavelength (LSPW). The molar extinction coefficient of the NRs, $\varepsilon$, was determined by using the correlation between the experimental LSPW values and the extinction coefficients obtained by Orendorff and Murphy for Au NRs with different aspect ratios.\textsuperscript{112} We used the Beer-Lambert law

$$A_{\text{LSPW}} = \varepsilon_{\text{LSPW}} [\text{NR}]_0 l$$  \hspace{1cm} (3)
where $A_{LSPW}$ is absorbance at the LSPW, $\varepsilon_{LSPW}$ is the molar extinction coefficient in $[\text{nM}^{-1}\text{cm}^{-1}]$ at the LSPW, $l$ is the path length in [cm], and $[\text{NR}]_0$ is the initial concentration of Au NRs in [nM], to calculate the $[\text{NR}]_0$ of the NR samples. The $\varepsilon_{LSPW}$ and $[\text{NR}]_0$ values for the SH-PS terminated Au NRs with different lengths are shown Table 2. The kinetic analysis was performed for the NRs with the lengths of 50 and 90 nm.

Table 2: Molar extinction coefficients and concentrations of Au NRs with different lengths.

<table>
<thead>
<tr>
<th>LSPW of NR (nm)</th>
<th>Length of NR (nm)</th>
<th>Molar Extinction Coefficient $\varepsilon_{LSPW}$ (nM$^{-1}$cm$^{-1}$)</th>
<th>Absorbance</th>
<th>Initial Concentration of NRs (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>685</td>
<td>35</td>
<td>2.326</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>822</td>
<td>50</td>
<td>5.600</td>
<td>0.5050</td>
<td>0.09018</td>
</tr>
<tr>
<td>939</td>
<td>65</td>
<td>8.396</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1079</td>
<td>80</td>
<td>11.742</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1185</td>
<td>95</td>
<td>14.276</td>
<td>1.3818</td>
<td>0.09680</td>
</tr>
</tbody>
</table>

The width of NRs was at 13 nm.

### 6.6 UV-vis spectra of the self-assembly of Au nanorods

Self-assembly reactions were performed for NRs with the lengths of 35, 50, 65, 80, and 95 nm. Here we show the results on the kinetic analyses of the self-assembly of 50- and 95-nm long Au NRs. Figure 19 (a) and (b) shows UV-vis spectra of the self-assembly reactions. The transverse plasmon wavelength peaks for both self-assembly reactions occurred at approximately 520 nm and they did not experience any shift during the course of self-assembly. The absence of shift in the transverse plasmon wavelength peaks indicated that no significant side-by-side assembly of NRs occurred. The longitudinal surface plasmon wavelengths (LSPWs) of NRs before the commencement of self-assembly ($t = 0$) appeared at 822 nm and 1185 nm for the self-assembly
reactions of 50-nm and 95-nm long NRs, respectively. During the self-assembly processes, the initial LSPW peaks at t = 0 underwent red-shifts due to surface plasmon coupling between the end-to-end assembled NRs.

After 136 h of self-assembly, the initial LSPW of 50-nm long NRs at 822 nm red-shifted to 978 nm (Figure 15 (a)). The primary absorbance peak developed a secondary peak after ~36 h of self-assembly of the 50-nm long NRs. The secondary peaks in the region from 900 to 978 nm gradually showed higher absorbance intensity than the primary peak at 822 nm. The initial LSPW of 95-nm long NRs appeared at 1185 nm (Figure 15 (b)). At the end of both of the self-assembly processes, the increase in the LSPW slowed down and the polymerized chains of NRs started precipitating. The final LSPW was 1428 nm.

The rate of red-shift for the 50-nm NRs was comparable to the rate for the 95-nm NRs. Figure 15 (c) shows the shift of LSPW as a function of self-assembly time. The total time of self-assembly was ~284 h and 22 days for the self-assembly processes of 50-nm and 95-nm NRs, respectively. For both processes, the rates of self-assembly were constant until ~50 h and ~150 h for 50-nm and 95-nm long NRs, but they gradually decreased. The initial rate for the 50-nm NRs was higher than that of the 95-nm NRs and indicated that the self-assembly of shorter NRs happened faster. For detailed analysis of the rates, TEM samples taken at different self-assembly times were imaged. The growth of NR chains was then described by the kinetics and statistics of step-growth polymerization and allowed the prediction of the degree of polymerization of NRs and their size distribution.
Figure 15. Absorbance spectra of the solution of (a) 50-nm NRs ([NR]₀ = 0.09018 nM) and (b) 95-nm NRs ([NR]₀ = 0.09679 nM) in a DMF-water mixture acquired at varying time intervals after the beginning of self-assembly of the NRs. C₀ = 15 wt%. (c) A graph showing the shifts in LSPW for the self-assembly of 50-nm and 95-nm NRs.

6.7 Imaging of the self-assembly of Au nanorods

To analyze the kinetics of the self-assembly processes, the TEM samples were collected periodically throughout the self-assembly processes. The NR solutions were dropped on the carbon-coated TEM grids. The hydrophobic nature of the grid allowed the liquid to flow away from the surface of the grid rapidly, rather than to evaporate slowly. No further self-assembly was assumed to occur during the preparation of TEM samples.

Figure 16 shows TEM images of the self-assembled 50-nm NRs at (a) 96 h and (b) 12 days and 95-nm NRs at the same self-assembly times (c, d). We observed that the mean number of NRs within NR chains and the size distribution of the NR chains increased with time. At 96 h, the
$\bar{X}_n$ values for the 50-nm NR and 95-nm NRs are 2.9 and 1.97, respectively. We saw a distribution of short NR chains, as well as a lot of monomers. After 12 days, the $\bar{X}_n$ values for 50-nm NRs and 95-nm NRs increased to 6.76 and 4.55, respectively, and the TEM images showed a larger distribution of chain lengths. Such a trend is analogous to step-growth polymerization. We characterized the evolution of the NR chains by the change in their number-average degree of polymerization ($\bar{X}_n$), weight-average degree of polymerization ($\bar{X}_w$), and PDI stated in Equation 1.\textsuperscript{93}

After obtaining TEM images at various self-assembly times, the individual NRs, as well as self-assembled NR chains were counted and the $\bar{X}_n$, $\bar{X}_w$, and PDI values were determined. For the sake of analyzing the kinetics in the next section, only the $\bar{X}_n$ values are discussed here.
6.8 The kinetics of step-growth polymerization of Au nanorods

In application of step-growth polymerization model to the self-assembly of Au NRs, individual NRs were treated as bifunctional monomers. The kinetic equation for step-growth polymerization of such units is of the form:

\[
\text{Reaction Rate} = -\frac{d[M]}{dt} = 2k[M]^2 \tag{4}
\]

where \([M]\) is the concentration of the SH-PS functional groups on the ends of NRs at any time in the self-assembly process, \(t\) is the time of reaction, and \(k\) is the rate constant.

Integration of (4) gives the following form:

\[
2kt = \frac{1}{[M]} - \frac{1}{[M]_0} \tag{5}
\]
where \([M]_0\) is the initial concentration of the SH-PS functional groups. To correlate the reaction rate equation (5) to the number average degree of polymerization \(\bar{X}_n\), it is necessary to relate (5) to the extent of reaction \(p\) (equation (2) in Section 4.4.1), which is the fraction of functional groups that have reacted after a time \(t\).

\[
p = 1 - \frac{[M]}{[M]_0}
\]  

(2)

\(\bar{X}_n\) and \(p\) have the following relationship:

\[
\bar{X}_n = \frac{1}{1 - p}
\]  

(6)

Substituting (2) to (5) gives:

\[
2k_t[M]_0 = \frac{1}{1 - p} - 1
\]  

(7)

The rate constant \(k\) can be found out for each of the self-assemblies if we know their slope of \(\bar{X}_n\) vs. \(t\) graph and the initial NR concentration. The initial concentration of NR before the commencement of self-assembly \([NR]_0\) in \([M]\) can be found out by UV-vis spectroscopy using the Beer-Lambert Law (equation (3) in Section 6.5).

\[
A_{LSPW} = \varepsilon_{LSPW} [NR]_0 l
\]  

(3)

The initial concentration of the PS functional groups at the two NR ends \([M]_0\) is

\[
[M]_0 = 2[NR]_0
\]  

(8)

where \([NR]_0\) is the initial concentration of nanorods. The number average degree of polymerization \(\bar{X}_n\) at any time point \(t\) during a self-assembly can be determined when equations (7) and (8) are incorporated into equation (6) as shown below:
\[ \bar{X}_n = 2[M]_0 kt + 1 = 4[NR]_0 kt + 1 \quad (9) \]

where k is the rate constant in \([\text{M}^{-1}\text{s}^{-1}]\) and t is the duration of self-assembly in seconds.

Taking the first derivative of the above equation with respect to t gives

\[ \frac{d\bar{X}_n}{dt} = 2[M]_0 k = 4[NR]_0 k \quad (10) \]

where \( \frac{d\bar{X}_n}{dt} \) is easily determined from the slope of the linear \( \bar{X}_n \) vs. t graph for self-assembly of each of the lengths. Isolating for k from equation (10) gives the final equation (11).

\[ k = \frac{\frac{d\bar{X}_n}{dt}}{2[M]_0} = \frac{\frac{d\bar{X}_n}{dt}}{4[NR]_0} \quad (11) \]

To determine the rate constants of the self-assembly reactions, \( \bar{X}_n \) values obtained by analyzing TEM samples obtained at various self-assembly times were plotted against time.

Figure 17 shows the variation of \( \bar{X}_n \) for the self-assembly of (a) 50-nm and (b) 95-nm NRs. The relationship between \( \bar{X}_n \) and t was linear, which confirmed the reactivity of the SH-PS functional group to be independent of the chain length [the Flory’s assumption\(^{92}\)]. The positive slope of the \( \bar{X}_n \) vs. t represented the growth of the NRs to chain per unit time or \( 4[NR]_0 k \) in the step-growth polymerization model. The y-intercept of the plot is the degree of polymerization \( \bar{X}_n \) at the self-assembly time of 0 and showed one corresponding to monomeric NRs. The slope of \( \bar{X}_n \) vs. t graphs (\( \frac{d\bar{X}_n}{dt} \)) indicated the rate of increase of the number average degree of
polymerization $\bar{X}_n$. The slopes of 50-nm long and 95-nm long NRs were 0.0202 h$^{-1}$ and 0.0125 h$^{-1}$, respectively (Figure 17 (c)). Also, the time required for $\bar{X}_n$ to increase by one unit $\frac{t}{\bar{X}_n}$ was 49.3 h and 80.0 h for 50-nm long and 95-nm long NRs, respectively, which indicated that the rate of self-assembly was higher for shorter NRs.

Figure 17. Variation in degree of polymerization of (a) 50-nm NRs, (b) 95-nm NRs and for (c) both.

To calculate rate constant values from the $\bar{X}_n$ vs. $t$ graphs, equation (12) was used. Table 3 shows the rate constants for Au NRs with different lengths. The rate constants for the self-assembly
reactions of the 50-nm long and 95-nm long NRs were $1.563\times10^4$ and $8.968\times10^3$ M$^{-1}$s$^{-1}$, which showed that the self-assembly of 95-nm long NRs was ~1.7 times slower than that of the short NRs.

Table 3: [$\text{NR}]_0$ values for Au NRs with the lengths of 50 and 95 nm.

<table>
<thead>
<tr>
<th>LSPW of NR (nm)</th>
<th>Length of NR (nm)</th>
<th>Molar Extinction Coefficient $\varepsilon_{\text{LSPW}}$ (nM$^{-1}$cm$^{-1}$)</th>
<th>Initial Concentration of NRs (nM)</th>
<th>$\frac{dX_n}{dt}$ (h$^{-1}$)</th>
<th>$\frac{t}{X_n}$ (h)</th>
<th>Rate Constant k (M$^{-1}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>822</td>
<td>50</td>
<td>5.5998</td>
<td>0.09018</td>
<td>0.0202</td>
<td>49.3</td>
<td>$1.563 \times 10^4$</td>
</tr>
<tr>
<td>1185</td>
<td>95</td>
<td>14.2755</td>
<td>0.09680</td>
<td>0.0125</td>
<td>80.0</td>
<td>$0.89768 \times 10^4$</td>
</tr>
</tbody>
</table>

The step-growth polymerization model was applicable to the self assembly of SH-PS tethered Au NRs with the mean lengths of 50 and 95 nm. We anticipate that the polymerization model can be applied to the reaction-controlled self-assembly of NRs in other length ranges or of other metals, such as palladium.

6.9 Conclusion

This work focused on studying the kinetics of self-assembly of Au NRs of different lengths and investigating the effect of length of monomeric NRs on the reactivity of SH-PS functional groups tethered to the ends of the NRs.
The results showed that the kinetics of self-assembly of Au NRs resembled that of the reaction-controlled step-growth polymerization for NRs in a wide length range. It also demonstrated that the reaction rate was indeed affected by the length of the monomeric NRs. The analysis of the kinetics of self-assembly processes demonstrated that the shorter the NRs, the higher the rate of self-assembly. The rates of self-assembly processes of NRs with different lengths were affected by the following factors: the excluded volume of NR chains, their reactive volume, and the surface charge of NRs with different lengths. The reactive volume $\phi_r$ (defined in Section 4.4.2 as $\phi_r = V_{\text{pol}}/(V_{\text{NR}}+V_{\text{pol}})$) decreased with increasing length and volume of NRs. The excluded volume of the NRs ($V_{\text{NR}}+V_{\text{pol}}$), which was proportional to the square of the length of NRs, was higher for longer NRs. Long NRs also had higher surface charge than short NRs and led to a higher degree of repulsion between CTAB-coated sides of the NRs. Hence, the low reactive volume and stronger repulsion between the NRs resulted in their longer time of bond formation.

Future work will involve investigation of zeta potential analysis of the Au NRs to study the effect of surface charge on the reactivity of SH-PS functional groups. Copolymerization of Au NRs with different lengths and copolymerization of Au and Pd NRs are also some of the areas of interest.
References


