Magnetic colloidal assemblies and their applications in bioscience

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Magnetic Colloidal Assemblies and Their Applications in Bioscience

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University in fulfillment of the requirement for the degree
of Doctor of Philosophy

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To my beloved parents

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Table of Contents

Acknowledgments.....	i
Table of Contents.....	iii
List of Figures.....	vi
List of Tables.....	xiii
Common Abbreviations.....	xiv
Abstract.....	xiv
 Chapter 1: Introduction.....	 1
1.1 Research progress of magnetic colloids in recent years.....	2
1.2 Interactions in magnetic colloidal systems.....	4
1.2.1 Gravitational interaction.....	5
1.2.2 Electrostatic interaction and van der Waal attraction.....	6
1.2.3 Magnetic interaction.....	8
1.2.4 Other interactions.....	9
1.3 Properties of magnetic colloids used in this thesis.....	10
1.4 Motivation and objectives.....	11
1.5 Organization of the thesis.....	12
 Chapter 2: Experimental Methods.....	 14
2.1 Experimental setup.....	14
2.1.1 Optical microscopy.....	14
2.1.2 Fluorescent microscopy.....	15
2.1.3 Magnetic field generator.....	15
2.2 Preparation of colloidal systems.....	16
2.2.1 Procedures of creating negatively-charged glass.....	16
2.2.2 Procedures of washing colloids.....	17
2.3 Data analysis.....	18

Chapter 3: Two-dimensional Colloidal Assemblies in a Confined Region.20

3.1 Introduction.....	20
3.2 Experimental details.....	21
3.3 Physical mechanism.....	23
3.4 Crystallization process in 2D colloidal system by adjusting the electrostatic interactions.....	28
3.5 Crystallization process in 2D colloidal system by adjusting the magnetic interactions.....	32
3.6 Order-disorder phase transition from 2D to 3D colloidal system.....	35
3.2 Summary.....	40

Chapter 4: Formation of Magnetic Colloidal Clusters in Evaporating Droplets.....41

4.1 Introduction.....	41
4.2 Experimental details.....	42
4.3 Characterization of formation of magnetic colloidal clusters in the evaporating droplets.....	44
4.4 Effect of magnetic field on the formation of colloidal clusters.....	48
4.5 Effect of salt on the formation of colloidal clusters.....	52
4.6 Possible physical mechanism.....	57
4.7 Summary.....	59

Chapter 5: Towards Controlling the Formation of Magnetic Colloidal Chains using Single-stranded DNA.....61

5.1 Introduction.....	61
5.2 Experimental details.....	62
5.3 Effect of the growth time on the colloidal chain length.....	64
5.4 Effect of the magnetic field on the colloidal chain length.....	66
5.5 Effect of the bead mixture on the colloidal chain length.....	68

5.6 Effect of the DNA density on the colloidal chain length.....	69
5.7 Scenario for magnetic colloidal chain formation bridged by single-stranded DNA.....	71
5.8 Summary.....	74
 Chapter 6: Colloidal Lithography of Protein Patterns.....	76
6.1 Introduction.....	76
6.2 Experimental details.....	78
6.3 Adsorption of bovine serum albumin on polyelectrolyte-coated glass substrate.....	82
6.4 Colloidal lithography of protein pattern.....	88
6.5 Influence of salt on colloidal lithography of protein.....	90
6.6 Summary.....	94
 Chapter 7 Conclusions and Future Perspective.....	96
 List of Author's Publications.....	99
 Bibliography.....	100

List of Figures

Figure 1.1 Schematic drawing of gravitational force acting on the colloidal particles.

Figure 1.2 Schematic drawing of electrical double layer near the surface of the colloidal particle.

(<http://www.hull.ac.uk/scg/paunov/paunov06536-2.pdf>)

Figure 1.3 The relation between the surface-to-surface distance and the interaction energy including Van der Waals attraction, repulsive electrostatic interaction and the total interaction energy.

(<http://www.hull.ac.uk/scg/paunov/paunov06536-3.pdf>)

Figure 1.4 Schematic drawing of dipolar magnetic interaction acting on the colloidal particles when they are placed in magnetic field.

Figure 2.1 Schematic drawing of the experimental setup.

Figure 3.1 Schematic drawing of the interactions involved in the colloidal systems. Top: thermal excitations from the collisions between the colloidal and liquid molecules; middle: the repulsive electrostatic interaction between the surfaces of the colloids; and bottom: repulsive magnetic interaction between paramagnetic particles.

Figure 3.2 Schematic drawing of the order-disorder transition. In absence of external field, the colloidal may be more or less randomly distributed. However, upon increasing the field, they tend to form a well-ordered colloidal array. Above a certain critical field, the dipolar attraction overcomes the gravitational force, and the colloids start to jump on top of each other, thus forming chains and clusters.

Figure 3.3 Colloidal assemblies with different area fractions and hence different

Coulomb coupling parameters. (a) The area fraction ϕ was 0.15, the average interparticle spacing d was $\sim 4.50 \mu\text{m}$, (b) was 0.30, d was $\sim 4.50 \mu\text{m}$, (c) was 0.50, d was $\sim 3.50 \mu\text{m}$ and (d) was 0.70, d was $\sim 3.30 \mu\text{m}$. The scale bar is $10 \mu\text{m}$.

Figure 3.4 The radial pair distribution function $g(r)$ of the 2D colloidal system. From the bottom up, the solid lines represent the $g(r)$ for area fraction ϕ was ~ 0.15 ; $g(r) + 1$ for area fraction ϕ was ~ 0.30 ; $g(r) + 2$ for area fraction ϕ was ~ 0.50 and $g(r) + 4$ for area fraction ϕ was ~ 0.70 .

Figure 3.5 Crystallization of dilute colloidal system with area fraction of 0.10 (interparticle spacing was $\sim 7.7 \mu\text{m}$) by adjusting the magnetic dipolar interaction. The strength of the magnetic field H was (a) 0 A/m^{-1} , (b) 180 A/m^{-1} , (c) 800 A/m^{-1} and (d) 2000 A/m^{-1} .

Figure 3.6 The radial distribution function of the dilute colloidal system with area fraction of 0.10 in different magnetic fields. (a) $g(r)$ at magnetic field $H = 0 \text{ A/m}$, (b) $g(r) + 0.5$ at magnetic field $H = 800 \text{ A/m}$, (c) $g(r) + 1$ at magnetic field $H = 800 \text{ A/m}$, and (d) $g(r) + 1.5$ at magnetic field $H = 2000 \text{ A/m}$.

Figure 3.7 Dilute system exposed to a magnetic field. (a) The magnetic field is much smaller than the critical field ($H = 80 \text{ A/m}$). (b) The magnetic field is close to the critical field ($H = 1200 \text{ A/m}$), and the colloids have been arranged into a more ordered structure. (c) Structure for a field just larger than the critical field ($H = 2800 \text{ A/m}$), where chains and clusters have started to form. The sharp, dark points are straight chains pointing in the z direction. (d) Focus plane was shifted to the top of the chains where the sharp, dark point now are the ends of the long chains, whereas the more grayish, blurred points are smaller chains and single particles.

Figure 3.8 Dense system exposed to a magnetic field. (a) Colloidal structure when the magnetic field is slightly smaller than the critical field ($h = 120 \text{ A/m}$).

(b and c) colloidal assembly at $H = 200$ A/m and 320 A/m, respectively. Note that as opposed to Figure 3.7 these images have been taken against a dark background to optimize the contrast between the close-packed colloids.

Figure 3.9 Measured critical field as a function of colloidal density ($\square$). The solid line shows a fit using eq 3.14 with $C = 4.2 \times 10^{12}$ A/m^{5/2}.

Figure 4.1 Schematic drawing of the basic experimental setup. The colloidal droplet is confined by the well on the glass slide. The magnetic field is generated and can be adjusted by a current in the coil.

Figure 4.2 Evolution process in a system of magnetic beads and formation of clusters. The left column is low-amplification images and the right is high-amplification images taken at the same time.

Figure 4.3 The radial distribution function $g(r)$ of the 2D colloidal system at different times after the droplet is injected into the well. The distance r_1 for the first peak of $g(r)$ is defined as the nearest most probable inter-particle spacing.

Figure 4.4 The smallest most probable inter-particle spacing distance (red) and distribution function at that distance (black) at different evolution times. (The solid lines are only a guide for the eyes.)

Figure 4.5 Evolution processes of magnetic colloidal assembly placed in different magnetic fields. The direction of the applied magnetic field was perpendicular to the 2D colloidal system, and the intensities of the magnetic field were 230 A/m (left) and 460 A/m (right), respectively.

Figure 4.6 The radial distribution function of the 2D colloidal system at different evolution times in the presence of a magnetic field of (a) 230 A/m, (b) 345 A/m, and (c) 460 A/m.

Figure 4.7 The nearest most probable inter-particle spacing distance vs. the

evolution time at different magnetic fields. This distance is the position of the first peaks of $g(r)$.

Figure 4.8 The radial distribution function at the nearest most probable inter-particle spacing distance vs. the evolution time at different magnetic fields. This is the value of the first peaks of $g(r)$.

Figure 4.9 Evolution processes of magnetic colloidal assembly in the presence of 0.2 mM of NaCl.

Figure 4.10 Evolution processes of magnetic colloidal assembly in the presence of 0.6 mM of NaCl.

Figure 4.11 Evolution processes of magnetic colloidal assembly in the presence of 1.0 mM of NaCl. Upper row is images in the center of the droplet; bottom row, images near the edges of the droplet.

Figure 4.12 Evolution processes of magnetic colloidal assembly in the presence of 10.0 mM of NaCl. Upper row is images in the center of the droplet; bottom row, images near the edges of the droplet.

Figure 4.13 The radial distribution function of 2D colloidal systems at different evolution times. NaCl concentration was (a) 0.2 mM, (b) 0.6 mM, and (c) 1.0 mM. For clarity, plots for different evolution times were shifted vertically from one another by 1 unit in (a) and (b), and by 0.5 units in (c).

Figure 4.14 The nearest most probable inter-particle spacing distance vs. the evolution time for a colloidal system in the presence of various salt concentrations.

Figure 4.15 The radial distribution function at the nearest most probable inter-particle spacing distance vs. the evolution time for a colloidal system in the presence of various salt concentrations.

Figure 4.16 Schematic drawing of the experimental setup in which the evaporation rate of the colloidal droplet can be controlled.

Figure 5.1 Schematic drawing of the basic experimental setup for fabricating the magnetic colloidal chains.

Figure 5.2 Images of magnetic colloidal chains formed under the magnetic field of 20 mT for different growth times.

Figure 5.3 The length distribution of colloidal chains for different growth times under the magnetic field of 20 mT. The distribution was calculated from various images taken in different area and each experiment was repeated 3 times.

Figure 5.4 The average length of the colloidal chains vs. growth time under the magnetic field of 20 ± 3 mT (with shaking and without shaking).

Figure 5.5 The average length of the colloidal chains vs. growth time under the magnetic field of 20 ± 3 mT and 30 ± 3 mT (without shaking).

Figure 5.6 Images of magnetic colloidal chains formed under different magnetic fields for >10 hours.

Figure 5.7 The length distribution of colloidal chains under different magnetic fields for >10 hours.

Figure 5.8 The average length of colloidal chains incubated for more than 10 hours under various magnetic fields.

Figure 5.9 Images of magnetic colloidal chains for different A:B ratios. The magnetic field was 20 mT; growth time, 10 h.

Figure 5.10 The length distribution of colloidal chains for various A:B ratios. The magnetic field was 20 mT; growth time, 10 h.

Figure 5.11 The average length of the colloidal chains for various A:B ratios.

The magnetic field was 20 ± 3 mT; growth time, 10 h.

Figure 5.12 The length distribution of colloidal chains for various DNA amount used. The magnetic field was 20 mT; growth time, 10 h.

Figure 5.13 The average length of the colloidal chains for various DNA amount used. The magnetic field was 20 ± 3 mT; growth time, 10 h.

Figure 5.14 Schematic drawing (not in scale) of a pair of beads covered by DNA.

Figure 5.15 Scenario for DNA-based magnetic colloidal chain formation between A-type and B-type beads.

Figure 5.16 Schematic drawing of controlling the DNA density by pre-occupying streptavidin sites on the bead surface using free biotin molecules.

Figure 6.1 Image showing the glass slides partially covered with Teflon and with each containing three uncovered glass wells as substrates. The green light is added to illustrate the fluorescent emission.

Figure 6.2 The procedure for adsorption of protein on glass substrate. Green color represents the biomolecules.

Figure 6.3 Schematic drawing of the basic steps for the two proposed lithography patterning processes (a) and (b). Green color represents the biomolecules.

Figure 6.4 Fluorescent images of the protein layer absorbed on the glass substrate when the concentration of the PAH is 0, 14, 28, 57, 143 and 286 μM (same exposure time and gain).

Figure 6.5 The relationship between the average relative intensities of the fluorescent images and the concentration of PAH (black squares). The solid line is fitted to the experimental data using the expression: $P_c = 1 - e^{-NE_s/k_B T}$.

Figure 6.6 Fluorescent images of the protein absorbed on the glass substrates when the concentration of SDS is 0.1, 1-8 and 10 mM.

Figure 6.7 The relationship between the concentration of SDS and the average relative intensities of fluorescent image, the solid line is only a guide for the eye.

Figure 6.8 Colloidal chains of paramagnetic polystyrene spheres on a substrate (a). After following the steps of Figure 6.3a, the FITC-albumin patterns corresponding to the original colloidal assembly was obtained (b).

Figure 6.9 Two-dimensional colloidal arrays of polystyrene sphere on a glass substrate (upper image and the corresponding BSA-FITC protein patterns. The scale bar is 10 μm .

Figure 6.10 Fluorescent patterns on a PAH-coated substrate observed after removing the colloid. The FITC-albumin was dissolved in 0 M (a) and 10^{-2} M NaCl (b), respectively. The contrast of the images has been enhanced for clarity.

Figure 6.11 Contrast as a function of NaCl concentration. Each measurement is an average over typically 30 patterns, with the error bars denoting the corresponding standard deviation.

Figure 6.12 Schematic drawing of the electrostatic trapping geometry, where an electrical field is set up between the positively charged PAH-coated glass substrate and the negatively charged polymer sphere. Also draw is the structure of PAH (on the left side of the picture).

List of Tables

Table 1.1 Types of colloids classified by different dispersed phase and dispersion medium.

Common Abbreviations

DNA	Deoxyribonucleic acid
DLVO	Derjaguin-Landau-Verwey-Overbeek
1D	One-dimensional
2D	Two-dimensional
3D	Three-dimensional
BSA	Bovine serum albumin
FITC	Fluorescein isothiocyanate
PAH	Poly (allylamine-hydrochloride)
SDS	Sodium dodecyl sulphate
CCD	Charged coupled device
DI	Deionized water

Abstract

Colloidal assemblies have many applications in science and technology, ranging from optics and photonics devices to bioscience and biotechnologies. For paramagnetic colloids, the interactions between colloidal particles can be manipulated by adjusting the magnitude of an external magnetic field. This thesis investigates the assemblies of micrometer-sized magnetic colloids and their applications in bioscience.

The phase ordering phenomena in a two-dimensional (2D) colloidal system by tuning the electro- and magnetostatic interparticle interactions were studied. A subtle competition between the repulsive interparticle interactions and the thermal excitations of the particles was found to determine the crystallization of the colloidal system. The order-disorder transition (from 2D to 3D) of the colloidal system was also observed, when the magnetic interaction between colloids was higher than a critical value.

The formation of 2D clusters of protein-coated colloids in evaporating aqueous droplets was observed, indicating the presence of attractive interactions between colloidal particles. The dynamical evolution of the cluster formation and the effects of different experimental parameters (e.g. magnetic field and salt concentration) on this process were quantitatively characterized by using the radial distribution function.

The magnetic colloidal chains bridged by short biotin-ended single-stranded DNA, and guided by the magnetic interactions between colloidal particles were fabricated. The relationship between the length of colloidal chains and different experimental parameters (e.g. magnetic field, time under magnetic field, DNA density on colloids' surface) was established.

The conditions for protein adsorption on glass substrates were investigated and

protein patterns were successfully created using colloidal lithography. The experimental results indicated that the adsorption of bovine serum albumin (BSA) protein on glass substrates can be controlled by (i) adjusting the pre-coated polyelectrolyte layer of poly (allylamine-hydrochloride) (PAH) molecules and (ii) adding the surfactant sodium dodecyl sulphate (SDS) or salt in the BSA solution.

Chapter 1 Introduction

A colloidal system is a small world of particles in the micrometer-to-nanometer scale, which cannot be viewed directly by our naked eyes. In short, a colloidal system is made up of a dispersed phase and a dispersion medium. A few examples can be found in Table 1.1. The history of the preparation and processing of colloids is very early, and can be dated back to the beginning of the human technology around 7000 BC.¹⁻⁷

Dispersed Phase	Dispersion medium	Common Name	Examples
Liquid	Gas	Aerosol	Fog
Solid	Gas	Aerosol	Smoke
Gas	Liquid	Micro-foam	Beer foam
Liquid	Liquid	Emulsion	Milk
Solid	Liquid	Sol	Paint, ink, clay
Gas	Solid	Solid micro-foam	Foam plastics
Liquid	Solid	Solid emulsion	Cheese, pearl
Solid	Solid	Micro composite	Injection-molded objects

Table 1.1 Types of colloids classified by different dispersed phase and dispersion medium.

Colloids have played a very important role in industries, as paint, ink, cosmetics, pharmaceutical, protein and tissue engineering, and biosensing during the last hundred years.⁸⁻¹⁸ Today, colloidal science and technologies offer exciting opportunities at the interfaces between chemistry, physics and biology. In fact, the study of colloids is throwing a new light not only on the fundamental problems, such as atomic and molecular interactions, plasma, condensed matter physics and biological science, but also on the technologies, such as the precise assembling of colloids, which can be used in important devices ranging from photonic devices to tunable biosensors.⁸⁻⁷⁹

1.1 Research progress of magnetic colloids in recent years

Magnetic colloidal particles can be distinguished from other kinds of colloidal particles by their strong interaction with magnetic fields. The interactions between the magnetic colloidal particles can be easily adjusted by applying an external magnetic field, and thus the colloidal assemblies can be easily manipulated and guided as well.^{32, 37, 51-52, 78, 80-97} Recently, magnetic colloidal particles have been employed in many research fields, such as magnetic recording media, photonic crystals, purification of biomaterials, bioarchitecture and clinical diagnostics.^{8, 15, 17-18, 54-55, 60, 77-78, 97-107, 229-231}

In the recent years, design and synthesis of colloidal magnetic particles have been well developed. A variety of magnetic colloids of nano-to-micrometer size, with or without polymer matrix, can be synthesized today.^{54, 108-115} As a result, they provide the potential that can spontaneously assembly into a great deal of structures with novel functional, mechanical, and optical properties.

Magnetic colloids have been used as tools in fundamental research. For instance, they can be used as an experimental model system for the study of crystal nucleation and structural phase transitions, since their crystallization phase behavior is analogous to that of atomic and molecular systems, while the structure and dynamics of colloidal system can be directly visualized using a microscope in real time and real space.^{32, 37, 51-52, 80-81, 116-118}

The most interesting colloidal phase is the colloidal crystals, which appear iridescent, as they Bragg scatter visible light. Usually, if the colloidal system is very dilute, i.e., the colloidal particles can move freely in every directions, such a colloidal system could be considered in gaseous state; as the concentration of the colloidal system is increased, the motion of each colloidal particle becomes more and more restricted due to the confining effect of the other particles, this kind of state could be considered as colloidal glass; if the colloidal system is

dense enough, the entropy causes the spontaneous formation of colloidal crystals, i.e., the 2D colloidal system tends to assemble into hexagonal order.²⁹⁸⁻³⁰¹ Colloidal crystals have potential in the application of photonic materials because they fulfill the following two requirements: first, a 2D ordered lattice structure of particles with different indices of refraction from the surrounding medium, and the lattice constant should be similar to the wavelength of the light.

Actually, these distinct phase occur in experiments are determined by the details of the interactions between colloidal particles. In this thesis, Magnetic dipolar interaction is used to help create long range ordered structure, which is very sensitive to the applied magnetic field. As the magnetic field was tuned carefully, the dilute system also could form long range ordered structure. Moreover, the magnetic interaction could be used to detect weak forces ranging from femtonewtons to piconewtons. This method can be applied to detect the interactions involved in many colloidal systems, especially in measuring the mechanical properties of biomolecules.^{82, 88, 90, 93, 99, 101, 119- 121, 232-234}

Magnetic colloids have been extensively used in bioscience and biotechnology in the past two decades. They are versatile tools in the separation of nucleic acids, proteins and other bio-macromolecules and cells, and they could be also used in molecular genetics and immunoassays because they meet the requirements for different bioassays. Moreover, the magnetic colloids have applications in electrochemical bio-sensing, and particular in DNA hybridization sensors. Generally, these techniques can be achieved with the immobilization of biomolecules on the surface of magnetic colloids.^{8, 17-18, 60, 77, 98-102, 104-107, 235}

There has been much progress in magnetic fluid rheology and flow in the recent years, carrying out in colloidal systems consisting of magnetic particles dispersed in a liquid. These studies also have applications in science and industrial engineering.^{88, 89, 108, 110-111, 236-240}

With the development of the lab-on-chip in recent years, the design and application of micro- and nanoscale devices gained a lot of interest. Such as micro-motors, which are propelled by self-generated forces and precisely guided by external magnetic field, and could be used in transport micro-objects in transportation and separation of biomolecular cargo in drug delivery system.^{78, 91, 97} The other kind application is in photonic band gap materials, which based on the anisotropic complex colloidal structures fabricated based on magnetic colloidal particles.²⁴² Moreover, paramagnetic colloidal particles dispersed in water and deposited above magnetic bubble domains of a uniaxial ferromagnetic garnet film could be used as microscopic stirrer when subjected to external rotating magnetic fields.⁹¹ Another kind of novel application of magnetic colloids is colloidal lithography, which does not employ high temperature, chemical solvent or UV radiation.^{125, 241}

1.2 Interactions in magnetic colloidal systems

There are different kinds of interactions governing a colloidal system, and the structure of the assembly changes accordingly.^{9, 31-34, 39, 41-45, 47-49, 51-53, 80-81, 95, 126-180, 228, 233-234, 236-237, 240, 243}

It should be noted that the particles interact with the surrounding water molecules, thus giving rise to Brownian motion: The Brownian motion is related to the thermal energy $k_B T$ of the surrounding molecules, where $k_B = 1.38 \times 10^{-23}$ J/K is Boltzmann's constant and T is temperature. In my experiments, the temperature was kept quasi constant around 300 K, so the thermal energy was about 4×10^{-21} J. Only in very dilute colloidal system, i.e. where the distance between two colloidal particles is large enough, the Brownian motion is not suppressed. However, if other interactions between particles are sufficiently strong, Brownian motion will be no longer apparent. Important external or interparticle forces are due to gravity, electrostatic and magnetostatic fields. Each of the most important interactions will be described as below.

1.2.1 Gravitational interaction

Colloidal particles settle down near the substrate, after the colloidal solution is injected into a container, due to the gravitational force acting on the particles as illustrated in Fig. 1.1.^{37, 125, 161-162, 164, 181, 243}

Figure 1.1 Schematic drawing of gravitational force acting on the colloidal particles.

The gravitational force acting on each particle is determined by the density and volume of the colloidal particle and is expressed as

$$F_g = \rho_p V_p g \quad (1.1)$$

where $\rho_p \sim 1.6 \times 10^3 \text{ kg/m}^3$ is the density of the colloidal particle, V_p is the volume of each particle and g is gravitational acceleration. Here the colloids are uniform spherical particles, so $V_p = (4\pi/3) a^3$, where a is the radius of particles. In the colloidal solution (water is used as the solvent here), the buoyancy force acting on the particles as shown in Fig. 1.1 should also be considered. The buoyancy force is given as

$$F_b = \rho_w V_p g \quad (1.2)$$

where $\rho_w \sim 1.0 \times 10^3 \text{ kg/m}^3$ is the density of water. So the effective gravitational force is found to be

$$F_e = (\rho_p - \rho_w) V_p g \quad (1.3)$$

1.2.2 Electrostatic interaction and Van der Waals attraction

Most colloidal particles carry surface charges in solution due to the dissociation of surface groups such as carboxylic groups or silanol groups, or by adsorption of charged molecules such as protein ions from surrounding solution. As a result, the surface potential will attract counter-ions from the surrounding solution and exclude co-ions as illustrated in Fig. 1.2. The region of the counter-ions is called electrical double layer.^{154, 163-171} There will be repulsive electrostatic interactions when colloidal particle coated with same kinds of charges approach each other, and there are also repulsive interactions between the colloids and the substrate if both of them are negatively-charged or positively-charged.

Figure 1.2 Schematic drawing of electrical double layer near the surface of the colloidal particle. (<http://www.hull.ac.uk/scg/paunov/paunov06536-2.pdf>)

The electrostatic interparticle interactions are very important because they determine the stability of the colloidal system.^{154, 163-171} According to Derjaguin's formula based on the DLVO theory,¹⁶³⁻¹⁶⁴ the interparticle electrostatic potential energy is given as

$$E_{ele} = 2\pi\epsilon a \psi_s^2 \ln\{1 + \exp[-(d - 2a)/\lambda_D]\}, \quad (1.4)$$

where ϵ is the dielectric constant of the fluid, a is the particle radius, ψ_s is the surface potential on each particle, d is the average inter-particle spacing, λ_D is the Debye length determined by

$$\lambda_D = \sqrt{(\epsilon k_B T)/(2e^2 n)} \quad , \quad (1.5)$$

where e is the electron charge, and n is the number density of ions in the solution. It is obvious that the interparticle electrostatic interactions can be controlled by modifying the electrolyte dissolved in liquid so as to have different ion densities.^{168, 171}

Figure 1.3 The relation between the surface-to-surface distance and the interaction energy including Van der Waals attraction, repulsive electrostatic interaction and the total interaction energy. (<http://www.hull.ac.uk/scg/paunov/paunov06536-3.pdf>)

The energy of van der Waals attraction between two identical spherical particles can be expressed as

$$E_{att} = - (A_H R)/(12D) \quad (1.6)$$

here A_H is a constant determined by the inherent properties of the colloidal particles, R is the radius of the sphere and D is the separation distance between closest surfaces of two spheres.²²⁸ The total interaction energy of the particles is a sum of the van der Waals attraction and repulsive electrostatic interaction and is illustrated in Fig. 1.3.

1.2.3 Magnetic interaction

When the magnetic colloidal particles are placed in a uniform magnetic field H , they will acquire dipole moments m which is aligned with the direction of the field as illustrated in Fig. 1.4.

Figure 1.4 Schematic drawing of dipolar magnetic interaction acting on the colloidal particles when they are placed in magnetic field.

The dipole moment can be given by

$$\vec{m} = \frac{4\pi}{3} a^3 \chi H \vec{e}_z \quad (1.7)$$

where a is the particle radius, χ is the susceptibility and $\vec{e}_z$ is a unit vector in z direction.^{30, 32, 37, 51-52, 81-82, 84-85, 93, 96, 155, 182} It should be noted that the dipole moment caused by the field from other particles did not considered here and it just proper only in the cases that the susceptibility of paramagnetic material is very small and the magnetic field applied is quite weak.

If a colloidal particle i can be considered as a point dipole of radius a and dipole moment m_i , the magnetic dipolar interaction energy between the particle i and j can be given by

$$\vec{E}_{ij} = \frac{\mu_0}{4\pi} \left(\frac{3(\vec{m}_i \cdot \vec{r}_{ij})(\vec{m}_j \cdot \vec{r}_{ij})}{r_{ij}^5} - \frac{\vec{m}_i \cdot \vec{m}_j}{r_{ij}^3} \right) \quad (1.8)$$

where μ_o is the permeability of surrounding medium and r_{ij} is the distance between the colloids.^{37, 51, 82, 84-85, 155, 182}

1.2.4 Other interactions

However, the DLVO theory cannot explain all colloidal phenomena, for example the like-charge attractions reported by certain research groups or the ordering processes of colloidal crystals in dilute dispersions.^{33, 39, 42, 143-144, 146-152}

In practice, colloidal clusters, which indicate strong attractive forces between colloidal particles, have been observed in many experiments, and which proved there are other kind of interactions in the colloidal system.^{39, 42, 143, 146, 148,}

The hydrodynamic interaction in colloidal system should be considered if they were immersed in a fluid and excite long-ranged flows as they move^{29, 32, 82,}

⁸⁸ By generating and reacting to a fluid's local velocity, colloidal particles experience hydrodynamic interactions with each other and with the walls of their container. So if the fluid's local velocity is quite small or almost no fluid motion, the hydrodynamic interactions between the colloidal particles could be omitted.

One kind of the attractive interaction between the colloidal particles is hydrophobic interaction. If hydrophobic molecules are inserted into water and some of them meet each other, they will associate with their hydrophobic surfaces to form complex. Another kind of the attractive interaction involved in colloidal system is famous depletion attraction. In this case, the small particles cannot penetrate into the gap between the surfaces of two big colloidal particles,

which will generate a negative osmotic pressure and a corresponding out-flux of solvent and leads to attraction between the two colloidal particles. Besides the depletion attraction, there are other kind of repulsive steric interactions involved in hard colloidal system due to the interparticle modulation.

Entropic forces, which are determined by the second law of thermodynamics, i.e., a system progresses to a state in which entropy is maximized, played important roles in some cases.²⁹⁸⁻³⁰¹ In the chapter 3 of this thesis, the transition of the 2D colloidal system from dilute gaseous state to ordered colloidal organization was investigated. However, here the entropic force was generated negatively by increasing the concentration of the colloidal particles, and therefore play less important role than other interactions, such as electrostatic and magnetic interactions involved. As a result, we did not discuss the entropic force in detail.

1.3 Properties of magnetic colloids used in this thesis

The magnetic colloids used in this thesis were Dynabeads. The beads are monosized and composed of polystyrene cross-linked with magnetic material, evenly distributed inside the beads. The properties of these colloids are as follows:

- (1) The size of the colloids is uniform, diameter of 1.0 μm or 2.8 μm (variations in diameter of about $\pm 0.2 \mu\text{m}$).
- (2) The colloids are covalently coupled with a monolayer of functional groups on their surfaces, such as carboxylic groups or streptavidin proteins. In the latter case, the colloids can be used as a solid-phase matrix for simple and efficient binding of biotinylated compounds.
- (3) The dynamics process of the colloidal system, such as pattern formation and its evolution, can be directly observed in real time because the size of the

colloids used in this thesis is from 1.0 to 2.8 μm .

(4) The magnetic colloids can be easily manipulated by an external magnetic field with very high accuracy, by which one can guide the colloidal assemblies, or detect the mechanical properties of the colloidal system.

1.4 Motivation and objectives

The low-dimensional colloidal systems, such as one-dimensional (1D) colloidal chains, or two-dimensional (2D) colloidal assemblies, have attracted much interest in the recent years due to their potentials of technological applications in optics, photonics, protein engineering, biosensor and bio-architecture. The structure of the colloidal assemblies or colloidal blocks are determined by the delicate balance between the attractive forces (e.g. Van der Waals force), repulsive forces (e.g. electrostatic force), and the Brownian force acting on the particles. Using magnetic colloids adds an extra parameter, since the interparticle magnetic interactions, regardless attractive or repulsive, can be easily adjusted by an applied external magnetic field.

In the research area of magnetic colloidal system, many investigations were carried out by different research groups before I started to pursue my PhD degree in 2005.^{8, 15, 30, 40, 51-52, 60, 64, 80, 82-83, 85, 98-99, 102, 108, 110, 119-120} However, problems were still left open in this research area, for examples, what factors rule the evolution of the magnetic colloidal assemblies confined in 1D or 2D systems, how to create real or imaginary protein micropattern by the magnetic colloidal lithography, how to detect the biomolecular interactions, shown in the hydrophobic effect of protein or some biological binding events at the surfaces of the colloidal particles, and the bio-architectures made up of colloidal particles and bonded by the biomolecules. Thus, the motivation in this thesis is to investigate these unresolved problems in order to better understand the magnetic colloidal system.

The objectives, concerning both fundamental research and applications, include four topics:

(1) To determine the important parameters quantitatively, which control the evolution of the magnetic colloidal assemblies, by experiments and theoretical analysis.

(2) To measure the weak interactions between the colloidal particles by magnetic dipolar interactions in the system evolution, especially during the colloidal pattern formation process.

(3) To control the unique colloidal chains, which were made up of magnetic colloidal particles with diameters of $1.05\mu\text{m}$, and bridged by single-stranded DNA molecules with 20 T sequences and two biotin ends.

(4) To optimize the parameters determining the quality of the protein microarray created by the magnetic colloidal lithography on the substrate.

1.5 Organization of the thesis

The thesis consists of seven chapters, and is organized as follows:

In chapter 1, I present a brief introduction on the research progress of the magnetic colloidal assemblies made in the recent years, including the studies on the preparations and properties of the magnetic colloids and their applications. Next, I discuss the important interactions that are involved in the magnetic systems used to control the colloidal assemblies. Finally, the motivation and objectives of my research are presented.

In chapter 2, I describe the experimental setup for imaging of colloidal systems and controlling of magnetic field. Next, I give a brief introduction on the preparation of colloidal systems, including procedures for creating

negatively-charged glass substrate and for washing the beads. At the end of this chapter, I introduce the algorithms used in data analysis.

In chapter 3, I present the studies on 2D colloidal assemblies that are confined in a small region. Two papers, “*Order-disorder transition in a quasi two-dimensional colloidal system*” and “*Crystallization of charged paramagnetic colloidal grains*”, are included.

In chapter 4, I present a study of cluster formation of magnetic protein-coated colloids in evaporating droplets. I show that the dynamical evolution of the cluster formation and the effects of different experimental parameters (e.g. magnetic field and salt concentration) on this process can be quantitatively characterized by using radial distribution function.

In chapter 5, I present evidence for the formation of magnetic colloidal chains bridged by short biotin-ended single-stranded DNA. The relationships between the length of colloidal chains and different experimental parameters (e.g. magnetic field, time under magnetic field, DNA density on colloids’ surface) are discussed.

In chapter 6, I present the colloidal lithography of protein patterns. Two papers, “*Adsorption of bovine serum albumin on polyelectrolyte-coated glass substrates: Applications to colloidal lithography*” and “*Influence of Salt on Colloidal Lithography of Albumin*”, are included.

In chapter 7, I summarize the main results of this thesis and present future perspectives.

Chapter 2 Experimental Methods

2.1 Experimental setup

The experimental setup used in this thesis for imaging of colloidal systems and controlling the magnetic field is illustrated in Fig. 2.1. There are mainly three parts in the setup: (1) the colloidal system, (2) the microscopy/fluorescent microscopy system, and (3) the magnetic field generation/control system.

Figure 2.1 Schematic drawing of the experimental setup.

2.1.1 Optical microscopy

The visible-light optical microscope uses a system of lenses to magnify images of small objects. This is an old technological method with a history of about four hundred years, but still has many applications in modern scientific research.

A microscope usually contains four components: (1) the objective lens - a cylinder containing one or more lenses, typically made of glass, to collect light from the sample; (2) the eyepiece - a cylinder containing two or more lenses to

focus the image for the eye; (3) the illumination source – providing and controlling light in a variety of ways; (4) the stage - a platform supporting the specimen being viewed. Typical magnification of a visible-light microscope is up to 1500x with a theoretical resolution limit of about 0.2 μm .

For better performance and convenience in use, a modern microscope is equipped with complex optical components, and the whole optical path is set up and controlled very accurately. With the development of electronic devices, a charged coupled device (CCD) camera can be used to record images from the microscope. Images can be shown on a computer screen and data can be stored directly in a computer.

In this thesis, we used two microscopes: (1) a Leica polarization microscope equipped with a black-white CCD camera, and (2) an inverted fluorescent microscope Nikon TE2000U equipped with a CCD camera (Nikon DS-5Mc) and used in optical light mode.

2.1.2 Fluorescent microscopy

Usually, the fluorescence microscope uses light of much higher intensity to illuminate the sample and the fluorescent species in the sample are excited, emitting light of a longer wavelength. Based on the light emanating from the fluorescent species, rather than that from the original light source, a fluorescence microscope can produce a magnified image of the sample.

In this thesis, we used an inverted fluorescence microscope Nikon TE2000U equipped with filters of appropriate wavelengths for detection, and the microscope is attached to a CCD camera.

2.1.3 Magnetic field generator

In this thesis, the magnetic field was produced mainly by two methods:

(1), The weak magnetic field was generated by using a copper coil placed on the platform of microscope, so the direction of magnetic field generated was perpendicular to the colloidal system. The copper coil was connected to a power source, and therefore the magnetic strength could be adjusted by tuning the intensity of the current applied. The diameter of the copper coil was about 10 cm and that of the colloidal system was less than 1 cm, so the magnetic field was uniform in the whole colloidal system when it was placed in the central region of the copper coil.

(2) The strong magnetic field was generated by one or two parallel rare-earth permanent magnets of about $2 \times 5 \times 10$ cm. In this method, the strength exerting on a colloidal system could be controlled by varying the distance between the magnet and the sample.

The relationship between the strength of the magnetic field and the intensity of the current or distance between the magnet and the sample was measured using Gauss-meter.

2.2 Preparation of colloidal systems

2.2.1 Procedures of creating negatively-charged glass

Unless otherwise specified, we used a glass slide (with a well on the surface) and a thin glass coverslip to create a container to confine the colloidal solution. The surface properties of the glass have important effects on the colloidal assemblies. The glass surface is negatively-charged at pH 7 due to the dissociation of silanol groups. The procedures for preparation of glass slides are described below (also see Ref. 183).

(1) Rinse the glass slides and coverslips with a lot of deionized (DI) water at room temperature.

(2) Immerse the slides and coverslips in 1 M NaOH solution at 90°C for 30

minutes. In this way, the hydroxide ions break silicon oxide bonds on the glass surface to form a silanol group (SiOH) and a silanol salt group (SiONa), through the reaction $\text{Si-O-Si} + \text{Na}^+ + \text{OH}^- \rightarrow \text{Si-OH} + \text{Si-ONa}$.

(3) Wash away the residual NaOH solution on the glass slides and coverslips with a lot of DI water.

(4) Immerse the glass slides and coverslips in 1 M HCl at room temperature for 10 minutes. In this way, the hydrogen ions in the hydrochloric acid displace the sodium ions and convert the silanol salt group (SiONa) into silanol group (SiOH) through the reaction $\text{Si-ONa} + \text{H}^+ + \text{Cl}^- \rightarrow \text{Si-OH} + \text{Na}^+ + \text{Cl}^-$.

(5) Rinse the glass slides and coverslips with a lot of DI water, and then store the glasses in pure DI water. At pH 7, the silanol groups are deprotonated, resulting in a negatively charged surface according to the reaction $\text{Si-OH} \rightarrow \text{Si-O}^- + \text{H}^+$.

2.2.2 Procedures of washing colloids

Protein-coated colloidal particles used here were stored in a buffer containing NaH_3 as a preservative. Before our experiments, the colloids were washed to remove NaH_3 . The procedures of washing colloids were facilitated by using a magnet and a bracket. The detailed procedures are given below.

(1) Shake the vial to make the colloidal solution homogeneous.

(2) Transfer an appropriate amount of colloidal solution to a tube.

(3) Dilute colloidal solution 100 times by adding DI water and then shaking the tube to obtain a homogeneous suspension.

(4) Put the tube containing the mixed colloidal suspension on a bracket, and put a permanent magnet under the tube with a small adjustable distance. In this way, we can make the magnetic colloidal particles sink to the bottom of the tube in about an hour.

(5) Carefully remove the solution contained in the upper part of the tube with a pipette.

(6) Wash the colloidal particles two more times by repeating the steps (3)-(5).

(7) Continue to wash the colloidal particles with a suitable buffer three to five times based on the specific applications. At the end of this procedure, keep a suitable volume of the mixture to obtain an expected concentration of colloids.

2.3 Data analysis

The software used in this thesis includes Matlab, image-J and Adobe Photoshop.

To locate the position of colloidal particle better, the images were optimized using Adobe Photoshop to make the positions of the particles well-resolved, and a center-locating algorithm composed with Matlab was used to determine the particle positions with an accuracy of 50 nm. The code was downloaded from (<http://www.physics.georgetown.edu/matlab/code.html>).

We also used a self-made program written on Matlab to compute the radial pair correlation function $g(r)$ of the colloidal distributions. The function $g(r)$ represents the probability of finding two particles separated in a distance r . For a 2D colloidal system, the radial (isotropic) pair correlation function is given as,

$$g(r) = \frac{n(r)}{2\pi r b \rho_{colloids}} \quad (2.1)$$

where $n(r)$ is the number of particles found in a ring with an inner radius of $r - 0.5b$ and an outer radius of $r + 0.5b$. In this thesis, we choose $b = 0.1d$, where d is the average interparticle spacing. $\rho_{colloids}$ is the number density of the particles in the bright field computed. This procedure was repeated using particles in the center region of field to avoid boundary effect and the results

were averaged.

In our experiments, we had two kinds of fluorescent protein patterns, one was uniform, and the other was heterogeneous, and accordingly we used two different methods to measure the fluorescent intensities of the protein patterns.

For the uniform protein patterns, we used a self-made program based on Matlab to measure the fluorescent intensities. We first confirmed a linear relationship between the recorded brightness scales and the fluorescent intensities; then, we measured the relative fluorescent values by finding the average gray level. The average relative intensity was obtained by analyzing six images taken on different substrates under the same experimental conditions.

For the heterogeneous protein patterns, we used the software ImageJ to analyze the images to obtain the intensity distribution/line profile, etc. ImageJ was downloaded from (<http://rsb.info.nih.gov/ij/>).

Chapter 3 Two-dimensional Colloidal Assemblies in a Confined Region

3.1 Introduction

Ordered colloidal systems play an important role in a number of applications, ranging from photonic devices to tunable biosensors.^{20, 34, 35, 153, 184, 191-199} The forces involved in colloidal system are complex and comprise of e.g. fluid drag, gravity, electrical and magnetic interactions, all of which can affect the assemblies of the colloidal system, and therefore give numerous possibilities for controlling colloidal phases.^{37, 80, 125, 137, 160, 170, 176, 178, 180, 186, 187, 199-203} In general, when the interactions between particles in two-dimensional (2D) system are purely repulsive and the forces acting on the entire system are cohesive, ordering will take place and crystallization will be developed when the inter-particle interaction strength increases.^{34, 37, 49, 80, 137, 170, 178, 180, 185} However, it has been shown that a vortex lattice in a superconductor can melt as the temperature in the material is lowered due to pinning of the individual vortices.²⁰⁴ This highly unusual behavior relies on an order-disorder transition by the inherent in the material.

Here we also show that dimensionality plays an important role in the rearrangement of colloidal crystals. Initially the system is entirely 2D because of the gravitational forces acting on the colloids and appears to form a more ordered array as we increased the magnetic field. However, above a critical magnetic field attractive dipolar interactions overcome the gravity, and the system tends to become more disordered, followed by cluster formation.

In this thesis, the 2D crystallization process of charged paramagnetic colloidal particles were experimentally investigated by adjusting the coupling

parameter $\Gamma = \frac{E}{k_B T}$, where k_B is the Boltzmann's constant, T is the temperature and E is the inter-particle repulsive potential energy. In this thesis, *the coupling parameter* was a sum of two terms, one intrinsically originated from the electrostatic potential energy of two spherical particles E_{ele} and the other from the magnetic dipolar interactions E_{mag} between two paramagnetic particles, which was adjustable. A variety of assemblies were observed when Γ was increased gradually, either by altering the distance between the particles when the surface potential of the particle was kept constant or by adjusting the external magnetic field when the colloidal density was unchangeable. The particle distribution was entirely random when Γ was small, but the colloidal system crystallized to form an ordered structure when the coupling parameter was increased above a critical value Γ_{cri} , which has been proved to be larger than 100 in gaseous media.^{137, 173, 175, 178, 185, 188} However, the pair potential between the colloidal particles is only several $k_B T$ when they dispersed in liquid media,¹⁶⁹ which means the coupling parameter would be much smaller than in gaseous media, it is about 10 in this thesis.

The interesting crystallization processes, especially order-disorder transition from 2D to 3D could help us obtain further insight into the phase behavior of colloidal particle arrays. Moreover, some novel applications, such as micro-optical filters and gratings are related to chain orientation, so manipulating the chains appropriately have of great significance.²⁰⁵

3.2 Experimental details

Carboxyl modified negatively charged paramagnetic polystyrene beads (Dynabeads M270 purchased from Dynal) with a radius $a = 1.4 \mu\text{m}$ and were coated with carboxylic acid (COOH^-) groups were dispersed in ultra-pure water and inserted into a circular well on the glass slide surface (coated with Teflon

everywhere except the well) of depth $\sim 0.1\text{mm}$ and diameter $\sim 8\text{mm}$. The well was enclosed by putting a cover glass of $18\times 18\text{ mm}$ over it to avoid external disturbances. The colloidal system was visualized by a Leica polarization microscope used in transmission mode and the images were captured by a CCD camera. The illustration of the experimental setup and the detail description of approach to obtain hydrophilic negatively charged surfaces are in Section 2.1 and Section 2.2.1 respectively. A copper coil connected to a current source was positioned on the microscope stage to generate magnetic fields and then the colloidal system was visualized by an inverted microscope (Nikon TE 2000 with a $60\times$ objective of numerical aperture 0.70) with attached CCD camera (Nikon DS-5Mc). Here the colloids are paramagnetic with initial magnetic susceptibility $\chi \sim 0.5$ and high-field susceptibility $\chi \sim 0.2$.⁹³ The temperature during the experiments was approximately $T=300\text{ K}$.

After letting the system equilibrate, one observes that the beads sink to the lower glass surface where they form a more or less ordered two-dimensional system depending on the concentration of colloids. Due to their negative charge, the colloids are repelled by the negatively charged glass surface and are therefore levitating a small distance ($<1\text{ }\mu\text{m}$) above it. Since all the colloids have the same negative charge, they repel each other and therefore move around randomly without forming clusters. A schematic drawing of how the configurations change with magnetic field is shown in Fig. 2. Upon applying a magnetic field H perpendicular to glass slide, the paramagnetic spheres will exhibit a magnetic moment and therefore repel each other. Upon increasing the magnetic field, the dipolar interactions become stronger, and the colloids form a 2D ordered lattice structure as drawn in Fig. 2. Normally one would expect that upon increasing the dipolar interactions the system would continue to crystallize forming a lattice that stiffens with increasing magnetic field. However, since the colloids are free to move in three dimensions this behavior is not seen here. Instead, we observe that at a certain critical magnetic field H_c some of the

particles overcome gravity and jump on top of each other in order to reduce their magnetic interaction energy. In this way they form chains in dilute systems or clusters in dense systems as illustrated in Fig. 2.

3.3 Physical mechanism

Figure 3.1 Schematic drawing of the interactions involved in the colloidal systems. Top: thermal excitations from the collisions between the colloidal and liquid molecules; middle: the repulsive electrostatic interaction between the surfaces of the colloids; and bottom: repulsive magnetic interaction between paramagnetic particles.

In order to get a better understanding of the crystallization process of the magnetic colloidal system, the interactions involved in colloidal system need to be understood. When the colloids confined in the glass well, they will settle down to form a 2D colloidal monolayer on the glass substrate due to gravitational force. Due to there are negative charges on their surfaces, the

colloids are repelled by the negatively charged glass surface and are therefore levitating a small distance ($< 1 \mu m$) above it. Since all the colloids have the same negative charge, they repel each other and therefore move around randomly without forming clusters. However, here the colloidal system is manipulated by the magnetic field, so the magnetic interaction, electrostatic interactions and thermal collisions were mainly three different kinds of forces involved in horizontal directions. Figure 3.1 illustrates these three kinds of interactions and all of them play the most important role for the crystallization.

The thermal excitations, which came from the collisions between the colloids and liquid molecules made the colloidal system randomly organized. This was determined by the temperature and was kept quasi constant as the temperature was kept at $\sim 300K$ during the experiments.

The repulsive electrostatic interactions between the surfaces of the colloids are inherent, which assist the colloidal system ordered organized and the energy of interaction between two particles is given by

$$E_{ele} = 2\pi\epsilon\epsilon_0\psi_s^2 \ln\{1 + \exp[-(d - 2a)/\lambda_D]\} \quad (3.1)$$

according to Derjaguin formula based on the DLVO theory,^{163, 164} where ϵ is the dielectric constant of the fluid (water in this paper), a is the radius of particle, ψ_s is the surface potential on each colloidal particle and kept constant during the experiment, d is the average inter-particle spacing and $d - 2a < 2a$ here, λ_D is the Debye length determined by

$$\lambda_D = \sqrt{(\epsilon k_B T)/(2e^2 n)} \quad (3.2)$$

where e is the electron charge, n is the density of ions.^{168, 171} When $\exp[-(d - 2a)/\lambda_D]$ is small enough, i.e. $(d - 2a)/\lambda_D \gg 1$, the formula can be simplified to

$$E_{ele} \approx 2\pi\epsilon a \psi_s^2 \exp[-(d-2a)/\lambda_D] \quad (3.3)$$

and when the d is very large, thus the colloidal particles like point charges, the Equation. (1) reduced to

$$E_{ele} \approx \frac{4\pi a^2 \epsilon \psi_s^2}{d} \exp[-(d-2a)/\lambda_D] \quad (3.4)$$

When a magnetic field H perpendicular to the 2D colloidal system is applied, each paramagnetic particle exhibited a magnetic moment

$$\vec{m} = (4\pi/3)a^3 \chi H \vec{e}_z \quad (3.5)$$

where χ is the magnetic susceptibility, H is the external magnetic field applied and $\vec{e}_z$ is a unit vector along the magnetic field direction.

Figure 3.2 Schematic drawing of the order-disorder transition. In absence of external field, the colloidal may be more or less randomly distributed. However, upon increasing the field, they tend to form a well-ordered colloidal array. Above a certain critical field, the dipolar attraction overcomes the gravitational force, and the colloids start to jump on top of each other, thus forming chains and clusters.

A schematic drawing of how the configurations change with magnetic field is shown in Fig. 3.2. Upon applying a magnetic field H perpendicular to glass slide, the paramagnetic spheres will repel each other and thus help the colloidal system to form ordered structure if the magnetic field is not too strong. Upon increasing the magnetic field, the dipolar interactions become stronger, and the colloids form a 2D ordered lattice structure as drawn in Fig. 3.2. Normally one would expect that upon increasing the dipolar interactions the system would continue to crystallize forming a lattice that stiffens with increasing magnetic field. However, since the colloids are free to move in three dimensions this behavior is not seen here. Instead, at a certain critical magnetic field H_c some of the particles overcome gravity and jump on top of each other in order to reduce their magnetic interaction energy. In this way they form chains in dilute systems or clusters in dense systems as illustrated in Fig. 3.2. Moreover, when the external magnetic field removed, the system loses its magnetic interactions and therefore diffuses back into a random distribution.

Here the magnetic field was uniform, and the corresponding repulsive inter-particle magnetic interaction between two closest particles can be expressed as

$$E_{mag} = 4\pi\mu\chi^2 a^6 H^2 / (9d^3) \quad (3.6)$$

where μ is the permeability of water.

The total magnetic interaction energy is a sum over all the dipolar pair interactions, where

$$E_d = -\mu \sum_{i,j} \vec{m}_i \cdot \vec{H}_j, \quad (3.7)$$

the magnetic field from the colloid is given by

$$\vec{H}_j = \frac{1}{4\pi} \left(\frac{3(\vec{m}_j \cdot \vec{r}_{ij})\vec{r}_{ij}}{r_{ij}^5} - \frac{\vec{m}_j}{r_{ij}^3} \right). \quad (3.8)$$

Note that the dipole approximation used in eq 2 is valid only when the magnetic moments are sufficiently far apart. However, it should be emphasized that we model the paramagnetic particles as point dipoles, which means that the magnetic moment is assumed to be located at the center of the particles. Thus, because the distance between point dipoles is at least 2.8 μm , eq 3.8 is a reasonable approximation. Moreover, all of the colloids have their magnetic moments in the z direction, and the particles are located on a nearly 2D lattice such that $\vec{m}_j \cdot \vec{r}_{ij} \approx 0$.

Both the Coulomb and magnetic coupling parameters are closely related with the average inter-particle spacing d , and therefore the total coupling parameter can be expressed as follows:

$$\Gamma_{tot} = \Gamma_{ele} + \Gamma_{mag}$$

$$\Gamma_{tot} = \frac{2\pi\epsilon a \psi_s^2 \ln\{1 + \exp[-(d - 2a)/\lambda_D]\}}{k_B T} + \frac{(4\pi\mu\chi^2 a^6 H^2)/(9d^3)}{k_B T} \quad (3.9)$$

In this thesis, the manipulation of d was achieved by adjusting the area fraction of colloidal system, since the average inter-particle spacing d between colloids is inversely proportional to the area fraction ϕ of colloids, i.e. $d^2 \sim 1/\phi$. From here we can get colloidal assemblies with ordering structures for each colloidal density by adjusting the d and H .

In addition, here the contribution from van der Waals attraction of the particle was negligibly small thus will not be considered in the discussion.¹⁶³

3.4 Crystallization process in 2D colloidal system by adjusting the electrostatic interactions

To understand the effect of electrostatic interactions on the colloidal crystallization process, an investigation of the relation between the inter-particle spacing d and the ordering of the colloidal system was carried out. Here the ions in the colloidal system came from two parts: the first part came from the silanol dissociation on the surface of glass substrate with an ionic strength around 10^{-6} mol/L,¹⁶⁹ and the other part came from the ionization of carboxylic acid coated on the surface of the colloids. The density of the carboxylic acid group is 150 $\mu\text{mol/g}$ colloids, so the negative charges on each beads is about 1.50×10^{-10} C/bead, and the bulk concentration was 6.15×10^{-5} mol/L when colloidal solution was diluted to 20-50 times during the investigation. However, only few of all acetic acid molecules were dissociated in water, in addition, the ionization constant of the carboxylic acid is 1.8×10^{-5} , consequently the concentration of $[\text{H}^+]$ at the surface of colloid local region was about 10^{-5} mol/L in our experiments, and the Debye length is thereby about 100nm. The Zeta potential ξ was measured in Brookhaven Instruments – PALS Zeta Potential Analyzer and was about -40 mV.

The colloidal assemblies with different area fractions are shown in Figure 3.3. In Figure 3.3a, the colloids were very dilute, the area fraction was ~ 0.15 and corresponding average inter-particle spacing d was about 6.5 μm . It is obvious that the distribution of colloidal system was entirely random, i.e. a dispersed phase, which means that the Coulomb coupling parameter Γ_{ele} should be much less than the critical coupling parameter Γ_{cri} at which the colloidal system displays lattice structure with long range ordering. When the area fraction of the colloidal system was increased to 0.30 and 0.50 as shown in Figure 3.3b and 3.3c, the corresponding inter-particle spacing d were decreased to 4.5 and 3.5 μm respectively. In these cases, the Coulomb coupling parameter Γ_{ele} increased with decreasing d , but was still less than Γ_{cri} as the distribution was still random.

When the area fraction was carefully increased to about 0.70, an excellent colloidal crystal was formed with hexagonal lattice as shown in Figure 3.3d. This means that the Coulomb coupling parameter Γ_{ele} was near the critical coupling parameter Γ_{cri} . In this case, the corresponding inter-particle spacing d was $\sim 3.35\mu\text{m}$, slightly bigger than the diameter of particle. This is reasonable since the colloids could not move freely and did not collide due to the electrostatic repulsion.

Figure 3.3 colloidal assemblies with different area fractions and hence different Coulomb coupling parameters. (a) The area fraction ϕ was 0.15, the average interparticle spacing d was $\sim 4.50\mu\text{m}$, (b) was 0.30, d was $\sim 4.50\mu\text{m}$, (c) was 0.50, d was $\sim 3.50\mu\text{m}$ and (d) was 0.70, d was $\sim 3.30\mu\text{m}$. The scale bar is $10\mu\text{m}$.

The detail characteristics for the crystallization of colloidal organizations were analyzed quantitatively by computing the radial pair correlation function $g(r)$ of the colloidal distributions with different Coulomb coupling parameters, where the function $g(r)$ is the probability of finding two particles separated by a distance r .^{189, 190} For a 2D colloidal system, the radial (isotropic) pair distribution function can be computed from

$$g(r) = \frac{n(r)}{2\pi b \rho_{colloids}} \quad (3.10)$$

where $n(r)$ is the number of particles found in a ring with an inner radius of $r - 0.5b$ and an outer radius of $r + 0.5b$, b is the width of the ring band $\sim 0.1d$, and $\rho_{colloids}$ is the density of the particles in the bright field computed. This procedure was repeated using particles in the center region of field (to avoid boundary effect) and the results were averaged. The images were analyzed using Adobe Photoshop and Matlab software. A center-locating algorithm was used to determine beads positions with an accuracy of 50nm.

Figure 3.4 The radial pair distribution function $g(r)$ of the 2D colloidal system. From the bottom up, the solid lines represent the $g(r)$ for area fraction ϕ was ~ 0.15 ; $g(r) + 1$ for area fraction ϕ was ~ 0.30 ; $g(r) + 2$ for area fraction ϕ was ~ 0.50 and $g(r) + 4$ for area fraction ϕ was ~ 0.70 .

Plots of $g(r)$ for 2D colloidal distributions with different area fraction and hence different Coulomb coupling parameters are shown in Figure 3.4. For the

dilute colloidal system, area fraction ϕ was about 0.15 ~ 0.30, the $g(r)$ was flat, which means the colloidal system is in a dispersed phase of random distribution. When increased the area fraction, the electrostatic interactions increased accordingly. As a result, the Coulomb coupling parameters became larger, and the colloidal particles were localized more easily with area fraction $\phi \sim 0.50$, ordering with short-range formed. This was proved by three peaks in $g(r)$ at $r/2a \sim 1.25, 2.30$ and 3.60 respectively. Good hexagonal structured colloidal crystals were formed when the area fraction is about 0.70. The corresponding $g(r)$ proved this structure by a large peak at the nearest neighbor inter-particle spacing d_0 , the second peak occurring at $r = \sqrt{3}d_0$ and the third peak at $2d_0$, where $d_0 \sim 1.20 \times (2a) \approx 3.35 \mu\text{m}$ was characterized by the first peak in $g(r)$.

As discussed in Section 3.3, the electrostatic repulsive interaction was sensitive to $d - 2a$. In the case of Figure 3d, the colloidal system meets the condition of $d - 2a \ll 2a$, but did not meet the condition of $(d - 2a)/\lambda_D \gg 1$ at the same time due to the long Debye length of $\sim 100\text{nm}$. Hence when $D - 2a < 5\lambda_D$, it is need to use Equation (1) to calculate the electrical interaction and the corresponding Coulomb coupling parameters. The 2D colloidal crystal was achieved when the inter-particle spacing was about $3.35 \mu\text{m}$, in which condition the $\Gamma_{ele} \approx \Gamma_{cri}$, and it was calculated to be 10 ± 2 accordingly. It is obvious that the $\Gamma_{cri} \sim 10$ is much less than the Γ_{cri} of the charged particles in gaseous medium.^{137, 173, 175, 178, 185, 188} When the inter-particle spacing was $\sim 3.60 \mu\text{m}$, the Coulomb coupling parameter Γ_{ele} was calculated to be about 1, which means the electrostatic interaction could not contribute much to the crystallization of the colloidal system when the inter-particle spacing is more than $3.60 \mu\text{m}$. However, the inter-particle repulsive force could be adjusted by using an external magnetic field on the paramagnetic colloidal particles.

3.5 Crystallization process in 2D colloidal system by adjusting the magnetic interactions

The novelty of the colloidal system is that the insufficiency of repulsive inter-particle potential energy generated from the electrostatic interaction could be offset by adjusting the repulsive magnetic dipolar interaction between colloids, and thus made the crystallization achieved in different colloidal systems from dilute to dense. Practically, both the electrostatic and magnetic interactions should be considered when the inter-particle spacing was between $3.35 - 3.60 \mu\text{m}$, in which the repulsive electrostatic potential contributed to the crystallization but not sufficient to crystallize it, and in these conditions, the critical coupling parameter could be expressed as $\Gamma_{crc} = \Gamma_{ele} + \Gamma_{mag}$ when crystallization is achieved. If the inter-particle spacing was more than $3.6 \mu\text{m}$, the contribution to the crystallization from the electrostatic interaction became smaller, the repulsive magnetic interaction played a more significant role in these cases.

Figure 3.5 Crystallization of dilute colloidal system with area fraction of 0.10 (interparticle spacing was $\sim 7.7 \mu\text{m}$) by adjusting the magnetic dipolar interaction. The strength of the magnetic field H was (a) 0 A/m^{-1} , (b) 180 A/m^{-1} , (c) 800 A/m^{-1} and (d) 2000 A/m^{-1} .

Figure 3.5 illustrated the crystallization process tuned by the repulsive magnetic interaction in dilute colloidal system of area fraction ~ 0.10 to make

the crystallization process clear. In Figure 3.5a, magnetic field $H = 0$, the distribution of the colloids was random, the corresponding $g(r)$ in Figure 3.6a was flat, which meant the coupling parameter was much less than the critical coupling parameter Γ_{cri} and the repulsive force caused by the electrostatic interaction was not strong enough to make the colloidal system form ordered structure.

Figure 3.6 The radial distribution function of the dilute colloidal system with area fraction of 0.10 in different magnetic fields. (a) $g(r)$ at magnetic field $H = 0$ A/m, (b) $g(r) + 0.5$ at magnetic field $H = 800$ A/m, (c) $g(r) + 1$ at magnetic field $H = 800$ A/m, and (d) $g(r) + 1.5$ at magnetic field $H = 2000$ A/m.

In fact, the electrostatic effect could be negligible here because the inter-particle spacing was much larger than $3.6 \mu\text{m}$. The distribution of the colloidal system went through an obvious change when the particles were separated from each other due to repulsive magnetic interaction as illustrated in Figure 3.5b when applied a magnetic field of 180 A/m . However, the colloids were still in random distribution and $g(r)$ was flat also as Figure 3.6b illustrated.

The inter-particle spacing became more uniform and ordering took place when we continued to increase the magnetic field to 800 A/m as seen in Figure 3.5c, characterized by the big peak in $g(r)$ at $r/2a \approx 2.80$ in Figure 3.6c. In Figure 3.5d, the magnetic field was ~ 2000 A/m, the distribution of colloidal system was in an ordered state, which was identified by three big peaks in $g(r)$ in Figure 3.6d, and the crystal unit cell with close-packed hexagonal structure which was emphasized in the black frame in Figure 3.5d.

In this case, the colloidal system could be considered near the crystallized state, and thus the critical coupling parameter can be determined because the repulsive potential energy can be calculated by the function of magnetic field and the inter-particle spacing. Note that the distribution of the dilute colloidal system is very sensitive to the inter-particle repulsive energy. When the inter-particle magnetic interaction is too strong, the particles will jump on top of each other to form multilayer due to the system energy-minimization, and if the energy is less than the critical energy, the ordered structure is difficult to form. As a result, it is not easy to make an excellent crystal with close-packed hexagonal structure in long range, so the crystal lattice was not as well-defined as illustrated in Figure 3.3d of dense colloidal system. All the experiments were carefully done to make colloidal system crystallized with the area fraction from 0.10 $\sim$ 0.70 and results indicated as the colloidal system are more denser, the more standard hexagonal crystal layer could be observed. Another novelty of the colloidal system is that the crystallization process is not only controllable, but reversible, i.e. when the external magnetic field is removed, the magnetic interactions disappear immediately and thus the colloids diffuse back into random distribution.

As discussed above, when the inter-particle spacing was more than 3.60 μm , the repulsive magnetic interaction played a more important role in the crystallization of the colloidal system and the effect from electrostatic

interaction became smaller, hence $\Gamma_{tot} \approx \Gamma_{mag}$. The critical magnetic fields H_c in which the colloidal systems crystallized for different area fraction are shown in Figure 3.7, where we used $A_0 H_c^2$ versus inter-particle space d , since according to Equation (3.9), that $A_0 H_c^2 \approx \Gamma_{cri} d^3$ could deduced, where $A_0 = (4\pi\mu\chi^2 a^6)/(9k_B T) \approx 10^{-21} \text{ A}^2 \text{ m}^5$. The solid curve shows a fit of the experimental data, the Γ_{cri} was determined to be $\sim 8 \pm 1$, which was reasonably consistent with the Γ_{cri} obtained from the electrostatic interaction in Section 4, but a little smaller. The deviation may be caused by omitting the electrostatic interaction when the inter-particle spacing is more than 3.60 μm , however, it still has slight effect on the particle organization. The critical coupling parameter here was much less than the theoretical value ~ 120 published for the particle crystallization in air.^{137, 173, 175, 178, 185, 188} In my experiments, charged paramagnetic colloids were immersed in water, the colloidal particle was surrounded by many water molecules, which number is about 1000 times of the air molecules, i. e. the viscosity of the water is much bigger than air thus resulting in the motion of colloidal particle dispersed in water to be reduced more significantly. Another reason is when the charged colloids were immersed in water, the large permittivity of water would cause a faster decay of the electrostatic force. As a result, the coupling parameter is only about ~ 10 and thus reasonable.

3.6 Order-disorder phase transition from 2D to 3D colloidal system

As discussed before, when the magnetic field more one critical value, the colloidal system will become form ordered 2D system to 3D disordered system. Details will be present in this section.

Experimental images of a dilute colloidal system are shown in Figure 3.7, where a colloidal concentration of 4×10^7 beads/mL was used. In Figure 3.7a,

the magnetic field is much smaller than the critical field. In Figure 3.7b, it is closer to the critical field, and the colloids have been arranged into a more ordered structure. Finally, Figure 3.7c and d shows the structure for a field larger than the critical field ($H = 2800$ A/m), where chains and clusters have formed. In Figure 3.7c, the focal plane is positioned at the colloids close to the glass plate such that the sharp, dark points are single particles, whereas the more grayish, blurred points are large straight chains pointing in the z direction. Any point of the chains could be visualized by shifting the focal plane of the objective lens. In Figure 3.7d, the focus plane was shifted to the top of the chains, where the sharp, dark points now are the ends of the long chains, whereas the more grayish, blurred points are smaller chains and single particles. In this way, some of the chains may have up to a few tens of particles, therefore extending nearly across the vertical direction of the cell.

Figure 3.7 Dilute system exposed to a magnetic field. (a) The magnetic field is much smaller than the critical field ($H = 80$ A/m). (b) The magnetic field is close to the critical field ($H = 1200$ A/m), and the colloids have been arranged into a more ordered structure. (c) Structure for a field just larger than the critical field ($H = 2800$ A/m), where chains and clusters have started to form. The sharp, dark points are straight chains pointing in the z direction. (d) Focus plane was shifted to the top of the chains where the sharp,

dark point now are the ends of the long chains, whereas the more grayish, blurred points are smaller chains and single particles.

Figure 3.8 Dense system exposed to a magnetic field. (a) Colloidal structure when the magnetic field is slightly smaller than the critical field ($h = 120$ A/m). (b and c) colloidal assembly at $H = 200$ A/m and 320 A/m, respectively. Note that as opposed to Figure 3.7 these images have been taken against a dark background to optimize the contrast between the close-packed colloids.

Experimental images of a high-density colloidal system are shown in Figure 3.8, where a colloidal concentration of 4.6×10^8 beads/mL was used. Figure 3a shows the colloidal assembly at fields right below the critical field $H_c = 200$ A/m, whereas b and c show the colloidal assembly at and above H_c , respectively. It should be noted that in Figure 3a the system is in a nearly hexagonally

close-packed state, whereas in b some of the long-range order has been destroyed by particles jumping out of the 2D formation. Finally, in Figure 3.8c the order is completely destroyed and a large variety of clusters and chains have formed. The process is therefore an order-disorder transition, where increasing dipolar interactions result in the destruction of long-range order.

The experiment were repeated with different concentrations ranging from 3.9×10^7 to 4.6×10^8 beads/mL and recorded the critical field H_c in each case. The results are shown in Figure 3.9, which displays the critical field H_c as a function of the measured colloidal density ρ_c (colloids per area). As we discussed in section 3.4, the critical could be approach to 0 A/m if the concentration of the colloidal system high enough. Here at large densities the critical field is about 200 A/m but increases to more than 1000 A/m as the bead separation doubles.

Figure 3.9 Measured critical field as a function of colloidal density ($\square$). The solid line shows a fit using eq 3.14 with $C = 4.2 \times 10^{12} \text{ A/m}^{5/2}$.

The average separation distance r_{av} between the colloids is inversely proportional to the colloids density (i.e., $\rho \propto 1/r_{ave}^2$), and then the total dipolar repulsive magnetic energy of the system should be

$$E_d \propto H_c^2 \rho_c^{3/2} \quad (3.11)$$

which follows from Eq. (3.8). In this 2D system, there are no attractive interactions. However, upon increasing the magnetic field, the colloids will, through a cooperative effect, push on each other such that one or more colloids will eventually be pushed out of the plane. Now the lattice will quickly rearrange while the colloids pushed out of the plane are attracted to the other colloids in the plane such that the interaction energy is minimized and chain formation takes place. An alternative way of thinking about this is in terms of the local nonuniform magnetic field produced by the paramagnetic spheres. This field strongly depends on the colloidal density and will facilitate chain formation in the z direction in order to minimize the magnetic energy. The magnetic dipolar energy of a chain depends only on the number of particles N in the chain:

$$E_{\text{chain}} = \frac{\pi}{9} \mu_0 N_{\text{eff}} a^3 \chi^2 H^2, \quad N_{\text{eff}} = \sum_{i=1}^{N-1} \sum_{j>i}^N \frac{1}{(i-j)^3} \quad (3.12)$$

When the field is just tuned to the critical field, a number of colloids jump on top of each other to form chains with $N=2$. The chain energy is therefore not depending on the density here, and may treat it as a constant. In addition to magnetic interactions, there are also gravitational forces acting on the colloids. When the colloids jump on top of each other to form chains, there is an increase in gravitational potential energy associated with each chain given by

$$E_g = \frac{4\pi}{3} (\sigma_c - \sigma_w) a^3 g \sum_{j=1}^N (2a)(j-1), \quad (3.13)$$

where $\sigma_c = 1600 \text{ kg/m}^3$ is the mass density of a single colloidal particle, $\sigma_w = 1000 \text{ kg/m}^3$ is the density of water and $g = 10 \text{ N/kg}$. It should be emphasized that at the transition a number of chains with $N=2$ are formed, and $N>2$ will only follow when the magnetic field is substantially larger than the critical field. Thus, at the transition both E_g and E_{chain} are independent of density. Moreover, the decrease

in magnetic interaction energy due to the formation of chains must be balanced by the increase in gravitational energy as the colloids are moving against gravity, and Eq. (3.12) suggests that the critical field scales with the density as follows:

$$H_c = \frac{C}{\rho_c^{3/4}} \quad (3.14)$$

In Figure 3.9, the Eq. (3.14) was plotted with $C=4.2 \times 10^{12} \text{ A/m}^{5/2}$. There is reasonable agreement between theory and experimental results, which suggests that the simple scaling argument above is indeed helpful in quantifying the critical field. However, it is also clear that further studies using e.g. dynamic simulations are needed to understand the detailed behavior of the melting transition.

3.7 Summary

In this chapter, we investigated the 2D colloidal crystallization processing of charged magnetic colloidal particles, from dispersed distribution to colloidal crystal, by adjusting the electro and magnetostatic interparticle interactions experimentally and quantitatively. In addition, the order-disorder transition of colloidal system would take place when the external magnetic field applied was more than a critical value. The relationship between the critical magnetic field and the density of colloids were determined experimentally and analyzed theoretically. These results can help us understand the ordering phenomena and to fabricate colloidal crystals.

Chapter 4: Formation of Magnetic Colloidal Clusters in Evaporating Droplets

4.1 Introduction

Colloidal particles, ranging from nanometer to millimeter scales, are under various forces involved in soft matter systems, such as electric and magnetic interactions between the colloidal particles or capillary forces, both on solid-liquid and air-liquid interfaces.^{7, 23-26, 29-32, 41, 49-52, 54, 80-97, 110, 116, 118-119, 133, 135, 138, 140-141, 153-156, 161-180, 182-184, 187-188, 199-203} In many cases, like-charged colloidal particles, suspended in liquid, repel each other through Coulomb interactions, and the corresponding interaction forces can be described by the famous theory of Derjaguin-Landau-Verwey-Overbeek, established more than 60 years ago.^{30-31, 163-166, 168-171, 244}

On the other hand, aggregation phenomena reported in the last two decades by several research groups, such as 2D colloidal cluster formation, have provided evidence for attractive interactions between the like-charged colloidal particles, and therefore led to an intense debate about the nature of such interactions.^{30, 33, 41-42, 48, 129, 132, 142-143, 146-152, 161} In fact, investigating the mechanisms of particle aggregation is very important in a variety of disciplines, e.g. manufacturing of paints and coatings as well as in fundamental research, where colloidal model systems are used to study crystal nucleation and structural phase transitions. In a colloidal model system, the colloidal crystallization phase diagram is analogous to that of atomic and molecular systems, but the process of phase transition can be visualized using microscopy thanks to the relatively larger sizes of colloidal particles. Recently, a great deal of effort has been put into the research on the mechanisms behind particle aggregation in colloidal systems, and several theories have been put forth in recent years.^{33-34, 39, 42-43, 45, 48, 132, 141-144, 146-152} However, the mechanism of particle aggregations has not been fully understood,

i.e. the origin of the attractive components of the interparticle interactions cannot be explained by the existing models, such as like-charge attraction, hydrophobic interactions or capillary attraction at the water-air or water oil interface.^{23-29, 142-146, 148, 151-152, 156-157, 161, 191, 217-220, 223-224, 227}

In this chapter, we report on direct imaging of the formation of colloidal cluster islands in aqueous droplet during the evaporation process in real time by means of microscopy. The colloids were micrometer-size negatively-charged magnetic polystyrene beads, coated with a monolayer of the streptavidin protein. The dynamical process of the cluster formation was characterized quantitatively in terms of the radial pair distribution function. The formation of clusters indicated the presence of attractive interactions between the colloidal particles. We also investigated the effects of magnetic field, salt and biotin concentration on the dynamical process of the colloidal cluster formation. Moreover, unique domain patterns were observed, ranging from colloidal islands to colloidal voids, when the repulsive interactions between the colloidal particles were varied by adjusting the external magnetic field.

4.2 Experimental details

Unless otherwise specified, monodisperse paramagnetic polystyrene beads coated with a monolayer of the protein streptavidin (Dynabeads M-270 Streptavidin, purchased from Invitrogen Dynal) were used in this chapter. The initial concentration of the colloids was 6.7×10^8 beads/ml, and the diameter of the bead was $(2.8 \pm 0.2) \mu\text{m}$. The initial magnetic susceptibility χ was about 0.5 and high-field susceptibility is 0.2 (ref. 93). The colloids were washed four times using deionized (DI) water and then diluted to a concentration of approximately 1.3×10^7 beads/ml.

The glass slides used in the experiments were purchased from Electron Microscopy Sciences. The whole slides were covered with Teflon except three

wells of depth ~ 0.1 mm and diameter ~ 8 mm. In order to generate negatively-charged substrate, the slides were first rinsed with 1 M NaOH for 30 minutes, followed by a water wash, then immersed in 1 M HCl for 10 minutes, and finally rinsed with water (see also refs. 181, 183, and 206).

Figure 4.1 Schematic drawing of the basic experimental setup. The colloidal droplet is confined by the well on the glass slide. The magnetic field is generated and can be adjusted by a current in the coil.

The schematic drawing of the basic experimental setup is shown in Fig. 4.1. In the experiment, a droplet containing colloids was inserted into a well on the horizontal glass slide. The droplet was circularly symmetric due to the geometrical confinement set up by the Teflon structured surface. A copper coil connected to a current source was positioned on the microscope stage to generate a magnetic field. The dynamics of colloidal particles and the pattern of cluster formation were visualized by an inverted microscope (Nikon TE 2000 with 10, 20 and 60 $\times$ objective of numerical aperture 0.70) with an attached CCD

camera (Nikon DS-5Mc), and the videos and images were stored in computer directly. The temperature was kept at $300 (\pm 3)$ K during the experiments.

4.3 Characterization of formation of magnetic colloidal clusters in evaporating droplets

Figure 4.2 Evolution process in a system of magnetic beads and formation of clusters. The left column is low-magnification images and the right is high-magnification images taken at the same time.

In the case of particles dissolved in DI water, the evolution process of the

colloid assembly on the glass slide without magnetic field could be divided into three stages before it dried up.

At the initial stage, the colloidal beads sank to the bottom of the well due to gravity. The beads were distributed randomly as illustrated in Fig. 4.2a. The time taken was dependent on the following factors: the volume of the droplet injected (from 10 μl to 100 μl); the effective gravitational interaction, i.e. the difference between the density of the colloidal particle and the density of the water; and the diameter of the colloidal particles. In our case, the volume of the colloidal solution for each experiment was 50 μl and the time required to finish the initial stage was about 10 minutes. At the end of the initial stage, most of the colloids sank to the bottom of the droplet and were levitated by a small distance ($<1 \mu\text{m}$) above the substrate due to the repulsion between the negatively-charged surfaces of beads and glass.

During the second stage, which lasted up to 20 minutes, colloidal particles were self-assembled into small clusters as shown in Fig. 4.2b, which indicated the presence of attractive interactions between the colloidal particles. As shown in the right image of Fig. 4.2b, the colloidal particles in the clusters were not yet well ordered.

During the third stage, most of the colloids aggregated into several big colloidal clusters as shown in Fig. 4.2c. In contrast to the second stage, colloids were now well ordered in each cluster. The ordered colloidal clusters could be called colloidal crystal islands; the distance between each pair of colloids was about 3.2 μm (or 1.1 times of their diameter) (Fig. 4.2c). Over time, two close colloidal islands could merge into one bigger cluster (Fig. 4.2d), giving it a circular shape.

To quantitatively characterize the colloidal assembly process, we computed the radial distribution function $g(r)$ of the colloidal system at different times. The

images were analyzed using the Adobe Photoshop and Matlab software. The location of the centers of beads could be determined by the center-locating algorithm with an accuracy ~ 50 nm. The details of $g(r)$ calculation were described in chapter 2 and in references 181, 189 and 190.

Figure 4.3 The radial distribution function $g(r)$ of the 2D colloidal system at different times after the droplet is injected into the well. The distance r_1 for the first peak of $g(r)$ is defined as the nearest most probable inter-particle spacing.

The radial distribution function $g(r)$ for the 2D colloidal assemblies at different evolution times are shown in Fig. 4.3. Three different stages can be distinguished based on the $g(r)$ function. At the first stage, i.e. 10 or 12 minutes after the droplet was injected into the well, $g(r)$ was almost flat, which implied that the colloidal distribution was random. At the second stage, the colloidal particles were driven closer together to form small clusters, i.e. the probability of finding other particles at a short distance from a test particle was increased (see Fig. 4.3, $g(r)$ plots at 16, 17 and 19 minutes). However, at this stage, there were still no ordered structures formed, and accordingly no discrete peaks were observed. At the third stage, e.g. at 24 or 35 minutes, clear long-range ordered structures were formed; discrete peaks were observed in the $g(r)$ plot. In fact, the

extent of ordered structure increased over time until a regular hexagonal colloidal assembly was formed (Fig. 4.2c), which could be seen in the corresponding $g(r)$ function with high and clear characteristic peaks (Fig. 4.3). It should be noted that for large values of r , the $g(r)$ function was calculated to be smaller than 1. This may be due to insufficient data points in the field of vision and difficulties in the colloidal density calibration (there were blank areas between colloidal islands).

Figure 4.4 The smallest most probable inter-particle spacing distance (red) and distribution function at that distance (black) at different evolution times. The lines are a guide for the eyes.

We defined the distance r_1 for the first peak of $g(r)$ as “the smallest (or nearest) most probable inter-particle spacing” (Fig. 4.3). To further explore the colloidal assembly process, we plotted this distance (r_1) and the distribution function at this distance ($g(r_1)$) as a function of time (Fig. 4.4, red and black curves respectively). The latter increased monotonously with time up to 35 minutes (Fig. 4.4) then gradually flattened when the formation of crystal clusters was completed (not shown). The former first decreased with time (particles moved closer to one another) until it reached a nearly constant value ($\sim 3.2 \mu\text{m}$).

4.4 Effect of magnetic field on the formation of colloidal clusters

To adjust the repulsive interactions between the magnetic beads, we varied the strength of the external magnetic field (perpendicular to the 2D colloidal system) by changing the current in the coil. Evolution processes of the colloidal assembly at two different external magnetic fields are illustrated in Fig. 4.5. The repulsive magnetic interactions generated between the magnetic colloidal particles were shown to significantly affect the colloidal assembly process.

Figure 4.5 Evolution processes of magnetic colloidal assembly placed in different magnetic fields. The direction of the applied magnetic field was perpendicular to the 2D colloidal system, and the intensities of the magnetic field were 230 A/m (left) and 460 A/m (right), respectively.

First, the aggregation process of the colloidal particles became slower than the case without the applied magnetic field. Second, voids structures were formed in the system rather than the colloidal clusters (islands). However, at the end of the whole evolution process, mainly colloidal clusters were formed in the system. Third, in the clusters, magnetic colloids were not well ordered as in the absence of magnetic field. Fourth, the colloids can jump on top of each other when the magnetic field exceeds a critical value. Of course, the repulsive interactions between the colloidal particles play important role, and which is the reason there are differences in the cases that with or without magnetic fields.

In order to obtain more quantitative characteristics of the colloidal assemblies, we calculated the radial distribution functions $g(r)$ at different evolution times for each magnetic field (Fig 4.6). The intensity of the applied magnetic field ranged from 230 A/m to 460 A/m. In general, $g(r)$ increased over time, but in contrast to the case when there was no magnetic field, only a few discrete peaks were observed over time (beads did not form ordered structure even in the clusters), which was caused partially by the distribution of colloidal particles over 3D instead of 2D (colloids jump on top of each other to form chains).

Figure 4.6 The radial distribution function of the 2D colloidal system at different evolution times in the presence of a magnetic field of (a) 230 A/m, (b) 345 A/m, and (c) 460 A/m.

The nearest most probable inter-particle spacing distance r_l was plotted as a function over the evolution time in Fig. 4.7. For each magnetic field, r_l decreased over time (beads getting closer to one another), approaching a final distance. However, the higher the applied magnetic field, the larger the value of

r_1 at each time point (clusters were formed at a slower rate). For example, the final distance of about $4.1 \mu\text{m}$ was observed for a magnetic field of 460 A/m , while this distance was $3.2 \mu\text{m}$ in the absence of magnetic field.

Figure 4.7 The nearest most probable inter-particle spacing distance vs. the evolution time at different magnetic fields. This distance is the position of the first peaks of $g(r)$.

Figure 4.8 The radial distribution function at the nearest most probable inter-particle spacing distance vs. the evolution time at different magnetic fields. This is the value of the first peaks of $g(r)$.

Plots of $g(r_1)$ as a function of time for different magnetic fields are shown in Fig. 4.8. For each magnetic field, $g(r_1)$ increased over evolution time. At higher magnetic fields, $g(r_1)$ increased at slower rates corresponding to slower rates of formation of colloidal clusters.

4.5 Effect of salt on the formation of colloidal clusters

At pH 7 both the surface of polystyrene colloidal particles and the coating protein streptavidin (isoelectric point 5) are negatively charged. To adjust the electrostatic interactions between the colloidal particles (and the interactions between the colloidal particles and the substrate), we investigated the effects of NaCl, in concentrations ranging from 0.2 mM to 40 mM, on the formation of colloidal clusters.

Figure 4.9 Evolution processes of magnetic colloidal assembly in the presence of 0.2 mM of NaCl.

Even at a very low NaCl concentration of 0.2 mM, the effect of salt was already obvious, as illustrated in Fig. 4.9. Here circular colloidal clusters still

could be formed, but the evolution process of the cluster formation was very slow and no ordered structures were observed within the clusters.

When the NaCl concentration was increased to 0.6 mM, the formation of colloidal clusters was further slowed down (Fig. 4.10). Some small colloidal clusters were observed in the system at 37 minutes, which grew larger over time. However, no clear colloidal islands were observed even after 100 minutes (Fig. 4.10).

Figure 4.10 Evolution processes of magnetic colloidal assembly in the presence of 0.6 mM of NaCl.

When the salt concentration reached 1.0 mM, in the central region of the well, no clear colloidal clusters were observed over time (Fig. 4.11, top row); however, in the outer region, close to the contact line at the edge of system, some loose colloidal clusters were formed after the evolution time of 60 min (Fig. 4.11, bottom row).

At the salt concentration of 10.0 mM, no colloidal clusters were observed in the whole droplet, even after 60 minutes of evolution time (Fig. 4.12). Further,

when the salt concentration reached 20 or 40 mM, the colloidal particles were stuck on the substrate immediately when they sank and touched the substrate (not shown).

Figure 4.11 Evolution processes of magnetic colloidal assembly in the presence of 1.0 mM of NaCl. Upper row is images in the center of the droplet; bottom row, images near the edges of the droplet.

Figure 4.12 Evolution processes of magnetic colloidal assembly in the presence of 10.0 mM of NaCl. Upper row is images in the center of the droplet; bottom row, images near the edges of the droplet.

The radial distribution function $g(r)$ of the 2D colloidal system in the presence of 0.2, 0.6 and 1.0 mM NaCl was calculated at different evolution times (Fig. 4.13). In each case, the formation of colloidal clusters can be seen in the $g(r)$

function by the appearance of a strong peak below 5 μm over time. However, high ordered structures of colloids were not seen (no discrete sharp peaks in $g(r)$ were observed as in the absence of salt).

Figure 4.13 The radial distribution function of 2D colloidal systems at different evolution times. NaCl concentration was (a) 0.2 mM, (b) 0.6 mM, and (c) 1.0 mM.

Figure 4.14 The nearest most probable inter-particle spacing distance vs. the evolution time for a colloidal system in the presence of various salt concentrations.

Figure 4.15 The radial distribution function at the nearest most probable inter-particle spacing distance vs. the evolution time for a colloidal system in the presence of various salt concentrations.

The inhibition of the colloidal clusters formation by the presence of salt could also be seen in the plots of the distance r_1 (smallest most probable inter-particle spacing) and radial distribution $g(r_1)$ as a function of time (Figs. 4.14 and 4.15). The inhibition caused by the presence of salt could attribute two reasons, one is that the electrostatic interaction between the colloidal particles were decreased, which in some cases is the origin of the attractive interactions between the like-charged colloidal particles (details are still in investigated), and the second reason is obvious and easy to understand, which is the presence of the salt decrease the repulsive interaction between the colloidal particles and the substrate, and therefore enhance the friction when colloidal particle move on the substrate.

4.6 Possible physical mechanism

The colloidal cluster formation can be described by the reaction-limited cluster aggregation model, i. e., colloidal particles can unbind from the cluster with a

certain probability and later rejoin the cluster during the aggregation process.³⁰²⁻³⁰⁵ However, the physical mechanism of the colloidal cluster formation observed here and the origin of all interactions in the system are not fully understood, but some insights have been gained from this work.

First, the micro fluidic flows, which are fuelled by the evaporation of the water, are necessary for the formation of the colloidal clusters. A preliminary experiment, in which the droplet was covered by a plastic tube to slow down the evaporation (Fig. 4.20), showed that the formation of colloidal clusters was also significantly “frozen”.

Second, the collisions between the colloidal particles assisted by the micro fluid flows would affect the distribution of the charges on the surface of the colloidal particles, and the two colloidal particles will stick together when they close enough and the charges on their surface would re-distribute simultaneously.

Third, the protein (Streptavidin) coating layer on the beads plays a role in the colloidal assembly. The protein streptavidin is macromolecules, and would form a soft thin shell on the surface of the colloidal particle, which could help to stabilize the colloidal clusters formed. In fact, if the colloidal particle coated with the small molecules, such as M-270 carboxylic beads which were coated with carboxylic functional groups, the formation of clusters was not observed (not shown). However, a treatment of the beads by salt (40 mM NaCl) (followed by washing with DI water) did not alter the capacity of the system to form colloidal clusters (not shown).

In the experiments, the colloidal aggregation always take place firstly on the outer region, and then in the central region of the droplet. In addition, the presence of an amount of salt affects the colloidal assembly behavior of the system because salt could screens the electrostatic interactions between colloids, and also between colloids and polymers (Streptavidin). The slowing down of the

colloidal assembly in the presence of high salt concentration might also partly be related to the evaporation of the droplet and to the ability of the colloids to move (freely) with the flows. Note that in these cases the beads were stuck on the substrate.

If the droplet of colloidal solution is uncovered, the colloidal particles in the inner region of the system will move outwards very quickly, forming a very thick circular band on the substrate at the end of the drying process. The dried colloidal systems could be used as the template to get some unique patterns, such as protein patterns described in the following chapter.

Figure 4.16 Schematic drawing of the experimental setup in which the evaporation rate of the colloidal droplet can be controlled.

4.7 Summary

In this chapter, we reported observations of the formation of the magnetic colloidal clusters in evaporating droplets. Here the colloidal particles were micrometer-sized paramagnetic beads coated with a monolayer of streptavidin. The pattern formation and the dynamics of the colloidal particles were directly observed by means of optical microscopy. The results on structures and dynamics of the colloidal clusters provided evidence that there were attractive interactions between the colloidal particles and that the cluster formation was assisted by fluid flows generated by the evaporation. We quantitatively

investigated the evolution process of the colloidal assembly at different applied magnetic fields, and different concentrations of the salt and biotin, through the radial distribution functions of the system. We also discussed possible physical mechanisms behind the cluster formation of our magnetic beads. Moreover, the estimation of the interaction based on the measurements at different magnetic fields is still in progress.

Chapter 5 Towards Controlling the Formation of Magnetic Colloidal Chains using Short Single-stranded DNA

5.1 Introduction

Colloidal materials, including 1D chains, 2D patterns and 3D sophisticated blocks, play important roles in the basic science research and technological applications, such as optics and photonics devices, biosensing and drug delivery systems.^{8-18, 54, 59-60, 69, 73, 78, 86, 91, 93, 95-96, 99, 101, 106, 119-120}

Recently, DNA has attracted much attention as versatile and convenient materials for nanotechnological applications^{207-213, 245-252}. For examples, DNA molecules were used as “glue” for self-assembly of colloids via bio-recognition is attracting great attention because of the many possibilities it offers for creating complex systems with molecular-level spatial precision and structural tenability, and therefore promise the potential in the design of new magnetic and plasmonic materials, cell-targeted delivery systems and biomolecular sensing.^{212-213, 245-252} To date, reported DNA-based colloidal assembly studies relied mainly on the formation of the Watson-Crick duplex.

In this chapter, we present evidence for the formation of chains of magnetic beads ($\sim 1.05 \mu\text{m}$) using short single-stranded DNA ($\sim 7.0 \text{ nm}$) as “glue”. Here the colloidal particle is of $\sim 1.05 \mu\text{m}$, which is smaller than the colloidal particles $\sim 2.8 \mu\text{m}$ used in chapter 3 and chapter 4. One reason is, the Brownian interactions imposed on the colloidal particles are proportional to the size of the colloidal particles, i. e., and it is easy to form colloidal chains for smaller colloidal particles. Another reason is it is better for the observation of the organization of the colloidal system if the colloidal particle is bigger.

Here we are interested in controlling (i) the length of the bead chains and (ii) the number of DNA molecules bridging each pair of beads. These are the initial steps to control the formation of colloidal assemblies using various DNA structures. These studies may allow us (i) to form new colloidal assembly architectures and also (ii) to study the structure, dynamics and other properties of the “DNA glue”.

5.2 Experimental details

Materials

Monodisperse paramagnetic polystyrene beads coated with streptavidin (Dynabeads Myone™ Streptavidin C1: supplied concentration, $7-12 \times 10^9$ beads/ml; magnetic susceptibility χ , 0.3; diameter, 1.05 μm) were purchased from Invitrogen Dynal. A 20-nt single-stranded DNA sequence containing biotin at two ends, 5’-/5Bio/TTT TTT TTT TTT TTT TT/3Bio/-3’, was purchased from 1st BASE (Singapore). Initial DNA concentration was 100 μM .

Experimental procedures and setup

Glass slides were prepared and streptavidin-coated magnetic beads were washed as described in Chapter 2.

For each experiment, the initial volume of the colloids was 2 μl , concentrated to 1 μl after washing and labeled as A. The colloids were supplied as a suspension of 10 mg/ml with binding capacity for free biotin of 2500 pmol /mg, and therefore 50 pmol of free biotin could bind to 2 μl of colloids.

The concentration of the Biotin-labeled DNA solution was 100 μM . In our experiments, the volume of Biotin-labeled DNA solution was within 0.3 to 3.0 μl range.

Biotin-labeled DNA was immobilized on the bead surface by mixing them for

5 minutes, and thereafter incubated for 120 minutes.

The beads were washed using DI water to remove the excess of DNA molecules after incubation. The water was removed at the end of this step to make the final volume about 1 μl , labeled as B.

The schematic diagram of the basic experimental setup for fabricating the colloidal chains is shown in Fig. 5.1. Two types of beads (A and B) were mixed in an equal amount, unless otherwise specified. The colloidal suspension was stored in a plastic tube, which was fixed in a bracket. A rare-earth permanent magnetic was placed under the tube to generate the magnetic field. The magnetic field was measured by a Gauss meter.

Figure 5.1 Schematic drawing of the basic experimental setup for fabricating the magnetic colloidal chains.

A droplet of colloidal solution was injected on a glass slide for the observation

by a Nikon microscope, as described in Chapter 2. We studied the effect of different parameters on the length of bead chain formed. Under appropriate experimental conditions, chains of up to six beads were observed.

5.3. Effect of the growth time on the colloidal chain length

At the magnetic field of (20 ± 3) mT, the length of the colloidal chains depends on the growth time, i.e. the time in which the magnetic beads are under the magnetic field. Examples are shown in Fig. 5.2.

Colloidal chains of different lengths were observed. Their length distributions for different growth times were plotted in Fig. 5.3. Longer chains were observed more often at longer growth time.

Figure 5.2 Images of magnetic colloidal chains formed under the magnetic field of 20 ± 3 mT for different growth times.

Figure 5.3 The length distribution of colloidal chains for different growth times under the magnetic field of 20 ± 3 mT. The distribution was calculated from various images taken in different area and each experiment was repeated 3 times.

Figure 5.4 The average length of the colloidal chains vs. growth time under the magnetic field of 20 ± 3 mT (with shaking and without shaking).

The increase in the number long chains over time could also be seen in the

plot of the average chain length vs. time (Fig. 5.4). During the preparation of bead chains, the solution could be shaken a few times to facilitate mixing of beads. However, this was found to only have a marginal effect on the length of the chains (Fig. 5.4).

5.4. Effect of the magnetic field on the colloidal chain length

A similar chain length and time-dependent behavior was also observed for the magnetic field of 30 ± 3 mT, as compared to 20 ± 3 mT (Fig. 5.5).

Attempts to form chains for over 10 hours at various magnetic fields showed that similar long chains could be formed under magnetic fields larger than 20 ± 3 mT, but not with the magnetic field of 10 mT (Fig. 5.6).

The length distributions of colloidal chains for different magnetic fields were plotted in Fig. 5.7. The distributions for magnetic fields larger than 20 ± 3 mT are similar, but very different from that of 10 ± 1 mT (Fig. 5.7). Similar conclusion can be drawn from the plot of the average chain length vs. magnetic field (Fig. 5.8).

Figure 5.5 The average length of the colloidal chains vs. growth time under the

magnetic field of 20 ± 3 mT and 30 ± 3 mT (without shaking).

Figure 5.6 Images of magnetic colloidal chains formed under different magnetic fields for >10 hours.

Figure 5.7 The length distribution of colloidal chains under different magnetic fields for >10 hours.

Figure 5.8 The average length of colloidal chains incubated for more than 10 hours under various magnetic fields.

5.5. Effect of the bead mixture on the colloidal chain length

Examples of colloidal chains formed for different A:B ratios are shown in Fig. 5.9. The A : B ratio of 1.0 is most favorable for formation of many long colloidal chains (Figs. 5.9-11).

Figure 5.9 Images of magnetic colloidal chains for different A:B ratios. The magnetic field was 20 ± 3 mT; growth time, 10 h.

Figure 5.10 The length distribution of colloidal chains for various A:B ratios. The magnetic field was 20 mT; growth time, 10 h.

Figure 5.11 The average length of the colloidal chains for various A:B ratios. The magnetic field was 20 ± 3 mT; growth time, 10 h.

5.6. Effect of the DNA density on the colloidal chain length

The DNA amount used for the DNA immobilization on the surface of beads B, hence the DNA density on the bead surface, was also found to affect the length

of the bead chains. However, adding more than 2.0 μl DNA solution did not further increase the length of colloidal chains (Figs. 5.12-13).

Figure 5.12 The length distribution of colloidal chains for various DNA amount used.

The magnetic field was 20 ± 3 mT; growth time, 10 h.

Figure 5.13 The average length of the colloidal chains for various DNA amount used.

The magnetic field was 20 ± 3 mT; growth time, 10 h.

5.7 Scenario for magnetic colloidal chain formation bridged by single-stranded DNA

From the experimental results described above, we could know that the several independent parameters, including the density of the DNA solution, the magnetic colloidal interactions, the growth time, and the ratio of A-type colloids with B-type colloids in the mixture, mainly determine the formation of the colloidal chains.

Figure 5.14 Schematic drawing (not in scale) of a pair of beads covered by DNA.

The density of the DNA solution determines the transition from A-type colloids to B-type colloids. According to the manufacturer, there are $\sim 10^5$ biotin binding sites available on the surface of each colloidal particle. Each bead can bind up to $\sim 10^5$ biotin molecules. However, the binding capacity of biotin-linked DNA on the bead surface is lower, e.g. each bead can only bind up to $\sim 10^4$ biotinylated 200-bp DNA. It is easy to understand that both biotin terminals of single-stranded DNA molecule will bind on the surface of the same particle if the DNA solution is quite dilute, and the increasing of the density of DNA solution will decrease the yield percentage of this kind of colloids, and

therefore increase the transition from A-type colloids to B-type colloids. As a result, the probability of the bridging events between two colloidal particles as illustrated in Fig. 5.14 will increase accordingly.

The formation process of colloidal chains is of course an aggregation process. There are mainly two kinds of aggregation process described in previous literature, one is diffusion-limited cluster aggregation and another is reaction-limited cluster aggregation. The first is observed when the aggregating clusters formation was caused by pure Brownian motion and form a stable bond as soon as physical contact between two colloidal particles is established. In reaction-limited aggregation, particles can unbind from the cluster with a certain probability and later rejoin the cluster, and in this way to produce the clusters. In fact, the aggregation process of the colloidal chains formation in this thesis cannot be described by the model of diffusion-limited cluster aggregation or reaction-limited cluster aggregation directly because here the aggregation process was mainly determined by the magnetic interactions between the colloidal particles and the chemical binding by DNA bridging.

Due to the electrostatic interactions between colloidal particles, there are no binding events take place after mixing A-type colloids with B-type colloids but did not apply magnetic field. In fact, there almost no aggregation of colloidal clusters because the attractive, mainly van der Waals attraction is quite small. If the strong magnetic field applied, there will be strong attractive interactions between colloidal particles and thus the colloidal chains are formed as illustrated in Fig. 5.15. The aggregation of the colloids chains is irreversible or not depends on the bridge types between the colloidal particles. If the DNA bridge A-type and B-type beads, which could call A-B bridging (Fig. 5.15), there will be the chemical binding formed by the single-stranded DNA molecules, and the aggregation is irreversible. The aggregation is reversible if the colloidal particles assemble as A-A or B-B order as illustrated in Fig. 5.15.

Figure 5.15 Scenario for DNA-based magnetic colloidal chain formation between A-type and B-type beads.

In principle, on the surface of B-type beads there should still be free binding sites. However, B-B bridging was almost not observed as only very few bead pairs were observed when there were only B-type beads in water solution. A possible reason could be a “brush” repulsion between DNA from two beads prevent them to approach each other (Fig. 5.14).

It is clear that the key point to form the colloidal chains is two colloidal particle bridged by the DNA molecules. As we discussed above, the attractive magnetic interaction could help overcome the repulsive interactions between the colloidal particles, and therefore to form the colloidal chains. The stronger the magnetic field applied, the closer the two colloidal particles are. So the weak magnetic field, i.e., 10 mt in our investigation, could not generate enough attractive interactions to make the inter-particle spacing less than 7 nm, and therefore the probability of the bridging events between two colloidal particles is small. However, the probability of the bridging events is also decreased if the magnetic applied is too strong, which because the DNA molecules could not stretch if the colloidal particles contact too close as shown in Fig. 5.6 and 5.8.

Figure 5.16 Schematic drawing of controlling the DNA density by pre-occupying streptavidin sites on the bead surface using free biotin molecules.

For B-type beads, the number of available binding sites on the bead surface is larger than the number of DNA-bound molecules; many DNA molecules might bind to the bead surface by their two biotin ends. Our preliminary results suggested that it was possible to control the number of available DNA-biotin ends, hence the connection strength of the A-B bridging by adjusting the amount free biotin added during the DNA immobilization step. These biotin molecules will occupy the binding sites on the surface of beads (Fig. 5.16). This approach may also be used to adjust the DNA density on the surface of beads.

5.8 Summary

In this chapter, the formation of magnetic colloidal chains bridged by 20-nt single-stranded DNA molecules was reported. The biomolecular recognition and magnetic interactions played important roles in the fabrication of the colloidal chains. Although the formation of colloidal chains reported here might depend somewhat on the experimental setup, the established effects of different parameters on the length of colloidal chains (including the time under the magnetic field, the strength of the magnetic field, the ratio between bead types A

and B, and the density of DNA on bead surface) are valuable, when we need to engineer different DNA-based colloidal structures. The proposed approach to adjust DNA density on the bead surface might be useful in the control of the number of DNA molecules, used as a “glue” to connect beads. In the future, we plan to study the structure, dynamics and other properties of this “DNA glue”.

Chapter 6 Colloidal Lithography of Protein Patterns

6.1 Introduction

Proteins are very important functional ingredients in a variety of food, health care and pharmaceutical products due to their ability to catalyze enzyme reactions, adsorb to surfaces, binding other molecules and form molecular aggregates.^{11, 70, 206, 253-256}

In recent years, a number of structures can now be routinely reproduced using various self-assembly techniques.^{51, 176, 191-193, 201, 280-283} Capillary, electric and magnetic forces have proven particularly useful for assembling structures ranging from nanometer to millimeter scale both on solid-liquid and air-water interfaces. To this end, colloidal lithography is a promising technique which has been used to pattern gold film, polymers and protein arrays.²⁸⁴⁻²⁸⁷ Colloidal lithography is able to create, e.g. microscopic rings and dots and therefore has a great potential as a technique for chemical pattern.²⁸⁴

Among different kinds of micropatterns and microarray in nano to micrometer scales, protein patterns have attracted much attention because they open up the possibilities for more sensitive fluorescence or electrical detection, and thereby play an important role in the development of biosensors, tissue engineering, and fundamental studies of cell biology.²⁵⁷⁻²⁶¹

The fundamental properties of the micropatterns and microarrays of protein are determined by the absorption of proteins to appropriate substrates, among which, adsorption on glass substrates is very important for biological detection, since in this way fluorescence microscopy can be easily used to do detection.^{206, 255, 257, 258, 261-264}

In this thesis, the absorption of protein on glass substrates using fluorescence microscopy was investigated. Bovine serum albumin (BSA) was used as a model protein because its structure and physico-chemical properties are well characterized, and can be bound to several small molecules such as fatty acids, dyes and amino acids.^{206, 254, 265-268} Positively charged substrates were produced by electrostatic self-assembly of a positively charged polyelectrolyte of poly(allylamine-hydrochloride) (PAH), which molecule has positively charged amino groups at pH 6-7.¹⁸³ Previous studies have shown that ionic surfactants may bind to protein molecules through a variety of physicochemical mechanisms in different concentration of surfactant.^{254, 262, 265, 266, 269-272} The BSA molecules were labeled with fluorescein isothiocyanate (FITC), and fluorescence microscopy was used to detect the amount of BSA absorbed.

In particularly, how the BSA protein molecule was adsorbed to a substrate coated with the positively charged polymer of PAH and covered by negatively charged colloids was investigated. BSA molecules were adsorbed to the polyelectrolytes, but were obstructed by the presence of the negatively charged colloids. Upon removing the colloids using ultrasound, it was observed that well-defined patterns could be created on otherwise uniformly coated substrates.

Furthermore, the adsorption of the protein and the protein pattern on substrate could be regulated by adjusting the salt or surfactant concentration of the solution containing the protein molecules.

The technique presented here reveals the conditions for optical adsorption of protein on glass substrate, which maybe have applications in biosensing system. Moreover, it could be applied to different biomolecules which can be immobilized by polyelectrolyte-coated substrate, and therefore offers a simple and cost-effective method for assembling biomolecular patterns without the use of expensive lithography techniques.

6.2 Experimental details

Materials: PAH (MW ~ 70000), FITC-albumin (Prod. No. A9771, albumin of MW 66000 labeled with FITC of MW 389.4), salt (NaCl, MW 58.4) and Polystyrene beads of radius $a=1.5\mu\text{m}$ were purchased from Sigma-Aldrich. SDS (MW 288.4) was purchased from Fisher Scientific. Carboxyl modified paramagnetic polystyrene beads of radius $a=1.4\mu\text{m}$ (Dynabeads M270) were purchased from Dynal. All of them were used as received, and the colloids were diluted to desired concentration using deionized (DI) water.

Equipments: The glass slides each containing 3/5 wells were purchased from Electron Microscopy Sciences as illustrated in Fig. 6.1. The surfaces of the glass slides are coated with Teflon everywhere except the wells. The diameter of the well is $\sim 8/7\text{ mm}$ and the depth is $\sim 100\mu\text{m}$. An inverted fluorescence microscope (Nikon TE2000U) equipped with proper wavelength filters for detection of FITC. The $60\times$ objective ($NA = 0.7$) and a Nikon DS-5Mc-US cooled CCD camera was used for imaging of the fluorescent patterns. The diffraction-limited resolution of the microscope is therefore about 370 nm.

Figure 6.1 Image showing the glass slides partially covered with Teflon and with each containing three uncovered glass wells as substrates. The green light is added to illustrate the fluorescent emission.

Treatment for glass substrate: The glass surface is negatively charged at neutral condition of $\text{pH} \sim 7$ due to the dissociation of silanol groups, but when this surface comes in contact with a polymer solution, positively charged molecules will be attracted to the surface forming an immobile layer. To get a glass surface covered uniformly with negatively charged ions, thus promoting polymer coating, the approach described in Section 2.2.1 were used (do not repeat to describe the details here).

Procedures for adsorption of protein: The basic procedures for absorption of protein on substrate are shown in Fig. 6.2. PAH was dissolved in DI water to the desired concentration, and then the resulting solution was injected into the well containing a negatively charged glass substrate (step 1). After 20 *min*, the slide was rinsed by DI water, and thus a thin layer of positively charged PAH thin film formed on the glass substrate due to the attractive electrostatic interactions (step 2). After this, 30 μl of 15 μM FITC-albumin solution was deposited on the glass well (step 3). About 5 *min* later, the slide was rinsed carefully in DI water, such that a thin layer of FITC-albumin was left on the PAH-coated substrate (step 4). In the investigation, these procedures were with different concentration of PAH from 14 to 286 μM at a constant concentration of FITC-albumin of 15 μM .

Figure 6.2 The procedure for adsorption of protein on glass substrate. Green color represents the biomolecules.

Mixing SDS with BSA: The serial experiments by mixing SDS with different concentrations and FITC-albumin with fixed concentration of $15\ \mu\text{M}$ were done. The concentration of the SDS varied between 0 to $10\ \text{mM}$, and the concentration of PAH solution used to treat the substrate in advance was kept as a constant of $286\ \mu\text{M}$. To check the influence of the SDS on the luminescent properties of FITC, the fluorescence spectra of BSA-FITC solutions mixed with different concentrations of SDS were measured by exciting it with a laser of $488\ \text{nm}$ wavelength and recorded the corresponding emission spectrum using an Ocean Optics spectrometer. The position of the emission peak is almost constant at $534\ \text{nm}$, and changing the concentration of SDS has only minor effects on the fluorescence emission intensity of FITC which do not influence our study of the SDS-controlled adsorption.

Procedures for colloidal lithography of protein patterns:

Two different procedures for lithographic protein patterning are shown in Fig. 6.3a and b. The first method is displayed in Fig. 6.3a. First the glass substrate well was covered with $10\ \text{g/L}$ PAH ($100\ \text{mg}$ PAH in $10\ \text{mL}$ deionized water) solution for $5\ \text{min.}$, followed by a thorough rinse in water. This left a thin layer of positively charged PAH on the glass slide (step 1). About $30\ \mu\text{L}$ of Dynabeads M270 solution was deposited on the horizontally aligned substrate. In less than $5\ \text{min.}$ the negatively charged colloids will eventually sink to the glass surface due to gravity where they are immobilized by the positively charged PAH-coated substrate. After waiting for $5\ \text{min.}$ the substrate was washed thoroughly in water (step 2). The upper surfaces of the colloids are negatively charged while the PAH-coated glass surface is positively charged, thus rendering a heterogeneously charged surface suitable for patterning of electrically charged molecules. The FITC-albumin was negatively charged, and therefore it is easily attached to the positively charged PAH-coated substrate. $30\ \mu\text{L}$ FITC-albumin $1\ \text{g/L}$ were deposited in the glass-well waited for about $5\ \text{min.}$ and gave the glass slide a thorough rinse of water. This procedure left a thin

layer of FITC-albumin on the PAH-coated areas not covered by colloids (step 3). This step (step 3) was repeated with FITC-albumin dissolved in different NaCl concentrations ranging from 0 M to 10^{-2} M NaCl. In the final step (step 4) we removed the paramagnetic colloids by sonication (5-10 min.), thus leaving areas not covered by albumin. The colloids could not be removed by a strong magnetic field gradient due to the strong interaction with the PAH-coated glass slide.

Figure 6.3 Schematic drawing of the basic steps for the two proposed lithography patterning processes (a) and (b). Green color represents the biomolecules.

The second method for generating lithographed area is shown in Fig. 6b. First, 30 μ L of polystyrene beads with concentration of 10^9 beads/ml. Within a few minutes the colloids sank to the glass surface due to gravity (step 1). After

waiting for several hours the water had evaporated and the particles formed a two-dimensional colloidal crystal. The substrate were washed thoroughly in water and covered the substrate and colloids with a PAH solution, followed by yet another thorough rinse in water (step 2). This left a thin layer of positively charged PAH on the parts of the glass slide not covered by colloids. The colloids were removed by sonication (step 3), thus leaving areas not covered by albumin open. Then we deposited $30\ \mu\text{L}$ of $15\ \mu\text{M}$ FITC-albumin in the glass-well, waited for about $5\ \text{min.}$ and gave the glass slide a thorough rinse of water (step 4). This procedure left a thin layer of FITC-albumin on the PAH-coated areas, while the negatively charged glass surface did not immobilize proteins.

6.3 Adsorption of bovine serum albumin on polyelectrolyte-coated glass substrate

Figure 6.4 Fluorescent images of the protein layer absorbed on the glass substrate when the concentration of the PAH is 0, 14, 28, 57, 143 and 286 μM (same exposure time and gain).

Figure 6.4 shows fluorescent images of the FITC-albumin layers absorbed on the glass substrates observed with the fluorescence microscope under the same

conditions (exposure time and gain). The camera was properly calibrated such that recorded brightness scales linearly with intensity. From the top down, the substrate was treated with the PAH solution at the concentration of 0, 14, 28, 57, 143 and 286 μM respectively. It was found that the intensity in each image was uniform, which means the thickness of the BSA layer absorbed on the substrate was homogeneous everywhere at different PAH solution concentration. When no PAH was added the fluorescent image is dark, which means there was no detectable BSA absorbed on the substrate. The other distinct feature of Fig. 6.4 is that the images became brighter when the concentration of PAH was increased, which means an increased absorption of FITC-albumin, since the intensity is directly proportional to the number of albumin molecules. Figure 6.4 also shows the difference between the two downmost images is small, which means that the amount of BSA absorbed to has reached saturation at about 286 μM .

Figure 6.5 The relationship between the average relative intensities of the fluorescent images and the concentration of PAH (black squares). The solid line is fitted to the experimental data using the expression: $P_c = 1 - e^{-NE_s/k_B T}$.

Quantitative analysis of the microscopy images was performed with MATLAB software whereby each of the average relative intensities were found by considering six different images taken at different substrates at the same PAH concentration. The relationship between the relative intensities of the fluorescent images and the concentration of the PAH is shown in Fig. 6.5 (dark squares) which is consistent with the trends seen by naked eyes in Fig 6.4. From Fig. 6.5, we can see that the saturated absorption of protein on the positively charged substrate took place when the concentration of PAH was $286 \mu M$.

Previous investigations have shown that the albumin has an isoelectric point at $pH \sim 5$, which means albumin can be absorbed on positively or negatively charged surface depending on the pH condition.²⁷³⁻²⁷⁶ At $pH \sim 7$, each albumin has a net charge of ~ 20 electrons,²⁷³⁻²⁷⁶ such that the electrostatic interaction between PAH and BSA molecules was attractive and therefore aids the absorption of BSA. This is consistent with our experimental results shown in Figs. 6.4 and 6.5. The thickness of the PAH layer was studied by atomic force microscopy and the maximum thickness is about 2 nm under the conditions used here,¹⁸³ which has also been demonstrated in other studies.^{277, 278} To obtain a semi-empirical model of the mechanism of absorption of protein on the substrate, we define a binding energy E_s between each single BSA molecule and PAH thin film. The probability of detachment of the BSA molecules from the PAH molecules is assumed to follow a Boltzmann distribution

$$P_d = e^{-NE_s/k_B T} \quad (6.1)$$

where N is the total number bonds, k_B is Boltzmann's constant and T is the temperature. Therefore, the probability of coverage of BSA is:

$$P_c = 1 - e^{-NE_s/k_B T} \quad (6.2)$$

The number of bonds is directly proportional to the concentration of the PAH, i.e. $N = a[\text{PAH}]$, where a is a constant. The solid line of Fig. 3 is fitted to the experimental data using Eq. (6.2), and $aE_s = 0.018 k_B T / \mu M$. The good agreement between experimental data and fitting result may suggest that the number of bonds is indeed proportional to the concentration of polyelectrolyte used to create the charged glass surface.

Figure 6.6 Fluorescent images of the protein absorbed on the glass substrates when the concentration of SDS is 0.1, 1-8 and 10 mM.

In order to further investigate how the protein coverage can be manipulated, how SDS influences the absorption of proteins on substrates was investigated. The previous studies demonstrated that there are strong bonding of ionic surfactants to protein by electrostatic interactions and the hydrophobic effect.^{254,}

262, 265, 267, 269-272 The interaction energy between the albumin charge and the electric field can be adjusted by changing the charge density of the surface of the BSA molecules. The FITC-albumin molecules were dissolved in SDS solution of different concentrations between 0 and 10 *mM*. After incubation for at least 1 hour, the mixture solutions were added into the wells of the substrate, which had been treated by PAH solution at the concentration of 286 μM in advance. The results shown in Fig. 6.6 illustrate that the fluorescent brightness, and therefore also the absorption of BSA molecules on the substrate, increased significantly when the concentration of SDS was increased from 0 to 3 *mM*. When the concentration of SDS was further increased, the absorption of BSA molecules decreased until almost no BSA molecules were observed at a concentration of 10 *mM*.

Figure 6.7 The relationship between the concentration of SDS and the average relative intensities of fluorescent image, the solid line is only a guide for the eye.

The measured relative intensities of the fluorescent images as function of SDS concentration are illustrated in Fig. 6.7. Since the SDS does not

significantly influence the fluorescence emission of light from FITC, we must look for alternative answers in order to explain why the relative intensity increases with SDS concentrations below 3 mM. At low concentrations of SDS the electrostatic interactions do not bind SDS and BSA's molecules together because both of them are negatively charged when $\text{pH} = 7$. However, the hydrocarbon tail of the surfactant can bind to the protein through hydrophobic attraction, which can slightly increase the negative charge on the surface of BSA and help the protein bind to the substrate.

Previous studies proved that this binding does not cause protein denaturation or aggregation but instead increases the thermal stability of the proteins.^{254, 265, 272} When the concentration was increased from 1 mM to 3 mM, the SDS continued to bind to the BSA molecules and caused the protein to be partially denatured. The denatured state of the protein can bind more surfactant molecules than the native state because the anionic head group is able to bind to the proteins' cationic group through electrostatic attraction, whereas the hydrocarbon tail of the surfactant can still bind to the protein's hydrophobic patch through hydrophobic attraction. In fact, each BSA molecule was surrounded by more than 10-15 SDS molecules in this range, so the SDS-BSA complex have stronger interaction with the PAH layer due to its increased negative charge.^{254, 272} Several investigations have concluded that the critical micelle concentration (CMC) of SDS in the buffer solution has important effects on the protein-surfactant interaction, which is about 2.5~3 mM.^{254, 267, 272} When the concentration of the SDS molecules was increased above 3 mM, the BSA was largely unfolded and that SDS continued to bind to the unfolded proteins to form micelle-like clusters. This causes the BSA molecules to conform to an extended structure with exposed hydrophobic residues.^{254, 272} At high concentration of SDS, the BSA molecules were completely extended, and a BSA-SDS complex of large volume could be formed, which would less efficiently adsorb to the polyelectrolyte-covered glass surface. Thus, from an

engineering perspective the maximum amount of BSA could be absorbed by mixing it with SDS at 2 mM.

6.4 Colloidal lithography of protein pattern

Figure 6.8 Colloidal chains of paramagnetic polystyrene spheres on a substrate (a). After following the steps of Figure 6.3a, the FITC-albumin patterns corresponding to the original colloidal assembly was obtained (b).

The conjunction of the technique of protein adsorption with the technique of the colloidal lithography could easily create charged surface and structuring of charged molecules. Because the polyelectrolyte immobilizes negatively charged colloids, thus it was rather straightforward to create one- and two-dimensional arrays.

In the presence of an external magnetic field $H \approx 3 \text{ kA/m}$ obtain by a small permanent magnet placed next to the glass slide during the deposition of the colloids; the colloidal chains of paramagnetic particles could be immobilized on the substrate as illustrated in Fig. 6.8a. By following the procedure of figure 6.3a, the one-dimensional fluorescent pattern as shown in fig. 6.8b was created.

Figure 6.9 Two-dimensional colloidal arrays of polystyrene sphere on a glass substrate (upper image and the corresponding BSA-FITC protein patterns. The scale bar is 10 μm .

In addition to using the paramagnetic colloids, other charged colloids could also be used for creating two-dimensional arrays. By following the procedure of figure 6.3b, one example could be got as shown in fig. 9. First, The PAH solution was added on the surface of the colloidal array and the substrate, and then remove the excess of the PAH by washing, followed by added BSA-FITC solution and rinsed the colloidal system 5 min later. At the end, the BSA-FITC pattern with heterogeneously charged surface was got by removing the colloidal particles by sonication.

There were no changes in the fluorescent patterns when the substrates were stored in darkness under dry conditions for several days, which means that the fluorescent patterns are stable.

6.5 Influence of salt on colloidal lithography of protein

Figure 6.10 Fluorescent patterns on a PAH-coated substrate observed after removing the colloid. The FITC-albumin was dissolved in 0 M (a) and 10^{-2} M NaCl (b), respectively. The contrast of the images has been enhanced for clarity.

Figure 6.10 shows typical images of the BSA-FITC patterns that could be observed with the fluoresce microscope when dissolving BSA-FITC in 0 M (a) and 10^{-2} M NaCl respectively. The areas not covered by FITC-albumin are dark, whereas the covered areas are bright green. It is seen that characteristic rings show up for 0 M NaCl, where the bright ring diameter is comparable to the diameter of the colloids with an average value $(2.5 \pm 0.4) \mu\text{m}$. These rings disappear when the salt concentration is 10^{-2} M, thus leaving only a dark hole. In the areas not originally covered by colloids (see Figure 6.10), we observed that the fluorescence intensity did not change with the salt concentration, thus suggesting that the amount of albumin adsorbed here was independent of the ions in the solution.

To this end, the fluorescent patterns observed in Figure 6.10a suggest that

many albumin molecules concentrate here to form a bright ring near the edge of the charged, spherical colloid. On the other hand, there are fewer albumin molecules directly under the colloid and far away from it. Thus, it is plausible that the molecules are trapped due to an electrostatic field gradient. In order to investigate whether electrostatic forces are responsible for the bright ring, a series of experiments that FITC-albumin dissolved in solutions of increasing NaCl concentrations were done, and the results indicated that the bright ring disappeared completely (see also Figure 6.10b) when the salt concentrations were above 5×10^{-3} M. It should be mentioned that, in order to display a clear image, we have enhanced the brightness and contrast of Figure 6.10b, and the gray levels seen should therefore not be compared to Figure 6.10a.

Figure 6.11 Contrast as a function of NaCl concentration. Each measurement is an average over typically 30 patterns, with the error bars denoting the corresponding standard deviation.

A measure of the ratio between the number of molecules near the boundary of the colloid to the number of molecules directly under the colloids (dark region) can be obtained by defining the contrast as the difference between peak intensity

(I_p) and center intensity (I_c) divided by the average of the two, i.e.,

$$C = 2 \frac{I_p - I_c}{I_p + I_c} \quad .$$

(6.3)

Figure 6.11 shows the contrast as a function of salt concentration ion concentration c . It is seen that the contrast initially decreases strongly with salt concentration, thus indicating that fewer and fewer albumin molecules are trapped in a ring formation as more salt is introduced. At a salt concentration of 5×10^{-2} M, the fluorescent patterns were seen to disappear completely, thus suggesting that the colloids no longer were able to obstruct the deposition of albumin.

Figure 6.12 Schematic drawing of the electrostatic trapping geometry, where an electrical field is set up between the positively charged PAH-coated glass substrate and the negatively charged polymer sphere. Also draw is the structure of PAH (on the left side of the picture).

The interaction between albumin and polyelectrolytes has been investigated in a number of studies, where it has been shown that albumin adsorbs to both positively and negatively charged surfaces.^{276, 293-297} Thus, trace amounts of

albumin molecules will also adsorb to the paramagnetic colloids covered with negatively charged carboxylic acid groups as well, in agreement with what we observed in our fluorescence studies. Thus, care should be taken, since albumin has both hydrophobic and hydrophilic sites, despite its net negative charge. Here, we will only be interested in how the albumin molecules distribute spatially on the PAH-coated substrate. For $\text{pH} \sim 7$, the molecular dimensions of BSA molecule are $5 \times 5 \times 9 \text{ nm}^3$, and it should be possible for this molecule to penetrate far under the spherical polymer spheres of diameter $2.8 \mu\text{m}$ if they were not influenced by any external forces. From the geometry of the system seen in Figure 6.12, it is obvious that a spherical molecule of diameter D will be geometrically obstructed at a distance $s \approx \sqrt{x^2 + y^2}$ approximately given by $(a-R)^2 + s^2 = a^2$, which in the case of $D \ll a$ gives $s \approx \sqrt{2aD}$.^{286, 287} A molecule of size 5 nm should therefore in principle be able to move to a position $s \approx 118 \text{ nm}$.

It should be noted that PAH is a positively charged polymer of molecular weight of about 70 000. Each of the about 750 monomers has a molecular weight of 93 and a typical length of 1 nm , with a calculated radius of gyration of about 50 nm .³⁰⁰ Once the positively charged polymer collapses on the negatively charged surface, it will comprise a thin film of nanometer thickness; see ref 183. However, it is reasonable to believe that the charge density is larger for small s , since the PAH chains will be drawn toward the carboxylic groups coating the colloids, thus extending slightly above the substrate as seen in Figure 6. 12. In the region where this happens, there is a larger density of positively charged ions, which may attract and stop the motion of negatively charged biomolecules.

In an electrolyte, the Debye length plays a natural role and is given by

$$l_D = \frac{1}{k} = \sqrt{\frac{\epsilon k_B T}{2e^2 n}}$$

(6.4)

where ϵ is the permittivity of water, k_B is Boltzmann's constant, T is the temperature, e is the electron charge, and n is the density of ions. The Debye length tells how effectively the charges of the albumin, PAH, and COOH^- are screened and is seen to decrease with increasing salt concentration, and is smaller than 100 nm at salt concentrations exceeding 10^{-5} M. It could be assumed that the rings occur due to capture of negatively charged albumin molecules by positively charged PAH molecules. Then, an increase in salt concentration would screen the charges and allow the albumin to penetrate further under the colloids before being captured. The bright rings disappeared at salt concentrations exceeding 5×10^{-3} M, and it is therefore reasonable to assume that this is due to the fact that electrostatic attraction has been reduced considerably due to electrostatic screening. Since the dimension of the albumin molecule is about 5 nm, it is allowed to move to a distance $s \approx 118$ nm before it is geometrically obstructed. The fact that the contrast goes to zero when the salt concentration increases above 5×10^{-2} M suggests that the colloid is not in contact with the glass slide, but is lifted a small distance above it due to the PAH coating. Thus, for larger salt concentrations the electrostatic attraction between albumin and PAH is screened out, and the albumin molecules can move more or less freely under the colloids.

6.6 Summary

In this chapter, the protein patterns based on adsorption of protein on substrate and colloidal lithography were reported. The experimental results indicate that the adsorption of protein of bovine serum albumin (BSA) on glass substrates can be controlled by adjusting the pre-coated polyelectrolyte layer of poly (allylamine-hydrochloride) molecules and adding the surfactant sodium

dodecyl sulphate or salt of NaCl in the BSA solution. Furthermore, the well-defined protein patterns based on the adsorption of protein on substrate and colloidal lithography were created successfully. The technique presented here can deepen the understanding of processing conditions and material composition on the absorption of protein on substrates, and therefore allow one to optimize such structures for biosensing applications. The technique can be applied to different biomolecules which can be immobilized by polyelectrolyte-coated substrates, and therefore offers a simple and cost-effective method for assembling biomolecular patterns without the use of expensive lithographic techniques.

Chapter 7 Conclusions and Future Perspectives

In this thesis, the assemblies of micrometer-size magnetic colloids and their applications in bioscience were investigated by microscopy. It explores four topics:

(1) We studied the phase ordering phenomena in a two-dimensional (2D) colloidal system by tuning the electro- and magnetostatic interparticle interactions. The crystallization process of the 2D colloidal system was characterized quantitatively in term of the radial pair distribution function. A subtle competition between the repulsive interparticle interactions and the thermal excitations of the particles was found to determine the crystallization of the colloidal system. We also observed the order-disorder transition (from 2D to 3D) of the colloidal system, when the magnetic interaction between colloids was higher than a critical value. The relationship between the critical magnetic field for phase transition and the density of colloids was determined experimentally and analyzed theoretically.

The method in our investigation of the 2D crystallization processes of charged magnetic colloidal particles can be applied to study a variety of phenomena in condensed matter physics, giving further insights in phase transitions of colloidal systems. For applications, the experimental principles presented here can be used to fabricate colloidal crystals; colloidal phase transitions can be used to monitor molecular interactions.

(2) We observed the formation of 2D clusters of protein-coated colloids in evaporating aqueous droplets, indicating the presence of attractive interactions between colloidal particles. The dynamical evolution of the cluster formation and the effects of different experimental parameters (e.g. magnetic field and salt concentration) on this process were characterized quantitatively using the radial

distribution function. Some unique domain patterns, from colloidal islands to colloidal voids could be created when the repulsive interactions between the colloids were adjusted by tuning the magnetic interactions.

Further experiments with varied experimental conditions can be performed to explain the origin of the attractive interactions between the colloids. For example, controls of evaporation rates will be required. The interactions between colloids can be derived from the record of their movements. For applications, the colloids can be coated with different biomolecules, such as membrane proteins, in order to mimic the interactions between cells.

(3) We formed magnetic colloidal chains bridged by short biotin-ended single-stranded DNA, and guided by the magnetic interactions between colloidal particles. The length distribution of magnetic colloidal chains could be controlled by adjusting the experimental parameters (e.g. magnetic field, time under magnetic field, DNA density on colloids' surface). A possible method to control the connection strength between two colloidal particles by pre-occupying the sites on the surface of the colloidal particles with biotin molecules has been proposed.

Formation of DNA-based magnetic colloidal chains is an initial step in our program to control the formation of colloidal assemblies using various DNA structures. These studies may allow us (i) to form new colloidal assembly architectures and also (ii) to study the structure, dynamics and other properties of the "DNA glue". DNA mediated magnetic colloidal chains or blocks can be used in bio-architecture or micro-devices.

(4) We investigated conditions for protein adsorption on glass substrates and successfully created protein patterns using colloidal lithography. The adsorption of BSA protein labeled with FITC on glass substrates could be controlled by (i)

adjusting pre-coated polyelectrolyte layer of PAH molecules, (ii) adding the surfactant of SDS or salt in the BSA solution.

Colloidal lithography of protein patterns can be used in the design of biochips for testing different biological activities. The protein adsorption techniques presented in our experiments can be extended to other biomolecules.

List of Author's Publications

1. **Y. H. Miao**, D. L. Geng and L. E. Helseth (2006) Order-Disorder Transition in a Quasi-Two-Dimensional Colloidal System, *Langmuir*, 22, 5572.
2. D. L. Geng, **Y. H. Miao** and L. E. Helseth (2007) Influence of salt on colloidal lithography of albumin, *Langmuir*, 23, 8480.
3. **Y. H. Miao** and Y. H. Liu (2008) Crystallization of charged paramagnetic colloidal grains, *Phys. Scr.*, 78, 035802.
4. **Y. H. Miao** and L. E. Helseth (2008) Adsorption of bovine serum albumin on polyelectrolyte-coated glass substrates: Applications to colloidal lithography, *Colloids and Surfaces B: Biointerfaces*, 66, 299.
5. **Y. H. Miao**, A. T. Phan, et al. (2010) Formation of magnetic colloidal clusters in evaporating droplets (in preparation).
6. **Y. H. Miao**, A. T. Phan, et al. (2010) Towards controlling the formation of magnetic colloidal chains using single-stranded DNA (in preparation).

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