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LABEL-FREE DETECTION OF BIOMOLECULES ON MICROARRAYS USING SURFACE-COLLOID INTERACTION

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Introduction

Microarrays are rapidly advancing the technology for analysis of expression of tens of thousands of genes in a single experiment by employing hybridization of target genes to probe molecules immobilized on solid surface [1–3]. DNA microarrays usually are small glass, plastic, silicon substrate, or nylon membrane onto which the probe molecules are tethered in the arrays of spots. Each array's spot carries probe molecules specific to one particular gene. The target molecules contained in a sample solution are captured on the array by hybridization to corresponding homologous probe molecules on the array surface. To quantify the number of captured targets on each array spot, the target molecules are usually modified (labeled) by chemical attachment of a fluorescent group [4,5]. The intensity of fluorescence detected from the array spot is the measure of gene expression, i.e., the measure of the abundance of the corresponding mRNA specie in sample solution [6]. Currently, the need for chemical modification of target molecules is a significant limitation of the microarray technology: (1) chemical modification of targets introduces inaccuracy of quantification due to labeling biases, (2) the chemical labeling requires expensive labeling reagents, and finally (3) the labeling process is time consuming and is often accompanied by a significant loss of sample materials during the post-labeling purification steps.

Recently we have reported development of the microarray system for gene expression analysis using cationic gold nanoparticles [7,8]. This approach is significantly different from the previous methods of using gold nanoparticles with oligonucleotides attached to the gold particles for sequence-specific recognition of targets [9,10]. In our approach there is no need to attach a sequence-specific target-recognition agent to the particle surface. The nanoparticles precipitate, under selected conditions, on the hybridized array spots due to electrostatic (e.g., ionic) attraction of the cationic particles and the anionic phosphate groups in the target DNA backbone. The most important aspect of this detection approach is that no covalent chemical modification of target molecules is required for detection. Furthermore, the number of hybridized molecules can be quantified by detecting absorption or light scattering by nanoparticles using a flatbed scanner instead of expensive confocal laser scanners required by conventional fluorescent microarray technologies. The ionic labeling by nanoparticles greatly simplifies procedures and reduces the cost of microarray analysis. In this report we follow up our previous demonstration of the method for label-free detection of DNA hybridization [8], and further investigate the physical chemistry of interaction of colloidal particles and the

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microarray surface. Semi-quantitative models are developed for optimization of reagents and reaction conditions for label-free detection of DNA, RNA, and proteins on microarrays.

Materials and Methods

The synthetic oligonucleotide probe is a non-modified oligonucleotide having the sequence (5'- caagcttatcactggctggtgttttacaacgtcgtgactgggaaaattct - 3'); this oligo was synthesized and PAGE-purified by AlphaDNA (Quebec, Canada). The 7,229-nt long M13mp18 phage DNA was purchased from Sigma (Cat. No. D-8410, Sigma-Aldrich, St. Louis, MO). The oligonucleotide and DNA were dissolved in a Corning spotting buffer (Corning Life Sciences, Acton, MA) and printed on a Corning UltraGAPS glass slide (Cat. No. 40016) or on a positively charged membrane (HybondTM-N+, Amersham Biosciences, UK) with the SpotBot personal array printer (TeleChem International, Inc., Sunnyvale, CA). The Corning UltraGAPS slides are uniformly coated by amine groups that carry positive charges in aqueous solution and ionically attract nucleic acids to the slide surface. The SpotBpt printer delivered 10 nl of spotting solution to each spot. After printing, the arrays were dried in a dessicator overnight. The nucleotides were then covalently bound to the substrate surface by exposure to 600-mJ UV light (UV Stratalinker 1800, Stratagene, La Jolla, CA). The cationic 40-nm and 250-nm gold particles are from the AuroGene detection kits (Cat. Nos. AG10 and AG12, Sci-Tec, Inc., Knoxville, TN). The solutions of colloidal particles were prepared in buffers at desirable pH and ionic strength as described below. All other reagents were of the biology grade and were used within the manufacturer's product expiration date.

Results

Models and Analysis

Surface-Colloid Interaction—The interaction between electrically charged colloidal particle and the microarray surface is driven by two main parameters: the net electric charge of the particle, Z_{Part} , and the local density of electric charge, σ_{Surf} , on the microarray surface in the area covered by the particle's footprint as illustrated in Figure 1A. In general, the density of electric charge on solid surface, either the particle or substrate, is given by the sum of electric charges of all ionized chemical groups within the surface unit area. With respect to microarrays manufactured on aminated glass substrates [11], in aqueous solutions the charge-determining ions are the negatively-charged ions of silicon oxide of the glass substrate SiO^- , the positively-charged amino-silane ions R-NH_3^+ , and the negatively-charged phosphate groups PO_4^- of the DNA backbone[12,13]:

The density of the electric charge on the array surface, σ_{Surf} , is given by the sum of the charges of positive and negative ions on the surface:

$$\sigma_{\text{Surf}} = e\Gamma_{\text{NH}_3^+} - e\Gamma_{\text{SiO}^-} - e\Gamma_{\text{PO}_4^-} \quad (4)$$

where $\Gamma_{\text{NH}_3^+}$, Γ_{SiO^-} , and $\Gamma_{\text{PO}_4^-}$ are the surface density of the respective ions, and e is the protonic charge. From the mass action law for protonation and deprotonation reactions:

$$\Gamma_{\text{NH}_2} / \Gamma_{\text{NH}_3^+} = 10^{-\text{pK}_{\text{NH}_3^+} + \text{pH}} \quad (5)$$

$$\Gamma_{\text{SiO}^-} / \Gamma_{\text{SiOH}} = 10^{-\text{pK}_{\text{SiO}^-} + \text{pH}} \quad (6)$$

$$\Gamma_{\text{PO}_4^-} / \Gamma_{\text{PO}_3\text{OH}} = 10^{-\text{pK}_{\text{PO}_4^-} + \text{pH}} \quad (7)$$

where pK is the logarithmic dissociation constant of the respective reaction and pH is the common logarithmic notation for activity of protons. For each type of ions on the surface:

$$\Gamma_{\text{Amin}} = \Gamma_{\text{NH}_2} + \Gamma_{\text{NH}_3^+} \quad (8)$$

$$\Gamma_{\text{Si}} = \Gamma_{\text{SiO}^-} + \Gamma_{\text{SiOH}} \quad (9)$$

$$\Gamma_{\text{DNA}} = \Gamma_{\text{PO}_4^-} + \Gamma_{\text{PO}_3\text{OH}} \quad (10)$$

where, with respect to amino-modified glass slides, $\Gamma_{\text{Amin}} = 90 \text{ pmole/cm}^2$ to 300 pmole/cm^2 is the range of densities of amine groups on amino-modified glass surface [11]; $\Gamma_{\text{Si}} = 1.3 \text{ nmole/cm}^2$ is the Si density on a glass surface [12]; and Γ_{DNA} is the density of nucleotide phosphate groups inside the particle footprint (see Figure 1). For the 20 nm colloidal particle in low ionic strength solutions, the particle's footprint area is equal to the particle cross-section: $\pi(20 \text{ nm}/2)^2 = 314 \text{ nm}^2$. At the typical density of probes immobilized on microarray surface of 1 pmole/cm^2 , the average distance between two neighboring probe molecules is 12.5 nm and is comparable to the size of the colloidal particle or particle footprint [14]. In absence of probe and target molecules on the substrate we have $\Gamma_{\text{DNA}}^{(I)} = 0$. For probe molecules of 50 bases long and the density of probes on the surface of 1 pmole/cm^2 the density of phosphate groups inside the particle footprint for 50-base long oligo is $\Gamma_{\text{DNA}}^{(II)} = \Gamma_{\text{Prob}} = 26 \text{ pmole/cm}^2$. Capturing target DNA of ~1200 bases long increases the number of phosphate groups inside the footprint area. Assuming the target has a globular shape on the surface, the density of phosphate groups on the surface with the bound target DNA is: $\Gamma_{\text{DNA}}^{(III)} = \Gamma_{\text{Targ}} = 660 \text{ pmole/cm}^2$. The Eqs. (4)–(10) can also be applied to estimate the density of the surface charges carried by colloidal particle. For a silicon-oxide particle modified by attaching positively charged polymer:

$$\sigma_{\text{SiO-Part}} = e\Gamma_{\text{NH}_3^+} - e\Gamma_{\text{SiO}^-} \quad (11)$$

where $\Gamma_{\text{NH}_3^+}$ is the density of ionized amino groups on the surface of colloidal particle. For gold colloidal particles encapsulated by a layer of positively charged polymer:

$$\sigma_{\text{Au-Part}} = e\Gamma_{\text{NH}_3^+} - \sigma_{\text{Au}} \quad (12)$$

where $\Gamma_{\text{NH}_3^+}$ is the density of ionized amino groups and σ_{Au} is the density of the negative charge carried by non-modified gold particle in aqueous solution [15]. For the 20 nm gold particle in aqueous solution having Zeta-potential of 30 mV, the surface charge density estimated from the Graham equation is $\sigma_{\text{Au}} = 89.6 \text{ mC/m}^2$ [12,15]. The energy, E , of interaction of the colloidal particle and the substrate surface is proportional to the multiplication of the electric charge densities of the substrate and the net charge of colloidal particle

$$E \sim \sigma_{\text{Surf}} Z_{\text{Part}} \quad (13)$$

The Eqs. (4)–(13) represent a “zero-order” approximation for modeling the interaction of colloidal particles with various areas on a microarray substrate. This simplified model does not include many important features such as activity factor for proton on surface (Gouy-Chapman model, [16]), the structure of the solid-liquid interface (Stern model, [17]), and redistribution of mobile charges in solution (Boltzman-Poisson equation [18]), which are described by the Gouy-Chapman-Stern-Graham (GCGS) model for modeling surface charge on a solid-liquid interface [12,16–18]. Nonetheless, Eqs. (4)–(13) introduce a helpful tool to illustrate the basic features of the binding colloidal particles on microarray surface. Figure 2A shows titration curves, i.e., dependence of the surface charge vs. the solution pH for three substrate areas: (I) the surface with no DNA molecules attached; (II) the substrate carrying a 50-base long probe molecule; and (III) the substrate carrying the probe hybridized with a 1200-base long target molecule and (IV) for gold colloidal particle encapsulated by NH_3^+ layer. As the density of Γ_{Amin} on the microarray substrate exceeds a certain threshold value, the substrate surface exhibits amphoteric behavior: at high pH the surface is negatively charged, and at low pH the surface carries positive charge. The pH value at which the surface has no net electric charge (Zero Charge Point) is marked in Figure 2A by pI. The relation between the Zero Charge Point values of the colloidal particle, pI_{Part} , the probe, pI_{Probe} , and target areas on the surface, $\text{pI}_{\text{Target}}$, plays the key role in determining the conditions at which colloidal particles can bind to microarray surface. Figure 2B shows the binding energy, E , of a colloidal particle and the array surface vs. the solution pH. In Figure 2B the $E > 0$ corresponds to the repulsive interaction of the surface and particle, which takes place when both the surface and particle carry the same sign electric charge. No particle-surface binding is expected at pH for which $E > 0$. The negative binding energy $E < 0$ in Figure 2B represents an attraction of the opposite electric charges carried by particle and array surface. Particles can bind to the surface if solution pH is in the range of $\text{pI}_{\text{Target}} < \text{pH} < \text{pI}_{\text{Part}}$ (see Figure 2B). Of special importance for microarray applications is the existence of experimental conditions at which colloidal particles bind to microarray substrate only if target molecules are present and are repelled from the surface carrying no DNA or having only probe molecules on the surface. This detection regime can be achieved under condition $\text{pI}_{\text{Target}} < \text{pI}_{\text{Probe}} \leq \text{pI}_{\text{Part}}$ by adjusting solution pH according to the requirement: $\text{pI}_{\text{Target}} < \text{pH} < \text{pI}_{\text{Probe}}$. The conditions for target-specific detection are universal and remain the same after including into consideration the surface potential and ion

mobility in solution. The parameters pI_{Target} , pI_{Probe} , and pI_{Part} are determined by surface chemical composition (i.e., pK value), the density of ionizable groups of probe/target complex on the microarray substrate and by the density of ionizable groups on the surface of colloidal particles. Amino-modified substrates from commercial vendors have been reported to have pI in the range of 6.5–7.5 and the density of amino-silane on surface of 90 pmole/cm² – 300 pmole/cm² [11]. Cationic gold particles can be prepared in broad range of pI_{Part} by using various activation reagents and by adjusting the concentration of the activation reagent [15]. Two other important parameters for surface-colloid interaction are the size of colloidal particles and the concentration of buffer ions in solution (e.g., solution ionic strength). However, for accurate consideration of the effects of these parameters on surface-colloid binding energy the model has to be refined to include the effect of redistribution of ion concentration near the charged microarray substrate and nanoparticle surface.

Gouy-Chapman-Stern-Graham model—The model given by Eqs. (4)–(13) can be generalized by substituting pH in Eqs. (5–7) to include redistribution of mobile ions in solution and the effects due to double-layer structure of the surface-liquid interface (Gouy-Chapman-Stern-Graham model [12]):

$$pH \rightarrow pH - (\psi_d e / k_B T) \ln(10) - (e \sigma_{\text{Surf}} / C_{\text{Surf}} k_B T) \ln(10) \quad (14)$$

where ψ_d is the potential of the diffuse layer on the array surface; $k_B T$ is the thermal energy; C_{Surf} is the capacitance of the surface-liquid interface; and σ_{Surf} is the charge density given by Eq. (4). To have a complete set of equations an additional functional relation between the potential of the diffuse layer, ψ_d and the surface charge, σ_{Surf} , is required, which can be obtained from the Poisson-Boltzman equation [16–18]. For the flat isolated surface the relation between ψ_d and σ_{Surf} , is given by Graham equation [17,18]:

$$\sigma_{\text{Surf}}(\psi_d) = (\epsilon_0 \epsilon k_B T \lambda_D / e) \sinh(\psi_d e / 2 k_B T) \quad (15)$$

where λ_D is the Debye screening length in solution: $\lambda_D = (\epsilon_0 \epsilon k_B T / 2 e^2 n)^{1/2}$, n is the concentration of small ions in bulk solution, and $\epsilon_0 \epsilon$ is the permittivity of the solution. The pH substitution and generalized Graham equation for colloidal particle of radius a [12]:

$$pH \rightarrow pH - (\psi_{\text{Part}} e / k_B T) \ln(10) - (e \sigma_{\text{Au-Part}} / C_{\text{Au-Part}} k_B T) \ln(10) \quad (16)$$

$$\sigma_{\text{Au-Part}}(\psi_{\text{Part}}) = (\epsilon_0 \epsilon k_B T / \lambda_D e) \{ \sinh(\psi_{\text{Part}} e / 2 k_B T) + (\lambda_D / a) \tanh(\psi_{\text{Part}} e / 2 k_B T) \} \quad (17)$$

In solutions, the binding energy, E , of the surface and charge q separated from the surface by distance x : $E(x) = q \psi_d e (-x / \lambda_D)$. For colloidal particles of the size $2a$ with uniformly distributed electric charge, the energy of interaction with the charged surface is given by:

$$E = \int q(x) \psi_d e (-x / \lambda_D) dx = 2\pi \sigma_{\text{Au-Part}} \psi_d a^2 e (-a / \lambda_D) \left\{ (2\lambda_D / a) + 4 / (a / \lambda_D)^3 \sinh(\lambda_D / a) - 4 / (a / \lambda_D)^2 \cosh(\lambda_D / a) \right\} \quad (18)$$

where $q(x)dx = 2\pi \sigma_{\text{Au-Part}}(x)^2 dx$ is the charge on the particle surface separated by x to $x+dx$ distance from the surface. Eqs.(4–12, 15–18) represent a complete set of equations which can be solved numerically for the surface charge, σ_{Surf} , and binding energy, E , vs. the composition and density of ionizable groups on the substrate, the size of colloidal particles, the composition and density of the positively charged groups on the colloidal particle, and the pH and concentration of ions in solution. Figure 3A,B shows example of titration curves and particle-surface binding energy calculated from Eqs. (4–12) and (15–18). The simulation parameters used in this model are summarized in Table I. The density of electric charge on surface according to the GCSG model is significantly lower and is about 1%–4% of the density calculated by neglecting redistribution of ions on surface given by simplified model of Eqs. (4–13). The most significant changes in the titration curves in Figure 3 vs. Figure 2 are observed at higher density of the surface charge (i.e., at low and high pH) due to the dominant contribution from the surface potential and the capacitive Stern term in Eqs. (14) and (16). Yet, the values for pI_{Target} and pI_{Probe} are the same in both models due to negligible contribution of the ion redistribution effect and capacitive term in the close proximity to Zero Charge Point, e.g., at $pH \approx pI$. Accordingly, in both models the binding of colloidal particle to the array surface is determined by the same set of conditions for the microarray and particle surface: $pI_{\text{Target}} < pI_{\text{Probe}} \leq pI_{\text{Part}}$. Target-specific detection can be achieved in solution with pH : $pI_{\text{Target}} < pH < pI_{\text{Probe}}$. Figure 4 shows calculation of pI_{DNA} vs. the DNA size on the substrate with the density of amino-groups on the substrate surface of 90 pmole/cm² – 300 pmole/cm² and the size of the particle's footprint $2a' = 40$ nm. In microarray experiments the size of oligonucleotide probes typically is less than 100-base long and the size of target mRNA and cDNA is often larger than 1200 bases. From the data in Figure 4 for substrate with the density of amino-groups of 90 pmole/cm² – 150 pmole/cm² we have $(pI_{\text{Probe}} - pI_{\text{Target}}) > 3.0$. An important implication of this result is that at the typical experimental conditions the $\Delta pH = (pI_{\text{Probe}} - pI_{\text{Target}})$ range is broad enough to be used for selective binding of nano-particles to target DNAs on microarray surface.

Selection of particle size—To select the size of colloidal particles for detection of target molecules on microarray two factors can be taken into consideration. First, the instrument chosen or available for reading microarray may impose certain requirements to the size of particle to be used. The previously reported microscope-based and flatbed scanner systems can operate in bright- and dark-field detection modes [6]. The bright-field detection mode is better to detect the absorbance of metal colloids having the size of less than 50 nm. The dark-field mode detects light scattering and requires the use of particles with the size larger than 50 nm. Here, the choice of imaging equipment, which can operate only in bright- or for dark-field detection mode, imposes certain requirement to the size of nanoparticles to be used. Second, the range ΔpH for target-specific detection is a function of the size of the colloidal particles, $2a$, and the concentration of small ions n (e.g., the ionic strength of reaction solution). Figure 5 shows the calculation of $\Delta pH(a, n) = (pI_{\text{Probe}} - pI_{\text{Target}})$ vs. the particle footprint size $2a'$ for detection of 1200-nt DNA target by 50-nt probe on microarray substrate with the density of amino-silane of (a) 90, (b) 150, and (c) 300 pmole/cm². From Figure 5, the particles with the footprint size of 25–35 nm providing pH range of $\Delta pH = (pI_{\text{Probe}} - pI_{\text{Target}}) > 4.0$, in which range the specific detection of target molecules can be achieved. For easy control of experimental conditions it is often desirable to have ΔpH range exceeding 1.0 which, from data in Figure 5, leads to the condition for particle footprint size of $2.7 \text{ nm} < 2a' < 47.9 \text{ nm}$. At the low ionic strength of solution where $n < 10 \text{ mM}$, the Debye length is larger than the lower limit for the particle footprint and we have $a \approx a'$, which leads to the low limit of the particle size of $2a > 2.7 \text{ nm}$. The footprint of large particles, for instance 250-nm particles, can be adjusted to the lower footprint size by increasing the solution ionic strength. However, in solution with the concentration of ions $n > 100 \text{ mM}$ the gold particles may become unstable due to detachment of the cationic reagent from the particle surface [10]. The requirement of low ionic strength of

solution $n < 100$ mM for stability of modified colloidal particles leads to the upper limit to the size of particles. The particle's footprint a' is given by:

$$a'^2 = (2\lambda_D a - \lambda_D^2) \quad (19)$$

which is valid at $\lambda_D < a$. Due to the limit to the maximum ionic strength of solution $n < 100$ mM we have the limit of $\lambda_D > 0.94$ nm for solutions, which can be used without compromising the stability of colloidal particles. Taking into consideration the requirement of $2a' < 47.9$ nm from Figure 5 and $\lambda_D > 0.94$ nm, from Eq. (19) we find that the upper limit of particle size is $2a < 1221$ nm.

In summary, the results of these simulations show that the target-selective detection of 1200-nt DNA targets can be achieved by using colloidal particles of the size $2.7 \text{ nm} < 2a < 1.2 \text{ }\mu\text{m}$ with the preferable particle footprint size of 25–35 nm. The larger particles can be used by adjusting the particle footprint by increasing the ionic strength of reaction solution. The experimental conditions can be further optimized for specific binding to target DNA and repulsion from the area covered only by probe molecules by adjusting the solution pH to the range of $3.5 < \text{pH} < 7.8$. Detection of DNA targets can be carried out on glass microarray substrates with the density of amino-groups of 90–300 pmole/cm² and by using gold colloidal particles with the density of amino-groups on the particle surface exceeding of $\sigma_{\text{Au}}/e = 89.6 \text{ mC/m}^2 / e = 93 \text{ pmol/cm}^2$. The nanoparticles of materials other than gold can be used under the condition the particles carry positive electric charge at pH range of $7.5 < \text{pH}$.

Experiment

In this study we have carried out two experiments: (1) to study detection sensitivity of the colloidal particles for nucleotide detection on glass and membrane substrates; and (2) to demonstrate detection of large DNA targets on a microarray substrate under experimental conditions at which no probe oligonucleotides with the size < 100 -nt are detectable on the substrate (i.e., target-specific detection). Demonstration of the method for detection of hybridization of cDNA on microarrays has been reported elsewhere [8].

Detection sensitivity has been investigated on two types of commercial substrates: amino-modified glass substrates (Ultr-GAPS, Corning Life Sciences) and positively charged nylon membrane (Hybond-N+, Amersham, Life Sciences). A serial dilution of a single stranded M13mp18 phage DNA (Sigma, Cat. No. D8410) was prepared in Corning's printing buffer (Corning Life Sciences). The DNA solution at six different concentrations with two-fold dilution steps and the maximum concentration of $1.0 \pm 0.05 \text{ ng/}\mu\text{l}$ was printed on glass and nylon substrate by SpotBot microarray printer (TeleChem, International, CA) using the Stealth SMP4 printing pin to deliver, on average, $10 \pm 2 \text{ nl}$ of printing solution per spot. Each of six DNA solutions have been printed in five replicates in four identical blocks consisting of total $4 \times (5 \times 6) = 120$ spots. After printing, the substrate with spotted DNA was incubated at room temperature in a desiccator overnight followed by UV cross linking at 600 mJ by UV Stratalinker 1800 (Stratagene, CA). The substrate was washed in distilled deionized water and dried by centrifugation (glass substrates) or in dust-free environment at room temperature (nylon substrates). The substrates subsequently were developed for 12 min in 40-nm cationic gold solution from the AG10 Staining Kit (Sci-Tec, Inc., Knoxville, TN) following the manufacturer's protocol. After washing in distilled deionized water, the substrates were dried and analyzed by an Epson Perfection 4870 scanner operating in bright-field detection mode and controlled by AuroGene 2.20 image acquisition software (Sci-Tec, Inc., Knoxville, TN). Figure 6 A,B shows images of (A) glass and (B) nylon substrate with DNA spots visible due to binding of 40 nm cationic gold particles. The diameter of an individual spot in Figure 6A is

220 ± 20 μm. The “butterfly” shape of the spots on the nylon substrate in Figure 6B reflects the shape of the tip of the Stealth SMP4 pin, which was used to print the spot. From the concentration of DNA solution and the amount of printing solution of 10 nl/spot, the amount of DNA/spot in Figure 6 is 10, 5, 2.5, 1.25, 0.61, and 0.0 pg/spot (i.e., 0.0 pg/spot is for printing buffer with no DNA). Figure 7 shows spot intensity vs. the amount of DNA per spot on glass and nylon substrates. The lowest detectable spot in Figure 6A corresponds to detection of 0.61 pg/spot; for the spot size of 220-μm this corresponds to detection of 16.1 pg/mm² of DNA. By calculating the amount of DNA per spot in high-density microarrays having the spot size of 120-μm the detection sensitivity of 40-nm cationic gold achieved in this experiment corresponds to detection of 0.18 ± 0.05 pg/spot of target DNA on the microarray surface both on glass and membrane substrate.

Target-specific detection has been investigated to demonstrate detection of large DNA targets without detection of small probe molecules present on microarray surface. A 7,229-nt M13mp18 phage DNA and 50-nt oligonucleotide probe each were spotted on Corning Ultra-GAPS slide at concentrations of spotting solution of 1, 0.3, 0.1 and 0.03 ng/μl. In this experiment each spot was produced by pipetting 100 nl of nucleotide in Corning’s spotting buffer. The sequence of 50-nt synthetic oligonucleotide probe has the same distribution of A,G,T, and C nucleotides as the 7,229-nt fragment of M13 phage DNA.

A solution of 250-nm cationic gold particles (Cat. No. AG-12, Sci-Tec, Inc., TN) at concentration of 1.4 × 10⁸ particles/ml was prepared in solutions with different pH values and ionic strengths adjusted by addition of HCl and NaCl. The concentration of Cl[−] in solution was kept constant at 10 mM [19]. The slide with probe and target nucleotides was developed for 15 min in 5 ml solution of the cationic gold particles at solution pHs of 3.0, 4.0, and 7.0. The microarray slide subsequently was washed in distilled deionized water, dried by centrifugation, and scanned by Epson 3200 flatbed scanner operating in dark-field detection mode. The image of the slide with nanoparticles bound to the surface is shown in Figure 8. Consistent with the data in Figure 3B and discussion above, no binding of nanoparticles was observed in Figure 8A at pH = 3.0. At pH = 4.0 nanoparticles bind selectively to the spots carrying target DNA molecules with virtually no binding observed in spots carrying 50-nt probe oligonucleotides. At pH = 7.0 the nanoparticles bind with about the same efficiency to the probe and target molecules tethered on the microarray. In this example, the solution at pH = 4.0 provides conditions for selective detection of target molecules on the substrate without undesirable detection of probe molecules present on the same spot. Importantly, the target-selective detection in this example is achieved without any modification/labeling of target molecules and without using sequence-specific agents or antibodies attached to nanoparticles as would be required by conventional techniques for discriminating targets by sequence or by employing antibody-antigen interaction for recognition of target molecules.

Discussion

The known methodologies for describing charge phenomena on solid-liquid interface can be adopted for analysis of interaction of colloidal particles and biopolymers tethered on the surface of a microarray. We have compared two models for describing surface charge on a microarray surface, one is based on the mass action law and the other is the adoption of the Gouy-Chapman-Stern-Graham model. Both models predict selective binding of colloidal particles to large (e.g., more than ~500 base long) target molecules at a certain range of solution pH and solution ionic strength. The more advanced Gouy-Chapman-Stern-Graham model however predicts a significantly lower overall electric charge on the surface and lower binding energy of colloidal particle and substrate with the maximum binding energy of the colloidal particle exceeding ~300kT (see Figure 3). The difference between charge and binding energy between the two models is attributed to the redistribution of free ions and Debye screening of charges in solution.

Yet, the simplified mass action low model provides a convenient and easy to use approach for initial analysis of the optimal pH range for selective binding of nanoparticle and bio-polymers on a solid substrate. Both models take into consideration the chemical composition and the density of chemical groups on the substrate, as well as the composition and the size of the probe and target molecules. The models can be applied for analysis of various experimental situations for detection of nucleic acids, proteins and other biopolymers using nano-size materials.

The results of theoretical analysis are in qualitative agreement with the experiment, in which selective detection of target phage DNA was observed by selecting the solution pH and ionic strength. The detection of nucleic acids on common types of substrates used in microarray experiments is sensitive and allows detection of a target amount on a substrate surface consistent with the requirements of a typical microarray experiment. The approach of using ionic interaction of nanoparticles and biopolymer targets on microarray surface provides a new alternative to the use of luminescent labels with the important benefit of reducing complexity, labeling bias, and the overall cost of microarray analysis.

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Figure 1.

(A) Interaction of the colloidal particle and the surface is determined by the net electric charge of the particle, and the density of the electric charge of the surface inside the particle's footprint. The footprint area is given by $\pi(2\lambda_D a - \lambda_D^2)$, where λ_D is the Debye length in solution and a is the particle radius. (B) Interaction of positively charged particle with three areas on a positively charged microarray substrate, where: (I) no DNA is present; (II) only a probe oligonucleotide is inside the particle footprint; and (III) a captured DNA molecule is inside the particle's footprint.

Figure 2.

(A) titration curves for (I–III) area on substrate surface covered by particle footprint and (IV) for gold nanoparticle carrying positive amino-groups on its surface, where (I) is the substrate with no probe and target molecules; (II) the substrate carries 50-nt probe molecules; (III) the substrate carries ~1250-base probe-target duplex.

(B) Energy of interaction, $E(\text{pH})/E(\text{pH} = 0)$, of nanoparticle and substrate surface vs. pH for (II) substrate carrying only probe molecules; and (III) for substrate carrying probe-target duplex.

Figure 3.

(A) Titration curves for substrate area covered by particle footprint (II) carrying probe molecule only; (III) carrying probe-target duplex; and (IV) for gold nanoparticle carrying positive amino-groups on the surface. The calculations are based on Gouy-Chapman-Stern-Graham model. (B) Energy of interaction of nanoparticle and the substrate surface (II) for substrate surface carrying only probe molecule; and (III) carrying probe-target duplex for titration curves shown in (A).

Figure 4. Isoelectric point of the substrate covered by particle footprint and carrying target molecule vs. the size of the target molecule at different density of amino-groups on the substrate: (a) 90 pmole/cm²; (b) 150 pmole/cm²; and (c) 300 pmole/cm².

Figure 5.

(A) The difference of ($pI_{\text{Probe}} - pI_{\text{Target}}$) vs. the particle footprint for three different substrates having the density of amino-groups of: (a) 90 pmole/cm²; (b) 150 pmole/cm²; and (c) 300 pmole/cm².

(B) The optimal pH = ($pI_{\text{Probe}} + pI_{\text{Target}}$)/2 of solution for target-specific detection vs. the size of particle footprint for substrate having the density of amino-groups of: (a) 90 pmole/cm²; (b) 150 pmole/cm²; and (c) 300 pmole/cm².

Figure 6. (A) Detection of M13mp18 DNA on amino-modified glass substrate and, (B), on a positive membrane. The glass substrate and membrane with immobilized DNA have been developed in solution of 40-nm cationic gold particles.

Figure 7. Intensity of spots in Fig. 5 vs. the amount of DNA immobilized on the substrate surface: (a) glass substrate; and (b) positively charged membrane.

Figure 8. Detection of M13 target DNA and 50-nt synthetic oligonucleotide on glass substrate vs. the solution pH: (A) pH = 3.0; (B) pH = 4.0; and (C) pH = 7.0.

Table I

Surface parameters used for simulation of surface charge and particle-surface binding energy.

		Value	Reference
Density of Si-groups on array	Γ_{SiO^-}	8.0 nm^{-2}	[12]
pK_{SiOH}	pK_{SiOH}	7.5	[12]
Density of amino-groups on array surface	$\Gamma_{\text{NH}_3^+}$	0.07–0.26	[11]
$\text{pK}_{\text{NH}_3^+}$	$\text{pK}_{\text{NH}_3^+}$	9.5	[20]
Substrate, surface capacitance	C_{surf}	0.29 F/m^2	[12,15] ^{*)}
Size of probe DNA		50-nt	
Size of target DNA		1200-nt	
$\text{pK}_{\text{PO}_4^-}$	$\text{pK}_{\text{PO}_4^-}$	2.5	[17]
Probe/target area capacitance	C_{DNA}	2.9 F/m^2	[12]

^{*)}Capacitance of hydrophobic substrate surface is assumed to be ten-fold lower than the capacitance of hydrophilic array spots carrying probe and target molecules.