Employing Double-Stranded Probes on Colloidal Particles for Nucleic Acid Detection

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DNA is a biological polymer

- sugar-phosphate backbone

http://www.accessexcellence.org/AB/GG/dna2.html
DNA is a biological polymer

- sugar-phosphate backbone
- one of four bases as side group

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DNA is a biological polymer

- sugar-phosphate backbone
- one of four bases as side group
- duplex of complementary strands

http://www.accessexcellence.org/AB/GG/dna2.html
DNA is a biological polymer

• sugar-phosphate backbone
• one of four bases as side group
• duplex of complementary strands
• thermally reversible at $T_m$

http://www.accessexcellence.org/AB/GG/dna2.html
DNA is a versatile, reversible assembly tool

Recognition-based assembly
• independent of material composition
• independent of particle size, etc.
DNA is a versatile, reversible assembly tool

Recognition-based assembly
- independent of material composition
- independent of particle size, etc.

Interactions between DNA-grafted surfaces can be “tuned”
- strand composition (A-T vs G-C)
- number of base pair matches
- strand concentration
- solution conditions (ionic strength)

Milam et al *Langmuir* 2003
## Advantages

- rapid interrogation of many samples (gene)
- commercially available

![DNA Microarray](image_url)

Picture by: Hanne Jarmer

Microarrays are a Popular Detection Platform

**Advantages**
- rapid interrogation of many samples (gene)
- commercially available

**Limitations**
- cost


Picture by: Hanne Jarmer
Microarrays are a Popular Detection Platform

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- rapid interrogation of many samples (gene)
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**Limitations**
- cost
- requires labeling of all candidate targets
Microarrays are a Popular Detection Platform

Advantages
- rapid interrogation of many samples (gene)
- commercially available

Limitations
- cost
- requires labeling of target of interest

Factors Hindering Fluorescence Signal
- probe density too high
- substrate interference
- other secondary structure

False Negatives
Microarrays are a Popular Detection Platform

Advantages
- rapid interrogation of many samples (gene)
- commercially available

Limitations
- cost
- requires labeling of target of interest

Factors Hindering Fluorescence Signal
- probe density too high
- substrate interference
- other secondary structure

Factors Favoring Fluorescence Signal
- nonspecific target association
- mismatched target hybridize

False Negatives
False Positives
Alternative Approach: Double-Stranded Probes

- labeled ds probe
- ss probe
- mismatched ds probe
- short ds probe

OR

toehold region
Alternative Approach: Double-Stranded Probes

Add unlabeled target

- ss probe
- mismatched short ds probe
- long labeled ds probe

OR

- short ds probe
- toehold region
Alternative Approach: Double-Stranded Probes

- Labeled ds probe
- Add unlabeled target
- Reporter strand displaced

OR

- ss probe
- Mismatched ds probe
- Short ds probe
- Toehold region
Alternative Approach: Double-Stranded Probes

Advantages
• No target labeling
• Reduce false positives
Alternative Approach: Double-Stranded Probes

**Advantages:**
- No target labeling
- Reduce false positives

**Dual Challenge:**
- Maximize stable ds probe formation
Alternative Approach: Double-Stranded Probes

**Advantages:**
• No target labeling
• Reduce false positives

**Dual Challenge:**
• Maximize stable ds probe formation
• Maximize displacement by appropriate target
Key Questions

- How does sequence design of dsProbe affect kinetics of reporter release?
Candidate Fluorescent dsProbes

Template μsphere

(spacer) CTC GTC ACA CTA TCA
AG TGT GAT AGT T- *

ds probe
P15:T11

Candidate Fluorescent dsProbes

Template μsphere

Spacer  Toehold  Original Duplex  Tag  ds probe

(\text{carbon12}) CTC GTC ACA CTA TCA
AG TGT GAT AGT T-\*  P15:T11

(\text{carbon12}) CTC GTC ACA CTA TCA
G CAG TGT GAT AGT T-\*  P15:T13

Mismatch

(\text{carbon12}) CTC GTC ACA CTA TCA
GAG CAG TCT GAT AGT T-\*  P15:T15m

(\text{carbon12}) CTC GTC ACA CTA TCA
GAG CAG TGT GAT AGT T-\*  P15:T15

Flow Cytometry Used to Measure Density of Labeled Duplexes on Microspheres
Candidate Fluorescent dsProbes

*Initial dsProbe Densities*

- **P15:T11**
  - 6,303 duplexes/µm²
- **P15:T13**
  - 11,816 duplexes/µm²
- **P15:T15m**
  - 6,392 duplexes/µm²
- **P15:T15**
  - 12,923 duplexes/µm²

**Template μsphere**

- (carbon12) CTC GTC ACA CTA TCA
- (carbon12) CTC GTC A CA CTA TCA
- (carbon12) CTC GTC ACA CTA TCA
  - mismatch
- (carbon12) CTC GTC ACA CTA TCA

**ds probe**

- AG TGT GAT AGT T- *
- G CAG TGT GAT AGT T- *
- GAG CAG TCT GAT AGT T- *
- GAG CAG TGT GAT AGT T- *

Explore ds probes with similar affinities, but different sequences
Candidate Fluorescent dsProbes

Target Sequence Used

**Target:** GAG CAG TGT GAT AGT

<table>
<thead>
<tr>
<th>ds probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>P15:T11</td>
</tr>
<tr>
<td>6,303 duplexes/µm²</td>
</tr>
</tbody>
</table>

**spacer**

**toehold**

**original duplex**

**tag**

(spacer toehold original duplex tag)

(CTC GTC ACA CTA TCA AG TGT GAT AGT T-*)

**ds probe**

P15:T15m

6,392 duplexes/µm²

**mismatch**

(CTC GTC ACA CTA TCA)

(GAG CAG TCT GAT AGT T-*)

(spacer toehold original duplex tag)

(CTC GTC ACA CTA TCA AG TGT GAT AGT T-*)

Target: GAG CAG TGT GAT AGT
Explore Responsiveness of dsProbes to Target DNA Release Profiles Measured Via Flow Cytometry

ds probes with similar affinities have different release profiles
Explore Responsiveness of dsProbes to Target DNA Release Profiles Measured Via Flow Cytometry

Target: GAG CAG TGT GAT AGT

Higher affinity ds probes do not reach equilibrium OR
DNA Release Profiles of Candidate dsProbes Measured Using Flow Cytometry

Highest affinity ds probes hinder competitive hybridization events

Target: GAG CAG TGT GAT AGT
DNA Release Profiles of Candidate dsProbes Measured Using Flow Cytometry

Three candidate ds probes chosen

Target: GAG CAG TGT GAT AGT

P15:T11
P15:T15m
P15:T13

P15:T15
Key Questions Explored

- How does sequence design of dsProbe affect kinetics of reporter release?
- What is the effect of embedding “recognition element” in longer target strands?
Effect of Embedding Recognition Element at Different Locations in Longer Target Strands

Prior Target: 3’ – GAG CAG TGT GAT AGT – 5’
Effect of Embedding Recognition Element at Different Locations in Longer Target Strands

Prior Target: 3’ – GAG CAG TGT GAT AGT – 5’

100 base-long targets
3’ – \( T_{100} \) – 5’ (100 thymine bases)
3’ – \( T_{43} \) GAG CAG TGT GAT AGT \( T_{42} \) – 5’
3’ – GAG CAG TGT GAT AGT \( T_{85} \) – 5’
3’ – \( T_{85} \) GAG CAG TGT GAT AGT – 5’

long targets
NC Target
Middle Target
3’ Target
5’ Target
Effect of Embedding Recognition Element at Different Locations in Longer Target Strands

Likely Secondary Duplex Formation

100 base-long targets

3′ – $T_{100}$ – 5′ (100 thymine bases)

3′ – $T_{43}$ GAG CAG TGT GAT AGT $T_{42}$ – 5′

3′ – GAG CAG TGT GAT AGT $T_{85}$ – 5′

3′ – $T_{85}$ GAG CAG TGT GAT AGT – 5′

long targets

NC Target

Middle Target

3′ Target

5′ Target
Embedded Recognition Element

**P15:T11 dsProbe**

**72 h**

*Significant dissociation occurs in the absence of target*

**First 6 h**

*no target*
Embedded Recognition Element

**P15:T11 dsProbe**

**First 6 h**

- 3’, 5’
- Middle
- NC
- no target

**72 h**

- 3’ Target, 5’ Target
- Middle Target
- NC Target
- no target

*Modest effect of location of recognition element*
Effects of dsProbe strand with weak stability

*P15:T11 dsProbe*

weak affinity ds probe

imperfect duplexes

“more” perfect duplexes
Effects of dsProbe strand with weak stability

P15:T11 dsProbe

weak affinity ds probe

imperfect duplexes

“more” perfect duplexes

reporter strand dissociated
Effects of dsProbe strand with weak stability

**P15:T11 dsProbe**

- Weak affinity ds probe
- Imperfect duplexes
- "More" perfect duplexes
- Reporter strand dissociated
- Add unlabeled target
- Signal changes results
- Reporter strand displaced
Two pathways for reporter strand release

\[ \text{Dissociation Pathway} \]

\[ \text{Displacement Pathway} \]

Two pathways for reporter strand release

**Dissociation Pathway**

\[ \text{dsProbe} \rightarrow P + T_1 + T_2 \]

**Displacement Pathway**

\[ P + T_1 + T_2 \rightarrow PT_1T_2 \rightarrow PT_2 + T_1 \]

Accounting Exclusively for Displacement Activity

P15:T11 dsProbe

Modest effect of location of recognition element
Accounting Exclusively for **Displacement Activity**

**P15:T15m dsProbe**

Displacement is qualitatively slower, but less thermal dissociation occurs
Accounting Exclusively for Displacement Activity

**P15:T15m dsProbe**

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>3' Target</th>
<th>5' Target</th>
<th>Middle Target</th>
<th>NC Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5.0e+4</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>1.0e+5</td>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>1.5e+5</td>
<td>0.6</td>
<td>0.8</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>2.0e+5</td>
<td>0.8</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>2.5e+5</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Compares rates of competitive displacement
Recognition Element Embedded at Different Locations
Effect of ds Probe sequence on displacement rate, $k_{\text{obs}}$

$$f(t) = f_0 + (f_\infty - f_0)(1 - \exp(-k_{\text{obs}} \cdot t))$$

Recognition Element Embedded at Different Locations
Effect of ds Probe sequence on displacement rate, $k_{obs}$

$$f(t) = f_0 + (f_\infty - f_0) \left(1 - \exp\left(-k_{obs} \cdot t\right)\right)$$

Displacement rate $k_{obs}$ (s$^{-1}$)

<table>
<thead>
<tr>
<th>Target</th>
<th>P15:T11 dsProbe</th>
<th>P15:T15m dsProbe</th>
</tr>
</thead>
<tbody>
<tr>
<td>3’ End</td>
<td>$2.0 \times 10^{-4}$</td>
<td>$2.3 \times 10^{-5}$</td>
</tr>
<tr>
<td>5’ End</td>
<td>$2.0 \times 10^{-4}$</td>
<td>$2.8 \times 10^{-5}$</td>
</tr>
<tr>
<td>Middle</td>
<td>$6.4 \times 10^{-5}$</td>
<td>$2.4 \times 10^{-5}$</td>
</tr>
</tbody>
</table>

Criteria for reporting $k_{obs}$ were R-squared value $> 0.9$

Reynaldo reports $k_{obs} \sim 10^{-6} - 10^{-5}$ (s$^{-1}$) for short, identical targets

Recognition Element Embedded at Different Locations
Effect of ds Probe sequence on displacement rate, $k_{obs}$

\[
f(t) = f_0 + (f_\infty - f_0)\left(1 - \exp\left(-k_{obs} \cdot t\right)\right)
\]

Displacement rate $k_{obs}$ (s$^{-1}$)

<table>
<thead>
<tr>
<th>Target</th>
<th>P15:T11 dsProbe</th>
<th>P15:T15m dsProbe</th>
</tr>
</thead>
<tbody>
<tr>
<td>3’ End</td>
<td>2.0 x 10$^{-4}$</td>
<td>2.3 x 10$^{-5}$</td>
</tr>
<tr>
<td>5’ End</td>
<td>2.0 x 10$^{-4}$</td>
<td>2.8 x 10$^{-5}$</td>
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<tr>
<td>Middle</td>
<td>6.4 x 10$^{-5}$</td>
<td>2.4 x 10$^{-5}$</td>
</tr>
</tbody>
</table>

Criteria for reporting $k_{obs}$ were R-squared value > 0.9

Overall, successful displacement occurs with all long targets
Key Questions Explored

- How does sequence design of dsProbe affect kinetics of reporter release?
- What is the effect of embedding “recognition element” in longer target strands?
- Can a dsProbe discriminate between complementary target and mismatched target?
Effect of Mismatch Location in Sequences

*P15:T15m*

5’ (carbon12) CTC GTC A\textcolor{red}{\text{CA}} CTA TCA

3’ – GAG CAG T\textcolor{red}{\text{CT}} GAT AGT T-\textcolor{green}{$\ast$}
Effect of Mismatch Location in Sequences

\[ P15:T15m \]

\[
\begin{align*}
5' &\text{(carbon12)} CTC GTC A\textcolor{red}{\text{CA}} CTA TCA \\
3' &\textcolor{red}{\text{mismatch}} \quad \text{GAG CAG \textcolor{red}{TCT} GAT AGT T-}^* \\
\end{align*}
\]

Note the lack of a toehold in this ds probe

Targets

\[
\begin{align*}
3' &\text{GAG CAG TGT GAT AGT} – 5' \quad T15 \\
3' &\text{GAG CAG TCT GAT AGT} – 5' \quad T15m \\
3' &\text{GAC CAG TGT GAT AGT} – 5' \quad T15x3 \\
\end{align*}
\]
Effect of Mismatch Location in Sequences
ds P15:T15m

Secondary duplexes formed via competition alone
P15:T15m dsProbe

Competitive displacement is successful, but does not discriminate targets.
Key Questions

- How does sequence design of dsProbe affect kinetics of reporter release?
- What is the effect of embedding “recognition element” in longer target strands?
- Can a dsProbe discriminate between complementary target and mismatch target?
- Could a reduced ds probe density AND reversal of the toehold region orientation improve discrimination capabilities for similar targets?
“Flip” the orientation of the toehold region

**P15:T13 versus Flip P15:T13**

spacer  toehold  original duplex  tag  ds probe

5’ (carbon12) CT C GTC ACA CTA TCA
G CAG TCT GAT AGT T-∗

P15:T13
“Flip” the orientation of the toehold region

**ds P15:T13 versus Flip P15:T13**

**ds probe**

<table>
<thead>
<tr>
<th>P15:T13</th>
<th>11,816 duplexes/µm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’ (carbon12)</td>
<td>CT C GTC ACA CTA TCA</td>
</tr>
<tr>
<td></td>
<td>G CAG TCT GAT AGT T-*</td>
</tr>
</tbody>
</table>

**Flip P15:T13**

<table>
<thead>
<tr>
<th>1,759 duplexes/µm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>3’ - TTT TTT ACT ATC ACA CTG CTC</td>
</tr>
<tr>
<td>5’ – TGA TAG TGT GAC G –</td>
</tr>
</tbody>
</table>
“Flip” the orientation of the toehold region
ds $P_{15}:T_{13}$ versus Flip $P_{15}:T_{13}$

Targets

- $5' - \text{TGA TAG TGT GAC GAG} \rightarrow 3'$
  - $T_{15}$
- $5' - \text{UGA UAG UGU GAC GAG} \rightarrow 3'$
  - $R_{15}$
- $5' - \text{TGA TAG TCT GAC GAG} \rightarrow 3'$
  - $T_{15m}$
- $5' - \text{TGA TAG TCT GAC CAG} \rightarrow 3'$
  - $T_{15x3}$

Flip $P_{15}:T_{13}$

1,759 duplexes/$\mu m^2$
Effect of Flipping Toehold Region

*Flip P15:T13*

![Graph showing the effect of flipping toehold region. The graph plots the fraction displaced against time (s). The legend includes lines marked as R15, T15, T15x3, and T15m.](image)
Effect of Flipping Toehold Region
*Flip P15:T13*

Choice in density & toehold orientation may affect ability to discriminate!
Alternative: Nanoparticle Fluorescent Tags
Alternative: Nanoparticle Fluorescent Tags

Soluble primary target

Immobilized primary target
Phase I: Colloid Assembly

High Oligonucleotide Density Microspheres

<table>
<thead>
<tr>
<th>Assembly Temp</th>
<th>P15: T11</th>
<th>P15: T13m</th>
<th>P15: T15m</th>
</tr>
</thead>
<tbody>
<tr>
<td>~ 22 °C</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>30 °C</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>40 °C</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Phase I: Colloid Assembly
High Oligonucleotide Density Microspheres

Here, high affinity sequences are least successful as colloidal assembly tools.
Phase I: Colloid Assembly

Low Oligonucleotide Density Microspheres

<table>
<thead>
<tr>
<th>Assembly Temp</th>
<th>P15: T11</th>
<th>P15: T13m</th>
<th>P15: T15m</th>
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<td><img src="image3" alt="Image" /></td>
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<td>30 °C</td>
<td><img src="image4" alt="Image" /></td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
</tr>
<tr>
<td>40 °C</td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
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</tbody>
</table>
Phase I: Colloid Assembly
Low Oligonucleotide Density Microspheres

Here, high affinity sequences are successful as colloidal assembly tools.
## Phase I: Colloid Assembly

<table>
<thead>
<tr>
<th>T15m Assemblies</th>
<th>HODM Template</th>
<th>LODM Template</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duplex Density</td>
<td>12,923 duplexes/µm²</td>
<td>4,321 duplexes/µm²</td>
</tr>
<tr>
<td>ssProbe Density</td>
<td>~4.58 x 10⁵ probe/µm²</td>
<td>~3.48 x 10⁵ probe/µm²</td>
</tr>
<tr>
<td>ssProbe Parking Area</td>
<td>2.18 nm²/probe</td>
<td>2.87 nm²/probe</td>
</tr>
</tbody>
</table>

*Lower DNA density on particles promotes assembly*
Phase II: Colloid Disassembly
Low Oligonucleotide Density Microspheres

P15:T11 linked particles Incubated with noncomplementary target
Phase II: Colloid Disassembly
Low Oligonucleotide Density Microspheres

P15:T11 linked particles
Incubated with noncomplementary target

Disassembly occurs in presence of longer complementary target
Phase II: Colloid Disassembly
Low Oligonucleotide Density Microspheres

Disassembly occurs in presence of complementary target
Conclusions

- Dye-labeled double-stranded probes on colloidal particles can also serve as fluorescence-based detection platform with potential for target discrimination
- Key challenge – maximize dsProbe formation while maintaining ability to displace reporter strand
- *Future optimization* – combine “flipped” toehold with off-center mismatches in ds probes
Acknowledgments

Funding

GA Tech start-up funds
Georgia Cancer Coalition Distinguished Scholars Program
Emory-GA Tech Cancer Center for Nanotechnology Excellence
NSF CAREER
Army Research Office
AFOSR
GAANN fellowship
Research Experience for Undergraduates
## Phase I: Colloid Assembly


<table>
<thead>
<tr>
<th>Strand</th>
<th>Sequence</th>
<th>Solution</th>
<th>Melt Temp.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probe (Template)</td>
<td>5’ – Amine-(carbon12) CTC GTC ACA CTA TCA – 3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T11</td>
<td>3’ – AG TGT GAT AGT (T-Fluor) – 5’</td>
<td></td>
<td>62.4 °C</td>
</tr>
<tr>
<td>T11m</td>
<td>3’ – AG TGT CAT AGT (T-Fluor) – 5’</td>
<td></td>
<td>43.0 °C</td>
</tr>
<tr>
<td>T13</td>
<td>3’ – G CAG TGT GAT AGT (T-Fluor) – 5’</td>
<td></td>
<td>67.8 °C</td>
</tr>
<tr>
<td>T13m</td>
<td>3’ – G CAG TGA GAT AGT (T-Fluor) – 5’</td>
<td></td>
<td>58.4 °C</td>
</tr>
<tr>
<td>T15</td>
<td>3’ – GAG CAG TGT GAT AGT (T-Fluor) – 5’</td>
<td></td>
<td>71.8 °C</td>
</tr>
<tr>
<td>T15m</td>
<td>3’ – GAG CAG TCT GAT AGT (T-Fluor) – 5’</td>
<td></td>
<td>59.5 °C</td>
</tr>
</tbody>
</table>


Phase I: Colloid Assembly

Representative Flow Cytometry Histogram

Prediction of the Duplex Density between Particles

Phase I: Colloid Assembly

Measured DNA duplex density on particle surface via flow cytometry.

<table>
<thead>
<tr>
<th>1° Target</th>
<th>High Oligo Density μsphere (HODM)</th>
<th>Low Oligo Density μsphere (LODM)</th>
<th>Calculated Duplex Number Between particles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HODM</td>
<td>LODM</td>
</tr>
<tr>
<td>TH18</td>
<td>9</td>
<td>14</td>
<td>NA</td>
</tr>
<tr>
<td>T11</td>
<td>6,303</td>
<td>2,719</td>
<td>5</td>
</tr>
<tr>
<td>T11m</td>
<td>309</td>
<td>365</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>T13</td>
<td>11,816</td>
<td>4,112</td>
<td>11</td>
</tr>
<tr>
<td>T13m</td>
<td>5,654</td>
<td>2,103</td>
<td>5</td>
</tr>
<tr>
<td>T15</td>
<td>12,923</td>
<td>4,321</td>
<td>13</td>
</tr>
<tr>
<td>T15m</td>
<td>6,392</td>
<td>2,879</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Bangs Laboratories Microspheres (LODM)</td>
<td>Invitrogen Microspheres (HOADM)</td>
<td></td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------------------------------------</td>
<td>---------------------------------</td>
<td></td>
</tr>
<tr>
<td>Concentration of Coupled DNA Strands</td>
<td>1.85 μM</td>
<td>2.38 μM</td>
<td></td>
</tr>
<tr>
<td>Total Number of Coupled DNA Strands</td>
<td>$4.18 \times 10^{14}$</td>
<td>$5.38 \times 10^{14}$</td>
<td></td>
</tr>
<tr>
<td>Number of Strands per Particle</td>
<td>$2.73 \times 10^7$</td>
<td>$3.74 \times 10^7$</td>
<td></td>
</tr>
<tr>
<td>Coupled Probe Density (per μm$^2$) Vendor Supplied Parking Area (Å$^2$/COOH group)</td>
<td>$3.48 \times 10^5$</td>
<td>$4.58 \times 10^5$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>44.5</td>
<td>50</td>
</tr>
<tr>
<td>COOH Groups per Particle</td>
<td>$1.76 \times 10^8$</td>
<td>$1.63 \times 10^8$</td>
<td></td>
</tr>
<tr>
<td>Coupling Efficiency</td>
<td>15.5%</td>
<td>22.3%</td>
<td></td>
</tr>
<tr>
<td>Parking Area of ssProbe (nm$^2$ / Probe strand)</td>
<td>2.87</td>
<td>2.18</td>
<td></td>
</tr>
</tbody>
</table>