Nanoparticle-based DNA multiplexed probes for pathogen detection using confocal raman microscopy

Specific Aims
- investigate the effectiveness of several fluorescent or non-fluorescent dyes as raman labels to be used as SERS tags
- synthesize SERS-DNA probes to detect species-specific DNA sequences of E. coli O157:H7, Campylobacter sp., Staphylococcus aureus, Listeria monocytogenes, and Salmonella sp. as targets
- develop a one-pot multiplex protocol using optimized SERS DNA probe to simultaneously detect E. coli O157:H7, Campylobacter sp., and Salmonella sp.

Funding: New Investigator Grant, Center for Food Safety Engineering

Procedures to fabricate SERS-DNA probes
- **Step 1:** Direct attachment of thiol modified oligos to gold nanoparticles
- **Step 2:** Identification of appropriate DNA probes
  - Direct attachment of non-fluorescent Raman tags to gold nanoparticles
- **Step 3:** Optimization step

Raman Spectra from DNA Probes Labeled with no glow tags

Multiplex Detection Schematic

Probe fabrication
- Au particle
- Raman tag
- dsDNA
- Streptavidin
- Biotin
- Magnetic particle
Characterization of the eight SERS-DNA probes

<table>
<thead>
<tr>
<th>Dye-1:DNA</th>
<th>Wavenumber (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1269, 1239, 1145, 1089, 1021, 959, 904, 853, 799, 753, 712, 690, 646, 494, 416</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dye-2:DNA</th>
<th>Wavenumber (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1537, 1480, 1425, 1319, 1271, 1151, 1098, 1016, 816, 781, 728, 651, 562, 483</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Dye-3:DNA</th>
<th>Wavenumber (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1579, 1518, 1471, 1419, 1378, 1334, 1234, 1186, 1098, 1000, 977, 870, 747, 726, 667, 569, 484, 441</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dye-4:DNA</th>
<th>Wavenumber (cm⁻¹)</th>
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</thead>
<tbody>
<tr>
<td>1689, 1559, 1470, 1439, 1381, 1315, 1251, 1216, 1158, 1063, 997, 960, 920, 853, 815, 710, 638, 565, 505, 438</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dye-5:DNA</th>
<th>Wavenumber (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1636, 1607, 1543, 1489, 1335, 1314, 1242, 1198, 1061, 1016, 968, 880, 843, 794, 733, 683, 660, 540, 506</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Dye-6:DNA</th>
<th>Wavenumber (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1579, 1518, 1471, 1419, 1378, 1334, 1234, 1186, 1098, 1000, 977, 870, 747, 726, 667, 569, 484, 441</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dye-7:DNA</th>
<th>Wavenumber (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1477, 1333, 1173, 1017, 956, 903, 844, 818, 738, 697, 630, 545, 495</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dye-8:DNA</th>
<th>Wavenumber (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1558, 1468, 1317, 1251, 1204, 1158, 1108, 1047, 1022, 1016, 999, 952, 910, 853, 828, 813, 784, 731, 675, 645, 555, 540, 498, 456</td>
<td></td>
</tr>
</tbody>
</table>

Raman Spectra from DNA Probes

Highlighted features in Raman Spectra from DNA Probes

Highlights

DNA detection using non-fluorescent Raman tags to multiplex up to 8 interactions

“Surface-Enhanced Raman Scattering Based Nonfluorescent Probe for Multiplex DNA Detection” Analytical Chemistry 79(11):3981-3988 (accelerated publication)

Featured article (June issue of ACS magazine). “No glow tags for Raman Spectroscopy”

Application: Sandwich Structure-based DNA Hybridization

1. Immobilize capturing strand (CS); 2. Immobilize 6-mercapto-1-hexanol to reduce non-specific binding; 3. Hybridize target (TS) to CS; 4. Hybridize DNA-AuP-RTag probe to overhanging TS; 5. Ag enhancement.

Multiplex DNA Hybridization

Raman Multiplexers for Alternative Gene Splicing (In review, NanoLetters)
**Sensitivity Analysis at 1 fM**

**Raman Multiplexers for Alternative Gene Splicing**

- **DNA-AuP-RTag-1**
  - 1 μM TS
  - 1 pM TS
  - 1 fM TS
  - Control

- **DNA-AuP-RTag-2**
  - 1 μM TS
  - 1 pM TS
  - 1 fM TS
  - Control

- **DNA-AuP-RTag-3**
  - 1 μM TS
  - 1 pM TS
  - 1 fM TS
  - Control

- **DNA-AuP-RTag-4**
  - 1 μM TS
  - 1 pM TS
  - 1 fM TS
  - Control

**Probe design and optimization**

1. Functionalize magnetic nanoparticles with L-aspartic acid (LAA);
2. Immobilize capturing strand (CS) onto LAA modified magnetic nanoparticles;
3. Hybridize target strand (TS) to CS;
4. Hybridize DNA-AuP-RTag probe to TS and separate the complex by external magnetic field;
5. Recover the DNA-AuP-RTag probe by heating the separated complex.

**TUBE FORMAT: Analysis Steps**

- Select Primers
- Probe design and optimization
- Amplification
- Detection

**Modified Analysis Steps**

- E. coli: Wec gene
- Staphy aureus: nuc gene
- Salmon ent: invA gene

**Table 1: Primer sequences for the detection of bacterial targets**

<table>
<thead>
<tr>
<th>Species/Genus</th>
<th>Target Gene</th>
<th>Primer sequence (5’-3’)</th>
<th>Amplification</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> O157:H7*</td>
<td>hlyA gene</td>
<td>GTAGGGAAGCGAACAGAG</td>
<td>361 bp</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>16S rRNA</td>
<td>GGATGACACTTTTCGGAGC</td>
<td>816 bp</td>
</tr>
<tr>
<td><em>Salmonella</em> sp</td>
<td>invA gene</td>
<td>TACTCGCCACGTTCGGGCAA</td>
<td>275 bp</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>nuclease gene</td>
<td>GCGATTGATGGTGATACGGTT</td>
<td>276 bp</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>LmoH gene</td>
<td>CGGAGGTTCCGCAAAAGATG</td>
<td>234 bp</td>
</tr>
</tbody>
</table>

**Modified Analysis Steps**

- E. coli: Wec gene
- Staphy aureus: nuc gene
- Salmon ent: invA gene
Single molecule binding experiments

Aptamers for Salmonella Typhimurium: Antibody vs Aptamer

Single molecule studies: Surface receptor - aptamer interaction

Aptamers for Salmonella Typhimurium: Antibody vs Aptamer

Single molecule binding experiments
Cell-Identity profiling: Back-scattering FESEM images

Yu, Nakshatri, Irudayaraj (Nanoletters, 2007)

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Chobi Debroy

Thank You