

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

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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.

Probe fabrication

Raman tag
 Au particle
 dsDNA
 Biotin
 Streptavidin
 Magnetic particle

Thiolated oligo sequence + Au → [Au]-S-S-Oligo
 Non-fluorescent Raman tags → [Raman tag]-S-S-Oligo

Multiplex Detection Schematic

SERS tag: CAAGGCTAGC, TTTCGAAGCT, CGAATTGCCG
 SERS DNA probe: CGATCGGAAC, TCGAAGCTTT, CCCGTTAAGC
 Sample: GCTAGCCTTG, AGCTTCGAAA, GGGCAATTCG

Excitation lasers: 633 nm, 514.5 nm, 514.5 nm

Measured by Raman spectrometer

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

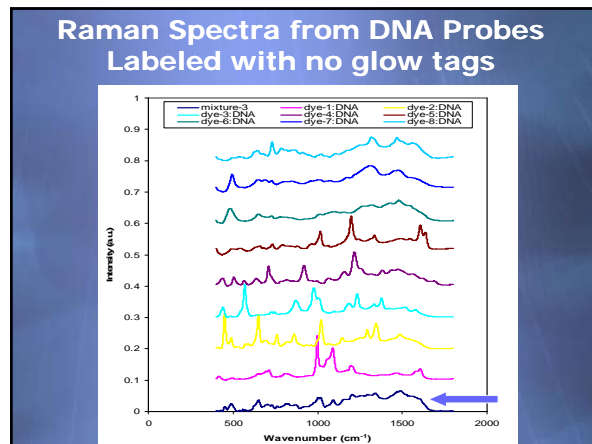
Raman Spectra from DNA Probes Labeled with no glow tags

Intensity (a.u.) vs Wavenumber (cm⁻¹)

- Dye-1: 4-Mercaptopyrindine
- Dye-2: 2-Thiazoline-2-thiol
- Dye-3: 4,6-Dimethyl-2-pyrimidinethiol
- Dye-4: 2-Thiouracil
- Dye-5: 1,2-Di(4-pyridyl)ethylene
- Dye-6: 3-Amino-1,2,4-triazole-5-thiol
- Dye-7: 1H-1,2,4-Triazole-3-thiol
- Dye-8: Pyrazinecarboxamide

Characterization of the eight SERS-DNA probes

Dye-1:DNA	1606, 1465, 1264, 1204, 1194, 1173, 1089, 1026, 998, 813, 712, 696, 660, 646, 494, 416
Dye-2:DNA	1487, 1345, 1293, 1255, 1192, 1145, 1069, 1021, 999, 959, 935, 862, 759, 730, 691, 650, 593, 575, 524, 489, 450, 413
Dye-3:DNA	1579, 1518, 1471, 1419, 1378, 1334, 1234, 1186, 1098, 1000, 977, 870, 747, 726, 667, 569, 484, 441
Dye-4:DNA	1689, 1559, 1470, 1439, 1381, 1315, 1251, 1216, 1158, 1063, 997, 960, 920, 853, 815, 710, 638, 565, 505, 438
Dye-5:DNA	1636, 1607, 1543, 1489, 1335, 1314, 1242, 1198, 1061, 1016, 968, 880, 843, 794, 733, 683, 660, 540, 506
Dye-6:DNA	1537, 1480, 1425, 1319, 1271, 1151, 1098, 1016, 816, 781, 728, 651, 562, 483
Dye-7:DNA	1477, 1320, 1173, 1072, 1017, 998, 981, 958, 848, 815, 728, 687, 651, 540, 495
Dye-8:DNA	1558, 1468, 1317, 1259, 1128, 1108, 1047, 1022, 1016, 999, 952, 910, 853, 828, 813, 784, 731, 675, 645, 555, 540, 498, 456



Characteristic peaks observed in mixture-3

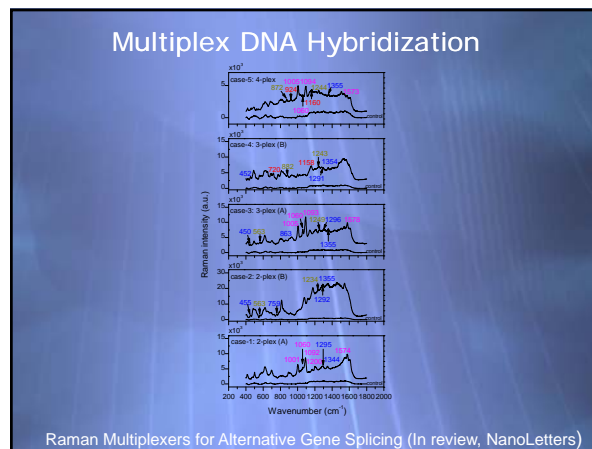
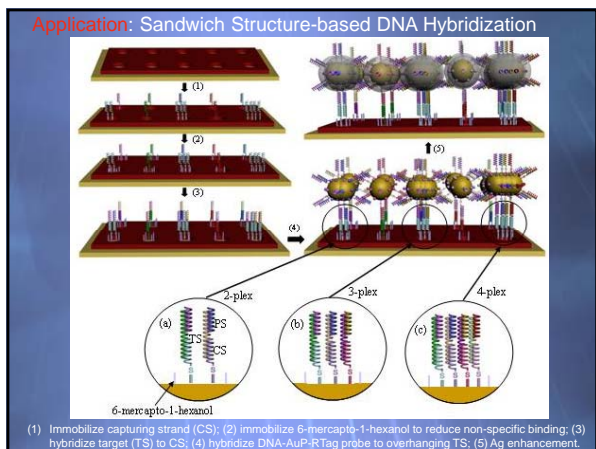
Bands from mixture-2	Assignment	Bands from dye-1:DNA	Bands from dye-2:DNA	Bands from dye-3:DNA	Bands from dye-4:DNA	Bands from dye-5:DNA	Bands from dye-6:DNA	Bands from dye-7:DNA	Bands from dye-8:DNA
451	dye-2:DNA	450							
489	dye-2:DNA	489							
571	dye-3:DNA		569						
677	dye-8:DNA								675
663	dye-3:DNA		663						
761	dye-2:DNA	759							
755	dye-5:DNA					754			
826	dye-8:DNA								828
868	dye-3:DNA			870					
923	dye-4:DNA				920				
981	dye-7:DNA							981	
1091	dye-1:DNA	1089							
1158	dye-4:DNA				1158				
1206	dye-1:DNA	1204							
1289	dye-6:DNA							1271	
1295	dye-2:DNA		1293						
1376	dye-3:DNA			1378					
1533	dye-6:DNA						1537		

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”



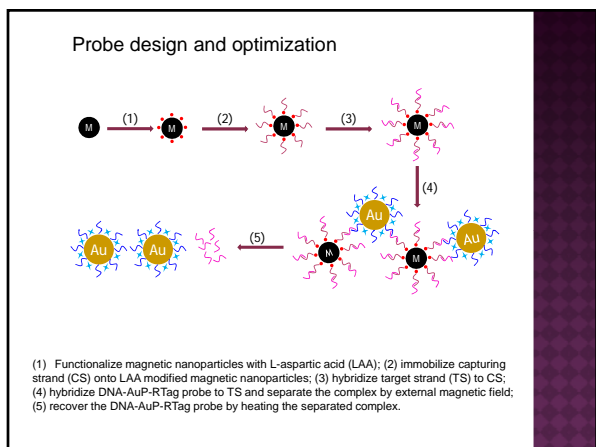
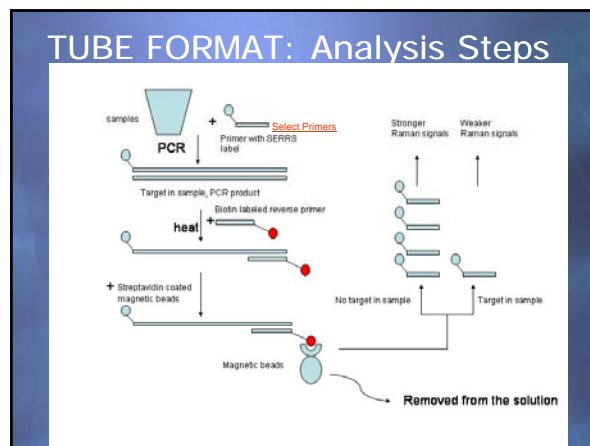
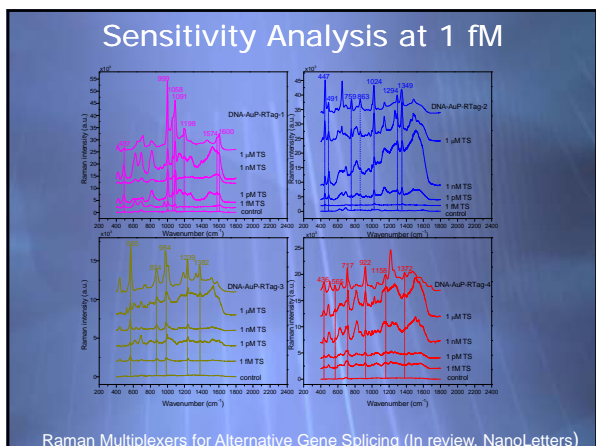
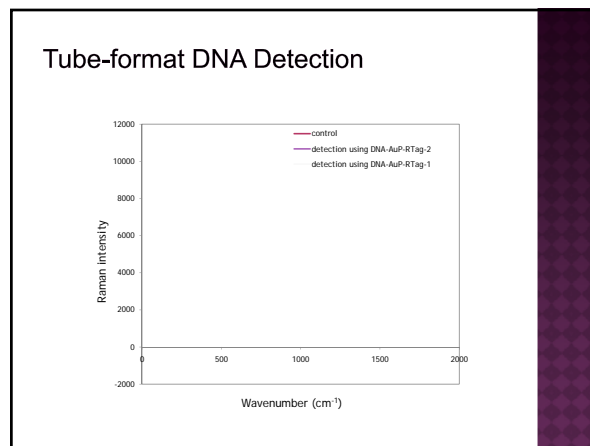
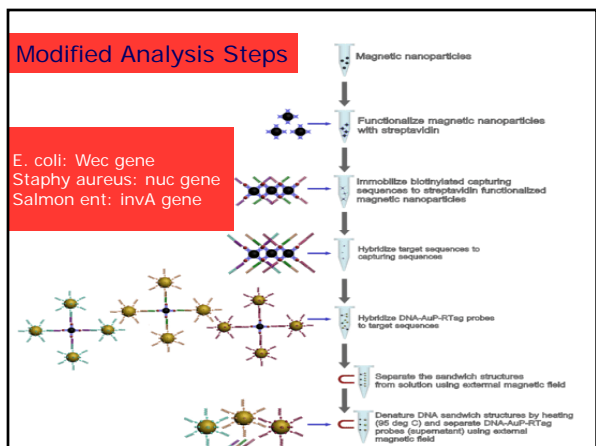


Table 1. Primer sequences for the detection of bacterial targets

Species	Target Gene	Primer sequence (5'-3')	Amplicon size
<i>E. coli</i> O157:H7	<i>hlyA</i> gene	GTAGGGGAAAGCGAACAGAG AAGCTCCGGTGCCCTGAA	361 bp
<i>Campylobacter</i> genus TM	16S rRNA	GGATGACACTTTCGGAGC CATGTAGCAGCTGTGTC	816 bp
<i>Salmonella</i> sp. ⁺	<i>InvA</i> gene	TATGCCACGTCGGGCAA TCCACCCTCAAGGAACC	275 bp
<i>Staphylococcus aureus</i> ⁺	nuclease gene	GGGATTGATGGTGATACGGTT CAAGAATTGACGAATAAAGC	276 bp
<i>Listeria monocytogenes</i> ⁺	hemolysin	CGGAGGTTCCGCAAAAGATG CCTCCAGATGATCGATGTT	234 bp



Deliverables

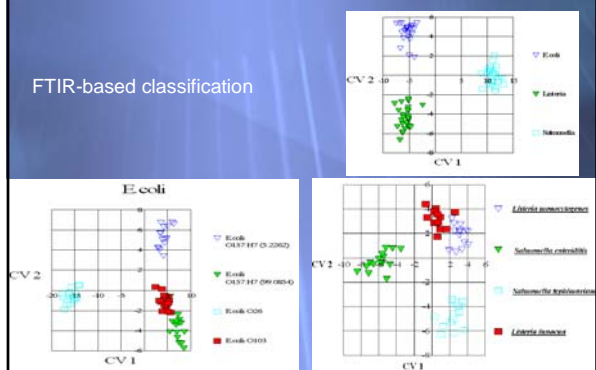
- ✦ Optimization of SERS effect of dye/gold particle size and excitation wavelength and concentrations with multiplexing
 - ✦ 8-plex system identified and demonstrated (manuscript published in Analytical Chemistry)
- ✦ Fabrication of SERS tagged DNA probes for multiplex DNA detection
 - ✦ A 4-plex nonfluorescent DNA detection protocol: in review, Nano Letters
- ✦ Implementation of a one-pot analysis assay for pathogen detection using SERS DNA probes

Summary

- ✦ One manuscript Published (ac)
- ✦ One manuscript in Review (nl)
- ✦ One manuscript expected (Dec-Jan)
 - ✦ Multiplexing and single basepair mismatch detection
- ✦ One Grant Proposal [Feb 2008]
 - ✦ Multiplexing (4-8 plex)
 - ✦ Quantification
 - ✦ SNPs
 - ✦ Pattern assessment

Biosensor-based Spectroscopy

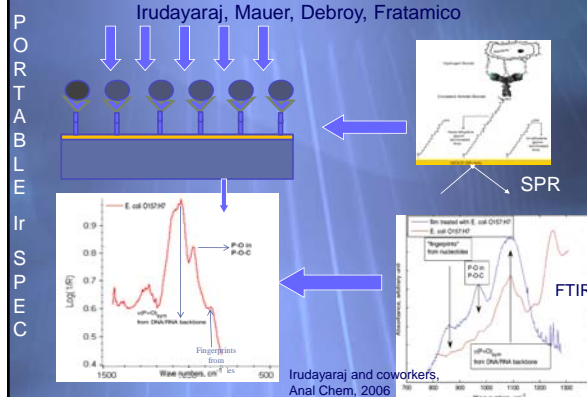
FTIR-based classification



Irudayaraj, Mauer, Debroy, Fraticco

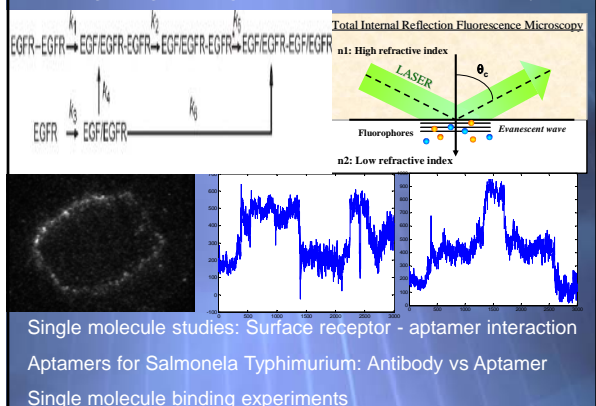
Biosensor-based Spectroscopy

Irudayaraj, Mauer, Debroy, Fraticco



Irudayaraj and coworkers, Anal Chem, 2006

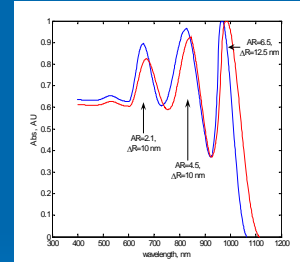
Ongoing: Single Molecule Microscopy



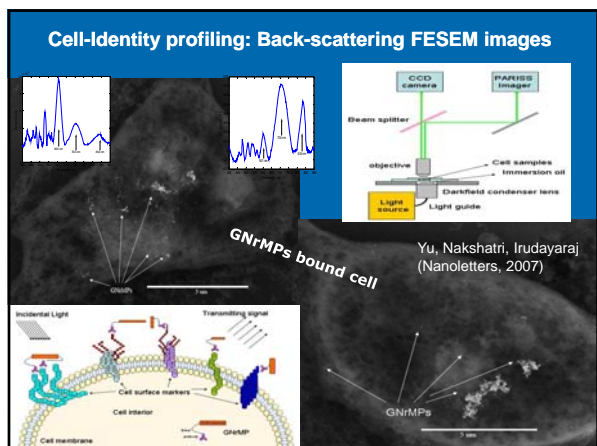
Quantitative target detection multiple targets in one sample

When the concentration of targets is within the dynamic range of the GNrMP system (10^{-9} to 10^{-7} M), multiple targets can be quantitatively measured simultaneously.

	Target 1	Target2	Target3
Real value	100 nM	20 nM	20 nM
Measured value	92.32 nM	19.14 nM	15.86 nM



Use of Multifunctional nanorods
 Yu and Irudayaraj (Anal Chem, 2007; Biophysical J, 2007; Langmuir, 2007)



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Thank You