DNA Biosensors using Morpholinos

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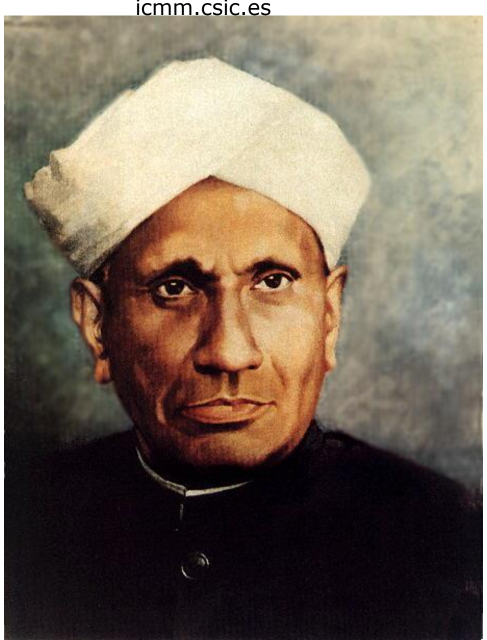
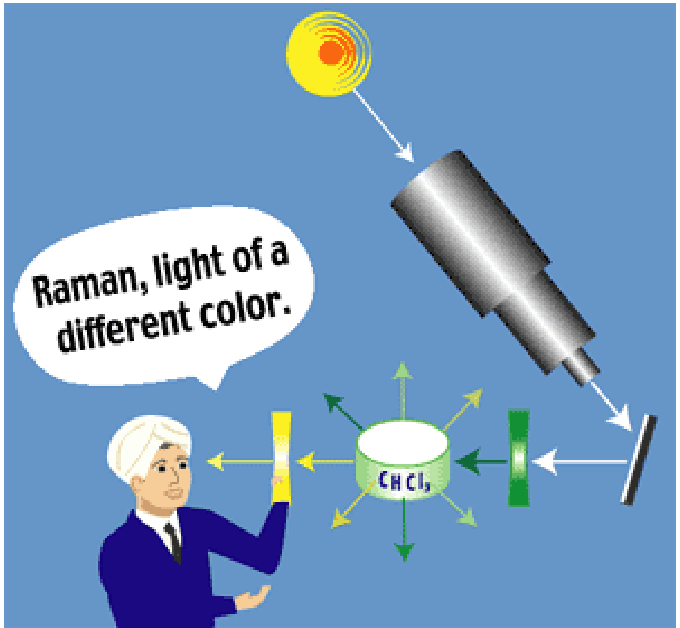
Introduction

- Raman Effect discovered in 1928
- Inelastic scattering of photons
- Surface Enhanced Raman Scattering (SERS), discovered in 1974
- Occurs on rough metal surfaces

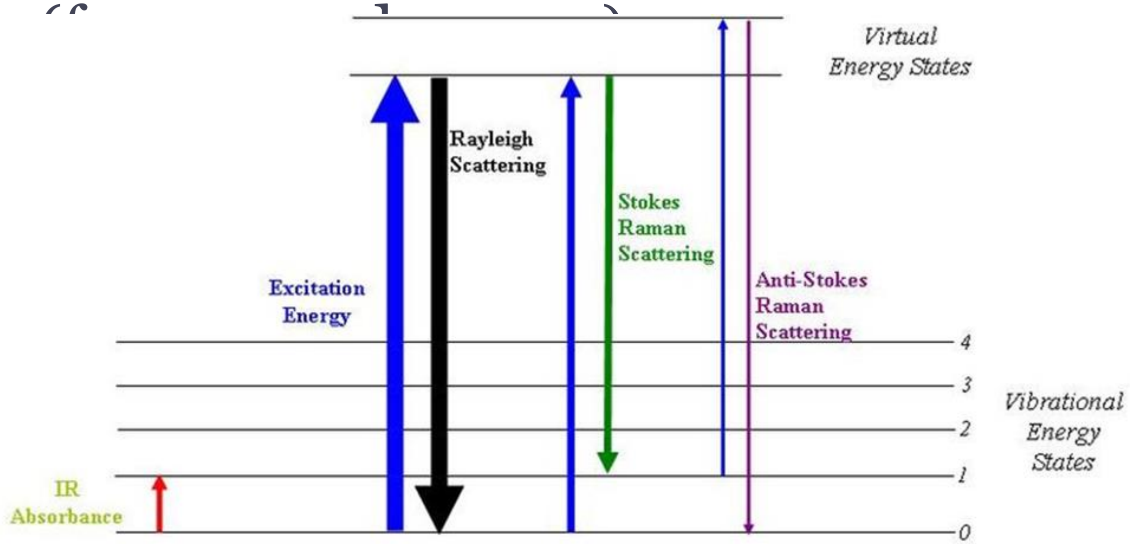
icmm.csic.es





Light Scattering

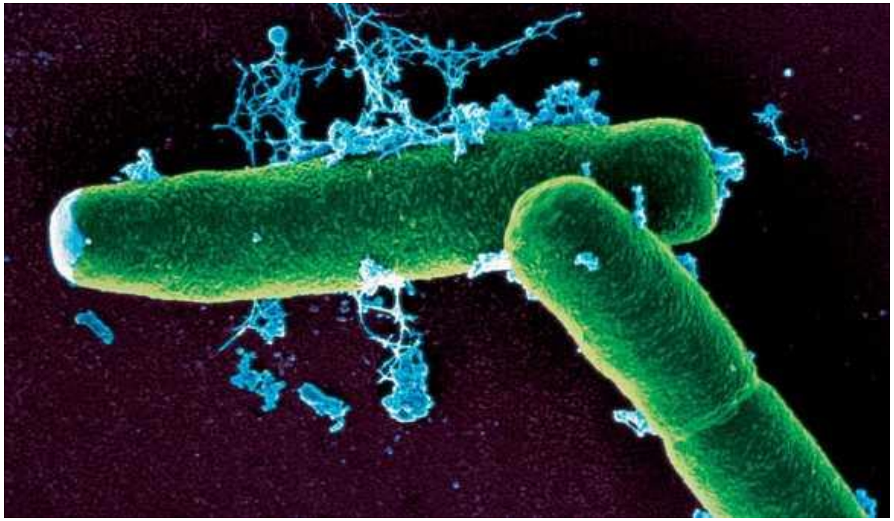
- Frequency shift is a change in the "color" of the scattered light
- Elastic Scattering
  - ΔE = 0 (no frequency shift)
- Inelastic scattering
  - ΔE > 0 (frequency increase)
  - ΔE <



Applications of SERS

- Diagnosis of infectious diseases.
- Biological warfare agents
- Protection against counterfeiting.
- Faster than other detection methods

www-cs-faculty.stanford.edu

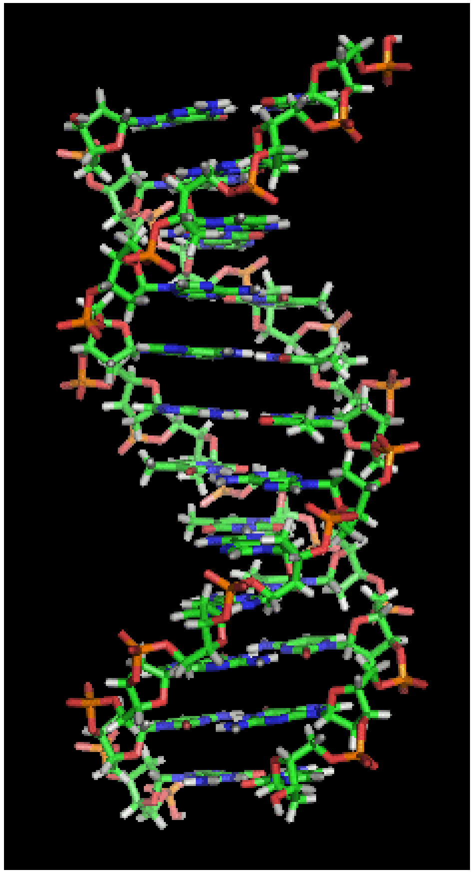


http://www.srs.dl.ac.uk/Annual_Reports/AnRep01_02/anthrax.htm

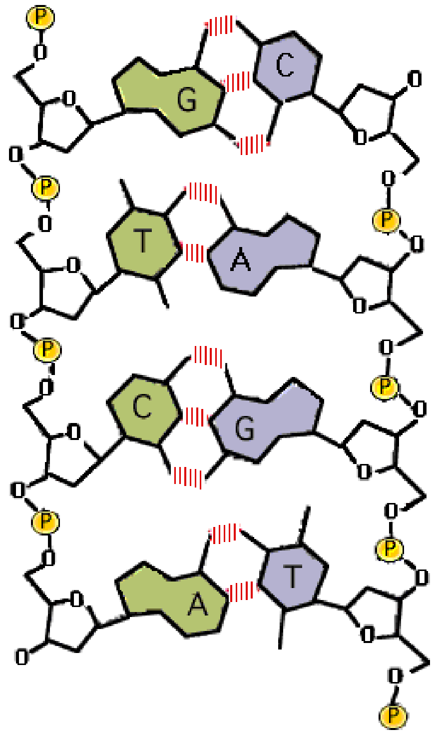
soupisnotafingerfood.wordpress.com

DNA

- Genetic material
- Four nucleotides
  - A, G, C, T
- Charged backbone
- Forms a double helix



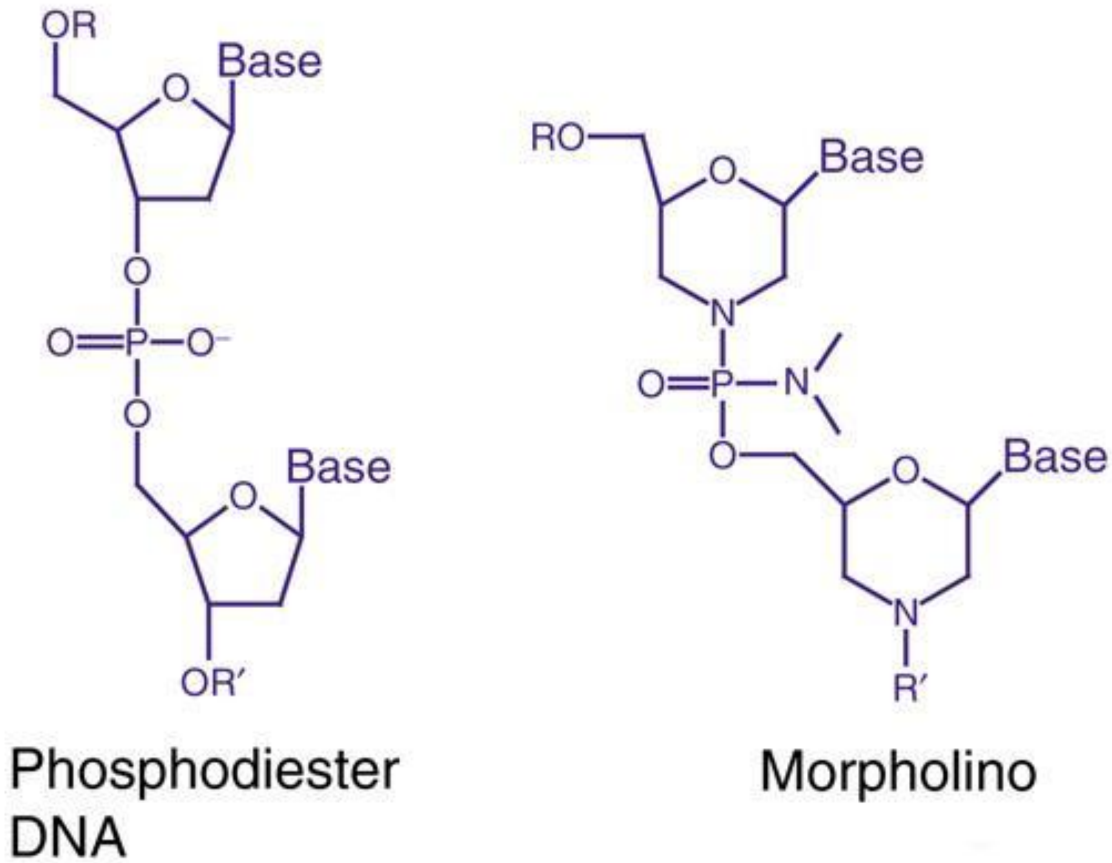
www-jmg.ch.cam.ac.uk



en.wikipedia.org

Morpholinos

- Nucleic acid analogue
- Uncharged backbone
- Morpholine ring in place of deoxyribose ring
- Binds strongly to DNA
- Immune to nuclease degradation

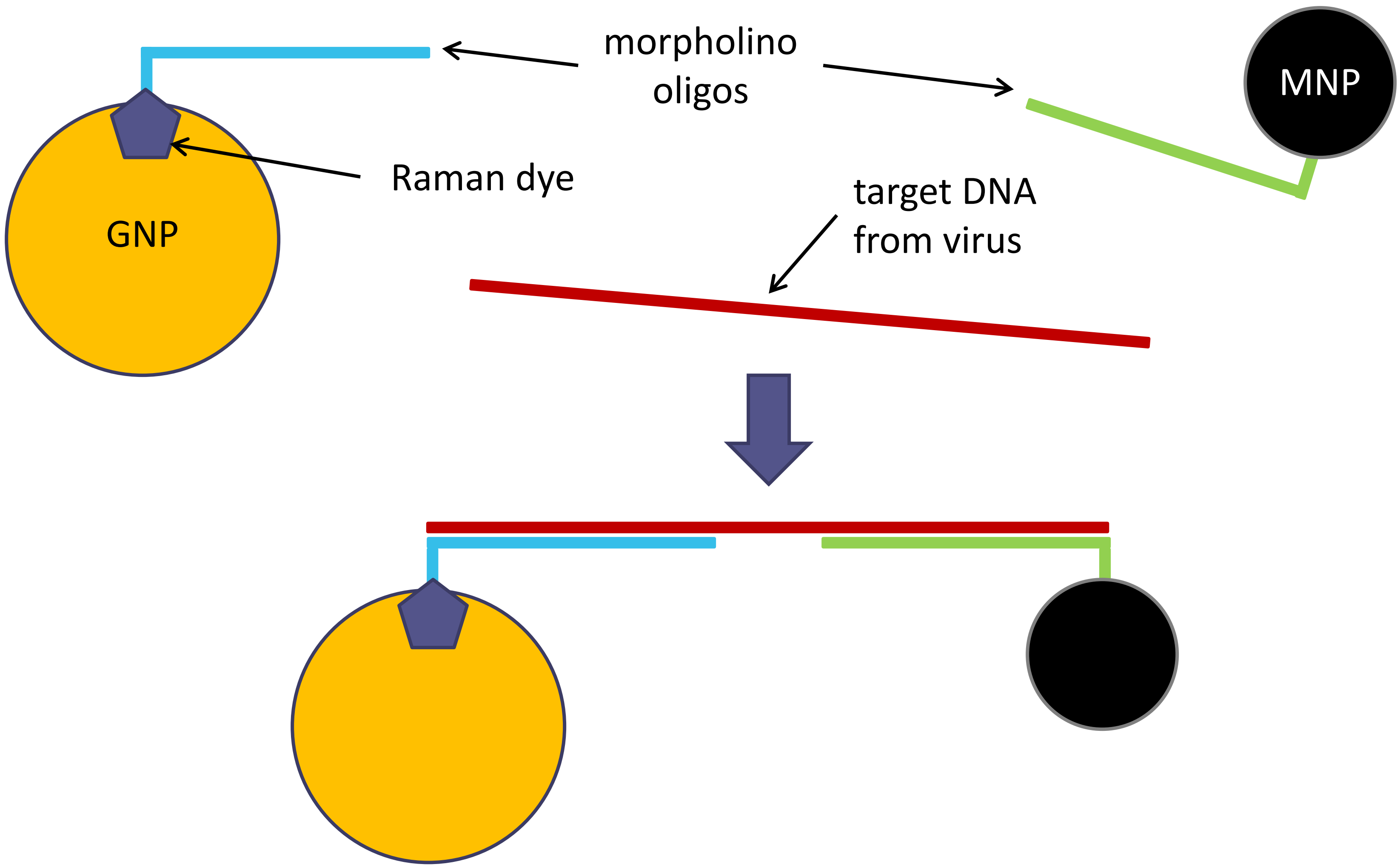


genomebiology.com

Research Objectives

- Incorporate morpholinos into our DNA sensing method
- Assess morpholino-DNA binding efficiency in low salt concentrations
- Test sensitivity of the DNA sensing method incorporating morpholinos

Mechanism of detection



Experimental

- Electrophoresis
  - Morpholino-DNA binding affinity was assessed using gel electrophoresis.
  - 10% Polyacrylamide gel used
  - 1M and 0.01M salt concentrations tested

Experimental

- Synthesis of magnetic nanoparticles
  - MNPs synthesized with the reverse micelles as nanoreactors
  - Fe(II) and Fe(III) were used in a 1:2 ratio in a water-xylene- NaDBS emulsion
  - MNPs were silica by adding TEOS and APTES to the reaction solution
  - MNPs were functionalized with morpholinos using SM-PEG under slightly basic conditions

Experimental

- DSNB synthesis
  - DCCD, NHS and DNBA added to THF and stirred magnetically for 12 hours.
  - Product was purified by dissolving obtained powder in a 1:1 mixture of hexanes and acetonitrile and cooling to -40^{o}C.
  - Product separated via centrifugation.

Experimental

- Surface functionalization of gold nanoparticles
  - DSNB added to GNP colloid under slightly acidic conditions
  - Morpholinos added to purified DSNB-GNPs under same conditions
  - SERS spectra of Functionalized GNPs obtained

Experimental

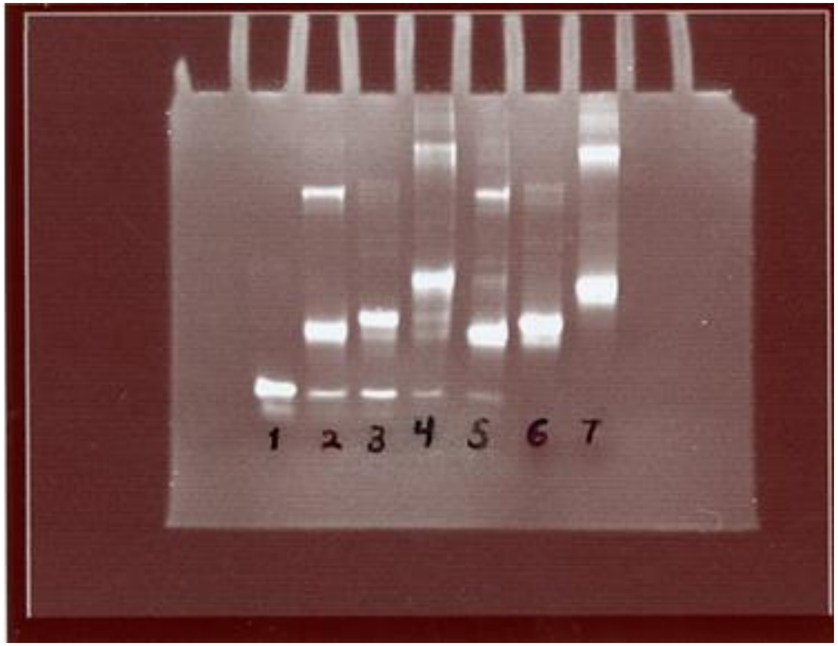
- Morpholino-DNA hybridization and SERS spectroscopy
  - Target DNA was added to a mixture functionalized MNPs and GNPs at in a 10mM buffer solution
  - Target DNA tested at 500nM, 250nM and 50nM concentrations
  - Hybridized particles collected magnetically and pipetted onto a small Neodymium magnet.
  - SERS spectra of of hybridized particles was obtained

Results

- Gel Electrophoresis yielded promising results
  - Binding efficiencies of morpholino-DNA hybrids were higher in low salt concentration lanes.
  - Confirms that Morpholinos do not require a strong buffer for hybridization
  - Provides strong evidence that morpholinos bind to DNA better in low salt concentrations

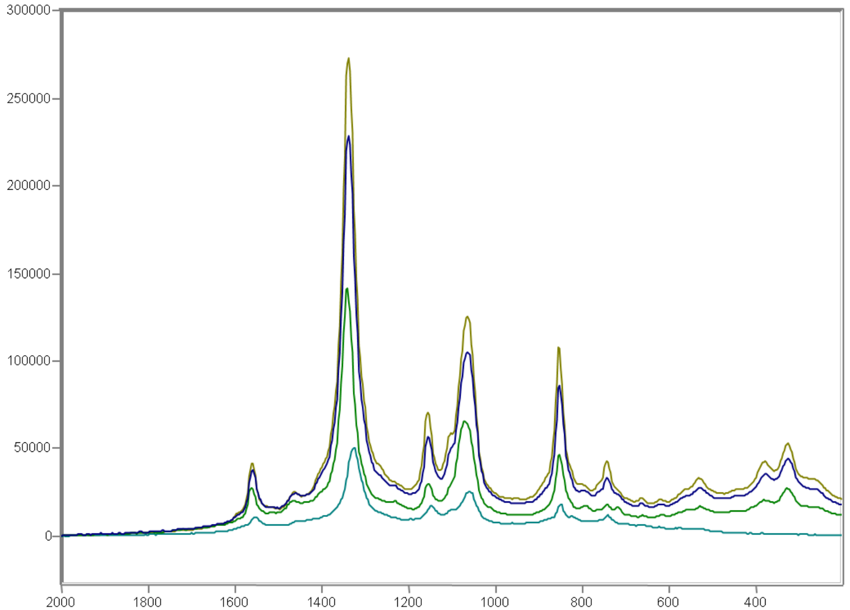
Results

- Lanes
  - 1: Target DNA (tDNA)
  - 2 and 5: tDNA + MNH5’
  - 3 and 6: tDNA + MSH3’
  - 4 and 7 tDNA + MNH5’ + MSH3’
  - Lanes 1-4 were hybridized in 1M PBS
  - Lanes 5-7 were hybridized at 10mM phosphate



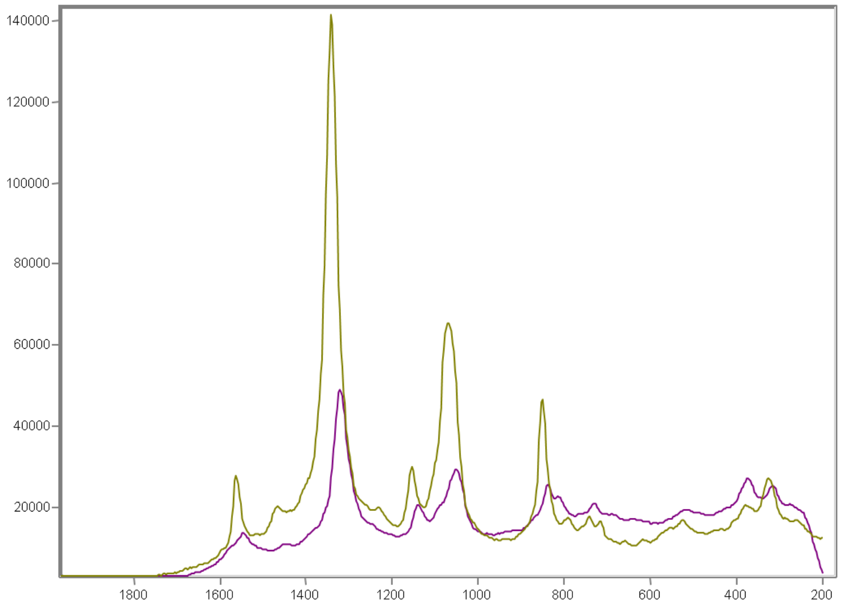
Results

- DSNB on colliodal gold exhibited distinctive, narrow peaks with a low baseline
- Spectra intensity varied widely
- Highest peak ranged from 50K to 275K arbitrary units



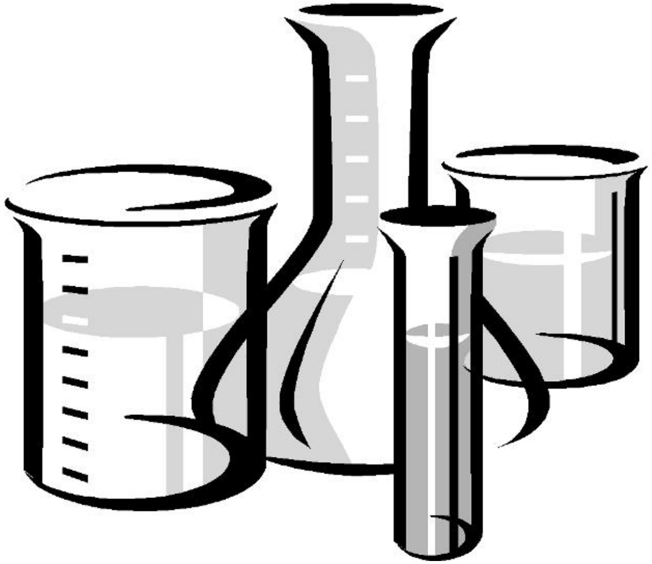
Results

- Successful hybridization of the functionalized GNPs and MNPs was accomplished in 10mM phosphate buffer with 500nM target DNA
  - Beige peak: DSNB on gold
  - Magenta peak: Hybridization
- Hybridizations at 250nM and 50nM DNA were not detected



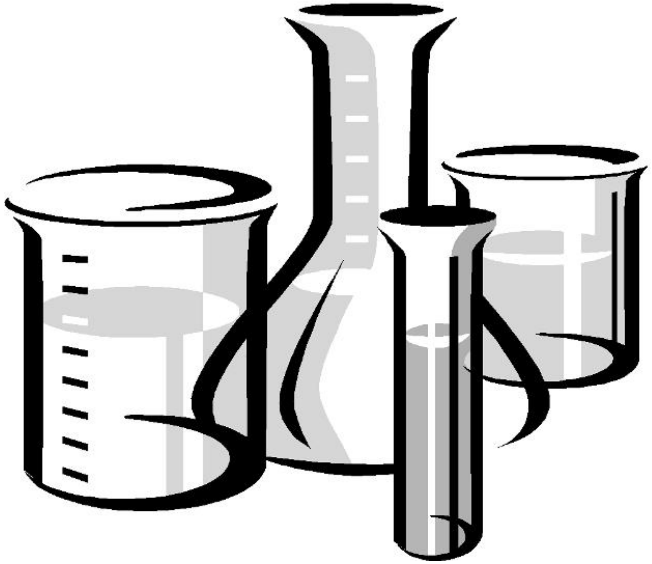
Conclusions

- Morpholinos do not require a strong buffer for hybridization
- Proof-of-concept of DNA sensing protocol
- Low sensitivity likely due to non-optimal morpholino surface concentration and MNP to GNP ratio



Future research

- Hybridization will be tested in purified H_2O
- Optimization of morpholino concentrations on nanoparticle surfaces
- Optimization of GNP to MNP ratio



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