

DNA Biosensors using Morpholinos

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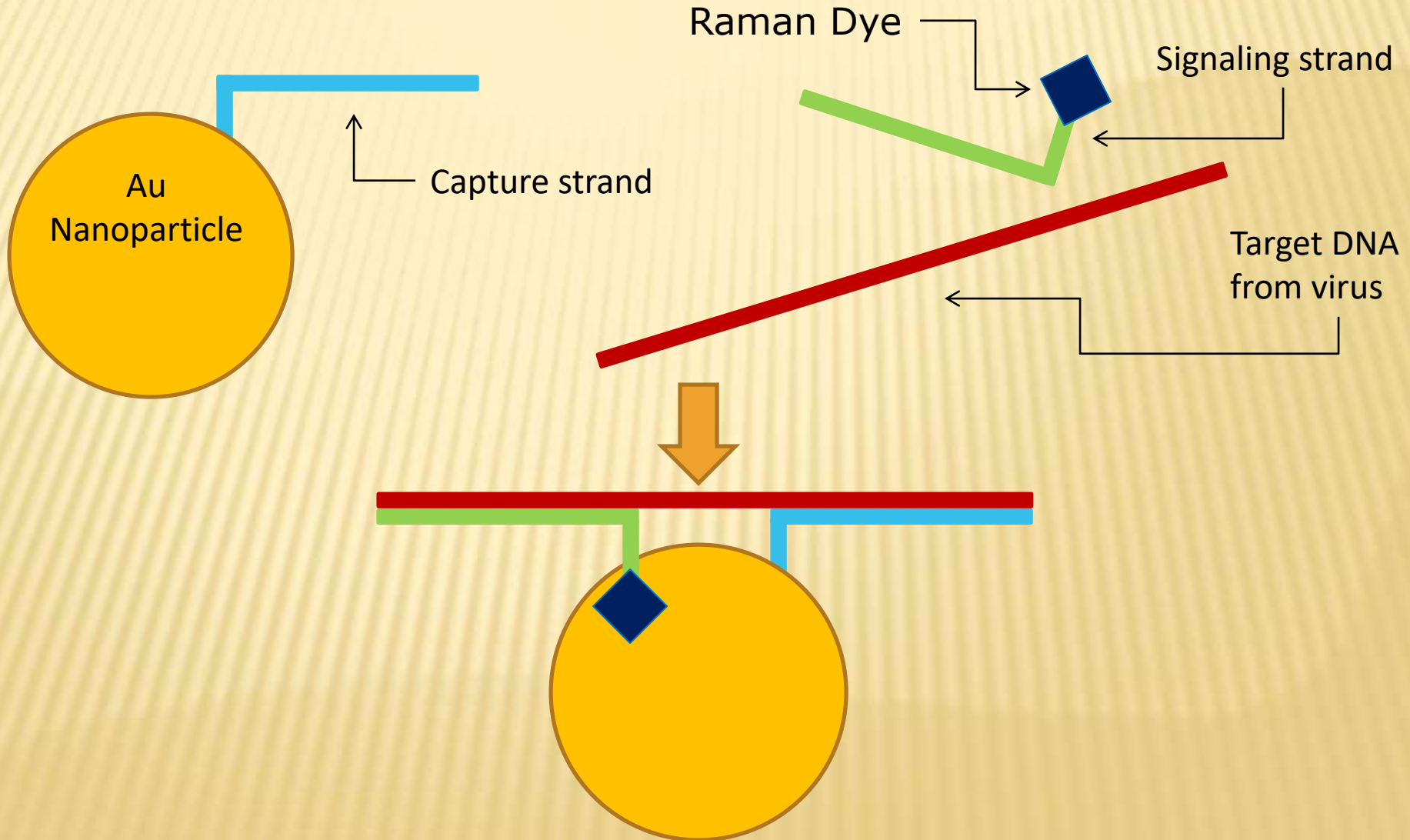
Graduate mentor: Hao Zhang

Applications of SERS Spectroscopy

- ✘ Surface Enhanced Raman Scattering (SERS)
- ✘ Highly sensitive and rapid
- ✘ Identification of nucleic acids
- ✘ Detection of biological warfare agents
- ✘ Many more applications exist



Mechanism DNA of detection



Research Objectives

- ✘ Compare Au and Ag nanoparticles for use in SERS-active surface fabrication
- ✘ Incorporate the use of morpholinos in DNA biosensor development
- ✘ Examine the effect of buffer strength on morpholino-DNA binding.

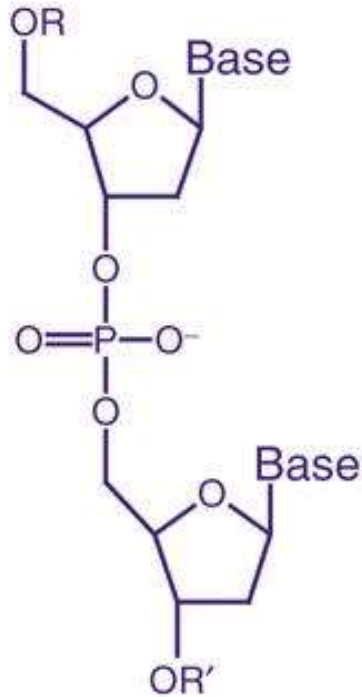
Hypothesis

- ✗ As a result of using morpholinos, detection of DNA will be enhanced
 - + Significantly Lower buffer strengths can be used effectively
 - + Decrease in background noise in SERS signal

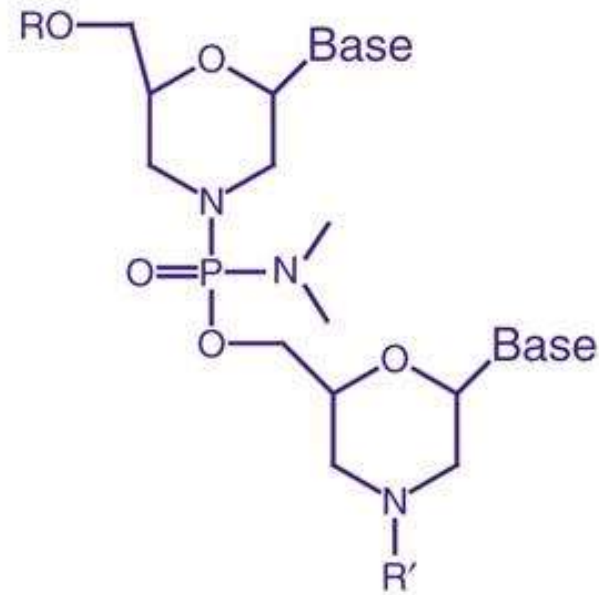


Morpholinos

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



Phosphodiester
DNA



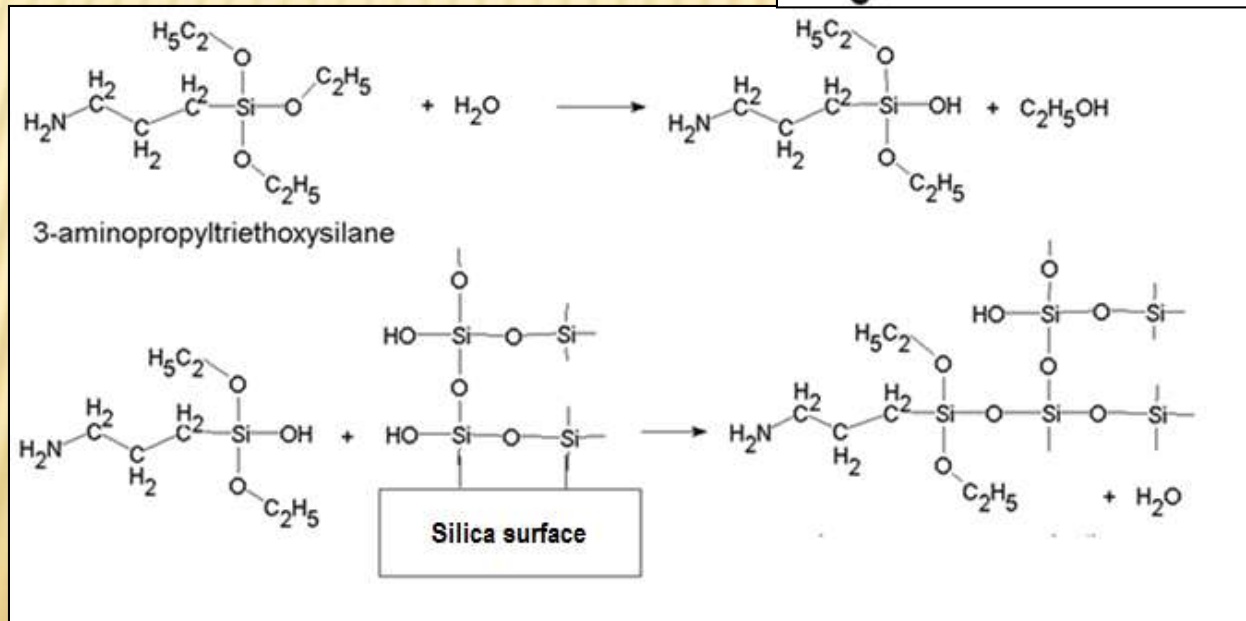
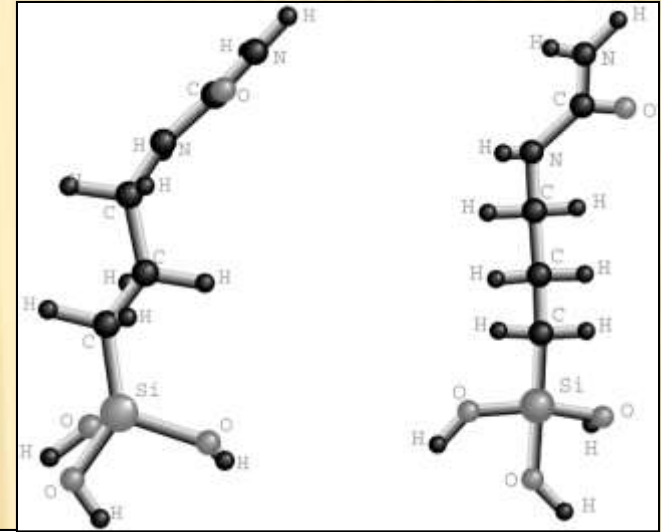
Morpholino

SERS-active Surface Fabrication

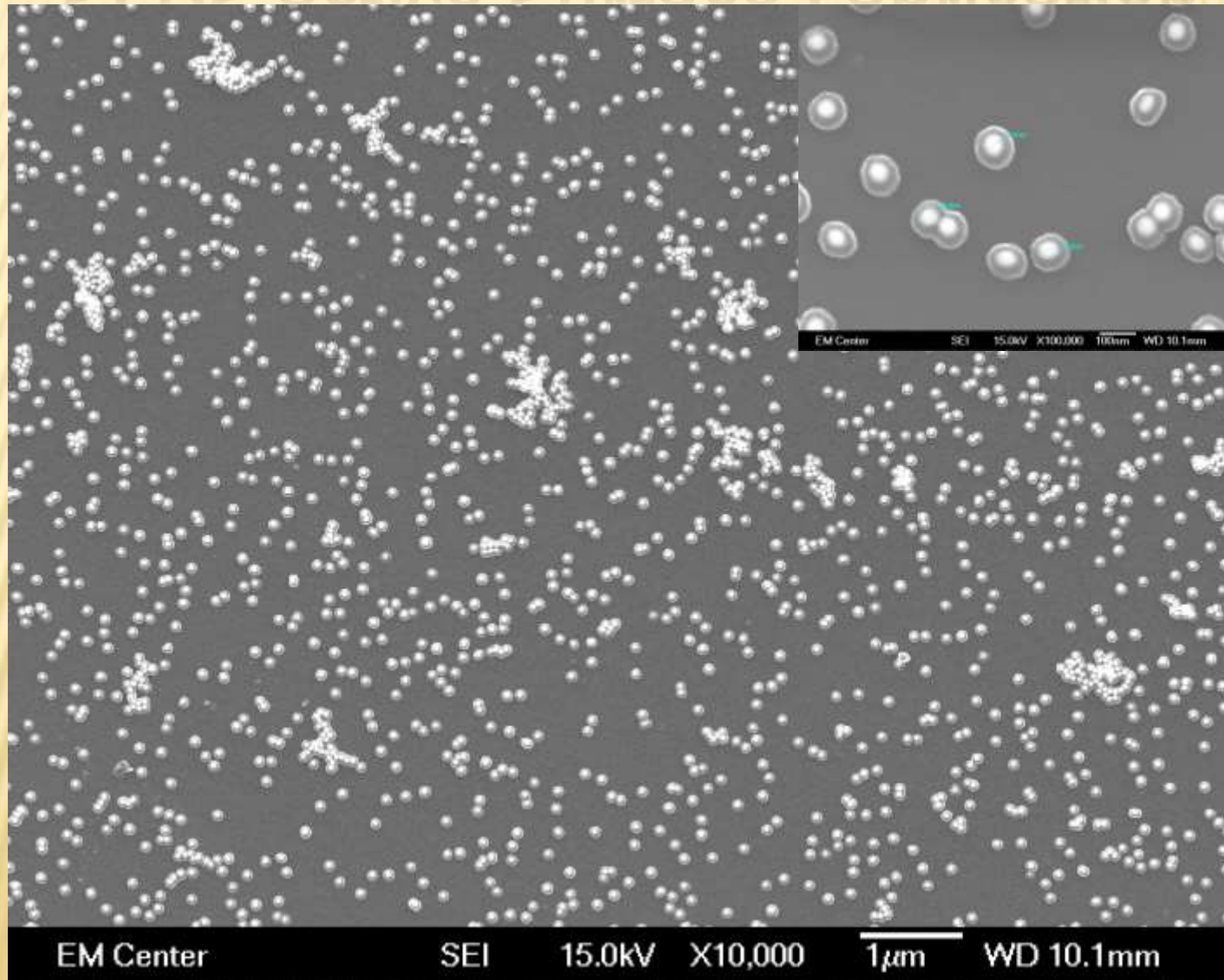
- ✘ Small silica squares used as a support for nanoparticle attachment
- ✘ SERS-active surfaces fabricated with 60nm Au and Ag nanoparticles

SERS-active Surface Fabrication

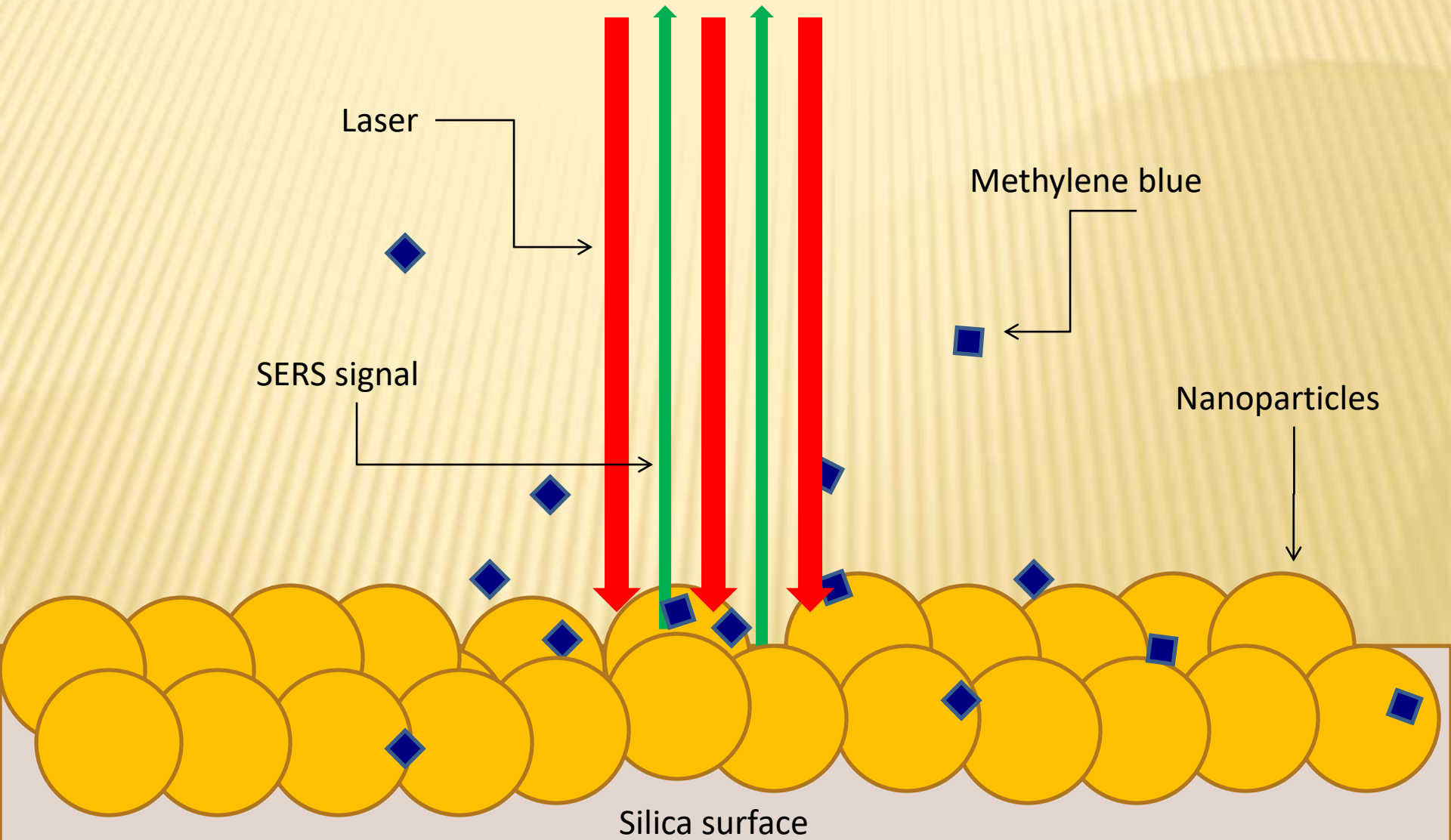
- ✗ Silica thoroughly cleaned
- ✗ Funtionalized with APTES (crosslinking agent)
- ✗ Rinsed and put in nanoparticle colloid



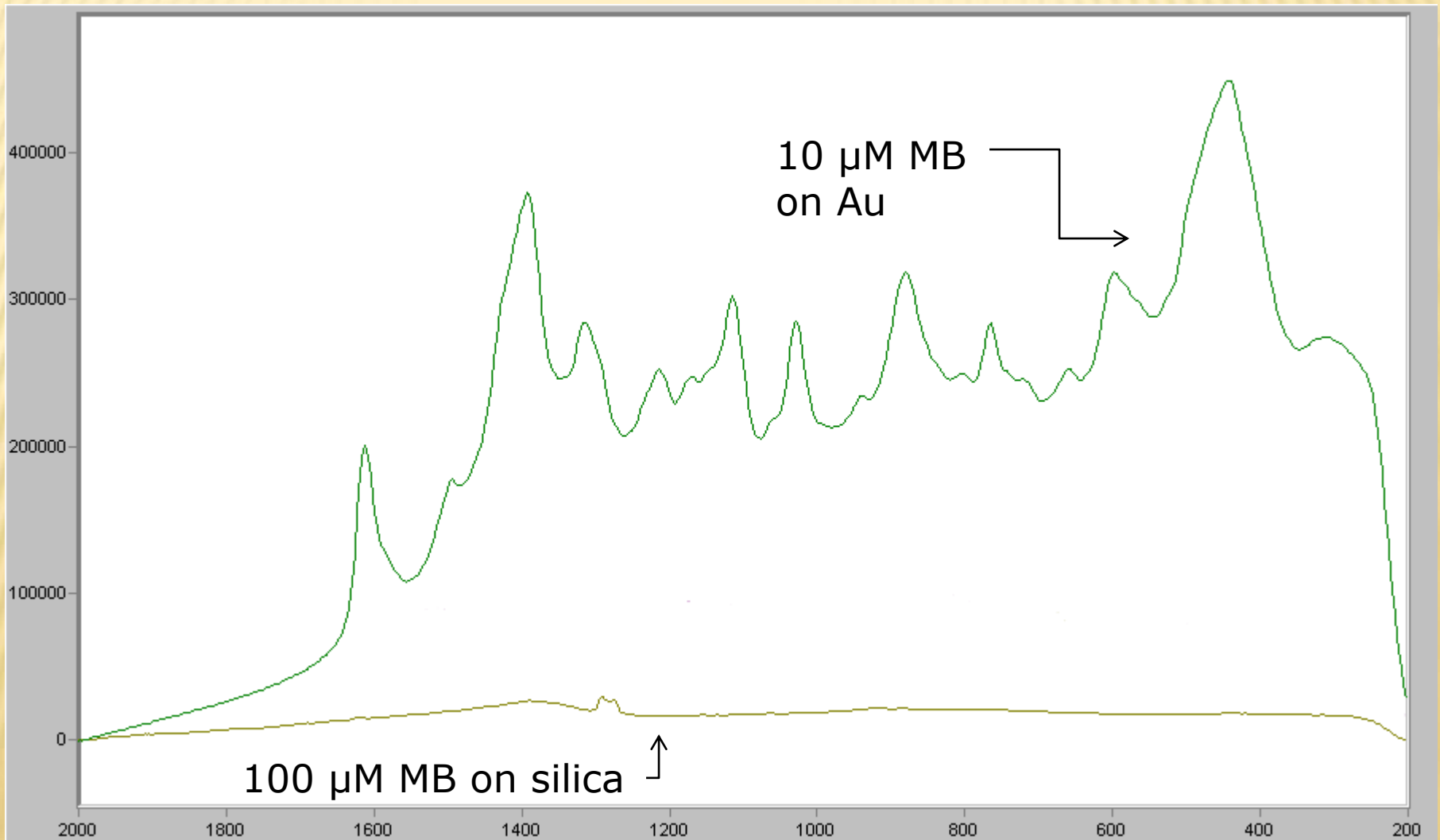
SERS-active Surface Fabrication



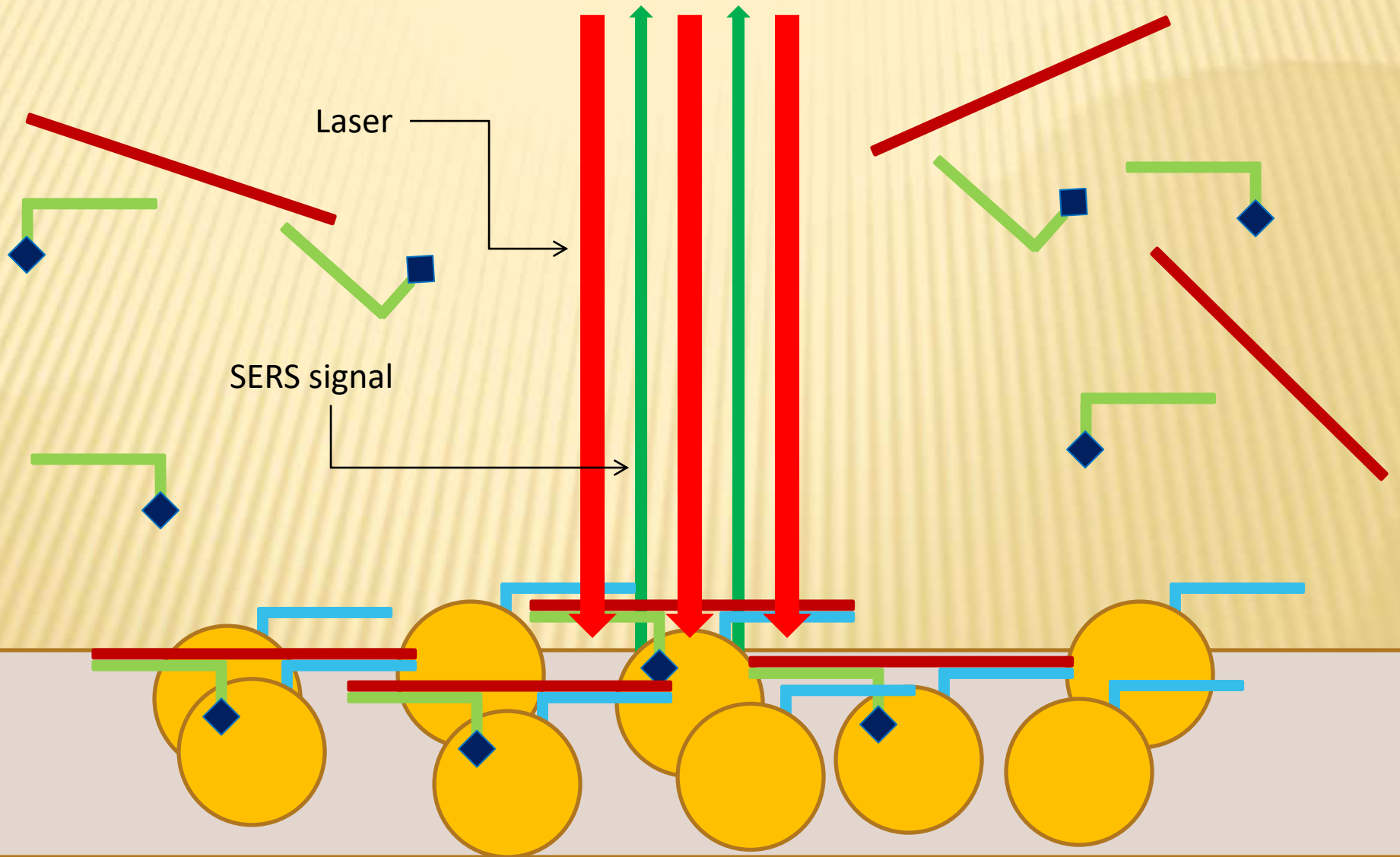
Preliminary experiments



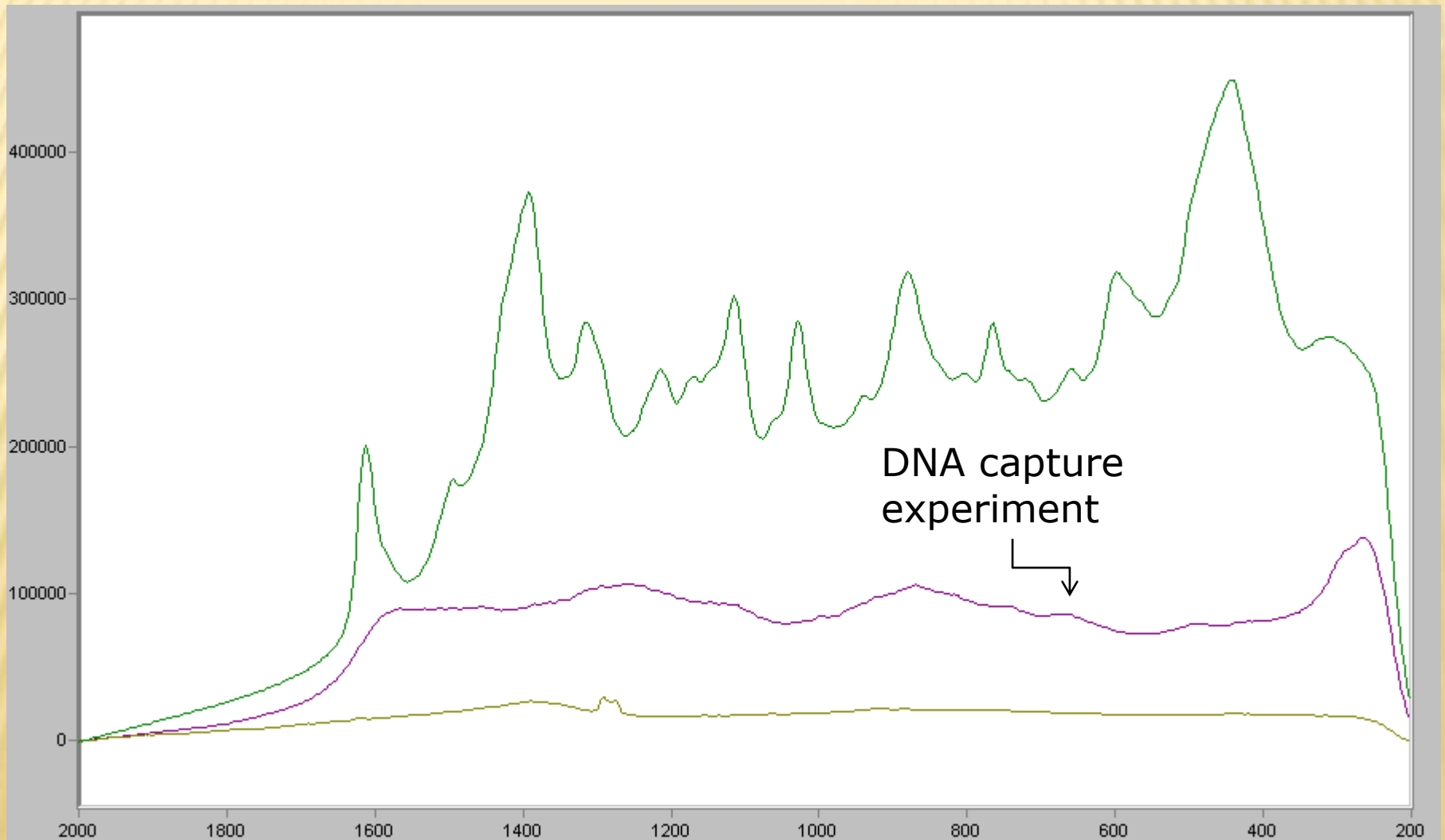
Results



DNA capture experiment



DNA capture Results



QCM-D

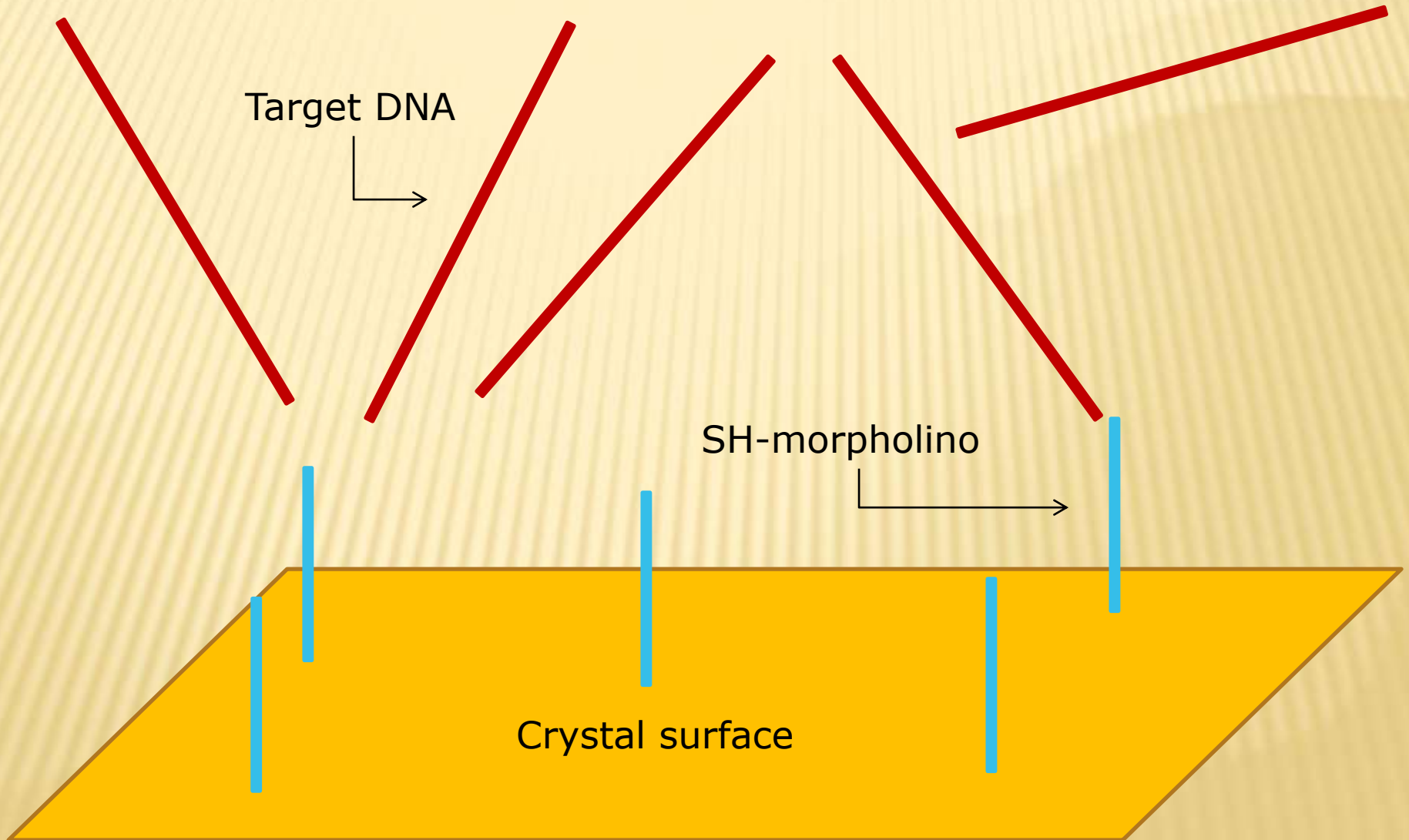
- ✘ Quartz Crystal Microbalance with Dissipation
- ✘ Detects both changes in mass and viscoelasticity
- ✘ Up to $0.5\text{ng}/\text{cm}^2$ sensitivity
- ✘ QCM-D used to examine DNA-morpholino binding



QCM-D experiments

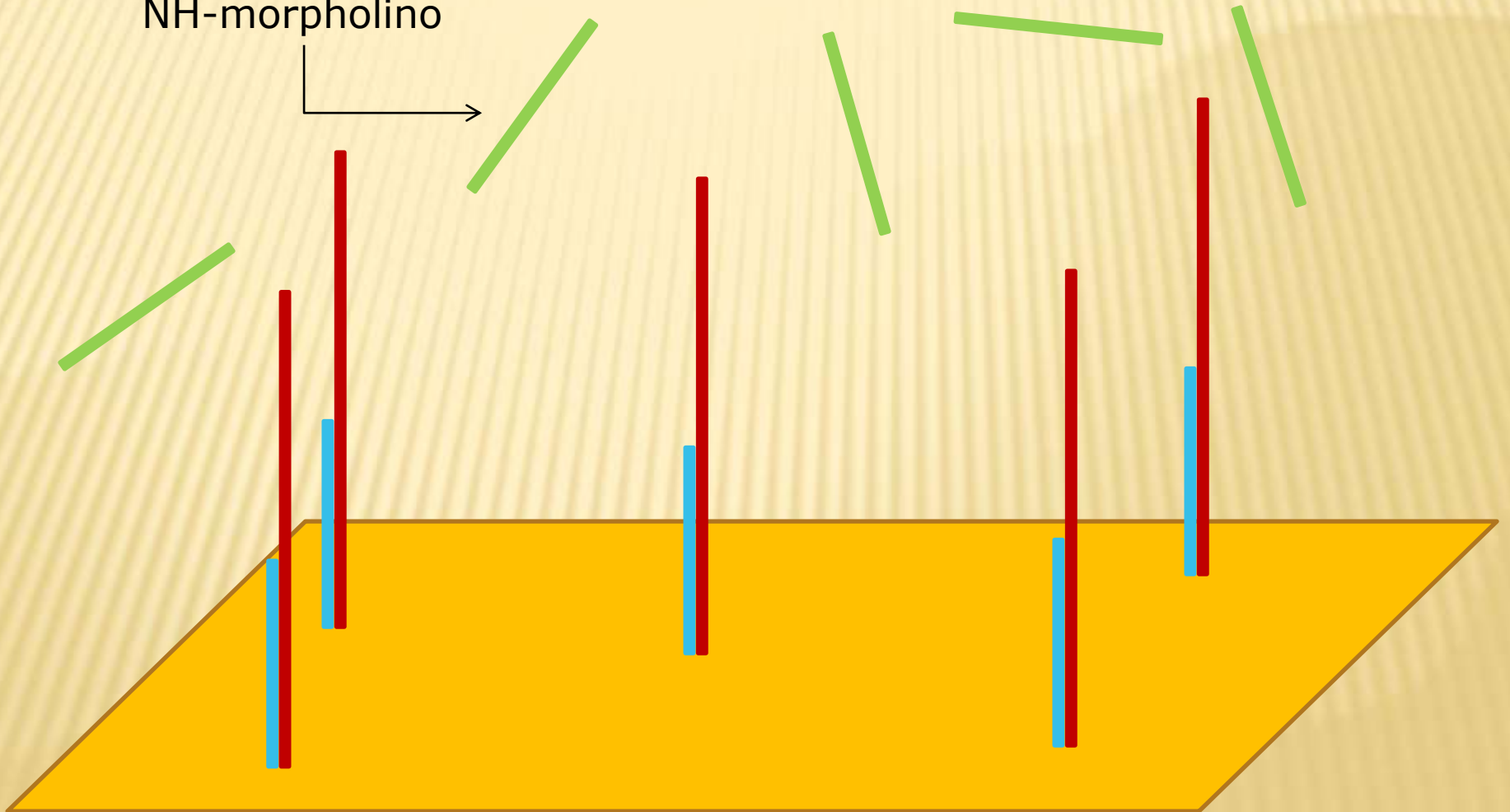
- ✘ QCM Crystals thoroughly cleaned
- ✘ Crystals submerged in thiol-functionalized morpholino solution
- ✘ Crystals placed in QCM-D modules
- ✘ Experiments run at 1.1M and 0.1M buffer strengths
- ✘ Control experiment used crystal without morpholinos bound to its surface.

QCM-D experiments

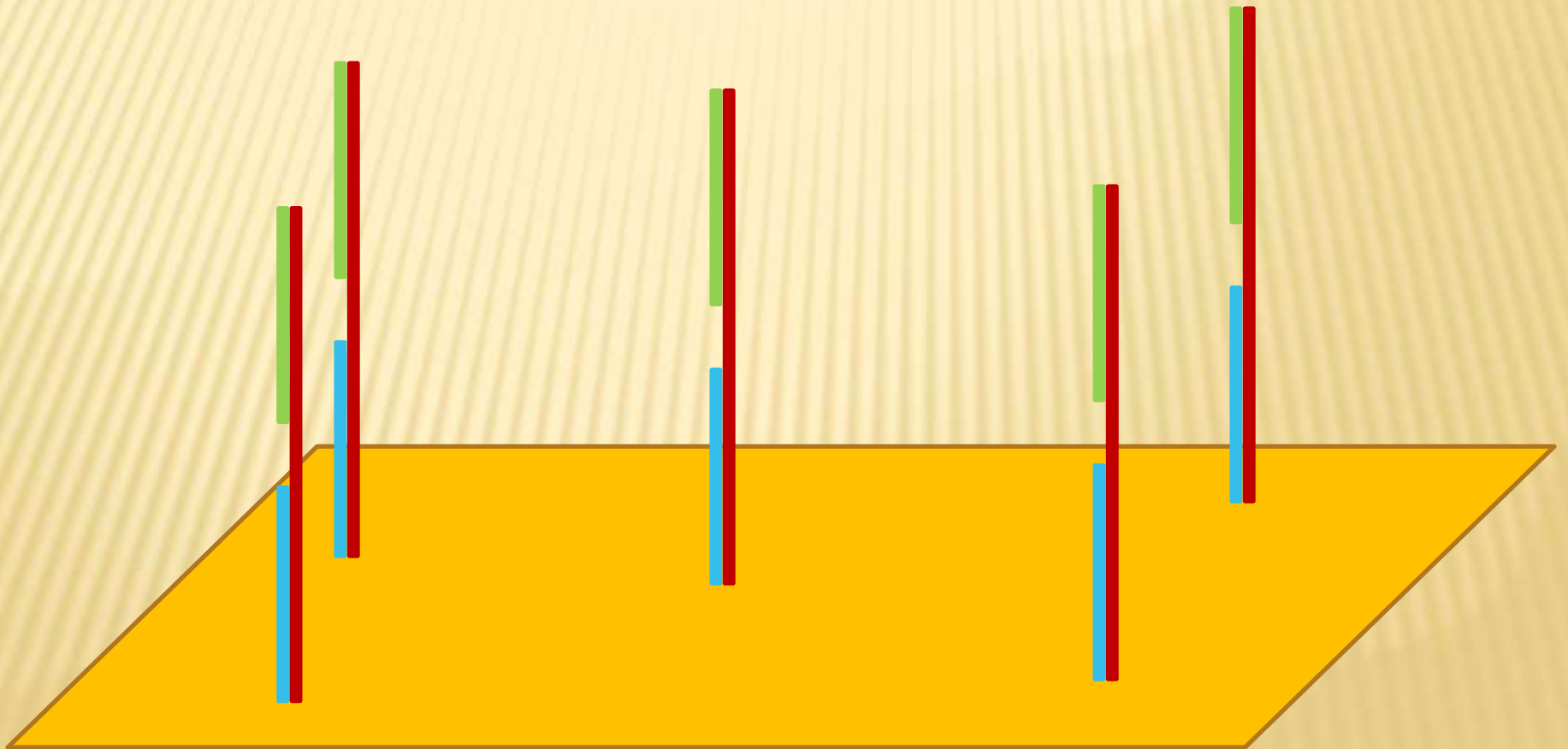


QCM-D experiments

NH-morpholino



QCM-D experiments



Results

- ✘ Enhancement factor of substrates 10^4 - 10^5
- ✘ Au substrates had the best long-term reliability
- ✘ Successful hybridization reaction on Au substrates
- ✘ QCM-D experiments showed mass adsorption under 0.1M buffer conditions was comparable to 1.1M buffer conditions.

Results

- ✘ Ag substrates oxidized over time
- ✘ Unsuccessful hybridization on Ag substrates
- ✘ Nanoparticle distribution on both types of substrates were inconsistent
- ✘ SERS spectra resulting from DNA hybridization experiment had poorly defined peaks.

Discussion

- ✘ Ag substrates failed to be reliable
- ✘ Inconsistency regarding nanoparticle distribution needs to be remedied
- ✘ Poorly defined peaks of DNA capture spectra possibly due to fluorescence or insufficient proximity of MB to the Au nanoparticles.
- ✘ Buffer concentration does not have a substantial effect on DNA-morpholino binding.
- ✘ Au substrates to be used in next experiments

Future research

- × SERS spectroscopy with morpholinos
- × Reaction with Nanoparticles in aqueous phase
- × Examine other Raman reporters
- × Development of new Raman surfaces

Acknowledgements

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