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Characterizing the interactions of gold nanoparticles with model cell membranes

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NSF CBET 0966496

How do NPs kill cells?

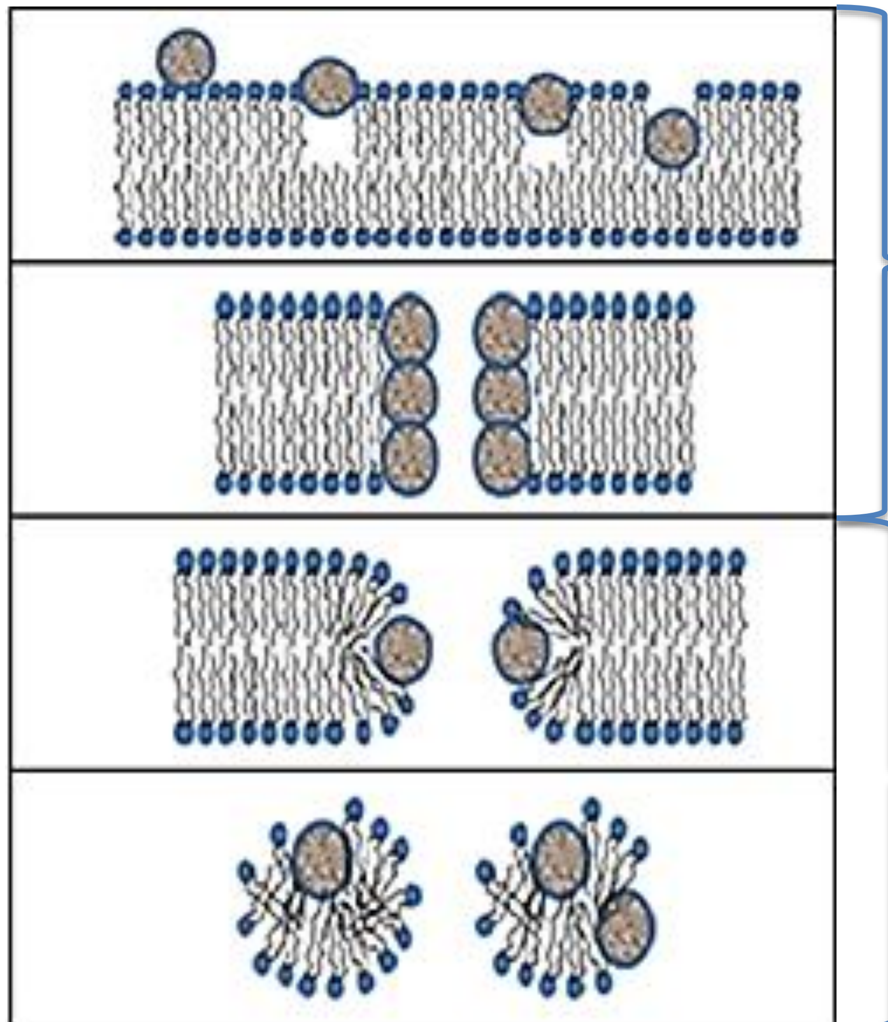
- Disruption of cell membrane integrity
- Cell damage by generation of reactive oxygen species (ROS)
- Damage to DNA
- Damage to the functionality of cellular proteins/enzymes
- Triggering of inflammation
- Damage to mitochondria

Mechanisms of NP Interaction with SLB

Adsorption onto bilayer

Pore formation

Lysis



Mass Increase

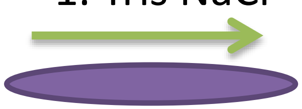
Mass Decrease

Quartz Crystal Microbalance with Dissipation (QCDM-D)

Q-sense.com

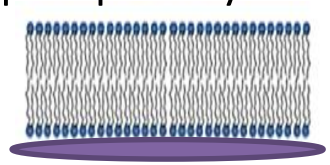
- Egg Phosphatidylcholine (PC) as a model membrane

1. Tris NaCl



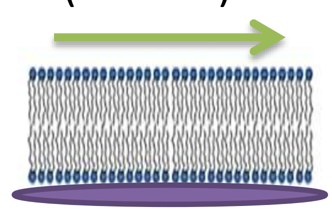
Sets Baseline

2. Add vesicles L-α-phosphatidylcholine



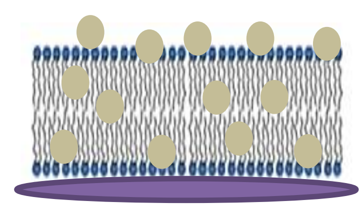
Creates Bilayer

3. Tris NaCl
4. Water
(5. PMA)



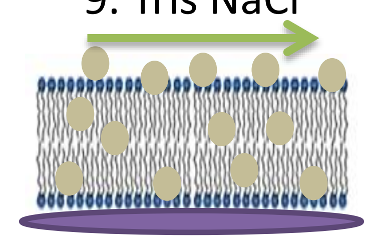
Stabilizes Bilayer
Creates similar viscosity to nanoparticle solution

6. Bare Gold Spherical Nanoparticles (2 nm, 5 nm, 10 nm, 40 nm)



Nanoparticles introduced to system

7. Water
(8. PMA)
9. Tris NaCl

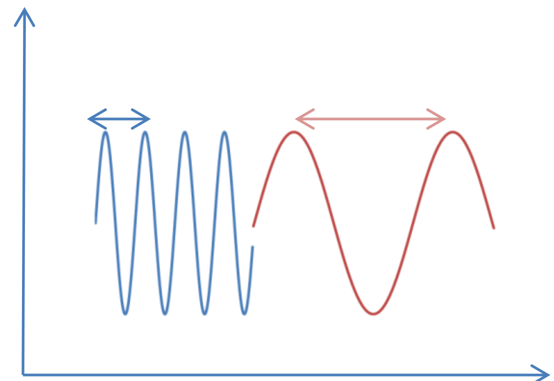


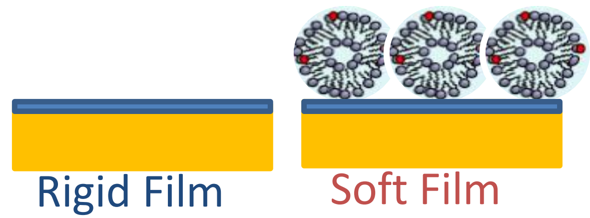
Gives comparison of bilayer after nanoparticles are introduced

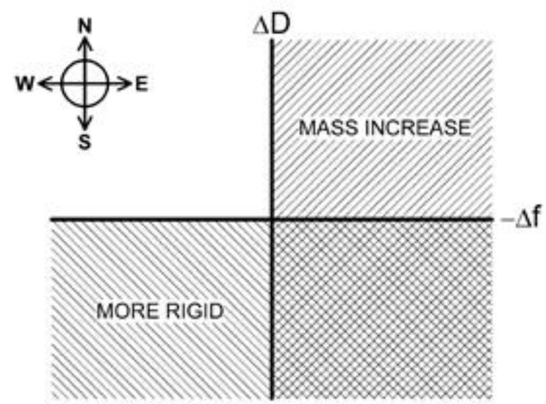
What does QCM-D show?

- Various overtones of the natural frequency (5 MHz) are measured
- Allows for characterization of processes in relation to distances from the sensor surface
  - **Higher** overtones related to processes **closer** to sensor surface[10]
  - **Similar overtone measurements**: similar structural characteristics across the membrane
    - Sauerbrey Equation is used to calculate mass changes from frequency changes

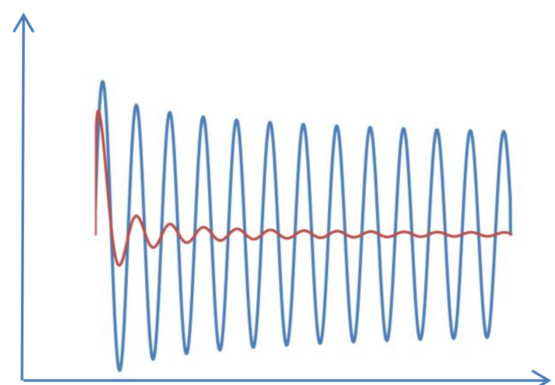
$$\Delta m = -\frac{C\,\Delta f}{n}$$





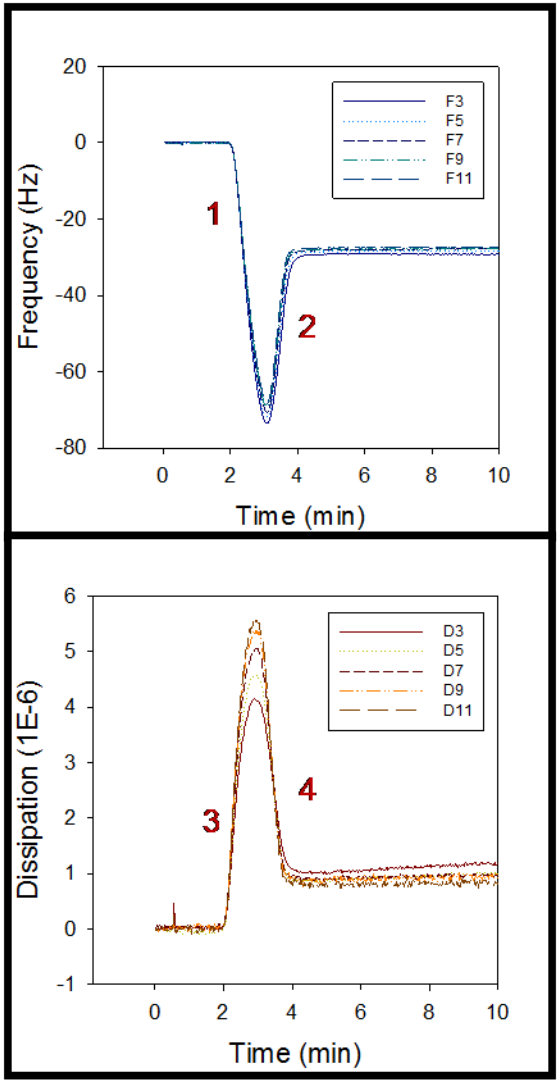


*Eur Biophys J (2011) 40:437–446



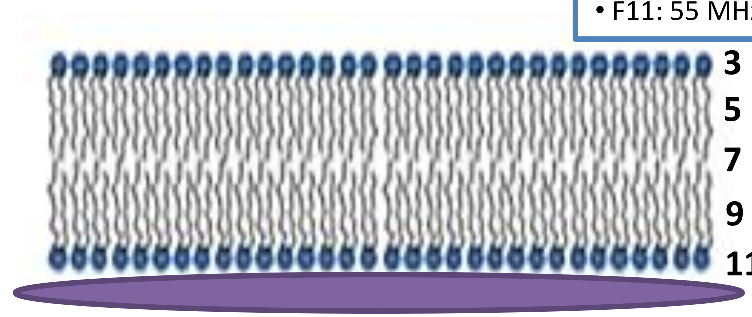
Bilayer Formation

[20]



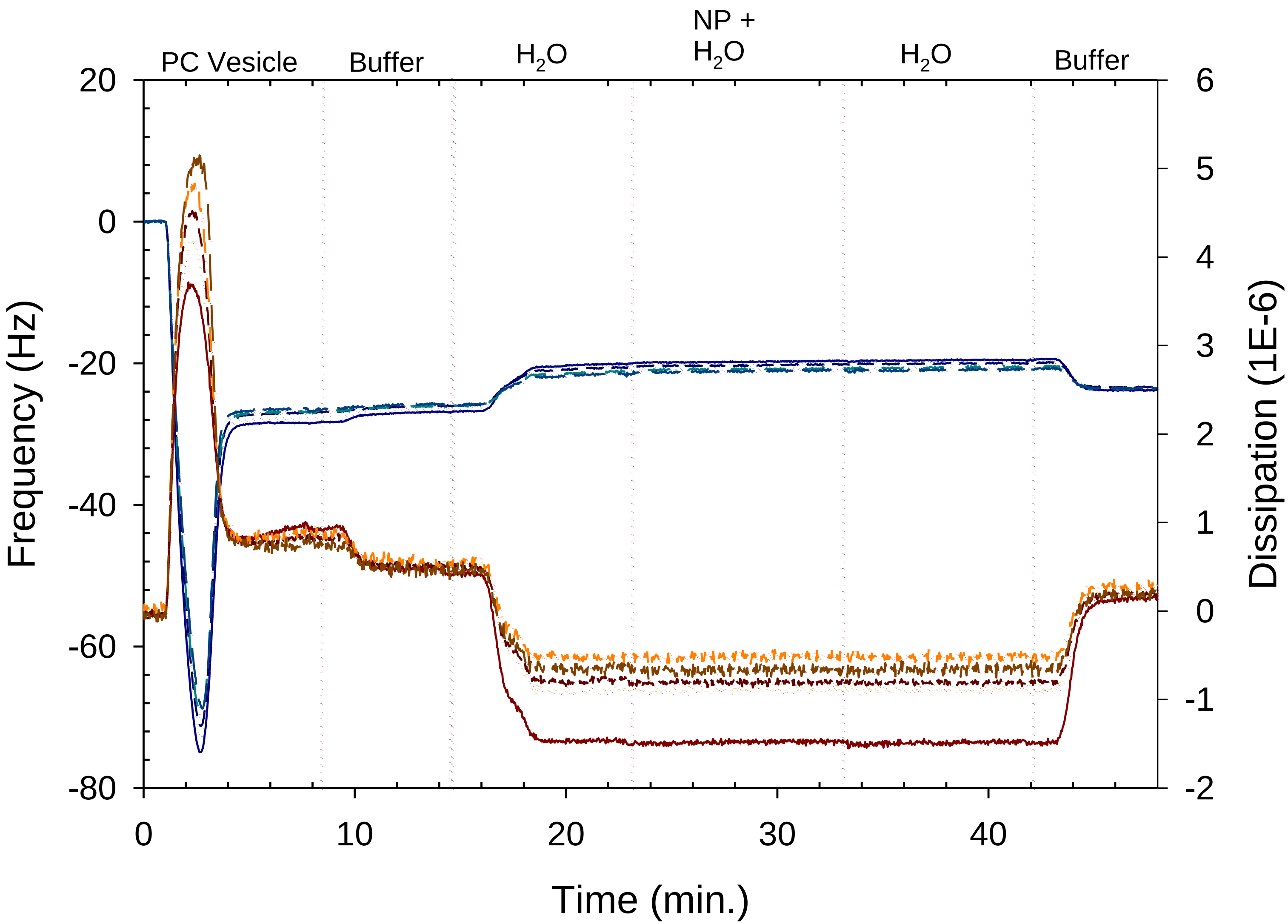
- **1)** Drop in Δ*f* corresponds to mass deposition
- **2)** Subsequent rise in Δ*f* corresponds to the release of fluid from ruptured vesicles
- **3)** Rise in Δ*D* due to presence of fluid in vesicles attached to surface
- **4)** Decrease in Δ*D* due to release of fluid and increased rigidity of mass

- F3: 15 MHz
- F5: 25 MHz
- F7: 35 MHz
- F9: 45 MHz
- F11: 55 MHz



[20] Brisson, IECB

Representative Data for Gold NPs in Water



Project Overview

Different Size

- 2 nm
- 5nm
- 10nm
- 40nm

Different Concentration

(2 nm nanoparticles coated in water)

- 5.0×10^{10} particles/mL
- 7.14×10^{10} particles/mL
- 5.0×10^{11} particles/mL
- 1.0×10^{12} particles/mL
- 1.0×10^{14} particles/mL
- 1.5×10^{14} particles/mL

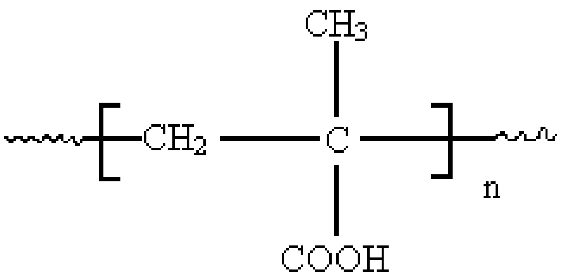
Different Environment

- Water
- PMA
- Swannee River Humic Acid

Natural Organic Matter (NOM)

Poly(methacrylic acid), PMA linear polyelectrolyte be a model to represent NOM

- MW = 6800 g/mol
- Charges due to carboxylic groups (Q=11.5 meq/g)



1 unit of PMA

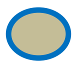
Suwannee River Humic Acid

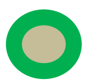
- Currently under study
- MW=1490 g/mol
- Q= 1.8 meq/g

Beckett et al. Environ. Sci. Technol. 1987, 21:289
Thurman, E. M. Organic Geochemistry of Natural Waters; Martinus Nijhoff/Junk: Dordrecht, The Netherlands, 1985.
Schnitzer, M.; Khan, S. U. Soil Organic Matter; Elsevier: Amsterdam, 1978.

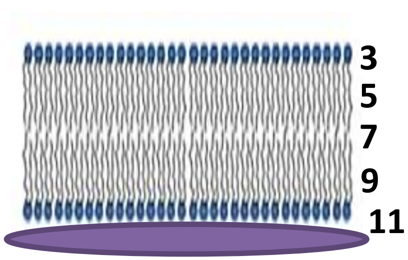
Chen and Schnitzer, Soil Soc. Am. J. 1976, 40:866
Vermöhlen et al., Colloids Surf. A 2000,163:45.

Mass Changes at all Overtones

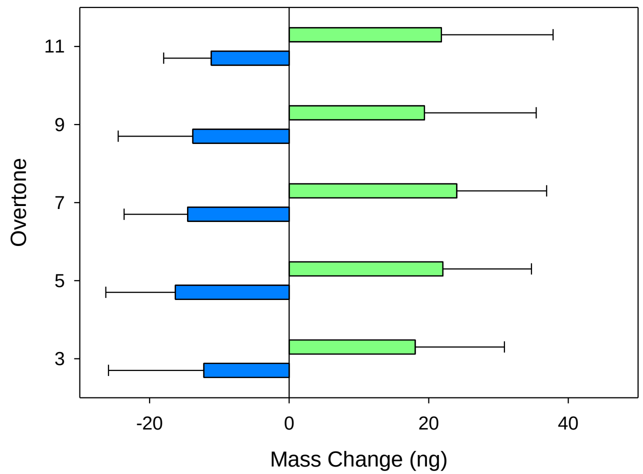
Water 

PMA 

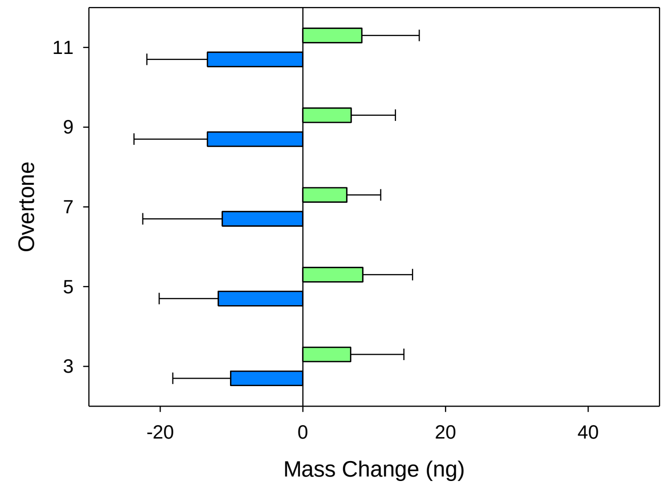
7.14×10^{10} $particles/mL$



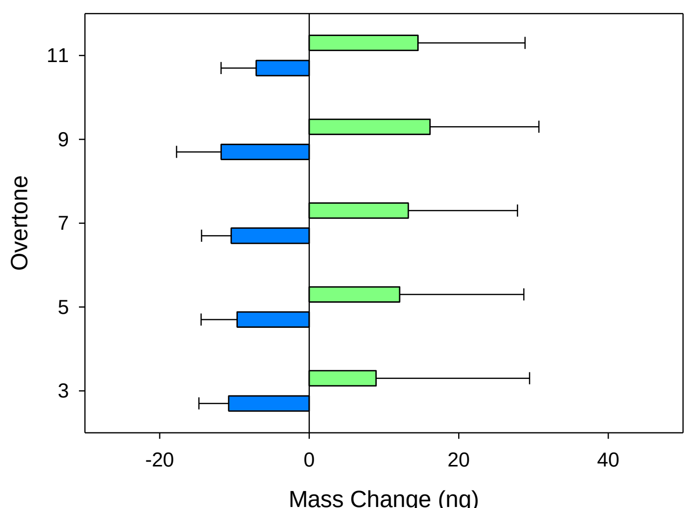
2 nm Gold Nanoparticles



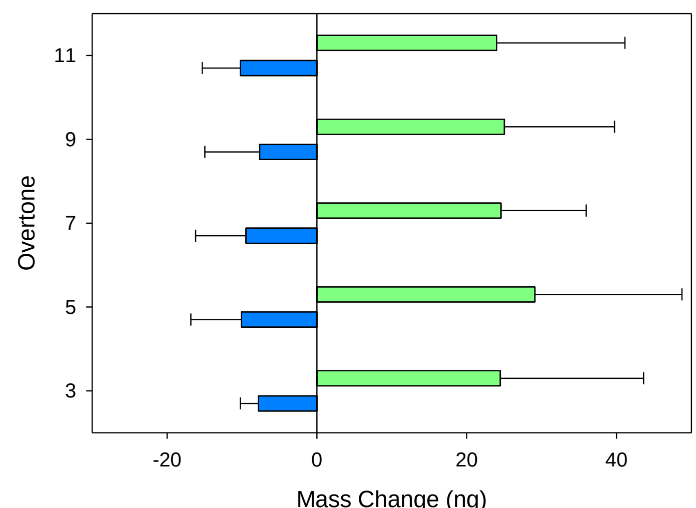
5 nm Gold Nanoparticles



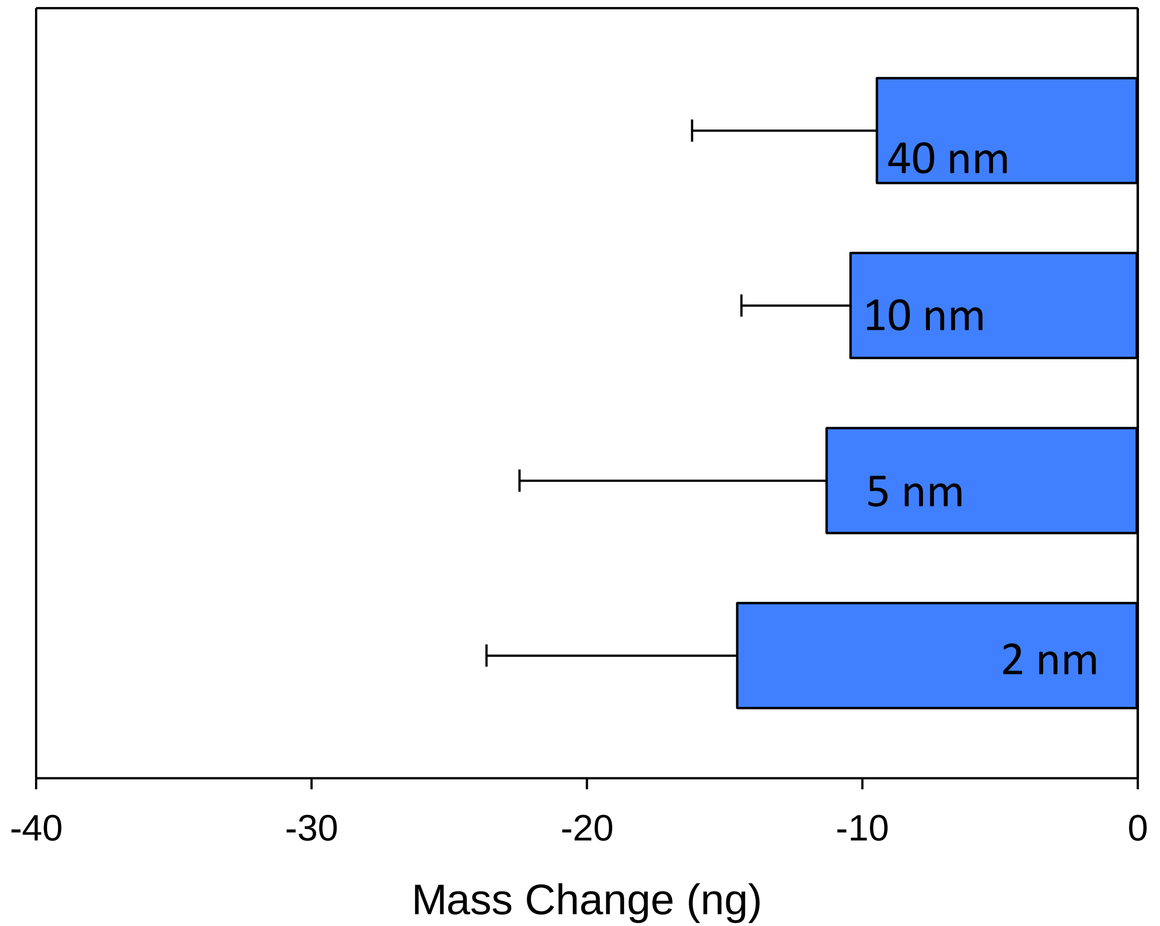
10 nm Gold Nanoparticles



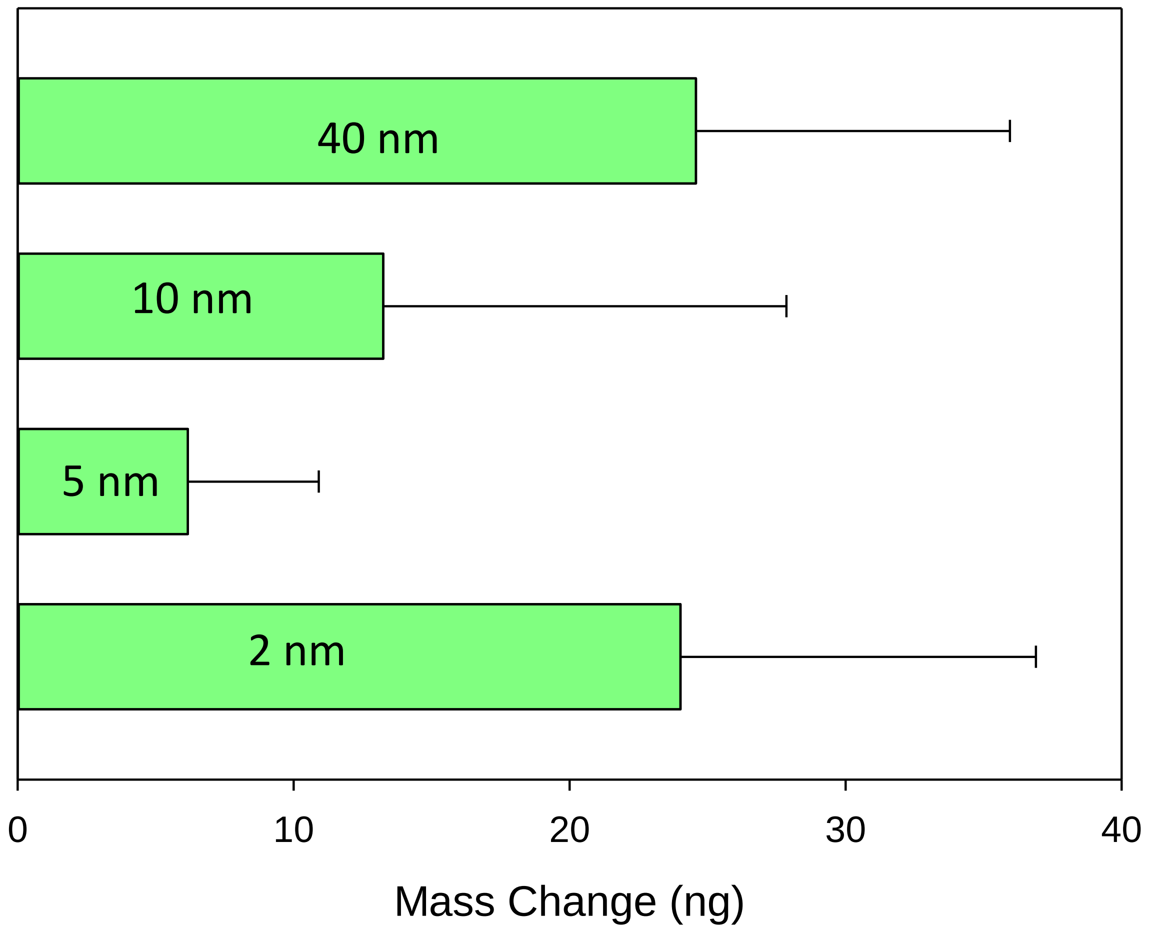
40 nm Gold Nanoparticles



Mass Change (7th Overtone) as a Function of NP Size: Water

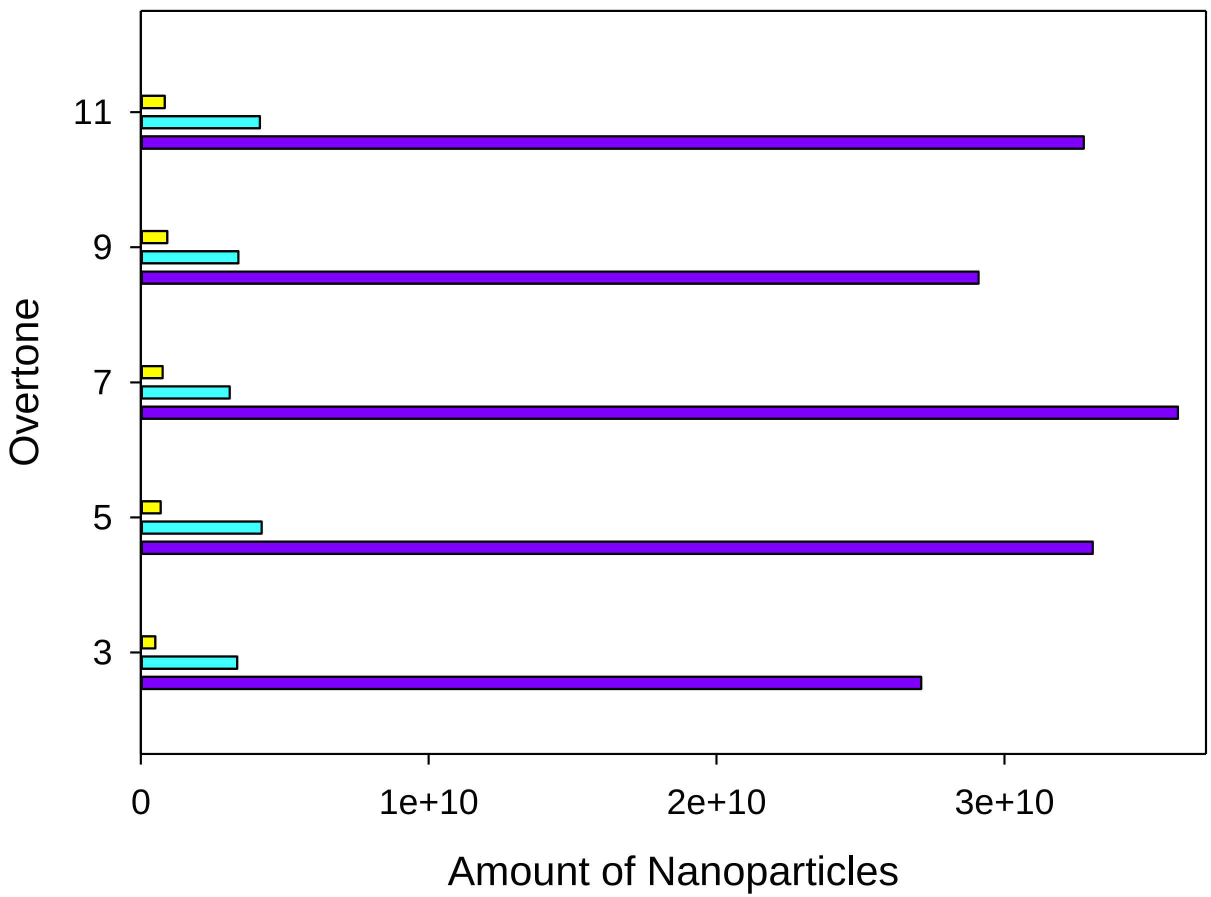


Mass Change as a Function of NP Size: Organic Polymer

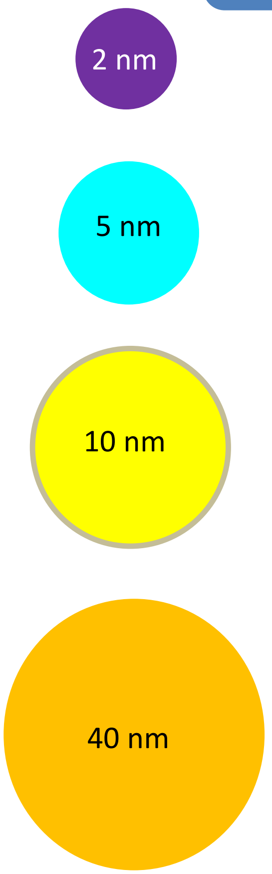


Results – Nanoparticle Insertion

Number of Nanoparticles that contribute to Mass Addition in Polymer



Percent Interaction



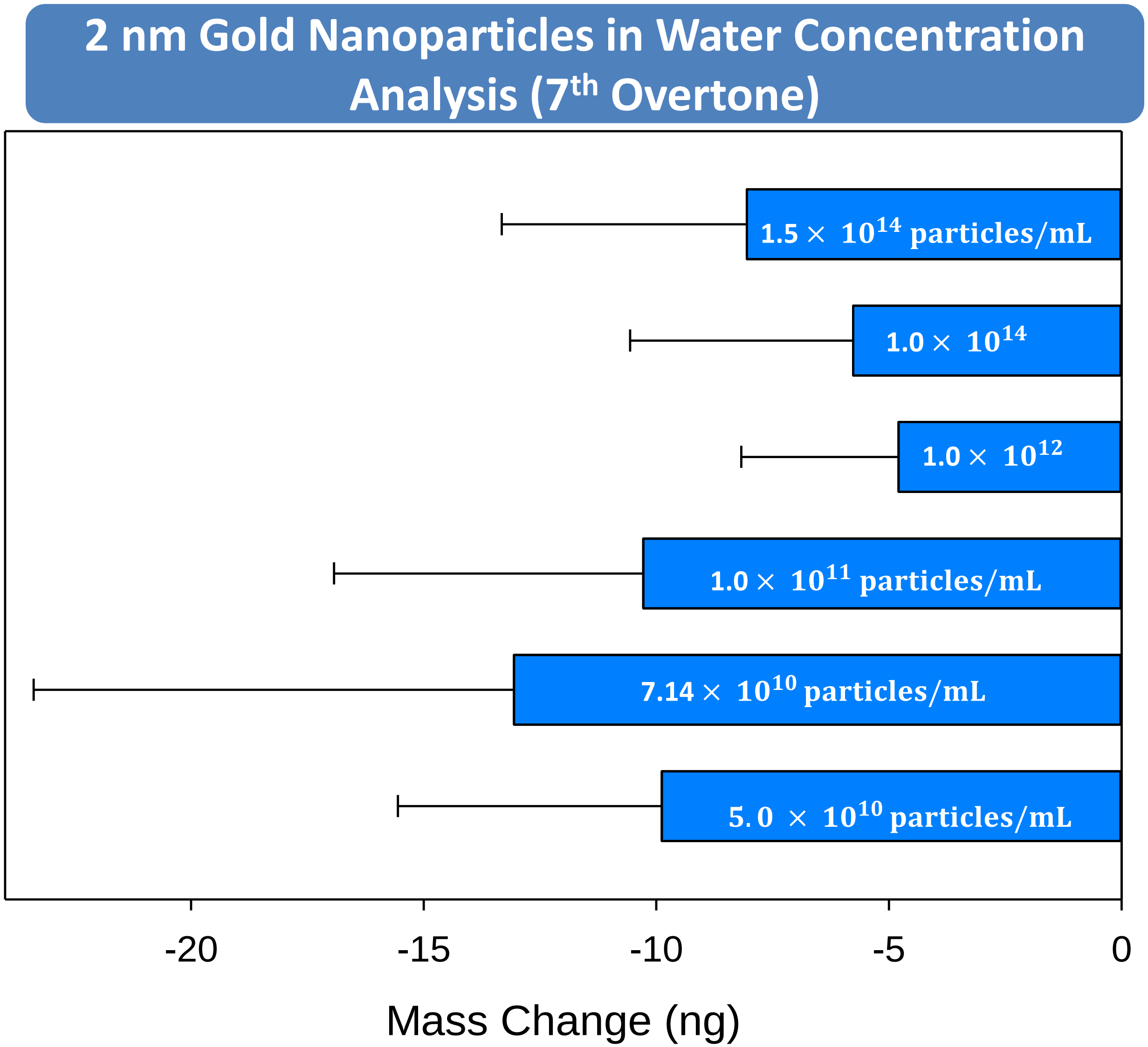
~33%

~ 3%

~0.70%

~0.020%

Results – Different Concentrations



Conclusions

Gold Nanoparticles in Water

- In water, nanoparticles remove mass from the lipid bilayer
- This indicates that the nanoparticles are compromising the bilayer, showing toxic effects

Gold Nanoparticles in Polymer

- In PMA, gold nanoparticles add mass to the cell membrane
- This indicates that the nanoparticles are inserting into the membrane and potentially forming pores. This is essential for drug delivery applications

Nanoparticle Size

- The 2 nm nanoparticles insert more nanoparticles into the membrane, and the 40 nm nanoparticles insert the least amount.
- This demonstrates a major difference between nanoparticle sizes and the way they interact

Nanoparticle Concentration

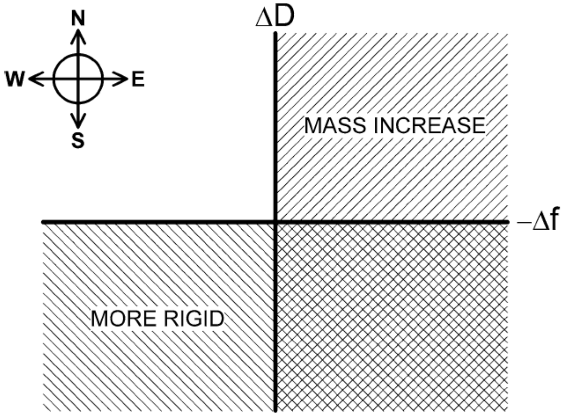
- Concentration appears to have an effect on the amount of mass change of the lipid bilayer for gold nanoparticles, and this will be further investigated for different sizes

Acknowledgements

- This work was supported in part by the National Science Foundation (CBET 0966496)
- A number of undergraduates assisted with these studies, including Yan Yan, Houssam Lazkhani, Andrew Carey, and Thomas Finelli.

Thank you for your attention!

D/F Plot



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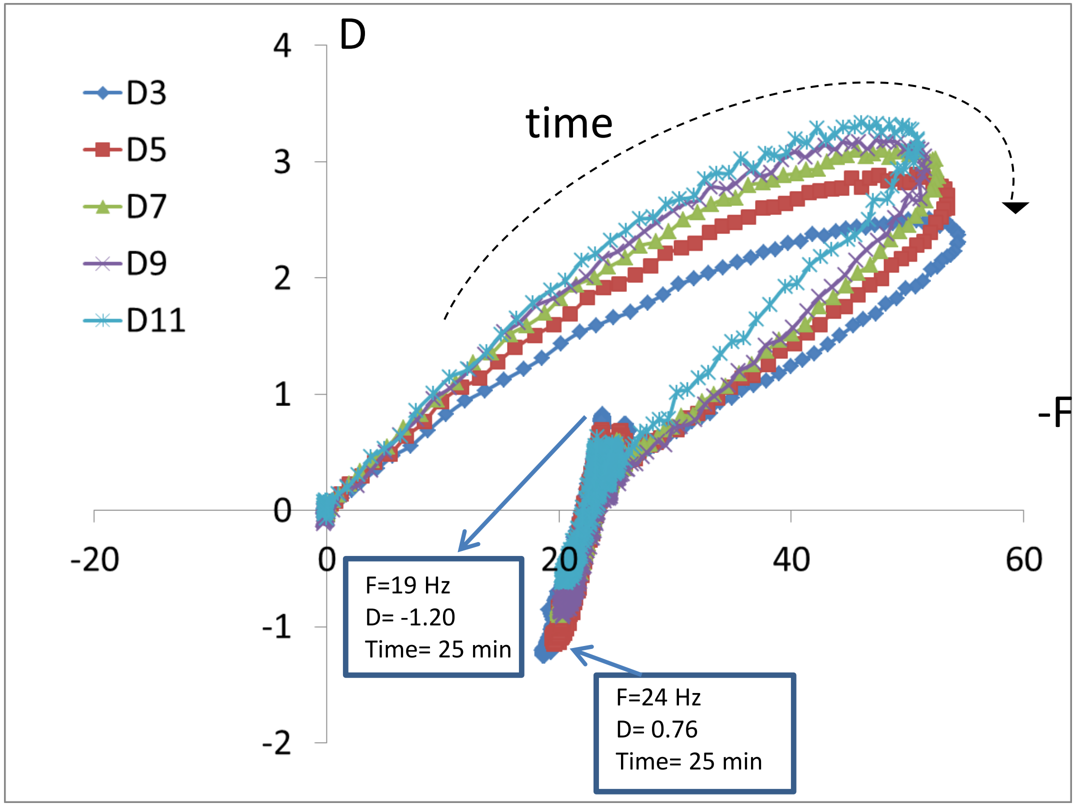
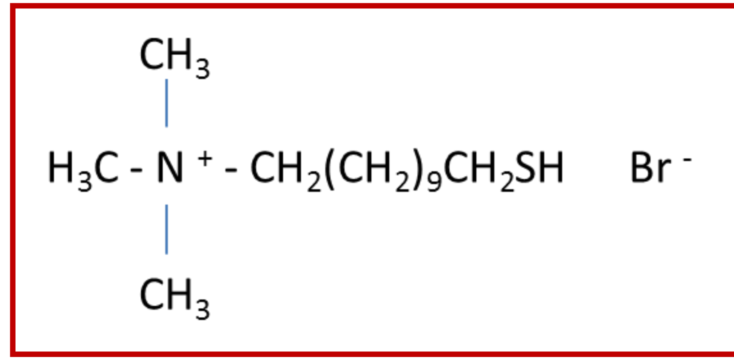
DOI 10.1007/s00249-010-0652-5



Characterizing the Gold Nanoparticles

Size (nm)	Coating	Zeta Potential (mV)
2	Sodium citrate/Tannic acid	-48.8
5	Sodium citrate/Tannic acid	-46.7
10	Sodium citrate/Tannic acid	-56.4
40	Sodium citrate/Tannic acid	ND

(11-Mercaptoundecyl)-N,N,N-trimethylammonium bromide
C14H32BrNS



- **Large organic molecule**

*previously used: 1-propanethiol, 2-mercaptoethanol, 3-mercaptopropionic acid, 2-aminoethanethiol

- **highly hydrophobic**
- **highly cationic**

*strong enough cation to overcome the negative charge of the gold nanoparticle itself

Membrane Lipid Composition

	Zwitterionic			Negatively Charged			
	PE	PC	SM	PS	PG	DPG	LPG
Erythrocyte membrane		50%	50%				
E coli (**Gram-negative**)	82%				6%	12%	
S aureus (**Gram-positive**)					57%	5%	38%

PE - phosphatidylethanolamine, PC - phosphatidylcholine,
SM - sphingomyelin, PS - phosphatidylserine, PG - phosphatidylglycerol,
DPG - diphosphatidylglycerol, LPG – lysophosphatidylglycerol

[9] Blondelle, S.E. et al. Biochim et Biophys Acta. **1462**: p89-108.

Nanoparticle Insertion Calculations

- Since mass is added when AuNPs are coated in PMA, the number of nanoparticles contributing to this mass increase can be calculated

General Equations

$$\text{Stock Concentration}\left(\frac{\text{particles}}{\text{mL}}\right) \times \text{Total Stock Volume (mL)} = \text{\# ofAuNPs in stock}$$

$$\text{Stock Concentration}\left(\frac{\text{mg}}{\text{mL}}\right) \times \text{Total Stock Volume (mL)} = \text{Mass of AuNPs in stock}$$

$$\frac{\text{Mass of AuNPs in stock}}{\text{\# of AuNPs in stock}} = \text{Mass of individual AuNP}$$

$$\frac{\text{Mass Change from Sauerbrey Equation}}{\text{Mass of individual particle}} = \text{\# of AuNPs interacted}$$

$$\text{Diluted Concentration of AuNP}\left(\frac{\text{particles}}{\text{mL}}\right) \times \text{Volume of solution(mL)} = \text{Total \# of AuNPs administered}$$

$$\text{\% of Nanoparticle Interaction} = \frac{\text{\# of AuNPs interacted}}{\text{Total \# of AuNPs adminsitered}} \times 100$$

2 nm 7th overtone AuNP Insertion Calculations

$$1.5 \times 10^{14}\ \frac{\text{AuNPs}}{\text{mL}} \times 20\ \text{mL} = 3.00 \times 10^{15}\ \text{AuNPs}$$

$$0.1\ \frac{\text{mg}}{\text{mL}} \times 20\ \text{mL} = 2\ \text{mg} = 2{,}000{,}000\ \text{ng}$$

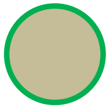
$$\frac{2{,}000{,}000\ \text{ng}}{3.00 \times 10^{15}\ \text{AuNPs}} = 6.66 \times 10^{-10}\ \frac{\text{ng}}{\text{AuNP}}$$

$$\frac{15.112\ \text{ng}}{6.66 \times 10^{-10}\ \frac{\text{ng}}{\text{AuNP}}} = 2.269 \times 10^{10}\ \text{AuNPs}$$

$$7.14 \times 10^{10}\ \frac{AuNPs}{mL} \times 1.5\ mL = 1.07 \times 10^{11}\ AuNPs$$

$$\frac{2.269 \times 10^{10}\ AuNPs}{1.07 \times 10^{11}\ AuNPs} \times 100 = 21.166\%$$

Nanoparticle Insertion Calculations



- Since mass is added when AuNPs are coated in PMA, the number of nanoparticles contributing to this mass increase can be calculated

$$\text{Stock Concentration}\left(\frac{\text{particles}}{\text{mL}}\right)\times\text{Total Stock Volume (mL)}=\text{\# ofAuNPs in stock}$$

$$\text{Stock Concentration}\left(\frac{\text{mg}}{\text{mL}}\right)\times\text{Total Stock Volume (mL)}=\text{Mass of AuNPs in stock}$$

$$\frac{\text{Mass of AuNPs in stock}}{\text{\# of AuNPs in stock}}=\text{Mass of individual AuNP}$$

$$\frac{\text{Mass Change from Sauerbrey}}{\text{Mass of individual particle}}=\text{\# of AuNPs interacted}$$

$$\text{Diluted Concentration of AuNP}\left(\frac{\text{particles}}{\text{mL}}\right)\times\text{Volume of solution(mL)}$$
$$=\text{Total \# of AuNPs administered}$$

$$\text{\% of Nanoparticle Interaction}=\frac{\text{\# of AuNPs interacted}}{\text{Total \# of AuNPs adminsitered}}\times 100$$