S[ASSAY]

GHB Detection for Drug Facilitated Sexual Assault Prevention

Melissa Ferranti, Yash Patel, Kara Walp

Molecular & Tissue Engineering, & Drug Delivery (Category 2)

Introducing The S[ASSAY] Team

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Computer Engineering

Minor in Mathematical Statistics

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Biomedical Engineering

Concentration in Machine Learning

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Concentration in Nanotechnology

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GHB

Properties
Prescribed to treat narcolepsy
Endogenous to the body

Short-Term Effects[17]
Drowsiness, dizziness
Lack of inhibition
Memory lapses
Hallucinations

Long-Term Effects[17]
Respiratory arrest
Overdose
Death

[17]

The Problem

Common **date-rape drug**

35.4% of sexual assault survivors are estimated to be victims of DFSA[25]

Sexual assault occurs **every 68 seconds** in the U.S.[23]

Nearly **1 in 5** Black women in the U.S. experience rape[24]
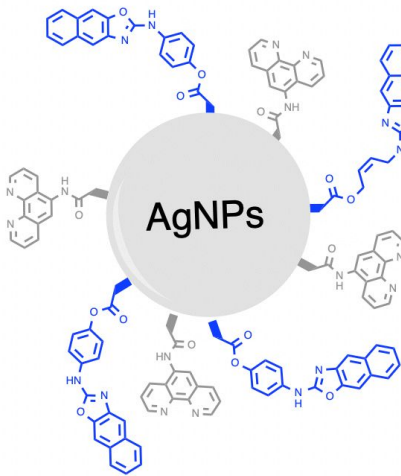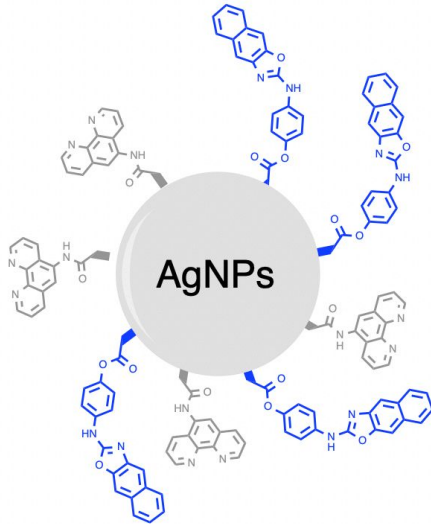
Less likely to report and seek help

The Solution

Allow users to detect a drink that has been spiked with GHB

Accurately, quickly, and discretely

Create a **safer environment** without placing the blame on the victim

Silver Nanoparticle Based Detection

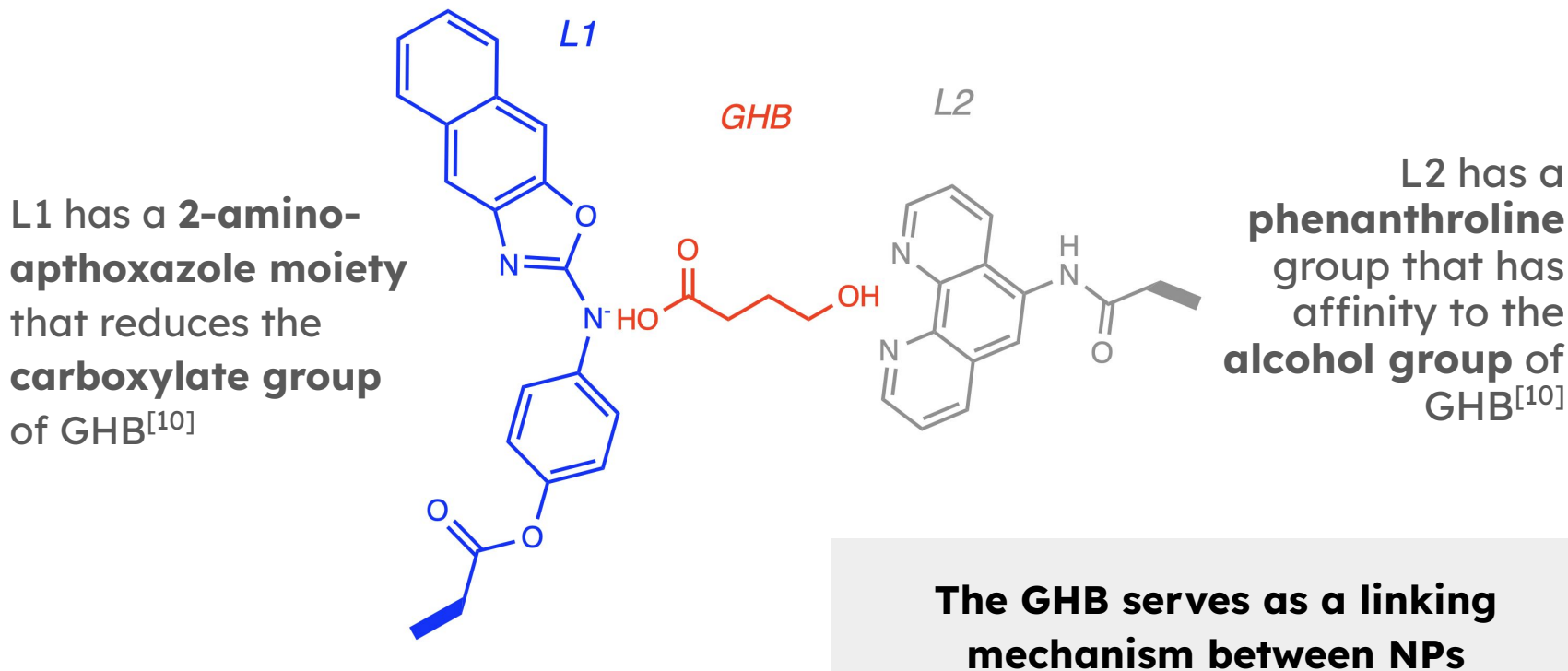GHB Triggers **Agglomeration of Silver Nanoparticles**
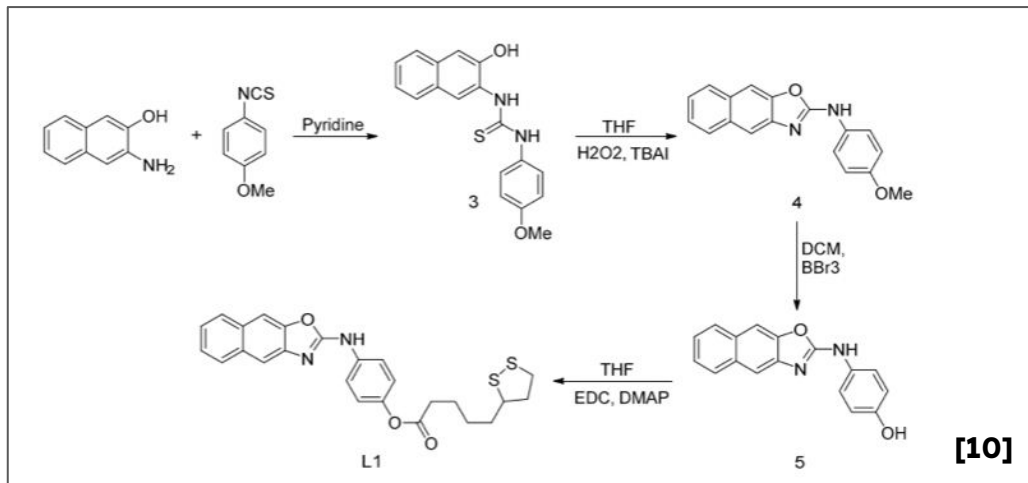
Increase in Effective Size

Red Shift in LSPR (Localized Surface Plasmon Resonance)

The Ligands and Their Production

L1

GHB

L2

L1 has a **2-amino-apthoxazole moiety** that reduces the **carboxylate group** of GHB[10]

L2 has a **phenanthroline** group that has affinity to the **alcohol group** of GHB[10]

The GHB serves as a linking mechanism between NPs

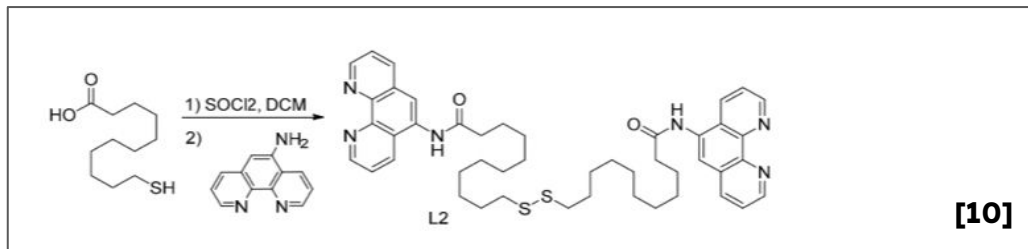# The Ligands and Their Production

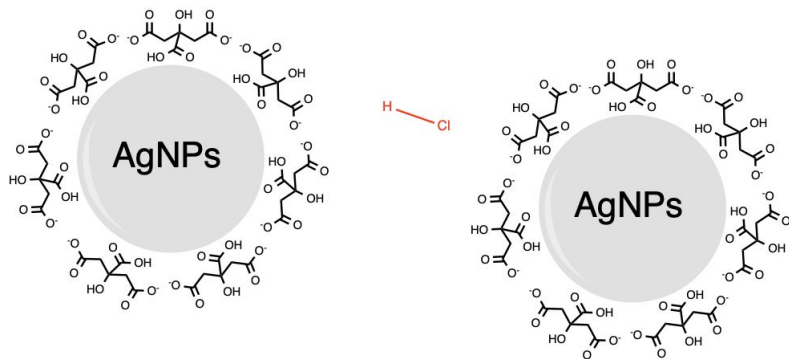Synthetic Pathway for **L1** Synthesis

[10]

Synthetic Pathway for **L2** Synthesis

[10]

The Prototype

Modeling Bifunctionalized AgNPs with Citrate Capped AgNPs

Detection of Analyte Using Citrate-Capped NPs

NP Size Distribution

LSPR Shift

Nanoparticle Diameter (nm)

No Analyte
With Analyte

40.8nm shift

No Analyte   With Analyte

Wavelength (nm)

Absorbance

ANOVA + Post HOC Tukey: α=0.05, p<0.000143

ANOVA + Post HOC Tukey: α=0.05, p<0.00001
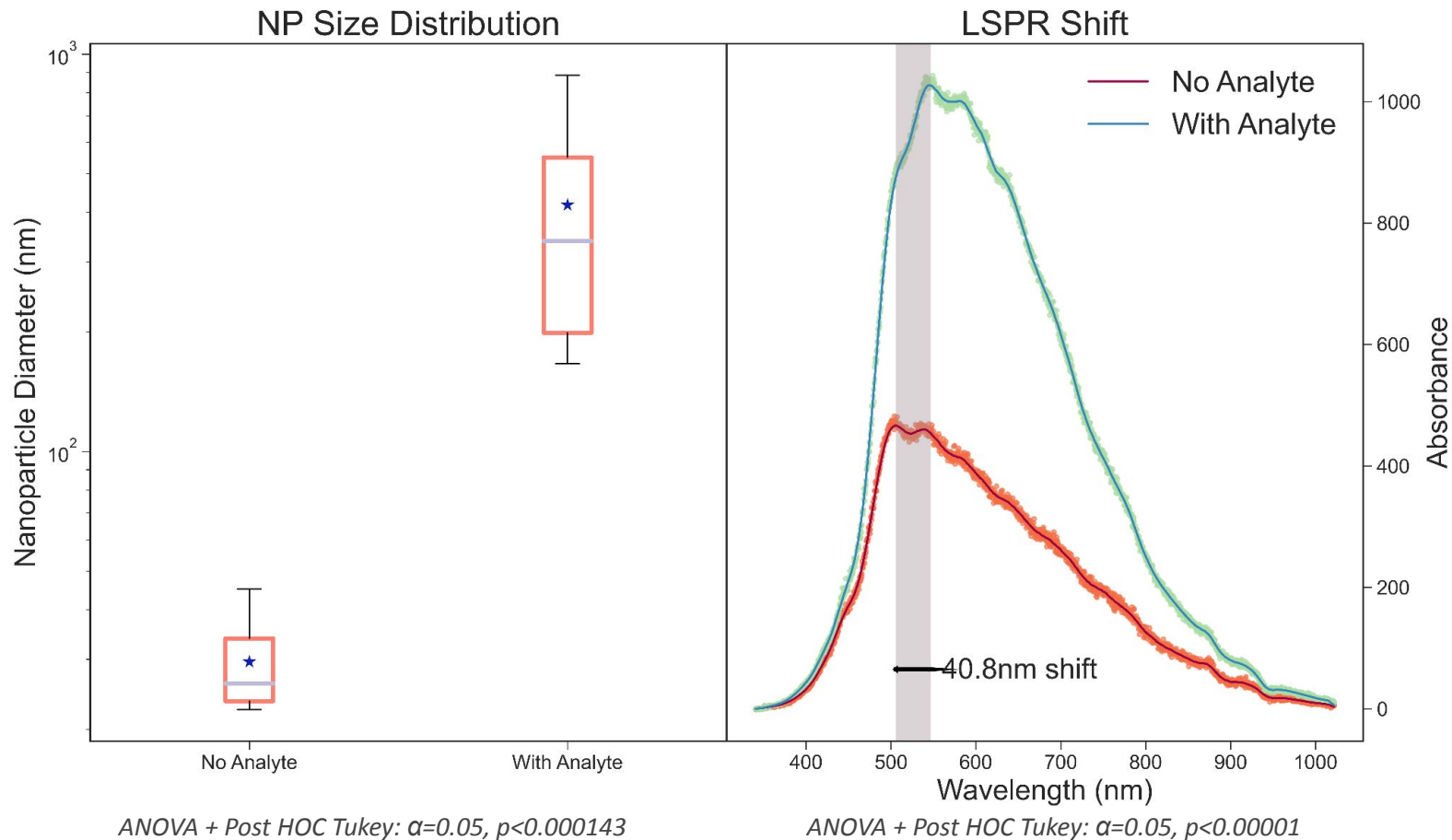
Transient Response of Absorbance Spectra After Introduction of Analyte
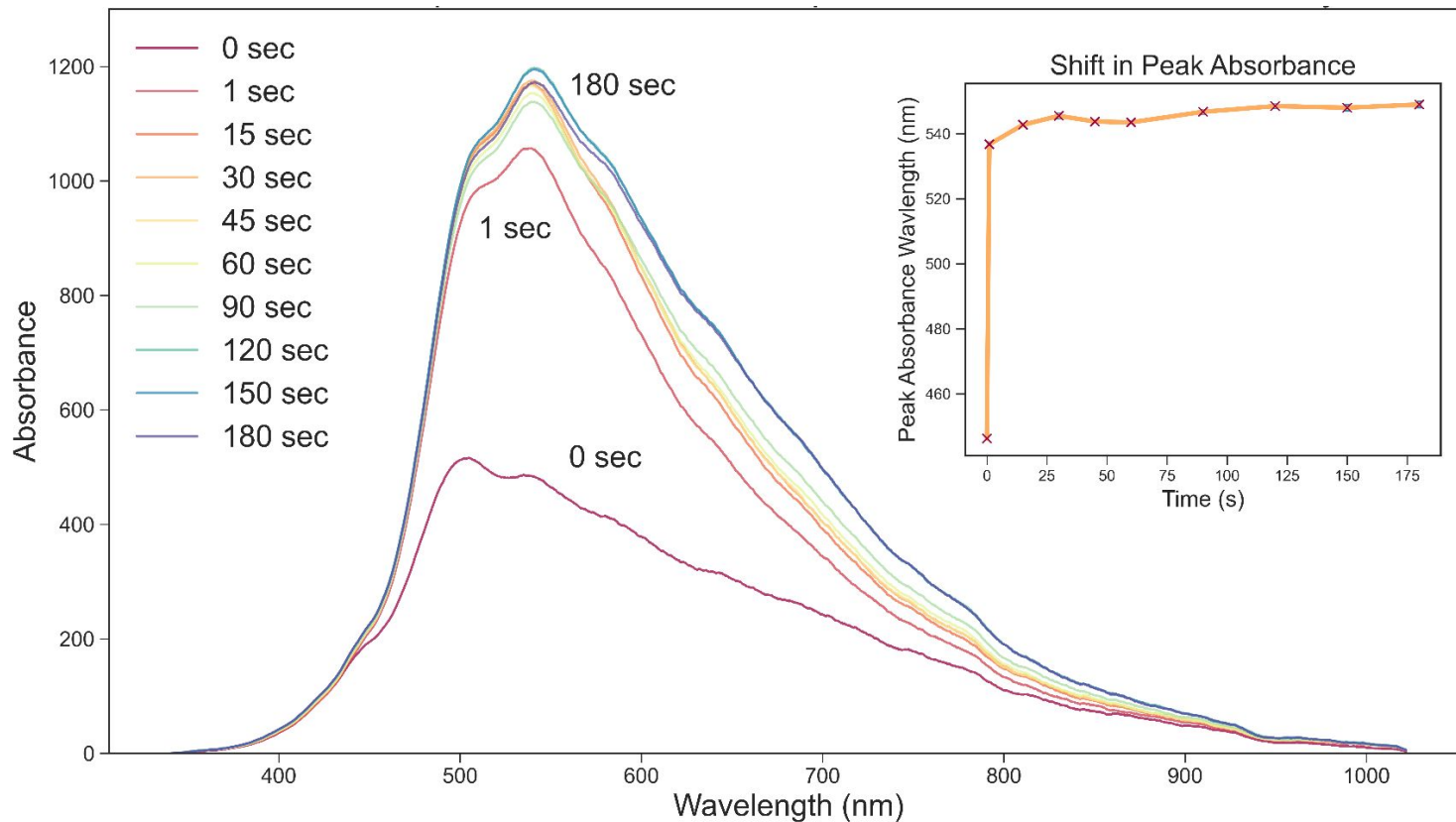
The Design

A Lateral Flow Assay (LFA) for Gamma Hydroxybutyric Acid (GHB) detection.

A lateral flow assay is a paper based microfluidic assay that required a small sample to detect the presence of a compound.
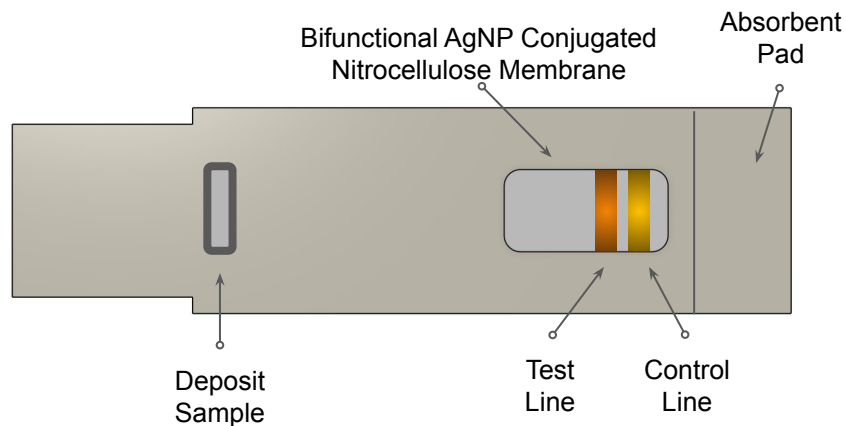

Bifunctional AgNP Conjugated Nitrocellulose Membrane

Absorbent Pad

Deposit Sample

Test Line

Control Line

LFA Design Using Biotin-Streptavidin Conjugation

L1

L2

AgNPs Stabilized with TrisC

Biotin-Streptavidin

Streptavidin-Biotin

Deposit Sample

Test Line   Control Line
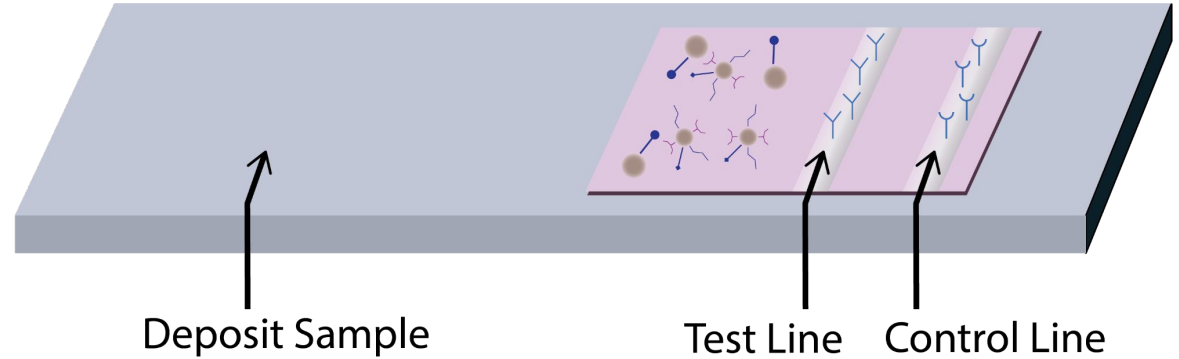
Sample Without GHB
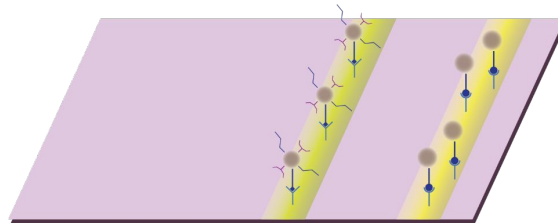
Sample With GHB
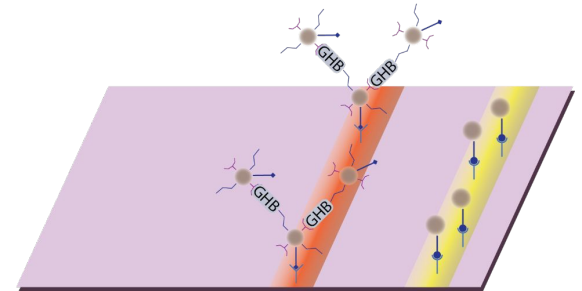
Our Product in the Market

Objectives	Our Solution
Accurate	> 98% Accuracy [10]
Rapid Results	< 2 minutes [6]
Portable	4.85 x 1.25 x 0.40 inches
Discrete	Indistinguishable from everyday item
Convenient	No specialty equipment required
Affordable	< $5 per test

[18]

[19]

Manufacturing & Production

Housing & Enclosure

2 hrs, 38.6g, $1.12 (PLA)

Synthesis of Bifunctional Ligands

$0.14 for 246mL of ligand solution

Synthesis of Functional LFA

$3.51 per assay

Scaled Manufacturing Costs

$22.18 for 1 device with 6 tests

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S[ASSAY]

Questions?

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AgNPs@C + HCl Methodology

Synthesize AgNPs with Citrate Cap

1. Boil 50 mL 0.5mM $AgNO_3$
2. Over a span of 4 mins add 4 mL of 1% Trisodium Citrate

NP synthesis should cause solution to turn yellow

NP size controlled by temperature, rate of addition of 1% TrisC solution, and stirring rate.

Further control can be achieved by addition of Tannic Acid solution, during nucleation reaction.

Adding HCl to Trigger Aggregation

1. Add 20µL of 0.8M HCl solution to 1 mL of AgNPs@C solution.

NP aggregation should trigger red shift in absorbance spectra

Collected Data

Absorbance Spectra
NP-Size (DLS)
Z-Potential (Surface Potential)

[20]

AgNPs @ L1&L2 + GHB Methodology 2

Synthesize Bifunctional AgNPs
AgNPs @ L1&L2

1. Mix Milli-Q water with 0.5M NaOH (200:1 volume ratio)
2. Add AgNPs
3. Add L1 (0.5mM) and L2 (0.32mM) simultaneously
4. Stir for 1 hour
5. Dilute with Milli-Q water
6. Centrifuge for 10 min (105e2 rpm)

[10]

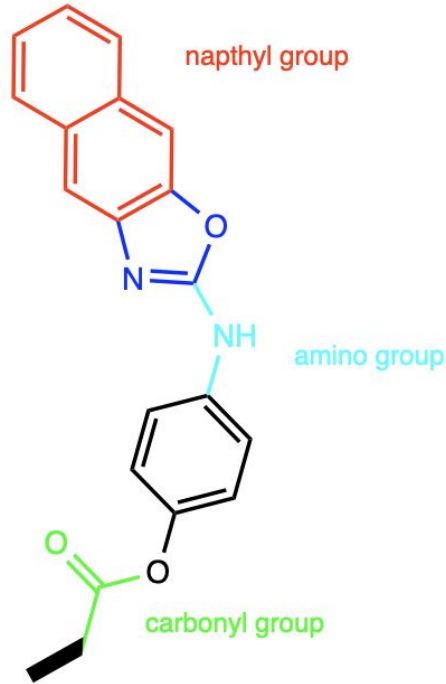## Conjugation of Bifunctional AgNPs to Nitrocellulose Membranes

1. Add 20µL of 0.8M HCl solution to 1 mL of AgNPs@C solution.

NP aggregation should trigger red shift in absorbance spectra

[10]

Ligand Chemistry Breakdown

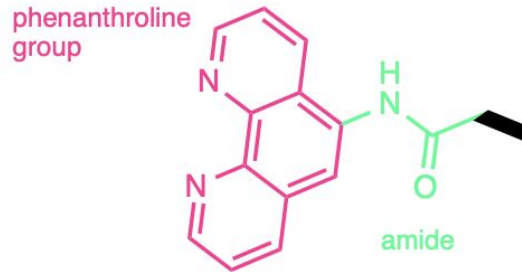L1

napthyl group

phenanthroline group

amino group

NH

O

O

carbonyl group

L2

amide

Carbonyl and **Amide** anchoring groups can be conjugated to both **noble metal (Au)** and **transition metal oxide (Ag)** nanoparticles[26]

Ligands previous designed for AuNPs can be applied to AgNPs
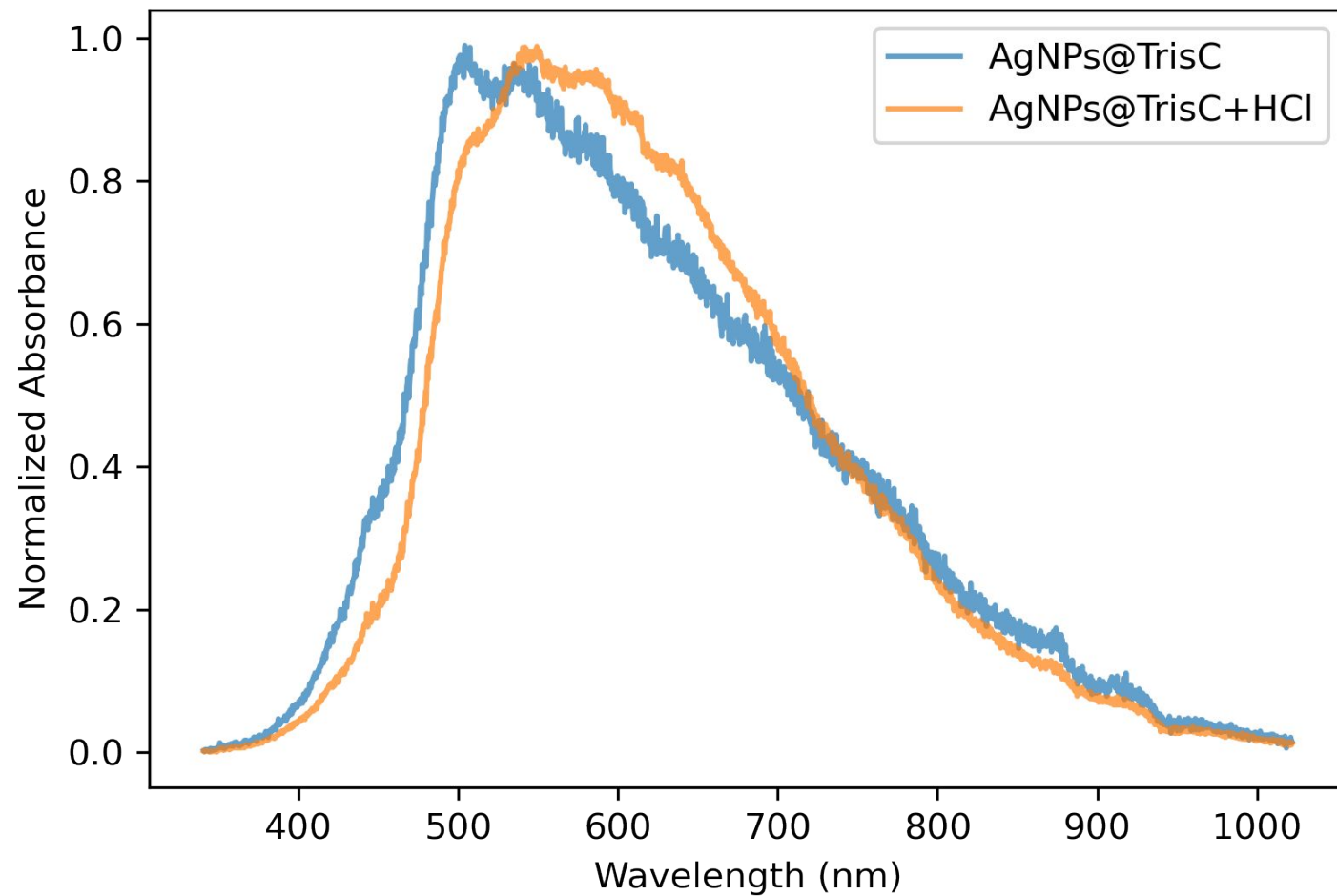
[10]

Final Materials Sourcing & Cost Calculations

| Product	Vendor	Price	Quantity Needed (g/peices)	Molar Mass	Price Per Mole	Concentration Required	Volume Required	Cost ($)
3-amino-2-naphthol	Sigma Aldrich	$38.00	5.00E+00	1.59E+02	4.77E-02	1.26E-03	2.00E-01	1.20E6-5
4-methoxyphenyl isothiocyanate	Sigma Aldrich	$108.00	5.00E+00	1.65E+02	1.31E-01	1.00E+00	1.80E-04	2.35E6-5
Boron Tribromide	Sigma Aldrich	$202.00	1.00E+02	2.51E+02	8.06E-03	8.30E-04	8.30E-04	5.55E6-9
α-Lipoic-acid	Sigma Aldrich	$620.00	5.00E+01	2.06E+02	6.01E-02	2.20E-04	4.60E+01	6.08E6-4
4-(Dimethylamino) pyridine	Sigma Aldrich	$572.00	5.00E+03	1.22E+02	9.36E-04	8.10E-05	1.00E+01	7.58E6-7
EDC	Sigma Aldrich	$173.00	5.00E-04	2.51E+02	1.38E+03	1.00E+00	4.00E-05	5.51E6-2
Tetrahydrofuran	Sigma Aldrich	$771.00	1.20E+03	7.21E+01	8.91E-03	1.00E+00	1.00E-01	8.91E6-4
11-mercaptoundecanoic acid	Sigma Aldrich	$345.00	2.50E+01	2.18E+02	6.32E-02	4.60E-04	1.00E+02	2.91E6-3
Thionyl Chloride	Sigma Aldrich	$168.00	1.00E+00	1.19E+02	1.41E+00	4.70E-04	4.00E-05	2.65E6-8
EDC	Sigma Aldrich	$173.00	5.00E-03	2.51E+02	1.38E+02	1.00E+00	2.00E-02	2.75E6+0
Acetylthiocholine iodide	Sigma Aldrich	$495.00	2.50E+01	2.89E+02	6.85E-02	4.70E-04	4.00E-05	1.29E6-9
1,10-phenanthrolin-5-amine	Sigma Aldrich	$550.00	5.00E+00	1.05E+02	1.05E+00	4.60E-04	9.00E+01	4.33E6-2
Silver Nitrate	Sigma Aldrich	$1,410.00	5.00E+02	1.69E+02	1.67E-02	5.00E-02	5.00E+01	4.17E6-2
Trisodium Citrate	Sigma Aldrich	$1,200.00	2.50E+04	2.94E+02	1.63E-04			0.00E6+0
Tannic Acid	Sigma Aldrich	$154.00	5.00E+02	1.70E+03	1.81E-04			0.00E6+0
Biotin	Sigma Aldrich	$1,530.00	2.50E+01	2.44E+02	2.51E-01			0.00E6+0
Biotin-Streptaviden Conjugated Nitrocellulose Membranes	EBAY	$200.00	3.00E+01		6.67E+00			2.67E6+1
Chromotography Paper	Amazon	$15.99	3.00E+02		5.33E-02			2.13E6-1
							Total Cost	$29.78
							for 6-strips	

Commercialization & Introduction to Market

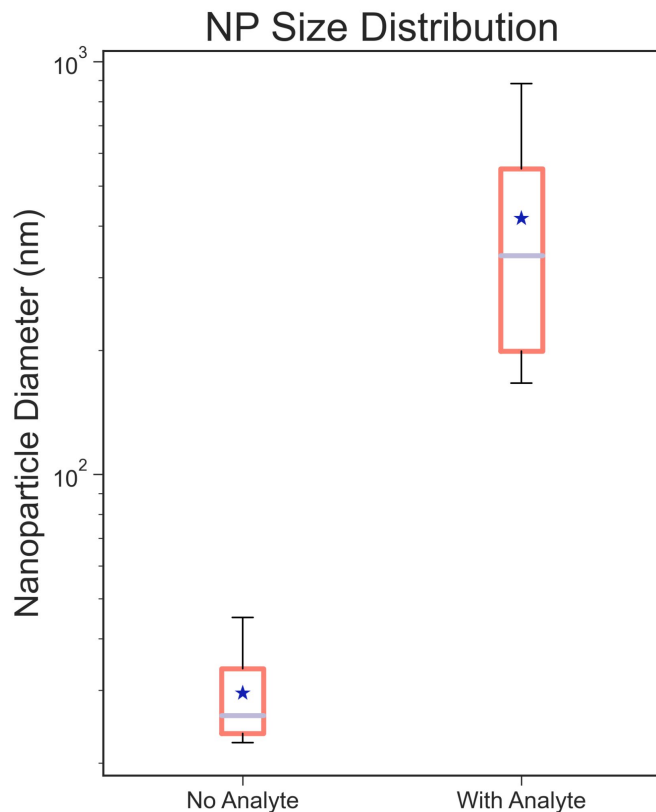$122K per Survivor amounting to $3.1 Trillion in costs per rape survivors [21]

Health care is 16% higher for women who were sexually abused as children and 36% higher for women who were physically and sexually abused as children [22]

Non-Normalized Average Absorbance Spectra (n=36)

NP Size Distribution
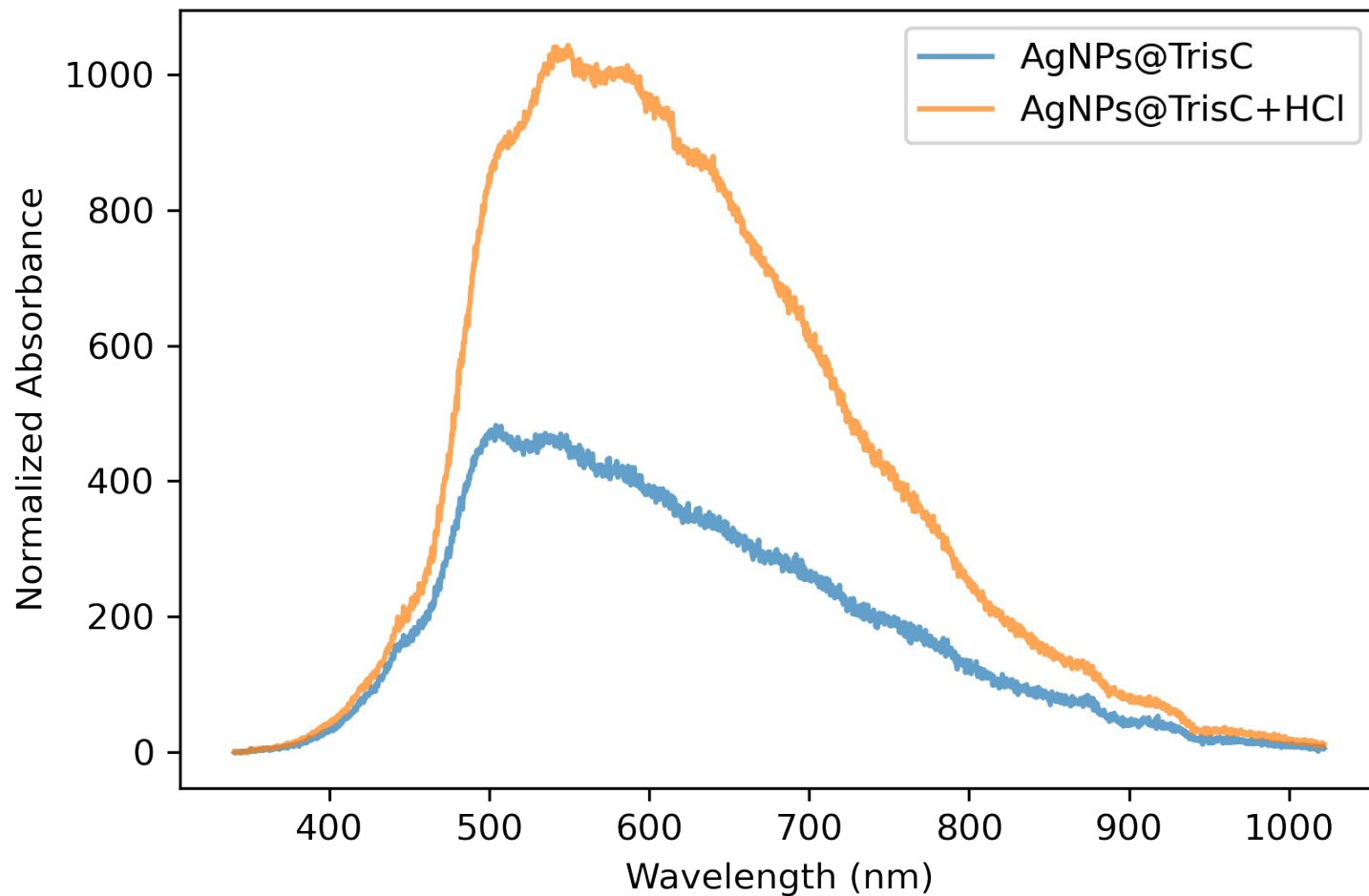
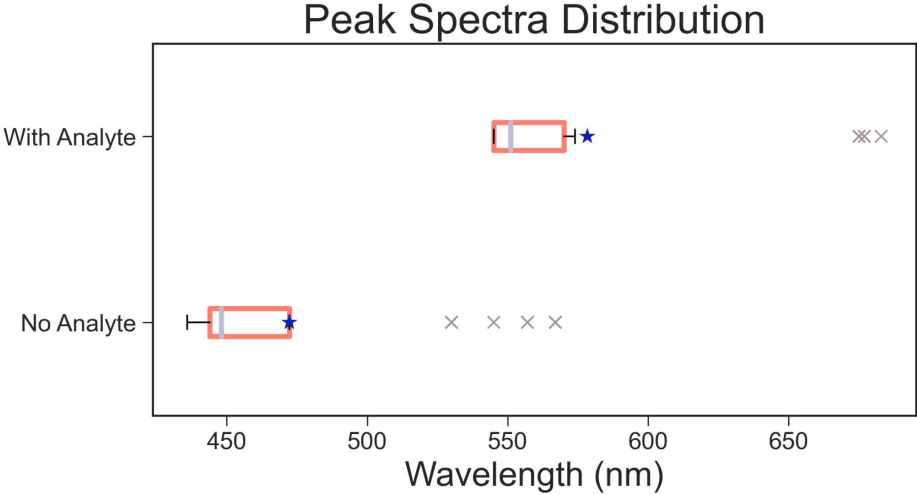**Summary of Data**

	Treatments					
	1	2	3	4	5	Total
N	9	9				18
ΣX	266.1	3755				4021.1
Mean	29.5667	417.2222				223.394
ΣX^2	8336.21	2006853				2015189.21
Std.Dev.	7.6528	234.5697				256.3199

Result Details

Source	SS	df	MS	
Between-treatments	676245.7339	1	676245.7339	F = 24.55437
Within-treatments	440652.0756	16	27540.7547	
Total	1116897.8094	17		

ANOVA & Post HOC Tukey Performed on NP Size Distribution

The f-ratio value is 24.55437. The p-value is .000143. The result is significant at p < .05.
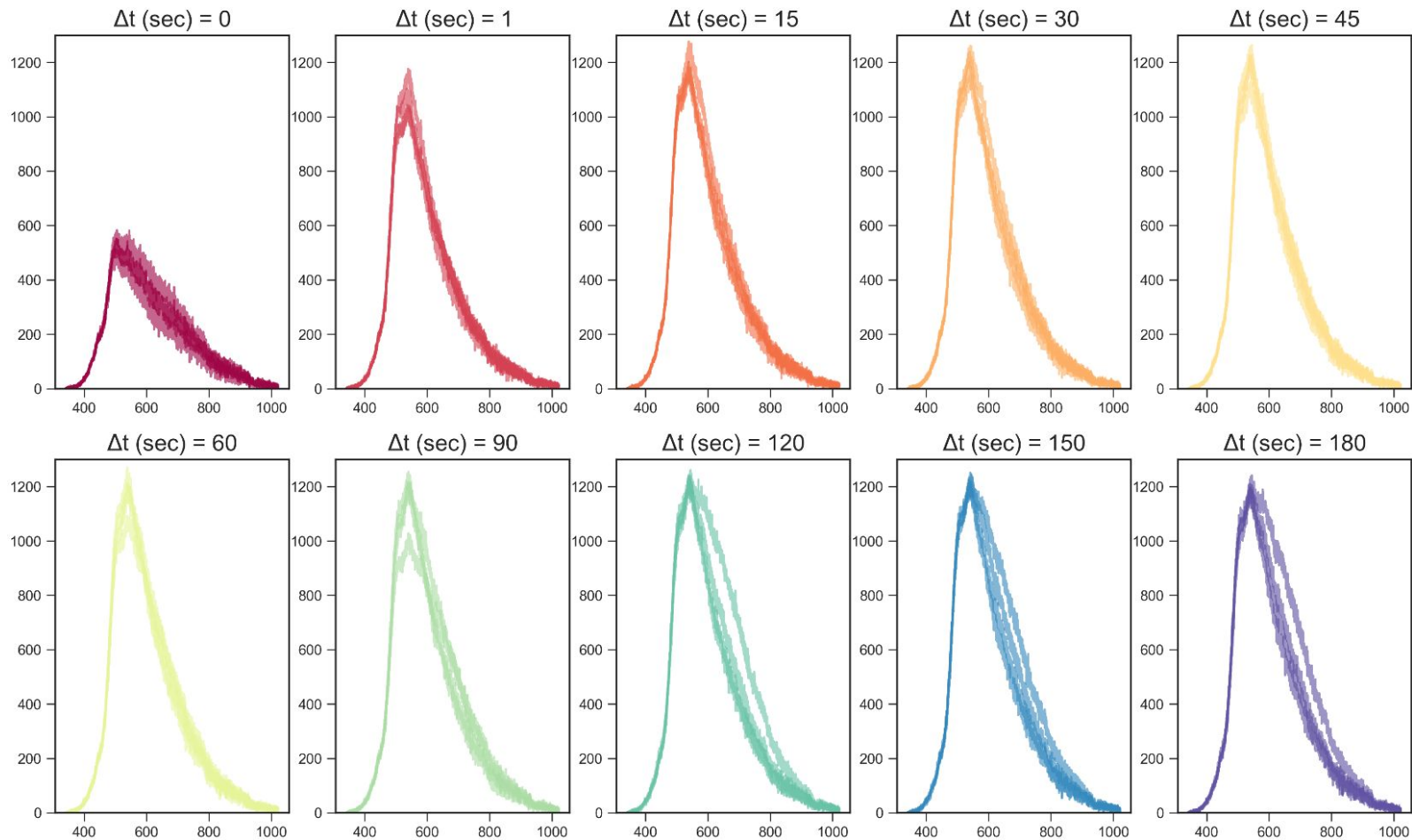
Peak Spectra Distribution

Summary of Data

	Treatments					
	1	2	3	4	5	Total
N	36	36				72
ΣX	17001	20822				37823
Mean	472.25	578.3889				525.319
ΣX^2	8103965	12130670				20234635
Std.Dev.	46.3659	49.9876				71.7466

Result Details

Source	SS	df	MS	
Between-treatments	202778.3472	1	202778.3472	$F = 87.24367$
Within-treatments	162699.3056	70	2324.2758	
Total	365477.6528	71		

ANOVA & Post HOC Tukey Performed on Calculated Peaks of Spectra Data
The f-ratio value is 87.24367. The p-value is < .00001. The result is significant at p < .05.

Shift in Absrobace Spectra Over 3 mins