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Therefore, this United States

Patent

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John A. Squires

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(12) **United States Patent**
Deng et al.

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(45) **Date of Patent: Oct. 28, 2025**

(54) **SILVER NANOPARTICLE SURFACE ENABLED SELF- ASSEMBLY OF ORGANIC DYE MOLECULES**

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(73) Assignee: **Morgan State University**, Baltimore, MD (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 896 days.

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G01N 1/30 (2006.01)
C09B 62/44 (2006.01)
C09K 11/06 (2006.01)

(52) **U.S. Cl.**
CPC ..... ***G01N 1/30*** (2013.01); ***C09B 62/44*** (2013.01); ***C09K 11/06*** (2013.01); (Continued)

(58) **Field of Classi cation Search**
CPC G01N 1/30; G01N 2001/302; C09B 62/44; C09K 11/06; C09K 2211/1007; C09K 2211/1018
See application le for complete search history.

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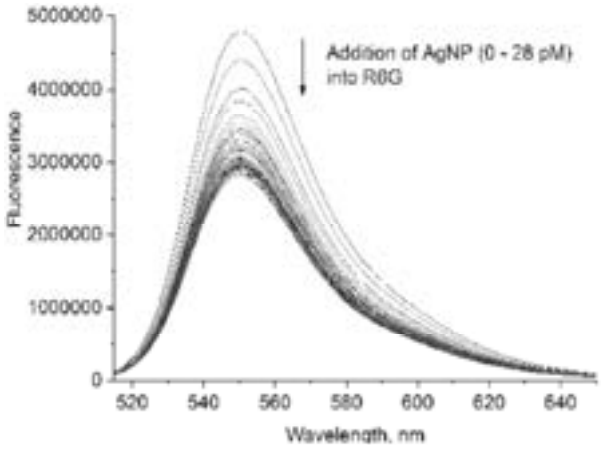
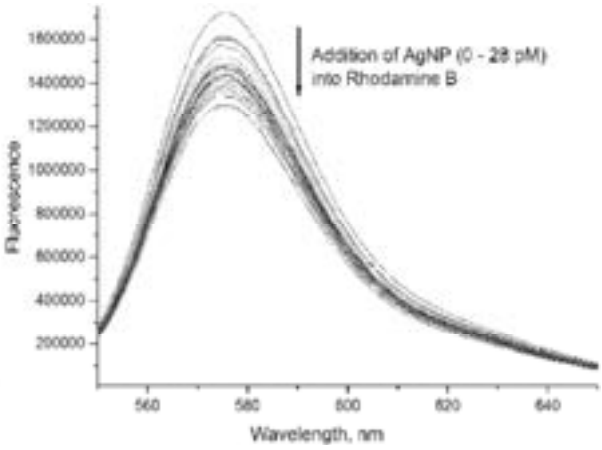
(57) **ABSTRACT**

Fluorescence titration of methylene blue, rhodamine B and rhodamine 6G (R6G) by silver nanoparticle (AgNP) all resulted in an initial steep quenching curve followed with a sharp turn and a much atter quenching curve. At the turn, there are about 200,000 dye molecules per a single AgNP, signifying self-assembly of approximately 36 layers of dye molecules on the surface of the AgNP to form a micelle-like structure. These uorescence-quenching curves t to a mathematical model with an exponential term due to molecular self-assembly on a AgNP surface, or self-assembly shielding effect , and a Stern-Volmer term (nanoparticle surface enhanced quenching). Such a super-quenching by AgNP can only be attributed to pre-concentration of the dye molecules on the nanoparticle surface that yields the formation of micelle-like self-assembly, resulting in great uorescence quenching. Overall, the uorescence quenching titration reveals three different types of interactions of dye molecules on AgNP surface: 1) self-assembly (methylene blue, rhodamine B and R6G), 2) absorption/tight interaction (tryptamine and uorescein), and 3) loose interaction (eosin Y). We attribute the formation of micelle-like self-assembly of these three dye molecules on AgNP to their positive charge, possession of nitrogen atoms, and with relatively large and at aromatic moieties.

3 Claims, 14 Drawing Sheets





(52) **U.S. Cl.**
CPC *C09K 2211/1007* (2013.01); *C09K 2211/1018* (2013.01); *G01N 2001/302* (2013.01)

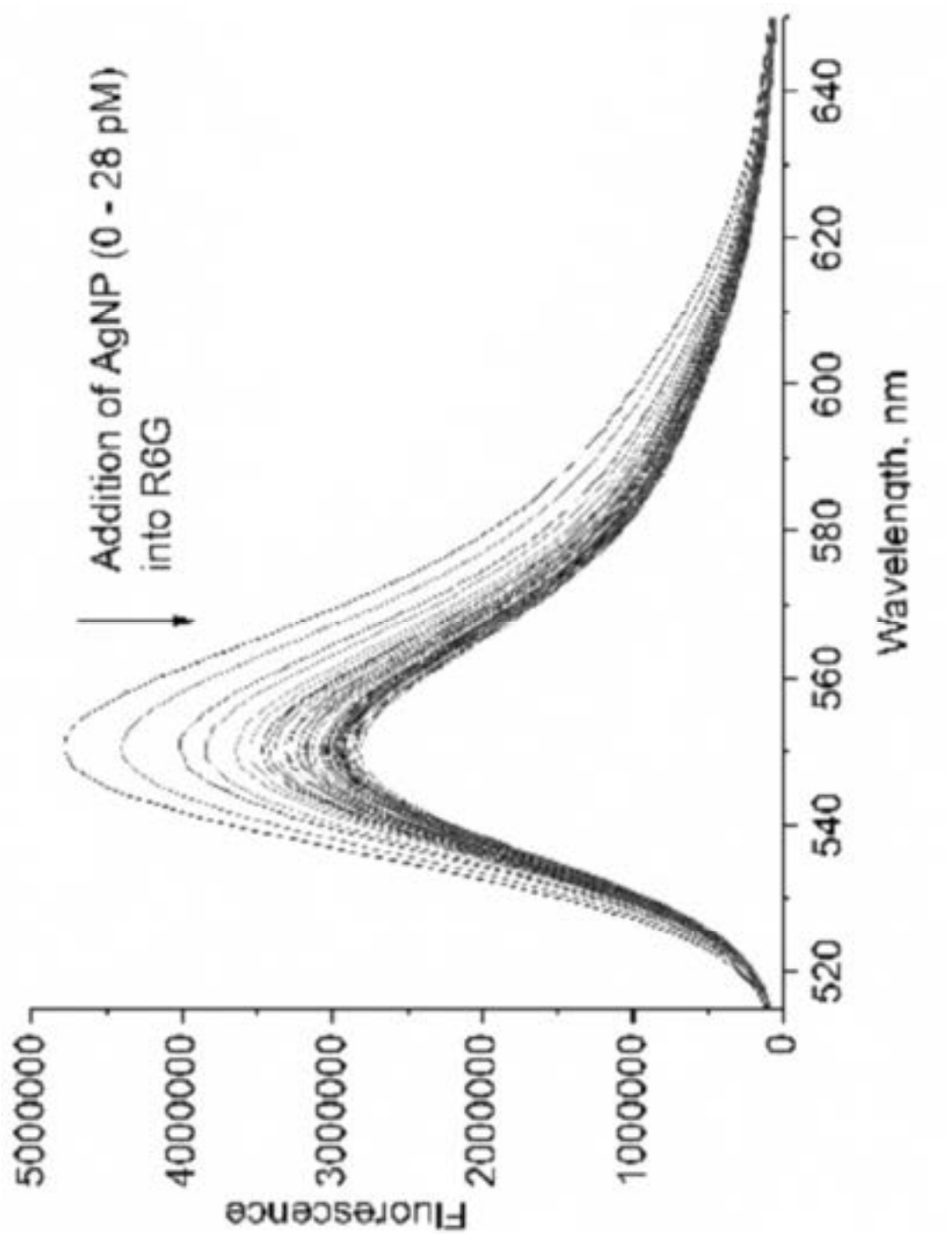

Addition of AgNP (0 - 28 pM)
into R6G
Fluorescence
5000000
4000000
3000000
2000000
1000000
0
520
540
560
580
600
620
640
Wavelength, nm

Figure 1A

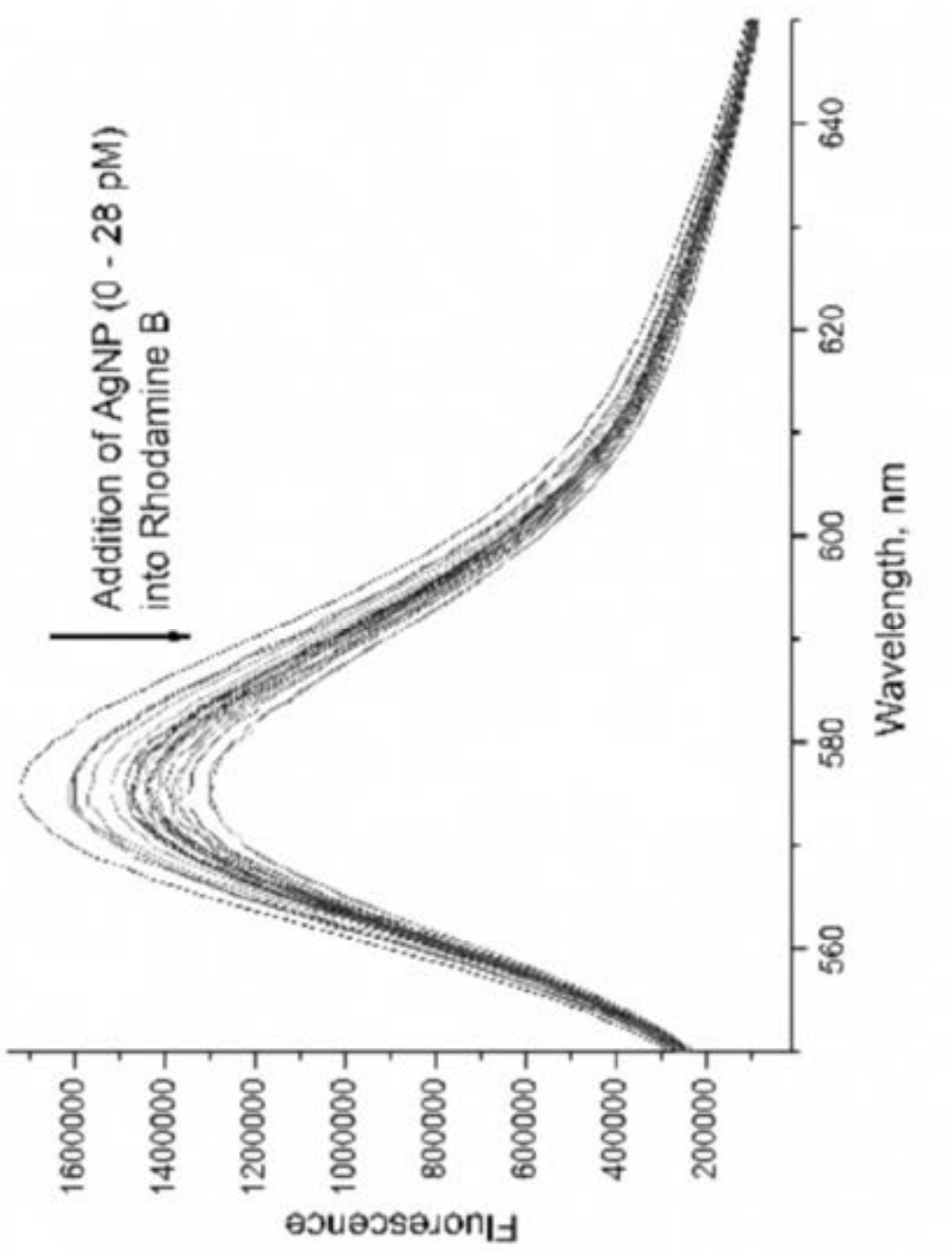

Addition of AgNP (0 - 28 pM)
into Rhodamine B
Fluorescence
1600000
1400000
1200000
1000000
800000
600000
400000
200000
560
580
600
620
640
Wavelength, nm

Figure 1B

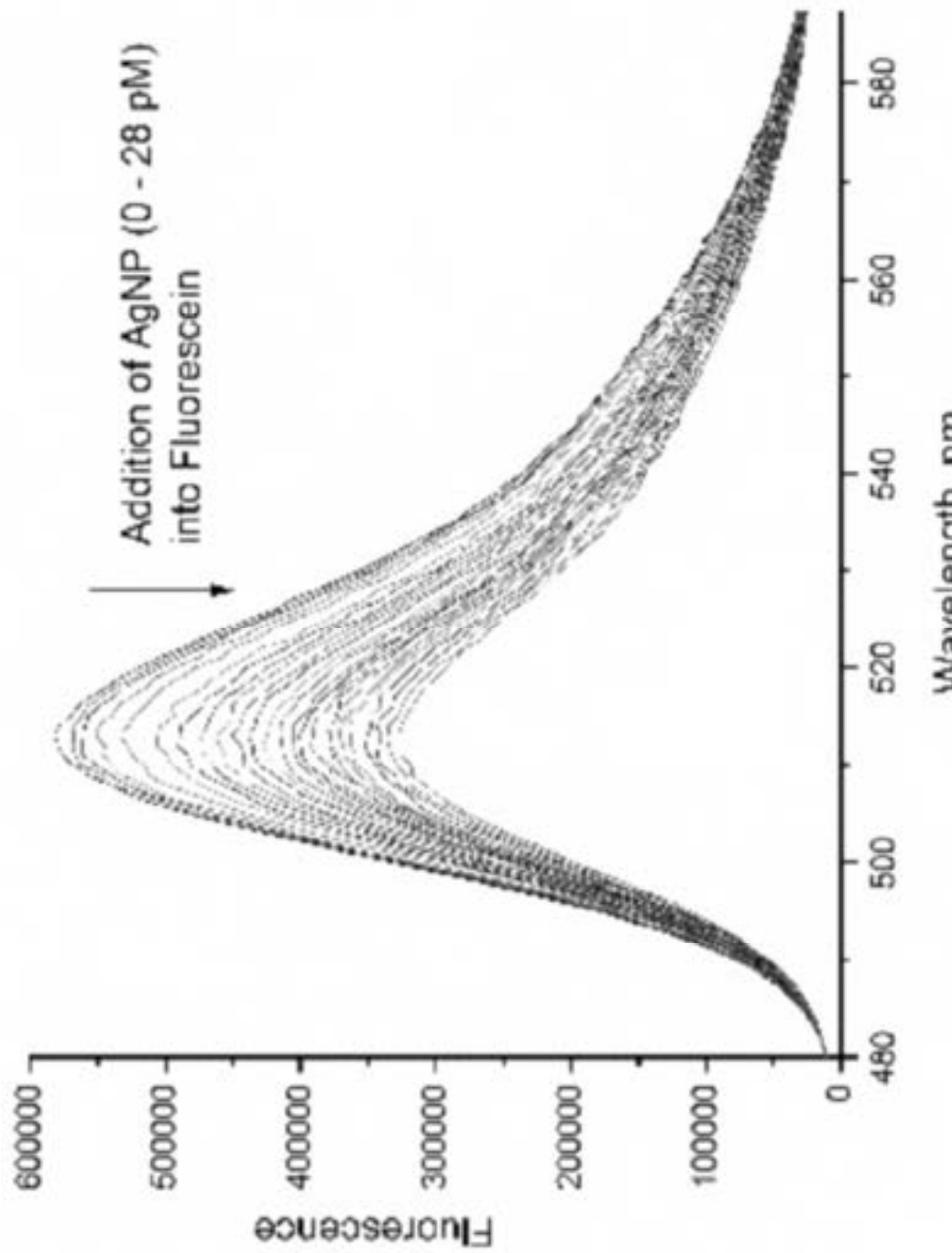

Addition of AgNP (0 - 28 pM)
into Fluorescein
Fluorescence
600000
500000
400000
300000
200000
100000
0
480
500
520
540
560
580
Wavelength, nm

Figure 1C

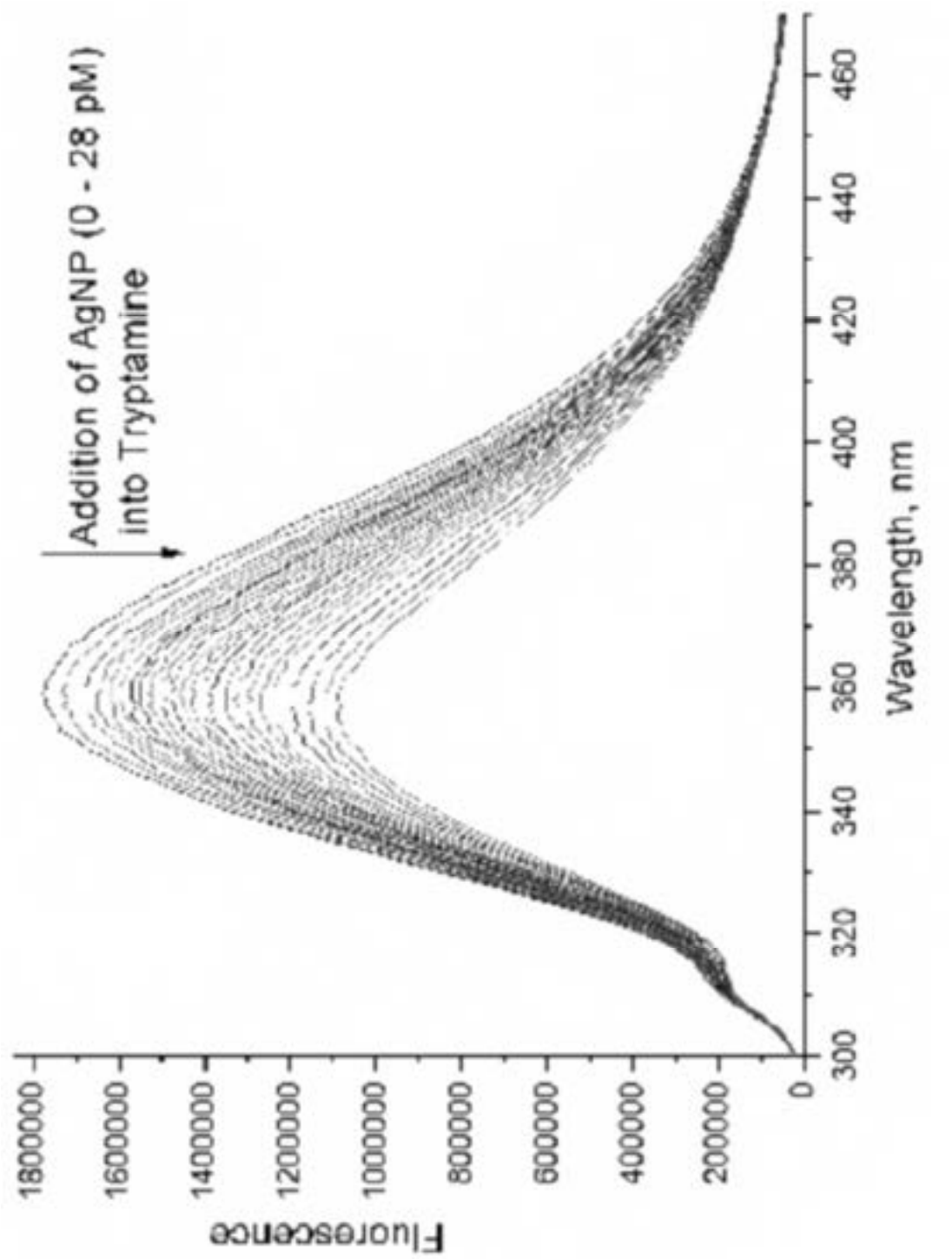

Addition of AgNP (0 - 28 pM)
into Tryptamine
Fluorescence
Wavelength, nm
0
200000
400000
600000
800000
1000000
1200000
1400000
1600000
1800000
300
320
340
360
380
400
420
440
460

Figure 1D

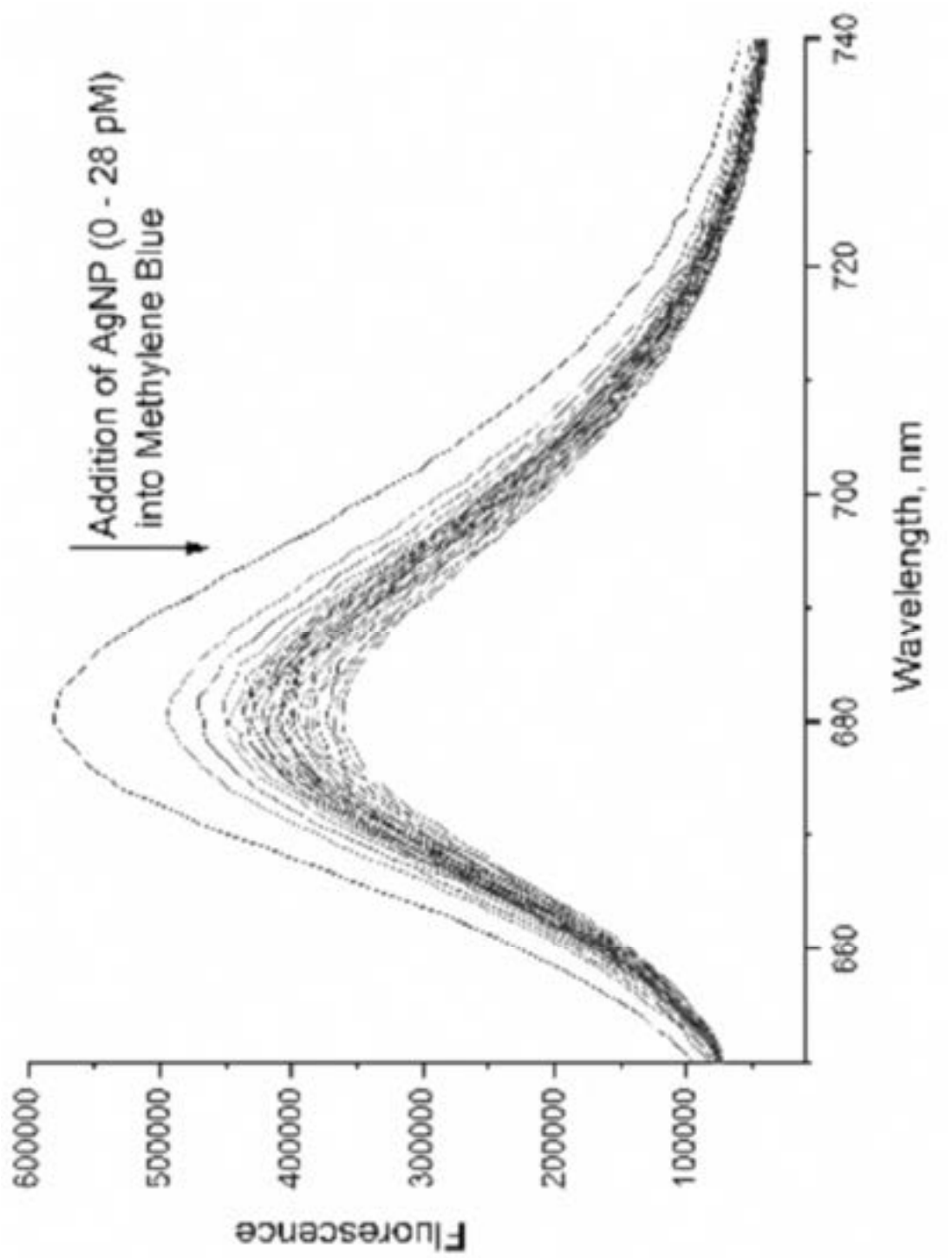

Addition of AgNP (0 - 28 pM)
into Methylene Blue
Fluorescence
600000
500000
400000
300000
200000
100000
660
680
700
720
740
Wavelength, nm

Figure 1E

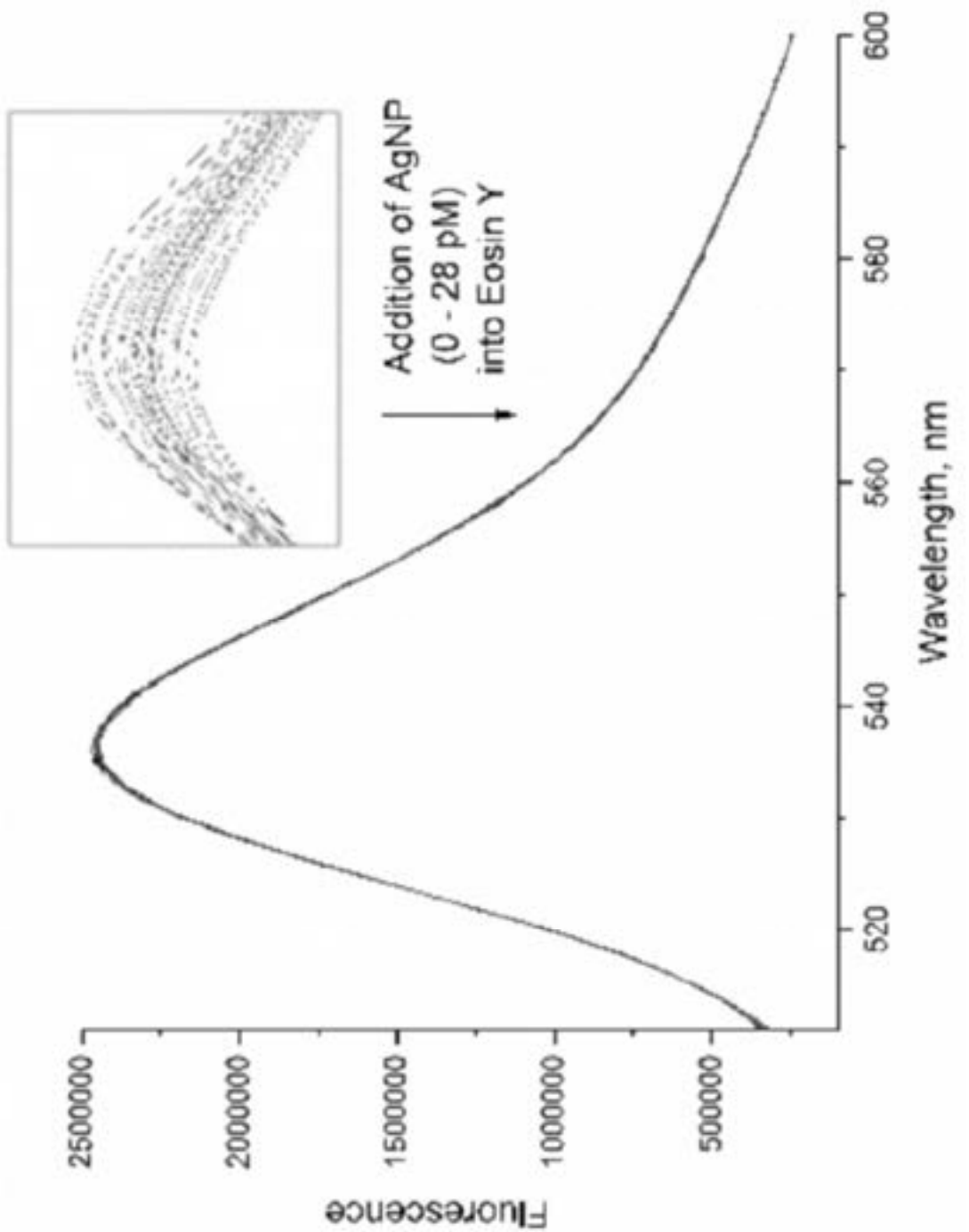

Addition of AgNP
(0 - 28 pM)
into Eosin Y
Fluorescence
2500000
2000000
1500000
1000000
500000
520
540
560
580
600
Wavelength, nm

Figure 1F

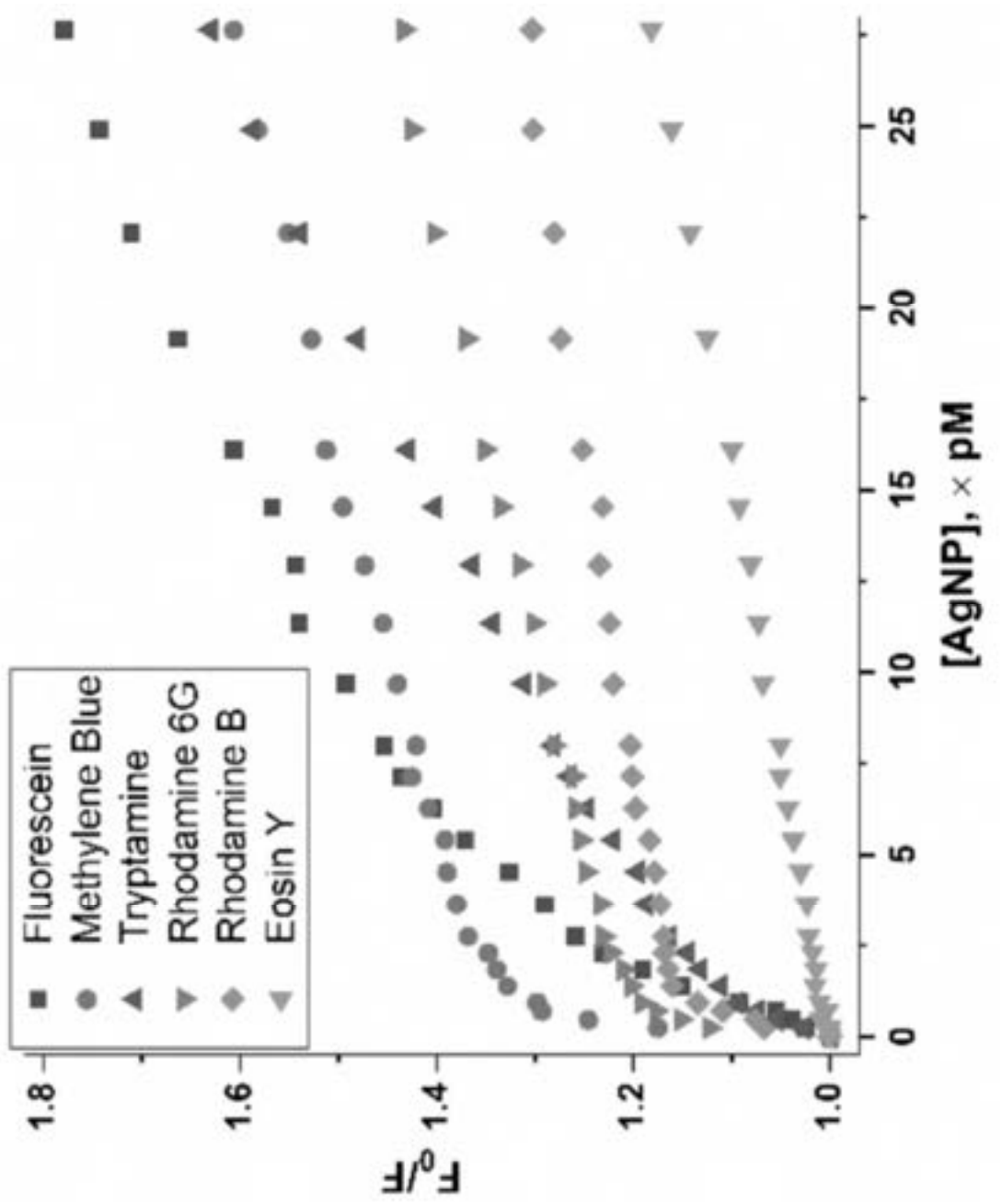

Fluorescein
Methylene Blue
Tryptamine
Rhodamine 6G
Rhodamine B
Eosin Y
F_0/F
1.8
1.6
1.4
1.2
1.0
0
5
10
15
20
25
[AgNP], × pM

Figure 2A

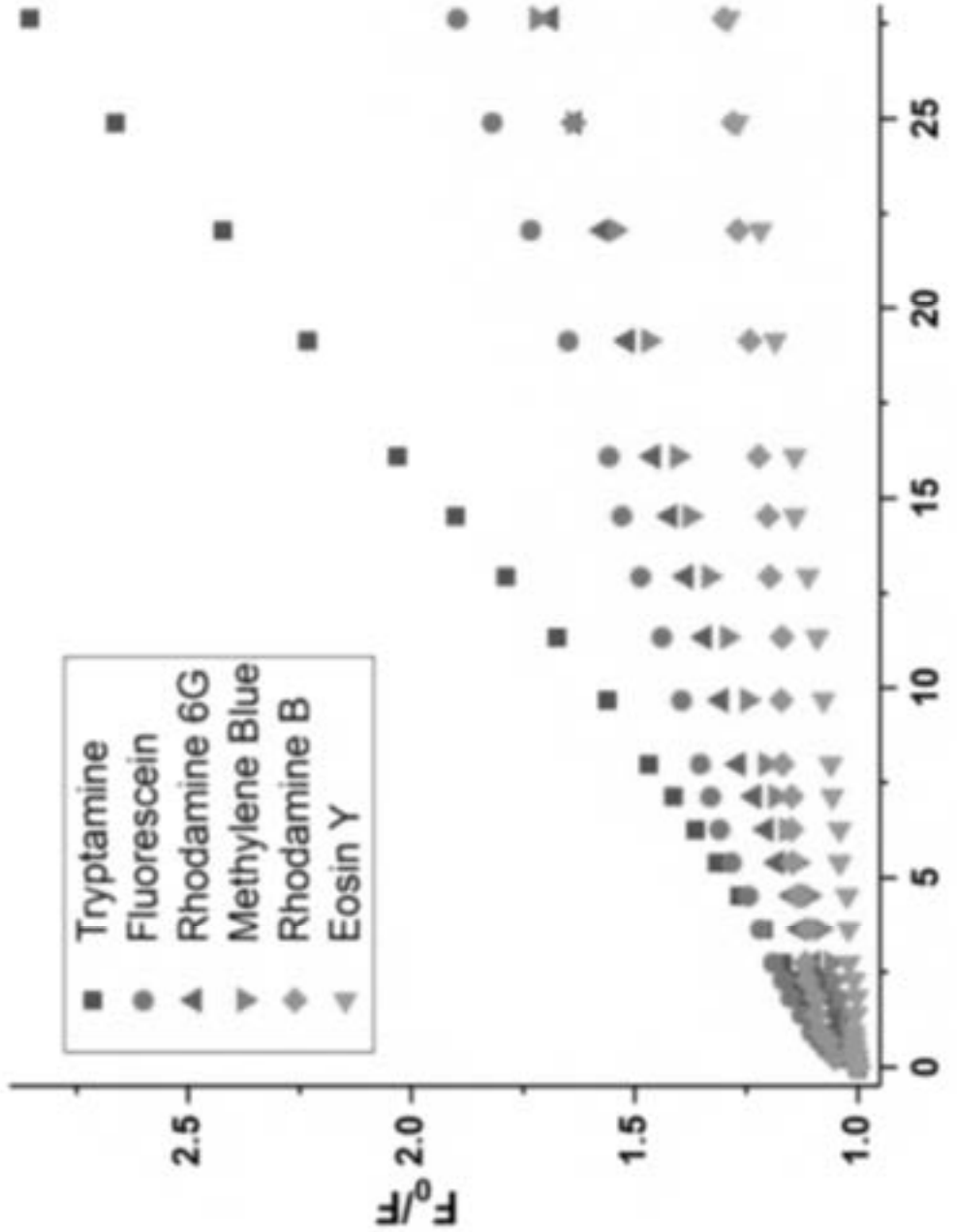

Tryptamine
Fluorescein
Rhodamine 6G
Methylene Blue
Rhodamine B
Eosin Y
I/I0
2.5
2.0
1.5
1.0
0
5
10
15
20
25
[AgNP], × 10 pM

Figure 2B

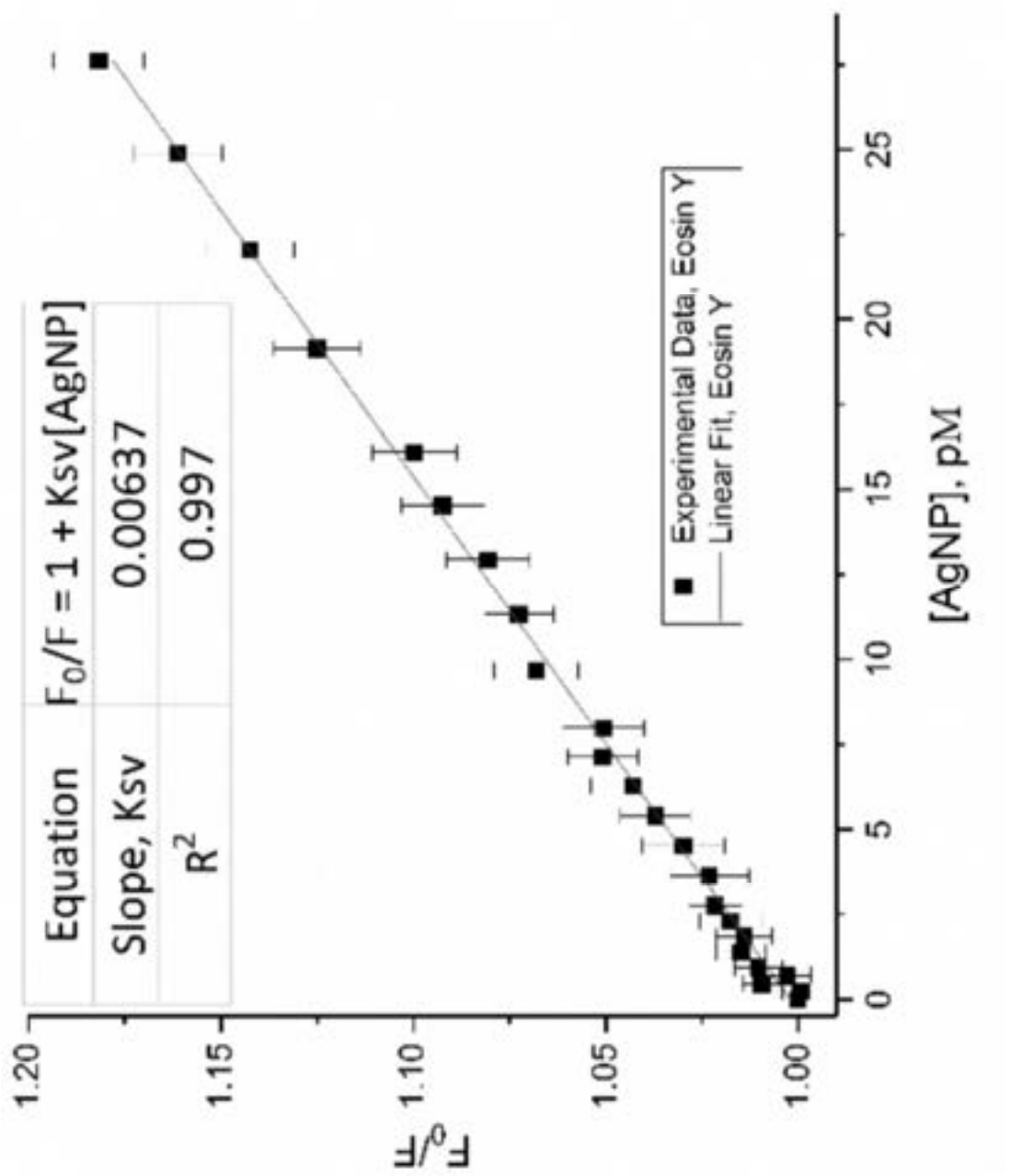

Equation $F_0/F = 1 + Ksv[AgNP]$
Slope, Ksv 0.00637
R^2 0.997
Experimental Data, Eosin Y
Linear Fit, Eosin Y
F_0/F
[AgNP], pM
1.20
1.15
1.10
1.05
1.00
0
5
10
15
20
25

Figure 3

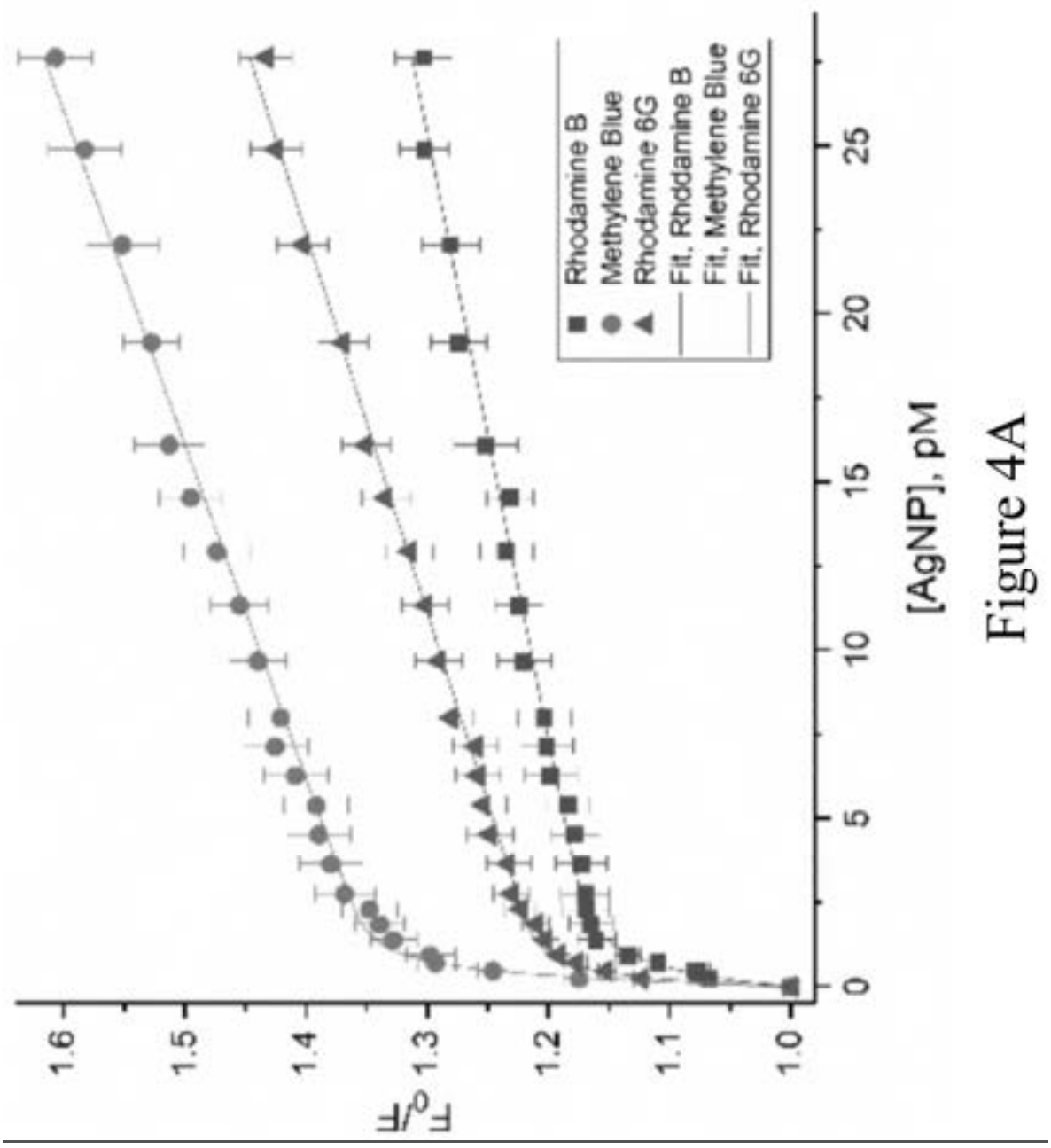

Rhodamine B
Methylene Blue
Rhodamine 6G
Fit, Rhddamine B
Fit, Methylene Blue
Fit, Rhodamine 6G
τ_0/τ
[AgNP], pM
1.0
1.1
1.2
1.3
1.4
1.5
1.6
0
5
10
15
20
25

Figure 4A

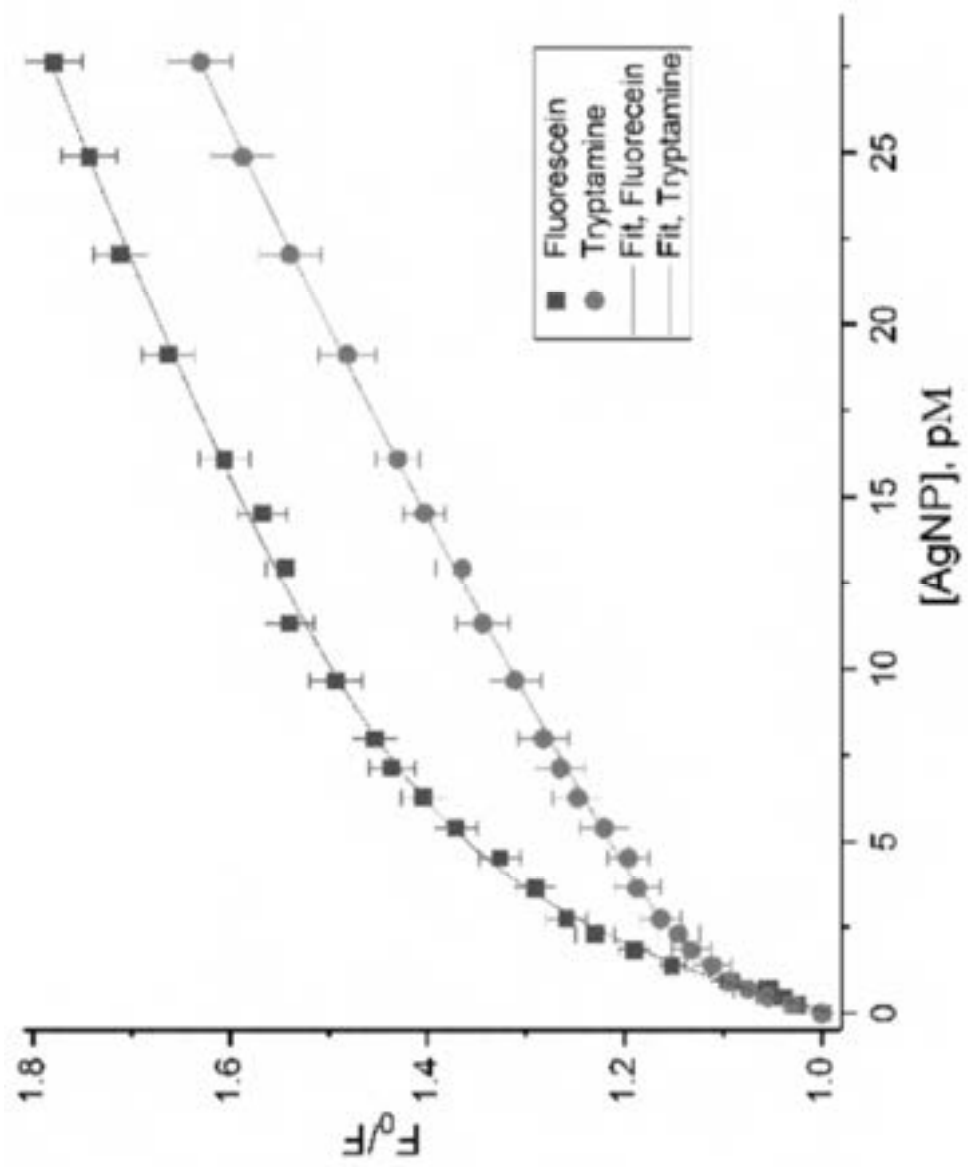

Fluorescein
Tryptamine
Fit, Fluorecein
Fit, Tryptamine
F_0/F
[AgNP], pM
1.0
1.2
1.4
1.6
1.8
0
5
10
15
20
25

Figure 4B

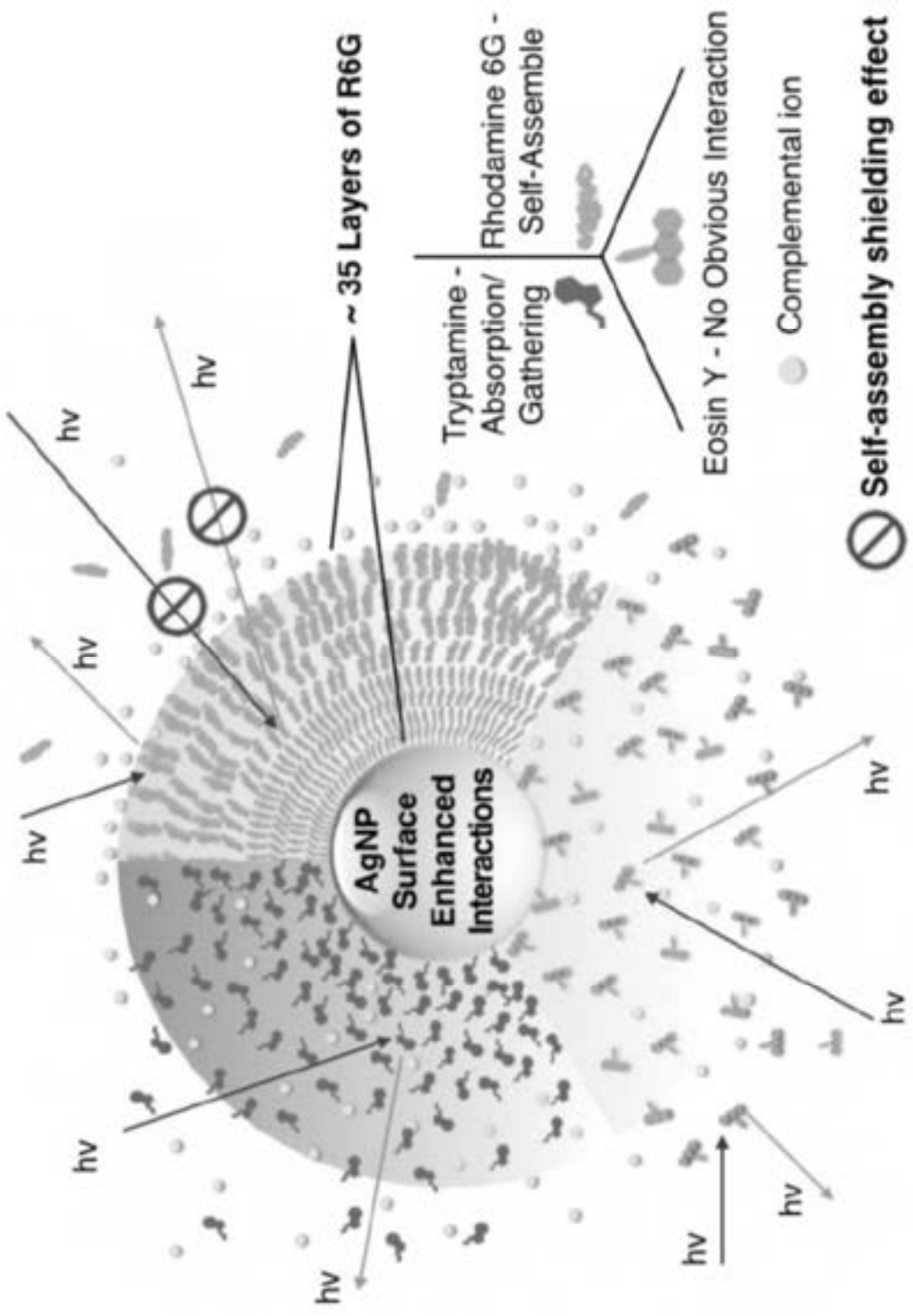

~ 35 Layers of R6G
Tryptamine - Absorption/ Gathering
Rhodamine 6G - Self-Assemble
Eosin Y - No Obvious Interaction
Complemental ion
Self-assembly shielding effect
AgNP Surface Enhanced Interactions
hv

Figure 5

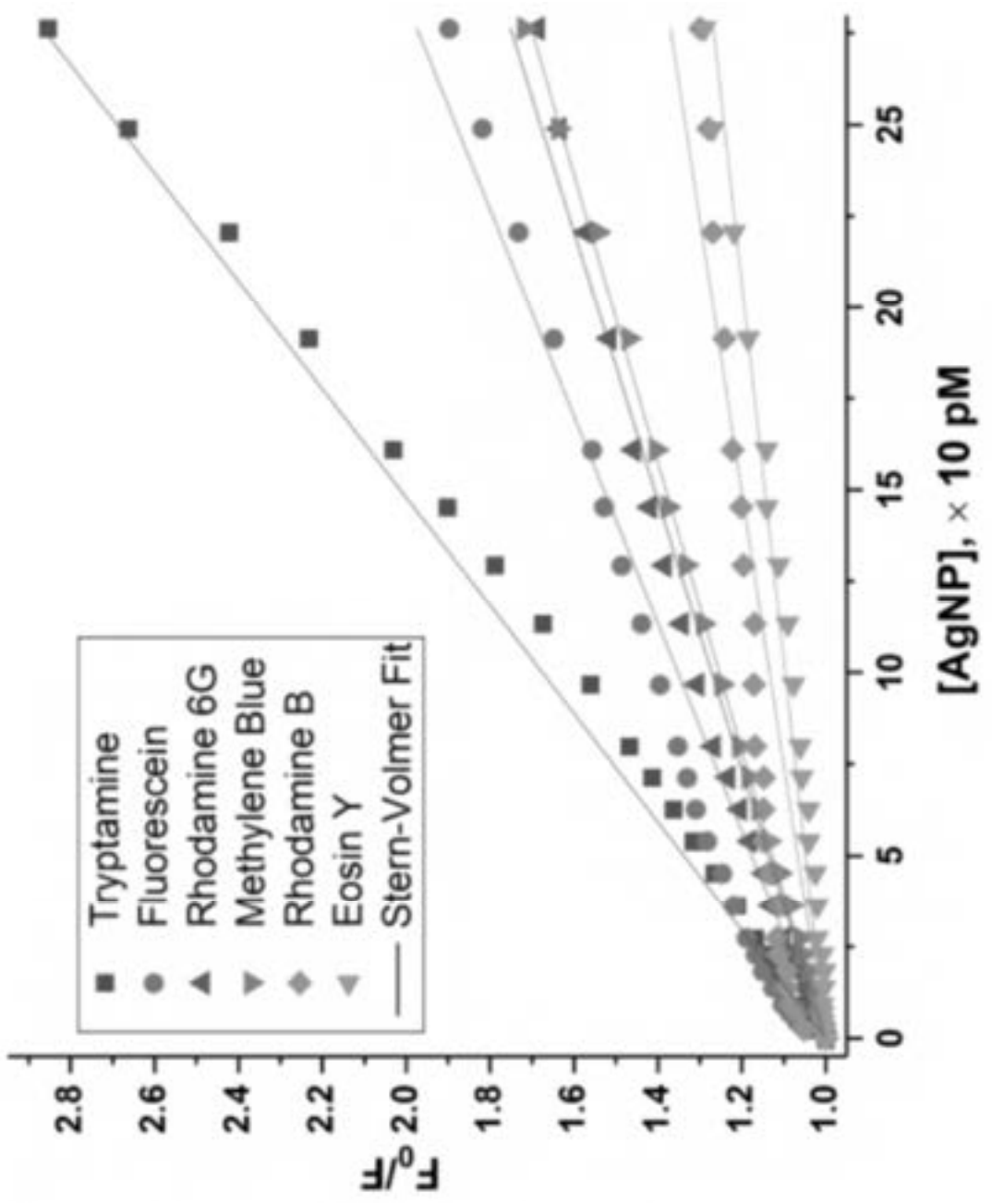

Tryptamine
Fluorescein
Rhodamine 6G
Methylene Blue
Rhodamine B
Eosin Y
Stern-Volmer Fit
I_0/I
[AgNP], × 10 pM
0
5
10
15
20
25
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8

Figure 6

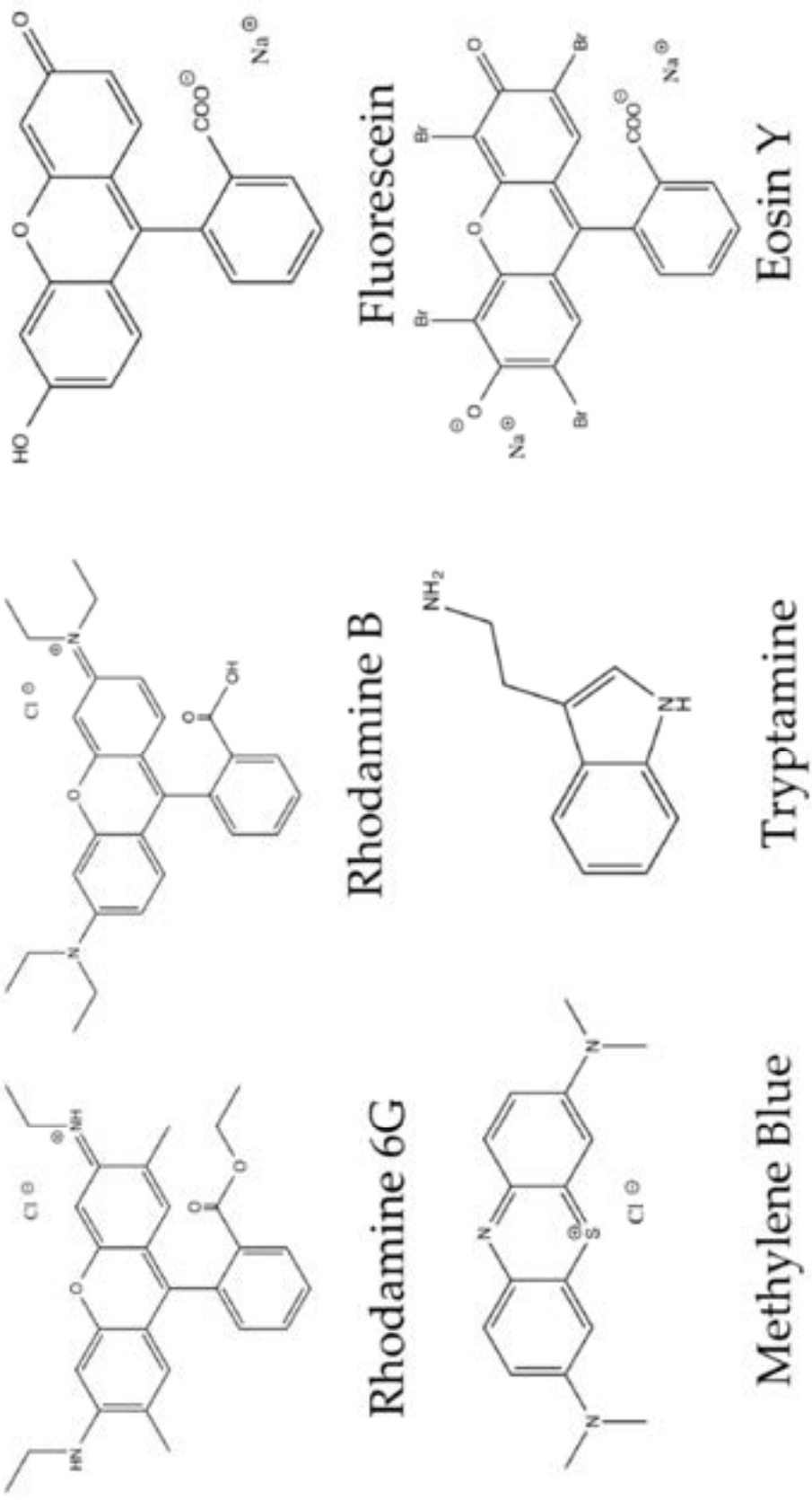

Fluorescein
Eosin Y
Rhodamine B
Tryptamine
Rhodamine 6G
Methylene Blue

Figure 7

SILVER NANOPARTICLE SURFACE ENABLED SELF- ASSEMBLY OF ORGANIC DYE MOLECULES

GOVERNMENT LICENSE RIGHTS

Portions of the invention described herein were made with government support from the United States Army Research Lab (W911NF-122-0022) and the National Science Foundation (Grant DMR-0611539). The U.S. government may have certain rights in the invention.

BACKGROUND OF THE INVENTION

Metal nanoparticles (NP) have shown great potential for use in catalysis [1], sensing [2], imaging [3,4] and antimicrobials [5,6]. Over 400 tons of silver nanoparticle (AgNP) have been produced annually and 30% of them are used in medical applications due to their antibacterial properties [7]. Most of these nanoparticle-containing products will be released to the environment and ultimately conduct unique interactions with biomolecules, such as the formation of protein and environmental corona [8,9]. These interactions are a result of the surface interactions between AgNP and ambient species or through surface-enabled molecular interactions. Hence, it is of interest to understand AgNP surface-enabled molecular interactions and reactions, which are also fundamental to the understanding of the toxicity of AgNP [10].

There have been reports on the study of the interaction between AgNP and biologically active small molecules such as dopamine [11], melamine [12], and pyronin Y [13], and organic molecules containing thiol groups [14]. In general, AgNPs have the highest binding affinity to thiol containing molecules [14,15]. Log K of SR to Ag(I) is 12, compared to 2.1 and 3.4 for OR and NH groups, respectively [16]. Munro et al. [17] studied the surface of citrate-coated AgNP using surface-enhanced Raman spectroscopy (SERS), they suggested that citrate s two carboxylate groups bind the surface Ag atoms of the AgNP while its tertiary hydroxyl group forms intermolecular hydrogen bonds, and with the third carboxylate acting as a reaction site. Petty et al. [18] con rmed that cysteine moieties are the main sites for AgNP attachment in DNAs. Zhao et al. [19] reported that rhodamine 6G (R6G) molecules can form dimers on AgNP surface, which is related to localized surface plasmon resonance. These ndings open some insights into the molecular interaction on the surface of AgNP.

Due to the lack of effective instruments or methods, it is difficult to obtain direct information on the interaction between AgNP and small molecules. To estimate the number of small molecules bound to AgNP, one has to go through a complex process: AgNP-organic molecule complexes are often separated from free organic molecules in solution using centrifugation, followed by mass spectroscopy or UV-vis spectrometry to examine the amount of organic molecules bound to AgNP surface. The number of molecules bound to each AgNP can be estimated by comparing the concentration differences of these molecules before and after mixing with AgNP [6,20,21]. X-ray structure was used to study the structure of the crystalized complex of a gold nanocluster (102 Au atoms) with p-mercaptobenzoic acid (p-MBA), which showed that 44 p-MBA molecules bind to 23 surface Au atoms [22]. The resolution was 1.1 . However, there have been no such studies for AgNP [23-25]. Currently, only surface plasmon resonance and SERS [12, 17] have been used to investigate AgNP interaction with small molecules, while theoretical calculations based on density functional theory or rst principles quantum theory [12,26] complement the experimental studies.

SUMMARY OF THE INVENTION

We studied the interactions of RG6, rhodamine B, methylene blue, uorescein, eosin Y and tryptamine with AgNP through uorescence titration, a method also used to investigate interactions between proteins and metal NP [27,28], and discovered that RG6, rhodamine B and methylene blue self-assemble around a single AgNP to form micelles and micelle agglomerates.

Accordingly, there is presented according to an embodiment of the invention, a self-assembling nano-particle-based micelle composition, comprising a silver nanoparticle core and a plurality of layers of positively charged aromatic nitrogen-containing compounds. According to various alternative embodiments, the silver nanoparticle may be citrate-coated; the silver nanoparticle may be present in solution at a concentration of about 1 M or less; the positively charged aromatic nitrogen-containing compounds may be present in solution at a concentration of about 1 M or less; the positively charged aromatic nitrogen-containing compounds may be dye compounds, including but not limited to Rhodamine 6G, Rhodamine B, and Methylene Blue.

There is further presented according to an embodiment of the invention a method for preparing self-assembling nanoparticle-based micelle composition, comprising a silver nanoparticle core and a plurality of layers of positively charged aromatic nitrogen-containing compounds, the method comprising gradually adding a 1 M solution of silver nanoparticle to a 1 M solution of positively charged aromatic nitrogen-containing compound. According to various embodiments of the invention, the silver nanoparticle may be citrate-coated; the silver nanoparticle may be present in solution at a concentration of about 1 M or less; the positively charged aromatic nitrogen-containing compounds may be present in solution at a concentration of about 1 M or less; the positively charged aromatic nitrogen-containing compounds may be dye compounds, including but not limited to Rhodamine 6G, Rhodamine B, and Methylene Blue.

There is also presented according to the invention, a method for aggregating positively charged aromatic nitrogen-containing compounds in solution, comprising adding silver nanoparticles to a solution containing said positively charged aromatic nitrogen-containing compounds. According to various embodiments of the invention, the silver nanoparticle may be citrate-coated; the silver nanoparticle may be present in solution at a concentration of about 1 M or less; the positively charged aromatic nitrogen-containing compounds may be present in solution at a concentration of about 1 M or less; the positively charged aromatic nitrogen-containing compounds may be dye compounds, including but not limited to Rhodamine 6G, Rhodamine B, and Methylene Blue.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**A. Fluorescence quenching of 1 M solution of R6G dye upon addition of various amounts of 240 pM AgNP.

FIG. **1**B. Fluorescence quenching of 1 M solution of rhodamine B upon addition of various amounts of 240 pM AgNP.

FIG. 1C. Fluorescence quenching of 1 M solution of uorescein upon addition of various amounts of 240 pM AgNP.

FIG. 1D. Fluorescence quenching of 1 M solution of tryptamine upon addition of various amounts of 240 pM AgNP.

FIG. 1E. Fluorescence quenching of 1 M solution of Methylene Blue upon addition of various amounts of 240 pM AgNP.

FIG. 1F. Fluorescence quenching of 1 M solution of eosin Y upon addition of various amounts of 240 pM AgNP. The insert shows the enlarged detail of uorescence peaks, where comparatively weak quenching is observed.

FIG. 2A. Plot of uorescence intensity ratio (F_0/F) versus AgNP concentration at dye concentration of 1 M. Average value from ve measurements are shown and error bars are removed for a better comparison.

FIG. 2B. Plot of uorescence intensity ratio (F_0/F) versus AgNP concentration at dye concentration of 10 M. Average value from ve measurements are shown and error bars are removed for a better comparison.

FIG. 3. Fit of F_0/F verse (AgNP) plot of eosin Y.

FIG. 4A. Fit of F_0/F verse [AgNP] plot of 1 M R6G, rhodamine B, and methylene blue.

FIG. 4B. Fit of F_0/F verse [AgNP] plot of 1 M uorescein and tryptamine.

FIG. 5. Schematic drawing of different types of interaction and behavior of various dye molecules, and self-assembly shielding effect caused by self-assemble of Rhodamine 6G molecules near AgNP surface.

FIG. 6. Fit of F_0/F verse [AgNP] plot of dye molecules in the case of 10 M. Error bars are removed for a better comparison.

FIG. 7. Structures of tested dye molecules.

DETAILED DESCRIPTION

Silver nitrate, sodium borohydride, sodium citrate, R6G, rhodamine B, methylene blue, uorescein, eosin Y, and tryptamine were purchased from Sigma Aldrich (St. Louis, MO). Nitric acid (trace metal grade, 67-70%) was from Fisher Scienti c (Houston, TX).

AgNPs were prepared following a previous reported method [6,29] using sodium borohydride to reduce silver nitrate, followed by citrate mediated reduction and growth [30,31]. Brie y, silver nitrate (17 mg) and sodium borohydride (15 mg) were each dissolved in 90 mL ice-chilled nanopure water. The two solutions were mixed slowly, and a yellowish color appeared. The mixture was left for 5 min under stirring followed by addition of 10 mL of sodium citrate (70 mg). The resultant solution was heated and kept under re ux at 90-95 C. for 30 min. After cooling to room temperature, the solution was centrifuged at 5000 rpm for 1 h and the pellet was collected and suspended into 20 mL water. Centrifugation was repeated twice to remove extra reactants. Finally, a further centrifugation at 2000 rpm for 30 min to remove the largest NPs was carried out and the supernatant was collected and kept in the refrigerator as AgNP stock solutions for further experiments.

The AgNP was characterized by UV-vis spectroscopy (UV-2600 spectrophotometer, Shimadzu, Kyoto, Japan) and Transmission Electron Microscopy (TEM, JOEL 2100, Tokyo, Japan). AgNPs have a characteristic extinction peak at 398 nm. The TEM images show that citrate-coated AgNPs have spherical morphology of an average diameter of 24±7 nm.

To determine the concentration of AgNP, its solution was added with nitric acid to completely oxidize all Ag atoms to Ag^+ ions. The resultant Ag^+ solution was diluted with nanopure water until the estimated Ag^+ concentration ranged from 20 to 100 ppb. It was then analyzed by ICP-MS (Varian 820-MS, Palo Alto, USA) to determine the total concentration of Ag^+ ion and thus the concentration of the AgNP in terms of Ag atoms.

The number of Ag atoms in each AgNP was estimated by the following equation [33,33],

$$N = \frac{\pi}{6}\frac{\rho d^3}{M}N_A = \frac{\pi N_A d^3}{6V_m} = 30.67d^3 \quad (1)$$

where ρ is the density of Ag (10.49 g/cm^3), M is the atomic weight of Ag (107.87 g/mol), V_m is molar volume (10.5 mL mol^{-1}), d is the diameter of AgNP in nm. It yields 420,000 Ag atoms per one 24 nm AgNP. Therefore, the concentrations in terms of Ag atoms that are obtained from ICP-MS can be converted to concentrations in terms of AgNP. E.g., 100 M in term of Ag atoms equals to 2.4×10^{-4} M or 240 pM in term of AgNP. Concentrations used below are in terms of AgNP.

Two concentrations of dye molecules, 1 M and 10 mM, were used for uorescence titrations. Fluorescence parameter of different dyes and instrumental setup are shown in Table 1. AgNP (240 pM or 2400 pM) were titrated into 2,000 L of dye solutions of 1 M and 10 M, respectively, with the following volumes of 0, 2.5, 2.5, 2.5, 2.5, 5.0, 5.0, 5.0, 5.0, 10.0, 10.0, 10.0, 10.0, 10.0, 10.0, 20.0, 20.0, 20.0, 20.0, 20.0, 40.0, 40.0, 40.0, and 40.0 L. Emission spectra were recorded and uorescence intensity values at the peak were collected and analyzed, using Fluoromax-4 spectro uorometer (Horiba Scienti c, Kyoto, Japan). The excitation spectra were also monitored upon addition of AgNP, following the same volume.

TABLE 1

Parameter and instrumental setup for uorescence titrations.

Dye	Excitation Wavelength, nm	Slit Width, nm, 1 m	Slit Width, nm, 10 M	Emission Peak, nm
Rhodamine 6G	500	1	1	554
Rhodamine B	540	2	1	580
Methylene Blue	640	5	4	688
Fluorescein	470	1	1	513
Eosin Y	505	2	1	539
Tryptamine	280	2	2	356

When AgNP solutions are gradually added into the 1 M or 10 M dye solutions, it causes dilution to the original dye solution and thus lower the uorescence intensity. The dilution factor, d_f, was used to correct the uorescence intensity before the plot of uorescence quenching curve, following Equations (2) and (3).

$$d_f = \frac{2000 + \Sigma_0^n V_i}{2000} \quad (2)$$

$$F_{actual} = F_{observed} \times d_f \quad (3)$$

V_i is the volume of AgNP solution added each time in the unit of L, df is the dilution factor. F_{actual} and $F_{observed}$ are uorescence intensities used for analysis and collected from

uorometer, respectively. Factual is the true uorescence and used as F in the further analysis. Concentration of Ag^+ ions released from AgNP is very low (around 100 nM level) [6] and their quenching to R6G uorescence is less than 1% [34]. This is con rmed by using $AgNO_3$ solution of equivalent concentration as a control quencher and it shows no effect on uorescence of dye molecules. Therefore, the role of Ag^+ ions on uorescence quenching is ignored in the current study.

DLS was employed to monitor the change of dynamic size and zeta-potential during titrations, using a Zetasizer Nano-ZS (Malvern, UK).

Effect of AGNPs on the Fluorescence of Dye Molecules

Fluorescence spectra of 1 M dye solutions upon addition of various amounts of the 240 pM AgNP solutions are shown in FIGS. **1**A-**1**F. As AgNP solutions are gradually added to the solution of the dyes, the uorescence intensity of the dyes decreases, an indication of quenching, although the rates of uorescence decrease are different for different dyes. The emission spectra for all the dyes remain the same during titration except tryptamine, whose emission maximum shifts to a shorter wavelength from 359 to 356 nm. The titration into the 10 M dye solutions also show similar uorescence quenching patterns (data not shown) except that emission peak of tryptamine has a larger shift from 359 nm to 345 nm.

The effect of the addition of AgNP on uorescence of various dye molecules in solution is analyzed by the Stern-Volmer plot, or plotting the uorescence intensity ratio (F_0/F) versus AgNP concentration. The AgNP concentration on the x-axis of titration for the 10 M dye solutions is also 10 times higher than that of the 1 M dye solution (FIGS. **2**A and **2**B). The higher the F_0/F ratio, the more uorescence is quenched.

In the case of 1 M dye solution, the F_0/F of methylene blue, RG6 and rhodamine B increases sharply at the initial stage followed by a distinct turn for a slower increase at the AgNP concentration around 1-2 pM, indicating that R6G, methylene blue and rhodamine B molecules self-assemble on the surface of a single AgNP under these conditions to form micelles. As for eosin Y, the uorescence quenching is at the very minimum. The plot for tryptamine and uorescein, whose uorescence ratios increase slowly, but steadily. Ultimately, they reach the highest quenching among all dyes tested.

When the concentration of the dye molecules is 10 the uorescence of all dye molecules in solution is also quenched, without the sharp turn on the plots for R6G, methylene blue and rhodamine B observed when the dye solution is 1 M. This indicates that the micelle-like self-assembly of these dye molecules on AgNP surface does not occur when the dye solution is at 10 M. Fluorescein and tryptamine exhibit a much smoother quenching curve, which is also distinct with those of 1 M dye solution. Eosin Y shows similar pattern since its quenching is weak.

Both absorption and excitation spectra were obtained for the titration. Due to low sensitivity of the photospectrometer, the absorption has to be done at 20 M (or above) of the dye molecules. When 2400 pM of AgNP solution were titrated into 50 M dye solution, R6G and methylene blue cause AgNP aggregation, while tryptamine, rhodamine B, uorescein and eosin Y do not. In terms of absorption intensity, tryptamine, rhodamine B, uorescein and eosin Y do not exhibit obvious decrease. The excitation spectra showed no shifts for all dye molecules.

Different Behaviors of the Dyes on AgNP Surface

Methylene blue, RG6 and rhodamine B as 1 M solutions show similar quenching behaviors, with a sharp quenching at initial additions of AgNP until a sharp turn, followed by a much slower quenching. This demonstrates that methylene blue, RG6 and rhodamine B have the same capability to self-assemble on the AgNP surface to form stable micelle-like structures.

Fluorescence of uorescein or tryptamine is gradually quenched with a smaller slope compared with R6G, rhodamine B and methylene blue, but lacks the turn. These results indicate that uorescein and tryptamine may gather around AgNP core in an absorption/tight interaction mode in a dynamic equilibrium but no stable micelle-like structure is formed since no obvious turn is observed (FIGS. **2**A and **2**B).

Eosin Y maintains a small but stable quenching slope during the whole titration.

These three types of molecular behaviors are further con rmed by the change of dynamic size and zeta-potential (Table 2). Rhodamine 6G and Rhodamine B have steady but dramatic change of both diameter and zeta-potential. Fluorescein and tryptamine have relative small size change with a difference around 34 nm and 36 nm, respectively. Size of AgNP in the presence of eosin Y is slightly increased due to pre-concentration that is further discussed below. Based on these interaction patterns, two mathematical models are used to t the plot of F_0/F versus [AgNP] using Origin 2017 SR2.

TABLE 2

Dynamic diameter and zeta-potential of AgNP coated by citrate and in presence of six dye molecules.

	AgNP with citrate	Rhodamine 6G	Rhodamine B	Methylene Blue *	Fluorescein	EosinY	Tryptamine
Size, nm	33.4 ± 1.6	318.5 ± 34.4	197.6 ± 62.1	/	67.1 ± 12.4	42.8 ± 3.9	69.2 ± 13.0
Potential, mV	–47.3 ± 1.2	21.5 ± 2.0	17.6 ± 1.8	/	–16.9 ± 2.2	–31.8 ± 1.6	8.9 ± 3.7

Notes:

Data at the titration point of 2 pM AgNP is shown. At this point, the corresponding dye reach equilibrium between stable micelle-like structures or complexes and free molecules in solution.

* DLS results of methylene blue are not available due to interference between laser source wavelength of DLS (633 nm) and absorption of methylene blue (peaked at 612 nm and 664 nm).

Stern-Volmer Fluorescence Quenching Model for Eosin Y

For eosin Y, its uorescence-quenching plot, F_0/F verse [AgNP], ts very well to the Stern-Volmer equation (FIG. **3**), with K_{sv} of 6.4 nM^{-1} of [AgNP], or 15,000 M^{-1} of [Ag] based on the estimate that one AgNP of 24 nm has about 420,000 Ag atoms.

Considering the Stern-Volmer equation,

$$\frac{F_0}{F} = 1 + k_q \tau_0 [AgNP] \tag{4}$$

the quenching rate constant k_q is comprised of two terms, the fraction of collisions that result in quenching f_Q, and the diffusion controlled bimolecular rate constant k_0.

$$k_q = f_Q k_o \tag{5}$$

$$k_0 = \frac{4\pi N_0}{1000\frac{\text{cm}^3}{\text{L}}}(r_f + r_q)(D_f + D_q) \tag{6}$$

where N_0 is Avogadro s number, r_f and r_q are the radii of the uorophore and quencher, and D_f and D_q are the diffusion coefficients of the uorophore and quencher, respectively. Based on the Stokes-Einstein equation

$$D = \frac{K_B \times T}{6\pi\eta r} \tag{7}$$

where K_B is Boltzmann constant, T is absolute temperature, η is the dynamic viscosity and r is the particle size. Diffusion coefficient (D) is mainly dependent on the particle size. Under current experimental conditions, eosin Y has a much smaller size than AgNP whose diffusion coefficient can be ignored when compared to that of eosin Y. Thus, Equation (4) can be rewritten as:

$$\frac{F_0}{F} = 1 + f_Q \tau_0 D_f \frac{4\pi N_0}{1000\frac{\text{cm}^3}{\text{L}}}(r_f + r_q)(AgNP) \tag{8}$$

Eosin Y has lifetime τ_0 of 2.50 ns [35] and small organic dye molecules normally have diffusion coefficients D_f around 420 $m^2\ s^{-1}$ [36]. Given approximate radius of eosin Y and AgNP as 0.8 nm and 12.0 nm, equation [8] yields an f_Q of 6.17×10^{-20}, demonstrating an extremely low percentage of collisions that cause uorescence quenching.

In contrast, eosin Y has an extremely high K_{sv} of 6.4 nM^{-1} of [AgNP], or 15,000 M^{-1} of [Ag], compared with the normal small molecule quenching, like the uorescence quenching of tryptamine by acrylamide in an aqueous solution, which is around 33 M^{-1} [37]. Thus, the terms of traditional dynamic/collisional quenching and static quenching are not proper to describe uorescence quenching by nanoparticles. Instead, we employed nanoparticle surface enabled uorescence quenching to describe uorescence quenching of eosin Y by AgNP.

Mathematics Model for the Molecular Self-Assembly on AgNP Surface

Plots of F_0/F of rhodamine B, R6G, and methylene blue versus [AgNP] all t very well to a function composed of an exponential term (self-assembly shielding effect) plus a Stern-Volmer term (nanoparticle surface enhanced uorescence quenching), shown in FIGS. **4**A and **4**B and Equation (9).

$$\frac{F_0}{F} = A\left(1 - e^{-\frac{(AgNP)}{B}}\right) + (1 + K_{SV}(AgNP)) \tag{9}$$

The resulting constants A, B, and K_{sv} are listed in Table 2 from the excellent ts.

The initial exponential term represents the rapid quenching through self-assembly shielding effect, where the internal dye molecules of the micelles cannot absorb light and thus not get to the excited state to uoresce, due to the self-assembly of the dye molecules on the surface of AgNP. Constant A corresponds to the AgNP concentration at which a stable micelle-like structure is in an equilibrium with the free dye molecules in solution. The higher is the A value, the lower is the concentration required for the corresponding dye to reach equilibrium between stable micelle-like structures and those free in solution. Thus, three dyes that form stable micelle-like structures follow a formation sequence from fastest to lowest: methylene blue>R6G>rhodamine B (A values of 0.33, 0.20 and 0.16, respectively).

Constant B represents the relative binding affinity between AgNP and dye molecules. Smaller B values indicate stronger interactions, and larger numbers of dye molecules gathering around each AgNP at equilibrium, and ultimately self-assembly shielding effect as well as uorescence quenching percentages. The three dyes follow an interaction affinity sequence of rhodamine B<methylene blue<R6G (B values of 0.59, 0.38 and 0.33, respectively).

The uorescence quenching of R6G, rhodamine B and methylene blue by AgNP have an obvious turning point where stable micelle -like structures are formed. Equation (5) is employed to estimate the number of dye molecules that assemble on the surface of each AgNP, assuming that all the uorescence quenching is caused by interactions between dye molecules and the AgNP.

$$\frac{\text{Quenched Dye}}{AgNP} = \frac{2000\ \mu\text{L} \times 1\ \mu\text{M} \times N_0 \frac{I}{I_0}}{240\ \text{pM} \times N_0 \Sigma_0^N V_i} = \frac{10^8 \times \frac{I}{I_0}}{12 \times \Sigma_0^n V_i} \tag{10}$$

V_i is the value of each titration volume, with unit of L. At the turning point, this ratio demonstrates the number of molecules in a single stable micelle-like structure, in other words, the number of dye molecules that a stable micelle can hold in a dynamic equilibrium. The estimated numbers are listed in Table 3, with 180,000, 200,000, and 220,000 for rhodamine B, methylene blue, and R6G, respectively, per AgNP. This number indicates that at dynamic equilibrium, the majority of the dye molecules in solution are assembled on AgNP. The remaining small portion is close to the minimum concentration of free dye molecules to maintain balance in the solution.

The Stern-Volmer term K_{sv}, representing the collisional quenching, has values for rhodamine B, R6G, and methylene blue of 5.5, 8.9 and 10 nM^{-1} of [AgNP], respectively, or 13,000, 21,000, and 24,000 M^{-1} of Ag atoms.

The uorescence quenching graphs for tryptamine and uorescein also t to Equation (9), but due to the lack of the sharp turn, there is no indication of a de nitive micelle formed. Therefore, there is no estimate made for the number of molecules per AgNP. The tting parameters A, B, and K_{sv} are on the same order of magnitude with those for methylene blue, rhodamine B and R6G, but the parameters B and K_{sv} values are obviously larger (Table 3). This demonstrates an absorption/tight interaction mode on AgNP surface and pseudo-micelles could be formed with these dyes (FIG. **5**).

TABLE 3

Mathematical tting results for all dye molecules.

$$\frac{F_0}{F} = A\left(1 - e^{-\frac{[AgNP]}{B}}\right) + (1 + K_{sv}[AgNP])$$

Dye	A	B	K_{sv}, nM^{-1} (AgNP)	K_{sv}, M^{-1} (Ag)	R^2	Dyes/ AgNP
Rhodamine B	0.16	0.59	5.5	13,000	0.992	180,000
Rhodamine 6G	0.20	0.33	8.9	21,000	0.996	220,000
Methylene Blue	0.33	0.38	10	24,000	0.995	200,000
Fluorescein	0.37	3.37	1.5	36,000	0.998	/
Tryptamine	0.12	1.27	1.8	43,000	0.999	/
Eosin Y	/	/	6.4	15,000	0.997	/
Tryptamine*	/	/	/	33*	/	/

*The K_{sv} for tryptamine quenching by acrylamide is 33 M^{-1} [38].

Super Stern-Volmer Fluorescence Quenching Constant

One interesting observation is that the Stern-Volmer quenching constant (nanoparticle surface enhanced uorescence quenching) for all the six dye molecules assembled on AgNP are generally three orders of magnitude higher than the normal small molecule quenching, like the uorescence quenching of tryptamine by acrylamide in an aqueous solution, which is around 33 M^{-1} [37], making AgNP a super-quencher [38]. One explanation is that the dye molecules, except those forming the micelles, are in close proximity of AgNP, or pre-concentrated on the AgNP surface. During this process, uorescence quenching is a complicated result caused by a combined self-assembly shielding effect and traditional static and even dynamic quenching. This again indicates that these dye molecules, charged or neutral, are in the proximity of the AgNP.

Self-Assembly Shielding Effect Caused Fluorescence Quenching

It is well known that aggregation, including self-assembly, causes uorescence quenching of dye molecules in aqueous solution (it is sometimes termed aggregation caused uorescence quenching, ACQ), owing to electron or energy transfer [39-41]. It occurs under relatively high concentrations for aggregation, at 10 M or higher [42,43]. In our case, the self-assembly of dye molecules on AgNP surface indicates that traditional electron or energy transfer (among dye molecules) causes uorescence quenching, even under 1 M for both the dye and AgNP. However, it cannot explain the sharp turn of the plot for R6G, methylene blue and rhodamine B.

Taking R6G as an example, 210,000 R6G molecules assemble on a single AgNP (24 nm) with an estimated 35 layers. As shown in FIG. **5**, the inner layers of R6G would be shielded from absorbing light and uoresce [43], termed self-assembly shielding effect to describe the unique uorescence quenching caused by self-assembly of dye molecules on AgNP surface. Considering that 35 layers of dye molecules, R6G, methylene blue or rhodamine B are tightly surrounding the AgNP core, the excitation light could penetrate approximately the outmost one to several layers (due to gaps among dye molecules). It cannot travel through many layers of self-assembled dye molecules to reach those of inner layers. Therefore, inner layers of dye molecules are shielded from excitation by light and thus would not generate any uorescence. This is a unique uorescence quenching mechanism caused by AgNP enabled self-assembly behavior, with a characteristic sharp turn on the plot of uorescence intensity ratio (F_0/F) versus AgNP concentration (FIGS. **2**A and **2**B).

For dye molecules that have loose or no obvious interaction with AgNPs, like eosin Y in FIG. **3**, the pathway of light is not completely blocked so that its uorescence quenching is mainly caused by electron transfer, F rster resonance energy transfer (FRET) and/or nanosurface energy transfer (NSET). Some organic molecules show a uorescence quenching efficiency between R6G and eosin Y, indicating they have a gathering around AgNP but it is not as dense/thick, nor as well-organized as that of R6G. Self-assembly shielding effect may also be involved but it is not that obvious.

Factors Affecting Molecular Interactions on AgNP Interface Concentration of Both Dyes and Nanoparticles

It is obvious that the self-assembly of dye molecules on AgNP surface is concentration dependent; and it only occurs at low concentrations for both AgNP and dyes. For the dye solutions of 10 all quenching plots t to a pseudo Stern-Volmer plot (FIG. **6**) with K_{sv} values of 10-68 nM^{-1} [AgNP] (Table 4), higher than those constants for dyes in the 1 M solutions. Such large Stern-Volmer quenching constants still indicate super-quenching. At the [AgNP]/[dye] ratio of 30, the quenching strength from the most to the least quenched dyes are: uorescein>tryptamine methylene blue>rhodamine 6G>rhodamine B>eison Y for 1 M dye solutions; and tryptamine> uorescein>methylene blue rhodamine 6G>rhodamine B≤eison Y for 10 M dye solutions. It is obvious that concentration plays an important role on uorescence quenching by AgNP.

TABLE 4

Stern-Volmer t results (K_{sv} in nM^{-1} of [AgNP]) with the concentration of the dyes at 10 M.

	Tryp-tamine	Fluorescein	R6G	Methylene Blue	Rhodamine B	Eosin Y
K_{sv}	68	35	27	25	13	10

Intrinsic Molecular Structure

The structures of all tested dyes are shown in FIG. **7** and their properties are listed in Table 5 with information on charge, functional groups and paired ions. Self-assembly is determined from interactions between AgNP and dye molecules at 1 M as shown in FIGS. **2**A and **2**B.

TABLE 5

Properties of dye molecules and their uorescence quenching behavior.

Dye	Charge	Functional Groups	Pair Ions	Self-Assembly
Rhodamine 6G	1+	NH , N═, O , COO	Cl^-	Yes
Rhodamine B	1+	N , N═, O , COO	Cl^-	Yes
Methylene Blue	1+	N , N═, S	Cl^-	Yes
Fluorescein	1–	OH, COO , O , ═O	Na^+	Weak
Eosin Y	2–	COO , Br , O , ═O	Na^+	No
Tryptamine	0	NH , NH_2	NA	Weak

Charge

All dye molecules forming the micelle-like self-assemblies on AgNP have a positive charge, indicating positive charge promotes the self-assembly of the dyes on AgNP. Surface of citrate-coated AgNP are negatively charged, thus AgNP favors binding to positively charged molecules. It is rather unusual that these positively charged aromatic dye molecules self-assemble on the AgNP surface to form large micelles.

Functional Groups

In addition to the positive charge, rhodamine B, R6G and methylene blue all have some nitrogen atoms in the molecule. We think that the nitrogen group also helps to facilitate the micelle formation. The other feature of these three molecules are the relatively large and at aromatic moieties, which might be necessary for the micelle formation. However, it is difficult to pinpoint the exact roles the functional groups play in the self-assembly, as well as the pre-concentration on the AgNP surface. These results are in agreement with previous reports that AgNP have preferable binding sites in terms of speci c functional groups and other protein components or secondary structure [12,15,44,45]

CONCLUSIONS

Furthermore, the self-assembly can only occur at low concentrations (at 1 M or lower) for both dye and AgNP, while at a higher dye concentration (10 M) yields pseudo Stern-Volmer quenching curves. Although the quenching of uorescein and tryptamine by AgNP also ts to the same two-term model, the quenching curve lacks the signature sharp turn for the formation of the micelle-like self-assembly. The quenching of eosin Y follows the Stern-Volmer quenching model very well without the exponential term. The Stern-Volmer quenching constants (K_{sv}) for these dyes are in the range of 1.5-10 nM^{-1} of [AgNP], or 13,000-43,000 M^{-1} of [Ag], three orders of magnitude higher than the value of the collisional quenching of tryptamine uorescence by acrylamide (33 M^{-1}).

Such a super-quenching by AgNP can only be attributed to pre-concentration of the dye molecules on the nanoparticle surface. The pre-concentration of methylene blue/R6G/rhodamine B near the surface of AgNP further yields the formation of micelle-like self-assembly, resulting in even greater uorescence quenching during the initial stage of the titration. Detailed mechanisms of self-assembly remain to be explored but the common features of all these dye molecules are the positive charge, the aromatic moiety, and the polar groups. The charge of the dye molecules seems to be the dominant factor. Negatively charged or neutral molecules are not likely to self-assemble on the AgNP surface. The uorescence quenching for Methylene Blue, Rhodamine B, and R6G all t to a mathematical model with an exponential term (self-assembly) and a Stern-Volmer term (nanoparticle surface enhanced quenching).

These ndings and models provide fundamental information of nanoparticle surface enabled interaction and molecular behavior, which could further facilitate the understanding of protein and environmental corona formation, and bene t engineering nanoparticle-based nanomaterials.

Having now fully set forth the preferred embodiments and certain modi cations of the concepts underlying the present invention, various other embodiments as well as certain variations and modi cations of the embodiments herein shown and described will obviously occur to those skilled in the art upon becoming familiar with said underlying concepts. If should be understood, therefore, that the invention may be practiced in ways other than explicitly set forth herein and still fall entirely within the scope of the present invention.

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The invention claimed is:

1. A self-assembling nano-particle-based micelle composition, comprising a silver nanoparticle core and a plurality of layers of positively charged aromatic nitrogen-containing compounds wherein the positively charged aromatic nitrogen-containing compounds are selected from the group consisting of Rhodamine B and Methylene Blue.

2. A self-assembling nano-particle-based micelle composition according to claim **1**, wherein the silver nanoparticle is citrate-coated.

3. A self-assembling nano-particle-based micelle composition according to claim **1**, wherein the silver nanoparticle is present in solution at a concentration of about 1 M or less, and the positively charged aromatic nitrogen-containing compounds are present in solution at a concentration of about 1 M or less.

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