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# An Analytical Method for Detecting Toxic Metal Cations Using Cyclotriveratrylene Derivative Capped Gold Nanoparticles

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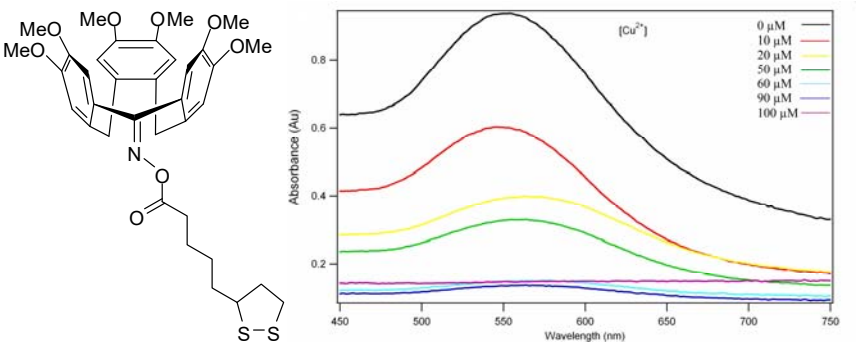



## Abstract

Cyclotriveratrylene-oxime (CTV-oxime) derivatives that terminate with a dithiolate linker were synthesized. The addition of the dithiolate linker enables the supramolecular scaffold to adhere to gold nanoparticles (AuNPs) with the bowl-shaped cavity of the CTV scaffold exposed for utilization in host-guest chemistry. Exposure of these CTV functionalized AuNPs to varying concentrations of di- and trivalent metal cations resulted in the formation of large CTV-AuNP polymeric clusters and an accompanying a shift in the plasmon resonance. These interactions between the CTV-AuNPs and the metal cations in solution provides proof-of-concept that supramolecular functionalized AuNPs can be used as a simple and straightforward, on-site detection system for toxic metal cations in solution.

## 1. Introduction

Due to the environmental and biological impact of metal ion contamination, the development of sensitive and selective detection systems for colorless metal ions is of great importance. The toxicity of lead and mercury ions is well known, and even copper ions are

highly toxic to algae, fungi, bacteria, and viruses. [1] High copper concentrations in children lead to cirrhosis of the liver, and may also play a role in neurodegenerative diseases such as Alzheimer's Disease. [2-5] A variety of analytical devices have been developed for the detection of metal ions, including systems based on chemosensors, [6] atomic fluorescence spectrometry (AFS), [7] inductively coupled plasma mass spectrometry (ICP-MS),[8] and atomic absorption spectroscopy (AAS). [9] Although these techniques offer a sensitive and selective analytical approaches they are expensive, require sophisticated equipment, and lack the portability for on-site detection. The use of surface bound ligands for detection of cations in solutions, as well as using an approach based on host-guest chemistry, where the optical absorbance of the system is correlated to the concentration of the analyte would productively impact the field of metal cation detection. Utilization of this approach affords a simple and straightforward field assay detection system

Cyclotriveratrylene (CTV, Figure 1) is a bowl-shaped supramolecular scaffold[10-15] that we have previously employed through apex derivatization to provide a supramolecular scaffold with the concave bowl receptor pointed *away* from the surface, enabling CTV to function as a gold surface-bound host molecule. [16] The crown and saddle conformers are comparable in energy and interconvert in solution.[17]

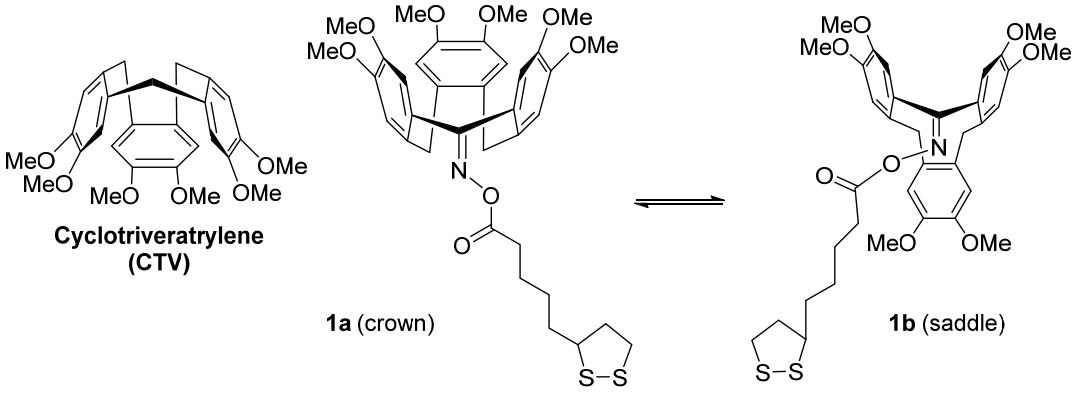



**Fig. 1.** Apex-modified dithiol CTV-oxime

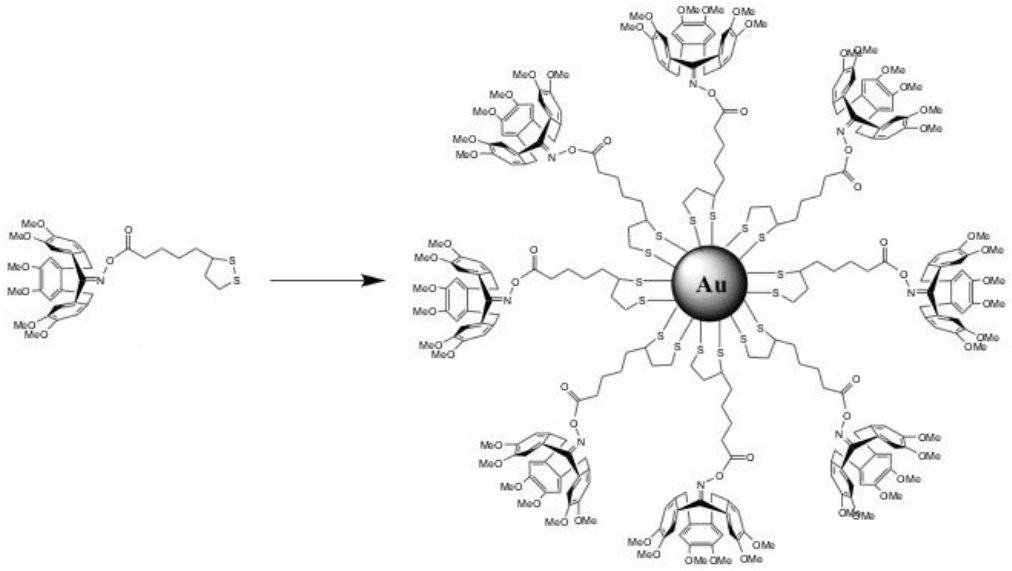
CTV derivatives have been shown to bind both divalent and trivalent metal ions with their peripheral ortho di-methoxy ether groups creating coordination polymers as well as

clatharate crystalline materials. [18-21] In a study conducted by Raston *et al*. [22] X-ray crystal structures were obtained of CTV bound to alkali-earth metal cations ($Na^+$, $K^+$, $Rb^+$, and $Cs^+$) forming large crystalline coordinate polymers through binding the methoxy group of CTV. Hardie *et al*. [14] revealed that lanthanide metal ions can also bind to CTV-methoxy groups. X-ray crystallographic data on CTV-lanthanide complexes indicated that the water molecules of the capped triangular dodecahedral $[Eu(H_2O)_9]^{3+}$ cations are at distances favorable for hydrogen bond formation with the CTV perimeter methoxy lone-pairs. Combining these technologies, we designed a colorimetric analytical device for the detection of di- and trivalent metal ions employing CTV bound to gold nanoparticles (AuNP). An apex-modified CTV supramolecular scaffold head group with a dithiolane tail for binding to a gold surface with the CTV bowl shaped cavity directed *away* from the surface was synthesized and bound to AuNPs. Unlike traditional organic fluorescent dyes, AuNPs have much stronger molar absorptivities that are 3 -5 orders of magnitude higher than organic fluorescent dyes. [23; 24] The optical properties of AuNPs are due to their unique surface plasmon resonance (SPR) where electrons on the surface of an AuNP are in collective oscillation and become in resonance with incident electro-magnetic radiation. [25; 26] Modified AuNPs make excellent colorimetric analytical platforms because NP aggregation due to analyte detection results in a distinct color change from red to blue. [27-34] CTV-functionalized AuNPs provide the basis for the design of an analytical tool that can be used for the detection of di- and trivalent metal ions in solution. Herein we report an analytical spectroscopic technique provides a new method for the use of host-guest interactions for the detection of environmentally toxic metal ions.

**Result and discussion**

With the successful design and synthesis of an apex modified CTV supramolecular scaffold head group with a dithiolane tail, 15 nm AuNPs were functionalized by the addition of a 2.7 mM solution of 1a/b in a 1% polysorbate 20 acetonitrile (ACN) solution (Scheme 1). [27] These samples were then vortexed for 20s to ensure homogeneity throughout the solution. The AuNP/CTV-dithiolane solution was allowed to sit at room temperature for ~24 hours to allow full functionalization of the AuNPs. In order to remove the excess CTV-dithiolane, the AuNPs were centrifuged at 14,000 rpm for ~30 min. The dense CTV-dithiolane functionalized AuNPs precipitated and the excess CTV-dithiolane remained in solution. The supernatant was carefully

removed and the functionalized AuNPs were resuspended in a 1 % polysorbate 20/ACN solution. This process was repeated three times to ensure complete removal of any excess CTV-dithiolane from the AuNP solution. Transmission electron microscopy (TEM) was used to confirm that the AuNPs were intact after functionalization with the CTV-dithiolate ligand (Figure 2). TEM images reveal that the CTV-functionalized AuNPs retain their roughly spherical structure. Due to the differences in the refractive indices between the water and acetonitrile solvents, the UV-Vis spectra were red shifted upon modification CTV-AuNPs in ACN. The shift has shifted SPR of the AuNP solution had shifted from 522 nm in water to the expected 550 nm in ACN solution (Figure 3). [35] The decrease in absorbance is due to the loss of nanoparticles from the washing and extracting of solvent to remove excess CTV after modification.




**Scheme 1.** Route to functionalizing the AuNPs with the CTV-dithiolate ligand.

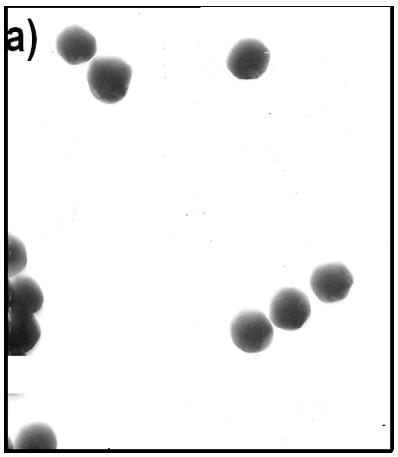



**Fig. 2.** TEM images a) unfunctionalized and b) of the CTV-dithiolate functionalized AuNPs show that after modification, the AuNPs retain their spherical shape and show that the CTV-lipoate AuNPs do not polymerize without the addition of metal cations.

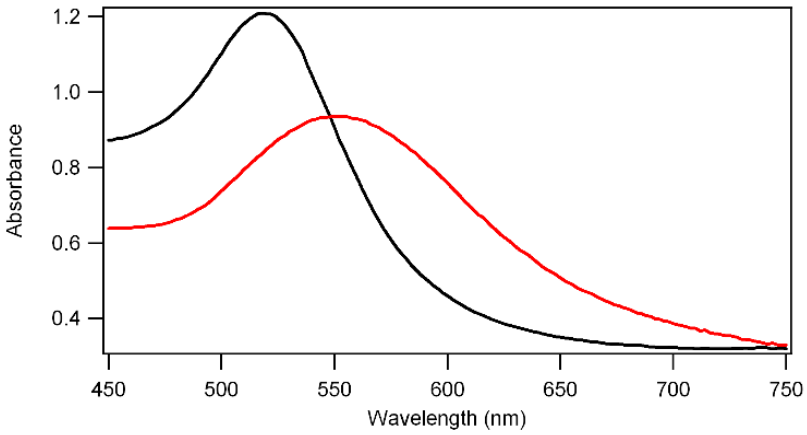
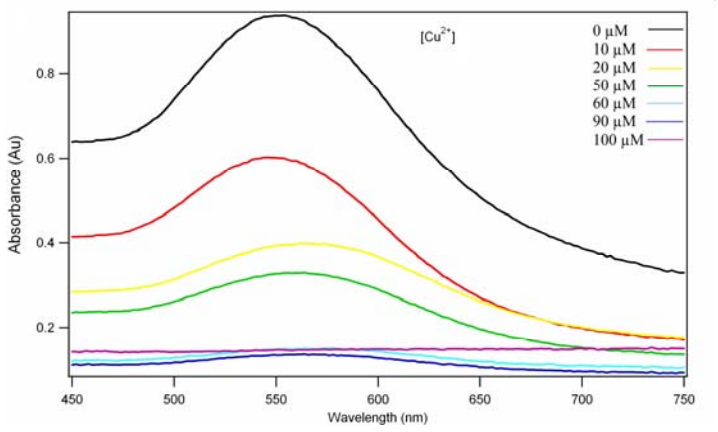



**Fig. 3.** UV-vis spectra of the unmodified nanoparticles purchased from Ted Pella (black) versus the CTV-lipoate modified AuNPs in 1% polysorbate 20/ACN (red) showing that modification of the AuNPs with the CTV-lipoate moiety and the subsequent solvent change results in a decrease and shift in absorbance from 522 nm to 550 nm.

With the successful functionalization of stable CTV-dithiolate 15 nm AuNPs, a series of colorless di- and trivalent metal ions ($Cu^{2+}$, $Pb^{2+}$, $Hg^{2+}$, $Zn^{2+}$, $Cd^{2+}$, and $Eu^{3+}$) were titrated into a solution of CTV-ditholate modified AuNPs in polysorbate 20/ACN. Various concentrations of each metal ion (0 µM, 10 µM, 20 µM, 50 µM, 60 µM, 90 µM, and 100 µM) in nanopure water were pipetted into the CTV-dithiolate AuNP solution. The solution was incubated at room temperature (~21 ºC) in order to allow for complexation between CTV-methoxy moieties and the divalent and trivalent metal cations, which resulted in the formation of a purple precipitate. The system was responsive to varying metal ion concentrations as evidenced by recording the UV-Vis spectrum of the supernatant between 450 – 750 nm. Typical UV-Vis spectra are shown in Figures 4a-c. The initial absorbance of the CTV-dithiolate functionalized 15 nm AuNPs which was monitored at 550 nm decreases accompanied by broadening as the metal ion concentration is increased from 10 to 100 µM. A decrease in absorbance at 550 nm and the observed precipitate are consistent with metal cation binding to CTV methoxy groups in an intra-NP fashion resulting in the formation of a metal ion assisted polymerization of the CTV-dithiolate modified AuNPs. Evidence of the formation of a metal ion-CTV-AuNP polymerization was obtained from TEM images (Figure 5).



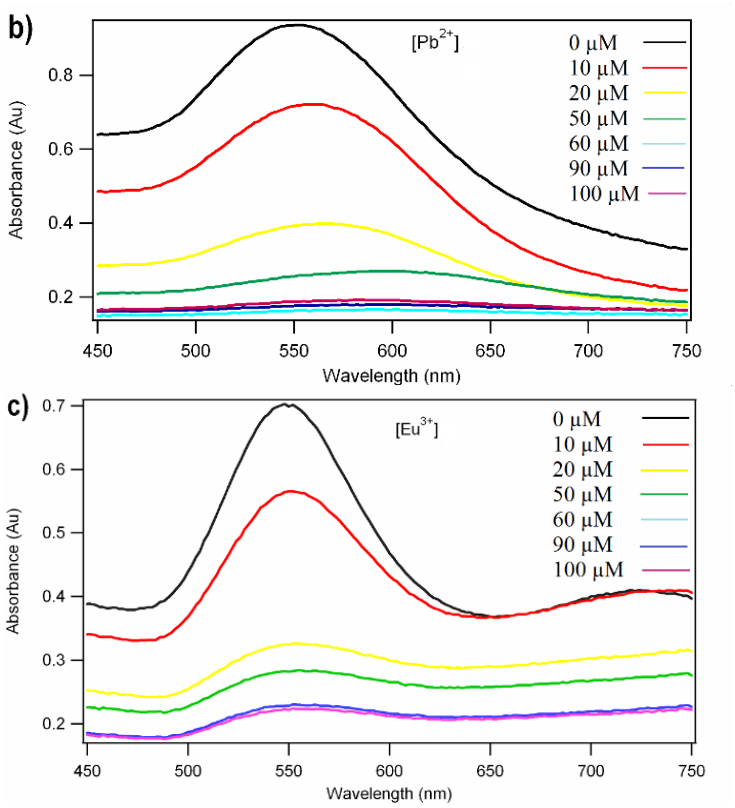



**Fig. 4.** Typical spectroscopic metal binding titrations of increasing a) [$Cu^{2+}$], b) [$Pb^{2+}$], and c) [$Eu^{3+}$] binding to 15 nm CTV-dithiolate functionalized AuNPs resulting in a decrease in absorbance after 24 hours.

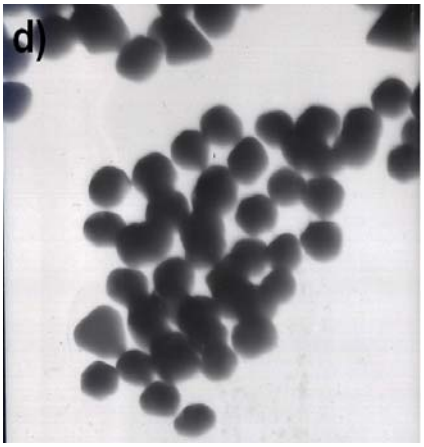



**Fig. 5.** TEM image of the observed CTV-lipoate AuNP precipitate upon metal complexation.

Metal cation affinities to the methoxy moieties of the CTV-dithiolate functionalized AuNPs were determined by titrating metal ions into a CTV-dithiolate functionalized AuNP solution and monitoring the decrease in absorbance at 550 nm (Figure 5a-c). Dissociation constants ($K_d$) for a single metal binding event were determined by fitting plots of absorbance vs. metal ion concentration to eq 1 [36]

$$r = pC_m/(K_d + C_m) \quad (1)$$

where $p$ is the number of sites for which the interaction with the metal cation is governed by the intrinsic dissociation constant, $K_d$, and $r$ is the binding function calculated by subtracting the metal titration absorbance, $Ab_m$, at 550 nm from the initial absorbance, $Ab_i$ using eq 2. $C_m$ is the concentration of the metal ion that was titrated into the AuNP solution.

$$r = Ab_i - Ab_M \quad (2)$$

A value for $K_d$ was obtained by fitting the data via an iterative process that allowed both $K_d$ and $p$ values to vary (Figure 6a-c). The best fits obtained provided $p$ values that ranged from 0.3 - 1.4 and $K_d$ values that range from 13 μM ± 1 for $Cd^{2+}$ to 60 μM ± 1 for $Cu^{2+}$ (Table 1). Based on these $K_d$ values, a binding affinity series was created represented by the following order: $Cu^{2+}$>$Zn^{2+}$>$Pb^{2+}$>$Hg^{2+}$>$Eu^{3+}$>$Cd^{2+}$.

**Fig. 6.** Plot of binding function $r$ vs $C_m$ (the concentration of the metal ions in the solution) for a) $Cu^{2+}$, b) $Pb^{2+}$, and c) $Eu^{3+}$ titrated into CTV-dithiolate AuNPs.

| Metal | $p$ | $K_d$ (μM) | $\sigma$ (±) | $\chi^2$ |
|---|---|---|---|---|
| $Pb^{2+}$ | 1.0 | 49 | 2 | 0.020 |
| $Cd^{2+}$ | 1.4 | 13 | 1 | 0.069 |
| $Zn^{2+}$ | 0.5 | 51 | 3 | 0.014 |
| $Cu^{2+}$ | 0.9 | 60 | 1 | 0.013 |
| $Hg^{2+}$ | 0.3 | 34 | 1 | 0.011 |
| $Eu^{3+}$ | 0.8 | 29 | 2 | 0.033 |

**Table 1.** Metal binding affinity data for varying metals titrated into CTV-lipoate AuNPs

Since the divalent metal ions used can adopt four, five, or six coordinate geometries in solution whereas $Eu^{3+}$ can exhibit coordination geometries of 8 or higher, we hypothesized that the metal ion's coordination sphere is made up of CTV-methoxy oxygens and water molecules. [37] Evidence for the existence of such a structure can be suggested from X-ray crystallographic data of a $Na^+$ bound CTV complex. [12] In this structure, each $Na^+$ ion coordinates to symmetry-equivalent CTV molecules resulting in the $Na^+$ cation residing in a highly distorted octahedral geometry where each CTV molecule chelates edge-bound to $Na^+$ through one dimethoxy moiety and *cis* water/hydroxy ligands. Likewise, the X-ray crystal structures of the $Cs^+$ and $Rb^+$ CTV complexes revealed that both of the larger monovalent cations bind to the dimethoxy moieties of CTV and two *cis* water/hydroxyl ligands forming a highly distorted six-coordinate complex in a similar fashion to $Na^+$. [22] Interestingly, the X-ray structure of the $Eu^{3+}$ CTV complex indicates that the methoxy moieties act as hydrogen bond acceptors with the aquo ligands of the $[Eu(H_2O)_9]^{3+}$ cations forming hydrogen-bonded superstructures. [14] The $[Eu(H_2O)_9]^{3+}$ cations reside in two distinct environments but both retain their water ligands and do not form direct bonding interactions with the methoxy moieties of CTV. We hypothesize that the CTV-functionalized AuNPs may form similar clusters.

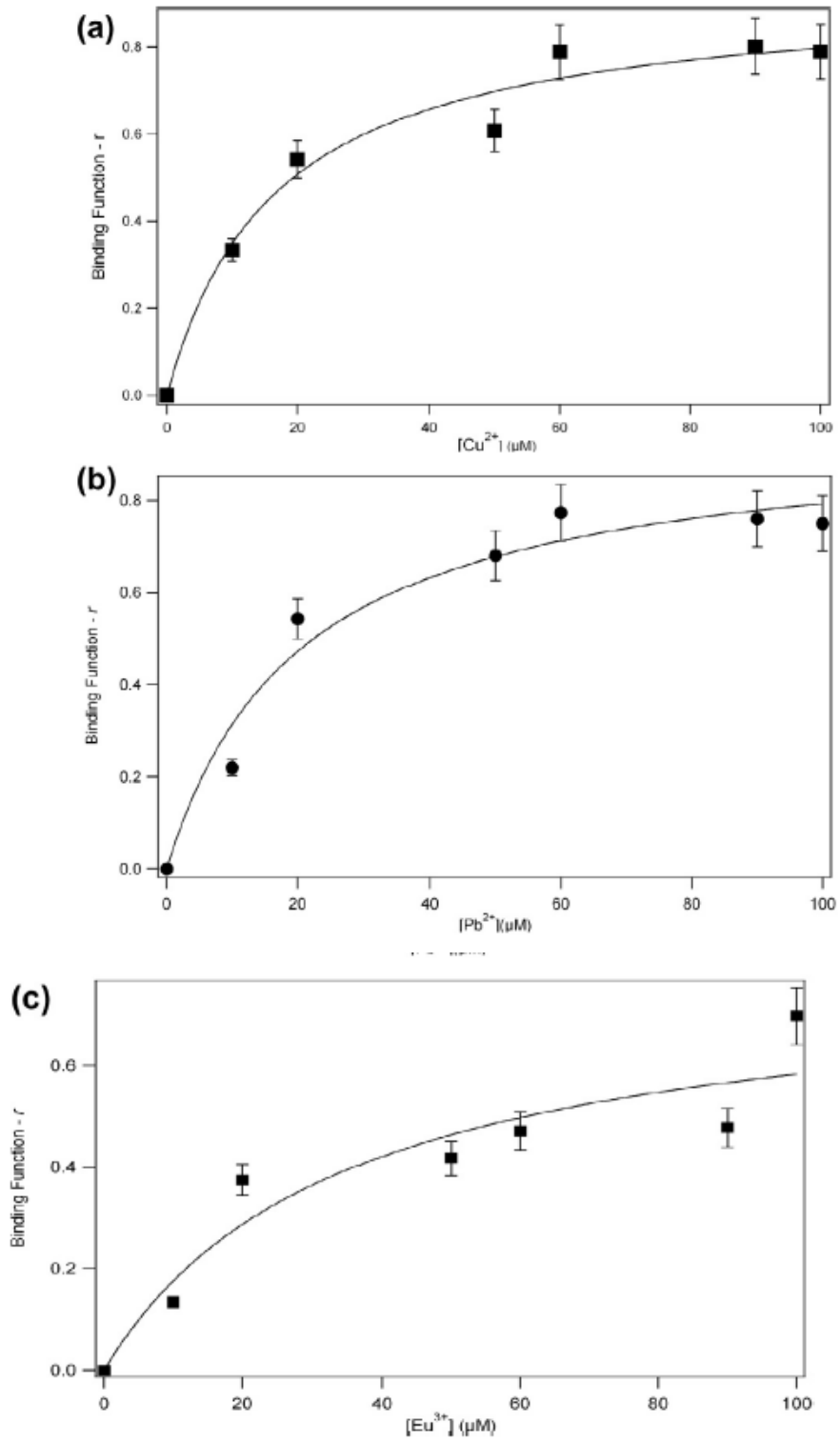



**Figure 6.** Plot of binding function r versus Cm (the concentration of the metal ions in the solution) for (a) Cu2+, (b) Pb2+, and (c) Eu3+ titrated with CTV-dithiolate AuNPs.

The binding mode observed for $Eu^{3+}$, while different from that observed for the alkali metal ions, is likely the result of the more Lewis acidic $Eu^{3+}$ ion vs. the $M^{2+}$ cations examined. Since methoxy moieties are not as strong a Lewis base as water molecules, the highly Lewis acidic $Eu^{3+}$ cation should preferentially bind to water over CTV methoxy groups. [38] Therefore, one would expect the $K_d$ value of 29 ± 2 μM observed for $Eu^{3+}$ results from $[Eu(H_2O)_9]^{3+}$ cation's hydrogen-bonding with the methoxy moieties of CTV, similar to the reported CTV-$Eu^{3+}$ X-ray crystal structure. For the remaining divalent transition metal complexes capable of forming octahedral complexes in solution ($Cd^{2+}$, $Pb^{2+}$, $Cu^{2+}$), all three are soft metal acids and exhibit $p$ values of ~1. The $K_d$ values for these three complexes increase with increasing hardness of these soft metal acids, suggesting that the softer the metal ion the better affinity for a methoxy ligand. Therefore, it was hypothesized that these three metal ions will be chelated by some combination of CTV-methoxy groups and water molecules (Figure 7), similar to the alkali metal cations. Likewise, the soft metal acids $Hg^{2+}$ and $Zn^{2+}$ likely bind to the CTV-methoxy groups and water molecules in some combination, similar to the alkali metal cations, since the softer $Hg^{2+}$ ion binds more tightly (34 ± 1nM) than $Zn^{2+}$ (51 ± 3 μM). However, the observed $p$ values for these two divalent metal ions are 0.3 and 0.5, respectively, indicating a difference in the type of polymeric structure formed upon binding. Given $Hg^{2+}$ and $Zn^{2+}$ preference for tetrahedral geometries, an altered superstructure is not unexpected.

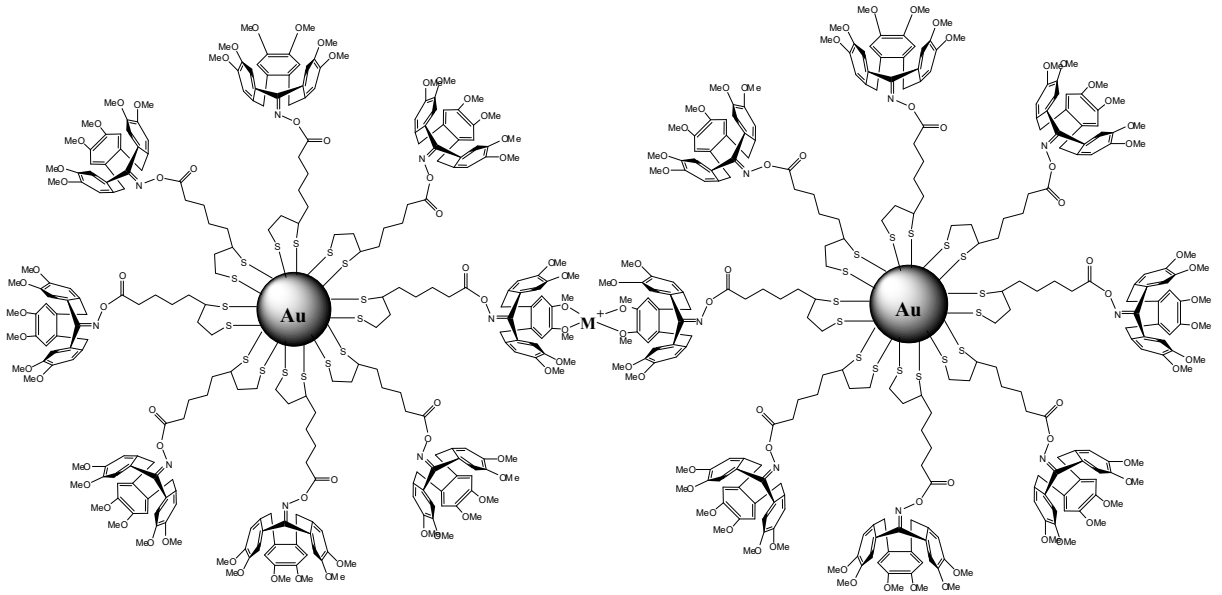


**Fig. 7.** Proposed binding interaction between a metal cation and the CTV-methoxy ligand in solution, forming insoluble AuNP based polymer aggregates.

In summary, an apex-modified CTV derivative containing a dithiolane tail was used to functionalize 15 nm AuNPs to afford a colorimetric analytical method to determine the metal binding of a series of di- and trivalent metal ions in solution. While different devices allow for the detection of metal ions in solution, no determination of metal ion binding constants was reported. The CTV-AuNP method that we report herein offers an advantage as being able to both detect and analytically quantify metal ion concentrations in the solution. This spectroscopic method provides a prototype for a new method to analyze metal cations in solution.

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