PEGylated N-Heterocyclic Carbene Anchors Designed To Stabilize Gold Nanoparticles in Biologically Relevant Media

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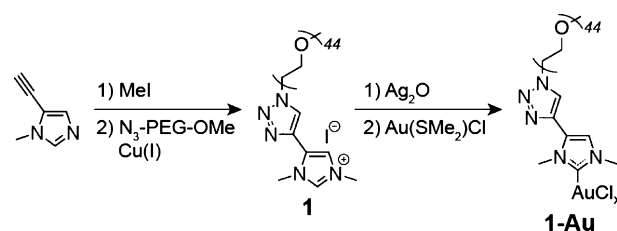
S *Supporting Information*

ABSTRACT: *N*-Heterocyclic carbenes (NHCs) have emerged as versatile ligands for surface functionalization. Their ease of synthesis and ability to form strong bonds with a range of substrates provide a unique complement to traditional surface modification methods. Gold nanoparticles (NPs) are a particularly useful class of materials whose applications intimately depend on surface functionalization. Here we report the development of PEGylated-NHC ligands for Au-NP surfaces and the first example of NHC-functionalized NPs that are compatible with biologically relevant conditions. Our PEGylated-NHC-Au-NPs are stable toward aggregation in aqueous solutions in the pH range of 3−14, in <250 mM electrolyte solutions, at high and low temperatures (95 and −78 °C), in cell culture media, and in aqueous H_2O_2 solutions. This work demonstrates for the first time that NHCs can serve as anchors for water-soluble Au-NPs and opens the door to potential biomedical applications of NHC surface anchors.

Thanks to their unique optical properties, high surface area, and biocompatibility, gold nanoparticles (Au-NPs) are versatile scaffolds for various biomedical applications including imaging, sensing, drug/gene delivery, and photothermal therapy.[1] These applications expose the NPs to variable pH, temperature, redox potential, and chemical diversity; surface ligands are required to ensure solubility and stability under such diverse conditions.[2] Though thiols are the standard ligands for covalent modification of Au-NP surfaces,[3,4] expanding the toolbox of functional surface anchors will provide unique opportunities for the construction of novel Au-NP interfaces.

N-Heterocyclic carbenes (NHCs) are versatile ligands for the functionalization of metallic NP and planar surfaces.[5] Some of the earliest reports on metal-NHC surface chemistry involved Au-NPs;[5a,b] in those cases only alkyl-substituted NHCs were used. In 2013, we demonstrated the first examples of stable, functional NHC monolayers on planar Au surfaces.[5f] In 2014, Crudden and Horton showed that planar NHC-Au monolayers could outperform thiols when exposed to various conditions such as high temperatures and dilute H_2O_2 solutions.[5j]

Based on these precedents, we reasoned that hydrophilic NHCs could potentially serve as stable anchors for Au-NPs in biomedical applications. However, to our knowledge, there is only one report on water-soluble NHC ligands for NP surfaces: Chaudret et al. described the synthesis of Pt-NPs coated with anionic sulfonate-NHCs.[6] In that system, flocculation was inhibited by ionic repulsion; the particles were used for Pt-surface-catalyzed reactions in aqueous media. Though excellent for heterogeneous catalysis, the use of small NHC ligands and ionic repulsion for colloidal stabilization may not be optimal for other applications.
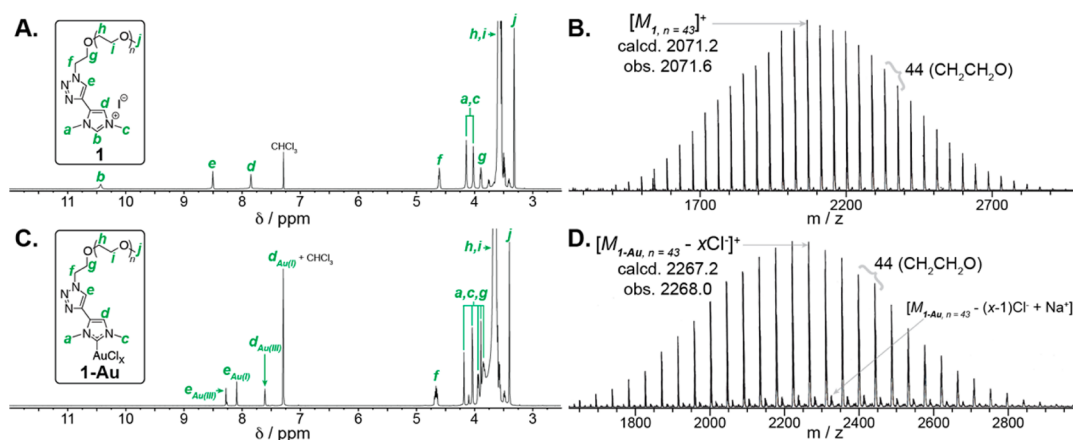
In an effort to develop charge-neutral, bulkier hydrophilic NHCs for steric stabilization of Au-NPs, we considered NHC-poly(ethylene glycol) (PEG) conjugates. PEG is a non-ionic polymer commonly used to impart biocompatibility and stealth to NP surfaces for biomedical applications.[1d,2d,3c] In the context of organometallic chemistry, PEGylated NHCs have been used to generate water-soluble metal complexes for catalysis in aqueous environments.[7] Here we report a novel PEGylated NHC precursor (1, Scheme 1) that was designed specifically for surface functionalization applications (*vide infra*). PEGylated NHC-Au-NPs prepared from 1 are stable under a range of aqueous conditions, which significantly expands the scope of NHC-Au surface chemistry.

Methylation of commercially available 5-ethynyl-1-methyl-1*H*-imidazole, followed by copper-catalyzed azide−alkyne cyclo-addition with 2 kDa N_3-PEG-OMe, provided PEGylated imidazolium salt 1 in 60% isolated yield after preparatory HPLC purification (Scheme 1; see Supporting Information for synthetic details). ^{1}H NMR spectroscopy (Figure 1A), matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (Figure 1B), and elemental analysis for 1 were consistent with the structure shown in Scheme 1.

Next, we sought to prepare complex 1-Au (Scheme 1), which we hypothesized could serve as a NP precursor. The *N*-methyl substituents of 1, and the attachment of PEG to the carbon backbone of the imidazolium ring, were chosen to limit steric

Scheme 1. Synthesis of PEGylated NHC Precursor 1 and Complex 1-Au[a]

[a]A mixture of Au(I) and Au(III) species with x = 1 or 3, respectively.

Figure 1. (A) ^{1}H NMR spectrum of PEGylated imidazolium salt **1**. (B) MALDI spectrum of **1**. (C) ^{1}H NMR spectrum of gold complex **1-Au**, obtained as a mixture of Au(I) and Au(III) complexes. Resonances from 7 to 8.5 ppm are assigned accordingly. Resonances from 3 to 5 ppm are not assigned to individual Au oxidation states due to peak overlap. (D) MALDI spectrum of **1-Au**.

interactions at the NP interface and thus facilitate the formation of a dense NHC layer.[4c,8] However, NHCs with small *N*-substituents tend to dimerize upon exposure of the carbene.[9] Thus, we employed transmetalation from the **1**-Ag complex to generate **1-Au**. Exposure of **1** to 2.5 equiv of Ag$_2$O followed by 2.5 equiv of Au(SMe$_2$)Cl gave complete conversion of **1** to a mixture of **1-Au(I)** and **1-Au(III)** complexes ("**1-Au**") in a 1:0.9 ratio, respectively, as shown by the two sets of triazole and imidazol-2-ylidene resonances in the ^{1}H NMR spectrum of the product mixture (Figure 1C). The MALDI spectrum of **1-Au** (Figure 1D) features a major distribution that corresponds to the calculated mass of **1-Au** minus the chloride ligand(s). A minor distribution corresponding to the mass of **1-Au** with one chloride ligand plus a sodium ion is also observed. Elemental analysis and gel permeation chromatography (Figure S1) further confirmed the identity of **1-Au**.

Our attempts to synthesize **1-Au** as a pure Au(I) complex failed. The use of 1 equiv of Ag$_2$O and Au(SMe$_2$)Cl gave incomplete conversion of **1**, which could not be separated from the desired product. Thus, excess Ag$_2$O and Au(SMe$_2$)Cl were required, which led to product disproportionation.[10] Our assignment of **1-Au** as a mixture of oxidation states is based on comparison of the ^{1}H and ^{13}C NMR spectra to related Au complexes[11] and the observation of Au(III) by cyclic voltammetry[12] (Figure S2; see Tables S1–S3 for comparisons between **1-Au** and representative NHC-Au complexes from the literature). We were, however, able to isolate triethylene glycol (TEG) derivative **1′-Au** (Figure 2) as a pure, mononuclear Au(I) chloride complex, allowing us to assign the ^{1}H NMR spectrum of **1-Au** by analogy. The single-crystal X-ray structure of **1′-Au** (Figure 2) revealed a carbene C–Au bond of 1.996(2) Å, typical for Au(I)-imidazolylidene complexes (Table S1).[13] Interestingly, the imidazolylidene and triazole rings of **1′-Au** are coplanar and oriented face-to-face in the solid state, suggesting the presence of π–π stacking interactions between adjacent **1′-Au** molecules (Figure 2, right). Furthermore, the TEG groups of adjacent **1′-Au** molecules are aligned in a lamellar morphology. Such intermolecular interactions, particularly between PEG chains of adjacent NHCs, could possibly play a role in surface stabilization in the same way that van der Waals interactions between alkyl groups of adjacent ligands are thought to stabilize thiol-Au[14] and alkyl-NHC-Au-NP interfaces.[5k]

We next turned to the synthesis of Au-NPs from **1-Au**. Exposure of **1-Au** to 2.0 equiv of *tert*-butyl amine borane complex
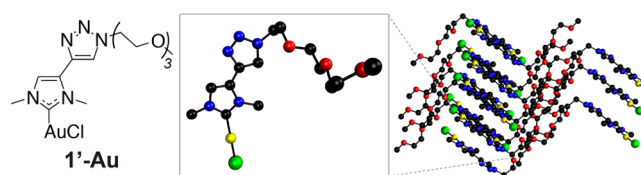

Figure 2. Schematic and single-crystal X-ray structures of **1′-Au**: black = C, blue = N, red = O, green = Cl, yellow = Au.

in tetrahydrofuran for 24 h followed by dialysis against water for 24 h (3500 MWCO) provided PEGylated-NHC-stabilized NPs **1-Au-NP** (Figure 3A).[5g,15] Transmission electron microscopy (TEM) images of films obtained via drop-casting an aqueous solution of **1-Au-NP** and drying revealed the presence of non-agglomerated, spherical Au-NPs with an average diameter (D_{TEM}) of 4.2 ± 0.7 nm (Figure 3B, inset). D_{TEM} corresponds to the diameter of the Au core within **1-Au-NP**. Given that the average end-to-end distance of a 2 kDa PEG chain in the mushroom or brush conformation is ~5.2 nm or ~8.3 nm, respectively, we would predict a core+shell diameter for **1-Au-NP** of ~14.6–20.8 nm. Dynamic light scattering (DLS) analysis of an aqueous solution of **1-Au-NP** (~1 mg/mL) revealed an average hydrodynamic diameter (D_{h}) of 16.2 ± 0.1 nm (Figure 3B). Thus, the TEM and DLS data provide strong evidence for the proposed core–shell structure of **1-Au-NP**. Furthermore, the small zeta potential for **1-Au-NP** (−0.027 ± 0.002 mV) is indicative of a neutral PEG shell. Lastly, thermogravimetric analysis suggested the presence of ~730 ligands per NP (Figure S3).

Spectroscopic data provide further support for the structure of **1-Au-NP**. The attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra of salt **1** and **1-Au-NP** are very similar, confirming the presence of the PEGylated ligand in **1-Au-NP** (Figure 3C). Despite this similarity, a key imidazolium vibrational band present at ~1576 cm^{-1} in **1** is not present in **1-Au-NP** (Figures 3C and S4), which strongly suggests that the NHC is coordinated to the Au surface.[16] The ^{1}H NMR spectrum of **1-Au-NP** in D$_2$O solvent shows a single set of triazole and imidazolylidene resonances (Figure 3D). Finally, the unique surface plasmon band (SPB) in the UV/vis spectrum of Au-NPs was observed for **1-Au-NP** (Figure 3E).

We next studied the stability of **1-Au-NPs** toward aggregation and decomposition in aqueous solutions under various
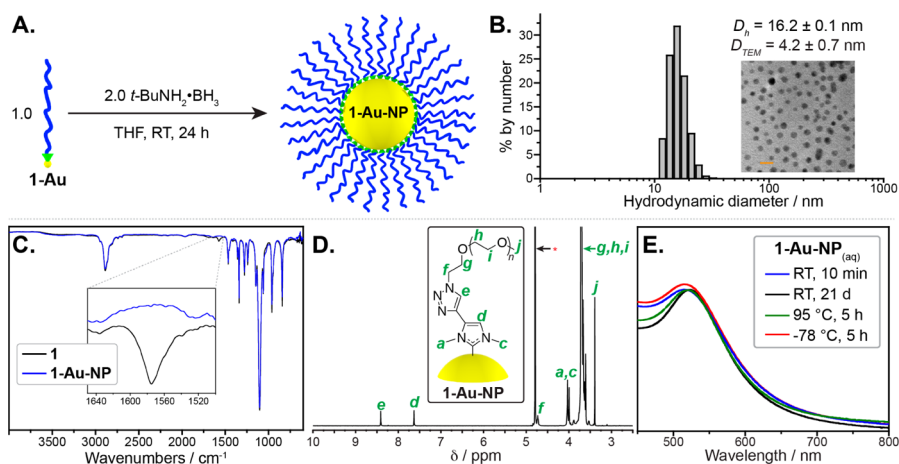
Figure 3. (A) Synthesis of PEGylated-NHC Au-NPs (**1-Au-NP**) via reduction of complex **1-Au**. (B) DLS histogram for aqueous solution of **1-Au-NP** (1 mg/mL). D_h is the average hydrodynamic diameter. Inset: TEM image of **1-Au-NP**. Scale bar = 10 nm. D_{TEM} is the average particle size as measured by TEM image analysis. (C) ATR-FTIR spectra for imidazolium salt **1** and **1-Au-NP**. Inset: Peak corresponding to the imidazolium heterocycle before and after coordination to Au. (D) ^{1}H NMR spectrum of **1-Au-NP** (RT, D_2O solvent, 400 MHz). * = residual solvent peak. (E) UV/vis spectra showing the surface plasmon band for **1-Au-NP**$_{(aq)}$ after exposure to various conditions.
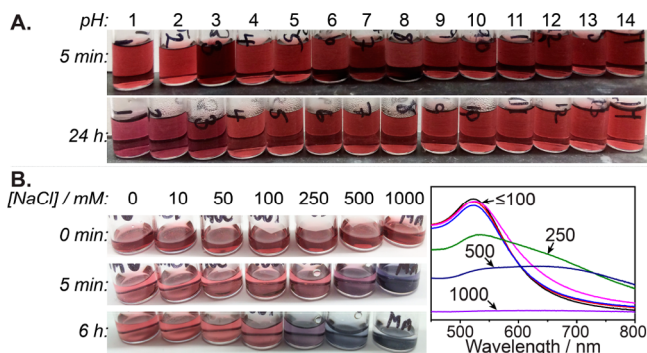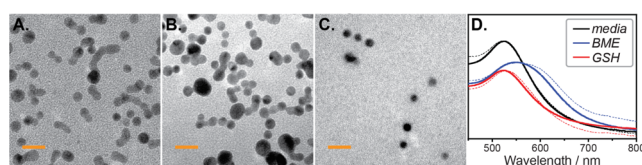

Figure 4. (A) Images of aqueous solutions of **1-Au-NPs** at varied pH. (B) UV/vis spectra showing the SPBs for **1-Au-NPs** exposed to NaCl$_{(aq)}$ for 6 h. Labels correspond to [NaCl] in mM.

Figure 5. TEM images of **1-Au-NPs** after 21 d exposure to (A) phosphate buffer, pH 9.0, (B) PBS buffer, pH 7.4, and (C) acetic acid buffer, pH 2.4. Scale bars = 20 nm. (D) UV/vis spectra showing SPBs for **1-Au-NPs** exposed to various conditions: "media" = cell culture media with fetal bovine serum for 5 min (—) and 6 h (···) at 37 °C, "BME" = 15.8 mM 2-mercaptoethanol for 5 min (—) and 3 h (···) at RT, "GSH" = 2 mM glutathione for 5 min (—) and 3 h (···).

conditions. The SPB is a convenient diagnostic handle for analysis of NP stability; a red shift, broadening, or loss of the SPB is indicative of NP aggregation.[17] **1-Au-NPs** were stable in water under ambient atmosphere for at least 3 months. Furthermore, the NPs were stable in aqueous solutions exposed to high and low temperatures (95 and −78 °C) for 5 h (Figure 3E).

We assessed the stability of **1-Au-NPs** in aqueous solutions with pH's from 1 to 14. Figure 4A shows images of these solutions 5 min and 24 h after preparation. After 1 d, all of the particles remained well dispersed, and there were only minor visible changes in the solution color for the most acidic solutions. After 2 d, all of the NPs were still homogeneously dispersed, except for the particles exposed to pH 1, where the NPs began to aggregate slightly on the vial sidewalls. After 8 weeks, the samples at pH 1 and 2 began to show significant reduction in the SPB and adhesion to the vial sidewalls; the sample at pH 3 showed a small decrease and red shift in the SPB (Figure S5). All samples at pH >3 remained essentially unchanged, which demonstrates that these PEGylated-NHC-coated Au-NPs are stable to a wide pH range.

Colloidal suspensions often undergo electrolyte-induced aggregation.[4b,18] We exposed **1-Au-NPs** to NaCl solutions of varied salt concentrations (Figure 4B). As can be seen both visually and in the UV/vis absorption spectra, the NPs began to aggregate within ~5 min at the highest NaCl concentrations

(>250 mM). After 6 h, particles in 1000 mM NaCl solution had fully sedimented. It should be noted that 2 kDa PEG-SH was reported to stabilize 12 nm Au-NPs in 1000 mM NaCl aqueous solution.[4c] The difference in stability toward NaCl between **1-Au-NPs** and thiolate-stabilized Au NPs could be due to the greater steric bulk of the NHC ligand. However, direct comparison is difficult since the Au core of **1-Au-NPs** is much smaller than 12 nm; smaller Au-NPs generally have more vacant sites and surface defects.[8b,19] Nevertheless, though it may be possible to improve the kinetic stability of NHC-Au-NPs in high [NaCl] solutions through the use of longer PEG chains or alternative NHC ligands, our first-generation NHC-Au-NPs presented here were stable for 6 h in <250 mM NaCl solutions, which are most relevant for biological applications.

When **1-Au-NPs** were exposed to acetic acid buffer at pH 3.2, PBS buffer at pH 7.4 (137 mM NaCl and 2.7 mM KCl), and phosphate buffer at pH 9.1 for 3 d, the NPs remained well-dispersed, but shifts in the UV/vis spectra suggested some nanoscale aggregation, which is known to occur in other Au-NP/electrolyte systems (Figure S6).[1c,2c] TEM imaging after 21 d in these buffers revealed minimal agglomeration (Figure 5A−C). After 7 weeks, the NPs were still homogeneously dispersed, with no signs of sedimentation or film formation on the vial walls.

We tested the stability of **1-Au-NPs** in response to compounds that could be encountered in *in vitro* and/or *in vivo* applications. First, we exposed the NPs to cell culture media with fetal bovine serum for 26 h at room temperature (RT) and 37 °C; there was

no significant change in the SPB (Figure 5D, black curves). Next, we treated **1-Au-NPs** with thiol-based reducing agents 2-mercaptoethanol (BME) and glutathione (GSH) for 3 h. UV/vis (Figure 5D, blue) and ^{1}H NMR (Figures S7 and S8) spectroscopies showed little changes within this time, though the sample exposed to BME showed some immediate broadening of the SBP. After 26 h, the NPs exposed to BME had completely aggregated; the NPs exposed to GSH showed only a slight SPB shift. The ^{1}H NMR spectra for the 26 h BME-treated NPs showed new aromatic resonances suggestive of the formation of NHC-Au complexes (Figure S8).

Exposure of **1-Au-NPs** to excess PEG-SH led to ligand exchange (Figure S9), which was also observed in other NHC-NPs stabilized in organic media.[5k] Again, it appears that **1-Au** complexes are the major product of this exchange (rather than free NHCs), typical for NHC-induced Au etching.[5a,i] The release of NHC-Au complexes in response to thiols, which are present in micromolar concentrations in blood but millimolar concentrations in the cytosol, could offer a novel drug delivery mechanism.[20] Finally, we note that **1-Au-NPs** are stable for up to 24 h in 0.15 and 1.8 M aqueous H_2O_2 (Figure S10).

We have described the first water-soluble Au-NPs stabilized by a PEGylated-NHC ligand. The stability of these Au-NPs under a wide range of conditions suggests that they could find applications in biomedicine. Furthermore, our modular PEGylated NHC design and simple synthesis will serve as a platform for the development of novel PEGylated-NHCs for function optimization. Efforts toward the development of next-generation NHC interfaces are ongoing in our laboratory.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supplementary figures, methods and materials, and characterization data. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b02452.

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Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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