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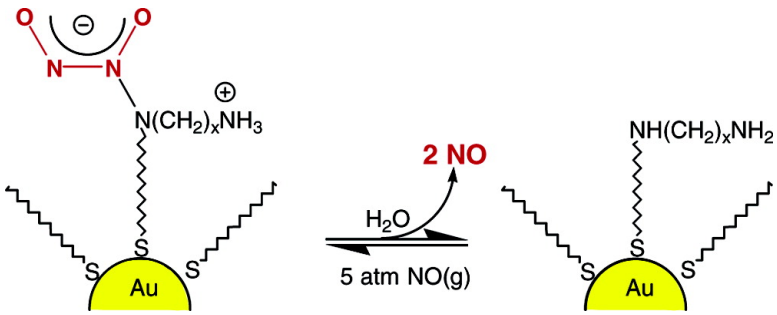

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Synthesis of Nitric Oxide-Releasing Gold Nanoparticles

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The astounding pace of discovery on the bioregulatory roles of nitric oxide (NO)[1] demands new methods for generating NO in a controlled manner to facilitate both an improved understanding of NO's function in physiology and the development of NO-associated therapies.[2] *N*-Diazeniumdiolate NO donors, small molecules synthesized by the reaction of amines with NO at elevated pressure, have proven particularly useful for spontaneously generating NO in aqueous solution.[3] Indeed, the rate of NO generation is easily tuned by varying the amine precursor structure, pH, and/or temperature. As such, polymers have been modified to release NO via doping or covalent attachment of the NO donor whereby low levels of NO release from the polymer interface mimics the endothelium of healthy blood vessels, preventing platelet adhesion/activation.[4,5] Such polymers have been employed successfully in the preparation of more thromboresistant sensors and extracorporeal heart bypass circuits.[6] The synthesis of NO-releasing fumed silica particles (amorphous silicon dioxide, 0.2−0.3 μm) was recently reported as an effective strategy for generating NO from within a polymer film.[7] The advantage of using *N*-diazeniumdiolate-modified fumed silica was the ease with which such particles could be embedded in a given polymer matrix and their ability to serve as both a reinforcing filler and a NO donor.

Monolayer-protected cluster (MPC) gold nanoparticles, or MPCs,[8] have received much attention due to their unique size (1−5 nm), stability, and highly functional design.[9] The exterior of MPCs is easily altered by place-exchanging in other thiols containing the desired functional groups.[10] Such modification has enabled the potential for employing gold nanoparticles as drug delivery vehicles and contrast agents.[9] Herein, we report on the synthesis of gold nanoparticles designed to controllably release NO. The unique functionality of these nanoparticles may represent a new platform for the targeted delivery of NO in vivo.

Gold nanoparticles were synthesized by the reaction of hydrogen tetrachloroaurate salt with hexanethiol in the presence of sodium borohydride.[11] After 30 min, the reaction was quenched with water. The nanoparticles were collected by filtration, washed with acetonitrile, and then functionalized with bromo-terminated alkanethiols by the place-exchange method (Figure 1).[10] Specifically, 11-bromo-1-undecanethiol[12] was added (3:1 ratio of bromo- to methyl-terminated alkanethiol) to a solution of gold nanoparticles in methylene chloride and stirred for 30 min. The solvent was removed by rotary evaporation, and the gold nanoparticles were purified with acetonitrile. The extent of ligand exchange, monitored by NMR, was easily controlled by varying the reaction time and/or concentration of bromoalkanethiol. The bromo-functionalized gold nanoparticles were then dissolved in toluene or methylene chloride and reacted with ethylenediamine, butylamine, hexanediamine, or diethylenetriamine. The disappearance of the $-C\mathbf{H}_2Br$ peak in the NMR spectra of the functionalized nanoparticles indicated the completion of the reaction (Supporting Information). The amine-functionalized gold nanoparticles were then suspended in a solution of methanol and sodium methoxide base, ultrasonic mixed for 1 min, and pressurized to 5 atm NO for 3 d with constant stirring to facilitate the synthesis of diazeniumdiolate NO donors[13] and prevent aggregation. The *N*-diazeniumdiolate-modified MPCs were filtered, washed (methanol), and stored at −4 °C until use.

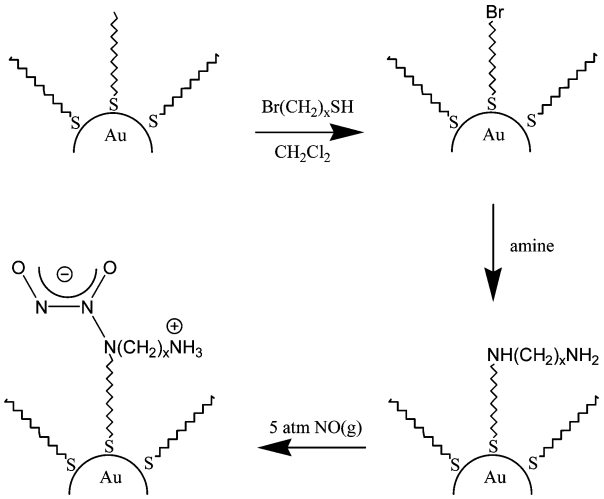

Figure 1. Synthesis scheme for preparing NO-releasing gold nanoparticles.

The size and stability of the gold nanoparticles were characterized using thermal gravimetric analysis (TGA), UV−vis spectroscopy, and transmission electron microscopy (TEM). The organic content of hexanediamine-modified gold nanoparticles was determined to be ∼22%, a value consistent with previous reports for hexanethiol MPCs composed of 140 gold atoms (core) protected by 53 thiol ligands.[11] Since NO is highly reactive and might disrupt gold−sulfur bonds,[3] the stability of the hexanethiol MPCs after exposure to high pressures of NO was evaluated using TGA and UV−vis spectroscopy to ensure that the conditions necessary for diazeniumdiolate formation did not compromise nanoparticle integrity. Both the organic content of the nanoparticles (as studied by TGA) and the UV−vis spectra (in toluene such that the absorbance was 1.0 at 400 nm) did not change following NO exposure, indicating negligible influence on monolayer stability. Transmission electron microscopy images further confirmed that the core diameter of the nanoparticles remained constant (2.1 ± 0.9 nm) regardless of amine derivatization or diazeniumdiolate formation. These studies suggest that the structural integrity of the MPC gold nanoparticles was not compromised by the conditions necessary to synthesize the NO donor and introduce NO-release capability.

Nitric oxide release was measured in phosphate-buffered saline solution at physiological temperature and pH using a Sievers NOA chemiluminescence nitric oxide analyzer (Boulder, CO). A constant stream of nitrogen bubbled through the PBS (200 mL/min) was used to carry the NO to the analyzer. As shown in Figure 2, the NO release for diazeniumdiolate-modified MPCs was tunable by varying the number and/or chemical structure of the substituted amine ligands. Increasing the concentration of ethylenediamine ligand from 14 to 21% led to a corresponding increase in total NO

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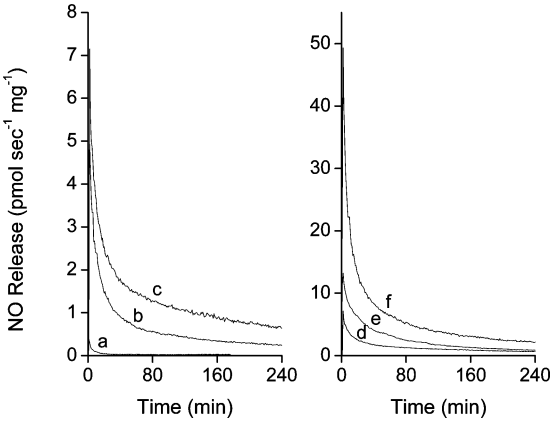

Figure 2. Nitric oxide-release profiles from gold nanoparticles derivatized with (a) 0% ethylenediamine, (b) 14% ethylenediamine, and (c) 21% ethylenediamine (varying the number of ligands), and (d) 21% ethylenediamine, (e) 21% diethylenetriamine, and (f) 21% hexanediamine (varying the structure of ligands). Release profiles were reproducible to within 10%.

Table 1. Nitric Oxide Release Properties of Amine-Derivatized Monolayer-Protected Gold Nanoparticles

ligand	% amine[a]	half-life (min)	release longevity (min)	total NO (pmol/mg)
hexane		2	5	400
butylamine	21	15	60	2 000
ethylenediamine	14	78	200	9 750
ethylenediamine	21	88	300	19 300
hexanediamine	21	68	600	87 000
diethylenetriamine	21	63	360	38 000

release (from 9750 to 19 300 pmol of NO/mg of MPC) and NO release duration (from 200 to 300 min). The elevated NO release is attributed to enhanced NO-donor formation due to a larger concentration of amines. A small amount of NO (400 pmol/mg) was also measured from the hexanethiol MPC controls. This NO release was negligible at periods >5 min, indicating that NO may intercalate within the hydrophobic alkyl chains under the conditions necessary for diazeniumdiolate synthesis (5 atm NO), but the amount of such NO is small and rapidly released upon solution immersion. The diazeniumdiolate-modified MPCs also released low levels of NO under a warm (37 °C) stream of nitrogen gas, suggesting a possible thermal dissociation mechanism. However, the level of NO release was greater in solution (data not shown), indicating that the *N*-diazeniumdiolate-modified nanoparticles undergo both thermal and proton-driven dissociation. The diazeniumdiolate-modified MPCs retained full NO-release characteristics when stored under nitrogen at −4 °C through 14 d.

The NO release from diazeniumdiolate-modified MPCs was also tunable by varying the amine precursor structure. Increasing the length of the alkyl chain separating the nitrogens from two to six methylene units led to an increase in the total amount of NO released (Figure 2d,f) (from 19 300 to 87 000 pmol of NO/mg of MPC for ethylenediamine- and hexanediamine-modified MPCs, respectively), suggesting a NO release/diazeniumdiolate structure relationship. Indeed, the half-life data (Table 1) show that separating the amines results in a more rapid release of NO as well, analogous to the dissociation behavior reported for small-molecule diazeniumdiolates.[13,14] The total amount of NO released from diethylenetriamine-modified MPCs (38 000 pmol of NO/mg) was between that measured for ethylenediamine- and hexanediamine-modified MPCs. The presence of an additional secondary amine in diethylenetriamine likely accounts for increased NO donor formation (and release capability) relative to ethylenediamine, even though the length of the alkyl chain separating the nitrogens remains short (two methylene units). Butylamine-modified MPCs, a secondary monoamine derivative, were characterized by the lowest total NO release of all the amine-modified MPCs studied. Such behavior was expected, however, as diazeniumdiolate formation is facilitated by the additional amine.[13,14] Notably, the diazeniumdiolate conversion efficiency for the amine-modified MPCs was calculated to be <1%, regardless of amine structure. Current efforts are focused on enhancing the conversion of diamines to NO donor to increase the amount and duration of NO release.

The synthesis of ∼2 nm NO-releasing gold nanoparticles represents an important step toward the development of a NO-delivery system that bridges small-molecule diazeniumdiolates and diazeniumdiolate-modified fumed silica particles (∼200 nm). The control over the type and amount of amine used during preparation of the nanoparticles allows for a range of NO-release properties. Further functionalization of the nanoparticles with receptor molecules to enable specific antibody−antigen or ligand−receptor interactions may allow for the targeting of specific tissues or cells. The size and stability of NO-releasing MPC gold nanoparticles may prove useful for a range of biomedical and pharmaceutical applications, including in vivo sensor design and topical creams to enhance wound healing and/or dilate blood vessels below the skin. Future studies will include determining the influence of amine precursor distance from the gold core on diazeniumdiolate formation and dissociation to NO.

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Supporting Information Available: Synthetic procedure for 11-bromo-1-undecanethiol and representative ^{1}H NMR spectra for amine-functionalized gold nanoparticles. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Marletta, M. A.; Tayeh, M. A.; Hevel, J. M. *BioFactors* **1990**, *2*, 219−225.
(2) Keefer, L. K. *Chemtech* **1998**, *28*, 30−35.
(3) Hrabie, J. A.; Keefer, L. K. *Chem. Rev.* **2002**, *102*, 1135−1154.
(4) Radomski, M. W.; Rees, D. D.; Dutra, A.; Moncada, S. *Br. J. Pharmacol.* **1992**, *107*, 745−749.
(5) Diodati, J. G.; Quyyumi, A. A.; Hussain, N.; Keefer, L. K. *Thrombosis Haemostasis* **1993**, *70*, 654−658.
(6) Frost, M. C.; Reynolds, M. M.; Meyerhoff, M. E. *Biomaterials* **2005**, *26*, 1685−1693.
(7) Zhang, H.; Annich Gail, M.; Miskulin, J.; Stankiewicz, K.; Osterholzer, K.; Merz, S. I.; Bartlett, R. H.; Meyerhoff, M. E. *J. Am. Chem. Soc.* **2003**, *125*, 5015−5024.
(8) Brust, M.; Walker, M.; Bethell, D.; Schiffrin, D. J.; Whyman, R. *J. Chem. Soc., Chem. Commun.* **1994**, 801−802.
(9) Feldheim, D. L., Foss, C. A., Eds. *Metal Nanoparticles—Synthesis, Characterization, and Applications*; Marcel Dekker: New York, 2000; p 360.
(10) Hostetler, M. J.; Templeton, A. C.; Murray, R. W. *Langmuir* **1999**, *15*, 3782−3789.
(11) Hostetler, M. J.; Wingate, J. E.; Zhong, C.-J.; Harris, J. E.; Vachet, R. W.; Clark, M. R.; Londono, J. D.; Green, S. J.; Stokes, J. J.; Wignall, G. D.; Glish, G. L.; Porter, M. D.; Evans, N. D.; Murray, R. W. *Langmuir* **1998**, *14*, 17−30.
(12) Troughton, E. B.; Bain, C. D.; Whitesides, G. M.; Nuzzo, R. G.; Allara, D. L.; Porter, M. D. *Langmuir* **1988**, *4*, 365−385.
(13) Hrabie, J. A.; Klose, J. R.; Wink, D. A.; Keefer, L. K. *J. Org. Chem.* **1993**, *58*, 1472−1476.
(14) Davies, K. M.; Wink, D. A.; Saavedra, J. E.; Keefer, L. K. *J. Am. Chem. Soc.* **2001**, *123*, 5473−5481.

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