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Photodelivery of Nitric Oxide from Water-Soluble Platinum Nanoparticles

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Research on compounds able to deliver nitric oxide (NO) has undergone a tremendous growth in recent years,[1] owing to the exciting discoveries related to the multiple roles NO plays in a broad array of physiological processes such as neurotransmission, hormone secretion, and vasodilatation.[2] Furthermore, NO has also recently proven to be an effective anticancer agent[3] as well as a chain breaking antioxidant in free radical-induced lipid oxidation.[4] In this context, dispenser substances releasing NO under light stimuli are more appealing than those based on spontaneous thermolysis. Indeed, the easy manipulation of light associated with the fast response of the photochemical reactions allows a precise spatial and temporal control of NO delivery. Although a number of NO photogenerators have appeared in the literature in recent years,[5] only a few examples have addressed their integration in appropriate materials. NO photorelease in a very small area has been in fact demonstrated in the case of polymer films,[6] hydrogels,[7] and self-assembled monolayers on two-dimensional (2D) gold surfaces.[8]

Thiol-stabilized metal nanoparticles, commonly referred to as monolayer-protected clusters (MPCs), are fascinating hybrid systems with intriguing properties and multifaceted applications in different fields.[9] In particular, the unique sizes, the excellent stability, and the easy modification of their exterior with the introduction of appropriate functional units have generated widespread interest in the use of MPCs in biomedicine spanning from biosensing to drug delivery.[9] Schoenfisch and co-workers have recently reported on the synthesis of NO releasing gold nanoparticles, ca. 2 nm in diameter.[10] On the basis of these properties, the achievement of metal nanoparticles with *photoactivable* NO release is a worthwhile objective to pursue that can represent a significant step forward in the perspective of biomedical applications requiring targeted delivery of NO. Construction of these 3D nanoarchitectures is also expected to offer a 2-fold advantage as compared to the 2D self-assembled monolayers on noble metals. In fact, unlike their bulk counterparts, they would exhibit improved light-harvesting efficiency as well as reduced quenching effects on the excited states of the photoactive units.[11] With this goal in mind, we report herein on the preparation of ultrasmall (ca. 1 nm) and water soluble monolayer-protected platinum nanoparticles MPC-**3** (Figure 1) as delivery vehicles able to supply NO under the control of visible-light stimuli.

Carboxy-terminated Pt nanoparticles, prepared according to our recently proposed photochemical methodology,[12] were used as suited building blocks to obtain MPC-**3**. This facile synthetic protocol allows obtaining in a single step the smallest (ca. 1 nm), water soluble and stable platinum nanoparticles known to date.

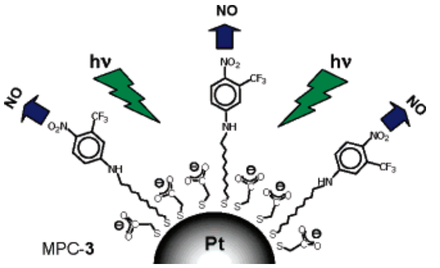

Figure 1. Idealized view of the NO photoreleasing MPC-**3**.

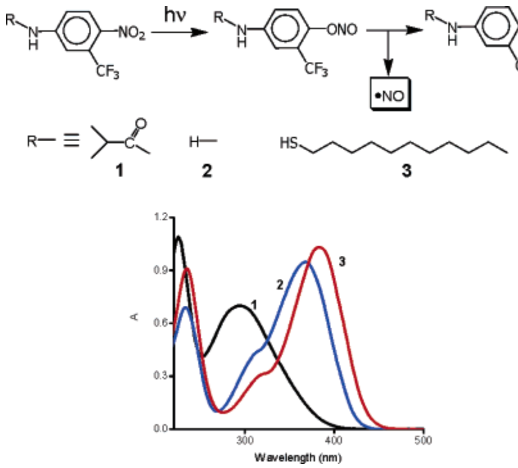

Figure 2. Predicted pathway for the NO photorelease from the nitroderivatives **1**−**3** and their absorption spectra of equimolar (0.1 mM) acetonitrile solutions.

In search of the suitable photoactive NO donor we were inspired by our recent work demonstrating the photorelease of NO by the thiol derivative of the nitro nitroamide **1** (Figure 2) self-assembled on a 2D gold surface.[8b] Our mechanistic studies on compound **1**,[13] demonstrated that the NO photorelease takes place through a pathway similar to that of some recently reported polycyclic nitroaromatics.[5c] In particular, the twisted geometry of the nitro group, induced by the CF$_3$ substituent, is crucial in triggering the NO photorelease accompanied by the concomitant formation of a phenoxyl radical (Figure 2).

With the aim to utilize a photoactive unit with spectroscopic characteristics better than **1**, we assessed the suitability of the commercial model compound **2** as possible NO photodonor. This compound is expected to retain the twisted geometry of the nitro group but, in view of the presence of the amine moiety in place of the amide at the para position, exhibits a desirable improvement over **1** in absorption features. As shown in Figure 2, compound **2** is characterized by a more intense absorption ca. 80 nm shifted toward longer wavelengths as compared to **1**. This represents an enhancement of more than 1 order of magnitude in the average absorption coefficient in the spectral region $>$400 nm, a desirable requisite for biological applications. Visible-light excitation of a phosphate-buffered solution of **2** at pH 7.4 results in a neat release of NO,[14] monitored by luminescent assay (Supporting Information). This finding encouraged us to synthesize the thiol **3** as an

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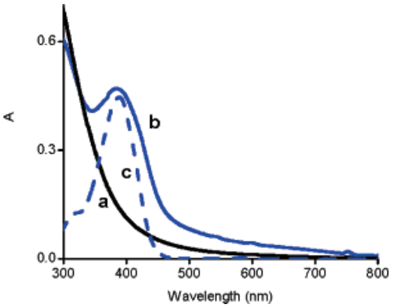

Figure 3. Absorption spectra of the carboxy-terminated Pt nanoparticles in aqueous phosphate buffer solution before (a) and after (b) the exchange reaction with **3**. The spectrum of the free **3** in acetonitrile (c) is also shown, in arbitrary absorbance, for sake of comparison.

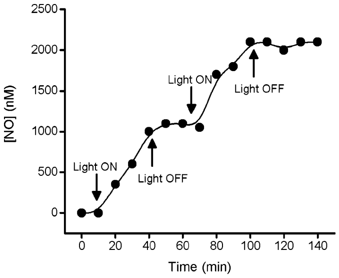

Figure 4. NO photorelease ($\lambda_{\mathrm{exc}} = 420$ nm) from a neutral buffered aqueous solution (pH 7.4) of MPC-**3** (40 μM).

appropriate photoactive agent with self-assembling characteristics. The presence of the long alkyl chain is not expected to influence the photochemical behavior with respect to the model compound **2** (vide infra), but rather, as shown in Figure 2, it is expected to lead to a further improvement of the absorption properties.

The carboxy-terminated metal nanoparticles were then partially modified to obtain MPC-**3** through the phase-exchange method.[9] Specifically, they (in the neutral form) were dissolved in an acetonitrile solution of **3** (0.5 mM) and stirred for 60 min at room temperature. Afterward the solvent was removed by rotary evaporation under vacuum, and the nanoparticles were redispersed in water at pH 7.4. The unbound **3**, totally insoluble in water, was removed through few cycles of redispersion−centrifugation. The extent of the exchange reaction was easily monitored by UV−vis spectroscopy.[15] As displayed in Figure 3, the absorption spectrum of the final aqueous solution of the Pt nanoparticles exhibits the metal plasmon absorption band associated with the typical absorption of **3**. In view of the insolubility of thiol **3** in the water medium, this observation provides unambiguous evidence for its partial incorporation into the carboxylate-terminated Pt nanoparticles.[16] From the absorbance values at 386 nm, the number of chemisorbed species was calculated to be ca. 6/cluster (Supporting Information).

High-resolution transmission electron microscopy (HRTEM) and UV−vis spectroscopy revealed that the exchange procedure does not alter the particles' characteristics, which retained their size and integrity for some days.

NO release from MPC-**3** was measured in buffered solution at neutral pH. Figure 4 shows that MPC-**3** are stable to the thermal release of NO but supply NO upon illumination with visible light. The release process is strictly dependent on the external light inputs as confirmed by the stop of NO production as the light is turned off. A rate of release of ca. 1.5 pmol s^{-1}, corresponding to a quantum yield $\Phi = 1.9 \times 10^{-3}$, can be estimated from the linear portion of the graphic. It is worthy noting that the photolysis profile of MPC-**3** is very similar to that of the model compound **2** under the same experimental conditions (Supporting Information) suggesting that neither the aliphatic chain nor the metal core alter the nature of the photoreactivity of the photoactive unit. A kinetic analysis of the photochemical profiles revealed only 30% quenching in the case of MPC-**3**.

In summary, we have reported the *first example* of NO photodelivery from *ad-hoc* modified metal nanoparticles. These hybrid nanosystems exhibit a combination of indispensable properties for biological applications, such as ultrasmall sizes, water solubility and thermal stability under physiological conditions, photoactivation with low-energy light, photogeneration of nontoxic intermediates.[17] These features make MPC-**3** very appealing *point sources* of NO that, hopefully, are expected to find wide applications in biomedical research where controlled release of small amounts of NO is required.

Acknowledgment. Financial support from University of Catania is greatly appreciated.

Supporting Information Available: Experimental procedures, TEM measurements, calculation of the surface coverage, and photochemical profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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