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## COMMUNICATION

## Selective colorimetric NO (g) detection with modified gold nanoparticles using click chemistry

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**A new colorimetric system for NO (g) detection is described. The detection method is based on the aggregation of modified AuNPs through a Cu(I) catalyzed click reaction promoted by the *in situ* reduction of Cu(II) by NO.**

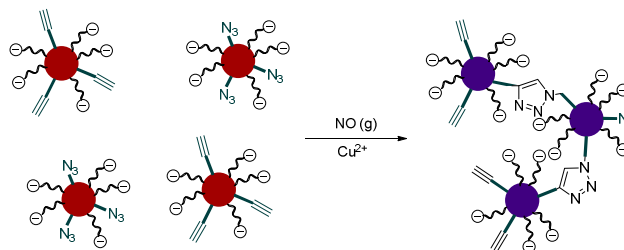
Nitric oxide (NO) is a gaseous free-radical species which exists in the atmosphere. NO is produced from the reaction of N<sub>2</sub> and O<sub>2</sub> during high-temperature combustion processes from car engines, power plants or industrial processes and naturally by lightning during a thunderstorm. NO is one of the components of the air pollutants known as nitrogen oxides (NO<sub>x</sub>), which are responsible for the acid rain and the smog pollution. High NO<sub>x</sub> levels in the atmosphere can cause serious diseases such as carcinogenesis and asthma.<sup>1</sup> NO is also a ubiquitous bioactive signalling molecule. Biological NO is generated in the oxidation of L-arginine to L-citrulline catalysed by nitric oxide synthases and participates in the regulation of a variety of physiological and pathological processes, such as in the control of vascular smooth muscle relaxation and vasodilation, platelet adhesion, regulation of the immune response and as neurotransmitter in the brain.<sup>2</sup>

Several analytical methods for NO detection have been developed such as electrophoresis, electron paramagnetic resonance (EPR) or GC-mass spectroscopies, chemiluminescence or electrochemical methods.<sup>1,3</sup> In comparison to these protocols, colorimetric and/or fluorescence-based techniques present a large number of advantages such as simple detection *in situ* or at site without any sample pre-treatment or use of low-cost, widely available equipment. Colorimetric detection is particularly appealing because it allows assays to be followed by the naked eye. However, whereas a large number of fluorescent probes for NO detection have been described,<sup>4</sup> studies on colorimetric detection of this gas are scarce.

In the last few years, functionalized gold nanoparticles (AuNPs) have attracted enormous interest in colorimetric sensing due to their

unique optoelectronic properties.<sup>5</sup> The detection strategy is based on the color change that arises from the interparticle plasmon coupling during analyte-induced aggregation or dispersion of AuNPs. The red color of dispersed nanoparticles turns into dark blue upon aggregation, and this color change can be observed by the naked eye even at nanomolar concentrations.<sup>6</sup> Recently, the copper(I)-catalyzed azide-alkyne cycloaddition (the archetypal “click” reaction), between alkyne- and azide-tagged AuNPs has been used in the design of colorimetric methods for the detection of Cu(II) ions in water.<sup>7</sup> The detection method is based on the *in situ* reduction of Cu(II) to Cu(I) in the presence of sodium ascorbate as the reductant. The Cu(I) catalyst promotes the “click” reactions on the surface of the AuNPs leading to interparticle-crosslinking aggregation.<sup>8</sup>

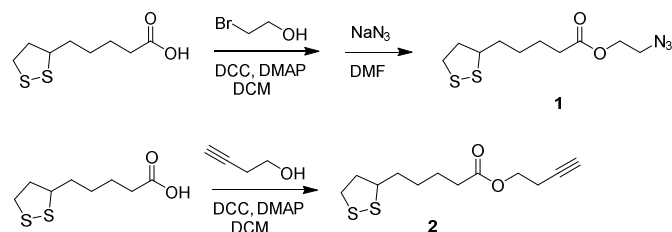
On the other hand it is well established that the copper centre in Cu(II) complexes undergo reduction to Cu(I) in the presence of NO,<sup>9</sup> and several Cu(II) complexes have been reported as colorimetric and/or fluorescent probes for nitric oxide detection.<sup>10</sup> Combining these two facts, herein we report a colorimetric method for the detection of NO (g) based on the aggregation-induced color change of a mixture of azide- and alkyne-functionalized AuNPs and Cu(II).



**Scheme 1.** Paradigm of the sensing mechanism: reaction of alkyne-AuNPs with azide-AuNPs by a Cu(I) catalyzed “click” reaction results in aggregation processes. The Cu(I) catalyst is generated *in situ* by the reduction of Cu(II) in the presence of NO (g).

The paradigm of the detection system is depicted in Scheme 1. The initial aqueous solution containing a mixture of azide- and terminal-alkyne functionalized AuNPs remains dispersed in the presence of Cu(II), with its characteristic red-wine color. However upon reduction of Cu(II) to Cu(I) by NO, the click reaction between azide and alkyne-terminated nanoparticles takes place leading to interparticle-crosslinking aggregation and subsequent change in the plasmon absorption band and in the color of the solution.

The synthesis of both, azide- and alkyne-terminated lipiolic acid derivatives **1** and **2**, used for the functionalization of the surface of the AuNPs, is depicted in Scheme 2.



**Scheme 2.** Synthesis of lipiolic acid derivatives **1** and **2**.

Compound **1** was obtained by the Steglich esterification of lipiolic acid with 2-bromoethanol, followed by reaction with sodium azide in 62% overall yield. Alkyne-terminated lipiolic acid derivative **2** was obtained in 65% yield from the esterification of lipiolic acid with 3-butyn-1-ol. The chemical structure and purity of compounds **1** and **2** were confirmed by spectroscopic techniques (see ESI).

Azide- and terminal alkyne-functionalized gold nanoparticles (**AuNP1** and **AuNP2** respectively) were synthesized by a two-step procedure. First, citrate-stabilized AuNPs were prepared by reducing tetrachloroauric acid with trisodium citrate in boiling water.<sup>11</sup> Then, by ligand-exchange reactions, citrate was displaced from the surface of the nanoparticles by a mixture of lipiolic acid (LA) and compounds **1** or **2** in an optimized 1:2 (LA/ligand) molar ratio. The functionalized nanoparticles were purified by repeated centrifugation and redispersion in water.

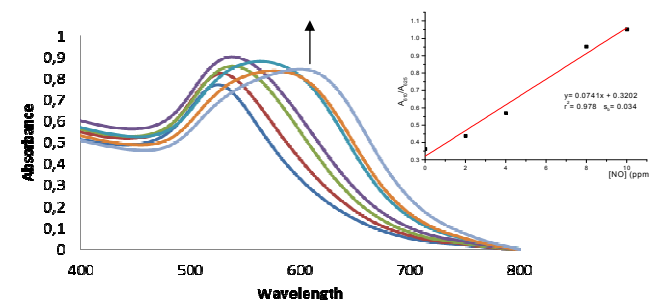
The resulting functionalized gold nanoparticles were characterized by UV-vis, transmission electron microscopy (TEM) and dynamic light scattering (DLS). Monodisperse stabilized AuNPs were obtained with an average size of 13 (**AuNP1**) and 13.5 nm (**AuNP2**), as determined by TEM. Both AuNPs displayed a surface plasmon absorption peak at 525 nm, in perfect agreement with the experimental data for particle sizes smaller than 25 nm.<sup>11</sup> The initial concentration of the AuNPs was calculated to be  $5.3 \cdot 10^{-9}$  M by UV-vis spectroscopy, from an estimated molar extinction coefficient of  $\epsilon = 2.5 \cdot 10^8 \text{ M}^{-1} \text{ cm}^{-1}$ .<sup>12</sup> The red wine aqueous dispersions of **AuNP1** and **AuNP2** remained stable in the fridge for more than 2 months, with no observable changes in their characteristic plasmon absorption band.

In order to verify the reaction of **AuNP1** with **AuNP2** under click conditions, a preliminary study was conducted with aqueous solutions containing equimolecular mixtures of both AuNPs ( $4 \cdot 10^{-10}$  M), copper (II) acetate (40  $\mu\text{M}$ ) and different amounts of sodium ascorbate as the reducing agent. The click reaction of the nanoparticles was monitored by UV-vis spectroscopy. In the absence of ascorbate, stable red-wine dispersions of the nanoparticles in

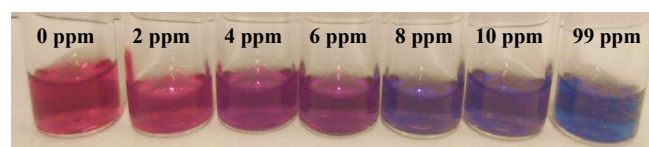
water were obtained, exhibiting the characteristic plasmon absorption band at 525 nm. Upon addition of increasing amounts of sodium ascorbate (from 4  $\mu\text{M}$  to 400  $\mu\text{M}$ ), and after an induction period of 5 min, the color of the solution gradually changed to dark blue, as was clearly appreciable by the naked-eye. The UV-vis spectra (see Fig. S4, ESI) were consistent with the observed color changes. Thus, the intensity of the surface plasmon peak at 525 nm ( $A_{525}$ ) corresponding to the monodispersed AuNPs gradually decreased and a new peak at ca. 627 nm ( $A_{627}$ ) characteristic of aggregated AuNPs appeared. The ascorbate-induced aggregation of the AuNPs was further confirmed by TEM analysis (see Fig. S5, ESI).

When the previous aqueous mixture of both AuNPs and Cu(II) was exposed to a  $\text{N}_2$  atmosphere containing low concentrations of NO (g) as the reductant, no change in the color of the solution was appreciated by the eye. Only when excess of NO was bubbled into the solution for 20 min, the color turned into blue, accompanied by the gradual appearance of a precipitate as a result of the aggregation process. However, when instead of water, a mixture of water/methanol (1:7, v/v) was used as a solvent a clear change in the color of the solution was observed after 5 min of exposure of the nanoparticles to low concentrations of NO (g) without any bubbling.<sup>13</sup>

To evaluate the minimum concentration of NO (g) detectable by our system, titration experiments were carried out by exposing a water-MeOH solution (1:7, v/v) containing a mixture of **AuNP1** and **AuNP2** ( $3.3 \cdot 10^{-10}$  M) and  $\text{Cu}(\text{AcO})_2$  (30  $\mu\text{M}$ ) to a  $\text{N}_2$  atmosphere containing increasing amounts of gaseous NO for 5 min, and then allowing the solution to stand in the air for other 5 min. The experiments were followed by UV-vis spectroscopy (see Figure 1).



**Figure 1.** Left: UV-vis spectra of titration of a mixture of **AuNP1**, **AuNP2** and  $\text{Cu}(\text{AcO})_2$  in  $\text{MeOH}:\text{H}_2\text{O}$  (7:1), upon exposition to increasing amounts of NO (g). Inset: Plot of  $A_{610}/A_{525}$  vs NO concentration (in ppm)



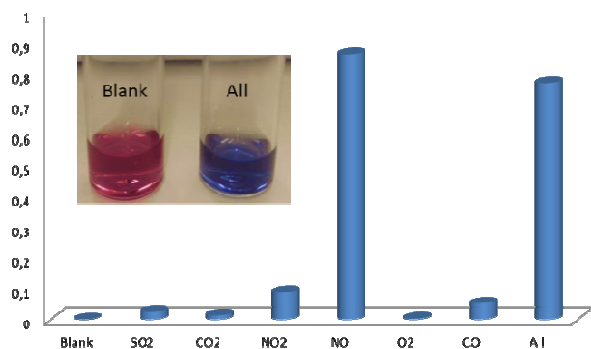
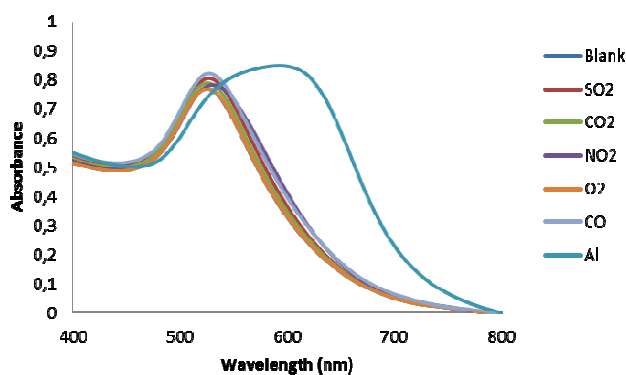
**Figure 2.** Photograph of solutions of **AuNP1**, **AuNP2** and Cu(II) in  $\text{MeOH}:\text{H}_2\text{O}$  (7:1) after exposure to a nitrogen atmosphere containing increasing amounts of NO (g)

The observed red shift from 525 to 610 nm in the surface plasmon band was indicative of the aggregation of the gold nanoparticles. The gradual change in the color of the solution from red to blue could be detectable by the naked eye for concentrations as low as 4 ppm of NO (g) (see Fig. 2). The interparticle-crosslinking aggregation was

also confirmed by TEM studies at different NO concentrations (see Fig. S7, ESI)

A detection limit of 1.4 ppm (v/v) for NO (g) was obtained from the plot of the ratio of the absorbance intensities at 525 and 610 nm ( $A_{610}/A_{525}$ ) versus NO concentration (Fig. 1, right).

Finally, to demonstrate the selectivity of the system, several possible interferents ( $\text{SO}_2$  (g),  $\text{CO}_2$  (g), CO (g) and  $\text{NO}_2$  (g), 50 ppm each) were tested by UV-vis spectroscopy following the same protocol. As can be observed in the UV-vis spectra (Fig. 3) only a modest response was observed in the presence of excess of  $\text{NO}_2$ . Furthermore, when all the gases and NO were exposed to the AuNPs dispersion, the change in color (Fig. 3, bottom and Fig. S8, ESI) and the red shift of the SPR absorption band clearly showed that this detection system is highly selective towards NO (g) with no appreciable interference from other common atmospheric gases.



**Figure 3.** Up: UV-vis spectra of a mixture of AuNP1, AuNP2 and Cu(II) in the presence of various interferents (50 ppm, v/v); Botom: Representation of  $A_{610}/A_{525}$  for the interferents and photograph of color change.

In summary, azide- and terminal alkyne-functionalized AuNPs in the presence of Cu (II) have demonstrated to be an interesting alternative for the direct colorimetric detection of NO (g) in aqueous media. The detection process is based on the aggregation of the AuNPs through a Cu(I) catalyzed click reaction promoted by the in situ reduction of Cu(II) by NO (g). With this method detection, limits of 1.4 ppm ( $\text{mL}/\text{m}^3$ ) have been achieved. This methodology can find uses in many scenarios where sensors for NO (g) are required. Furthermore, the biocompatibility of the AuNPs allows the possibility of extending their applications for NO detection in bioassays.

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## Notes and references

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Electronic Supplementary Information (ESI) available: Materials and synthetic procedures <sup>1</sup>H- and <sup>13</sup>C-NMR spectra, procedures for the titration and interference studies, calculation of the detection limit, supplemental spectra. See DOI: 10.1039/c000000x/

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