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Fluorogenic dansyl-ligated gold nanoparticles for the detection of sulfur mustard by displacement assay[†]

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The dansyl fluorophore ligated to gold nanoparticles *via* imidazole and amine groups affords conjugates capable of detecting micromolar concentrations of the chemical warfare agent sulfur mustard by a fluorescence switching 'ON' displacement assay.

The colorimetric and fluorescent sensing of chemical warfare agents (CWAs) has been largely dominated by the use of reaction chemistries that generate new molecular species.¹ This includes cyclisation approaches for organophosphorus G-agent analogue detection,² and the use of amine-based PET sensors for the detection of alkylating agents as simulants for bis-2-chloroethyl-sulfide, or sulfur mustard (referred to hereafter as HD).³

Conversely, the sensing of CWAs through supramolecular recognition has been reported only sporadically. As a necessity, it often relies upon the use of low toxicity simulants rather than the agents themselves for proof-of-concept. In the case of organophosphorus nerve agents, sensing systems reported include the recognition of pinacolyl methylphosphonate by molecular imprinted polymers⁴ and ligand displacement *via* $P=O\cdots$ lanthanide coordination.^{5,6} More recently the binding of the nerve agent GD, or Soman, by a series of hydrogen bond donating receptors has been shown.⁷

The recognition of HD, by virtue of its limited functionality for the formation of non-covalent bonds, is almost unknown. In work which has implications for HD sensing, Tilley reported the stability of Ag(1) salts of 2-chloroethyl methyl sulfide (CEMS), HD and diethylsulfide in polar organic solvents.⁸ Recently, the sensing of HD has been demonstrated through the formation of $R_2S \cdots Cu$ charge transfer complexes in a copper acetate



impregnated silica sol-gel matrix.⁹ A rare organic-based sensor was reported by Hunt and Alder who demonstrated the quenching of sodium fluorescein by HD,¹⁰ however, the mechanism for this quenching process is unclear.

Herein we describe a proof-of-concept method for recognizing and sensing sulfur mustard *via* a novel fluorescence switching 'ON' displacement assay which exploits the hierarchy of binding energies of different ligands with gold nanoparticle surfaces. The displacement sensing assay concept is illustrated in Fig. 1. The weak donor atom attachment of appropriately functionalised fluorophores in close proximity to a gold nanoparticle surface results in the fluorophore being quenched through an energy transfer process.¹¹ Exposure to a target sulfur-containing CWA interferes with the relatively weak surface interaction between the metal nanoparticle surface and functional group of the appended fluorophore, triggering the displacement of the fluorophore from the surface and a switching 'ON' of the fluorescence signal results.

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We have recently demonstrated the capability of the imidazole ligand to stabilise gold nanoparticle materials.¹² Due to the 'hardness' of nitrogen donor ligands when compared to the relatively 'soft' nature of sulfur donor compounds, we hypothesised that upon treatment of dansyl fluorophore appended imidazole or amine ligand stabilised gold nanoparticle materials with a bis alkyl sulfide such as HD, the sulfur containing analyte will compete with the respective nitrogen stabilising ligand for the gold nanoparticle and displace the quenched 'OFF' dansyl fluorophore from the surface leading to a switching 'ON' of the dansyl fluorescence.

The syntheses of the dansyl functionalised ligands were accomplished using commercially available reagents. Ligand 1 was prepared by condensing dansyl chloride with *N*-amino-propyl imidazole in dichloromethane in 77% yield. The amine derivative was prepared by mono-BOC protection of ethylene-diamine and subsequent reaction with the sulfonyl chloride to yield the proligand. Deprotection with trifluoroacetic acid in dichloromethane (1 : 4 v/v) afforded ligand 2 (see ESI[†] for X-ray-single crystal structure) in an overall yield of 54% (Scheme 1).

Both ligands were ligated to gold nanoparticles using a modified Brust method,¹³ using HAuCl₄ and three equivalents of the appropriate dansyl ligand. Reduction with NaBH₄ in dichloromethane–toluene afforded the target dansyl-functionalised gold nanoparticles (GNPs) **Au-1** and **Au-2** which were characterised by TEM, elemental analysis, UV-vis and ¹H NMR (see ESI[†]). The GNPs exhibited characteristic UV-vis absorption bands assignable to the ligand n- π * transition centred around 350 nm and broad maxima at 520–530 nm attributed to the Surface Plasmon Resonance (SPR) band of the gold nanoparticles. Analysis of the TEM imaging data gave mean particle diameters of 3.1 ± 0.7 nm and 3.2 ± 0.7 nm for the imidazole and amine functionalised nanoparticles, respectively.

In order to test the displacement assay concept (Fig. 1) a qualitative ¹H NMR analysis was conducted initially using **Au**·1 and the mustard simulant CEMS in CDCl₃ solution. As expected, the ¹H NMR spectrum of **Au**·1 reveals very broad resonances (see ESI[†]), consistent with chemical shift anisotropy of the bound species as a result of being chemisorbed to the gold surface.¹⁴ Upon addition of 10 equivalents of CEMS new sharp resonances associated with the dansyl moiety were seen (Fig. 2). This observation is evidence of the liberation of the free ligand species 1 by the aurophilic mustard simulant.



Fig. 2 ¹H NMR spectra of (top) $Au \cdot 1$ and (bottom) $Au \cdot 1$ in the presence of ten equivalents of CEMS (CDCl₃, 500 MHz, 298 K). ¹³C satellites marked with an asterisk.

Studies of these materials by UV/Vis absorption spectroscopy provide further evidence of the occurrence of surfacebound molecular events following exposure to both CEMS and HD. The SPR bands of both $Au \cdot 1$ and $Au \cdot 2$ in CHCl₃ were observed to undergo significant bathochromic shifts from 528 to 536 nm and 520 to >600 nm,¹⁵ respectively, upon addition of CEMS. Similarly, upon exposure to HD shifts of 528 to 540 nm for $Au \cdot 1$ and 520 to 540 nm for $Au \cdot 2$ were observed (see ESI[†]). This may indicate particle agglomeration following displacement of the stabilising dansyl ligands. Indeed a significant change in colour of the respective nanoparticle solution from brown to clear was observed with the formation of a grey precipitate upon addition of CEMS and HD (Fig. 3).



Fig. 3 2.5 mL of a 0.04 mg mL⁻¹ solution of Au·2 in CHCl₃ (left), 30 minutes after addition of 50 μ L CEMS (right).

Both GNPs exhibited weak fluorescence from the ligated dansyl group with emission maxima around 500 nm. In order to probe the fluorogenic properties of the GNPs and their response to the addition of the sulfides, qualitative fluorescence measurements were undertaken in chloroform. The response of solutions of Au·1 and Au·2 upon addition of either CEMS or HD was monitored over five minute intervals (Fig. 4). Addition of either sulfide species resulted in a significant increase in the fluorescence intensity of the sample. This increase in fluorescence is explained by the highly distantdependant nature of the non-radiative processes responsible for the quenching of the fluorescent dansyl group. Proximity of the fluorophore to the metal surface in the initial GNP conjugate results in weak fluorescence due to efficient quenching processes between the dansyl group and the gold surface, attributed to through-space relaxation by Nanosurface Energy



Fig. 4 Fluorescence spectra of a 0.04 mg mL⁻¹ solutions of **Au-1** (left) and **Au-2** (right) upon addition of CEMS (50 μ L) in CHCl₃ at 298 K (λ_{ex} = 345 nm).

Transfer (NSET).¹¹ Substitution of the dansyl ligand by the sulfide analyte at the gold surface releases the dansyl group into solution and results in the loss of the NSET through-space quenching process. Displacement for both analytes with both nanoparticles occurred almost instantaneously, with the maximum increase in fluorescence occurring within five minutes for both samples. Importantly analogous titration experiments of $Au \cdot 1$ and $Au \cdot 2$ with octanol and di-*n*-butyl ether gave no significant fluorescence increase. The selective sulfide fluorescence response together with the rapid kinetics of displacement suggests that conjugates $Au \cdot 1$ and $Au \cdot 2$ are promising prototype sensory materials for mustard detection.¹⁶

In order to probe the lower detection limit of the GNPs towards HD the surface coverage of $Au \cdot 2$ was calculated using sulfur elemental analysis data, with surface coverage determined to be 13.5% ligand by weight. Titrations were conducted using a 0.04 mg mL⁻¹ solution of GNPs and the fluorescence changes monitored upon addition of 0–10 molar equivalents of either CEMS or HD (Fig. 5). As can be seen from the spectra,



Fig. 5 Fluorescence titration of a 0.04 mg mL^{-1} solution of Au-2 with HD in CHCl₃ at 298 K (λ_{ex} = 345 nm).

the ligand-nanoparticle conjugates are shown to be highly sensitive to the presence of the mustard derivative, displaying a response to micromolar concentrations of sulfur mustard.

Assuming the displacement is a 1 : 1 ligand exchange process with one guest sulfide molecule displacing one surface stabilised ligand, and ignoring any cooperativity effects between surface ligand exchange sites, SPECFIT analysis¹⁷ of the fluorescence titration data gave association constants log $K = 4.65 \pm 0.06$ and log $K = 4.19 \pm 0.05$ for CEMS and HD, respectively, for Au·2.

As expected, the relative surface binding affinities of the two sulfide analytes in the surface displacement of the amine stabilised dansyl ligand of $Au \cdot 2$ are of similar magnitude.

In summary, we have demonstrated a proof-of-concept capability of imidazole and amine dansyl-ligated gold nanoparticles to detect the chemical warfare agent sulfur mustard. The mechanism of detection occurs *via* the displacement of a nanoparticle surface energy transfer quenched fluorophore, resulting in a 'switching-on' fluorescence sensing response.

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Notes and references

- 1 K. Kim, O. G. Tsay, D. A. Atwood and D. G. Churchill, *Chem. Rev.*, 2011, **111**, 5345–5403.
- 2 S. W. Zhang and T. M. Swager, J. Am. Chem. Soc., 2003, 125, 3420–3421. 3 S. Tal, H. Salman, Y. Abraham, M. Botoshansky and Y. Eichen,
- Chem.-Eur. J., 2006, **12**, 4858-4864.
- 4 L. Malosse, P. Palmas, P. Buvat, D. Ades and A. Siove, *Macromolecules*, 2008, **41**, 7834–7842.
- 5 D. Knapton, M. Burnworth, S. J. Rowan and C. Weder, *Angew. Chem., Int. Ed.*, 2006, **45**, 5825–5829.
- 6 J. Massue, S. J. Quinn and T. Gunnlaugsson, J. Am. Chem. Soc., 2008, 130, 6900–6901.
- 7 M. R. Sambrook, J. R. Hiscock, A. Cook, A. C. Green, I. Holden, J. C. Vincent and P. A. Gale, *Chem. Commun.*, 2012, **48**, 5605–5607.
- 8 R. I. Tilley, Aust. J. Chem., 1990, 43, 1573-1576.
- 9 J. F. Brinkley, M. L. Kirkey, A. D. S. Marques and C. T. Lin, *Chem. Phys. Lett.*, 2003, **367**, 39–43.
- 10 A. L. Hunt and J. F. Alder, Anal. Chim. Acta, 1999, 387, 207-215.
- 11 T. L. Jennings, M. P. Singh and G. F. Strouse, J. Am. Chem. Soc., 2006, 128, 5462–5467.
- 12 C. J. Serpell, J. Cookson, D. Ozkaya and P. D. Beer, *Nat. Chem.*, 2011, 3, 478–483.
- 13 M. Brust, M. Walker, D. Bethell, D. J. Schiffrin and R. Whyman, J. Chem. Soc., Chem. Commun., 1994, 801–802.
- 14 R. H. Terrill, T. A. Postlethwaite, C. H. Chen, C. D. Poon, A. Terzis, A. D. Chen, J. E. Hutchison, M. R. Clark, G. Wignall, J. D. Londono, R. Superfine, M. Falvo, C. S. Johnson, E. T. Samulski and R. W. Murray, *J. Am. Chem. Soc.*, 1995, 117, 12537–12548.
- 15 The broadness of the SPR band prevented an accurate maximum being measured.
- 16 Although other sulfur containing species may give a similar fluorescence response, it is highly unlikely that such interferences will be present under the typical conditions in which this sensor is likely to be used.
- 17 SPECFIT, 2.02, Spectrum Software Associates, Chapel Hill, NC, USA.