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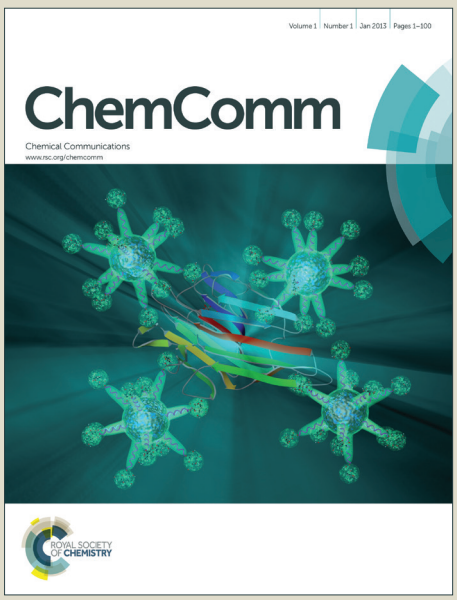

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ARTICLE TYPE

A tailor-made nucleoside-based colourimetric probe of formic acid

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A ratiometric, specific probe of formic acid has been developed. It is based on intermolecular nucleobase-pairing of inosine-capped plasmonic nanoparticles to form nucleoside channels, which are destabilised by the analyte.

There are a considerable number of specific probes of ions, but this rarely applies to small organic molecules. Gold nanoparticles (AuNPs) are of particular interest in colourimetric sensing. On the one hand, the large surface-to-volume ratio of spherical NPs can be used advantageously for placing a considerable number of specific functionalities on their periphery, i.e., it makes it possible to have a high local concentration of a functional group in an otherwise very diluted solution. On the other hand, nanometre-sized AuNPs display intense colour, thus being clearly detectable by the naked eye at nanomolar concentrations, and, in addition, they exhibit strong distance-dependent optical properties. Thus, in the aggregated state, they form blue colloidal solutions, which change to intense burgundy (a typical plasmonic band at λ_{max} ca. 530 nm) when the NPs are well-dispersed.[1,2,3] Consequently, AuNPs are being applied as colourimetric probes by making use of analyte-induced changes in their aggregation state. Usually, large biomolecules, such as oligonucleotides, DNA, and proteins at the NP periphery are used to obtain systems that are responsive to small molecules, which induce the assembly of the NPs.[4,5,6,7,8,9,10,11, 12,13,14]Alternatively, aggregated NPs can be disassembled by metal ions or other analytes and the colour of the colloid gradually changes from blue to red.[7]

Probes based on disassembly of metal NPs capped with small ligands are uncommon.[15] In addition, it is hard to design colourimetric probes exhibiting selective molecular recognition of small molecules (one or two carbon atoms) from compounds of a higher molecular weight of the same chemical family. We have recently reported a colourimetric method for sensing of methanol and ethanol in an organic media based on AuNPs decorated with adenosine (AD) derivatives (Au@S-AD).[15] Au@S-AD aggregates were insensitive to n-propanol, i-propanol, n-butanol, and t-butanol.

These unprecedented results prompted us to explore the usefulness of the particular features of the inosine (IN) nucleobase, specifically its extraordinary capacity for self-association in low-polar solvents (690 ± 100 M^{-1} in chloroform),[16] to build up a highly selective probe of formic acid on the basis of a demanding unzipping of the nucleoside channels of inosine-capped AuNP (Au@S-IN) aggregates (Fig. 1).

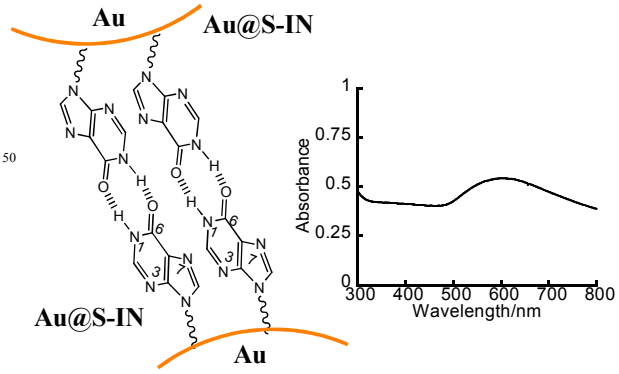

Fig.1: Left: Schematic representation of the formation of Au@S-IN aggregates by hydrogen-bonding. Right: UV-Visible absorption spectrum of Au@S-IN aggregates in chloroform

We demonstrate here that Au@S-IN aggregates possess an exceptional capacity for the specific sensing of formic acid. The aggregates remain stable in the presence of methanol, ethanol, and alcohols of a higher weight, as well as in the presence of other carboxylic acids (even acetic acid or more acidic compounds, such as chloroacetic and trifluoroacetic acids).

Theoretical calculations[15] have shown that adenine-capped AuNPs (Au@S-AD) give rise to nucleoside channels in low polar solvents due to the supramolecular assembly of the AuNPs by means of hydrogen bonds (HBs) between the adenine moieties belonging to different nanoparticles. The sensing selectivity to small alcohols, such as methanol and ethanol, has been attributed to the penetration of the small alcohols into the nucleoside channels created in the structure, combined with the anchoring of the alcohol to the N7 basic centre of the nucleobase; this centre enhances the methanol basicity. Consequently, the molecular recognition capacity of the Au@S-AD hybrids has been ascribed to size exclusion effects combined with "allosteric" weakening of the inter-nanoparticle HBs. Taking into account the variety of nucleobases available, this strategy could be expanded to detect selectively other analytes by making use of the particular features of the nucleobases in respect to i) the relative position of their hydrogen donor/acceptor centres, ii) the HB strength of their nucleobase-nucleobase pairing, and/or iii) the size of the resulting channels after Au@nucleobase aggregation.

Inosine, which is commonly found in tRNAs and is essential for proper translation of the genetic code in wobble base pairs, is a

nucleoside comprising hypoxanthine attached to a ribofuranose via a β-N9-glycosidic bond.[17] IN self-associates in chloroform, but the associates differ from those of AD in two key ways: i) AD can coexist in a variety of isomeric forms, but IN exists in only one[20] and ii) the IN[16] association constant (K) in chloroform is considerably greater than that of AD[18,19] (690 ± 100 M^{-1} and 1.6 ±0.2 M^{-1}, respectively). Consequently, Au@S-IN nanohybrids would form stronger aggregates in chloroform than Au@S-AD hybrids and, therefore, they would be considerably more difficult to disrupt.

To test our hypothesis, first we prepared oleylamine-stabilized AuNPs (Au@OA), with an average core diameter of 10.0±0.4 nm. These AuNPs gave rise to burgundy-coloured solutions in chloroform and their UV-Vis absorption spectrum exhibited the typical plasmon band at λ_{max} 525 nm. Au@OA nanoparticles reacted with 5′-(11-mercaptoundecamido)-5′-deoxy-2′,3′-O-isopropylideneinosine (HS-IN) to give rise to Au@S-IN nanoparticles (see all the experimental details and spectra of Au@OA , HS-IN, Fig. S1-S4, in the supporting information). The red-shift of the plasmon peak, accompanied by a considerable broadening of the spectrum (Fig. 1), evidenced the decrease in the interparticle distance and suggested HB interactions between the nucleobase moieties located around the periphery of different NPs (Fig. 1). The aggregates, which are typically blue, proved stable in chloroform. As previously reported for IN [12], such interaction would involve the NH1---O=C6 hydrogen bonding between the IN units. The alignment of such units, belonging to different nanohybrids, would induce an antiparallel arrangement between the nanoparticles and create a series of nucleoside channels (Fig. 1). This would permit the movement of molecules of the right size within the channels. The disassembly of the Au@S-IN aggregates would be induced through destabilisation of the inter-particle HBs by increasing the concentration of the appropriate analyte (Fig. 2 A).

Unlike the case of the Au@S-AD aggregates, neither methanol nor ethanol produced disassembly of the Au@S-IN aggregates. Assuming that IN-IN pairing produces nucleoside channels of a similar size to those of adenosine, this result was compatible with the incapacity of the alcohols to destabilise the strong binding between the IN units. In addition, an important difference between AD and IN aggregates is the absence of an available NH10 centre to coordinate methanol once it binds to the basic N7 centre of the nucleobase. Such interaction enables the cooperative weakening of the intermolecular HBs in the Au@S-AD aggregates. Hence, the coordination of methanol to the N7 centre of IN did not induce the cleavage of the strong IN-IN bond in the Au@S-IN aggregates.

The coordination of a sufficiently strong acid to the N7 centre (pKa ca. 1.06 in aqueous solvent)[20] could be decisive in the weakening of the IN-IN HB in the organic solvent. Therefore, we tested the effect of small-sized carboxylic acids, namely formic and acetic acid, on Au@S-IN aggregates.

Addition of increasing amounts of formic acid (aliquots of 10 µl) to the IN-capped NPs in 1 mL of $CHCl_3$ caused different states of disassembly of the NPs, evidenced by the blue-shift of the absorption band up to ca. 530 nm with a concomitant narrowing of the spectrum (Fig. 2B). Interestingly, colour changes occurred rapidly and, therefore, each UV/Vis spectrum was registered at ca. 1 minute after the addition of each formic aliquot.

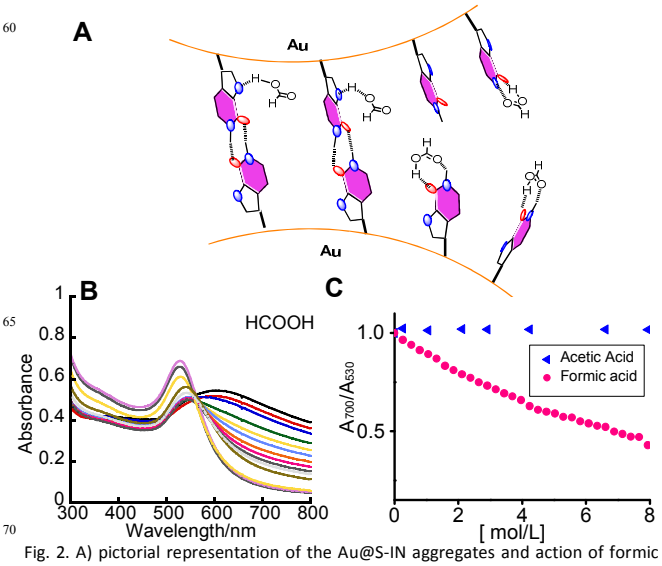

Fig. 2. A) pictorial representation of the Au@S-IN aggregates and action of formic acid on the IN nucleobase thereby causing unzipping of the nucleoside channels; B) UV-Visible absorption spectra of Au@S-IN aggregates in chloroform in the absence and in the presence of formic acid; the arrows indicate the absorbance trend with increasing [HCOOH]; C) Plots of the A_{700}/A_{530} ratio vs [HCO_2H] and [H_3CCO_2H] for Au@S-IN aggregates in chloroform.

A reliable method to analyse the capacity of an analyte to induce the disassembly is by monitoring the ratio between the absorbance at 700 nm and that of the NP plasmon peak maximum (ca. 530 nm); a low ratio is associated with dispersed red NPs and a high ratio is associated with blue aggregates. Ratiometric probes are suitable for practical applications since they are less vulnerable to fluctuations of monitoring conditions.

Fig. 2C shows the A_{700}/A_{530} ratio dependence for Au@S-IN aggregates on acid (formic and acetic acids) concentration up to 8 M. The minimum detectable limit of formic acid was lower than 5 µl (in 1 mL $CHCl_3$). An exponential dependence of the A_{700}/A_{530} ratio on formic acid concentration was found whereas huge amounts of acetic acid (1 mL) did not cause any detectable change. Assays with chloroacetic and trifluoroacetic acid did not cause significant colour changes either (not shown). Consequently, Au@S-IN aggregates proved extremely specific in formic acid sensing.

The naked eye assay after addition of methanol, ethanol, and even acetic acid to Au@S-IN aggregate is shown in Fig. S5; it shows that blue became fainter upon addition of increasing amounts of the analyte, due to the dilution effect. By contrast, a blue-to-red change was detected after addition of ca. 120 µL of formic acid. These assays showed that the sensing capacity of the Au@S-IN aggregates can alternatively be analysed by measuring the absorbance at a specific wavelength.

The response of Au@S-IN aggregates in different organic solvents was also tested (see Fig. S6 in the supporting information). These nanohybrids proved dispersible in medium polar to polar solvents and showed a response to formic acid in all of them, though with different sensitivity. In addition, the assay is reversible as proved by de-agglomeration upon addition of formic acid, again followed by agglomeration of the nanoparticles after addition of chloroform, and subsequent de-aggregation of the nanoparticles after addition of formic acid (see Figure S7 in the supporting information).[21]

Despite the small differences in their size, formic and acetic acids display dissimilar properties. Thus, the relative permittivity and acidity of formic acid are considerably higher than those of acetic acid.[22] Moreover, while liquid acetic acid contains basically cyclic dimers, formic acid forms linear associates. Therefore, the results were consistent with the peculiarities of formic acid.

For comparative purposes, we performed assays to determine the response of Au@S-AD aggregates to formic and acetic acids (Fig. S8 in the supporting information). Contrary to the Au@S-IN aggregates, Au@S-AD aggregates were sensitive to both acetic and formic acids. Consequently, the considerable strength of the HB between the IN units played a key role in the response of the Au@S-IN aggregates to formic and acetic acids.

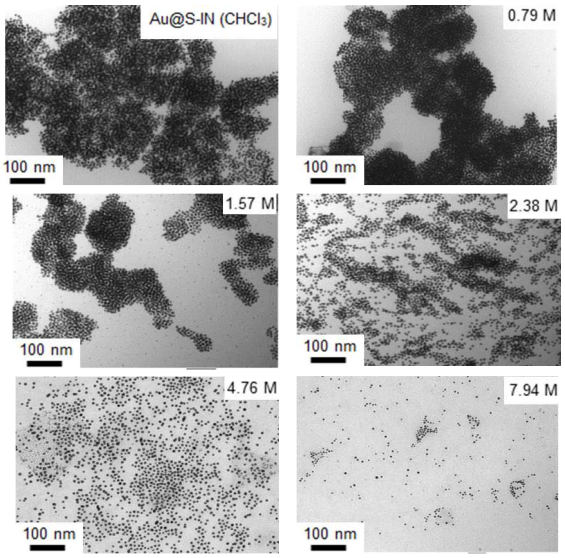

Fig. 3. TEM images (scale bar of 100 nm) of Au@S-IN in $CHCl_3$ in the presence of increasing amounts of formic acid (0, 30, 60, 90, 180, and 300 µl; molar concentrations at the top right-hand side of each image).

Transmission electron microscopy (TEM) images of the Au@S-IN aggregates in chloroform (1mL) in the absence of HCOOH and those in the presence of up to 100 µL of HCOOH showed that the acid led to disassembling of the Au@S-IN and that nanoparticle core fusion did not occur during their aggregation in chloroform (Fig. 3). Moreover, the images of the AuNPs before and after addition of 30, 60, 90, 180, and 300 µl of HCOOH were consistent with the dependence of the A_{700}/A_{530} ratio of the colloidal solution on the acid concentration. Thus, a progressive disassembling of the aggregates was observed when increasing amounts of HCOOH (from ca. 0.79 to ca. 7.94 M) were added. Also dynamic light scattering spectra of chloroform solutions of Au@S-IN AuNPs (1 mL), registered before and after adding up to 100 µL of HCOOH, i.e., up to ca. 7.94 M, were also consistent with the dependence of the aggregation degree of the NPs in chloroform on the formic acid concentration (Fig. 4 and Fig. S9).

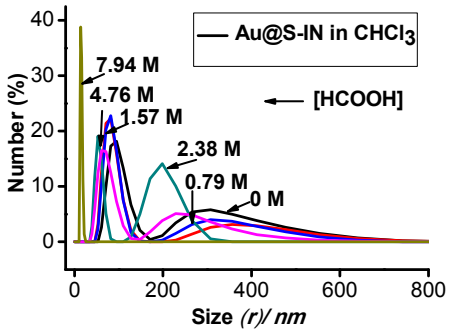

Fig. 4. Dynamic light scattering spectra of Au@S-IN in 1 mL of chloroform compared with those obtained after addition of increasing concentrations of formic acid; number vs. size.

We suggest the following mechanism for the disassembly of Au@S-IN aggregates in chloroform. Within the nucleoside channel, formic acid interacts via H-bonding with the more basic centre, i.e., N7, (Fig. 2A), thus weakening the nucleobase-pair bond and leading to aggregate disassembly. Eventually, HCOOH successfully competes with inosine binding at high concentrations by forming more stable complexes than those between the nucleobases. The non-response of the aggregates to acetic acid could be due to its lower acidity combined with the considerable strength of the IN-IN HB. In addition, the transit through the channels would be impeded for carboxylic acids such as chloroacetic and trifluoroacetic acid, and consequently they would not be operative in the disassembling in spite of their stronger acidity than formic acid.

Formic acid is a very small and important chemical of application as an antibacterial agent in animal food, an environmentally friendly bleaching agent in the paper industry, a miticide, an intermediate in the production of many of pharmaceuticals, flavours, perfumes, and as a catalyst in the manufacturing of phenolic resins, among others.[23] Formic acid is also promising for direct formic acid fuel cells which are a potential challenger to direct methanol technologies.[24,,] While up to now most applications are related to the detection of very low concentrations of formic acid, or a mixture of carboxylic acids, probes operative in higher analyte concentrations are currently relevant or can become relevant for future applications.[25,26,27]

In summary, we have shown here that AuNPs decorated merely with a nucleobase can lead to unique nanohybrids which specifically recognise formic acid vs other carboxylic acids and also alcohols. These structures make use of the synergistic cooperation between the plasmonic nanoparticle and the nucleoside, namely inosine, to build a colourimetric probe based on the formation, in medium polar to polar solvents, of nucleoside channels, whose size and strength play a key role in molecular recognition. To our knowledge, this is the first example of a specific colourimetric probe of the lightest member

of a family of organic compounds.

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Notes and references

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Electronic Supplementary Information (ESI) available: See DOI: 10.1039/c000000x/- experimental details; synthesis of Au@OA, HS-IN, Au@IN; visual colorimetric detection of formic acid; response of Au@S-IN aggregates to formic acid in different organic solvents; response of Au@AD to acetic and formic acids.

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