Accepted Manuscript

Title: Selective and sensitive colorimetric detection of the neurotransmitter serotonin based on the aggregation of bifunctionalised gold nanoparticles

Authors: Tania M. Godoy-Reyes, Antoni Llopis-Lorente, Ana M. Costero, Félix Sancenón, Pablo Gaviña, Ramón Martínez-Máñez

Please cite this article as: Tania M.Godoy-Reyes, Antoni Llopis-Lorente, Ana M.Costero, Félix Sancenón, Pablo Gaviña, Ramón Martínez-Máñez, Selective and sensitive colorimetric detection of the neurotransmitter serotonin based on the aggregation of bifunctionalised gold nanoparticles, Sensors and Actuators B: Chemical https://doi.org/10.1016/j.snb.2017.11.181

Selective and sensitive colorimetric detection of the neurotransmitter serotonin based on the aggregation of bifunctionalised gold nanoparticles

Tania M. Godoy-Reyes [a,b,d], Antoni Llopis-Lorente [a,b,d], Ana M. Costero [a,b,c], Félix Sancenón [a,b,d], Pablo Gaviña [a,b,c],* and Ramón Martínez-Máñez [a,b,d],*

[a] Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Universitat Politècnica de València, Universitat de València, Spain
[b] CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain
[c] Departamento de Química Orgánica, Universitat de València, Doctor Moliner 50, Burjassot, 46100, Valencia, Spain
[d] Departamento de Química, Universitat Politècnica de València, Camino de Vera s/n, 46022, Valencia, Spain
* Corresponding authors. E-mail addresses: pablo.gavina@uv.es (P. Gaviña), rmaez@qim.upv.es (R. Martínez-Máñez).

Graphical abstract
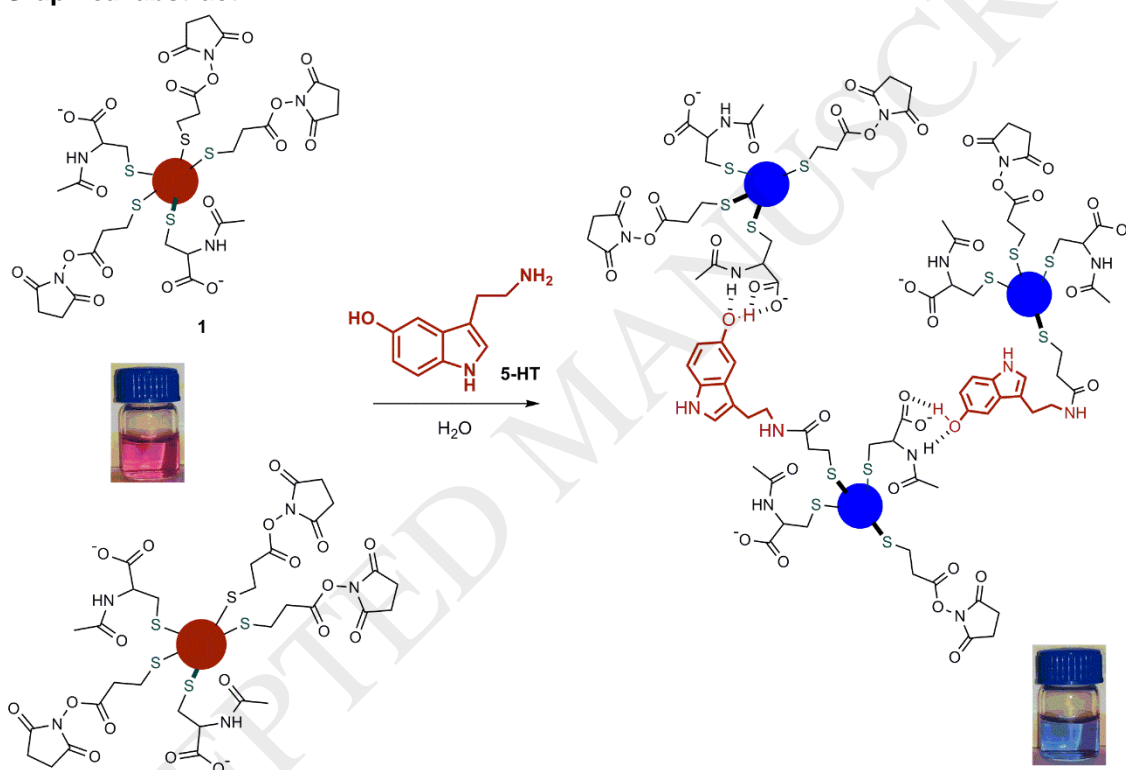
Abstract: We report a simple, sensitive and selective method for the colorimetric detection of serotonin (5-HT) in aqueous media using bifunctionalized gold nanoparticles (AuNPs). The probe **(1)** consisted of AuNPs functionalised with dithiobis(succinimidylpropionate) (DSP) and N-acetyl-L-cysteine (NALC). DSP was chosen to react with the amino group of 5-HT, whereas NALC was chosen to bind the hydroxyl group in 5-HT through hydrogen bonding and electrostatic interactions. A double interaction between nanoparticles and the hydroxyl and the amino group of serotonin led to interparticle-crosslinking aggregation. This, resulted in a colour change from red to blue that can be observed by the naked eye. The probe was selective to 5-HT and no colour modulation was observed in the presence of other neurotransmitters (i.e.

dopamine, epinephrine, norepinephrine), selected biomolecules (i.e. L-tyrosine, gamma-aminobutyric acid, L-cysteine, uric acid, oxalic acid, aspartic acid and glutamic acid) and common inorganic species. A limit of detection as low as 0.1 μM was determined in buffered water at pH 7 by UV-vis titrations. Similar response of the probe to 5-HT was observed in simulated blood serum, with a limit of detection of 0.12 μM, and a linear response within the 0-3 μM concentration range, which is within the range of the 5-HT concentrations of clinical interest. Finally, the performance of probe (**1**) in real human blood samples was evaluated, and showed a remarkable ability to distinguish between normal 5-HT levels and 5-HT levels indicative of disease.

Keywords: serotonin (5-HT) • gold nanoparticles • colorimetric detection • aggregation • neurotransmitters

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT), commonly known as the molecule of happiness, is an important neurotransmitter that plays a key role in the regulation of various cognitive and behavioural functions such as sleep, mood, pain, anxiety, appetite control, sexual activity and learning [1]. Serotonin is produced in several locations including the brain, to transport information along the central nervous system, and also in the gastrointestinal track (GIT) to regulate intestinal movement [2-3]. Abnormal 5-HT levels have been related with a number of disorders like depression, migraines, ADHD, autism and inflammatory syndromes [4-5]. Moreover, carcinoid tumours, which usually start in the GI tract, secrete large amounts of 5-HT. This leads to high 5-HT levels in the blood of patients and causes flushing of the skin, diarrhoea and breathing problems. In fact, evaluating 5-HT blood levels, together with 5-hydroxyindoleacetic acid (a metabolite of 5-HT), is the principal laboratory test to diagnose carcinoid syndrome [6-7]. Although how the serotoninergic system operates exactly is still not well-known, scientists are attempting to elucidate the possible hormonal role of serotonin in neurodegenerative disorders like Parkinson's [8-9] and Alzheimer's disease [10]. All these previous results have suggested the potential use of 5-HT as a biomarker in a number of diseases.

In this scenario, the development of methods to detect 5-HT is a field of interest. Reported methods for 5-HT detection include liquid chromatography [11-17] and electrochemical techniques [18-24]. For example, the oxidation/reduction of 5-HT and dopamine using modified glassy carbon electrodes has been reported as a suitable electrochemical approach for 5-HT detection [23-24]. Enzymatic [25] and solid surface-room temperature phosphorescence assays have also been reported [26]. Other reported methods include fluorometric [27-29], flow cytometry [30], immunocytochemical and immunohistochemical [31-33] techniques. Although most of these methods can accurately measure 5-HT, they require sophisticated high cost equipment and are time-consuming (especially liquid chromatography). However, designing simple procedures to detect 5-HT remains a challenge due to the complexity of the molecule and common interference from other neurotransmitters with similar chemical structures such as dopamine and epinephrine.

As an alternative to traditional instrumental methods, chromogenic probes offer certain advantages as they are usually cheap, easy to use, require simple instrumentation or not instrumentation at all, and allow in situ and at-site detection [34]. An appealing approach to develop colorimetric probes is to use gold nanoparticles (AuNPs) given their unique optoelectronic properties [35-37]. When using AuNPs, the detection strategy is usually based on colour changes that arise from interparticle plasmon coupling during analyte-induced aggregation of nanoparticles. It is known that the red colour of dispersed AuNPs of a certain size turns dark blue upon aggregation, and this colour change can usually be observed by the naked eye, even at very low analyte concentrations [38-43]. For instance, Tian and co-workers have reported the use of functionalised AuNPs for the colorimetric detection of dopamine. A double interaction between the nanoparticles and the diol and the amino group of dopamine triggered the aggregation of AuNPs that results in a clear colour modulation [44].

For all the above reasons, and given our current interest in developing probes to detect bio-relevant molecules, we report herein a simple, sensitive and selective method for the colorimetric detection of 5-HT using bifunctionalised AuNPs. The recognition paradigm is shown in Scheme 1. Probe **(1)** consists of AuNPs of ca. 15 nm, which were functionalised dithiobis(succinimidylpropionate) (DSP) and N-acetyl-L-cysteine (NALC). DSP was chosen as a suitable group because it is able to react with the amino group in 5-HT [45], whereas NALC

plays a double role: (i) it acts as a stabiliser for AuNPs thanks to its negative charge at a neutral pH; (ii) is also able to bind the hydroxyl group in 5-HT through hydrogen bonding and electrostatic interactions [46]. Whereas, functionalised AuNPS **1** would not aggregate, we expected the double interaction between 5-HT and **1** to lead to interparticle-crosslinking aggregation, which would result in a colour change from red to blue.
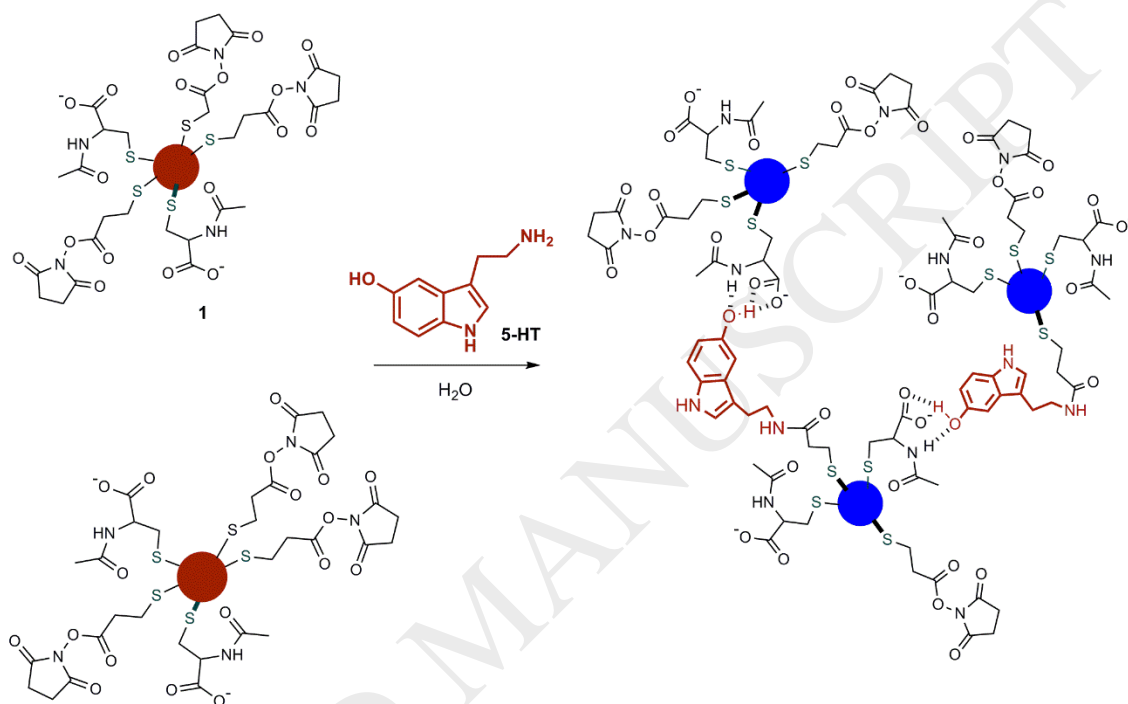
Scheme 1. Sensing paradigm of the colorimetric detection of serotonin (5-HT) based on gold nanoparticles bifunctionalised with NALC and DSP (probe **1**).

2. Experimental

2.1. Chemicals

Chloroauric acid (HAuCl$_4$·3H$_2$O), sodium citrate dihydrate, N-acetyl-L-cysteine (NALC), dithiobis succinimidyl propionate (DSP), serotonin (5-HT), dopamine (DA), epinephrine (Epy),

norepinephrine (NE), L-tyrosine (L-Tyr), gamma-aminobutyric acid (GABA), uric acid (AU), oxalic acid (AO), aspartic acid (A.A) and glutamic acid (GA) were commercially available, and were used without purification.

2.2.   General Methods

UV-Vis absorption spectra were recorded using a 1-cm path length quartz cuvette on a Shimadzu UV-2101PC spectrophotometer. All the measurements were taken at room temperature. To verify the serotonin-induced aggregation of the gold nanoparticles probe, high-resolution transmission electron microscopy (JEOL-1010 transmission electron microscopy operating at 100 kV) was used. Z potential and hydrodynamic diameter values were measured in a Malvern Zetasizer ZS 3 times in 10-25 cycles. Fourier-transform infrared spectroscopy spectra were recorded with a Cary 630 FT-IR spectrometer within the wavenumber range of 648-4000cm^{-1} at a resolution of 8 cm^{-1}. Proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) spectra were recorded with a Bruker DRX-500 Spectrometer (500 MHz, 1024 scans).

2.3.   Synthesis of citrate-capped AuNPs

Citrate-capped AuNPs with a diameter ca. 15 nm, were synthesised as previously reported [46]. Briefly, 5 mL of aqueous 13.61 mM trisodium citrate solution were added to an aqueous boiling solution of HAuCl$_4$ (95 mL, 0.23 mM) and the resulting solution was allowed to boil for 30 min until a red solution was obtained. The solution was cooled to room temperature. Then the mixture was purified by filtering through a 0.22 μM membrane and the filtrate was stored in a refrigerator at 4°C until it was used.

2.4.   Functionalisation of AuNPs

Probe **1** was prepared by modifying the citrate-capped AuNPs by ligand-exchange reaction, which was performed at room temperature by mixing 95 mL of the as-prepared AuNPs with 190 μL of 2 mM aqueous solution of NALC and 190 μL of DSP (2 mM in DMF). Ligands were added simultaneously and the solution was stirred for 1 h with magnetic stirring. To purify **1**, the mixture was centrifuged for 10 min at 11,000 rpm and the supernatants were decanted twice.

2.5.   Sensing studies

For the sensing studies, serotonin solutions were freshly prepared in 50 mM Tris buffer, pH 7.0, at room temperature. Next 500 µL of **1** (4.37 x 10^{-10} M) and 500 µL of the 5-HT solution at an appropriate concentration were placed to obtain a final volume of 1 mL. Samples were incubated for 3 min before taking measurements in the spectrophotometer. The procedure was the same for the interferences. Simulated blood serum (SBS) [47] was prepared as follows: 8.036 g of NaCl, 0.352 g NaHCO$_3$, 0.225 g of KCl, 0.230 g of K$_2$HPO$_4$·3H$_2$O, 0.311 g MgCl$_2$·6H$_2$O, 40 mL of HCl 1M, 0.293 g of CaCl$_2$, 0.072 g of Na$_2$SO$_4$ and 6.063 g of Tris were all dissolved in 1 L of miliQ water and adjusted to pH 7.4. Next 100 mL of this solution and 900 mL of miliQ water were used to prepare the corresponding SBS solutions of 5-HT. Then 500 µL of **1** were mixed with 500 µL of SBS solutions of 5-HT at an appropriate concentration, incubated for 3 min, and analysed by UV-vis spectrophotometry. Real human blood samples from a healthy volunteer were obtained thanks to the support of our university health centre. Firstly, red blood cells were separated from serum by centrifugation. Next, serum samples were further centrifuged (15 min, 11000 rpm) using Amicon Ultra-05 centrifugal filters units with Ultracel-10 membranes to remove proteins, and were mixed (1:1) with 50 mM Tris Buffer. Finally, 100 µL of **1** and 100 µL of the resulting serum samples (which either contained spiked 5-HT or did not) were incubated for 3 min and measured in the spectrophotometer (see the illustrated steps in Figure S13).

3. Results and Discussion

In order to prepare probe **1**, the citrate-stabilised AuNPs were firstly obtained following the Turkevich-Frens method, by reducing tetrachloroauric acid with trisodium citrate in boiling water [48-50]. Then, citrate was displaced from the surface of the nanoparticles in a ligand-exchange reaction by the simultaneous addition of NALC and DSP (1:1 molar ratio). Finally, the functionalised AuNPs **1** were purified by repeated centrifugation and redispersion in water.

The resulting bifunctionalised AuNPs were characterised by UV-Vis, transmission electron microscopy (TEM), dynamic light scattering (DLS), Fourier-transform infrared spectroscopy (FTIR) and proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) studies. The monodisperse bifunctionalised nanoparticles **(1)** were obtained with an average size of 15 nm as determined by TEM (Figure S1). The aqueous suspensions of **1** were red wine-coloured and showed a surface plasmon absorption peak at 521 nm, which perfectly agrees with the

typical colour and SPR band shown by those AuNPs smaller than 25 nm [51-52]. Typical concentrations of final probe **1** were calculated by UV-vis spectroscopy to be ca. 8.74 x 10^{-10} M from an estimated molar extinction coefficient of ε = 3.97 x 10^8 M^{-1} cm^{-1} [53]. The FTIR studies showed the appearance of absorption bands which were attributed to NALC (C=O stretching at 1649 cm−1, N-H bending at 1543 cm−1 and N-H stretching at 3352 cm−1), as reported in other studies [54], and DSP's C=O stretching also appeared at 1720 cm$^{−1}$ [55] (see Figure S3). ^{1}H-NMR spectra also showed the appearance of signals, which were attributed to the functionalisation with NALC and DSP. (see Figure S4). The red wine-coloured aqueous dispersions of **1** remained stable in the refrigerator for more than 1 month, and no changes were observed on the characteristic plasmon absorption band.

In order to evaluate the sensing capabilities of the prepared nanoparticles, buffered aqueous suspensions of **1** at pH 7.0 (Tris, 50 mM) were studied in the absence and presence of 5-HT (100 µM). Whereas the suspension of the nanoparticles remained red in the absence of 5-HT, addition of 5-HT induced a remarkable shift of the surface plasmon absorption from 521 nm to 605 nm, which resulted in a colour change from red to blue that could be observed by the naked eye, and indicated aggregation of AuNPs (see Figure 1). Aggregation of nanoparticles was not only confirmed by the instantaneous colour change, but also by TEM (vide infra) and DLS (see Figure S2). From the latter, it was found that the hydrodynamic diameter of the nanoparticles increased from 16.5 nm for **1** to 704 nm when 5-HT was added. The zeta potential reduced from -17.8 mV (**1**) to -6.14 mV (**1**+5-HT).
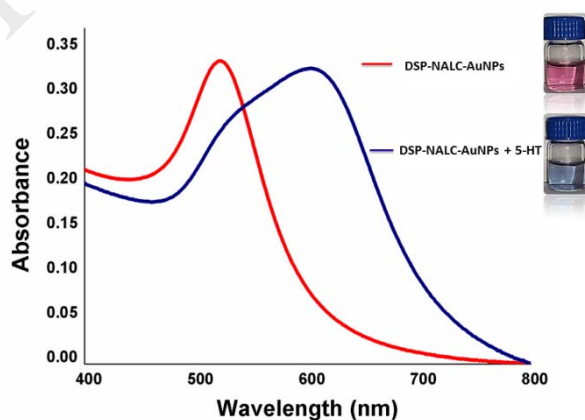
Figure 1. UV-Vis of **1** in the absence (red) and presence of serotonin (100 µM) (blue) at pH 7.0 (Tris, 50 mM).

In another step, the selectivity of the prepared functionalised AuNPs towards 5-HT was evaluated. The response of **1** in the presence of other neurotransmitters, e.g., dopamine (DA), epinephrine (Epy) and norepinephrine (NE), and of other biomolecules, e.g., L-tyrosine (L-Tyr, a precursor for stimulatory neurotransmitters), gamma-aminobutyric acid (GABA, an inhibitory neurotransmitter), L-cysteine (L-Cys), aspartic acid (AA) and glutamic acid (GA) (excitatory amino acids), uric acid (UA, described recently as a potential neurotransmitter) and oxalic acid (OA) was studied. Whereas a remarkable colour change was observed in the presence of 5-HT, negligible colour modulations and negligible changes in the A_{605}/A_{521} ratio (A_{605} = absorbance at 605 nm, A_{521} = absorbance at 521 nm) were observed upon the addition of potential interferents (see Figure 2a and 2b and Figure S5). In an additional competitive experiment, the response of **1** in a mixture that contained 5-HT and DA, Epy, NE, L-Tyr, GABA, L-Cys, UA, OA, AA and GA (500 µM of each) was also tested. As seen in Figure 2b, the observed response was extremely similar to that found when probe **1** was used alone with 5-HT. All these data indicate that the bifunctionalised AuNPs **1** are able to selectively detect 5-HT in buffered media, and in the presence of other neurotransmitters and selected biomolecules. One possible explanation for the good selectivity for 5-HT versus catecholamines (dopamine, epinephrine and norepinephrine) could be the different ionisation equilibria of the biogenic phenolic amines and catecholamines in biological media, with different proportions of zwitterionic species [55-56]. The selectivity of the probe in the presence of common inorganic cations and anions was also tested. Probe **1** remained stable in the presence of the different species individually and in a mixture that contained them all. Remarkably, the probe retained its capability to recognise 5-HT in the presence of all the inorganic species (see Figure S6).
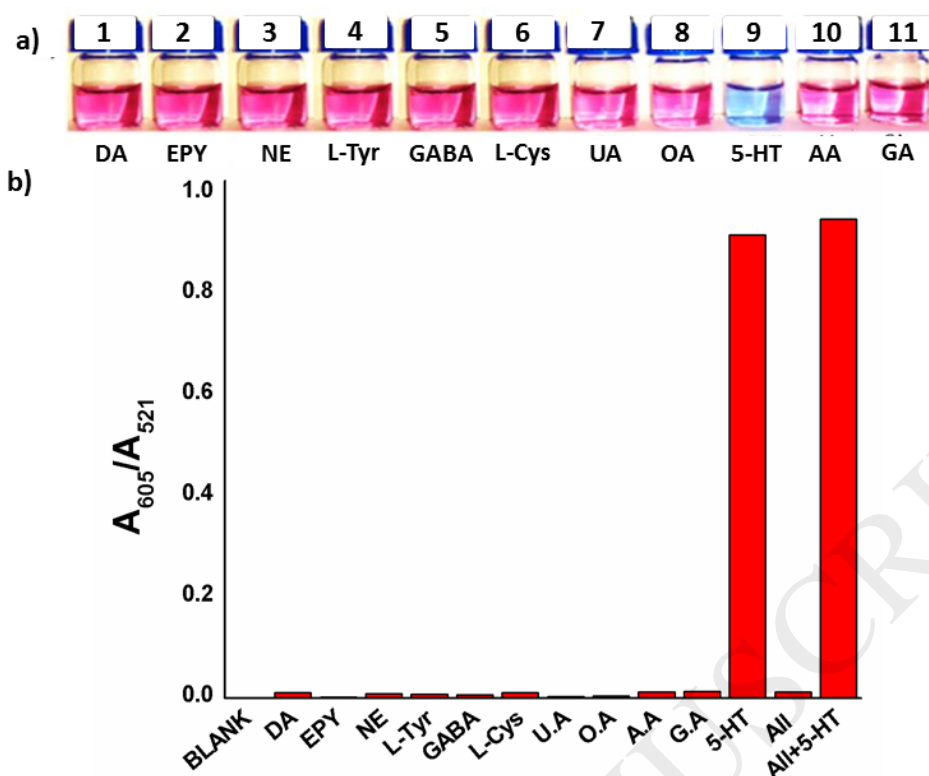
Figure 2. Response of **1** in the presence of different molecules (500 μM) at pH 7.0 (Tris, 50mM). **a)** Vials with the different tested molecules. **b)** Representation of A_{605}/A_{521} for interferences.

Furthermore, the sensitivity of **1** to 5-HT was evaluated. In this experiment, the colour changes of aqueous suspensions of **1** in the presence of different amounts of 5-HT were studied. As seen in Figure 3, a gradual colour modulation from red wine to purple, and finally to blue, which was dependent on the 5-HT concentration, was observed. This change coincides with the proposed sensing protocol that involves the 5-HT-induced aggregation of the bifunctionalised AuNPs. The changes in colour shown in Figure 3a agree with changes in the UV-vis bands observed for the different prepared suspensions of **1** in the presence of increasing amounts of 5-HT (see Figure 3b). An increase in the aggregation of AuNPs, according to the 5-HT concentration, was confirmed by the TEM studies (see Figure 3c). From the A_{605}/A_{521} *vs.* 5-HT concentration plot, a linear response within the 0-1 μM 5-HT concentration range was noted. From these studies a limit of detection (LOD) as low as 0.1 μM (17 ng mL^{-1}) was determined (see Figure S7). The calculated LOD indicates the noteworthy sensitivity of the probe, and confers our system a competitive basis to detect 5-HT at relevant clinical concentrations. In particular, reported studies have determined that 5-HT levels above 500 ng mL^{-1} (2.80 μM) in

blood serum can be indicative of carcinoid tumours, while values above 1,000 ng mL^{-1} are indicative of metastasis [58-62]. Other studies have found that 5-HT rises from a mean value of 767 ng mL^{-1} for those patients with carcinoid tumours to 1,720 ng mL^{-1} for the patients with carcinoid tumours who are also affected by heart disease [63]. Given the importance of determining the 5-HT concentration in blood serum, further studies into the response of probe **1** in simulated blood serum [40] were carried out. Probe **1** in simulated serum showed similar behaviour as described above in water when increasing amounts of 5-HT were added (see the Supplementary Data). From the A_{605}/A_{521} vs 5-HT concentration plots, a linear response in simulated serum within the 0 to 3 µM 5-HT concentration range was observed, and a LOD of 0.12 µM (23 ng mL^{-1}) was determined in this medium, which was lower than the normal range for adults of 50-300 ng mL^{-1} (0.28 to 1.70 µM) in blood [64-65]. Finally, encouraged by these results, we decided to test the performance of nanoparticles **1** in real human blood samples. Samples were taken from a healthy volunteer, and were centrifuged to remove red blood cells and proteins (see the Experimental section and Figure S13 for details). Nanoparticles were stable in serum samples and led to a change that was observed by the naked eyed when serum was spiked with 1 mM of 5-HT (see Figure S14 A). A remarkably significant increase in the A_{605}/A_{521} values was observed from normal serum to serum containing 10 µM of 5-HT (indicative of carcinoid tumours) (see Figure S14-B).
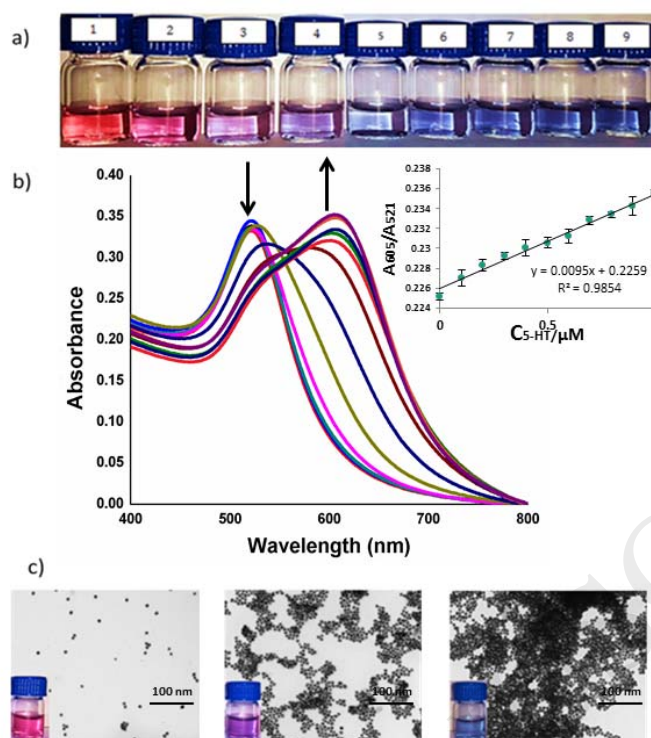
Figure 3. a) Colorimetric visualisation of probe **1** when 5-HT was added at concentrations of 0, 0.9, 10, 20, 30, 40, 50, 70, 100 μM (1-9, respectively). **b)** Changes in the UV-Vis spectra of probe when 5-HT was added at concentrations of 0, 0.9, 10, 20, 30, 40, 50, 70, 100 μM. Inset: plot of the A_{605}/A_{521} vs. 5-HT concentrations within the 0-1 μM range. **c)** TEM images of **1** with 0, 40 and 150 μM of 5-HT, respectively. Experiments were performed at a buffered pH 7.0 (Tris, 50 mM).

4. Conclusions

In summary, we report herein a new method for the colorimetric detection of 5-HT using 15 nm AuNPs bifunctionalized with dithiobis(succinimidylpropionate) and N-acetyl-L-cysteine. The prepared nanoparticles, **1**, selectively detect 5-HT in water at pH 7 (Tris, 50mM) via the 5-HT-induced aggregation of the bifunctionalised AuNPs which results in a remarkable colour change. The probe is selective to 5-HT and no colour modulation was observed in the presence of other neurotransmitters (i.e. dopamine, epinephrine, norepinephrine) and selected biomolecules (i.e. L-tyrosine, gamma-aminobutyric acid, L-cysteine, uric acid, oxalic acid, aspartic acid and glutamic acid). A linear response within the 0-1 μM 5-HT concentration range and a LOD as low as 0.1 μM were determined in buffered water at pH 7 (Tris, 50 mM). A similar

response of probe **1** to 5-HT was observed in simulated blood serum with a linear response within the 0-3 μM 5-HT concentration range and with a LOD as low as 0.12 μM. Finally, the probe was capable of detecting 5-HT in human blood samples and of distinguishing between normal 5-HT levels and 5-HT levels that are indicative of disease. Given the importance of serotonin in different diseases, we expect that our findings could help to elucidate the role played by serotonin and to develop innovative tools for early point-of-care or personalised diagnosis applications.

Appendix A. Supplementary Data

Supplementary data associated with this article can be found, in the online version, at…

References

[1] T. Canli, K.P. Lesch, Long story short: the serotonin transporter in emotion regulation and social cognition, Nat. Neurosci. 10 (2007) 1103-1109.

[2] J. M. Yano, K. Yu, G. P. Donaldson, G. G. Shastri, P. Ann, L. Ma, C. R. Nagler, R. F. Ismagilow, S. K. Mazmanian, E. Y. Hsiao, Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis, Cell 161 (2015) 264-276.

[3] M. Camilleri, Serotonin in the gastrointestinal tract, Curr. Opin. Endocrinol. Diabetes Obes. 16 (2009) 53-59.

[3] J. R. Lacasse, J. Leo, Serotonin and depression: a disconnect between the advertisements and the scientific literature, PLoS Med. 2 (2005) e392.

[4] E. H. Cook Jr, B. L. Leventhal, The serotonin system in autism, Curr. Opin. Pediatr. 8 (1996) 348-354.

[5] J. M. Zuetenhorst, B. G. Taal, Neuroendocrine tumors in decompensated liver disease, Oncologist 10 (2005) 123-131.

[6] J. M. Feldman, Urinary serotonin in the diagnosis of carcinoid tumors, Clin. Chem. 32 (1986) 840-844.

[7] S. H. Fox, R. Chuang, J. Brotchie, Serotonin and Parkinson's disease: On movement, mood, and madness, Mov. Disord. 24 (2009) 1255-1266.

[8] M. Politis, F. Niccolini, Serotonin in Parkinson's disease, Behav. Brain Res. 277 (2015) 136-145.

[9] M. Butzlaff, E. Ponimaskin, The role of serotonin receptors in Alzheimer's disease, Opera Med. Physiol., 2 (2016) 77-86.

[10] M. Maillet, S. J. Robert, F. Lezoualc'h, New insights into serotonin 5-HT4 receptors: a novel therapeutic target for Alzheimer's disease?, Curr. Alzheimer Res. 1 (2004) 79-85.

[11] C. P. Bearcroft, M. J. G. Farthing, D. Perrett, Determination of 5-hydroxytryptamine, 5-hydroxyindoleacetic acid and tryptophan in plasma and urine by HPLC with fluorimetric detection, *Biomed. Chromatogr.* 9 (1995) 23-27.

[12] A. Gironi, G. Seghieri, M. Niccolai, P. Mammini, Simultaneous liquid-chromatographic determination of urinary vanillylmandelic acid, homovanillic acid, and 5-hydroxyindoleacetic acid, Clin. Chem. 34 (1998) 2504-2506.

[13] O. C. Ingebretsen, A. N. Bakken, M. Farstad, Liquid chromatography of serotonin and adenine nucleotides in blood platelets, illustrated by evaluation of functional integrity of platelet preparations, Clin. Chem. 31 (1985) 695-698.

[14] P. C. Gunaratna, K. K. Cadle, C. B. Kissinger, An improved liquid chromatographic method with electrochemical detection for direct determination of serotonin in microdialysates from Caudate-putamen and pineal gland regions of rat brain, J. Neurosci. Methods 155 (2006) 143-148.

[15] B. A. Patel, M. Arundell, K. H. Parker, M. S. Yeoman, D. O'Hare, Simple and rapid determination of serotonin and catecholamines in biological tissue using high-performance liquid chromatography with electrochemical detection, J. Chromatogr. B 818 (2005) 269-276.

[16] A. Vaarmann, A. Kask, U. Maeorg, Novel and sensitive high-performance liquid chromatographic method based on electrochemical coulometric array detection for simultaneous determination of catecholamines, kynurenine and indole derivatives of tryptophan, J. Chromatogr. B 769 (2002) 145-153.

[17] F. Mashige, Y. Matsushima, C. Miyata, R. Yamada, H. Kanazawa, I. Sakuma, N. Takai, N. Shinozuka, A. Ohkubo, K. Nakahara, Simultaneous determination of catecholamines, their basic metabolites and

serotonin in urine by high-performance liquid chromatography using A mixed-mode column and an eight-channel electrochemical detector, Biomed. Chromatogr. 9 (1995) 221-225.

[18] K. Wu, J. Fie, S. Hu, Simultaneous determination of dopamine and serotonin on a glassy carbon electrode coated with a film of carbon nanotubes, Anal. Biochem. 318 (2003) 100-106.

[19] B. E. K. Swamy, B. J. Venton, Carbon nanotube-modified microelectrodes for simultaneous detection of dopamine and serotonin in vivo, Analyst 132 (2007) 876-884.

[20] J. Li, X. Lin, Simultaneous determination of dopamine and serotonin on gold nanocluster/overoxidized-polypyrrole composite modified glassy carbon electrode, Sens. Actuators B Chem. 124 (2007) 486-493.

[21] R. N. Goyal, V. K. Gupta, M. Oyama, N. Bacheti, Gold nanoparticles modified indium tin oxide electrode for the simultaneous determination of dopamine and serotonin: Application in pharmaceutical formulations and biological fluids, Talanta, 72 (2007) 976-983.

[22] Y. Li, X. Huang, Y. Chen, L. Wang, X. Lin, Simultaneous determination of dopamine and serotonin by use of covalent modification of 5-hydroxytryptophan on glassy carbon electrode, Microchim. Acta 164 (2009) 107-112.

[23] Y. Sun, J. Fei, J. Hou, Q. Zhang, Y. Liu, B. Hu, Simultaneous determination of dopamine and serotonin using a carbon nanotubes-ionic liquid gel modified glassy carbon electrode, Microchim. Acta 165 (2009) 373-379.

[24] S. K. Kim, S. Jeon, Simultaneous determination of serotonin and dopamine at the PEDOP/MWCNTs–Pd nanoparticle modified glassy carbon electrode, J. Nanosci. Nanotechnol. 12 (2012) 1903-1909.

[25] M. I. Nichkiva, H. Huisman, P. M. Wynveen, D. T. Marc, k. L. Olson, G. H. Kellermann, Evaluation of a novel ELISA for serotonin urinary serotonin as potential biomarker for depression, Anal. Biochem. Chem. 402 (2012) 1539-1600.

[26] T. Ramon-Marquez, A. Medina-Castillo, A. L. Fernandez-Gutierrez, J. F. Fernandez-Sanchez, A novel optical biosensor for direct and selective determination of serotonin in serum by Solid Surface-Room Temperature Phosphorescence, Biosens. Biolectron. 82 (2016) 217-223.

[27] J. H. Thompson, C. A. Spezia, M. Angulo, Fluorometric detection of serotonin using o-phthaldialdehyde: an improvement, Experientia. 26 (1970) 327-329.

[28] P Frattini, M. L. Cucchi, G. Santagostino, G. L. Corona, A sensitive fluorimetric method for determination of platelet-bound and plasma free serotonin, Clin. Chim. Acta. 92 (1979) 353-360.

[29] R. M. Fleming, W. G. Clark, Single extraction method for the simultaneous fluorometric determination of serotonin, dopamine and norepinephrine in brain, Anal. Chem, 37 (1965) 692-696.

[30] G. Gobbi, P. Mirandola, P. L. Tazzari, F. Ricci, L. Caimi, A. Cacchioli, M. Vitale, Flow cytometry detection of serotonin content and release in resting and activated platelets. Br. J. Haematol 121 (2003) 892-896.

[31] Consolazione, C. Milstein, B. Wright, A. C. Cuello, Immunocytochemical Detection of Serotonin with Monoclonal Antibodies, J. Histochem. Cytochem, 29 (1981) 1425-1430.

[32] C. S. Kim, M. C. McNamara, J. M. Lauder, E. E. Lawson, Immunocytochemical detection of serotonin content in raphe neurons of newborn and young adult rabbits before and after acute hypoxia, Int. J. Dev. Neurosci 12 (1994) 499-505.

[33] C. Sur, H. Betz, P. Schloss, Immunocytochemical detection of the serotonin transporter in rat brain. Neuroscience 73 (1996), 217-231.

[34] L. E. Santos-Figueroa, M. E. Moragues, E. Climent, A. Agostini, R. Martínez-Mañez, F. Sancenón, Chromogenic and fluorogenic chemosensors and reagents for anions. A comprehensive review of the years 2010–2011, Chem. Soc. Rev. 42 (2013) 3489-3613.

[35] K. Saha, S. S. Agasti, C. Kim, X. Li, V. M. Rotello, Gold nanoparticles in chemical and biological sensing, Chem. Rev. 112 (2012) 2739-2779.

[36] K. M. Mayer, J. H. Hafner, Localized surface plasmon resonance sensors, Chem. Rev. 111 (2011) 3828-3857.

[37] E. Boisselier, D. Astruc, Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity. Chem. Rev. 38 (2009) 1759-1782.

[38] K. A. Rawat, J. R. Bhamore, R. K. Singhal, S. K. Kailasa, Microwave assisted synthesis of tyrosine protected gold nanoparticles for dual (colorimetric and fluorimetric) detection of spermine and spermidine in biological samples, Biosens. Bioelectron. 88 (2017) 71-77.

[39] K. A. Rawat, R. K Singhal, S. K. Kailasa, Colorimetric and fluorescence "turn-on" methods for the sensitive detection of bromelain using carbon dots functionalized gold nanoparticles as a dual probe, RSC Adv. 6 (2016) 32025-32036.

[40] K. A. Rawat, S. K Kailasa, 2, 3, 4-Trihydroxy benzophenone as a novel reducing agent for one-step synthesis of size-optimized gold nanoparticles and their application in colorimetric sensing of adenine at nanomolar concentration. RSC Adv. 6 (2016) 11099-11108.

[41] A. Martí, A. M. Costero, P. Gaviña, M. Parra, Selective colorimetric NO (g) detection based on the use of modified gold nanoparticles using click chemistry, Chem. Commun. 51 (2015) 3077-3079.

[42] J. V. Rohit, S. K. Kailasa, Simple and selective detection of pendimethalin herbicide in water and food samples based on the aggregation of ractopamine-dithiocarbamate functionalized gold nanoparticles, Sens Actuators B Chem. 245 (2017) 541-550.

[43] L. Chena, W. Lua, X. Wanga, L. Chen, A highly selective and sensitive colorimetric sensor for iodide detection based on anti-aggregation of gold nanoparticles, Sens Actuators B Chem. 182 (2013) 482-488.

[44] B. Kong, A. Zhu, Y. Luo, Y. Tian, Y. Yu, G. Shi, Sensitive and selective colorimetric visualization of cerebral dopamine based on double molecular recognition, Angew. Chem. 123 (2011) 1877-1880.

[45] H. Su, Q. Zheng, H. Li, Colorimetric detection and separation of chiral tyrosine based on N-acetyl-L-cysteine modified gold nanoparticles, J. Mater. Chem. 22 (2012) 6546-6548.

[46] V. A. Turek, M. P. Cecchini, J. Paget, A. R. Kucernak, A. A. Kornyshev, J. B. Edel, Plasmonic ruler at the liquid–liquid interface, ACS nano 6 (2012) 7789-7799.

[47] A. Oyane, H. M. Kim, T. Furuya, T. Kokubo, T. Miyazaki, T. Nakamura, Preparation and assessment of revised simulated body fluids, J. Biomed. Mater. Res. Part A 65 (2003) 188-195.

[48] P. Zhao, N. Li, D. Astruc, State of the art in gold nanoparticle synthesis, Coord. Chem. Rev. 257 (2013) 638-665.

[49] J. A. Turkevich, A study of the nucleation and growth processes in the synthesis of colloidal gold Discuss. Faraday Soc. 11 (1951) 55-75.

[50] G. Frens, Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions, Nature 241 (1973) 20-22.

[51] W. Haiss, N. T. K. Thanh, J. Aveyard, D. G. Fernig, Determination of size and concentration of gold nanoparticles from UV− Vis spectra, Anal. Chem. 79 (2007) 4215-4221.

[52] S.-Y. Lin, Y.-T. Tsai, C.-C. Chen, C.-M. Lin, C.-H. Chen, Two-step functionalization of neutral and positively charged thiols onto citrate-stabilized Au nanoparticles, *J. Phys. Chem. B* 108 (2004) 2134-2139.

[53] X. Liu, M. Atwater, J. Wang, Q. Huo, Extinction coefficient of gold nanoparticles with different sizes and different capping ligands, Colloids Surf. B 58 (2007) 3-7.

[54] Y. Zhang, M. Yan, S. Wang, J. Jiang, P. Gao, G. Zhang, C. Dong, S. Shuang, Facile one-pot synthesis of Au(0)@Au(I)-NAC core-shell nanoclusters with orange-yellow for cancer cell imaging, RSC Adv. 6 (2016) 8612-8620.

[55] C. S. Stan, C. Albu, A. Coroaba, M. Popa, D. Sutiman, One step synthesis of fluorescent carbon dots through pyrolysis of N-hydroxysuccinimide. J. Mater. Chem. C. 3 (2015) 789-795.

[56] J. Armstrong, R. B. Barlow, The ionization of phenolic amines, including apomorphine, dopamine and catecholamines and an assessment of zwitterion constant, Br J Pharmacol. 57(1976) 501-516.

[57] G. Rudnick, K. L. Kirk, H. Fishkes, S. Schuldine, Zwitterionic and anionic forms of a serotonin analog as transport substrates, J. Biol. Chem., 264(1989), 14865-14868.

[58] P. C. Emson, R. F. Gilbert, H. Martensson, A. Nobin, Elevated concentrations of substance p and 5-HT in plasma in patients with carcinoid tumors, Cancer 54 (1984) 715-718.

[59] E. Pussard, N. Guigueno, O. Adam, J.-F. Giudicelli, Validation of HPLC-amperometric detection to measure serotonin in plasma, platelets, whole blood, and urine, *Clin. Chem.* 42 (1996) 1086-1091.

[60] D. O'Toole, M. Ducreux, G. Bommelaer, J.-L. Wemeau, O. Bouché, F. Catus, J. Blumberg, P. Ruszniewski, Treatment of carcinoid syndrome, Cancer 88 (2000) 770-776.

[61] R. H. Resnick, S. J. Gray, Serotonin metabolism and the carcinoid syndrome: a review, Med. Clin. North Am., 44 (1960) 1323-1339.

[62] F. V. Plapp, ClinLab Navigator, http://www.clinlabnavigator.com/carcinoid-syndrome.html, (accessed March 2017).

[63] P. A. Robiolio, V. H. Rigolin, J. S. Wilson, J. K. Harrison, L. L. Sanders, T. M. Bashore, J. M. Feldman, Carcinoid heart disease, Circulation 92 (1995) 790-795.

[64] Laura J. Martin, MedlinePlus, https://medlineplus.gov/ency/article/003562.htm, (accessed July 2017).

[65] Mayo Clinic, http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/84373, (accessed July 2017).

Author Biographies

Tania M. Godoy-Reyes was born in 1993 in Cuyamel, Honduras. She graduated in Natural Science from the National Pedagogical University Francisco Morazán (UPNFM) in 2014. She received a Master's Degree in Sensors for Industrial Applications in 2016 from the Polytechnic University of Valencia (UPV). She received a PhD grant from Generalitat Valenciana "Santiago Grisolía fellowship". Currently, she is a PhD candidate at IDM Research Institute at the Polytechnic University of Valencia (UPV). Her main area of research interest is the development of new fluoro-chromogenic sensors for the detection of neurotransmitters in biological fluids.

Antoni Llopis-Lorente studied Chemistry at the University of Valencia and graduated with honors in 2013. Currently, he is a PhD candidate at the Polytechnic University of Valencia (UPV) under the supervision of Prof. Ramón Martínez-Mañez. He has carried out research stays at the Complutense University of Madrid (Spain) and the Radboud University (the Netherlands) in the group of Prof. Reynaldo Villalonga and the group of Prof. Daniela Wilson, respectively. His main research interests are to develop enzyme-based nanodevices and nanoparticles for sensing, drug delivery and chemical communication.

Ana M. Costero received her PhD in 1982 in Valencia (Spain). After a postdoctoral appointment in Pittsburg (USA) in the Rebek group, she began her independent research career. She became a full professor in 1994. She is a member of the Interuniversity Research Institute for Molecular Recognition and Technological Development (IDM) of UV-UPV. Her research interest includes design, synthesis and evaluation of chemosensors for specific recognition of cations, anions and neutral biologically or environmentally important species.

Félix Sancenón graduated in chemistry at the University of Valencia (UV) in 1991, and he received his PhD in 2003 at the Polytechnic University of Valencia (UPV). After a postdoctoral period at the University of Pavia (Italy), he joined the Department of Chemistry at the UPV in 2005. He became lecturer in 2006. His main areas of interest fall in the field of chromo-fluorogenic sensors and molecular probes and in the development of hybrid organic-inorganic materials with applications in recognition processes.

Pablo Gaviña is associate professor of Organic Chemistry at the University of Valencia since 2008. After receiving his PhD from the University of Valencia in1994, he joined the Sauvage group at Strasbourg, (France) as a postdoctoral researcher (1995–1997). In 2010, he joined the group headed by A. Costero. He is a member of the Interuniversity Research Institute for Molecular Recognition and Technological Development (IDM) of UV-UPV. His current research interests include host-guest chemistry, molecular recognition and the development of chemical sensors and probes for ions and neutral biologically relevant molecules.

Ramón Martínez-Mañez graduated in chemistry from the University of Valencia (UV) in 1986, and received his PhD in 1990 at the same university. After a postdoctoral period at Cambridge (UK), he joined the Department of Chemistry at the UPV. He became a full professor in 2002. His main areas of interest lie in the field of chromo-fluorogenic probes for anions, cations and neutral chemical species and the design of nanoparticles for controlled release of drugs.