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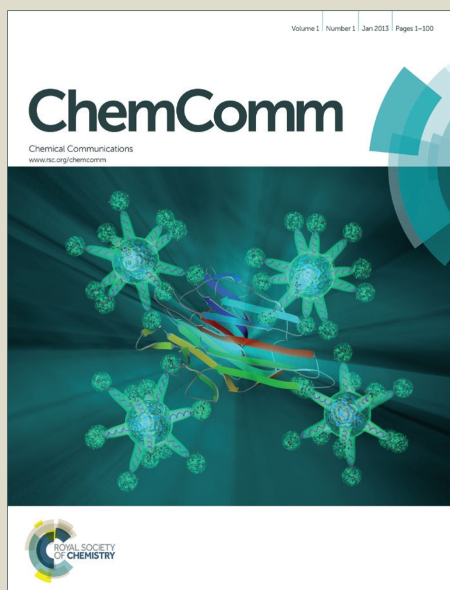

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COMMUNICATION

A fluorescent probe for ecstasy

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A nanostructure formed by the insertion in silica nanoparticles of a pyrene-derivatized cavitand, able to specifically recognize ecstasy in water, is presented. The absence of effects from interferents and an efficient electron transfer process occurring after complexation of ecstasy, makes this system an efficient fluorescent probe for this popular drug.

Amphetamine-type-stimulants (ATS), of which 3,4-methylenedioxy-methamphetamine (MDMA, widely known as "ecstasy") is the most popular version, are synthetic psychoactive compounds altering the serotonin and dopamine levels in the brain synapses. Their addictiveness can be dramatically enhanced by tinkering with their molecular structure in key places.[1] The synthetic permutations not only boost psychotropic properties, but allow also to circumvent existing regulations and drug tests. Therefore the consumption of ATS and of the related "designer drugs" has become a social problem of utmost relevance. The 2014 World Drugs Report[2] states that the 5.2 per cent of the world population had used a substance belonging to the cannabis, opioid, cocaine or ATS group (including MDMA), at least once in the previous year.

Design of portable sensors for a rapid on-site detection and quantification of these analytes is therefore a timely and crucial task, and two issues should be considered to address it: the identification of 'designer drugs' along with that of banned ones and the problem of the interferents. The first challenge is related to the diffusion of these new synthetic analogues manufactured in clandestine laboratories that is very large (348 such substances in December 2013) clearly exceeding the number of psychoactive substances controlled at the international level (234 substances).[2]

For this reason, the ability to detect chemical similarity of the controlled drugs is a fundamental asset for a sensor. The other issue is that the "street samples", the real substances that are sold on the streets, are cut with excipients that can act as interfering agents in the recognition process. So far, MDMA recognition has been mainly based on immunoassay methods,[3] with very few attempts to use artificial hosts.[4]

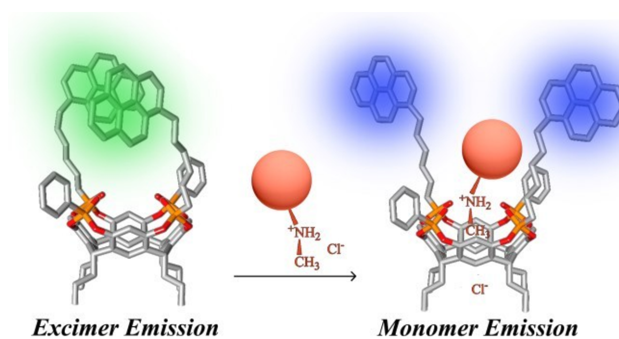

Fig. 1 Proposed recognition mode of the designed chemosensor.

In this paper, we report a new nanoarchitecture based on tetraphosphonate cavitand embedded in nanoparticles, able to sense the whole class of methamphetamines and related designer drugs in water, but giving a unique, distinctive, response towards MDMA.

The ability of the **Tiiii** cavitand receptor to recognize specifically the *N*-methyl ammonium hydrochloride group in water has been recently demonstrated both for drugs,[5] and *N*-methylated amino acids.[6] In order to create a more specific receptor, we designed a new chemosensor able to differentiate methamphetamines from other

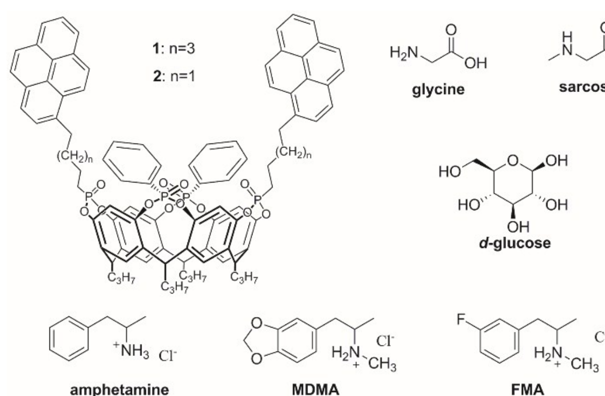

Chart 1 Chemical structures of the synthesized **Tiiii** receptors and tested analytes (drugs and interferents).

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N-methylated ammonium molecules, such as sarcosine, exploiting the bulky groups characteristic of methamphetamines. To this purpose, we synthesized a couple of tetraphosphonate cavitands, each one equipped with two pyrene moieties on the top of the cavity in distal position. Proximity and orientation of the fluorophores are designed to yield optimal overlap for the formation of the excimer acting as transducer element.[7] In this way, the inclusion inside the cavitand of a proper bulky *N*-methyl ammonium guest perturbs the excimer formation causing the monomer emission (Figure 1). The choice of the length of the tethers is pivotal for the selectivity of the chemosensor. Following molecular modelling, we designed two cavitand probes, featuring respectively hexyl (1) and butyl (2) chains as tether (Chart 1).

The preparation required a convergent synthesis: two fragments were synthesized, diphosphonate resorcinarene (**VIII**) and the phosphonic dichloride bridging reagent (**VI** or **XVI**), that were joined together through a stereoselective reaction using as templating agent *N*-methyl pyrrolidine (see ESI for the synthesis).[8]

Fluorescent spectra (λ_{exc} 330 nm) of **2** in various solvents, however, showed only the monomer emission band (Figure 2 black line), indicating that this tether is not sufficiently long to promote the π-stacking of the pyrene groups and the consequent formation of the excimer. The fluorescence spectra in dichloromethane (λ_{exc} 330 nm, Figure 2, red line) of **1** instead validated our design, since in this case the emission band of the excimer, between 450 and 550 nm, can be clearly observed.

A drawback of **1** is its insolubility in water: due to its marked hydrophobicity, it forms an unstable dispersion of aggregates that tend to precipitate in few hours. To overcome this problem, taking profit of the possibilities offered by nanotechnology in sensing applications,[9] we loaded cavitand **1** in multi-compartment water soluble organo-silica core modified Pluronic® F127-Silica NanoParticles (PluS NPs). These nanoparticles present, together with an organo-silica core and an outer hydrophilic PEG shell, responsible for the stability of their dispersion, an enhanced hydrophobic internal compartment. This is similar to the one that has been already used to host hydrophobic dyes,[10] photo-switches[11] and other chemical sensors[12] for different applications.[13] The use of a confined environment in this case was also conceived to favor the formation of an intramolecular excimer.[14] A significant advance in the hosting ability of PluS NPs towards hydrophobic molecules was obtained by employing a modified silica precursor (1,2-bis(triethoxysilyl)ethane), in combination with the commonly used tetraethoxysilane (TEOS) with a 3:1 molar ratio. The cavitand is indeed expected to be hosted in the accessible hydrophobic region of Plus NPs, far from water, i.e. in the innermost region of the PEG shell, close to the PPO brushes, which are in turn embedded into the impenetrable silica nuclei. We found that increasing the hydrophobicity of the organo-silica matrix resulted in an enhanced efficiency of the cavitand inclusion process. Dynamic light scattering (DLS) data and the fluorescence spectra of a dispersion of an equimolar solutions of **1** and PluS NPs (**1@PlusNPs**) showed that they are stable for days, while a lower stability was observed using the NPs prepared using TEOS as a precursor.

The much higher stability of **1** in the presence of PluS NPs suggests that a substantial inclusion of cavitand in the NPs has taken place. Further proof of the absence of free aggregates of **1** in water were obtained from DLS measurements of **1@PlusNPs** (see ESI, Figure S1) that showed only the typical, very narrow peak of the NPs, indicating the absence in these conditions of self-aggregation of **1** due to its loading into the nanoarchitecture. Furthermore, we demonstrated that cavitand **1** is hosted by PluS NPs by using rhodamine doped PluS NPs:[15] in this system, efficient energy transfer was observed from **1** to the embedded rhodamine, which resulted in substantial quenching of the excimer emission (see ESI, Figure S3). At the low dye concentration used in this study, occurring FRET proves that donor (**1**) and acceptor (rhodamine) are embedded in the same nanostructure, only few nanometers apart.

As it can be observed from Figure 2, the inclusion of **1** in PluS NPs leads to another significant advantage, i.e. a striking enhancement of the excimer formation and emission, ascribable to polarity and confinement effects. The fluorescence spectrum of **1@PlusNPs** in water clearly shows a large contribution from the excimeric emission, indicating that most of the excited states of pyrene decay to their ground state through the formation of excimers.

These results suggest that NPs synthesized using 1,2-bis(triethoxysilyl)ethane provide the best starting condition for fluorescence sensing: the aggregation of the chemosensor is satisfactorily avoided, and a stable and intense excimer emission is obtained.

We tested chemosensor **1** in aqueous solution towards amphetamine and MDMA hydrochlorides, as ATS archetypes, and 3-fluoro-methamphetamine (FMA) hydrochloride, as "designer drugs". The FMA analysis was performed directly on the seized street sample, containing ≈45% of drug, the remaining being glucose.[5] We then selected three control compounds in order to rule out possible interferences in the sensing procedures: (i) glucose, since it is commonly used as the excipient in street samples, (ii) glycine, as an amine which is not hosted by the cavitand, and (iii) sarcosine as

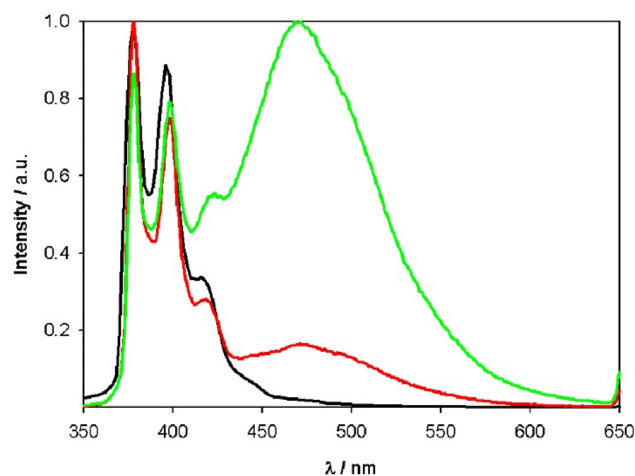

Fig. 2 Fluorescence spectra (λ_{exc} 330 nm) of chemosensor **2** (butyl tethers) 2x10^{-6} M in DCM (black line), of **1** (hexyl tethers) 2x10^{-6} M in DCM (red line) and of a dispersion of **1@PlusNPs** in H_2O ([**1**] = 1x10^{-6} M, green line).

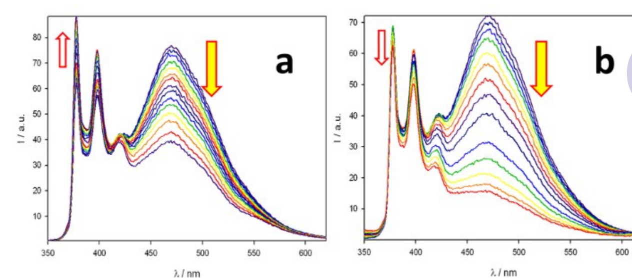

Fig. 3 Fluorescence spectra (λ_{exc}=330 nm) of **1@PlusNPs** in H_2O ([**1**]=1x10^{-6} M) upon addition of increasing amounts of (a) FMA and (b) of MDMA hydrochlorides (0-0,0025 M).

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molecule that is recognized by the receptor,[6] but should be unable to trigger any response, lacking a bulky unit.

Upon addition of FMA (Figure 3a), amphetamine, and MDMA (Figure 3b) hydrochlorides to a solution of **1@PlusNPs**, we observed large spectral changes, while control compounds sarcosine, glucose and glycine did not substantially affect the emission signal of the pyrene moieties (Figure 4a). In particular, FMA and amphetamine deplete the excimer emission of **1@PlusNPs** and enhance the monomeric emission accordingly. This can be attributed to the bulkiness of the aromatic moiety of methamphetamines which, hosted in the cavity, reduce the rate of formation of excimers. The lifetime of residual excimeric emission is not affected, suggesting that the bulky guest hampers the excimer formation but not its relaxation to the ground state. Noteworthy, MDMA induces a much stronger quenching, accompanied by a concomitant dramatic decrease of the luminescence lifetime, observed both on the excimer and on the monomer emissions: monomer decay is in fact shortened from 7.2 ns to 2.6 ns, while excimer decay is depleted from 17.7 ns to 4.2 ns (Figure 4b). Such observations prove the onset of an efficient deactivation pathway which quenches both the excimer and monomer excited states, and which thus operates beyond the simple hindrance of the guest. Since the energy transfer process from pyrene to MDMA is thermodynamically forbidden, such quenching pathway can be attributed to an efficient electron-transfer process involving the benzodioxole unit present in the MDMA molecule.

The presence of two different signals -monomeric and excimeric emissions- increase the information content that can be obtained from the analysis of fluorescence spectra. First, the ratiometric spectral response (see ESI, Figure S4) allows for calibrationless measurements; furthermore, the specific monomer-excimer ratio can discriminate various analytes and in particular MDMA. It is to be underlined that the system **1@PlusNPs** does not respond to sarcosine, which is strongly bonded by tetraphosphonate cavitands,[6] supporting the proposed detection mechanism.

All the results presented so far indicate that the system **1@PlusNPs** is able to sense in water different methamphetamines, but gives a unique, distinctive, response towards MDMA. The presence of MDMA can be clearly identified by looking at the intensity of the excimeric emission and, as additional signal, by the analyses of the excited state lifetime. In all other cases the presence of the drug can be identified looking at the increase of the intensity of the monomeric band, with possibility to use the excimer band, through a ratiometric approach, to avoid any calibration needs.

In conclusion, a new pyrene-functionalized cavitand has been synthesized to detect illicit drugs. This probe merges the complexation properties of the **Tiiii** cavitand with the unique luminescent properties of the pyrene excimer that acts as transducer element. As a consequence, we boosted the selectivity of the cavitand introducing steric hindrance as an additional discrimination point. The cavitand has been successfully loaded in PluS NPs to form a nanoarchitecture, which has been tested in water with three different illicit drugs containing *N*-methylated groups and three different interferents. These last species did not change the emission of the excimer. Of particular interest is the fact that sarcosine, although strongly complexed by the cavitand, does not bring apart the pyrene moieties since it does not possess a bulky group in its skeleton, confirming the effectiveness of the design. By contrast, the fluorescence experiments demonstrated that the inclusion of drugs depleted the excimer emission due to their steric hindrance. Particularly appealing is the response towards MDMA. This molecule showed the stronger effect due to the combination of its steric hindrance and an efficient electron-transfer process involving the aromatic units of the pyrene and the benzodioxole moiety, demonstrating that **1** is an efficient probe for MDMA.

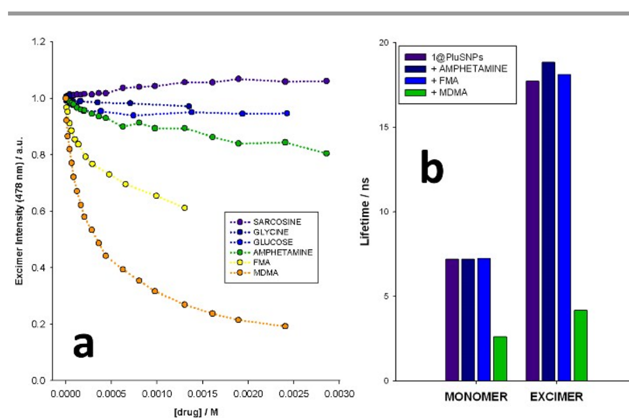

Fig. 4 (a) Fluorescence intensity in the excimer band (λ_{exc}=330 nm; λ_{em}=475 nm) of **1@PlusNPs** in H_2O ([**1**]=1x10^{-6} M) upon addition of increasing amounts of guests as hydrochloride. (b) Lifetimes of monomer and excimer emission before and after addition of amphetamine, FMA or MDMA.

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Notes and references

1 E. Underwood, *Science*, 2015, **347**, 469.
2 http://www.unodc.org/documents/wdr2014/World_Drug_Report_2014_web.pdf
3 D. Butler, M. Pravda and G. G. Guilbault, *Anal. Chim. Acta*, 2006, **556**, 333; I. Vikholm-Lundin, S. Auer and A.-C. Hellgren, *Sens. Actuator B,* 2011, **156**, 28; Y.-C. Tseng, J.-S. Chang, S. Lin, S. D. Chao and C.-H. Liu, *Sens. Actuator A,* 2012, **182**, 163.
4 D. Moreno, B. Díaz de Greñu, B. García, S. Ibeas and T. Torroba, *Chem. Commun.,* 2012, **48**, 2994; F. Reviriego, P. Navarro, E. García-España, M. T. Albelda, J. C. Frías, A. Domènech, M. J. R. Yunta, R. Costa and E. Ortí, *Org. Lett.* 2008, **10**(*22*), 5099.
5 E. Biavardi, S. Federici, C. Tudisco, D. Menozzi, C. Massera, A. Sottini, G. G. Condorelli, P. Bergese and E. Dalcanale, *Angew. Chem., Int. Ed.,* 2014, **53**, 9183.
6 E. Biavardi, C. Tudisco, F. Maffei, A. Motta, C. Massera, G. G. Condorelli and E. Dalcanale, *Proc. Natl. Acad. Sci USA,* 2012, **109**(*7*), 2263.
7 H. Matsumoto and S. Shinkai, *Tetrahedron Lett.*, 1996, **37**, 77-80; J. S. Kim and D. T. Quang, *Chem. Rev.,* 2007, **107**, 3780; M. H. Filby, S. J. Dickson, N. Zaccheroni, L. Prodi, S. Bonacchi, M. Montalti, M. J. Paterson, T. D. Humphries, C. Chiorboli and J. W. Steed, *J. Am. Chem. Soc.*, 2008, **130**, 4105-4113; J. Shirtcliff, H. Xu, F. Diederich *Eur. J. Org. Chem.,* **2010**, 846; L. You, D. Zha and E. V. Anslyn, *Chem. Rev.,* 2015, **115**, *in press* (DOI:10.1021/cr5005524).
8 P. Delangle and J. P. Dutasta, *Tetrahedron Lett.,* 1995, **36** 9325.

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9 S. Yang, J. Sun, P. He, X. Deng, Z. Wang, C. Hu, G. Ding and X. Xie, *Chem. Mater.* 2015, **27**, 2004–2011; T. Zhang, S. Yang, J. Sun, X. Li, L. He, S. Yan, X. Kang, C. Hu and F. Liao, *Synthetic Metals*, 2013, **181,** 86– 91; M. Montalti, E. Rampazzo, N. Zaccheroni and L. Prodi, *New J. Chem.*, 2013, **37**, 28-34; S. Yang, J. Sun, X. Li, W. Zhou, Z. Wang, P. He, G. Ding, X. Xie, Z. Kang and M. Jiang, *J. Mater. Chem. A*, 2014, **2**, 8660–8667; F. Liao, X. Song, Siwei Yang, C. Hu, L. He, Shuai Yan and G., *J. Mater. Chem. A*, 2015, **3**, 7568–7574.
10 M. Montalti, L. Prodi, E. Rampazzo and N. Zaccheroni, *Chem. Soc. Rev.*, 2014, **43**, 4243.
11 D. Genovese, M. Montalti, L. Prodi, E. Rampazzo, N. Zaccheroni, K. Altenhöner, F. May and J. Mattay, *Chem. Commun.*, 2011, **47**, 10975.
12 E. Rampazzo, S. Bonacchi, D. Genovese, R. Juris, M. Sgarzi, M. Montalti, L. Prodi, N. Zaccheroni, G. Tomaselli, S. Gentile, C. Satriano and E. Rizzarelli, *Chem. Eur. J.*, 2011, **17**, 13429; C. Bazzicalupi, C. Caltagirone, Z. Cao, Q. Chen, C. Di Natale, A. Garau, V. Lippolis, L. Lvova, H. Liu, I. Lundström, M. C. Mostallino, M. Nieddu, R. Paolesse, L. Prodi, M. Sgarzi and N. Zaccheroni, *Chem. Eur. J.*, 2013, **19**, 14639.
13 A. Burns, H. Owb and U. Wiesner, *Chem. Soc. Rev.*, 2006, **35**, 1028; C. Caltagiron, A. Bettoschi, A. Garau and R Montis, *Chem. Soc. Rev.*, 2015, *Advance Article*, DOI: 10.1039/C4CS00270A.
14 E. Rampazzo, S. Bonacchi, M. Montalti, L. Prodi and N. Zaccheroni, *J. Am. Chem. Soc.,* 2007, **129**, 14251.
15 E. Rampazzo, S. Bonacchi, R. Juris, M. Montalti, D. Genovese, N. Zaccheroni, L. Prodi, D. C. Rambaldi, A. Zattoni and P. Reschiglian, *J. Phys. Chem. B,* 2010, **114**(*45*), 14605.

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