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Supramolecular Sensing with Phosphonate Cavitands

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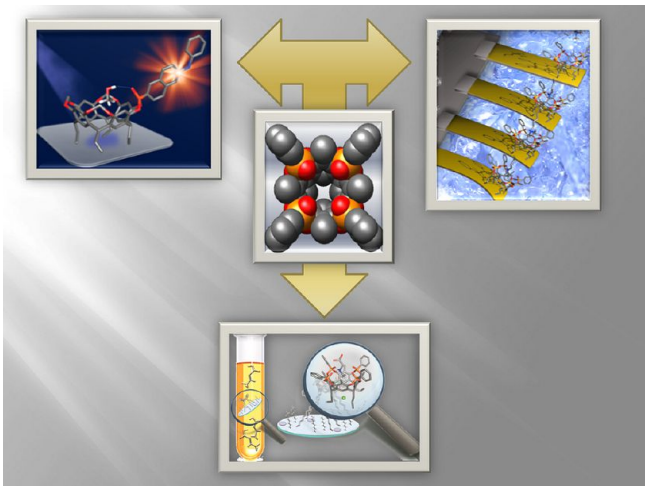

Molecular recognition is a recurrent theme in chemical sensing because of the importance of selectivity for sensor performances. The popularity of molecular recognition in chemical sensing has resulted from the progress made in mastering weak interactions, which has enabled the design of synthetic receptors according to the analyte to be detected.

However, the availability of a large pool of modular synthetic receptors so far has not had a significant impact on sensors used in the real world. This technological gap has emerged because of the difficulties in transferring the intrinsic molecular recognition properties of a given receptor from solution to interfaces and in finding high fidelity transduction modes for the recognition event. This Account focuses on the ways to overcome these two bottlenecks, and we recount our recent efforts to produce highly selective supramolecular sensors using phosphonate cavitands as receptors. Through two examples, we present an overview of the different operating strategies that are implemented depending on whether the interface is vapor–solid or liquid–solid.

First we describe the selective detection of short chain aliphatic alcohols in the vapor phase. In this example, we solved a key issue common to all sensors for organic vapors: the dissection of the specific interaction (between cavitand and the alcohol) from ubiquitous nonspecific dispersion interactions (between the analytes and interferents in the solid layer). We removed responses resulting from the nonspecific interactions of the analytes with interferents by directly connecting the recognition event at the interface to the transduction mechanism (photoinduced charge transfer).

The second example addresses the specific detection of sarcosine in urine. Recent research has suggested that sarcosine can serve as reliable biomarker of the aggressive forms of prostate cancer. Tetraphosphonate cavitands can complex *N*-methyl ammonium salts with impressive selectivity in solution, and we used this property as a starting point. The sensor implementation requires that we first graft the cavitand onto silicon and gold surfaces as monolayers. The exclusive recognition of sarcosine by these supramolecular sensors originates from their operation in aqueous environments, where synergistic multiple interactions with the phosphonate cavitand are possible only for *N*-methyl ammonium derivatives. We couple that selectivity with detection modes that probe the strength of the complexation either directly (microcantilever) or via exchange with molecules that have comparable affinity for the cavity (fluorescence dye displacement).

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Introduction

The demand for fast and reliable detection of biological and chemical hazards as well as rapid and accurate diagnosis of diseases is continuously rising, because of their foremost relevance in global health.[1] Optimal sensor and assay technologies for environmental, security, and biomedical applications must be responsive enough to allow detection of the target analyte at low concentration, selective to respond primarily to a single chemical species in presence of interferents, and rugged to withstand the widest range of operating conditions.[2] Nevertheless, present chemical sensors are prone to false positive and false negative responses, since they often suffer from limited selectivity to identify the analyte of interest in the presence of interfering molecules. Boosting selectivity in supramolecular sensors requires that the specific receptor–analyte binding interactions dominate over nonspecific ones. To accomplish this demanding task, not only do the molecular receptors have to be devised according to the target analyte, but they also need to be structured in architectures which retain the designed molecular recognition functionalities in the real-world working conditions. Finally, the chosen transduction mode must translate the desired molecular recognition event into a readable signal with high fidelity.

Chemists synthesize molecular receptors that mimic the exquisite specificity of biological receptors, exploiting the concepts of shape recognition and binding site complementarity. The degree of sophistication achieved in controlling weak host–guest interactions is such to allow the rational design of synthetic receptors according to the analyte to be detected. For this reason, synthetic receptors have a great potential in sensing and more in general in analytical chemistry.[3] However, harnessing their potential in sensing requires mastering weak interactions at the solid–liquid or at the solid–gas interfaces.[4]

Supramolecular sensing can be approached in two ways: specific sensing[4] and differential sensing.[5] While specific sensing aims at detecting a single analyte in a given environment, differential binding, akin to the mammalian sense of smell, strives for the simultaneous detection of multiple analytes in a mixture. The two approaches are mutually compatible and can be implemented together to improve selectivity in both fields. Specificity toward a single analyte or a single class of analytes is the ultimate goal of selective binding, calling for a minimal degree of cross-reactivity toward interferents. Instead, differential sensing relies on the use of large numbers of partially selective receptors, whose significant cross-reactivity is dealt with pattern recognition protocols. Admittedly, specific sensing is more demanding in terms of receptor performances.

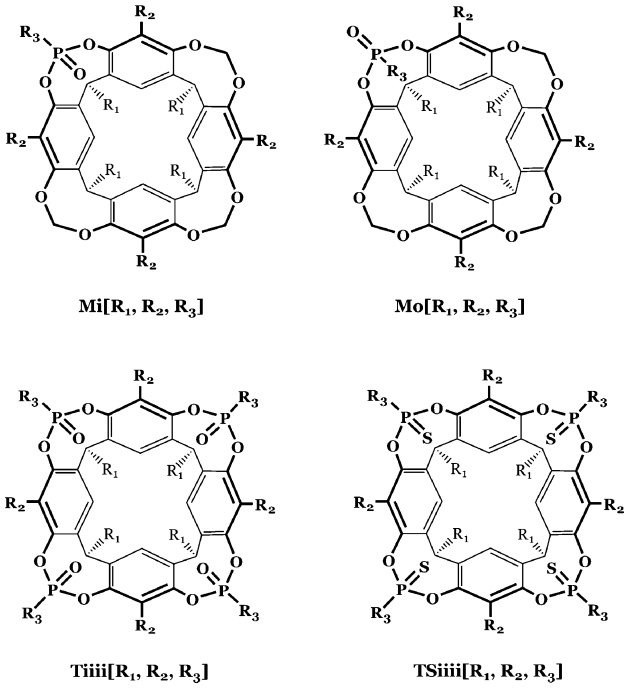

FIGURE 1. Phosphonate cavitands nomenclature.

In this Account, we report our recent efforts to produce selective supramolecular sensors using phosphonate cavitands as receptors. The implementation of the molecular recognition paradigm throughout the whole sensing chain will be highlighted through selected examples from our own work. Particular emphasis will be given to strategies to remove the two bottlenecks hindering the exploitation of synthetic receptors in supramolecular sensing, namely, the precise transfer of the intrinsic molecular recognition properties at the gas–solid and liquid–solid interfaces and the high fidelity transduction of the interfacial molecular recognition events.

Phosphonate Cavitands

Cavitands, defined as synthetic organic compounds having enforced cavities of molecular dimensions, are well-known molecular receptors.[6] In the design of cavitands, the choice of the bridging groups connecting the phenolic hydroxyls of the resorcinarene scaffold is pivotal, since it determines shape, dimensions and complexation properties of the resulting cavity. Phosphonate cavitands,[7] presenting one to four H-bonding acceptor P=O groups at the upper rim of the cavity, are particularly appealing for the complexation of molecules capable of H-bonding. The presence of P(V) stereocenters brings configurational properties into play, since the relative orientation of the P=O groups with respect to the cavity (inward or outward)[8] determines the number of possible stereoisomers. Figure 1 reports a survey of the

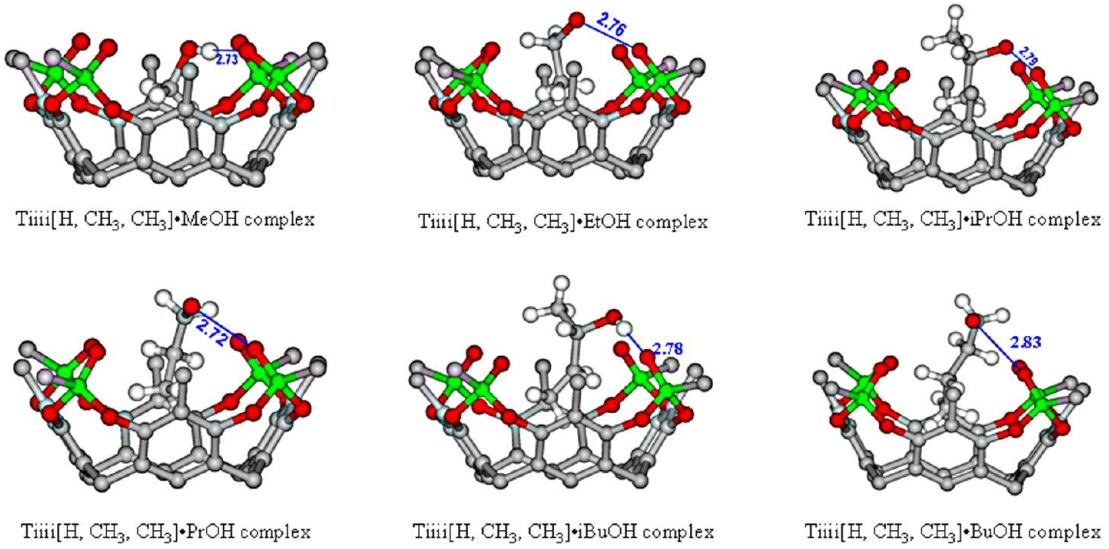

FIGURE 2. Side views of the crystal structures of Tiiii[H, CH_3, CH_3] with six C_1-C_4 alcohols.

structures discussed in this Account with their general acronym below.[7] The first capital letter defines number of the bridges, the second lower case letters define the in−out stereochemistry at each P(V) center, R_1, R_2, and R_3 in brackets define, respectively, the substituents at the lower rim, in the apical positions, and on the P(V) stereocenters (Figure 1). Only the inward facing stereoisomers (i), having the P═O groups pointing toward the cavity, are efficient receptors. A stereoselective procedure for the preparation of in isomers via oxidation of the corresponding P(III) precursors is available.[9]

The complexation properties of phosphonate cavitands toward organic guests have been extensively studied in the solid state,[10,11] in solution,[12] and in the gas phase.[13] The main specific interactions responsible for the recognition of neutral and charged guests evidenced by these studies are H-bonding, $CH_3-\pi$ and cation−dipole interactions.

Phosphonate Cavitands as Receptors for Gas Sensing: The Alcohols Case

The design of supramolecular receptors for vapor sensing requires the appropriate choice of the weak interactions to be implemented according to the analytes to be detected. A synergistic two-point interaction was identified in phosphonate cavitands for the complexation of short chain alcohols: the introduction of a single P═O unit as bridging group at the upper rim of a rigid methylene-bridged cavitand (Mi and Mo cavitands of Figure 1)[14] allows the cooperative formation of an H-bond between the P═O and the alcoholic OH and CH$-\pi$ interactions between the methyl residue of the alcohol and the π-basic cavity beneath. The well-defined spatial orientation of the P═O group with respect to the cavity determines the complexation properties of these cavitands. The two-point interaction with an alcohol is possible only for the Mi cavitand, while in the Mo isomer the two interactions are disconnected. The introduction of more inward facing P═O bridges does not change this pattern, it only stabilizes entropically the complex by increasing the number of energetically equivalent H-bonding options available to the alcohol.[10,15]

The intrinsic molecular recognition properties of phosphonate cavitands toward alcohols can be inferred from the sequence of crystal structures of Figure 2. Tiiii cavitand binds linear and branched short chain alcohols with the same two-point interaction mode described above. The cutoff of the receptor binding is at the C_5 level, as evidenced by the lack of guest inclusion in the crystallization with 1-pentanol and by the weakening of the H-bond in the case of 1-butanol.

The performances of phosphonate cavitands as sensing layers have been assessed in mass sensors. QCMs (quartz crystal microbalances) are the easiest choice as transducers for supramolecular sensors, because they do not require receptor derivatization for their operation modes, like pendant groups for surface grafting or fluorescent probes for optical detection. QCMs measure the mass uptake of a spin-coated sensing layer deposited on their surface when exposed to vapors; however, they are totally unselective, since they translate whatever mass change occurring on their surface into a frequency variation.[16] Contrary to the expectations, the responses of Tiiii-coated QCM do not reflect the

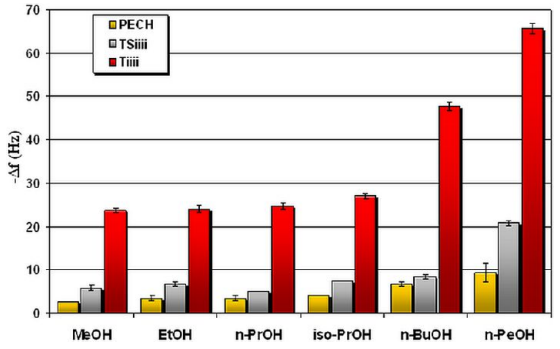

FIGURE 3. QCM responses of Tiiii[$C_{11}H_{23}$, H, Ph], TSiiii[$C_{11}H_{23}$, H, Ph] and PECH (reference polymer) to C_1-C_5 linear alcohols (25 ppm each). Adapted from ref 10.

intrinsic binding properties of phosphonate cavitands for C_4 and C_5 alcohols (Figure 3). The deviation is particularly significant for 1-pentanol, which ideally should not be detected.

The influence of nonspecific physisorption on the sensor responses is evidenced by comparing the behavior of Tiiii and TSiiii. The structural similarity of the two cavitands allows a proper comparison of the sensor data, without any bias due to different solid state packing and coating morphology. Substitution of the four P═O groups with the P═S analogues eliminates the H-bonding interactions, making TSiiii ineffective as alcohol receptor. The increased weight of dispersion interactions, associated with alcohol chain length, determines the general enhancement of the responses exhibited by both cavitands. For 1-butanol and 1-pentanol, the extra cavity physisorption contributes significantly to the overall QCM response. In all other cases, the response is comparable, suggesting that the inclusion mode is the same for short chain alcohols. This behavior can be rationalized recalling that gaseous species experience a net gain in nonspecific dispersion interactions upon moving to the solid state,[17] which often override weak host–guest interactions.[18]

The relative contributions of intracavity complexation and extracavity adsorption to the overall QCM responses of Tiiii[H, CH_3, Ph] and TSiiii[H, CH_3, Ph] receptor layers to ethanol were determined using adsorption isotherms.[19] In this case, the undecyl chains at the lower rim of the two cavitands, introduced to boost layer permeability and reduce QCM response times, were removed to minimize nonspecific adsorption. Linear adsorption isotherms are typical of nonspecific physisorption processes (Figure 4a, black trace), while Langmuir-type isotherms indicate preferential analyte/layer interactions, particularly at low concentrations (Figure 4a, red trace). At higher concentrations, when the available receptor sites are saturated, the isotherms flatten out, returning to the nonspecific regime. The overall trend for a truly selective layer in a wide analyte concentration range is shown in Figure 4a, blue trace.[20]

As expected, only for Tiiii, a Langmuir type isotherm was observed at low analyte concentrations, while for TSiiii only a linear isotherm was recorded (Figure 4b). Moreover, the Tiiii curve resembles nicely the theoretical one of Figure 4a, thus confirming the dual mode analyte adsorption: cavity inclusion dominates at low analyte concentration, while extra cavity nonspecific adsorption operates above 50 ppm.

The unavoidable presence of extra cavity adsorption has detrimental effects on the sensor selectivity, since the interaction of interferents with the layer produces a frequency change of the QCM. These adsorptions dilute or, even worse, override the specific ones due to complexation. A transduction mode activated exclusively by the molecular recognition event is therefore necessary to harness the intrinsic molecular recognition properties of phosphonate cavitands toward alcohols at the gas–solid interface.

Following this idea, we designed a monophosphonate cavitand incorporating a fluorophore directly connected to the P═O bridge (Figure 5).[21] A derivative of 2-anilinonaphthalene-6-sulfonic acid (2,6-ANS) was chosen, because of the charge-transfer character of its excited state. The rationale of this design was based on the assumption that the formation of the hydrogen bond between the P═O and the OH of the alcohol could decrease the electronic density on the phosphorus atom, leading to a red-shift of the emission band of the fluorophore.

A single P═O unit was introduced on the cavitand to funnel the H-bond perturbation on a single site, in order to maximize the desired red-shift. Moreover, monophosphonate cavitands are water insensitive, as at least two P═O units are necessary to bind water efficiently.[10] This strongly improves sensor robustness, because water is the most common interferent in gas monitoring.

PVC thin films containing 0.2% w/w of cavitands Mi and Mo were deposited on glass substrates by spin coating. The measurements were made exposing the Mi/Mo-functionalized glass substrates to the vapors of different short alcohols in a suitably equipped cell. Upon excitation at 350 nm, the Mi film showed an intense band emission with a maximum at 414 nm, which was red-shifted by 5 nm in the presence of C_1-C_4 alcohols. The relative fluorescence intensity changes at 460 nm, where the difference is more pronounced, are plotted in Figure 6. Maximum red-shift and intensity changes were comparable for all the alcohols studied in this series

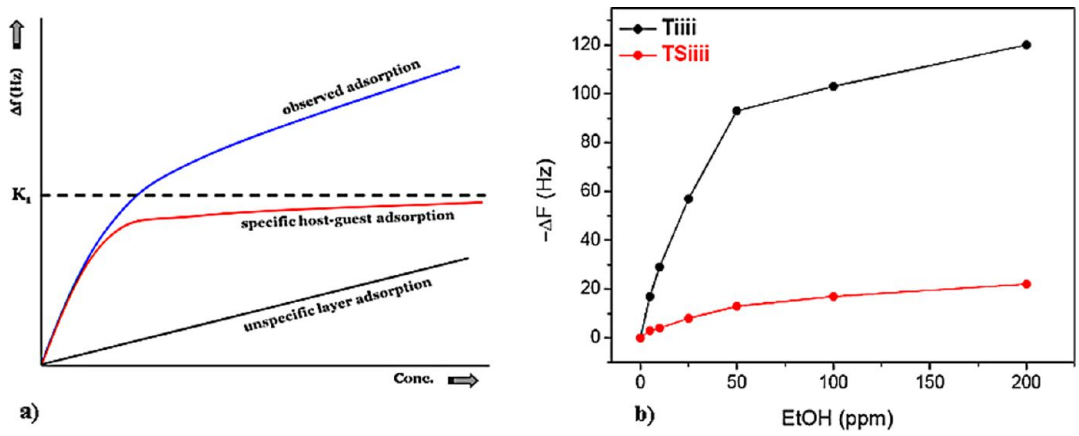

FIGURE 4. (a) Theoretical isotherms for specific and nonspecific analyte adsorption on solid receptor layer in mass sensors. Adapted from ref 20. (b) Experimental isotherms for cavitand-coated QCM sensors Tiiii[H, CH_3, Ph] and TSiiii[H, CH_3, Ph] in the presence of ethanol.

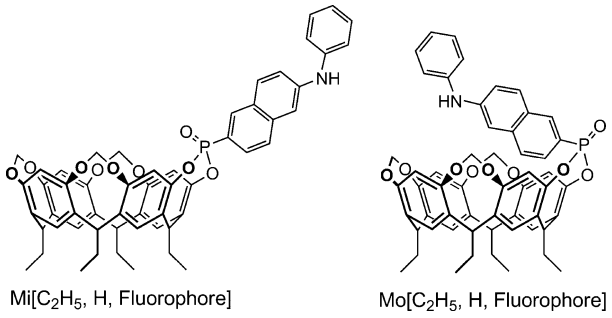

FIGURE 5. Structure of the fluorescent cavitands Mi[C_2H_5, H, fluorophore] and Mo[C_2H_5, H, fluorophore].

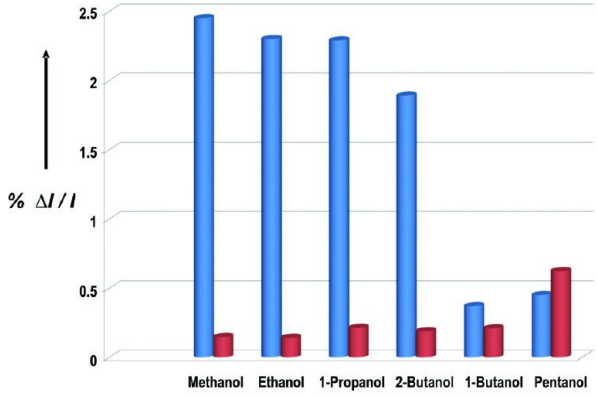

FIGURE 6. Relative fluorescence intensity changes (λ_{exc} = 350 nm, λ_{em} = 460 nm) of PVC films containing the receptor Mi[C_2H_5, H, fluorophore] (blue) or Mo[C_2H_5, H, fluorophore] (red) exposed to different alcohols in N_2 (500 ppm each). Detection limit: 40 ppm.

with the exception of 1-butanol and 1-pentanol, which caused lower responses. Under the same conditions, films of Mo showed negligible changes in the emission maximum, as expected owing to the disconnection of the two interaction modes. The original maximum is restored by flushing pure N_2 into the chamber. The fluorescent responses nicely reflect the intrinsic molecular recognition properties of phosphonate cavitands toward alcohols. The sensitivity is such that the fluorescent probe registers also the weaker H-bond of 1-butanol to the P═O compared to that of the shorter alcohols. The remarkable sensor selectivity is demonstrated by the very low responses registered by exposing the Mi layer to high concentrations of water, acetone and hydrocarbons.

The ubiquitous nonspecific layer adsorptions, being luminescence silent, do not contribute to the overall response, as they did in QCM devices (Figure 3). In this way, the responses owing to nonspecific interactions of the analytes and competitive binding by interferents have been almost completely removed. These results demonstrate that it is possible to achieve molecular level resolution in chemical vapor sensing by harnessing the binding specificity of a cavitand receptor.

Phosphonate Cavitands as Receptors for Liquid Sensing: The *N*-Methyl Ammonium Salts Case

Phosphonate cavitands are capable of binding different classes of guests in different phases.[22] In solution, Tiiii cavitands present remarkable molecular recognition properties toward *N*-methyl ammonium salts.[23] The origin of Tiiii selectivity toward *N*-methyl ammonium salts, can be attributed to the presence of three interaction modes: (i) $N^+\cdots$ O═P cation−dipole interactions; (ii) $CH_3-\pi$ interactions of the acidic $^+N-CH_3$ group with the π basic cavity; (iii) two simultaneous hydrogen bonds between two adjacent P═O bridges and the two nitrogen protons. Within the ammonium salt series, the bias toward the monomethylated

moiety over di- and trimethylated ones is determined by the number of H-bonds formed with Tiiii, while ammonium ions are disfavored by the lack of $CH_3-\pi$ interactions. This peculiar affinity of Tiiii cavitands toward the $H_2N^+-CH_3$ group is potentially valuable for the detection of a broad range of biologically active compounds containing this residue, like synthetic drugs, cancer biomarkers, and neurotransmitters.

In order to take advantage of this selective recognition mode for sensing, the Tiiii receptor must be grafted onto surfaces. Organic monolayers hosted on inorganic surfaces have the advantage of reducing or even eliminating nonspecific interactions which often mask the recognition events. Silicon is a particularly attractive inorganic platform, as it offers the possibility to make robust and durable devices by forming thermally and hydrolytically stable Si−C covalent bonds. The easiest and mildest way to graft cavitands on silicon wafers is the photochemical hydrosilylation.[24] The key requirements of the grafting procedure are an hydrogenated silicon surface and at least one terminal alkene in the organic molecules to be attached. The extension of this procedure to the grafting of phosphonate cavitands on silicon has been reported by us, using Tiiii cavitands equipped with ω-decene feet (Tiiii[$C_{10}H_{19}$, H, Phl]).[11] 1-Octene (Oct) was used as a spectator spacer in the mixed monolayers with our Tiiii cavitand to improve the passivation of the Si surface, thus minimizing substrate oxidation due to aging.[25]

Sensing analytes at the solid−liquid interface brings solvation into play. This is particularly true for this case, since isothermal titration calorimetry (ITC) measurements showed that the complexation is not only enthalpy but also entropy driven. The positive entropic contribution highlights the pivotal role played by desolvation in the complexation process. Indeed, the K_a values are strongly influenced by the solvent, increasing by 2 orders of magnitude upon moving from methanol to chlorinated solvents.[26] The unbiased transfer of the molecular recognition properties of Tiiii from solution to the silicon surface was proven using two independent techniques, namely, X-ray photoelectron spectroscopy (XPS) and fluorescence spectroscopy. Halogen-marked and fluorescent-tagged guests were prepared for XPS and fluorescence detection, respectively (Figure 7).

The outcome of XPS-monitored surface complexation cycle is shown in Figure 8. The sequence complexation-exchange-decomplexation demonstrated that all three interaction modes of the host available for the complexation of *N*-methyl ammonium derivatives are operative on the silicon surface without interference.[11] In particular, the two

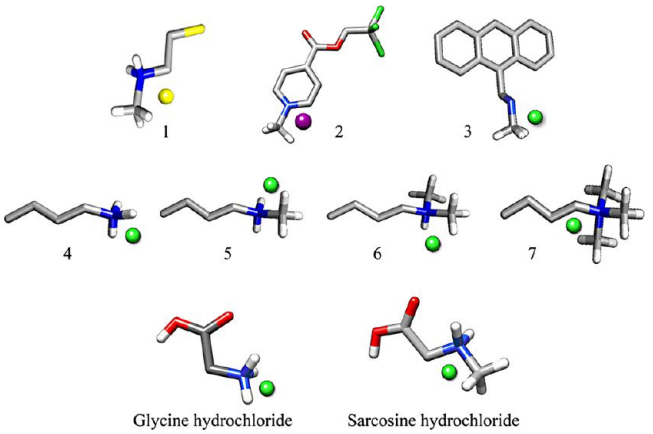

FIGURE 7. Analytes used in the Si-Tiiii and MC-Tiiii sensing: *N*-methyl(2-bromo-ethyl) ammonium bromide **1**, 1-methyl-4-((2,2,2-trichloroethoxy)carbonyl) pyridinium iodide **2**, *N*-(9-anthrylmethyl)-methyl ammonium chloride **3**, butyl ammonium chloride **4**, *N*-methyl-butylammonium chloride **5**, *N*,*N*-dimethylbutylammonium chloride **6**, *N*,*N*,*N*-trimethylbutylammonium chloride **7**, and glycine and sarcosine hydrochlorides.

additional H-bonds experienced by the brominated guest **1** are sufficient to completely replace the chlorinated methyl-pyridinium derivative **2** on the Si-Tiiii surface. Removal of the ammonium complex by deprotonation with DBU resets the original surface cleanly and efficiently.

A very important advantage of the surface grafting is the extension of the molecular recognition event to otherwise inaccessible solvents, precluded by Tiiii insolubility in them. Water, for its biological relevance, is the paradigmatic case. The selective complexation of *N*-methyl ammonium salts on Si-Tiiii surface is effective in water, as in organic solvents.

The missing item for harnessing the molecular recognition properties of Tiiii on surfaces is an effective and reliable transduction mode. Two options have been considered and tested so far: fluorescence dye displacement on silicon wafers[27] and nanomechanical detection with microcantilevers.[28] As playground to verify the prowess of Tiiii in sensing, we chose the detection of sarcosine in water and urine. The reason of this choice was due to the appearance of a recent paper in Nature proposing sarcosine as biomarker of the aggressive forms of prostate cancer.[29]

The challenges of detecting sarcosine in urine are twofold: (i) the presence of overwhelming amounts of glycine and other potential intereferents such as ammonium, sodium, potassium, magnesium, and calcium salts; (ii) operation in aqueous environment, where the H-bonding between host and guest can be severely weakened. Effective discrimination between sarcosine and glycine is the overriding need, since glycine is the precursor of sarcosine in the biochemical

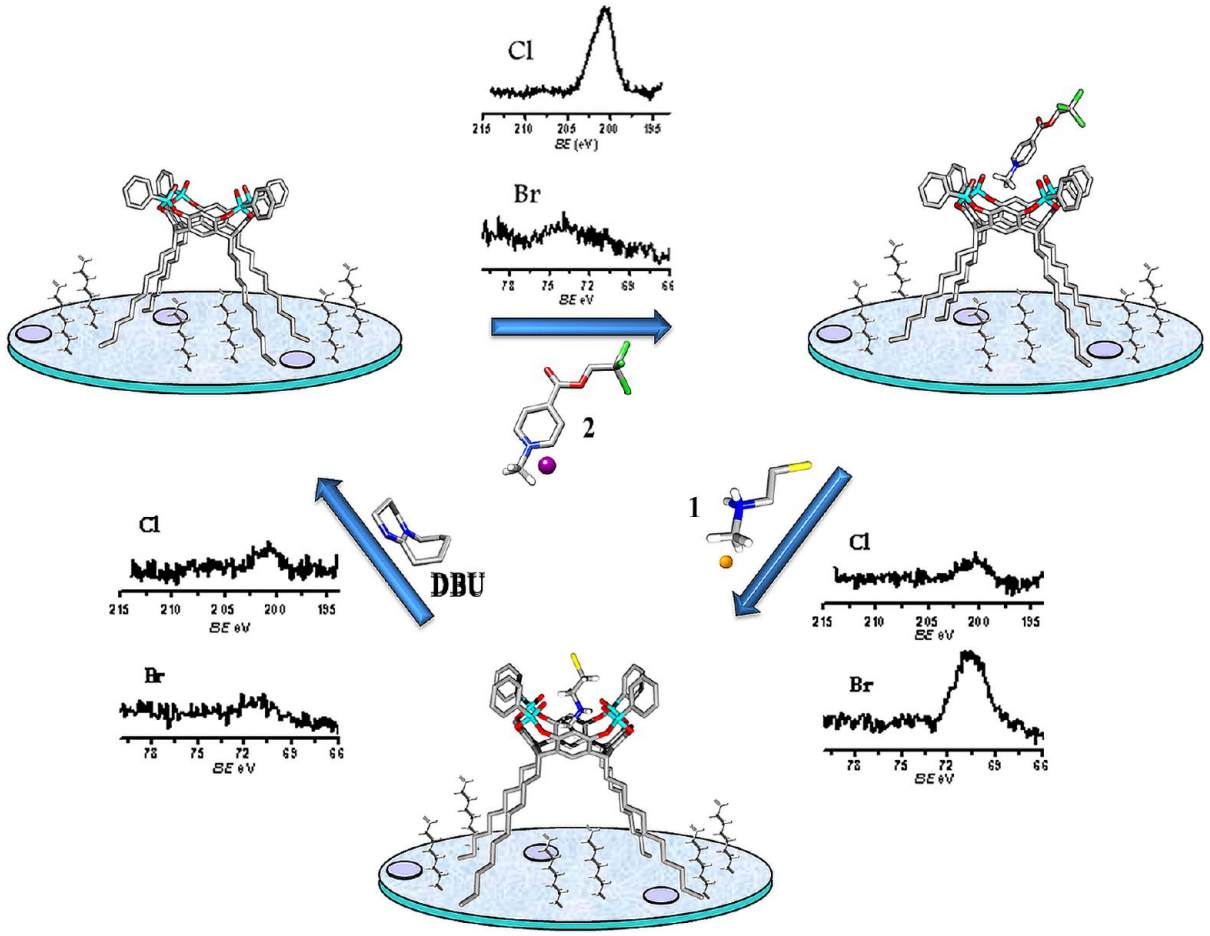

FIGURE 8. Si-Tiiii surface complexation loop: XPS signatures of the halogen-tagged guests **1** and **2**.

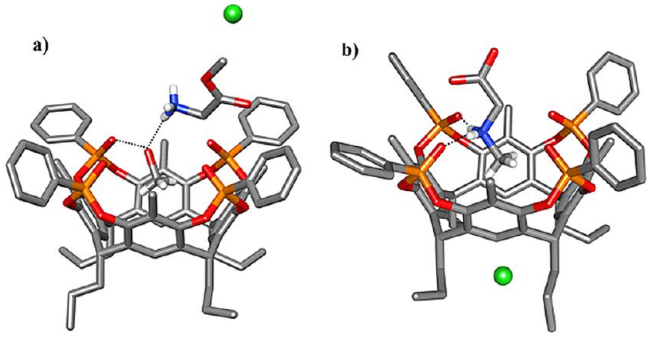

FIGURE 9. (a) Crystal structures of complexes Tiiii[C_3H_7, CH_3, Ph]·methanol·glycine methyl ester hydrochloride; (b) Tiiii[C_3H_7, CH_3, Ph]·sarcosine hydrochloride. C, gray; O, red; P, orange; N, blue; Cl, green; H, white; H-bonds, black dotted lines. Reprinted from ref 27. Copyright 2012 PNAS.

pathway and the dominant amino acid metabolite in urine. The preference of Tiiii for sarcosine over glycine is testified by the crystal structures of the corresponding complexes reported in Figure 9.

The difference in the binding mode is evident: the glycine hydrochloride complexation is mediated by a molecule of methanol which occupies the cavity, while sarcosine hydrochloride is directly bound to Tiiii through all three interaction modes described above. The different behavior of the two guests toward Tiiii is due to the presence of the methyl residue on the nitrogen in the sarcosine. Its $CH_3-\pi$ interaction with the cavity triggers the formation of the two H-bonds and the setting of cation−dipole interactions, which further stabilize the complex. The pivotal role of the methyl residue in directing the binding is experimentally supported by ITC complexation measurements in methanol for both guests ($K_a = 6.8 \pm 0.5 \times 10^4$ M^{-1} for sarcosine methyl ester hydrochloride versus a K_a too low to be detected for glycine methyl ester hydrochloride) and theoretically evaluated in 3.8 kcal mol^{-1} by density functional theory (DFT) calculations.[27]

The presence of water completely shuts down glycine uptake without affecting sarcosine complexation, as demonstrated by NMR biphasic extraction experiments.[27] This result can be rationalized by recalling that water severely impairs H-bonding, the only interaction present between

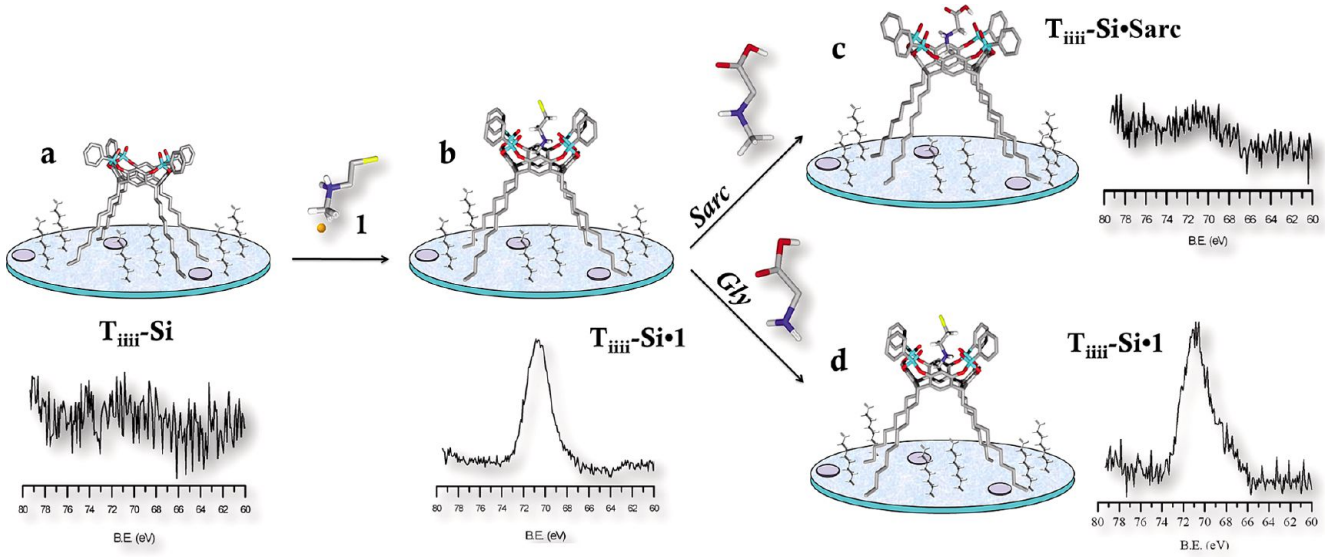

FIGURE 10. XPS analysis of Br 3d region along all steps of the sarcosine recognition protocol in water. (a) pristine Si-Tiiii wafer, (b) Si-Tiiii · **1** wafer after exposure to a water solution of **1**; (c) Si-Tiiii · Sarc wafer after exposure to a water solution of sarcosine; (d) Si-Tiiii · **1** wafer after exposure to a water solution of glycine. Reprinted from ref 27. Copyright 2012 PNAS.

Tiiii and glycine, while it does not influence CH–π interactions. The sarcosine complexation studies at the solid–water interface were performed employing the Si-Tiiii wafers, adopting the bromine marked guest **1** as XPS probe. The results are visually summarized in Figure 10.

Sarcosine replaces **1** completely on the surface as demonstrated by the absence of the Br 3d signal in the XPS surface analysis (Figure 10c). Under the same experimental conditions, glycine was totally ineffective (Figure 10d). Therefore, the behavior of Tiiii at the silicon–water interface reflects exactly its conduct at the organic–water interface.

Fluorescence Dye Displacement on Silicon Wafers for Sarcosine Detection in Urine

XPS is an effective tool to assess molecular recognition on surfaces, but it cannot be employed as a transducer in sensing. Fluorescence dye displacement[30] turned out to be a viable option for sarcosine detection in urine. *N*-(9-Anthrylmethyl)methyl ammonium chloride **3** was used as an indicator dye (Figure 7), since it binds efficiently to Si-Tiiii, retaining its fluorescence. The introduction of an *N*-methyl ammonium moiety on the indicator is dictated by the need to displace the dye only with molecules forming energetically equivalent complexes with Tiiii (sarcosine in this case).

Sarcosine was added to a human urine sample, to simulate its biological occurrence due to prostate cancer. Si-Tiiii · **3** wafers were exposed to three portions of human urine sample containing different amounts of sarcosine (pristine, 1 mM, and 0.1 mM). Both sarcosine added samples displaced fluorescent guest **3** (traces c and d of the graph in Figure 11), while the untreated one did not (trace b).[27] The fluorescence results were confirmed by XPS experiments performed under the same conditions, using bromine-tagged guest **1** as indicator. As in the case of gas sensing, a specific fluorescence detection mode allows the direct, unbiased readout of the outstanding molecular recognition properties of phosphonate cavitands at the liquid–solid interface. The fluorescent dye displacement approach is facilitated by the large host–analyte association constant, avoiding the need of covalent modification of the cavitand.

Microcantilever Detection of Sarcosine versus Glycine

Microcantilevers (MCs)[31] represent an innovative route to translate surface molecular recognition into nanomechanical work. The free energy released by a host–guest interaction confined at a solid–solution interface splits into chemical and mechanical surface work, with the latter determined by the work that the host performs to "accommodate" the guest at the solid–solution interface.[32] This work appears as a variation of the surface stress (surface pressure) that the MC balances by bending. MC nanomechanical transduction is therefore "energy-based" and occurs regardless of the analyte mass in a label-free fashion. In addition, MC detection is performed in real-time, while the MC architecture is amenable to implementation for parallel analysis. At present, MC sensors are limited by the availability

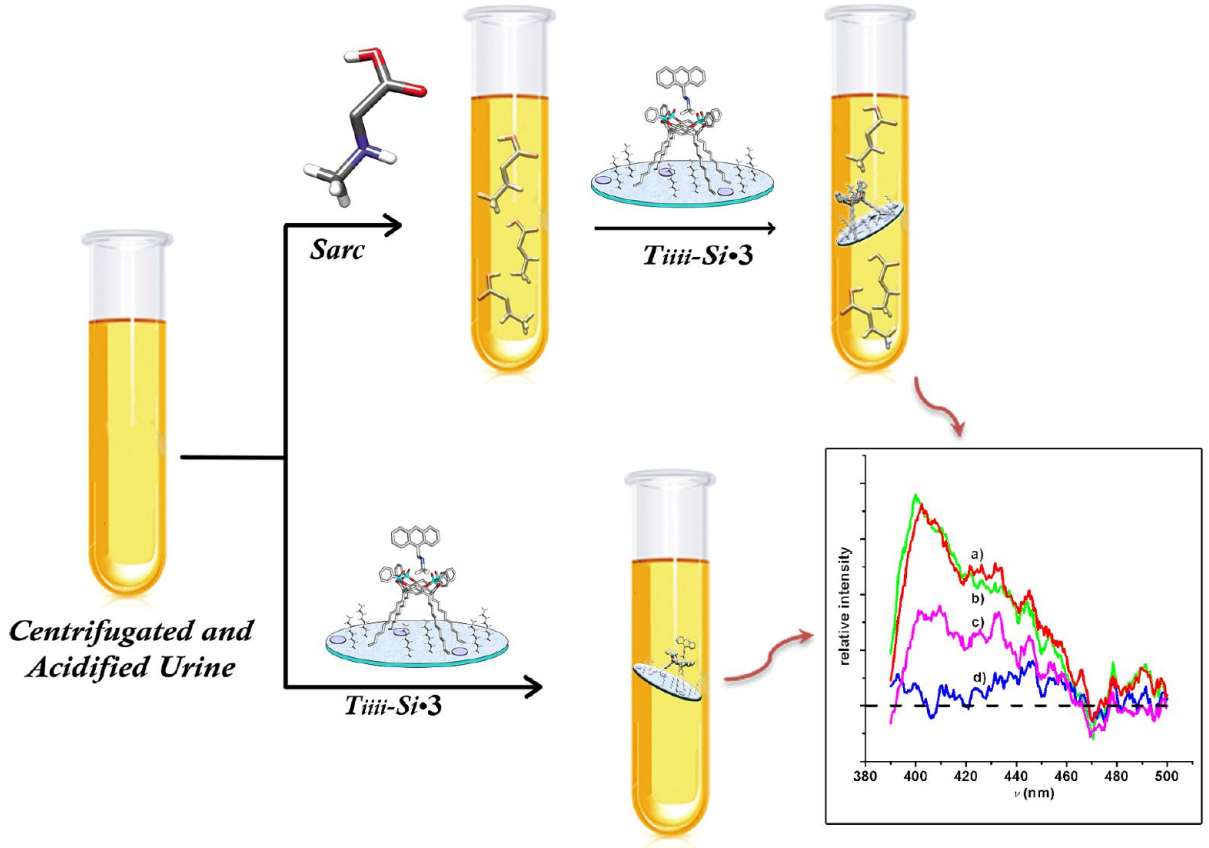

FIGURE 11. Luminescence spectra of Si-Tiiii·**3**: (a) before urine exposure (red line); (b) after dipping in urine (green line); (c) after dipping in 0.1 mM sarcosine-added urine (magenta line); (d) after dipping in 1 mM sarcosine added urine (blue line). Detection limit = 0.1 mM in urine.

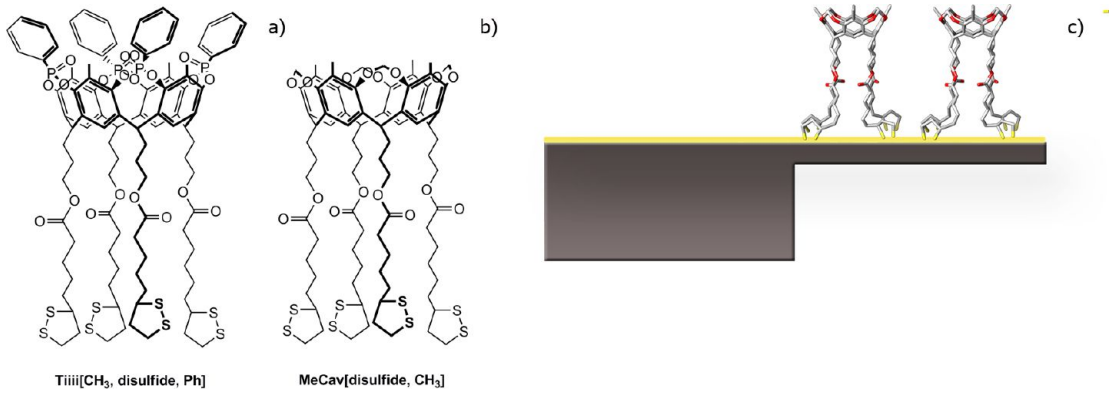

FIGURE 12. (a) Active Tiiii[disulfide, CH_3, Ph] cavitand and (b) reference MeCav[disulfide, CH_3] cavitand used for the MC experiments; (c) Si MCs (500 × 100 × 1 μm^3) with the top faces coated by a 20 nm Au functionalized with Tiiii[disulfide, CH_3, Ph].

of coatings that interact exclusively and selectively with the analyte of interest.

The decoration of the MC surface with cavitands relies on the presence of a 20 nm Au layer on the top face of the MC. The introduction of four lipoic acid units at the lower rim of Tiiii[disulfide, CH_3, Ph] cavitand (Figure 12a) allows the functionalization of the MC with all cavities pointing toward the liquid phase (Figure 12c). The structurally similar but complexation inefficient MeCav[disulfide, CH_3] (Figure 12b) was also synthesized, deposited on Au, and used as reference MC to rule out nonspecific responses due to physisorption.

The molecular recognition properties of MC-Tiiii were tested toward the *N*-methyl ammonium salts series **4**–**7** (Figure 7). Arrays of eight MCs were exposed to methanol solutions of the four chosen guests at a molar concentration

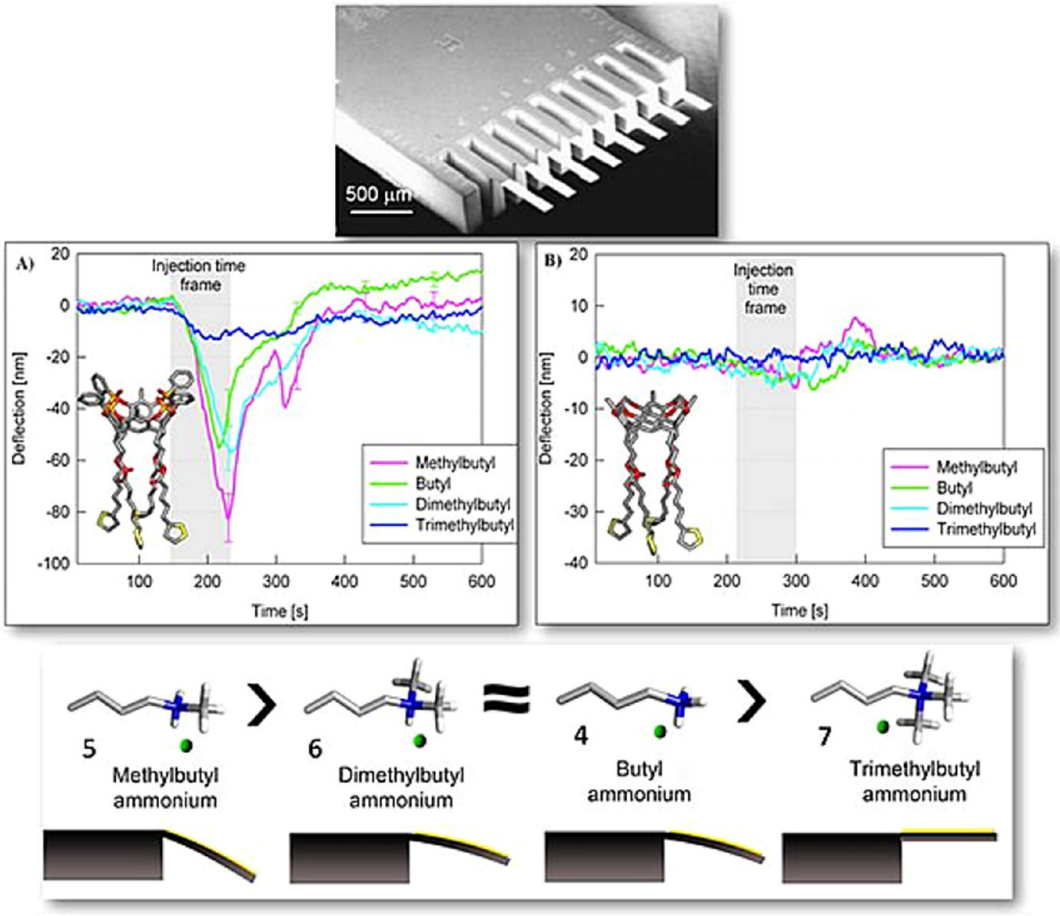

FIGURE 13. MC-Tiiii absolute deflections during the injection of a 1×10^{-5} M methanol solution of methyl−ammonium guests (gray area). Each line represents the mean deflection of the eight MCs. (A) MCs functionalized with the active cavitand Tiiii[disulfide, CH_3, Ph]. (B) MCs functionalized with the control cavitand MeCav[disulfide, CH_3].

of 1×10^{-5} M. The mean signal of the eight MCs was monitored during the flow of each guest through the microfluidic chamber (Figure 13).

The biggest deflection curve of the MC-Tiiii was in presence of guest **5**, while the lowest was registered in presence of guest **7.** In presence of guest **4** and **6**, an intermediate value of curve deflection was registered. No deflection was registered for the reference MC-MeCav in the presence of any guests. The observed complexation trend, $\mathbf{5} > \mathbf{6} \approx \mathbf{4} \gg \mathbf{7}$, was independently confirmed and rationalized by ITC measurements, performed both as direct titration and displacement titration experiments. The ITC measured K_a trend nicely matches the MC-Tiiii deflections as shown in Figure 14. This trend is consistent with the number of interactions established by each guest with the host; in particular, for guest **5**, all three interaction modes available with Tiiii are operating. MC-Tiiii achieved an unprecedented performance toward screening across the *N*-methylammonium salt series with differences between each other only by a few methyl groups (15 Da each). Therefore, the ability of MC-Tiiii to discriminate mass differences as minute as a methyl group is due to the fact that the deflection response of the MCs to the cavitand−guest recognition event is related to the energy of the event rather than to the mass of the guest.

The influence of the solvent on the MC-Tiiii sensing performances was determined assaying sarcosine and glycine methyl ester hydrochlorides in water and methanol as solvents. The insolubility of glycine in methanol precluded the direct comparison of the parent amino acids. In methanol, both analytes were detected by MC-Tiiii, the difference being in the higher response to the sarcosine derivative (Figure 15A). Also in this case, the MC deflections correlate with the K_a values measured for the two compounds in methanol via ITC.[28] In water, the cantilevers were totally insensitive to glycine methyl ester hydrochloride and showed a deflection only when the corresponding sarcosine derivative was introduced in the chamber (Figure 15B). The same trend was observed in water for the parent amino

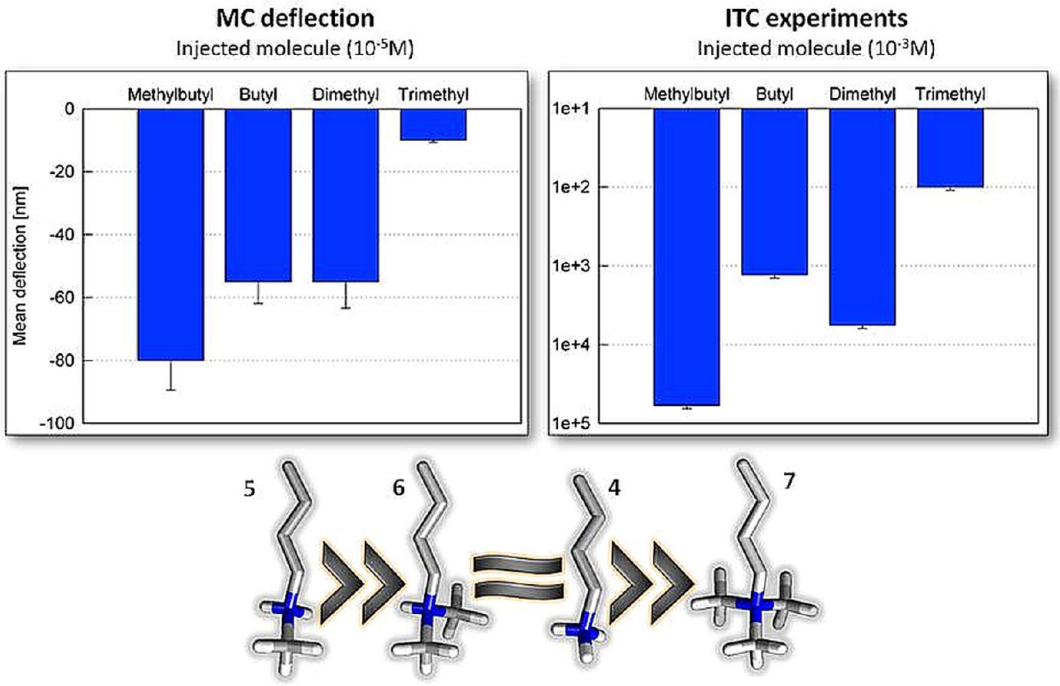

FIGURE 14. Left: bar chart of MC deflections in MeOH. Right: bar chart of the K_a evaluated by ITC in MeOH.

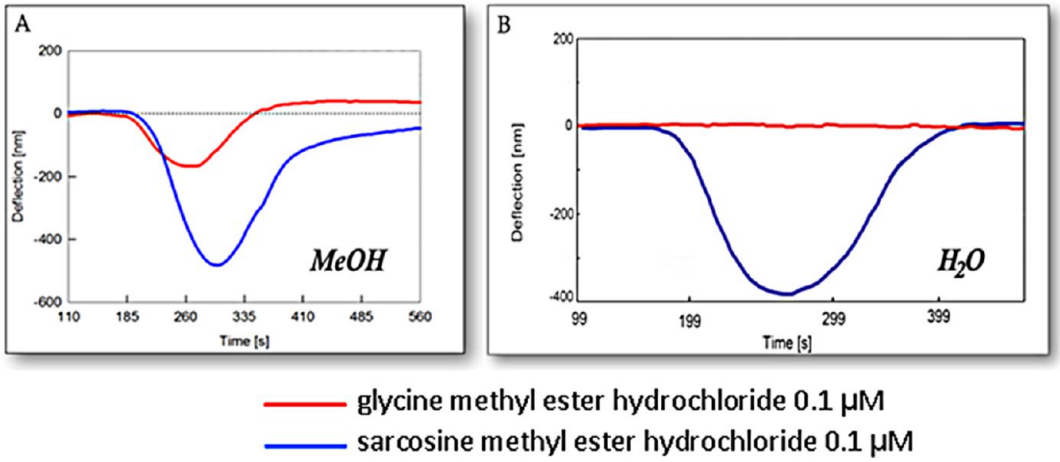

FIGURE 15. MC-Tiiii average deflection during the injection of 0.1 μM sarcosine and glycine methyl ester hydrochloride solutions in MeOH (A) and water (B).

acids.[28] This behavior pinpoints the pivotal role played by the liquid phase in supramolecular sensing. As discussed above for the silicon wafer detection, water interferes with H-bonding when it is the dominant host–guest interaction (Figure 9a). The presence of additional, synergic hydrophobic CH_3–π interactions, offers to sarcosine and methyl ammonium derivatives in general a handle to retain the other two binding modes in water, namely, cation–dipole interactions and H-bonding (Figure 9b). The overall result is a dramatic enhancement of Tiiii selectivity at the water–solid interface, pivotal for sensing in biological fluids.[27]

Conclusions

Turning molecular recognition into specific sensing requires an integrated approach, where the transfer of the intrinsic binding properties of the chosen receptor at the interface and the high fidelity transduction of the recognition event are dealt with. As already discussed,[4] the mere presence of a molecular receptor in the sensitive layer does not guarantee sensing selectivity.

Different operating strategies have to be implemented depending on the analytes phase. For vapors, the main issue is to introduce transduction mechanisms turned on exclusively

by the desired complexation mode with the analyte. For liquids, the key parameter is the modulation of the desired host−analyte complexation with respect to interactions with interferents. The modulation can be tuned at two levels: (i) the choice of the solvent to boost the preference of the host for the target analyte with respect to interferents; (ii) the use of transduction modes registering the strength of the host-analyte complexation and not the inset of a recognition event.

The following factors turned out to be essential for the selective detection of C_1-C_4 alcohol vapors: (i) synergy of H-bonding with the PO group and $CH_3-\pi$ interaction with the cavity; (ii) a rigid cavity which provides a permanent free volume for the analyte around the inward facing PO groups, pivotal for effective H-bonding;[4] (iii) the direct connection of the P═O group with a suitable chromophore, whose photoinduced charge transfer is activated exclusively by the molecular recognition event.

The exclusive recognition of sarcosine by Si-Tiiii and MC-Tiiii originates from their operation in an aqueous environment, where synergistic multiple interactions with Tiiii are possible only for *N*-methyl ammonium derivatives, coupled with detection modes probing the strength of the complexation either directly (MC) or via exchange only with molecules having comparable affinity for the cavity (fluorescence dye displacement).

The supramolecular sensing strategies delineated above are not limited to cavitands, but can be implemented with a wide variety of synthetic receptors, opening the way for the rational design of highly selective sensing materials according to the analytes to be detected and their environment. On the other hand, cavitands are ideal receptors for supramolecular sensing thanks to their exquisite versatility both in the binding modes, through the proper choice of the bridging groups at the upper rim,[33] and in the transducer integration,[34] through the manifold functionalization options at the lower rim. In the future, synthetic receptors could be embedded into a polymer matrix, as alternative to synthetic antibodies.[35]

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BIOGRAPHICAL INFORMATION

Roberta Pinalli received her M.S. in Industrial Chemistry in 1998 from the University of Parma. After a 2 year stint in Antibioticos SpA, as a process chemist, she obtained her Ph.D. in Chemistry from the same University in 2002, under the supervision of Prof. Dalcanale, working on nanosize coordination cages. During her Ph.D. tenure, she spent a semester at the University of Twente, in the group of Prof. D. N. Reinhoudt. After a 3 year experience as senior researcher in Bracco Imaging SpA, she rejoined Dalcanale's group. Actually she is Assistant Professor at the University of Parma. Her research interests are centered on supramolecular sensors and materials.

Enrico Dalcanale graduated in Industrial Chemistry (cum laude) at the University of Bologna in 1981. After working as research scientist at the Donegani Research Institute of Montedison in Novara from 1982 to 1990, he joined the Faculty of the Department of Organic and Industrial Chemistry of the University of Parma, where he is currently Associate Professor. In 1985−1986, he worked as postdoctoral fellow the group of D. J. Cram at UCLA. In 2004, he has been visiting professor at Naval Research Laboratory (Washington, D.C.). He is presently the Scientific Director of the Functional Materials Section of the Italian University Consortium for Materials Science (INSTM). His research interests include molecular recognition, supramolecular sensors, self-assembly of nanostructures, and supramolecular polymers.

FOOTNOTES

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The authors declare no competing financial interest.

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