UNIVERSITAT POLITÈCNICA DE VALÈNCIA

DEPARTAMENTO DE QUÍMICA

CENTRO DE RECONOCIMIENTO MOLECULAR
Y DESARROLLO TECNOLÓGICO

Silica nanoparticles as inorganic scaffolds for the preparation of hybrid materials for the optical detection of anions

PhD. THESIS

Submitted by

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Valencia, February 2016

A mis padres,
y a los hombres de mi vida,
Manuel, Álvaro y Pablo.

La vida no es fácil, para ninguno de nosotros. Pero... ¡qué importa! Hay que perseverar y, sobre todo, tener confianza en uno mismo. Hay que sentirse dotado para realizar alguna cosa y que esa cosa hay que alcanzarla, cueste lo que cueste.

-Marie Curie-

ACKNOWLEDGEMENTS- AGRADECIMIENTOS

Ha pasado mucho tiempo desde que inicie esta andadura del doctorado; y han sido muchas, las personas que han contribuido en mayor o menor medida, y sin las que sin duda el resultado habría sido diferente. Muchas gracias a todos por haber hecho posible que hoy este escribiendo estas palabras.

En primer lugar, quisiera dar mis más sinceras gracias a mis directores de Tesis, Dr. Ramón Martínez y Dr. Félix Sancenón. Gracias Ramón por confiar en mí, por tu tiempo, tu dedicación y tu apoyo siempre que lo he necesitado; y gracias Félix por tus conocimientos, tus orientaciones, tu paciencia y tu motivación, sin los cuales esta Tesis no se habría concluido. A Félix amigo, ¿qué te puedo decir?. ¡Hace casi una vida que nos conocemos!. Muchas gracias por tus ratos de conversación, por enseñarme, por guiarme, por animarme y por tranquilizarme en todo este proceso de escritura-exposición; muchas gracias por todo.

Muchas gracias a Loles y a Luis, por enseñarme y aconsejarme sobre materiales y sus caracterizaciones. Muchas gracias a Juan por sus consejos y ayuda con los cálculos de constantes y distancias; y muchas gracias al resto de profesores por aconsejarme en todo momento.

También quiero dar las gracias a una persona que de manera indirecta ha contribuido en esta Tesis, Dr. Patricia Schubert. Gracias por

tenderme la mano en mis primeros pasos como científica, por iniciarme, guiarme y ensenarme el camino de la I+D, por tu seriedad y tu rigurosidad académica.

Por supuesto, dar las gracias a tod@s los “Ramones” a los que me acogieron a la llegada al lab.: a J.V., a Bea, a Hanoi, a María, a Rosa, y a Carmen, por hacer que los comienzos en el laboratorio y en una nueva ciudad fueran más sencillos y cómodos. Y a los que se fueron incorporando: a Elena, Santi, Merche, Inma Campos, Andrea, Estela, Cristina, Tati, Nuria…Gracias a Bea y a Elena por ayudarme con la síntesis de algún que otro colorante; gracias a Maria y a Rosa por enseñarme los “trucos” de la funcionalización; Gracias Carmen por compartir los imidazoles conmigo y gracias J.V. por ayudarme a ver e interpretar los datos con otra mirada; Gracias a Andrea por su alegría y su sonrisa; gracias a Estela por su dulzura y su gran apoyo; gracias a Cris por su creatividad; y gracias Tati por ser como eres, ¡autentica!. Muchas gracias a tod@s por hacer agradable el día a día, por vuestro apoyo y vuestra amistad.

No puedo olvidarme de los compañeros del 2.2, 2.5 y primera planta: Raúl, Carlos, Nacho, Pavel, Carmela, Belén, Esther, Sergio, Mireia, Roberto, Maykel, Nuria, Eva, M. José, Jesús, Esther,… Gracias a todos por vuestra ayuda y por los ratos en la cocina.

Gracias también a Inma, Quique y Arancha, por su ayuda en el papeleo del doctorado-Tesis; en especial a Arancha, por su amistad, y sus ánimos en las horas bajas; y gracias a Loli y a Enrique por sus ánimos.

Gracias al servicio de microscopia, a Manolo y a Merche, por su inestimable ayuda con la caracterización de mis sólidos.

También quiero dar las gracias a l@s que en un primer momento fueron compañer@s, pero que con el tiempo se convirtieron en amig@s y en un apoyo inestimable en tiempos complicados; muchas gracias Marisa, Pilar, Sol, Yolanda, Esther, Fernando y Jesús, tengo mucha suerte de teneros como amigos. A mi amiga Paqui, por haberme acompañado y haber estado a mi lado durante todos estos años. A los Rambleños, Marta, Raúl, Rafa, Conchi, Manolo, Trini; a Flori y a Encarni; a Pedro; a JoseVi, y Amelia, a Juan y Lola, a Ángel, a Margarit y a Patricia, con la que siempre estaré en deuda. ¡Muchas gracias a todos!

Y..., por supuesto, gracias de corazón a toda mi familia, y a esa familia que se elige, a Lola, Manolo, Marta y Blanca; a Carmen, Felipe, Felipillo y Carmen; a Juan Manuel, Ana, JuanMa y Carmen. A mis sobrinas Laura y Cristina, a Yolanda, a Manolo y Fina.

Sin lugar a dudas, muchas gracias a mis padres, por quererme, apoyarme y animarme durante toda mi vida, por guiar mis pasos, por ayudarme a levantarme cada vez que he caído, porque gracias a ellos soy lo que soy. A mi hermana, por estar siempre a mi lado. A mi abuelo, al que le hubiera encantado ver concluida esta Tesis y conocer a mis dos luceros; te echamos mucho de menos. A mi abuela, por ser una segunda madre, por echarme un cable, por quererme y cuidarme, por su apoyo incondicional…A mi abuela Pilar, por enseñarnos a luchar por lo que creemos, por su apoyo y por querernos; y es que, si el amor de unos padres es impagable, el de los abuelos también. A Manolo, el pilar fundamental de mi vida, por quererme, por darme la mano y caminar junto a mí, por esa habilidad para iluminar la oscuridad más absoluta, por calmarme..; y, …lo más importante, por esos dos soles que dan sentido a mi vida.

Y por último, gracias al Ministerio de Educación y Ciencia por la ayuda concedida dentro del programa Torres Quevedo PTQ-06-2-0936, 2008-2012.

Muchas gracias a todos.

RESUMEN

La presente Tesis Doctoral titulada "*Nanopartículas de sílice como soporte inorgánico para la preparación de materiales híbridos para la detección óptica de aniones*" se basa en el empleo de conceptos de química supramolecular y de química de materiales para la preparación de sistemas sensores híbridos con capacidad para reconocer iones mediante cambios de color y de fluorescencia.

El primer material sensor desarrollado esta basado en nanopartículas de sílice funcionalizadas con espirobenzopiranos (unidad indicadora) y con tioureas (unidad coordinante). En las nanopartículas bifuncionalizadas el espiropirano está en su forma polar abierta (merocianina de color rojo). Cuando coordinan las tioureas con carboxilatos de cadena larga (octanoato, decanoato y dodecanoato) se forma un entorno apolar alrededor de la unidad indicadora que favorece su transformación de la forma abierta a la apolar espirocíclica cerrada con el consiguiente cambio de color.

En la segunda parte de la Tesis se han preparado nanoparticulas de sílice funcionalizadas con antraceno, como unidad indicadora, y con dos diferentes tioureas, como unidades coordinantes de aniones. Suspensiones de las nanopartículas bifuncionalizadas en acetonitrilo muestran la típica emisión estructurada del antraceno. Al añadir diferentes aniones a la suspensión de las nanoparticulas se obiene un

aumento de fluorescencia (con Cl^-, Br^-, $H_2PO_4^-$, acetato y benzoato) o una desactivación de la misma (F^- y CN^-).

Por último se prepararon nanopartículas de sílice funcionalizadas con terpiridinas, como unidad coordinante, y con sulforodamina B, como unidad indicadora. La coordinación de cationes metálicos de transición (Fe^{3+}, Hg^{2+}, Cu^{2+}, Ni^{2+} y Pb^{2+}) con las terpiridinas dio lugar a una desactivación importante de la emisión de la sulforodamina B anclada (95% de la inicial). En un segundo paso se estudio la capacidad de ciertos aniones ($H_2PO_4^-$, HSO_4^-, F^-, Cl^-, Br^-, I^- y NO_3^-) de desplazar al metal coordinado con la subsiguiente regeneración de la emisión de la sulforodamina. Aplicando el análisis de componentes principales (PCA) se consiguió la discriminación de todos los aniones empleados. Además, las nanopartículas tratadas con el catión Pb^{2+} dieron una respuesta selectiva con el anión $H_2PO_4^-$.

ABSTRACT

The PhD Thesis entitled "*Silica nanoparticles as inorganic scaffolds for the preparation of hybrid materials for the optical detection of anions*" deals with the combination of supramolecular and material chemistry concepts to prepare hybrid sensing materials with the ability to detect selected ions through color and emission changes.

The first hybrid material prepared is based in the use of silica nanoparticles as inorganic scaffold functionalized with spirobenzopyrans (signaling unit) and with thiourea moieties (binding unit). In the final nanoparticles the spirobenzopyran signaling unit is in its open polar conformation (red color merocyanine structure). Coordination of long chain carboylates (octanoate, decanoate and dodecanoate) with the thiourea moieties induced the formation of a dense hydrophobic monolayer around the signaling unit. This non-polar monolayer induced a change in the conformation of the spirobenzopyran to the closed spirocyclic form with a subsequent color change.

The second part of this PhD Thesis was related with the preparation of silica nanoparticles functionalized with anthracene, as signalling subunit, and two different thioureas, as anion binding sites. Acetonitrile suspensions of the bifunctionalized nanoparticles showed the typical structured emission band of the anthracene fluorophore. Addition of certain anions to acetonitrile suspensions of the nanoparticles induced an enhancement of the emission intensity (Cl^-, Br^-, $H_2PO_4^-$, acetate and benzoate) or a marked quenching (F^- and CN^-).

Finally, the last part of this PhD Thesis deals with the preparation of silica nanoparticles functionalized with terpyridine binding sites and sulforhodamine B as signaling subunit. Coordination of transition metal cations (Fe^{3+}, Hg^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+}) with the terpyridine bindind sites induced a marked quenching of the emsission intensity (*ca.* 95%) of the sulforhodamine B fluorophore. The prepared materials were used for the fluorogenic recognition of anions. At this respect, addition of $H_2PO_4^-$, HSO_4^-, F^-, Cl^-, Br^-, I^- and NO_3^-anions induced different degrees of cation displacements with the subsequent enhancement of the emission intensity of the fluorophore. Principal component analysis (PCA) allowed the discrimination of all the anions tested. Besides, only $H_2PO_4^-$ anion was able to induce the displacement of Pb^{2+} cation with the subsequent emission enhancement.

RESUM

La present Tesi Doctoral titulada "*Nanopartícules de sílice com a suport inorganic en la preparació de materials hibrids per a la detecció optica de anions*" es basa en l'ús de conceptes de química supramolecular i de química de materials per a la preparació de sistemes sensors híbrids amb capacitat per a reconèixer ions mitjançant canvis de color i de fluorescència.

El primer material sensor desenvolupat està basat en nanopartícules de sílice funcionalitzades amb espirobenzopirans (unitat indicadora) i amb tiourees (unitat coordinant). A les nanopartícules bifuncionalitzades l'espiropirà està en la seua forma polar oberta (merocianina de color vermell). Quan coordinen les tiourees amb carboxilats de cadena llarga (octanoat, decanoat i dodecanoat) es forma un entorn apolar al voltant de la unitat indicadora que afavoreix la seua transformació de la forma oberta a l'apolar espirocíclica tancada amb el conseqüent canvi de color.

A la segona part de la Tesi s'han preparat nanoparticules de sílice funcionalitzades amb antracè, com a unitat indicadora, i amb dos tiourees diferents, com unitats coordinants d'anions. Suspensions de les nanopartícules bifuncionalitzades en acetonitril mostren la típica emissió estructurada del'antracè. En afegir diferents anions a la suspensió de les nanoparticules s'obté un augment de fluorescència

(amb Cl^-, Br^-, $H_2PO_4^-$, acetat i benzoat) o una desactivació de la mateixa (F^- i CN^-).

Finalment es prepararen nanopartícules de sílicie funcionalitzades amb terpiridines, com a unitat coordinant, i amb sulforodamina B, com a unitat indicadora. La coordinació de cations metàl·lics de transició (Fe^{3+}, Hg^{2+}, Cu^{2+}, Ni^{2+} i Pb^{2+}) amb les terpiridines va donar lloc a una desactivació important de l'emissió de la sulforodamina B unida covalentment al material (95% de la inicial). En un segon pas, es va estudiar la capacitat de certs anions ($H_2PO_4^-$, HSO_4^-, F^-, Cl^-, Br^-, I^- i NO_3^-) de desplaçar al metall coordinat amb la subsegüent regeneració de l'emissió de la sulforodamina. Aplicant l'anàlisi de components principals (PCA) es va aconseguir la discriminació de tots els anions estudiats. A més, les nanopartícules tractades amb el catió Pb^{2+} van donar una resposta selectiva amb l'anió $H_2PO_4^-$.

PUBLICATIONS

Results of this Thesis and other contributions have resulted in the following publications:

1. Pilar Calero, Elena Aznar, J. Manuel Lloris, Maria D. Marcos, Ramón Martínez-Máñez, José V. Ros-Lis, Juan Soto, and Félix Sancenón. ***Chromogenic silica nanoparticles for the colorimetric sensing of long-chain carboxylates***. Chem. Commun., 2008, 1668–1670.

2. Pilar Calero, Ramón Martínez-Máñez, Félix Sancenón and Juan Soto. ***Synthesis, characterization and optical properties of silica nanoparticles coated with anthracene fluorophore and thiourea hydrogen bonding subunits***. Eur. J. Inorg. Chem. 2008, 36, 5649-5658.

3. Estela Climent, Pilar Calero, M. Dolores Marcos, Ramón Martínez-Máñez, Félix Sancenón and Juan Soto. ***Selective Chromofluorogenic Sensing of Heparin by using Functionalised. Silica Nanoparticles Containing Binding Sites and a Signalling Reporter.*** Chem. Eur. J. 2009, 15, 1816–1820.

4. M. Manuela M. Raposo, Beatriz García-Acosta, Tatiana Abalos, Pilar Calero, Ramón Martínez-Máñez, Félix Sancenón and Juan Soto. ***Synthesis and Study of the Use of Heterocyclic Thiosemicarbazones as Signaling Scaffolding for the Recognition of Anions.*** J. Org. Chem. 2010, 75, 2922–2933.

5. Pilar Calero, Mandy Hecht, Ramón Martínez-Máñez, Félix Sancenón, Juan Soto, José L. Vivancos and Knut Rurack. ***Silica nanoparticles functionalised with cation coordination sites and fluorophores for the differential sensing of anions in a quencher displacement assay (QDA).*** Chem. Commun., 2011, 47, 10599–10601.

6. Inmaculada Candel, Pilar Calero, Ramón Martínez-Máñez, Félix Sancenón, María D. Marcos, and Juan Soto***. Sensing properties of silica nanoparticles functionalized with anion binding sites and sulforhodamine B as fluorogenic signalling unit.*** Inorg. Chim. Acta, 2012, 381, 188-194.

ABBREVIATIONS AND ACRONYMS

(L, a, b)	Coordinates of color
AS-30	Silica colloidal comercial 20nm
ATP	Adenosinetriphosphate
AuNPs	Gold nanoparticles
BODIPY	4,4-difluoro-4-bora-3a
CHEF	Chelation-enhanced fluorescence
CIE 1976Lab	Color space
DMSO	Dimethylsulfoxide
E	The emission intensity of fluorescent nanoparticles and anions
E_0	The emission intensity of fluorescent nanoparticles
F	Fluorophore
FRET	Förster resonance energy transfer
HEPES	4-(2-Hydroxyethyl)-1-Piperazineethanesulfonic
IDAs	Indicator displacement assays
Imd	Imidazolium
LUDOX	Silica colloidal comercial

MCM-41	Mobil Composition of Matter-Mesoporous materials
MCM-48	Mobil Composition of Matter-Mesoporous materials
MHA	16-mercaptohexadecanoic acid
NA	Nanoparticles with anthracene
NA$_1$-Bt$_1$	Nanoparticles with anthracene and N-butyl-N'-(propyl)thiourea
NA$_1$-Bt$_3$	Nanoparticles with anthracene and N-butyl-N'-(propyl)thiourea
NA$_1$-Pt$_1$	Nanoparticles with anthracene and N-phenyl-N'-(propyl)thiourea
NA$_1$-Pt$_3$	Nanoparticles with anthracene and N-phenyl-N'-(propyl)thiourea
NA-Bt$_3$	Nanoparticles with anthracene and N-butyl-N'-(propyl)thiourea
NA-Pt$_3$	Nanoparticles with anthracene and N-phenyl-N'-(propyl)thiourea
NA$_X$-Bt$_Y$	Nanoparticles with anthracene and N-butyl-N'-(propyl)thiourea
NA$_X$-Pt$_Y$	Nanoparticles with anthracene and N-phenyl-N'-(propyl)thiourea
NMR	Nuclear Magnetic Resonance
PCA	Principal Component Analysis
PET	Photoinduced electron transfer
Q	Quenching metal ion

QDA	Quencher displacement approach
R	Receptor
RFNs	Receptor- and fluorophore-functionalised nanoparticles
Rh	Sulforhodamine B
S	Signaling fluorophores
S_1	Nanoparticles funcionalized with Rh and Imd
S_2	Nanoparticles funcionalized with Rh and guanidinium
SAM	Self-assembled monolayer
SBA-15	Santa Barbara Amorphous material. Mesoporous silica
SEM	Scanning Electron Microscope
SEM-WDS	Wavelength-Dispersive X-Ray Spectroscopy
S_G	Nanoparticles funcionalized with guanidinium
S_N	Nanoparticles funcionalized with aminopropyl
T	Metal ion binding sites
TEM	Transmission Electron Microscopy
TEOS	Tetraethylorthosilicate
TGA/ SDTA	Thermogravimetric analysis
TMOS	Tetramethylorthosilicate

TNT	Trinitrotoluene, 2-Methyl-1,3,5-trinitrobenzene
TSNP-M^{n+}	The bi-functionalized terpyridine–sulphorhodamine nanoparticles with metal
TSNP-Q	The bi-functionalized terpyridine–sulphorhodamine nanoparticles with Q = Cu^{2+}, Fe^{3+}, Hg^{2+}, Ni^{2+} and Pb^{2+}
TSNPs	The bi-functionalized terpyridine–sulphorhodamine nanoparticles
UV-Vis	Ultraviolet-Visible
NIR	Near-infrared spectroscopy
ΔE	Difference between two any colours. Euclidean distance.
SEM-EDX	Energy Dispersive X-Ray Spectroscopy

TABLE OF CONTENTS

OBJECTIVES

- ✓ Design and synthesis of hybrid silica nanoparticles functionalized with anion binding sites and optical signaling units (chromophores and/or fluorophores).
- ✓ Characterization of the prepared hybrid nanoparticles using stardard techniques of materials chemistry.
- ✓ Study of the optical behavior (colour and/or emission changes) of the prepared hybrid silica nanoparticles in the presence of selected anions.

CHAPTER 1

INTRODUCTION

1.1. SUPRAMOLECULAR CHEMISTRY

A vast number of molecules of different sizes and structure are known, ranging from the simple hydrogen molecule to the infinitely more complex biological proteins and nucleic acids. We may therefore be tempted to believe that the structures and properties of these materials and compounds can be directly related to those of the individual molecules that comprise them in a straightforward way. Unfortunately, this notion is not correct. However, deeply we understand the nature of individual molecules, this knowledge is not enough to explain the structures, functions and micro-macroscopic properties of materials and molecular assemblies, which are derived as a result of organizing individual molecules. The discipline that provides us the tools to understand how simple molecules organize themselves to build-up more complex units is *Supramolecular Chemistry*.[1]

Supramolecular Chemistry appears during the decade of 1960 with the pioneering works of Pedersen, Cram and Lehn, who received the Nobel Prize in 1987 for their contributions to the development of this new area inside chemistry.

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[1] J. M. Lehn, *Angew. Chem., 1988, 100, 91.*

Development of Supramolecular Chemistry was inspired primarily by Nature, which displays a wide variety of complex nanostructures originated during processes that occur in biology, such as substrate binding to a receptor protein, enzymatic reactions, assembling of protein-protein complexes, immunological antigen-antibody association, intermolecular reading, translation, etc.

Supramolecular Chemistry may be defined as *"the chemistry beyond the molecule"*, focusing on the organized entities of higher complexity that result from the association of two or more chemical species held together by intermolecular forces.[2] Therefore this area of chemistry has a deep effect on how efficiently chemists prepare structures of different sizes and shapes with dimension in the range of 1 to 100 nm. Thus, this new branch of chemistry deals mainly with how discrete molecules can cooperate through non-covalent interactions as hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, π-π interactions and electrostatic effects[3], to easily generate unique nanostructured supermolecules that present different properties, often better than the sum of the properties of each individual component.[4]

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[2] J. M. Lehn*, Supramolecular Chemistry, Ed. VCH, 1995; J.-M. Lehn, Nobel lecture, 1987.*

[3] Taylor & Francis Group, *Encyclopedia of Supramolecular Chemistry, Vols. 1, 2 (Ed.: Atwood, J.L.; Steed, J.W.), LLC, New York, 2004.*

[4] K. Ariga, T. Kunitake, *Supramolecular Chemistry-Fundamentals and application, Springer-Verlag Heidelberg.*

Due to this fact, incorporation of molecules onto materials offers new developments in the area of nanoscience and nanotechnology.

Bearing in mind the infinite number of possibilities given by the non-covalent bond chemistry, Supramolecular Chemistry can be divided in two main areas:

- ***molecular recognition chemistry***, wich is the chemistry associated with a molecule recognizing a partner molecule, also defined as host-guest chemistry.

- ***self-assembly chemistry,*** which is the chemistry of molecular assembly of many molecules.

The present PhD Thesis deals with molecular recognition chemistry in liquid-solid interface. For this reason, the basis of molecular recognition chemistry will be discussed below.

1.1.1. MOLECULAR RECOGNITION CHEMISTRY

Through supramolecular studies, nowadays we know that all biological functions are associated with a selective recognition between two or more molecules through specific interactions. This process is known as "molecular recognition". More specifically, molecular recognition is considerated the way that molecules recognize each other to form complex structures through a privileged relationship resting on the mutual interest of interacting partners possessing special qualities. Usually, the interaction between these partners (host-guest) is of kind of non-covalent interactions as hydrogen bonding, metal coordination, hydrophobic forces, Van der Waals forces and π–π interactions.[5]

The first and simplest model of molecular recognition is the lock-key principle suggested by Dr. Hermann Emil Fischer in 1894.[6] In this model, an enzyme (host) can discriminate among different substrates (guests) through the specific geometric complementarity between host and guest. Thus, only one guest fits exactly into one host like only a key enters in a specific lock. Knowledge of this simple principle allowed explaining the enzymatic catalysis, the compression of many complex

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[5] a) F. P. Schmidtchen, *Chem. Soc. Rev., 2010, 39, 3916; b) D. K. Smith, J. Chem. Edu., 2005, 82, 393.*

[6] E. Fischer, *Ber. Dtsch. Chem. Ges., 1895, 28, 1429.*

biological processes and setting up the foundation for the preparation and optimization of new synthetic hosts (receptors).[7]

Chemical receptors are particular molecules designed and optimized for molecular recognition of a given substrate (Figure 1).

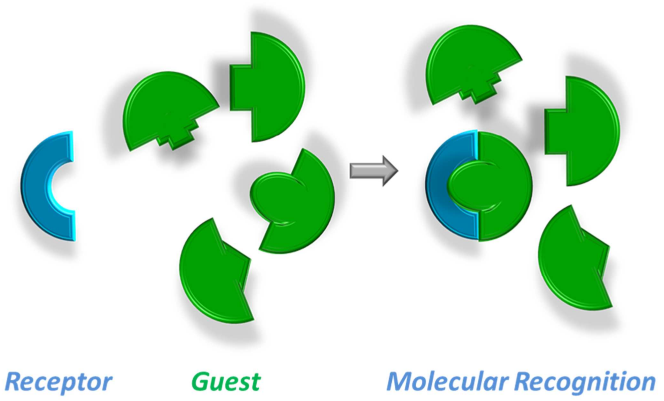

Figure 1. *Scheme of molecular recognition event by a specific host-guest interaction.*

For this, in the design and preparation of chemical receptors, it has to be taken into account the physical and chemical characteristics of the substrate in order to ensure the greatest possible complementarity in the host-guest pair. During the design of a molecular receptor it is

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[7] a) F. W. Lichtenthaler, *Angew. Chem., 1992, 104, 1577*; b) D. E. Koshland, *Angew. Chem., 1995, 33, 2375.*

necessary an extensive evaluation of the size, shape, geometry, charge, hydrophilic/lipophilic character and other physico-chemical characteristics of the guest-analyte pair to provide the best and largest amount of intermolecular forces between the host and the guest that are possible. In addition, an assessment of the environment in which molecular recognition takes places is very important when designing chemical receptors due to the influence of intermolecular processes such as solvation and electrostatic interactions between receptor, medium and analyte.[8]

Moreover, the effectiveness of a chemical receptor is associated to the degree of complementarity between host and guest, which promotes the action of one or more intermolecular forces during the recognition process. Furthermore, the receptor efficiency is determined by its ability to selectively recognize a specific guest, including the presence of other substrates with similar features. Therefore, it is intended that the receptor has more than one form of interaction type with the analyte, such as biological receptors whose complexity and complementarity with certain substrates ensures the activation of biological processes in a consistent and very specific manner.[9]

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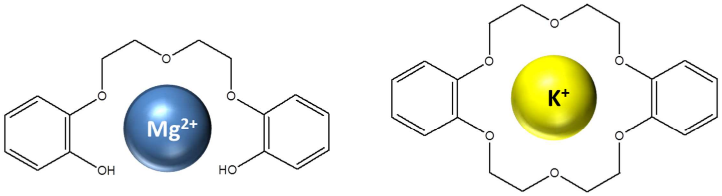
[8] a) G. V. Oshovsky, D. N. Reinhoudt, W. Verboom, *Angew. Chem. Int. Ed. 2007, 46, 2366*; b) S. Kubik, *Chem. Soc. Rev., 2010, 39, 3648*.

[9] L. A. Joyce, S. H. Shabbir, E. V. Anslyn*, Chem. Soc. Rev., 2010, 39, 3621*.

Villiers conducted the first studies with biological receptors in 1891 with cyclodextrins.[10] However, the skills of cyclodextrins as receptors were not studied until 1935 when Pringsheim demonstrated their ability to form complexes with certain specific substrates.[11] The first synthetic receptors were reported in 1960 by Lehn,[12] Cram[13] and Pedersen[14] who prepared crown ethers and cryptands capable of recognizing certain metal cations by coordination processes (Figure 2).



1 **2**

Figure 2. *Metal complex based on crown ethers. Pederson´s complexes of divalent cations (**1**) and K^+ (**2**) with crown ethers.*

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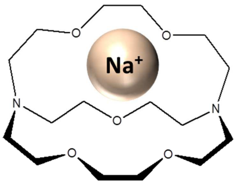
[10] A. Villiers, *Compt. Rend. Fr. Acad. Sci. 1891, 112, 435*.

[11] H. Pringsheim, *Chemistry of the Sacchaides; McGraw-Hill: New York, 1932; p 280.*

[12] a) J. M. Lehn*, Acc. Chem. Res.,1978,11, 49*; b) J. M. Lehn, *Pure Appl. Chem., 1978, 50, 9, 871.*

[13] D. J. Cram*, J. Am. Chem. Soc. 1978, 100, 8190.*

[14] a) C. J. Pedersen, *J. Am. Chem. Soc. 1957, 79, 2295*; b) C. J. Pedersen*, J. Am. Chem. Soc. 1967, 89, 7017.*



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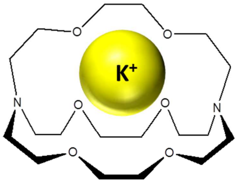

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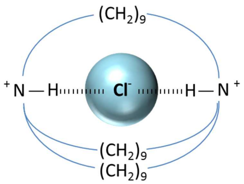
Figure 3. *Structures of metal complex of Na$^+$ (**3**) and K$^+$ (**4**) with two of the first synthetic cryptands.*

Later on, at the end of the same decade, the first anion receptors were reported by Biallas[15] and by Simmons (Figure 4).[16]



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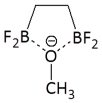

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Figure 4. *Representation of the first anion receptors. Left: chelated complex (**5**) formed between macrobicyclic amines and Cl$^-$ anion, reported by Park and Simmons. Right: chelated complex (**6**) formed between bidentade 1,2-bis(difluoroboryl)ethane and methoxide anion, reported by Shriver and Biallas.*

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[15] D. F. Shriver, M. J. Biallas, *J. Am. Chem. Soc., 1967, 89, 1078.*

[16] C. H. Park, H. E. Simmons, *J. Am. Chem. Soc., 1968, 90, 2429.*

1.1.2. MOLECULAR SENSORS

In the Supramolecular Chemistry field, receptors specifically designed for sensing purposes are generally called chemosensors. [17] A chemosensor is a molecule that produces a detectable and easy-to-measure signal, revealing the presence of the guest, as a result of molecular recognition processes. Signals widely used to detect the presence of certain guest molecules are changes in color, [18] fluorescence[19] or modulations in electrochemical properties.[20] In this sensing process, information at the molecular level, such as the presence or not of a certain guest in solution, is amplified to a macroscopic level; hence, sensing might open the door to the determination (qualitative or quantitative) of certain analytes. In this sense, one interesting goal in the development of sensors deals with the synthesis of highly selective systems.[21] Reversibility and fast response will be also appealing features for an applicable sensor to be taken into account in its design.

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[17] Chemosensors for Ion and Molecule Recognition, Ed. J. P. Desvergne, A. W. Czarnik, *NATO Asi Series, Series C., 1997, Kluwer Academic Publishers.*

[18] a) H.-G. Löhr, F. Vögtle, *Acc. Chem. Res., 1985, 18, 65*. b) M. Takagi, K. Ueno, *Top. Curr. Chem., 1984, 121, 39.*

[19] A. W. Czarnik*, Acc. Chem. Res., 1994, 27, 302.*

[20] a) P. D. Beer*, Chem. Commun., 1996, 689*. b) P. D. Beer, *Coord. Chem. Rev., 2000, 205, 131.*

[21] a) R. Martínez-Máñez, F. Sancenón, *Chem. Rev., 2003, 103, 4419*. b) M. Moragues, R. Martínez-Máñez, F. Sancenón, *Chem. Soc. Rev., 2011, 40, 2593.*

Whereas chemical sensors or chemosensors usually refer to systems that typically used coordinative forces for guest binding, the term reagents or chemodosimeter is related with the use of specific irreversible reactions involving guests.

A molecular chemical sensor or chemosensor is composed by two units:

- ***Binding subunit*** which is the unit responsible of the recognition of the substrate. The process of recognition depends on the host molecule characteristics. The binding subunit is designed in order to achieve a selective coordination via a suitable receptor-guest complementarity.

- ***Signalling subunit*** which acts as a signal transducer and informs of the recognition process that occurs at molecular level through changes in a measurable macroscopic signal.

Among changes that could be observed, modulations in colour and fluorescence are especially appealing. While colorimetric sensors can be used to the naked eye detection of analytes, fluorogenic sensors have in general a high degree of sensitivity. Chromo-fluorogenic chemosensors are normally constructed by one of following approaches (Figure 5).

a) ***Binding site-signalling subunit approach.*** The chemosensor is formed by “binding sites” and “signaling units” that are covalently linked. The coordination of the guest with the binding site changes physical properties of the signaling subunit giving rise to variations in either, the color (chromogenic chemosensor) or in the fluorescence behavior (fluorogenic chemosensor).[22]

b) ***The displacement approach.*** This approach uses binding sites and signaling subunits forming a coordination complex (molecular ensemble) not covalently linked. This approach relies in a displacement reaction when the target molecule is coordinated with the binding site and the signaling subunit returns to the solution. A suitable signal is observed when the color or emission of the signaling subunit in the molecular ensemble is different than that present when it is free in solution.[23]

c) ***The ‘‘chemodosimeter’’ approach.*** This is related with the use of specific irreversible reactions involving hosts and guests,

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[22] R. A. Bissell, P. de Silva, H. Q. N. Gunaratne, P. L.M. Lynch, G. E. M. Maguire, K. R. A. S. Sandanayake, *Chem. Soc.Rev., 1992, 21, 187.*

[23] a) S. L. Wiskur, H. Ait-Haddou, J. J. Lavigne, E. V. Anslyn, *Acc. Chem. Res., 2001, 34, 963*. b) B. T. Nguyen, E. V. Anslyn*, Coord. Chem. Rev., 2006, 250, 3118*.

which are coupled to a color or emission variations.[24] If the chemical reaction is irreversible, the use of the term chemosensor cannot strictly be used and we will refer to these systems as chemodosimeters or chemoreactants.[25]

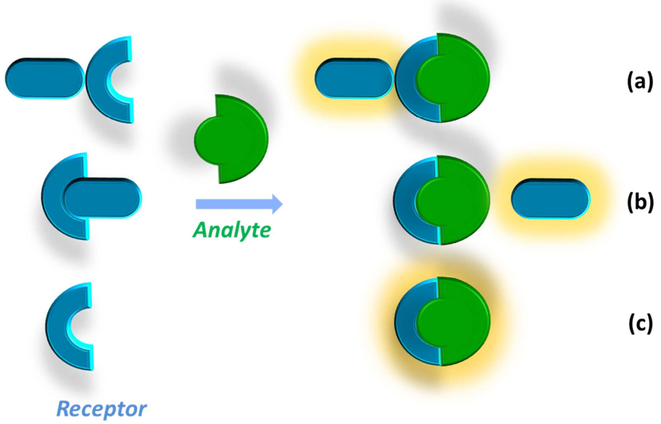

Figure 5. *Representative scheme of the three main approaches used in the development of optical chemosensors. a) Binding site-signalling subunit; b) Displacement protocol; c) Chemodosimeter approach.*

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[24] a) M.-Y. Chae, A. W. Czarnik, *J. Am. Chem. Soc., 1992, 114, 9704*. b) V. Dujols, F. Ford, A. W. Czarnik, *J. Am. Chem. Soc., 1997, 119, 7386.*

[25] Z. Xu, X. Chen, H. N. Kim, J. Yoon*, Chem. Soc. Rev., 2010, 39, 127*.

1.2. HETERO-SUPRAMOLECULAR CHEMISTRY

Very recently, two major research areas have transformed our vision of the chemistry of molecules as well as material science. One of them, Supramolecular Chemistry, was established in the 1980ies and is concerned, as explained above, with the study of the interaction between molecules. The other one, nanotechnology[26], emerged in the 1990ies and involves research and development of technology at the nanometric level (1–100 nm).[27]

In many respects, Supramolecular Chemistry still largely utilizes molecular organic components so that it has traditionally been poorly interrelated with the chemical concepts of nanoscopic (and mainly

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[26] A brief history of Nanotechnology.

On December 29, 1959, physicist Richard Feynman gave a radical lecture at an American Physical Society meeting at Caltech titled " There´s Plenty of Room at the Bottom".Feynman suggested that it should be possible to make machines at a nano-scale that "arrange the atoms the way we want", and do chemical synthesis by mechanical manipulation. This lecture was the birth of idea and study of nanotechnology.

Professor Norio Taniguchi of the Tokyo Science University introduced the term "nanotechnology" in 1974. He described nanotechnology as the processing of separation, consolidation, and deformation of materials by one atom or by one molecule.

In the 1980´s, Dr K.Eric Drexler, promoted nanoscale phenomena through books: "Engines of creation: The coming era of Nanotechnology", "Nanosystems: Molecular machinery, manufacturing, and computation". He was ultimately responsible for the term nanotechnology to acquire its current sense. He was presented the frist PhD. in nanotechnology.

[27] K.E. Drexler, Engines of Creation, Anchor Books/Doubleday, 1986. For a www version of the book see http://www.foresight.org/EOC/.

inorganic) solids. Supramolecular achievements have been obtained by the stepwise synthesis of molecular architectures via the successive formation of covalent bonds.[28] Alternative routes utilize the generation of supramolecular forms by the self-assembly of (supra)molecular components.[29] Despite the design of entirely organic superstructures for various purposes,[28] recent years saw the development of metal organic frameworks[30] and coordination polymers[31] by combining inorganic and organic building blocks into larger networks.

An attractive alternative to achieve organised hybrid systems, i.e., inorganic-organic supramolecular ensembles with very diverse yet sophisticated performances, is to use pre-organised nanoscopic solid-state structures and attach, arrange or assemble functional molecular units of varying complexity to or at the inner and/or outer surface of the inorganic scaffold. Recent examples suggest that the wise combination of supramolecular principles and solid nanoscopic structures harbours an unprecedented tunability of the properties of the solids and opens up new perspectives of the applicability of supramolecular concepts. What distinguishes these “hetero-supramolecular” functionalities and what

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[28] (a) T.R. Kelly *(Ed.) Top. Curr. Chem.2005, 262*, 1; (b) B.W. Purse, J. Rebek, *Jr.Proc. Natl. Acad. Sci. USA2005, 102, 10777*; (c) A.C. Grimsdale, K. Müllen, *Angew. Chem. Int. Ed.2005, 44, 5592.*

[29] (a) S. Uppuluri, L.T. Piehler, J. Li, D.R. Swanson, G.L. Hagnauer, D.A. Tomalia*, Adv. Mater.2000, 12, 796*; (b) K. Haupt, *Chem. Commun.2003, 171.*

[30] S. Kaskel, F. Schüth, M. Stöcker, *Micropor.Mesopor.Mater.2004, 73, 1.*

[31] S. Kitagawa, R. Kitaura, S. Noro, *Angew. Chem. Int. Ed. 2004, 43, 2334.*

renders them especially attractive is the appearance of synergistic effects that are hardly achievable in molecular-based systems or in nanoscopic solids alone.

The next points will give an introduction to the main concepts of enhanced binding by pre-organization of active organic functionalities on inorganic surfaces, the unique recognition and signalling properties of functionalized bulk or porous nanoparticles and signal amplification features.

1.2.1. ENHANCED COORDINATION BY PREORGANIZATION OF BINDING SITES

Pre-organization is one of the key concepts in supramolecular recognition. It determines the tendency of a host molecule and its coordination sites to assume a conformation that is ideally suited to bind a guest molecule, leading to a complex with increased stability. Moreover, in general, a structurally rigid ligand or receptor, which is in the optimum conformation to complex a guest, is more pre-organized than a flexible ligand.[32] This design principle has a strongimpact on many different areas of application and advances. However, increasing the amount of macrocyclic structural elements, which can easily get synthetically very demanding, is not the only way to obtain highly pre-

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[32] D. P. Cormode, J. J. Davis, P. D. Beer, *J. Inorg. Organomet. Polym., 2008, 32.*

organized recognition elements with improved binding ability. Increased pre-organization can also be achieved by immobilizing (simple) ligands on solid supports like nanoparticles, whether bulk or mesoporous, or other nano-structured solids[33] With respect to particulate objects, today, gold and silica are typically used as solid supports in such approaches because of their well-known surface functionalization chemistries.[34]

In essence, the pre-organizational features provided by the attachment of chemical entities on a surface avoid demanding ligand design and allow for a combinatorial approach to functionalization of the solid supports.[35] The synergistic effect achieved by the organization and the structural rigidity of hosts on the solid surface is the key element for enhanced recognition of a guest, often resulting in an improved selectivity and sensitivity compared with the free molecular hosts.[36] The modification of surface functionalities plays a decisive role in the development of such systems and has an important impact on the application of these hybrid materials in areas ranging from sensing to biotechnology and catalysis.

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[33] J. V. Ros-Lis, R. Casasús, M. Comes, C. Coll, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós, J. El. Haskouri, N. Garró,K. Rurack, *Chem. Eur. J., 2008, 14, 8267.*

[34] X. Feng, G. E. Fryxell, L.-Q. Wang, A. Y. Kim, J. Liu, K. M. Kemner, *Science, 1997, 276, 923.*

[35] H. Y. Lee, D. R. Bae, J. C. Park, H. Song, W. S. Han, J. H. Jung, *Angew. Chem. Int. Ed., 2009, 48, 1239.*

[36] S. A. El-Safty, D. Prabhakaran, A. A. Ismail, H. Matsunaga, F. Mizukami, *Chem.Mater.,2008, 20, 2644.*

1.2.1.1. GOLD SUPPORTED PREORGANIZATION

A pioneering study introducing the principle of enhanced recognition through pre-organization of a self-assembled monolayer (SAM) of thiol-functionalized ligands on gold nanoparticles (AuNPs) was reported by Major and Zhu, who investigated the formation of Cu^{2+}complexes with SAMs of 16-mercaptohexadecanoic acid (MHA) on gold surfaces in relation to using molecular mono- and bidentate carboxylates.[37] They found that the formation constant for the Cu^{2+}complex with the pre-arranged MHA was 119 times greater than with succinic acid, and 213 times greater than with glutaric acid in aqueous solutions. Since bidentate ligands generally form more stable complexes than monodentate ligands, the authors proposed that the monodentate MHAs are pre-organized on the “flat” gold surface and act as a multidentate coordinating layer, able to form a more stable Cu^{2+}complex than bidentate carboxylates (Figure 6). Accordingly, this behaviour has been termed the *surface chelate effect*.

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[37] R. C. Major, X.-Y. Zhu, *J. Am. Chem. Soc., 2003, 125, 8454.*

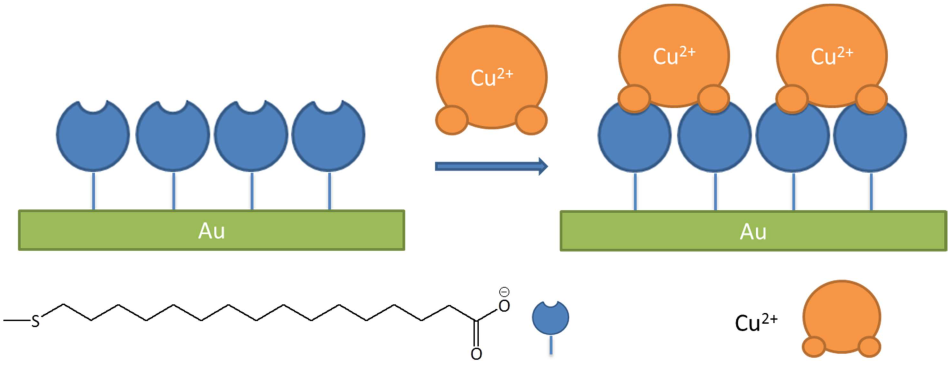

Figure 6. *Schematic illustration of the surface chelate effect.*

In other example, Beer and co-workers attached zinc metalloporphyrins to AuNPs (Figure 7).[38] These hybrid AuNPs are able to bind chloride and dihydrogen phosphate in DMSO with association constants log$K$ of 4.3 and 4.1, respectively, which are two orders of magnitude larger than the log$K$ measured for the free zinc metalloporphyrin receptor and chloride (log$K$< 2) or dihydrogen phosphate (log$K$ = 2.5) in solution. It is obvious that the step from the molecule itself to the hetero-supramolecular ensemble results in new properties, which are not simply an extrapolation of the solution conduct to the surface, but to own unique features.

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[38] P. D. Beer, D. P. Cormode, J. J. Davis, *Chem. Commun., 2004, 414.*

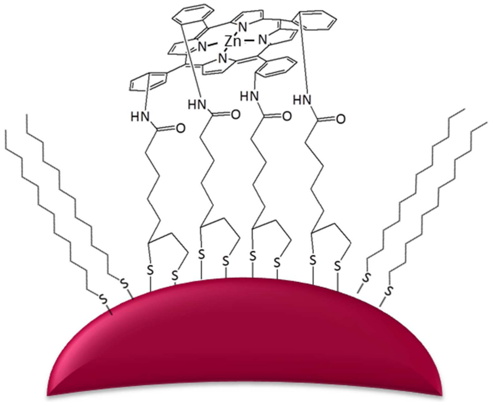

Figure 7. *Porphyrin-gold nanoparticles hybrid used for the enhanced recognition of anions.*

A nice example of the use of multi-functionalised nanoscopic surfaces for improved recognition involving multiple binding interactions employs AuNPs that contain diacyldiaminopyridinehydrogen-bonding moieties and pyrene aromatic stacking elements for flavin binding.[39] In this hybrid material, the chemical amplification of the recognition of flavins is facilitated by the synergistic effects of multiple non-covalent interactions, i.e., hydrogen-bonding and π-stacking (Figure 8).

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[39] (a) A.K. Boal, V.M. Rotello, *J. Am. Chem. Soc. 2000, 122, 734*; (b) A.K. Boal, V.M. Rotello, *J. Am. Chem. Soc. 1999, 121, 4914.*

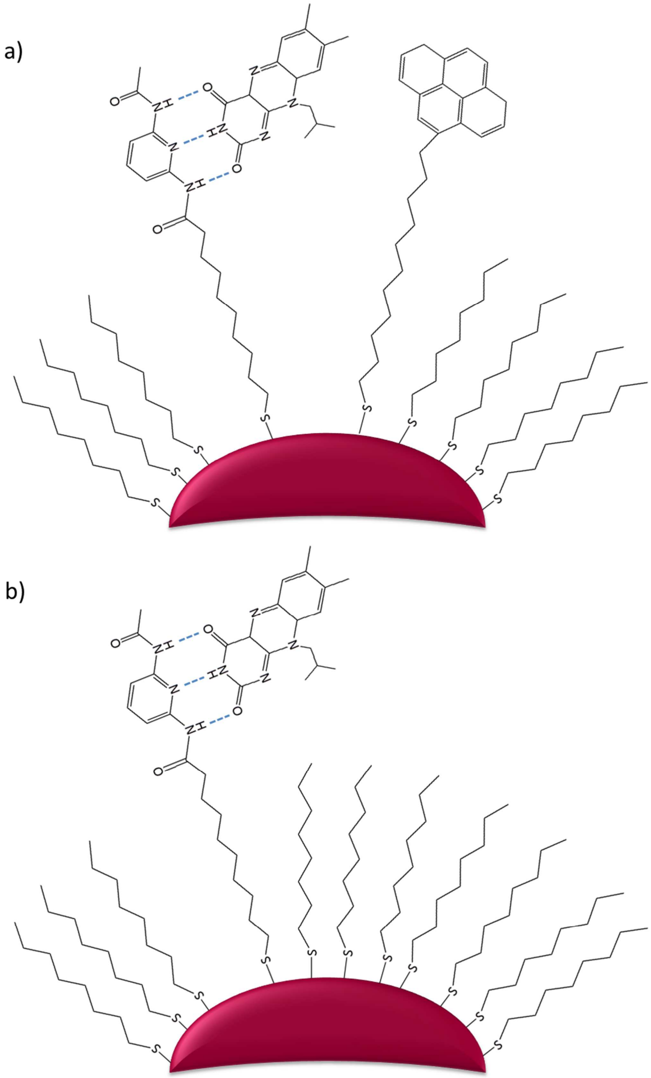

Figure 8. *(a) Gold nanoparticles functionalized with diacyldiaminopyridine hydrogen bonding moieties and pyrene units to enhance flavin recognition through arene–π stacking perform better than (b) mono-receptor-functionalized gold nanoparticles lacking the pyrene support.*

The observed association constant K of colloid in Figure 8a with flavin (K = 323 M^{-1}) is distinctly higher than that observed for the colloid/flavin system (K = 196 M^{-1}), showed in Figure 8b, where only hydrogen bonding can occur.

Watanabe and co-workers[40] designed a system for the optical detection of anions based on AuNPs surface-modified with amide ligands. The detection limit is increased by about three orders of magnitude higher than that originally expected from the anion binding ability of neutral amide ligands. The addition of anions to the solution of material caused dramatic changes in the plasmon band. When 0,5 equivalents of anion were added the plasmon band decreased by 45–55% in intensity with a slight red shift in wavelength. The marked decrease in extinction would be due to anion-induced aggregation through hydrogen-bond formation between the anions and the interparticle amide ligands (Figure 9). The authors also showed, using coupled dipole approximations, that a dramatic red shift occured in the plasmon band when the interparticle distance in the aggregates decreaseed to less than the average particle radius.[41] When an excess of $H_2PO_4^-$, HSO_4^- or AcO^- was added to the solution, the plasmon band was restored to the original state (Figure 9).

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[40] S. Watanabe, M. Sonobe, M. Arai, Y. Tazume, T. Matsuo, T. Nakamuraand K. Yoshida, *Chem. Commun. , 2002, 2866.*

[41] A. A. Lazarides and G. C. Schatz, *J. Chem. Phys., 2000, 112, 2987.*

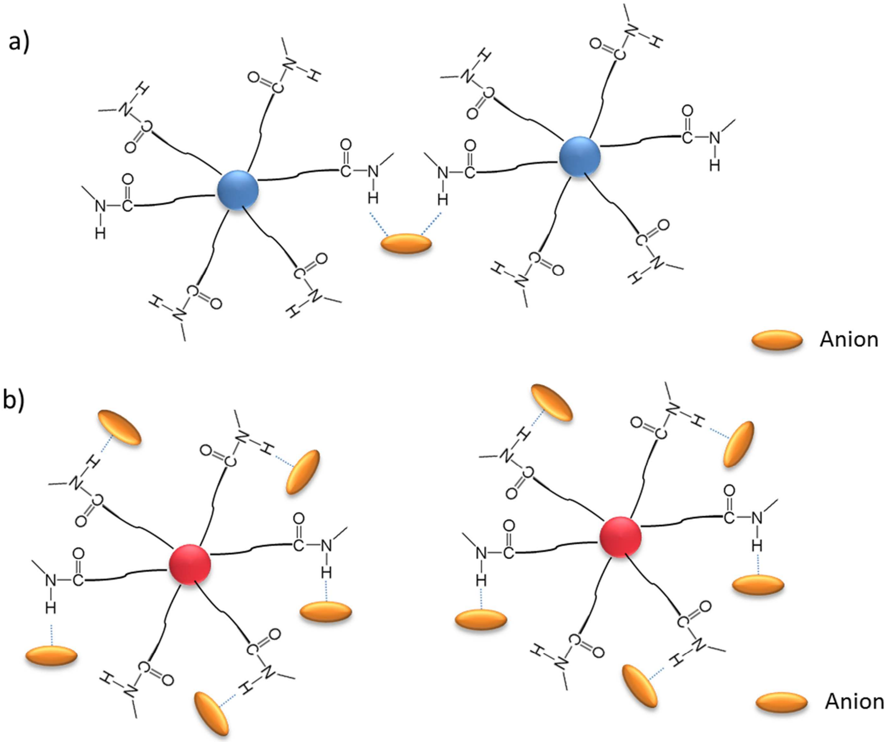

Figure 9. *(a) functionalized AuNPs aggregates via hydrogen-bond formation between amide and anions. (b) functionalized AuNPs disaggregate when the anion concentration further increases.*

In other example Lin et al.[42] developed a new hybrid material based on AuNPs functionalized with 15-crown-5 capable to recognice K^+ in an aqueous matrix in the presence of Li^+, Cs^+, NH^{4+}, Mg^{2+}, Ca^{2+}, and excess amounts of Na^+. By adding K^+, the colloidal solution changes color from

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[42] S. Lin, S. Liu, C. Lin, C. Chen, *Anal. Chem. 2002, 74, 330-335.*

red to blue, in response to surface plasmon absorption of dispersed and aggregated nanoparticles (Figure 10).

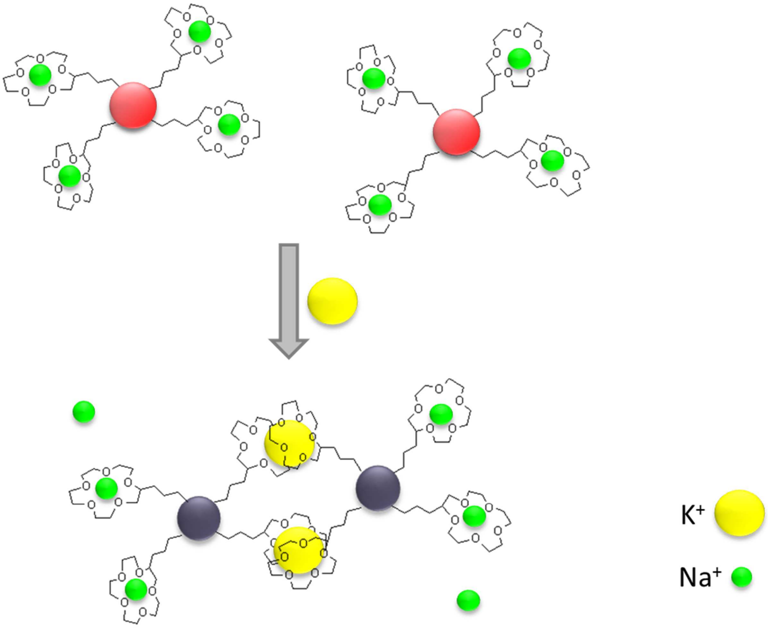

Figure 10. *Recognition of K^+ by 15-crown-5 functionalized AuNPs in the presence of Na^+.*

The concentration ranges of K^+ detected in this study are 0.099-0.48 mM and 7.6 μM-0.14 mM, when concentrations of colloidal gold are 54.9 and 7.1 nM, respectively. Crown ethers are known for the unusual property of forming stable complexes with alkali metals. The stability of these complexes is related to how it fits the cation in the cavity of the crown.[43]

In another example, the group of Lin reported an opposite transformation in which the AuNPs are initially aggregated and promptly become dispersive in response to an analyte.[44] To achieve rapid sensing, the interaction for the initial aggregation should be weaker than the interaction of the analyte with the nanoparticules. AuNPs[45] were bifunctionalized by thioctic acid and 15-crown-5. Initially the AuNPs are aggregated due to hydrogen bond interactions between carboxylates.[46] Then, the addition of Pb^{2+},that it is hosted by the 15-crown-5, produces electrostatic repulsion and resulted in dispersive AuNPs due to the positive charge of metal ions (Figure 11). The authors also found that a

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[43] (a) D. Masilamani and M. E. Lucas, *in Fluorescent Chemosensors for Ion and Molecule Recognition, Ed. A. W. Czarnik, American Chemical Society: Washington, DC, 1992*. (b) Y. Inoue, G.W. Gokel, *Cation Binding by Macrocycles: Complexation of Cationic Species by Crown Ethers, Ed. Dekker: New York, 1990.* (c) J.S. Bradshaw, K.E. Krakowiak, R.M. Izatt, *Aza-Crown Macrocycles; Ed. John Wiley and Sons: New York, 1993.* (d) T. Toupance, H. Benoit, D. Sarazin, J. Simon, *J. Am. Chem. Soc. 1997, 119, 9191-9197*. (e) A. Boldea, I.Levesque, M. Leclerc, *J. Mater. Chem. 1999, 9, 2133-2138.* (f) S. Flink, B.A. Boukamp, A. van den Berg, F. C. J. M. van Veggel, D. N. Reinhoudt, *J. Am. Chem. Soc. 1998, 120, 4652-4657*. (g) S. Flink, F. C. J. M. van Veggel, D. N. Reinhoudt, *J. Phys. Chem. B 1999,103, 6515-6520*. (h) K. Kimura, H. Harino, E. Hayata, T. Shono, *Anal. Chem. 1986, 58, 2233-2237.*

[44] S. Lin, S. Wu, C. Chen, Angew. Chem. Int. Ed. 2006, 45, 4948–4951

[45] (a) K. C. Grabar, R. G. Freeman, M. B. Hommer,M. J. Natan, *Anal. Chem. 1995, 67, 735–743*. (b) S. Lin, Y. Tsai, C. Chen, C. Lin, C. Chen, *J. Phys. Chem. B 2004, 108, 2134–2139*. (c) S. Lin, C. Chen, M. Lin, H. Hsu, *Anal. Chem. 2005, 77, 4821–4828.*

[46] (a) H. Schmitt, A. Badia, L. Dickinson, L. Reven, R. B. Lennox, *Adv. Mater. 1998, 10, 475–480*. (b) S. Chen, K. Kimura, *Langmuir 1999, 15, 1075–1082*. (c) J. Simard, C. Briggs, A. K. Boal, V. M. Rotello, *Chem. Commun. 2000, 1943–1944*.

suspension of nanoparticles functionalized with thioctic acid alone remained blue upon Pb^{2+} addition.

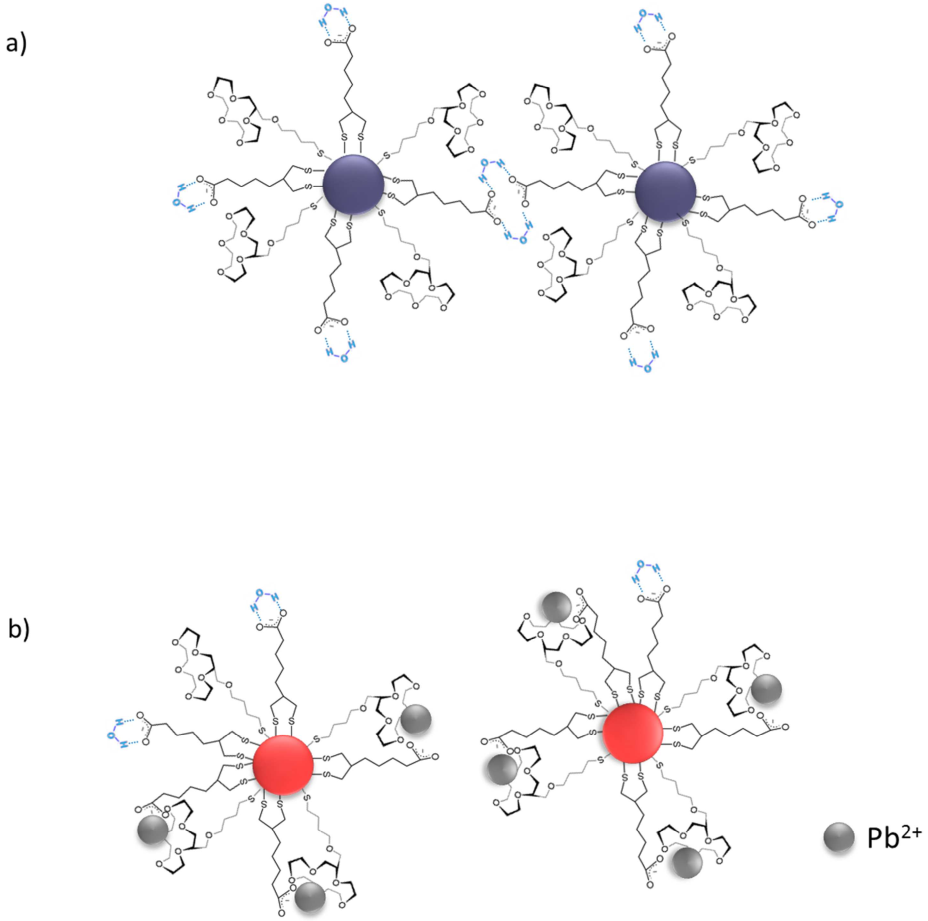

Figure 11. *(a) AuNPs aggregate by hydrogen bonds. (b) Recognition of* Pb^{2+} *by AuNPs.*

1.2.1.2. SILICA SUPPORTED PREORGANIZATION

Surfactant-templated mesoporous silica materials have stimulated the research area of supramolecular nanomaterials because of their ability to interact with atoms, ions, and molecules on their external and internal surface when adequately functionalized. The possibilities of specific surface modification of the supports and the unique character of the pore-confined “active” inner surface allow for enhanced performance with respect to selectivity and sensitivity, again largely through the pre-organization of the recognition elements on the surface. The same features could be expected when using silica nanoparticles as inorganic supports.

An early example was based in the covalent grafting of alkylaminoanthracene groups to a mesoporous MCM-41-type silica material (Figure 12).[47] The organic probe moiety contains both (i) an amine that acts as an anion coordination site in its protonated form and (ii) an anthracene group, bound to the amino moiety through a methylene spacer that acts as the signalling unit. In aqueous solution at *ca*. pH 2.8, the ammonium residues of the organic groups are able to interact with the target ATP through electrostatic and hydrogen bonding forces. Moreover, since ammonium groups are unable to quench

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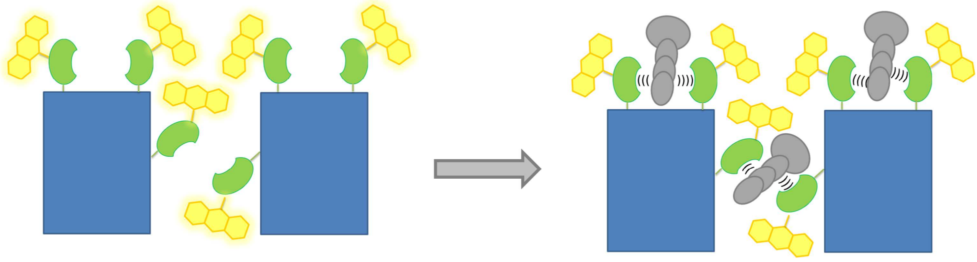
[47] A. B. Descalzo, D. Jiménez, M. D. Marcos, R. Martínez-Máñez, J. Soto, J. El Haskouri, C. Guillem, D. Beltrán, P. Amorós, M. V. Borrachero, *Adv. Mater., 2002, 14, 966.*

anthracene, the hybrid sensing material was highly fluorescent in the absence of the analyte. Binding of ATP to the anchored probes then led to remarkable fluorescence quenching, because of the interaction of the electron-rich adenine moiety with the anthracene units. At the same time, π stacking interactions between adenine and anthracene presumably stabilize the supramolecular complex. Moreover, the importance of the confinement of the probe molecules in the nanoscopic pores, i.e., the mesoporous structure of the MCM-41 material, was verified by comparative studies with surface-bound alkylaminoanthracene groups attached to a locally flat and non-porous fumed silica support. The association constant for ATP measured for the mesoporous MCM-41 material was significantly larger than that obtained for the functionalised fumed silica, and much larger than the association constant found for the corresponding molecular aminomethylanthracene probe in solution. This enhanced ATP response in the ordered mesoporous solid is clearly due to the surface chelate effect.



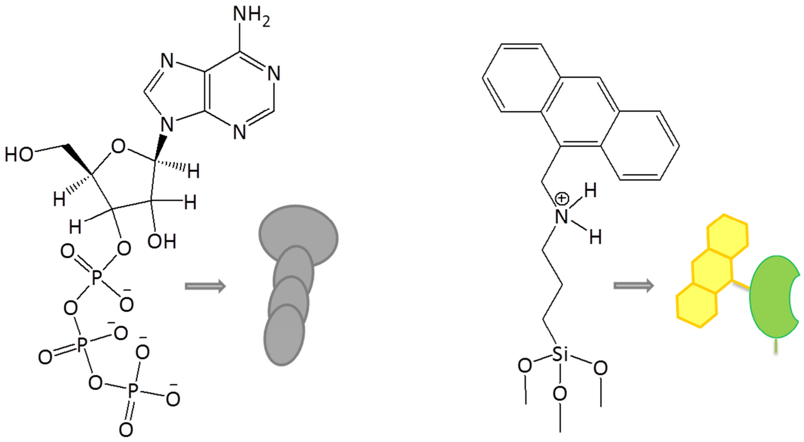


Figure 12. *Enhanced recognition of ATP with protonated aminoalkylanthracene hosts on the MCM-41 surface.*

A naked-eye dual functional hybrid material for detection and removal of Pb^{2+} ions from water and blood was reported by Jung and co-workers.[48, 36] A BODIPY derivative was selected as fluorescent host and signal-transducing unit and was anchored on a nanoparticle consisting of a magnetic nickel core coated with a silica shell (Figure 13). The hosts on the silica surface were virtually non-fluorescent due to an efficient photoinduced electron transfer (PET) process from the lone electron pair of the aniline nitrogen to the excited fluorophore. Upon the addition of Pb^{2+}, a large chelation-enhanced fluorescence (CHEF) was

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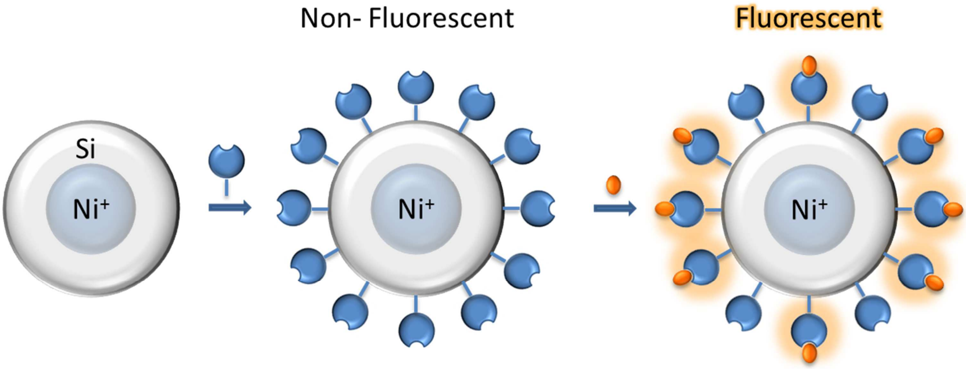
[48] H. Y. Lee, D. R. Bae, J. C. Park, H. Song, W. S. Han, J. H. Jung, *Angew. Chem. Int. Ed., 2009, 48, 1239.*

observed due to coordination of Pb^{2+} to the nitrogen atom, which resulted in blocking the PET process. An overall Pb^{2+}-induced emission change of approximately 8-fold was observed. The selectivity of the particle-appended BODIPY host for Pb^{2+} detection was approximately 12-fold higher compared to the free host. Moreover the response of the sensing material in the presence of various metal ions such as Li^{+}, Na^{+}, Mg^{2+}, K^{+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Ag^{+}, Cd^{2+}, and Hg^{2+} was investigated in aqueous solution, but only Pb^{2+}induced a large CHEF effect. The authors assumed that the chemical detection of $Pb^{2+}$was facilitated by the cooperative effect of multiple noncovalent interactions as a result of the pre-organization of the hosts on the solid surface. Magnetic field-assisted removal of Pb^{2+} from water and blood with the hybrid material rendered remediation yields of up to 97 %.



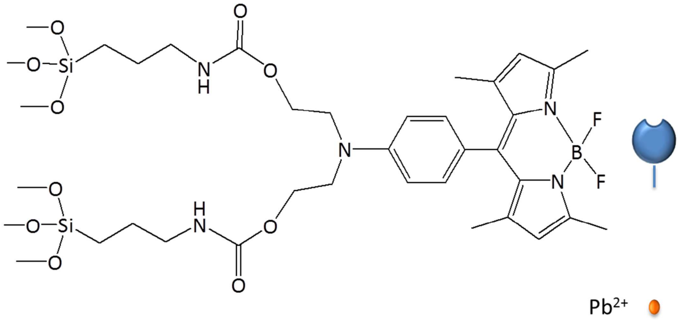


Figure 13. *Preparation of BODIPY-functionalized magnetic silica nanoparticles with a nickel core and subsequent Pb^{2+} ion detection through fluorescence enhancement.*

Apart from the previous examples, the recent literature shows an increasing number of reports dealing with the design of hybrid mesoporous silicas containing chromogenic or fluorogenic probes for the indication of metal ions. Most of the reported examples involve the use of MCM-41, MCM-48, hydrothermally stable mesoporous molecular sieves (SBA-15) or silica nanotube matrices. Additionally, the very low detection limits observed for some of these systems suggests that the surface chelate effect also plays a role here. Examples have been reported for the signalling of Hg^{2+},[49] Cu^{2+},[50] Fe^{3+},[51] Zn^{2+},[52] Cd^{2+},[53] Bi^{3+},[54]

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[49] (a) M. H. Lee, S. J. Lee, J. H. Jung, H. Lim, J. S. Kim, *Tetrahedron, 2007, 63, 12087*; (b) R. Métivier, I. Leray, B. Lebeau, B. Valeur, *J. Mater. Chem., 2005, 15, 2965;* (c) S. J. Lee, J. E. Lee, J. Seo, I. Y. Jeong, S. S. Lee, J. H. Jung, *Adv. Funct. Mater., 2007, 17, 3441*; (d) S. A. El-Safty, D. Prabhakaran, Y. Yoshimichi, F. Mizukami, *Adv. Funct. Mater., 2009, 18, 1739.*

[50] (a) L. Gao, J. Q. Wang, L. Huang, X. X. Fan, J. H. Zhu, Y. Wang, Z. G. Zou, *Inorg. Chem., 2007, 46, 10287*; (b) H. Zhang, P. Zhang, K. Ye, Y. Sun, S. Jiang, Y. Wang, W. Pang, *J. Lumin., 2006, 117, 68;* (c) L. L. Li, H. Sun, C. J. Fang, J. Xu, J. Y. Jin, C. H. Yan, *J. Mater. Chem., 2007, 17, 4492.*

and Pb^{2+}.[55] Also, optical pH sensors using mesoporous structures have been reported.[56]

A different approach that also uses the concept of enhanced coordination by pre-organization and mesoporous solids as the inorganic framework is the design of abiotic binding pockets for selective colorimetric displacement assays. Here, the protocol involves several steps: (i) the pores are functionalized with adequate binding sites, (ii) the pores are loaded with dyes that coordinate to these anchored sites and (iii) a target analyte which forms a stronger complex with the binding sites displaces the dye and enables the colorimetric or fluorescence detection of the guest (Figure 14).

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[51] J. Q. Wang, L. Huang, M. Xue, Y. Wang, L. Gao, J. H. Zhu, Z. Zou, *J. Phys. Chem. C, 2008, 112, 5014.*

[52] L. Gao, Y. Wang, J. Wang, L. Huang, L. Shi, X. Fan, Z. Zou, T. Yu, M. Zhu, Z. Li, *Inorg. Chem., 2006, 45, 6844.*

[53] (a) T. Balaji, M. Sasidharan, H. Matsunaga, *Anal. Bioanal.Chem., 2006, 384, 488*; (b) T. Balaji, S. A. El-Safty, H. Matsunaga, T. Hanaoka, F. Mizukami, *Angew. Chem. Int. Ed., 2006, 45, 7202;* (c) S. A. El-Safty, A. A. Ismail, H. Matsunaga, F. Mizukami, *Chem. Eur. J., 2007, 13, 9245*; (d) S. A. El-Safty, D. Prabhakaran, A. A. Ismail, H. Matsunaga, F. Mizukami, *Adv. Funct. Mater., 2007, 17, 3731.*

[54] S. A. El-Safty, A. A. Ismail, H. Matsunaga, H. Nanjo, F. Mizukami, *J. Phys. Chem. C, 2008, 112, 4825.*

[55] (a) S. A. El-Safty, D. Prabhakaran, A. A. Ismail, H. Matsunaga, F. Mizukami, *Chem. Mater., 2008, 20, 2644*; (b) S. A. El-Safty, A. A. Ismail, H. Matsunaga, T. Hanaoka, F. Mizukami, *Adv. Func. Mater., 2008, 18, 1485.*

[56] G. Wirnsberger, B. J. Scott, G. D. Stucky, *Chem. Commun., 2001, 119.*

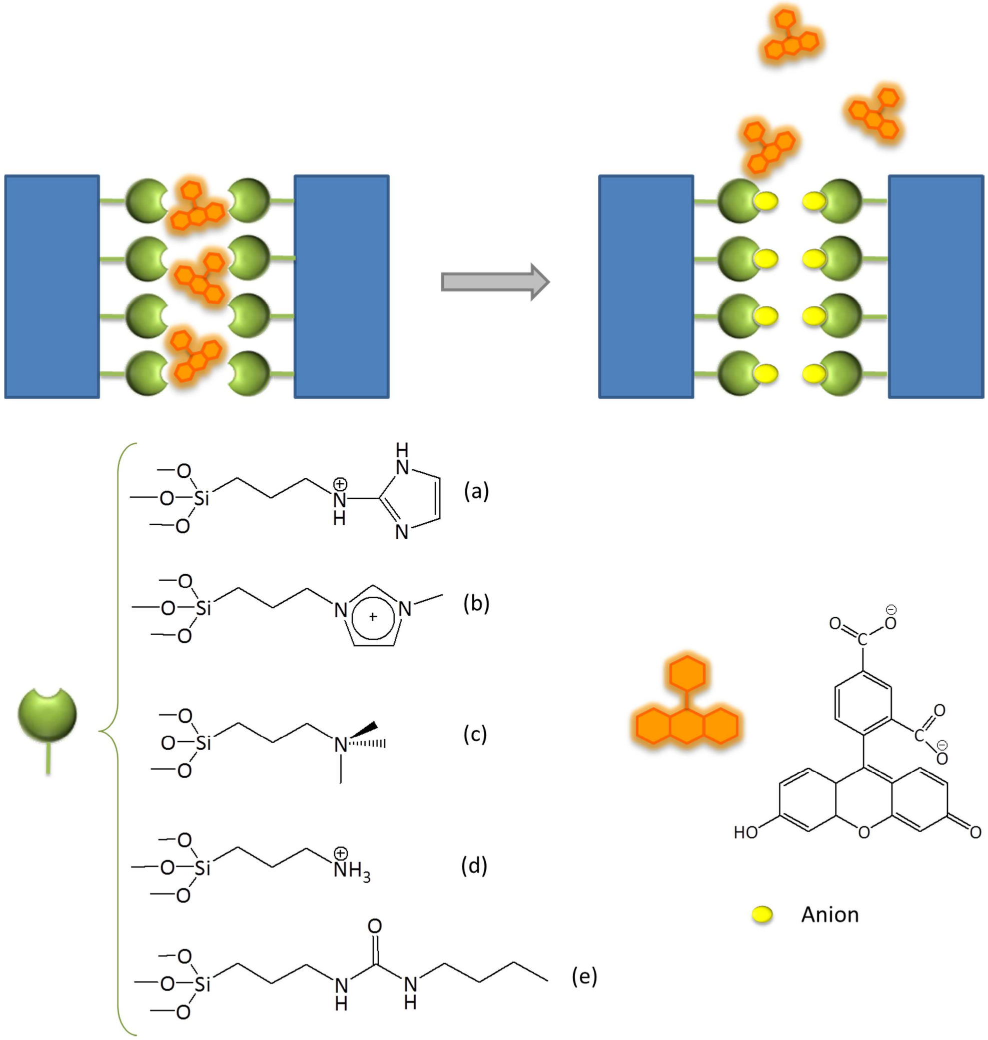

***Figure 14**. Hybrid system for anion recognition according to a dye displacement protocol using mesoporous supports.*

A first example relies on a mesoporous MCM-41-type solid, containing guanidinium groups as binding sites within the mesopores and methylthymol blue as the dye.[57] Guanidinium groups are known to give hydrogen-bonding interactions with carboxylate groups and the present material was found to respond selectively to the tricarboxylate citrate (Figure 14) which was the unique anion able to displace the dye.

In a continuation of their work, the same authors used mesoporous hybrid materials containing nanoscopic pockets functionalized with amino, imidazolium, urea or trimethylammonium groups. These hybrid solids were loaded with 5-carboxyfluorescein and were used in displacement assays with a family of carboxylates (acetate, citrate, lactate, succinate, oxalate, tartrate, malate, mandelate, glutamate) and certain nucleotides in water at pH 7.[58] In a related study, a solid functionalized with amines and filled with carboxyfluorescein was found to be a selective optical sensing material for phosphate in water (Figure 14).[59]

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[57] M. Comes, G. Rodríguez-López, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, L. A. Villaescusa, P. Amorós, D. Beltrán, *Angew. Chem. Int. Ed., 2005, 44, 2918.*

[58] M. Comes, E. Aznar, M. Moragues, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, L. A. Villaescusa, L. Gil, P. Amorós, *Chem. Eur. J., 2009, 15, 9024.*

[59] M. Comes, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, L. A. Villaescusa, P. Amorós, *Chem. Commun., 2008, 3639.*

1.2.2. ENHANCED SIGNALLING BY PREORGANIZATION

Besides the improvement of the classical supramolecular host–guest formation, i.e., the enhancement of the recognition features as such as described in the previous section, a major issue and continuing challenge of modern supramolecular chemistry is the design of advanced functional materials such as sensors and molecular machines that are characterized by the generation and/or transduction of an extraordinary strong signal, i.e., that go beyond the traditional equation of one input producing one output signal. Such features are inherently difficult to achieve for conventional molecular design. Here, the case is often defined by one indicator binding one analyte, the input, generating one output signal, for instance, a photon. Quite recently, the combination of nanomaterials like nanoparticles or hybrid analogues as solid supports with the controlled positioning of molecules and assemblies on their surface has resulted in multifunctional platforms with amplified signal generation. In addition to the controlled assembly of recognition sites on these nanoscopic objects or nano-structured surfaces, signalling units can also be utilized in these hybrids, eventually leading to signal amplification. Especially organically functionalized monodisperse silica spheres are widely used as organic–inorganic hybrid materials in the approaches discussed in this section, mainly because of their unique chemical and physical properties.

The synthetic procedure to produce virtually monodisperse silica nanoparticles has been known since the late 1960s from the works of Stöber,[60] which were refined by Van Blaaderen in the late 1980s/early 1990s.[61,49,50] Silica nanoparticles possess a wealth of advantageous features such as pronounced chemical stability, high surface-to-volume ratio, and superior optical properties as well as facile preparation and good biocompatibility, the latter basically because they are hydrophilic and readily form dispersions in water. Concerning system design, the major advantage of silica nanoparticles compared with, for instance, polymer nanoparticles is the simplicity of the introduction of functional groups on the surface by modification of surface hydroxyl groups with silane-coupling reagents. Based on the aforementioned, functionalized

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[60] The Stöber process is aphysical chemistry process for the generation of monodisperse particles of silica. This process has been widely researched. The hydrolysis and condensation TEOS occurs in an alcoholic solution with ammonia as a catalyst and stabilizer. The resulting silica particles have diameters between 50 and 2000 nm depending on type of silicate ester used, type of alcohol used and volume ratios.

The chemical reaction is expressed as follows:

$$Si\ (OC_2H_5)_4 + xH_2O \rightarrow Si\ (OC_2H_5)_{4-x}\,(OH)_x + xC_2H_5OH$$

Following the hydrolysis reaction, the condensation reaction occurs immediately. Where the hydroxyl group of intermediate $[Si\ (OC_2H_5)_{4\text{-}x}(OH)_x]$ reacts with either the ethoxy group of other TEOS “alcohol condensation” or the hydroxyl group of another hydrolysis intermediate “water condensation” to form Si-O-Si bridges.

$$\equiv Si - OC_2H_5 + HO - Si \equiv \rightarrow \equiv Si - O - Si \equiv + C_2H_5OH$$

$$\equiv Si - OH + HO - Si \equiv \rightarrow \equiv Si - O - Si \equiv + H_2O$$

The overall reaction is expressed as follows:

$$Si\ (OC_2H_5)_4 + 2\ H_2O \rightarrow SiO_2 + 4C_2H_5OH$$

[61] (a) W. Stöber, A. Fink, E. Bohn, *J. Colloid Interface Sci., 1968, 26, 62;* (b) A. Van Blaaderen, A. Vrij, *Langmuir, 1992, 8, 2921.*

silica nanoparticles as organic–inorganic hybrid materials are ideal to establish advanced fluorescent chemosensors relying on strong analyte-induced signal modulations. The popularity of this approach is apparent from the large number of examples that have been published in the last decade.

An illustrative example utilizes silica nanoparticles that were covered with dansyl (5-dimethylamino-1-naphthalenesulfonyl) moieties on the surface and served as pH sensors.[62] Here, the protonation of few dansyl groups resulted in a remarkable quenching of both the protonated and surrounding unprotonated groups, showing clear features of signal amplification. A similar protocol was established for enhanced signalling of certain metal ions. In this case, silica nanoparticles were functionalized with dansyl-polyamine molecules. The addition of Cu^{2+}, Co^{2+} and Ni^{2+} resulted in a strong quenching of the fluorescence at nanomolar concentrations, the coordination at a single probe molecule being sufficient to quench several other dansyl units in close proximity (Figure 15).[63] In these cases, signal modulation involves a larger number of units than those actually coordinated, i.e., binding of one target amplifies the output signal.

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[62] M. Montalti, L. Prodi, N. Zaccheroni, G. Falini, *J. Am. Chem. Soc., 2002, 124, 13540.*

[63] M. Montalti, L. Prodi, N. Zaccheroni, *J. Mater. Chem., 2005, 15, 2810.*

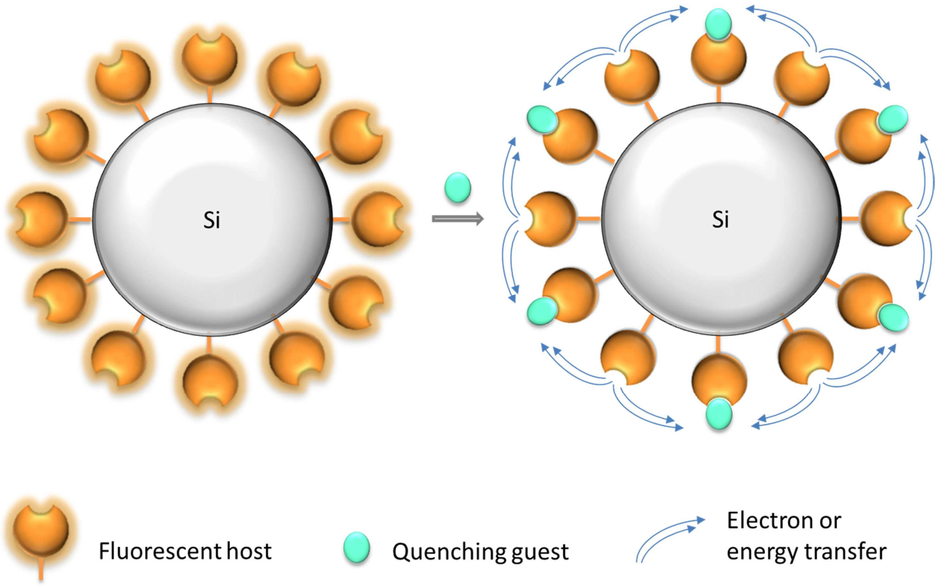

Figure 15. *General scheme for amplified quenching on a nanoparticle surface densely covered with probe molecules.*

In order to translate this approach into a more modular or even combinatorial system design, several researchers began to anchor binding sites and signalling units independently in close proximity to the surface, hoping for additional cooperative effects. If the distance between a receptor site and its next-neighbour fluorophores is close enough, long-range processes such as electron or energy transfer should still be operative and all signalling units within the active interaction distance might funnel their energy to the bound receptor. This approach also permits to adjust the ratio of grafted ligand to grafted fluorophore in a straightforward manner if for instance different concentration ranges have to be assessed in different analytical applications.

In addition to modularity, such an approach would also overcome synthetic problems connected to the conventional preparation of complex receptors. Among the first to establish this strategy were Tecilla and Tonellato. They developed a fluorescent sensor for Cu^{2+} using silica nanoparticles functionalized with both ligands for the selective coordination of Cu^{2+} and fluorescent dyes which can be quenched in the presence of the paramagnetic guest.[64] This comparatively simple system indeed allowed the detection of Cu^{2+} concentrations down to the nanomolar level in DMSO-water solutions. The authors demonstrated that coordination of a single metal ion led to the quenching of up to 10 fluorescent groups, i.e., to amplified signalling (Figure 16). An analogous system was developed for the sensing of Pb^{2+} by the independent anchoring of thiol groups (binding sites) and dansyl fluorophores (signalling units) onto silica nanoparticles.[65]

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[64] (a) E. Rampazzo, E. Brasola, S. Marcuz, F. Mancin, P. Tecilla, U. Tonellato, *J. Mater. Chem., 2005, 15, 2687*; (b) E. Brasola, F. Mancin, E. Rampazzo, P. Tecilla, U. Tonellato, *Chem. Commun., 2003, 3026.*

[65] M. Arduini, F. Mancin, P. Tecilla, U. Tonellato, *Langmuir, 2007, 23, 8632.*

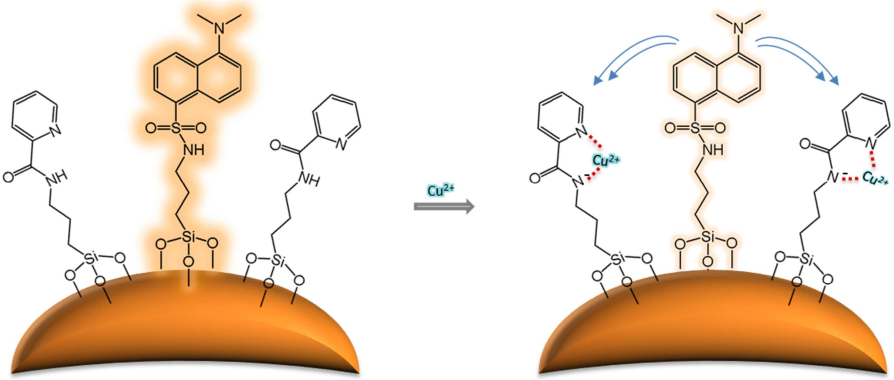

Figure 16. *Cu^{2+}-amplified quenching of dansylated silica nanoparticles.*

In a recently published work, the same authors prepared silica nanoparticles coated with a 6-methoxy-8-(p-toluensulfonamido)-quinolin derivative which displayed a selective emission enhancement.[66] The selective emission shows a 30-fold increase, accompanied by a 6 nm redshift of the maximum in the presence of Zn^{2+} cation in ethanol–water 1:1 v/v mixtures.

However, not only metal ions can be targeted with this approach but also organic quenchers such as TNT. For this purpose, aminopropyltriethoxysilane and a fluorescein derivative were grafted

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[66] P. Teolato, E. Rampazzo, M. Arduini, F. Mancin, P. Tecilla, U. Tonellato, *Chem. Eur. J. 2007, 13, 2238–2245.*

onto the surface of silica nanoparticles (Figure 17).[67] Aqueous suspensions of these hybrid nanoparticles showed the typical fluorescein emission that was quenched in the presence of TNT, due to the formation of a Meisenheimer complex between TNT and amine groups. The spatial proximity of the TNT-amine complex and fluorescein led to enhanced quenching through Förster resonance energy transfer (FRET), enabling the ultrasensitive and selective detection of the explosive.

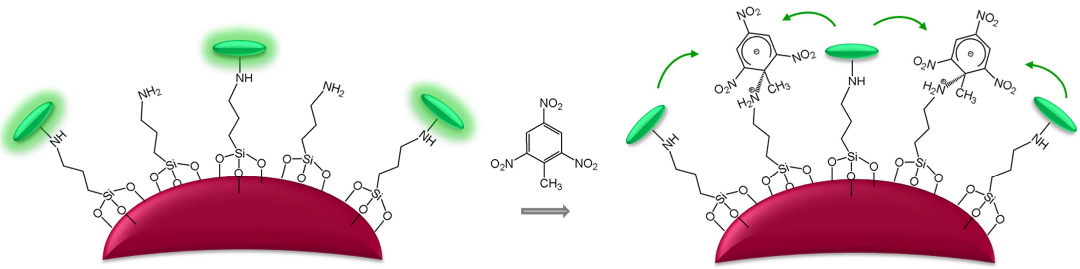

__Figure 17.__ Schematic illustration of the FRET-based silica nanoparticle probe for TNT detection.

The last example presented in this section was reported by Martínez-Mañez’s group and involved the independent anchoring of signalling and binding units on silica nanoparticles to take advantage of the ability of the nanoparticles to induce cooperative effects. In particular the

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[67] D. Gao, Z. Wang, B. Liu, L. Ni, M. Wu, Z. Zhang, *Anal. Chem., 2008, 80, 8545.*

authors functionalised silica nanoparticles[68] with sulforhodamine B dye (Rh) as signalling unit, and imidazolium (Imd) (**S1**) or guanidinium (**S2**) groups as binding sites (Figure 18).

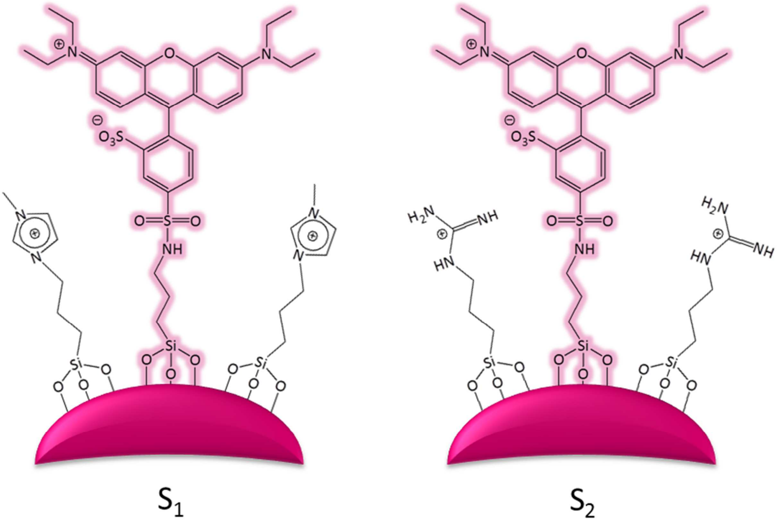

Figure 18. *Schematic representation of the hybrid nanoparticles **S1** and **S2** coated with imidazolium groups and sulforhodamine (left) and with guanidinium groups and sulforhodamine (right).*

The functionalization of the nanoparticle surface was carried out by two different approaches. The first approach relies in the simultaneous grafting of the two subunits. In this protocol, the nanoparticles were

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[68] I. Candel, P. Calero, R. Martínez-Máñez, F. Sancenón, M. D. Marcos, T. Pardo, J. Soto, *Inorg.Chimica Acta, 381, 2012, 188–194.*

functionalized simultaneously with **Rh** and **Imd** (in different ratios) leading to the preparation of **S1**. The second approach relies in the consecutive grafting of the biding site and the signalling subunit. In a typical experiment, the surface of the silica nanoparticles (Figure 19) was grafted with (3-aminopropyl) trimethoxysilane yielding solid **S_N**. Then, the amino groups on the surface were transformed into guanidinium binding sites by reaction with pyrazole carboxamidine obtaining solid **S_G**. Finally, the grafting of **Rh** yielded the hybrid nanoparticles **S2**.

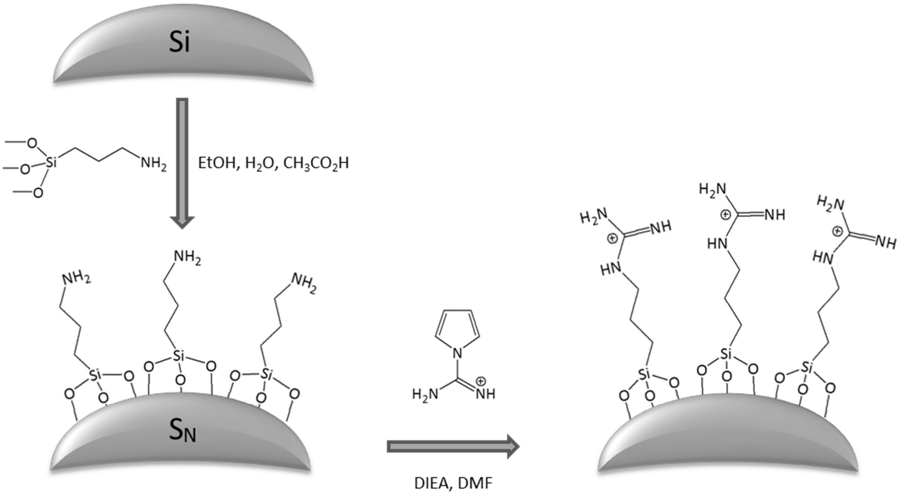

***Figure 19**. Scheme of Synthesis and structure of solids **S_N** and **S_G**.*

Typical sulforhodamine B absorption and emission bands were observed in acetonitrile suspensions of **S1** and **S2**. The study of the response of these nanoparticles functionalized against target anions (organic such as acetate, benzoate and inorganic such as F^-, Cl^-, Br^-, CN^-, NO_3^-, HSO_4^- and $H_2PO_4^-$) was carried out in acetonitrile and acetonitrile:water mixtures. Upon coordination of anions with the anchored binding sites, a modulation of the emission behaviour of the sulforhodamine groups was induced, via energy or electron transfer process from the anion to the photo-excited fluorophore. Nanoparticles functionalized with imidazolium (**S1**) cations favoured the selective response to iodide and benzoate. Addition of iodide induced a moderate quenching, by about 7 % with 12 equivalents, whereas addition of benzoate induced an enhancement of the emission intensity by about 20% with 12 equivalents. On the other hand, nanoparticles functionalized with guanidinium (**S2**) moieties gave selective response in the presence of hydrogen sulphate that resulted in a significant quenching of the emission intensity.

The effect of improved signalling by independent pre-organization of ligands and signalling units is not unique for silica nanoparticles but it has also been reported for polymeric nanoparticles,[69] micellar systems,[70]

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[69] (a) R. Méallet-Renault, R. Pansu, S. Amigoni-Gerbier, C. Larpent, *Chem. Commun., 2004, 2344*; (b) F. Gouanvé, T. Schuster, E. Allard, R. Méallet-Renault, C. Larpent, *Adv. Funct. Mater., 2007, 17, 2746.*

[70] E. L. Doyle, C. A. Hunter, H. C. Philips, S. J. Webb, N. H. Williams, *J. Am. Chem. Soc., 2003, 125, 4593.*

silica nanotubes[71] and extended surfaces. In the latter case, self-assembled monolayers (SAMs) on gold or glass can be created from two different precursors and form bi-functionalized 2D surfaces where directional pre-organization facilitates communication between the binding group and signalling subunit in a similar way as described above. In this respect, the works of Crego-Calama and Reinhoudt were remarkable and have led to the development of chemosensing materials for cations and anions by using a combinatorial approach in which glass monolayers were functionalized with different fluorescent groups and certain types of binding sites.[72]

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[71] (a) S. J. Lee, S. S. Lee, M. S. Lah, J. M: Hong, J. H. Jung, *Chem. Commun., 2006, 4539*; (b) L. Mu, W. Shi, J. C. Chang, S. T. Lee, *Nano Lett., 2008, 8, 104.*

[72] (a)M. Crego-Calama, D. N. Reinhoudt, *Adv. Mater., 2001, 13, 1171*; (b) L. Basabe-Desmonts, J. Beld, R. S. Zimmerman, J. Hernando, P. Mela, M. F. García Parajó, N. F. van Hulst, A. van den Berg, D. N.Reinhoudt, M. Crego-Calama, *J. Am. Chem. Soc., 2004, 126, 7293*; (c) R. S. Zimmerman, L. Basabe-Desmonts, F. van der Baan, D. N. Reinhoudt,M. Crego-Calama, *J. Mater. Chem., 2005, 15, 2772.*

CHAPTER 2

CHROMOGENIC SILICA NANOPARTICLES FOR THE COLORIMETRIC SENSING OF LONG-CHAIN CARBOXYLATES

CHROMOGENIC SILICA NANOPARTICLES FOR THE COLORIMETRIC SENSING OF LONG-CHAIN CARBOXYLATES

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Received: 3rd December 2007

Accepted: 22nd January 2008

First published on the web: 5th February 2008

*Chemical Communications, **2008**, 41, 1668–1670*

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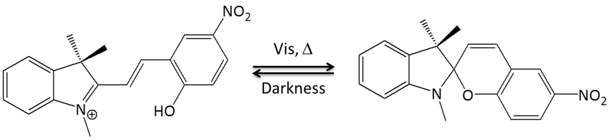
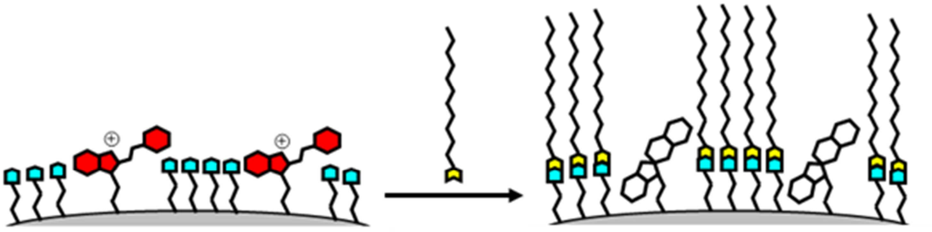
Silica nanoparticles functionalized with chromogenic spirobenzopyran and thiourea subunits show selective colour changes in the presence of certain long-chain carboxylates.





2.1. INTRODUCTION

In recent years the blending of supramolecular and material chemistry has lead to the preparation of novel hybrid systems showing synergic effects that are hardly achievable within molecular-based systems or solids alone.[1] In this area prominent examples involving the combination of supramolecular ideas and nanoscopic scaffoldings, have recently been

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[1] A. B. Descalzo, R. Martínez-Máñez, F. Sancenón, K, Hoffman, K. Rurack, *Angew. Chem. Int. Ed., 2006, 45, 5924*.

reported.[2] One of the fields in which these hybrid materials is ensured to have promising applications is related to the development of optical chemical sensors.[3] Among different nanoscopic scaffoldings the use of quantum dots[4] and gold or silica nanoparticles are especially appealing due to their easy preparation, straightforward surface functionalization and high stability in water.[5] For instance, shift of plasmon band of crownether-modified gold nanoparticles due to aggregation processes has been recently used for the selective recognition of metal cations in water.[6] Also, silica nanoparticles functionalized with organic fluorophores have

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[2] (a) F. Corbellini, A. Mulder, A. Sartori, M. J. W. Ludden, A. Casnati, R. Ungaro, J. Huskens, M. Crego-Calama, D. N. Reinhoudt, *J. Am. Chem. Soc., 2004, 125, 17050*; (b) I. A. Banerjee, L. Yu, H. Matsui*, J. Am. Chem. Soc., 2003, 125, 9542*; (c) R. Casasús, M. D. Marcos, R. Martínez-Máñez, J. V. Ros-Lis, J. Soto, L. A. Villaescusa, P. Amorós, D. Beltrán, C. Gullem, J. Latorre*, J. Am. Chem. Soc., 2004, 126, 8612*; (d) R. Hernández, H. –R. Tseng, J. W. Wong, J. F. Stoddart, J. I. Zink*, J. Am. Chem. Soc., 2004, 126, 3370*; (e) S. Giri, B. G. Trewyn, M. P. Stellmaker, V. S. –Y. Lin, *Angew. Chem. Int. Ed., 2005, 44, 5038.*

[3] (a) A. B. Descalzo, D. Jiménez, M. D. Marcos, R. Martínez-Máñez, J. Soto, J, El Haskouri, C. Gullem, D. Beltrán, P. Amorós, M. V. Borrachero, *Adv. Mater., 2002, 14, 966*; (b) A. B. Descalzo, K. Rurack, H. Weisshoff, R. Martínez-Máñez, M. D. Marcos, P. Amorós, K. Hoffmann, J. Soto, J. Am. *Chem. Soc., 2005, 127, 184*; (c) M. Comes, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, L. A. Villaescusa, P. Amorós, D. Beltrán*, Adv. Mater., 2004, 16, 1783*; (d) M. Comes, G. Rodríguez-López, M. D. Marcos, R. Maerínez-Máñez, F. Sancenón, J. Soto, L. A. Villaescusa, P. Amorós, D. Beltrán, *Angew. Chem. Int. Ed., 2005, 44, 2918*; (e) R. Casasús, E. Aznar, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós, *Angew. Chem. Int. Ed., 2006, 45, 661;* (f) C. Coll, R. Martínez-Máñez, M. D. Marcos, F. Sancenón, J, Soto, *Angew. Chem. Int. Ed., 2007, 46, 1675.*

[4] (a) L. Basabe-Desmonts, D. N. Reinhoudt, M. Crego-Calama, *Chem. Soc. Rev., 2007, 36, 993*; (b) F. M. Raymo, I. Yildiz, *Phys. Chem. Chem. Phys., 2007*, 9, 2036; (c) J. M. Costa-Fernández, R. Pereiro, A. Sanz-Medel, *Trends Anal. Chem., 2006, 25, 207*; (d) C. J. Murphy, *Anal. Chem., 2002, 74, 520A*.

[5] (a) R. Shenhar, V. M. Rotello, *Acc. Chem. Res., 2003, 36, 549*; (b) C. Beck, W. Härtl, R. Hempelmann, *Angew. Chem. Int. Ed., 1999, 38, 1297.*

[6] (a) *Sui-Yi Lin, Shi-Wei Liu, Chia-Mei Lin, and Chun-hsien Chen, Anal. Chem., 2002, 74, 330*; (b) Shu-Yi Lin, Chun-hsien Chen, Meng-Chieh Lin, and Hsiu-Fu Hsu*, Anal. Chem., 2005, 77, 4821*.

been used for the fluorescent recognition of certain metal cations.[7] Despite these encouraging examples, the use of nanoparticle-based hybrid sensors for the specific optical recognition of anions has been much less explored and only certain examples dealing with the use of colour changes induced by anion-induced aggregation processes in gold nanoparticles have been described.[8] In contrast, as far as we know, silica nanoparticles have been never used in the development of hybrid materials for optical anion recognition.

Following our interest in the search of new concepts for anion sensing, we focused our attention towards silica nanoparticles and the use of the spiropyran photochrome as suitable signalling unit. The later is a well-known bistable system than can be transformed reversibly between two different molecular entities; when the spirocyclic form is irradiated with UV light, or put in the darkness, it isomerizes to the merocyanine form which is positively charged at neutral pH or a zwitterion at high pH (see Scheme 1).[9] Additionally, the spyropyran-merocyanine transformation can be followed by changes from the

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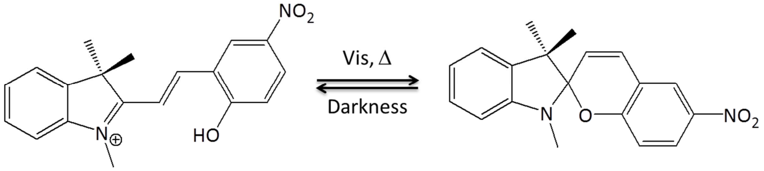
[7] (a) M. Montalti, L. Prodi, N. Zacheronni, G. Falini, *J. Am. Chem. Soc., 2002, 124, 13540*; (b) E. Brasola, F. Mancin, E. Rampazzo, P. Tecilla, U. Tonellato, *Chem. Commun., 2003, 3026*; (c) E. Rampazzo, E. Brasola, S. Marcuz, F. Mancin, P. Tecilla, U. Tonellato, *J. Mater. Chem., 2005, 15, 2687*.

[8] (a) S. Watanabe, M. Sonobe, M. Arai, Y. Tazume, T. Matsuo, T. Nakamura, K. Yoshida, *Chem. Commun., 2002, 2866*; (b) K. Kubo, S. Uchida, Y. Kemmochi, T. Okubo, *Tetrahedron Lett., 2005, 46, 4369*; (c) P. D. Beer, D. P. Cormode, J. J. Davis, *Chem. Commun., 2004, 414*; (d) H. Itoh, K. Naka, Y. Chujo, *J. Am. Chem. Soc., 2004, 126, 3026*.

[9] G. Berkovic, V. Krongauz, V. Weiss*, Chem. Rev., 2000, 100, 1741.*

reddish colour of the merocyanine to the pale colour of the cyclic form.[10] This particular behaviour of the spiropyran-merocyanine couple has been widely used for the preparation of light-switchable systems.[11]



Scheme 1 *Merocyanine-spiropyran transformation.*

However, we have focused here in a less common approach to achieve the merocyanine-spiropyran transformation that entails changes in the environment polarity; i.e. the spirocyclic is formed favourably in highly hydrophobic environments whereas the more polar merocyanine derivative is stabilized in hydrophilic media.[12] Following this concept, a solid **N1** was prepared to asses as a proof-of-concept the possibility of developing new chromogenic sensing protocols based on a merocyanine-spiropyran anion-controlled transformation. The sensing approach is schematically shown in Scheme 2. It involves the independent anchoring

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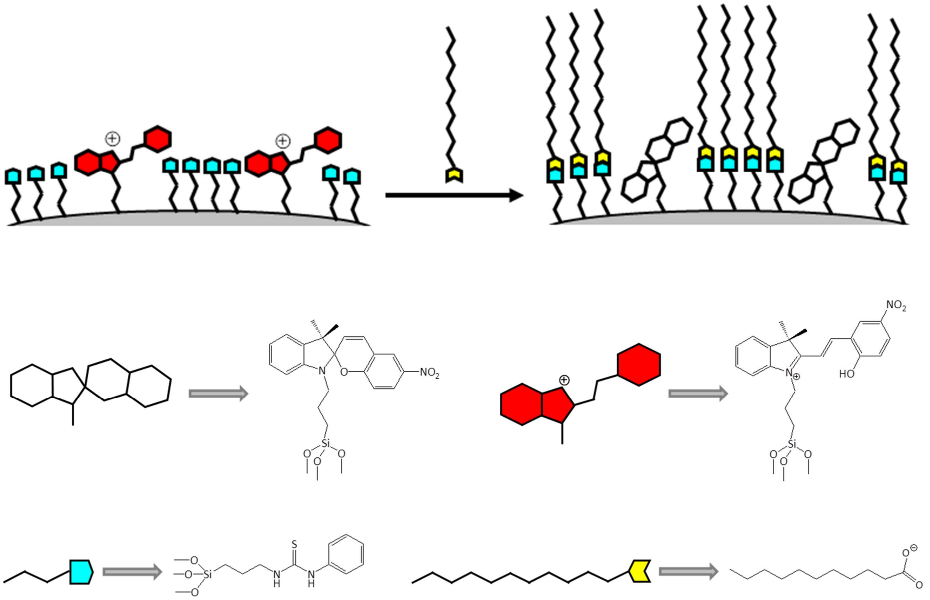
[10] E. Aznar, R. Casasús, B. García-Acosta, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós*, Adv. Mater., 2007, 19, 2228*.

[11] V. I. Minkin, *Chem. Rev., 2004, 104, 2751*.

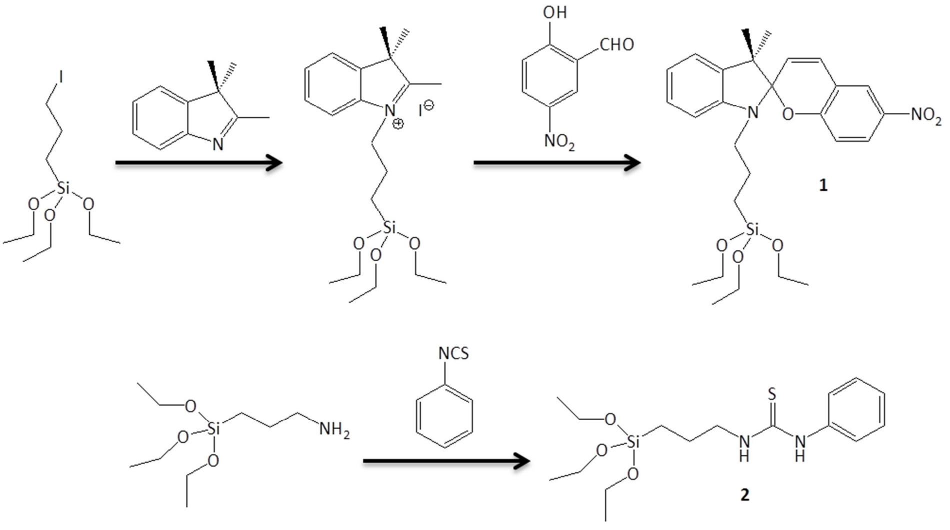
[12] I. Vlassiouk, C. –D. Park, S. A. Vail, D. Gust, S. Smirnov, *Nano Lett., 2006, 6, 1013*.

of anion binding sites (thiourea) and the merocyanine signalling groups in close proximity in the surface of a silica nanoparticle. The signalling event is based on the concept that coordination of anions with different hydrophobicity (for instance certain carboxylates, see below) would modify the merocyanine-spiropyran ratio via an anion-induced polarity modulations near the signalling groups, therefore resulting in a colour change (see Scheme 2).



***Scheme 2** Signalling protocol. Left, the anion binding sites (thiourea) and the signalling unit that is in its stable form in water; i.e. merocyanine form. Right, coordination of large carboxylates on the binding site induce remarkable changes in polarity around the signalling group inducing cyclization to the spirocyclic form and colour modulation from pink-red to pale yellow.*

The binding site and signalling units anchored to the silica nanoparticles for the preparation of **N1** are shown in Scheme 3. The spirobenzopyran derivative 1'-(3-triethoxysilanpropyl)-3'-3'-dimethyl-6-nitrospiro[2H-1]benzopyran-2,2'-indoline (**1**) was synthesised in two steps[10] whereas the *N*-phenyl-*N'*-[3-(triethoxysilyl)propyl]thiourea (**2**) was prepared by direct reaction between phenylisothiocyanate and aminopropyltriethoxyxilane.



***Scheme 3** Synthesis of compounds **1** and **2**.*

These two compounds were attached to the nanoparticle surface through the following procedure: 5 mL of a 30 % suspension of LUDOX silica nanoparticles AS-30 (20 nm average diameter) were added to a solution containing 20 mL of acetic acid, 40 mL of ethanol, 0.2 mmol of **1** and 1.2 mmol of **2**. The crude reaction was heated at 80 °C for 24 h, the ethanol was evaporated and then the acetic acid solution neutralized with a saturated solution of sodium hydrogen carbonate. The functionalized nanoparticles (**N1**) precipitated and were isolated by filtration, washed with water and acetone and dried at 70 °C. Also, and by a similar procedure, nanoparticles functionalized only with the spiropyrane derivative **1** were synthesized (**N2**) as a model derivative for the elucidation of the role played by the anionic binding site.

N1 and **N2** materials were characterized by following standard techniques. From thermogravimetric, elemental analyses and X-ray absorption microanalyses, contents of 16 mmol of thiourea/mol SiO_2 and 4 mmol of spiropyrane/mol SiO_2 in **N1** were found. The spiropyrane content in **N2** was found to be 11 mmol/mol SiO_2. Additionally **N1** and **N2** show a very homogenous particle size around 20 nm (see TEM image in the ESI). As a rough estimate, each coated nanoparticle bears up to 1500 attached subunit molecules. The average distance between two subunits amounts to 10.3 Å.

In a first step it was demonstrated that the polarity-induced merocyanine-spiropyran switching still remains after the grafting of the signalling unit onto the nanoparticles. This can be seen in Fig. 1 which shows the colour of precipitated **N1** nanoparticles after being in contact with water (left) or hexane (right). This colour modulation shows that the red merocyanine form in polar media (water) is readily transformed into the corresponding pale yellow spirocyclic form in lipophilic environment (hexane).

***Figure 1** Changes in the colour of nanoparticles **N1** after being in contact with water (left) and in hexane (right).*

Stimulated by these favorable features, the potential sensing behaviour of **N1** in water (HEPES 0.001 mol dm^{-3} at pH 7) was studied against a family of carboxylates of increasing hydrophobicity, i.e. acetate, butanoate, hexanoate, octanoate, decanoate and dodecanoate. In a typical experiment, 20 mg of **N1** were added to 2 ml of the respective carboxylate solution ($C_{carboxylate}$ = 1 x 10^{-3} mol dm^{-3}) in HEPES (1 x 10^{-3} mol dm^{-3} at pH 7) and then the nanoparticles were suspended by using a rotor-stator system (Ultra-Turrax) for 4 min. and then ultrasonicated at 25 ^{o}C.

As an example, Fig. 2 shows the different colour of precipitated **N1** nanoparticles after their suspension in aqueous solutions containing hexanoate or dodecanoate (C = 1 x 10^{-3} mol dm^{-3}). As can be seen in Fig. 2 there is a gradual change in colour from red, for solid **N1** alone in water, to pale red in the presence of hexanoate and to pale yellow in the presence of dodecanoate. This behaviour clearly resembles that shown by **N1** nanoparticles in water/hexane suspensions (vide ante) and is in accordance with carboxylate coordination to the binding sites (thiourea) that results in a polarity-induced merocyanine-to-spirobenzopyran transformation.

***Figure 2** Changes in colour observed in **N1** nanoparticles in the presence of certain carboxylates. From left to right: no carboxylate, hexanoate and dodecanoate*

For the other carboxylates tested a gradual colour change from magenta/red to light pink clearly linked with the length of the alkyl chain was observed. The polarity-controlled mechanism was assessed by the fact that the addition of certain inorganic anions (fluoride, chloride, bromide, iodide, cyanide, sulfate, phosphate and nitrate) induced negligible colour changes in water-**N1** suspensions. In order to quantify the colour modulation upon addition of different carboxylates, the colour of the water-**N1** suspensions were measured by using an optical fibre UV-visible spectrophotometer and characterised on the CIE 1976 (L, a, b) colour space.[13] This system is one of the most complete colour mode conventionally used to describe visible colours to the human eye. It consist of three coordinates that represent the lightness of the colour (L) and its position between magenta/green and yellow/blue (a and b

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[13] M. T. Fairchild, "Color Appearance Models", *Wiley, 2005.*

respectively). In this systems each colour is a point in three dimensional space and the relative difference between two any colours is given by ΔE which represents the Euclidean distance between them.

The difference between colours as ΔE values of **N1** and **N1** nanoparticles upon addition of the respective carboxylate are plotted in Fig. 3. As could be seen there is a gradual increment in the value of ΔE upon the increase in the length of the alkyl chain of the carboxylate. Changes in ΔE correlated well with the coordination of the carboxylates to the thiourea moieties and therefore formation of self assembled monolayer of different degrees of hydrophobicity around the signalling reporter.

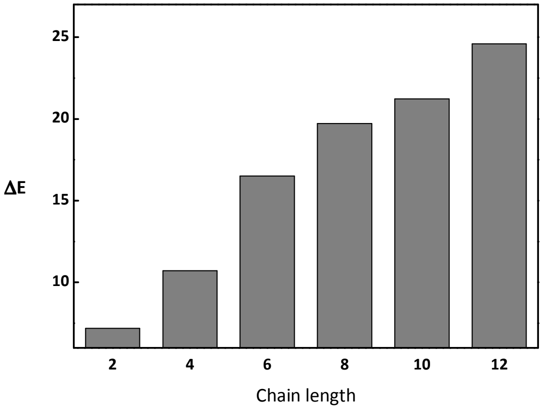

Figure 3 *ΔE values for* ***N1*** *nanoparticles in the presence of carboxylates in water ($C_{carboxylate}$ = 1 x 10^{-3} mol dm^{-3}, HEPES, pH 7).*

The interaction of **N1** nanoparticles with hexanoate and docecanoate was studied in more detail using elemental and thermogravimetric analysis after the interaction of **N1** with neutral solutions of these carboxlates. From these analyses it was evident that both carboxylates trend to coordinate on the surface of the **N1** nanoparticles. For instance, contents in the range of ca. 14-17 mmol of carboxylate/mol SiO_2 were found to be anchored to the **N1** nanoparticles. These values are in good agreement with the thiourea content in **N1** strongly suggesting the formation of a dense, highly hydrophobic monolayer around the signalling reporter that is responsible for the signalling event.

In order to elucidate if the colour changes observed with **N1** nanoparticles were only induced by the environment polarity a test with lauryl sulfate (a lipophilic anion) was carried out. Addition of lauryl sulfate solutions ($C = 1 \times 10^{-3}$ mol dm^{-3}) in HEPES (1×10^{-3} mol dm^{-3} at pH 7) to a stirred suspension of **N1** nanoparticles (20 mg in HEPES 0.001 mol dm^{-3} at pH 7) does not induce any noticeable colour change. This result points toward the relevance, not only of the environment polarity around the colorimetric probe generated upon coordination, but also of the chemical nature of the binding site. Thiourea bind carboxylates strongly whereas it is unable to coordinate with the sulfate group of the lauryl sulfate. The usefulness of this approach, towards the development of novel sensing systems, is remarkable because, additionally to the polarity control, the selectivity of the material could be tuned simple by changing the chemical structure of the binding site.

Finally, we were concerned with the possibility that the observed effect might be caused by simple interactions of the carboxylates with the silica surface. To eliminate this possibility the experiments were repeated using an aqueous suspension of the **N2** nanoparticles (i.e. functionalized with the signalling reporter but not containing thiourea groups). These nanoparticles do not show response, showing the relevance that the presence of the thiourea groups has in relation to the development of the colour modulation and strongly suggesting that colour modulations are not due to carboxylate interaction with the nanoparticle silice surface, but most likely due to polarity-induced effects described above.

In conclusion, we have synthesized hybrid silica-based nanoparticles by anchoring independent spiropyran groups as signalling unit and thiourea moieties as binding sites for the colorimetric signalling of carboxylates in pure water via polarity-induced colour modulations. Further studies with other carboxylates and supports are been carried out, seeking the applicability of this concept. The possibility of using different molecular entities sensitive to polarity changes combined with anion binding sites anchored on supports, makes the results we show here of interest in relation to the opening of new prospects in tailor-made materials for advanced chromo-fluorogenic sensing.

We thank the Ministerio de Educación y Ciencia (MEC) (project CTQ2006-153456-C04-01/BQU) for support. EA and JMLl thank the MEC for a Fellowship and Ramón y Cajal contract, respectively. JVRL thanks Generalitat Valenciana for a Postdoctoral Fellowship.

SUPPORTING INFORMATION

CHROMOGENIC SILICA NANOPARTICLES FOR THE COLORIMETRIC SENSING OF LONG-CHAIN CARBOXYLATES

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2.2. EXPERIMENTAL PROCEDURES

2,3,3-trimethylindolenine, phenylisothiocyanate, (3-iodopropyl) trimethoxysilane, (3-aminopropyl)triethoxysilane, 2-hidroxy-5-nitrobenzaldehyde, triethylamine, 30 % suspension of LUDOX silica nanoparticles AS-30 Colloidal Silica were purchased from Sigma-Aldrich and were used without any further purification. The solvents were absolute grade and were purchased from Scharlab. Anion stock solutions (C_{anion} = 1 x 10^{-3} mol dm^{-3}) were prepared in HEPES (0.001 mol dm^{-3} at pH 7). The buffer 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma) was used as supplied by the manufacturers.

^{1}H and ^{13}C NMR spectra were recorded using a Varian Gemini 300 MHz NMR spectrometer. Chemical shifts are reported relative to internal Me_4Si. Multiplicity is given as usual. Thermogravimetric analysis were carried out in a Mettler Toledo TGA/SDTA 851^{e}. Transmission Electron Microscopy (TEM) images of the particles were obtained with a Philips CM10 operating at 20 KeV. Samples for TEM were prepared by spreading a drop of nanoparticles solution in HEPES onto standard carbon-coated copper grids (200 mesh). SEM microanalyses were obtained using a JEOL 6300 with a detector WDS of Oxford Instruments. UV-Vis absorption measurements were performed by using an optical fibre Avantes VIS/NIR AvaSpec-2048 spectrophotometer with light source tungsten-halogen Avalight-HAL and characterised on the CIE 1976 (L, a, b) colour space.

2.3. SYNTHESIS AND CHARACTERIZATION

2.3.1 SYNTHESIS OF 1'-(3-TRIETHOXYSILANPROPYL)-3'-3'-DIMETHYL-6-NITROSPIRO[2H-1]BENZOPYRAN-2,2'-INDOLINE (1)

The spirobenzopyran derivative 1'-(3-triethoxysilanpropyl)-3'-3'-dimethyl-6-nitrospiro[2*H*-1]benzopyran-2,2'-indoline (**1**) was synthesised by a two step procedure (see Scheme 1) via reaction of 2,3,3-trimethylindolenine (1.96 mL, 12 mmol) and (3-iodopropyl) triethoxysilane (4.96 mL, 24 mmol) in refluxing ethanol (40 mL) for 24h.[14] The product 1-(3-triethoxysilanpropyl)-2,3,3-trimethyilindolenine precipitates from ethyl ether (ca. 3 mL). In a further step, reaction of 1-(3-triethoxysilanpropyl)-2,3,3-trimethyilindolenine (2.55 g, 7 mmol) with 2-hydroxy-5-nitrobenzaldehyde (1.50 g, 9 mmol) and triethylamine (9 mL) in ethanol (135 mL). The triethoxysilyl derivative **1** was purified in a silica gel column using ethyl acetate-hexane 2:1 as eluent (0.85 g, 17% yield).^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.55 (t, 2H, H-3), 1.16 (t, 9H, H-1), 1.25 (s, 6H, H-10 and H-11), 1.72 (qn, 2H, H-4), 3.75 (q, 6H, H-2), 3.81 (t, 2H, H-5), 5.84 (d, 1H, H-12), 6.58 (d, 1H, H-9), 6.73 (d, 1H, H-16), 6.84 (t, 1H, H-7), 6.87 (d, 1H, H-13), 7.06 (d, 1H, H-6), 7.14 (t, 1H, H-8), 7.97 (s, 1H, H-14), 8.00 (d, 1H, H-15). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$) δ: 10.5 ($-\underline{C}H_2-$

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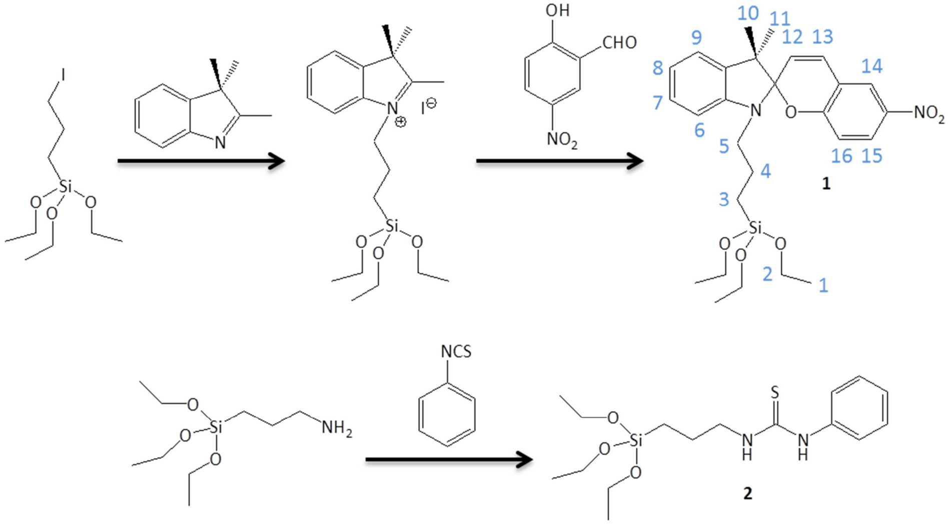
[14] E. Aznar, R. Casasús, B. García-Acosta, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós, *Adv. Mater., 2007, 19, 2228.*

Si-), 20.1 ($\underline{C}H_3$-CH_2-O-Si-), 23.5 (($\underline{C}H_3)_2$-C), 26.2 (-$\underline{C}H_2$-CH_2-Si-), 27.7 (($\underline{C}H_3)_2$-C), 44.6 (($CH_3)_2$-$\underline{C}$), 46.4 (N-$\underline{C}H_2$-CH_2-CH_2-Si), 52.9 (CH_3-$\underline{C}H_2$-O-Si-), 106.9 (-N-$\underline{C}$-O-), 115.8 ($\underline{C}_6H_4$), 118.7 ($\underline{C}$H=CH), 119.3 ($\underline{C}_6H_3$), 119.7 ($\underline{C}_6H_4$), 122.2 ($\underline{C}_6H_4$), 123.1 ($\underline{C}_6H_3$), 126.1 ($\underline{C}_6H_3$), 127.9 ($\underline{C}_6H_4$), 128.4 ($\underline{C}_6H_3$), 130.1 ($\underline{C}_6H_4$), 131.9 (CH=$\underline{C}$H), 141.1 ($\underline{C}_6H_3$), 147.3 ($\underline{C}_6H_4$), 159.8 ($\underline{C}_6H_3$). Elemental analysis, calculated for $C_{27}H_{36}O_3SiN_2O_6$: C, 63.3; H, 7.1; N, 5.5; found: C, 63.1; H, 7.2; N, 5.6.

2.3.2 SYNTHESIS OF N-PHENYL-N'-[3-(TRIETHOXYSILYL)PROPYL]THIOUREA (2)

N-phenyl-*N'*-[3-(triethoxysilyl)propyl]thiourea (**2**) was synthesized by the reaction between 3-(aminopropyl)triethoxysilane (4.77 mL, 27 mmol) and phenylisothiocyanate (3.27 mL, 27 mmol) in CH_2Cl_2 (10 mL) and with few drops of triethylamine (see scheme 1). After 24 hours the solvent was removed by evaporation and the product isolated as yellowish oil (7.6 g, 90% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.55 (t, 2H, -C$\underline{H}_2$-Si-), 1.60 (t, 2H, -C$\underline{H}_2$-CH_2-Si-), 3.47 (s, 9H, $\underline{C}H_3$-O-), 3.60 (t, 2H, -NH-C$\underline{H}_2$-CH_2-), 6.20 (s, 1H, -CO-N$\underline{H}$-CH_2-), 7.12 (m, 5H, $C_6\underline{H}_5$-NH-), 7.78 (s, 1H, C_6H_5-N$\underline{H}$-CO-). ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$) δ: 6.2 (-$\underline{C}H_2$-Si-), 22.1(-$\underline{C}H_2$-CH_2-Si-), 47.8 (-NH-$\underline{C}H_2$-CH_2-), 50.5 ($\underline{C}H_3$-O-), 125.3 ($\underline{C}_6H_5$-NH-), 127.2 ($\underline{C}_6H_5$-NH-), 130.1 ($\underline{C}_6H_5$-NH-), 136.0 ($\underline{C}_6H_5$-NH-), 181.0 (-NH-$\underline{C}$O-NH-). Elemental analysis, calculated for $C_{16}H_{28}O_3SiN_2S$: C, 53.9; H, 7.9; N, 7.9; S 9.0; found: C, 53.8; H, 7.8; N, 8.0; S, 9.1.



***Scheme 1**. Synthesis of compounds **1** and **2**.*

2.3.3 PREPARATION OF COATED SILICA NANOPARTICLES

The preparation of the photochromic silica nanoparticles (**N1**) was carried out by the grafting of compounds **1** and **2** by using standard procedures.[15]In a typical reaction 5 mL of a 30 % suspension of LUDOX silica nanoparticles AS-30 Colloidal Silica were added to a solution

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[15] (a) M. Montalti, L. Prodi, N. Zacheronni, G. Falini*, J. Am. Chem. Soc., 2002, 124, 13540*; (b) E. Brasola, F. Mancin, E. Rampazzo, P. Tecilla, U. Tonellato, *Chem. Commun., 2003, 3026*; (c) E. Rampazzo, E. Brasola, S. Marcuz, F. Mancin, P. Tecilla, U. Tonellato, *J. Mater. Chem., 2005, 15, 2687.*

containing 20 mL of acetic acid and 40 mL of ethanol. Then 0.2 mmol of **1** and 1.2 mmol of **2** (dissolved in chloroform) were added to the nanoparticle solution. The crude reaction was heated at 80 °C for 24 hours, the ethanol was evaporated and then the acetic acid solution neutralized with a saturated solution of sodium hydrogencarbonate. The functionalized nanoparticles (**N1**) precipitated and were isolated by filtration, washed with water and acetone and dried at 70 °C.

Also, and by a similar procedure, nanoparticles functionalized only with the spiropyrane derivative **1** were synthesized (**N2**) as model derivative for the elucidation of the role played by the anionic binding site. 5 mL of a 30 % suspension of LUDOX silica nanoparticles AS-30 were added to a solution containing 20 mL of acetic acid and 40 mL of ethanol. Then 0.2 mmol of **1** (also dissolved in chloroform) were added to the nanoparticle solution. The crude reaction was heated at 80 °C for 24 hours, the ethanol was evaporated and then the acetic acid solution neutralized with a saturated solution of sodium hydrogencarbonate. The functionalized nanoparticles (**N2**) precipitated and were isolated by filtration, washed with water and acetone and dried at 70 °C. The contents in thiourea and spiropyrane were determinated from thermogravimetric, elemental analyses and X-ray absorption microanalyses. The results obtained are shown in Table 1.

***Table 1**. Typical contens of thiourea and spiropyrane in **N1** and **N2**.*

	*Content of **1** (mmol/mol SiO_2)*	*Content of **2** (mmol/mol SiO_2)*
N1	4	16
N2	11	-

Thermogravimetric analyses were carried out under a flow of air and with a heating rate of 10 °C/ minute in the 30-1000 °C interval. The final solid was maintained at 1000 °C for 30 minutes. In the obtained thermograms of **N1** three clearly defined zones are observed; (i) from 30 °C to 150 °C which was assigned to loss of water and organic solvents (2.44 %), (ii) from 150 °C to 800 °C which was assigned to the organic matter attached into the nanoparticle surface (5.97 %) and, finally (iii) from 800° to 1000° C a third step was assigned to condensation of silanol groups (0.44 %). The silica residue amounts to 91.15 %.

The diameter of **N1** and **N2** nanoparticles was determined by TEM. TEM images show a very homogenous particle size around 20 nm (see Figure 1 for N1) for both materials.

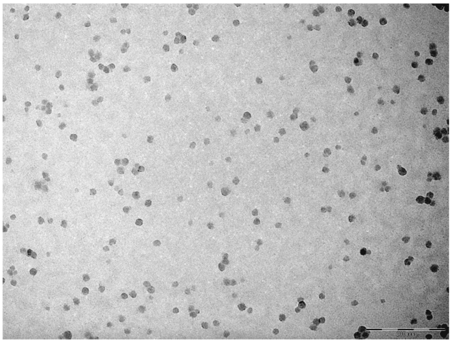

***Figure 1**. TEM images of functionalized nanoparticles **N1**, average diameter = 20 nm.*

2.4. CARBOXYLATE SENSING

The potential sensing behaviour of **N1** nanoparticles in water (HEPES 0.001 mol dm^{-3} at pH 7) was studied against a family of carboxylates of increasing hydrophobicity (acetate, butanoate, hexanoate, octanoate, decanoate and dodecanoate), certain inorganic anions (fluoride, chloride, bromide, iodide, cyanide, sulfate, phosphate and nitrate) and lauryl sulfate. In a typical experiment, 20 mg of **N1** were added to a 2 ml solution of the respective anion (C_{anion} = 1 x 10^{-3} mol dm^{-3}) in HEPES (1 x 10^{-3} mol dm^{-3} at pH 7) and were suspended with a rotor-stator system (Ultra-Turrax) for 4 minutes and ultrasonicated at 25 °C. Then, and in order to quantify the colour modulation upon addition of different anions, the colour of the water-**N1** suspensions were measured by using an optical fibre UV-visible spectrophotometer and characterised on the

CIE 1976 (L, a, b) colour space.[13] This system is one of the most complete colour mode conventionally used to describe visible colours to the human eye. It consist of three coordinates that represent the lightness of the colour (L) and its position between magenta/green and yellow/blue (a and b, respectively). In this systems each colour is a point in a three dimensional space and the relative difference between two any colours is given by ΔE that represents the Euclidean distance between them.

$$\Delta E_{ab} = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

Of all the anions tested only carboxylates are able to induced changes in the colour of **N1** nanoparticles (from magenta/red to light pink) whereas the addition of inorganic anions and lauryl sulfate induced negligible changes in the colour of **N1** nanoparticle suspension. The coordinates L, a and b and the difference between colours as ΔE values of **N1** and **N1** nanoparticles upon addition of the respective carboxylate are listed in table 2. As could be seen there is a gradual increment in the value of ΔE upon the increase in the length of the alkyl chain of the carboxylate. Changes in ΔE correlated well with the coordination of the carboxylates to the thiourea moieties and therefore formation of self assembled monolayer of different degrees of hydrophobicity around the signalling reporter.

Table 2. *Quantify of colour modulation upon addition of different carboxilates characterised on the CIE 1976 (L, a, b) colour space. The coordinates represent the lightness of the colour (L) and its position between magenta/green and yellow/blue (a and b respectively). The relative difference between two any colours is given by ΔE.*

	L	*a*	*b*	ΔE
No carboxylate	77,590	37,77	-0,340	
Acetate	79,780	31,80	3,020	7,196
Butanoate	81,650	29,45	5,060	10,721
Hexanoate	87,575	26,76	6,820	16,498
Octanoate	90,483	23,84	4,997	19,717
Decanoate	90,430	22,09	5,980	21,229
Dodecanoate	93,457	21,43	8,925	24,587

The gradual colour change from magenta/red to light pink upon carboxylate coordination is clearly linked with the length of the alkyl chain. The polarity-controlled mechanism was assessed by the fact that the addition of the inorganic anions (fluoride, chloride, bromide, iodide, cyanide, sulfate, phosphate and nitrate) and lauryl sulfate induced negligible colour changes in **N1** aqueous suspension.

The same carboxylate sensing experiments were carried out with aqueous suspensions of **N2** nanoparticles (i.e. functionalised with the signalling reporter but no containing thiourea groups). Again 20 mg of **N2** were added to a 2 ml solution of the respective anion (C_{anion} = 1 x 10^{-3} mol dm^{-3}) in HEPES (1 x 10^{-3} mol dm^{-3} at pH 7) and were suspended with a rotor-stator system (Ultra-Turrax) for 4 minutes and ultrasonicated at 25 ^{o}C. Then, the colour of the water-**N2** suspensions were measured by using an optical fibre UV-visible spectrophotometer and characterised again on the CIE 1976 colour space. These nanoparticle suspensions do not show any noticeable colour change (reflected in the L, a, b values) in the presence of all the anions tested (acetate, butanoate, hexanoate, octanoate, decanoate, dodecanoate, fluoride, chloride, bromide, iodide, cyanide, sulfate, phosphate, nitrate and lauryl sulfate) pointing out the relevance that the presence of the thiourea moieties has in relation to the development of the colour modulation and suggesting that this colour change is not due to carboxylate interaction with the nanoparticle surface but most likely due to polarity-induced effects.

Titration experiences with **N1** nanoparticles and hexanoate and dodecanoate were carried out. In these experiences several UV-visible measurements of **N1** nanoparticles with different amounts of hexanoate and dodecanoate (0, 0.3, 0.5, 0.7, 1.0, 2.0 and 3.0 equivalents related to the content in thiourea moiety in the nanoparticles) were performed. In a typical experiment, 20 mg of **N1** were added to solutions of hexanoate and dodecanoate (C_{anion} = 3 x 10^{-3} mol dm^{-3}) in HEPES (1 x 10^{-3} mol dm^{-3} at pH 7) and were suspended with a rotor-stator system (Ultra-Turrax) for 4 minutes and ultrasonicated at 25 ºC. Then, and in order to quantify the colour modulation upon addition of different quantities of hexanoate and dodecanoate, the colour of the water-**N1** suspensions were measured by using an optical fibre UV-visible spectrophotometer and characterised on the CIE 1976 (L, a, b) colour space. The titration curves obtained are shown in Figure 2.

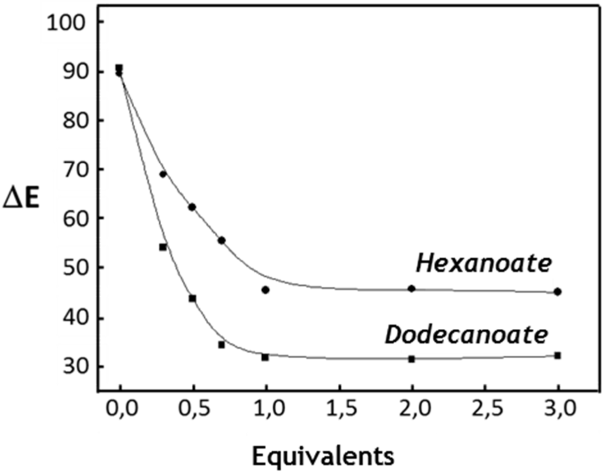

***Figure 2**. Titration curves of functionalized nanoparticles **N1** obtained upon addition of increasing quantities of hexanoate and dodecanoate.*

Also the colorimetric response of water-**N1** suspensions in the presence of certain inorganic anions (fluoride, chloride, bromide, iodide, cyanide, sulfate, phosphate and nitrate) has been studied. The sensing procedure is the same described above for the sensing of linear alkyl carboxylates. In a typical experiment, 20 mg of **N1** were added to a 2 ml solution of the respective inorganic anion (C_{anion} = 1 x 10^{-3} mol dm^{-3}) in HEPES (1 x 10^{-3} mol dm^{-3} at pH 7) and were suspended with a rotor-stator system (Ultra-Turrax) for 4 minutes and ultrasonicated at 25 ^{o}C. Then, and in order to quantify the colour modulation upon addition of different anions, the colour of the water-**N1** suspensions were measured by using an optical fibre UV-visible spectrophotometer and characterised on the CIE 1976 (L, a, b) colour space. The presence of this inorganic anions induced negligible changes neither in the colour nor in the CIE Lab coordinates in water-**N1** suspension. This fact points directly to a polarity-controlled mechanism as a responsible of the colour changes observed upon addition of certain linear alkyl carboxylates.

CHAPTER 3

SYNTHESIS, CHARACTERIZATION AND OPTICAL PROPERTIES OF SILICA NANOPARTICLES COATED WITH ANTHRACENE FLUOROPHORE AND THIOUREA HYDROGEN BONDING SUBUNITS

SYNTHESIS, CHARACTERIZATION AND OPTICAL PROPERTIES OF SILICA NANOPARTICLES COATED WITH ANTHRACENE FLUOROPHORE AND THIOUREA HYDROGEN BONDING SUBUNITS

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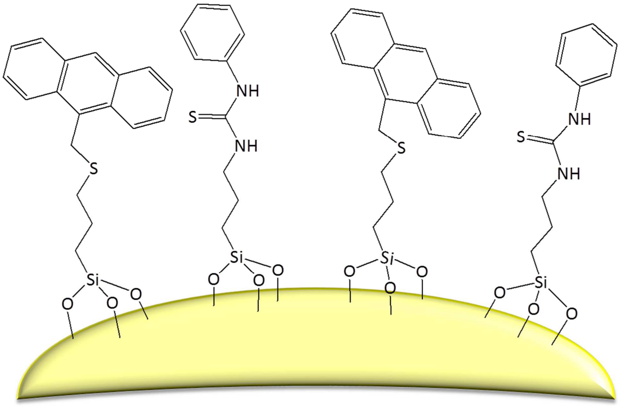
Received: 22 July 2008

First published on the web: 12 November 2008

Eur. J. Inorg. Chem. ***2008****, 5649–5658*

Eur. J. Inorg. Chem. 2008, 5649-5658

Synthesis, characterization and optical properties of silica nanoparticles coated with anthracene fluorophore and thiourea hydrogen bonding subunits.



3.1. ABSTRACT.

Bifunctionalised hybrid silica nanoparticles have been synthesised and characterised, and their optical emission properties in the presence of certain anions in acetonitrile solutions have been studied. The alkoxysilane derivatives *N*-butyl-*N'*-[3-(trimethoxysilyl)propyl]thiourea (**1**), *N*-phenyl-*N'*-[3-(trimethoxysilyl)propyl]thiourea (**2**) and 3-((anthracen-10-yl)methylthio)propyltriethoxysilane (**3**) were prepared and used to functionalise uncoated LUDOX silica nanoparticles with a

mean diameter of 18 ± 2 nm. The functionalization of the nanoparticle surfaces was carried out by two different approaches. The first approach relies on the consecutive grafting of the two subunits. In this protocol, the nanoparticles were first functionalised with anthracene derivative **3** (solid **NA**) and then treated with the corresponding binding sites **1** or **2** to result in solids **NA-Pt$_3$** and **NA-Bt$_3$**. The second approach deals with the simultaneous grafting **1** or **2** and the signalling subunit **3** in different ratios. This method was used for the preparation of the **NA$_1$Pt$_1$**, **NA$_1$Bt$_1$**, **NA$_1$Pt$_3$** and **NA$_1$Bt$_3$** nanoparticles. The bifunctionalised silica nanoparticles were characterised by using standard techniques. Acetonitrile suspensions of **NA** nanoparticles (5 mg in 20 mL) showed anthracene bands centred at ca. 350, 370 and 390. Upon excitation at 365 nm, a typical emission band with fine structure in the 390-450 nm range was observed. Similar absorption and emission spectra were found for the bifunctionalised nanoparticles. The work is completed with a prospective study of the fluorescence of the prepared nanoparticles in the presence of organic (acetate, benzoate) and inorganic (F^-, Cl^-, Br^-, CN^-, HSO_4^- and $H_2PO_4^-$) anions. The apparent binding constants (adsorption constants) for the interaction of **NA-Pt$_3$** with anions in acetonitrile were determined by performing a Langmuir-type analysis of fluorescence titration data.

3.2. INTRODUCTION

There is an increasing interest in the development of new sensing methods and protocols for target guests, and a number of recent advances have been described in this area. In a classical design approach, chemosensors are built up by the combination of two groups, i.e. the binding site and the signalling unit, connected through covalent bonds. The binding site is usually designed or selected bearing in mind the size, shape and nature of the chemical species to be coordinated and ideally results in selective interactions with certain target guests. On the other hand, the signalling unit transforms the host-guest interaction into suitable chromo-fluorogenic changes[1] or redox potential shifts.[2] Usually only one type of signalling unit is used in chemosensing although some few examples are known where chemosensors contain more than one type of signalling subunits.[3]

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[1] See for instance: a) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev., 1997, 97, 1515-1566*; b) R. Martínez-Máñez, F. Sancenón, *Chem. Rev., 2003, 103, 4419-4476*; c) P. D. Beer, P. A. Gale*, Angew. Chem. Int. Ed., 2001, 40, 486-516*; d) R. Martínez-Máñez, F. Sancenón*, Coord. Chem. Rev., 2006, 250, 3081-3093*; e) B. Valeur, I. Leray, *Coord. Chem. Rev., 2000, 205, 3-40.*

[2] See for instance: a) P. D. Beer, *Chem. Commun., 1996, 689-696*; b) P. D. Beer, Coord. *Chem. Rev., 2000, 205, 131-155*; c) M.E. Padilla-Tosta, R. Martínez-Máñez, T. Pardo, J. Soto, M.J.L. Tendero, *Chem. Commun. 1997, 887-888*; d) J.M. Lloris, R. Martínez-Máñez, M.E. Padilla-Tosta, T. Pardo, J. Soto, P.D: Beer, J. Cadman, D.K. Smith, J. *Chem. Soc. Dalton Trans, 1999, 2359-2369*; e) M.J.L. Tendero, A. Benito, J. Cano, J.M. Lloris, R. Martínez-Máñez, J. Soto, P.R. Raithby, M.A. Rennie*, J. Chem. Soc. Dalton Trans, 1995, 1643-1644.*

[3] See for instance: a) F. Zapata, A. Caballero, A. Espinosa, A. Tárraga, P. Molina, *Org. Lett., 2008, 10, 41-44*; b) M. Schmittel, H. –W. Lin, *Angew. Chem. Int. Ed., 2007, 46, 893-896*; c) F. Otón, A.

Among different chemosensing systems, those relaying on transformations of the emission fluorescence behaviour are especially appealing. By following this approach, an amazing quantity of fluorescent chemosensors for the detection of metal cations,[4] organic and inorganic anions[5] and neutral species[6] have been synthesised and tested. In spite of these examples, many fluorescent chemosensors show several limitations such as lack of selectivity. Recently, as an alternative for the development of classical fluorogenic probes, self-assembled structures on surfaces have attracted increasing attention in chemistry.[7] Many of these systems involve the use of a number of relatively simple chemical units which are attached to the surface of nanoparticles or nanostructured solids. As a result of the molecular

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Espinosa, A. Tárraga, C. Ramírez de Arellano, P. Molina, *Chem. Eur. J., 2007, 13, 5742-5752*; d) F. Otón, A. Tárraga, A. Espinosa, M. D. Velasco, P. Molina*, J. Org. Chem., 2006, 71, 4590-4598*; e) D. Jiménez, R. Martínez-Máñez, F. Sancenón, J. V. Ros-Lis, J. Soto, A. Benito, E. García-Breijo, *Eur. J. Inorg. Chem., 2005, 2393-2403*; f) T. Ghosh, B. G. Maiya, M. W. Wong*, J. Phys. Chem. A, 2004, 108, 11249-11259*; g) F. Sancenón, A. Benito, F.J. Hernández, J.M. Lloris, R. Martínez-Máñez, T. Pardo, J. Soto, *Eur. J. Inorg. Chem. 2002, 866-875*; h) J. Maynadie, B. Delavaux-Nicot, S. Fery-Forgues, D. Lavabre, R. Mathieu*, Inorg. Chem. 2002, 41, 5002-5004*; i) B. Delavaux-Nicot, S. Fery-Forgues, *Eur. J. Inorg. Chem. 1999, 1821-1825*; j) P. D. Beer, A. R. G. Graydon, A. O. M. Johnson, D. K. Smith, *Inorg. Chem., 1997, 36, 2112*.

[4] a) L. Prodi, F. Bolletta, M. Montalti, N. Zaccheroni, *Coord. Chem. Rev., 2000, 205*, 59-83; b) A. Ikeda, S. Shinkai, *Chem. Rev., 1997, 97, 1713-1734*; c) J. F. Callan, A. P. de Silva, D. C. Magri, *Tetrahedron, 2005, 61, 8551-8588.*

[5] a) L. Fabbrizzi, M. Lichelli, G. Rabaioli, A. Taglietti, *Coord. Chem. Rev., 2000, 205, 85-108*; b) S. L. Wiskur, H. Aït-Haddou, J. J. Lavigne, E. V. Anslyn, *Acc. Chem. Res., 2001, 43, 963-972.*

[6] See for example: a) N. A. Rakow, A. Sen, M. C. Janzen, J. B. Ponder, K. S. Suslick, *Angew. Chem. Int. Ed., 2005, 44, 4528-4532*; b) P. L. McGrier, K. M. Solntsev, S. Miao, L. M. Tolbert, O. R. Miranda, V. M. Rotello, U. H. F. Bunz, *Chem. Eur. J., 2008, 14, 4503-4510*; c) G. Lu, J. E. Grossman, J. B. Lambert, *J. Org. Chem., 2006, 71, 1769-1776*; d) M. H. Lim, S. J. Lippard, *Acc. Chem. Res., 2007, 40, 41-51.*

[7] a) L. Basabe-Desmonts, D. N. Reinhoudt, M. Crego-Calama, *Chem. Soc. Rev., 2007, 36, 993-1017*; b) M. J. W. Ludden, D. N. Reinhoudt, J. Huskens, *Chem. Soc. Rev., 2006, 35, 1122-1134*.

organization into a solid support, novel functional properties have been reported in hybrid materials, which show enhanced applications potential. [8] Additionally, from a supramolecular viewpoint, the functionalization of nanostructured solids with certain groups to enhance recognition or switching effects is particularly appealing.[9] Thus, the functionalization of inorganic supports with receptors and fluorescent dyes can overcome synthetic problems which arise when following the classical “biding site–signalling unit” approach and provide an efficient strategy for the preparation of fluorogenic sensing systems.

The organisation of photoactive molecules and coordinating units onto the surface of nanoparticles is still a relatively recent field that could lead to the design of novel hybrid materials for use as labels and/or sensors.[10] Besides the reported examples of dye adsorption onto nanoparticle surfaces, covalent grafting is necessary in order to obtain stable arrangements and avoid structural reorganisations due to either, redistribution of the adsorbed dye on the surface or between the

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[8] A. B. Descalzo, R. Martínez-Máñez, F. Sancenón, K. Hoffmann, K. Rurack, *Angew. Chem. Int. Ed., 2006, 45, 5924-5948.*

[9] a) M. Comes, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, L. A. Villaescusa, P. Amorós, D. Beltrán, *Adv. Mater., 2004, 16, 1783-1786*; b) C. Coll, R. Martínez-Máñez, M. D. Marcos, F. Sancenón, J. Soto, *Angew. Chem. Int. Ed., 2007, 46, 1675-1678*; c) R. Casasús, E. Aznar, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós, *Angew. Chem. Int. Ed., 2006, 45, 6661-6664*; d) M. Comes, G. Rodríguez-López, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J.Soto, L. A. Villaescusa, P. Amorós, D. Beltrán, *Angew. Chem. Int. Ed., 2005, 44, 2918-2922*; e) C. Coll, R. Casasús, E. Aznar, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós, *Chem. Commun., 2007, 1957-1959.*

[10] a) A. Burns, H. Ow, U. Wiesner, *Chem. Soc. Rev., 2006, 35, 1028-1042*; b) L. Prodi, New. *J. Chem., 2005, 29, 20-31*; c) A. P. R. Johnston, B. J. Battersby, G. A. Lawrie, M. Traut, *Chem. Commun., 2005, 848-850*; d) E. Katz, I. Willner, *Angew. Chem. Int. Ed., 2004, 43, 6042-6108.*

surface and the solution.[11] In addition, silica nanoparticles can be synthesised easily, and their surface can be modified by the use of well-know alcoxysilane chemistry.[12] This versatility makes silica nanoparticles a good choice as scaffoldings for the grafting of dyes and coordinating units, especially when considering that these materials are transparent to visible light and inert as far as energy- and electron-transfer processes are concerned. This approach also induces the generation of amplification and cooperative effects associated with the independent anchoring of binding site and fluorophores in close proximity to the surface support.[13] For instance, this close arrangement allows intercommunication between both subunits without the need of a direct covalent link between them. Also, the grafting of the receptor and the fluorophores onto the nanoparticle surface can induce cooperative processes such as enhanced binding constants.[14]

This strategy has been successfully employed in the development of fluorescent silica nanoparticles for the recognition of certain metal cations. Tecilla, Tonellato and co-workers prepared silica nanoparticles functionalised with trialkoxysilane derivatives of picolinamides as

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[11] M. Arduini, E. Rampazzo, F. Mancin, P. Tecilla, U. Tonellato, *Inorg. Chim. Acta, 2007, 360, 721-727*

[12] a) G. Kickelbick, *Angew. Chem. Int. Ed., 2004, 43, 3102-3104*; b) G. Schulz-Ekloff, D. Wöhrle, B. van Duffel, R. A. Schoonheydt, *Micropor. Mesopor. Mat., 2002, 51, 91-138.*

[13] a) M. Montalti, L. Prodi, N. Zaccheroni, A. Zattoni, P. Reschiglian, G. Falini, *Langmuir, 2004, 20, 2989-299*1; b) M. Montalti, L. Prodi, N. Zaccheroni*, J. Mater. Chem., 2005, 15, 2810-2814.*

[14] K. Ariga, A. Vinu, J. P. Hill, T. Mori, *Coord. Chem. Rev., 2007, 251, 2562-2591.*

receptors and dansylamides as fluorescent reporters for the fluorescent sensing of Cu^{2+}.[15] The picolinamide ligand complexes the Cu^{2+} ion, and the bound ion quenches the dansyl emission substantially in DMSO. In further studies the same authors used a combinatorial approach to functionalise silica nanoparticles with other ligands and fluorophores in various ratios.[16] The cooperative and collective effects are achieved by the organisation of the components on the particle surface to form multivalent binding sites with an increased affinity for Cu^{2+} ions. The binding of a single metal cation leads to the quenching of up to ten fluorescent groups that are surrounding a receptor unit with the corresponding signal amplification. The same authors, in a recent work, prepared fluorescent silica nanoparticles coated with a 6-methoxy-8-(*p*-toluensulfonamido)-quinoline derivative that showed a selective emission enhancement in the presence of Zn^{2+} cations in ethanol/water (1:1 v/v) mixtures.[17]

Also improved signalling by independent preorganisation of receptors and signalling units has been observed for extended surfaces and self-assembled monolayers (SAMs). SAMs on glass are examples of difunctionalized surfaces where directional preorganization facilitates

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[15] E. Brasola, F. Mancin, E. Rampazzo, P. Tecilla, U. Tonellato, *Chem. Commun., 2003, 3026-3027.*

[16] E. Rampazzo, E. Brasola, S. Marcuz, F. Mancin, P. Tecilla, U. Tonellato, *J. Mater. Chem., 2005, 15, 2687-2696.*

[17] P. Teolato, E. Rampazzo, M. Arduini, F. Mancin, P. Tecilla, U. Tonellato, *Chem. Eur. J., 2007, 13, 2238-2245.*

communications between the binding group and the signalling subunits. One of the first examples was described by Crego-Calama et al.[18] In a first step a monolayer of {3-[(2-amino-ethyl)propyl}trimethoxysilane was formed on a glass substrate and then treated with dansyl chloride and succinimidyl 7-(dimethylamino)coumarin-4-acetate in order to attach two distinct fluorophores. Then the residual amino groups were converted into amide and urea groups that would act as binding sites. The addition of certain metal cations (Pb^{2+}, Cd^{2+} and Zn^{2+}) to the prepared hybrid materials induced several degrees of quenching of the appended fluorophore. The best results were obtained for Pb^{2+}. In a further evolution the same research group prepared chemosensor materials for both cations and anions by using a combinatorial approach where glass monolayers were functionalised with fluorescent groups (rhodamine derivatives) and coordinating units (amino, arylurea, alkylurea, arylamide, alkylamide, sulphonamide, urea and thiourea).[19]

Thus, the use of silica supports, as nanoparticles or locally “flat” surfaces, has led to new organic-inorganic hybrid materials with enhanced recognition features. However, as far as we know, only one example dealing with hybrid materials using silica nanoparticles for anion sensing has been described. This hybrid system is based on the

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[18] M. Crego-Calama, D. N. Reinhoudt, *Adv. Mater., 2001, 13, 1171-1174.*

[19] L. Basabe-Desmonts, J. Beld, R. S. Zimmerman, J. Hernando, P. Mela, M. F. G. Parajó, N. F. van Hulst, A. van den Berg, D. N. Reinhoudt, M. Crego-Calama, *J. Am. Chem. Soc., 2004, 126, 7293-7299.*

bifunctionalisation of silica nanoparticle surface with a spiropyran photochrome as chromogenic reporter and a thiourea derivative as binding site.[20] The merocyanine form is selectively transformed into the corresponding spirocyclic structure in the presence of long-chain carboxylates because of a change in the polarity on the nanoparticle surface.

Bearing these facts in mind, we report herein the synthesis of novel hybrid materials based on silica nanoparticles functionalised with two subunits: a binding site (thiourea based receptor) and a signalling fluorescent subunit (anthracene). The synthesis, characterisation and studies on the emission behaviour of suspensions of the prepared nanoparticles in the presence of certain organic and inorganic anions have been carried out.

3.3. RESULTS AND DISCUSSION

3.3.1 DESIGN, SYNTHESIS AND CHARACTERIZATION

The binding sites and the signalling subunit used for the preparation of the hybrid nanoparticles are shown in Scheme 1. Binding molecules **1** and **2** were prepared by the reaction between (3-aminopropyl)-

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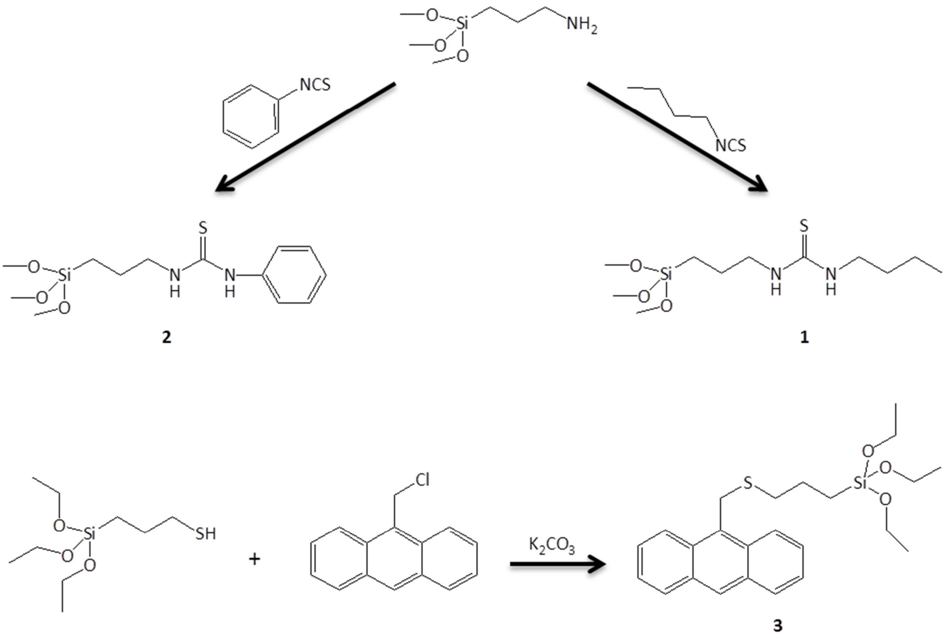
[20] P. Calero, E. Aznar, J. M. Lloris, M. D. Marcos, R. Martínez-Máñez, J. V. Ros-Lis, J. Soto, F. Sancenón, *Chem. Commun., 2008, 1668-1670.*

trimethoxysilane and butyl isothiocyanate (for **1**) and phenyl isothiocyanate (for **2**) in dichloromethane in the presence of catalytic amounts of triethylamine. The final products were isolated in nearly quantitative yields by evaporation of dichloromethane and characterised by standard techniques. The ^{1}H NMR spectrum of compound **1** shows the signals of the N-H thiourea protons at δ= 6.20 and 6.37 ppm. The most important features of the ^{1}H NMR spectrum of compound **2** was the apparition of the aromatic signals in the δ= 7.05-7.20 nm range and signals of the N-H protons of the thiourea moiety centred at δ= 6.27 and 8.41 ppm. The downfield shift observed for the thiourea protons in receptor **2** relative to receptor **1** is related to the presence of the aromatic ring in the structure of the former. The formation of the thiourea moieties in **1** and **2** was also confirmed by the presence of the thiourea quaternary carbon signal centred at δ= 181 nm in the ^{13}C-NMR spectrum.

The synthesis of the anthracene derivative **3** was achieved by nucleophilic substitution reaction between (3-mercaptopropyl) triethoxysilane and 9-chloromethylanthracene in dry acetonitrile and potassium carbonate in a nearly quantitative yield. The ^{1}H NMR spectrum of compound **3** displays the typical signals of the anthracene moiety, i.e. two triplets centred at δ= 7.45 and 7.55 ppm, two pairs of doublets at δ= 8.00 and 8.33 ppm and a singlet centred at δ= 8.40 ppm. The spectrum is completed with a singlet at δ= 4.73 ppm assigned to the methylene group located between the anthracene unit and the sulphur atoms and two triplets at δ= 2.70 and 0.78 ppm assigned to the methylene moieties between the sulphur and silicon atoms.



Scheme 1. *Synthesis and structure of trialcoxysilane derivatives**1**-**3**.*

Silica nanoparticles were selected as inorganic scaffoldings for the grafting of anion binding sites and signalling subunits due to their locally flat surfaces, known functionalisation reactions and easy dispersion in organic solvents. Coated silica nanoparticles were prepared using the trialcoxysilyl derivatives **1**-**3** applying reported procedures by Montalti and co-workers.[21] In this synthetic protocol, commercially available silica nanoparticles were heated at 70 °C in a mixture of water/ethanol/acetic acid (1:2:1) in the presence of the coating subunits. Subsequent purification involved the evaporation of the ethanol, neutralisation of the acetic acid with sodium hydrogencarbonate, centrifugation and washing of the nanoparticles with acetone.

The functionalisation of the nanoparticle surface was carried out by two different approaches. The first approach relies on the consecutive grafting of the two subunits. In this protocol, the nanoparticles are first functionalised with anthracene moieties (solid **NA**) and then treated with the corresponding binding sites **1** or **2** to the result in the **NA-Bt$_3$** and **NA-Pt$_3$** solids (the dash in this nomenclature indicates the two-step grafting process). The second approach deals with the simultaneous grafting **1** or **2** and the signalling subunit **3** in different ratios. Different nanoparticles were simply prepared by modulation of the concentration of the alkoxysilane derivatives in the reaction mixture. This approach resulted in the preparation of nanoparticles identified as **NA$_x$Bt$_y$** and **NA$_x$Pt$_y$**, where the absence of the dash between the signalling and binding subunits indicates the simultaneous grafting process and the values of *x* and *y* specify the signalling/binding subunits molar ratios used in the reaction mixture. The **NA$_1$Bt$_1$**, **NA$_1$Pt$_1$**, **NA$_1$Bt$_3$** and **NA$_1$Pt$_3$** nanoparticles were prepared according to this procedure. A schematic

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[21] M. Montalti, L. Prodi, N. Zaccheroni, G. Falini, *J. Am. Chem. Soc., 2002, 124, 13540-13546.*

representation of the hybrid fluorescent nanoparticles is shown in Figure 1. The amounts of binding sites and signalling subunit finally anchored on the different materials were determined through thermogravimetic analysis and SEM microanalysis (Table 1).

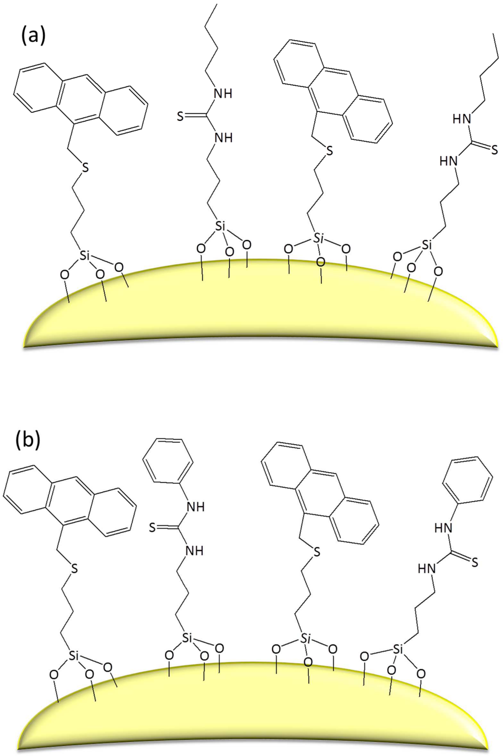

Figure 1. *Schematic representation of the hybrid nanoparticules coated with butylthiourea and anthracece (a) and with phenylthiourea and anthracene (b).*

***Table 1.** Contents of signalling subunit and binding site in hybrid nanoparticles.*

	1(mol/mol SiO_2)	**2**(mol/mol SiO_2)	**3** (mol/mol SiO_2)	Distance (Å)
NA	-	-	0.0042	22.1
NA-Pt_3	-	0.0125	0.0042	11.7
NA-Bt_3	0.0100	-	0.0042	12.8
NA_1Pt_1	-	0.0086	0.0086	11.6
NA_1Bt_1	0.0130	-	0.0130	9.4
NA_1Pt_3	-	0.0052	0.0017	19.0
NA_1Bt_3	0.0078	-	0.0026	14.8

As stated above two different synthetic routes for the preparation of the bifunctionalised silica nanoparticles were studied. In the two-step grafting procedure, the anthracene functionalised solid **NA** was first synthesised. A solid with a final content of 0.0042 mol **3**/mol SiO_2 was obtained. Transmission electron microscopy (TEM) measurements were carried out in order to determine the size and aggregation of **NA** nanoparticles. Uncoated LUDOX silica nanoparticles showed a mean diameter of 18 ± 2 nm that changed to 20 ± 2 nm when are grafted with **3** (solid **NA**). This significant increase in the mean diameter was attributed to the coating of nanoparticle surfaces with the anthracene moiety. The number of anthracene moieties present on a single nanoparticle can be roughly estimated on the basis of three parameters: the anthracene content (determined by thermogravimetry and SEM microanalysis), the particle radii and the surface of LUDOX nanoparticles. Through the approximation of a single monolayer of subunits on a smooth sphere, an average distance between two anthracenes of about 22.1 Å was determined.[22]

For the preparation of the **NA-Pt$_3$** and **NA-Bt$_3$** solids, the **NA** nanoparticles were treated with **1** and **2**, respectively, by using a 3:1 binding site/signalling subunit molar ratio. This second grafting was accomplished in acetonitrile heated at reflux for 24 hours. Thermogravimetric and elemental analyses were carried out in order to determine the amount of binding site grafted onto the surface of the **NA-Pt$_3$** and **NA-Bt$_3$** nanoparticles. Content ratios of 0.0100 and 0.0125 mmol binding site/mmol SiO_2 for butylthiourea- and phenylthiourea-functionalised materials were determined in the prepared solids, respectively. The final molar ratio between binding site and signalling subunit in **NA-Bt$_3$** amounts to 2.4, whereas in the case of **NA-Pt$_3$** this

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[22] Taking into account the data of mmol of organic moieties per gram of SiO_2 and the value of specific surface of the nanoparticle scaffolding, the average coverage (β_A in groups per nm^2) of the surface by the correspondingorganic moiety in the different nanoparticles prepared were calculated using equation (9).

$$\beta_A = \alpha_A \cdot 10^{-3} \cdot S^{-1} \cdot 10^{-18} \cdot N_A = \alpha_A \cdot S^{-1} \cdot 602.3 \qquad (9)$$

where α_A is the organic moiety content (mmol/g SiO_2), S is the specific surface (230 m^2 g^{-1}) of the nanoparticles and N_A is Avogadro´s number.

ratio is 3.0. TEM images show a mean diameter of 20 ± 2 nm for both types of hybric nanoparticles. These bifunctionalised nanoparticles bear up to 1100 attached molecules (the sum of binding sites and signalling subunits). The average distance between two subunits is 12.8 and 11.7 Å for **NA-Bt$_3$** and **NA-Pt$_3$**, respectively.

In order to study the effect exerted by the grafting procedure on the properties of the hybrid materials, a second approach based on the simultaneous grafting of the binding and signalling subunit has been used. Thus, coated nanoparticles characterised by different binding site/signalling subunit ratios were prepared by using compounds **1** and **2** and anthracene derivative **3** simply by modulation of the concentration of the alkoxysilane derivatives in the reaction mixture. With this experimental procedure four coated nanoparticles were prepared. When the molar ratio was set to 1, the coated **NA$_1$Pt$_1$** and **NA$_1$Bt$_1$** nanoparticles were obtained. The composition of the coating for these nanoparticles, bearing in mind thermogravimetric data and TEM microanalyses, were always found to be close to that of the reaction mixtures. The mol anthracene/mol SiO_2 and mol thiourea/mol SiO_2 ratios in **NA$_1$Pt$_1$** were both 0.0086 whereas for the **NA$_1$Bt$_1$** nanoparticles these ratios were both 0.0130 (see Table 1). The differing amount of anchored groups in the two solids can be attributed to the bulkiness of the butyl subunit when compared with the phenyl one. The average distance between two molecules in **NA$_1$Pt$_1$** amounts to 11.6 Å, whereas the distance between two subunits in **NA$_1$Bt$_1$** is 9.4 Å. This clearly indicates the formation of a more dense monolayer on the nanoparticle surface with butylthiourea binding sites. Again TEM measurements were carried out in order to determine the mean diameter of the bifunctionalised nanoparticles. From these measurements both **NA$_1$Pt$_1$** and **NA$_1$Bt$_1$** nanoparticles presented nearly the same mean diameter (20 nm). Figure 2 shows an image of both hybrid nanoparticles.

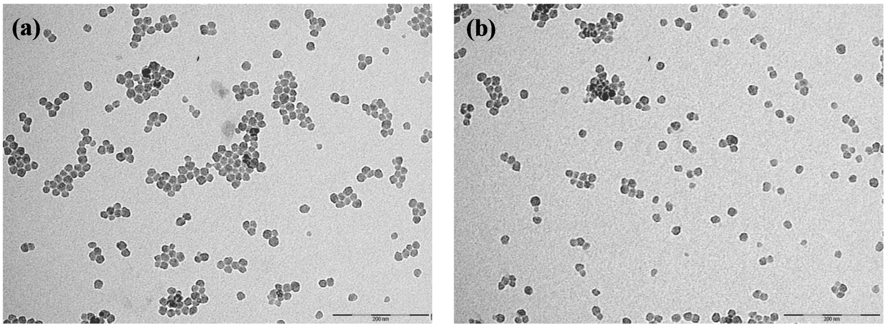

Figure 2. *TEM images of hybrid nanoparticles* ***NA$_1$Bt$_1$*** *(a) and* ***NA$_1$Pt$_1$*** *(b). In both images the bars correspond to 200 nm.*

The coated **NA$_1$Pt$_3$** and **NA$_1$Bt$_3$** nanoparticles were prepared by the same grafting procedure described above but changing the molar fraction of the components. In these materials the molar ratio of binding site/signalling subunit was fixed to 3:1. The composition of the coating for these nanoparticles, bearing in mind the thermogravimetric analysis and TEM microanalyses, was found to be relatively close to that present in the reaction mixtures. Also TEM measurements were carried out in order to determine the mean diameter of the bifunctionalised nanoparticles. TEM images showed mean diameters of 20 nm for both **NA$_1$Bt$_3$** and **NA$_1$Pt$_3$** solids.

As a conclusion, both grafting procedures lead to the preparation of hybrid bifunctionalised silica nanoparticles with well-defined binding site and signalling subunit contents. We think that one advantage of the second approach lies in the fact that only one step of functionalisation is required, which ensures faster synthetic procedures when compared with the sequential grafting of the two subunits. Also, the homogeneity of the monolayer formed with the capped ligands was higher when a one-step grafting procedure was used instead of the consecutive grafting procedure.

3.3.2. EMISSION BEHAVIOUR

This family of coated nanoparticles bearing anthracene as fluorescent signalling subunits and thiourea groups as binding sites are prospective sensory hybrid materials that have the potential ability to recognize anions through changes in fluorescence. The underlying idea was that coordination of certain anions with the thiourea subunit, for instance through hydrogen-bonding interactions, might induce some changes in the emission properties of the anthracene unit by energy- or electron-transfer processes from the anion to the excited anthracene fluorophore.[23,24] As we have cited in the introduction, and as far as we

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[23] a) G. De Santis, L. Fabbrizzi, M. Licchelli, A. Poggi, A. Taglietti, *Angew. Chem. Int. Ed., 1996, 35, 202-204*; b) L. Fabbrizzi, G. Francese, M. Licchelli, A. Perotti, A. Taglietti, *Chem. Commun., 1997, 581-582*; c) L. Fabbrizzi, I. Faravelli, *Chem. Commun., 1998, 971-972*; d) I. Bruseghini, L. Fabbrizzi, M. Licchelli, A, Taglietti, *Chem. Commun., 2002, 1348-1349*; e) L. Fabbrizzi, M.

know, only one example dealing with the use of hybrid materials based on silica nanoparticles for anion sensing has been described.

In order to check the ability of the prepared hybrid materials for the selective recognition of anionic species UV/Vis measurements were carried out. Acetonitrile suspensions of **NA** nanoparticles (5 mg in 20 mL) showed the typical anthracene bands centred at ca. 350, 370 and 390 nm. This indicates that the grafting procedure used for the preparation of bifunctionalised nanoparticles did not fundamentally change the photochemical features of the anthracene moiety. The same UV/Vis features were observed in acetonitrile suspensions (5 mg of nanoparticles in 20 mL of dry acetonitrile) of **NA-Bt$_3$**, **NA-Pt$_3$**, **NA$_1$Bt$_1$**, **NA$_1$Pt$_1$**, **NA$_1$Bt$_3$** and **NA$_1$Pt$_3$** that showed in all cases the characteristic bands of the anthracene groups.

The fluorescence of the acetonitrile suspensions of **NA** nanoparticles was measured. Upon excitation at 365 nm, the typical broad emission band with fine structure in the 390-450 nm range was observed. The emission spectrum was also characterised by the absence of emission

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Licchelli, F. Mancin, M. Pizzeghello, G. Rabaioli, A. Taglietti, P. Tecilla, U. Tonellato, *Chem. Eur. J., 2002, 8, 94-101.*

[24] a) T. Gunnlaugsson, A. P. Davis, J. E. O´Brien M. Glynn, *Org. Lett., 2002, 4, 2449-2452*; b) T. Gunnlaugsson, A. P. Davis, G. M. Hussey, J. Tierney, M. Glynn, Org. Biomol. *Chem., 2004, 2, 1856-1863*; c) T. Gunnlaugsson, M. Glynn, G. M. Tocci, P. E. Kruger, F. M. Pfeffer, Coord. *Chem. Rev., 2006, 250, 3094-3117*; d) S. E. García-Garrido, C. Caltagirone, M. E. Light, P. A. Gale, *Chem. Commun., 2007, 1450-1452.*

bands in the 450-600 range typical of excimer/exciplex formation. This absence of redshifted bands indicates that there are no interactions between two closely located anthracene subunits (the distance between two anthracene moieties is ca. 22.1 Å). When taking into account the necessary distance for excimer formation in solution, estimated to be ca. 8 Å (in chloroform),[25] the absence of this band in solid **NA** seems to be logical. Again the same emission bands and the absence of redshifted bands were found in acetonitrile suspensions (5 mg in 20 mL) for the bifunctionalised hybrid **NA-Bt$_3$**, **NA-Pt$_3$**, **NA$_1$Bt$_1$**, **NA$_1$Pt$_1$**, **NA$_1$Bt$_3$** and **NA$_1$Pt$_3$** nanoparticles. Figure 3 shows the emission spectrum for **NA-Pt$_3$** nanoparticles in acetonitrile (λ_{exc}= 365 nm).

In order to check the response towards a certain organic (acetate, benzoate) and inorganic anions (fluoride, chloride, bromide, cyanide, dihydrogen phosphate and hydrogen sulfate) a series of fluorescence measurements were carried out. Suspensions of the corresponding coated nanoparticles (5 mg) in dry acetonitrile (20 mL) were prepared and then fluorimetric titrations with the corresponding anions were carried out. The first material tested was **NA**. This solid does not contain binding sites and is a suitable model. The addition of increasing quantities of the target anions to acetonitrile suspension of **NA** induced negligible changes in the intensity of the anthracene emission bands (data not shown). The negative response observed was a clear

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[25] T. Kobayashi, S. Nagakura, M. Szware, *Chem. Phys., 1979, 39, 105.*

consequence of the absence of any binding site on the surface of **NA** nanoparticles.

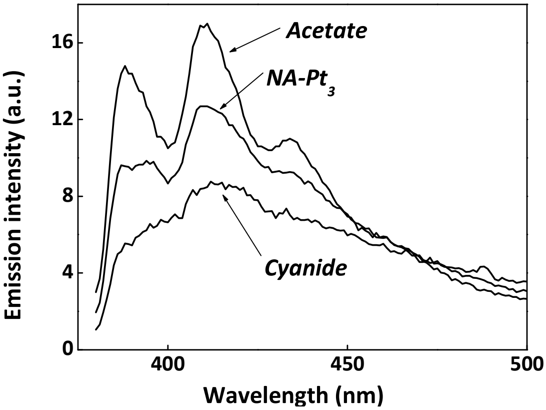

Figure 3. *Fluorescence spectra of* ***NA-Pt*** $_3$ *hybrid nanoparticles suspended in acetonitrile (*λ_{exc}*= 365 nm) alone and in the presence of cyanide or acetate.*

In a second step we proceeded to study the emission behaviour of the bifunctionalised nanoparticles. The general trends in the emission intensity observed upon addition of target anions could be summarised by the detailed study of the behaviour of acetonitrile suspensions of **NA-Pt**$_3$ nanoparticles. Therefore fluorescent titrations upon addition of anions to acetonitrile suspensions (5 mg in 20 mL of dry acetonitrile) of **NA-Pt**$_3$ were carried out. As a representative example Figure 4 shows the relative emission changes as difference between the emission intensity of **NA-Pt**$_3$ nanoparticles (5 mg in 20 mL) in the presence of 7 equiv. of the corresponding anion (E) and the nanoparticles alone (E_0), in acetonitrile. Three main behaviours were observed (see Figure 4): (i) poor change, (ii) fluorescence enhancement and (iii) fluorescence enhancement coupled with quenching upon reaching a certain quantity of anion equivalents. Thus, the addition of increasing quantities of sulfate anions did not induce substantial changes in either the intensity or in the position of the emission bands. On the other hand, the addition of increasing quantities of phosphate, acetate, benzoate, chloride and bromide anions induced a moderate increase (about 1.4-fold) in the anthracene emission intensity. We attributed this enhancement to coordination of the cited anions with the phenylthiourea binding sites on the surface of the nanoparticles, which resulted in restricting the mobility of the excited anthracene fluorophore disabling the possibility of de-excitation by non-emissive channels, and thus leading to an increase in the emission intensity. The third behaviour was observed upon addition of cyanide and fluoride anions. In this case, the addition of increasing quantities of both anions (up to 5 equiv.) to acetonitrile suspensions of **NA-Pt$_3$** nanoparticles induced an enhancement in the emission intensity, and then further addition of anion equivalents induced a moderate quenching of the emission intensity (see Figure 4 for the response observed after the addition of 7 equiv. of anions with respect to the total number of binding sites in the nanoparticles). This enhancement/quenching of the emission intensity of the excited anthracene could be explained bearing in mind the fact that cyanide and fluoride are the most basic of all the anions tested. Again the first emission enhancement could be assigned to a certain restriction in the mobility of the excited anthracene fluorophore upon anion binding, whereas addition of more equivalents of the cited basic anions would deprotonate the N-H moiety attached directly to the phenyl ring. This proton-transfer reaction would create a negative charge on the binding site that would be the responsible for the quenching of the emission intensity through a PET process. These coordination/deprotonation processes of urea and/or thiourea binding sites are well known and, when coupled with dyes or fluorescent units, have been extensively employed for the optical sensing of basic anions in organic solvents.[26] Figure 3 shows the emission spectrum for **NA-Pt$_3$** nanoparticles in acetonitrile (λ_{exc}= 365 nm) in the presence of acetate and sulfate. However, the moderate changes in the emission intensity observed are indicative of weak perturbations of the electronic states of the anthracene fluorophore. These changes are a direct consequence of the weak interactions present between the surface-grafted thiourea binding sites and the target anions.

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[26] See for example: a) V. Amendola, D. Esteban-Gómez, L. Fabbrizzi, M. Licchelli, *Acc. Chem. Res., 2006, 39, 343-353*; b) S. Camiolo, P. A. Gale, M. B. Hursthouse, M. E. Light, *Org. Biomol. Chem., 2003, 1, 741-744;* c) M. Vázquez, L. Fabbrizzi, A. Taglietti, R. M. Pedrido, A. M. González-Noya, M. R. Bermejo, *Angew. Chem. Int. Ed., 2004, 43, 1962-1965*; d) M. Boiocchi, L. Del Boca, D. Esteban-Gómez, L. Fabbrizzi, M. Licchelli, E. Monzani, *J. Am. Chem. Soc., 2004, 126, 16507-16514*; e) L. S. Evans, P. A. Gale, M. E. Light, R. Quesada, *Chem. Commun., 2006, 965-967*; f) A. D. P. Ali, P. E. Kruger, T. Gunnlaugsson, *New J. Chem., 2008, 32, 1153-1161.*

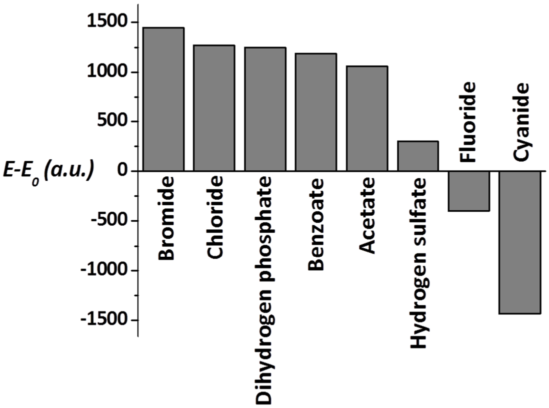

Figure 4. *Relative emission changes, as difference between the emission intensity of the* ***NA-Pt₃*** *nanoparticles (5 mg in 20 mL) in the presence of 7 equiv. of the corresponding anion (E) and the nanoparticles alone (E_0), in acetonitrile (mol-equiv. with respect to the number of binding sites).*

In order to complete the characterisation of the interaction of the nanoparticles with anions, the apparent binding constants (adsorption constants) for the interaction of **NA-Pt₃** with anions in acetonitrile were determined by performing a Langmuir-type analysis of fluorescence titration data using Equation (1).

$$I = I_L \frac{Kc}{1+Kc} \quad ; \quad c = \frac{-\left\{\frac{1}{K}+\frac{n_M}{V}-c_0\right\}+\sqrt{\left(\frac{1}{K}+\frac{n_M}{V}-c_0\right)^2+\frac{4c_0}{K}}}{2} \qquad (1)$$

Here, K is an adsorption constant that accounts for the interaction between the corresponding anion and the recognition centres anchored to the solid, and I and I_L are the observed intensities at a specific anion concentration and in the presence of an excess of anion (the limiting value), respectively. Additionally, c_0 is the total added salt (in mol L^{-1}) and n_M is the maximum amount of anion (in mol) that could be adsorbed in the monolayer. Finally, V is the volume (in L) in which the experiment was carried out. The formalism that leads to the deduction of Equation 1 is given in the Experimental Section. This equation can be easily applied to the study of the interaction of certain guest with functionalised nanoparticles or other functionalised solid supports.

By using this equation and the experimental titration data, adsorption constants of for the acetate, Cl^-, Br^-, benzoate, $H_2PO_4^-$ and HSO_4^- anions have been calculated and are shown in Table 2. Figure 5 shows the emission intensity of solid **NA-Pt$_3$** in the presence of increasing amounts of acetate and HSO_4^- in acetonitrile and the fitting of the experimental data with Equation (1). As can be seen in Table 2, the interaction of the anions with the nanoparticles results in adsorption constants that range from the value observed for acetate (K = 2340) to that found for HSO_4^- (K = 178). The effect of the binding site is reflected in the stability constant found for acetate. In fact this anion forms the more stable thermodynamic interaction with the nanoparticles and displays the largest adsorption constant. This was expected bearing in mind the structural design of the thiourea moiety, which improves carboxylate binding through the formation of Y shaped complexes.

Additionally, whereas log *K* for acetate is 3.37 this value is one order of magnitude lower for benzoate (log *K* = 2.49). This difference was due to the larger size of benzoate anion, when compared with acetate, which imposes some steric hindrance to the coordination with the thiourea moiety. The constant observed for acetate is quite high. However this constant is lower than that reported for the interaction of ATP with MCM41-type solids containing anthracene as signalling units.[27]

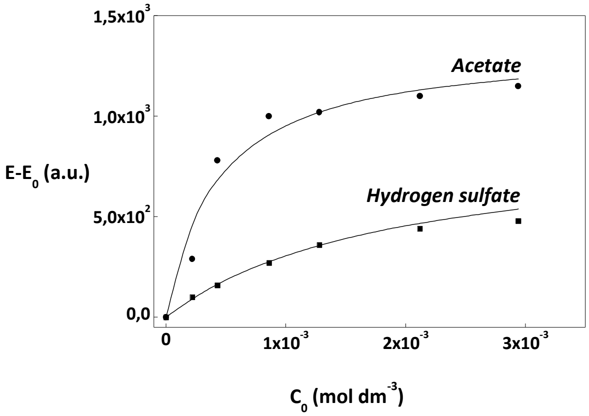

Figure 5. *Changes in the emission intensity (λ_{em} = 411 nm) of the anthracene unit of the **NA-Pt₃** nanoparticles in the presence of increasing amounts of acetate and hydrogensulfate. The emission intensity has been normalised to 1. The solid line shows the fit obtained with Equation (1).*

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[27] a) A.B. Descalzo, M.D. Marcos, R. Martínez-Máñez, J. Soto, D. Beltrán, P. Amorós, *J. Mater. Chem. 2005, 15, 2721-2731*; b) A.B. Descalzo, K. Rurak, H. Weisshoff, R. Martínez-Máñez, M.D. Marcos, P. Amorós, K. Hoffmann, J. Soto. *J. Am. Chem. Soc., 2005, 127, 184-200*; c) A.B. Descalzo, D. Jiménez, M.D. Marcos, R. Martínez-Máñez, J. Soto, J. El Haskouri, C. Guillém, D. Beltrán, P. Amorós, V. Borrachero, *Adv. Mater., 2002, 14, 966-969.*

Table 2*. log K values obtained for the interaction of* ***NA-Pt$_3$*** *nanoparticles with the anions in acetonitrile.*

	Log K [a]
Acetate	3.37
Cl^-	2.91
Br^-	2.27
Benzoate	2.27
$H_2PO_4^-$	2.47
HSO_4^-	2.25

[a] Log *K* values have been calculated using titration curves and Equation (1).

3.4. CONCLUSIONS

Silica nanoparticles have been treated with the alkoxysilane derivatives *N*-butyl-*N′*-[3-(trimethoxysilyl)propyl]thiourea (**1**), *N*-phenyl-*N′*-[3-(trimethoxysilyl)propyl]thiourea (**2**) and 3-((anthracen-10-yl) methylthio)propyltriethoxysilane (**3**) in order to prepare nanoparticles functionalised with both thiourea and anthracene subunits. The functionalisation process was carried out by two different procedures through (i) consecutive or (ii) simultaneous grafting of both subunits. Fluorescence studies on the hybrid nanoparticles were carried out. Typical anthracene absorption and emission bands were observed. The addition of target anions to suspensions of the hybrid nanoparticles in acetonitrile induced moderate changes in the emission intensity with modest patterns of selectivity. The apparent binding constants (adsorption constants) for the interaction of **NA-Pt$_3$** with anions in acetonitrile were determined by performing a Langmuir-type analysis of fluorescence titration data. Despite these results, this procedure is highly modular because it allows the functionalisation of the surface with several binding sites and signalling subunits by routes with a relatively low synthetic effort. We are currently developing novel protocols using nanoparticles for the development of new chromofluorogenic chemosensors for anions.

3.5. EXPERIMENTAL SECTION

3.5.1 GENERAL REMARKS

Phenylisothiocyanate, butylisotiocyanate, (3-aminopropyl) trimethoxysilane, (3-mercaptopropyl)triethoxysilane, 9-(chloromethyl) anthracene and 30 % suspension of LUDOX silica nanoparticles AS-30 Colloidal Silica were purchased from Sigma-Aldrich and were used without any further purification. The solvents were absolute grade and were purchased from Scharlab. The anions were used as tetrabutylammonium salts and were purchased from Aldrich (Cl^-, Br^-, AcO^-, BzO^-, CN^-, HSO_4^- and $H_2PO_4^-$) and Fluka (F^-).

3.5.2 PHYSICAL MEASUREMENTS AND INSTRUMENTATION

^{1}H and ^{13}C NMR spectra were recorded using a Varian Gemini 300 MHz NMR spectrometer. Chemical shifts are reported relative to residual $CHCl_3$. Multiplicities are given as usual. Thermogravimetric analyses were carried out in a Mettler Toledo TGA/SDTA 851^{e}. Transmission Electron Microscopy (TEM) images of the particles were obtained with a Philips CM10 operating at 20 KeV. Samples for TEM were prepared by spreading a drop of nanoparticles solution in HEPES onto standard carbon-coated copper grids (200 mesh). SEM microanalyses were obtained with a JEOL 6300 with a detector WDS of Oxford Instruments. UV/Vis absorption measurements were measured with a Perkin-Elmer Lambda-35 spectrometer. The fluorescence behaviour was studied with an FS900CDT Steady State T-Geometry Fluorimeter from Edinburgh Analytical Instruments. All solutions for photophysical studies were previously degassed. Anion stock solutions for photophysical studies (C = 1 x 10^{-2} mol dm^{-3}) were prepared in dry acetonitrile.

3.5.3 SYNTHESIS

The syntheses of the alkoxyxilane derivatives were carried out according to known procedures. The reaction of (3-aminopropyl) trimethoxysilane with butyl isothiocyanate and phenyl isotiocyanate afforded the trialcoxysilane derivatives **1** and **2** in high yields. Trialcoxysilane derivative **3** was prepared by a nucleophilic substitution reaction between (3-mercaptopropyl)triethoxysilane and 9-(chloromethyl)anthracene.

3.5.3.1 N-BUTYL-N′-[3-(TRIMETHOXYSILYL)PROPYL]THIOUREA (1)

N-butyl-*N′*-[3-(trimethoxysilyl)propyl]thiourea was synthesised by the reaction between (3-aminopropyl)trimethoxysilane (1.75 mL, 10 mmol) and butyl isothiocyanate (1.27 mL, 10 mmol) in CH_2Cl_2 (10 mL) and with few drops of triethylamine (see Scheme 1). After 24 h, the solvent was removed by evaporation, and the product isolated as yellowish oil (2.80 g, 95% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.60 (t, J = 7.8 Hz, 2H, -C$\underline{H}_2$-Si-), 0.92 (t, J = 6.6 Hz, 3H, C$\underline{H}_3$-CH_2-CH_2-), 1.35 (m, 2H, CH_3-C$\underline{H}_2$-CH_2-), 1.55 (m, 2H, CH_3-CH_2-C$\underline{H}_2$-), 1.61 (t, J = 7.8 Hz, 2H, -C$\underline{H}_2$-CH_2-Si-), 3.38 (m, 4H, -NH-C$\underline{H}_2$-CH_2-), 3.50 (s, 9H, C$\underline{H}_3$-O-), 6.20 (s, 1H, -CO-N$\underline{H}$-CH_2-), 6.37 (s, 1H, -CH_2-N$\underline{H}$-CO-). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$) δ: 6.5 (-$\underline{C}H_2$-Si-), 14.0 ($\underline{C}H_3$-CH_2-CH_2-), 20.3 (CH_3-$\underline{C}H_2$-CH_2-), 22.5 (-$\underline{C}H_2$-CH_2-Si-), 31,4 (CH_3-CH_2-$\underline{C}H_2$-), 47.6 (-NH-$\underline{C}H_2$-CH_2-), 50.8 ($\underline{C}H_3$-O-), 181 (-NH-$\underline{C}$O-NH-). $C_{11}H_{26}N_2O_3SSi$ (294.49): calcd. C 44.86, H 8.90, N 9.51, S 10.89; found C 44.78, H 8.92, N 9.45, S 10.71.

3.5.3.2 N-PHENYL-N′-[3-(TRIMETHOXYSILYL)PROPYL]THIOUREA (2)

N-phenyl-*N′*-[3-(trimethoxysilyl)propyl]thiourea was synthesised by the reaction between (3-aminopropyl)trimethoxysilane (4.77 mL, 27 mmol) and phenyl isothiocyanate (3.27 mL, 27 mmol) in CH_2Cl_2 (10 mL) with a few drops of triethylamine (see Scheme 1). After 24 h, the solvent was removed by evaporation and the product isolated as yellowish oil (6.00 g, 95% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.55 (t, *J* = 7.1 Hz, 2H, -$C\underline{H}_2$-Si-), 1.60 (t, *J* = 7.1 Hz, 2H, -$C\underline{H}_2$-CH_2-Si-), 3.47 (s, 9H, $C\underline{H}_3$-O-), 3.60 (t, *J* = 7,1 Hz, 2H, -NH-$C\underline{H}_2$-CH_2-), 6.27 (s, 1H, -CO-N$\underline{H}$-CH_2-), 7.20-7.27(m, 5H, $C_6\underline{H}_5$-NH-), 8.41 (s, 1H, C_6H_5-N$\underline{H}$-CO-). ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$) δ: 6.5 (-$\underline{C}H_2$-Si-), 22.4 (-$\underline{C}H_2$-CH_2-Si-), 47.6 (-NH-$\underline{C}H_2$-CH_2-), 50.8 ($\underline{C}H_3$-O-), 125.4 ($\underline{C}_6H_5$-NH-), 127.2 ($\underline{C}_6H_5$-NH-), 130.3 ($\underline{C}_6H_5$-NH-), 136.0 ($\underline{C}_6H_5$-NH-), 180.7 (-NH-$\underline{C}$O-NH-). $C_{13}H_{22}N_2O_3SSi$ (314.47): calcd. C 49.65, H 7.05, N 8.91, S 10.20; found C 49.72, H 7.15, N 8.78, S 9.96.

3.5.3.3 3-((ANTHRACEN-10-YL)METHYLTHIO)PROPYLTRIETHOXYSILANE (3)

9-(Chloromethyl)anthracene (1.0 g, 4.4 mmol) and (3-mercaptopropyl)triethoxysilane (1.06 mL, 4.4 mmol) were dissolved in dry acetonitrile (50 mL). Potassium carbonate (1.8 g, 13 mmol) was added, and the mixture was heated to reflux for 24 hours. The crude mixture was filtered off in order to remove the excess of potassium carbonate and the acetonitrile evaporated to give **3** as yellowish oil (1.73 g, 95 % yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.78 (t, *J* = 7.8 Hz, 2H, -$C\underline{H}_2$-Si-), 1.20 (t, *J* = 7.1 Hz, 9H, $C\underline{H}_3$-CH_2-O-), 1.84 (t, *J* = 7.8 Hz, 2H, -$C\underline{H}_2$-CH_2-Si-), 2.70 (t, *J* = 7.8 Hz, 2H, -S-$C\underline{H}_2$-CH_2-CH_2-Si), 3.81 (q, *J* = 7.1 Hz, 6H, CH_3-$C\underline{H}_2$-O-), 4.73 (s, 2H, -S-$C\underline{H}_2$-Ar), 7.45 (t, *J* = 6.5 Hz, 2H, Ar), 7.55 (t, *J* = 6.5 Hz, 2H, Ar), 8.0 (d, *J* = 6.7 Hz, 2H, Ar), 8.33 (d, *J* = 6.7 Hz, 2H, Ar), 8.40 (s, 1H, Ar). $^{13}C\{^{1}H\}$ NMR (75 MHz, $CDCl_3$) δ: 8.0 (-$C\underline{H}_2$-Si-), 18.3 ($C\underline{H}_3$-CH_2-O-), 23.3 (-$C\underline{H}_2$-CH_2-Si-), 35.2 (-S-$C\underline{H}_2$-CH_2-CH_2-Si), 38.3 (-S-$C\underline{H}_2$-Ar), 53.3 (CH_3-$C\underline{H}_2$-O-), 124.1 (Ar), 124.8 (Ar), 126.0 (Ar), 127.0 (Ar), 129.1 (Ar), 130.2 (Ar), 131.5 (Ar), 131.8 (Ar). $C_{24}H_{32}O_3SSi$ (428.66): calcd. C 67.25, H 7.52, S 7.48; found C 67.37, H 7.60, S 7.34.

3.5.3.4 SYNTHESIS OF FUNCTIONALISED SILICA NANOPARTICLES

The functionalisation of silica nanoparticles with the signalling subunit and the binding site was carried out according to two distinct approaches. The first approach was based on the consecutive grafting of the two subunits. In the first step nanoparticles functionalised with antracene moieties were prepared (**NA**) and then the corresponding binding sites (3-fold molar excess based on the content in anthracene) were incorporated onto the nanoparticle surface (**NA-Bt$_3$**, **NA-Pt$_3$**, the dash in this nomenclature indicates the two step grafting process). The second approach deals with the functionalisation of nanoparticles by the grafting of both subunits (in different molar ratios) in only one synthetic step. These nanoparticles were designated by **NA$_x$Bt$_y$** and **NA$_x$Pt$_y$**, where the absence of the dash between the signalling and binding subunits indicates the simultaneous grafting process and the values of *x* and *y* indicates the molar ratios between the signalling and binding subunits.

3.5.3.5 NANOPARTICLES FUNCTIONALISED WITH ANTHRACENE (NA)

Compound **3** (0.32 g, 0.75 mmol) was placed in a round bottom flask and then dissolved with ethanol (100 mL), water (50 mL) and acetic acid (50 mL). Then a water suspension of silica nanoparticles (12 mL LUDOX AS-30 colloidal silica) was added. The reaction mixture was warmed at 80°C while being stirred for 48 h. After this time, the ethanol was evaporated under reduced pressure, and solid $NaHCO_3$ was added to the suspension until a pH value between 5 and 6 was reached. The silica nanoparticles were isolated by centrifugation and washed with water and acetone. The solid was dried at 70 ^{o}C during 16 h.

3.5.3.5 NANOPATICLES FUNCTIONALISED WITH BUTYLTHIOUREA AND ANTHRACENE (NA-Bt_3)

NA nanoparticles (0.75 g) and **1** (0.12 g, 0.7 mmol) were added to dry acetonitrile (50 mL). The resulting mixture was heated at reflux for 24 hours. The solid nanoparticles were obtained by centrifugation and were washed several times with water and acetone. The final solid was dried at 70 ^{o}C for 16 h.

3.5.3.6 NANOPARTICLES FUNCTIONALIZED WITH PHENYLTHIOUREA AND ANTHRACENE (NA-Pt_3)

NA nanoparticles (0.75 g) and **2** (0.12 g, 0.4 mmol) were added to dry acetonitrile (50 mL). The resulting mixture was heated at reflux for 24 h. The solid nanoparticles were obtained by centrifugation and were washed several times with water and acetone. The final solid was dried at 70 ^{o}C for 16 h.

3.5.3.7 NANOPARTICLES FUNCTIONALIZED WITH BUTYLTHIOUREA AND ANTHRACENE (NA_1Bt_1)

A colloidal dispersion of LUDOX AS-30 silica nanoparticles (5 mL) was added to a solution containing ethanol (40 mL), water (20 mL) and acetic acid (20 mL). Then compounds **1** (0.084 mmol) and **3** (0.084 mmol) dissolved in dry acetonitrile (5 mL) were added to the nanoparticle suspension and the resultanting mixture was at reflux for 48 hours. Then the ethanol was removed in a rotary evaporator and the pH of the water suspension was lowered to 5 by the addition of sodium hydrogencarbonate. The functionalised **NA_1Bt_1** nanoparticles were isolated by centrifugation at 10000 r.p.m., washed with water and acetone and dried at 70 ^{o}C overnight.

3.5.3.8 NANOPARTICLES FUNCTIONALIZED WITH PHENYLTHIOUREA AND ANTHRACENE (NA_1Pt_1)

A colloidal dispersion of LUDOX AS-30 silica nanoparticles (5 mL) was added to a solution containing ethanol (40 mL), water (20 mL) and acetic acid (20 mL). Then compounds **2** (0.084 mmol) and **3** (0.084 mmol) dissolved in dry acetonitrile (5 mL) were added to the nanoparticle suspension and the resultanting mixture was heated at reflux for 48 h. Then the ethanol was removed in a rotary evaporator, and the pH of the water suspension was lowered to 5 by the addition of sodium hydrogencarbonate. The functionalised **NA_1Pt_1** nanoparticles were isolated by centrifugation at 10000 r.p.m., washed with water and acetone and dried at 70 ^oC overnight.

3.5.3.9 NANOPARTICLES FUNCTIONALIZED WITH BUTYLTHIOUREA AND ANTHRACENE (NA_1BT_3)

A colloidal dispersion of LUDOX AS-30 silica nanoparticles (5 mL) was added to a solution containing ethanol (40 mL), water (20 mL) and acetic acid (20 mL). Then compounds **1** (0.084 mmol) and **3** (0.028 mmol) dissolved in dry acetonitrile (5 mL) were added to the nanoparticle suspension, and the mixture was heated at reflux for 48 h. Then the ethanol was removed in a rotary evaporator and the pH of the water suspension was lowered to 5 by the addition of sodium hydrogencarbonate. The functionalised **NA_1Bt_3** nanoparticles were isolated by centrifugation at 10000 r.p.m., washed with water and acetone and dried at 70 °C overnight.

3.5.3.10 NANOPARTICLES FUNCTIONALIZED WITH PHENYLTHIOUREA AND ANTHRACENE (NA_1PT_3)

A colloidal dispersion of LUDOX AS-30 silica nanoparticles (5 mL) was added to a solution containing ethanol (40 mL), water (20 mL) and acetic acid (20 mL). Then compounds **2** (0.084 mmol) and **3** (0.028 mmol) dissolved in dry acetonitrile (5 mL) were added to the nanoparticle suspension, and the resulting mixture was heated at reflux for 48 h. Then the ethanol was removed in a rotary evaporator, and the pH of the water suspension was lowered to 5 by the addition of sodium hydrogencarbonate. The functionalised **NA_1Pt_3** nanoparticles were isolated by centrifugation at 10000 r.p.m., washed with water and acetone and dried at 70 °C overnight.

3.5.4 DEDUCTION OF EQUATION (1)

The affinity of the solid **$NA\text{-}Pt_3$** nanoparticles for different anions has been studied by using as starting model the well-known Langmuir isotherm for adsorption processes on surfaces. In the Langmuir isotherm the coverage θ is a function of both the temperature and the adsorbate concentration. If the temperature is constant, the coverage is only a function of the concentration and follows the Equation (2).

$$\theta = \frac{n_A}{n_M} = \frac{I}{I_L} = \frac{Kc}{1+Kc} \qquad (2)$$

where θ is the fraction of adsorbed anion (n_A, in mol) vs. the maximum amount of anion (n_M, in mol) that could be adsorbed in the monolayer. This is equivalent to the ratio between the measured fluorescence intensity at a certain concentration (I) and the fluorescence signal obtained upon saturation with the analyte (I_L). Alternatively, θ can be expressed by the Langmuir adsorption constant K and the concentration c (in mol L^{-1}) of the anion in the equilibrium. Thus, after rearrangement Equation (3):

$$n_A = n_M\theta = \frac{n_M Kc}{1+Kc} \qquad (3)$$

Additionally, taking into account that the total number of moles of anion (n_0) is the sum of the moles adsorbed on the monolayer (n_A) and the moles of anion remaining in the solution (n); i.e. $n_0 = n + n_A$, Equation (3) is then transformed into Equation (4):

$$n_0 = n + \frac{n_M Kc}{1+Kc} \qquad (4)$$

Dividing by the volume (solution volume V in L) we obtain Equation (5)

From eqn. (2) we obtain Equation (8)

$$I = I_L \frac{Kc}{1+Kc} \tag{8}$$

$$c_0 = c + \frac{\frac{n_M}{V} Kc}{1+Kc} \tag{5}$$

and finally Equations (6) and (7):

$$Kc^2 + (1 + K\frac{n_M}{V} - Kc_0)c - c_0 = 0 \tag{6}$$

And

$$c = \frac{-\left\{\frac{1}{K} + \frac{n_M}{V} - c_0\right\} + \sqrt{\left(\frac{1}{K} + \frac{n_M}{V} - c_0\right)^2 + \frac{4c_0}{K}}}{2} \tag{7}$$

and by substitution of Equation (7) into Equation (8), the final Equation (1), used for fitting of the titration curves is obtained.

Acknowledgments

The authors wish to express their gratitude to the Ministerio de Ciencia y Tecnología (projects CTQ2006-15456-C04-01), for financial *support. P. C. thanks the Ministerio de Educación y Ciencia for a Torres Quevedo contract.*

CHAPTER 4

SILICA NANOPARTICLES FUNCTIONALISED WITH CATION COORDINATION SITES AND FLUOROPHORES FOR THE DIFFERENTIAL SENSING OF ANIONS IN A QUENCHER DISPLACEMENT ASSAY (QDA)

SILICA NANOPARTICLES FUNCTIONALISED WITH CATION COORDINATION SITES AND FLUOROPHORES FOR THE DIFFERENTIAL SENSING OF ANIONS IN A QUENCHER DISPLACEMENT ASSAY (QDA)

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Received: 24th May 2011

Accepted: 11th August 2011

*Chemical Communications, **2011**, 47, 10599–10601.*

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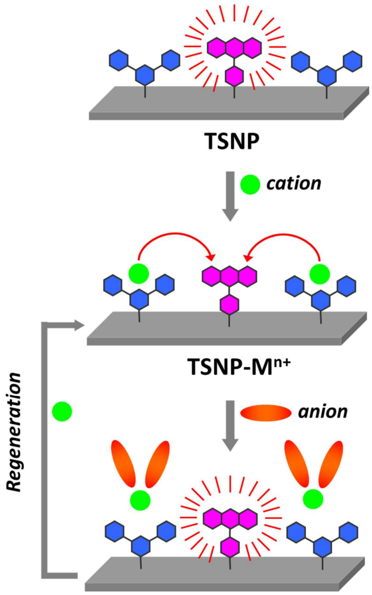
Chem. Comm., 2011, 47, 10599–10601

In conjunction with quenching metal ions, silica nanoparticles carrying terpyridine coordination sites and sulphorhodamine B signalling units were employed for the differential fluorometric recognition of anions.



The development of chromo- and fluorogenic chemosensors for anions has received growing interest during the past years due to the fundamental roles that anions play in chemical and biological processes.[1] The majority of these chemosensors incorporate molecular

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[1] F. P. Schmidtchen and M. Berger, *Chem. Rev., 1997, 97, 1609*; P. D. Beer and P. A. Gale, *Angew. Chem. Int. Ed., 2001, 40, 486.*

receptors of supramolecular chemistry design. Traditionally, supramolecular anion sensing utilizes either colour and/or fluorescence changes in probes with a "binding site–signalling subunit" architecture or undergoing chemodosimetrical reactions and indicator release in displacement assays (IDAs) for signal transduction.[2, 3] However, recently a novel trend in chemosensors development has emerged which uses organic-inorganic hybrid materials, in many cases nanoparticles.[4] These hybrid systems often show synergistic effects which are hardly achievable with molecular systems or inorganic solids alone.[5]

Among different inorganic supports silica nanoparticles have been extensively used for hybrid sensing materials due to their straightforward preparation and surface functionalization and high stability in aqueous suspensions.[6] As an alternative to chromo- and fluorogenic probes, self-assembled structures on surfaces in particular have attracted increasing attention recently. Especially the independent

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[2] T. Gunnlaudsson, M. Glynn, G. M. Tocci, P. E. Kruger and F. M. Pfeffer, *Coord. Chem. Rev., 2006, 250, 3094*; V. Amendola, D. esteban-Gómez, L. Fabbrizzi and M. Licchelli, *Acc. Chem. Res., 2006, 39, 343*; S. L. Wiskur, H. Aït-Haddou, J. J. Lavigne and E. V. Anslyn, *Acc. Chem. Res., 2001, 34, 963*.

[3] R. Martínez-Máñez and F. Sancenón, *Chem. Rev., 2003, 103, 4419*; R. Martínez-Máñez and F. Sancenón, *J. Fluoresc., 2005, 15, 267*; M. E. Moragues, R. Martínez-Máñez and F. Sancenón, *Chem. Soc. Rev., 2011, 40, 2593.*

[4] The Supramolecular Chemistry of Organic-Inorganic Hybrid Materials, ed. K. Rurack and R. Martínez-Máñez, *Wiley, 2010.*

[5] A. B. Descalzo, R. Martínez-Máñez, F. Sancenón, K. Hoffmann and K. Rurack, *Angew. Chem. Int. Ed., 2006, 45, 5924*; R. Martínez-Máñez and F. Sancenón, *Coord. Chem. Rev., 2006, 250, 3081.*

[6] R. Shenhar and V. M. Rotello, *Acc. Chem. Res., 2003, 36, 549*; C. Beck, W. Härtl and R. Hempelmann, *Angew. Chem. Int. Ed., 1999, 38, 1297.*

functionalization of inorganic surfaces with receptors and fluorophore units is an appealing approach that overcomes the synthetic problems usually immanent to the classical “binding site–signalling subunit” architecture.[7] Moreover, signal amplification and cooperative binding effects associated with the independent anchoring of receptor and fluorophores in close proximity to each other and to the surface of the support are noticeable features of this strategy, allowing for instance intercommunication between both subunits without the need for a direct covalent link between them. Employing silica nanoparticles functionalised with fluorophores and suitable coordination sites, this approach has recently been used for the selective recognition of metal cations.[8] In contrast, hybrid nanoparticles for the optical recognition of anions are much less explored.[9]

Our present hybrid system for the optical differential recognition of anions is based on the competitive coordination to a metal centre. Since one of our interests in such assays is the facile regeneration of the

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[7] M. J. W. Ludden, D. N. Reinhoudt and J. Huskens, *Chem. Soc. Rev., 2006, 35, 1122*; P. M. Mendes, *Chem. Soc. Rev., 2008, 37, 2512.*

[8] E. Rampazzo, E. Brasola, S. Marcuz, F. Mancin, P. Tecilla and U. Tonellato, *J. Mater. Chem., 2005, 15, 2687*; M. Crego-Calama and D. N. Reinhoudt, *Adv. Mater., 2001, 13, 1171*.

[9] P. Calero, E. Aznar, J. M. Lloris, M. D. Marcos, R. Martínez-Máñez, J. V. Ros-Lis, J. Soto and F. Sancenón, *Chem. Commun., 2008, 1668*; P. Calero, R. Martínez-Máñez, F. Sancenón and J. Soto, *Eur. J. Inorg. Chem., 2008, 5649*; L. Basabe-Desmonts, J. Beld, R. S. Zimmerman, J. Hernando, P. Mela, M. F. G. Parajó, N. F. van Hulst, A. van den Berg, D. N. Reinhoudt and M. Crego-Calama*, J. Am. Chem. Soc., 2004, 126, 7293*.

sensing material,[10] we modified the conventional IDA concept. Instead of displacing an indicator dye with the analyte from a binding site, which requires a two-step regeneration procedure of analyte removal and subsequent indicator reloading (and sometimes also the spatial separation of indicator from the host–guest complex for detection), we attached a fluorophore (F) and a receptor (R), which does not bind to the analyte but to a mediator, separately to the support. A quenching metal ion (Q), which is at the same time a good binder for the target anion, then served as the mediator, i.e., as the displaceable species, arriving at a quencher displacement assay (QDA, Scheme 1). In the first step of the protocol, the R units of the highly fluorescent receptor- and fluorophore-functionalised nanoparticles (RFNs) are loaded with Q, switching off the hybrid's fluorescence. Second, addition of the target analyte, which shows a higher affinity for Q than R does, displaces Q from R, leading to revival of the fluorescence of the RFNs. In a third step, regeneration of the material is simply accomplished by reloading Q onto the RFNs. For the title material, terpyridine units as R for the quencher metal ions Q and sulphorhodamine B as signalling units F were independently anchored onto the surface of the silica nanoparticles.

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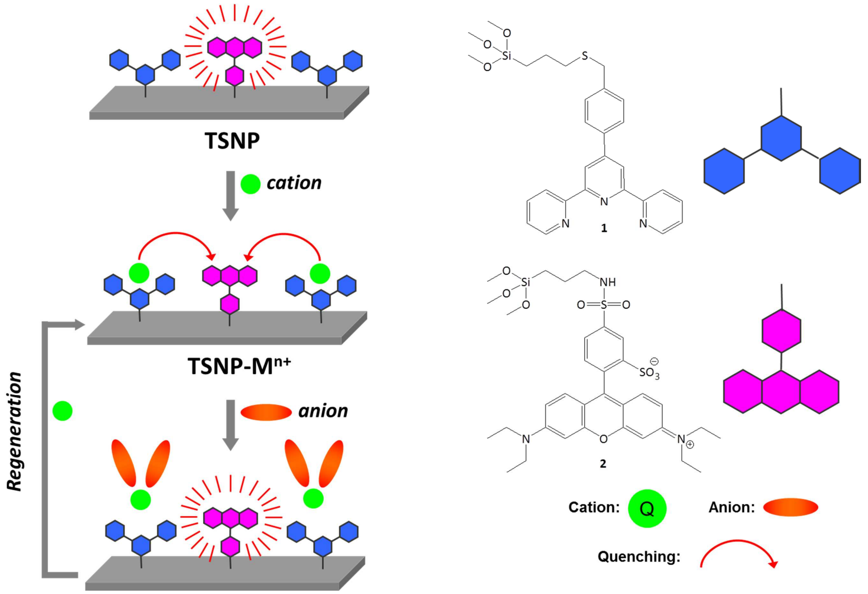
[10] J. V. Ros-Lis, M. D. Marcos, R. Martínez-Máñez, K. Rurack and J. Soto, *Angew. Chem., Int. Ed., 2005, 44, 4405*; J. V. Ros-Lis, R. Casasús, M. Comes, C. Coll, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós, J. El Haskouri, N. Garró and K. Rurack, *Chem. Eur. J., 2008, 14, 8267.*



Scheme 1** Design concept of the quencher displacement assay (QDA) involving terpyridine-sulforhodamine-functionalised nanoparticles (**TSNPs**) and metal ion quenchers **(Q).

The synthesis of the reactive precursors **1** and **2** (Scheme 1) and the functionalised nanoparticles is described in the ESI. The contents of terpyridine and rhodamine amounts to 30.84 and 3.9 mmol (mol/SiO_2) in the terpyridine-sulforhodamine functionalised nanoparticles (**TSNP**) and that of rhodamine to 3.9 mmol (mol/SiO_2) in the nanoparticles functionalised only with **2** (**SNPs**). Both, **TSNP** and **SNP** show a homogenous particle size of 20± 2 nm (Fig. 1). As a rough estimate, each coated **TSNP** carries up to 2100 attached molecules. The average distance between two subunits thus amounts to *ca*. 8 Å.

The first step toward a successful assay is evident from the observation that the emission of the sulphorhodamine fluorophore was retained after the grafting of the signalling units onto the surface of the silica nanoparticles. Excitation of acetonitrile suspensions of **TSNPs** at 575 nm gave rise to an emission band at 580–660 nm, similar to that obtained for sulphorhodamine B chloride in acetonitrile (Figure 1).

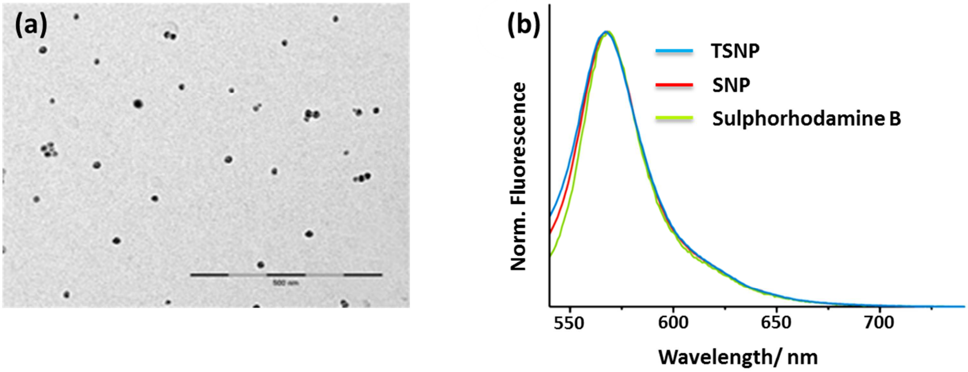


Figure 1. *a) TEM image of* ***TSNP****s, average diameter= 20 nm; b) fluorescence spectra of* ***TSNP*** *and* ***SNP*** *suspended and sulphorhodamine B dissolved in acetonitrile.*

Fluorescence lifetime measurements revealed that the decay times of the dye in the **TSNPs** (τ_f = 2.21 ns; and **SNPs** τ_f = 2.21 ns) is virtually identical to that of the isolated molecule in solution (τ_f = 2.24 ns), stressing the fact that the single dye molecules do not interact with each other when attached to the surface and that grafting has also no influence on the fluorophore's properties.

In the second step, various heavy and transition metal ions were tested as quenchers in CH_3CN. In a typical experiment, suspensions of **TSNP** (2.5 mg in 20 mL) were titrated with Fe^{3+}, Hg^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+} (as perchlorates). As would be expected for a rather non-selective ligand, terpyridine binds all these metal ions. Moreover, in all the cases, a severe quenching of 94–99 % of the rhodamine emission was found, suggesting that the average distance between R and F is sufficiently short to activate the transduction channel. Depending on the metal ion bound at the terpyridine unit, the process responsible for the quenching of the dye's fluorescence involves an energy or electron transfer or enhanced spin–orbit coupling because of the heavy atom effect.[11] At saturation quenching, the major lifetime components in the complexed hybrids amount to 20–45 ps for **TSNP**–Fe^{3+}, **TSNP**–Hg^{2+}, **TSNP**–Pb^{2+} and **TSNP**–Cu^{2+} and 0.11 ns for **TSNP**–Ni^{2+}. In contrast to metal ions bound directly at a fluorophore, for which paramagnetic ions such as Cu^{2+} usually show static quenching, i.e., a non-emissive complex, and heavy metal ions like Hg^{2+} a complex with a reduced lifetime, here, due to the separation of **R** and **F**, all metal ions lead to a reduction of the lifetime of **F**, stressing the fact that a distance-dependent process is active.

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[11] K. Rurack, *Spectrochim. Acta, Part A, 2001, 57, 2161.*

The critical role of the terpyridine receptor was demonstrated in parallel studies using the model **SNP**s that are only functionalised with **F** but do not contain **R**. The addition of metal ions to acetonitrile suspensions of **SNP**s induced negligible changes in the emission of the dye, indicating that the presence of the R units in **TSNP** is critical for the quenching mechanism. Only when coordinated to terpyridine are the metal ions in a spatial proximity with the fluorophore that is close enough to induce fluorescence quenching.

Having established the signalling mechanism the corresponding **TSNP–Q** hybrid materials (Q = Cu^{2+}, Fe^{3+}, Hg^{2+}, Ni^{2+} and Pb^{2+}) were tested as sensing systems for anions. The underlying idea of our approach relies on the ability of the anion to successfully compete with the terpyridine units for the metal ion and displace the latter form the **R** units, resulting in an enhancement of the fluorescence. This enhancement should be a direct consequence of a delicate balance between the binding strength of the metal ion with terpyridine and the affinity of the cation for the added anion.

To test the ability of the **TSNP–Q** ensembles for differential recognition of small monovalent anions, acetonitrile solutions of **TSNP–Q** chemosensors were prepared by adding 1 equivalent (with respect to the 2.0×10^{-4} mmol R units contained, see ESI for experimental details) of the corresponding metal cation to quench the rhodamine emission. Then, 0.1 to 10 equivalents of the anions $H_2PO_4^-$, HSO_4^-, F^-, Cl^-, Br^-, I^- and NO_3^- were added to the **TSNP–Q** suspensions. Except in one case (see below), no uniquely selective fluorescence changes were found. However, as a general trend the addition of $H_2PO_4^-$ and F^- induced a remarkable fluorescence enhancement, addition of Cl^-, Br^- and HSO_4^- induced a moderate increase of the emission whereas the addition of I^- and NO_3^- induced only poor fluorescence changes. In addition, despite this general observation a clear modulation of the relative response in the presence of anions was found, depending on the metal ion employed.[12] This behaviour is reminiscent of other probe–metal ion–anion ternary complex systems[13] and is a well-suited prerequisite for differential recognition.[14] Qualitative analysis of this differential response for anions using PCA (Principal Component Analysis) algorithms yielded the results summarized in Figure 2. As can be deduced from the figure, recognition patterns can be identified for all the anions studied even for those that display a rather poor change in the fluorescence of the fluorophore. Fluorescence lifetime studies of the ternary systems revealed that upon addition of anions, the lifetime features of the **TSNP–M**$^{n+}$ ensembles are gradually turned into the features of **TSNP**, i.e., the major 2.2 ns component of the

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[12] Higher valent anions naturally induce similar changes as verified with $[(CH_3)_4N]_2(SO_4)$. However, because of the significantly lower solubility of such salts in CH_3CN, the interference is negligible.

[13] B. García-Acosta, X. Albiach-Martí, E. García, L. Gil, R. Martínez-Máñez, K. Rurack, F. Sancenón and J. Soto, *Chem. Commun., 2004, 774*; B. García-Acosta, R. Martínez-Máñez, F. Sancenón, J. Soto, K. Rurack, M. Spieles, E. García-Breijo and L. Gil, *Inorg. Chem., 2007, 46, 3123.*

[14] A. P. Umali, E. V. Anslyn, *Curr. Opin. Chem. Biol., 2010, 14, 685.*

sulphorhodamine is recovered, pointing to an actual displacement of the metal ion from the terpyridine units.

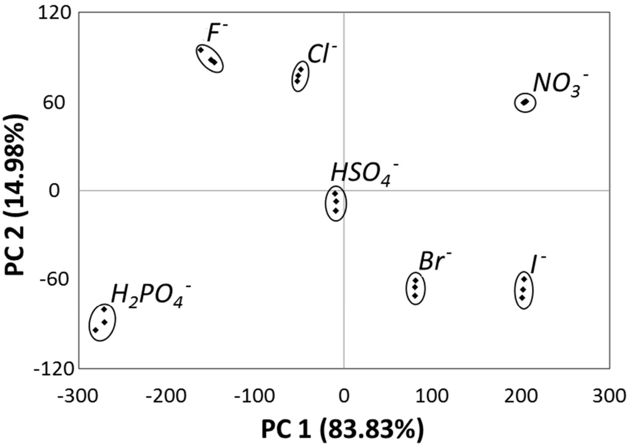

Figure 2. *Principal component analysis (PCA) score plot for the anions as indicated using* ***TSNP****–Q(Q = Fe^{3+}, Hg^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+}) ensembles. Data shown from three different trials and 1:5* ***TSNP****–Qto anion ratios. PC axes are calculated to lie along lines of diminishing levels of variance in the data set.*

Whereas the **TSNP–Q** systems, with Q = Cu^{2+}, Fe^{3+}, Hg^{2+} or Ni^{2+}, show a differential but no selective response in the presence of small inorganic anions, **TSNP–Pb^{2+}** behaves different, i.e., only the addition of $H_2PO_4^-$ revives the sulphorhodamine fluorescence whereas the other anions remain largely passive. (Figure 3). Using this procedure, concentrations as low as 5 ppm of $H_2PO_4^-$ can be detected.

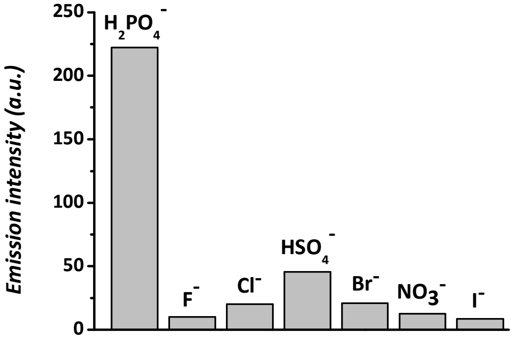

***Figure 3**. Emission intensity of acetonitrile suspensions of **TSNP**–Pb^{2+} in the presence of 5 equivalents (1.0×10^{-3} mmol) of the anions indicated.*

A final control experiment revealed that the addition of anions to acetonitrile suspensions of **TSNP**s (in the absence of **Q**) or **SNP**s did not result in changes of the dye´s fluorescence, pointing out the importance of the quenching metal ion as the mediator in the sensing paradigm. A further benefit of the system is obvious from Scheme 1. Once an analytical reaction has been performed and Q displaced, regeneration is easily accomplished by addition of an appropriate metal ion salt with a non-coordinating anion like perchlorate.

In summary, we have prepared a hybrid organic-inorganic sensing ensemble by grafting fluorophores and receptors onto the surface of silica nanoparticles and its use in anion recognition.

Coordination of metal ions at the terpyridine units induced the quenching of the neighbouring fluorophores. Upon addition of anions, coordination to the metal centres and a partial recovery of the fluorescence was observed. The final response of a certain **TSNP–Q** system to a particular anion is a delicate balance between the binding strength of the metal ion with the terpyridine and the affinity of the cation for the added anion. This allows differential recognition of small inorganic anions in a quencher displacement assay. Furthermore, regeneration of the system is possible in a straightforward manner. The facile and independent functionalisation of silica surface with various chemical entities and the possible use of a number of different coordination units and metal ions make this approach highly appealing for the search for new chemosensors for anions.

Financial support from the Spanish Government (project MAT2009-14564-C04-01), the Generalitat Valencia (project PROMETEO/2009/016) and the Innovationsfonds (BAM/Bundesministerium für Wirtschaft und Technologie) is gratefully acknowledged.

SUPPORTING INFORMATION

SILICA NANOPARTICLES FUNCTIONALISED WITH CATION COORDINATION SITES AND FLUOROPHORES FOR THE DIFFERENTIAL SENSING OF ANIONS IN A QUENCHER DISPLACEMENT ASSAY (QDA)

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[b] Instituto Tecnológico de la Construcción (AIDICO), Avenida Benjamín Franklin 17, 46980, Paterna (Valencia), Spain.

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4.1. EXPERIMENTAL PROCEDURES.

4.1.1 CHEMICALS

30 % suspension of LUDOX silica nanoparticles AS-30 Colloidal Silica were purchased from Sigma-Aldrich and the nanoparticles were used without any further purification. The solvents were absolute grade and were purchased from Scharlab. Anions stock solutions of fluoride, chloride, bromide, hydrogen sulfate, nitrate and dihydrogen phosphate solutions were prepared from tetrabutyl ammonium salts and, in the case of the representative divalent sulphate anion, from the bis(tetramethylammonium) salt, all obtained from Aldrich. Cations Fe^{3+}, Hg^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+} (as perchlorate salts) were also purchased from Aldrich. For the terpyridine derivative **1**, *p*-tolualdehide, 2-acetylpyridine, ammonium acetate, *N*-bromosuccinimide and (3-mercaptopropyl)triethoxysilane all again from Aldrich, were used. For compound **2**, sulphorhodamine B chloride (from Aldrich) and (3-aminopropyl)triethoxysilane were used.

4.1.2 GENERAL TECHNIQUES

TG analysis, transmission electron microscopy, elemental analysis, nuclear magnetic resonance, and fluorescence spectroscopy techniques were employed to characterize the materials. Thermo-gravimetric analysis were carried out on a TGA/SDTA 851e Mettler Toledo equipment, using an oxidant atmosphere (Air, 80 mL/min) with a heating program consisting on a heating ramp of 10 °C per minute from 393 K to 1273 K and an isothermal heating step at this temperature during 30 minutes. Transmission Electron Microscopy (TEM) images of the particles were obtained with a Philips CM10 operating at 20 KeV. Samples for TEM were prepared by spreading a drop of nanoparticles solution in H_2O onto standard carbon-coated copper grids (200 mesh). ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were acquired with Varian 300 spectrometer (Sunnyvale, CA, USA). Steady-state fluorescence measurements were carried out on a Felix 32 Analysis Version 1.2 (Build 56) PTI (Photon Technology International) and a FluoroMax-4 Spectrofluorometer from Horiba Jobin-Yvon. Fluorescence lifetimes were determined with a unique customized laser impulse fluorometer with picosecond time resolution described in detail in 15, 16. The fluorescence was collected at right angles (polarizer set at 54.7°; monochromator with spectral bandwidths of 8 nm) and the fluorescence decays were recorded with a modular single photon timing unit described in [16]. Typical instrumental response functions were of 25–30 ps full width at half maximum, the time division was 4.8 ps channel^{-1} and the experimental accuracy amounted to ±3 ps, respectively. The

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[15] U. Resch and K. Rurack, *Proc. SPIE-Int. Soc. Opt. Eng., 1997, 3105, 96.*

[16] Z. Shen, H. Röhr, K. Rurack, H. Uno, M. Spieles, B. Schulz, G. Reck, N. Ono, *Chem. Eur. J., 2004, 10, 4853.*

fluorescence lifetime profiles were analyzed with a PC using the software package Global Unlimited V2.2 (Laboratory for Fluorescence Dynamics, University of Illinois).

4.1.3 SYNTHESIS OF 1 AND 2

1: A mixture of bromomethylterpyridine[17] (0.6 g, 1.5 mmol), potassium carbonate (2.0 g, 14 mmol) and 3-(mercaptopropyl) trimethoxysilane (381.6 μL, 1.5 mmol) were dissolved in dichloromethane (75 mL), stirred and heated to reflux, under an Ar atmosphere, for 4 days. After cooling at room temperature, the resulting suspension was filtered, the organic solvent removed with a rotary evaporator and the crude obtained was purified by column chromatography (silica gel, dichloromethane-ethyl acetate 9:1 v/v as eluent). The final product (**1**) was obtained as a yellow oil (44 % yield).

^{1}H NMR ($CDCl_3$): δ (ppm) = 0.78 (t, 2H, $–CH_2–Si–$), 1.20 (t, 9H, $CH_3–CH_2–O–Si–$), 1.84 (t, 2H, $CH_2–CH_2–Si–$), 2.70 (t, 2H, $S–CH_2–CH_2–CH_2–Si–$), 3.81 (q, 6H, $CH_3–CH_2–O–$), 3.91 (s, 2H, $–S–CH_2–$tpy), 7.32-7.38 (m, 2H, tpy), 7.55-7.75 (d, 4H, Ar), 7.82-7.88 (m, 2H, tpy), 8.59-8.62 (m, 2H, tpy), 8.71-8.75 (m, 2H, tpy), 8.75 (s, 2H, tpy). ^{13}C NMR (DMSO-D6): δ (ppm) = 8.5, 22.5, 34.2, 35.7, 50.5, 118.7, 121.3, 127.4, 129.4, 136.8, 137.0,

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[17] W. Spahni and G. Calzaferri, *Helv. Chim. Acta, 1984, 67, 450.*

148.1, 149.1, 155.8, 156.6. m/z (relative intensity): 559 (5), 354 (20), 323,10 (40), 322,10 (30), 162,8 (25), 119 (20), 79,10 (100),67 (20).

2: The synthesis of rhodamine derivative **2** from 3 (aminopropyl)triethoxysilane and sulforhodamine B acid chloride was published else where.[18]

4.2. PREPARATION AND CHARACTERIZATION OF COATED SILICA NANOPARTICLES.

Coated silica nanoparticles **TSNP** were prepared using the corresponding trialcoxysilyl derivatives (**1** and **2**) following reported procedures by Montalti and coworkers.[19] LUDOX silica nanoparticles AS-30, 20 nm average diameter, (5 mL) were added to a solution containing acetic acid (20 mL), water (40 mL) and ethanol (40 mL). Then a mixture of **1** (1 mmol) and **2** (0.125 mmol) were added to the nanoparticle suspension. The crude reaction was heated at 80 °C for 48 hours, ethanol was evaporated and the acetic acid solution neutralized with a saturated solution of ammonium acetate. Solvent was removed and nanoparticles were washed with acetone. The functionalized nanoparticles (**TSNP**) were precipitated and isolated by filtration, washed with water and acetone and dried at 70 °C. This method would

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[18] M. Ganschow, M. Wark, D. Wöhrle and G. Schulz-Ekloff, *Angew. Chem. Int. Ed., 2000, 39, 161.*

[19] M. Montalti, L. Prodi, N. Zacheronni and G. Falini, *J. Am. Chem. Soc., 2002, 124, 13540.*

allow obtaining a uniform distribution of both functional groups on the silica surface.

Thermogravimetric analyses were carried out under a flow of air and with a heating rate of 10 °C/ minute in the 30-1000 °C interval. The final solid was maintained at 1000 °C for 30 minutes. In the thermograms of TSNP three clearly defined zones are observed; (i) from 30 °C to 150 °C which was assigned to loss of water and organic solvents (2.032 %), (ii) from 150 °C to 800 °C which was assigned to the organic matter attached onto the nanoparticle surface (19.001 %) and, finally (iii) from 800° to 1000° C a third step was assigned to condensation of silanol groups (0.330 %). The diameter of **TSNP** nanoparticles was determined to 20 ± 2 nm by TEM; the particle size was very homogenous.

Coated silica nanoparticles **SNP** were prepared using trialcoxysilyl derivative **2** following reported procedures by Montalti and coworkers[19]. LUDOX silica nanoparticles AS-30, 20 nm average diameter, (5 mL) were added to a solution containing acetic acid (20 mL), water (40 mL) and ethanol (40 mL). Then **2** (0.175 mmol) was added to the nanoparticle suspension. The crude reaction was heated at 80 °C for 48 hours, ethanol was evaporated and then the acetic acid solution neutralized with a saturated solution of ammonium acetate. Solvent was removed and nanoparticles were washed with acetone. The functionalized nanoparticles (**SNP**) were precipitated and isolated by filtration, washed with water and acetone and dried at 70 °C. This method would allow obtaining a uniform distribution of the functional groups on the silica surface.

Thermogravimetric analyses were carried out under a flow of air with a heating rate of 10 °C/ minute in the 30-1000 °C interval. The final solid was maintained at 1000 °C for 30 minutes. In the thermograms of **SNP** obtained three clearly defined zones were observed; (i) from 30 °C to 150 °C which was assigned to loss of water and organic solvents (1.7398 %), (ii) from 150 °C to 800 °C which was assigned to the organic matter attached onto the nanoparticle surface (7.5896 %) and, finally (iii) from 800° to 1000° C a third step was assigned to condensation of silanol groups (0.806 %). The diameter of **SNP** nanoparticles was determined to 20 ± 2 nm by TEM the particle size was very homogenous.

4.3. FLUORESCENCE STUDIES WITH TSNP IN THE PRESENCE OF METAL CATIONS AND ANIONS

TSNP nanoparticles (2.5 mg) were suspended in acetonitrile (20 mL) and then ultrasonicated during 5-8 minutes in order to obtain a homogeneous suspension. At the same time acetonitrile solutions of the metal cations (1.0×10^{-2} mol dm^{-3}) and anions (2.0×10^{-2} mol dm^{-3}) were prepared. The emission intensity of **TSNP** nanoparticles at 581 nm (excitation at 550 nm) was first measured in the presence of increasing quantities of metal cations (Fe^{3+}, Hg^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+}). A complete quenching was observed upon addition of 1 equivalent of metal cation (with respect to the content of **1**). Then, 20 μL (2.0 x 10^{-4} mmol) of the acetonitrile solutions of metal cations were added to 3 mL of **TSNP** suspensions (containing 1.9 10^{-4} mmol of **1**). To this suspension increasing quantities of the corresponding anions were added and the emission intensity at 581 nm measured (excitation at 550 nm).

CONCLUSION AND PERSPECTIVES

The development of hybrid sensory materials has recently attracted significant attention because the wise combination of inorganic scaffolds with organic functionalities lead to novel recognition protocols with enhanced features in terms of selectivity and sensibility. This PhD Thesis has intended to contribute to this field developing new hybrid materials based on silica nanoparticles, as inorganic scaffold, functionalized with anion binding sites and optical (chomo-fluorogenic) signaling units.

The first chapter of this PhD Thesis deals with description of the principles and concepts of supramolecular chemistry. Also, the synergic effects obtained upon the attachment of supramolecular entities onto solid supports were commented. Finally, the use of pre-organised nanoscopic solid structures in sensing protocols was also reviewed.

The second chapter of this PhD Thesis reports the preparation of silica nanoparticles functionalized with spirobenzopyrans (signaling unit) and thiourea (binding site) moieties and its use as chromogenic sensors for long chain carboxylates. The color modulations observed in the presence of long chain carboxylates is ascribed to the formation of a dense hydrophobic monolayer around the signaling unit that changes its conformation.

The third chapter reports the preparation and fluorogenic behavior on silica nanoparticles functionalized with anthracene fluorophores and thiourea anion binding sites. Addition of selected anions induced emission enhancements (Cl^-, Br^-, $H_2PO_4^-$, acetate, benzoate) or emission quenching (F^-, CN).

Finally, in the fourth chapter, silica nanoparticles functionalized with terpyridine binding sites and the sulforhodamine B fluorophore were prepared and used in a displacement assay for the sensing of anions. Coordination of metal cations with terpyridine binding sites induced a marked quenching of the sulforhodamine emission. However, upon addition of selected anions, a recovery of the emission was observed.

Principal component analysis allowed the discrimination of selected anions.