ACCOUNTS
of chemical research

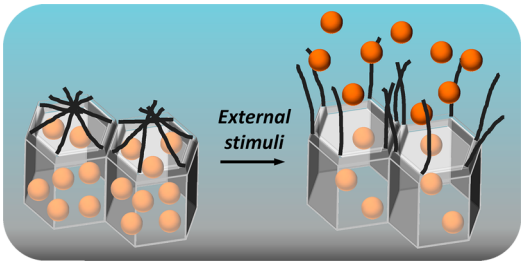
Gated Silica Mesoporous Supports for Controlled Release and Signaling Applications

CARMEN COLL,[†,‡,§] ANDREA BERNARDOS,[†,‡]
RAMÓN MARTÍNEZ-MÁñEZ,[*,†,‡,§] AND FÉLIX SANCENÓN[†,‡,§]

[†]*Centro de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Unidad Mixta Universidad Politécnica de Valencia - Universidad de Valencia, Spain,* [‡]*Departamento de Química, Universidad Politécnica de Valencia, Camino de Vera s/n. E-46022, Valencia, Spain, and* [§]*CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN)*

RECEIVED ON MAY 15, 2012

CONSPECTUS



Blending molecular and supramolecular advances with materials science has resulted in recent years in the development of new organic–inorganic hybrid materials displaying innovative functionalities. One appealing concept in this field is the development of gated nanodevices. These materials are prepared by grafting molecular or supramolecular caps onto the external surface of mesoporous inorganic scaffolds loaded with a particular cargo. The caps or "gates" can then be opened and the cargo delivered at will upon the application of a given stimulus.

In this Account, we report some of the recent advances we have made in designing such materials for drug delivery and as new chromo-fluorogenic probes. For controlled release applications, we have prepared capped hybrid mesoporous supports capable of being selectively opened by applying certain physical and chemical stimuli. We report examples of gated materials opened by changes in pH (using polyamines as caps), light (employing spiropyran derivatives or gold nanoparticles), and temperature (using selected paraffins). We also report gated materials opened by enzymes that cleave capping molecules based on lactose, hydrolyzed starch, and peptides. The use of enzymes is especially appealing because molecular caps built of enzyme-specific sequences made of peptides or other cleavable molecules could allow on-command delivery of drugs and biomolecules in specialized contexts.

In the second part of the manuscript, we revisit the possibility of using hybrid gated nanomaterials as sensory systems. In such systems, when target analytes interact with the cap, their presence triggers the transport of a dye from pores to the solution, resulting in a chromo-fluorogenic signal that allows their detection. Two approaches are possible. In the first one, pores remain open and the dye can diffuse into the solution, until the presence of a target analyte binds to receptors in the caps and closes the gate. In the second approach, the caps are closed and the presence of a target analyte induces pore opening and dye delivery. One of the most interesting properties of these sensory hybrid materials is their inherent amplification features, because few target analyte molecules can modulate the transport of a significant amount of dye molecules within the porous network. We describe such systems for the recognition and sensing of anionic (ATP, long-chain carboxylates, anionic surfactants, borate, and oligonucleotides), cationic (methylmercury), and neutral (nerve agent simulants and sulfathiazole) species.

Introduction

In the last ten years, anchoring organic molecules or supermolecules onto certain inorganic scaffoldings has led to the development of hybrid materials, which has resulted in cooperative functional supramolecular behaviors that are not found in unanchored molecules or in the unfunctionalized solids alone. One appealing concept in this novel field relates to the development of gated nanodevices for

Published on the Web 12/06/2012 www.pubs.acs.org/accounts
10.1021/ar3001469

delivery applications. These functional materials contain "molecular gates", which are the switchable entities controlling either the on-command release of confined guests or the on-command entrance of molecular species to certain sites (see Figure 1).[1,2] These gated materials usually contain two components: (i) a switchable "gate-like" ensemble capable of being "opened" or "closed" upon the application of certain external stimuli; (ii) a suitable inorganic support acting as a nanocontainer (for loading the carrier), to which gate-like molecules can be easily grafted.

Both components are important, and their selection determines the controlled release performances of the hybrid support. Mesoporous silicas of different pore sizes and morphologies are selected and used as inorganic scaffolds in gated ensembles.[3] Mesoporous supports can be prepared in different forms (from micrometric to nanometric) with tailor-made pores of around 2−10 nm. They show a very large specific surface area (up to 1200 m^2/g) and have, therefore, a large load capacity, homogeneous porosity, and high inertness and are easy to functionalize. In relation to the gated ensemble, several molecular and supramolecular systems have been developed that are able to trigger at will the delivery of the entrapped cargo using several external stimuli such as light, pH, changes in redox potential, temperature, and the presence of certain ions, molecules, or biomolecules.[4−10] The design of gated mesoporous materials proves to be a promising starting point for applying the versatility of supramolecular ideas to the design of nanoscopic gating solids, and a way of studying the factors that can influence the design of molecular gating functions based on molecular, biomolecular, or supramolecular concepts.

This Account shows our recent results in the design and preparation of capped materials and their use in on-command controlled release applications, including delivery of drugs in cells. In addition, it reviews our pioneering work on the application of gated materials in sensing protocols.

Nanoscopic Molecular Gates for Controlled Release Applications

The inclusion of ionizable organic moieties anchored onto the pore outlets of mesoporous supports, which might change in size or shape upon protonation or deprotonation processes, is a usual procedure followed to prepare materials capable of controlling mass transport by pH modulations. One of the first examples of an ionically controlled gated support described in the literature which, at the same time, is the first reported capped example working in an aqueous environment, is based on an MCM-41 mesoporous support containing linear polyamines grafted onto the outer surface

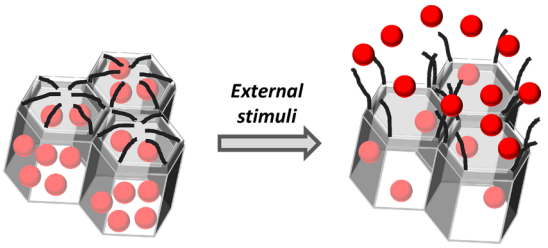


FIGURE 1. Scheme of a nanoscopic "molecular gate".

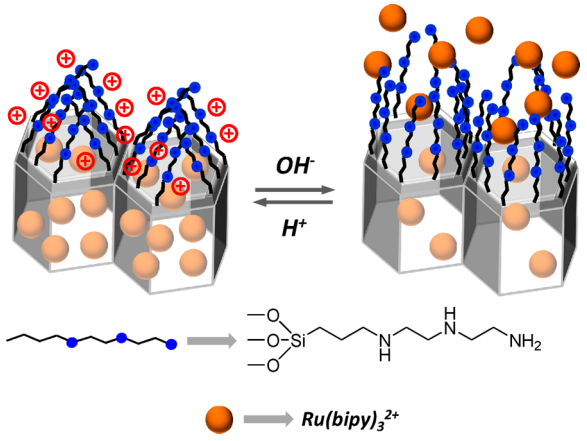


FIGURE 2. Schematic representation of S2, an ionically controlled nanoscopic molecular gate.

and mercaptopropyl chains anchored onto inner pores (solid S1).[11] Whereas most of the reported gated materials are used for delivery applications, S1 was designed to demonstrate the control of the entrance of an external molecule into pores, which is, squaraine dye. The open−close protocol in S1 is studied by means of the well-known reaction between the squaraine dye (blue) and the thiol groups located inside the pores that yield a colorless derivative. At a neutral pH, addition of squaraine to the water suspensions of S1 induces a complete bleaching of the solution due to the free access of the dye to pores, whereas at an acidic pH, the nitrogen atoms of the polyamine are protonated and adopt a rigid-like conformation (due to the Colulombic repulsions between the ammonium groups), resulting in a partial pore blockage and the inhibition of squaraine access to the thiol moieties (the suspension remains blue). Additionally, a synergic anion-controlled outcome results from the interaction between protonated amines and certain anions. For instance, the presence of anions such as ATP (that displays a strong coordination with the amine/ammonium groups) also helped to inhibit squaraine access to pores.

In a further evolution, solid S2 consisting of a mesoporous material loaded with the $[Ru(bipy)_3]^{2+}$ dye and functionalized

with linear polyamines on the external surface was prepared (see Figure 2).[12] As noted with solid S1, a pH-driven open/close mechanism is also observed. At a neutral pH, polyamines are mostly unprotonated, allowing the release of the entrapped dye. However at an acidic pH, nitrogen atoms are protonated and result in a partial closing of pores with a subsequent dye release inhibition.

In addition to the pH-driven protocol, the opening/closing of the gate-like ensemble in S2 is also modulated by an anion-controlled mechanism. The influence of different anions in the delivery process at a certain pH has been studied by using a range of anions with different structural dimensions and charges, including chloride, sulfate, phosphate, and ATP. Different behaviors are observed, which basically range from no action (chloride) to a complete (ATP) or partial (sulfate and phosphate) pore blockage depending on the pH. The remarkable anion-controllable response of the gate-like ensemble can be explained in terms of anion complex formation with the tethered polyamines. The molecular dynamics calculations are also in agreement with the proposed mechanism.

In order to test the potential applicability of gated pH-controlled mesoporous materials as drug delivery systems, a new solid loaded with vitamin B_2 and capped with polyamines is prepared (S3).[13] Suspensions of S3 at pH 2.0 in the presence of certain anions show no vitamin B_2 release, whereas complete delivery is noted at pH 7.0. This solid has been suggested to be a suitable prototype for the development of orally applicable delivery systems designed to have the particular ability to protect the cargo from acidic stomach conditions (acidic pH, gate closed) but can release them in the intestine (basic pH, gate open).

Light is another popular external trigger used for governing mass transport in certain capped hybrid materials. By using light-driven gated systems, one can particularly control cargo release spatially and temporally by finely tuning the area and time of the light stimulus. In this area, the gated material S4 is prepared, which is able to release an entrapped cargo in water by application of two distinct external stimuli, irradiation with visible light and pH changes. S4 consists of a mesoporous support loaded with $[Ru(bipy)_3]^{2+}$ and is functionalized on the external surface with a photoswitchable spiropyran derivative.[14] As an additional component, 1.5 poly(amidoamine) (G1.5 PAMAM) dendrimers are used as molecular caps. Aqueous (pH 7.2) suspensions of S4 irradiated with UV light in the presence of the G1.5 PAMAM dendrimers show a negligible dye release due to the photoisomerization of the neutral spiropyran isomer to

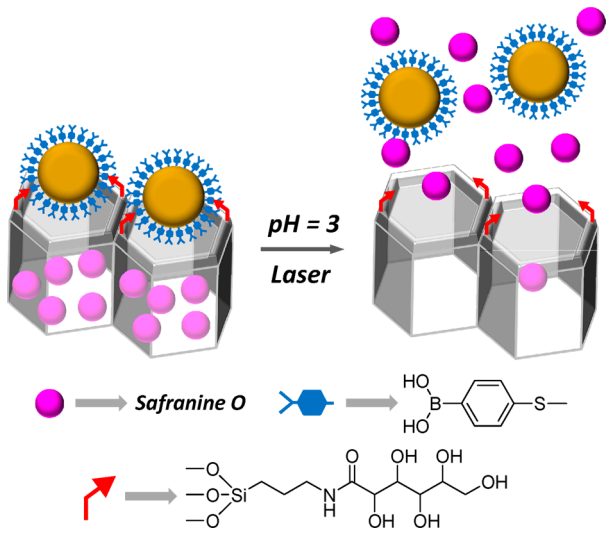


FIGURE 3. Scheme of the photo- and pH-switched S5 material.

the positively charged merocyanine, and the subsequent formation of a supramolecular ensemble through electrostatic interactions with the negatively charged dendrimers. Through irradiation with visible light, the positively charged merocyanine isomerizes to the neutral spirocyclic form, which has no affinity for the dendrimers, with the subsequent cargo release. In addition, delivery of the dye can also be achieved by simply changing the pH; at an acidic pH, the G1.5 PAMAM dendrimers do not act as stoppers because the protonation of their carboxylic groups prevents an interaction with the merocyanine isomer.

In another work, the photo- and pH-switched hybrid material S5 is prepared. In this case, the mesoporous support is loaded with safranin O dye and functionalized on the external surface with a gluconamide derivative. Gold nanoparticles (AuNPs) of a suitable size, functionalized with 4-mercaptophenylboronic acid, act as nanoscopic caps due to the formation of boroester bonds with the anchored saccharide (see Figure 3).[15] At pH 5.0 or at a higher pH, no dye delivery is detected due to an effective pore blockage with the AuNPs, whereas a release at pH 3.0 is observed due to the hydrolysis of the boroester bonds at an acidic pH, which detaches the AuNPs from the surface. According to the reversibility of the boroester formation, we demonstrate that cargo delivery in small portions can be controlled at will through pH-changing cycles. This hybrid material is also able to release the entrapped cargo by plasmonic heating upon laser irradiation thanks to a thermal cleavage of the boroester bonds. It has also been demonstrated that a partial dye release from S5 in a pulsating mode can be obtained by controlling the laser irradiation time.

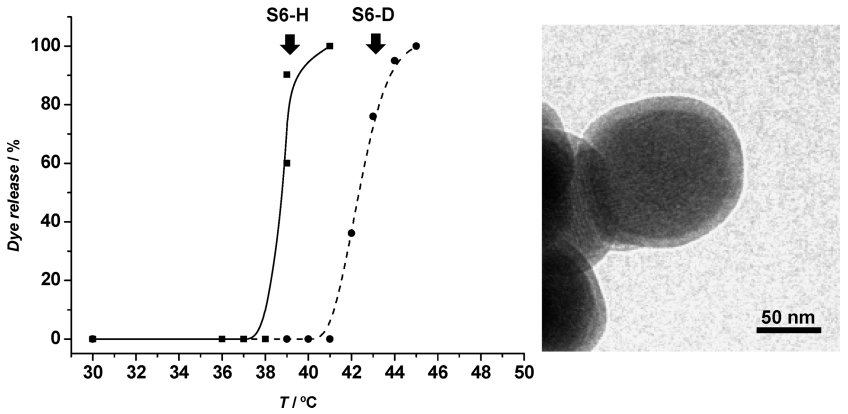


FIGURE 4. (left) Release profiles of solids S6-H and S6-D at different temperatures (release data were normalized to the maximum delivery obtained for each solid). (right) TEM image of S6-D showing the typical porosity of the MCM-41 mesoporous matrix and paraffin coating.

Another physical stimulus that will be the object of interesting applications in the field of controlled release is temperature. In this area, we recently focused on designing tailor-made temperature-responsive gated mesoporous materials based on using paraffins as capping agents. Nanoparticles consist of a mesoporous support loaded with a dye (safranin O) that is then functionalized with octadecyltrimethoxysilane to give solid S6. This solid still allows the cargo delivery, whereas the final capped material (S6-D) is prepared by coating S6 with docosane (melting point of 42 °C) through van der Waals interactions between the paraffin and the octadecyl chain covalently linked to the inorganic support.[16] The water suspensions of S6-D at 40 °C (below the paraffin melting point) show negligible dye release, while the same suspensions heated at 44 °C display a clear cargo release due to the melting of the paraffin coating (see Figure 4). It has been additionally demonstrated that the release temperature can be finely tuned by the selection of an appropriate paraffin. To investigate the biological application of these coated materials as drug nanocarriers for an intracellular temperature-controlled release, the S6-H solid capped with heneicosane (melting point of 39 °C) and loaded with the chemotherapeutic agent doxorubicin is prepared. HeLa human cancer cells are incubated in duplicate in the presence of solid S6-H at 37 and 42 °C. The sample incubated at 42 °C exhibits features of doxorubicin-induced cell death, whereas that incubated at 37 °C shows healthy cells.

As seen in the examples cited above, several physical and chemical stimuli have been used to trigger the delivery of an entrapped guest in mesoporous gated materials. As a further step, silica mesoporous materials designed to trigger cargo release (e.g., a drug) in the presence of certain biomolecules have been reported as systems displaying good potential applicability for on-command delivery in cells, tissues, and model animals.[17] In particular, the use of enzymes is especially appealing if we take into account the possibility of synthesizing tailor-made enzyme-specific sequences as molecular caps. One of the first examples of gated mesoporous materials capable of delivering an entrapped cargo in the presence of target enzymes is S7. This hybrid material consists of a mesoporous support loaded with the $[Ru(bipy)_3]^{2+}$ dye and capped with a covalently anchored lactose derivative.[18] Dye delivery from aqueous suspensions of S7 is negligible because of the formation of a dense disaccharide network in which the lactose groups are most likely linked through hydrogen-bonding interactions around pore outlets. Addition of β-D-galactosidase enzyme induces progressive cargo release, which is clearly related to the enzymatic hydrolysis of the glycosidic bond in the disaccharide lactose.

In a further evolution of S7, in which commercially available hydrolyzed starch (Glucidex 47, 39, and 29) is used as molecular cap, new solids are prepared. In order to prepare final material S8-*n* (where *n* indicates the number of the Glucidex derivative used as a gate), the pores of the nanoparticle mesoporous supports are loaded with $[Ru(bipy)_3]^{2+}$ and capped with the trialkoxysilane derivative of the corresponding hydrolyzed starch.[19] Solids S8-*n* release less than 2% of the entrapped dye after 5 h in water, whereas in the presence of pancreatin, S8-47, S8-39, and S8-29 release 63%, 48%, and 31% of the cargo, respectively. It is clear that a simple choice of the hydrolysis degree of starch has a dramatic influence on the delivery profile, that is, the lesser the hydrolysis of starch, the lower the delivery rate. Moreover, solid S8-47 has been selected to study the controlled release of the dye in the intracellular media. It has been demonstrated that the "saccharide"-functionalized nanoparticles are efficiently taken up by both tumoral (HeLa) and

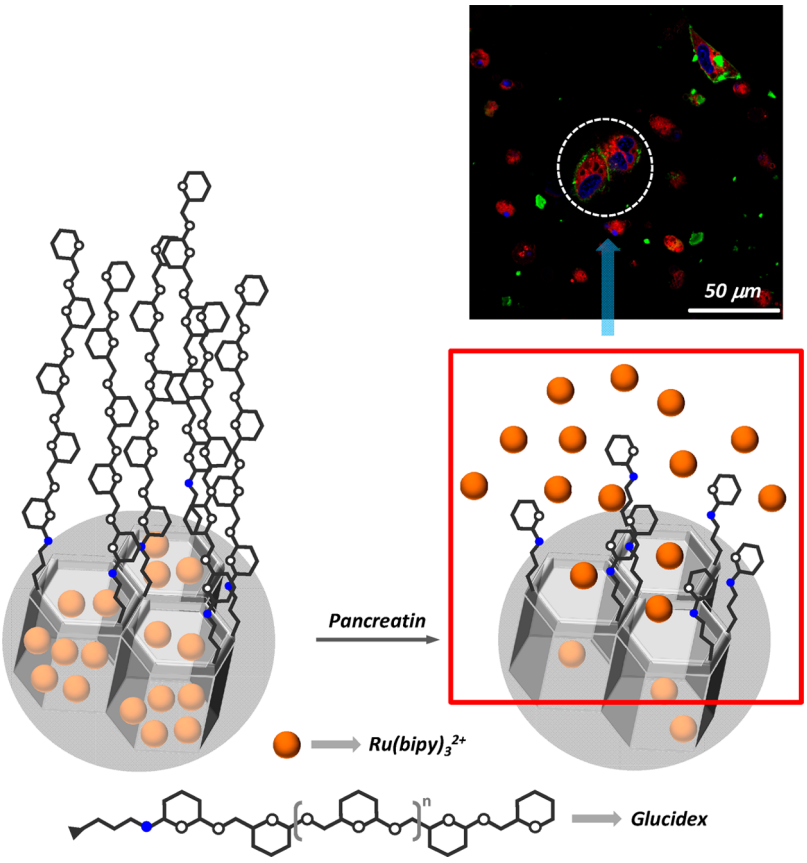


FIGURE 5. Schematic representation of solid S8-*n* capped with hydrolyzed starch and a confocal microscopy photograph of HeLa cells treated with S8-47 loaded with doxorubicin. As we can observe, cells show a red diffuse pattern of doxorubicin-associated fluorescence.

nontumoral (LLC-PK1) cells and that they are devoid of nonspecific cell toxicity. The cellular uptake of nanoparticles has been demonstrated to occur via endocytosis, targeting them to autolysosomes, where the capping polysaccharides are hydrolyzed by lysosomal enzymes and result in a concomitant cargo delivery. In addition, the possible application of "saccharide"-functionalized nanoparticles as delivery systems of chemotherapeutic agents in cells has been demonstrated, and a substantial reduction of cell viability has been found in HeLa cells treated with S8-47 loaded with doxorubicin. As far as we are aware, this was the first example of enzyme-induced delivery in cells using capped silica mesoporous nanoparticles (See Figure 5).

In another proof-of-concept work, we have verified that it is possible to anchor complex peptide sequences onto silica supports with a view to developing nanodevices showing zero release that are specifically opened in the presence of targeted proteolytic enzymes. Specifically, the modular peptide H-GGDEVDGGDEVDGGDEVD-OH is employed as a molecular gate. It has been designed to act as a substrate of the proteolytic enzymes obtained from *Streptomyces griseus*, which are able to perform a cleavage at the C terminus of the amide bond from the negatively charged aspartic (D) and glutamic (E) amino acids.[20] In line with this, the peptide, which is attached to azide-functionalized mesoporous nanoparticles (S9), is able to hamper the release of the $[Ru(bipy)_3]^{2+}$ dye, whereas delivery is triggered in the presence of a protease. This work not only demonstrates the efficiency of the "click" chemistry reaction in anchoring the peptide sequence to the mesoporous support but suggests that an enzyme-responsive capped materials design is a promising area of biomedical relevance for the design of custom-made nanodevices for advanced drug delivery and regenerative medicine applications.

Another step in this field should contemplate that the flow of the defined biological process relies on biochemical networks in which multiple enzyme-dependent stages are involved. Based on this concept, we have attempted to demonstrate the possible design of dual or multiple enzyme-responsive delivery systems by using gated mesoporous supports via the preparation of capping molecules containing different enzyme hydrolyzable groups to provide specific-release nanodevices showing different delivery profiles controlled by defined enzyme combinations. With this

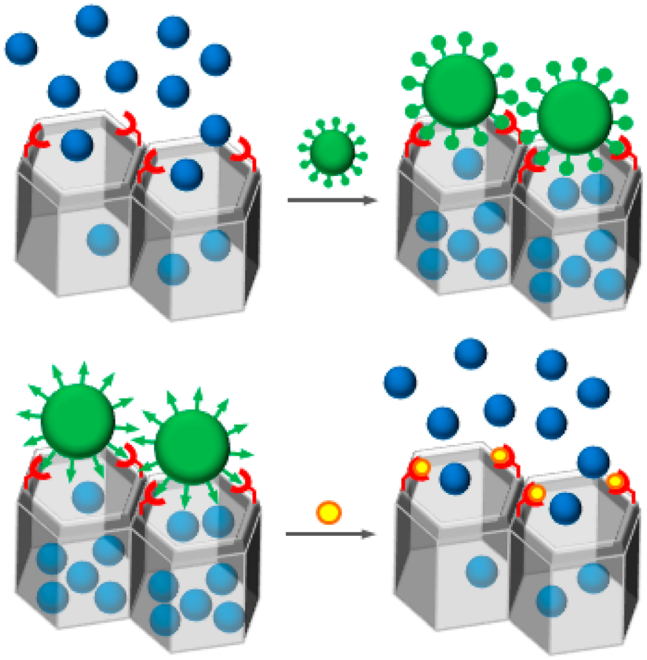


FIGURE 6. Scheme of the recognition paradigm using nanoscopic gate-like scaffoldings: (top) inhibition of dye release due to the coordination of the selected analyte with the anchored binding sites; (down) uncapping pores by an analyte-induced displacement reaction.

idea in mind, we have designed the S10 nanoparticles, which are capped with a bulky molecule containing the amide and urea hydrolyzable groups located at a predefined position.[21] A different remarkable delivery profile of the entrapped $[Ru(bipy)_3]^{2+}$ dye is observed according to the enzyme used. Amidase induces the hydrolysis of the amide bonds located far away from the inorganic support, thus allowing not only an immediate but not complete dye release, whereas urease hydrolyzed the urea bond, located more deeply inside the capping molecule and closer to the silica nanoparticle surface, allowing a near total cargo release but delayed in time. Simultaneous treatment with both enzymes displayed a synergistic effect, and a delivery profile showing a fast, complete payload release is observed. In order to test the possible applicability of this dual-enzyme triggered material, a new solid functionalized with the same molecular gate but loaded with the chemotherapeutic agent camptothecin has been prepared. This gated material is internalized by HeLa cells and a significant reduction in cell viability is observed after 24 h.

Recognition and Signaling Using Nanoscopic Gate-like Scaffoldings

The above-mentioned examples show how silica mesoporous materials bearing gate-like ensembles can be used for on-command delivery applications in the presence of different physical or (bio)chemical stimuli. In the particular case of chemical stimuli, the reported examples typically utilize highly nonspecific chemical species; for instance, protons (changes in pH) and reducing agents (to open redox-active capped supports). Moreover, we envision the possibility of designing gated materials capable of responding specifically to a certain target molecule as a suitable method for developing new protocols for sensing applications. The underlying idea here is that the coordination or reaction of the target analyte with the binding sites could modulate the transport of the dye from pores to the solution and would result in a chromo-fluorogenic signal. This idea translated to the world of analytical chemistry offers two possible situations (see Figure 6). In one, pores remain open and the indicator can diffuse into the solution, whereas in the presence of a target analyte this molecule or ion can bind to receptors and close the gate. In a second approach, the starting material is capped and the presence of a target analyte induces pore opening and dye delivery. One of the advantages of both approaches is the potential existence of amplification features. In particular, the presence of a few analyte molecules may induce the inhibition or the release of a relatively high amount of entrapped dye. However, of these approaches, the second is perhaps the most valuable from an analytical point of view, because the generation of a signal (release of a dye in the presence of the target species, which induced color or emission appearance, that is, off−on behavior) is easier to measure than an inhibition of dye delivery (i.e., on−off behavior). Given the possible use of different porous supports, diverse guest-selective gate-like systems, and a wide range of indicator dyes, this strategy, which has still to be fully studied, displays enormous potential for the development of novel signaling systems.

We herein report examples of both analyte-induced pore blockage and pore-opening protocols. As a first proof-of-concept of the former, we used the previously described solid S2. This consists in an MCM-41 mesoporous material loaded with the $[Ru(bpy)_3]^{2+}$ indicator and polyamine moieties, which act as receptors.[22] At pH 7.8, the water suspensions of S2 show an intense yellow color because polyamines are unprotonated, the molecular gate is open, and the ruthenium complex is released into the solution. However, the presence of ATP and ADP allowed selective inhibition the indicator release by the formation of strong complexes with tethered polyamines through hydrogen bonding and electrostatic interactions. Other anions, such as chloride, sulfate, or GMP, are too small or form complexes that are too weak to effectively

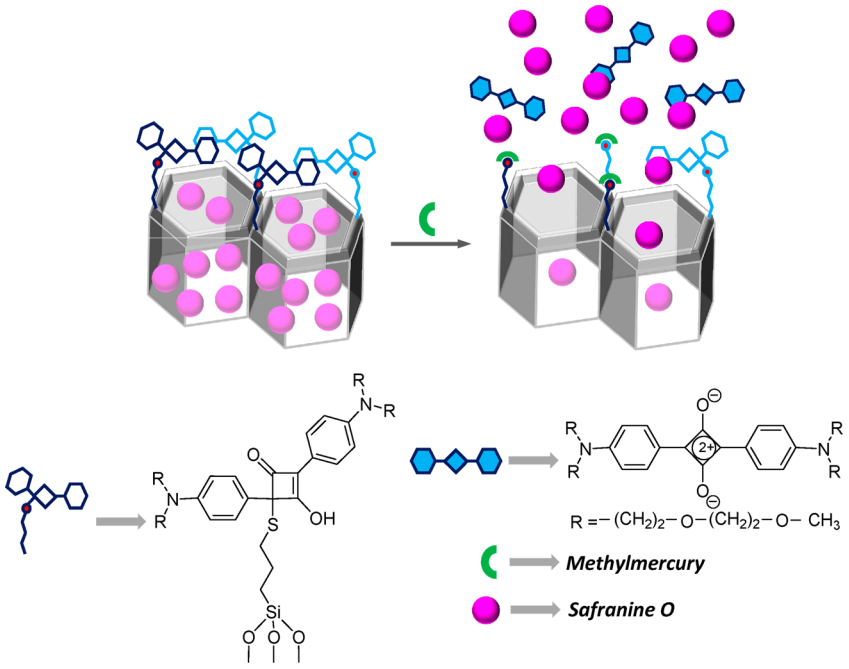


FIGURE 7. Chromo-fluorogenic detection of methylmercury using solid S15.

close pores, and they cannot stop the $[Ru(bpy)_3]^{2+}$ dye from leaching.

Gated nanodevices for sensing applications display interesting features that frequently are barely achievable by classic molecular-based receptors. For instance, the discrimination of certain selected members in a similar family of compounds was achieved by using mesoporous supports loaded with $[Ru(bipy)_3]^{2+}$ and functionalized on the external surface with imidazolium (S11), thiourea (S12), or urea (S13) binding sites. The controlled release of the entrapped guest from these gated materials has been tested in the presence of selected linear carboxylates in aqueous media at a neutral pH.[23] Short carboxylates (acetate, butanoate, hexanoate, and octanoate), which are added to the aqueous suspensions of the three sensing materials, are unable to induce pore blockage, whereas addition of larger carboxylates (decanoate and dodecanoate) inhibits dye release due to the formation of a dense hydrophobic monolayer around pore outlets. Despite this similar behavior, solids S12 and S13 show a gradual inhibition of the complex release as carboxylate length increases, while a rather "on/off" behavior is noted for S11.[24] Differences in sensing performance should be ascribed to the different nature of the binding sites. Moreover, it has also been demonstrated that S11 is able to selectively detect anionic surfactants in an aqueous environment if a similar protocol is followed.

Following this pore-blockage paradigm, detection of the small anion borate is achieved by using material S14, which consists of a mesoporous support loaded with $[Ru(bipy)_3]^{2+}$ and functionalized on the external surface with saccharide derivative *N*-(3-triethoxysilylpropyl)gluconamide (similar to solid S5 but with a different dye loaded in the pore network).[25] Dye release is observed for aqueous suspensions of S14 at a neutral pH, while an inhibited dye delivery is noted in the presence of borate due to the formation of boroester bonds with the hydroxyl moieties of the grafted saccharide. The chromo-fluorogenic system is selective for borate detection, and the other tested anions (i.e., carbonate, chloride, bromide, nitrate, sulfate, and phosphate) are unable to induce pore blockage. Following this approach, a detection limit as low as 70 ppb of boron can be achieved. The experimental borate control of mass transport has also been studied by means of molecular dynamic calculations using force field methods.

The above-described examples are all based on analyte-induced pore blockage, whereas the examples below follow a pore-opening protocol. The first example has been designed for the selective detection of methylmercury. The sensing gated material S15 consists in a mesoporous support loaded with safranin O dye and capped with 2,4-bis-(4-dialkylaminophenyl)-3-hydroxy-4-alkylsulfanylcyclobut-2-one (APC) groups (formed by the reaction of a squaraine dye and surface grafted thiols; see Figure 7).[26] Studies were carried out in acetonitrile–toluene 4:1 v/v mixtures in order to achieve discrimination between CH_3Hg^+ and Hg^{2+} due to the low solubility of the latter in this medium.The acetonitrile–toluene

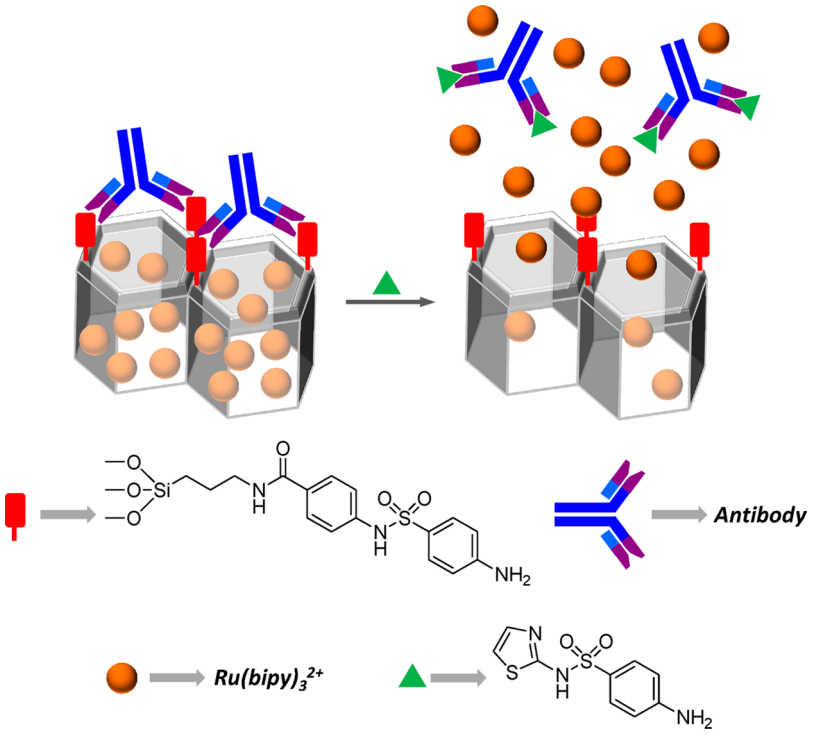


FIGURE 8. Schematic representation of gated material S17 capped with antibody and chemical structures of the anchored hapten and of sulfathiazole.

4:1 v/v suspensions of solid S15 remain capped given the presence of the bulky APC groups. However, addition of methylmercury induces the uncapping of pores with the subsequent release of entrapped safranin O. This uncapping process derives from the reaction of methylmercury with the thiol group on APC moieties, resulting in the coordination of the cation to thiols and in the release of the squaraine chromophore. An inherent feature of such capped systems is the potential achievement of signal amplification. In this particular system, for instance, the reaction of one equivalent of methylmercury with the APC groups leads to the delivery of one squaraine and of around 200 safranin O molecules. By employment of this procedure, a method has been successfully developed to determine methylmercury in fish samples by using a simple extraction procedure with toluene that achieved discrimination of CH_3Hg^+ from the Hg^{2+} by lipophilicity partitioning.

The selective chromo-fluorogenic sensing of nerve agent simulants has also been achieved by using capped mesoporous supports. Nerve agents (i.e., sarin, soman and tabun) are highly toxic organophosphorous compounds that can have severe effects on human health and have been used in terrorist attacks. Given the high toxicity of these chemicals, simulants diethyl chlorophosphate (DCP), diisopropyl fluorophosphate (DFP), and diethyl cyanophosphate (DCNP), which present similar reactivity but are much less toxic, are usually employed to design sensing systems. In this case, the mesoporous support is loaded with $[Ru(bipy)_3]^{2+}$ and capped with bis(2-hydroxyethyl)aminopropyltriethoxysilane (HET) groups (S16).[27] These HET moieties form a thick hydrogen-bonding network around pore outlets in aprotic solvents (such as acetonitrile), which inhibit dye delivery. Addition of nerve agent simulants induces the selective release of the indicator. This release is ascribed to the rupture of the dense hydrogen-bonding network as a result of the nucleophilic attack of the hydroxyl groups on the electrophilic phosphorus atom present in the simulants. S16 has also been used for the detection of nerve agent mimics in the gas phase with good results.

To conclude, we provide two more examples following the analyte-induced pore-opening protocol, which uses biomolecules as capping systems. The first is based on the functionalization of the mesoporous support with a certain hapten at the pore outlets and an antibody that acts as a nanoscopic cap. In our particular case, the mesoporous support is loaded with $[Ru(bipy)_3]^{2+}$ and derivative 4-(4-amino-benzenesulfonylamino)-benzoic acid is anchored onto the outer surface; finally, pores are capped with a polyclonal antibody for sulfathiazole (S17; see Figure 8).[28] Delivery of the entrapped ruthenium complex from S17 in the presence of a family of sulfonamides has been studied in

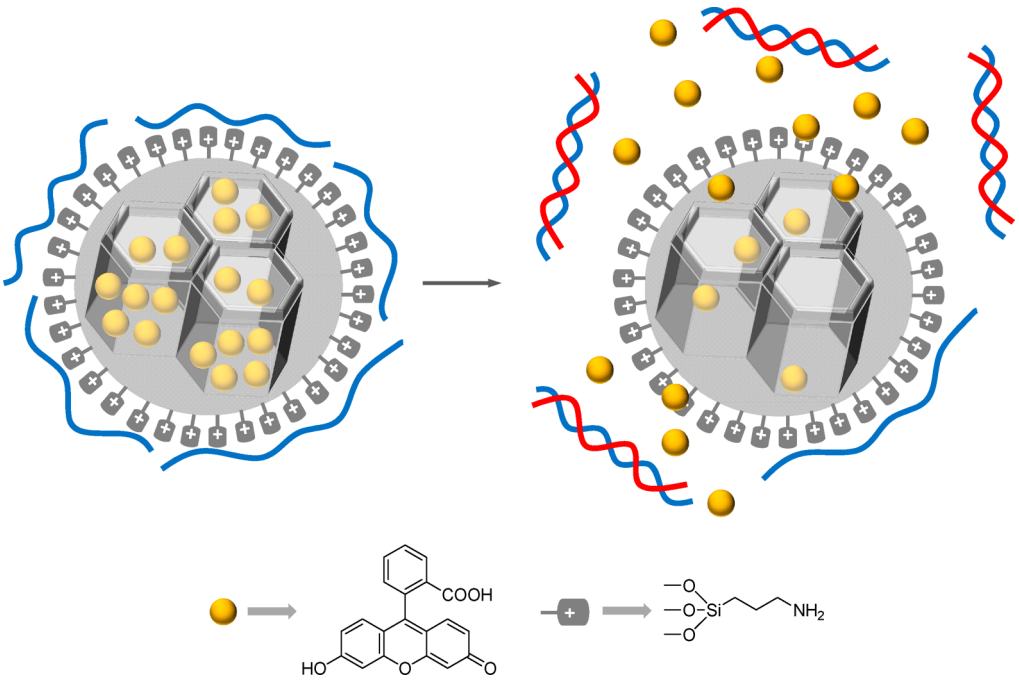


FIGURE 9. Representation of gated material S18 functionalized with 3-aminopropyltriethoxysilane and capped with a single-stranded oligonucleotide. Delivery of fluorescein is selectively accomplished in the presence of the complementary oligonucleotide.

phosphate-buffered saline (PBS 1×, pH 7.5) solutions. A remarkable and highly selective uncapping of pores and the subsequent dye delivery is seen for sulfathiazole. Concentrations as low as 100 ppb of sulfathiazole are able to start uncapping mesopores. Moreover a cargo delivery proportional to the sulfathiazole concentration is observed in agreement with uncapping due to an antibody displacement.

The second example using a biomolecule as a cap has been designed to detect oligonucleotides. The sensing material is prepared with mesoporous nanoparticles loaded with fluorescein, functionalized on the outer surface with (3-aminopropyl)triethoxysilane, and capped with oligonucleotide sequence 5′-AATGCTAGCTAATCAATCGGG-3′, which remains attached to the nanoparticle surface via electrostatic interactions with the partially protonated amines (S18) (see Figure 9).[29] The aqueous suspensions of the S18 nanoparticles (pH 7.5) have been demonstrated to be selectively open in the presence of the complementary single strand due to the hybridization of both single oligonucleotide sequences with the concomitant cargo release.

Conclusions and Outlook

This Account reports our recent research into the design of gated mesoporous siliceous materials for cargo-controlled release using different stimuli. Applications for these capped materials can be found in the design of novel, creative ways to deliver drugs. In fact, mesoporous supports display characteristics that are not usually found in classic drug delivery systems (such as polymers, dendrimers, micelles, etc.) owing to their unique properties. Moreover, we and others have shown how these supports can be functionalized with molecular or supramolecular ensembles on their external surface in order to develop gated materials showing "zero delivery" that are capable of an on-command release of their cargo in response to external stimuli. Capped systems displaying delivery upon pH modulations, light, temperature and selected enzymes have been shown. Despite some recent advances made in this field, the design of smart mesoporous delivery systems for their application in nanomedicine is still in its very early stages. Future work in the field would most likely be directed to the preparation of modular multifunctional nanoparticles functionalized with molecular gates (for the controlled release of entrapped guests), bearing targeting (bio)molecules (able to direct the nanodevices to the desired cells) and incorporating contrast agents (in order to study the traceability of the nanoparticles in the cells and in animals). Moreover additional studies regarding the toxicity and the pharmacokinetics of these novel nanodevices and the design of target nanoparticles for more realistic applications will also be important. In addition, we also demonstrate that capped mesoporous supports can be used to design conceptually new protocols for sensing applications, and examples of the detection of ATP, fatty acids, anionic surfactants, borate, methylmercury,

nerve gas simulants, sulfathiazole, and oligonucleotides have been described. It is noteworthy that this paradigm relies on a different approach and that it displays new possibilities of modulation and design that cannot be considered in classic receptors. For instance, selection of pore size in the mesoporous support, the nature of appended binding or reactive sites, and the loaded signaling dye are easily modified here. Moreover, the intrinsic amplification features of these sensory materials (few molecules of target analyte may modulate the release/inhibition of high amounts of the entrapped dye) will find some new analytical applications. These findings open up new perspectives relating to the applicability of mesoporous hybrid solids with gating functionalities.

We thank the Spanish Government (Projects MAT 2009-14564-C04 and MAT 2012-38429-C04-01) and the Regional Valencian Government (Project PROMETEO/2009/016) for support.

BIOGRAPHICAL INFORMATION

Carmen Coll was born in 1980 in Quart de Poblet, Valencia, Spain. She graduated in Chemistry at the University of Valencia in 2003. She received her Ph.D. degree in 2010 with Professor Martínez-Máñez at the Polytechnic University of Valencia on the development of new hybrid materials for the colorimetric detection and removal of environmental anions in natural waters. Currently, she is a member of the IDM institute (with a post-doctoral contract), and her current research interests comprise the development of new molecular gates for delivery applications in the nanomedicine field.

Andrea Bernardos was born in 1981 in Barcelona, Spain. She graduated in Chemistry in 2006 at the Autonomous University of Madrid. She received her Ph.D. degree in 2011 with Professors J. Barat and R. Martínez-Máñez at the Polytechnic University of Valencia on the development and synthesis of hybrid materials for the controlled release of bioactive molecules. Currently, she has joined the Czech University of Life Sciences in Prague with a postdoctoral contract to work in the controlled release of bioactive molecules in foods. Her research interest comprises the development of gated nanomaterials for the controlled release of biomolecules in food technology and in nanomedicine.

Ramón Martínez-Máñez was born in Valencia, Spain. He received his Ph.D. in Chemistry from the University of Valencia in 1986 and was a postdoctoral fellow at Cambridge University, U.K. He is a full professor in the Department of Chemistry at the Polytechnic University of Valencia. Presently, he is the director of the IDM Research Institute at the Polytechnic University of Valencia. He is the coauthor of more than 200 research publications and nine patents. He is a member of the American Chemical Society. His current research interest involves developing new sensing methods for different chemicals of interest, including anions, cations, and neutral species, such as explosives and chemical warfare agents. He is also involved in designing gated hybrid materials for on-command delivery applications.

Félix Sáncenón was born in 1968 in Manises, Valencia, Spain, and graduated in Chemistry in 1991. He received his Ph.D. degree in 2003 with Professor R. Martínez-Máñez at the Polytechnic University of Valencia on the development of chromogenic and fluorogenic chemosensors for cations and anions. Afterwards, he obtained a Marie-Curie contract from the E.U. and worked with Professor L. Fabbrizzi at the Universitá di Pavia on the synthesis of chromogenic receptors for ion-pairs. Then, he joined the Department of Chemistry at the Polytechnic University of Valencia with a Ramón y Cajal contract. He became a lecturer in 2006. His current research interest comprises the use of hybrid materials for the development of sensors and for the construction of molecular gates.

FOOTNOTES

*To whom correspondence should be addressed. Phone: 34-963877343. Fax: 34-963879349. E-mail: rmaez@qim.upv.es.
The authors declare no competing financial interest.

REFERENCES

1 Aznar, E.; Martínez-Máñez, R.; Sancenón, F. Controlled release using mesoporous materials containing gate-like scaffoldings. *Expert Opin. Drug Delivery* **2009**, *6*, 643–655.

2 Cotí, K. K.; Belowich, M. E.; Liong, M.; Ambrogio, M. W.; Lau, Y. A.; Khatib, H. A.; Zink, J. I.; Khashab, N. M.; Stoddart, J. F. Mechanised nanoparticles for drug delivery. *Nanoscale* **2009**, *1*, 16–39.

3 Kresge, C. T.; Leonowicz, M. E.; Roth, W. J.; Vartuli, J. C.; Beck, J. S. Ordered mesoporous molecular sieves synthesized by a liquid-crystal template mechanism. *Nature* **1992**, *359*, 710–712.

4 Nguyen, T. D.; Leung, K. C.-F.; Liong, M.; Liu, Y.; Stoddart, J. F.; Zink, J. I. Versatile supramolecular nanovalves reconfigured for light activation. *Adv. Funct. Mater.* **2007**, *17*, 2101–2110.

5 Trewyn, B. G.; Slowing, I. I.; Giri, S.; Chen, H. T.; Lin, V. S.-Y. Synthesis and functionalization of a mesoporous silica nanoparticle based on the sol-gel process and applications in controlled release. *Acc. Chem. Res.* **2007**, *40*, 846–853.

6 Slowing, I. I.; Trewyn, B. G.; Giri, S.; Lin, V. S.-Y. Mesoporous silica nanoparticle for drug delivery and biosensing applications. *Adv. Funct. Mater.* **2007**, *17*, 1225–1236.

7 Trewyn, B. G.; Giri, S.; Slowing, I. I.; Lin, V. S.-Y. Mesoporous silica nanoparticle based controlled release, drug delivery, and biosensor systems. *Chem. Commun.* **2007**, 3236–3245.

8 Saha, S.; Leung, K. C.-F.; Nguyen, T. D.; Stoddart, J. F.; Zink, J. I. Nanovalves. *Adv. Funct. Mater.* **2007**, *17*, 685–693.

9 Klajn, R.; Stoddart, J. F.; Grzybowski, B. A. Nanoparticles functionalized with reversible molecular and supramolecular switches. *Chem. Soc. Rev.* **2010**, *39*, 2203–2237.

10 Yang, Y.-W. Towards biocompatible nanovalves based on mesoporous silica nanoparticles. *Med. Chem. Commun.* **2011**, *2*, 1033–1049.

11 Casasús, R.; Marcos, M. D.; Martínez-Máñez, R.; Ros-Lis, J. V.; Soto, J.; Villaescusa, L. A.; Amorós, P.; Beltrán, D.; Guillem, C.; Latorre, J. Toward the development of ionically controlled nanoscopic molecular gates. *J. Am. Chem. Soc.* **2004**, *126*, 8612–8613.

12 Casasús, R.; Climent, E.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Amorós, P.; Cano, J.; Ruiz, E. Dual aperture control on pH- and anion-driven supramolecular nanoscopic hybrid gate-like ensembles. *J. Am. Chem. Soc.* **2008**, *130*, 1903–1917.

13 Bernardos, A.; Aznar, E.; Coll, C.; Martínez-Máñez, R.; Barat, J. M.; Marcos, M. D.; Sancenón, F.; Soto, J. Controlled release of vitamin B_2 using mesoporous materials functionalized with amine-bering gate-like scaffoldings. *J. Controlled Release* **2008**, *131*, 181–189.

14 Aznar, E.; Casasús, R.; García-Acosta, B.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Amorós, P. Photochemical and chemical two-channel control of functional nanogated hybrid architectures. *Adv. Mater.* **2007**, *19*, 2228–2231.

15 Aznar, E.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Amorós, P.; Guillem, C. pH- and Photo-switched release of guest molecules from mesoporous silica supports. *J. Am. Chem. Soc.* **2009**, *131*, 6833–6843.

16 Aznar, E.; Mondragón, L.; Ros-Lis, J. V.; Sancenón, F.; Marcos, M. D.; Martínez-Máñez, R.; Soto, J.; Pérez-Payá, E.; Amorós, P. Finely tuned temperature-controlled cargo release

using paraffin-capped mesoporous silica nanoparticles. *Angew. Chem., Int. Ed.* **2011**, *50*, 11172–11175.

17 Taylor-Pashow, K. M. L.; Della Rocca, J.; Huxford, R. C.; Lin, W. Hybrid nanomaterials for biomedical applications. *Chem. Commun.* **2010**, *46*, 5832–5849.

18 Bernardos, A.; Aznar, E.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Barat, J. M.; Amorós, P. Enzyme-responsive controlled release using mesoporous silica supports capped with lactose. *Angew. Chem., Int. Ed.* **2009**, *48*, 5884–5887.

19 Bernardos, A.; Mondragón, L.; Aznar, E.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Barat, J. M.; Pérez-Payá, E.; Guillem, C.; Amorós, P. Enzyme-responsive intracelular controlled release using nanometric silica mesoporous supports capped with "saccharides". *ACS Nano* **2010**, *4*, 6353–6368.

20 Coll, C.; Mondragón, L.; Martínez-Máñez, R.; Sancenón, F.; Marcos, M. D.; Soto, J.; Amorós, P.; Pérez-Payá, E. Enzyme-mediated controlled release systems by anchoring peptide sequence on mesoporous silica supports. *Angew. Chem., Int. Ed.* **2011**, *50*, 2138–2140.

21 Agostini, A.; Mondragón, L.; Coll, C.; Aznar, E.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Pérez-Payá, E.; Amorós, P. Dual enzyme-triggered controlled release on capped nanometric silica mesoporous supports. *ChemistyOpen* **2012**, *1*, 17–20.

22 Casasús, R.; Aznar, E.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Amorós, P. New methods for anion recognition and signalling using nanoscopic gatelike scaffoldings. *Angew. Chem., Int. Ed.* **2006**, *45*, 6661–6664.

23 Coll, C.; Aznar, E.; Martínez-Máñez, R.; Marcos, M. D.; Sancenón, F.; Soto, J.; Amorós, P.; Cano, J.; Ruiz, E. Fatty acid carboxylate- and anionic surfactant-controlled delivery systems that use mesoporous sílica supports. *Chem.—Eur. J.* **2010**, *16*, 10048–10061.

24 Coll, C.; Casasús, R.; Aznar, E.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Amorós, P. Nanoscopic hybrid systems with a polarity-controlled gate-like scaffolding for the colorimetric signalling of long-chain carboxylates. *Chem. Commun.* **2007**, 1957–1959.

25 Aznar, E.; Coll, C.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Amorós, P.; Cano, J.; Ruíz, E. Borate-driven gatelike scaffolding using mesoporous materials functionalised with saccharides. *Chem.—Eur. J.* **2009**, *15*, 6877–6888.

26 Climent, E.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Rurack, K.; Amorós, P. The determination of methylmercury in real samples using organically capped mesoporous inorganic materials capped of signal amplification. *Angew. Chem., Int. Ed.* **2009**, *48*, 8519–8522.

27 Candel, I.; Bernardos, A.; Climent, E.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Costero, A.; Gil, S.; Parra, M. Selective opening of nanoscopic capped mesoporous inorganic materials with nerve agente simulants; an application to design chromofluorogenic probes. *Chem. Commun.* **2011**, *47*, 8313–8315.

28 Climent, E.; Bernardos, A.; Martínez-Máñez, R.; Maquieira, A.; Marcos, M. D.; Pastor-Navarro, N.; Puchades, R.; Sancenón, F.; Soto, J.; Amorós, P. Controlled delivery systems using antibody-capped mesoporous nanocontainers. *J. Am. Chem. Soc.* **2009**, *131*, 14075–14080.

29 Climent, E.; Martínez-Máñez, R.; Sancenón, F.; Marcos, M. D.; Soto, J.; Maquieira, A.; Amorós, P. Controlled delivery using oligonucleotide-capped mesoporous silica nanoparticles. *Angew. Chem., Int. Ed.* **2010**, *49*, 7281–7283.