VIP Programmable Host–Guest Systems

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A Programmable DNA-Based Molecular Valve for Colloidal Mesoporous Silica**

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In memory of Victor S.-Y. Lin

Recently, stimuli-responsive gatekeepers or valves for the controlled release of guest molecules from mesoporous silica hosts have been developed.[1–4] Controlled release mechanisms are of great interest in many different fields, such as drug delivery, detergent design, or additives in polymers. For example, inorganic nanoparticles, large molecules, and also polymers have been applied to prevent guest molecules from leaving the pore system.[5–7] The release mechanisms include those based on changes in the reduction potential, pH, UV irradiation, or temperature.[8–10] For instance, gold nanoparticles were functionalized with photocleavable linkers and subsequently adsorbed onto the surface of MCM-41. Other systems utilize the external presence of enzymes for the controlled release of incorporated guests.[11,12] One approach uses α-cyclodextrins fixed with ester-bound adamantyl stoppers. Opening was achieved by the addition of porcine liver esterase, leading to a removal of the stoppers.[12] Our research has recently centered on systems using biomolecules as components for the design of the molecular valves. For example, we recently demonstrated the applicability of the biotin–avidin complex as a biomolecule-based valve on novel core–shell-functionalized colloidal mesoporous silica, which opens by enzyme action or at high temperature.[3]

The molecular valves reported to date are commonly based on one specific opening mechanism, which is determined by the chemical nature of the system. Herein we demonstrate that a molecular valve system can be made programmable by encoding the desired behavior in a DNA sequence that is part of the responsive system. We demonstrate this concept with the programmable opening temperature of a molecular valve on the pore mouths of novel core–shell colloidal mesoporous silica. We have recently developed the controlled molecular core–shell functionalization of colloidal mesoporous silica, which is a prerequisite for the complex assembly of the different functionalities at different locations of the nanoscale porous particles.[13,14]

As described below, we have selectively attached biotin-labeled DNA double strands to the pore mouths of the core–shell mesoporous nanoparticles. This procedure allows a subsequent closing of the pores by the protein avidin whilst leaving open the internal pore volume of the mesoporous nanoparticle. The opening of the valve is achieved by DNA strand melting at the specific melting temperature of the oligonucleotide (see Figure 1).

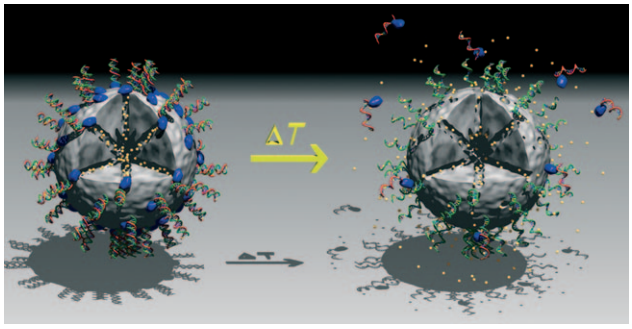

Figure 1. Concept of the programmable molecular valve system. The avidin caps are opened by melting the DNA linkers at specifically encoded temperatures.

Double-stranded alkyne-modified oligodeoxyribonucleotides (DNA oligomers) of two different lengths (15 mer and 25 mer) were applied as linkers between avidin and the silica surface. Covalent immobilization of such strands to the selectively functionalized outer silica surface (pore mouths) was achieved by a click chemistry approach. The applied DNA oligomers bear an alkyne functionality at their 5′ end. Specifically, a C_8-alkyne-desoxyuridine-phosphordiamidite that features a C_8-alkyne linker at the 5-position of the base was incorporated at the 5′ end of the DNA strands. This method was recently introduced by some of us.[15,16] The functionalized DNA oligomers were then pre-hybridized with complementary strands carrying a biotin functionality at their 3′ end by briefly heating a 1:1 mixture of the strands to 95 °C and slow cooling to room temperature in a 500 mM sodium

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chloride solution. For our experiments, two different double strands were synthesized, a 25 mer and a 15 mer. Along with the described alkyne and biotin functionality, the 15 mer is modified with two dyes (Cy3, Cy5), one on each end of the respective complementary oligomers. This allows for a fluorescence resonance energy transfer (FRET) between the two strands to further characterize the hybridization state of the DNA at different temperatures.[17] Furthermore, the synthesized DNA strands were characterized by MALDI-TOF mass spectrometry. An overview of the DNA oligomers that were applied is given in Table 1.

Table 1: Sequences and MALDI-TOF analysis of the applied DNA strands.[a]

Applied DNA double strands
5′-XCA CGT CGC ATC TTG GCC TAC GCC C-3′
M_{calcd} = 7616.4 M_{found} = 7617.1
3′-Bio-AGT GCA GCG TAG AAC CGG ATG CGG G-5′
M_{calcd} = 8230.9 M_{found} = 8231.4
5′-XCA CGT CGC ATC TTT Cy3-3′
M_{calcd} = 5404.8 M_{found} = 5405.6
3′-Bio-AGT GCA GCG TAG AAA Cy5-5′
M_{calcd} = 5902.3 M_{found} = 5902.8

[a] X: dU-alkyne, Bio: biotin modification.

Colloidal mesoporous silica (CMS) with chloropropyl functionality exclusively on the outer shell of the nanoparticles was synthesized by using our recently developed delayed co-condensation method for the controlled synthesis of functionalized core–shell mesoporous nanoparticles.[13] This approach avoids uncontrolled pore clogging of the nanoparticles upon the attachment of large organic moieties to the surface.

Herein, the inner pore walls remain unfunctionalized, leaving the whole pore volume available for loading with guest molecules, such as drugs or other organic molecules. A reaction mixture containing tetraethyl orthosilicate (TEOS), cetyltrimethylammonium bromide (CTAB), and triethanolamine (TEA) results in the spontaneous generation of seeds that grow further radially. After 30 min, a 1:1 mixture of 3-chloropropyltrimethoxysilane and TEOS was added to the reaction to yield organic functionality exclusively at the outer shell of the nanoparticles. CMS with particle sizes of about 50 nm were thus obtained as determined by transmission electron microscopy (TEM). A TEM image of the sample CMS-Cl can be found in the Supporting Information.

The functionalized mesoporous particles feature pore sizes of 3.8 nm and a pore volume of 0.99 $cm^3 g^{-1}$, as determined from nitrogen sorption isotherms with non-local density functional theory (NLDFT). The corresponding isotherm can be found in the Supporting Information. The colloids were then transferred into dry *N*,*N*-dimethylformamide. The remaining water from the pore system was removed under mild conditions according to our previously published vapor extraction procedure for 3 h at 90 °C using molecular sieves.[18] This approach protects the particles from temperature-induced agglomeration. The resulting water-free suspension was then saturated with sodium azide and heated to 85 °C for 5 h to give an azide-carrying surface (sample CMS-N_3). The successful conversion of the surface was confirmed by IR spectroscopy, which showed the characteristic azide stretch signal at 2105 cm^{-1} (Figure 2).

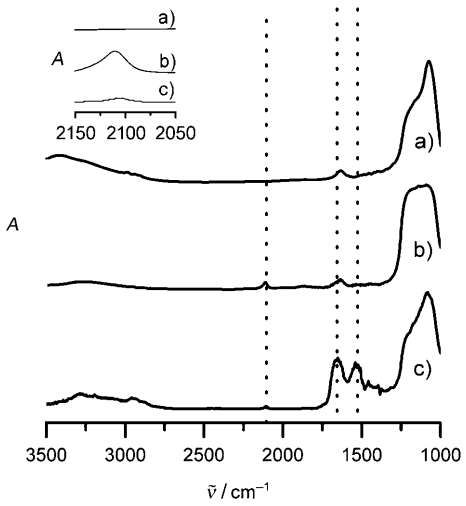

Figure 2. Infrared spectra of the samples: a) CMS-Cl, b) CMS-N_3, and c) CMS-DNA_{15}-AVI. Inset: a magnification of the azide stretching region; •••••= guiding lines at $\tilde{\nu}$ = 1551, 1650, and 2105 cm^{-1}.

The DNA oligomers were then attached to the particles using a click chemistry approach. The applicability of click chemistry on mesoporous silica surfaces was recently demonstrated by our group.[19] Samples were synthesized that carry the two different dsDNA strands introduced in Table 1 (CMS-DNA_{15} and CMS-DNA_{25}). The sequence of building up the molecular DNA valve was followed by IR spectroscopy (Figure 2). Whereas the sample CMS-Cl (Figure 2a) only shows the silica framework vibrations, an azide band occurs in sample CMS-N_3 at 2105 cm^{-1} (Figure 2b). This band is strongly reduced in intensity upon DNA attachment during the click reaction. We take this result as evidence for the covalent DNA binding to the CMS sample. As can be seen in Figure 2, the sample CMS-DNA_{15}-AVI shows the typical amide vibrations at 1551 cm^{-1} and 1650 cm^{-1} of the attached protein, along with the silica vibrations. The weak signals between 1500 cm^{-1} and 1300 cm^{-1} in sample CMS-DNA_{15}-AVI can be attributed to the carbonyl vibrations of the desoxyribose backbone and the amino groups of the incorporated DNA.

As described above, the attached strands have a biotin functionality that is now positioned closely to the surface of the nanoparticle pore mouth. The intact hybridization of the strands after the click reaction is indicated by a successful measurement of the FRET signal in the sample CMS-DNA_{15}-AVI (Figure 3). In analogy, it is assumed that the longer 25 mer is also in a hybridized state after the click reaction. A corresponding reference FRET experiment using the pure as-received DNA can be found in the Supporting Information. The samples CMS-DNA_{15} and CMS-DNA_{25} were then loaded

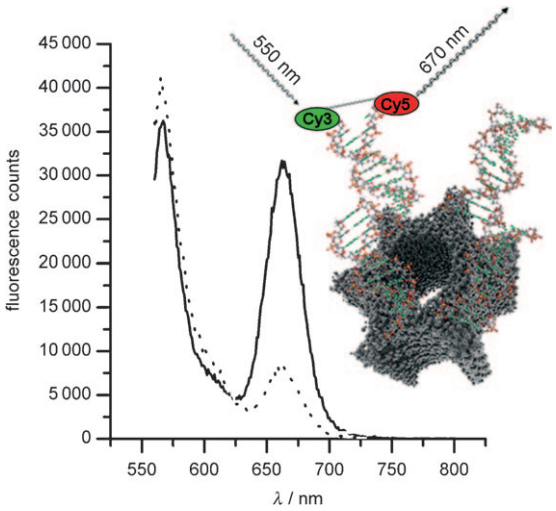

Figure 3. FRET measurements of the sample CMS-DNA$_{15}$-AVI in the closed state (——) and in the open state (•••••).

with fluorescein as a model compound for guest molecules. To achieve the loading, 1 mg of each sample was stirred in 1 mL of a solution containing 2 mg fluorescein in water at room temperature for 2 h. Subsequently, 1 mg avidin from egg white was added and stirring was continued for another two hours. This leads to a coordination of the biotinylated DNA with the avidin protein and thus should close the pores of the colloidal particles.

The subsequent release of fluorescein can be easily detected and quantified by fluorescence spectroscopy, as we have demonstrated earlier.[3] After incorporation of the dye, the particles were centrifuged in a 1.5 mL vial and redispersed in water (pH 7). Thereafter three washing cycles were applied to remove free dye from the suspension, yielding the samples CMS-DNA$_{15}$-AVI and CMS-DNA$_{25}$-AVI. The samples were then transferred into a two-compartment fluorescence cuvette, which can be closed by a dialysis membrane, and features a sample volume of 200 μL.[3] A picture of this cuvette can be found in the Supporting Information. With the two-compartment temperature-controlled fluorescence cuvette, the release of small fluorescent molecules can be detected by fluorescence spectroscopy. The silica nanoparticles are too large to diffuse through the dialysis membrane (featuring a molecular weight cut-off of 16000 g mol^{-1}).

The opening mechanism of the molecular valve was examined with FRET measurements. As described above, the sample CMS-DNA$_{15}$-AVI is equipped with the FRET pair Cy3-Cy5 on the DNA strands (Table 1). Cy3 has an absorption maximum around 550 nm and an emission at 570 nm that tails into the red. Cy5 is excited at 650 nm and emits at 670 nm. With the attached double strand in a hybridized state, an emission at 670 nm can be achieved upon excitation at 550 nm, owing to the energy transfer occurring between the two dyes in close contact (less than 10 nm; see Figure 3).[17,20] After the thermoresponsive release experiment (see below), the sample was centrifuged and then redispersed in 2 mL of water. As can be seen in Figure 3, the FRET emission at 670 nm decreases substantially in intensity after the thermoresponsive release, indicating the opening of the molecular valve by separation of the DNA strands.

Finally, the programmed thermoresponsive opening of the molecular DNA-avidin-biotin valves was demonstrated by quantification of the released fluorescein molecules with fluorescence spectroscopy in the two-compartment cuvette described above (Figure 4). All intensity values are normalized to the highest obtained fluorescent count of the respective sample. Therefore, the highest released amount of fluorescein is set to 100% relative release.

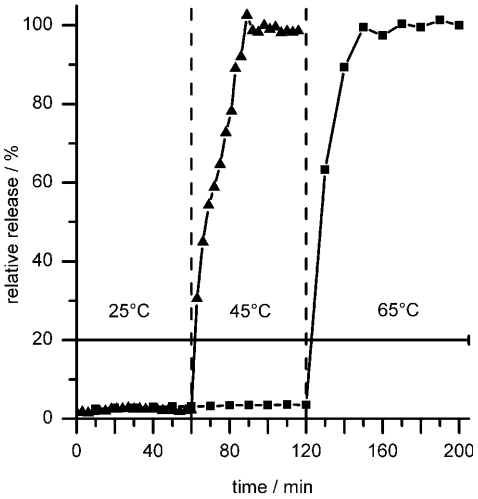

Figure 4. Release curves (normalized fluorescence intensity of fluorescein) of the samples CMS-DNA$_{15}$-AVI (▲) and CMS-DNA$_{25}$-AVI (■).

The temperature-dependent release curves show the striking effect of the DNA-linker on the valve-opening behavior. Whilst the 15 mer opens at 45 °C, the longer 25 mer is still in a tightly sealed state and thus keeps the avidin firmly bound to the CMS pore mouth. The sample CMS-DNA$_{25}$-AVI does not convert into the open state until further heating to 65 °C. Quantification of the released amount of fluorescein by UV/Vis spectroscopy gives comparable amounts of dye released in both samples. Specifically, the sample CMS-DNA$_{15}$-AVI released 0.033 mg dye/mg CMS, whilst the sample CMS-DNA$_{25}$-AVI released 0.040 mg dye/mg CMS (for details, see the Supporting Information).

In summary, we have shown how the incorporation of double-stranded DNA into a novel molecular valve can impart programmable thermoresponsive release behavior at moderate temperatures. The successful assembly of this multifunctional nanodevice builds on the novel multifunctional core–shell colloidal mesoporous silica host, free available pore volume inside the host, selective functionalization of the pore mouths with dsDNA linker bearing two dyes for FRET monitoring, and biotin–avidin coupling for valve closure. These nanodevices allow the release temperature of incorporated guest molecules to be adjusted precisely for a desired application. The general concept of programmed release will be of significant importance in fields such as

targeted drug delivery, detergents, or encapsulation of polymer hardeners.[21] A further potential application is targeted drug release, although toxicological aspects have to be considered here as well.

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