Communication

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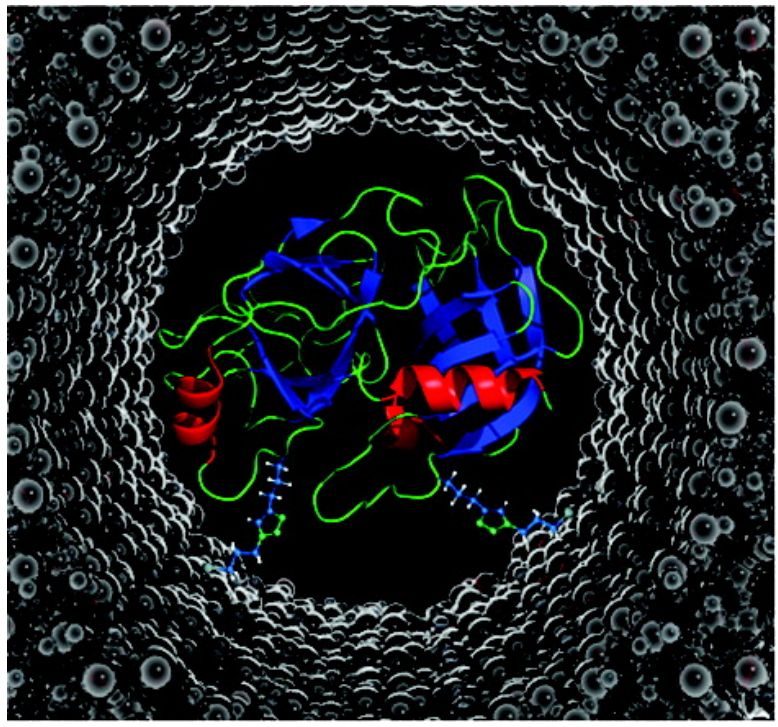

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Click Chemistry for High-Density Biofunctionalization of Mesoporous Silica

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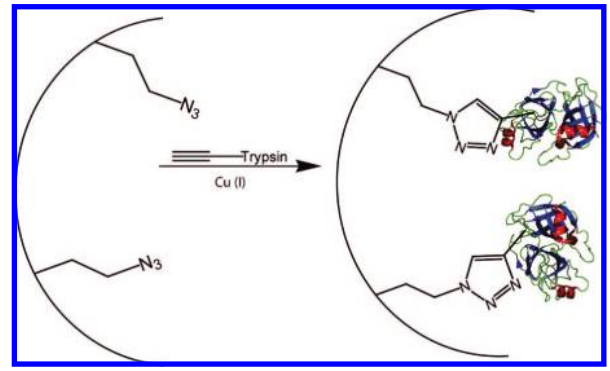
Surface-based click chemistry is attracting increasing attention.[1−4] The mild reaction conditions of the copper(I)-catalyzed azide-alkyne cycloaddition and its very high yield make this reaction suitable for the functionalization of surfaces with biomolecules.[2] Supported enzymes on mesoporous solids have many possible applications in biotechnology, including synthesis and purification of fine chemicals and catalysis for green chemistry.[5] It has been shown that the stability and recoverability of enzymes benefit from encapsulation in mesoporous hosts.[6] In earlier work, lysozyme was absorbed into the pores of SBA-15.[7] However, the noncovalent interactions between mesoporous silica materials and proteins are too weak to prevent the absorbed biomolecules from leaching out of the pore system during further washing and reaction steps, thus leading to relatively low loading levels.[8] Organic modification of the pore walls of mesoporous hosts or glutaraldehyde-based cross-linking of absorbed proteins can sometimes prevent the absorbed enzymes from leaching out of the pore system.[6,9] However, these methods are typically limited to certain proteins and carry the risk of deactivating the enzyme.

First attempts at covalent enzyme immobilization in mesoporous silica have claimed the binding of penicillin G acylase onto glycidopropyl-functionalized mesoporous silica, although no proof of covalent attachment was provided.[10]

Here we present the stable covalent immobilization of the protease trypsin in the channel system of a large-pore SBA-15 host by a click chemistry approach. This versatile and mild method for the covalent immobilization of functional biomolecules on mesoporous supports allows for unprecedented functionalization densities while retaining enzyme activity and preventing protein leaching from the mesoporous support.

Large-pore SBA-15 was synthesized according to previously published procedures.[11] Tetraethyl orthosilicate (TEOS) was hydrolyzed in a reaction mixture containing Pluronic 123 as template, cetyltrimethylammonium bromide (CTAB) as cotemplate, mesitylene as swelling agent, potassium chloride, and hydrochloric acid. Removal of the template was achieved by calcination at 550 °C for 5 h (heating rate 1 °C/min). Mesoporous silica spheres featuring diameters of 6 μm with a surface area of 700 m^2/g and pore sizes of ∼9 nm were obtained. The silica surface was functionalized with (3-chloropropyl)trimethoxysilane (sample SBA-Cl) using a postsynthetic grafting approach. The mesostructure of the resulting materials was demonstrated by small-angle X-ray diffraction (see Supporting Information). By a substitution reaction with sodium azide (NaN_3) in *N*,*N*-dimethylformamide (DMF), an azide-functionalized mesoporous silica surface was created (SBA-N_3). Acetylene-modified trypsin (sp-trypsin) was synthesized according to previously reported procedures,[12] using 4-pentynoic acid as reagent and *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDC) as an activating agent.

Scheme 1. Covalent Attachment of Trypsin into the Mesoporous System of Large Pore SBA-15 Achieved by Click Chemistry



Subsequently, SBA-N_3 was reacted with sp-trypsin in a copper(I)-catalyzed Huisgen reaction (Click reaction) at 4 °C in aqueous medium followed by rigorous washing steps, resulting in trypsin-functionalized mesoporous silica (SBA-trypsin, Scheme 1). A detailed Experimental Section can be found in the Supporting Information. The result of the click reaction and the presence of trypsin in the sample were monitored by IR spectroscopy (Figure 1). The almost complete loss of intensity of the azide stretch (2105 cm^{-1}, Figure 1a,b) indicates the covalent attachment of trypsin to the surface. The emerging strong absorption bands located at 1667 and 1531 cm^{-1} are assigned to the amide bonds of the attached protein (Figure 1b,c).

Nitrogen sorption measurements of samples SBA-15, SBA-Cl, and SBA-trypsin confirm the presence of the large trypsin molecules

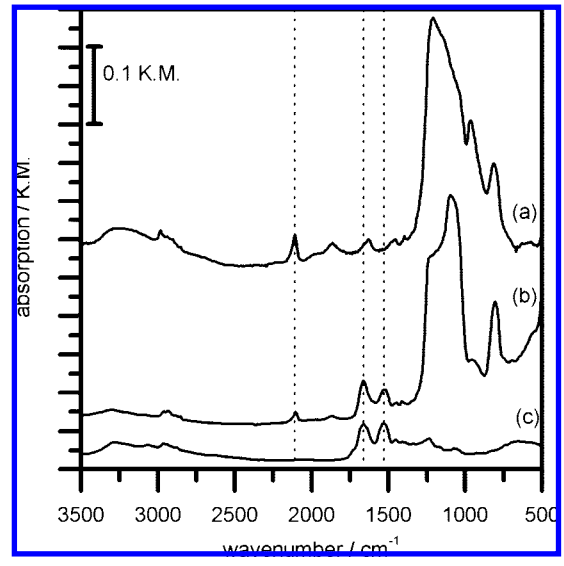


Figure 1. IR spectra of the samples (a) SBA-N_3, (b) SBA-trypsin, and (c) trypsin.

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Table 1. Nitrogen Sorption Data of the Synthesized Samples

sample name	BET surface [$m^2\ g^{-1}$]	pore size [nm]	pore volume [$cm^3 g^{-1}$]
SBA-15	701	8.91	1.89
SBA-Cl	651	8.40	1.69
SBA-trypsin	430	6.95	0.73

attached to the pore walls (Table 1). The considerable decrease in surface area, NLDFT pore volume, and pore diameter in sample SBA-trypsin clearly indicates functionalization with the enzyme on the inner surface.

The functionalization density was determined by thermogravimetric analysis (TGA, Figure 2) and confirmed by BCA assay (bicinchoninic acid). While the unfunctionalized SBA-15 only shows a minor mass loss of less than 1% (Figure 2a), the mass loss of 5.0% in sample SBA-Cl corresponds to 0.68 mmol g^{-1} of chloropropyl residues bound to the silica surface (Figure 2b). Based on the mass loss of 15% in the sample SBA-trypsin (Figure 2c), the amount of bound enzyme was calculated to be 12 wt%. This value was confirmed by BCA assay quantification of the remaining protein in the supernatant of the synthesis solution. A detailed analysis of the TGA and BCA assay data can be found in the Supporting Information. Based on these results, the protein activity of the surface-bound enzyme in SBA-trypsin was determined.

In order to prove the retention of the enzymatic activity, the assay developed by Hummel et al. was applied.[13] The performance of trypsin, acetylene-modified trypsin (sp-trypsin), and silica-supported trypsin (SBA-trypsin) was photometrically quantified by the release of Nα-*p*-tosyl-L-arginine from Nα-*p*-tosyl-L-arginine methyl ester hydrochloride (TAME). In the following, one unit is defined as the amount of enzymatic activity releasing 1 μmol of Nα-*p*-tosyl-L-arginine per minute. Both sp-trypsin and SBA-trypsin remain biologically active. The activity found for trypsin was 346 units/mg, while sp-trypsin showed an activity of 334 units/mg. The high activity of SBA-supported trypsin was calculated to be 7.9 units/mg (host + enzyme), corresponding to 65.6 units/mg (enzyme), or a retention of ~20% of enzyme activity relative to aqueous solution. As a reference, nonporous silica aerogel was functionalized the same way, resulting in an enzymatic activity of 80 units/mg (enzyme) or 10.8 units/mg (host + enzyme). In comparison, the reported activity of chymotrypsin is reduced to 1.7% upon cross-linking with glutaraldehyde in SBA-15.[9,14] Our results show that porous SBA-15 causes no substantial diffusional limitations compared to nonporous silica. The remaining pore diameter of ~7 nm still allows efficient substrate transport to the reactive site of the enzyme molecules. The stabilizing role of SBA-15 is demonstrated by the exceptional conservation of activity during multiple recovery cycles (see Supporting Information). Leaching experiments were performed in order to show the stability of the covalent enzyme attachment. The amount of 1.1 mg of sample SBA-trypsin was stirred in 2 mL of tris(hydroxymethyl)aminomethane buffer (pH 8.1) for 4 h at 25 °C. The solid was then separated from the supernatant by centrifugation. Subsequently, the biological activity of the supernatant was tested by TAME hydrolysis without showing any measurable activity. BCA assay quantification shows no detectable enzyme in solution. These data clearly prove that no leaching occurred under the experimental conditions.

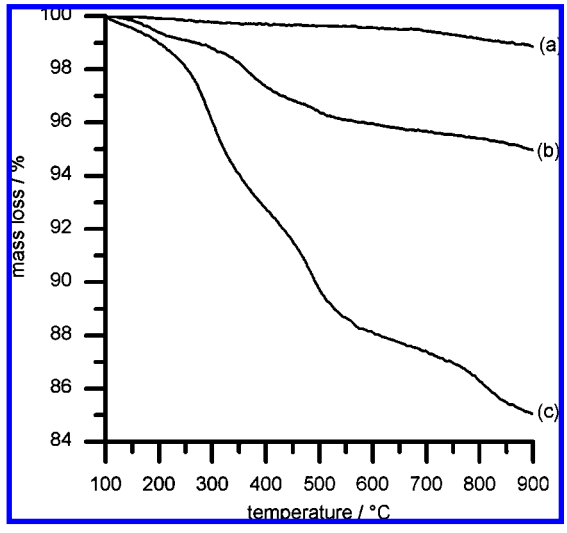

Figure 2. TGA data of the samples (a) SBA-15, (b) SBA-Cl, and (c) SBA-trypsin.

In summary, we present a novel approach for the covalent modification of mesoporous silica surfaces with intact enzymes. The high covalent enzyme functionalization density under simultaneous retention of enzyme activity and the absence of leaching demonstrates the promising potential of this approach.

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Supporting Information Available: Detailed experimental section; aerogel reference experiments; XRD diffractograms; nitrogen sorption isotherms; data on enzyme activity and quantification; TGA calculations; SEM micrographs. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Lummerstorfer, T.; Hoffmann, H. *J. Phys. Chem. B* **2004**, *108*, 3963–3966.
(2) Gallant, N. D.; Lavery, K. A.; Amis, E. J.; Becker, M. L. *Adv. Mater.* **2007**, *19*, 965–969.
(3) Kacprzak, K. M.; Maier, N. M.; Lindner, W. *Tetrahedron Lett.* **2006**, *47*, 8721–8726.
(4) Prakash, S.; Long, T. M.; Selby, J. C.; Moore, J. S.; Shannon, M. A. *Anal. Chem.* **2007**, *79*, 1661–1667.
(5) Yiu, H. H. P.; Wright, P. A. *J. Mater. Chem.* **2005**, *15*, 3690–3700.
(6) Yiu, H. H. P.; Wright, P. A.; Botting, N. P. *J. Mol. Catal. B: Enzym.* **2001**, *15*, 81–92.
(7) Vinu, A.; Murugesan, V.; Hartmann, M. *J. Phys. Chem. B* **2004**, *108*, 7323–7330.
(8) Hartmann, M. *Chem. Mater.* **2005**, *17*, 4577–4593.
(9) Kim, M. I.; Kim, J.; Lee, J.; Shin, S.; Na, H. B.; Hyeon, T.; Park, H. G.; Chang, H. N. *Microporous Mesoporous Mater.* **2008**, *111*, 18–23.
(10) Lu, Y.; Lu, G.; Wang, Y.; Guo, Y.; Guo, Y.; Zhang, Z.; Wang, Y.; Liu, X. *Adv. Funct. Mater.* **2007**, *17*, 2160–2166.
(11) Katiyar, A.; Yadav, S.; Smirniotis, P. G.; Pinto, N. G. *J. Chromatogr., A* **2006**, *1122*, 13–20.
(12) Gole, A.; Murphy, C. J. *Langmuir* **2008**, *24*, 266–272.
(13) Hummel, B. C. W. *Can. J. Biochem. Physiol.* **1959**, *37*, 1393–1399.
(14) Kim, J.; Delio, R.; Dordick, J. S. *Biotechnol. Prog.* **2002**, *18*, 551–555.

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