

VIP **A Nanoporous Reactor for Efficient Proteolysis****Liang Qiao,^[a] Yun Liu,^[a] Sarah P. Hudson,^[b] Pengyuan Yang,^[a] Edmond Magner,^[b] and Baohong Liu*^[a]**

Abstract: A nanoreactor based on mesoporous silicates is described for efficient tryptic digestion of proteins within the mesochannels. Cyano-functionalized mesoporous silicate (CNS), with an average pore diameter of 18 nm, is a good support for trypsin, with rapid in situ digestion of the model proteins, cytochrome c and myoglobin. The generated peptides were analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Pro-

teolysis by trypsin-CNS is much more efficient than in-solution digestion, which can be attributed to nanoscopic confinement and concentration enrichment of the substrate within the mesopores. Proteins at concentrations of $2 \text{ ng } \mu\text{L}^{-1}$ were successfully identified after digestion for 20 min. A biological

complex sample extracted from the cytoplasm of human liver tissue was digested by using the CNS-based reactor. Coupled with reverse-phase HPLC and MALDI-TOF MS/MS, 165 proteins were identified after standard protein data searching. This nanoreactor combines the advantages of short digestion time with retention of enzymatic activity, providing a promising way to advance the development of proteomics.

Keywords: mass spectrometry • mesoporous materials • nanoreactors • nanostructures • proteolysis

Introduction

There has been an explosion of interest in the detection and identification of proteins, using the techniques of mass spectrometry and database searching, with the aim of establishing links to pathological conditions.^[1] One of the preferred approaches for protein identification in the ever-growing field of proteomic research is peptide mass fingerprinting by using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). This technique involves the sequence-specific enzymatic cleavage of a protein by a protease (for example, trypsin) obtained after two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) liquid chromatography, or capillary zone electropho-

resis separation steps, and the subsequent identification of the protein by using MALDI-TOF mass spectra by comparing the peptide fragments to a theoretical digestion pattern in a database.

A critical step in the detection and identification of proteins is protein digestion prior to MS analysis. Conventional in-solution or in-gel digestion is prone to such intrinsic limitations as prolonged digestion time, autolysis, sample loss, and so on, with negative effects on comprehensive proteomic profiling.^[2] Furthermore, the abundance distribution of different proteins in a complex biological system is very broad and still presents a major challenge. The use of immobilized enzymatic reactors has been proposed to address these issues. Enzymes immobilized in confined zones are more stable and provide increased cleavage efficiency.^[3–9] Sol-gels, polymers, and inorganic materials are attractive candidates for the immobilization of enzymes due to their resistance to microbial contamination and good chemical, mechanical, and thermal stability.^[10–12] We have previously described the immobilization of trypsin on nanozeolite, polysaccharide, or gold nanoparticle assembled microchannels and have demonstrated that these systems provide rapid protein digestion.^[13] Peterson and co-workers^[14] reported that trypsin immobilized on porous polymer monoliths by the formation of stable covalent bonds between azo-

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lactone functional groups on the polymer and amine groups of the enzyme achieved high proteolytic activity.

Features such as high surface area and large pore diameters have made mesoporous materials attractive as supports in a range of applications such as catalysis, adsorption, sensors, and drug delivery.^[15,16] Functionalized mesoporous silicates (MPS) can provide a rigid, uniform and well-defined open-pore microstructure, with the ability to adsorb proteins with retention of physiological function.^[17–20] In particular, the MPS pore dimensions are readily adjustable to accommodate proteins of disparate sizes and the internal surface can also show a specific affinity for the reactants.^[5,6] The use of the nanoscale volumes of the inner pores of these materials as confined nanoreactors to perform enzyme-catalyzed processes opens up promising possibilities in chemical engineering and biotechnology.

In this study, a novel nanoreactor based on an MPS with cyano functional groups (CNS) is described for the enzymatic digestion of proteins. CNS is a mesoporous material with an average pore diameter of 18 nm, a size sufficiently large to accommodate proteins and enzymes. We have previously shown that trypsin is readily adsorbed onto CNS, with up to 18 μmol trypsin adsorbed per gram CNS.^[6] There have been reports on the adsorption, desorption, activity, and stability of MPS-immobilized enzymes.^[5–9,21,22] However, few reports on the use of the inner lumens of MPS as nanoconfined reactors for protein digestion have been published. With its unique microscopic confinement, CNS is capable of performing the multiple steps of protein capture and rapid in situ digestion. Such a strategy can be applied to many techniques in protein profiling, resulting in a CNS-based enzymatic nanoreactor for protein digestion. We used trypsin-catalyzed protein digestion as a model reaction inside the CNS mesopores. Compared with in-solution digestion, this nanoreactor combines the advantages of shortened digestion time (20 min) with the retention of enzymatic activity. To examine the practical feasibility of the nanoreactor, proteolysis of a biological complex sample, a protein mixture extracted from the cytoplasm of human liver tissue, was exam-

ined. Coupled with RP-HPLC and MALDI-TOF MS, 165 proteins were identified.

Results and Discussion

Figure 1 displays the process of nanopore-based digestion. Trypsin was first adsorbed in the nanopores of CNS by mixing with a suspension of CNS in potassium phosphate buffer. At pH 6.5, trypsin is positively charged and is rapidly captured in the negatively charged nanochannels of CNS. It has been reported that when the mesopore diameter is sufficiently large to accommodate biomolecules, proteins penetrate into the mesoporous networks, as well as being adsorbed onto the external surface.^[7,21,22] Nitrogen adsorption analysis indicates the presence of pores with an average diameter of 18 nm and a total pore volume of $1.26 \text{ cm}^3 \text{ g}^{-1}$, (Figure 1b). The pore entrances of CNS are much larger than the diameter of trypsin molecules ($\approx 4 \text{ nm}$)^[23] enabling both trypsin and protein substrates to be entrapped within the mesopores.

Enzyme autolysis is one of the most important drawbacks of in-gel or in-solution proteolysis, as it can suppress the MS signals of proteins and interfere in the positive identification of proteins. Thus, the concentration of trypsin should be as low as practicable to reduce the number of protease autolysis peptides. The isoelectric point of trypsin is about 8.23 and the digestion occurs in the medium buffer (pH 7.8). Thus, during proteolysis, trypsin is likely to be attached to the inner walls of the mesochannels of CNS by electrostatic forces, restricting its degrees of freedom and ability to diffuse and concomitant autolysis. Experiments show that no trypsin autolysis could be observed when the enzyme/substrate ratio was as high as 1:3. However, in-solution digestion should be performed at an enzyme/substrate ratio of 1:30 to avoid enzyme autolysis, a ratio that is widely accepted as standard in protein digestion.^[24,25]

Cytochrome c and myoglobin, in the concentration range of $2 \approx 200 \text{ ng } \mu\text{L}^{-1}$, were selected as model substrates. Myo-

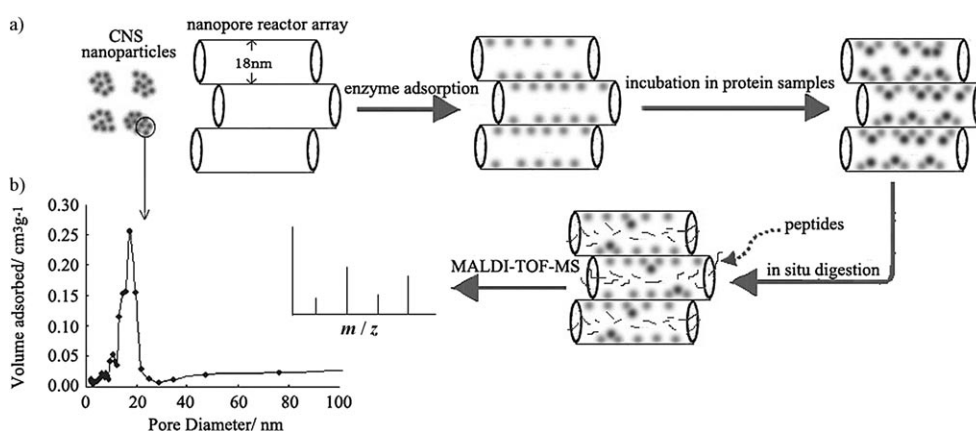


Figure 1. a) Schematic representation of in-nanopore substrate entrapment, in situ proteolytic digestion, and subsequent MS identification. b) Pore size distribution of CNS.

globin is known as a protein that is resistant to tryptic digestion, whereas cytochrome c is much easier to digest.^[2] Efficient tryptic digestion could take place after trypsin-CNS was placed into a protein solution for 20 min at 37°C. The resulting tryptic products generated from trypsin-CNS were analyzed by using MALDI-TOF mass spectrometry. Proteolysis efficiency was evaluated based on the results of protein identification by using peptide-mass fingerprinting (PMF).

As the amount of adsorbed trypsin could affect subsequent protein identification, the optimal enzyme loading was determined. Five enzyme loadings, ranging from 25 to 430 $\mu\text{g mg}^{-1}$ (maximum load^[6]) were prepared. Digestion of cytochrome c (5 $\text{ng } \mu\text{L}^{-1}$) was carried out at a protease/substrate ratio of 1:3 at pH 7.8. The results demonstrate that

the CNS nanoreactor with a trypsin amount of 200 $\mu\text{g mg}^{-1}$ had the highest proteolytic efficiency (Table 1 and Figure 2), with 11 peptide fragments from cytochrome c successfully

Table 1. Comparison of the proteolytic efficiency of CNS-trypsin nanoreactors with different trypsin loadings.

Trypsin loading [$\mu\text{g}(\text{mg CNS})^{-1}$]	Protein score	Amino acid sequence coverage [%]	Peptide matches
430	162	47	7
200	184	63	11
100	174	47	8
50	128	42	9
25	failed	failed	4

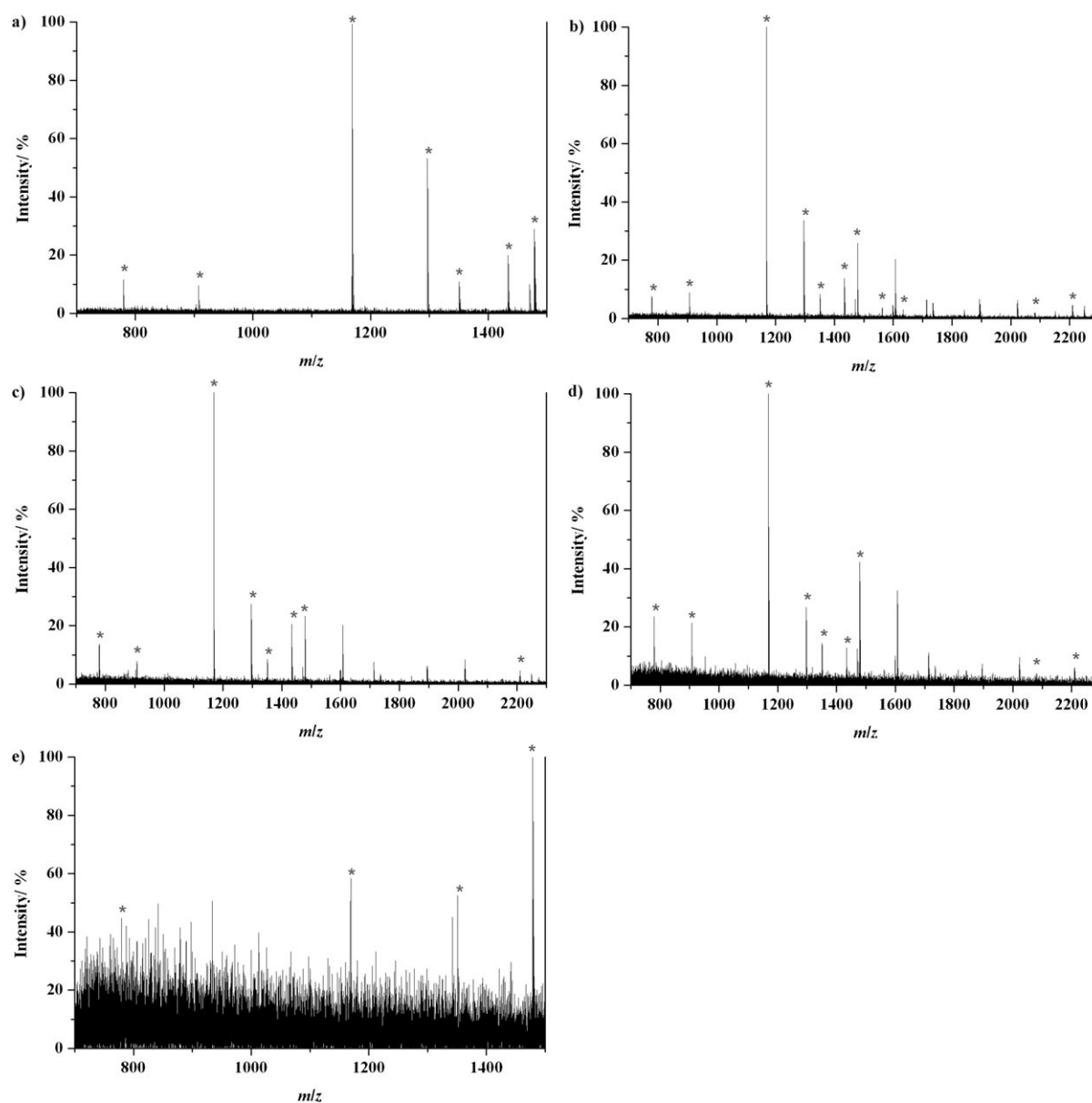


Figure 2. PMF spectra of cytochrome c (5 $\text{ng } \mu\text{L}^{-1}$) digested in CNS-trypsin nanoreactors with different trypsin loading amounts: a) 430 μg trypsin on 1 mg CNS; b) 200 μg trypsin on 1 mg CNS; c) 100 μg trypsin on 1 mg CNS; d) 50 μg trypsin on 1 mg CNS; e) 25 μg trypsin on 1 mg CNS.

identified. Moreover, the MOWSE (MOlecular Weight SEarch) score and amino acid sequence coverage also demonstrated that the nanoreactor with this loading had optimal proteolytic efficiency. By using these criteria, a loading of $200 \mu\text{g mg}^{-1}$ was selected to test the proteolytic ability of the CNS-trypsin nanoreactor.

The PMF results of peptides obtained after proteolysis for 20 min inside the mesopores of CNS are much better than those obtained by in-solution digestion (20 min) and are comparable to the results obtained after proteolysis for 12 h (Figures 3a–e). These results demonstrate that the reaction rate can be significantly accelerated when proteolysis takes

place within the confined CNS nanoreactor. For myoglobin ($200 \text{ ng } \mu\text{L}^{-1}$, without denaturation), the PMF spectrum of protein digest generated in the trypsin-CNS shows confident identification of 12 peptides with a MOWSE score of 222. In contrast, the in-solution digestion (at an enzyme/substrate ratio of 1:30) yields only one peptide peak at a very low signal/noise (S/N) ratio after a reaction time of 20 min. After overnight incubation, 12 peptides could be identified, comparable to those obtained after proteolysis for 20 min by using the CNS-based nanoreactor. Similarly, digestion of cytochrome c ($200 \text{ ng } \mu\text{L}^{-1}$) in the CNS reactor yielded an amino acid sequence coverage of 63 % and a protein score

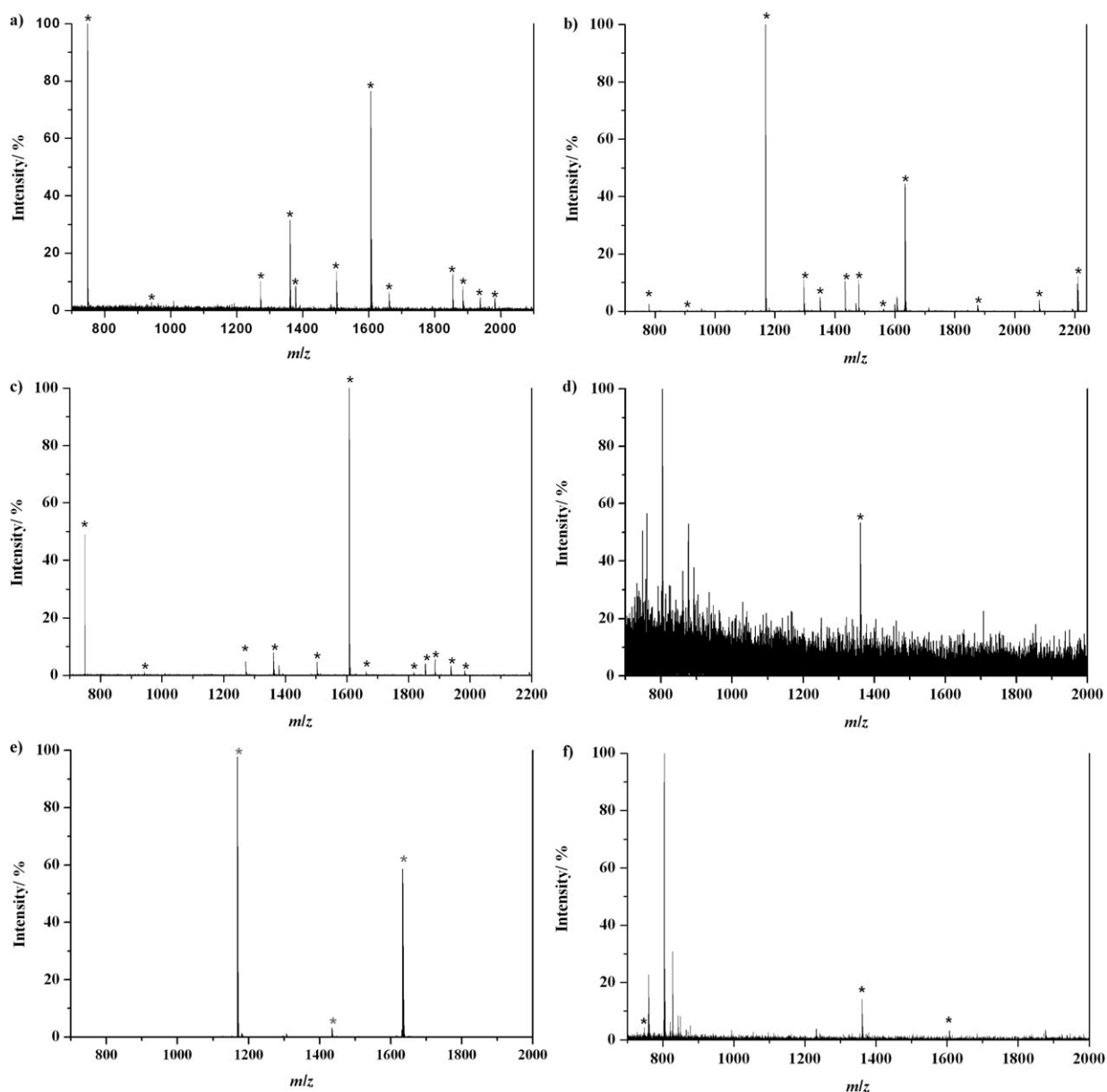


Figure 3. PMF spectra of myoglobin ((a) $200 \text{ ng } \mu\text{L}^{-1}$) and cytochrome c ((b) $200 \text{ ng } \mu\text{L}^{-1}$) proteolysis products from in-CNS-nanoreactor digestion (20 min), myoglobin ($200 \text{ ng } \mu\text{L}^{-1}$) proteolysis products from in-solution digestion for 12 h (c) and 20 min (d), cytochrome c (e) $200 \text{ ng } \mu\text{L}^{-1}$, some identified peaks are too small to be assigned) proteolysis products from in-solution digestion (12 h), and myoglobin (f) $200 \text{ ng } \mu\text{L}^{-1}$ proteolysis products from HMS-based (pore size 3.0 nm) reactor digestion (20 min).

of 155, whereas a sequence coverage of 59% and a protein score of 108 were obtained after overnight in-solution digestion. The results show that when trypsin is confined within the nanopores of CNS it retains its activity and can have an increased efficiency of digestion on different substrates.

As previously reported, the proteolysis rate depends on the concentrations of enzymes and substrates.^[26] In the CNS nanoreactor, trypsin and its substrates accumulate in the nanoporous spaces at local concentrations which are dramatically increased in comparison with those in solution. Thus, the digestion rate could be increased when the substrates are confined in the inner pores. In this experiment, the proteolysis rate in the mesopores of CNS is 35–70 times faster than the in-solution digestion according to the PMF results and the digestion time required to reach equilibrium. It has also been reported that substrate unfolding in nanopores can also facilitate proteolysis in MPS-based nanoreactors.^[12] Due to these effects, location of the protease inside the confined spaces of MPS can achieve far more rapid and effective proteolysis.

To test whether the tryptic digestion occurred inside the nanopores, a second MPS with smaller pore diameter (≈ 3 nm) was selected for the immobilization of trypsin. Hexagonal mesoporous silicate (HMS) has an average pore size of approximately 3 nm, which is too small for trypsin (≈ 4 nm) and proteins to diffuse into the channels. Proteolysis yielded only one peptide peak at high signal-to-noise ratio ($S/N > 80$) and two peptide peaks at very low S/N (15, 18), as shown in Figure 3f. Due to the small entrance size of the HMS, trypsin can only be adsorbed on the outer surface, and the proteolytic efficiency of the HMS-trypsin is only a little higher than in bulk solution (Figure 3d), but much lower than in the nanopores (Figure 3a). Thus, the nanoporous confinement is an important factor contributing to the high proteolytic efficiency of the CNS-trypsin, where enzymes and protein samples are trapped in the CNS nanochannels and the followed digestion occurs in confined zones.

The low efficiency of protein digestion at low concentrations is an unfavorable factor encountered in proteomics which arises from the inherent kinetic limitations of most proteases in solution.^[27] On the basis of the unique mesoscopic structure of CNS, the designed nanoreactor could not only largely reduce the digestion time, but also avoid enzymatic autolysis. In this case, trypsin may be attached to the inner walls of the mesochannels of CNS by electrostatic forces restricting its degrees of freedom and concomitant autolysis. With these advantages, trypsin-CNS can overcome the major challenge of digestion of proteins at low concentrations. Proteolysis is still efficient when the concentration of proteins is reduced to $2 \text{ ng}\mu\text{L}^{-1}$ (Figure 4a and b), with six peptides of myoglobin and six peptides of cytochrome c identified after a digestion time of 20 min.

Proteolysis of a complex sample, a protein mixture extracted from the cytoplasm of human liver tissue, was utilized to examine the practical feasibility of the system. After digestion for 20 min, the tryptic products resulting from the

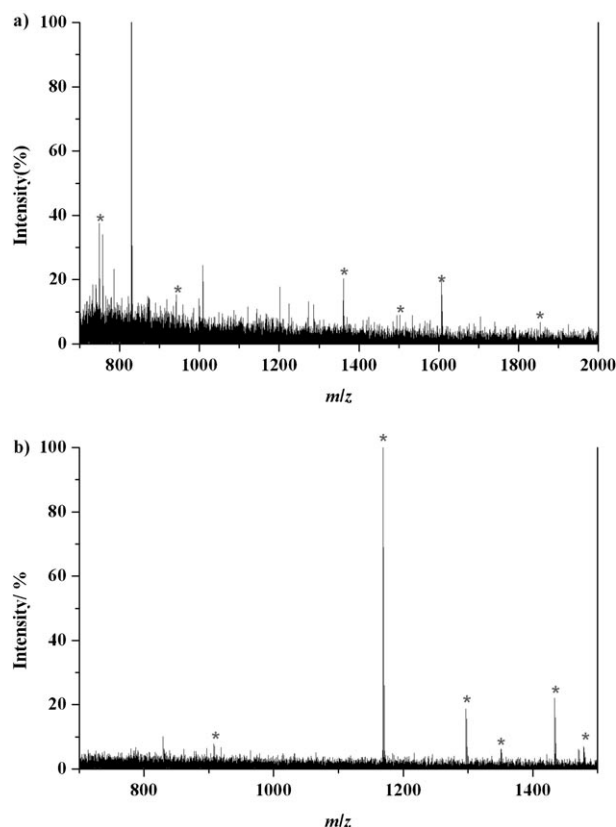


Figure 4. PMF spectra of myoglobin ((a) $2 \text{ ng}\mu\text{L}^{-1}$) and cytochrome c ((b) $2 \text{ ng}\mu\text{L}^{-1}$) proteolysis products from in-CNS-nanoreactor digestion (20 min).

digestion of $5 \mu\text{g}$ protein mixture were separated on a $\mu\text{-C}_{18}$ column followed by MALDI-TOF/TOF MS analysis. A total of 165 proteins were unambiguously identified, with each protein having at least two matched peptides (Supporting Information, Table S1). The molecular weight of the majority of the identified proteins was between 20000 and 200000 and their isoelectric points ranged from 5 to 10 (Figure 5), suggesting that this CNS-trypsin nanoreactor could be applied to a large range of substrates. Compared with the con-

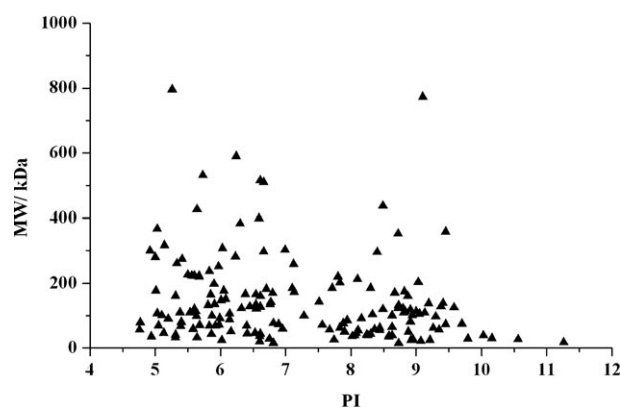


Figure 5. Plot of PI of the identified proteins as a function of molecular weight.

ventional in-solution digestion time of approximately 12 h, the trypsin-CNS reactor required only 20 min for the whole digestion procedure, indicating that the mesopore-based digestion protocol is a promising approach in the sensitive and comprehensive analysis of cellular protein mixtures.

Conclusion

In summary, a CNS-based reactor for proteolysis has been shown to be effective in proteomic analysis, with both nanoscale confinement and protein enrichment effects being responsible for the highly efficient proteolysis. Furthermore, when the concentration of protein was reduced to below micromolar levels, the CNS-based reactor could efficiently digest the substrates due to protein enrichment within the nanopores of CNS. Our findings also suggest that this reactor can be used for the detection of proteins from biological samples. This versatile system is a good example of proteolytic reactions occurring in mesoporous silicates, where CNS not only provides a support for the biocatalysts, but also acts as a nanoreactor to facilitate protein digestion.

Experimental Section

2-Cyanoethyltriethoxysilane (CEOS, 98%) and tetraethoxysilane (TEOS, 98%) were purchased from Lancaster. Cetyltrimethylammonium bromide (CTAB, 99%), *n*-hexadecylamine (98%), myoglobin (from horse heart, 95%), cytochrome *c* (from horse heart, 95%), trypsin (from bovine pancreas), α -cyano-4-hydroxycinnamic acid (CHCA, 99%), dithiothreitol (DTT 99%), iodoacetamide (IAA 99%), ammonium bicarbonate and potassium phosphate buffer were obtained from Sigma-Aldrich. Acetonitrile (ACN, 99.9%) and trifluoroacetic acid (TFA, 99.8%) were purchased from Merck. This base was obtained from Pharmacia Biotech. Ammonium hydroxide solution (25 \approx 28%), isopropanol (\geq 99.5%) and hydrochloric acid (36–38%) was obtained from Shanghai No.4 Reagent Company, Kunshan. All reagents were used as received without further purification. Deionized water (18.4 M Ω cm) used for all experiments was obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

Synthesis and characterization of MPS: CNS was synthesized as reported previously.^[6,7] CTBA (50 g) was mixed with water (450 g) and the mixture was heated to 30 °C until all the surfactant was dissolved. Then, TEOS (50 mL), CEOS (6.25 mL), and NH₄OH (2.5 mL) were added into the solution. After heating at 105 °C for 24 h, the mixture was refluxed in a solution containing 1.5% HCl and 2.5% H₂O in methanol to remove the surfactant until no change in mass was observed. The solid product was calcined (ramp rate 1 °C min⁻¹) and held at 650 °C for 6 h under a stream of air (100 cm³ min⁻¹). Hexagonal mesoporous silicate (HMS) was synthesized according to a published method.^[28] A homogenous solution was prepared by dissolving *n*-hexadecylamine in a solution of isopropanol (100 mL), H₂O (90 mL), and NH₄OH (1.4 mL, 28%). Then, TEOS (12 mL) was added, and the mixture was kept at ambient temperature overnight. The resulting solid was extensively washed with deionized water and was then calcined at 600 °C for 6 h to remove the surfactant.

Nitrogen gas adsorption/desorption isotherms were obtained by using a Micromeritics Gemini ASAP 2000 system. Samples were pretreated by heating at 150 °C under vacuum for 1 h to remove H₂O. The thermodynamics-based Barrett–Joyner–Halenda (BJH) method was used to analyze the pore size data by using the desorption branch of the isotherm and the Brunauer–Emmett–Teller (BET) method was employed to measure surface areas.

Enzyme adsorption: The CNS-trypsin nanoreactors were generated by mixing different amounts of trypsin (25–430 μ g trypsin on 1 mg CNS) with the CNS suspension (final concentration of 1 mg mL⁻¹, in potassium phosphate (25 mM), pH 6.5) in a beaker at room temperature for 16 h.^[6,7] The suspension was centrifuged to remove excess trypsin and was then washed (3 times) with NH₄HCO₃ (10 mM, pH 7.8) to remove externally or weakly bound trypsin.

Preparation of the human liver cytoplasm sample: Samples were obtained from the Human Liver Proteome Project (HLPP), which is one of the initiatives launched by the Human Proteome Organization (HUPO) and the first initiative on human tissues or organs.^[29] The human liver cytoplasm sample was dissolved in a buffer containing urea (8 M) and a mixture of protease inhibitors and phosphatase inhibitors (1 mM PMSF, 0.2 mM Na₂VO₃, and 1 mM NaF), and was then vortexed for 30 min. The suspension was centrifuged at 18000 g for 1 h (4 °C) and the extracted proteins in the supernatant were reduced with DTT (20 mM) at 37 °C for 30 min and then alkylated with IAA (25 mM) for an additional 30 min at room temperature in the dark. After these procedures, the samples were freeze dried and stored in a refrigerator before use.

Nanopore-based digestion: CNS-trypsin particles were first immersed in a solution (10 mM, NH₄HCO₃, pH 7.8) containing protein substrates (myoglobin or cytochrome *c*) at different concentrations (2–200 ng μ L⁻¹) for 20 min at 37 °C (the protease/substrate ratio was 1:3 (w/w)). The solution was centrifuged at 14000 rpm for 3 min. The supernatant was collected and directly used for subsequent MS analysis. The procedure for nanopore proteolysis of the liver samples was similar, with the exception that the concentration of protein was 1 μ g μ L⁻¹. For comparison, the proteins were digested in solution. Various amounts of myoglobin and cytochrome *c* were dissolved in ammonium bicarbonate buffer (10 mM, pH 7.8) and denatured at 100 °C for 5 min. The solutions were incubated for 12 h at 37 °C with trypsin at an enzyme/substrate ratio of 1:30 (w/w).

Capillary LC separation of nanopore-based digests of the biological sample: Capillary LC separation of the digest of the liver cytoplasm sample from the CNS-trypsin nanoreactor was performed by using an Agilent 1100 series capillary pumping system (DE, Germany) equipped with the AccuSpot microfraction collector for MALDI plate spotting. Samples (5 μ L) were injected and captured on a trap column (Vydac C₁₈, 5 μ m, 300 Å , 300 μ m i.d. \times 5 mm), were then eluted and separated on an analytical LC column (Vydac C₁₈, 5 μ m, 300 Å , 300 μ m i.d. \times 100 mm) by using 0.1% TFA in water as mobile phase A and 0.1% TFA in acetonitrile as mobile phase B. The gradient was fixed at 5% B for 10 min to elute salts in the sample, and then ramped linearly from 5 to 50% B over 30 min, then 50 to 75% B over the next 15 min, and 75 to 95% over the following 10 min. At the end, the solvent composition was returned to the start point in 5 min. The flow rate was 3 μ L min⁻¹. Automatic MALDI plate spotting commenced when the gradient change began, and the time interval between two drops was 24 s.

Sample preparation and identification: The digestion products were spotted on the MALDI sample plate (0.4 μ L for each drop) and dried at room temperature. Then, CHCA matrix (0.4 μ L) was dropped and dried under the same conditions. The CHCA matrix was a mixture (1:1 (v/v)) of diammonium citrate (0.4 mg) in a solution of ACN/H₂O/TFA (50/49.9/0.1% (v/v)) (1 mL) and CHCA (8 mg) in a solution of ACN/H₂O/TFA (50/49.9/0.1% (v/v)) (1 mL). Before sample identification, the MS instrument was calibrated in external calibration mode by using tryptic peptides of myoglobin. All tryptic digests were analyzed on an Applied Biosystems 4700 proteomics analyzer.

For standard proteins, proteolysis efficiency was evaluated by PMF (peptide-mass fingerprinting), and the spectrum of each spot was obtained by accumulation of 2000 laser shots by using a laser power set to 10% above the threshold for ion formation. For the liver sample, an MS/MS method (3800 laser shots per analysis, laser power set to 30% above the threshold for ion formation) was used, and only peaks with S/N ratios above 20 from the PMF spectrum were selected. GPS Explorer software from Applied Biosystems with Mascot as a search engine and SwissProt (Version 050303) as a database was employed for protein identification. The peptide mass tolerance was set to 80 ppm, and the tandem mass tolerance was set to 0.5 Da.

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- [1] H. L. Shen, G. Cheng, H. Z. Fan, J. Zhang, X. M. Zhang, H. J. Lu, C. L. Liu, F. X. Sun, H. Jin, X. J. Xu, G. B. Xu, S. Wang, C. Y. Fang, H. M. Bao, Y. Wang, J. Wang, H. Zhong, Z. I. Yu, Y. K. Liu, Z. Y. Tang, P. Y. Yang, *Proteomics* **2006**, *6*, 528–537.
- [2] Z. Y. Park, D. H. Russell, *Anal. Chem.* **2000**, *72*, 2667–2670.
- [3] K. Mukhopadhyay, S. Phadtare, V. P. Vinod, A. Kumar, M. Rao, R. V. Chaudhari, M. Sastry, *Langmuir* **2003**, *19*, 3858–3863.
- [4] X. Y. Yang, Z. Q. Li, B. Liu, A. K. Hofmann, G. Tian, Y. F. Feng, Y. Ding, D. S. Su, F. S. Xiao, *Adv. Mater.* **2006**, *18*, 410–414.
- [5] D. Goradia, J. Cooney, B. K. Hodnett, E. Magner, *Biotechnol. Prog.* **2006**, *22*, 116–123.
- [6] D. Goradia, J. Cooney, B. K. Hodnett, E. Magner, *J. Mol. Catal. B* **2005**, *32*, 231–239.
- [7] J. Deere, E. Magner, J. G. Wall, B. K. Hodnett, *J. Phys. Chem. B* **2002**, *106*, 7340–7347.
- [8] J. M. Gómez, J. Deere, D. Goradia, J. Cooney, E. Magner, B. K. Hodnett, *Catal. Today* **2003**, *88*, 183–186.
- [9] J. Deere, E. Magner, J. G. Wall, B. K. Hodnett, *Catal. Today* **2003**, *85*, 19–23.
- [10] I. Gill, A. Ballesteros, *J. Am. Chem. Soc.* **1998**, *120*, 8587–8598.
- [11] H. L. Wu, Y. P. Tian, B. H. Liu, H. J. Lu, X. Y. Wang, J. J. Zhai, H. Jin, P. Y. Yang, Y. M. Xu, H. H. Wang, *J. Proteome Res.* **2004**, *3*, 1201–1209.
- [12] J. Fan, W. Q. Shui, P. Y. Yang, X. Y. Wang, Y. M. Xu, H. H. Wang, X. Chen, D. Y. Zhao, *Chem. Eur. J.* **2005**, *11*, 5391–5396.
- [13] a) Y. H. Zhang, Y. Liu, J. L. Kong, P. Y. Yang, Y. Tang, B. H. Liu, *Small*, **2006**, *10*, 1170–1173; b) Y. Liu, W. Zhong, S. Meng, J. L. Kong, H. J. Lu, P. Y. Yang, H. H. Girault, B. H. Liu, *Chem. Eur. J.* **2006**, *12*, 6585–6591; c) Y. Liu, Y. Xue, J. Ji, X. Chen, J. L. Kong, P. Y. Yang, H. H. Girault, B. H. Liu, *Mol. Cell. Proteomics* **2007**, *6*, 1428–1436.
- [14] a) D. S. Peterson, T. Rohr, F. Svec, J. M. J. Frechet, *Anal. Chem.* **2002**, *74*, 4081–4088; b) D. S. Peterson, T. Rohr, F. Svec, J. M. J. Frechet, *J. Proteome Res.* **2002**, *1*, 563–568.
- [15] J. Fan, C. Z. Yu, F. Gao, J. Lei, B. Z. Tian, L. M. Wang, Q. Luo, B. Tu, W. Z. Zhou, D. Y. Zhao, *Angew. Chem.* **2003**, *115*, 3254–3258; *Angew. Chem. Int. Ed.* **2003**, *42*, 3146–3150.
- [16] Y. F. Zhu, J. L. Shi, W. H. Shen, X. P. Dong, J. W. Feng, M. L. Ruan, Y. S. Li, *Angew. Chem.* **2005**, *117*, 5213–5217; *Angew. Chem. Int. Ed.* **2005**, *44*, 5083–5087.
- [17] C. H. Lei, Y. S. Shin, J. Liu, E. J. Ackerman, *J. Am. Chem. Soc.* **2002**, *124*, 11242–11243.
- [18] S. Hudson, E. Magner, J. Cooney, B. K. Hodnett, *J. Phys. Chem. B* **2005**, *109*, 19496–19506.
- [19] S. Hudson, J. Cooney, B. K. Hodnett, E. Magner, *Chem. Mater.* **2007**, *19*, 2049–2055.
- [20] J. Deere, E. Magner, J. G. Wall, B. K. Hodnett, *Chem. Commun.* **2001**, *5*, 465–466.
- [21] J. F. Diaz, K. J. Balkus, *J. Mol. Catal. B* **1996**, *2*, 115–126.
- [22] K. J. Balkus, J. F. Diaz, *Abstr. Pap. Am. Chem. Soc.* **1996**, *212*, 231-INOR.
- [23] G. T. P. Saccone, J. D. Skinner, L. A. Burgoyne, *FEBS Lett.* **1983**, *1*, 111–114.
- [24] S. Feng, M. Ye, X. Jiang, W. Jin, H. Zou, *J. Proteome Res.* **2006**, *5*, 422–428.
- [25] H. Jiang, H. Zou, H. Wang, J. Ni, Q. Zhang, Y. J. Zhang, *J. Chromatogr. A* **2000**, *903*, 77–84.
- [26] P. Atkins, *Physical Chemistry*, 6th ed., Freeman, New York, **1998**.
- [27] D. Craft, A. Doucette, L. Li, *J. Proteome Res.* **2002**, *1*, 537–547.
- [28] M. Grun, C. Buchel, D. Kumar, K. Schumacher, B. Bidlingmaier, K. K. Unger, *Stud. Surf. Sci. Catal.* **2000**, *128*, 155–165.
- [29] F. C. He, *Mol. Cell. Proteomics* **2005**, *4*, 1841–1848.

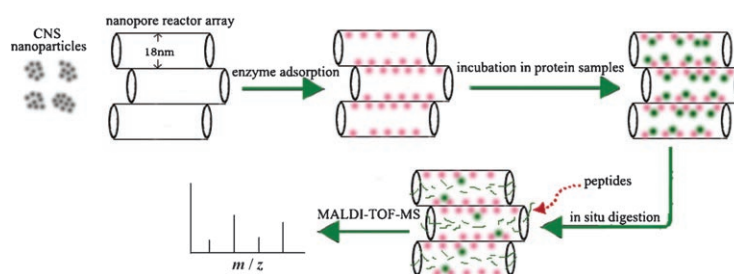
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VIP A Nanoporous Reactor for Efficient
Proteolysis



Prot(e)o-types: Mesoporous silicates with cyano functional groups for accommodating trypsin have been proposed as a nanopore-based bioreactor

to favor highly efficient protein digestion inside the mesochannels (see graphic).