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A Selective Artificial Enzyme Inhibitor Based on Nanoparticle-Enzyme Interactions and Molecular Imprinting

Cheng Zheng, Xiao-Long Zhang, Wei Liu, Bo Liu, Huang-Hao Yang, Zi-An Lin,* and Guo-Nan Chen*

Enzymes, as super-catalysts, play prominent roles in regulation of numerous important biological processes, including cellular processes and metabolic exchange.[1–3] The discovery of efficient enzyme inhibitors as drug candidates is of critical relevance to be exploitable for disease treatment.[4–7] Nowadays, small-molecule enzyme inhibitors account for a large scale of the pharmaceutical market.[8,9] A common problem with conventional small inhibitors, however, is that they not only inhibit the target enzyme but may also block other enzymes, or act indiscriminately on healthy and sick cells. For the purpose to minimize unwanted side effects and quest for selective enzyme inhibition, this field has been further advanced by the recent discovery of several selective chemical inhibitors, e.g., octahedral metal complexes,[10] polymetallic clusters,[11] carboranes[12] and DNA aptamers.[13] Although substantial progress has been made, the generality of producing such selective chemical inhibitors remains a problem since the refined X-ray crystal structures of the target enzymes always should be known. Furthermore, different designs are required for different enzymes. Also, the preparation processes of such selective chemical inhibitors are relatively complicated and time-consuming.

Rapid advances in nanotechnology have demonstrated that nanoparticles are attractive biomaterials due to their unique properties such as simple and low-cost fabrication, size comparability and biocompatibility.[14–17] In recent years, several nanoparticles are found to be potent enzyme inhibitors, such as carbon nanotubes,[18] graphene oxide,[19] gold nanoparticles,[20,21] dendrimers[22] and amphiphilic polymer nanoparticles.[23] Unfortunately, such nanoparticle inhibitors may not be developed as drug candidates due to their poor target-selectivity. Since the enzyme inhibition is mostly attributed to the electrostatically driven interactions between nanoparticles and enzymes, nanoparticle inhibitors could not discriminate the enzymes having the same charge.

Molecular imprinting technology (MIT) has been accepted as a cost-effective approach to synthesize artificial antibodies with specific molecular recognition properties of the target molecules,[24–27] including low molecular weight compounds[28–33] and biological macromolecules.[34–36] Currently, molecular imprinted polymers (MIPs) used for the recognition and detection of proteins in biological samples have attracted much attention.[37,38] Up to now, some remarkable achievements have been realized to prepare protein-imprinted polymers, which have wide-ranging applications in separation,[39–42] biosensors,[43–45] mimetic enzymes,[46] protein crystallization[47] and enzyme inhibitors.[48,49] Haupt et al. pioneered MIP microgels as enzyme inhibitor by employing a known small-molecule enzyme inhibitor as functional monomer.[48] The inhibition constant of MIP microgels is almost three orders of magnitude lower than that of the free small-molecule inhibitor. However, a known small-molecule enzyme inhibitor for the target enzyme should be required, which may limit the general applicability of the strategy. In this work, we combined nanoparticle-enzyme interactions with molecular imprinting for the first time and described a novel and general strategy to design selective artificial enzyme inhibitor.

Herein, we have chosen the protease α-chymotrypsin (ChT) as the target enzyme. ChT, whose active site is surrounded by some positive residues,[50] is known as a suitable enzyme for studying the biomacromolecule surface recognition owning to its well-characterized geometry and associated enzymatic activity. In previous report, carbon nanotubes can inhibit the activity of ChT through electrostatic interactions with the cationic patch around the enzyme active site.[51] Dopamine (DA) is a melanin-like mimic of mussel adhesive proteins. It has been reported that self-polymerization of DA can produce a surface-adherent polydopamine (PDA) nanolayer deposited on the surface of multifarious materials at weak alkaline pH.[52] Nowadays, PDA has been successfully implemented for surface modification of nanomaterials[53,54] and surface molecular imprinting[29,55] due to its high stability, hydrophilicity and biocompatibility. **Scheme 1**a describes the process flow of the experiment we conducted to synthesize ChT-selective inhibitor. As template molecule, ChT was firstly physically adsorbed on the surface of carboxylic acid-functionalized multiwalled carbon nanotubes (MWCNT) through electrostatic interactions in buffered solution to form the MWCNT-ChT complex. Then an adherent PDA layer was coated on the surface of MWCNT via polymerization of DA using oxidizing agent under neutral pH conditions. Finally, MWCNT-MIP was obtained after the removal of the embedded enzyme molecules.

C. Zheng, X.-L. Zhang, W. Liu, B. Liu, Prof. H.-H. Yang, Prof. Z.-A. Lin, Prof. G.-N. Chen
The Key Lab of Analysis Technology for Food Safety of the MOE, College of Chemistry and Chemical Engineering, Fuzhou University
Fuzhou, 350108, P. R. China
E-mail: hhyang@fio.org.cn; zianlin@fzu.edu.cn

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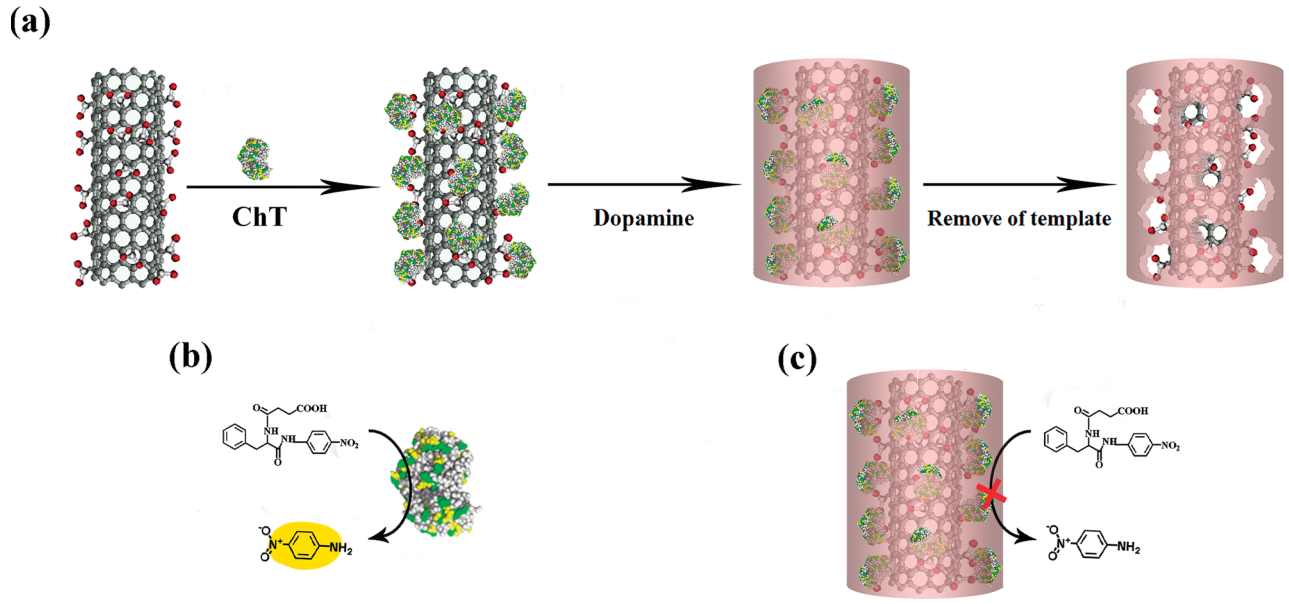

Scheme 1. (a) Schematic illustration of MWCNT-MIP as ChT-selective inhibitor prepared via surface molecular imprinting. (b) Enzymatic hydrolysis of the chromogenic substrate by free ChT. (c) Inhibition of the adsorbed ChT by MWCNT-MIP.

In this case, MWCNT serve as not only a support material for the preparation of the MIP layer, but also as functional monomer to participate in binding the active sites of template ChT molecules and generating the recognition cavities. Due to the creation of specific binding cavities, the resulting MWCNT-MIP would achieve the selective recognition as well as the selective activity inhibition of ChT over other biological macromolecules (Scheme 1b,c).

The surface morphology of MWCNT and the as-synthesized MWCNT-MIP were characterized with transmission electron microscopy (TEM). The average diameter of MWNT was about 20 nm (**Figure 1**a). And a grey PDA layer with well-defined configuration and homogeneity was readily observed on the MWCNT surface as shown in the TEM image of MWCNT-MIP (Figure 1b). The high-resolution TEM images shown in insets reveal that the thickness of the PDA layer was about 4 nm.

The X-ray photoelectron spectroscopy (XPS) was employed to further ascertain the formation of the PDA layer. Figure 1c,d displays XPS survey spectra of MWCNT before and after coating a PDA layer. It can be observed that the N1s peak appeared at about 400.16 eV in the spectrum of MWCNT-MIP (Figure 1d), which is attributed to the nitrogen element of amines in the dopamine. While there was no nitrogen signal detected for MWCNT (Figure 1c). Thermogravimetric analysis (TGA) was performed on the basis of the different thermal stability between MWCNT and PDA layer. From the TGA results (see Supporting Information, Figure SI-1), MWCNT was thermally stable up to 600 °C with negligible weight change, yet MWCNT-MIP showed a significant weight loss below 600 °C because of the thermal degradation of the PDA layer. The zeta-potential measurements indicated that MWCNT exhibited a zeta-potential of about –60.3 mV at pH 7.4. After imprinting, the zeta-potential of MWCNT-MIP was increased to –40.0 mV. The results above support that a uniform and nanoscale PDA layer is successfully polymerized and anchored on the surface of MWCNT.

To evaluate the imprinting effect of MIPs, the binding isotherm is often carried out to get the imprinting factor (IF) and the specific adsorption capacity. The adsorption isotherms of MWCNT-MIP and control MWCNT-NIP were investigated by a batch binding approach with different initial concentrations of ChT molecules (**Figure 2**a). The adsorption capacity of ChT molecules to MWCNT-MIP increased with the increasing of the initial ChT concentration and came to equilibrium over 0.6 mg mL^{-1}. Nevertheless, only weak adsorption of ChT molecules to MWCNT-NIP was observed, which may be the result of nonspecific interactions between the polymer matrix and ChT molecules. Under this condition, the IF, which is defined as the ratio of binding capacity of the MIPs with respect to that of the NIPs, is about 6. It confirms that MWCNT-MIP has high affinity for ChT molecules. Moreover, a good-linear curve fitting of a single-site Langmuir-type binding isotherm for MWCNT-MIP is shown in inset using Scatchard plot equation. According to the Scatchard analysis, the equilibrium dissociation constant K_d and the maximum number of binding cavities B_{max} are calculated to be 3.65 μM and 134.67 μmol g^{-1}, respectively. The above data imply that highly dense recognition cavities are successfully formed in the imprinted polymer layer by molecular imprinting. From the binding isotherm of ChT on MWCNT (see Supporting Information, Figure SI-2), MWCNT exhibited higher adsorption capacity of ChT molecules than MWCNT-MIP.

The binding kinetics given in Figure 2b describes the time-dependent evolution of the ChT amount bound by MWCNT-MIP and MWCNT-NIP. MWCNT-MIP reached the maximum adsorption capacity within 1 h, revealing a rapid adsorption of ChT molecules into MWCNT-MIP. Given the fact that the thickness of the PDA layer is comparable to the hydrodynamic radius of ChT (2.5–2.8 nm),[56,57] template ChT molecules are

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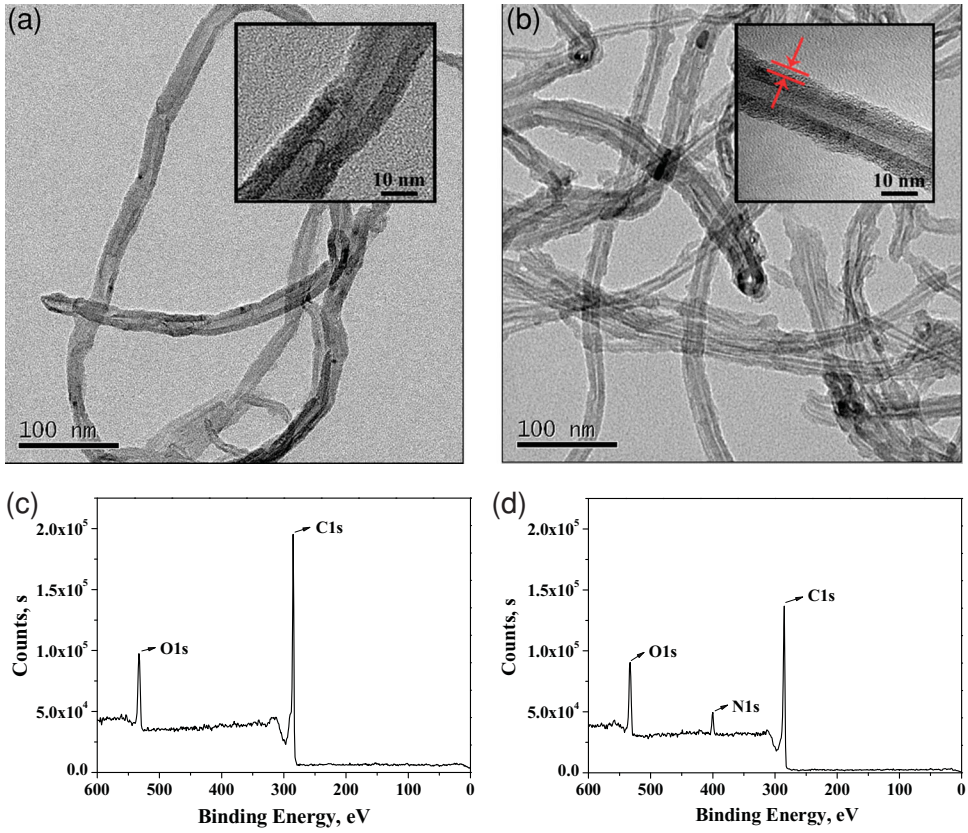

Figure 1. TEM images of MWCNT (a) and MWCNT-MIP (b), respectively. The insets show the high-resolution TEM images, which indicate that the MIP layer was uniform and nanoscale. XPS patterns of MWCNT (c) and MWCNT-MIP (d), respectively. A distinct N1s peak was observed in the spectrum of MWCNT-MIP.

presumed to be not fully encapsulated in the PDA layer. So, the ease of template removal as well as rebinding can be improved. It is also implied that there should be monolayer coverage of the recognition cavities on the MWCNT surface. Furthermore, MWCNT participates in binding the active sites of template ChT molecules in the conduction of the molecular recognition cavities. So in the rebinding process, the active sites of the adsorbed ChT molecules are presumed to direct towards the MWCNT surface. As a result, the activity of the adsorbed ChT molecules would be inhibited.

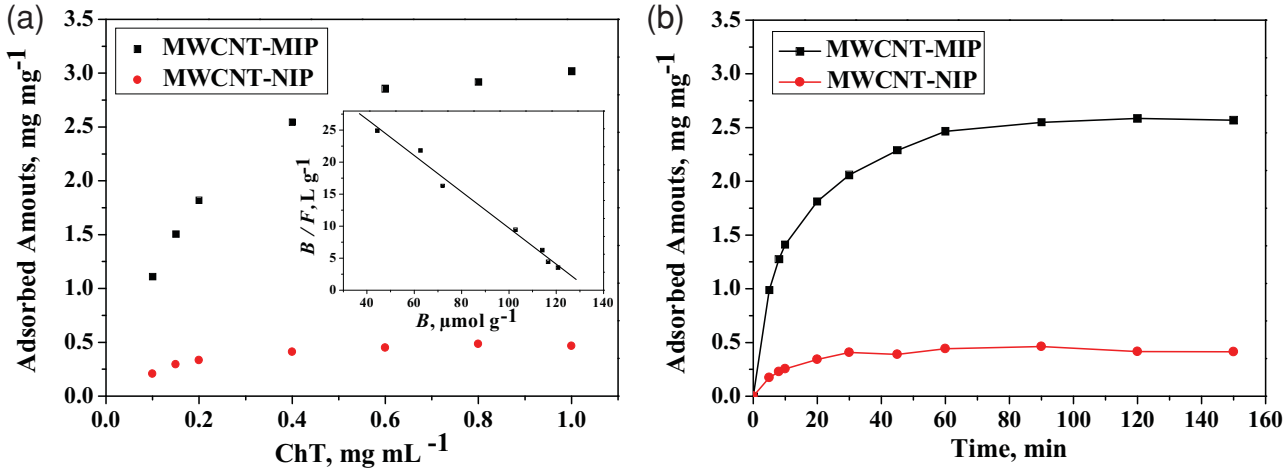

Figure 2. (a) Binding isotherms of ChT (0.1–1.0 mg mL^{-1}) on MWCNT-MIP (■) and MWCNT-NIP (●). The inset shows Scatchard analysis of the binding capacity of ChT to MWCNT-MIP. (b) Binding kinetics of ChT (0.4 mg mL^{-1}) on MWCNT-MIP (■) and MWCNT-NIP (●).

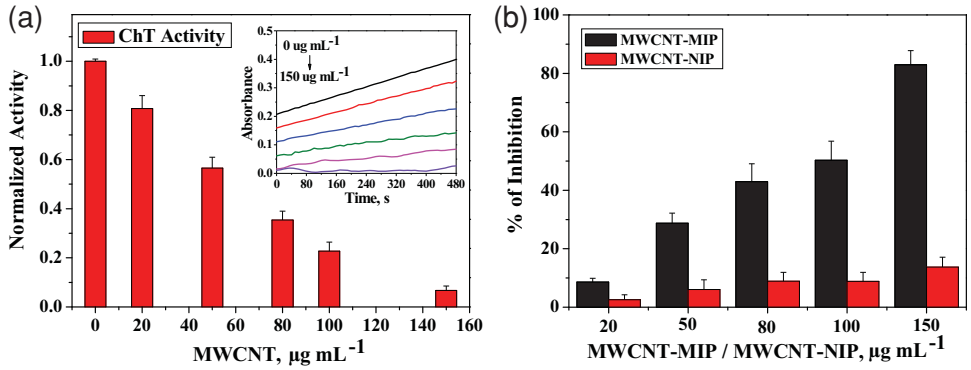

Figure 3. (a) Activity of ChT (500 μg mL^{-1}) plotted as a function of MWCNT concentration. The inset shows UV-vis absorbance change of the system at 405 nm with the time after incubation with different concentrations of MWCNT (0–150 μg mL^{-1}). (b) Degrees of inhibition of the ChT activity after incubating ChT (500 μg mL^{-1}) with different concentrations of MWCNT-MIP and MWCNT-NIP (0–150 μg mL^{-1}). The activities were normalized to that of free ChT. The data shown here represented the means and standard deviations of three independent experiments.

In general, ChT hydrolyses polypeptides at the carboxyl-side of a Tyr, Trp or Phe residue. The ChT activity is commonly detected with chromogenic substrate, N-succinyl-L-phenylalanine-p-nitroanilide (SPNA), to provide color readout. The enzyme-inhibition experiment was performed to evaluate the inhibition efficiency of MWCNT by the ChT-catalyzed hydrolysis of SPNA after incubating ChT with various concentrations of MWCNT. As shown in **Figure 3**a, the rate of enzymatic hydrolysis decreased upon addition of MWCNT, and the strongest inhibitory potency was around 92% ChT activity suppression at a MWCNT concentration of 150 μg mL^{-1}.

In the case of MWCNT-MIP (Figure 3b), the inhibition tendency was similar to that of MWCNT. The ChT activity was profoundly affected by MWCNT-MIP with a loss of 85% activity at concentrations up to 150 μg mL^{-1}. On the other hand, the activity of ChT only decreased 13% when the dosage of MWCNT-NIP increased to 150 μg mL^{-1}. It is apparent that MWCNT-MIP provides much higher inhibition efficiency than MWCNT-NIP at equivalent dose. It is attributed to the recognition cavities within MIP layer created by template ChT molecules. On the contrary, NIP layer without recognition cavities could prevent ChT molecules to attach to the MWCNT surface. Therefore, inhibitory potency of MWCNT-NIP is low. In the meantime, MWCNT-MIP has higher inhibitory potency in comparison with the reported MIP microgels.[48] We attribute the higher inhibitory potency of MWCNT-MIP to two features. First, MWCNT-MIP has good site accessibility towards the target enzyme via surface molecular imprinting. Second, nanoscale MWCNT-MIP has extremely high surface-to-volume ratio, which provides larger enzyme binding capacity and higher inhibition efficiency.

High target-selectivity has been regarded as one of the most fascinating properties of MIPs. Four non-template proteins with the different isoelectric points (pI) and molecular weights (Mw), namely, trypsin, cytochrome c (Cyt), bovine serum albumin (BSA), myoglobin (Mb), were subjected as the controls to study the binding selectivity of MWCNT-MIP. It is seen from **Figure 4**a that MWCNT-MIP exhibited a high adsorption capacity of ChT molecules. However, the binding capacity of other proteins to MWCNT-MIP was very low. Based on the above results, it is demonstrated that MWCNT-MIP has considerably high selectivity towards ChT molecules, which involves multiple weak interactions provided by functional monomers and stereo-shape complementarities.

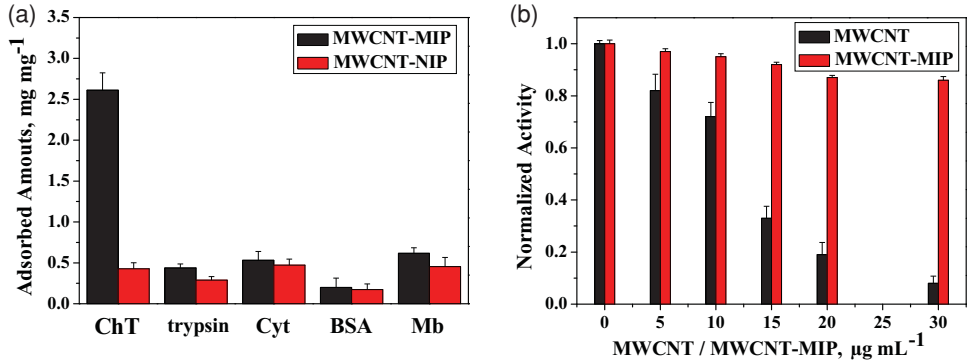

Figure 4. (a) Selective adsorption of ChT (Mw = 25,000, pI = 9.1), compared with trypsin (Mw = 23 800, pI = 10.5), Cyt (Mw = 12 400, pI = 10.2), BSA (Mw = 66 000, pI = 4.8), Mb (Mw = 17 600, pI = 7.1), on MWCNT-MIP and MWCNT-NIP. (b) Activity of trypsin (15 μg mL^{-1}) plotted as a function of MWCNT and MWCNT-MIP concentrations (0–30 μg mL^{-1}). The activities were normalized to that of free trypsin. The above data shown here represented the means and standard deviations of three independent experiments.

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After the selective molecular recognition property of MWCNT-MIP was confirmed, its selective inhibitory response for ChT was further investigated. Trypsin was selected as a control enzyme. Trypsin is also a serine protease with similar molecular weight and isoelectric point compared to ChT. Some basic residues, for example, Lys^{97}, Lys^{75}, Arg^{62} and Arg^{96}, distribute over the trypsin surface.[58] Obviously, MWCNT could deactivate trypsin (Figure 4b). In marked contrast, MWCNT-MIP only showed slight inhibitory activity towards trypsin. This observation can be explained by the fact that the recognition cavities within MIP layer do not fit the size and shape of trypsin. The inhibition studies above expressly verify that the selective feature of the nanoparticle inhibitor is significantly improved when combined with molecular imprinting.

In conclusion, we have developed a novel type of selective artificial enzyme inhibitors based on nanoparticle-enzyme interactions and molecular imprinting. Nanoparticles, such as MWCNT, are considered as non-selective inhibitors towards enzymes, given that the electrostatic interactions are mainly contributed to the enzyme inhibition. By taking advantage of the selective molecular recognition property of MIPs, MWCNT-MIP has been proved to exhibit significantly enhanced selectivity of the enzyme inhibition compared with MWCNT itself. Our approach appears to be more general than that for recently reported selective inhibitors. It requires only a proper nanoparticle inhibitor for the target enzyme, and could be easily expanded to other enzymes by changing the surface modifications of nanoparticles. Moreover, nanoparticle-MIP can be simply fabricated by coating a nanoscale MIP layer on the surface of nanoparticle. And there is no requirement for the precise knowledge of the crystal structure of the target enzyme. Additionally, several biodegradable nanoparticles, such as ZnO quantum dots and SiO_2, can be also developed as nanoparticle-MIP inhibitors to overcome the problem of the potential toxicity of carbon nanotubes. Taken together, these features suggest that the proposed approach would represent a novel and general way to pursue selective enzyme inhibition. Meanwhile, it has implications for the future design and development of new enzyme inhibitors as drug candidates for clinical therapeutics.

Experimental Section

Materials, details on synthesis of MWCNT-MIP, procedures for binding and activity assays are included in the Supporting Information.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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[1] M. Uttamchandani, C. H. S. Lu, S. Q. Yao, *Acc. Chem. Res.* **2009**, *42*, 1183.
[2] B. A. Gibson, W. L. Kraus, *Nat. Rev. Mol. Cell Biol.* **2012**, *13*, 411.
[3] K. Marschner, K. Kollmann, M. Schweizer, T. Braulke, S. Pohl, *Science* **2011**, *333*, 87.
[4] A. G. Kazantsev, L. M. Thompson, *Nat. Rev. Drug Discov.* **2008**, *7*, 854.
[5] P. Workman, P. A. Clarke, F. I. Raynaud, R. L. M. van Montfort, *Cancer Res.* **2010**, *70*, 2146.
[6] E. Meggers, *Angew. Chem. Int. Ed.* **2011**, *50*, 2442.
[7] C. Bailly, *Chem. Rev.* **2012**, *112*, 3611.
[8] S. Touchet, F. Carreaux, B. Carboni, A. Bouillon, J.-L. Boucher, *Chem. Soc. Rev.* **2011**, *40*, 3895.
[9] T. M. Gloster, D. J. Vocadlo, *Nat. Chem. Biol.* **2012**, *8*, 683.
[10] L. Feng, Y. Geisselbrecht, S. Blanck, A. Wilbuer, G. E. Atilla-Gokcumen, P. Filippakopoulos, K. Kräling, M. A. Celik, K. Harms, J. Maksimoska, R. Marmorstein, G. Frenking, S. Knapp, L.-O. Essen, E. Meggers, *J. Am. Chem. Soc.* **2011**, *133*, 5976.
[11] E. L.-M. Wong, R. W.-Y. Sun, N. P.-Y. Chung, C.-L. S. Lin, N. Zhu, C.-M. Che, *J. Am. Chem. Soc.* **2006**, *128*, 4938.
[12] P. Farràs, E. J. Juàrez-Pérez, M. Lepšík, R. Luque, R. Núñez, F. Teixidor, *Chem. Soc. Rev.* **2012**, *41*, 3445.
[13] Y. Kim, J. A. Phillips, H. Liu, H. Kang, W. Tan, *Proc. Natl. Acad. Sci. USA.* **2009**, *106*, 6489.
[14] J.-M. Nam, C. S. Thaxton, C. A. Mirkin, *Science* **2003**, *301*, 1884.
[15] Z. J. Deng, M. Liang, M. Monteiro, I. Toth, R. F. Minchin, *Nat. Nanotechnol.* **2011**, *6*, 39.
[16] M. Mahmoudi, I. Lynch, M. R. Ejtehadi, M. P. Monopoli, F. B. Bombelli, S. Laurent, *Chem. Rev.* **2011**, *111*, 5610.
[17] M. Swierczewska, G. Liu, S. Lee, X. Chen, *Chem. Soc. Rev.* **2012**, *41*, 2641.
[18] B. Zhang, Y. Xing, Z. Li, H. Zhou, Q. Mu, B. Yan, *Nano Lett.* **2009**, *9*, 2280.
[19] M. De, S. S. Chou, V. P. Dravid, *J. Am. Chem. Soc.* **2011**, *133*, 17524.
[20] C.-C. You, M. De, G. Han, V. M. Rotello, *J. Am. Chem. Soc.* **2005**, *127*, 12873.
[21] O. R. Miranda, H.-T. Chen, C.-C. You, D. E. Mortenson, X.-C. Yang, U. H. F. Bunz, V. M. Rotello, *J. Am. Chem. Soc.* **2010**, *132*, 5285.
[22] A. Klaikherd, B. S. Sandanaraj, D. R. Vutukuri, S.Thayumanavan, *J. Am. Chem. Soc.* **2006**, *128*, 9231.
[23] B. S. Sandanaraj, D. R. Vutukuri, J. M. Simard, A. Klaikherd, R. Hong, V. M. Rotello, S. Thayumanavan, *J. Am. Chem. Soc.* **2005**, *127*, 10693.
[24] G. Wulff, A. Sarhan, *Angew. Chem.* **1972**, *84*, 364; *Angew. Chem. Int. Ed. Engl.* **1972**, *11*, 341.
[25] G. Vlatakis, L. I. Andersson, R. Müller, K. Mosbach, *Nature* **1993**, *361*, 645.
[26] L. Ye, K. Mosbach, *Chem. Mater.* **2008**, *20*, 859.
[27] L. Chen, S. Xu, J. Li, *Chem. Soc. Rev.* **2011**, *40*, 2922.
[28] H.-J.Wang, W.-H. Zhou, X.-F. Yin, Z.-X. Zhuang, H.-H. Yang, X.-R. Wang, *J. Am. Chem. Soc.* **2006**, *128*, 15954.
[29] R. Ouyang, J. Lei, H. Ju, Y. Xue, *Adv. Funct. Mater.* **2007**, *17*, 3223.
[30] M. Riskin, R. Tel-Vered, O. Lioubashevski, I. Willner, *J. Am. Chem. Soc.* **2009**, *131*, 7368.
[31] H.-F. Wang, Y. He, T.-R. Ji, X.-P. Yan, *Anal. Chem.* **2009**, *81*, 1615.

[32] G. Pan, Y. Zhang, Y. Ma, C. Li, H. Zhang, *Angew. Chem. Int. Ed.* **2011**, *50*, 11731.
[33] P. Çakir, A. Cutivet, M. Resmini, B. T. S. Bui, K. Haupt, *Adv. Mater.* **2013**, *25*, 1048.
[34] Y. Hoshino, H. Koide, T. Urakami, H. Kanazawa, T. Kodama, N. Oku, K. J. Shea, *J. Am. Chem. Soc.* **2010**, *132*, 6644.
[35] S. Shinde, A. Bunschoten, J. A. W. Kruijtzer, R. M. J. Liskamp, B. Sellergren, *Angew. Chem. Int. Ed.* **2012**, *51*, 8326.
[36] K. Ren, R. N. Zare, *ACS Nano* **2012**, *6*, 4314.
[37] Y. Ge, A. P. T. Turner, *Trends Biotechnol.* **2008**, *26*, 218.
[38] M. J. Whitcombe, I. Chianella, L. Larcombe, S. A. Piletsky, J. Noble, R. Porter, A. Horgan, *Chem. Soc. Rev.* **2011**, *40*, 1547.
[39] L. Qin, X.-W. He, W. Zhang, W.-Y. Li, Y.-K. Zhang, *Anal. Chem.* **2009**, *81*, 7206.
[40] A. Menaker, V. Syritski, J. Reut, A. Öpik, V. Horváth, R.E. Gyurcsányi, *Adv. Mater.* **2009**, *21*, 2271.
[41] S.-H. Lee, Y. Hoshino, A. Randall, Z. Zeng, P. Baldi, R. Doong, K. J. Shea, *J. Am.Chem. Soc.* **2012**, *134*, 15765.
[42] J. Bognár, J. Szücs, Z. Dorkó, V. Horváth, R. E. Gyurcsányi, *Adv. Funct. Mater.* DOI: 10.1002/adfm.201300113.
[43] D. Cai, L. Ren, H. Zhao, C. Xu, L. Zhang, Y. Yu, H. Wang, Y. Lan, M. F. Roberts, J. H. Chuang, M. J. Naughton, Z. Ren, T. C. Chiles, *Nat. Nanotechnol.* **2010**, *5*, 597.
[44] G. Lautner, J. Kaev, J. Reut, A. Öpik, J. Rappich, V. Syritski, R. E. Gyurcsányi, *Adv. Funct. Mater.* **2011**, *21*, 591.
[45] D. Dechtrirat, K. J. Jetzschmann, W. F. M. Stöcklein, F. W. Scheller, N. Gajovic-Eichelmann, *Adv. Funct. Mater.* **2012**, *22*, 5231.
[46] Z. Chen, L. Xu, Y. Liang, M. Zhao, *Adv. Mater.* **2010**, *22*, 1488.
[47] E. Saridakis, S. Khurshid, L. Govada, Q. Phan, D. Hawkins, G. V. Crichlow, E. Lolis, S. M. Reddy, N. E. Chayen, *Proc. Natl. Acad. Sci. USA.* **2011**, *108*, 11081.
[48] A. Cutivet, C. Schembri, J. Kovensky, K. Haupt, *J. Am. Chem. Soc.* **2009**, *131*, 14699.
[49] Y.-J. Liao, Y.-C. Shiang, C.-C. Huang, H.-T. Chang, *Langmuir* **2012**, *28*, 8944.
[50] H. Tsukada, D. M. Blow, *J. Mol. Biol.* **1985**, *184*, 703.
[51] S. S. Karajanagi, A. A. Vertegel, R. S. Kane, J. S. Dordick, *Langmuir* **2004**, *20*, 11594.
[52] H. Lee, S. M. Dellatore, W. M. Miller, P. B. Messersmith, *Science* **2007**, *318*, 426.
[53] S. M. Kang, S. Park, D. Kim, S. Y. Park, R. S. Ruoff, H. Lee, *Adv. Funct. Mater.* **2011**, *21*, 108.
[54] Q. Ye, F. Zhou, W. Liu, *Chem. Soc. Rev.* **2011**, *40*, 4244.
[55] W.-H. Zhou, C.-H. Lu, X.-C. Guo, F.-R. Chen, H.-H. Yang, X.-R. Wang, *J. Mater. Chem.* **2010**, *20*, 880.
[56] M. De, C.-C. You, S. Srivastava, V. M. Rotello, *J. Am. Chem. Soc.* **2007**, *129*, 10747.
[57] B. J. Jordan, R. Hong, G. Han, S. Rana, V. M. Rotello, *Nanotechnology* **2009**, *20*, 434004.
[58] R. Huber, W. Bode, *Acc. Chem. Res.* **1978**, *11*, 114.

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