

## Highly Specific Dual Enzyme-Mediated Payload Release from Peptide-Coated Silica Particles

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**Abstract:** Stimuli-responsive gate mechanisms offer potential for the controlled passage of payload molecules from a porous carrier vehicle on-demand. We describe a method for the enzyme-mediated release of macromolecular guest molecules from inorganic silica particles coated with a bioactive peptide shell, synthesized precisely by Fmoc chemistry. Specific enzymatic hydrolysis of the peptide shell removes the bulky peptide-terminated Fmoc groups, permitting the selective release of previously entrapped guest molecules.

### Introduction

The physical properties of stimuli-responsive materials may be modified when triggered by a targeted external stimulus.<sup>1</sup> Enzyme-responsive materials (ERMs) are a class of responsive material that are expected to play a key role in a number of biomedical applications such as regenerative medicine, medical diagnostics, and drug delivery due to their high selectivity and biocompatibility.<sup>2</sup> In this instance, the material properties are altered when reacted with target enzyme molecules. Enzymes offer key advantages as release triggers; they are not biologically disruptive, function under mild conditions, and possess a high degree of selectivity.

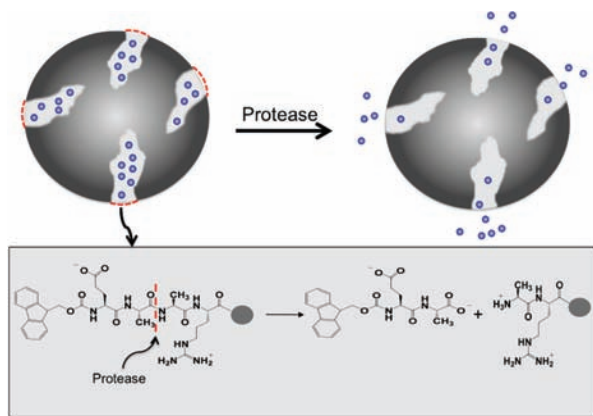
The use of ERMs for the selective release of therapeutic agents has gained considerable attention in recent years.<sup>3</sup> Such systems offer spatiotemporal control of drug release triggered by particular enzymes, or combinations of enzymes, which are (over)expressed in affected regions in most disease states.<sup>4</sup> Common approaches have placed emphasis upon the release of covalently attached drug molecules from prodrug carriers through selective enzymatic hydrolysis reactions.<sup>5</sup> In an alterna-

tive approach, the release of noncovalently encapsulated guest molecules has also been successfully demonstrated, offering a release mechanism in which the release rate is less dependent on the rate of enzyme activity, and chemical modification of the therapeutic agent is not required.<sup>6</sup> In an example of the latter approach, fluorenylmethoxycarbonyl (Fmoc) chemistry was utilized to generate specific zwitterionic peptide sequences that, when successfully cleaved by a target enzyme, developed a net charge, prompting the hydrogel to swell through the electrostatic repulsion between adjacent charged groups. While results were highly promising, the release mechanism is only possible using hydrogel polymers with the capability to swell/collapse upon charge generation/removal. We therefore propose an alternative enzyme-responsive biogate mechanism which maintains material response specificity, chemical simplicity, and biocompatibility, with potential to be readily applied to a range of porous carriers.

Gated release mechanisms have recently been reported that offer the selective release of guest molecules upon changes in local pH, temperature, light and chemical environment.<sup>7</sup> In addition, novel mechanisms that employ enzymes to trigger the opening of a molecular gate have been devised and demonstrated with some encouraging results being generated.<sup>8</sup> We report a novel enzyme-mediated release mechanism that takes advantage of well understood, and chemically straightforward, Fmoc chemistry to generate specific gatekeepers from a selection of the 20 natural amino acids. Highly selective payload release of noncovalently encapsulated unmodified macromolecules is

- (1) (a) Mohammed, J. S.; Murphy, W. L. *Adv. Mater.* **2009**, *21*, 2361. (b) Hendrickson, G. R.; Lyon, L. A. *Soft Matter* **2009**, *5*, 29. (c) Mano, J. F. *Adv. Eng. Mater.* **2008**, *10*, 515. (d) Hempenius, M. A.; Cirmi, C.; Song, J.; Vancso, G. J. *Macromolecules* **2009**, *42*, 2324. (e) Cai, K.; Luo, Z.; Hu, Y.; Chen, X.; Liao, Y.; Yang, L.; Deng, L. *Adv. Mater.* **2009**, *21*, 4045. (f) Banerjee, S. S.; Chen, D. H. *Nanotechnology* **2009**, *20*, 185103. (g) Alexander, C. *Exp. Opin. Drug Delivery* **2006**, *3*, 573. (h) Tang, L. Y.; Wang, Y. C.; Li, Y.; Du, J. Z.; Wang, J. *Bioconjugate Chem.* **2009**, *20*, 1095. (i) Bysell, H.; Schmidtchen, A.; Malmsten, M. *Biomacromolecules* **2009**, *10*, 2162.
- (2) (a) Ulijn, R. V. *J. Mater. Chem.* **2006**, *16*, 2217. (b) Anderson, D. G.; Burdick, J. A.; Langer, R. *Science* **2004**, *305*, 1923. (c) Alexander, C.; Shakesheff, K. M. *Adv. Mater.* **2006**, *18*, 3321. (d) Duxbury, C. J.; Hilker, I.; de Wildeman, S. M. A.; Heise, A. *Angew. Chem., Int. Ed.* **2007**, *46*, 8452. (e) Yang, Z.; Liang, G.; Wang, L.; Xu, B. *J. Am. Chem. Soc.* **2006**, *128*, 3038. (f) Yang, Z.; Ma, M.; Xu, B. *Soft Matter* **2009**, *5*, 2546. (g) Todd, S. J.; Scurr, D. J.; Gough, J. E.; Alexander, M. R.; Ulijn, R. V. *Langmuir* **2009**, *25*, 7533. (h) Thornton, P. D.; McConnell, G.; Ulijn, R. V. *Chem. Commun.* **2005**, *47*, 5913.
- (3) (a) Kumashiro, Y.; Ooya, T.; Yui, N. *Macromol. Rapid Commun.* **2004**, *25*, 867. (b) Li, C.; Madsen, J.; Armes, S. P.; Lewis, A. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 3510. (c) Law, B.; Weissleder, R.; Tung, C. H. *Biomacromolecules* **2006**, *7*, 1261. (d) Lee, M.; Baek, K.; Jin, H. J.; Jung, Y.; Shin, I. *Angew. Chem., Int. Ed.* **2004**, *43*, 1675.

- (4) (a) Plunkett, K. N.; Berkowski, K. L.; Moore, J. S. *Biomacromolecules* **2005**, *6*, 632. (b) Vartak, D. G.; Gemeinhart, R. A. *J. Drug Targeting* **2007**, *15*, 1. (c) McDonald, T. O.; Qu, H.; Saunders, B. R.; Ulijn, R. V. *Soft Matter* **2009**, *5*, 1728. (d) Raemdonck, K.; Demeester, J.; De Smedt, S. *Soft Matter* **2009**, *5*, 707. (e) Azagarsamy, M. A.; Sokkalingam, P.; Thayumanavan, S. *J. Am. Chem. Soc.* **2009**, *131*, 14184.
- (5) (a) Duncan, R. *Nat. Rev. Drug Discovery* **2003**, *2*, 34. (b) Tauroa, J. R.; Leec, B. H.; Lateef, S. S.; Gemeinhart, R. A. *Peptides* **2008**, *29*, 1965. (bb) Shabat, D. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 1569. (c) Duncan, R.; Vicent, M. J.; Greco, F.; Nicholson, R. I. *Endocr. Relat. Cancer* **2005**, *12*, S189.
- (6) Thornton, P. D.; Mart, R. J.; Webb, S. J.; Ulijn, R. V. *Soft Matter* **2008**, *4*, 821.



**Figure 1.** Schematic representation of highly selective protease-mediated release following the cleavage of specifically designed peptide linkers consisting of Fmoc-terminated glutamic acid separated from particle-coupled arginine by a dialanine enzyme-cleavable linker. Macromolecular release through the mesoporous silica structure is only possible upon the enzyme-mediated removal of Fmoc protecting groups that act to partially cap the particle pores.

dependent on the peptide sequence/target enzyme selected. The system allows the vast current knowledge of enzyme–substrate pairs for proteases and peptides to be utilized, offering the system versatility to potentially respond independently to a number of targeted proteases dependent on the peptide sequence selected. This offers a chemically simplistic, highly tunable release mechanism capable of releasing macromolecular therapeutic agents exclusively in response to particular, targeted, proteolytic enzymes.

Intermolecular interactions between peptide sequences that possess Fmoc protecting groups have been reported previously for the self-assembly of Fmoc protected peptides.<sup>9</sup> We hypothesized that particle-coupled peptide sequences capped with terminal Fmoc protecting groups would interact through  $\pi$ – $\pi$  interactions to provide a molecular gate, restricting the passage of loaded molecules through the pores of the carrier (Figure 1, left). Successful enzymatic cleavage of the peptide sequence removes the Fmoc group, enabling the peptide fragments

remaining to become spatially independent of adjacent chains, therefore opening the gate to enable the diffusion of payload molecules from the particle core (Figure 1, right). Electrostatic repulsion generated following enzymatic hydrolysis induces maximum chain separation, offering the payload sufficient area through which to pass. Payload entrapment prior to enzyme-stimulated release is maintained either by  $\pi$ – $\pi$  interactions between adjacent Fmoc groups causing the gate to “close”, or simply due to the presence of bulky Fmoc groups that physically prevent the passage of guest molecules from the particle core through steric hindrance. The concept offered combines notions of molecular and supramolecular chemistry to produce a result of some significance within the biomedical field.

In our proof-of-concept study, amine functionalized silica particles (5  $\mu$ m diameter, 300 Å pores) were employed as carrier vehicles. Silica mesoporous supports offer biocompatibility, chemical stability, and relatively large load capacities.<sup>10</sup> In addition, solid phase peptide synthesis and subsequent enzymatic hydrolysis reactions have been successfully demonstrated using silica-based particles which are compatible with aqueous and organic solvents.<sup>11</sup> Pioneering work by the group of Victor Lin has extensively demonstrated the use of functionalized mesoporous silica spheres as carrier vehicles in a stimuli-responsive gated release mechanism.<sup>12</sup> Cadmium sulfide nanoparticles (CdS) were used as caps to encapsulate drug molecules/neurotransmitters.<sup>13</sup> Reduction of the disulfide bond between the CdS caps and the silica host particles by molecules such as dithiothreitol and mercaptoethanol triggered the intracellular delivery of previously entrapped payload molecules. The work validates the use of silica particles as host materials in a stimuli-responsive gated release mechanism for use within a biomedical context.

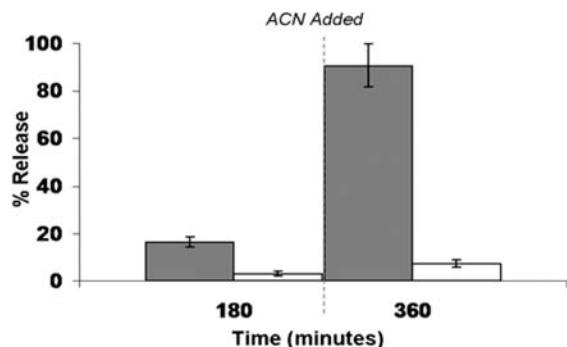
The particles that we used in this study had a loading value of 1 mmol/g (as determined by high performance liquid chromatography (HPLC) analysis of cleaved Fmoc groups by piperidine). We believe that the distance between the particle-functionalized free amine groups is suitable for the generation of intermolecular interactions between Fmoc groups present on adjacent particle-coupled short peptide chains.

## Results and Discussion

Initially the enzyme-stimulated release of fluorescein isothiocyanate (FITC)-conjugated 4 kDa dextran molecules from peptide modified particles was monitored in response to a target enzyme (thermolysin, 38 kDa) and a nonspecific enzyme for the designed peptide sequence (chymotrypsin, 25 kDa). Thermolysin possesses the correct specificity to cleave the Ala~Ala bond of the tetrapeptide sequence Fmoc-Asp(–)-Ala-Ala-Arg(+) (1) to a high efficiency whereas chymotrypsin does not (90.0  $\pm$  6.2% cleavage by thermolysin compared to 9.3  $\pm$  1.1% cleavage achieved by chymotrypsin as analyzed by HPLC after 3 h reaction). Particles possessing this peptide sequence were produced and loaded (50 mg particles per experiment) with FITC-labeled dextran (1.5 mg) as described in the experimental

- (7) (a) Angelos, S.; Yang, Y. W.; Patel, K.; Stoddart, J. F.; Zink, J. I. *Angew. Chem., Int. Ed.* **2008**, *47*, 2222. (b) Casasús, R.; Climent, E.; Marcos, M. D.; Martínez-Mañez, R.; Sancenón, F.; Soto, J.; Amorós, P.; Cano, J.; Ruiz, E. *J. Am. Chem. Soc.* **2008**, *130*, 1903. (c) Bernardos, A.; Aznar, E.; Coll, C.; Martínez-Mañez, R.; Barat, J. M.; Marcos, M. D.; Sancenón, F.; Benito, A.; Soto, J. *J. Controlled Release* **2008**, *131*, 181. (d) Aznar, E.; Coll, C.; Marcos, M. D.; Martínez-Mañez, R.; Sancenón, F.; Soto, J.; Amorós, P.; Cano, J.; Ruiz, E. *Chem.—Eur. J.* **2009**, *15*, 6877. (e) Aznar, E.; Marcos, M. D.; Marcos, M. D.; Martínez-Mañez, R.; Sancenón, F.; Soto, J.; Amorós, P.; Guillem, C. *J. Am. Chem. Soc.* **2009**, *131*, 6833. (f) Ito, T.; Yamaguchi, T. *Langmuir* **2006**, *22*, 3945. (g) Hernandez, R.; Tseng, H. R.; Wong, J. W.; Stoddart, J. F.; Zink, J. I. *J. Am. Chem. Soc.* **2004**, *126*, 3370. (8) (a) Patel, K.; Angelos, S.; Dichtel, W. R.; Coskun, A.; Yang, Y. W.; Zink, J. I.; Stoddart, J. F. *J. Am. Chem. Soc.* **2008**, *130*, 2382. (b) Bernardos, A.; Aznar, E.; Marcos, M. D.; Martínez-Mañez, R.; Sancenón, F.; Soto, J.; Barat, M.; Amorós, P. *Angew. Chem., Int. Ed.* **2009**, *48*, 5884. (c) Schlossbauer, A.; Kecht, J.; Bein, T. *Angew. Chem., Int. Ed.* **2009**, *48*, 3092. (d) Corma, A.; Díaz, U.; Arrica, M.; Fernández, E.; Ortega, I. *Angew. Chem., Int. Ed.* **2009**, *48*, 6247. (9) (a) Tang, C.; Smith, A. M.; Collins, R. F.; Ulijn, R. V.; Saiani, A. *Langmuir* **2009**, *25*, 9447. (b) Williams, R. J.; Smith, A. M.; Collins, R. F.; Hodson, N.; Das, A. K.; Ulijn, R. V. *Nat. Nanotechnol.* **2009**, *4*, 19. (c) Yang, Z.; Xu, B. *J. Mater. Chem.* **2007**, *17*, 2385. (d) Wang, W.; Yang, Z.; Patanavanich, S.; Xu, B.; Chau, Y. *Soft Matter* **2008**, *4*, 1617. (e) Mahler, A.; Reches, M.; Rechter, M.; Cohen, S.; Gazit, E. *Adv. Mater.* **2006**, *18*, 1365. (f) Orbach, R.; Adler-Abramovich, L.; Zigerson, S.; Mironi-Harpaz, I.; Seliktar, D.; Gazit, E. *Biomacromolecules* **2009**, *10*, 2646.

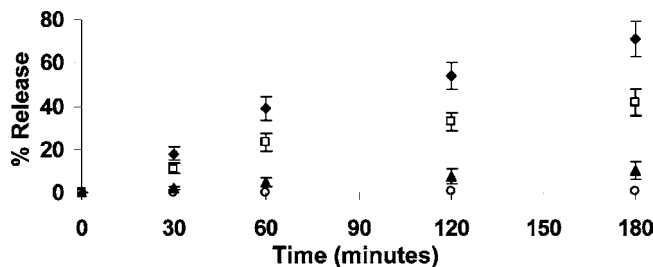
- (10) (a) Aznar, E.; Martínez-Mañez, R.; Sancenón, F. *Expert Opin. Drug. Delivery* **2009**, *6*, 643. (b) Slowing, I. I.; Vivero-Escoto, J. L.; Wu, C. W.; Lin, V. S. Y. *Adv. Drug Delivery Rev.* **2008**, *60*, 1278. (11) Basso, A.; De Martin, L.; Ebert, C.; Linda, P.; Gardossi, L.; Ulijn, R. V.; Flitsch, S. L. *Tetrahedron Lett.* **2003**, *44*, 6083. (12) (a) Trewyn, B. G.; Slowing, I. I.; Giri, S.; Chen, H. T.; Lin, V. S. Y. *Acc. Chem. Res.* **2007**, *40*, 846. (b) Radu, D. R.; Lai, C. Y.; Wiench, J. W.; Pruski, M.; Lin, V. S. Y. *J. Am. Chem. Soc.* **2004**, *126*, 1640. (13) Trewyn, G. G.; Jęftinija, D. M.; Jęftinija, K.; Xu, S.; Jęftinija, S.; Lin, V. S. Y. *J. Am. Chem. Soc.* **2003**, *125*, 4451.



**Figure 2.** Release of 4 kDa dextran from thermolysin (gray) and chymotrypsin (white) (enzyme concentrations 1 mg/mL) treated particles (1). Thermolysin adhesion to the carrier particle restricts complete payload diffusion from the particle core. Acetonitrile (ACN) was added after 180 min, at which point the particles were agitated for a further 180 min before payload diffusion was measured after 360 min. The results confirm that control over the opening of peptide gates is achievable in this mechanism but consideration of the size of the chosen enzyme is of importance to minimize the blocking of particle pores following proteolysis ( $n = 3$ ).

section. The chosen enzyme solutions (1.5 mL, 1 mg/mL) were independently added to the particles which were then left to react at room temperature under agitation. After 180 min of reaction there is a significant difference between the extent of release from particles reacted with thermolysin compared to those reacted with chymotrypsin. It appeared that thermolysin obstructed the particle pores, since washing with acetonitrile allows unrestricted release from the particles as analyzed by fluorescence spectrophotometry (Figure 2, washing was done before the beads were agitated for a further 180 min and the extent of release analyzed). The different specificities that the two enzymes possess for cleavage of the tetrapeptide sequence are shown in the variation in release rates of dextran molecules. The results show a marked difference between the rate and extent of release in each case, confirming that the successful removal of the Fmoc protecting group by thermolysin has triggered the release of guest molecules.

The triggered release of 4 kDa dextran molecules in response to a second proteolytic enzyme with a lower molecular weight, elastase (25 kDa), was then tested to show that extensive protease-triggered release can occur via the proposed mechanism without the requirement of washing the particles with acetonitrile following proteolysis. Furthermore, material response to a second proteolytic enzyme demonstrates the versatility of the system. Elastase-induced release mechanisms have been reported previously due to the correlation between increased levels of elastase and specific biological events concerning disease or injury.<sup>14</sup> Previous studies have shown that elastase has the correct specificity to cleave the Ala~Ala amide bond of particular tripeptide<sup>15</sup> and tetrapeptide sequences.<sup>16</sup> Particles were modified with the peptide linker Fmoc-Glu(-)-Ala-Ala-Arg(+) (2) and loaded with 4 kDa dextran solution. Following the washing of particles with water they were independently reacted with elastase solutions of varying concentrations. The extent of release from each reaction was monitored by fluorescence spectrophotometry and the results are presented in Figure

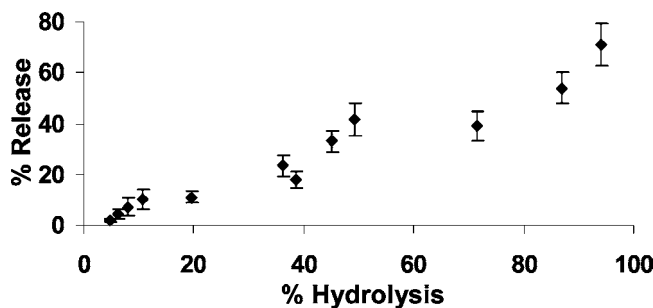


**Figure 3.** Release of 4 kDa dextran from peptide modified silica particles (2) upon incubation with elastase in solution at the following concentrations: (◆) 1 mg/mL, (□) 0.1 mg/mL, (▲) 0.01 mg/mL, (○) 0.001 mg/mL. Extensive release was observed at 1 mg/mL elastase concentration without the requirement of washing the particles with acetonitrile following the enzymatic reaction ( $n = 3$ ).

**Table 1.** Extent (%) of Peptide Hydrolysis of (2) When Reacted with Various Concentrations of Elastase<sup>a</sup>

	1 mg/mL	0.1 mg/mL	0.01 mg/mL
30 min	38.5 ± 5.9	19.6 ± 2.8	4.8 ± 0.9
60 min	71.4 ± 6.3	36.1 ± 5.1	6.3 ± 0.8
120 min	86.9 ± 8.1	45.1 ± 3.7	8.1 ± 1.1
180 min	94.2 ± 6.6	49.2 ± 9.6	10.7 ± 1.4

<sup>a</sup> Results show that the extent of proteolysis is dependent on the concentration of elastase used.



**Figure 4.** Correlation between the extent of peptide hydrolysis by different concentrations of elastase, and the amount of 4 kDa dextran consequently released from the particle core ( $n = 3$ ). The result shows a correlation ( $R^2 = 0.938$ ) between the extent of enzyme activity and the degree of payload release.

3. Both the rate and extent of payload release are dependent on the concentration of elastase used in the proteolytic reaction.

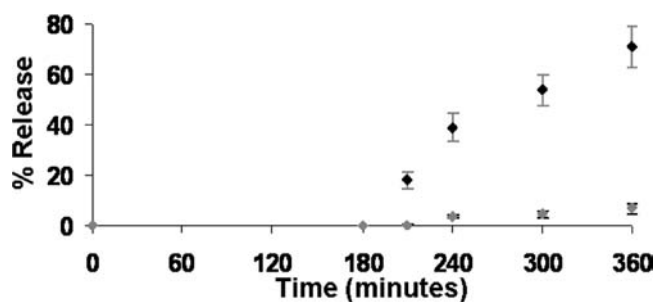
In addition, the hydrolysis kinetics by elastase of unloaded particles modified with the peptide linker (2) were monitored by HPLC. The reactions involving various concentrations of elastase were terminated at specific time points and the supernatant analyzed to determine the extent of peptide cleavage by the enzyme. The results (Table 1) reveal that the extent and rate of proteolysis is strongly dependent on enzyme concentration. Further studies using the same particles reacted with elastase in a solution of concentration 1  $\mu\text{g/mL}$  found that no detectable hydrolysis of the peptide linker occurred, and consequently payload release was prevented. The correlation between the extent of peptide hydrolysis and the amount of payload release is provided in Figure 4. While the relationship between proteolysis and payload release is not perfectly linear ( $R^2 = 0.938$ ), there is clearly a correlation between the number of Fmoc protecting groups removed from the peptide linkers, and the extent of release of guest molecules. Extensive hydrolysis produces a vast number of open molecular gates for the guest molecules to diffuse through, increasing the rate of

(14) (a) Aimetti, A.; Tibbitt, M. W.; Anseth, K. S. *Biomacromolecules*. **2009**, *10*, 1484. (b) Owen, C. A.; Campbell, E. J. *J. Leukocyte Biol.* **1999**, *65*, 137.

(15) Birchall, L. S.; Ulijn, R. V.; Webb, S. J. *Chem. Commun.* **2008**, *25*, 2861.

(16) Thornton, P. D.; Mart, R. J.; Ulijn, R. V. *Adv. Mater.* **2007**, *19*, 1352.





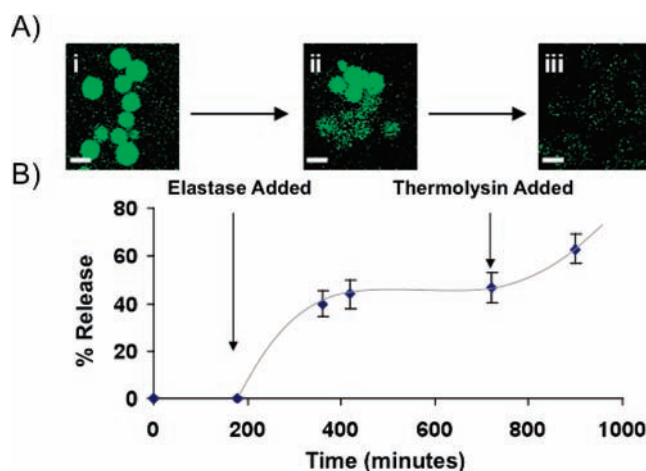
**Figure 5.** Release profiles corresponding to the release of 4 kDa dextran from particles modified with peptide linkers (2) (black  $\blacklozenge$ ) and (3) (gray  $\blacklozenge$ ) when reacted with elastase (1 mg/mL). The result highlights the potential to design specific peptide linkers to respond exclusively to a target enzyme. Substantial release occurred without the requirement of washing the particles with acetonitrile to remove partially release prohibiting enzyme molecules ( $n = 3$ ).

diffusion compared to more limited release when the extent of proteolysis is restricted.

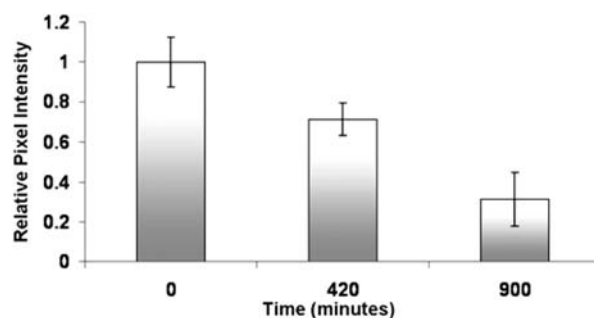
The ability of the described mechanism to withhold, and selectively release, guest molecules of a range of sizes was tested by independently loading particles modified with the peptide sequence (2) with rhodamine B (479.0 Da) and fluorescein (332.3 Da) and reacting the particles with elastase (1 mg/mL) to trigger payload release. In each case significant release of the loaded material was observed in the washing stages prior to incubation with elastase. Analysis of the supernatant from each wash cycle by fluorescence spectroscopy revealed that 36% of rhodamine and 31% of fluorescein were expelled from the peptide-modified particles during the washing stage and >95% of each initially loaded solution was expelled from the particle core within an hour of elastase reaction. The result suggests that the control that the release mechanism offers is dependent on the size of the loaded molecules. The system allows the constant diffusion of smaller molecules (such as antimicrobial agents<sup>17</sup>) out of the particle core, but will only release the payload, of greater molecular weight, specifically upon interaction with a targeted protease enzyme.

To demonstrate the ability to design peptide linkers capable of responding exclusively to a specified enzyme, Fmoc-Glu(-)-Ala-Ala-Arg(+) (2) and Fmoc-Glu(-)-Gly-Gly-Arg(+) (3) coupled silica particles were reacted with elastase which has the correct specificity to cleave between Ala~Ala of (2) ( $97.1 \pm 2.2\%$ , 24 h reaction. Elastase concentration: 1 mg/mL) but demonstrates little activity toward (3) ( $8.0 \pm 1.4\%$ , 24 h reaction. Elastase concentration: 1 mg/mL). The particles were loaded with FITC-dextran (4 kDa) and stored in water for 3 h with release being monitored by fluorescence spectroscopy. Upon elastase addition extensive release is observed from (2) but not from (3) (Figure 5) with 70% of payload release (2) compared to less than 10% release from (3) after 3 h reaction. Greater release compared to thermolysin-triggered release is attributed to less obstruction of particle pores by smaller elastase molecules. The results demonstrate the high degree of specificity offered by the release mechanism, displaying the capability to design peptide linkers to respond specifically to targeted enzymes.

Finally, the selectivity that the release mechanism possesses toward different proteolytic enzymes was highlighted by the two-stage release of 4 kDa FITC-dextran molecules. Particles



**Figure 6.** (A) Confocal microscopy images showing the amalgamation of FITC-dextran loaded particles (2) and (3) (i) prior to elastase addition, (ii) after reacting with elastase for 180 min, and (iii) following the addition of both enzymes after 900 min. (B) Release profile corresponding to the reaction events. Enzyme concentrations used were 1 mg/mL. Acetonitrile was not used in at any stage to wash the particles in this experiment. Scale bars represent 5  $\mu\text{m}$  ( $n = 3$ ).



**Figure 7.** Pixel intensity measurements obtained from images of a mixture of beads modified with peptide linkers (2) and (3) reacted with (i) no enzyme (0 min), (ii) elastase for 3 h and consequently washed (420 min), and (iii) elastase and thermolysin.

coupled with (2) were combined with particles coupled with (3). Twenty-five milligrams of each type of functionalized particles were combined following the loading of payload molecules, and the amalgamation was stored in water for 3 h before being reacted with elastase for 3 h. Following this the beads were washed for an hour and left in water where diffusion was monitored for a further 6 h. Subsequently, thermolysin was added to the mixture and further release was monitored (Figure 6). Release occurred in two distinct stages corresponding to particle reactions initially between (2) and elastase, and consequently unreacted (2) and (3) with thermolysin. Image analysis was done on 10 particles chosen at random from 30 images at each of the 3 stages of the experiment. The pixel intensity of the particles was measured using Image J analysis software and is shown in Figure 7. There is clearly two stages of release that correspond to the initial addition of elastase to the particle mixture, and then the subsequent addition of thermolysin. These results demonstrate the ability of the methodology to selectively release controlled portions of payload molecules dependent upon interactions with a number of targeted enzymes.

## Conclusion

The work detailed in this article suggests and validates a novel release mechanism based on the selective cleavage of peptide

(17) Izquierdo-Barba, I.; Vallet-Regí, M.; Kupferschmidt, N.; Terasaki, O.; Schmidtchen, A.; Malmsten, M. *Biomaterials* **2009**, *30*, 5729.

chains bearing terminal Fmoc protecting groups that act to cap the pores of peptide-modified silica particles. Successful enzymatic hydrolysis enables the passage of freely loaded guest molecules through the newly created spaces, offering the system highly selective responsiveness to release payload molecules. While this work has initially been demonstrated using amine functionalized silica microparticles, it is hoped that the methodology is transferable for use on a host of inorganic and organic supports of varying size ranges. Successful progress offers the potential to possibly generate longer Fmoc protected peptide chains capable inducing a material response following interac-

tions with more complex proteolytic enzymes of greater biological relevance.

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**Supporting Information Available:** Full experimental details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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