

Maltodextrin-Modified Magnetic Microspheres for Selective Enrichment of Maltose Binding Proteins

Jin Zheng,[†] Chongjun Ma,[‡] Yangfei Sun,[‡] Miaorong Pan,[†] Li Li,^{||} Xiaojian Hu,[‡] and Wuli Yang^{*,†}

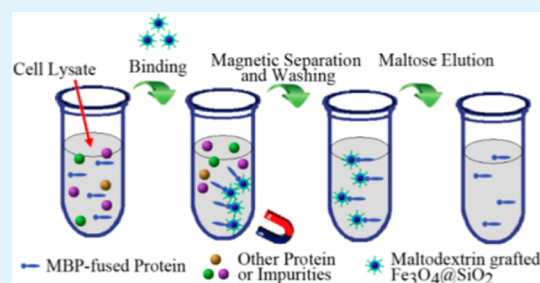
[†]State Key Laboratory of Molecular Engineering of Polymers and Department of Macromolecular Science, Fudan University, No. 220 Handan Road, Shanghai 200433, China

[‡]School of Life Sciences, Fudan University, No. 220 Handan Road, Shanghai 200433, China

^{||}The Emerther Company, No. 526 Ruiqing Road, Shanghai 2001201, China

ABSTRACT: In this work, maltodextrin-modified magnetic microspheres $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -Maltodextrin ($\text{Fe}_3\text{O}_4@\text{SiO}_2$ -MD) with uniform size and fine morphology were synthesized through a facile and low-cost method. As the maltodextrins on the surface of microspheres were combined with maltose binding proteins (MBP), the magnetic microspheres could be applied to enriching standard MBP fused proteins. Then, the application of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -MD in one-step purification and immobilization of MBP fused proteins was demonstrated. For the model protein we examined, $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -MD showed excellent binding selectivity and capacity against other *Escherichia coli* proteins in the crude cell lysate. Additionally, the maltodextrin-modified magnetic microspheres can be recycled for several times without significant loss of binding capacity.

KEYWORDS: magnetic microspheres, Fe_3O_4 core-shell, maltodextrin, MBP, separation



1. INTRODUCTION

Proteins are composed of amino acids which participate in virtually all aspects of cell function, and proteins are important raw materials of variant biomedical reagents, industrial products, and so on.¹ However, target proteins in cells are mixed with numerous biological components, such as different proteins, DNAs, RNAs, lipids, etc. These components will complicate the functional analysis of target proteins and restrict the uses of target proteins in biomedical or industrial fields. So protein purification is a prerequisite for further investigations and applications of target proteins. Besides, an efficient protein purification tool is necessary to enrich and purify target proteins from limited materials, which is essential for subsequent biochemical studies, and purer protein products will diminish the interference of contaminants, providing reliable products for basic researches and industrial productions.

Fusion protein technology is a significant advance in protein engineering.² Usually, fusion proteins, by fusing the genes encoding two or more different proteins, include an affinity tag linked to a target protein for a specific purpose.³ A wide range of protein fusion tags have been developed such as His-tag,⁴ GST tag,⁵ SNAP tag,⁶ etc. to provide several benefits such as simplifying the purification, facilitating expression, detection and tracing of recombinant proteins, improving solubility and stability, and enhancing potency by increasing valency.⁷ Among all the protein fusion tags, the maltose binding protein (MBP) tag, a 42 kDa protein part of the maltose/maltodextrin system of *Escherichia coli*, is often fused to relevant proteins to improve its yield, to protect the target protein from intracellular

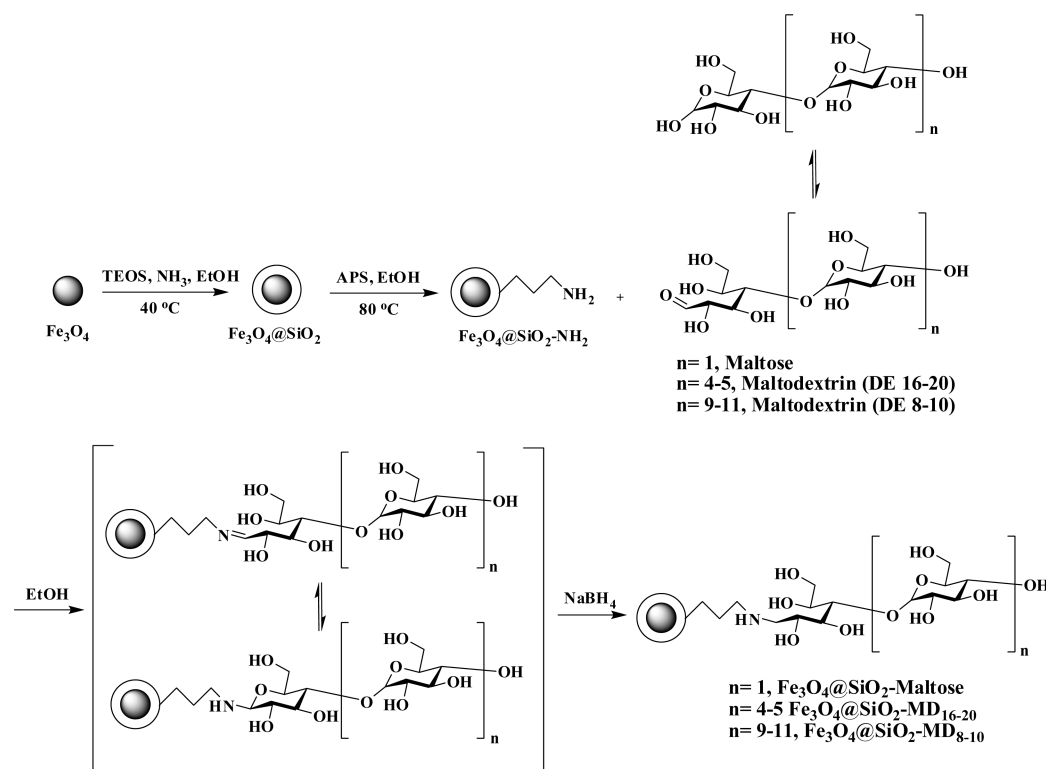
proteolysis, to enhance protein solubility effectively, and to simplify the purification.^{8–11} More and more proteins with difficultly independent crystallization have been reported on crystallization and the following structure for fusion with large-affinity MBP tag.^{12–15} Therefore, a cost-effective approach for the separation and purification of MBP tag recombinant proteins is urgently needed.^{16,17}

Functionalized magnetic microspheres, owing to their excellent magnetic responsiveness, easy manipulation and recovery have been extensively investigated in a wide variety of fields,^{18–23} including as superior materials for protein purification.^{24–32} In the past few years, many efforts have been paid to develop magnetic microsphere-based systems for the purification of recombinant proteins. For example, Xu and his co-workers developed a magnetic nanoparticles with poly(2-hydroxyethyl methacrylate) brushes and subsequently functionalized with nitrilotriacetate- Ni^{2+} to capture His-tagged protein directly from cell extracts.³¹ Zhang and co-workers developed a magnetic microsphere of $\text{Fe}_3\text{O}_4/\text{poly}(N,N'$ -methylenebisacrylamide-co-glycidyl methacrylate)/IDA- Ni^{2+} with a core of magnetic particle and a shell of hydrophilic polymer. The core-shell structured microspheres were successfully applied to selective enrichment of His-tagged cellulytic proteins.³³ Pan and her co-workers synthesized glutathione (GSH)-decorated iron oxide nanoparticles as a general agent to separate a GST-tagged protein fused with human α 1-glutathione S-transferase

Received: December 15, 2013

Accepted: January 9, 2014

Published: January 9, 2014

Scheme 1. Synthesis of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-Maltose}$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-MD}$ 

(GST) and to manipulate live cells.^{32,34} However, less attention has been paid to the preparation of magnetic microspheres for purification and enrichment of MBP fusion proteins. Zhou developed maltose-modified magnetic microspheres which can be used for affinity adsorption with MBP-Heparinase I fusion proteins, and used carboxyl PEO as the linkers and coupled maltose at the end to capture the MBP fusion proteins.³⁵

Herein, we reported a facile method to obtain maltodextrin-modified magnetic microspheres for MBP fusion proteins. Low-cost, soluble and stable maltodextrins (MD) which could effectively bind with MBP fusion proteins⁸ were chosen to modify the magnetic microspheres. The MD-modified magnetic microspheres had a higher magnetic response, better purification, and enrichment abilities. We then demonstrated the use of the MD-modified magnetic microspheres in one-step purification and immobilization of MBP fusion proteins for biotechnological applications. NON-YELLOWING 1 (NYE1) as the key protein of regulating Chlorophyll degradation³⁶ was chosen as a model protein in our research. MBP tag fused NYE1 in *E. coli* expression system was favorable on improving solubility and purification.³⁷ For the MBP-NYE1 we examined, the MD-modified magnetic microspheres showed an excellent binding selectivity against other *E. coli* proteins in the crude cell lysate. The MD-modified magnetic microspheres could be recycled for many times without significant loss of binding capacity to proteins.

2. EXPERIMENTAL SECTION

2.1. Chemicals and Reagents. Iron(III) chloride (FeCl_3), aqueous ammonia solution (25%), tetraethyl orthosilicate (TEOS), sodium acetate (NaAc), ethylene glycol, trisodium citrate dihydrate, 3-aminopropyltriethoxysilane (APS), sodium borohydride (NaBH_4), maltose, and anhydrous ethanol were purchased from Sinopharm Chemical Reagent Co. Ltd. Maltodextrin (MD), including maltodextrin (DE = 8-10, MD₈₋₁₀) and maltodextrin (DE = 16-20, MD₁₆₋₂₀)

(DE value is the reducing sugar percentage of syrup of dry matter), were purchased from Shandong Xiwang pharmaceutical Co, Ltd. DL-Dithiothreitol (DTT), Tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl) and other biological reagents were purchased from Sigma-Aldrich. Standard MBP protein (MBP-mCherry) was supplied by professor Ding's group in Fudan University. Other chemicals were of reagent grade and were used without further purification. Deionized water was used in all experiments.

2.2. Preparation of Fe_3O_4 Particles. The magnetic Fe_3O_4 particles were prepared by a modified solvothermal reaction.³⁸ Briefly, 0.65 g of FeCl_3 , 0.24 g of sodium citrate and 1.2 g of NaAc were dissolved in 20 mL of ethylene glycol under vigorous stirring for 30 min. Then the resulting solution was transferred into a Teflon-lined stainless-steel autoclave with a capacity of 50 mL. The autoclave was sealed and heated at 200 °C for 10 h. Then it was cooled to room temperature. The black products were washed with ethanol and deionized water for several times and collected with magnet. The final products were dispersed in 10 mL ethanol for further use.

2.3. Preparation of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ Microspheres. The sol-gel approach was applied to prepare core-shell structured $\text{Fe}_3\text{O}_4@\text{SiO}_2$. Typically, 250 mg of Fe_3O_4 particles were stirred in 100 mL of deionized water, 400 mL of ethanol and 5 mL of aqueous ammonia. Then, 10 mL of TEOS was added. The dispersion was continuously stirred at 40 °C for 6 h. After that, the products were washed with ethanol and deionized water for several times to remove the unreacted TEOS. The products were collected with magnet. The final products were dispersed in 10 mL ethanol for further use.

2.4. Preparation of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ Microspheres. $\text{Fe}_3\text{O}_4@\text{SiO}_2$ microspheres (50 mg) were stirred in 100 mL of ethanol. Then, 25 μL of APS was added to the dispersion. The dispersion was continuously stirred at 60 °C for 6 h. After that, the products were washed several times with ethanol, collected with a magnet and finally dried under vacuum at 40 °C for 12 h to obtain $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$.

2.5. Preparation of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-MD}$ Microspheres and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-Maltose}$ Microspheres. $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ microspheres (25 mg) were stirred in 100 mL of ethanol. Then 10 mg of MD were added into the above dispersion. The dispersion was continuously stirred at 70 °C for 6 h. Then, the dispersion was stirred

at room temperature, and 1 mg of NaBH_4 were added and reacted for another 12 h. The products were washed with ethanol and deionized water for several times, and collected with a magnet to obtain $\text{Fe}_3\text{O}_4@$ SiO_2 -MD. $\text{Fe}_3\text{O}_4@$ SiO_2 -Maltose was prepared using a process similar to the preparation of $\text{Fe}_3\text{O}_4@$ SiO_2 -MD.

2.6. Characterization. Transmission electron microscopy (TEM) images were obtained on a Hitachi H-600 transmission electron microscope. Hydrodynamic diameter and zeta potential were measured by using a dynamic light scattering (DLS) particle size analyzer (Malvern Nano-ZS90) at scattering angle of 90° . Magnetic characterization was carried out with a vibrating sample magnetometer (VSM) on a Model 6000 physical property measurement system (Quantum, USA) at 300 K. FT-IR spectra were measured on a Nicolet Nexus-440 FTIR spectroscopy. All measured samples were dried, and the powders were mixed with KBr and pressed to a plate for measurement. Thermo gravimetric analysis of microspheres was performed on a Pyris 1 TGA instrument with a heating rate of $20^\circ\text{C}/\text{min}$ under air environment.

2.7. Expression and Purification of MBP-Tagged Proteins. NYE1 amino acids 1-220 were cloned into reconstructive pMAL-c2x with NotI and XhoI for *E. coli* expression. The recombinant MBP-NYE1 was transformed into BL21 (DE3) plysS cell strain. BL21 (DE3) plysS cells containing this plasmid were grown in LB medium at 37°C to an OD_{600} of 0.5. The temperature of culture was lowered to 20°C and expression was induced by the addition of 0.2 M IPTG for 16 h. Two liters of the medium were pelleted, the cells were lysed with sonication and the debris was removed by centrifugation.

The lysate was mixed and shaken with the magnetic microspheres at 0°C for a certain time. After affinity adsorption, the excess protein solution was removed with magnet, and the protein-immobilized magnetic microspheres were washed with buffer solution to remove any proteins which were not adsorbed by affinity adsorption. Briefly, $100\ \mu\text{L}$ of magnetic microspheres (2 mg/mL) were aliquoted to a sterile microcentrifuge tube. $500\ \mu\text{L}$ of MBP binding buffer (200 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, 1 mM DTT, pH 7.4) was added and a magnet was applied for 30 seconds to pull magnetic microspheres to the side of the tube and supernatant was decanted. Then $500\ \mu\text{L}$ of cell culture supernatant were added to sterile microcentrifuge tube, mixed thoroughly and incubated at 4°C with agitation for 1 h. A magnet was applied and supernatant was decanted to another microcentrifuge tube. The magnetic microspheres were washed for three times by MBP binding buffer. Then $50\ \mu\text{L}$ of MBP binding buffer containing 10 mM maltose (elution buffer) was added to the bead pellet and incubated for 10 minutes at 4°C with agitation. Magnet was applied and the eluted MBP-fused protein was pipeted into a clean microcentrifuge tube. Protein analysis was performed on 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).³⁹ The protein concentration was measured according to the Bradford method using the Quick Start Bradford Protein Assay Kit 3.

3. RESULTS AND DISCUSSION

3.1. Preparation of $\text{Fe}_3\text{O}_4@$ SiO_2 -MD. The preparation process of $\text{Fe}_3\text{O}_4@$ SiO_2 -MD was illustrated in Scheme 1. The magnetic particles (Fe_3O_4) were prepared by a modified solvothermal reaction.⁴⁰ The silica coating was fabricated by a sol-gel process to form core-shell structures ($\text{Fe}_3\text{O}_4@$ SiO_2).⁴¹ Amino groups were modified onto the surface to obtain $\text{Fe}_3\text{O}_4@$ SiO_2 - NH_2 by reacting with APS.⁴² Finally, $\text{Fe}_3\text{O}_4@$ SiO_2 - NH_2 reacted with maltodextrin (MD) in ethanol at 70°C . The amino groups on the surface of the microspheres reacted with the aldehyde groups of MD or maltose to form the Schiff base which was an unstable intermediate. After reduced by NaBH_4 , Schiff base was reduced to form a stable amino structure and MD or maltose was grafted onto the microspheres to produce $\text{Fe}_3\text{O}_4@$ SiO_2 -MD or $\text{Fe}_3\text{O}_4@$ SiO_2 -Maltose.^{43,44} MD is typically composed of a mixture of polysaccharides and the chain length of these polysaccharides

varies from three to seventeen glucose units. MD is also classified by the dextrose equivalent (DE) value which stands for the percentage of the reducing sugar (glucose) of syrup in the dry matter as mentioned above. Thus, different DE values mean different chain lengths. The higher DE value is, the shorter MD chain is.⁴⁵ Maltodextrin (DE = 8-10, MD₈₋₁₀) and maltodextrin (DE = 16-20, MD₁₆₋₂₀) were chosen to graft onto the magnetic microspheres ($\text{Fe}_3\text{O}_4@$ SiO_2 -MD₈₋₁₀ and $\text{Fe}_3\text{O}_4@$ SiO_2 -MD₁₆₋₂₀) and the resulting MD-modified magnetic microspheres could be used as the support for MBP-NYE1 immobilized by affinity adsorption. Meanwhile, maltose-coated magnetic microspheres ($\text{Fe}_3\text{O}_4@$ SiO_2 -Maltose) were prepared by the similar process for comparison.

Figure 1 showed representative TEM images of Fe_3O_4 , $\text{Fe}_3\text{O}_4@$ SiO_2 , and $\text{Fe}_3\text{O}_4@$ SiO_2 -MD. Figure 1a displayed well

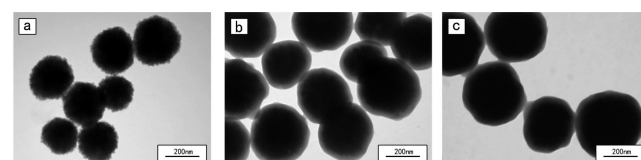


Figure 1. TEM images of (a) Fe_3O_4 , (b) $\text{Fe}_3\text{O}_4@$ SiO_2 , and (c) $\text{Fe}_3\text{O}_4@$ SiO_2 -MD.

mono-dispersed Fe_3O_4 particles with the mean diameter of 250 ± 25 nm. X-ray diffraction (XRD) measurements were carried out to determine the composition of Fe_3O_4 particles. According to the Scherrer equation, the average primary crystallite size which was calculated based on the XRD pattern (311), was approximate 6.5 nm. All the diffraction peaks in the XRD patterns were indexed and assigned to the typical cubic structure of Fe_3O_4 (JCPDS 75-1609). In contrast, Figure 1b showed $\text{Fe}_3\text{O}_4@$ SiO_2 microspheres with the mean diameter of 320 ± 30 nm. Specifically, the silica shell was formed by the hydrolysis and subsequent condensation of TEOS in a basic alcohol/water solution, according to the well-known Stöber method. The dark core area was Fe_3O_4 , while the light shell was SiO_2 layer. Figure 1c showed that $\text{Fe}_3\text{O}_4@$ SiO_2 -MD was similar as $\text{Fe}_3\text{O}_4@$ SiO_2 . The hydrodynamic diameter of Fe_3O_4 was 297 nm and the ζ potential was -21.7 mV because of the existence of citrate groups on the surface of Fe_3O_4 (Table 1). After coated with SiO_2 , the hydrodynamic diameter of $\text{Fe}_3\text{O}_4@$ SiO_2 was increased to 368 nm and the zeta potential was -43.7 mV because of the presence of silanol groups on the surface.

Table 1. Particle Size and ζ Potential of Fe_3O_4 , $\text{Fe}_3\text{O}_4@$ SiO_2 , $\text{Fe}_3\text{O}_4@$ SiO_2 - NH_2 , $\text{Fe}_3\text{O}_4@$ SiO_2 -Maltose, and $\text{Fe}_3\text{O}_4@$ SiO_2 -MD^a

sample	ζ potential (mV)	hydrodynamic diameter (nm) ^b	PI ^c
Fe_3O_4	-21.7	297	0.18
$\text{Fe}_3\text{O}_4@$ SiO_2	-43.7	368	0.13
$\text{Fe}_3\text{O}_4@$ SiO_2 - NH_2	19.8	595	0.27
$\text{Fe}_3\text{O}_4@$ SiO_2 -Maltose	-4.8	580	0.28
$\text{Fe}_3\text{O}_4@$ SiO_2 -MD ₁₆₋₂₀	-4.9	513	0.22
$\text{Fe}_3\text{O}_4@$ SiO_2 -MD ₈₋₁₀	-4.3	557	0.22

^aAll the data was measured at pH = 7.4 and 0.15 M sodium phosphate buffer solution. ^bThe diameter was determined at 25°C by DLS. ^cPI, polydispersity index, $\text{PI} = \langle \mu_2 \rangle / \Gamma^2$.⁴⁶

Because of successful functionalization with amino groups, the ζ potential of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-NH}_2$ was increased to 19.8 mV.⁴² After they were modified with maltose, MD₁₆₋₂₀ or MD₈₋₁₀, the ζ potential of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-Maltose}$, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$ were decreased to -4.8, -4.9, and -4.3 mV, respectively. The hydrodynamic diameters of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-Maltose}$, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$, and $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$ were 580, 513, and 557 nm, respectively. The microspheres showed good stability and dispersity (low PI) because of the steric stabilization of maltose and MD. Additionally, the hydrodynamic sizes of the microspheres were larger than that showed in TEM images because of the presence of hydrate layer outside of microspheres in aqueous environment.

The functional groups of the microspheres surface were further proved by FT-IR. After they were coated with SiO_2 , the

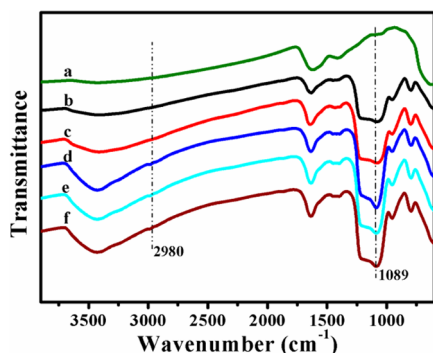


Figure 2. FT-IR spectra of (a) Fe_3O_4 , (b) $\text{Fe}_3\text{O}_4@/\text{SiO}_2$, (c) $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-NH}_2$, (d) $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-Maltose}$, (e) $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$, and (f) $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$.

absorption peak for the $\text{Fe}_3\text{O}_4@/\text{SiO}_2$ microspheres at 1089 cm^{-1} was assigned to the Si-O-Si vibration. After modified with MD or maltose, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-Maltose}$, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$, and $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$ all had a peak at 2980 cm^{-1} which was assigned to the C-H stretching vibrations for maltose or MD. The thermogravimetric analysis (TGA) of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-NH}_2$, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-Maltose}$, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$, and $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$ microspheres were also performed (Figure 3). The TGA curves demonstrated that the weight loss of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-NH}_2$ was 8.9%, while the weight loss of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-Maltose}$, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$, and $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$ were 11.3%, 17.8%, and 22.4% at 800 $^\circ\text{C}$, respectively, which indicated that as the sugar chain length increased, the weight loss also increased. The MD or maltose groups on the microsphere surface were also further proved by

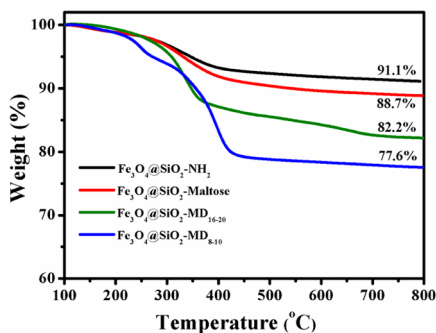


Figure 3. TGA curves of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-NH}_2$, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-Maltose}$, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$, and $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$.

elementary analysis using XPS in Table 2. The percentage content for C, Si, and O were 33.3%, 19.0%, and 47.0%,

Table 2. Surface Element Percentage of $\text{Fe}_3\text{O}_4@/\text{SiO}_2$, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-NH}_2$, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-Maltose}$, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$, and $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$ by X-ray Photoelectron Spectroscopy

sample code	C (%)	Si (%)	O (%)
$\text{Fe}_3\text{O}_4@/\text{SiO}_2$	33.3	19.0	47.0
$\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-NH}_2$	32.4	20.1	19.8
$\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-Maltose}$	44.9	13.9	39.7
$\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$	55.3	8.7	35.2
$\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$	56.2	6.2	36.9

respectively for $\text{Fe}_3\text{O}_4@/\text{SiO}_2$. Before coating of MD or maltose, the percentage content for C, Si, and O were 32.4%, 20.1%, and 19.8%, respectively, for $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-NH}_2$. After coating, the percentage content of C increased significantly from 32.4% to 44.9%, 55.3% and 56.2% while the ratio of Si decreased dramatically from 20.1% to 13.9%, 8.7% and 6.2% for $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-Maltose}$, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$, and $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$ respectively.

The magnetic properties of Fe_3O_4 , $\text{Fe}_3\text{O}_4@/\text{SiO}_2$, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-NH}_2$, and $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$ were determined by VSM (Figure 4). The saturation magnetization of Fe_3O_4 was 60.7

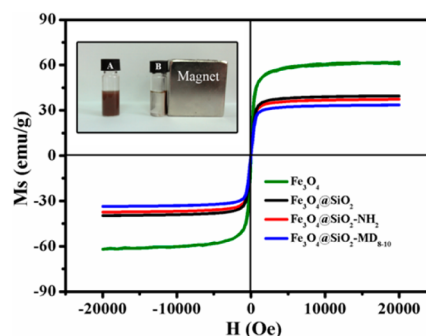
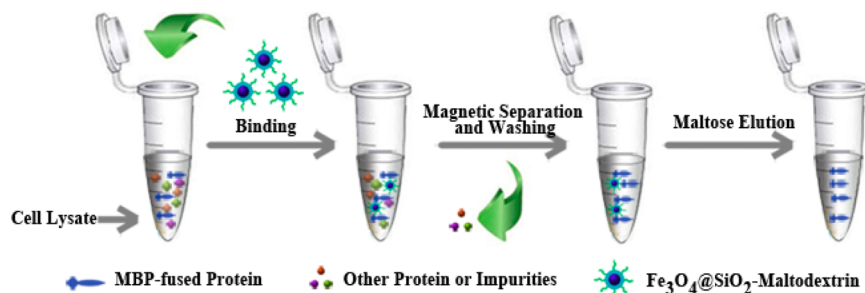


Figure 4. Magnetic hysteresis curves of Fe_3O_4 , $\text{Fe}_3\text{O}_4@/\text{SiO}_2$, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-NH}_2$, and $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$. The inset photograph showed two bottles of the sample $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$ in the same concentration of the aqueous solution: (A) well-dispersed in water and (B) attracted particles to the side wall by a magnet. After they were shaken slightly, the particles could be dispersed again in water.

emu/g. After it was coated with SiO_2 , the saturation magnetization of $\text{Fe}_3\text{O}_4@/\text{SiO}_2$ decreased to 39.6 emu/g. After it was grafted with MD, the saturation magnetization of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$ turned out to be 33.6 emu/g. Although the saturation magnetization value of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$ microspheres decreased significantly compared with the original Fe_3O_4 particles, the magnetic property was still strong enough to ensure the microspheres to be magnetic separated rapidly, as demonstrated by the insert photograph in Figure 4. All of the magnetic microspheres could be attracted to the side wall which was close to a magnet within 30 s. The VSM research also demonstrated that the samples had no obvious hysteresis at 300 K, indicating that the magnetic microspheres were superparamagnetic.⁴¹ So the magnetic microspheres could be separated by an external magnetic field within 30 s and also can be re-dispersed immediately once the external magnetic field was removed.

Scheme 2. Representation of MBP-Fusion Protein Separation/Purification



3.2. Separation and Purification of MBP-Fusion Protein.

To realize the integration of purification/separation and immobilization of MBP-NYE1, we established a protocol to prepare the novel MD-modified magnetic support $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}$. To immobilize MBP-NYE1 onto $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}$, the raw proteins produced by *E. coli* were mixed and shaken with the magnetic support at room temperature for a certain time. After the affinity adsorption between MD and MBP-NYE1, the excessive protein solution was removed with a magnet. The protein-immobilized magnetic support was washed several times using a buffer solution. Then, a maltose buffer was used to wash the magnetic support immobilized MBP-NYE1. After washing, the resulting buffer was analyzed by SDS-PAGE (Scheme 2). Two types of MD-coated magnetic microspheres ($\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$ and $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$) and maltose-coated magnetic microspheres were chosen for comparison purpose because they had different chain lengths.

The results of separation/purification of MBP-fusion protein were shown in Figure 5. Compared to the marker in lane M,

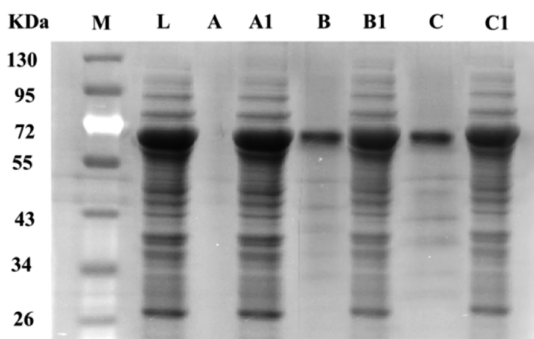


Figure 5. SDS-PAGE analysis: lane M, marker; L, total proteins of lysate; A, maltose washing buffer for $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-Maltose}$; A1, the supernatant solution after the immobilization of crude MBP-NYE1 of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-Maltose}$; B, maltose washing buffer for $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$; B1, the supernatant solution after the immobilization of crude MBP-NYE1 of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$; C, maltose washing buffer for $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$; C1, the supernatant solution after the immobilization of crude MBP-NYE1 of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$.

the crude MBP-NYE1 (with M_w about 64 kDa) mixture solution (lane L) contained proteins with various molecular weights. After immobilization and separation, the MBP-NYE1 immobilized on the magnetic support was washed with the maltose buffer. The resulting maltose buffer solution of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-Maltose}$ (lane A) had no band of MBP-NYE1. While the maltose buffer solution for $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$ (lane B) and $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$ (lane C) contained MBP-NYE1 with high purity. These observations indicated that MBP-NYE1 could be specifically immobilized onto the MD-

modified magnetic support but not be immobilized onto the maltose-modified magnetic support, which may be because the chain lengths of MD₁₆₋₂₀ and MD₈₋₁₀ were long enough to have multi-valent interactions with the proteins.^{47,48} Protein adsorption capacity was measured by Bradford protein assay.⁴⁹ As shown in Figure 6, the protein adsorption of

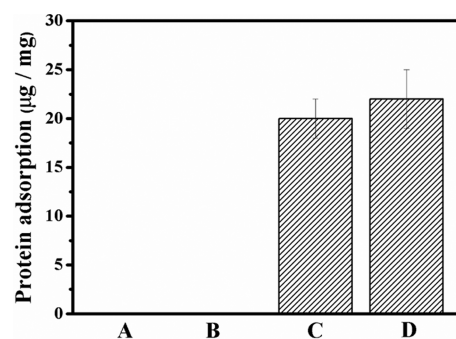


Figure 6. Protein adsorption capacity for A, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-NH}_2$; B, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-Maltose}$; C, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$; D, $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$.

$\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-NH}_2$ was zero which indicated that the $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-NH}_2$ had no non-specific binding. The protein adsorption of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-Maltose}$ was zero while the protein adsorption on $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{16-20}$ was $20 \pm 2 \mu\text{g/mg}$ and on $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$ was $22 \pm 3 \mu\text{g/mg}$. Both results were higher than the binding capacity of amylose magnetic beads from NEW ENGLANG Biolabs Inc ($10 \mu\text{g/mg}$). We confirmed the binding efficiency of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$ with a standard MBP protein (MBP-mCherry) at different concentrations. The protein maximum adsorption of $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$ was around $25 \mu\text{g/mg}$ for MBP-mCherry. When the protein content was less than the maximum adsorption, the magnetic microsphere presented high binding efficiency (more than 70%). And the binding efficiency decreased when the protein content exceed the maximum adsorption.

The relation between binding time and enrichment efficiency was also investigated and the results were shown in Figure 7. The enrichment content and efficiency at pH 7.4 first increased sharply till 30 min (about $22 \pm 3 \mu\text{g/mg}$) and then flattened, which implied that the $\text{Fe}_3\text{O}_4@/\text{SiO}_2\text{-MD}_{8-10}$ microspheres had a rapid enriching behavior within the first 30 min. The recyclability of the magnetic microspheres in MBP-tagged protein separation was tested (Figure 8). After each separation, the magnetic microspheres were rinsed with buffer solution (200 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, 1 mM DTT, pH 7.4) for three times to wash out the excessive maltose and clean the surface of the microspheres. Recycling experiments

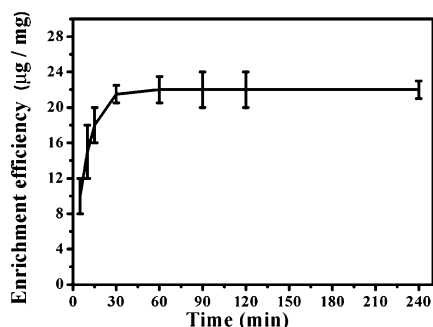


Figure 7. Enrichment efficiency of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-MD}_{8-10}$ microspheres at pH 7.4 at different binding time.

were carried out for five times. The recyclability of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-MD}_{8-10}$ was evaluated by comparing the amount of collected MBP-NYE1 each time by normalizing the amount of MBP-NYE1 isolated in the first cycle. After five separation cycles, the separation capacity of the MBP-tagged protein was about 100%, 98%, 95%, 92%, and 85%, respectively (Figure 8b), which showed excellent recyclability for these composite microspheres in the separation of MBP-tagged proteins. As we reduced the loading volume, less trace was also observed in Figure 8a compared with Figure 5. All of the above research indicated that $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-MD}$ was suitable for the integration of MBP-fusion protein separation/purification and immobilization.

4. CONCLUSIONS

In summary, $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-MD}$ was successfully fabricated by a facile method with low-cost raw materials. The resulting $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-MD}$ had a well-defined core/shell structure and a high magnetization and it also exhibited excellent performance in the separation of MBP-fusion proteins with a binding capacity as high as 22 $\mu\text{g}/\text{mg}$. A practical application of the maltodextrin-modified magnetic microspheres was successfully demonstrated by separating MBP-NYE1 directly from *E. coli* lysate. Considering the simple synthesized method, the process was readily adaptable for industrial scales. Furthermore, this technology was not limited to MBP-NYE1 and such a magnetic support may have wide applications in other MBP-fusion proteins as well.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +86-21-65642385. Fax: +86-21-65640293. E-mail: wlyang@fudan.edu.cn.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful for the support of the National Science Foundation of China (Grant No. 51273047) and the “Shu Guang” project (12SG07) supported by Shanghai Municipal Education Commission and Shanghai Education Development Foundation.

REFERENCES

- Perrett, D. *Proteom. Clin. Appl.* **2007**, *1*, 720–738.
- Walsh, G. *Nat. Biotechnol.* **2010**, *28*, 917–924.
- Carter, P. J. *Exp. Cell Res.* **2011**, *317*, 1261–1269.
- Banks, C. A. S.; Kong, S. E.; Washburn, M. P. *Protein Expr. Purif.* **2012**, *86*, 105–119.
- Young, C. L.; Britton, Z. T.; Robinson, A. S. *Biotechnol. J.* **2012**, *7*, 620–634.
- Kepler, A.; Pick, H.; Arrivoli, C.; Vogel, H.; Johansson, K. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 9955–9959.
- Kontermann, R. E. *Biodrugs* **2009**, *23*, 93–109.
- Bellot, P.; Tiels, P.; Melkebeek, V.; Devriendt, B.; Goddeeris, B. M.; Cox, E. *Vet. Immunol. Immunopathol.* **2013**, *152*, 101–108.
- Butt, T. R.; Jonnalagadda, S.; Monia, B. P.; Sternberg, E. J.; Marsh, J. A.; Stadel, J. M.; Ecker, D. J.; Crooke, S. T. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2540–2544.
- Fox, J. D.; Kapust, R. B.; Waugh, D. S. *Protein Sci.* **2001**, *10*, 622–630.
- Kapust, R. B.; Waugh, D. S. *Protein Sci.* **1999**, *8*, 1668–1674.
- Liu, Y. F.; Manna, A.; Li, R. G.; Martin, W. E.; Murphy, R. C.; Cheung, A. L.; Zhang, G. Y. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 6877–6882.
- Chao, J. A.; Prasad, G. S.; White, S. A.; Stout, C. D.; Williamson, J. R. *J. Mol. Biol.* **2003**, *326*, 999–1004.
- Ullah, H.; Scappini, E. L.; Moon, A. F.; Williams, L. V.; Armstrong, D. L.; Pedersen, L. C. *Protein Sci.* **2008**, *17*, 1771–1780.
- Kobe, B.; Center, R. J.; Kemp, B. E.; Pountourios, P. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4319–4324.
- Bhunia, S. K.; Jana, N. R. *ACS Appl. Mater. Interfaces* **2011**, *3*, 3335–3341.

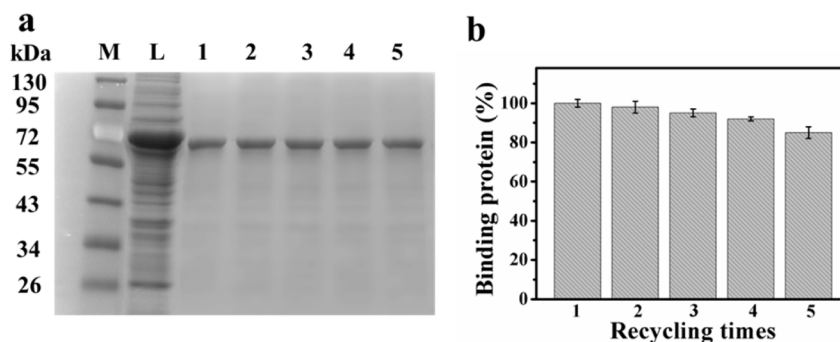


Figure 8. (a) SDS-PAGE analysis of proteins released from the $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-MD}_{8-10}$ reused up to five times (lanes 1–5). Lane M, molecular weight marker; Lane L, the total proteins of lysate; (b) Recycling of the $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-MD}_{8-10}$ in the separation of MBP-NYE1.

- (17) Zhen, G. L.; Falconnet, D.; Kuennemann, E.; Voros, J.; Spencer, N. D.; Textor, M.; Zurcher, S. *Adv. Funct. Mater.* **2006**, *16*, 243–251.
- (18) Lu, A. H.; Salabas, E. L.; Schueth, F. *Angew. Chem., Int. Ed.* **2007**, *46*, 1222–1244.
- (19) Lim, E. K.; Huh, Y. M.; Yang, J.; Lee, K.; Suh, J. S.; Haam, S. *Adv. Mater.* **2011**, *23*, 2436–2442.
- (20) Jeong, U.; Teng, X.; Wang, Y.; Yang, H.; Xia, Y. *Adv. Mater.* **2007**, *19*, 33–60.
- (21) Gutfleisch, O.; Willard, M. A.; Bruck, E.; Chen, C. H.; Sankar, S. G.; Liu, J. P. *Adv. Mater.* **2011**, *23*, 821–842.
- (22) Ge, J.; Huynh, T.; Hu, Y.; Yin, Y. *Nano Lett.* **2008**, *8*, 931–934.
- (23) Gao, J.; Gu, H.; Xu, B. *Accounts Chem. Res.* **2009**, *42*, 1097–1107.
- (24) Ma, W. F.; Zhang, Y.; Li, L. L.; You, L. J.; Zhang, P.; Zhang, Y. T.; Li, J. M.; Yu, M.; Guo, J.; Lu, H. J.; Wang, C. C. *ACS Nano* **2012**, *6*, 3179–3188.
- (25) Zhang, Y.; Li, L.; Ma, W.; Zhang, Y.; Yu, M.; Guo, J.; Lu, H.; Wang, C. C. *ACS Appl. Mater. Interfaces* **2013**, *5*, 614–621.
- (26) Xu, S.; Song, X.; Guo, J.; Wang, C. C. *ACS Appl. Mater. Interfaces* **2012**, *4*, 4764–4775.
- (27) Pan, M.; Sun, Y.; Zheng, J.; Yang, W. *ACS Appl. Mater. Interfaces* **2013**, *5*, 8351–8358.
- (28) Chen, H.; Deng, C.; Zhang, X. *Angew. Chem., Int. Ed.* **2010**, *49*, 607–611.
- (29) Liu, S.; Chen, H.; Lu, X.; Deng, C.; Zhang, X.; Yang, P. *Angew. Chem., Int. Ed.* **2010**, *49*, 7557–7561.
- (30) Zhang, Y. H.; Wang, X. Y.; Shan, W.; Wu, B. Y.; Fan, H. Z.; Yu, X. J.; Tang, Y.; Yang, P. Y. *Angew. Chem., Int. Ed.* **2005**, *44*, 615–617.
- (31) Xu, F.; Geiger, J. H.; Baker, G. L. *Langmuir* **2011**, *27*, 3106–3112.
- (32) Pan, Y.; Long, M. J. C.; Lin, H.; Hedstrom, L.; Xu, B. *Chem. Sci.* **2012**, *3*, 3495–3499.
- (33) Zhang, Y.; Yang, Y.; Ma, W.; Guo, J.; Lin, Y.; Wang, C. C. *ACS Appl. Mater. Interfaces* **2013**, *5*, 2626–2633.
- (34) Pan, Y.; Long, M. J. C.; Li, X.; Shi, J.; Hedstrom, L.; Xu, B. *Chem. Sci.* **2011**, *2*, 945–948.
- (35) Zhou, L. L.; Wu, J. J.; Zhang, H. J.; Kang, Y.; Guo, J.; Zhang, C.; Yuan, J. Y.; Xing, X. H. *J. Mater. Chem.* **2012**, *22*, 6813–6818.
- (36) Ren, G. D.; An, K.; Liao, Y.; Zhou, X.; Cao, Y. J.; Zhao, H. F.; Ge, X. C.; Kuai, B. K. *Plant Physiol.* **2007**, *144*, 1429–1441.
- (37) Smyth, D. R.; Mrozkiewicz, M. K.; McGrath, W. J.; Listwan, P.; Kobe, B. *Protein Sci.* **2003**, *12*, 1313–1322.
- (38) Ma, W. F.; Xu, S. A.; Li, J. M.; Guo, J.; Lin, Y.; Wang, C. C. *J. Polym. Sci., Polym. Chem.* **2011**, *49*, 2725–2733.
- (39) Laemmli, U. K. *Nature* **1970**, *227*, 680–685.
- (40) Xuan, S. H.; Wang, Y. X. J.; Yu, J. C.; Leung, K. C. F. *Chem. Mater.* **2009**, *21*, 5079–5087.
- (41) Deng, H.; Li, X. L.; Peng, Q.; Wang, X.; Chen, J. P.; Li, Y. D. *Angew. Chem., Int. Ed.* **2005**, *44*, 2782–2785.
- (42) Chang, B. C.; Guo, J.; Liu, C.; Qian, J.; Yang, W. L. *J. Mater. Chem.* **2010**, *20*, 9941–9947.
- (43) Ueda, T.; Tomita, K.; Notsu, Y.; Ito, T.; Fumoto, M.; Takakura, T.; Nagatome, H.; Takimoto, A.; Mihara, S. I.; Togame, H.; Kawamoto, K.; Iwasaki, T.; Asakura, K.; Oshima, T.; Hanasaki, K.; Nishimura, S. I.; Kondo, H. *J. Am. Chem. Soc.* **2009**, *131*, 6237–6245.
- (44) van Wijk, A.; Siebum, A.; Schoevaart, R.; Kieboom, T. *Carbohydr. Res.* **2006**, *341*, 2921–2926.
- (45) Boos, W.; Shuman, H. *Microbiol. Mol. Biol. R.* **1998**, *62*, 204–229.
- (46) Chu, B.; Wang, Z. L.; Yu, J. Q. *Macromolecules* **1991**, *24*, 6832–6838.
- (47) Danishefsky, S. J.; McClure, K. F.; Randolph, J. T. *Science* **1993**, *260*, 1307–1309.
- (48) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. *Science* **2001**, *291*, 1523–1527.
- (49) Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248–254.