Supporting Information

Click and Release: Fluoride Cleavable Linker for Mild Bioorthogonal Separation

Elia M. Schneider, Martin Zeltner, Vladimir Zlateski, Robert N. Grass, Wendelin J. Stark*

Institute for Chemical and Bioengineering, ETH Zurich, Vladimir-Prelog-Weg 1, 8093 Zurich, Switzerland

*E-mail: Wendelin.stark@chem.ethz.ch
1. General information

Carbon-coated cobalt nanoparticles were suspended by the use of an ultrasonic bath and subjected to various reactions (see below). After a reaction or a pre-treatment step, nanoparticles were recovered from the reaction mixture with the aid of a conventional magnet (neodymium based magnet, N48, W-12-N, Webcraft GmbH, side length 12 mm). The nanoparticles were analyzed by Fourier transform infrared spectroscopy (FT-IR) 5 wt% nanoparticles in KBr with a Tensor 27 Spectrometer (Bruker Optics) equipped with a diffuse reflectance accessory (DiffuseIR, Pike Technologies) and elemental microanalysis (ELEMENTAR, Elementar Analysensysteme). Transmission electron microscopy (TEM) was measured with a CM12 (Philips, operated at 120 kV). The nanomaterial was further characterized by magnetic hysteresis susceptibility as a powder in a gelatin capsule (vibrating sample magnetometer, VSM, Princeton Measurements Corporation, model 3900)

Commercially available reagents were used as received from Sigma-Aldrich, TCI Deutschland, Alfa Aeasar and Kerafast. All air-sensitive reactions were carried out under nitrogen atmosphere. HPLC measurements were conducted on an AGILENT 1100 (WATERS Column, particle size, 5 μm; column size, 2.1 × 150 mm2). GC–MS measurements were performed (capillary, 30 m × 250 × 0.5 μm2) with split injection (250 °C; ratio = 10:1; injection volume, 1 μL) and a temperature program (80 °C for 2 min; increase at 20 °C min⁻¹ until 300 °C). UV Spectroscopy was done using a Thermo Scientific NANODROP 2000c spectrometer.
2. Experimental procedures

C/Co@starter 1 was prepared according to literature.\textsuperscript{[12b]} It should be noted that only fresh 2-Bromo-2-methylpropionyl bromide should be used to generate an active starter moiety.

FT-IR: 2980 cm\(^{-1}\), 2931 cm\(^{-1}\), 1733 cm\(^{-1}\), 1277 cm\(^{-1}\), 1162 cm\(^{-1}\).
Elemental microanalysis: \([C]: 10.7\%\), \([N]: 0.13\%\), \([Br]: 0.62\%\).

2.1 Synthesis of C/Co@PEGMA 2

All reaction steps were performed under protective nitrogen atmosphere. Poly(ethylene glycol)methacrylate (PEGMA, \(M_n\) 360, 5 mL) was filtered over a basic aluminum oxide column in order to remove the inhibitor (MEHQ), then mixed with 25 mL of solvent (MeOH : H\(_2\)O, 4:1) and degassed with nitrogen by bubbling through for 30 min. CuBr\(_2\) (10 mg, 0.045 mMol), bipyridine (52 mg, 0.033 mMol) and L-ascorbic acid (60 mg, 0.34 mMol) were added to the mixture and degassed for additional 5 minutes. Meanwhile, C/Co@starter 1 (900 mg) was dispersed in 10 mL of solvent (MeOH : H\(_2\)O, 4:1) and degassed with nitrogen for 30 min. Subsequently, the monomer/catalytic system-solution was added to the particles and sonicated at 20 °C overnight. Upon completion of the reaction the polymerized particles were recovered from the reaction mixture with the aid of a permanent magnet and washed with H\(_2\)O (4x), EtOH (2x) and acetone (1x). The nanoparticles were dried in a vacuum oven at 50 °C for 16 h.

FT-IR: 2920 cm\(^{-1}\), 2876 cm\(^{-1}\), 1725 cm\(^{-1}\), 1455 cm\(^{-1}\), 1105 cm\(^{-1}\).
Elemental microanalysis: \([C]: 35.08\%\), \([N]: 0.04\%\).
2.2 Synthesis of C/Co@PEGMA-Si-Octyne 3

All reaction steps were performed under protective nitrogen atmosphere. (1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethanol (49 mg, 0.32 mMol) and 4-dimethylaminopyridine (27 mg, 0.22 mMol) were degassed 3 times in a 10 mL Schlenk flask and dissolved in 1 mL dry DMF. The solution was cooled in an ice-bath and triethylamine (75 μL, 0.74 mMol) was added. Subsequently, dichlorodisopropylsilane (69 μL, 0.35 mMol) was added dropwise. After 6 hours, the reaction mixture was allowed to warm to room temperature.

C/Co@PEGMA 2 (200 mg) were washed with dry DMF (3x) and dispersed in 10 mL dry DMF. Subsequently, the in situ-generated mono-chlorinated silane solution was added and the resulting dispersion stirred overnight. Upon completion of the reaction the functionalized particles were recovered from the reaction mixture with the aid of a permanent magnet and washed with DMF (3x), EtOH (3x) and H₂O (2x). For analysis, a fraction of the nanoparticles was dried in a vacuum oven at 40 °C for 24 h.

FT-IR: 2929 cm⁻¹, 2871 cm⁻¹, 1731 cm⁻¹, 1463 cm⁻¹, 1113 cm⁻¹. 
Elemental microanalysis: [C]: 40.33%, [N]: 0.00%.
2.3 Synthesis of C/Co@PEGMA-Si-Naphthol

All reaction steps were performed under protective nitrogen atmosphere. 1-naphthol (400 mg, 2.7 mMol), 4-dimethylaminopyridine (180 mg, 1.3 mMol) were degassed 3 times in a 10 mL Schlenk flask and dissolved in 1 mL dry DMF. The solution was cooled in an ice-bath and triethylamine (0.7 mL, 6.9 mMol) was added. Subsequently, dichlorodiisopropylsilane (0.5 mL, 2.7 mMol) was added dropwise. After 8 hours, reaction was allowed to warm to room temperature. GC-MS found: m/z 292 (monochlorinated adduct)

C/Co@PEGMA 2 (500 mg, different batch, carbon mass content = 20.1 %) were washed with dry DMF (2x) and dispersed in 20 mL dry DMF. Subsequently, the in situ-generated mono-chlorinated silane solution was added and the resulting dispersion stirred overnight. Upon completion of the reaction the functionalized particles were recovered from the reaction mixture with the aid of a permanent magnet and washed with DMF (2x) and EtOH (2x). For analysis, a fraction of the nanoparticles was dried in a vacuum oven at 40 °C for 24 h.

FT-IR: 2868 cm\(^{-1}\), 1731 cm\(^{-1}\), 1643 cm\(^{-1}\), 1460 cm\(^{-1}\).
Elemental microanalysis: [C]: 22.12\% (different precursor 20.1 \% C), [N]: 0.00\%.
2.4 Functionalization of α-Chymotrypsin with azide

α-Chymotrypsin (8 mg, 0.3 μMol) was dissolved in 2 mL of 0.2 M NaHCO₃ in an Eppendorf tube. Azide-PEG-NHS (5 EQ, 0.57 μL) was added and the solution shaken (500 RPM) at RT for 16 h. Purification of the protein was done using Amicon-Ultra centrifugal filter units, diluting with Milli-Q water (4 mL, MWCO 10000, 6000 RPM, 20 min, 3 times).

2.5 Standard procedure for TAMRA-Azide dipolar cycloaddition (click)

C/Co@PEGMA-Si-Octyne 3 (10 mg) was dispersed in 1 mL H₂O using a 2 mL Eppendorf tube. TAMRA-azide solution (40 μL, 0.01 mM) was added and the solution was shaken for 3 h. The reaction was monitored by UV-spectroscopy (550 nm). Particles were separated using a permanent magnet.

2.6 Standard procedure for enzyme click

C/Co@PEGMA-Si-Octyne 3 (10 mg) was dispersed in 1 mL H₂O using a 2 mL Eppendorf tube. α-Chymotrypsin-Azide solution (100 μL, 0.001 mM) was added and the solution was shaken for 16 h (500 RPM). Particles were separated using a permanent magnet.

2.8 Standard procedure for enzymatic assay with α-Chymotrypsin

The activity of the protease was determined via UV monitoring of the cleavage of N-benzoyl-L-tyrosine p-nitroanilide. Therefore, a standard enzymatic assay was performed based on the enzyme catalyzed hydrolysis of the peptide and monitored by UV-spectroscopy at 390 nm over a defined amount of time (i.e., 10 min). 2 mL of Tris/HCl buffer pH 7.8 was added to a PS-cuvette, followed by 80 μL 2 M CaCl₂ solution, 100 μL substrate (1.4 mM, DMSO : H₂O, 1:1) and at t = 0 100 μL sample. Measurements were taken each 30 s.
2.7 Standard procedure for cleavage reaction using buffered oxide etch

Particles were dispersed in 0.9 mL destilled water using an Eppendorf tube. Subsequently, 0.1 mL buffered oxide etch (0.13 M) was added and the tube shaken at RT. Detailed preparation and handling of buffered etch can be found in literature.[10e]

3. TEM micrographs

![Image of TEM micrographs](image)

Fig. 1. TEM micrographs of the magnetic particles.
4. Cleave test with 1-naphthol

Fig. 2. Deprotection analysis using different buffers and concentrations of buffered oxide etch. Conditions: 1 mL of solvent (buffer, water or BOE) and 10-20 mg of 1-naphthol functionalized magnetic particles.
5. Unspecific protein adsorption

Fig. 3. Chart depicting the unspecific protein adsorption. Rhodamine labelled BSA was dissolved in water (0.5 nMol mL$^{-1}$) and the absorption at 555 nm was measured using naked particles, 2 or 3 (20 mg each).
### 6. Elemental analysis overview

Table 1. Elemental analysis results

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>C (%)</th>
<th>N (%)</th>
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<tr>
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<tr>
<td>2</td>
<td>2</td>
<td>35.08</td>
<td>0.04</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>38.14</td>
<td>0.00</td>
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<td>4</td>
<td>3 w/ enzyme</td>
<td>37.78</td>
<td>0.14</td>
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<tr>
<td>5</td>
<td>3 after cleavage</td>
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<td>0.04</td>
</tr>
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</table>
7. Vibrating sample magnetometer measurements (VSM)

7.1 C/Co

7.2 C/Co@PEGMA 2
7.3 C/Co@PEGMA-Si-Octyne 3
8. XRD Spectra

8.1 C/Co

8.2 C/Co@PEGMA-Si-Octyne 3