Supporting information for

Polymer Brush-Modified Magnetic Nanoparticles for His-Tagged Protein Purification

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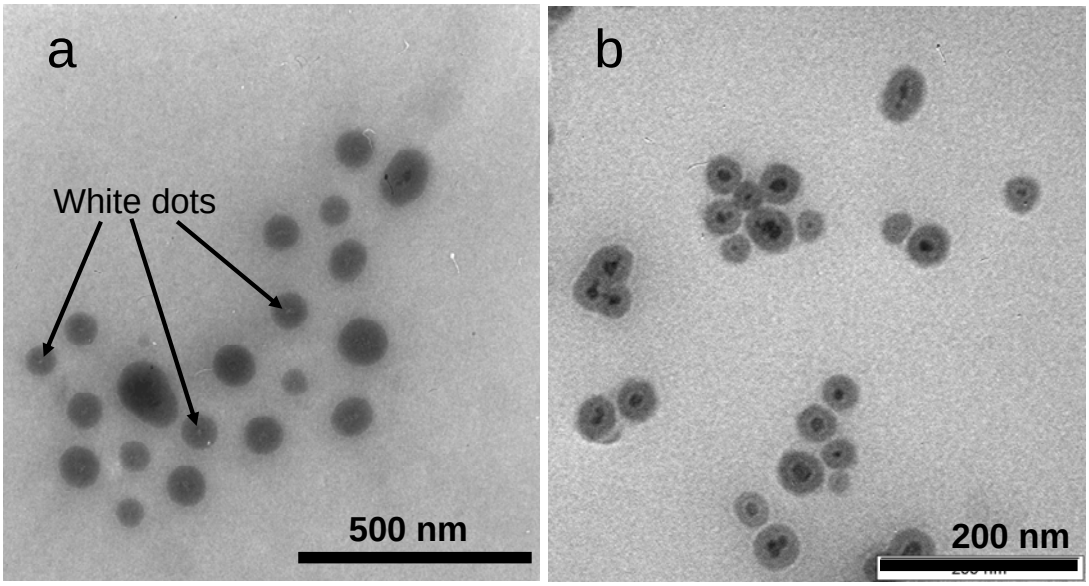


Figure S1. TEM images of initiator-modified SiO_2-Fe_3O_4 particles prepared (a) without and (b) with nitrogen bubbling during initiator attachment.

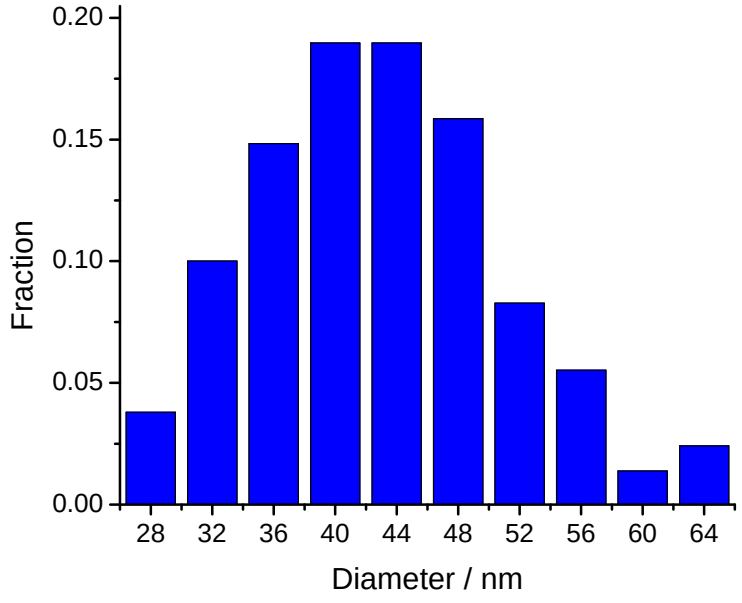


Figure S2. Size distribution of SiO_2-Fe_3O_4 particles in TEM images.

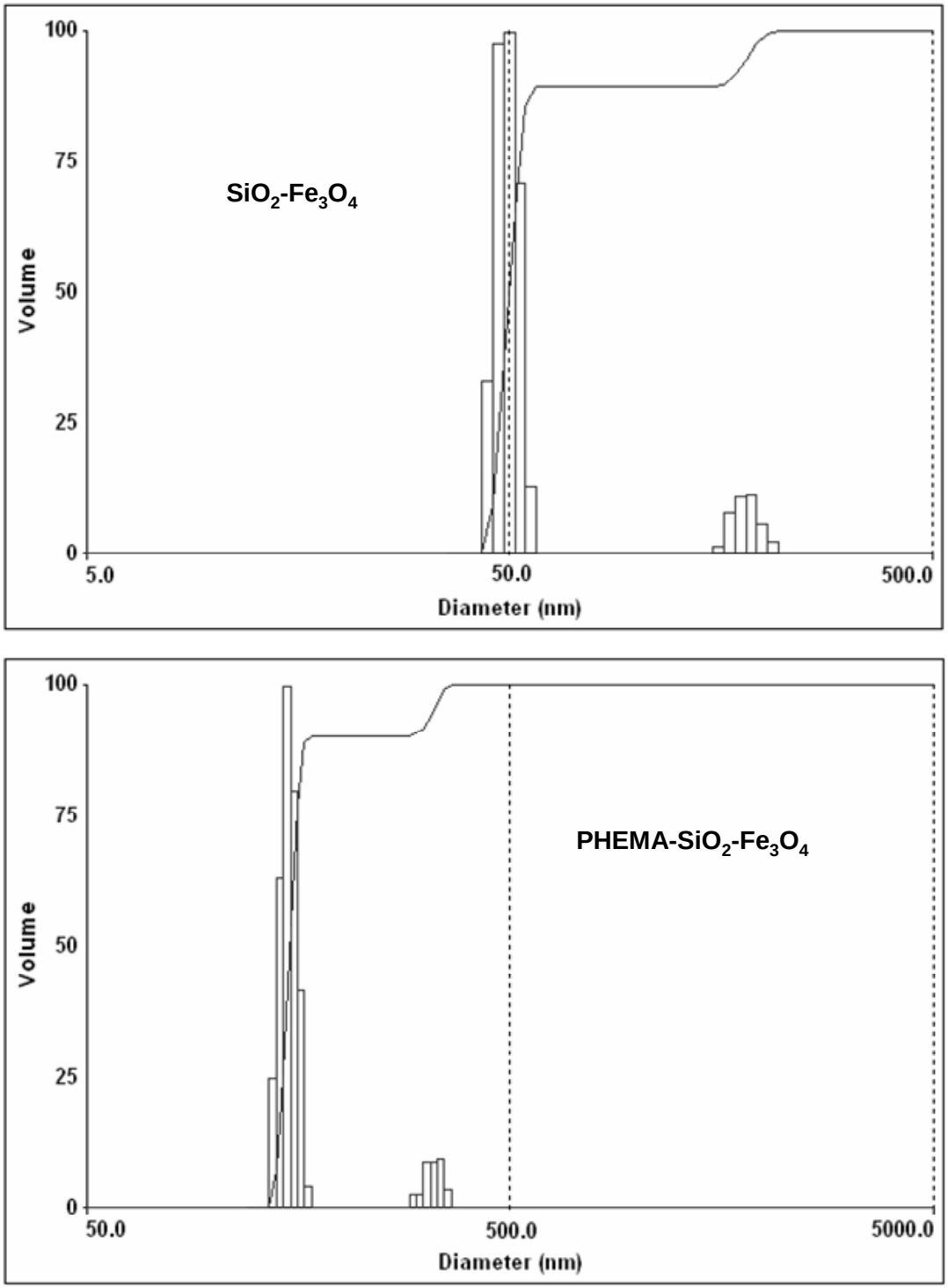


Figure S3. Nanoparticle size distribution determined from light scattering data, SiO_2-Fe_3O_4 (top) and PHEMA-SiO_2-Fe_3O_4 (bottom).

Estimation of the Extent of Reaction of PHEMA with SA

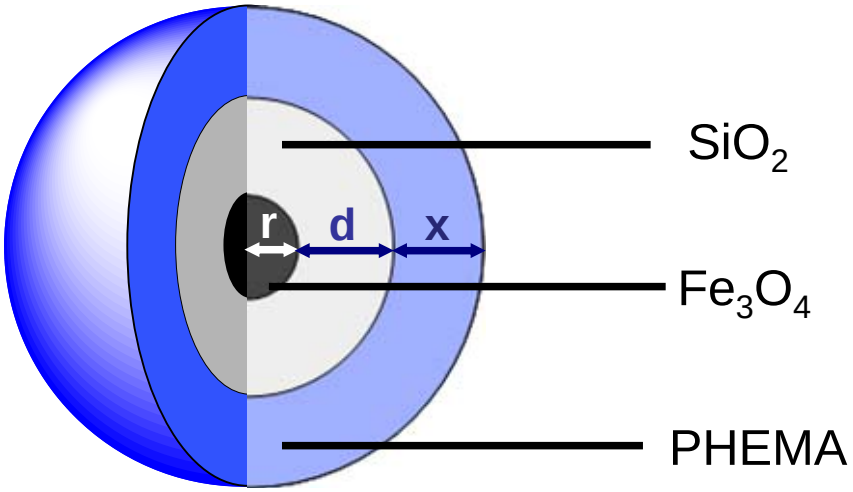
To estimate the percent yield of the reaction of PHEMA with SA, suppose we start from 1.00 g of initiator-Silica-MNPs for all samples. TGA analysis shows that such a sample yields approximately 0.89 g of residue. Added polymer and its SA derivatives should completely decompose, so the weight of residue should be constant at 0.89 g for all the samples. To convert the initial mass for a sample of arbitrary mass to a normalized initial mass based on starting from 1.00 g of initiator-Silica-MNPs, we simply divide 0.89 g by the weight fraction remaining after TGA. Subtraction of the 1.00 g of initiator-Silica-MNPs from the normalized initial mass gives the mass of added polymer. Table S1 shows the masses of PHEMA and PHEMA-SA attached to initiator-Silica-MNPs. Based on the molecular masses of PHEMA (130 g/mol) and PHEMA-SA (230 g/mol), the PHEMA and PHEMA-SA masses determined from TGA suggest that the reaction with SA occurs in 105% yield. Thus, the reaction proceeds essentially to completion.

Table S1. Calculated Masses (based on TGA) of PHEMA and PHEMA-SA Formed on Initiator-Silica-MNPs. The masses are normalized to samples starting with 1.00 g of initiator-Silica-MNPs with 0.89 g residue remaining.

sample	% mass of residue from TGA	Initial Mass of sample/g	Mass of polymer/g
Initiator-Silica-MNPs	89	1.00	0
PHEMA-MNPs	29	3.1	2.1
SA-PHEMA-MNPs	18	4.9	3.9

Estimation of PHEMA thickness from TGA data.

Scheme S1 illustrates the composition of a PHEMA-SiO_2-Fe_3O_4 bead. The thickness of the polymer brushes can be calculated from the mass of PHEMA as follows.



Scheme S1. Structure of PHEMA-SiO_2-Fe_3O_4.

Roughly:

Mass(PHEMA)/Mass(initiator+SiO_2+Fe_3O_4) = 2.1 from TGA data in Table S1

Density of polymer (ρ_{PHEMA}): 1.2 mg/cm^3; thickness: x nm

Density of silica (ρ_{SiO2}): 2 mg/cm^3; thickness (d): 16.5 nm

Density of magnetite (ρ_{Fe3O4}): 5 mg/cm^3; radius (r): 5 nm

Noting that mass equals the production of density, ρ, and volume, V,

$$\frac{\rho_{PHEMA} \times V_{PHEMA}}{\rho_{silica} \times V_{silica} + \rho_{Fe_3O_4} \times V_{Fe_3O_4}} = 2.1$$

Using the formulae for the volumes of spherical shells,

$$\frac{\rho_{PHEMA} \times \frac{4}{3} \times \pi \times [(x+d+r)^3 - (d+r)^3]}{\rho_{SiO_2} \times \frac{4}{3} \times \pi \times [(d+r)^3 - r^3] + \rho_{Fe_3O_4} \times \frac{4}{3} \times \pi \times r^3} = 2.1$$

$$\frac{1.2\times\frac{4}{3}\times\pi\times[(x+16.5+5)^3-(16.5+5)^3]}{2\times\frac{4}{3}\times\pi\times[(16.5+5)^3-5^3]+5\times\frac{4}{3}\times\pi\times5^3}=2.1$$

Solving the simple equation, x=14 nm. This calculation neglects the thickness of the initiator (less than 0.5 nm based on TGA data).

Calculation of the Mass of Protein in a BSA monolayer on a SiO_2-Fe_3O_4 Particle with a Diameter of 43 nm

To calculate the mass of a BSA monolayer on a SiO_2-Fe_3O_4 Particle (43 nm in diameter), we assume that a 4 nm thick BSA monolayer[1] (d_{BSA}) forms on the outside of a single bead with a surface area of S_{bead}. (The assumption of a monolayer thickness of 4 nm and a film density of 1 g/cm^3 may be a slight overestimation because of incomplete packing.)

$$m_{bead}=m_{silica}+m_{Fe_3O_4}=\rho_{SiO_2}\times V_{SiO_2}+\rho_{Fe_3O_4}\times V_{Fe_3O_4}$$
$$=\frac{4}{3}\times\pi\times\{\rho_{SiO_2}\times[(d+r)^3-(r)^3]+\rho_{Fe_3O_4}\times r^3\}$$
$$=\frac{4}{3}\times\pi\times\{2\times[(21.5e-7)^3-(5e-7)^3]+5\times(5e-7)^3\}$$
$$=(8.5e-17)g$$
$$m_{monolayerBSA/bead}=\rho_{BSA}\times d_{BSA}\times S_{bead}=\rho_{BSA}\times d_{BSA}\times 4\pi\times(d+r)^2$$
$$=1\times(4e-7)\times 4\times\pi\times(21.5e-7)^2$$
$$=(2.3e-17)g$$

Calculation of the mass of a fully modified bead

The above calculations show that the mass of a single SiO_2-Fe_3O_4 particle is 8.5 x 10^{-17} g. The TGA data in Table S1 show that there are 2.1 g of polymer per g of initiator-modified SiO_2-Fe_3O_4. Assuming complete derivatization of each PHEMA

repeat unit with SA and aminobutyl NTA-Cu^{2+}, the molar mass of the repeat unit will increase from 130 to 538 g/mol. Thus, after modification there will be 8.7 g of Cu^{2+}-NTA-SA-PHEMA per g of initiator-SiO_2-Fe_3O_4. Hence, neglecting the initiator mass, which should be negligible, a single, fully modified bead will have a mass of 8.2 x 10^{-16} g. (Note that we neglect the initiator in part because its TGA data are difficult to interpret as the the silane will become part of the residue.)

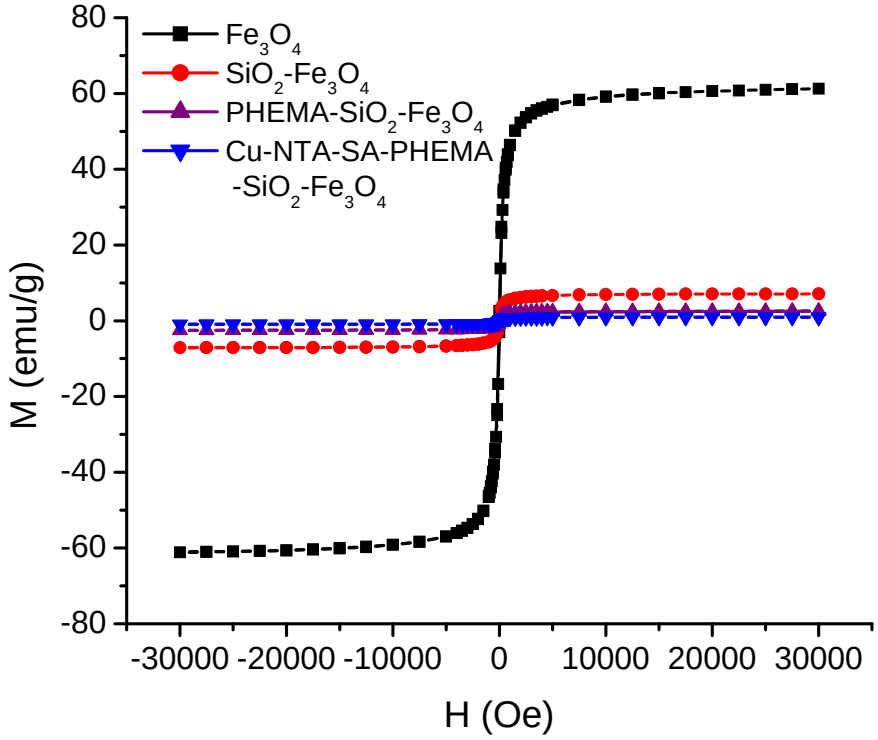


Figure S4. Magnetization curves for Fe_3O_4 (Black), SiO_2-Fe_3O_4 (red), PHEMA-SiO_2-Fe_3O_4 (purple), and Cu^{2+}-NTA-SA-PHEMA-SiO_2-Fe_3O_4 (blue) nanoparticles.

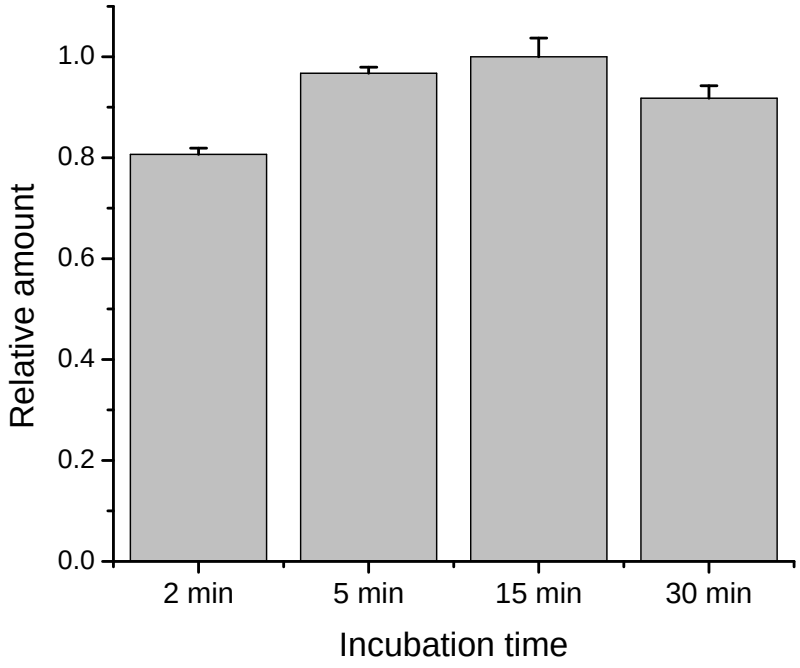


Figure S5. Bradford assay analysis of the amount of His-CRALBP eluted from Ni^{2+}-NTA-SA-PHEMA-SiO_2-Fe_3O_4 beads after incubation of the beads in a cell lysate for various times. (The beads were washed prior to elution.) The amounts are normalized to the concentration with the 15-min incubation, and the error bars are the standard deviations of three measurements of the concentration in a single experiment.

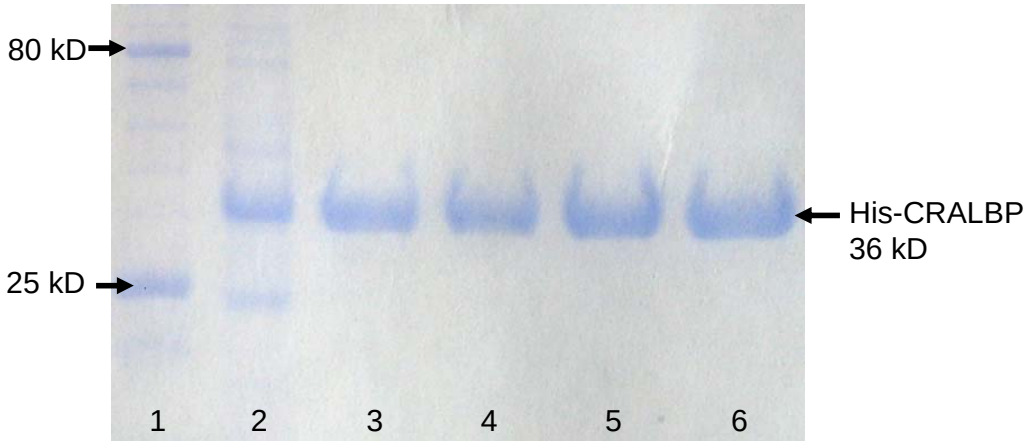


Figure S6. SDS-PAGE analysis (Coomassie staining) of a cell lysate containing overexpressed His-tagged CRALBP before (lane 2) and after (lane 3-6) purification through adsorption on Ni^{2+}-NTA-SA-PHEMA-SiO_2-Fe_3O_4 beads with various incubation times (lane 3: 2 min; lane 4: 5 min; lane 5: 15 min; lane 6: 30 min). Prior to analysis, the purified protein was eluted from washed beads using 0.5 M imidazole in buffer. Lane 1 shows a standard protein ladder.

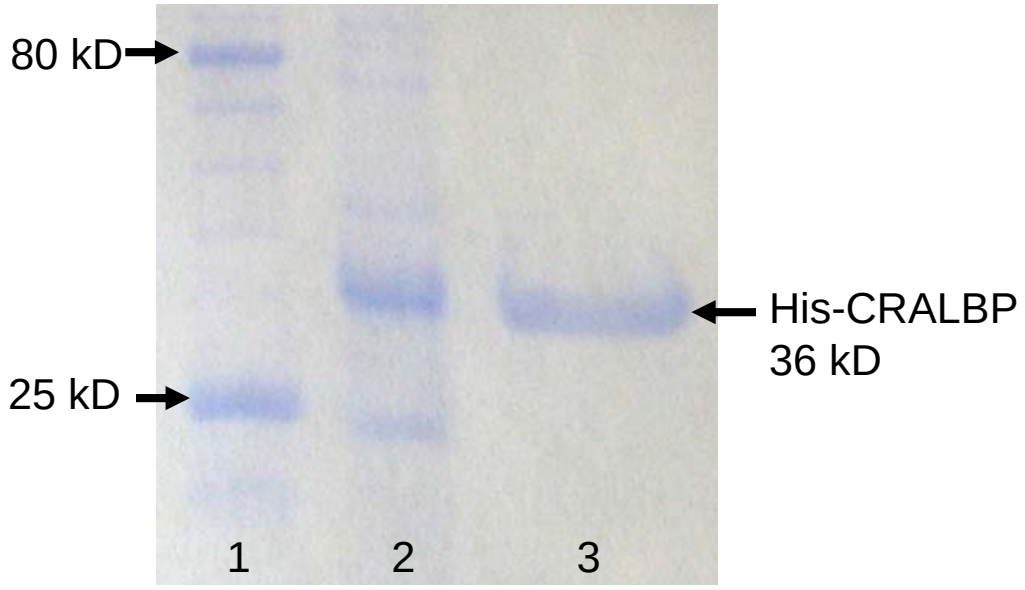


Figure S7. SDS-PAGE analysis (Coomassie staining) of a cell lysate containing overexpressed His-tagged CRALBP before (lane 2) and after (lane 3) purification using Ni^{2+}(reloaded)-NTA-SA-PHEMA-SiO_2-Fe_3O_4 beads. Prior to analysis, the purified protein was eluted from washed beads using 0.5 M imidazole in buffer. Lane 1 shows a standard protein ladder.

(1) Tsuneda, S.; Saito, K.; Furusaki, S.; Sugo, T. *J. Chromatogr., A* **1995**, *689*, 211-218.