Electronic Supplementary Information:

Task-specific membranes for the isolation of recombinant proteins with peptide-tags

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Experimental

Materials

N-(5-Amino-1-carboxypentyl)iminodiacetic acid was purchased from Dojindo Laboratories (Kumamoto, Japan). 1-Bromhexadecane and diethyl iminodiacetate were purchased from TCI (Tokyo, Japan). Glutathione (oxidized form) and 2-mercaptethanol were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). FITC-albumin and TRITC were purchased from Sigma (St. Louis, MO) and Invitrogen (Carlsbad, CA), respectively. Cellulose diacetate (substitution ratio 2.38) was purchased from Eastman Chemical Japan, Inc. (Tokyo, Japan). Other chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Polystyrene nanospheres (50, 100 and 300 nm in diameter) were purchased as a suspension from Duke Scientific (Palo Alto, CA).

His-tag GFP (hexahistidine-tagged enhanced green fluorescent protein) was prepared as previously reported.[11] Briefly, the gene fragment coding N-terminus His-tagged GFP was cloned into the pBAD/thio-TOPO vector (Invitrogen). Protein expression was induced in *E. coli* strain Top10 (Invitrogen) by addition of 0.1% L-arabinose and subsequent incubation for 16 h at 27 °C. The His-tag GFP was purified using TALON metal affinity resin (Clontech).

Glutathione *S*-transferase (GST) was expressed in the *E. coli* strain BL21(DE3) and was purified from the cell lysate on a GST affinity gel column (GSTrap HP, GE Healthcare).[12] To label GST with tetramethylrhodamine-5-(and 6)-isothiocyanate (TRITC), a 0.4 ml GST solution (2 mg/ml, pH 9.2 carbonate buffer) was mixed with a 100 µl DMSO solution containing TRITC 0.12 mg at 4 °C overnight. The solution was dialyzed using an ultrafiltration membrane of MWCO 20,000 Da.

NTA-surfactant (oleoyl AB-NTA): Oleic acid (1 mmol) was esterified with *N*-hydroxysuccinimide (1 mmol) using *N,N'*-Dicyclohexylcarbodiimide (1.1 mmol) in DMF (1 ml) for 20 h at room temperature. After filtration of the solution, the oleic acid NHS ester (0.11 mmol) and *N*-(5-amino-1-carboxypentyl)iminodiacetic acid (0.1 mmol, AB-NTA) were dissolved in a mixture of 470 µl DMF, 200 µl triethylamine and 30 µl water. After the reaction for 20 h at room temperature, the reaction solution was evaporated and then dissolved in chloroform. The chloroform solution was washed twice with water and twice using a 0.1 M HCl solution. This process was followed by evaporation. The resultant powder was dispersed in *n*-hexane and filtrated to obtain oleoyl AB-NTA. Oleoyl AB-NTA was identified by ^{1}H-NMR (500 MHz, DMSO-d_6 containing 1% v/v TMS)) using Bruker Avance 500 and MALDI TOF/MS (m/z = 524.7).

Glutathione-surfactant (*N*-hexadecyl glutathione): The oxidized form of glutathione (0.5 mmol), 1-bromohexadecane (0.55 mmol) and potassium carbonate (0.75 mmol) were mixed in 10 ml DMF. The mixture was refluxed at 50 ºC for 24 h under N_2. After the reaction, the mixture was evaporated and the resultant residue was washed twice with diethyl ether. The residue was dissolved in chloroform and washed twice with a 1

M HCl solution and water. The chloroform phase was evaporated and dried in vacuo to obtain *N*-hexadecyl glutathione (oxidized). *N*-Hexadecyl glutathione (oxidized) was identified by H-NMR (500 MHz, DMSO-d_6 containing 1% v/v TMS. The reduced form was identified by ESI-MS (m/z =587.2).

^{1}H NMR assignments

Oleoyl AB-NTA: 0.853 ppm (t, 3H, C**H$_3$**-) (J=6.31, 7.25 Hz), 1.293 ppm (m, 32H, -C**H$_2$**-), 1.991 ppm (m, 4H, -CH-C**H$_2$**-), 2.981 ppm (m, 1H, - N-C(COOH)-C**H**-), 3.442 ppm (m, 4H, -N-C**H$_2$**-COOH), 5.322 ppm (t, 2H, -C**H**-CH_2-) (J=4.57, 4.61 Hz)

Glutathione-surfactant (oxidized form): 0.88 ppm (t, 6H, -CH_2-C**H$_3$**) (J=6.46, 6.07 Hz), 1.28-1.56 ppm (m, 60H, -C**H$_2$**-), 1.85 ppm (m, 2H, - CO-C**H**(CH_2)-NH_2-), 2.78 ppm (d, 4H, -CH(NH)-C**H$_2$**-S-) (J=4.49 Hz), 2.90 ppm (s, 4H, -NH-C**H$_2$**-COOH), 3.40 ppm (m, 4H, -CO-CH_2-C**H$_2$**-), 3.64 ppm (t, 4H, - CO-C**H$_2$**-CH_2-) (J=5.99, 6.86 Hz), 4.16 ppm (t, 2H, -CH_2-C**H**(NH)-COOH) (J=6.42, 6.86 Hz)

Membrane preparation

Cellulose diacetate (CDA, 2.3 g) was mixed with triethyleneglycol (7.7 g) at 150 ºC. The synthesized ligand-surfactant (1 wt%) was then dissolved in the CDA solution. The CDA solution was placed between two pieces of a glass plate (preheated at 150 ºC) with 100 µm clearance, followed by quenching at room temperature (approx. 10 ºC/min) to induce phase separation. After solidification of the polymer, a membrane was immersed in water to remove triethyleneglycol. The membrane was cut to a 4.9 cm^2 circle and placed in a membrane filter holder (Swinnex®, Millipore). In the case of NTA-functionalized membranes, a 0.1 M $NiSO_4$ solution (5 ml) was filtered through a

membrane using a syringe to load Ni^{2+} ions. Water (5 ml) was then filtrated through the Ni^{2+}-loaded membrane three times. A phosphate buffer (10 ml, 20 mM, pH 7.4) containing 0.5 M NaCl was filtrated to equilibrate the membrane surface. A phosphate buffer solution (2 ml) containing the protein (*e.g.* 45 μg/ml His-tag GFP) was applied to the membrane using a syringe at a flow rate of approx. 1 drop/s. The protein-loaded membrane was filtrated using 10 ml phosphate buffer and bound protein was eluted by a phosphate buffer (5 ml) containing 0.5 M NaCl and 500 mM imidazole, pH 7.4).

In the case of GST, the membrane was equilibrated by filtrating a phosphate buffer solution (10 ml, 20 mM, pH 7.4) containing 0.5 M NaCl. A phosphate buffer solution (2 ml) containing protein (*e.g.* 45 μg/ml His-tag GFP) was applied to the membrane. The protein-loaded membrane was also filtrated by a 10 ml phosphate buffer solution and bound protein was eluted by a 5 ml Tris-HCl buffer solution (pH 7.3, 50 mM) containing 10 mM glutathione.

The concentrations of GFP and TRITC-labeled GST were measured by a fluorescence spectrophotometer (LS55, Perkin Elmer).

Membranes were observed by a field-emission scanning electron microscope (FE-SEM, JSM-7500F, JEOL, Tokyo, Japan).

The Ni^{2+}-binding capacity of an NTA-functionalized membrane was evaluated as follows. The Ni^{2+}-loaded membrane was immersed in 50 mM EDTA solution for 2 h and the EDTA solution was subjected to ICP-AES analysis (SPS3100, SII Technologies, Japan).

SDS-PAGE analysis

SDS polyacrylamide gel electrophoresis was carried out using ready-made slab gels (Pagel, Atto Corp., Tokyo, Japan). Proteins were stained using the Silver Stain Kit (Wako Pure Chemical Industries, Ltd.).