

Affinity ionic liquid

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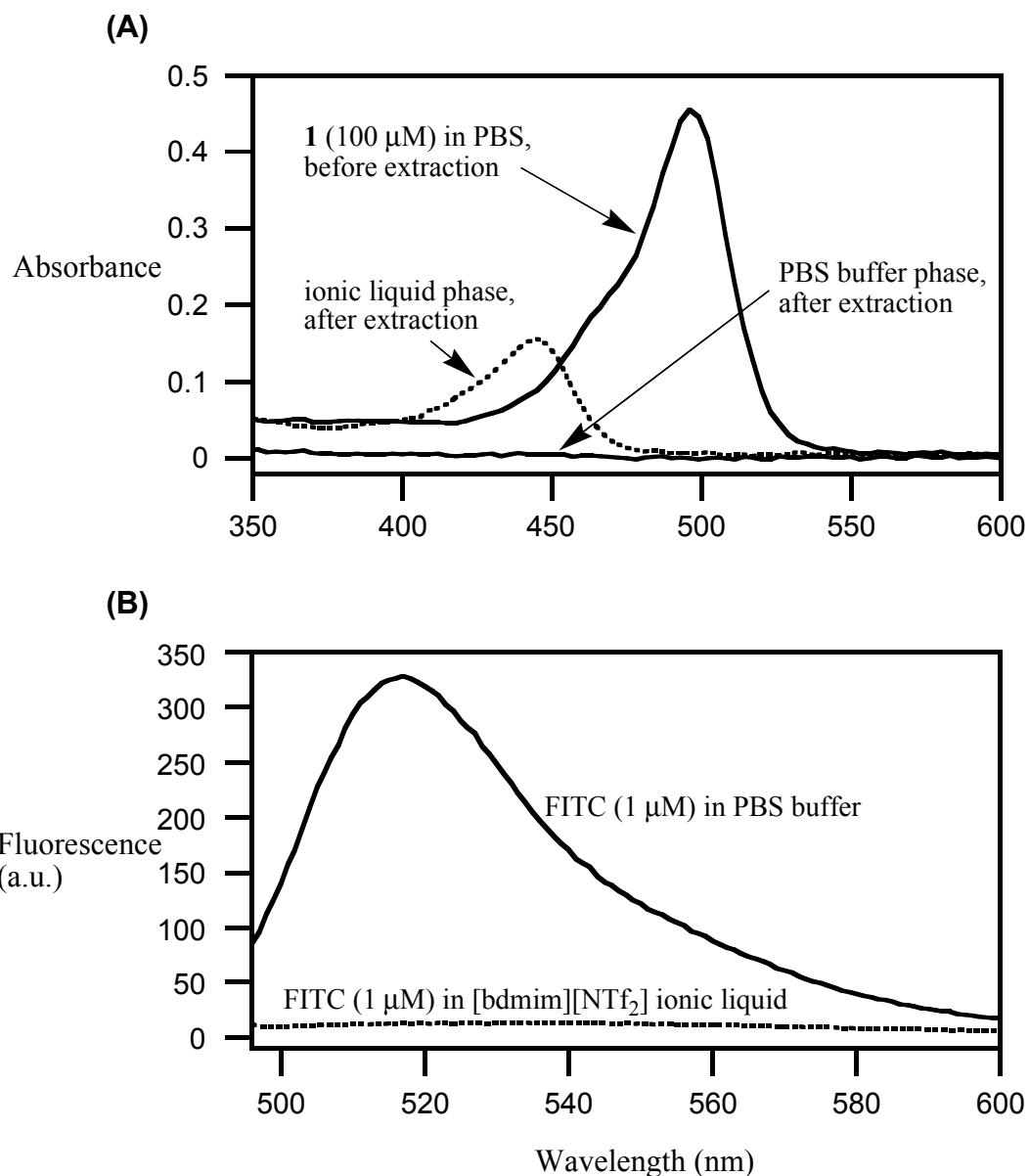


Fig. S1 (A) UV-vis absorption spectra of extraction of FITC-(His)₆-NH₂ peptide **1** (100 M) in PBS buffer by AIL **2** solubilized in [bdmim][NTf₂] ionic liquid **8**. Note that, upon affinity extraction, a significant blue shift in wavelength for **1** from PBS layer (495 nm) to ionic liquid phase (445 nm) was observed. The intensity of absorption for **1** in ionic liquid **8** was also greatly decreased. (B) The steady-state fluorescence emission spectra of FITC (1 M) in PBS buffer and

the ionic liquid resulting from 488-nm excitation. The fluorescence of FITC in ionic liquid **8** was essentially quenched.

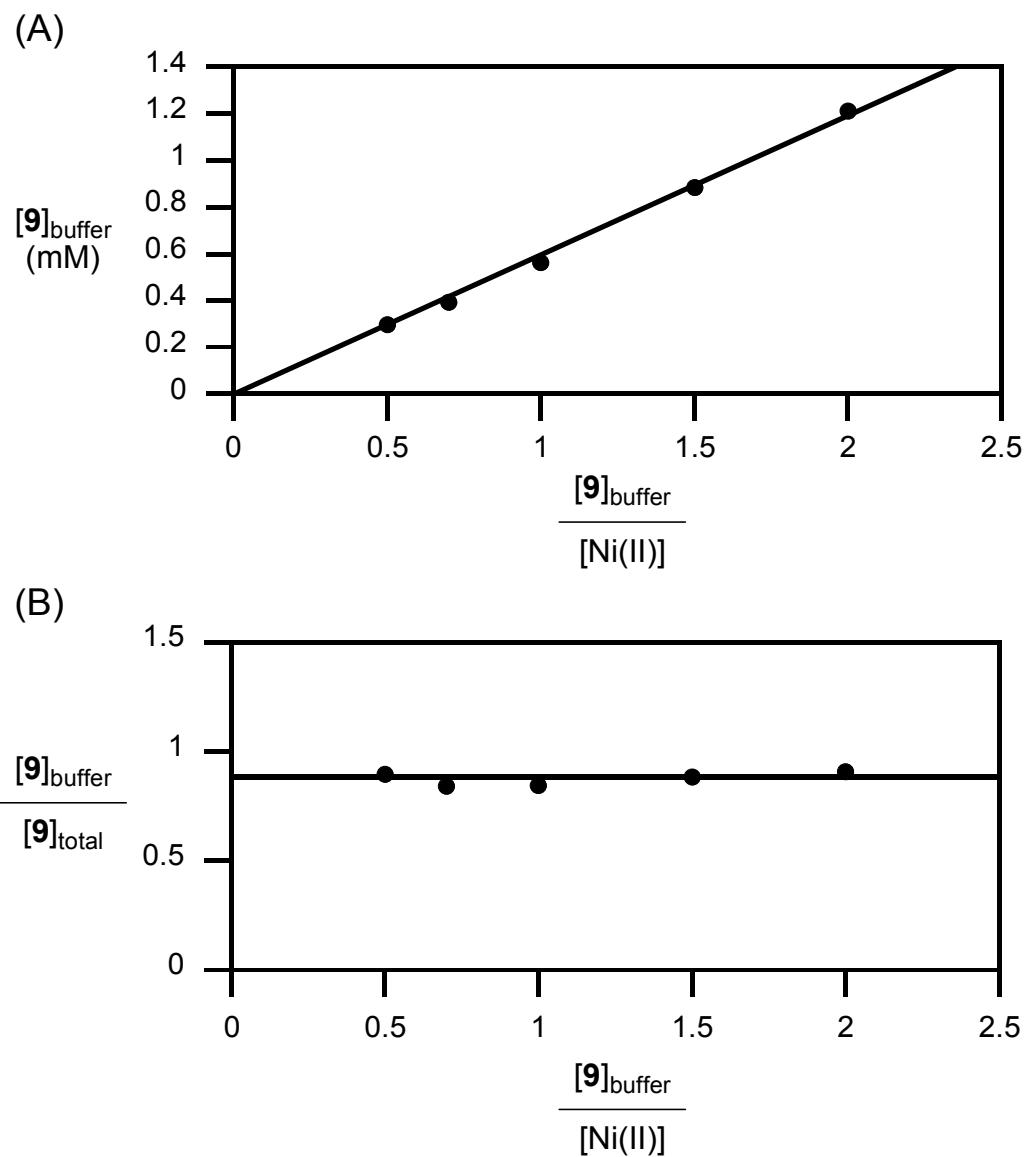


Fig. S2 Extraction of the peptide **9** in PBS buffer by Ni(II) cation in ionic liquid **8**. Experimental condition was identical to the Fig. 2. The result of (A) indicates simple partitioning of peptide **9** between aqueous and IL phases, and (B) suggests that, upon extraction, peptide **9** preferentially resides in aqueous PBS buffer (the preliminary result indicates that, under the experimental condition, partitioning coefficients of **9** range from 0.84 to 0.91).

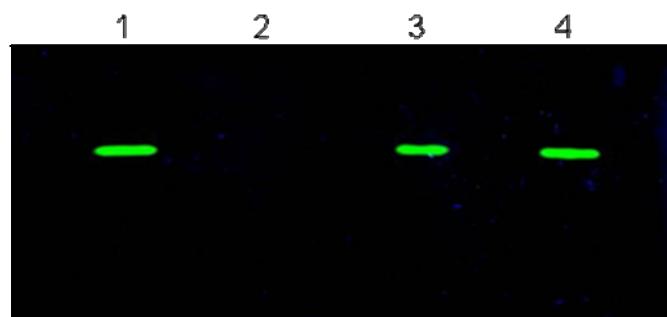


Fig. S3 A fluorescence photographic image from native polyacrylamide gel electrophoresis of extraction of the His-tag GFP by the Ni(II)-chelated AIL **2**. Lane 1, the GFP in buffer after extraction by ionic liquid **8** containing AIL **2** but without NiCl_2 ; lane 2, the GFP in buffer after affinity extraction by Ni(II)-chelated AIL **2** in **8**; lane 3, the competitive extraction of the GFP in ionic liquid layer to aqueous buffer phase by imidazole; lane 4, purified GFP (2.5 μg). Electrophoresis was performed under non-denaturing condition on 12% polyacrylamide gel.

*Synthesis of 1-(6-carboxylhexyl)-2,3-timethylenimidazolium bis(trifluoromethanesulfonimide) (the precursor ionic liquid acid **6**).* Into an ice-cooled solution of 3-chloropropionitrile **3** (10 g, 97 mmol) and methanol (3.4 g, 107 mmol) in ether (20 mL) was bubbled HCl(g) until white solid was precipitated. The solution was allowed to stand at -20 °C for 24 h, the solvent and excess HCl was removed under reduced pressure to give a white salt. The salt was successively washed with ether and dried under reduced pressure to give 3-chloropropioimidate hydrochloride **4** (14.9 g, 90 % yield).

A mixture of the imidate **4** (7.2 g, 42 mmol) and aminoacetaldehyde dimethyl acetal (4.0 g, 38.2 mmol) and triethylamine (12.7 g, 126 mmol) in dichloromethane was heated to reflux for 2 h. Dichloromethane and excess triethylamine was removed under reduced pressure. A solution of crude amidine in formic acid (3 mL) was heated at 80°C for 20 h. Fomic acid was removed *in vacuo*, benzene was added to the residue, and the mixture was evaporated to dryness. The residue was poured water (5 mL) and solid sodium bicarbonate to raise the pH to 8. The solution was extracted with dichloromethane (3 × 30 mL). The extracts were dried over anhydrous Na₂SO₄ gave pure yellowish solid **5** (3 g, 66 % yield in 2 steps).

To a round-bottomed flask containing 6,7-dihydro-5H-pyrrolo[1,2-*a*]imidazole **5** (120 mg, 1.11 mmol) was added benzyl 6-bromohexanoate (320 mg, 1.13 mmol). The mixture was stirred and heated at 80 °C for 2 h. The reaction solution was mixed with water (3 mL) and washed with ethyl acetate (3 × 3 mL). The water was removed *in vacuo* to obtain the yellow liquid.

The crude product was then dissolved in methanol and catalytic amount of Pd(OH)₂/C was added. The reaction mixture was bubbled with hydrogen. Reaction was carried out at room temperature for 1 h. Pd(OH)₂/C was filtered off, and the methanol was removed under reducing pressure to give the product. The lithium salt of bistrifluoromethanesulfonimide (320 mg, 1.11 mmol) and water were then poured into the solution. The mixture was allowed to proceed the ion exchange for 12 h at room temperature. The resulting solution was diluted with dichloromethane (5 mL) and then washed with water (3 × 3 mL). Removal of the solvent under reduced pressure afforded the 1-(6-carboxylhexyl)-2,3-timethylenimidazolium bistrifluoromethanesulfonimide **6** with good isolated yield (520 mg, 93% yield in 3 steps).

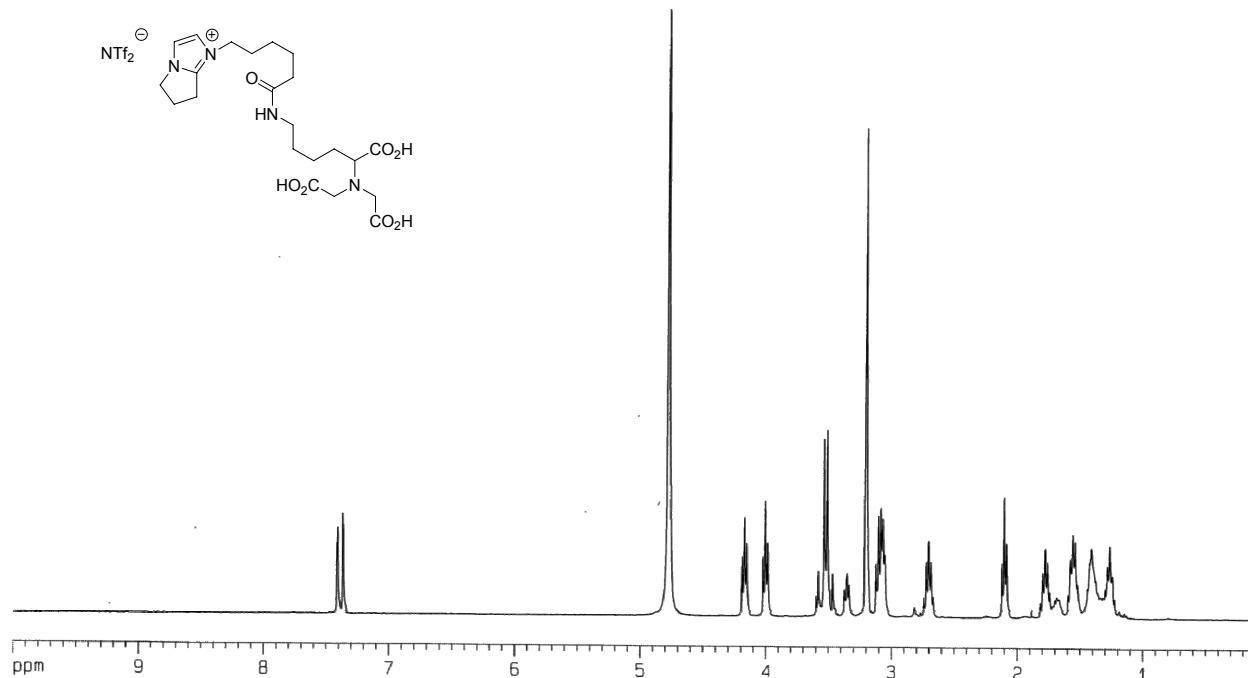
Synthesis of AIL 2. NTA (100 mg, 0.38 mmol) was dissolved in 20 mL toluene, *p*-toluenesulfonic acid (160 mg, 0.84 mmol) and excess benzyl alcohol (410 mg, 3.8 mmol) were added. The solution was reflux and azeotropically removed water for 10 h. Toluene was removed in vacuo and washed by adding cold ether. After removal of diethyl ether the crude product was then purified by silica gel column chromatography with 5 % methanol/dichloromethane as the solvent to afford the pure product (196 mg, 73 % yield) as a viscous liquid **7**.

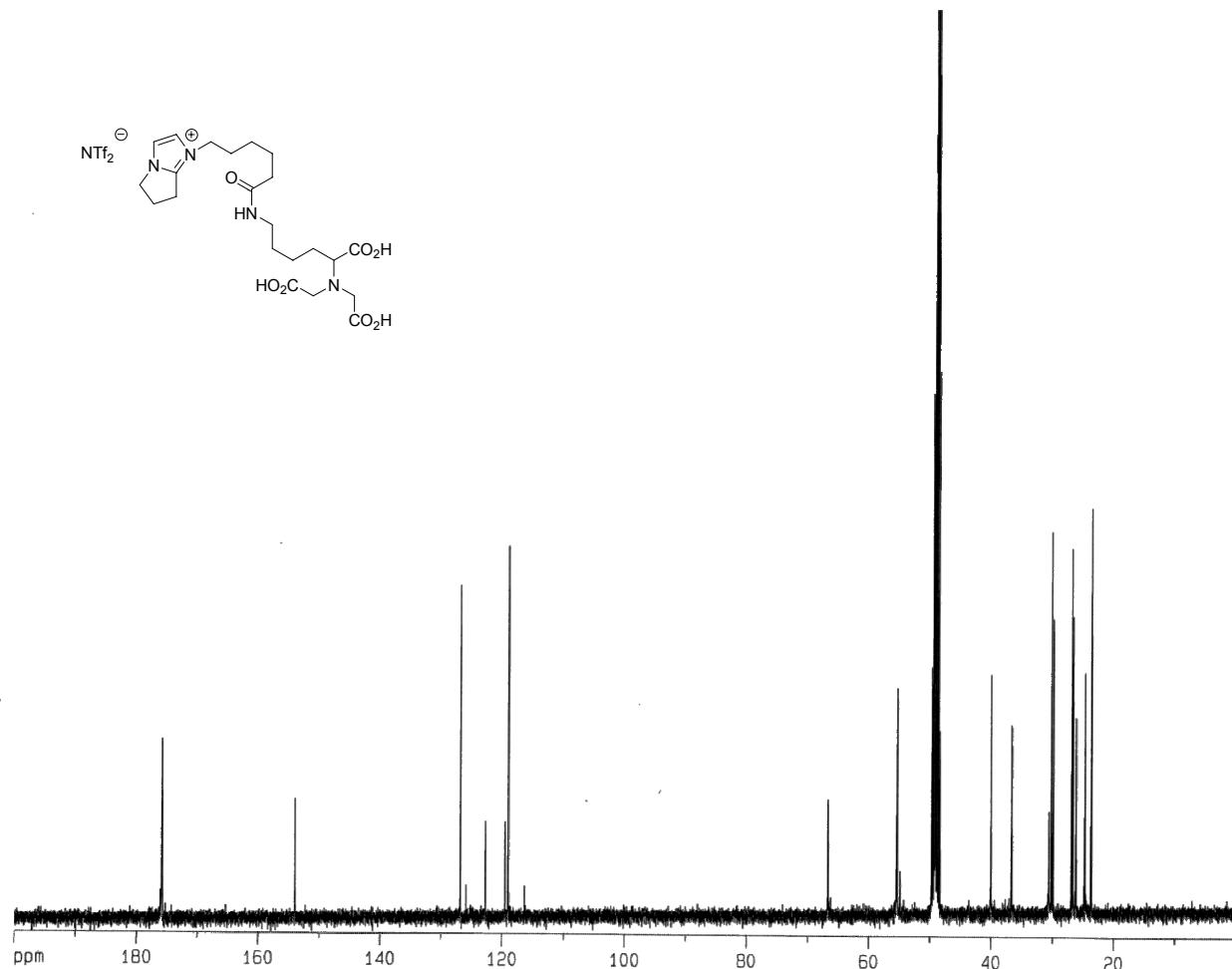
To a solution of **6** (70 mg, 0.14 mmol) and EDC (37 mg, 0.19 mmol) in dichloromethane, was added solution of TsOH • NTA-OBzI **7** (90 mg, 0.12 mmol) and DIEA (50 mg, 0.39 mmol) in dichloromethane (5 mL). The reaction was allowed to stand for 5 h at room temperature. And then the solution was washed with citric acids (3 × 5 mL, 10 %, w/w), water, sodium bicarbonate

(3 × 5 mL, 10%, w/w) and dried with Na₂SO₄. The solvent was evaporated to provide crude product. This crude product was chromatographed over silica gel with 5 % methanol/dichloromethane as the solvent to obtain the desired tribenzyl ester product (101 mg, 78% yield).

The tribenzyl ester was then dissolved in methanol and catalytic amount of Pd(OH)₂/C was added. The reaction mixture was bubbled with hydrogen. Reaction was carried out at room temperature for 1 h. Pd(OH)₂/C was filtered off, and the methanol was removed in vacuo to give the final product as a viscous liquid, AIL **2** (72 mg, 97% yield).

¹H NMR (400 MHz, CD₃OD) δ 1.27 (p, *J* = 8.0 Hz, CH₂, 2H), 1.37-1.44 (m, 2CH₂, 4H), 1.56 (p, *J* = 7.8 Hz, 2CH₂, 4H), 1.76 (p, *J* = 7.5 Hz, CH₂, 2H), 2.11 (t, *J* = 7.2 Hz, NCOCH₂, 2H), 2.71 (p, *J* = 7.6 Hz, CH₂, 2H), 3.05-3.10 (m, N=CCH₂, CONCH₂, 4H), 3.35 (t, *J* = 6.4 Hz, NCH, 1H), 3.51 (s, NCH₂, 2H), 3.54 (s, NCH₂, 2H), 4.01 (t, *J* = 7.2 Hz, C=NCH₂, 2H), 4.17 (t, *J* = 7.2 Hz, NCH₂C, 2H), 7.39 (dd, *J* = 2.0, 18.0 Hz, 2C=CH, 2H); ¹³C NMR (100 MHz, CD₃OD) δ 23.6, 24.7, 26.1, 26.6, 26.9, 29.9, 30.2, 30.6, 36.6, 40.0, 55.4, 66.7, 119.0, 121.2 (q, *J*_{CF} = 319 Hz, CF₃), 126.8, 153.9, 175.8; FAB-HRMS m/z [M]⁺ calcd for C₂₂H₃₃N₄O₇ 467.2506, found 467.2493.





*Solid-phase synthesis of hexapeptides (**1**, **9**, and **10**).* Solid-phase peptide synthesis was carried out on the PAL resin (Advanced ChemTech, Louisville, KY, USA) using standard Fmoc (N-(9-fluorenyl)methoxycarbonyl) chemistry. The purity and integrity of peptides were verified on a HPLC system (Hitachi L7420/L7100) with Mightysil RP-C18 GP column (250 mm × 4.6 mm, 5 m) using a 30 min linear gradient of 0–100% acetonitrile (0.1% TFA) in water (0.1% TFA), as monitored at 220 nm at a flow rate 1 mL/min. FAB-HRMS m/z [M]⁺ calcd for the Fmoc-(His)₆-NH₂ (**9**) 1062.4559, found 1062.4572; calcd for Fmoc-(Gly)₆-NH₂ (**10**) 582.2312, found 582.2322; calcd for FITC-(His)₆-NH₂ (**1**) 1229.4236, found 1229.4248.

*Stoichiometric titration of the Ni(II)-chelated AIL **2** with Fmoc-His-NH₂ (**9**).* Stoichiometric titrations were performed by using reaction mixtures containing 1 mM AIL **2** (20 L, in [bdmim][NTf₂] ionic liquid **8**). The AIL **2** was activated by addition of 20 L NiCl₂ (20 mM in PBS buffer, pH 7.2); after removing the aqueous layer, the activated AIL **2** was titrated with hexapeptide solution (30 L) containing Fmoc-(His)₆-NH₂ (0.067–1.33 mM) and Fmoc-(Gly)₆-NH₂ (0.167 mM). After extraction, the aqueous layers were analyzed with HPLC (Hitachi L7420/L7100) using an A Mightysil RP-C18 GP column (250 mm × 4.6 mm, 5 m). Elution was performed with a 15 min linear gradient (0–15% acetonitrile in water in the presence of 0.1% TFA) at 1 mL/min. Absorbance at 280 nm was monitored. The area ratio of Fmoc-(His)₆-NH₂ (**9**) and Fmoc-(Gly)₆-NH₂ (**10**) were calculated and transferred into concentration of unbound

Fmoc-(His)₆-NH₂ (**9**). The concentrations of free peptide **9** in buffer phase were plotted vs. [9]_{total}/[Ni(II)-chelated AIL **2**].

*Affinity extraction of FITC-His₆-NH₂ (**1**)*. Peptide **1** (3 mg, 0.004 mmol) was dissolved in 200 L of [bdmim][NTf₂] ionic liquid **8**, and then NiCl₂ (200 L, 50 mM in PBS buffer, pH 7.2) was added to saturate the AIL **2**. After removal of the aqueous NiCl₂ solution, 100 M of FITC-His₆-NH₂ (200 L in PBS buffer) was poured into the vial and shaked. After removal of aqueous layer, imidazole solution (500 mM, in 20 mM phosphate buffer, pH 7.9) was added to back-extract **1**.

Construction of expression vector for His-tag green fluorescence protein (His-tag GFP). To obtain N-terminal in-frame His-tag green fluorescence protein (GFP), the pEGFP-N3 plasmid (Clontech) was digested with *Bam*HI and *Not*I to obtain the DNA fragment containing enhanced green fluorescence protein. The DNA fragments containing enhanced green fluorescence protein were ligated with *Bam*HI and *Not*I linearized pET-28a vector (Novagen), and the resulting mixture was used to transform competent *E. coli* BL21(DE3). The resulting plasmid, pET-EGFP, encodes the His-tagged–green fluorescence protein.

Production and purification of His-tag green fluorescence protein (His-tag GFP). The *E. coli* BL21(DE3) carrying pET-EGFP was grown in the Luria–Bertani (LB) broth medium (100 mL) supplemented with kanamycin (25 µg/mL). The inoculum was grown at 37 °C to an OD600 of 11

0.8, and isopropyl- β -D-thiogalactoside (IPTG) was added to a final concentration of 1 mM. After 3 hour of induction at 37 °C, the cells were harvested by centrifugation. Cell pellets were resuspended in 2 mL PBS buffer (2.7 mM KCl, 1.4 mM KH₂PO₄, 4.3 mM Na₂HPO₄, 137 mM NaCl) and were disrupted with sonication. Cellular debris was removed by centrifugation (9000 × g, 20 min), and the supernatant was applied to a Ni Sepharose (Amersham Bioscience) (50 × 25mm) previously equilibrated with PBS buffer and washed with PBS buffer (50 mL). After washing the column consecutively with 50 mL each of PBS buffer containing 5 mM, 10 mM, 20 mM and 50 mM imidazole, the desired His-tag green fluorescence protein was eluted with PBS buffer containing 500 mM imidazole. Fractions (2 mL each) containing the recombinant His-tag green fluorescence protein were analyzed for purity by SDS-PAGE with the pure fractions pooled and dialyzed against PBS buffer. The overall yield of pure His-tag green fluorescence protein obtained from the culture of induced *E. coli* BL21(DE3) was 2.5 mg/mL.

Affinity extraction of His-tag GFP. For purification of His-tag GFP from total cell lysate, AIL **2** (2 mg) was dissolved thoroughly in 30 L [bdmim][NTf₂] ionic liquid **8**. The ionic liquid solution was added 25 L NiCl₂ (100 mM, in PBS buffer, pH 7.2) to activate the AIL **2**. After removal of aqueous layer, additional PBS buffer (2 × 50 L) was added to wash unbound NiCl₂. Then, 10 uL of recombinant His-tag GFP crude extract and additional 20 L PBS were added. Subsequently, the aqueous layer was removed and the ionic liquid layer was washed with PBS buffer (3 × 20 L). Finally, 500 mM imidazole (3 × 20 L) were poured into the vial to back-extraction of His-tag GFP.

All of the aqueous layers were electrophoresed on 12 % SDS-PAGE gel.

Native polyacrylamide gel electrophoresis. 50 L of “extractant” solution (20 mM, 50 L), prepared by dissolving AIL **2** in [bdmim][NTf₂] ionic liquid **8**, were treated with NiCl₂ solution (25 L, 100 mM in PBS buffer, pH 7.2) to activate AIL **2**. After removal of aqueous layer, additional PBS buffer (2 × 50 L) was added to wash unbound NiCl₂. Then, 10 L His-tag GFP (1 mg/mL) were added and the mixture was adequately shocked. The aqueous layer was then separated and collected. The ionic liquid layer was treated by 2 × 10 L elution buffer (500 mM imidazole in 20 mM phosphate buffer, pH 7.9) to back-extract the GFP. The aqueous layers were electrophoresed on a 12% non-denaturing PAGE.

Notes and references

1. This binding of hexahistidine tag (His-tag) to Ni(II) chelate of nitrilotriacetic acid (NTA) has been widely used as a powerful and universal means for the rapid one-step affinity purification of recombinant proteins. For recent reviews, see: (a) A. C. A. Roque, C. S. O. Silva and M. A. Taipa, *J. Chromatogr. A*, 2007, **1160**, 44; (b) G. S. Chaga, *J. Biochem. Biophys. Methods*, 2001, **49**, 313; (c) P. N. Hengen, *Trends Biochem. Sci.*, 1995, **20**, 285; (d) F. H. Arnold, *BioTechnology*, 1991, **9**, 151.
2. There are, to our knowledge, no reports in literature showing effective affinity extraction of proteins from aqueous buffer into ionic liquid based on affinity ligand-receptor interactions,

although (a) a crown ether-mediated extraction of the Lys-rich cytochrome *c* into ionic liquid has been reported: K. Shimojo, N. Kamiya, F. Tani, H. Naganawa, Y. Naruta and M. Goto, *Anal. Chem.*, 2006, **78**, 7735 and (b) an aqueous two-phase partitioning of bacterial alcohol dehydrogenases from the salt-rich phase into an oligo(ethylene glycol)-based quaternary ammonium chloride (Ammoeng110TM)-rich phase was recently reported: S. Dreyer and U. Kragl, *Biotechnol. Bioeng.*, 2008, **99**, 1416.

3. Proteins and enzymes are usually insoluble in ionic liquids, leading to biologically active suspensions, and, when dissolved, often are denatured, primarily due to their major conformational changes in ionic liquids. For references, see: (a) F. van Rantwijk and R. A. Sheldon, *Chem. Rev.* 2007, **107**, 2757; (b) Z. Yang and W. Pan, *Enzym. Microb. Biotechnol.*, 2005, **37**, 19; (c) M. B. Turner, S. K. Spear, J. G. Huddleston, J. D. Holbrey and R. D. Rogers, *Green Chem.*, 2003, **5**, 443 (d) S. Park and R. J. Kazlauskas, *Curr. Opin. Biotechnol.*, 2003, **14**, 432.