## **Supporting Information**

## Ionic Liquid Modified Magnetic Microspheres for Isolation of Heme Protein with High Binding Capacity

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**Supplementary Figures** 



Figure S1. TEM images of Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub> microspheres.



**Figure S2.** a) The FT-IR spectra of  $Fe_3O_4@SiO_2$  and  $Fe_3O_4@SiO_2@IL$ . b) The FT-IR spectra of  $Fe_3O_4@SiO_2$  and  $Fe_3O_4@SiO_2@IL$  from 1300 cm<sup>-1</sup> to 2000 cm<sup>-1</sup>. Small differences in spectra were observed in relation to the wavenumbers and intensities of the absorption bands for  $Fe_3O_4@SiO_2$  and  $Fe_3O_4@SiO_2@IL$ . In spectrum of  $Fe_3O_4@SiO_2@IL$  functionalized silica, one important band at 1540 cm<sup>-1</sup> is attributed to the characteristic frequencies of cationic imidazole group.





The result of recycle experiment was shown in Fig.S3. The experiment condition is same as experiment **3.3.1** in this paper, pH 6.8. Compared to the actual bioseparations, sample concentration is very high. But the adsorption efficiencies maintained at more than 80% after 8 recycled use. So our resin would have a relatively long lifetime in the practical application.

resin	Separation	n capacity	size	company
	time			
HIS-Select nickel magnetic beads $^1$	30 min	>10 mg/g	20-75µm	Sigma
Glutathione High Capacity	30min	≥12 mg/g	50µm	Sigma
Magnetic Agarose Beads <sup>2</sup>				
MagPrep <sup>®</sup> P-25 Protein A Particles <sup>3</sup>		> 220 µg human	25nm	Novagen
		IgG/mg		
Ni-NTA magnetic agarose beads <sup>4</sup>	30min	0.25-1 mg/g	20-70µm	QIAGEN
IL resin (This work)	15min	2.15 g Hb per g	~270nm	
		resin		

## Table S1. The separation efficiency of the IL resin compare to some commercial resins.

protein	Size	molecular weight	maximum	
	(dimensions)		adsorption number	
Hb	$5.3 \times 5.4 \times 6.5$ nm <sup>5</sup>	64500	3.33×10 <sup>-5</sup> mol/g	
Lys	$3.0 \times 3.0 \times 4.5$ nm <sup>6</sup>	14388	1.39×10 <sup>-6</sup> mol/g	

**Table S2**. The comparison of size, molecular weight and maximum adsorption number between Hb and Lys.

Seen from the table 1 below, the gap of size is not conspicuous relative to molecular weight. But the magnitude of adsorption number is different, which proved the selective affinities for hemoglobin.

## References

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- 2 http://www.sigmaaldrich.com/catalog/product/sigma/g0924?lang=en&region=CA.
- 3 http://www.merckmillipore.com/china/life-science-research/magprep-p-25-protein-a-p articles/EMD\_BIO-72189/english/p\_uDGb.s1O2tQAAAEjYRp9.zLX?WFSimpleSearch\_Na meOrID=+beads&BackButtonText=search+results
- 4 http://www.qiagen.com/Products/Protein/Purification/QIAexpressProteinPurificationSys tem/Ni-NTAMagneticAgaroseBeads.aspx?r=609
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