

1 Supporting information

2 **Development of purification process for dual-function recombinant human heavy-chain ferritin**  
3 **by the investigation of genetic modification impact on conformation**

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5 Shuang Yin<sup>1</sup>,

6 Bingyang Zhang<sup>1</sup>,

7 Jianying Lin<sup>2</sup>

8 Yongdong Liu<sup>3</sup>,

9 Zhiguo Su<sup>3</sup>,

10 Jingxiu Bi<sup>1</sup>.

11 1. School of Chemical Engineering & Advanced Materials, Faculty of Engineering, Computer and  
12 Mathematical Sciences, University of Adelaide, Adelaide, Australia

13 2. College of Biomedical Engineering, Taiyuan University of Technology

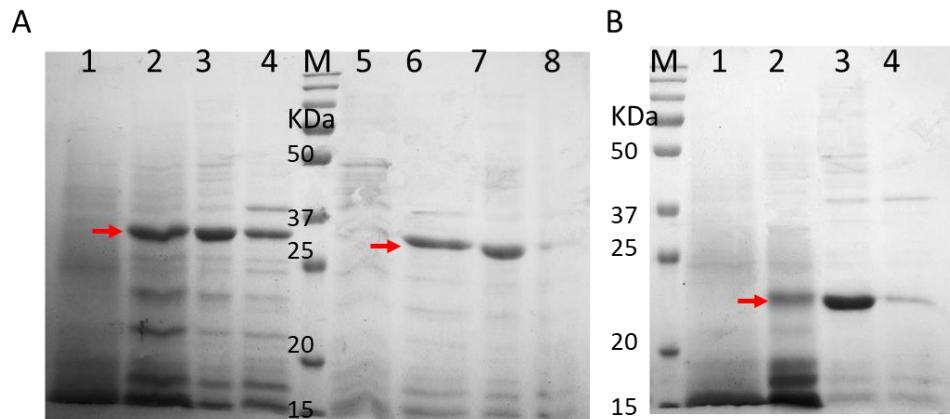
14 3. State Key Laboratory of Biochemistry Engineering, Institute of Process Engineering, Chinese  
15 Academy of Sciences, Beijing, China

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17 **Correspondence:** A/Prof Jingxiu Bi ([jingxiu.bi@adelaide.edu.au](mailto:jingxiu.bi@adelaide.edu.au)). School of Chemical Engineering  
18 &Advanced Materials, Faculty of Engineering, Computer and Mathematical Sciences, University of  
19 Adelaide, North terrace, SA5005, Adelaide, Australia.

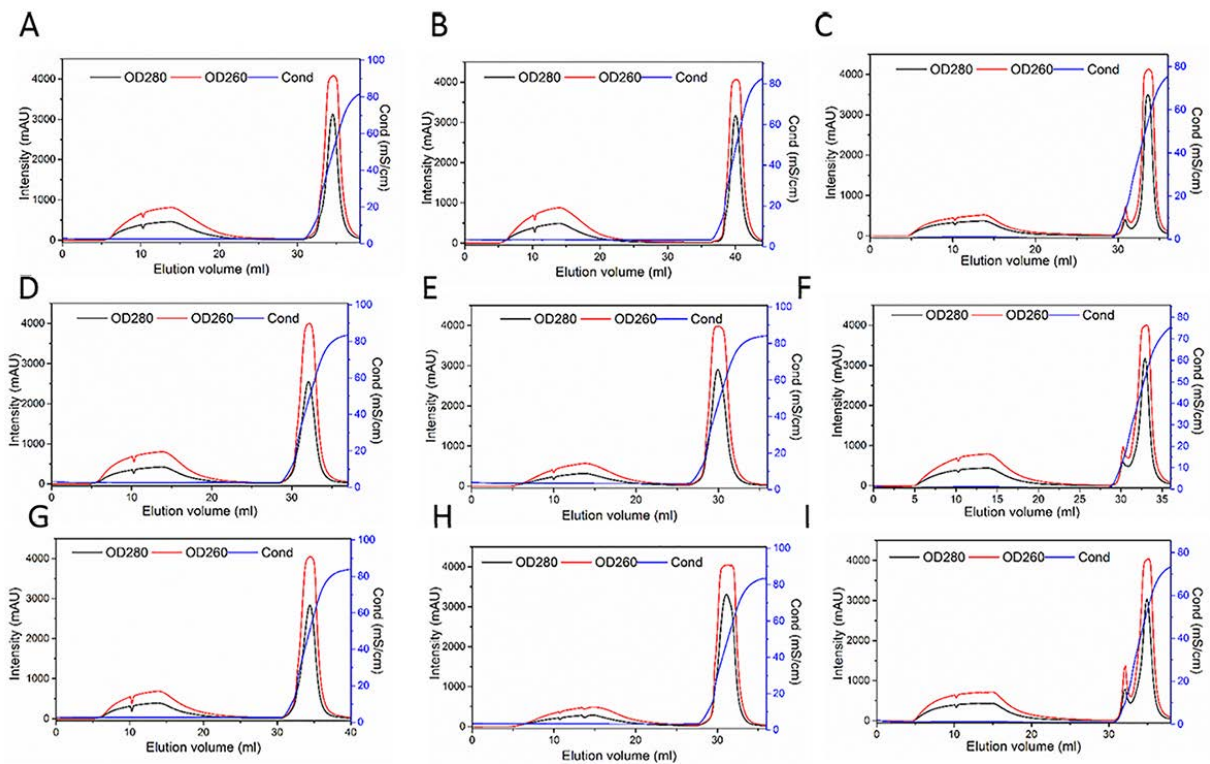
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1 **Figure S1.** 12 % reducing SDS-PAGE images of expression results of HFn and two modified  
2 HFns.



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4 **A**, expression results of HFn-PAS and HFn-PAS-RGDK, lane 1: bacterial for HFn-PAS-RGDK before  
5 IPTG induction; lane 2: bacterial for HFn-PAS-RGDK after IPTG induction; lane 3: supernatant of  
6 bacterial lysate; lane 4: sediment of bacterial lysate; M:protein marker, lane 5: bacterial for HFn-PAS  
7 before IPTG induction; lane 6: bacterial for HFn-PAS after IPTG induction; lane 7: supernatant of  
8 bacterial lysate; lane 8: sediment of bacterial lysate. M: protein marker. **B**, expression result of HFn,  
9 lane 1: bacterial before IPTG induction; lane 2: bacterial after IPTG induction; lane 3: supernatant of  
10 bacterial lysate; lane 4: sediment of bacterial lysate. M: protein marker. Red arrows indicate target  
11 protein bands.  
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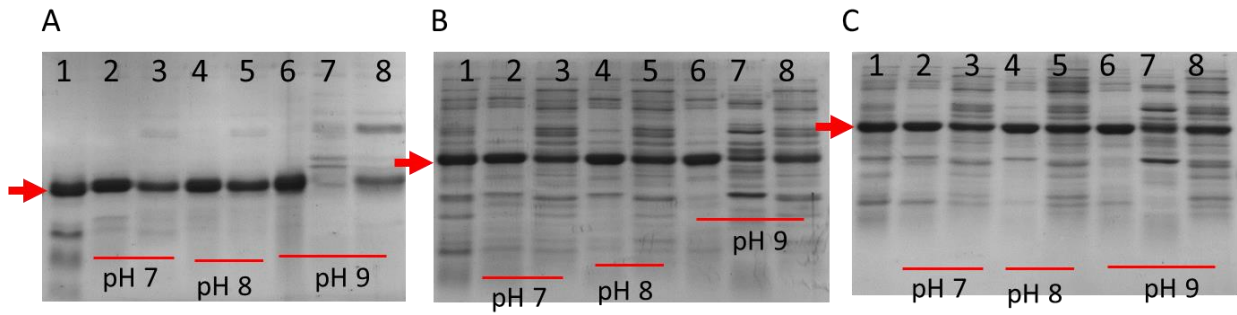
1 **Figure S2.** Q FF chromatograms of HFn and modified HFns.



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3 **A,** pH 7 HFn chromatogram. **B,** pH 8 HFn chromatogram. **C,** pH 9 HFn chromatogram. **D,** pH 7 HFn-  
4 PAS chromatogram. **E,** pH 8 HFn-PAS chromatogram. **F,** pH 9 HFn-PAS chromatogram **G,** pH 7 HFn-  
5 PAS-RGDK chromatogram. **H,** pH 8 HFn-PAS-RGDK chromatogram. **I,** pH 9 HFn-PAS-RGDK  
6 chromatogram.  
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1 **Figure S3.** 12 % reducing SDS-PAGE images of Q FF chromatography



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3 **A,** 12 % reducing SDS-PAGE results of HFn by Q FF chromatography. **B,** 12 % reducing SDS-PAGE  
4 results of HFn-PAS by Q FF chromatography. **C,** 12 % reducing SDS-PAGE results of HFn-PAS-  
5 RGDK by Q FF chromatography. Lane 1: heat-acid precipitation supernatant; lane 2: pH 7 flow through  
6 peak; lane 3: pH 7 eluted peak 1; lane 4: pH 8 flow through peak; lane 5: pH 8 eluted peak 1; lane 6:  
7 pH 9 flow through peak; lane 7: pH 9 eluted peak 1; lane 8: pH 9 eluted peak 2. Red arrows indicate  
8 target protein bands.

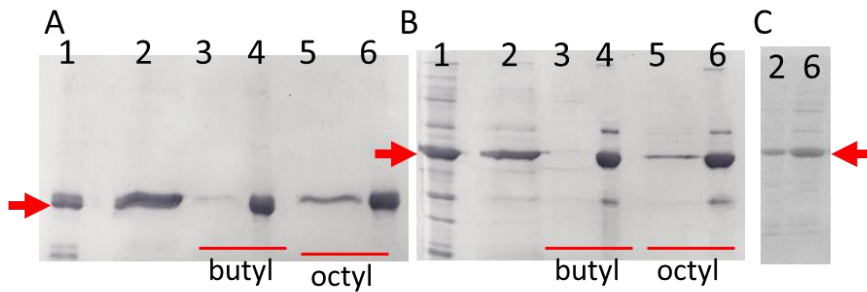
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13 **Figure S4.** 12 % reducing SDS-PAGE images of HIC



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15 **A,** 12 % reducing SDS-PAGE results of peaks from HFn HIC. **B,** 12 % reducing SDS-PAGE results of  
16 peaks from HFn-PAS-RGDK HIC. **C,** 12 % reducing SDS-PAGE results of peaks from HFn-PAS butyl  
17 FF HIC. Lane 1: heat-acid precipitation supernatant; lane 2: Loading sample for HIC; lane 3: flow  
18 through peak of butyl FF; lane 4: eluted peak of butyl FF; lane 5: flow through peak of octyl FF; lane  
19 6: eluted peak of octyl FF. Red arrows indicate target protein bands.

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1 **Table S1.** Purity and recovery yield (step) in heat-acid precipitation

Protein	Conditions	50 °C pH	50 °C pH	50 °C pH	60 °C pH	60 °C pH
		4.0	4.5	5.0	4.5	5.0
HF <sub>n</sub>	Purity (%)	<b>64.77</b>	55.77	47.87	<b>65.50</b>	58.92
	Recovery yield (%)	88.70	91.58	93.82	<b>99.69</b>	99.22
HF <sub>n</sub> -PAS	Purity (%)	27.31	41.43	<b>50.77</b>	15.02	45.01
	Recovery yield (%)	4.98	13.14	<b>68.41</b>	2.26	8.56
HF <sub>n</sub> -PAS-	Purity (%)	40.85	43.98	<b>50.41</b>	9.50	41.10
RGDK	Recovery yield (%)	0.32	12.55	<b>61.45</b>	0.88	9.19

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3 Recovery yield (step) calculation example:

4 After 5 min heating at 60 °C pH 4.5, HF<sub>n</sub> recovery yield (step) was:

5 Recovery yield (step) (%) = the amount HF<sub>n</sub> in heat-acid supernatant/ the amount of HF<sub>n</sub> in  
6 sample before heat-acid precipitation = (purity of HF<sub>n</sub> in heat-acid supernatant × total protein  
7 amount of heat-acid supernatant)/(purity of HF<sub>n</sub> in bacterial lysate (supernatant) × total protein  
8 amount of sample before heat-acid precipitation = (65.503 % × 0.5 mL × 2.058  
9 mg/mL)/(33.806 % × 0.5 mL × 4.000 mg/mL) = 99.69 %

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1 **Table S2.** Purity, recovery yield (step) and nucleic acid removal of peaks in Q FF IEC

Protein	Q FF Peak	pH 7	pH 7	pH 8	pH 8	pH 9	pH 9	pH 9
		FT	P1	FT	P1	FT	P1	P2
HFn	Purity (%)	94.27	85.69	95.56	95.08	<b>97.98</b>	18.00	67.73
	Recovery yield (%)	82.12	16.34	80.07	18.47	<b>91.68</b>	0.14	2.01
	Nucleic acid removal (%)	/	/	/	/	<b>46.00</b>	/	/
HFn-	Purity (%)	70.96	50.45	70.95	44.95	<b>80.13</b>	16.41	42.06
PAS	Recovery yield (%)	81.40	17.17	68.93	21.33	<b>79.06</b>	1.59	13.83
	Nucleic acid removal (%)	/	/	/	/	<b>65.17</b>	/	/
HFn-	Purity (%)	69.92	51.72	75.60	49.40	<b>83.96</b>	32.75	45.96
PAS-	Recovery yield (%)	63.47	23.17	69.17	22.21	<b>70.25</b>	2.67	13.55
RGDK	Nucleic acid removal (%)	/	/	/	/	<b>46.81</b>	/	/

2 10 mg protein loaded in each run. FT: flow through peak. /: no measurement

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1 **Table S3.** Purity, recovery yield (step) and nucleic acid removal of peaks in HIC

Protein	Chromatography peak	Butyl FF	Butyl FF	Octyl FF	Octyl FF
		FT	P1	FT	P1
HF <sub>n</sub>	Purity (%)	96.09	<b>95.24</b>	98.02	95.94
	Recovery yield (%)	3.18	<b>93.87</b>	7.13	75.65
	Nucleic acid removal (%)	/	<b>99.93</b>	/	99.95
HF <sub>n</sub> -PAS	Purity (%)	83.48	<b>82.32</b>	/	/
	Recovery yield (%)	5.05	<b>93.46</b>	/	/
	Nucleic acid removal (%)	/	<b>99.81</b>	/	/
HF <sub>n</sub> -PAS-	Purity (%)	80.78	<b>83.74</b>	83.75	82.83
RGDK	Recovery yield (%)	3.68	<b>94.54</b>	8.79	76.66
	Nucleic acid removal (%)	/	<b>99.81</b>	/	99.78

2 10 mg protein loaded in each run. FT: flow through peak. /: no measurement.

3 Purity of FT peak is not as highly accurate as eluted peak purity because of the much lighter bands in  
4 gel.

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7 **Table S4.** IC<sub>50</sub> values of all groups against MCF7

Group	IC <sub>50</sub> (μg/mL)
Doxorubicin	0.20±0.03
DOX/HF <sub>n</sub>	1.31±0.22
DOX/HF <sub>n</sub> -PAS	0.98±0.14
DOX/HF <sub>n</sub> -PAS-RGDK	0.59±0.05

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