Supporting Information

Effective Protein Separation by Coupling Hydrophobic Interaction and Reverse Phase Chromatography for Top-down Proteomics

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**Supplemental Table**

Table S-1. Protein identification from in *E. coli* lysate through HIC-RPC-MS/MS. Only HIC fraction 2 is shown demonstrating the proof-of-principle.

| No. | No. of Matching Fragments | Molecular Weight (Da) | P Value | E Value | Accession No. | Protein Name |
|-----|---------------------------|-----------------------|---------|---------|---------------|--------------|
| 1   | 64                        | 13472.1               | 5.4E-40 | 5.4E-40 | YP_003001796 | Hypothetical protein B21_04075 |
| 2   | 73                        | 11772.9               | 1.9E-38 | 1.8E-38 | YP_003001789 | Cytochrome b562 (soluble) Transcriptional dual regulator HU-alpha (HU-2), subunit of HU transcriptional dual regulator |
| 3   | 71                        | 9529.1                | 2.2E-37 | 2.7E-37 | YP_003001562 | Protein |
| 4   | 42                        | 7145.6                | 3.6E-36 | 3.6E-36 | YP_002998197 | Protein |
| 5   | 49                        | 11855.8               | 2.2E-36 | 2.2E-36 | YP_002999764 | Transcriptional dual regulator HU-beta, NS1 (HU-1), subunit of HU transcriptional dual regulator |
| 6   | 62                        | 9219.9                | 4.3E-34 | 4.3E-34 | YP_002998253 | Hypothetical protein B21_01767 |
| 7   | 57                        | 12354.3               | 4.5E-29 | 4.5E-29 | YP_002999561 | Superoxide dismutase (Mn) Superoxide dismutase precursor (Cu-Zn) Regulator of phosphatidyl-ethanolamine synthesis |
| 8   | 47                        | 17427.4               | 7.8E-29 | 5.7E-27 | YP_003001476 | Protein |
| 9   | 60                        | 15726.7               | 1.3E-26 | 1.3E-25 | YP_002999407 | Protein |
| 10  | 39                        | 6571.4                | 3.5E-25 | 3.5E-25 | YP_002998836 | Protein |
| 11  | 29                        | 7701.9                | 4.3E-25 | 7.3E-24 | YP_002998149 | Protein |
| 12  | 22                        | 10380.5               | 3.9E-17 | 7.9E-17 | YP_003001699 | Protein |
| 13  | 25                        | 10455.5               | 2.3E-19 | 1.8E-18 | YP_002998539 | Protein |
| 14  | 15                        | 6222.8                | 1.2E-12 | 5.8E-12 | YP_003001781 | Protein |
| 15  | 28                        | 12407.4               | 4.1E-14 | 7.7E-11 | YP_002999974 | Protein |
| 16  | 14                        | 8320.0                | 1.8E-10 | 1.8E-10 | YP_003001605 | Stress response protein |
| 17  | 20                        | 10722.8               | 3.9E-12 | 7.0E-10 | YP_002999417 | Superoxide dismutase (Fe) Hypothetical protein B21_03627 |
| 18  | 13                        | 10455.5               | 2.3E-11 | 2.1E-08 | YP_003001364 | Superoxide dismutase (Mn) ytflQ, subunit of galactose ABC transporter |
| 19  | 44                        | 22932.5               | 5.5E-25 | 3.8E-20 | YP_003001476 | Superoxide dismutase (Mn) ytflQ, subunit of galactose ABC transporter |
| 20  | 29                        | 32083.5               | 4.7E-17 | 9.2E-14 | YP_003001781 | Stress response protein |
Supplemental Figure

Figure S-1. Representative mass spectrum of myoglobin in HIC buffers, 1.8 M ammonium sulfate (a), 1.8 M ammonium tartrate (b), desalted by ultra-centrifugal device and RPC. RPC conditions, same as shown in Figure 4.