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Analytical and Bioanalytical Chemistry  
Electronic Supplementary Material

Determination of the protein content of complex samples  
by aromatic amino acid analysis, liquid  
chromatography-UV absorbance, and colorimetry

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## 1 Protein characterization

To characterize the six test proteins, SDS-PAGE and MALDI-TOF MS measurements were performed (Figure S6, S7).

A high-purity, low endotoxin, low IgG, monomeric bovine serum albumin (BSA) crystallized powder preparation with near native albumin characteristics was used. An aqueous solution of this BSA is termed high-purity BSA thereafter and was used as a secondary reference (1.07 mg/mL determined by AAAA(Phe)). The expected mass is 66.4 kDa, which was confirmed by MALDI-TOF MS measurements, and also the SDS-PAGE showed a single protein band at  $\sim 65$  kDa. The integrated LC-220 chromatogram gave a purity result of 98.8% (related to the UV signal). Of note is that the LC-220 protein purity does not account for any inorganic or other non-absorbing material.

Chicken avidin forms a strong non-covalent complex with biotin, and is often used in biochemical assays such as ELISA or western blot [1]. Avidin is a basic protein with an isoelectric point of 10.0-10.5, and approx. 10% of its total mass results from carbohydrates. The expected mass from the protein sequence is 13.9 kDa, and MALDI-TOF MS gave a mass of 15.7 kDa. SDS-PAGE showed one protein band at  $\sim 17$  kDa. The integrated LC-220 chromatogram gave a purity result of 87%.

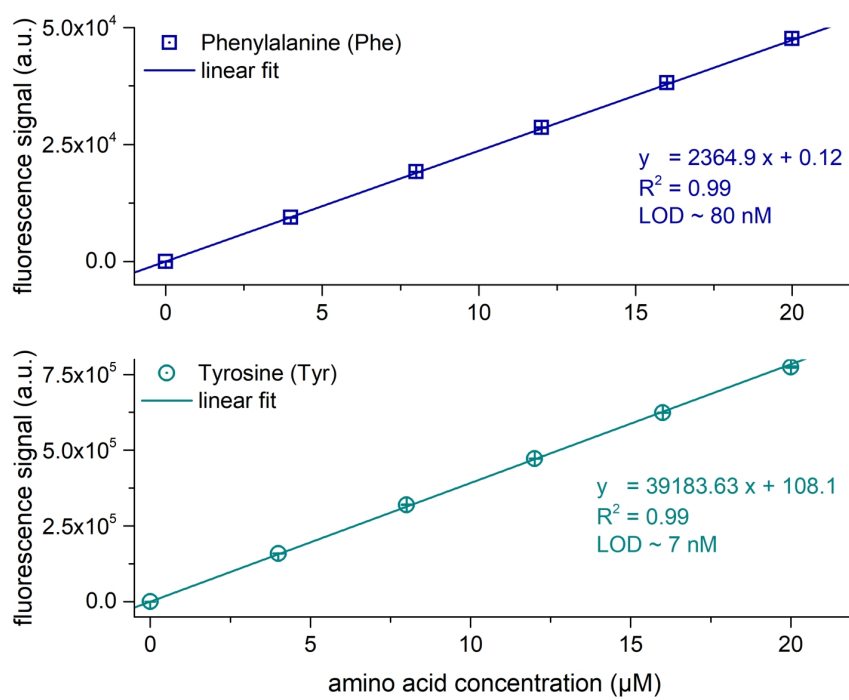
Myoglobin is an essential hemoprotein in striated muscle [2]. Myoglobin is a single chain heme protein with no disulfide bridges or free SH-groups and an iron content of 0.25-0.32% (information of the manufacturer). The expected mass is 16.9 kDa, which was confirmed by MALDI-TOF MS measurements; the SDS-PAGE showed one protein band at  $\sim 16$  kDa. The integrated LC-220 chromatogram gave a purity result of 96.6%.

Jacalin, a galactose-binding lectin from jackfruit seeds is able to bind to O-linked glycoproteins, particularly human IgA, which makes it useful for isolating plasma glycoproteins, investigating IgA nephropathies, and tumor detection [3]. The expected mass is 16.2 kDa, which was confirmed by MALDI-TOF MS. The SDS-PAGE showed two protein bands between  $\sim 15$ -17 kDa. The integrated LC-220 chromatogram gave a purity result of 82.3%.

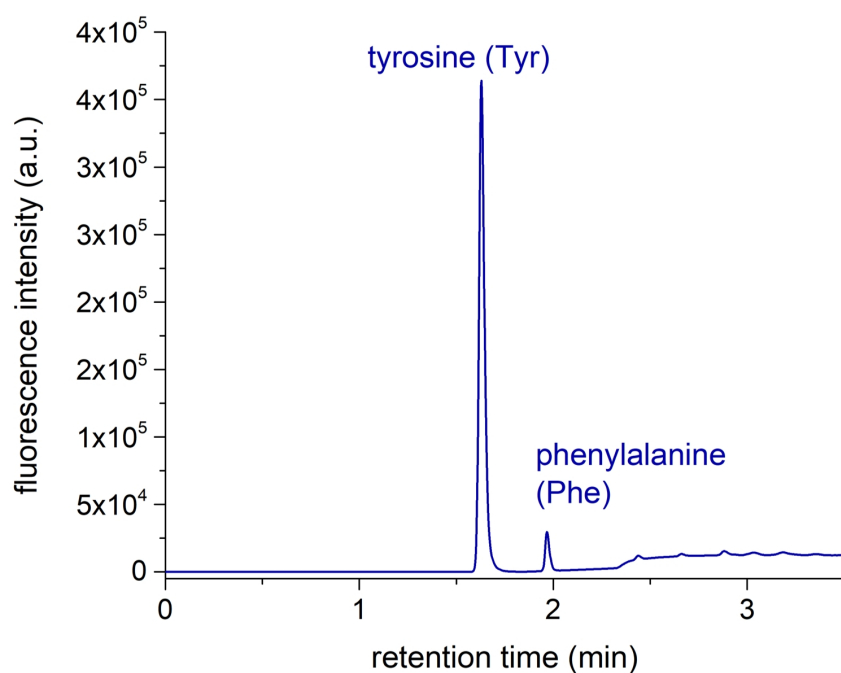
Transferrins are iron-transport proteins [4]. The iron-deficient transferrin is called apotransferrin. We used bovine apotransferrin (Apo) in form of a sterile filtered lyophilized sample with a purity of 95% and an iron content  $< 40$  ppm (information of the manufacturer). The expected mass is 75.8 kDa, and the measured MALDI-TOF MS gave a mass of 77.6 kDa. The SDS-PAGE showed two protein bands between  $\sim 50$ -80 kDa. The integrated LC-220 chromatograms gave a purity result of 96.5%.

Protein G is a single non-glycosylated protein, which can bind to a broad range of mouse and human IgG subclasses. In our study, the recombinant form of Protein G is used [5]. The expected mass of recombinant protein G (rPG) is 21.8 kDa, which was confirmed by MALDI-TOF MS measurements, and the SDS-PAGE showed several protein bands between  $\sim 28$ -35 kDa. The integrated LC-220 chromatogram gave a purity result of 99.1%.

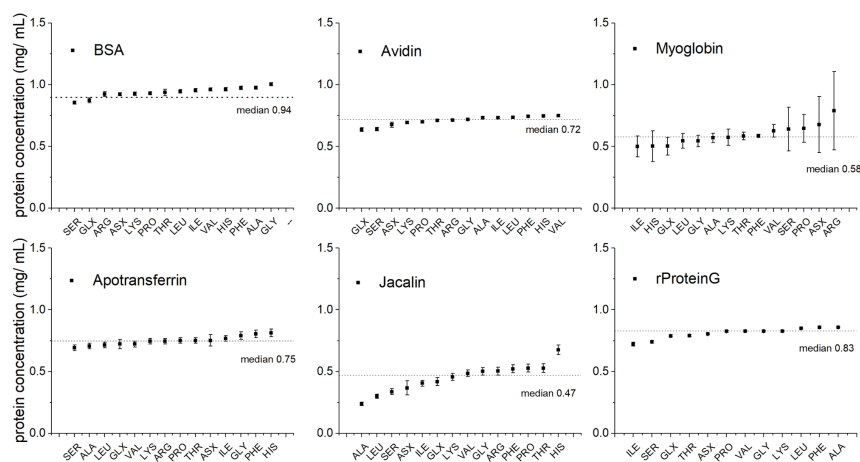
According to our quality evaluation of the investigated proteins (SDS-PAGE, MALDI-TOF MS, LC-220), aliquots of the same aqueous high-purity BSA solution were used as our calibration solution with a concentration of  $1.07 \pm 0.03$  mg/mL determined by AAAA(Phe). If not stated differently, all method calibrations were done with this high-purity BSA solution.



**Fig. S1** Phenylalanine and tyrosine calibration lines for AAAA(Phe) and AAAA(Tyr). Fluorescence of Tyr and Phe were detected at 272 nm excitation/303 nm emission and 260 nm excitation/280 nm emission wavelengths, respectively.



**Fig. S2** UHPLC-FLD measurement for AAAA(Phe) and AAAA(Tyr). Phenylalanine (Phe) and tyrosine (Tyr) standards had a concentration of  $20 \mu\text{M}$ . Fluorescence of Tyr and Phe was detected at 272 nm excitation/303 nm emission and 260 nm excitation/280 nm emission wavelengths, respectively.



**Fig. S3** Protein concentrations determined by AAA calculated for each amino acid

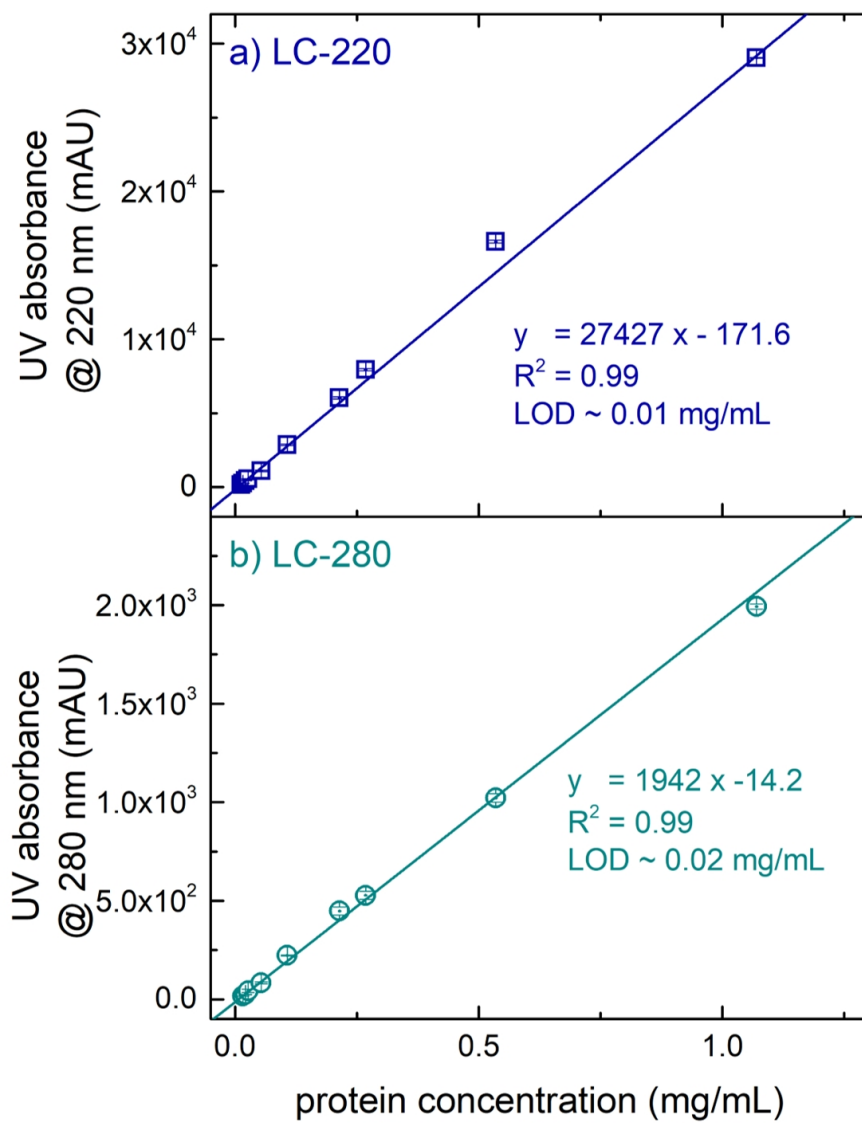
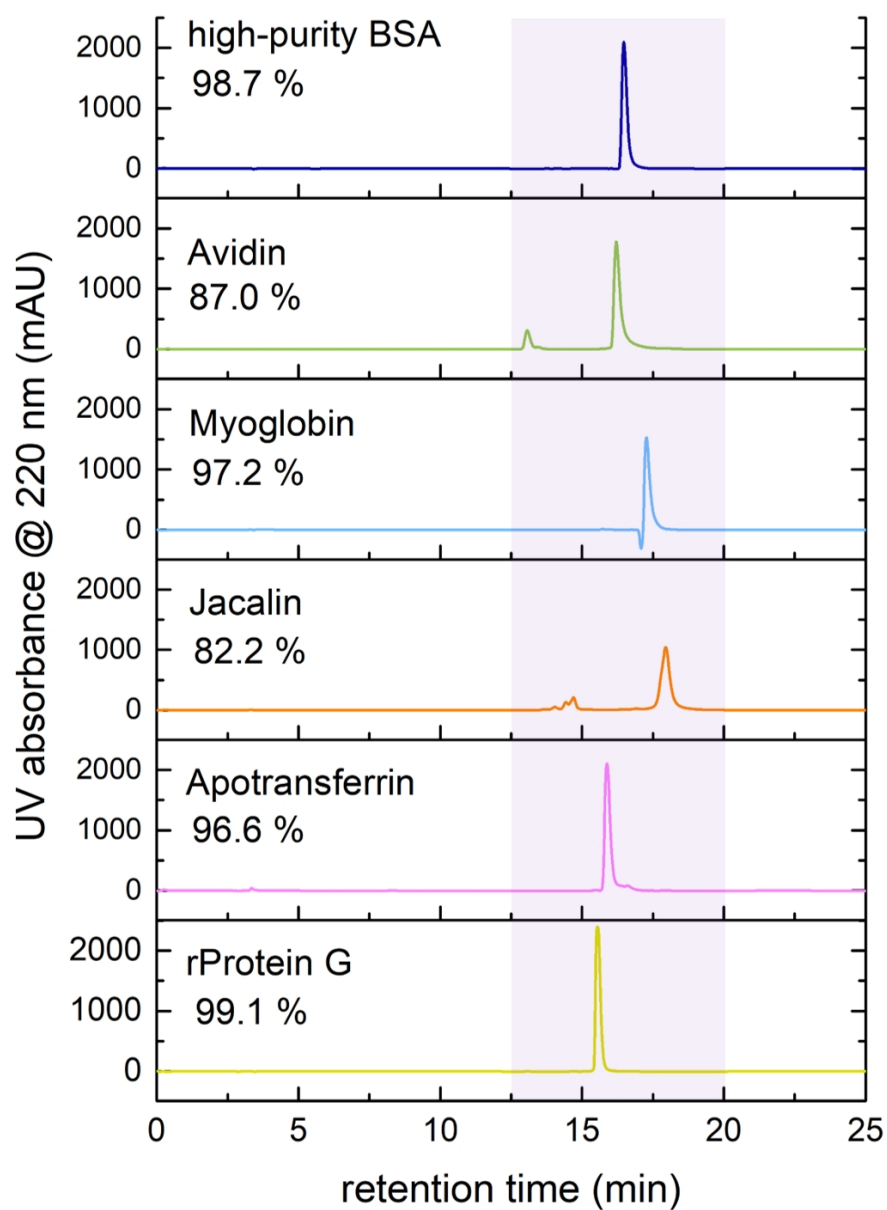
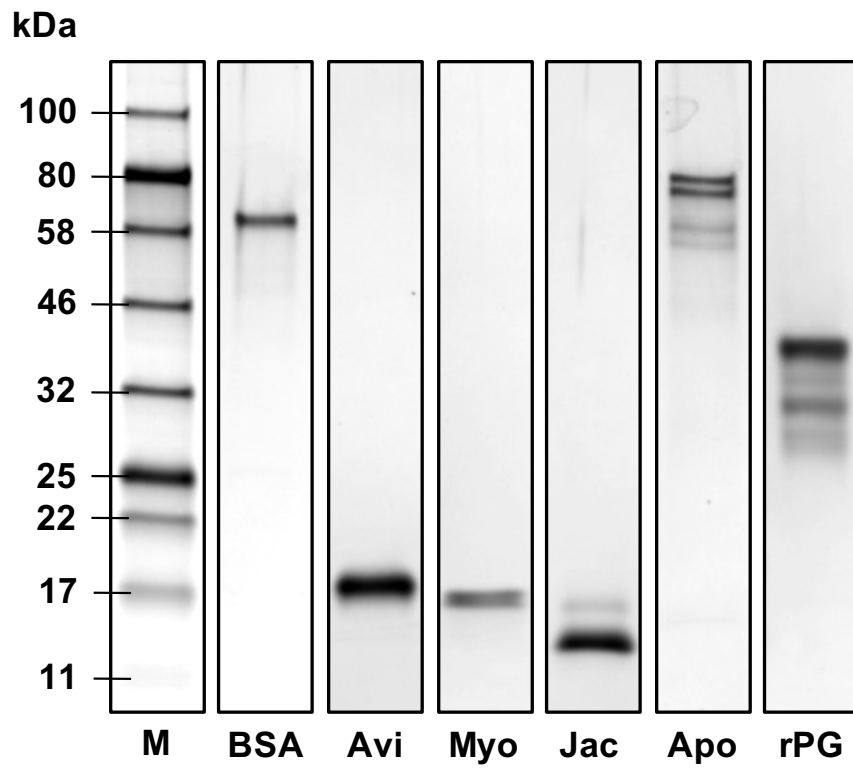


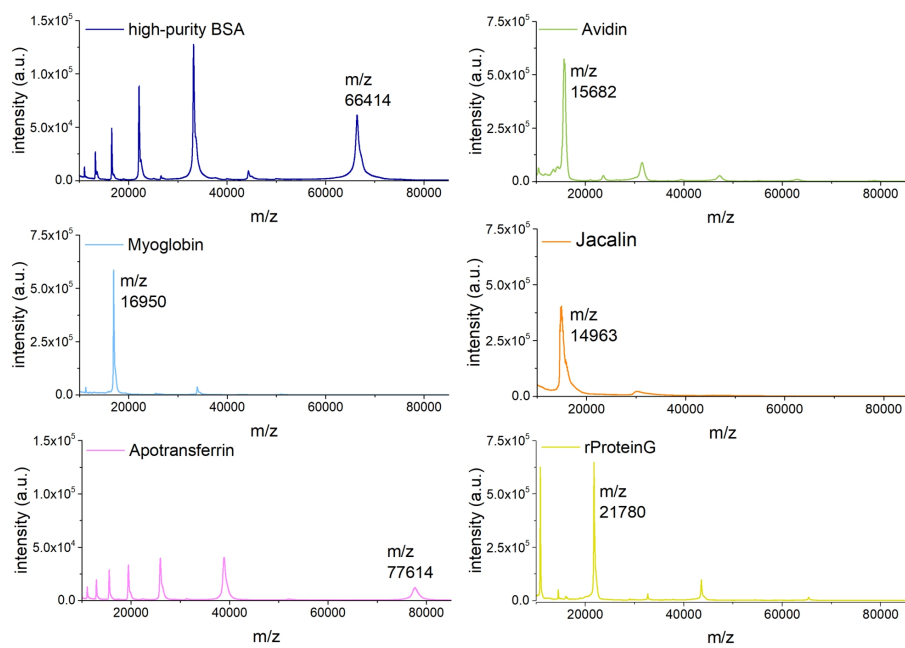
Fig. S4 Bovine serum albumin (BSA) calibration lines for LC-220 (a) and LC-280 (b)



**Fig. S5** Reversed-phase chromatograms at 220 nm (LC-220) of the tested proteins. The main peak area of the integrated chromatograms was used for the purity calculation. All protein peaks had retention times between 12.5 and 20 min (shaded area).



**Fig. S6** SDS-PAGE gel of the six test proteins. Marker (M), bovine serum albumin (BSA), avidin (Avi), myoglobin (Myo), jacalin (Jac), apotransferrin (Apo), recombinant protein G (rPG)



**Fig. S7** MALDI-TOF-MS spectra of six test proteins: high-purity bovine serum albumin (BSA), avidin, myoglobin, jacalin apotransferrin, recombinant protein G.



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## References

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