Supplementary Information for

Charge manipulation using solution and gas-phase chemistry to facilitate analysis of highly heterogeneous protein complexes in native mass spectrometry

Yang Yang, Chendi Niu, Cedric E. Bobst, and Igor A. Kaltashov

Department of Chemistry, University of Massachusetts-Amherst, 240 Thatcher Road, Amherst, MA 01003

Contents

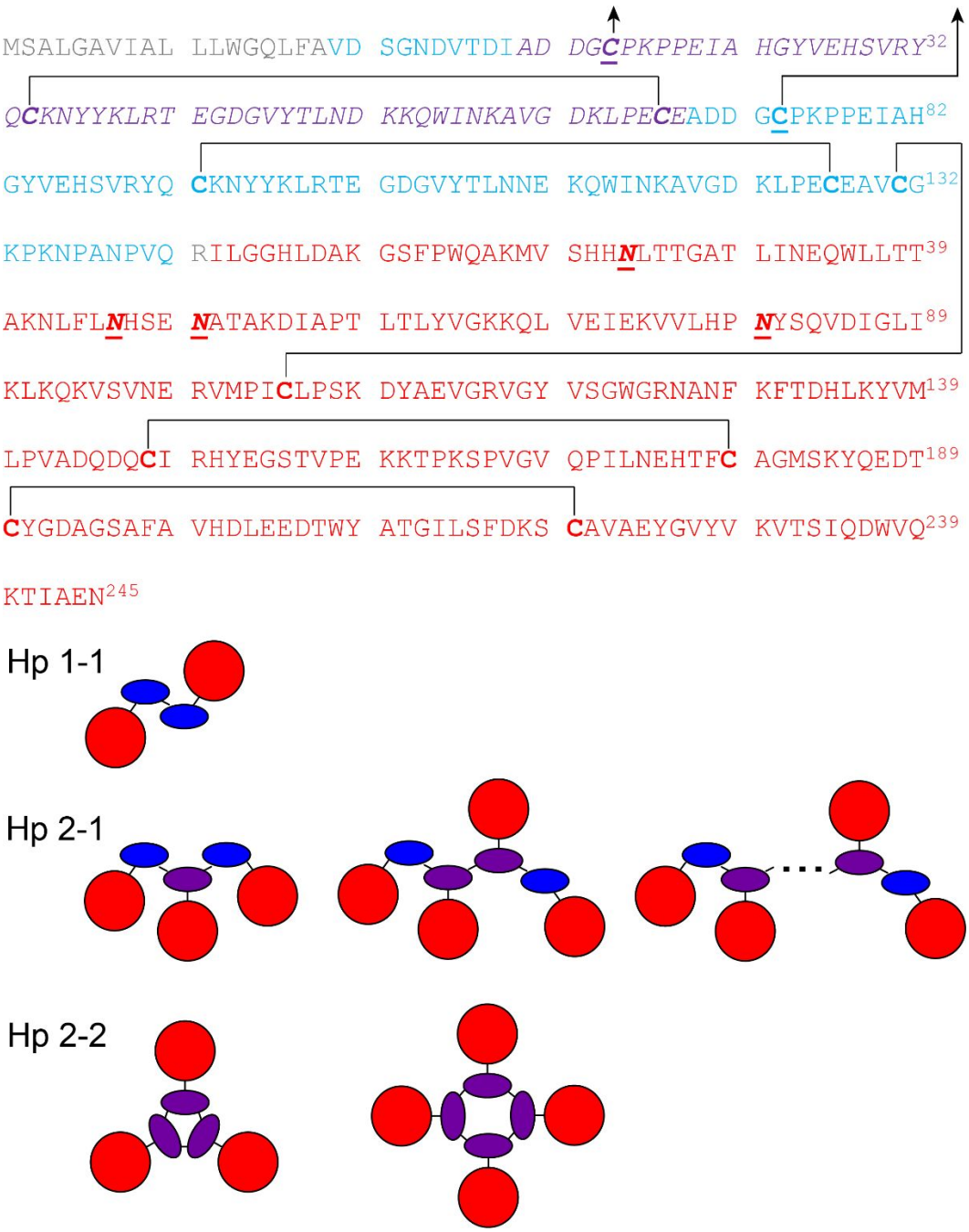

Figure S1. Top: the amino acid sequence of human haptoglobin pre-protein (P00738). The sequences of the light (L) and heavy (H) chains are highlighted in blue and red, respectively. The segment highlighted in purple corresponds to the additional insert present in the light chain L*. The black brackets show the disulfide bonds; arrows indicate the external disulfide bonds (L-L, L-L* and L*-L*). Bottom: schematic representations of the quaternary organization of haptoglobin oligomers.

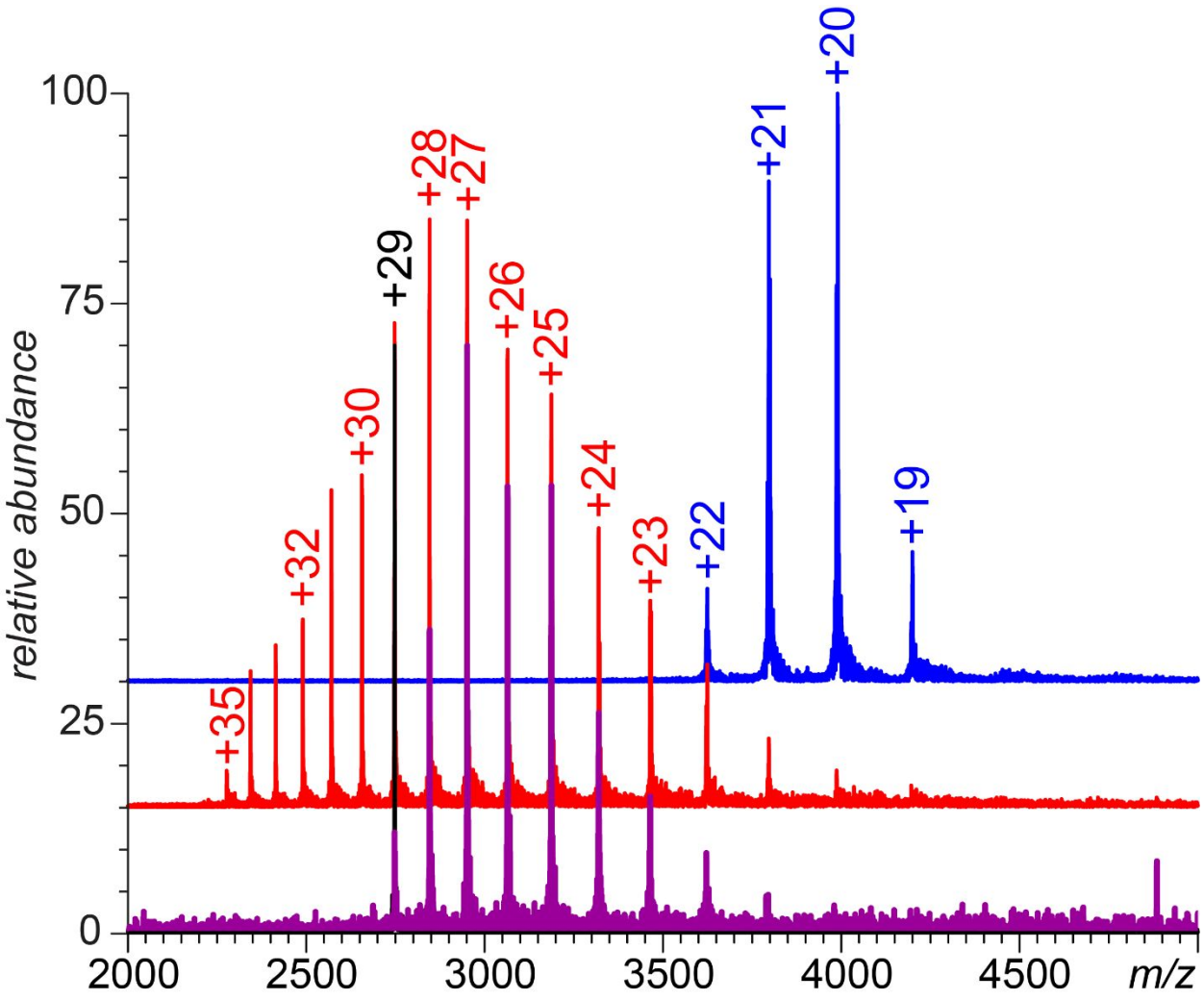

Figure S2. ESI mass spectra of 5 μM aqueous (150 mM ammonium acetate) solution of *h*Tf before (the blue trace) and after modifying the solvent with of 6% mNBA (the red trace). The "supercharged" protein ions at charge state +29 were isolated (the black trace) and subjected to limited charge reduction producing a well-defined ladder extending to the charge state +21 (the purple trace).

Table S1. Measured masses of the most abundant ionic species representing different Hp/Hb complexes.

	Hp 1-1		Hp 2-1	
$(\alpha^*\beta^*)_2H_2L_2$	Expected mass[1.1] 158.1 kDa	Measured mass w/ supercharging 160±2.9 kDa	Expected mass[1.1] 153.9 kDa	Measured mass w/ supercharging 154±2.5 kDa
	Expected mass[2] 158.0 kDa		Expected mass[1.2] 155.9 kDa	
$(\alpha^*\beta^*)_3H_3L_2L^*$	N/A	N/A	Expected mass[1.1] 236.5 kDa	Measured mass w/ supercharging 241±3.9 kDa
	N/A		Expected mass[1.2] 240.5 kDa	
$(\alpha^*\beta^*)_4H_4L_2L^*_2$	N/A	N/A	Expected mass[1.1] 319.3 kDa	Measured mass w/ supercharging 336±4.3 kDa
	N/A		Expected mass[1.2] 325.2 kDa	

[1.1] Limited charge reduction results provided by Yang, Y.; Pawlowski, J. W.; Carrick, I.; Kaltashov, I. A. Evaluation of the extent of haptoglobin glycosylation using orthogonal intact-mass MS approaches. *ChemRxiv***2020**, doi:10.26434/chemrxiv.13518512.v1.

[1.2] Cross-path reactive chromatography results provided by Yang, Y.; Pawlowski, J. W.; Carrick, I.; Kaltashov, I. A. Evaluation of the extent of haptoglobin glycosylation using orthogonal intact-mass MS approaches. *ChemRxiv***2020**, doi:10.26434/chemrxiv.13518512.v1.

[2] Pawlowski, J. W.; Carrick, I.; Kaltashov, I. A. Integration of On-Column Chemical Reactions in Protein Characterization by Liquid Chromatography/Mass Spectrometry: Cross-Path Reactive Chromatography. *Anal. Chem*.**2018**, 90, 1348-1355.