Supporting Information

MALDI-ISD Mass Spectrometry Analysis of Hemoglobin Variants: a top-down approach to the characterization of hemoglobinopathies

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Supplemental Tables 1 and 2.
Supplemental Figures 1 to 7.
Supplemental Figure 1. MALDI-ISD mass spectrum of the beta chain of wild-type hemoglobin obtained from diluted whole blood in sDHB matrix. Inset shows an expanded view of m/z 3550-3620 showing the $\beta_{c34}$ fragment ion at m/z 3584.98. Greater than 80% sequence coverage was obtained using BioTools 3.2 and BUPID Top-Down software and is illustrated on the sequence inset. Assignments correspond to values shown in Supplemental Table 1. * indicates matrix adducts.
Supplemental Figure 2. MALDI-ISD mass spectrum of the alpha chain of wild-type hemoglobin obtained from diluted whole blood in sDHB matrix. Greater than 80% sequence coverage was obtained using BioTools 3.2 and BUPID Top-Down software and is illustrated on the sequence inset. Assignments correspond to values shown in Supplemental Table 1. * indicates matrix adducts.
Supplemental Figure 3. Comparison of $\beta_{c34}$ calculated isotope pattern (a) and the observed isotope pattern (b) at $m/z$ 3584-3592 in the MALDI-ISD of diluted whole blood obtained with sDHB matrix.
Supplemental Figure 4. Expanded view of $m/z$ 6900-6970 from the MALDI-ISD mass spectrum of (a) wild-type hemoglobin obtained from diluted whole blood in sDHB matrix, (b) sickle cell ($\beta$-E6V) heterozygote hemoglobin in sDHB matrix. Monoisotopic $m/z$ values are shown here and the table inset.

<table>
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<th>Hb</th>
<th>Ion</th>
<th>Measured m/z</th>
<th>Calculated m/z</th>
<th>Error (Da)</th>
<th>$\Delta$mass E6V</th>
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<tr>
<td>$\beta$</td>
<td>c$64$</td>
<td>6948.65</td>
<td>6948.60</td>
<td>-0.05</td>
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<tr>
<td>$\beta$(E6V)</td>
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<td>6918.62</td>
<td>0.02</td>
<td>obs. 30.04</td>
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<tr>
<td>$\alpha$</td>
<td>c$65$</td>
<td>6925.58</td>
<td>6925.54</td>
<td>-0.04</td>
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Supplemental Figure 5. Expanded view of \(m/z\) 4675-4770 from the MALDI-ISD mass spectrum of (a) wild-type and (b) sickle cell (\(\beta6\) Glu\(\rightarrow\)Val) heterozygote hemoglobin in sDHB matrix.
Supplemental Figure 6. Nanospray ESI Mass Spectrum of Hb Westmead (α122 His→Gln) obtained using a 12-T Qh/FT-ICR hybrid MS (SolariX, Bruker, Billerica, MA, USA). The spectra were acquired over the range m/z 172–3000 during a transient for which 1Mpoints provided a mass resolving power around 67,000 (at m/z 800), after FFT processing (total time per scan was 2 s). External calibration was carried out using sodium trifluoroacetate clusters.
Supplemental Figure 7. Expanded views from the MALDI-ISD mass spectra of normal hemoglobin obtained from diluted whole blood in sDHB matrix of (a) $m/z$ 2965-3005 and (b) $m/z$ 3685-3717, indicating regions (*) where potential diagnostic $\beta_y^{28}$ and $\beta_y^{34}$ ions for Hb D-Los Angeles ($\beta_{121}$ Glu→Gln) would be observed.