Electronic Supplementary Information For

Separation and characterization of KRas protein and tryptic peptides using enhanced fluidity liquid chromatography tandem mass spectrometry

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Peptide Digestion Procedure.

Reduction of disulfide bonds and alkylation of cysteine residues in protein

1. Conduct buffer exchange of the protein sample solution to 50 mM ammonium bicarbonate (pH 7.8).
2. Add dithiothreitol (DTT) to a final concentration of 5 mM and incubate at 37 °C for 1 hour.
3. Add freshly prepared iodoacetamide (IAA) to a final concentration of 15 mM and incubate at room temperature in the dark for 30 min.

Digestion of protein and clean up of peptides

1. Add trypsin at a 1:50 ratio trypsin : protein ratio, mix gently and incubate at 55 °C for 4 hours.
2. Clean up peptide sample using C18 solid phase extraction using 0.1% TFA in water as washing solution and 0.1% TFA in water/acetonitrile (40/60) as elution solution.
3. Eluted peptide sample was stored at -20 °C until LC-MS analysis.

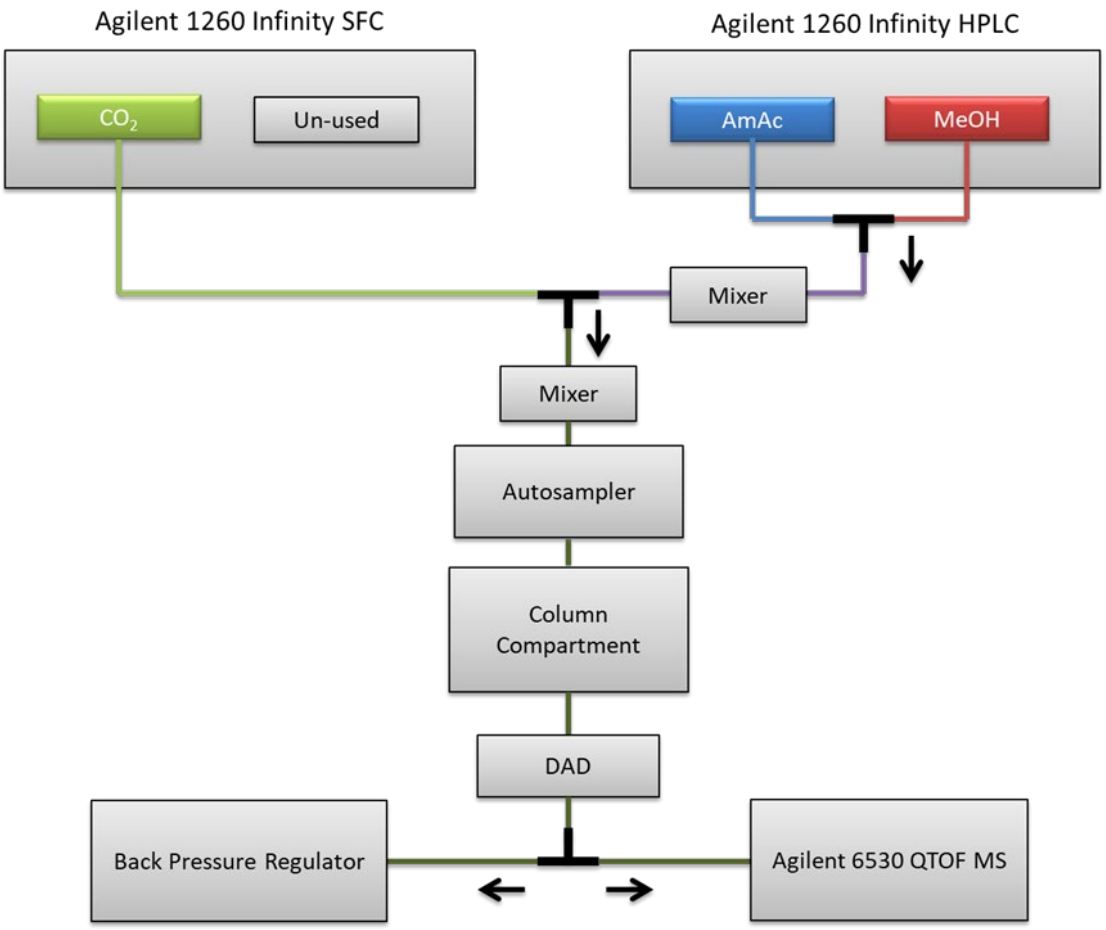

Fig. S1 Instrumentation Setup.

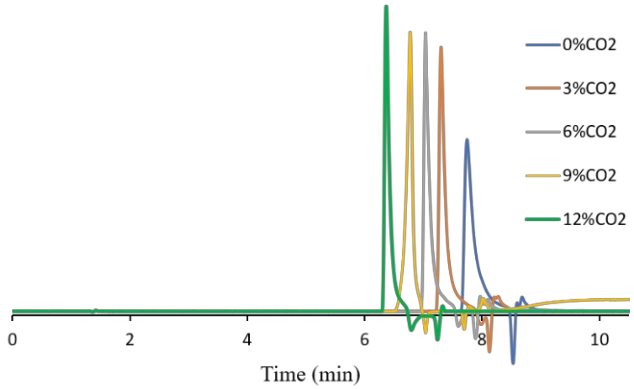

Fig. S2. DAD chromatograms at 280 nm for isocratic elution of myoglobin on XBridge C4 column with addition of liquefied CO_2. Volume percentage of CO_2 in H_2O/MeOH (40:60) solvent system: (blue —) 0%, (orange —) 3%, (grey —) 6%, (yellow —) 9%, (green —) 12%.