



Figure S1. Workflow of the mass spectrometry approach. Lysine residues in FVIII are chemically modified by TMT-126 and the lysine residues in dis-FVIIIa with TMT-127. Labelled FVIII and dis-FVIIIa are pooled in an equal molar ratio and the proteins are cleaved by chymotrypsin. CID and HCD spectra of the peptide ions are subsequently acquired on a nanoLC-LTQ Orbitrap XL mass spectrometer. Both CID and HCD spectra of the same peptide ion allows for identification of the peptides. HCD spectra include the intensities of the TMT-126 and TMT-127 reporter groups. The TMT-127/TMT-126 ratio between these intensities reveals whether a peptide is more prone to chemical modification in dis-FVIIIa or in FVIII (36,38).