Supporting Information

Direct Monitoring of Protein-Protein Inhibition Using Nano Electrospray Ionization Mass Spectrometry

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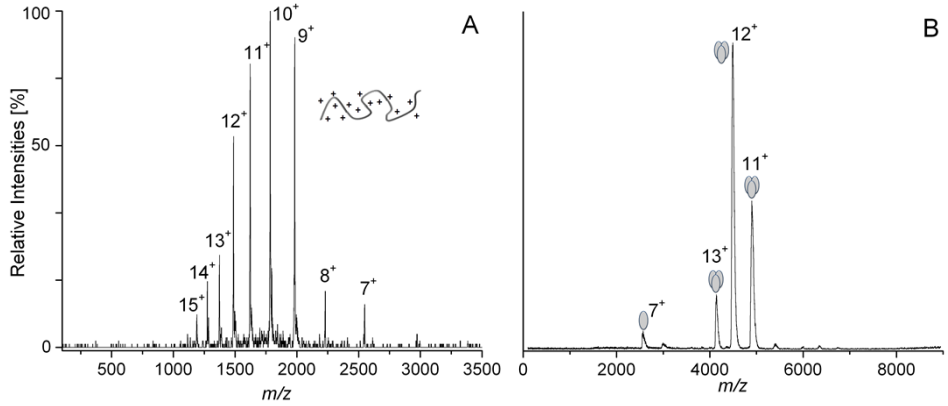

Figure S1. NanoESI mass spectra of TNF-alpha showing **A.** unfolded monomer measured under denaturing conditions and **B.** native intact trimer using 50 mM ammonium acetate buffer in 1 % DMSO at pH = 7.7

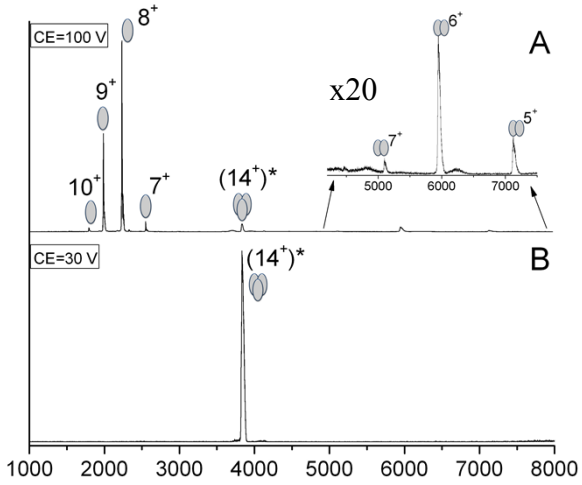

Figure S2. CID experiments of the trimeric TNF-alpha: **A.** For the MS/MS measurements the +14- charged protein precursor ions (*) were selected. The collision energy offset was set to 30 V; **B.** At the collision energy offset of 100 V the selected trimeric +14 ions dissociate into monomers and dimers.

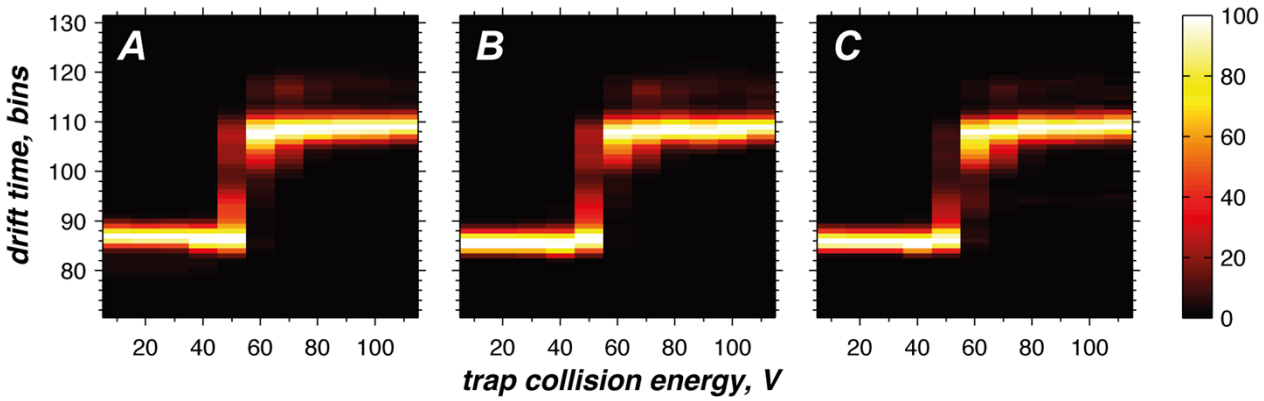

Figure S3. Dependence of the drift time distribution of TNF-alpha trimer 13+ ion on the trap collision energy applied. **A**. 4.5 µM TNF-alpha in 75 mM ammonium acetate buffer pH 7.7. **B.** Same as in A, in the presence of 1 % (vol.) DMSO. **C**. Same as in A, in the presence of 1 % (vol.) DMSO and 100 µM SPD304. The peak intensity is normalized.

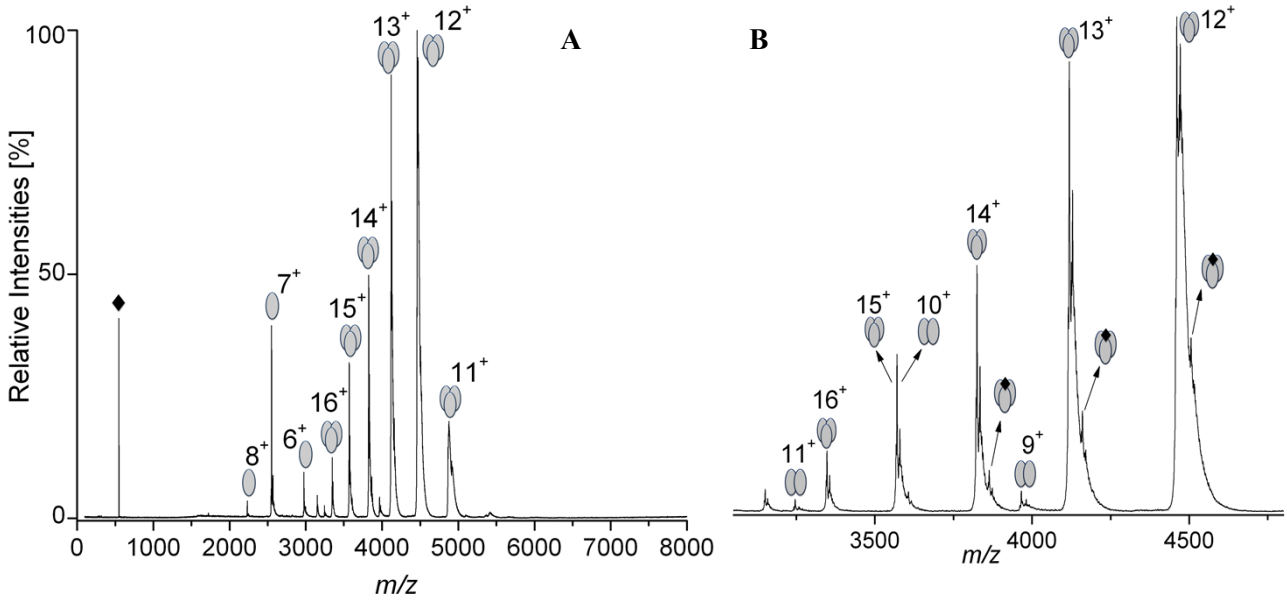

Figure S4. A. Representative nanoESI mass spectra of 4.5 µM TNF-alpha in the presence of 100 µM SPD304 under "native" conditions at pH = 7.7 in 50 mM ammonium acetate and in 1 % DMSO; binding of SPD304 (filled rhombus) dissociates the trimeric protein into dimers and monomers. B. Zoom of the spectrum; dimer peaks and binding of SPD304 to the trimeric TNF-alpha is detected.