Supporting Information

Figure S1.

In-spray supercharging ESI FT-ICR MS of myoglobin using A) 1% sulfolane solution, myoglobin to sulfolane solutions flow ratio of 1/1; B) 1% sulfolane solution, myoglobin to sulfolane solutions flow ratio of 1/2; C) 5% sulfolane solution, myoglobin to sulfolane solutions flow ratio of 1/1. The abundances of myoglobin signals decrease with an increase of the amount of sulfolane mixed with myoglobin in the Taylor cone.

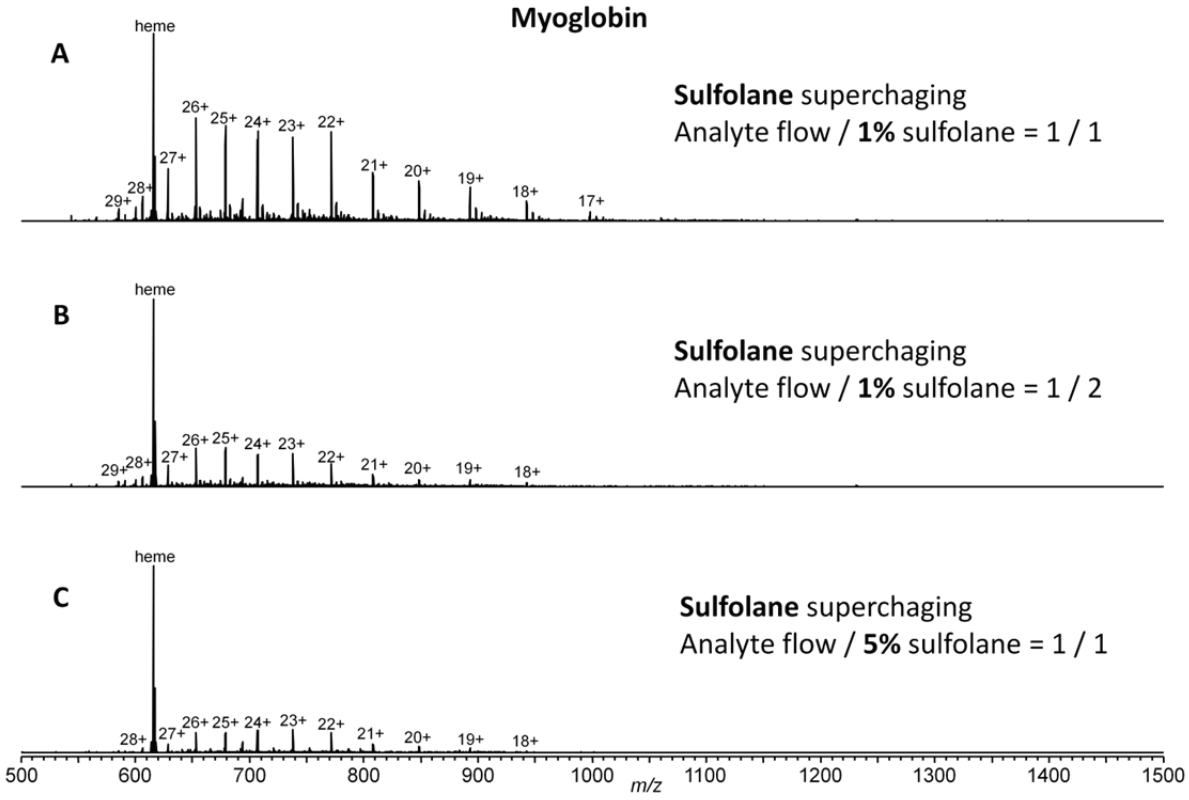

Table S1. Signal variation (in %) for in-spray supercharged myoglobin in comparison with ionization without supercharging.

Myoglobin	***m*-NBA**	**DMSO**	**sulfolane**
Average intensity variation	-3.49E+01	3.43E+01	-7.48E+01
Average norm intensity variation	-4.36E+01	1.63E+01	-7.89E+01
Average top 3 peaks intensity variation	-3.08E+01	5.08E+01	-7.96E+01

Table S2. Signal variation (in %) for in-spray supercharged substance P in comparison with ionization without supercharging.

Subtance P	***m*-NBA**	**DMSO**	**sulfolane**
Average intensity variation	1.75E+01	-3.13E+01	-2.42E+01
Average norm intensity variation	-2.76E+00	-5.82E+01	-3.31E+01
Top peak intensity variation	6.31E+01	-1.60E+01	-4.36E+01

All the reported intensity variations are derived from FT-ICR MS data. For both a mid-size protein and a short peptide we compared the intensity values of analytes supercharged by in-spray addition of different reagents with the corresponding analytes sprayed by using standard acidic H_2O/MeOH (50:50, V/V) solution. The intensity of a certain charge state referred to the intensity of the most intense isotopomer of each isotopic distribution.

The *average intensity variation* was calculated considering the intensities of each single charge state detected. All the intensity values were summed and divided over the number of observed charge states. What we report is the percent variation when *m*-NBA, DMSO and sulfolane are compared with H_2O/MeOH solution.

We also report the *average normalized intensity variation*, which is based on intensity values normalized over the number of charges of each detected charge state. This second parameter is needed considering the principle on which FTMS is based (i.e., induced current is proportional to the charge state of the ions).

From the point of view of proteomic applications of in-spray supercharging of peptides and proteins, we also reported the intensity variation for the most intense detected charge states. We considered the *top peak intensity variation* for substance P, and the *average top 3 peaks variation* for myoglobin. When performing MS/MS in a data-dependent fashion those are the values that are generally used for triggering the MS/MS scan event.