Figure legend (Supplementary material)

**Supplementary Figure 1:** Comparison of nanoelectrospray MS/MS spectra for the doubly-protonated ion at m/z 785.8 from Glu-fibrinopeptide B at concentrations of 5 (a and b) and 30 fmol/µL (c and d) in 50 % aqueous methanol with FAIMS nanoelectrospray at a CV of -25.5 V (a and c) and conventional interface (b and d). Conditions as for Figure 1.

**Supplementary Figure 2:** Reproducibility of CV stepping during nanoLC-FAIMS-MS analyses of 200 ng inj. of human monoblastic U937 protein extracts. (a) Scatter plot representing the distribution of ion intensities for triplicate nanoLC-FAIMS-MS experiments using CV values of -22 V, -26 V, -30 V, -34 V, -38 V, and -42 V (1s/CV step). (b) Scatter plots of intensity values obtained using CV stepping compared to those acquired using fix CV conditions. A good correlation of ion intensities was obtained between these experiments with RSD values within 45 % for 95 % of the ion population. Elution conditions as indicated in Experimental section.

**Supplementary Figure 3:** Comparison of nanoLC-MS/MS analyses of 200 ng inj. of human monoblastic U937 protein extract with and without FAIMS. Extracted ion chromatograms for m/z 628.65 with (a) and without FAIMS. The intensity of the corresponding ions is shown in the top right corner of each panel. (b). Extracted mass spectrum for peak eluting at 39.0 min in nanoLC-MS/MS analyses with (c) and without FAIMS (d). MS/MS spectrum of precursor ion m/z 628.65 using FAIMS (e). No MS/MS spectrum was acquired using conventional nanoLC-MS/MS. Precursor ion m/z 628.65
was assigned to peptide FQDGDLTLYQSNTILR from Glutathione S-transferase P. A 3.7 fold increase in intensity was observed for FAIMS (CV = -42 V). Elution conditions as indicated in Experimental section.

**Supplementary Figure 4:** Comparison of nanoLC-MS/MS analyses of 200 ng inj. of human monoblastic U937 protein extract with and without FAIMS. Extracted ion chromatograms for m/z 611.98 with (a) and without FAIMS. The intensity of the corresponding ions is shown in the top right corner of each panel. (b). Extracted mass spectrum for peak eluting at 29.0 min in nanoLC-MS/MS analyses with (c) and without FAIMS (d). MS/MS spectrum of precursor ion m/z 611.98 using FAIMS (e) and conventional nanoelectrospray (f). Precursor ion m/z 611.98 was assigned to peptide IISNASCTTNCLAPLAK from Glyceraldehyde-3-phosphate dehydrogenase. A 5.1 fold increase in intensity for FAIMS (CV = -34 V). Elution conditions as indicated in Experimental section.

**Supplementary Figure 5:** Comparison of nanoLC-MS/MS analyses of 200 ng inj. of human monoblastic U937 protein extract with and without FAIMS. Extracted ion chromatograms for m/z 614.63 with (a) and without FAIMS. The intensity of the corresponding ions is shown in the top right corner of each panel. N.D.: Not detected (b). Extracted mass spectrum for peak eluting at 42.4 min in nanoLC-MS/MS analyses with FAIMS (c). An extracted mass spectrum taken at 42.4 min is also presented in (d) for the conventional nanoelectrospray analysis although this peptide at m/z 614.63 was not
detected. MS/MS spectrum of precursor ion m/z 614.63 using FAIMS (e). No MS/MS was acquired for this ion using conventional nanoelectrospray as this peak was not detected. Precursor ion m/z 614.63 was assigned to STGEAFVQFASQEIAEK from heterogeneous nuclear ribonucleoprotein H1 (CV = -38 V). Elution conditions as indicated in Experimental section.

**Supplementary Figure 6:** Comparison of nanoLC-MS/MS analyses of 200 ng inj. of human monoblastic U937 protein extract with and without FAIMS. Extracted ion chromatograms for m/z 650.37 with (a) and without FAIMS. The intensity of the corresponding ions is shown in the top right corner of each panel. N.D.: Not detected. (b). Extracted mass spectrum for peak eluting at 42.7 min in nanoLC-MS/MS analyses with FAIMS (c). An extracted mass spectrum taken at 42.7 min is also presented in (d) for the conventional nanoelectrospray analysis although this peptide at m/z 650.37 was not detected. MS/MS spectrum of precursor ion m/z 650.37 using FAIMS (e). No MS/MS was acquired for this ion using conventional nanoelectrospray. Precursor ion m/z 650.37 was assigned to peptide GVVQELQQAI SK from heterogeneous eukaryotic translation elongation factor 1 delta isoform 1 (CV = -30 V). No MS/MS spectrum for this ion was acquired in the conventional nanoelectrospray experiment. Elution conditions as indicated in Experimental section.
Supplementary Figure 1

a) FAIMS spiked Glu-Fib B 5 fmol

b) Non-FAIMS spiked Glu-Fib B 5 fmol

c) FAIMS spiked Glu-Fib B 30 fmol

d) Non-FAIMS spiked Glu-Fib B 30 fmol
Supplementary figure 2

a)

b)
Supplementary Figure 6

(a) FAIMS

(b) No FAIMS

(c) [M+2H]^2

(d)

(e) KS QAQ LEQ VVG

KS 1 AQ Q L E Q V VG

m/z 234.1 347.4 418.3 546.4 674.2 916.55 1044.29 1144.51

Relative intensity (%)