

Supplementary Material for

**Characterization of a PEGylated protein therapeutic by ion exchange chromatography
with on-line detection by native ESI MS and MS/MS**

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Figure S1. Purity assessment of PEG-IFN β by SEC (top) and reversed-phase HPLC (bottom).

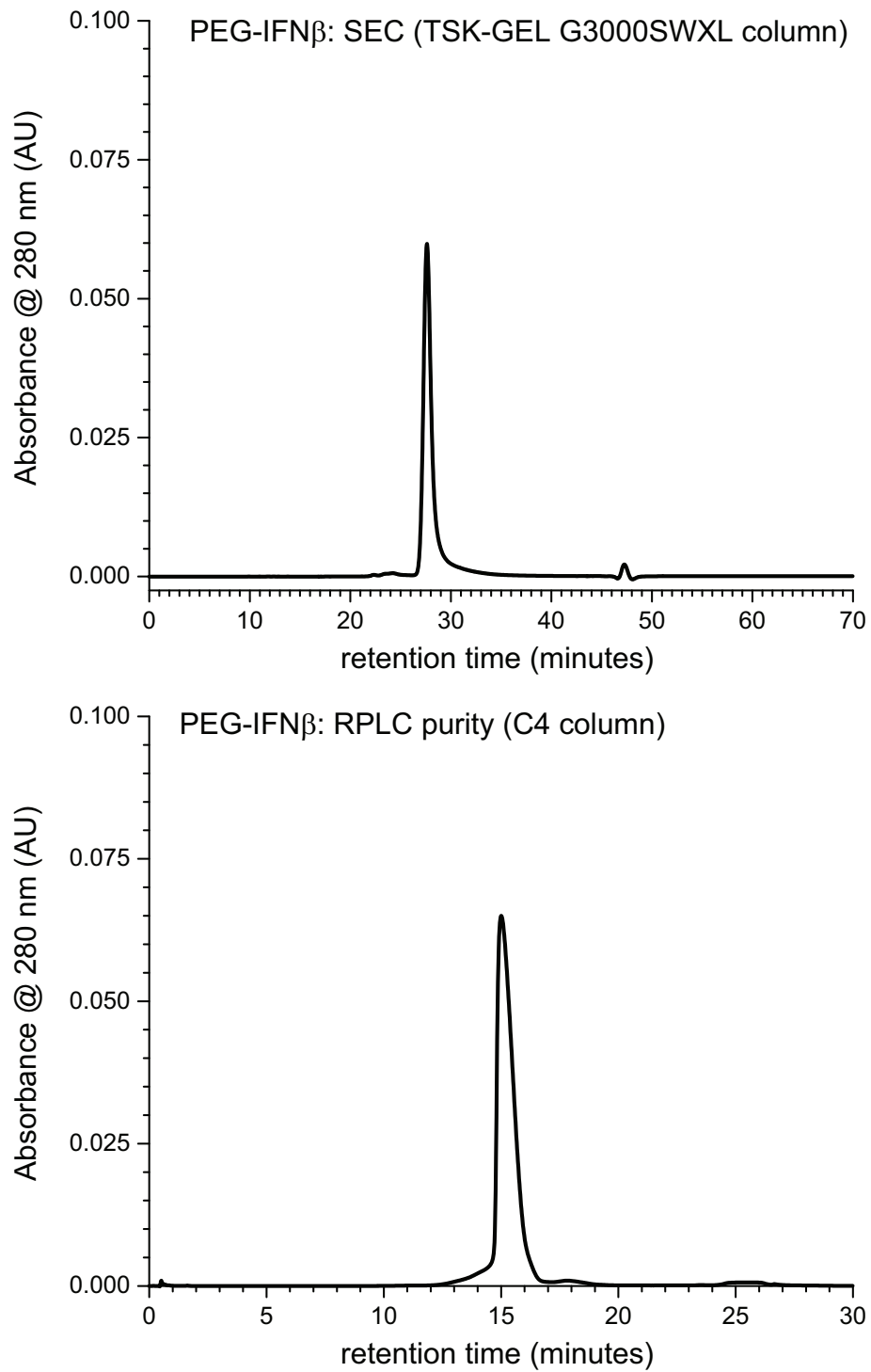


Table S1. Distribution of PEG-IFN β glycoforms based on peptide mapping of unfractionated sample (relative peak areas of extracted ion chromatograms of glycopeptides in Lys-C digest) and on fluorescence measurements of released glycans labeled with 2-aminobenzoic acid. Glycoforms detected in the present work (on-line IXC/ESI/MS/MS) are highlighted in gray.

Carbohydrate composition	Peptide mapping (MS)	Released glycans (fluorescence)
Non-glycosylated	0 %	n/a
BiNA0	2 %	1.2 %
BiNA1	21 %	9.3 %
BiNA2	68 %	58.9 %
TriLAcNA3	3 %	10.5 %
TriNA3	5%	18.4 %
BiNA3	1 %	1.8 %

Figure S2. A zoomed view of a mass spectrum of PEG-IFN β ions produced by limited charge reduction of ESI-generated ions at m/z 1000 and calculated isotopic distributions for several hypothetical PEG-IFN β ions representing different protein glycoforms, lengths of PEG chain and composition of cationizing agents.

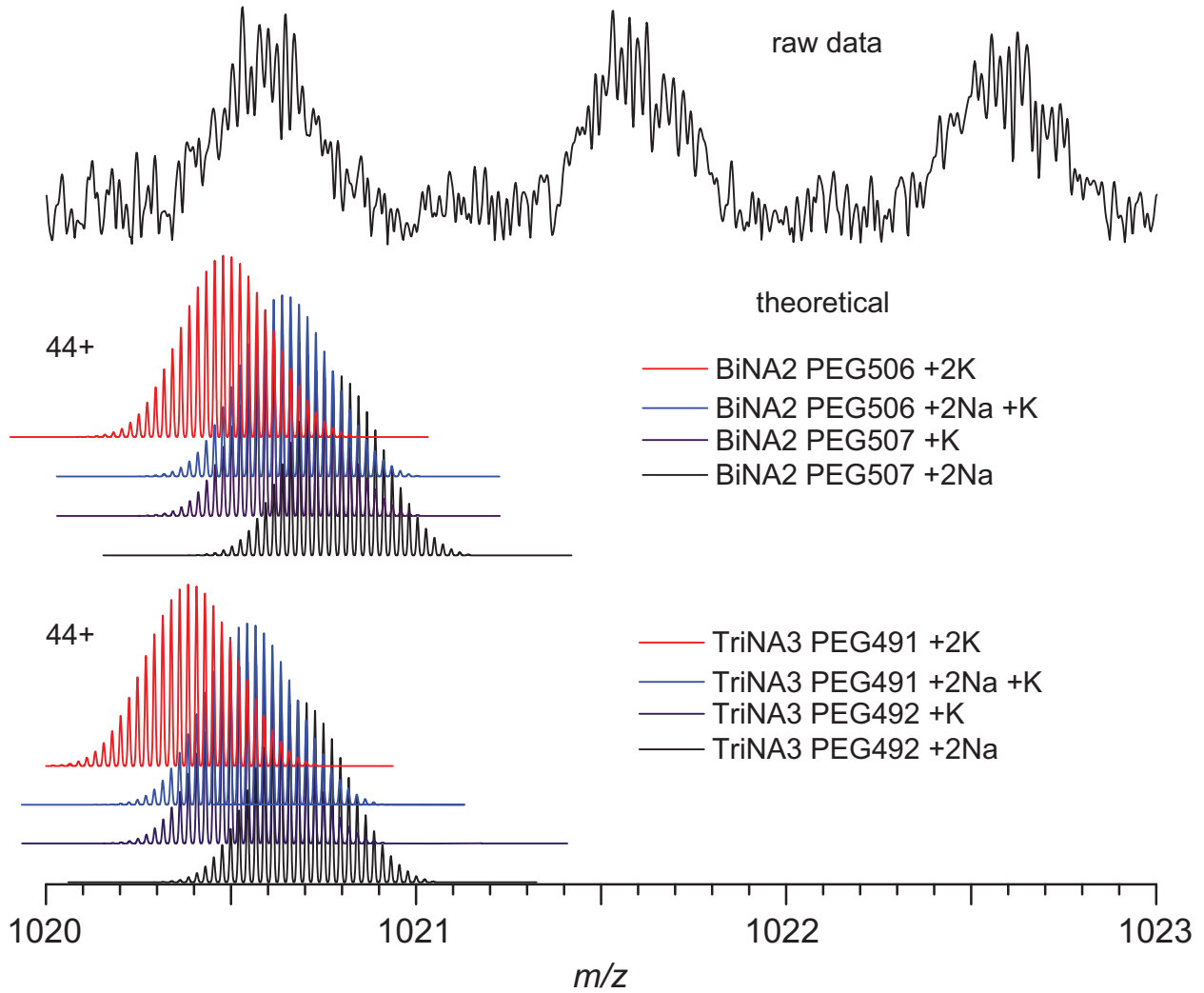


Figure S3. Mass spectra of PEG-IFN β ions produced by limited charge reduction of ESI-generated ions at m/z 1150 in on-line IXC/MS experiment (traces 1-3) and off-line without any chromatographic separation (gray-filled trace). The labels indicate the masses calculated for different ions.

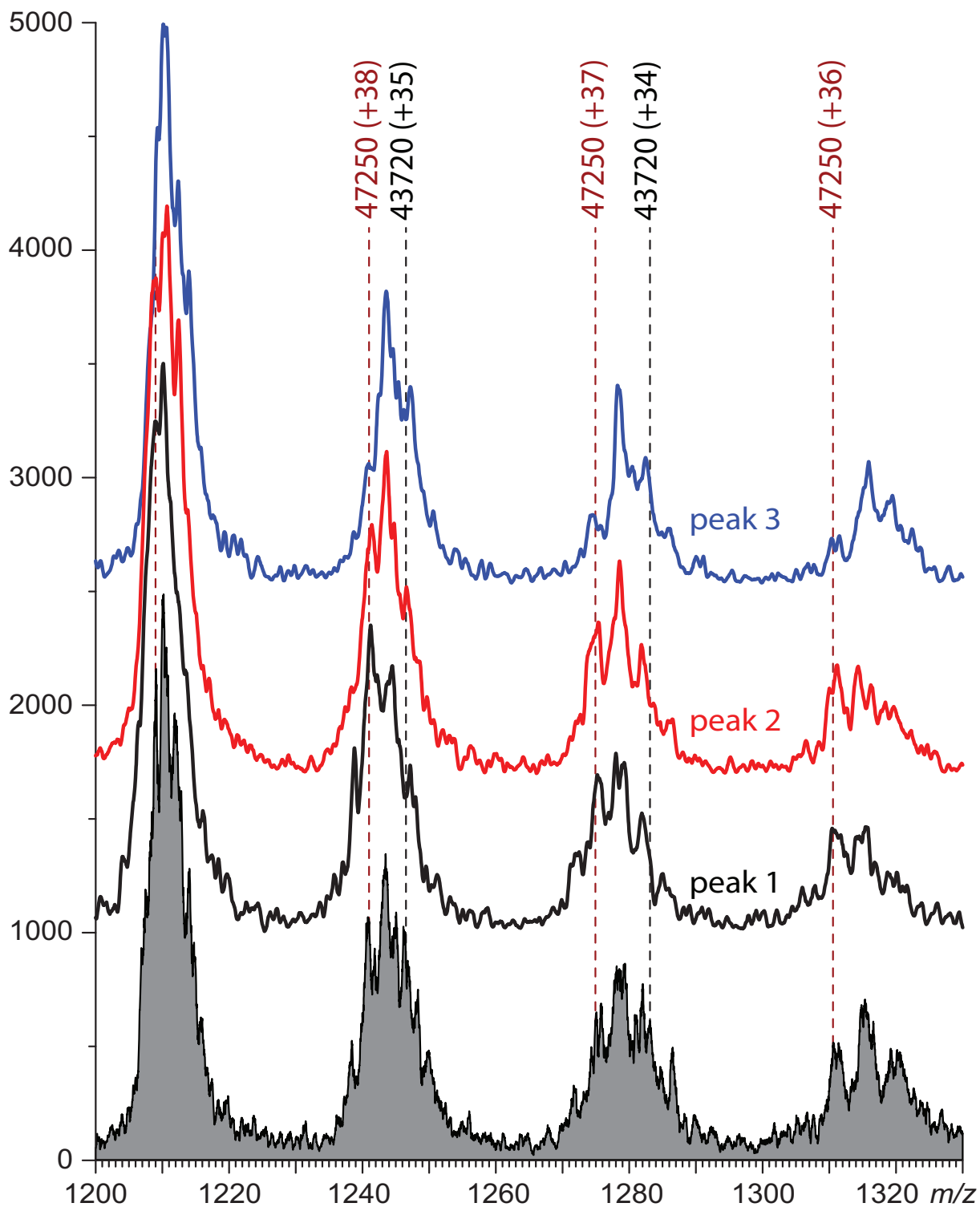


Figure S4. A zoomed view of a low- m/z region of the on-line IXC/MS/MS spectrum averaged across the 31.9-33.5 min elution time window. The colored lines with arrows indicate spacing equal to 44 Da ($\text{CH}_2\text{CH}_2\text{O}$ unit) adjusted for the charge state of small PEG fragments.

