An MS-based middle-up approach for the allotype-specific analysis of human plasma IgG Fc *N*-glycosylation

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Introduction

Current approaches to study polyclonal human immunoglobulins G (IgG) glycosylation imply protein digestion or glycan release. Although these approaches allow high-throughput analysis, their use inevitably brings a considerable loss of information, particularly regarding the allotypes or the interdependence of different post-translational modifications (PTM). However, due to the complexity and inherent variability of polyclonal IgGs, their intact analysis is still not feasible. We here propose a new middle-up strategy for the analysis of the fragment crystallizable (Fc)-region obtained from human plasma IgGs, with the aim of acquiring an integrated overview of the glycosylation and other PTMs of each specific allotype.
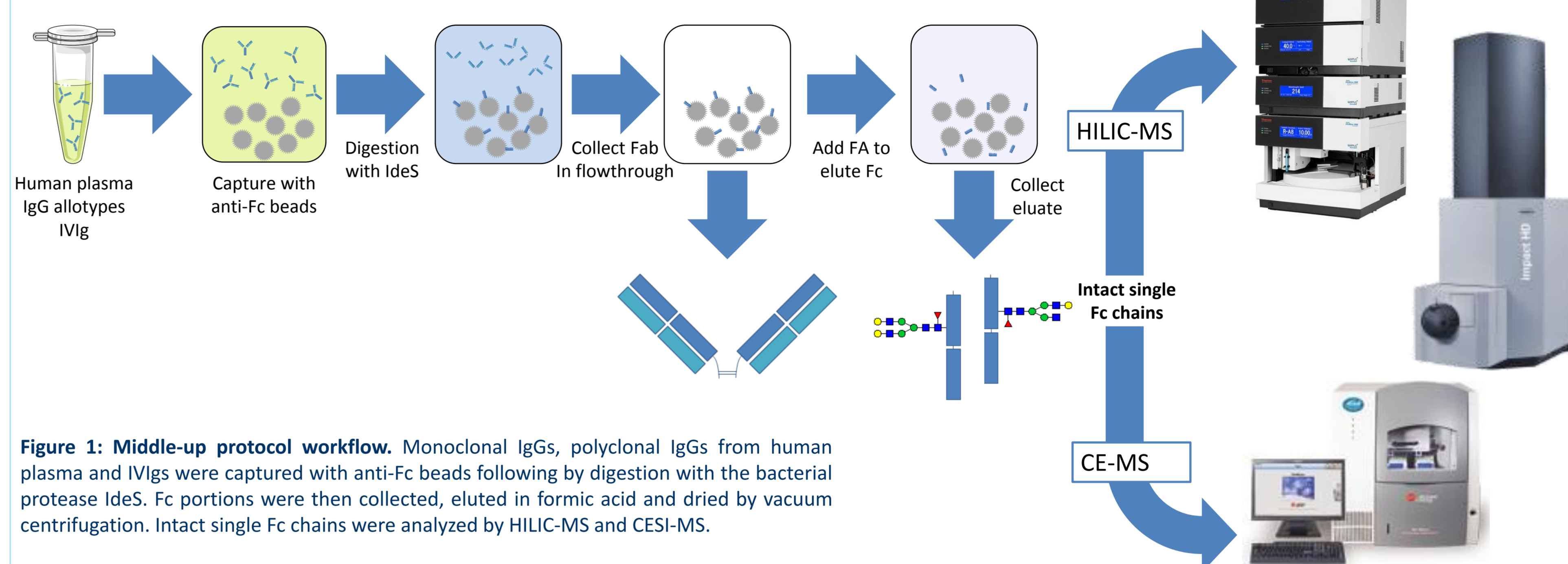
Workflow

Figure 1: Middle-up protocol workflow. Monoclonal IgGs, polyclonal IgGs from human plasma and IVIgs were captured with anti-Fc beads following by digestion with the bacterial protease IdeS. Fc portions were then collected, eluted in formic acid and dried by vacuum centrifugation. Intact single Fc chains were analyzed by HILIC-MS and CESI-MS.

Results

HILIC-MS vs CE-MS: complementarity of the two techniques
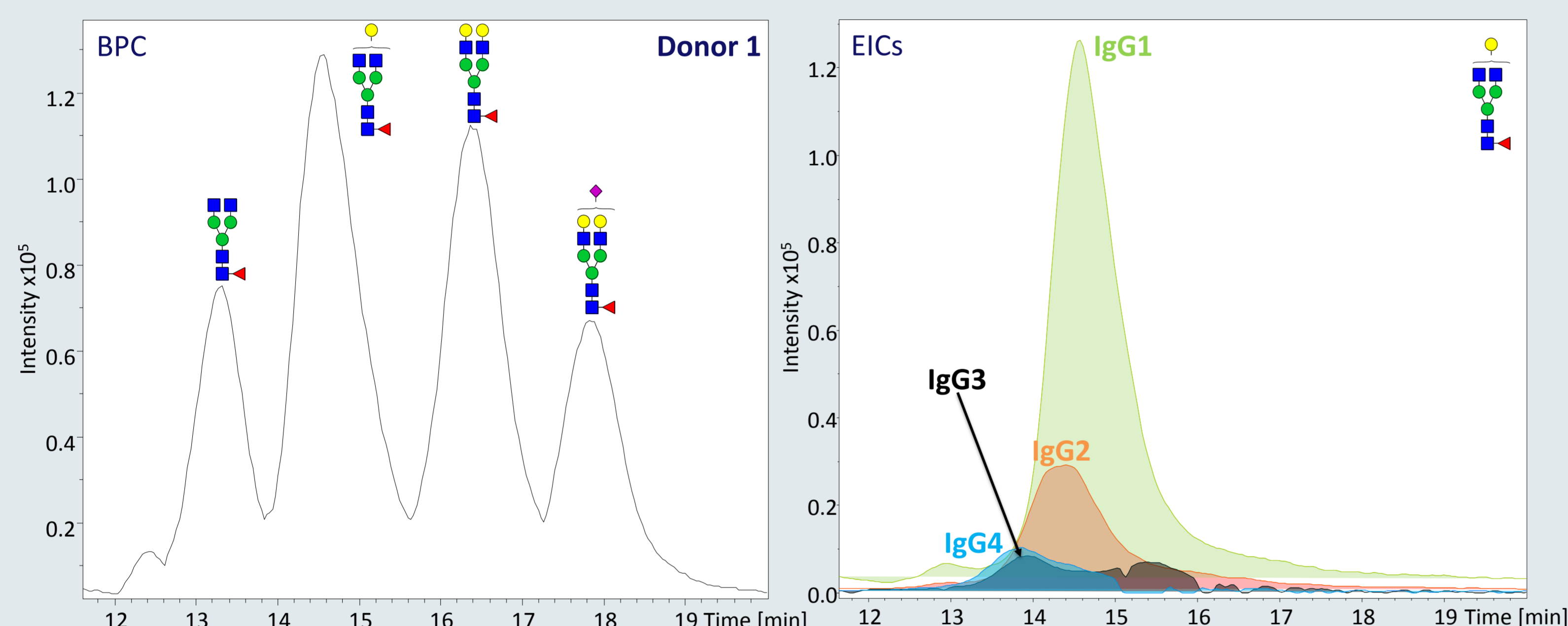

Figure 2: HILIC-MS analysis of Human plasma (donor 1). Fc portions were separated based on their different glycan composition (see base peak chromatogram (BPC)), while the differences on the amino acid backbone barely impact the separation (see extracted ion chromatograms (EICs) of the G1F peak). Conditions: amideHILIC column; mobile phases, A: 98% ACN, 2% water, 0.1% TFA, B: 10% 2-propanol, 2% ACN, 0.1% TFA.
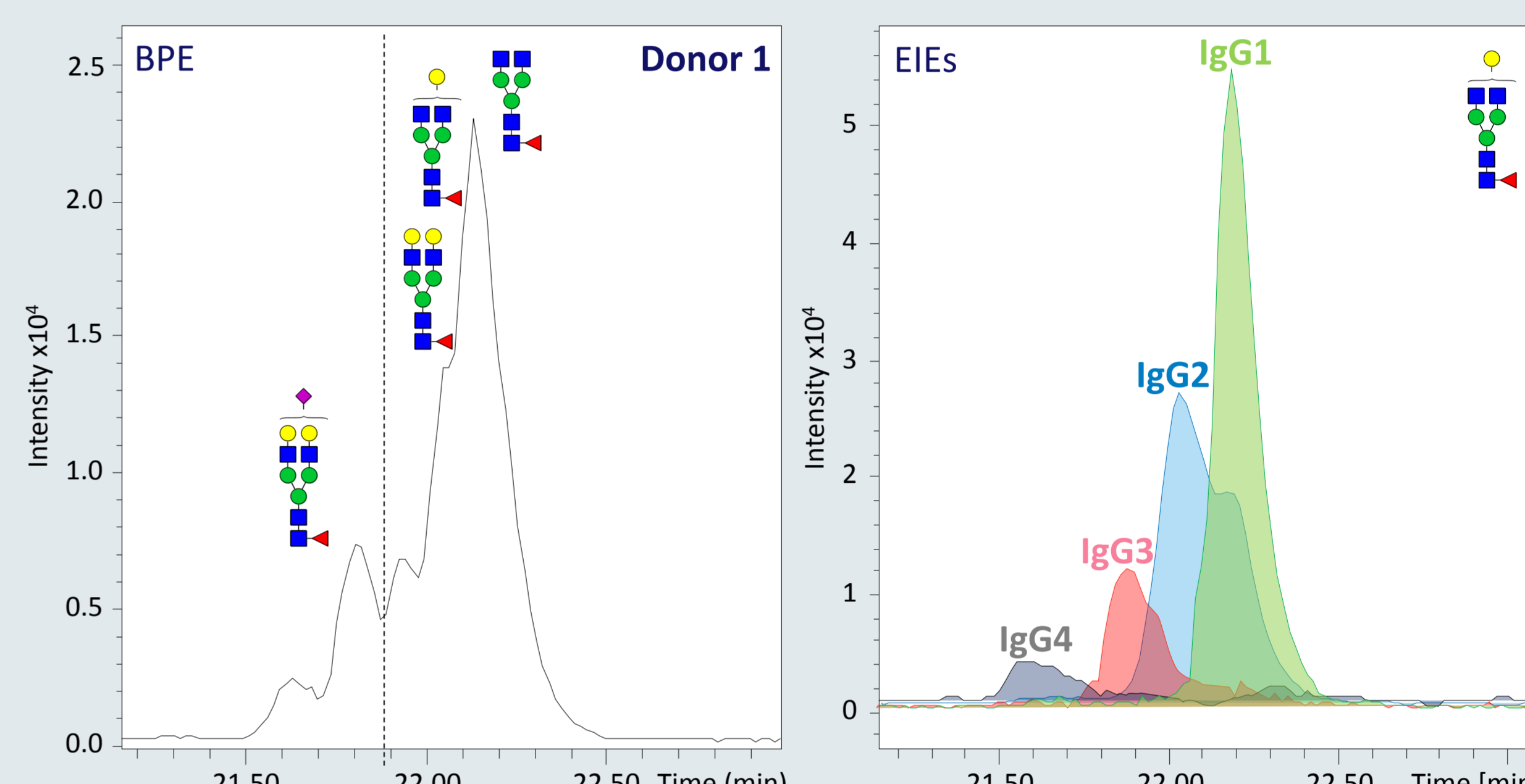

Figure 3: CE-MS analysis of Human plasma (donor 1). Fc portions were separated based on the charges present on both the amino acid sequence and the sialic acids (see base peak electropherogram (BPE)), while the amino acidic differences are clearly distinguishable (see extracted ions electropherograms (EIEs) of the G1F peak) and result in the separation of the subclasses. Conditions: PEI-coated capillary ; BGE: 20% Acetic Acid + 10% MeOH; -20 kV, 20 ºC.
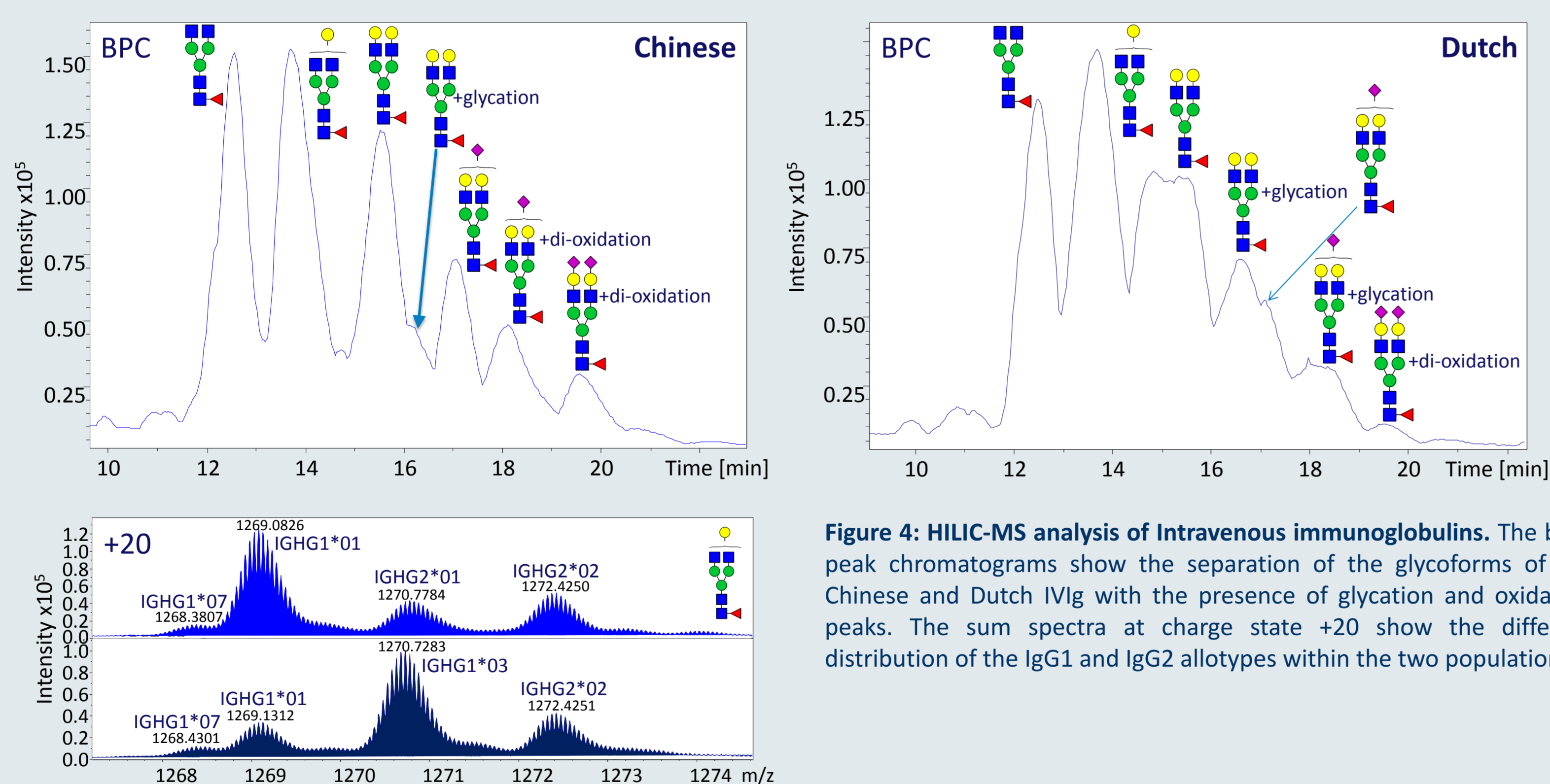
HILIC-MS of IVIgs

Figure 4: HILIC-MS analysis of Intravenous immunoglobulins. The base peak chromatograms show the separation of the glycoforms of the Chinese and Dutch IVIg with the presence of glycation and oxidation peaks. The sum spectra at charge state +20 show the different distribution of the IgG1 and IgG2 allotypes within the two populations.

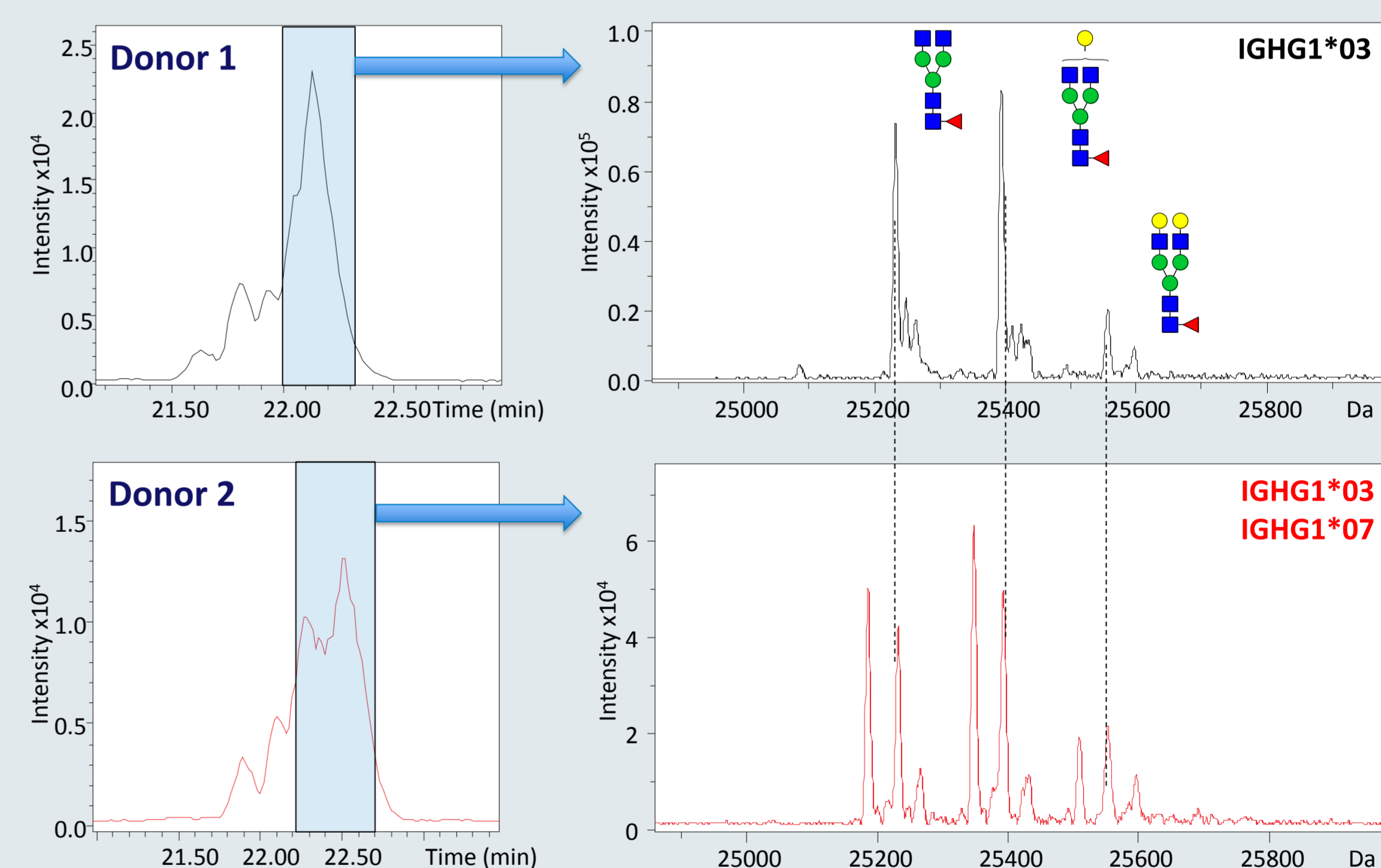### CESI-MS of Fc from different donors

Figure 5: CE-MS analysis of human plasma. The deconvoluted spectra of the neutral glycoforms peaks allow to see the difference between the donor 1 who carries only one allotype of IgG1 and donor 2 who carries two allotypes of IgG1.

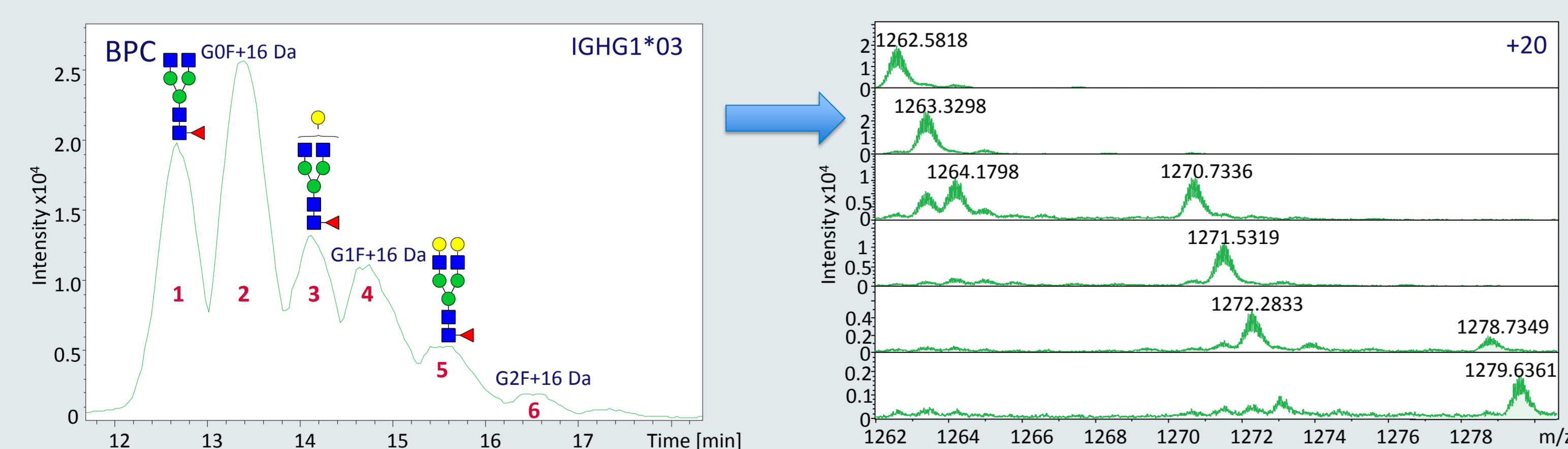### HILIC-MS of monoclonal standard IgG allotypes

Figure 6: HILIC-MS analysis of monoclonal standard IgG allotype. BPC shows the separation of the glycoforms as well as the oxidations. The sum spectra at the charge state +20 of each peak show G0F, G1F, G2F with their oxidized forms (+16 Da and +32 Da).

Fc/2 mass (non reduced + lysine-clipped)			
Assignment	Experimental mass	Theoretical mass	Δ (Da)
G0F	25216.4309	25216.4276	0.0033
G0F +16 Da	25232.4250	25232.4226	0.0024
G0F +32 Da	25248.4198	25248.4175	0.0023
G1F	25378.4934	25378.4804	0.0130
G1F +16 Da	25394.4848	25394.4754	0.0094
G1F +32 Da	25410.4790	25410.4703	0.0087
G2F	25540.5578	25540.5332	0.0246
G2F +16 Da	25556.5732	25556.5282	0.0450

Conclusion and perspectives

➢ The proposed middle-up approach is a promising solution for the analysis of the intact Fc part of polyclonal IgGs.
➢ The orthogonality of HILIC and CE separations allows the identification of several PTMs and allotypes.
➢ The next steps in our method development will be the exploitation of MS/MS to further confirm the mass assignments and the relative quantitation of different proteoforms to prove an allotype-specific glycosylation profile.

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