Supplemental data

Investigating the interaction between the neonatal Fc receptor and monoclonal antibody variants by hydrogen/deuterium exchange mass spectrometry

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Figure S1. (A-B) Intact mass analysis of reduced Ab\textsubscript{wt} (A) and Ab\textsubscript{degly} (B). For Ab\textsubscript{wt} the HCs are detected with three different glycoforms and in Ab\textsubscript{degly} the HCs are detected without glycosylation. (C-E) Mass analysis of Ab\textsubscript{wt} glycopeptides. (C) Extracted ion chromatogram of GlcNAc (m/z 204.09) and GlcNAc + Hex (m/z 366.14). Deconvolution of peak at retention time 12.8 min of glycopeptide with 1 missed cleavage (D) and at retention time 13.7 min of glycopeptide without missed cleavage (E). The monoisotopic masses of the intact glycopeptide (N-glycan + peptide) are shown above the peaks. Hex = Hexose, GlcNAc = N-Acetylhexosamine, dHex = Fucose. (F) Relative abundance of glycans on Ab\textsubscript{wt} as detected by peptide mapping of glycopeptides (C-E).
Figure S2. Intact mass analysis of reduced FcRn (A) and deglycosylated FcRn (B). Characterization of FcRn glycosylation by peptide mapping (C-H). (C) Extracted ion chromatogram of GlcNAc (m/z 204.09) and GlcNAc + Hex (m/z 366.14). Deconvolution of peak at retention time 71.6 min (D), 72.6 min (E), 73.3 min (F) and 73.9 min (G). The monoisotopic masses of the intact glycopeptide (N-glycan + peptide) are shown above the peaks. Hex = Hexose, GlcNAc = N-Acetylhexosamine, dHex = Fucose, Neu5Ac =...
N-acetylneuraminic acid, S = Sulfate. (H) Relative abundance of glycans on FcRn as detected by peptide mapping of glycopeptides.

Figure S3. HDX-MS peptide map Ab$_{wt}$ and Ab$_{degly}$. Peptic peptides from which productive HDX-MS data could be obtained are illustrated by white bars for the HC (A) and LC (B). Regions showing differential deuterium incorporation in the two IgG variants are marked by red boxes.
Figure S4. Comparison of the HDX of Abwt and Abdegly. HDX plots of heavy chain peptides 243-260, 249-260 and the glycopeptide 286-308. Grey curves display the HDX of Abwt and red curves display the HDX of Abdegly. For peptide 286-308 the three main glycoforms are displayed by dashed black (G0F), grey (G1F) and orange (G2F). The deuterium incorporation was monitored in triplicates at 1 min, 10 min, 1 h, 2.5 h and 5 h. The full deuterium level (at 90% D2O) is shown in black at the 5 h time point. HDX-MS results are mapped onto a homology model of the 3D structure of Abwt. Ab regions that display altered HDX upon deglycosylation are indicated by red. N-linked glycosylations are shown in light blue.
Figure S5. HDX plots of Abwt (grey), Abdegly (red). The full deuterium level measured in control experiments (at 90% D₂O) is shown in black at the 5 h time point.
Figure S6. HDX plots of Ab<sub>wt</sub> in the absence (grey) and presence (red) of FcRn. The full deuterium level measured in control experiments (at 90% D<sub>2</sub>O) is shown in black at the 5 h time point.
Figure S7. Comparison of the interaction of FcRn with Abwt and Ustekinumab (A) Chromatograms of Abwt (red) and Ustekinumab (blue) following FcRn affinity chromatography. (B) Bar chart of the
deuterium uptake in percent (relative to the 90% control) of $\text{Ab}_{\text{wt}}$ (grey), $\text{Ab}_{\text{wt}}$ with FcRn (red), Ustekinumab (black) and Ustekinumab with FcRn (blue) after 1h labeling. Error bars show the standard deviation of three replicate experiments. The deuterium uptake is shown for peptides in the Fab heavy chain and light chain along with the antibody sequences in these regions.

Figure S8. Bar chart of the deuterium uptake of $\text{Ab}_{\text{wt}}$ in the absence (grey) or presence of either FcRn (red) or deglycosylated FcRn (orange) at the 1 h time point. The standard deviations of three replicate experiments are show by error bars.
Figure S9. HDX analysis of peptide 441-454 of Ab<sub>wt</sub> by ETD. The bar chart shows the deuterium uptake of the precursor ion and the c4-ion when unbound (grey) or bound to FcRn (red). Error bars show standard deviation of three replicate measurements.
Figure S10. HDX plots of Ab$\text{degly}$ in the absence (grey) and the presence of FcRn (red). At 300 min the 90% D$_2$O control is plotted for Ab$\text{degly}$ (black).
Figure S11. Bar charts of the deuterium uptake in percent (relative to the 90% control) of peptide 249-260 (A) and peptide 286-308 (B) of Ab<sub>wt</sub> (grey), Ab<sub>wt</sub> with FcRn (red), Ab<sub>degly</sub> (black) and Ab<sub>degly</sub> with FcRn (blue) at four time points: 1 min, 1 h, 2.5 h and 5 h. Error bars show standard deviation of three replicate measurements.
Figure S12. Comparison of FcRn binding of Ab\textsubscript{wt} and Ab\textsubscript{degly} by surface plasmon resonance (A) and (B) and analytical FcRn affinity chromatography (C). (A) SPR of Ab\textsubscript{wt} binding to FcRn (10µg/ml) at 500nM (pink), 250nM (light blue), 125nM (yellow), 62.5nM (dark blue), 31nM (green) and 15nM (orange). Black curves show the 1:1 Langmuir fit. (B) SPR showing binding of Ab\textsubscript{wt} (red) and Ab\textsubscript{degly} (blue) (250 nM) to FcRn (10µg/ml). The half-life (T\textsubscript{1/2}) was calculated for antibody dissociation. (C) Chromatograms of Ab\textsubscript{wt} (red) and Ab\textsubscript{degly} (blue) by analytical FcRn chromatography.
Figure S13. HDX-MS peptide map of FcRn. Peptic peptides from which productive HDX-MS data could be obtained are shown as white bars for the α-chain (A) and β-2-microglobulin (B). Regions showing reduced deuterium uptake upon Ab_{wt} and Ab_{degly} binding are indicated by red boxes.
Figure S14. HDX plots of FcRn in the absence (grey) and presence of Ab<sub>wt</sub> (red) or Ab<sub>degly</sub> (blue). The full deuterium level measured in control experiments (at 90% D<sub>2</sub>O) is shown in black at the 5 h time point.