Supplemental Information

Identification of a direct interaction between the Fab domains of IgG antibodies and human FcRn upon IgG-FcRn complex formation

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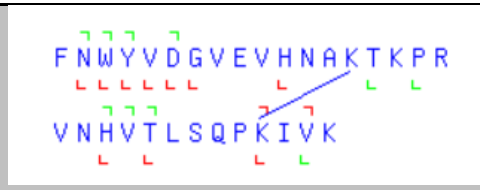
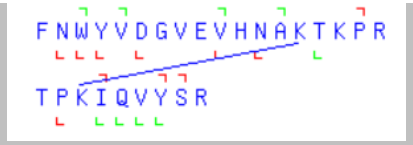
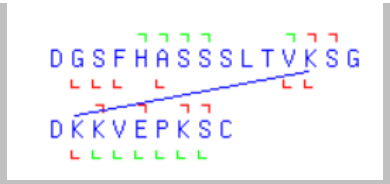
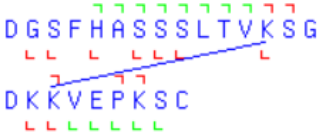
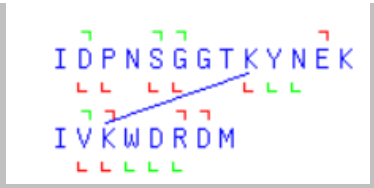
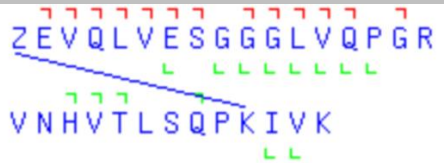
Supplemental Methods

Calculation and visualization of surface charge: Surface charge analysis was done based on the crystal structures of the Fab domains of H-mAb and C-mAb (PDB ID: 4NYL and 1NGP). The surface charge maps were generated by the pdb2pqr (3.6.1) and APBS (3.4.1) servers (https://server.poissonboltzmann.org/pdb2pqr) with standard settings. The protonation states of side chains were calculated by the PROPKA algorithm and the PARSE forcefield was used. The resulting surface potential maps where visualised at $\pm 3k_BT/e$ in PyMOL (2.5.2). The sequence based pH-dependent net charge of the Fab domains of H-mAb and C-mAb were calculated using the Emboss iep calculator (http://www.bioinformatics.nl/cgi-bin/emboss/iep?_pref_hide_optional=0).[1] All cysteines were assumed to form disulfide bridges and the Fab domiains were set to contain 2 N-terminals and 1 C-termini.

In-gel digestion of C-mAb: The SDS-PAGE band corresponding to monomeric C-mAb was sliced of the gel, cut into smaller pieces, placed in a Eppendorf tube, solvated with 200µL 25 mM 25mM NH_4HCO_3 in 50:50 MQ water:acetonitrile, and incubated at 37 ºC under shaking for 30 min. The supernatant was discarded and the process was repeated twice to de-stain the gel pieces. The gel pieces were dehydrated with 100µL acetonitrile, washed with 100µL MQ water, dehydrated with 100µL acetonitrile before addition of 100µL 6.5 mM dithiothreitol in MQ water and incubated at 60 ºC under shaking for 60 min to ensure efficient reduction of C-mAb. The solvent was discarded and the gel pieces were dehydrated in 100µL acetonitrile before addition of 100µL 54 mM iodoacetamide in MQ water and incubated at 25 ºC in the dark under shaking for 30 min to alkylate C-mAb. The solvent was discarded and the gel pieces was dehydrated with 100µL acetonitrile and hydrated with 50mM NH_4HCO_3 in MQ water. This process was repeated 3 times, and the gel pieces were dehydrated a final time in 100µL acetonitrile. The supernatant was discarded and the dehydrated gel pieces were hydrated in 100µL of 10ng/µL trypsin solution in 50mM NH_4HCO_3. The gel pieces were incubated overnight at 37 ºC under shaking. The next morning the supernatant was collected. The gel pieces were covered in 40µL 0.1 % formic acid in MQ water and incubated for 15 min at room temperature under shaking. The supernatant was collected. The gel pieces were covered in 40µL of a 50:50 mixture of 0.1 % formic acid in MQ water and acetonitrile and incubated for 15 min at room temperature under shaking. The supernatant was collected. The gel pieces were covered in 40µL acetonitrile and incubated for 15 min at room temperature under shaking. All collected supernatants were pooled and evaporated to dryness in a vacuum centrifuge. The samples were re-suspended in 20µL SEC running buffer, purified by SEC and analyzed by LC-MS as described for XL-MS samples in the methods section.

Supporting Table S1. Table of identified cross-links including overview of identified fragment ions. A "Z"-residue denotes the end amino group on the N-terminal of a protein chain (cross-link number 8).

	Residue 1	Residue 2	Peptide 1	Peptide 2	Id-Score	Euclidean Cα-Cα distance (Å)	Identified fragments ions Red: fragment ion containing cross-linker, Green: Fragment ions not containing cross-linker
1	β2m - K91	Fc HC - K292	VNHVTLSQP**K**IVK	FNWYVDGVEVHNAKTKPR	34.11	16.8	
2	β2m - K91	Fc HC - K294	VNHVTLSQPKIVK	FNWYVDGVEVHNAKTKPR	36.36	20.5	
3	β2m - K6	Fc HC - K292	TPKIQVYSR	FNWYVDGVEVHNAKTKPR	33.26	24.0	
4	α3 - K243	Fab HC - K217	DGSFHASSSLTVKSG	DKKVEPKSC	36.16		

5	α3 - K243	Fab HC - K218	DGSFHASSSLTVKSG	DKKVEPKSC	40.56	
6	β2m - K91	Fab HC - K59	VNHVTLSQPKIVK	IDPNSGGTKYNEK	33.41	
7	β2m - K94	Fab HC – K59	IVKWDRDM	IDPNSGGTKYNEK	37.86	
8	β2m - K91	Fab HC – E1 (N-term)	VNHVTLSQPKIVK	EVQLVESGGGLVQPGR	32.60	

Supporting Table S2. Identification of overlength cross-links between Fab domains and the Fc part of C-mAb. C-mAb was cross-linked with DSS as described in the methods section. After cross-linking the cross-linking products where seperatated on an SDS-PAGE as described in the methods section. The band corresponding to monomeric C-mAb was sliced out of the gel and in-gel digestion with LysC and trypsin was performed, see supporting methods. The released peptides were analysed by LC-MS as described in the methods section. 5 overlenght cross-links where identified highlighting the flexibility of the Fab domains in C-mAb. The Euclidan distances where determined in Pymol on the homology model of C-mAb. The highest Id-score observed across all data sets is shown.

Residue 1	Residue 2	Id-Score	Replicates	Euclidean Cα-Cα distance (Å)
non overlength XL				
HC - 137	LC - 207	25.19	1/2	11.1
HC - 137	HC - 218	36.4	2/2	19.9
HC - 137	HC - 222	28.98	2/2	10.6
HC - 218	LC - 207	32.8	1/2	24.5
HC - 364	HC - 418	39.9	2/2	7.8
Overlength XL				
HC - 59	HC - 344	31.75	1/2	88.3
HC - 59	HC - 418	37.59	2/2	79.5
HC - 137	HC - 330	32.54	2/2	39.8
LC - 207	HC - 344	37.94	1/2	60.0
HC - 63	HC - 418	32.22	1/2	76.0

Supporting Table S3. Cross-links only observed in a single experimental condition or only observed in the FcRn:mutC-mAb complex. The abbreviation in the "Residue 1 and 2" columns refers to the domains of FcRn (α1, α2, α3 or β2m) and IgGs (LC or HC). The "Complex" column refers to in which complex the cross-link have been identified. The "Chemistry" column refers to the cross-linking chemistry applied. The "Enzymes" column refers to the used proteases. The "Id-Score / FDR" refers to the Id-score in xQuest, while the FDR was calculted in xProphet. The "n-seen" column refers to number of spectrums, where the cross-link was identified.

	Residue 1	Residue 2	Complex	Chemistry	Enzymes	Id-Score / FDR	n-seen
1	β2m - 6	HC - 292/294	FcRn:H-mAb	DSS	LysC + Tryp	33.26	1
2	α2 - 146	HC - 76	FcRn:H-mAb	DSS	LysC + Tryp	40.74	3
3	β2m - 91	HC - 418	FcRn:H-mAb, FcRn:C-mAb, FcRn:mutC-mAb	DSS	LysC + Tryp	36.08	1
4	β2m - 91	LC - 145	FcRn:H-mAb	DSS	LysC + Tryp	26.16	1
5	α2 - 146	HC - 418	FcRn:H-mAb	DSS	LysC + Tryp	26.83	1
6	α2 - 146	LC - 1	FcRn:H-mAb	DSS	LysC + Tryp	37.38	1
7	β2m - 91	LC - 107	FcRn:H-mAb	DSS	LysC + Tryp	37.4	1
8	β2m - 91	LC - 188	FcRn:H-mAb	DSS	LysC + Tryp	39.09	1
9	α2 - 177	LC - 1	FcRn:H-mAb	DSS	LysC + Tryp	30.76	1
10	α1 - 59	HC - 74	FcRn:C-mAb	DSS	LysC + Tryp	25.11	2
11	β2m - 91	HC - 278	FcRn:H-mAb	DSS	AspN	36.12	3
12	β2m - 41	HC - 343	FcRn:H-mAb	DSS	AspN	25.63	1
13	β2m - 94	HC - 278	FcRn:H-mAb	DSS	AspN	27.71	1
14	β2m - 94	HC - 46	FcRn:H-mAb	PDH-ZL	LysC + Tryp	23.24 / 0	2
15	α2 - 146	LC - 207	FcRn:mutC-mAb	DSS	LysC + Tryp	29.28	1
16	β2m - 48	LC - 72	FcRn:mutC-mAb	DSS	AspN	28.16	1
17	β2m - 91	HC - 217/218	FcRn:mutC-mAb	DSS	AspN	27.01	1
18	α1 - 67	LC - 206	FcRn:mutC-mAb	XP	LysC + Tryp	37.77	1
19	α2 - 168	LC - 163	FcRn:mutC-mAb	XP	LysC + Tryp	36.52	1
20	α2 - 178	LC71	FcRn:mutC-mAb	XP	LysC + Tryp	27.43	1
21	β2m - 77	HC - 46	FcRn:mutC-mAb	XP	LysC + Tryp	37.93	1
22	α1 - 54/62	HC - 237	FcRn:mutC-mAb	XP	AspN	32.05	2
23	β2m - 38	HC - 262/269	FcRn:mutC-mAb	PDH	LysC + Tryp	29.43	2
24	α1 - 71	LC -71	FcRn:mutC-mAb	XP-ZL	LysC + Tryp	40.88 / 0	2
25	β2m - 50/53	HC - 217/218	FcRn:mutC-mAb	XP-ZL	LysC + Tryp	37.36 / 0	1
26	β2m - 50	HC - 330	FcRn:mutC-mAb	XP-ZL	LysC + Tryp	37.29 / 0	1
27	β2m - 53	HC - 330	FcRn:mutC-mAb	XP-ZL	LysC + Tryp	33.76 / 0	1
28	β2m - 48	LC - 206	FcRn:mutC-mAb	XP-ZL	LysC + Tryp	33.56 / 0	1
29	β2m - 94	LC - 201/206	FcRn:mutC-mAb	XP-ZL	LysC + Tryp	31.95 / 0	2
30	α1 - 71	LC - 80	FcRn:mutC-mAb	XP-ZL	LysC + Tryp	31.57 / 0	1
31	α2 - 178	HC - 418	FcRn:mutC-mAb	XP-ZL	LysC + Tryp	30.09 / 0.05	2
32	α2 - 145	HC - 67	FcRn:mutC-mAb	XP-ZL	LysC + Tryp	26.89 / 0	1
33	α2 - 165	HC - 330	FcRn:mutC-mAb	XP-ZL	LysC + Tryp	31.24 / 0	3
34	β2m - 50	HC 324/326	FcRn:mutC-mAb	PDH-ZL	LysC + Tryp	24.37 / 0	4

Supporting Table S4. Identified inter protein cross-links bewteen IdeZ-treated mAbs and FcRn. The identified cross-links below all belong to the IdeZ treated mutC-mAb in complex with FcRn, confirming the experimental conditions. As no interprotein cross-links have been identified between the Fc-part of an mAb and FcRn in a AspN digest in the current study this is expected. The Euclidan distances where determined in Pymol on the homology model of C-mAb. The highest ld-score observed across all data sets is shown

	Residue 1	Residue 2	ld-Score	Euclidean Cα-Cα distance (Å)
1	β2m - K91	Fc HC - K292	34.37	16.8
2	β2m - K91	Fc HC - K294	36.59	20.5
3	β2m - K6	Fc HC - K294	35.79	24.0

Supporting Table S5. Overview of recorded HDX data. The numbers in the table refers to the number of replicate(s) of recorded HDX data for every single time point at every condition. N/A: Not applicable.

Experiment	Protein	Ligand	Molar Excess of Ligand	13 s	25 s	38 s	2.09 min	6min 17s	25min 7s	3t 25min 45s	4t 11min 11s	1d 1t 7min 8s	Maximally labelled samples
I	FcRn	Not present	N/A				3	3	3		3	3	3
	FcRn	H-mAb	7 times				3	1	1		1	1	
	FcRn	C-mAb	7 times				3	3	3		3	3	
II	FcRn	Not present	N/A	3	3	3	3						3
	FcRn	H-mAb	7 times	3	3	3	3						
	FcRn	C-mAb	7 times	3	3	3	3						
III	H-mAb	Not present	N/A				3	1	1	1			1
	H-mAb	FcRn	7 times				3	1	1	1			
IV	C-mAb	Not present	N/A				3	3	3		3	3	
	C-mAb	FcRn	7 times				3	3	3		3	3	3

A)

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Identities:391/451(87%), Positives:415/451(92%), Gaps:1/451(0%)

H-mAb  1    EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSAITWNSGHIDY  60
            +VQL + G  LV+PG S++LSC ASG+TF  Y MHWV+Q PG+GLEW+  I  NSG   Y
C-mAb  1    QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGRGLEWIGRIDPNSGGTKY  60

H-mAb  61   ADSVEGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVSYLSTASSLDYWGQGTLVTVS  120
             +  + + T++ D   ++ Y+Q++SL +ED+AVYYCA+  Y   +S  DYWGQGT +TVS
C-mAb  61   NEKFKSKATLTVDKPSSTAYMQLSSLTSEDSAVYYCARYDYYG-SSYFDYWGQGTTLTVS  120

H-mAb  121  SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS  180
            SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
C-mAb  121  SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS  180

H-mAb  181  SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG  240
            SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
C-mAb  181  SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG  240

H-mAb  241  GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY  300
            GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
C-mAb  241  GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY  300

H-mAb  301  NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD  360
            NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
C-mAb  301  NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD  360

H-mAb  361  ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR  420
            ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
C-mAb  361  ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR  420

H-mAb  421  WQQGNVFSCSVMHEALHNHYTQKSLSLSPG  450
            WQQGNVFSCSVMHEALHNHYTQKSLSLSPG
C-mAb  421  WQQGNVFSCSVMHEALHNHYTQKSLSLSPG  450
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B)

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Identities:206/209(99%), Positives:207/209(99%), Gaps:0/209(0%)

H-mAb  240  GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ  299
            GGPSVFLFPPKPKDTL I+R PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
Fc-YTE 236  GGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ  295

H-mAb  300  YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR  359
            YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
Fc-YTE 296  YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR  355

H-mAb  360  DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS  419
            DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
Fc-YTE 356  DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS  415

H-mAb  420  RWQQGNVFSCSVMHEALHNHYTQKSLSLS  447
            RWQQGNVFSCSVMHEALHNHYTQKSLSLS
Fc-YTE 416  RWQQGNVFSCSVMHEALHNHYTQKSLSLS  444
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Supporting Figure S1. Alignment of HCs of H-mAb with C-mAb (A) and HC of H-mAb with Fc-YTE (B). Residues marked in bold are directly implicated in the binding of Fc-YTE to FcRn according to the crystal structure solved by Oganesyan et al..[2] The alignment was performed by the BLAST algorithm.[3,4]

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Identities:87/215(40%), Positives:123/215(57%), Gaps:11/215(5%)

H-mAb  1    DIQMTQSPSSLSASVGDRVTITCRASQG---IRNYLAWYQQKPGKAPKLLIYAASTLQSG  57
                   S+L+ S G+ VT+TCR+S G     NY  W Q+KP      LI   +    G
C-mAb  1    -QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNNRAPG  59

H-mAb  58   VPSRFSGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQGTKVEI-KRTVAAPSVF  116
            VP+RFSGS  G    LTI+  Q ED A Y+C  +    + FG GTK+ +  +  ++PSV
C-mAb  60   VPARFSGSLIGDKAALTITGTQTEDEAMYFCALWYSNHWVFGGGTKLTVLGQPKSSPSVT  119

H-mAb  117  IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST--YS  174
            +FPPS E+L++  A++VC + +FYP    V WKVD    +  +Q   T Q SK S   Y
C-mAb  120  LFPPSSEELETNKATLVCTITDFYPGVVTVDWKVDG---TPVTQGMETTQPSKQSNNKYM  176

H-mAb  175  LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC  214
             SS LTL+   +E+H  Y+C+VTH+G +  V KS +R +C
C-mAb  177  ASSYLTLTARAWERHSSYSCQVTHEGHT--VEKSLSRADC  215
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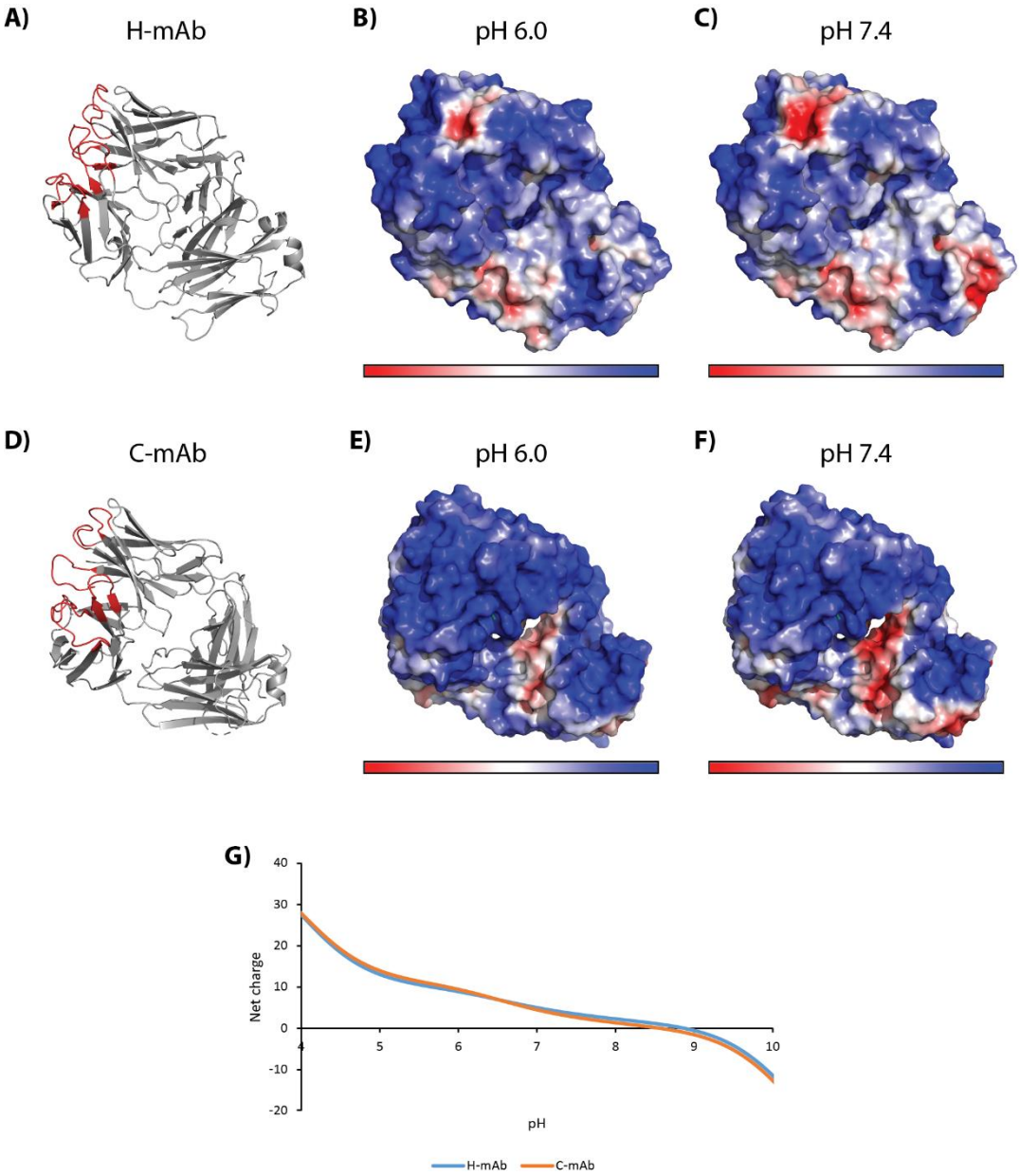
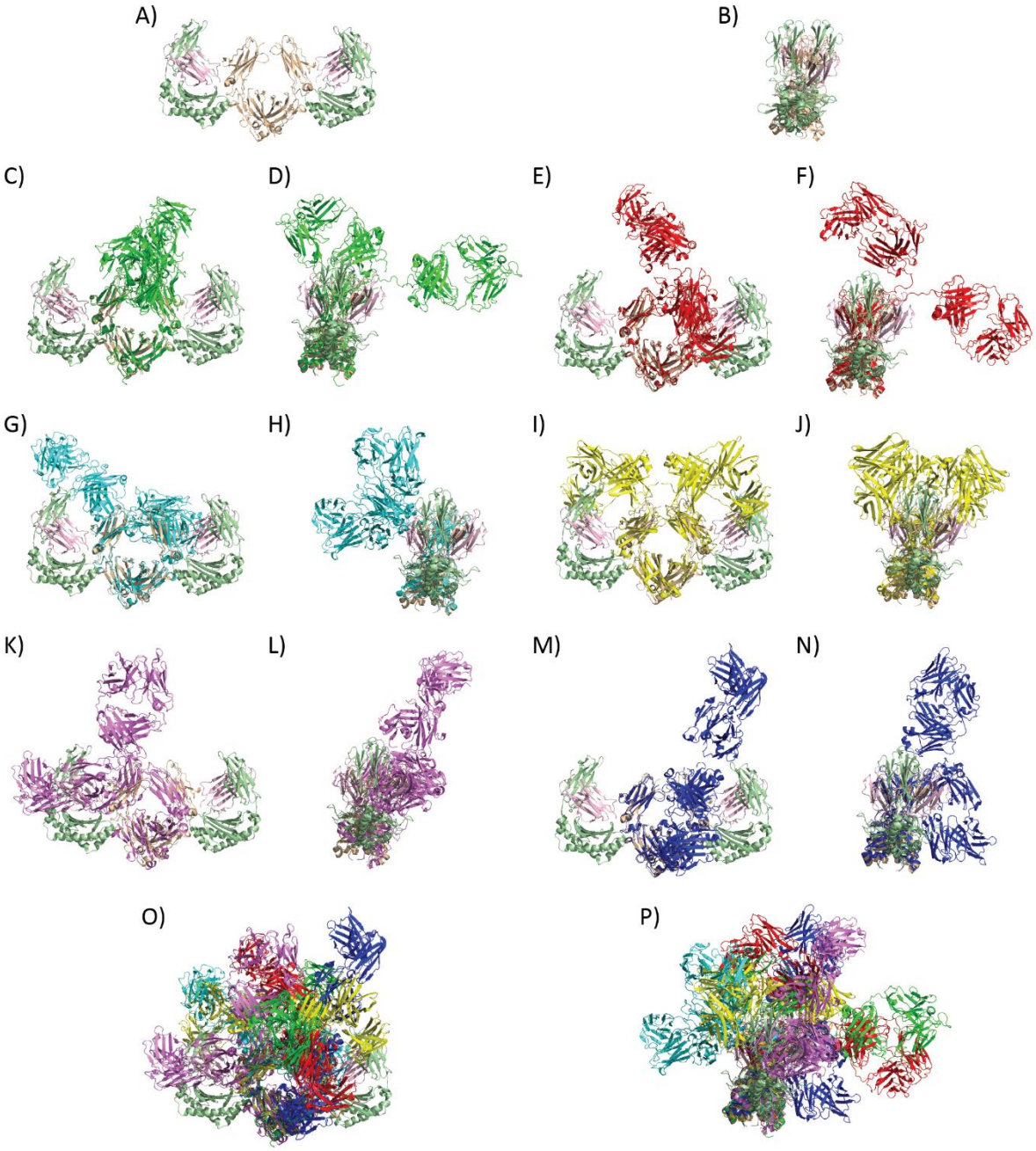
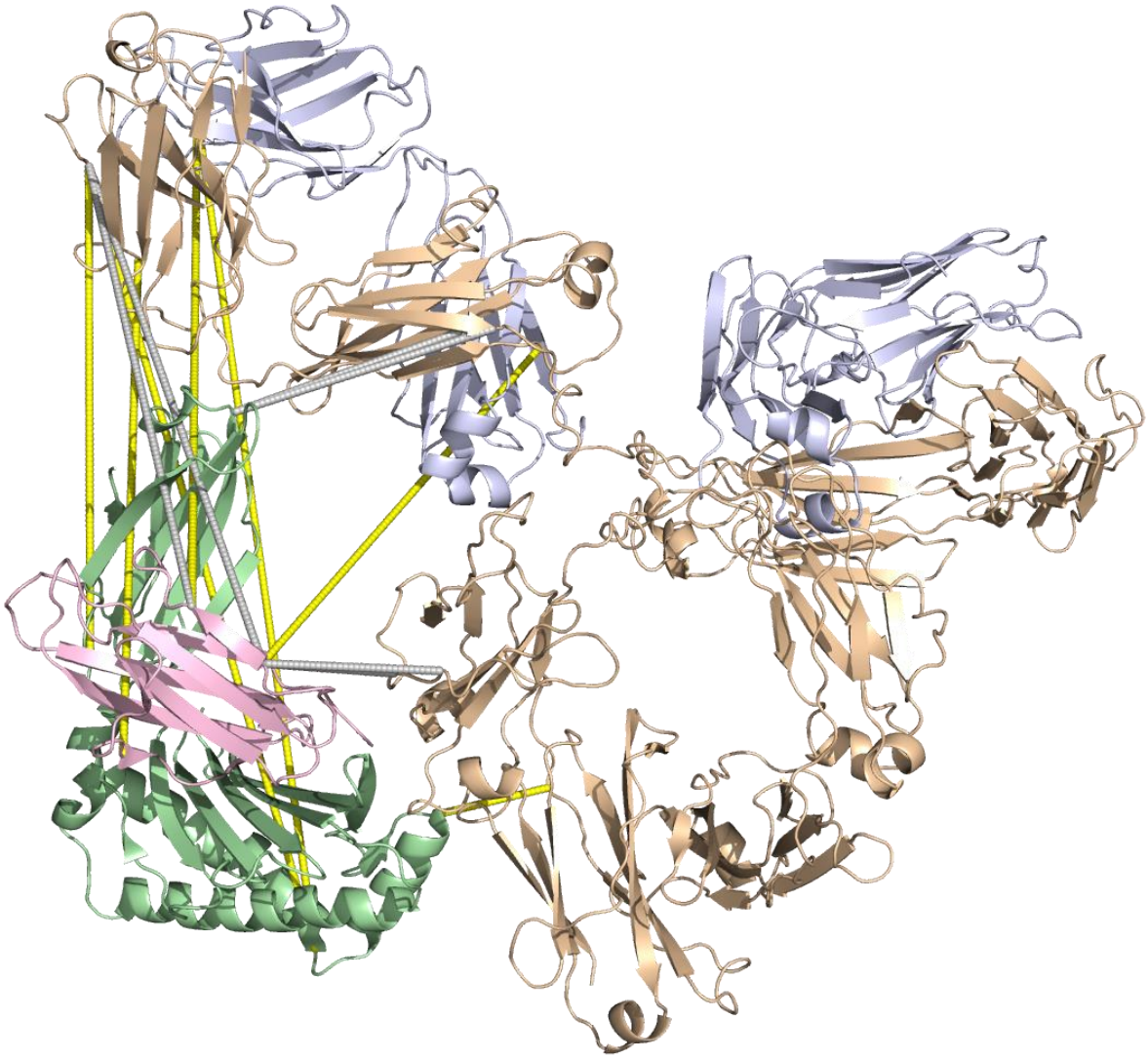
Supporting Figure S2. Alignment of LCs of H-mAb and C-mAb. The alignment was performed by the BLAST algorithm.[3,4]



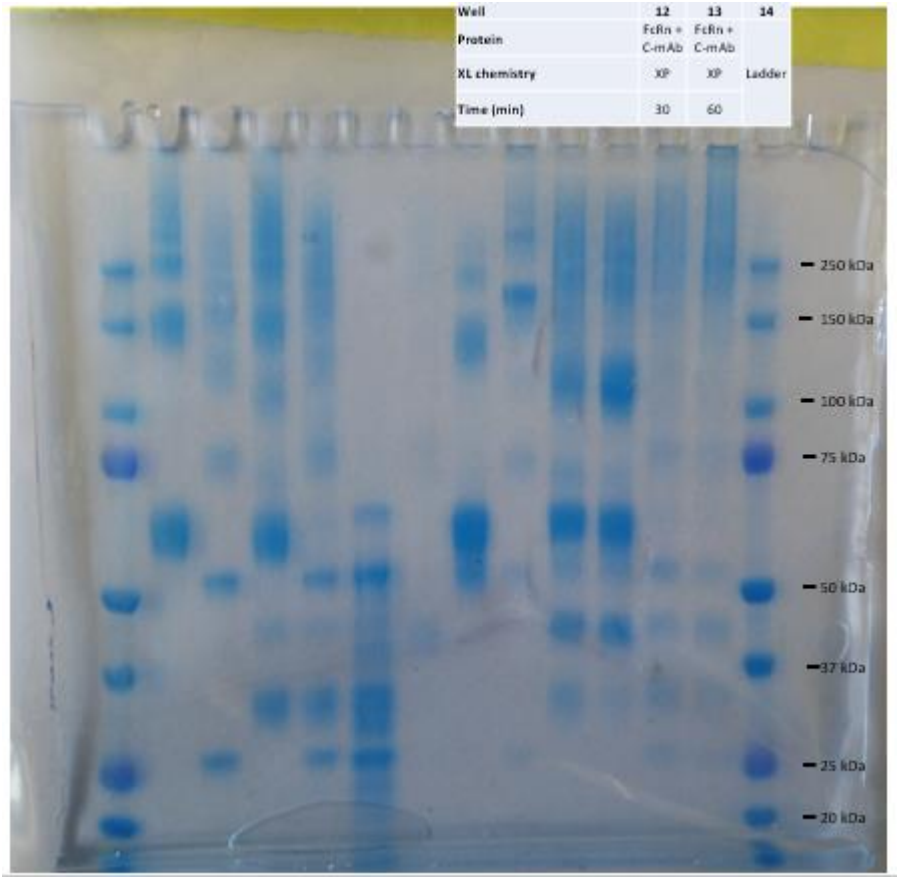
Supporting Figure S3. Charge distribution and pH-dependent net charge of the Fab domains of H-mAb and C-mAb. Crystal structure of the Fab domains of H-mAb (A) and C-mAb (D) (PDB ID:4NYL and 1NGP). CDRs are highlighted in red. B-C) Isoelectrical surface maps of H-mAb at pH 6.0 and pH 7.4 contured at $\pm 3k_BT/e$, red; negative and blue: positive. E-F) Isoelectrical surface maps of C-mAb at pH 6.0 and pH 7.4 contured at $\pm 3k_BT/e$, red; negative and blue: positive. G) pH-dependent sequence based net charge of the Fab domains of H-mAb (*blue* line) and C-mAb (*orange* line). See supplemental methods for further details.



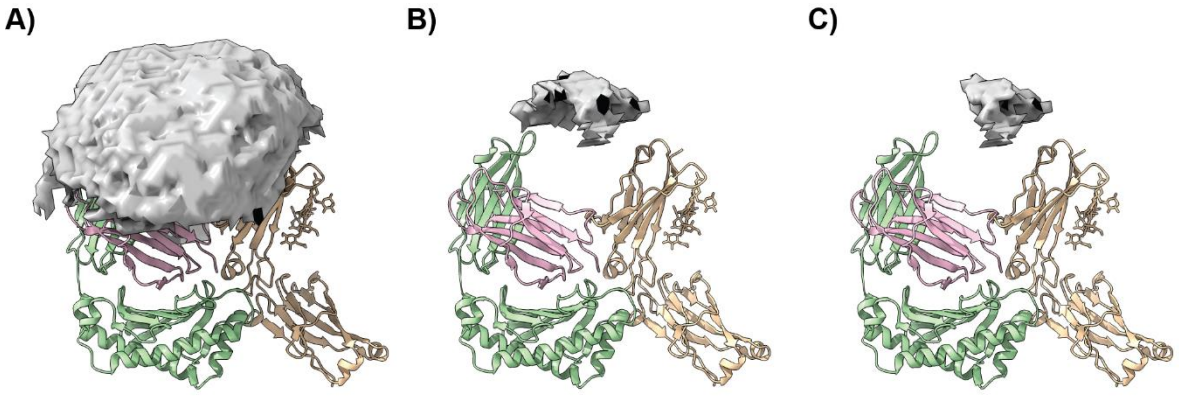
Supporting Figure S4: Conformational flexibility of full-length IgGs. Six crystal structures of full-length IgGs (PDB ID: 1IGT, 1HZH, 6GFE, 1MCO, 5FK3, and 1IGY) were aligned with the Fc part of the crystal structure of Fc-YTE in complex with FcRn (PDB ID: 4N0U, human serum albumin was omitted to simplify the figure).A-B) FcRn in complex with Fc-YTE (PDB ID: 4N0U) C-N) Individual IgGs shown with FcRn in complex with Fc-YTE. O-P) All IgG structures are shown at the same time. *Red*: 1IGT, *green*: 1HZH, *blue*: 6GFE, *yellow*: 1MCO, *violet*: 5DK3, *cyan*: 1IGY, *pale green*: FcRn, *light pink*: β-2-microglobulin, *wheat*: Fc-YTE. All figures are presented in two different views: a front view (A, C, E, G, I, K, M and O) and a view where the front view is rotated 90° counter clockwise along the horizontal axis (B, D, F, H, J, L, N and P).



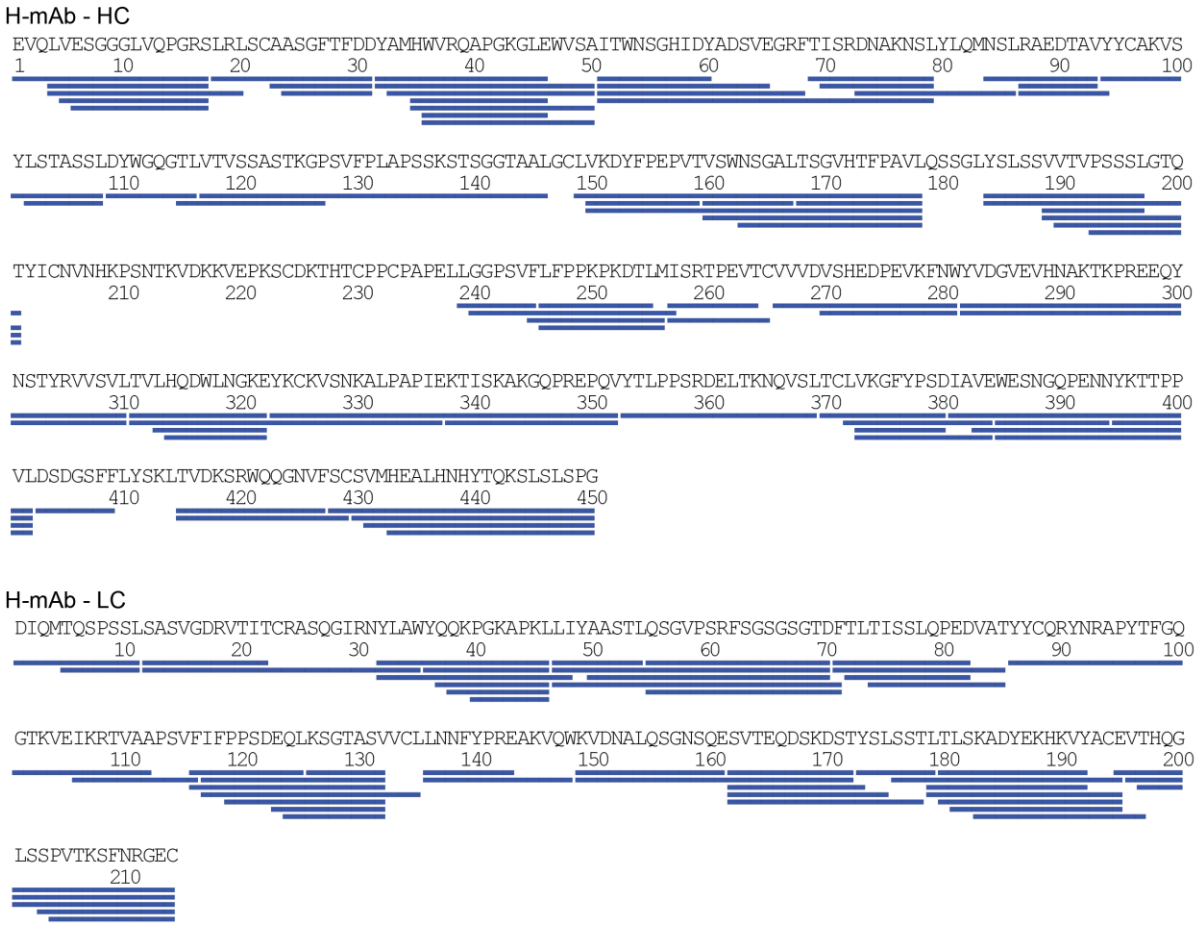
Supporting Figure S5: Visualization of cross-links identified in the complex between mutC-mAb and FcRn. Cross-links also found in the complex of C-mAb and FcRn are colored gray, while the cross-links only identified in the mutC-mAb:FcRn complex are colored yellow. The cross-links between mutC-mAb and FcRn illustrates several different binding modes not in line with the published crystal structure of FcRn:Fc-YTE complex (PDB ID: 4N0U). *Pale green*: α domains of FcRn, *light pink*: β2m, *wheat*: HC of mutC-mAb, *light blue*: LC of mutC-mAb.



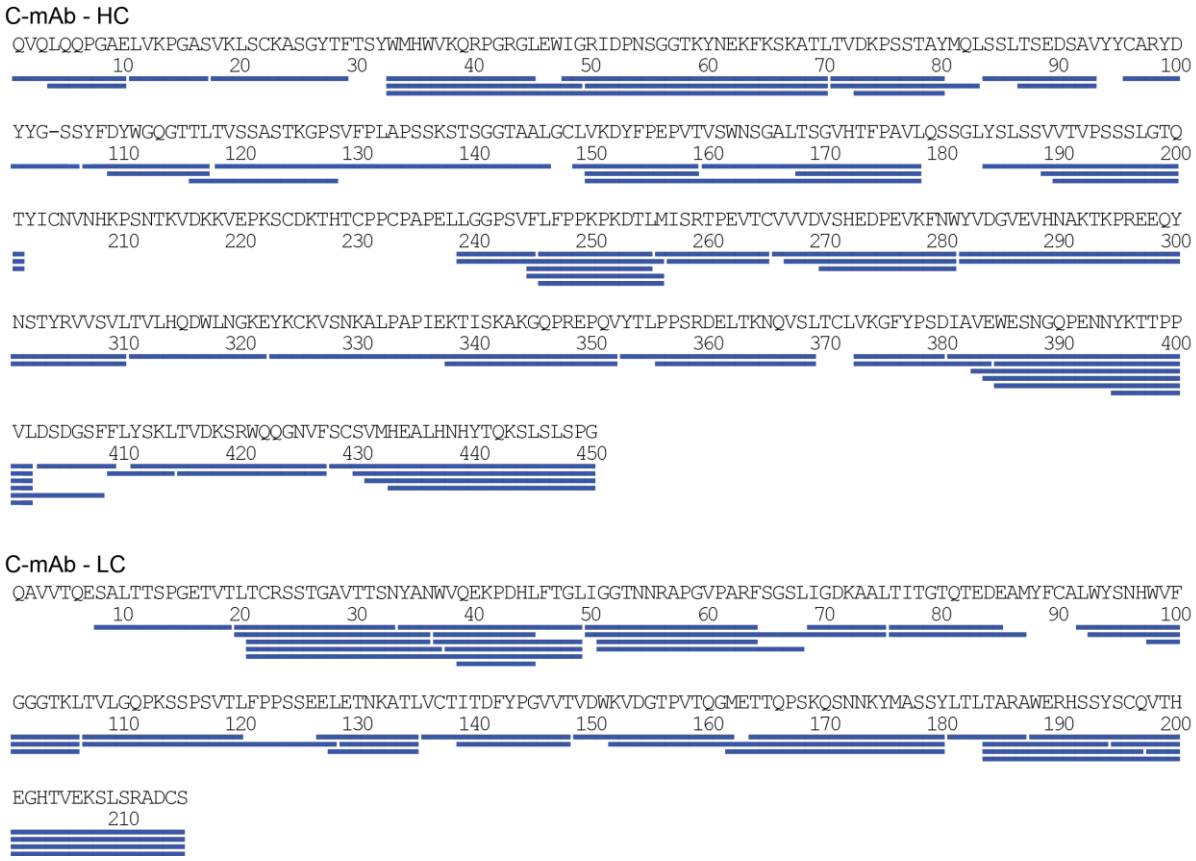
Supporting Figure S6: Observation of higher order oligomers after cross-linking a solution of FcRn and C-mAb with XPlex cross-linking (XP).



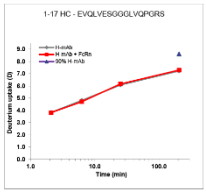
Supporting Figure S7: DisVis analysis of mAbs Fab domain interaction with FcRn. A) Interaction space of the Fab domain of H-mAb defined by the identified cross-links between H-mAb Fab domains and FcRn. B) Interaction space of the C-mAb Fab domain defined by the identified cross-links between C-mAb Fab domains and FcRn. C) Interaction space of a generic Fab domain defined by combining all cross-links between the Fab domains of both C-mAb and H-mAb and FcRn. *Pale green*: α domains of FcRn, *light pink*: β2m, *wheat*: Fc-YTE.

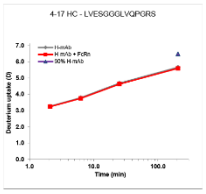


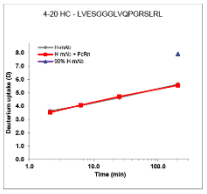
Supporting Figure S8: Effective HDX-MS coverage map of H-mAb. HDX data were obtained for 77 peptides covering 89.1% of the HC and for 51 peptides covering 100% of the LC.

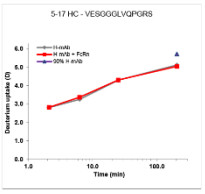


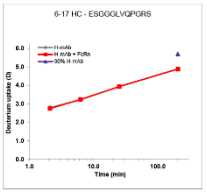
Supporting Figure S9: Effective HDX-MS coverage map of C-mAb. HDX data were obtained for 61 peptides covering 88.4% of the HC and for 39 peptides covering 94.9% of the LC.

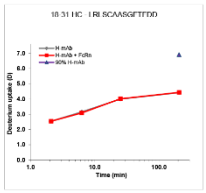

1-17 HC - EVQLVESGGGLVQPGRS

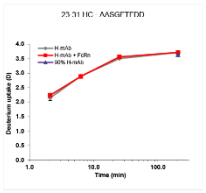

4-17 HC - LVESGGGLVQPGRS

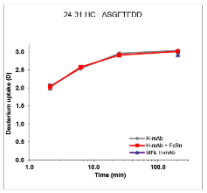

4-20 HC - LVESGGGLVQPGRSLRL

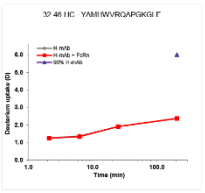

5-17 HC - VESGGGLVQPGRS

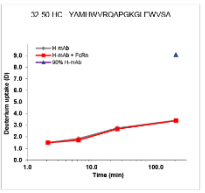

6-17 HC - ESGGGLVQPGRS

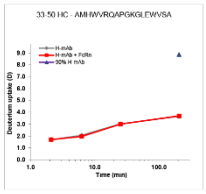

18-31 HC - LRLSCAASGFTFDD

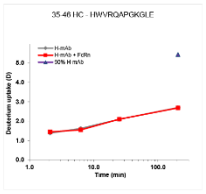

23-31 HC - AASGFTFDD

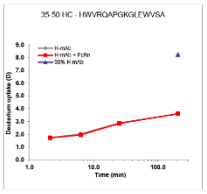

24-31 HC - ASGFTFDD

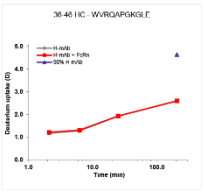

32-46 HC - YAMHWVRQAPGKGLE

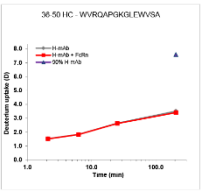

32-50 HC - YAMHWVRQAPGKGLEWVSA

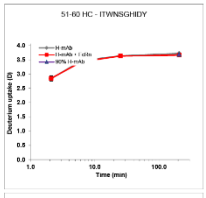

33-50 HC - AMHWVRQAPGKGLEWVSA

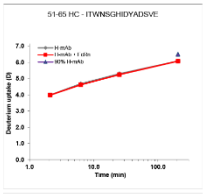

35-46 HC - HWVRQAPGKGLE

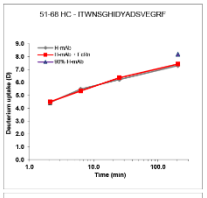

35-50 HC - HWVRQAPGKGLEWVSA

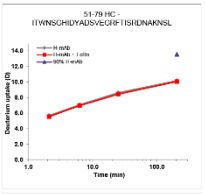

36-46 HC - WVRQAPGKGLE

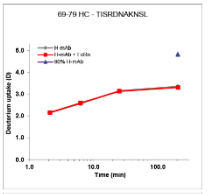

36-50 HC - WVRQAPGKGLEWVSA

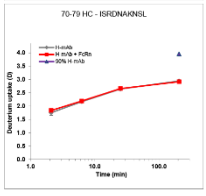

51-60 HC - ITWINSGHIDY

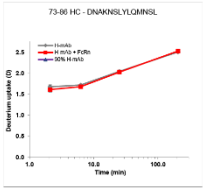

51-65 HC - ITWINSGHIDYADSVE

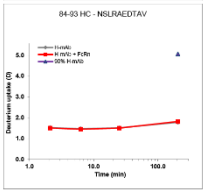

51-68 HC - ITWINSGHIDYADSVEGRF

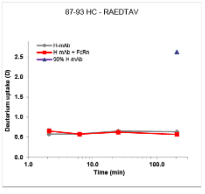

51-79 HC -
ITWINSGHIDYADSVEGRFTISRDNAKNSL

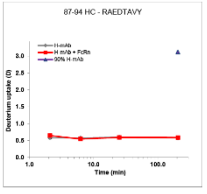

69-79 HC - TISRDNAKNSL

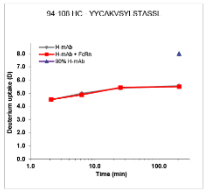

70-79 HC - ISRDNAKNSL

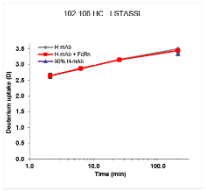

73-86 HC - DNAKNSLYLQMNSL

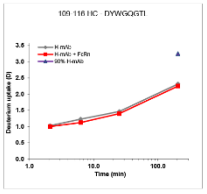

84-93 HC - NSLRAEDTAV

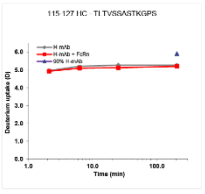

87-93 HC - RAEDTAV

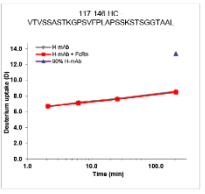

87-94 HC - RAEDTAVY

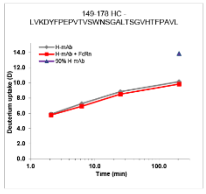

94-106 HC - YYCAKVSYLSTASSI

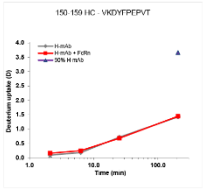

102-106 HC - LSTASSI

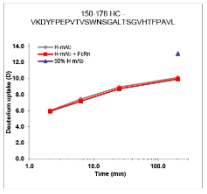

109-116 HC - DYWGQGTI

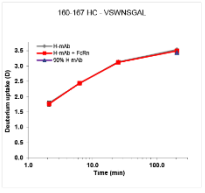

115-127 HC - TLTVSSASTKGPS

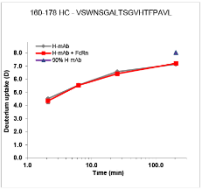

117-146 HC -
VTVSSASTKGPSVFPLAPSSKSTSGGTAAL

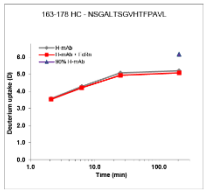

149-178 HC -
LVKDYFPEPVTVSWNSGALTSGVHTFPAVL

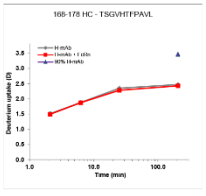

150-159 HC - VKDYFPEPVT

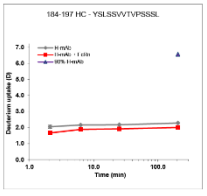

150-178 HC -
VKDYFPEPVTVSWNSGALTSGVHTFPAVL

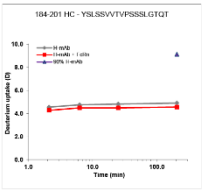

160-167 HC - VSWNSGAL

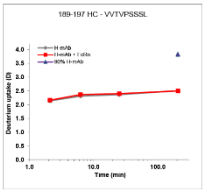

160-178 HC - VSWNSGALTSGVHTFPAVL

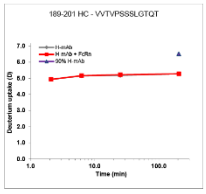

163-178 HC - NSGALTSGVHTFPAVL

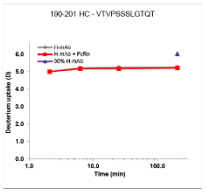

168-178 HC - TSGVHTFPAVL

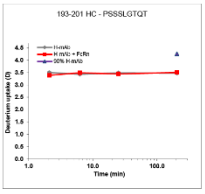

184-197 HC - YSLSSVVTVPSSSL

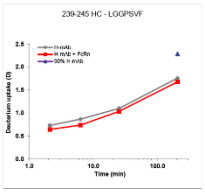

184-201 HC - YSLSSVVTVPSSSLGTQT

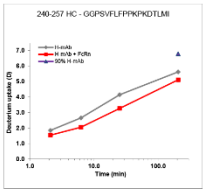

189-197 HC - VVTVPSSSL

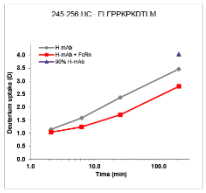

189-201 HC - VVTVPSSSLGTQT
Deuterium uptake (D)
Time (min)

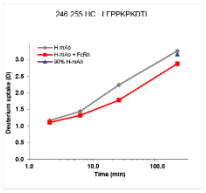

190-201 HC - VTVPSSSLGTQT
Deuterium uptake (D)
Time (min)

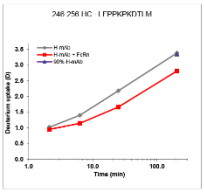

193-201 HC - PSSSLGTQT
Deuterium uptake (D)
Time (min)

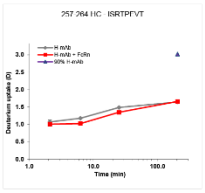

239-245 HC - LGGPSVF
Deuterium uptake (D)
Time (min)

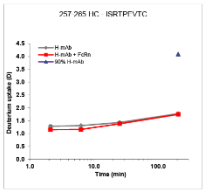

240-257 HC - GGPSVFLFPPKPKDTLMI
Deuterium uptake (D)
Time (min)

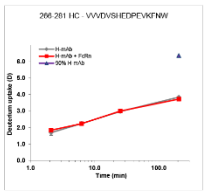

245-256 HC - FLFPPKPKDTLM
Deuterium uptake (D)
Time (min)

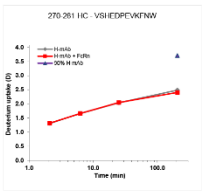

246-255 HC - LFPPKPKDTL
Deuterium uptake (D)
Time (min)

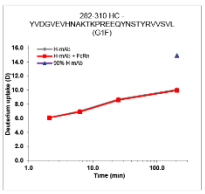

246-256 HC - LFPPKPKDTLM
Deuterium uptake (D)
Time (min)

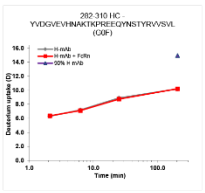

257-264 HC - ISRTPEVT
Deuterium uptake (D)
Time (min)

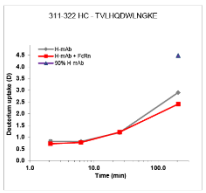

257-265 HC - ISRTPEVTC
Deuterium uptake (D)
Time (min)

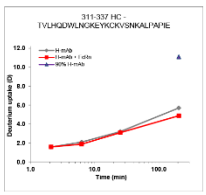

266-281 HC - VVVDVSHEDPEVKFNW
Deuterium uptake (D)
Time (min)

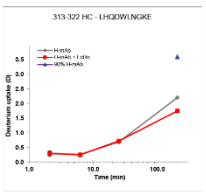

270-281 HC - VSHEDPEVKFNW
Deuterium uptake (D)
Time (min)

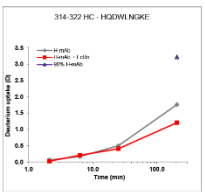

282-310 HC -
YVDGVEVHNAKTKPREEQYNSTYRVVSVL
(G1F)
Deuterium uptake (D)
Time (min)

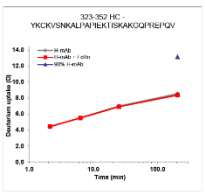

282-310 HC -
YVDGVEVHNAKTKPREEQYNSTYRVVSVL
(G0F)
Deuterium uptake (D)
Time (min)

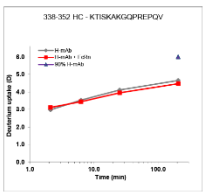

311-322 HC - TVLHQDWLNGKE
Deuterium uptake (D)
Time (min)

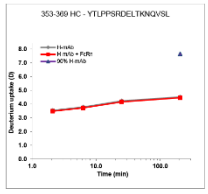

311-337 HC -
TVLHQDWLNGKEYKCKVSNKALPAPIE
Deuterium uptake (D)
Time (min)

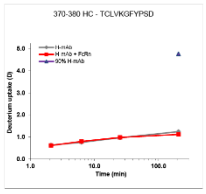

313-322 HC - LHQDWLNGKE
Deuterium uptake (D)
Time (min)

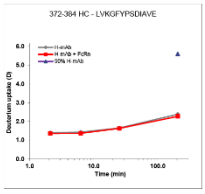

314-322 HC - HQDWLNGKE
Deuterium uptake (D)
Time (min)

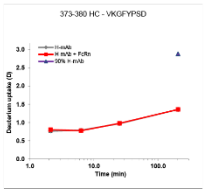

323-352 HC -
YKCKVSNKALPAPIEKTISKAKGQPREPQV
Deuterium uptake (D)
Time (min)

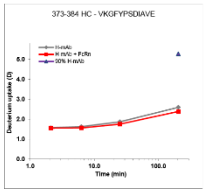

338-352 HC - KTISKAKGQPREPQV
Deuterium uptake (D)
Time (min)

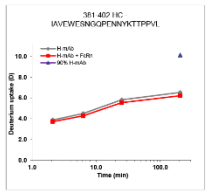

353-369 HC - YTLPPSRDELTKNQVSL
Deuterium uptake (D)
Time (min)

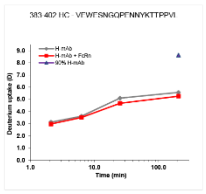

370-380 HC - TCLVKGFYPSD
Deuterium uptake (D)
Time (min)

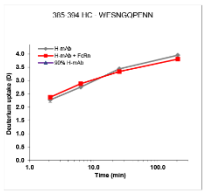

372-384 HC - LVKGFYPSDIAVE
Deuterium uptake (D)
Time (min)

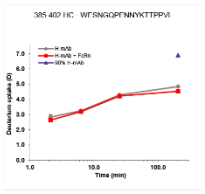

373-380 HC - VKGFYPSD
Deuterium uptake (D)
Time (min)

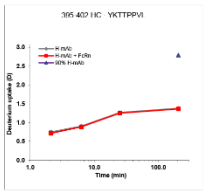

373-384 HC - VKGFYPSDIAVE
Deuterium uptake (D)
Time (min)

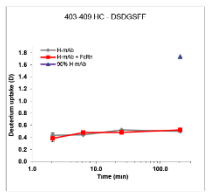

381-402 HC -
IAVEWESNGQPENNYKTTPPVL
Deuterium uptake (D)
Time (min)

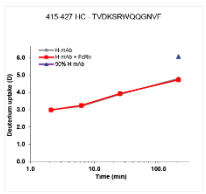

383-402 HC - VEWESNGQPENNYKTTPPVL
Deuterium uptake (D)
Time (min)

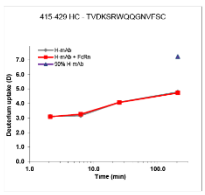

385-394 HC - WESNGQPENN
Deuterium uptake (D)
Time (min)

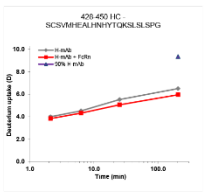

385-402 HC - WESNGQPENNYKTTPPVL
Deuterium uptake (D)
Time (min)

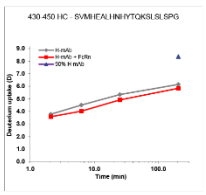

395-402 HC - YKTTPPVL
Deuterium uptake (D)
Time (min)

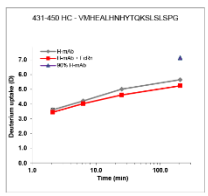

403-409 HC - DSDGSFT
Deuterium uptake (D)
Time (min)

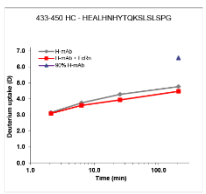

415-427 HC - TVDKSRWQQGNVF
Deuterium uptake (D)
Time (min)

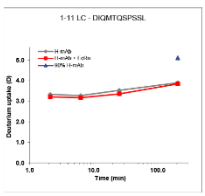

415-429 HC - TVDKSRWQQGNVFSC
Deuterium uptake (D)
Time (min)

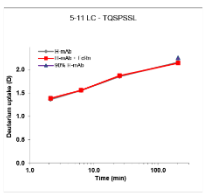

426-450 HC -
SCSVMHEALHNHYTQKSLSLSPG
Deuterium uptake (D)
Time (min)

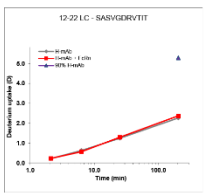

430-450 HC - SVMHEALHNHYTQKSLSLSPG
Deuterium uptake (D)
Time (min)

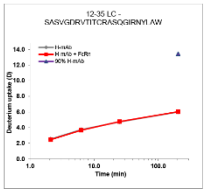

431-450 HC - VMHEALHNHYTQKSLSLSPG
Deuterium uptake (D)
Time (min)

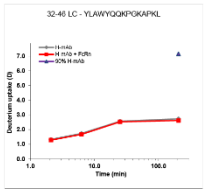

433-450 HC - HEALHNHYTQKSLSLSPG
Deuterium uptake (D)
Time (min)

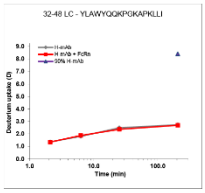

1-11 LC - DIQMTQSPSSL
Deuterium uptake (D)
Time (min)

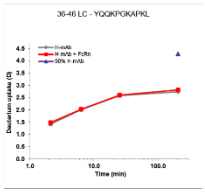

5-11 LC - TQSPSSL
Deuterium uptake (D)
Time (min)

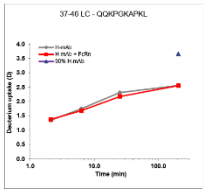

12-22 LC - SASVGDRVTIT
Deuterium uptake (D)
Time (min)

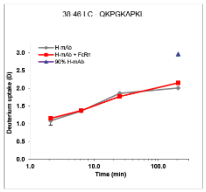

12-35 LC -
SASVGDRVTITCRASQSIRNYLAW
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

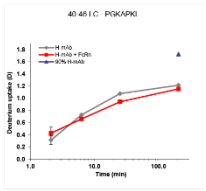

32-46 LC - YLAWYQQKPGKAPKL
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

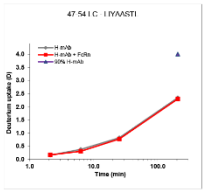

32-48 LC - YLAWYQQKPGKAPKLLI
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

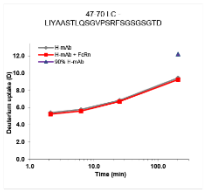

36-46 LC - YQQKPGKAPKL
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

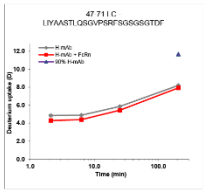

37-46 LC - QQKPGKAPKL
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

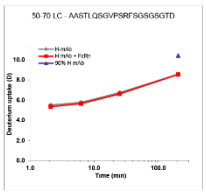

38-46 LC - QKPGKAPKL
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

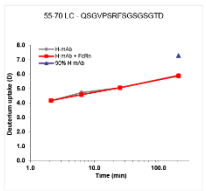

40-46 LC - PGKAPKL
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

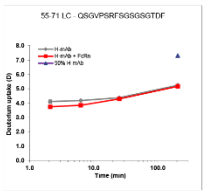

47-54 LC - LIYAASTL
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

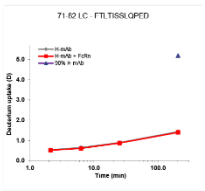

47-79 LC -
LIYAASTLQSGVPSRFSGSGSGTD
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

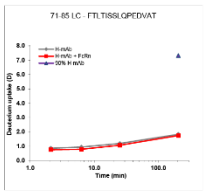

47-711 LC -
LIYAASTLQSGVPSRFSGSGSGTDF
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

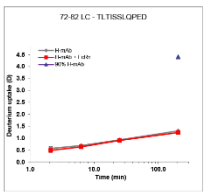

50-70 LC - AASTLQSGVPSRFSGSGSGTD
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

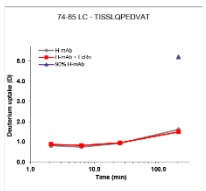

55-70 LC - QSGVPSRFSGSGSGTD
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

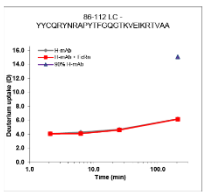

55-71 LC - QSGVPSRFSGSGSGTDF
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

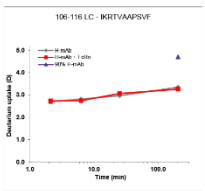

71-82 LC - FTLTISSLQPED
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

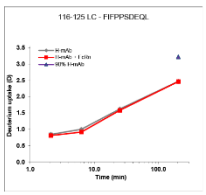

71-85 LC - FTLTISSLQPEDVAT
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

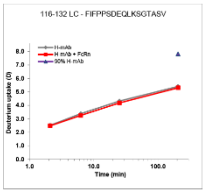

72-82 LC - TLTISSLQPED
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

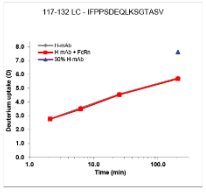

74-85 LC - TISSLQPEDVAT
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

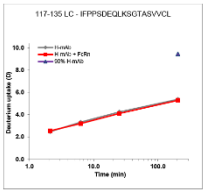

86-112 LC -
YYCQRYNRAPYTFGQGTKVEIKRTVAA
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

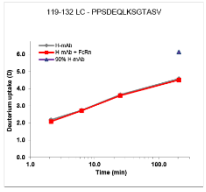

106-116 LC - IKRTVAAPSVF
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

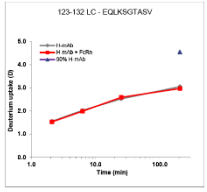

116-125 LC - FIPPSDEQL
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

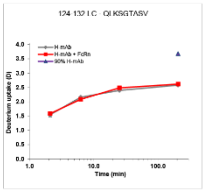

116-132 LC - FIPPSDEQLKSGTASV
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

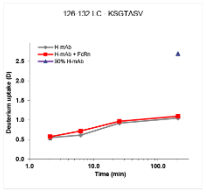

117-132 LC - IFPPSDEQLKSGTASV
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

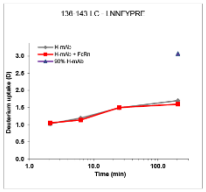

117-135 LC - IFPPSDEQLKSGTASVVCL
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

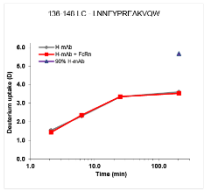

119-132 LC - PPSDEQLKSGTASV
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

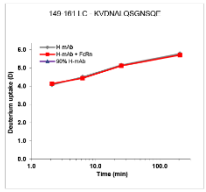

123-132 LC - EQLKSGTASV
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

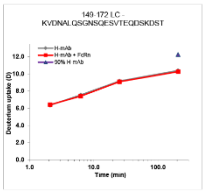

124-132 LC - QLKSGTASV
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

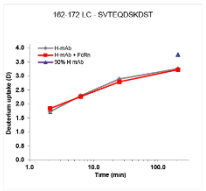

126-132 LC - KSGTASV
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

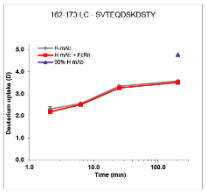

136-143 LC - LNNFYPRE
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

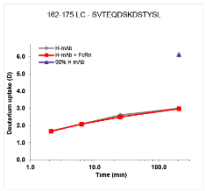

136-146 LC - LNNFYPREAKVQW
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

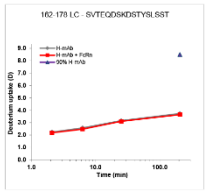

149-161 LC - KVDNALQSGNSQE
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

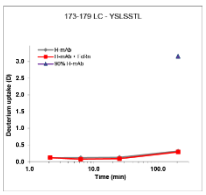

149-172 LC -
KVDNALQSGNSQESVTEQDSKDST
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

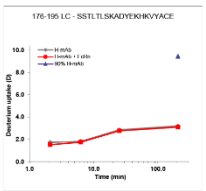

162-172 LC - SVTEQDSKDST
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

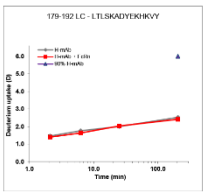

162-173 LC - SVTEQDSKDSTY
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

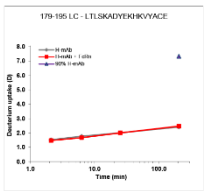

162-175 LC - SVTEQDSKDSTYSL
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

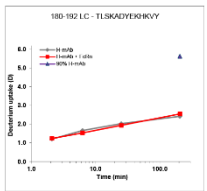

162-178 LC - SVTEQDSKDSTYSLSST
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

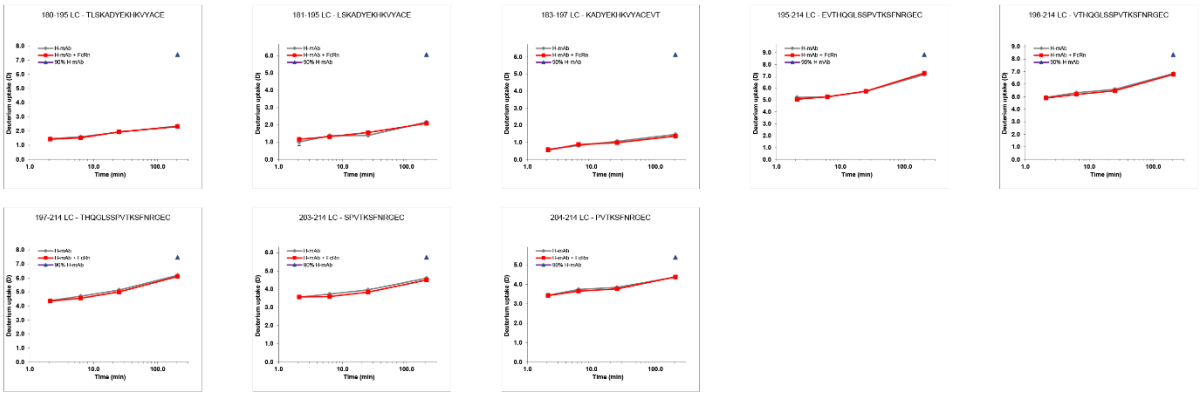

173-179 LC - YSLSSTL
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)


176-195 LC - SSTLTLSKADYEKHKVYACE
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

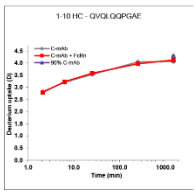

179-192 LC - LTLSKADYEKHKVY
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

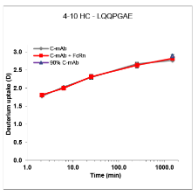

179-195 LC - LTLSKADYEKHKVYACE
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

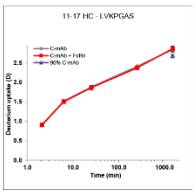

180-192 LC - TLSKADYEKHKVY
H mAb
H mAb + FcRn
90% H mAb
Deuterium uptake (D)
Time (min)

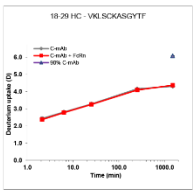


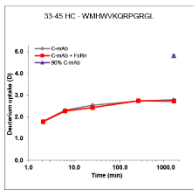
Supporting Figure S10: HDX plots of H-mAb in the absence and presence of FcRn. Absolute deuterium incorporation is plotted as a function of time for H-mAb (*grey* curves) and H-mAb in the presence of FcRn (*red* curves). Maximally labelled (90 %) control samples are plotted as *purple* triangles at the longest time points. SD is plotted as error bars (only slightly visible). (n = 1 for all data points, except the first time point, where n=3).

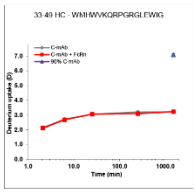

1-10 HC - QVQLQQPGAE
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

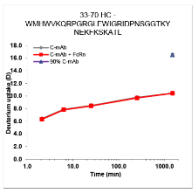

4-10 HC - LQQPGAE
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

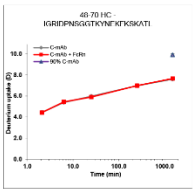

11-17 HC - LVKPGAS
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

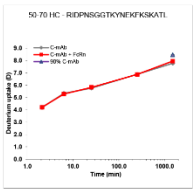

18-29 HC - VKLSCKASGYTF
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

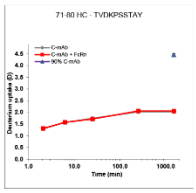

33-45 HC - WMHWVKQRPGRGL
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

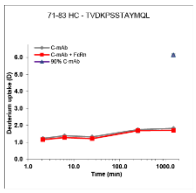

33-49 HC - WMHWVKQRPGRGLEWIG
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

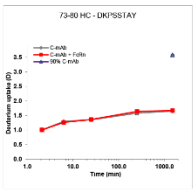

33-70 HC -
WMHWVKQRPGRGLEWIGRIDPNSGGTKY
NEKHKSKATL
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

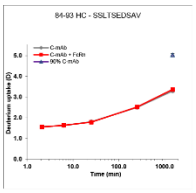

46-70 HC -
IGRIDPNSGGTKYNEKFKSKATL
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

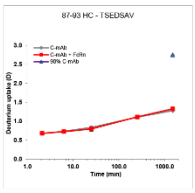

50-70 HC - RIDPNSGGTKYNEKFKSKATL
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

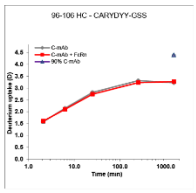

71-80 HC - TVDKPSSTAY
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

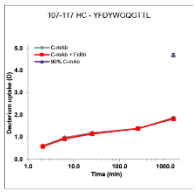

71-83 HC - TVDKPSSTAYMQL
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

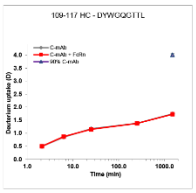

73-80 HC - DKPSSTAY
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

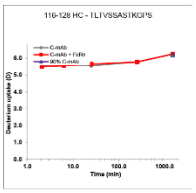

84-93 HC - SSLTSEDSAV
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

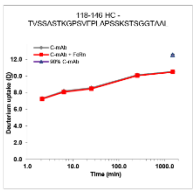

87-93 HC - TSEDSAV
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

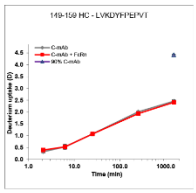

96-106 HC - CARYDYY-GSS
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

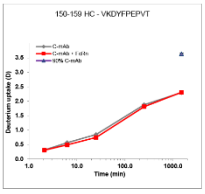

107-117 HC - YFDYWGQGTTL
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

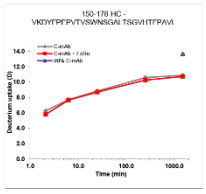

109-117 HC - DYWGQGTTL
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

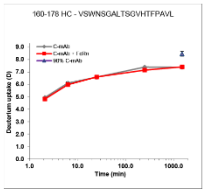

116-128 HC - TLTVSSASTKGPS
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

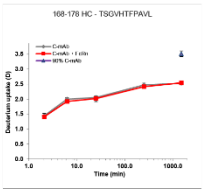

118-146 HC -
TVSSASTKGPSVFPLAPSSKSTSGGTAAL
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

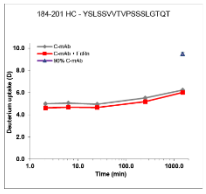

149-159 HC - LVKDYFPEPVT
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

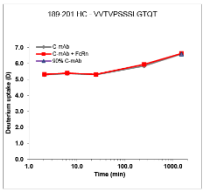

150-159 HC - VKDYFPEPVT
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

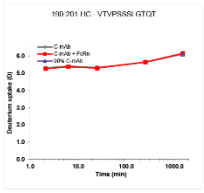

150-178 HC -
VKDYFPEPVTVSWNSGALTSGVHTFPAVL
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

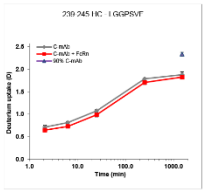

160-178 HC - VSWNSGALTSGVHTFPAVL
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

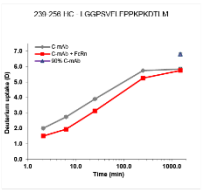

168-178 HC - TSGVHTFPAVL
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

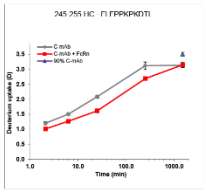

184-201 HC - YSLSSVVTVPSSSLGTQT
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

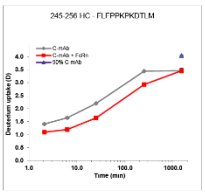

189-201 HC - VVTVPSSSLGTQT
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

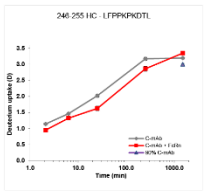

190-201 HC - VTVPSSSLGTQT
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

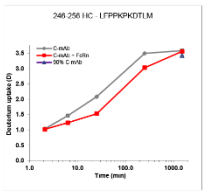

239-245 HC - LGGPSVF
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

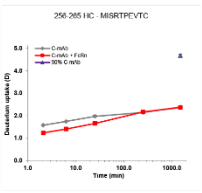

239-256 HC - LGGPSVFLFPPKPKDTLM
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

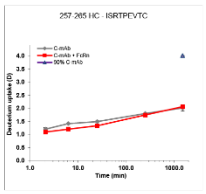

245-255 HC - FLFPPKPKDTL
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

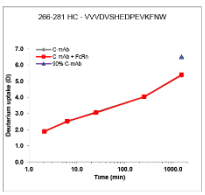

245-256 HC - FLFPPKPKDTLM
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

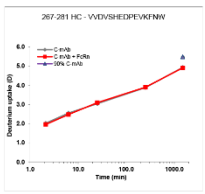

246-255 HC - LFPPKPKDTL
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

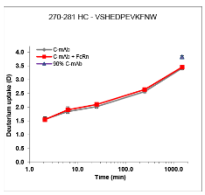

246-256 HC - LFPPKPKDTLM
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

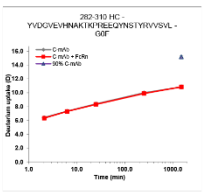

256-265 HC - MISRTPEVTC
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

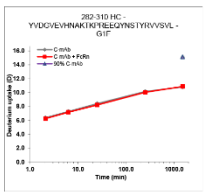

257-265 HC - ISRTPEVTC
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

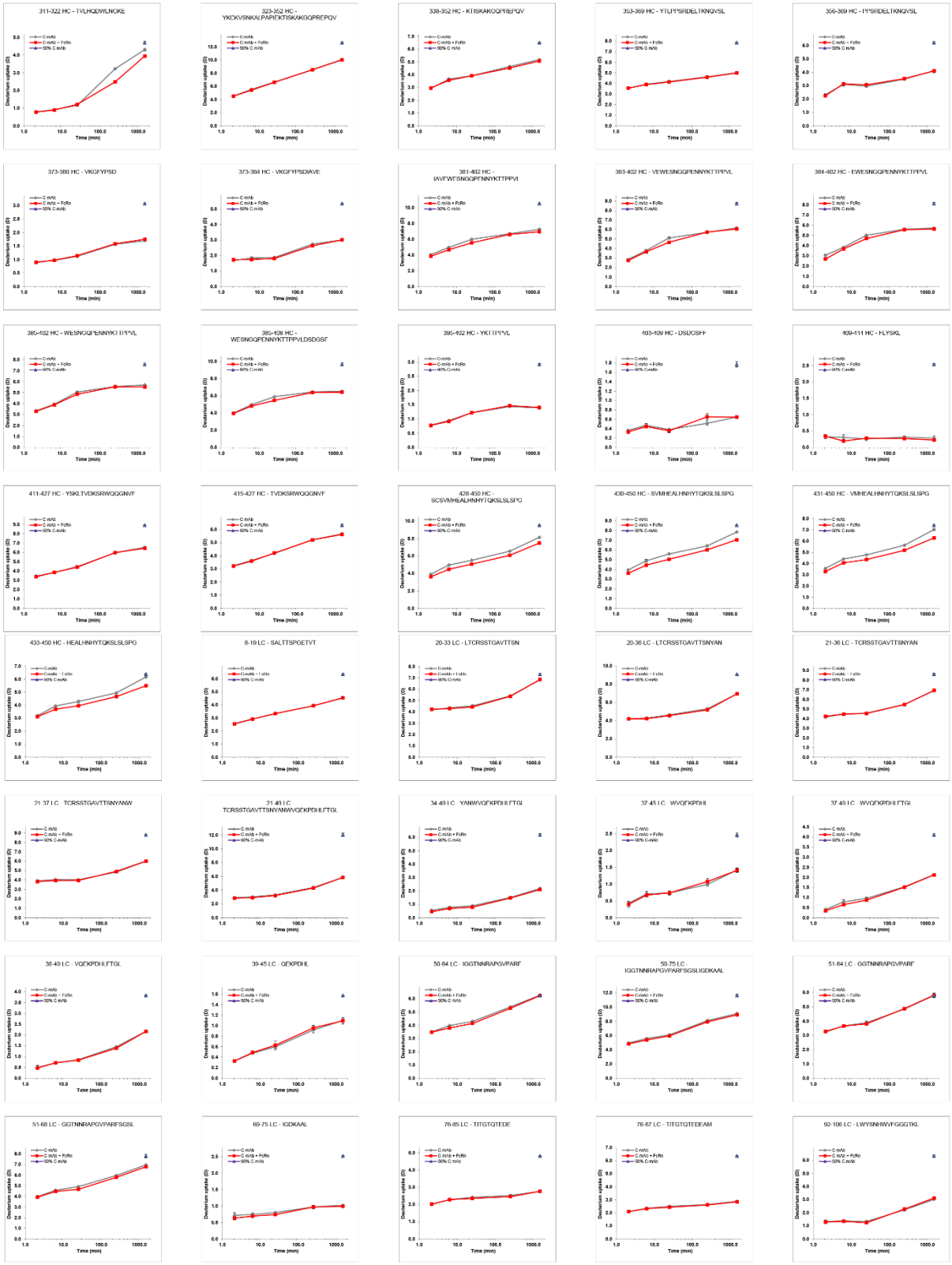

266-281 HC - VVDVSHEDPEVKFNW
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

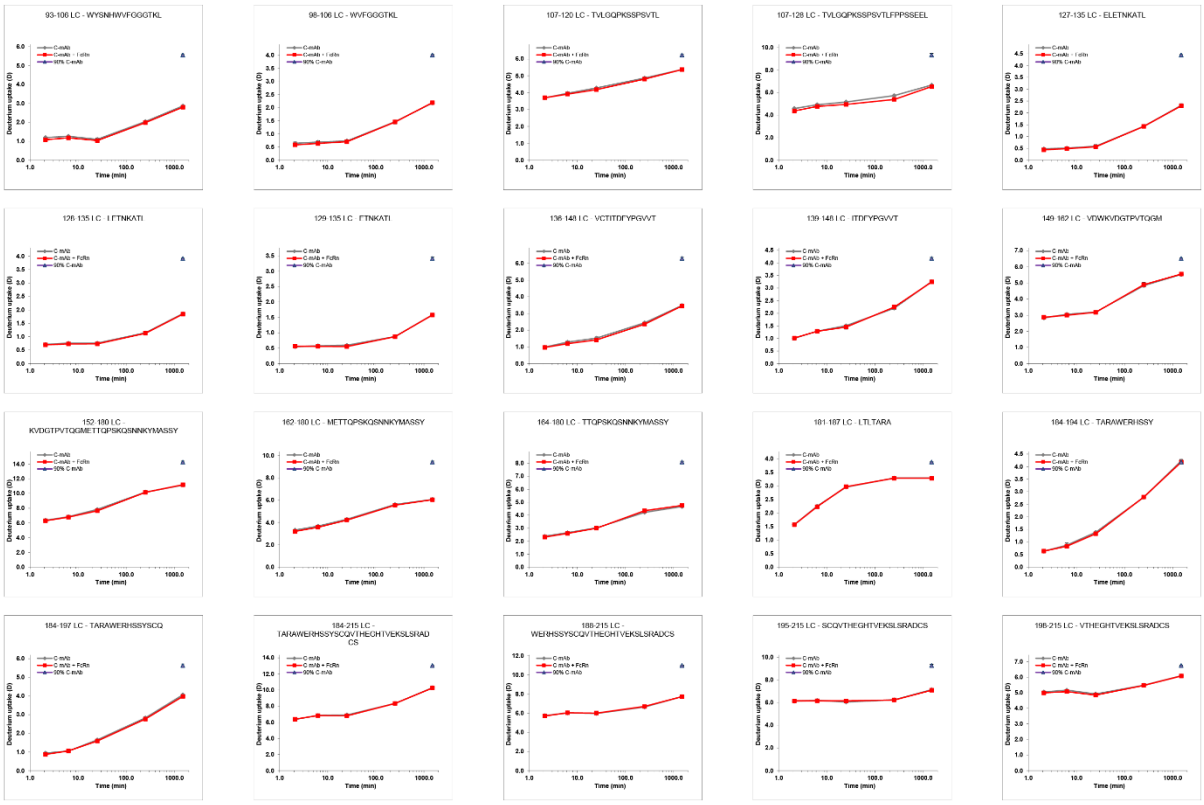

267-281 HC - VVDVSHEDPEVKFNW
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)


270-281 HC - VSHEDPEVKFNW
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

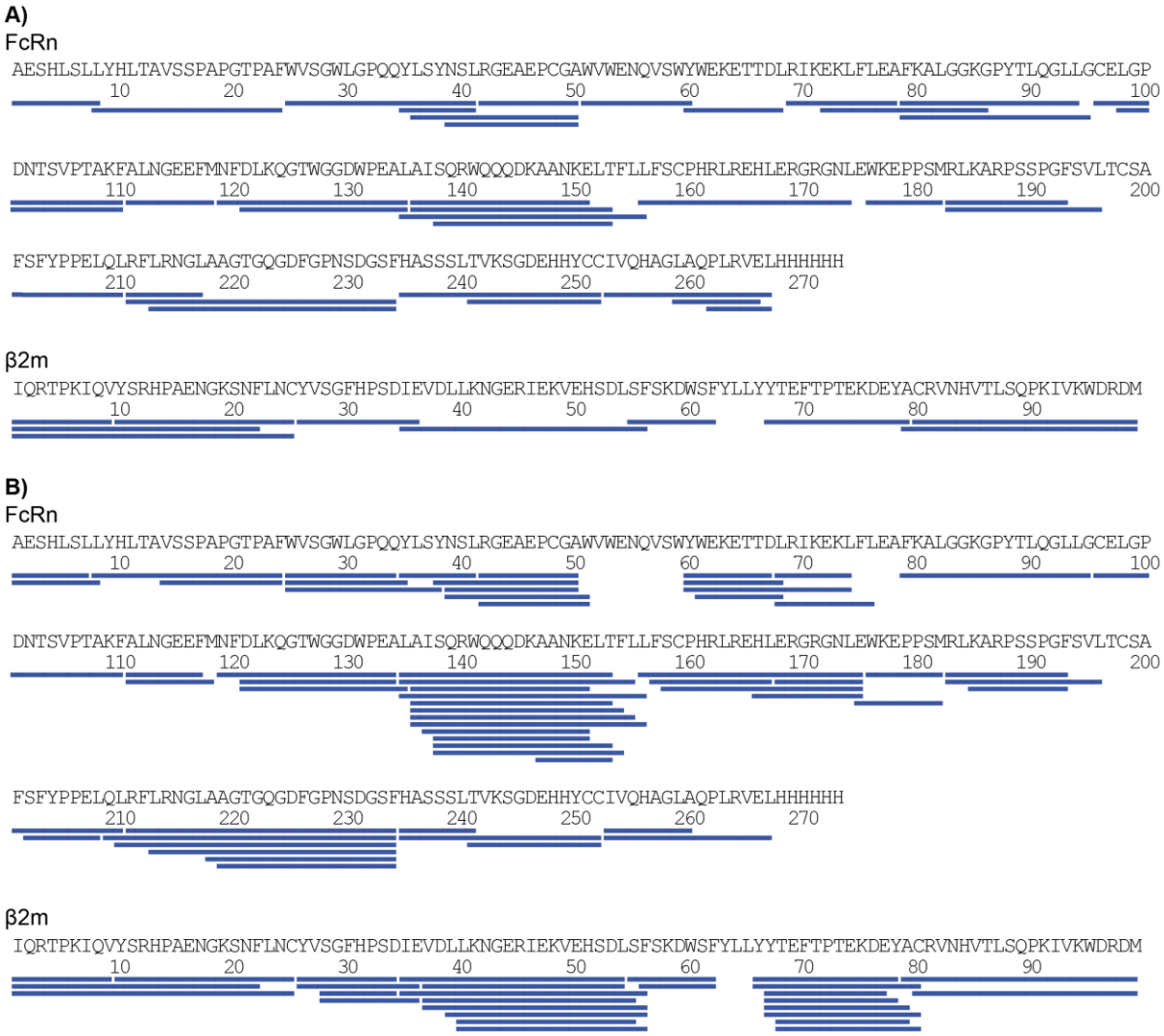

282-310 HC -
YVDGVEVHNAKTKPREEQYNSTYRVVSVL -
GIF
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)


282-310 HC -
YVDGVEVHNAKTKPREEQYNSTYRVVSVL -
GIF
C-mAb
C-mAb + FcRn
90% C-mAb
Deuterium uptake (D)
Time (min)

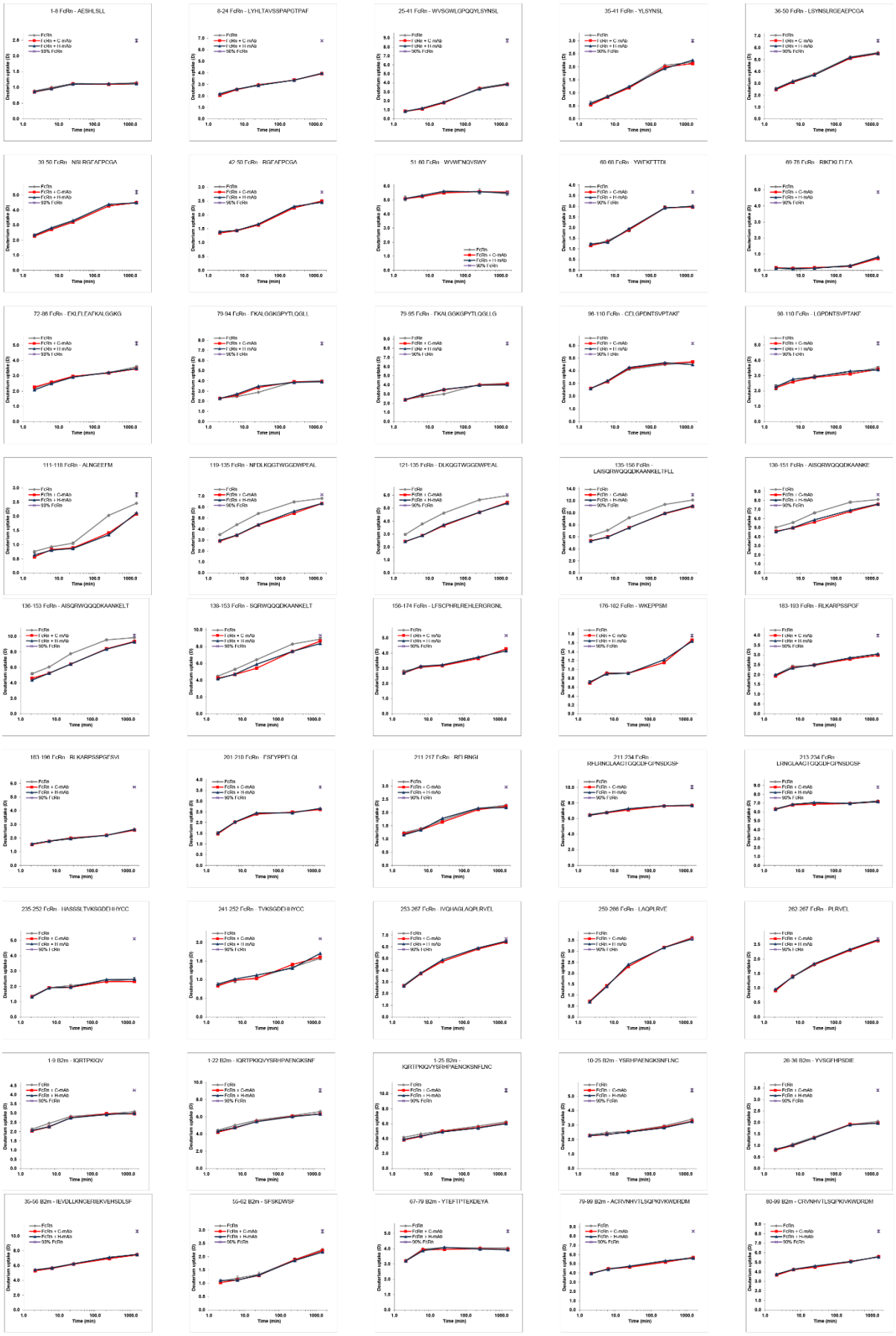

311-322 HC - TVLHQDWLNGKE
323-352 HC - YKCKVSNKALPAPIEKTISKAKGQPREPQV
338-352 HC - KTISKAKGQPREPQV
353-369 HC - YTLPPSRDELTKNQVSL
356-369 HC - PPSRDELTKNQVSL
373-380 HC - VKGFYPSD
373-384 HC - VKGFYPSDIAVE
381-402 HC - IAVEWESNGQPENNYKTTPPVL
383-402 HC - VEWESNGQPENNYKTTPPVL
384-402 HC - EWESNGQPENNYKTTPPVL
385-402 HC - WESNGQPENNYKTTPPVL
385-428 HC - WESNGQPENNYKTTPPVLDSDGSF
396-402 HC - YKTTPPVL
403-409 HC - DSDGSFF
409-414 HC - FLYSKL
411-427 HC - YSKLTVDKSRWQQGNVF
415-427 HC - TVDKSRWQQGNVF
428-450 HC - SCSVMHEALHNHYTQKSLSLSPG
430-450 HC - SVMHEALHNHYTQKSLSLSPG
431-450 HC - VMHEALHNHYTQKSLSLSPG
433-450 HC - HEALHNHYTQKSLSLSPG
8-19 LC - SALTTSPGETVT
20-33 LC - LTCRSSTGAVTTSN
20-36 LC - LTCRSSTGAVTTSNYAN
21-36 LC - TCRSSTGAVTTSNYAN
21-37 LC - TCRSSTGAVTTSNYANW
21-49 LC - TCRSSTGAVTTSNYANWVQEKPDHLFTGL
34-49 LC - YANWVQEKPDHLFTGL
37-45 LC - WVQEKPDHL
37-49 LC - WVQEKPDHLFTGL
38-49 LC - VQEKPDHLFTGL
39-45 LC - QEKPDHL
50-64 LC - IGGTNNRAPGVPARF
50-75 LC - IGGTNNRAPGVPARFSGSLIGDKAAL
51-64 LC - GGTNNRAPGVPARF
51-68 LC - GGTNNRAPGVPARFSGSL
69-75 LC - IGDKAAL
76-85 LC - TITGTQTEDE
76-87 LC - TITGTQTEDEAM
92-106 LC - LWYSNHWVFGGGTKL



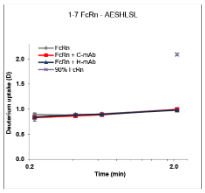
Supporting Figure S11 HDX plots of C-mAb in the absence and presence of FcRn. Absolute deuterium incorporation is plotted as a function of time for C-mAb (*grey* curves) and C-mAb in the presence of FcRn (*red* curves). Maximally labelled (90 %) control samples are plotted as *purple* triangles at the longest time points. SD is plotted as error bars (only slightly visible). (n=3).

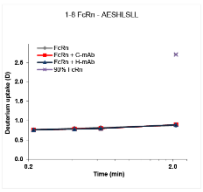


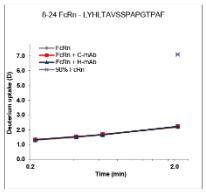
Supporting Figure S12: Effective HDX-MS coverage map of FcRn. Two separate data sets investigating two different exchange windows of FcRn in presence and absence of C-mAb and H-mAb were obtained. The first data set covered exchange times from 2.09 min to 1507.13 min (A), while the second data set covered exchange time from 13 s to 2.09 min (B). A) HDX data were obtained for 35 peptides covering 96.0% of the FcRn HC and for 10 peptides covering 96.0% of the β2m sequence. B) HDX data were obtained for 62 peptides covering 92.7% of the FcRn HC and for 28 peptides covering 97.0% of the β2m sequence.

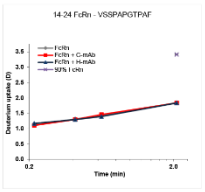

1-8 FcRn - AESHLSLL
8-24 FcRn - LYHLTAVSSPAPGTPAF
25-41 FcRn - WVSGWLGPQQYLSYNSL
35-41 FcRn - YLSYNSL
36-50 FcRn - LSYNSLRGEAEPCGA
39-50 FcRn - NSLRGEAEPCGA
42-50 FcRn - RGEAEPCGA
51-80 FcRn - WVWTNQVSWY
60-68 FcRn - YWTKFTTDI
69-78 FcRn - RIKTKLTLFA
72-86 FcRn - EKLFLEAFKALGGKG
79-94 FcRn - FKALGGKGPYTLQGLL
79-95 FcRn - FKALGGKGPYTLQGLLG
96-110 FcRn - CELGPDNTSVPTAKF
98-110 FcRn - LGPDNTSVPTAKF
111-118 FcRn - ALNGEEFM
119-135 FcRn - NFDLKQGTWGGDWPEAL
121-135 FcRn - DLKQGTWGGDWPEAL
135-156 FcRn - LAISQRWQQQDKAANKELTFLL
136-151 FcRn - AISQRWQQQDKAANKE
136-153 FcRn - AISQRWQQQDKAANKELT
138-153 FcRn - SQRWQQQDKAANKELT
156-174 FcRn - LFSCPHRLREHLERGRGNL
176-182 FcRn - WKEPPSM
183-193 FcRn - RLKARPSSPGF
183-196 FcRn - RLKARPSSPGFSVI
201-210 FcRn - FSFYPPTLQI
211-217 FcRn - RFLRNGI
211-234 FcRn - RFLRNGLAAGTGQGDFGFNSDGSF
213-234 FcRn - LRNGLAAGTGQGDFGFNSDGSF
235-252 FcRn - HASSSLTVKSGDEHHYCC
241-252 FcRn - TVKSGDEHHYCC
253-267 FcRn - IVQHAGLAQPLRVEL
259-266 FcRn - LAQPLRVE
262-267 FcRn - PLRVEL
1-9 B2m - IQRTPKIQV
1-22 B2m - IQRTPKIQVYSRHPAENGKSNF
1-25 B2m - IQRTPKIQVYSRHPAENGKSNFLNC
10-25 B2m - YSRHPAENGKSNFLNC
26-36 B2m - YVSGFHPSDIE
35-56 B2m - IEVDLLKNGERIEKVEHSDLSF
50-62 B2m - SFSKDWSF
67-79 B2m - YTEFTPTEKDEYA
78-99 B2m - ACRVNHVTLSQPKIVKWDRDM
80-99 B2m - CRVNHVTLSQPKIVKWDRDM

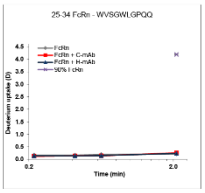
Supporting Figure S13: HDX plots of FcRn in the absence and presence of either H-mAb or C-mAb (exchange time: 2.09 min to 1507.13 min). Absolute deuterium incorporation is plotted as a function of time for FcRn (*grey* curves), FcRn in the presence of C-mAb (*red lines*) and FcRn in the presence of H-mAb (*blue* curves). Maximally labelled (90 %) control samples are plotted as *purple* crosses at the longest time points. SD is plotted as error bars (only slightly visible). (n = 3 for all data points, except the last four time points of FcRn in presence of H-mAb, where n=1).

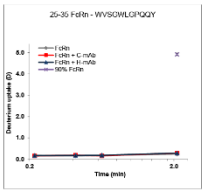

1-7 FcRn - AESHLSL
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

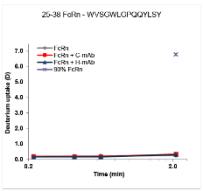

1-8 FcRn - AESHLSLL
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

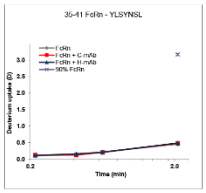

8-24 FcRn - LYHLTAVSSPAPGTPAF
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

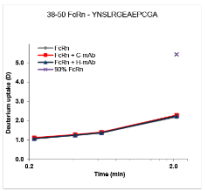

14-24 FcRn - VSSPAPGTPAF
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

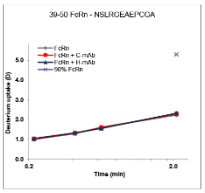

25-34 FcRn - WVSGWLGPQQ
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

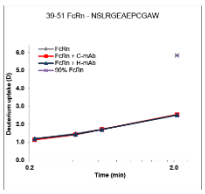

25-35 FcRn - WVSGWLGPQQY
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

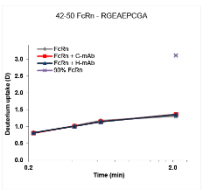

25-38 FcRn - WVSGWLGPQQYLSY
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

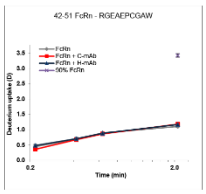

35-41 FcRn - YLSYNSL
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

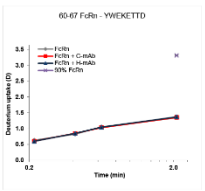

38-50 FcRn - YNSLRGEAEPCGA
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

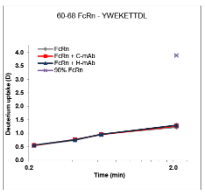

39-50 FcRn - NSLRGEAEPCGA
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

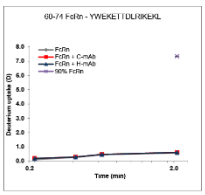

39-51 FcRn - NSLRGEAEPCGAW
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

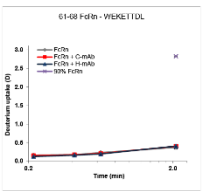

42-50 FcRn - RGEAEPCGA
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

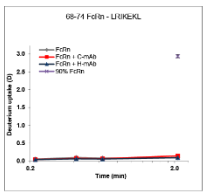

42-51 FcRn - RGEAEPCGAW
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

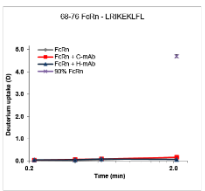

60-67 FcRn - YWEKETTD
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

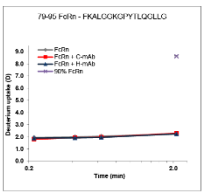

60-68 FcRn - YWEKETTDL
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

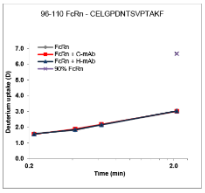

60-74 FcRn - YWEKETTDLRIKEKL
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

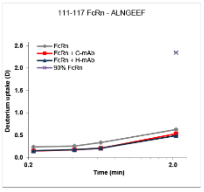

61-68 FcRn - WEKETTDL
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

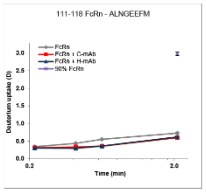

68-74 FcRn - LRIKEKL
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

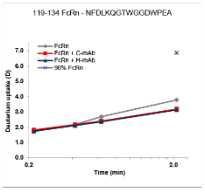

68-76 FcRn - LRIKEKLFL
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

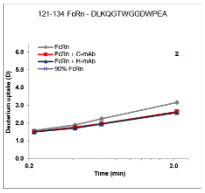

79-95 FcRn - PKALCGKGPYTLQGLLG
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

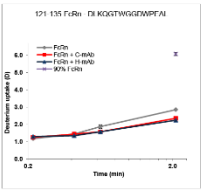

96-110 FcRn - CELGPDNTSVPTAKF
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

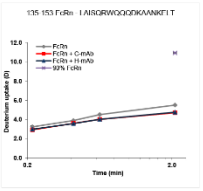

111-117 FcRn - ALNGEEF
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

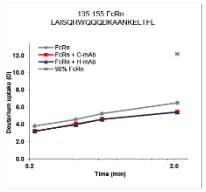

111-118 FcRn - ALNGEEFM
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

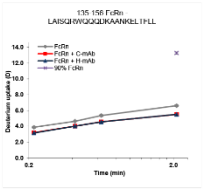

119-134 FcRn - NFDLKQGTWGGDWPEA
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

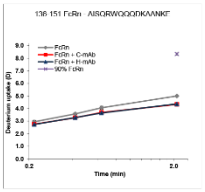

121-134 FcRn - DLKQGTWGGDWPEA
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

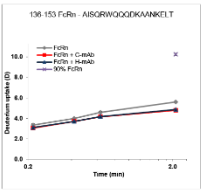

121-135 FcRn - DLKQGTWGGDWPEAI
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

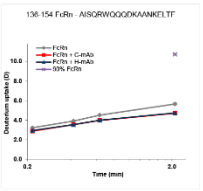

135-153 FcRn - LAISQRWQQQDKAANKELT
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

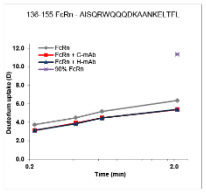

135-155 FcRn
LAISQRWQQQDKAANKELTFL
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

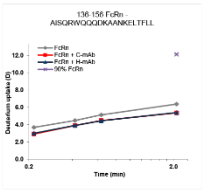

135-156 FcRn
LAISQRWQQQDKAANKELTFLL
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

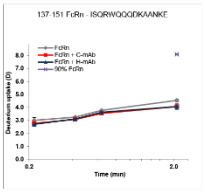

136-151 FcRn - AISQRWQQQDKAANKE
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

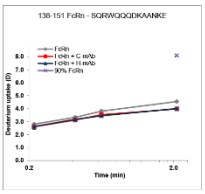

136-153 FcRn - AISQRWQQQDKAANKELT
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

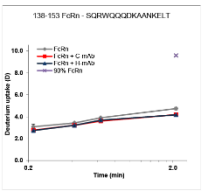

136-154 FcRn - AISQRWQQQDKAANKELTF
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

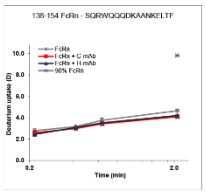

136-155 FcRn - AISQRWQQQDKAANKELTFL
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

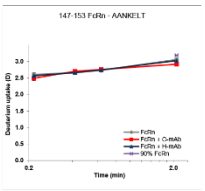

136-156 FcRn
AISQRWQQQDKAANKELTFLL
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

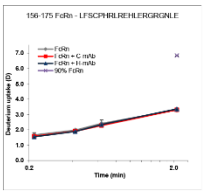

137-151 FcRn - ISQRWQQQDKAANKE
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

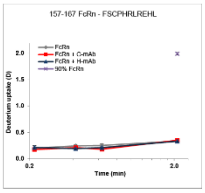

138-151 FcRn - SQRWQQQDKAANKE
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

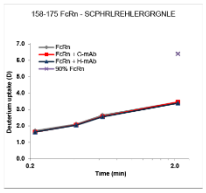

138-153 FcRn - SQRWQQQDKAANKELT
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

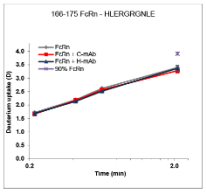

138-154 FcRn - SQRWQQQDKAANKELTF
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

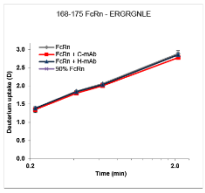

147-153 FcRn - AANKELT
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

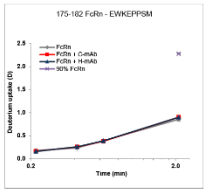

156-175 FcRn - LFSCPHRLREHLERGRGNLE
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn
Deuterium uptake (D)
Time (min)

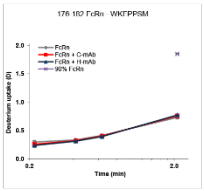

157-167 FcRn - FSCPHRLREHL
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

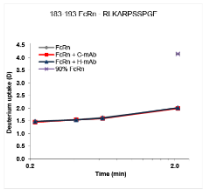

158-175 FcRn - SCPHRLREHLERGRGNLE
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

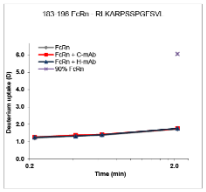

166-175 FcRn - HLERGRGNLE
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

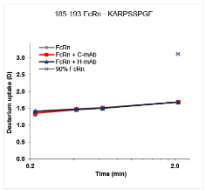

168-175 FcRn - ERGRGNLE
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

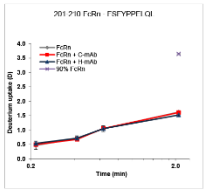

175-182 FcRn - EWKEPPSM
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

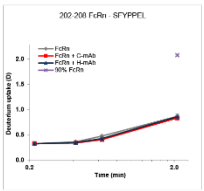

176-182 FcRn - WKEPPSM
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

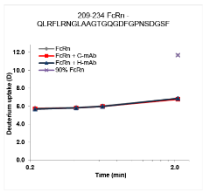

183-193 FcRn - RILKARPSSPGFSV
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

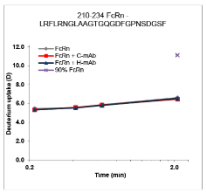

183-198 FcRn - RILKARPSSPGFSVYLQL
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

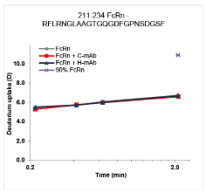

185-193 FcRn - KARPSSPGF
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

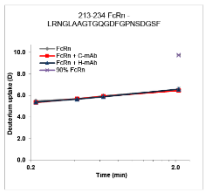

201-210 FcRn - FSTYPPTLQL
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

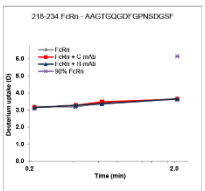

202-208 FcRn - SFYPPTL
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

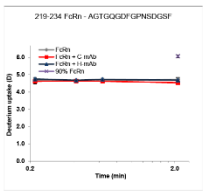

209-234 FcRn - QLRFLRNGLAAGTGQGDFGPNSDGSF
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

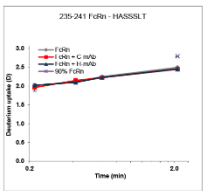

210-234 FcRn - LRFLRNGLAAGTGQGDFGPNSDGSF
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

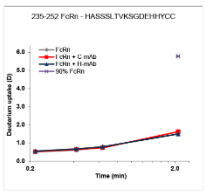

211-234 FcRn - RFLRNGLAAGTGQGDFGPNSDGSF
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

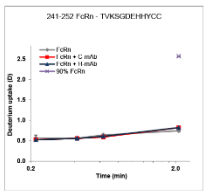

213-234 FcRn - LRNGLAAGTGQGDFGPNSDGSF
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

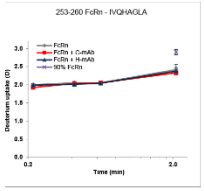

218-234 FcRn - AAGTGQGDFGPNSDGSF
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

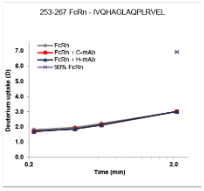

219-234 FcRn - AGTGQGDFGPNSDGSF
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

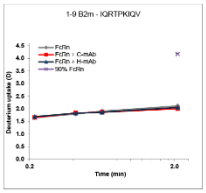

235-241 FcRn - HASSSLT
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

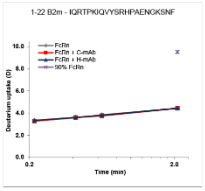

235-252 FcRn - HASSSLTVKSGDEHHYCC
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

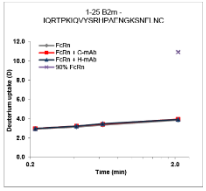

241-252 FcRn - TVKSGDEHHYCC
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

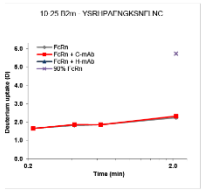

253-260 FcRn - IVQHAGLA
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

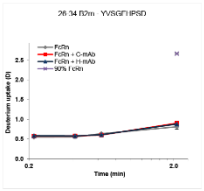

253-267 FcRn - IVQHAGLAQPLRVEL
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

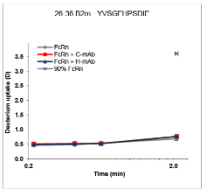

1-9 B2m - IQRTPKIQV
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

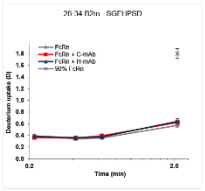

1-22 B2m - IQRTPKIQVYSRHPAENGKSNF
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

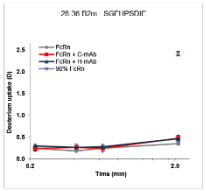

1-25 B2m - IQRTPKIQVYSRHPAENGKSNFLNC
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

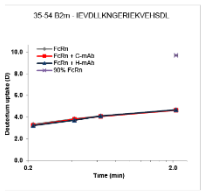

10-25 B2m - YSRHPAENGKSNFLNC
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

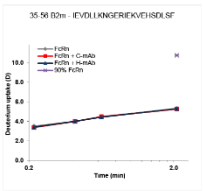

26-34 B2m - YVSGFHPSDI
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

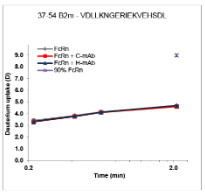

26-36 B2m - YVSGFHPSDIEV
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

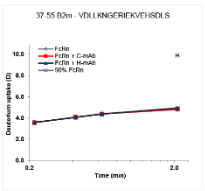

26-34 B2m - SGFHPSDI
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

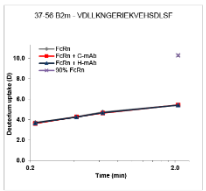

26-36 B2m - SGFHPSDIEV
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

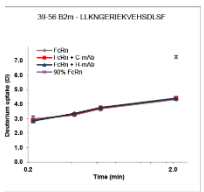

35-54 B2m - IEVDLLKNGERIEKVEHSDL
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

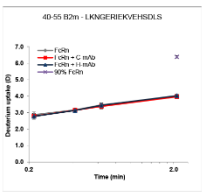

35-56 B2m - IEVDLLKNGERIEKVEHSDLSF
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

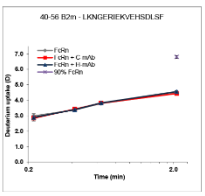

37-54 B2m - VDLLKNGERIEKVEHSDL
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

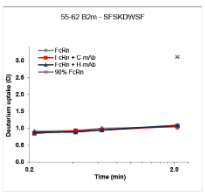

37-55 B2m - VDLLKNGERIEKVEHSDLS
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

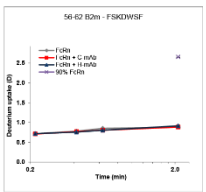

37-56 B2m - VDLLKNGERIEKVEHSDLSF
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

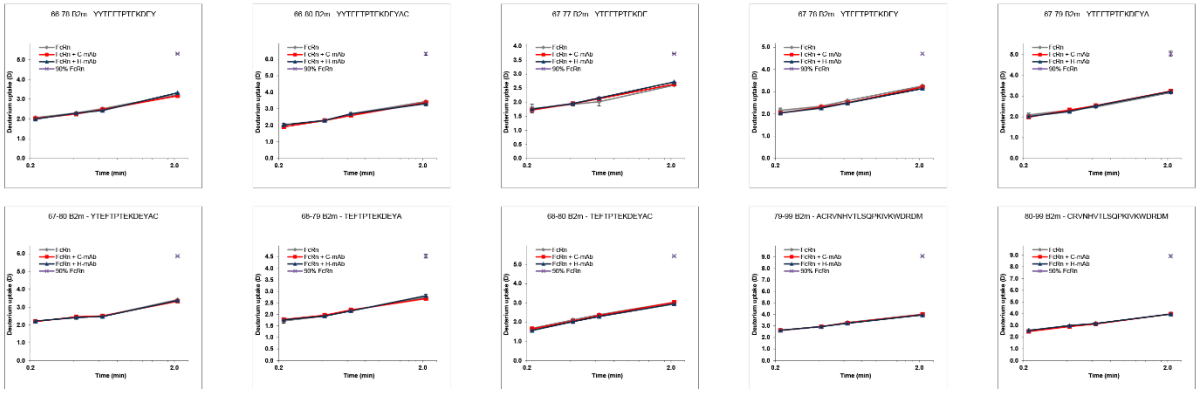

39-56 B2m - LLKNGERIEKVEHSDLSF
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn


40-55 B2m - LKNGERIEKVEHSDLS
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

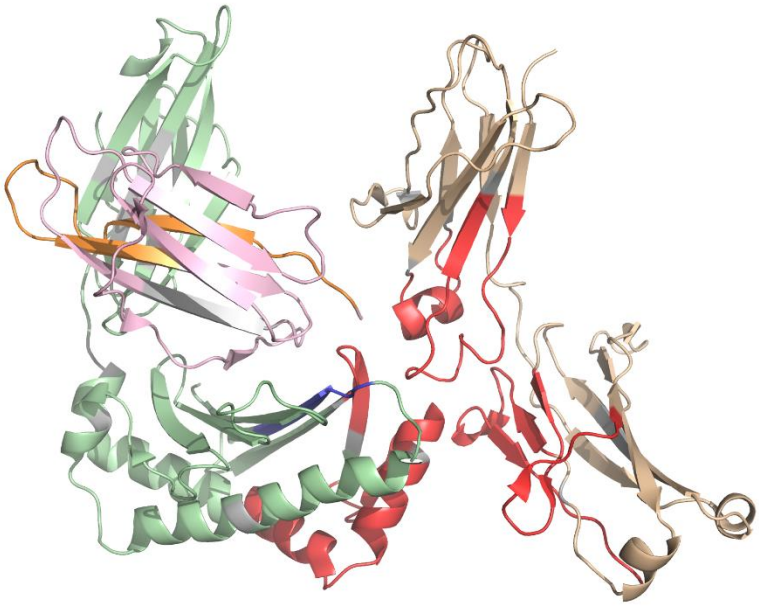

40-56 B2m - LKNGERIEKVEHSDLSF
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn


55-62 B2m - SFSKDWSF
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn

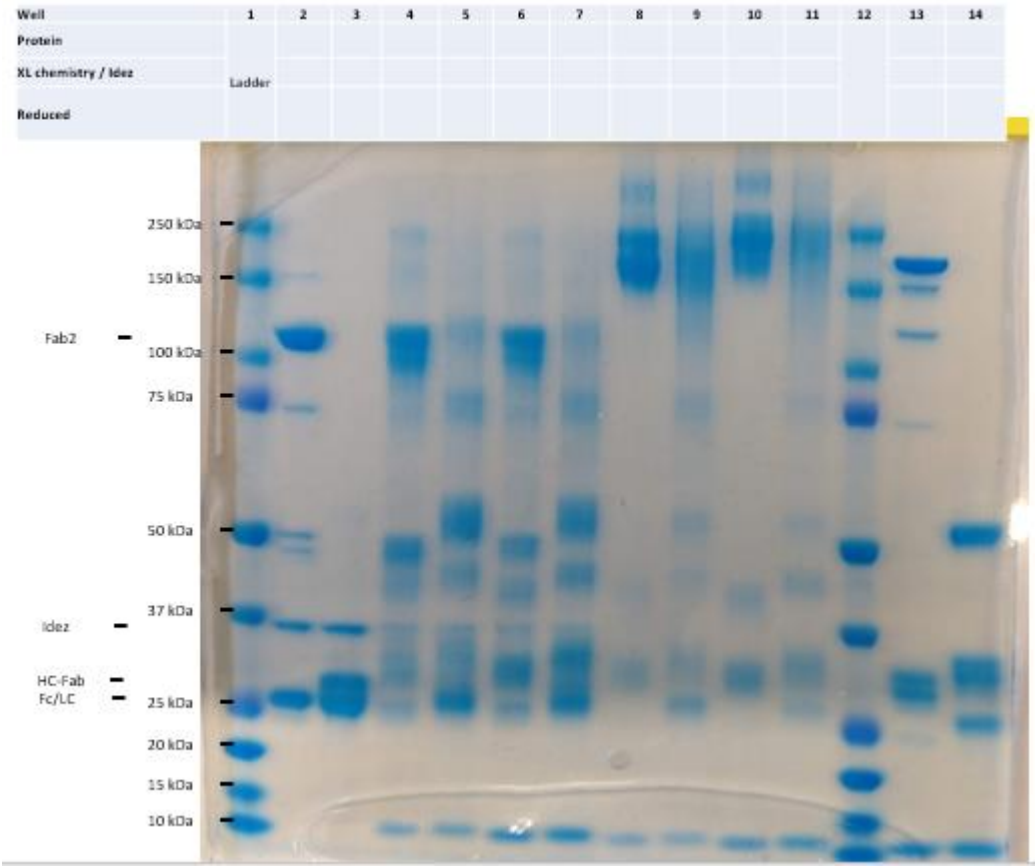

56-62 B2m - FSKDWSF
Deuterium uptake (D)
Time (min)
FcRn
FcRn + C-mAb
FcRn + H-mAb
90% FcRn



Supporting Figure S14: HDX plots of FcRn in the absence and presence of either H-mAb or C-mAb (exchange time: 13 s to 2.09 min). Absolute deuterium incorporation is plotted as a function of time for FcRn (*grey lines*), FcRn in the presence of C-mAb (*red lines*) and FcRn in the presence of H-mAb (*blue lines*). Maximally labelled (90 %) control samples are plotted as *purple crosses* at the longest time points. SD is plotted as error bars (only slightly visible). (n = 3 for all data points).



Supporting Figure S15: Conformational response of complex formation between FcRn and C-mAb. Differences in HDX mapped onto the crystal structure of FcRn in complex with Fc-YTE (α-domain: *pale green*, β2m: *light pink*, Fc-YTE: *wheat*, PDB ID: 4N0U). Regions in the α-domain and β2m of FcRn displaying a significant protection from exchange in the presence of H-mAb or C-mAb are colored *orange* and *red*, respectively. Regions displaying a significant deprotection from exchange in the presence of H-mAb or C-mAb are colored *blue.* Regions in the Fc-part of C-mAb displaying a significant protection from exchange in the presence of FcRn are colored *red*, *while* regions for which no HDX information could be obtained are colored *grey*.



Supporting Figure S16: SDS-PAGE of IdeZ treated C-mAb. Well 2 (non-reduced conditions) and 3 (reduced conditions) illustrate the successful cleavage of C-mAb by IdeZ.

Supplemental References

1. Rice, P., Longden, I. & Bleasby, A. EMBOSS: the European Molecular Biology Open Software Suite. *Trends Genet* **16**, 276–7 (2000).

2. Oganesyan, V. *et al.* Structural insights into neonatal Fc receptor-based recycling mechanisms. *J Biol Chem* **289**, 7812–7824 (2014).

3. Altschul, S. F. *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–402 (1997).

4. Altschul, S. F. *et al.* Protein database searches using compositionally adjusted substitution matrices. *FEBS J* **272**, 5101–9 (2005).