

SUPPLEMENTAL INFORMATION

ASSESSING THE HETEROGENEITY OF THE FC-GLYCAN OF A THERAPEUTIC ANTIBODY USING AN ENGINEERED Fc γ RECEPTOR IIIA-IMMOBILIZED COLUMN.

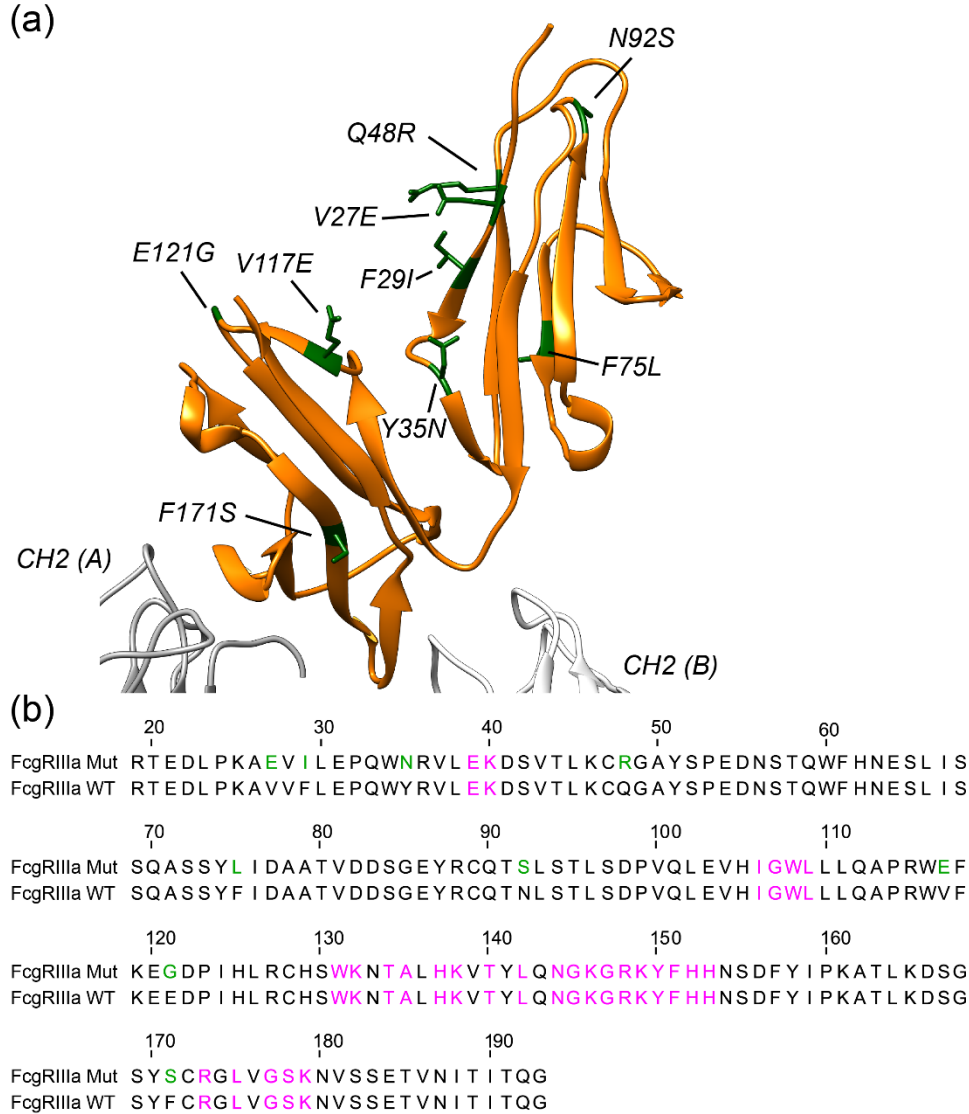
Keywords: Method, Glycosylation, Effector Function, mAbs, Fc γ RIIIa column.

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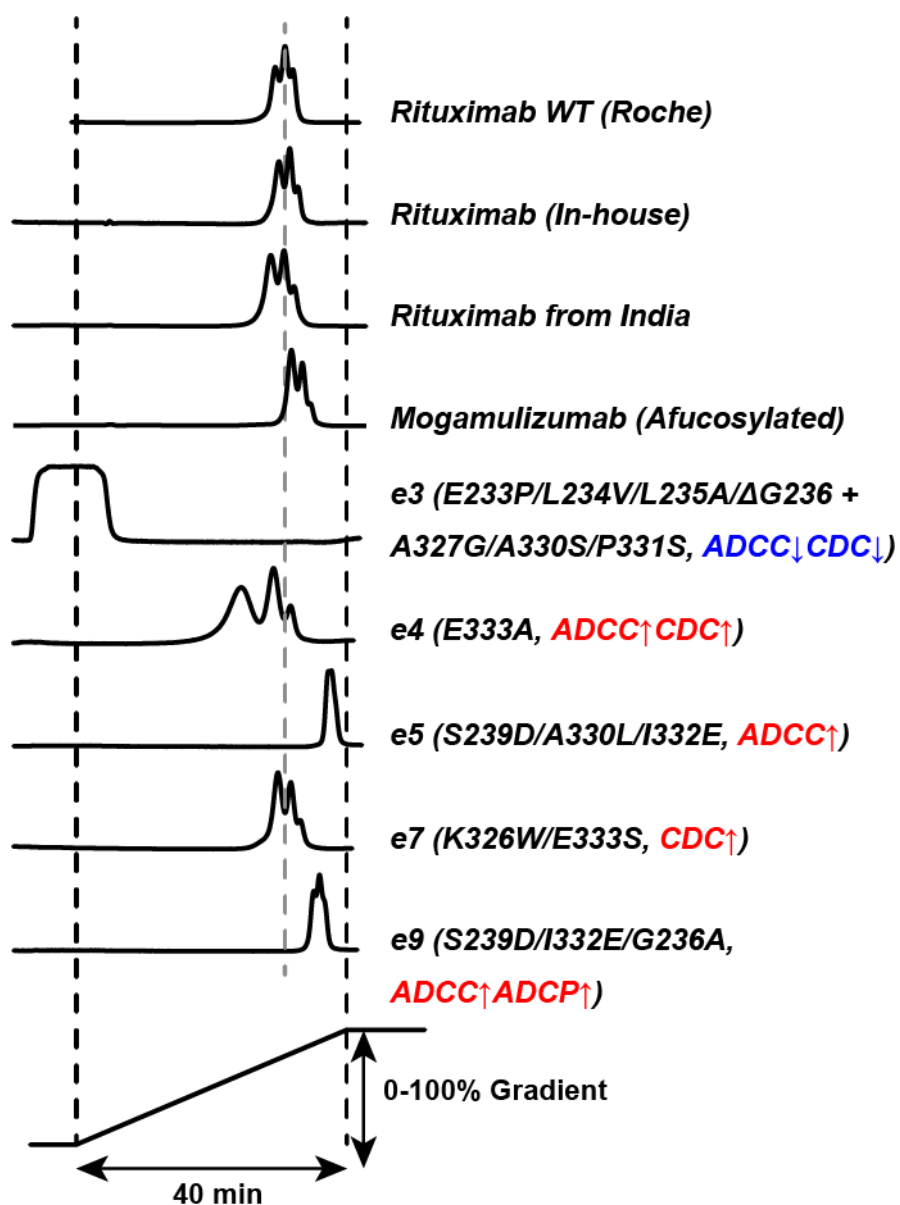
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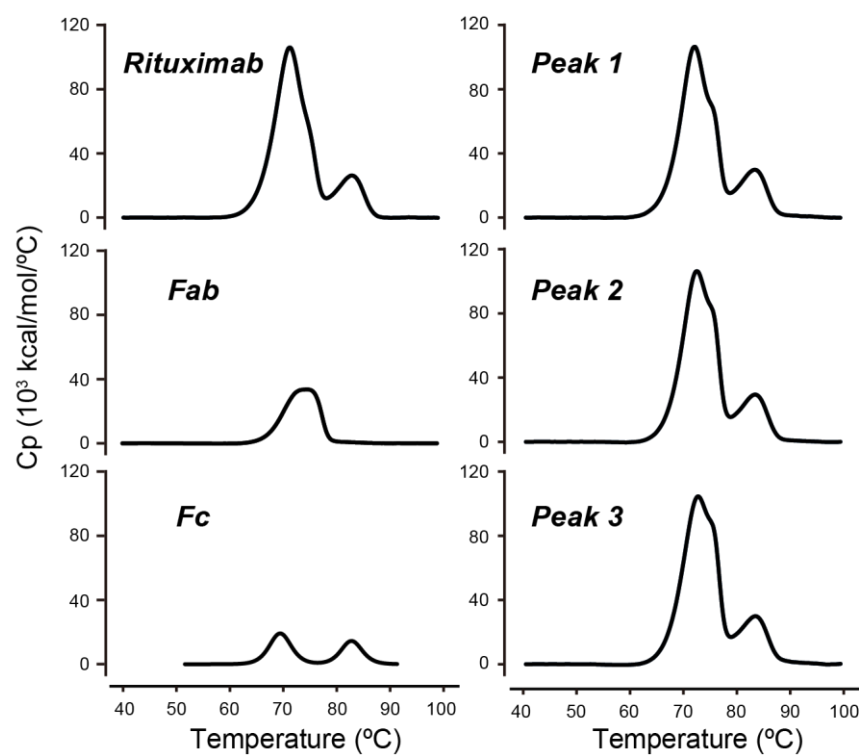
Supplemental Figure 1. Mutated residues in FcγRIIIa.

(a) FcγRIIIa, chain A of CH2, chain B of CH2 are colored in orange, gray, and white, respectively. The mutated residues are depicted in green. (b) The sequence alignment of Mut FcγRIIIa of this study and that of wild type. The sequence number was adopted from the Uniprot database. The mutated residues are colored in green. Residues interacting with Fc are colored in magenta.



Supplemental Figure 2. Analytical chromatography using FcγRIIIa-immobilized column.

The mutation information was derived from InvivoGen website.



Supplemental Figure 3. DSC analysis of each IgG fraction. Thermal stability of each IgG glycovariant was determined using DSC. The thermograms of IgG before the chromatographic step is shown in the top-left. We observed differences in the partially overlapped peak at around 76 °C next to the first transition.

Supplemental Table 1. Kinetic parameters of binding between antibody and WT FcγRIIIa, and Mut FcγRIIIa.

WT FcγRIIIa (glycosylated)			
Protein	$k_{\text{on}} (\times 10^4 \text{ M}^{-1} \text{ s}^{-1})$	$k_{\text{off}} (\times 10^{-2} \text{ s}^{-1})$	$K_{\text{D}} (\text{nM})$
Peak 1	8.2 ± 0.04	1.5 ± 0.03	180
Peak 2	7.2 ± 0.04	9.3 ± 0.02	130
Peak 3	6.7 ± 0.03	6.4 ± 0.02	95

Mut FcγRIIIa (non-glycosylated)			
Protein	$k_{\text{on}} (\times 10^4 \text{ M}^{-1} \text{ s}^{-1})$	$k_{\text{off}} (\times 10^{-2} \text{ s}^{-1})$	$K_{\text{D}} (\text{nM})$
Peak 1	5.2 ± 0.18	7.8 ± 0.23	1490
Peak 2	3.7 ± 0.10	3.8 ± 0.10	1010
Peak 3	9.0 ± 0.19	6.3 ± 0.14	701

Supplemental Table 2. Data collection and refinement statistics.
Statistical values given in parenthesis refer to the highest resolution bin.

Data Collection	Fc + Mut-FcγRIIIA (non-glycosylated)
Space Group	P 2 ₁ 2 ₁ 2 ₁
Unit cell	
a, b, c (Å)	72.8, 101.4, 122.6
α , β , γ (°)	90.0, 90.0, 90.0
Resolution (Å)	46.9 – 2.71
Wavelength	1.000
Observations	151,026 (20,452)
Unique reflections	25,395 (3,324)
R_{merge}	0.131 (0.848)
$R_{p.i.m.}$	0.058 (0.370)
Half-set correlation CC _{1/2}	0.995 (0.842)
$I / \sigma(I)$	9.6 (2.0)
Multiplicity	5.9 (6.2)
Completeness (%)	100 (100)
Refinement Statistics	
Resolution (Å)	46.9 – 2.71
R_{work} / R_{free} (%)	20.9 / 27.3
No. protein chains	3
No. atoms	
Protein	4,751
Carbohydrate	209
Other	1
Water	35
B-factor (Å ²)	
Protein	55.2
PPIX	61.9
Others	77.8
Water	40.0
Ramachandran Plot	
Preferred (%)	90.6
Allowed (%)	9.6
Outliers (%)	0
RMSD Bond (Å)	0.012
RMSD Angle (°)	1.67
PDB identification code	5YC5

Supplemental Table 3. Thermal stability of purified IgG evaluated by DSC.

Protein	T_{M1} (°C)	T_{M2} (°C)	T_{M3} (°C)	ΔH (kcal/mol)
Rituximab	71.6 ± 0.02	75.5 ± 0.06	83.1 ± 0.04	9.8×10^2
Peak 1	72.1 ± 0.02	75.8 ± 0.03	83.2 ± 0.03	9.8×10^2
Peak 2	72.5 ± 0.03	75.7 ± 0.03	83.3 ± 0.03	9.9×10^2
Peak 3	72.6 ± 0.04	75.7 ± 0.03	83.4 ± 0.04	10.0×10^2