Supporting Information

Chemoenzymatic Synthesis and Fcγ Receptor Binding of Homogeneous Glycoforms of Antibody Fc Domain. Presence of a Bisecting Sugar Moiety Enhances the Affinity of Fc to FcγIIIa Receptor

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M E T D T L L L W V L L L W V P G S T G
*DAADIQHSGGRS*SEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK
LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*SLEGPRFE*GKPIPNPLLGLDSTRTGHHHHHH

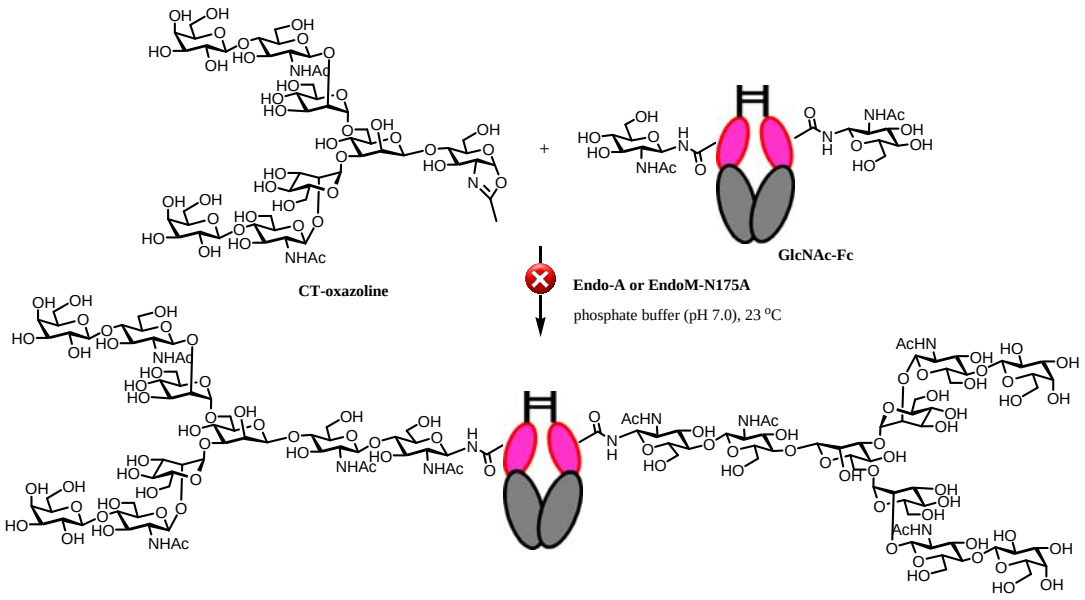
Figure S1. The amino acid sequence of the recombinant human IgG1-Fc. The Ig K signal peptide were underlined and in bold; the conserved N-glycosylation sequence was marked red; the two segments that are italized and in blue were amino acids introduced by mutiple cloning sites; the V5 tag from simian virus 5 (SV5) was italized and underlined.

Calculations of the molecular mass for the Fc glycoforms

Number of residues = 276
Reduced, monomeric Fc, MW = 30863.62 Da (average)
Fc homodimer: MW = 61713 Da (with 7 disulfide bonds)

GlcNAc-Fc, calcd. M = 62119 Da
Man9GlcNAc2-Fc, calcd. M = 65444 Da
Fc-1, calcd. M = 63905 Da
Fc-2, calcd. M = 64229 Da
Fc-3, calcd. M = 63822 Da
Fc-4, calcd. M = 63498 Da
Fc-5, calcd. M = 63498 Da
Fc-6, calcd. M = 62849 Da

A.



B.

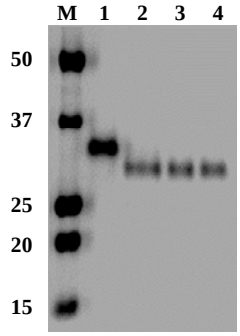


Figure S2. Analysis of the transglycosylation of GlcNAc-Fc with complex type N-glycan oxazoline by Endo-A and Endo-M mutant (EndoM-N175A). The reaction was performed as follows: A solution of GlcNAc-Fc (5 nmol) complex-type glycan oxazoline (CT-oxazoline) (100 nmol) in a phosphate buffer (50 mM, pH 7.0, 50 μL) was incubated with Endo-A (2 μg) or EndoM-N175A (5 μg) 23^{o}C. Aliquots were taken and analyzed by SDS-PAGE.

Panel A, the reaction scheme. Panel B, SDS-PAGE: Lane M, protein marker; lane 1, HM-Fc; lane 2, GlcNAc-Fc; lane 3, incubation of GlcNAc-Fc, CT-oxazoline, and Endo-A at 23 ^{o}C for 3 h; lane 4, incubation of GlcNAc-Fc, CT-oxazoline, and EndoM-N175A at 23 ^{o}C for 3 h. No transglycosylation was observed between GlcNAc-Fc and the complex-type glycan oxazoline under the given conditions.