Crystal structures of PF-06438179/GP1111, an infliximab biosimilar

BioDrugs – Electronic Supplemental Material

Thomas F. Lerch*, Penelope Sharpe, Stephen J. Mayclin, Thomas E. Edwards, Sharon Polleck, Jason C. Rouse, Qin Zou, and Hugh D. Conlon

* Analytical Research and Development, Biotherapeutics Pharmaceutical Sciences, Pfizer Inc., Chesterfield, MO 63017

Email: thomas.lerch@pfizer.com

Tel: (636) 247-0236

Figure S1

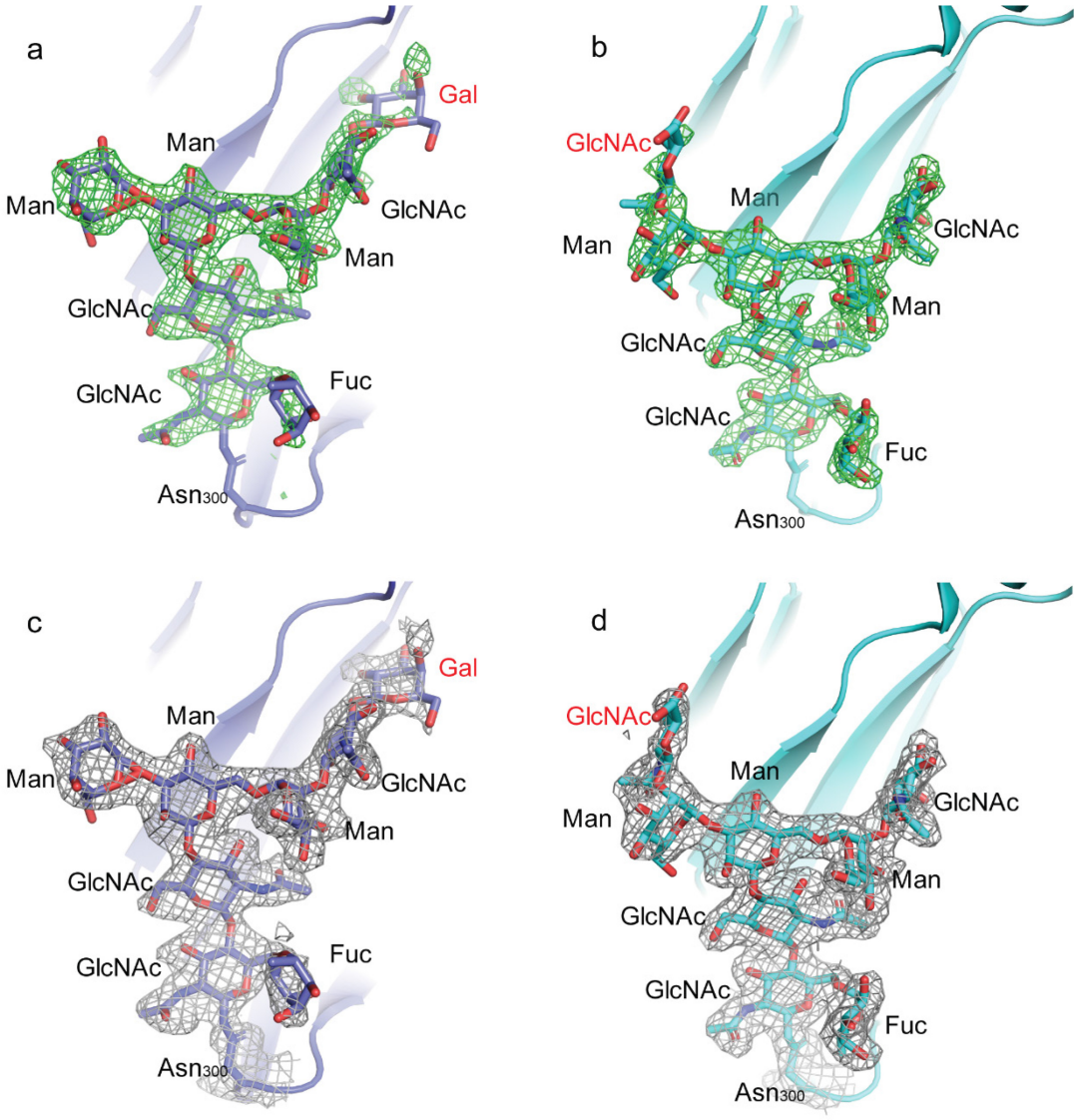

Figure S1. Electron density surrounding the PF-06438179/GP1111 Asn_{300}-linked glycans. The $C222_1$ crystal form is shown in panels a and c (blue), and the $P2_12_12_1$ form is shown in panels b and d (cyan). The Fc protein region is shown as a cartoon representation, and the glycans are shown as sticks, with nitrogen atoms shown in blue and oxygen atoms shown in red. The green mesh in a and b shows the mFo-DFc difference map calculated in the absence of the glycans and contoured at 3.0 σ. The gray mesh in c and d shows the final 2mFo-DFc map (within 2Å of the Asn_{300} and glycan atoms) contoured at 1.0 σ. Glycan residues uniquely-resolved in each crystal form are labeled in red. The electron density around the glycans is consistent with the G1F minus GlcNAc and G0 glycan structures modeled in the $C222_1$ and $P2_12_12_1$ forms, respectively. Man = mannose, Fuc = fucose, Gal = galactose, GlcNAc = N-acetyl glucosamine.

Analytical Ultracentrifugation – Sedimentation Equilibrium

Analytical ultracentrifugation sedimentation equilibrium (AUC-SE) was performed to determine the equilibrium dissociation constant(s) among the predominant species in the concentration range of 0.07 to 0.6 mg/mL. The AUC-SE experiments were conducted as described previously [1]. Briefly, infliximab was reconstituted to approximately 10 mg/mL, and subsequently dialyzed against 5 mM sodium phosphate pH 7.2, 0.0025% polysorbate-80, 2.5% sucrose. Samples were then diluted to 0.07, 0.2, and 0.6 mg/mL in the dialysate buffer and analyzed using a Beckman Coulter Analytical Ultracentrifuge Proteome XL/I at 7500, 10000, 12500 and 15000 rpm at 20°C using the UV absorption optical system. Equilibrium was monitored using the WinMatch program (http://www.biotech.uconn.edu/auf). Data from different rotor speeds (7500, 10000, 12500, and 15000 rpm) and different concentrations were fit simultaneously (i.e., globally) using SedAnal software.

The data were fit to the monomer-dimer equilibrium model to obtain the monomer-dimer dissociation constant (K_D). To reduce the number of fitting parameters, the sequence-based molecular weight for both the monomer and the dimer were fixed. The experimental and fitted AUC-SE profiles are shown in Figure S2. The fitted curves overlay well with the experimental profiles, with acceptable residual distribution, indicating that the equilibrium equations used by the software were adequate and the results obtained by these analyses were reliable.

Figure S2

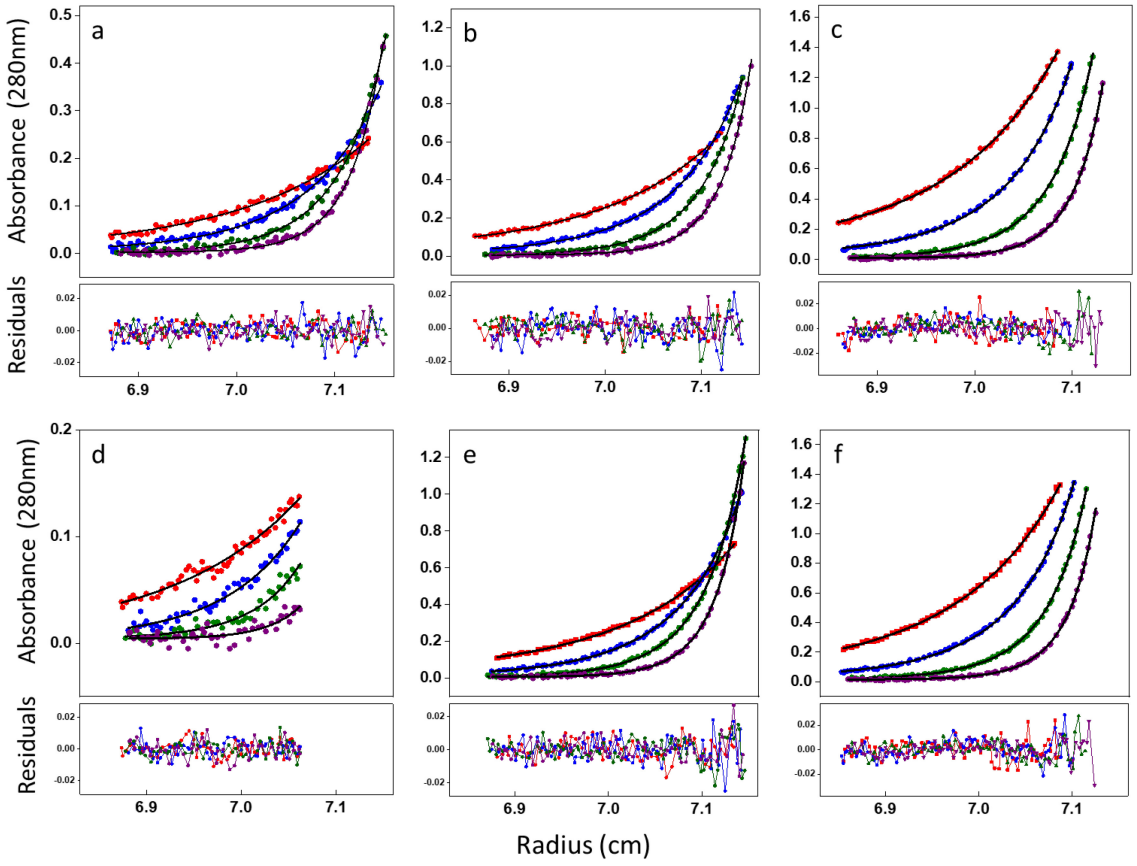

Figure S2. AUC-SE experimental data (scatter plots) were fit to a monomer-dimer equilibrium model (C(cal), solid lines) to determine the equilibrium dissociation constant (K_D), as described previously [1]. Panels a-c correspond to PF-06438179/GP1111, and panels d-f correspond to Remicade purchased from the EU market (Remicade-EU). Samples were evaluated at 0.07 mg/mL (a and d), 0.2 mg/mL (b and e), and 0.6 mg/mL (c and f). Samples were run at rotor speeds of 7500 (red), 10000 (blue), 12500 (green) and 15000 rpm (purple).

Table S1 – Crystallographic Data Collection and Refinement Statistics – Remicade-EU

	Overall (Highest shell)	**Overall (Highest shell)**	**Overall (Highest shell)**
Infliximab Fragment	Fab	Fab	Fc
Crystallization conditions	2.16 M sodium malonate, 4% pentaerythritol ethoxylate (cryo: 20% glycerol)	20% PEG 3350, 0.2 M potassium citrate tribasic (cryo: 20% EG)	4% isopropanol, 0.2 M zinc acetate, 0.1 M sodium cacodylate pH 6.8 (cryo: 20% EG)
Beam line	APS LS-CAT 21 ID-G	APS LS-CAT 21 ID-G	APS LS-CAT 21 ID-G
$\Delta\phi$	1.0 °	1.0 °	1.0 °
Images	150 (150 °)	150 (150 °)	150 (150 °)
Exposure	1.5 s	1 s	1 s
Detector Distance	210 mm	200 mm	270 mm
Wavelength	0.97872 Å	0.97872 Å	0.97872 Å
Space Group	$I2_12_12_1$	$C222_1$	$C222_1$
Unit Cell	a = 90.66 Å, b = 93.30 Å, c = 315.66 Å; α = β = γ = 90°	a = 87.26 Å, b = 138.15 Å, c = 195.31 Å; α = β = γ = 90°	a = 50.34 Å, b = 148.65 Å, c = 75.98 Å; α = β = γ = 90°
Solvent content	66 %	60 %	51.0 %
V_m	3.60 Å^3/Da	3.10 Å^3/Da	2.51 Å^3/Da
Resolution	50-2.4 Å (2.46-2.40 Å)	50-2.2 Å (2.24-2.20 Å)	50-2.1 Å (2.15-2.10 Å)
I/σ	12.66 (2.68)	17.28 (3.29)	15.26 (3.28)
Completeness	99.9% (99.9%)	99.5% (100%)	99.9% (100%)
R_{merge}	0.126 (0.790)	0.093 (0.600)	0.086 (0.558)
CC ½	99.6 (79.1)	99.8 (85.0)	99.8 (87.1)
Multiplicity	6.2 (6.3)	6.3 (6.3)	6.1 (6.2)
Reflections	52,789 (3,860)	59,825 (4,392)	17,056 (1,213)

Mosaicity	0.2-0.4	0.2	0.2
Refinement			
R	0.155 (0.210)	0.172 (0.211)	0.181 (0.216)
R_{free}	0.210 (0.254)	0.218 (0.275)	0.228 (0.310)
FOM	0.86	0.85	0.83
Coordinate error	0.25 Å	0.25 Å	0.22 Å
Wilson B-factor	31.5 $Å^2$	27.5 $Å^2$	28.2 $Å^2$
Validation			
Ramachandran favored	98.03%	97.6%	100%
Ramachandran outliers	0%	0%	0%
Rotamer outliers	0.94%	0.95%	0%
Clash score	2.01 (100^{th} percentile)	1.42 (100^{th} percentile)	2.08 (100^{th} percentile)
Molprobity score	0.97 (100^{th} percentile)	0.97 (100^{th} percentile)	0.98 (100^{th} percentile)

EU = European Union, Fab = fragment antigen binding, Fc = fragment crystallizable, EG = ethylene glycol, APS LS-CAT = Advanced Photon Source Life Sciences Collaborative Access Team, V_m = Matthews coefficient, CC = correlation coefficient, FOM = figure of merit

Table S2 – Crystallization and unit cell parameters for infliximab Fc fragment

Infliximab Fc Fragment *C*222$_1$ Crystal Form			
	PF-06438179/GP1111	Infliximab-US [1]	Infliximab-EU
Crystallization conditions	10% isopropanol 0.2 M zinc acetate 0.1 M sodium cacodylate pH 6.5 (cryo: 20% EG)	10% isopropanol 0.2 M zinc acetate 0.1 M sodium cacodylate pH 6.5 (cryo: 20% EG)	4% isopropanol 0.2 M zinc acetate 0.1 M sodium cacodylate pH 6.8 (cryo: 20% EG)
Unit Cell	*a* = 50.09 Å *b* = 148.09 Å *c* = 75.79 Å α = β = γ = 90°	*a* = 50.16 Å *b* = 148.03 Å *c* = 75.90 Å α = β = γ = 90°	*a* = 50.34 Å *b* = 148.65 Å *c* = 75.98 Å α = β = γ = 90°

US = United States, EU = European Union, EG = ethylene glycol

Table S3 – Crystallization and unit cell parameters for infliximab Fab fragment

Infliximab Fab Fragment $I2_12_12_1$ Crystal Form			
	PF-06438179/GP1111	Infliximab-US [1]	Infliximab-EU
Crystallization conditions	2.16 M sodium malonate 4% t-butanol (cryo: 20% EG)	2.16 M sodium malonate 10 mM NAD (cryo: 20% glycerol)	2.16 M sodium malonate 4% pentaerythritol ethoxylate (cryo: 20% glycerol)
Unit Cell	a = 90.60 Å b = 93.33 Å c = 316.56 Å α = β = γ = 90°	a = 90.85 Å b = 93.61 Å c = 316.07 Å α = β = γ = 90°	a = 90.66 Å b = 93.30 Å c = 315.66 Å α = β = γ = 90°
Infliximab Fab Fragment $C222_1$ Crystal Form			
	PF-06438179/GP1111	Infliximab-US [1]	Infliximab-EU
Crystallization conditions	19.5% PEG 10,000 0.1 M Hepes pH 7.8 (cryo: 20% EG)	20% PEG 3350 0.2 M potassium citrate tribasic (cryo: 20% EG)	20% PEG 3350 0.2 M potassium citrate tribasic (cryo: 20% EG)
Unit Cell	a = 87.21 Å b = 139.09 Å c = 195.68 Å α = β = γ = 90°	a = 86.58 Å b = 138.82 Å c = 195.46 Å α = β = γ = 90°	a = 87.26 Å b = 138.15 Å c = 195.31 Å α = β = γ = 90°

US = United States, EU = European Union, EG = ethylene glycol, PEG = polyethylene glycol, NAD = nicotinamide adenine dinucleotide

References

1. Lerch TF, Sharpe P, Mayclin SJ, Edwards TE, Lee E, Conlon HD et al. Infliximab crystal structures reveal insights into self-association. mAbs. 2017;9(5):874-83. doi:10.1080/19420862.2017.1320463.