

Supplemental Material to:

Ian C. Wilkinson, Susan B. Fowler, LeeAnn Machiesky, Kenneth Miller, David B. Hayes, Morshed Adib, Cheng Her, M. Jack Borrok, Ping Tsui, Matthew Burrell, Dominic J. Corkill, Susanne Witt, David C. Lowe and Carl I. Webster

Monovalent IgG4 molecules: Immunoglobulin Fc mutations that result in a monomeric structure

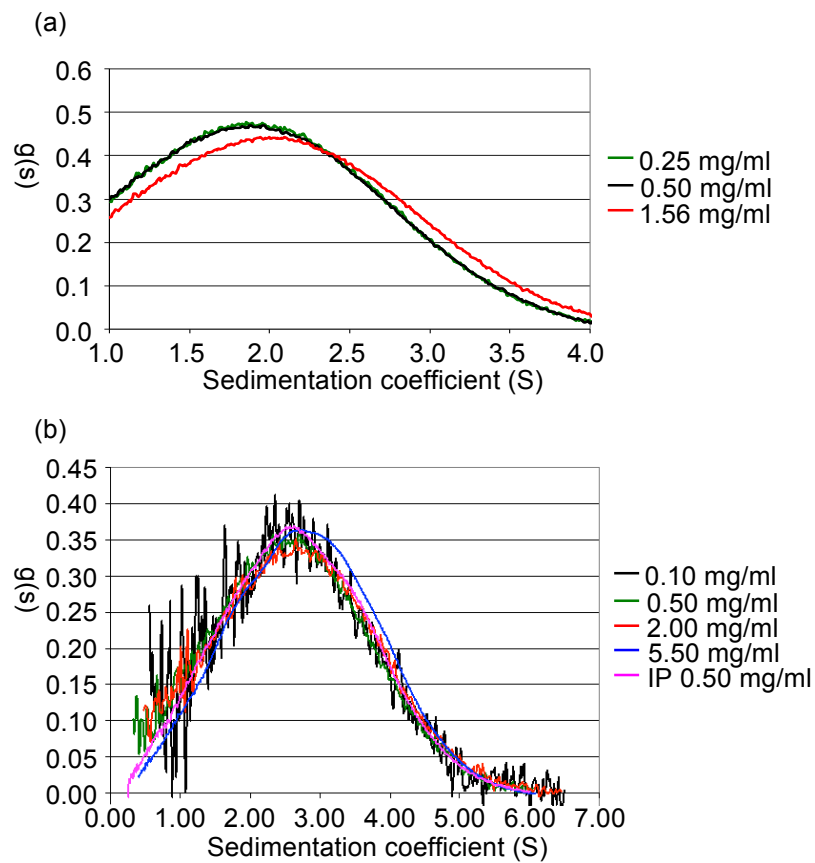
2013; 5(3)

<http://dx.doi.org/10.4161/mabs.23941>

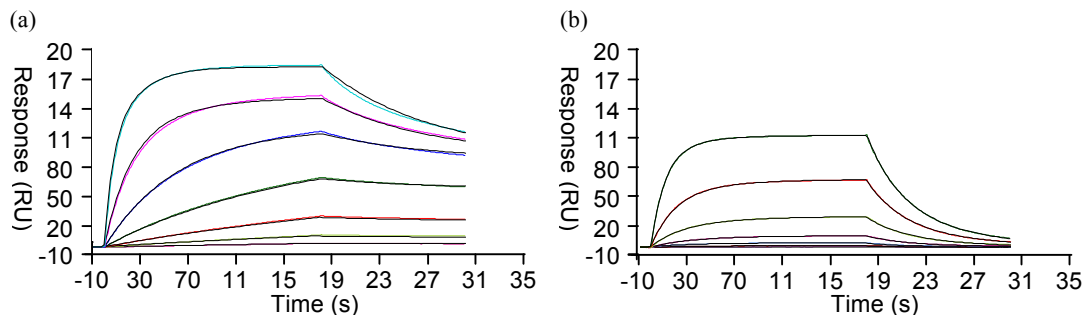
<http://www.landesbioscience.com/journals/mabs/23941/>

Mutant	IgG4 hingeless Fc	IgG1 hingeless Fc	IgG1 K409R hingeless Fc
Y407D	✓	X	-
T394R	✓	X	-
T366R	✓	X	X
R409W	✓	X	-
E357W	✓	-	-
Y407R	✓	✓	-
D399R	✓	X	✓
T366RY407R	✓	X	-
F405AY407A	✓	-	-
T366QF405QY407Q	✓	-	-
T394D	✓	✓	✓
F405Q	✓	✓	-
S364R	✓	X	✓
L351DT394D	✓	✓	-
L368R	✓	✓	✓
L351D	✓	X	✓
S364RL368R	✓	✓	-
L351R	✓	X	-
F405R	✓	-	-
L351RT394R	✓	✓	-
S364WL368W	✓	✓	-
E357R	✓	-	-
D399RK439D	✓	X	-
E356RD399R	✓	✓	-
T366WL368W	✓	✓	-
L351RS364RT394R	✓	✓	-

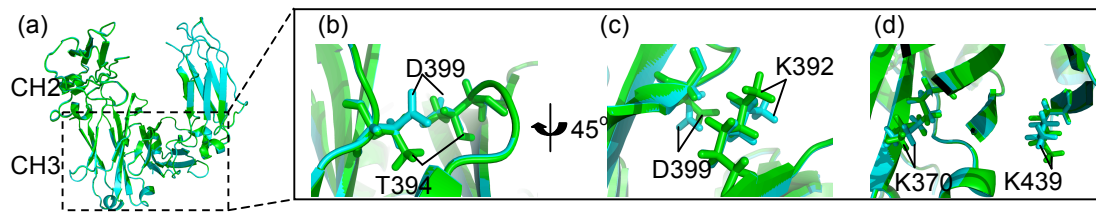
Supplementary Table 1. An overview of the monomeric CH3 domain mutants for IgG4 and IgG1 subtypes. A monomeric species, as determined by HPLC, is represented by a tick (✓), with mutants that are dimeric or in monomer-dimer equilibrium represented by a cross (x) and mutants for which there is no data a dash (-).



Supplementary Figure 1. SV-AUC profiles showing sedimentation coefficient distribution at a range of protein concentrations. (a) L368R Fc domain mutant at 0.25, 0.50 and 1.56 mg/ml. Reversible self-association is observed at 1.56 mg/ml. (b) T394D Fc domain mutant at 0.1, 0.50, 2.00 and 5.50 mg/ml. No self-association is observed within the limitations of this technique.



Supplementary Figure 2. Sensorgram data generated on T200 biosensor fit to a heterogeneous ligand model. a) Wild-type IgG4 and b) aglycosylated monovalent IgG4 binding to mouse FcRn at pH 6.0 with a sample concentration range of 1 mM to 1.37 nM. Black line on each curve represents fit of kinetic model to sensorgram data.



Supplementary Figure 3. Modelling of a monomeric Fc domain incorporating the T394D mutation. (a) Overlay of cartoon representations of models for a wild type IgG4 Fc domain in green and a monomeric Fc domain in cyan. (b) View centred on position 394. T394D mutation places two aspartate residues opposite each other and impacts side chain packing at the interface. (c and d) Adjustment of side chain orientation of charged amino acids D399, K392, K370 and K439.