Electronic Supplementary Material (ESI) for Chemical Science.

Not all therapeutic antibody isotypes are equal: The case of IgM versus IgG in Pertuzumab and Trastuzumab

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Table S1: T-test values for SKBR3 cell count assays

	Control vs Pert IgM	Control vs Trast IgM	Control vs Pert IgG1	Control vs Trast IgG1
T-test values	0.000203672	0.000336721	0.006840331	4.35325E-05

Pert IgM vs Pert IgG1	Trast IgM vs Trast IgG1
0.002390305	0.000863381

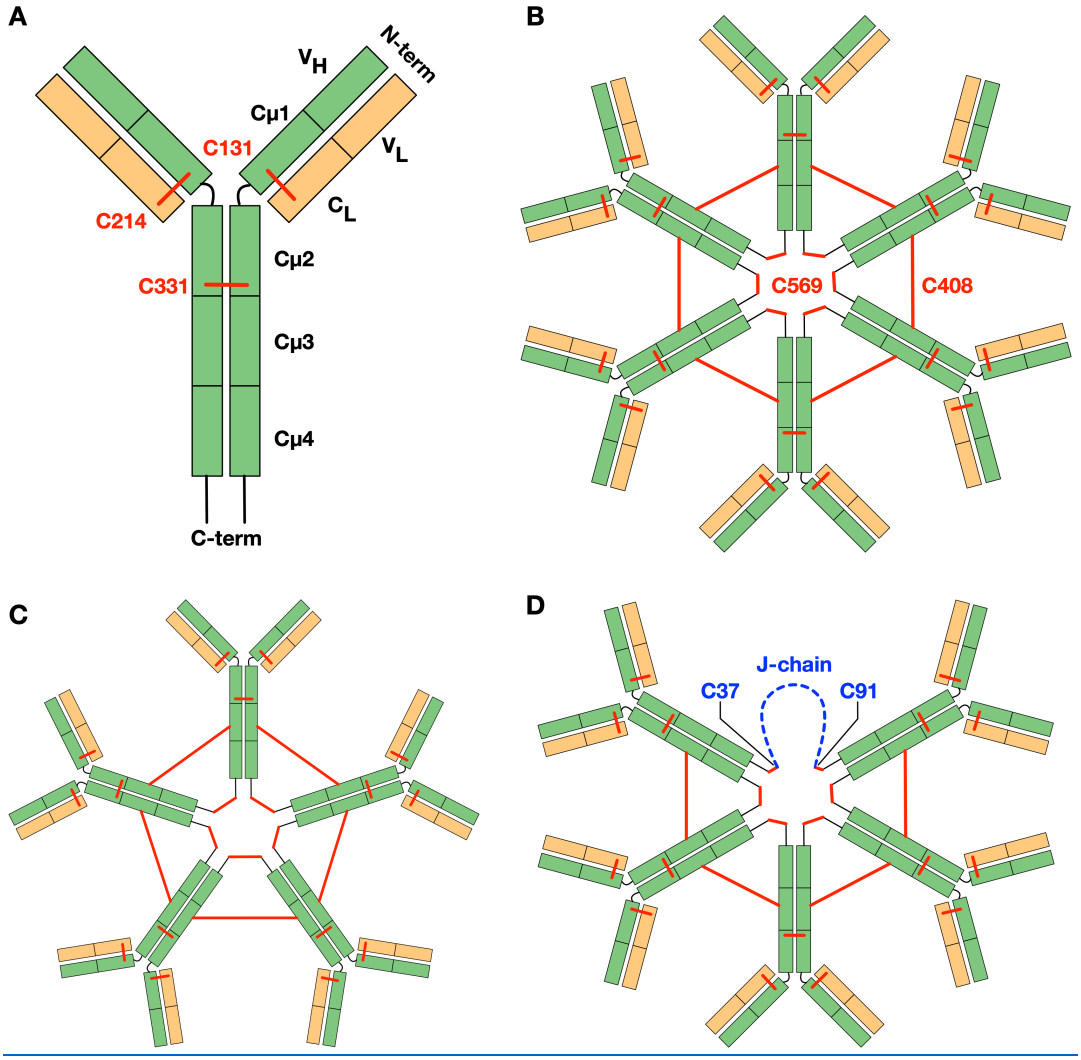

Figure S1. Schematics of IgM and the positions of interchain disulfide bridges. (A) The IgM protomer consists of two heavy chains (green) and two light chains (orange). The former is made of five immunoglobulin (Ig) domains (heavy chain constant domains Cμ1-4 and heavy chain variable domain VH), whilst the latter is comprised of two domains: light chain constant (CL) and light chain variable (VL). The two heavy chains are covalently linked by a disulfide bond between the Cμ2 domains, and the light chains are connected to adjacent heavy chains via a disulfide bond in their C-termini. **(B)** IgM hexamers consists of six units of IgM, each linked to another via two disulfide bonds: one on the Cμ3 domains and the other on the C-terminus of the heavy chain. **(C)** IgM pentamers consists of five constant regions of IgM, which are connected to each other via the same disulfide bridges as the hexamer. **(D)** Pentameric IgM with a J-chain is an asymmetric pentagon, whereby the J-chain replaces the sixth IgM constant region and connects to the neighboring IgM via disulfide bonds on their C-terminus.

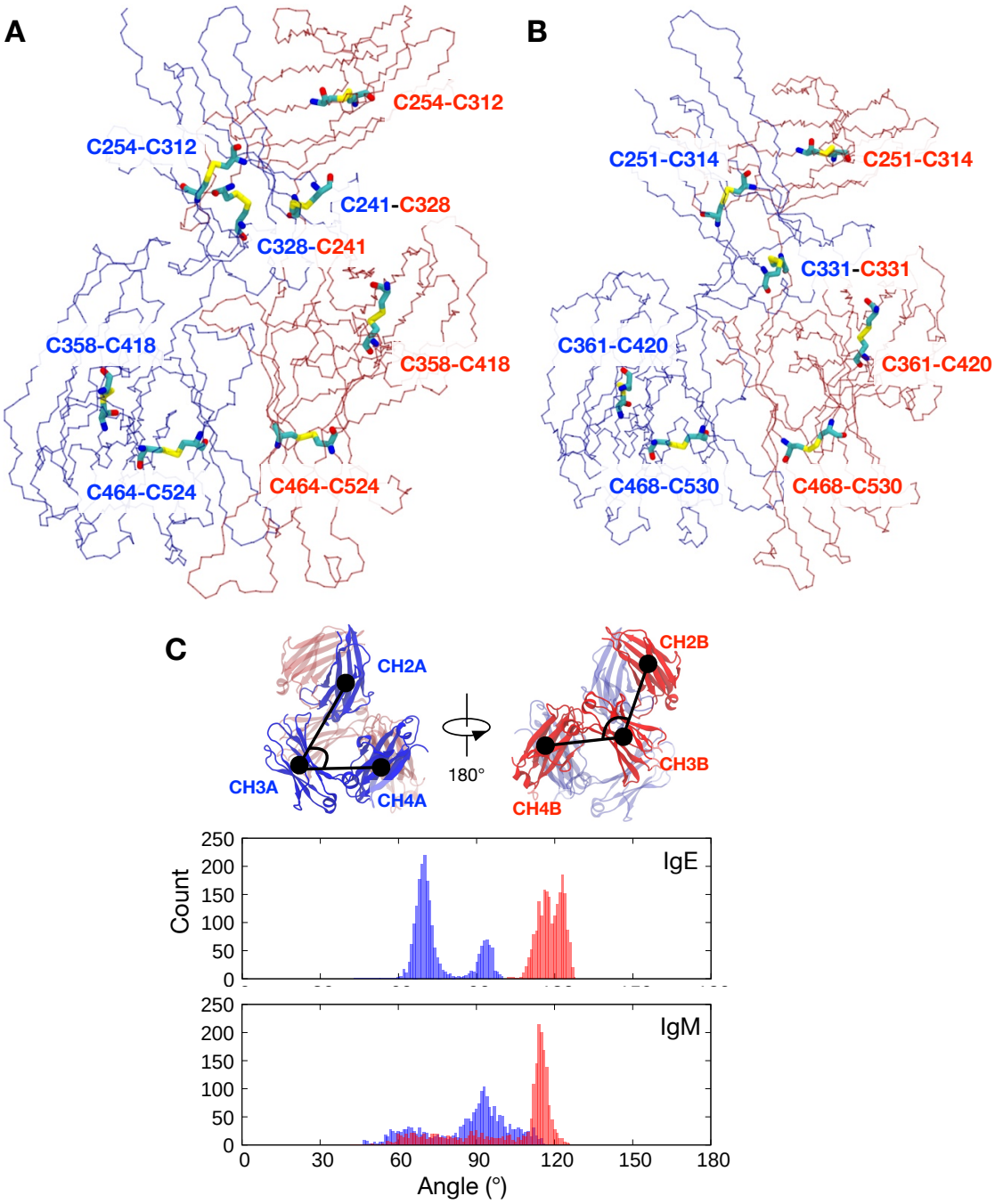

Figure S2: Comparison of IgM and IgE Fc dynamics. (A) Crystal structure of IgE Fc (PDB: 1O0V). The two chains are coloured red and blue and only the backbone is shown for clarity. Disulfide bonds are labelled and shown in stick representation. (B) A homology model of Pertuzumab IgM Fc built based on alignment to the IgE Fc structure. (C) The distribution of two Fc bend angles, one for each heavy chain, defined by two vectors connecting the centers of mass of CH2 and CH3, as well as CH3 and CH4.

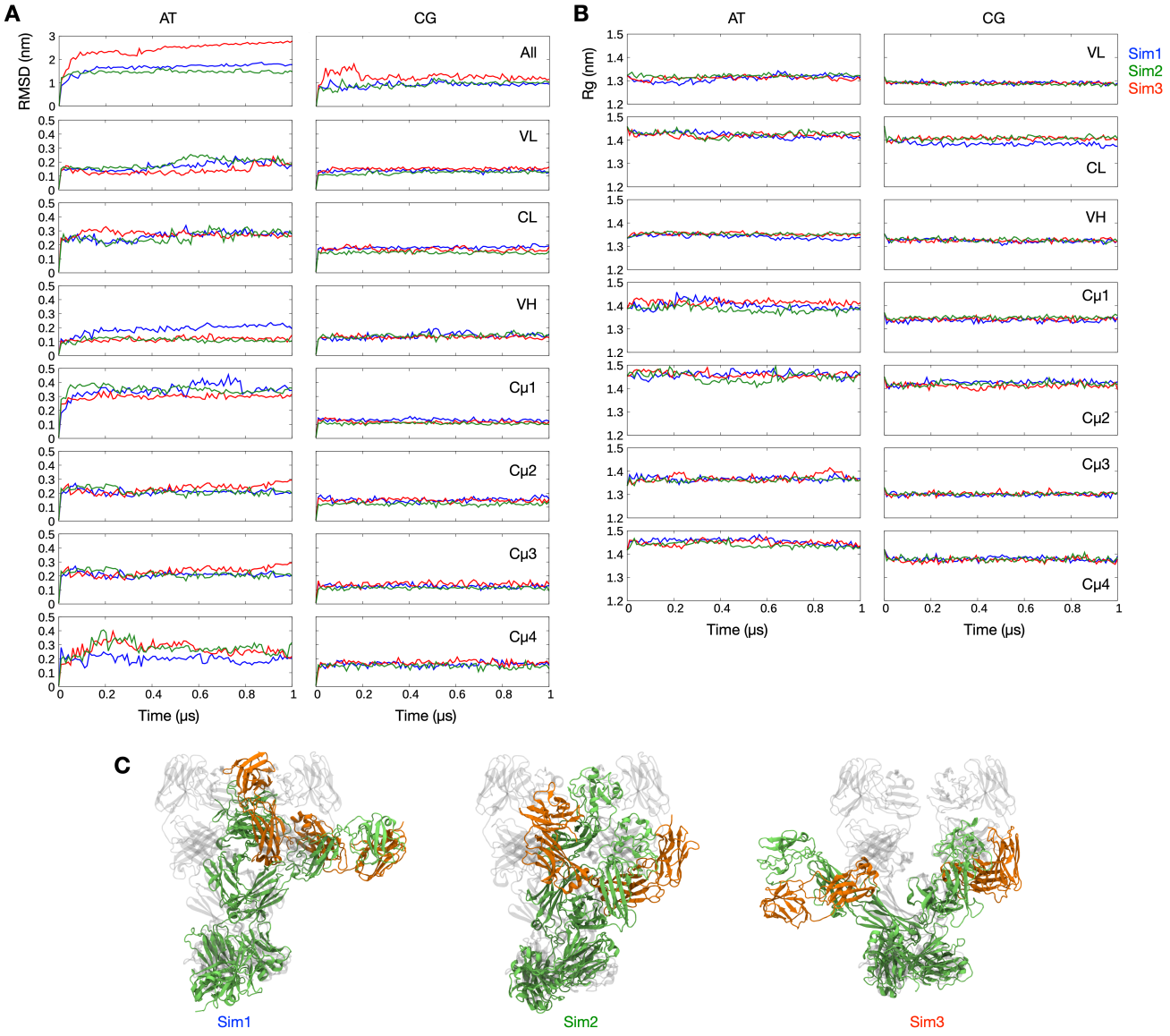

Figure S3: Structural stability of the homology models of IgM protomers in all-atom and coarse-grained simulations. (A) Backbone root mean square deviations (RMSD) for Pertuzumab from three independent atomistic (left) and coarse-grained (right) simulations for the whole antibody and each Ig domain. **(B)** The radius of gyration of each Ig domain of Pertuzumab from three independent atomistic (left) and coarse-grained (right) simulations. **(C)** Snapshots at the end of the three atomistic simulations showing IgM in cartoon representation with heavy chains in green and light chains in orange. The structure of the model at the beginning of the simulation is shown in grey for comparison.

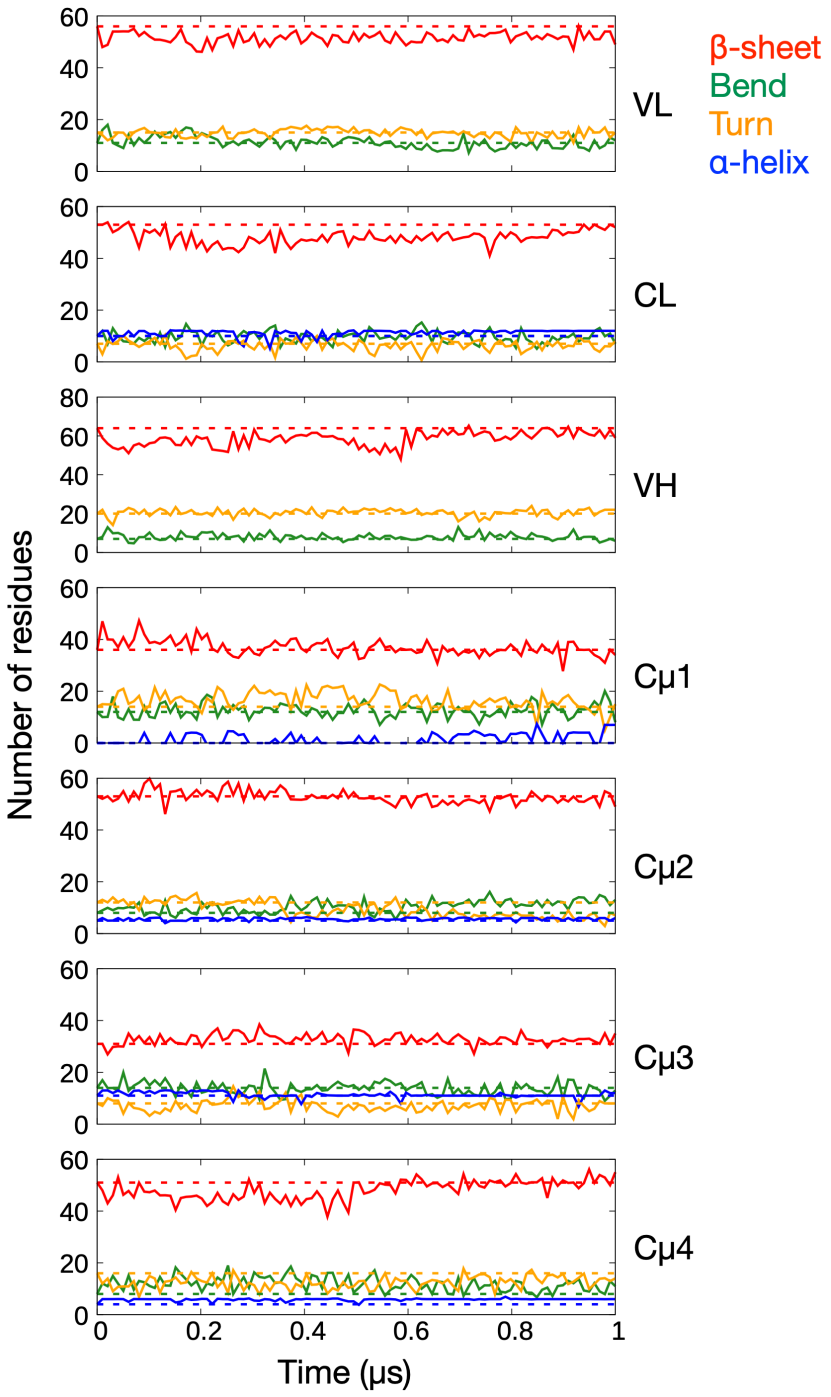

Figure S4: Secondary structure preservation of each Ig domain in all-atom simulations. Average number of residues forming β-sheets, bends, turns and α-helices throughout three independent 1 μs simulations. Dotted lines show the number of residues forming these secondary structures at the beginning of the simulations.

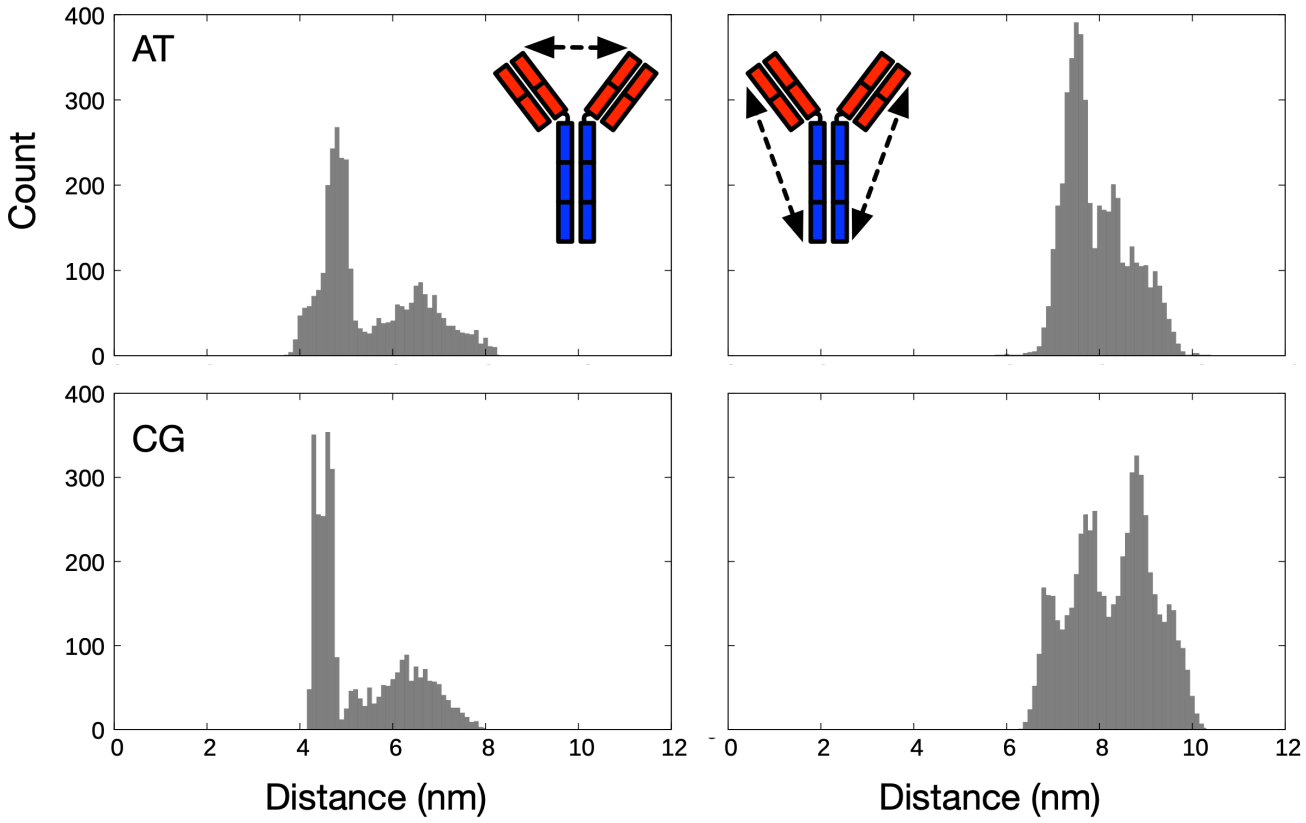

Figure S5: The distribution of distances from three 1 μs atomistic (top) and coarse-grained (bottom) simulations between the centers of mass of the two Fab domains in Pertuzumab (left), as well as between each Fab domain and the Cμ4 domain (right).

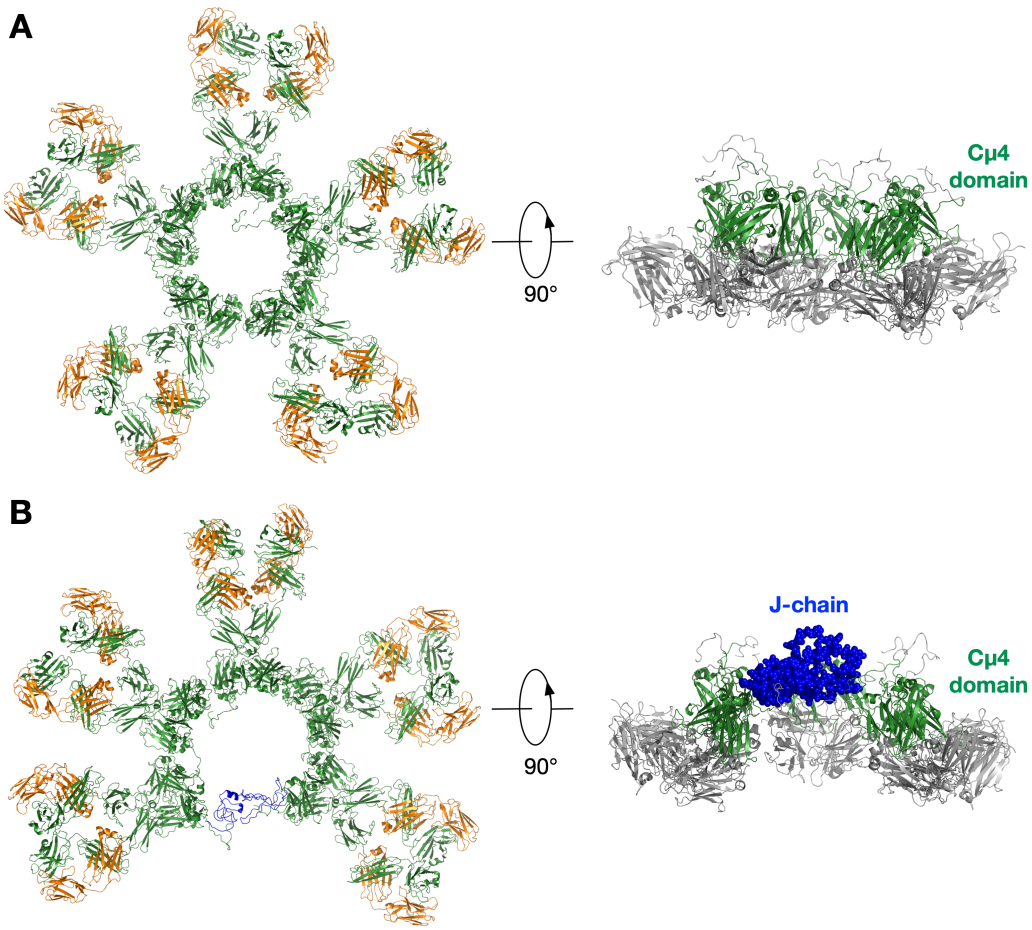

Figure S6: Pentameric Pertuzumab IgM. The model is shown **(A)** without and **(B)** with a J-chain in cartoon representation. Heavy chains are in green, light chains are in orange, and the J-chain is in blue. Side views highlighting the protruding Cμ4 domain and the J-chain are shown on the right.

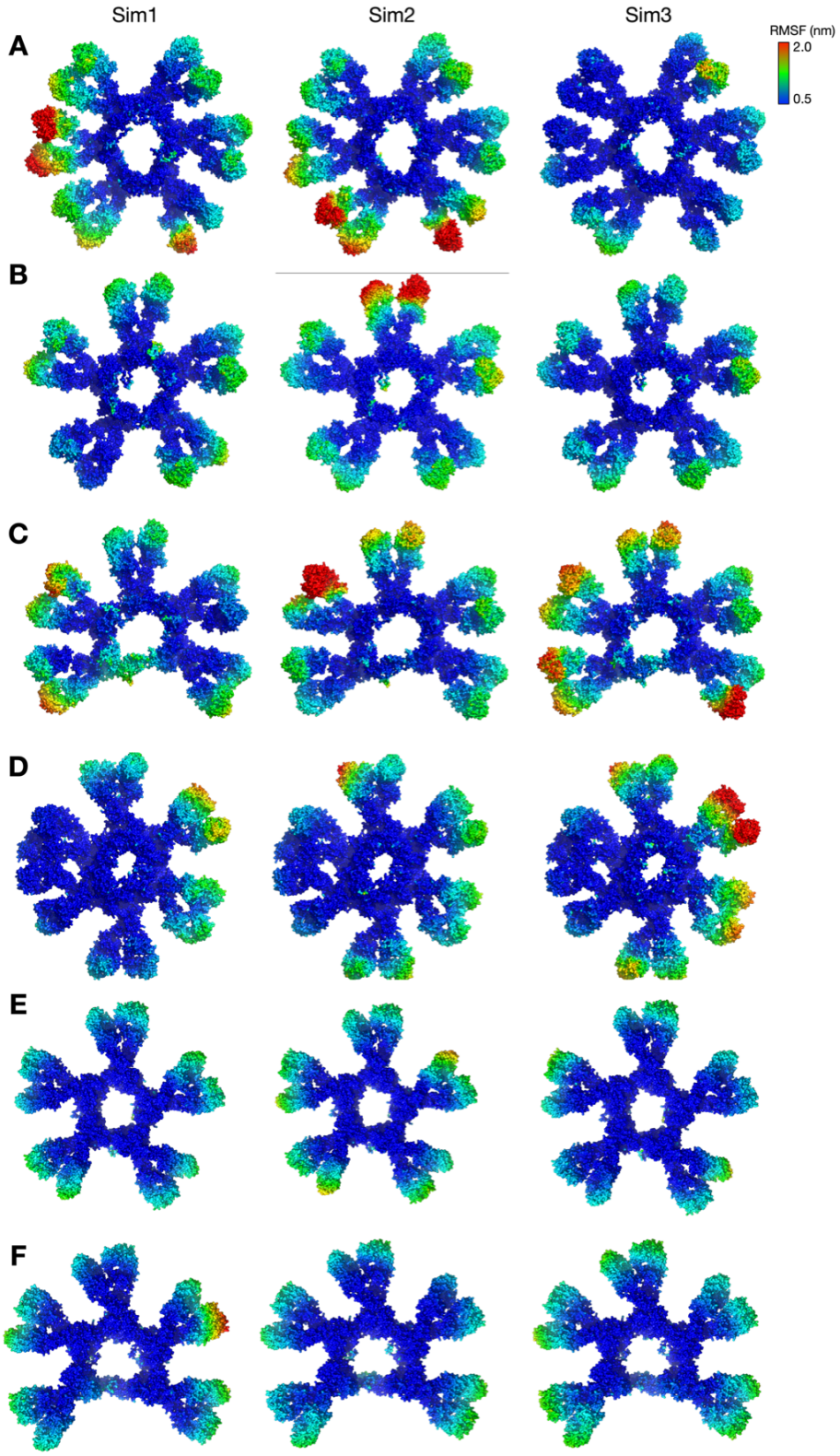

Figure S7: **Flexibility of the Fab domain in IgM multimers.** RMSF from three 10 μs simulations values mapped onto the protein surface for: **(A)** Pertuzumab IgM hexamer; **(B)** Pertuzumab IgM pentamer; **(C)** Pertuzumab IgM pentamer with a J-chain; **(D)** Trastuzumab IgM hexamer; **(E)** Trastuzumab IgM pentamer; **(F)** Trastuzumab IgM pentamer with a J-chain.

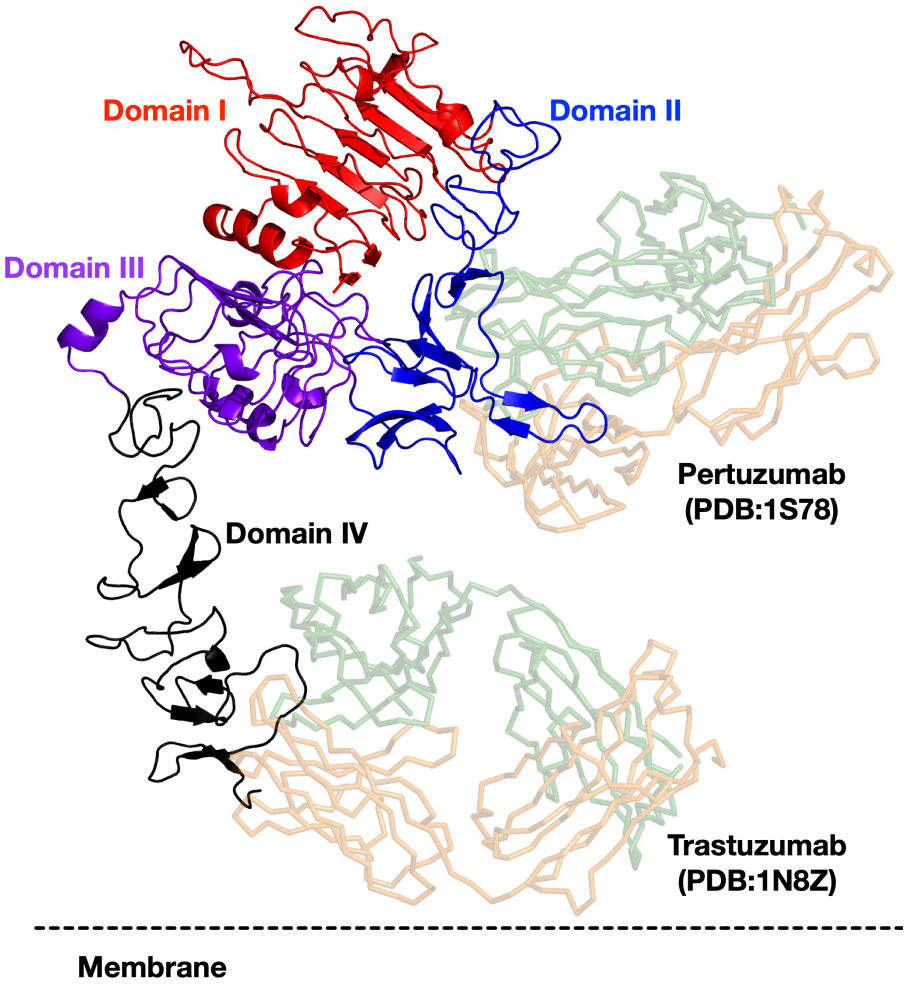

Figure S8: The binding sites of Pertuzumab and Trastuzumab on HER2 extracellular domain (ECD) as elucidated by X-ray crystallography (PDB: 1S78 and 1N8Z, respectively). The HER2 ECD is shown in cartoon representation and coloured based on its subdomains. The antibodies are shown in ribbon representation with the heavy and light chains in green and orange, respectively.

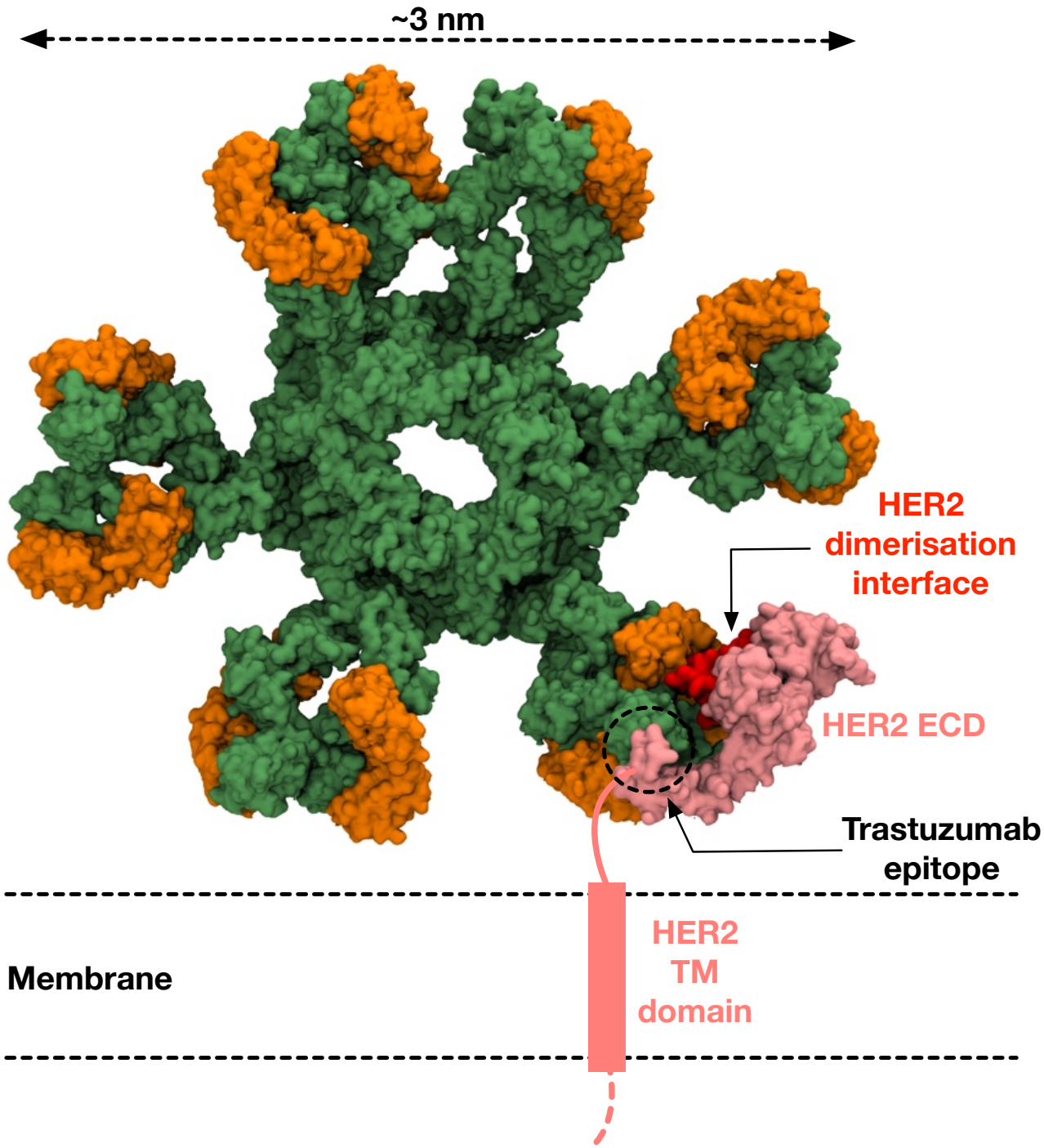

Figure S9: Modelling the binding of a single HER2 ECD to Trastuzumab IgM. Both HER2 and IgM are shown in surface representation. IgM heavy chains are shown in green and light chain in orange. HER2 ECD is shown in pink with the dimerisation interface highlighted in red and Trastuzumab binding epitope is circled. The approximate position of the membrane and HER2 transmembrane (TM) domain is indicated schematically. Due to the large size of IgM, a single binding of Trastuzumab IgM to HER2 ECD may block the dimerisation interface of the latter to prevent receptor dimerisation.

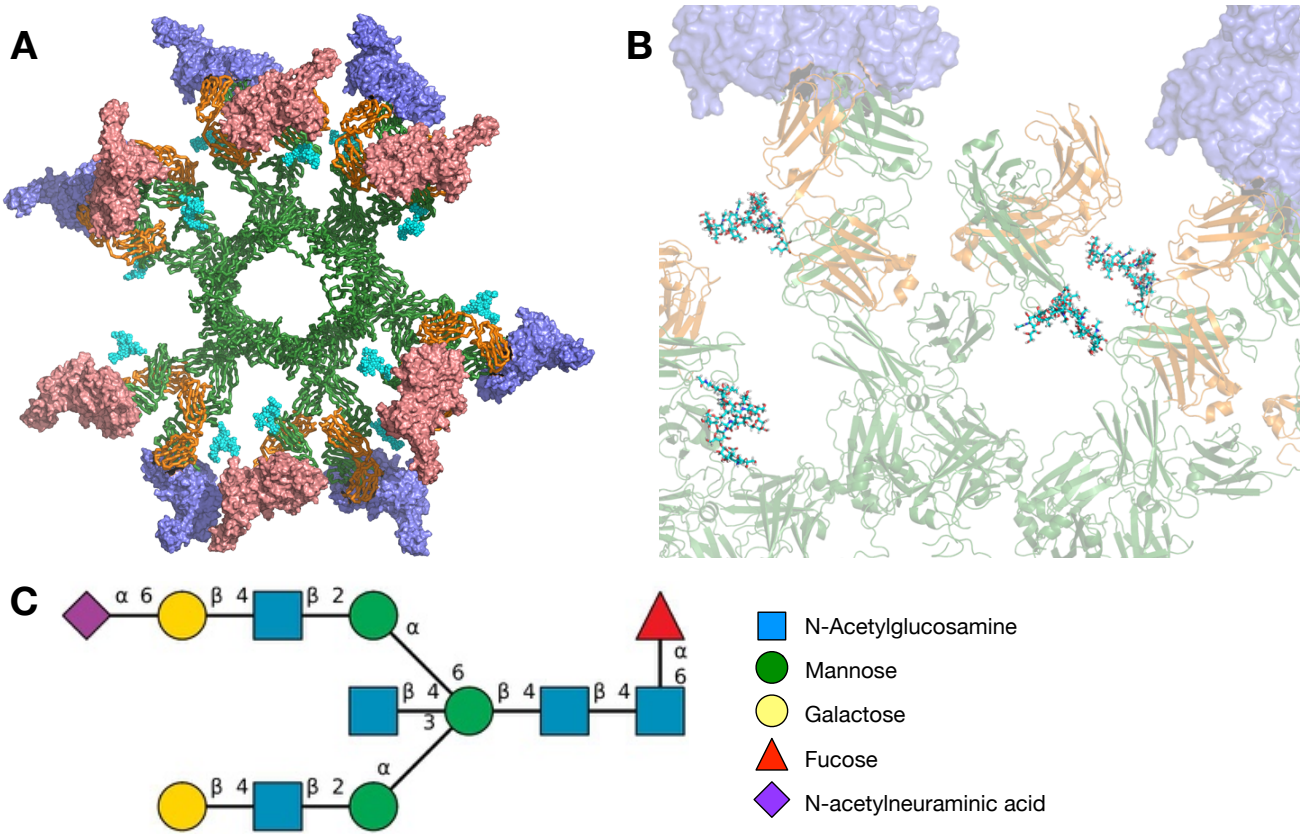

Figure S10: Modelling N-glycosylation on the Cμ1 domain of hexameric IgM. (A) The IgM hexamer (heavy chain in green and light chain in orange) is shown in ribbon representation, whilst HER2 ECDs (pink and purple) are shown in surface representation. Complex glycans on residue N163 of each of the heavy chains are shown in cyan using van der Waals representation. (B) Enlarged image of the structure highlighting the glycans in stick representation. The rest of the complex is shown in transparent format. (C) A schematic of the monosialylated complex glycan structure modeled on to the hexamer.