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Supporting information for article:

Structural and biophysical characterization of an epitope-specific engineered Fab fragment and complexation with membrane proteins: implications for co-crystallization

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Table S1 Fab/EE heavy chains (chain H and A) Kabat numbering and modelled residues. Residues in white were modelled in both chains. Residues in light grey were not modelled in chain H. Residues in dark grey were not modelled in chains H or A.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	35A	35B	49																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
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82	80383	80384	80385	80386	80387	80388

Table S2 Fab/EE light chains (chain L and B) Kabat numbering and modelled residues. Residues in white were modelled in both chains. Residues in light grey were not modelled in chain B. The residue in pale blue was not modelled in chain L. Residues in dark grey were not modelled in chains L or B.

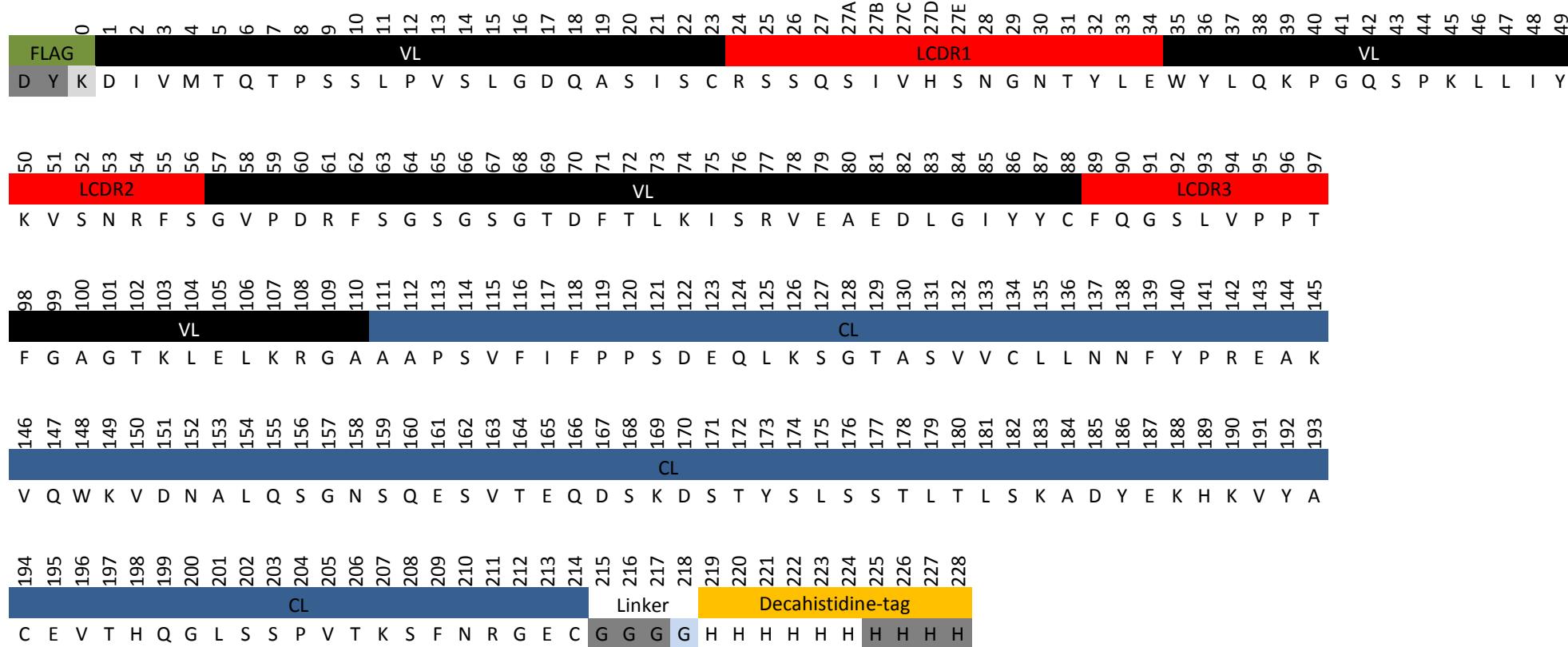


Table S3 Primers used for molecular biology and sequencing

Primer purpose	Forward primer	Reverse primer
Fab/EE sequencing	5'-GTGAGCGGATAACAATTCAC ACAGG	5'-GAGAAGGAGATATACATATGAAG TCG
SDM for A ₂ aR-GFP-EE	5'-CGACGACAGCTGAAGGGTAGT GAATATATGCCAATGGAAGGTAG TCAGATGGAGAGCCAG	5'-CAGCTGTCGCGCCGCCAGGA AGATCCG
pITy-A2aR-GFP-EE colony screening	5'-GGTTTGATTGTCTTGTGGC (5'prepro)	5'-CTACCTCCATTGGCATATATTG
pITy-A2aR-GFP-EE sequencing	5'-CACGACTTCTTCAAGTCCG 5'-GAGAGCAGGTCAGCCTCC	5'prepro,5'-GCCATCCGCGGCTTGTAC AGCTGTCCAT
SDM for intimin-EE2	5'-CGGCTACTTCCGTATGAGTGGT TGGCATCGGCCGGAATACATGCC CATGGAAGCGCGGATTACGATG AACGCCCGCAAATGGC	5'- GCCATTGCCGGCGTTCATCGT AATCCGCCGCTTCCATGGCATGTA TTCCGCCGCATGCCAACCAACTCATA CGGAAGTAGCCG

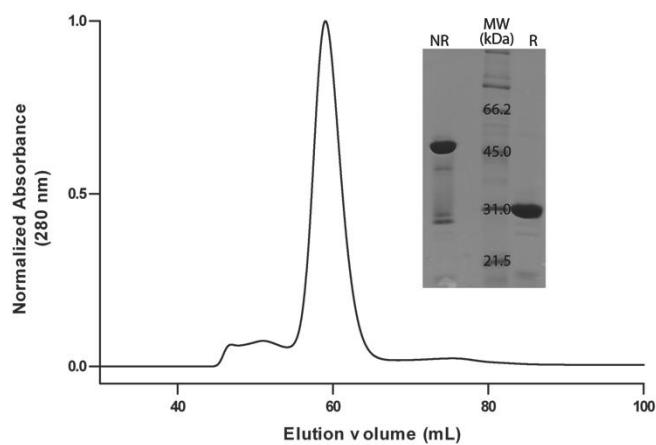


Figure S1 Fab/EE purification. Purified from the *E. coli* periplasm by Ni^{2+} -affinity followed by SEC, Fab/EE elutes from an analytical gel filtration column as a single peak at the expected elution volume. *Inset*, non-reducing (NR) and reducing (R) SDS-PAGE gel of a fraction taken from the major peak predominantly shows a single dominant band with >95% purity ~50 kDa and 31 kDa for non-reducing and reducing samples, respectively.

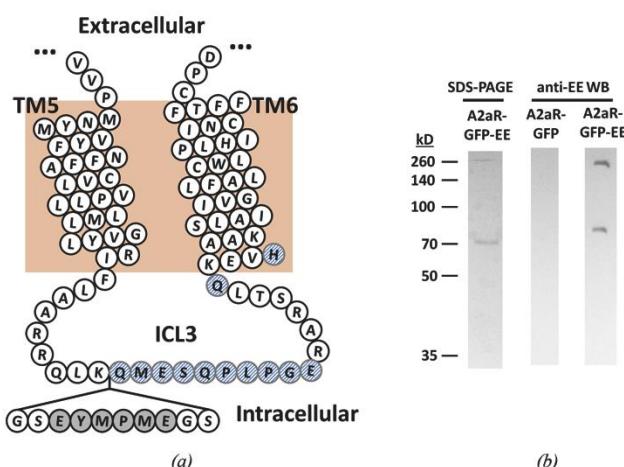


Figure S2 Design and purification of A_{2a}R-GFP-EE. (a) Collier de perles diagram of the A_{2a}R transmembrane regions 5-6 (TM5-6) depicting the insertion location for the EE peptide (shaded) with flexible linker residues. Residues homologous to those forming the Fab epitope in the Fab:GPCR co-crystal structure of β₂ adrenergic receptor (PDB code 2R4S) are shown using hatched circles. (b) SDS-PAGE gel with silver staining shows single band at the expected monomer size for A_{2a}R-GFP-EE after purification by Ni²⁺ affinity and SEC. Western blot of the same fractions using commercial anti-EE antibody confirms the presence of the EE peptide. The lower band represents monomer and the upper band dimer at the expected sizes.

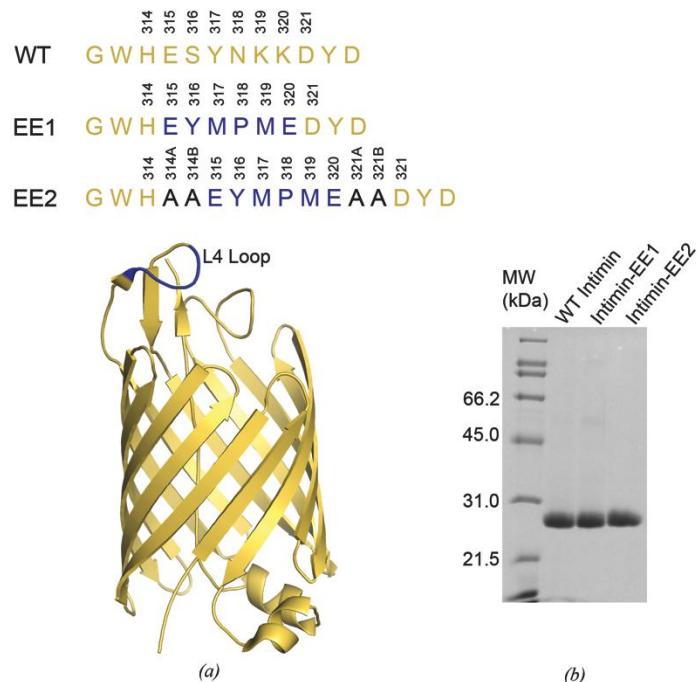


Figure S3 Construction and purification of EE-tagged intimin. (a) Residues of WT intimin loop L4 (yellow) were mutated to the EE epitope (blue) for intimin-EE1. Two alanine residues (black) were inserted on each side of the EE peptide for intimin-EE2. Numbering is as in Figure 4e-h. (b) Each intimin variant (WT, -EE1 and -EE2) were purified by Ni^{2+} affinity and SEC. Reducing SDS-PAGE shows a single band ~31 kDa for each variant.

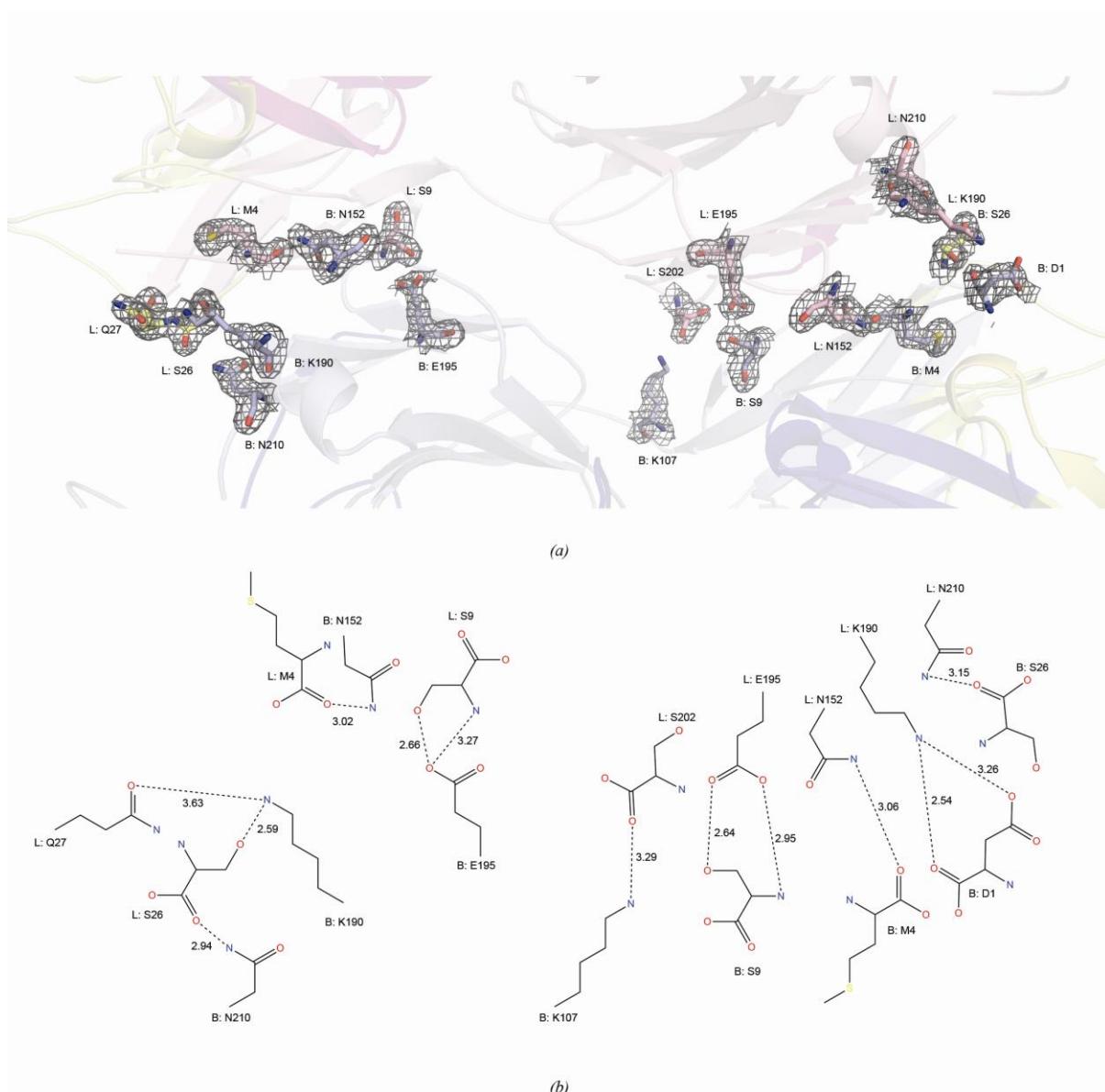


Figure S4 Interactions and final $2F_o - F_c$ electron density for crystal contact ID2 (see Table 3). (a) Electron density for residues involved in crystal contact, contoured to 1σ . (b) Interactions between light chain (chains L and B) residues in contact ID2 depicted as dashed lines, with distances (\AA) according to PISA analysis (Table 3).

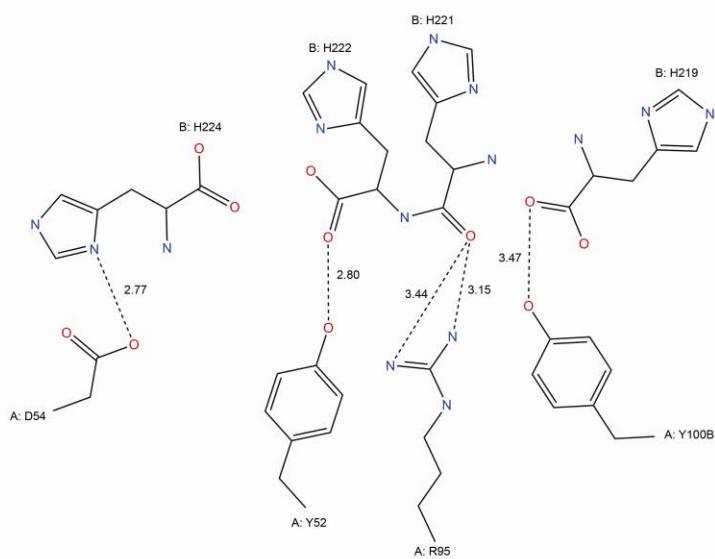
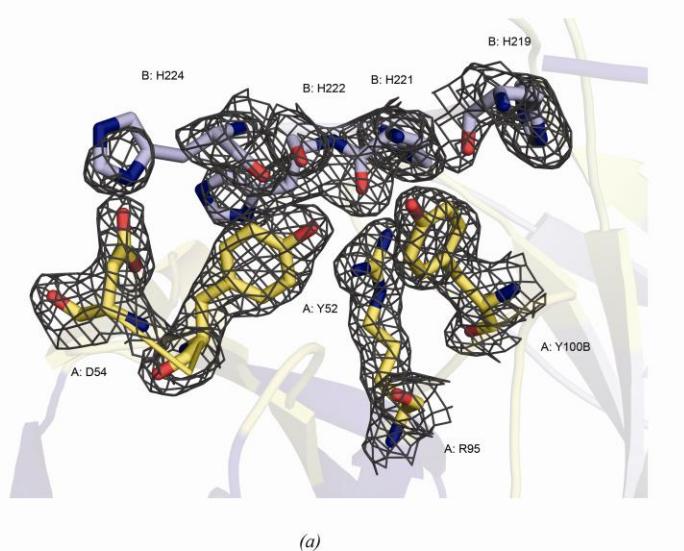


Figure S5 Interactions and final $2F_o - F_c$ electron density for crystal contact ID5 (see Table 3). (a) Electron density for residues involved in crystal contact from chains A and B, contoured to 1σ . (b) Interactions between the histidine-tag on the C-terminus of the light chain (chain B) and the CDR of the heavy chain of a symmetry related molecule (chain A). Interactions are depicted as dashed lines, with distances (\AA) according to PISA analysis (Table 3).