## Supporting Material

# NMR Observable-Based Structure Refinement of DAP12-NKG2C Activating Immunoreceptor Complex in Explicit Membrane

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**Table S1** System information

| System | # of water molecules | # of detergents/ lipids | # of ions | # of atoms | Box size ($Å^3$) |
|---|---|---|---|---|---|
| Micelle | 16,476 | 13 SDS, 130 TPC | 55 $K^+$, 44 $Cl^-$ | 60,230 | 83.8×83.8×83.8 |
| Bilayer | 7,411 | 129 DMPC | 19 $K^+$, 18 $Cl^-$ | 38,937 | 65.6×65.6×87.3 |

**Table S2** Orientation of DAP12-NKG2C transmembrane helix complex in terms of helix-helix distance (*D*) and crossing angle (Ω).

| Structural element | PDB:2L35 | | Micelle | | Bilayer | |
|---|---|---|---|---|---|---|
| | *D* (Å) | Ω (°) | *D* (Å) | Ω (°) | *D* (Å) | Ω (°) |
| DAP12-1 and NKG2C | 9.6±0.4 | 3.6±1.8 | 9.9±0.5 | 3.9±1.8 | 9.9±0.3 | 4.0±2.2 |
| DAP12-2 and NKG2C | 11.0±0.4 | 10.9±2.8 | 10.6±0.6 | 11.8±2.5 | 10.4±0.7 | 10.7±2.2 |
| DAP12-1 and DAP12-2 | 9.2±0.4 | 12.5±1.7 | 9.4±0.6 | 10.8±2.8 | 9.7±0.4 | 11.3±0.3 |

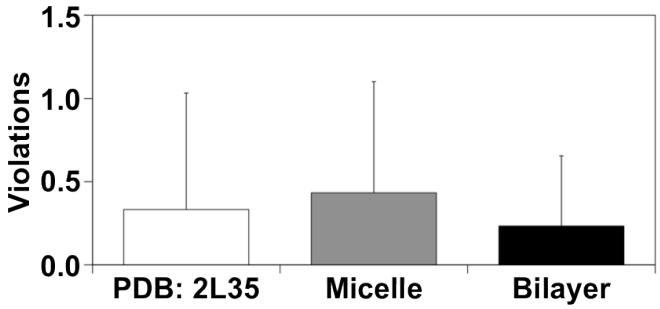


**Figure S1** The average number of violated NOE distance restraints in the different systems with a cutoff value of 0.5 Å. 15 structures with the least violations were selected for the restrained MD simulations.

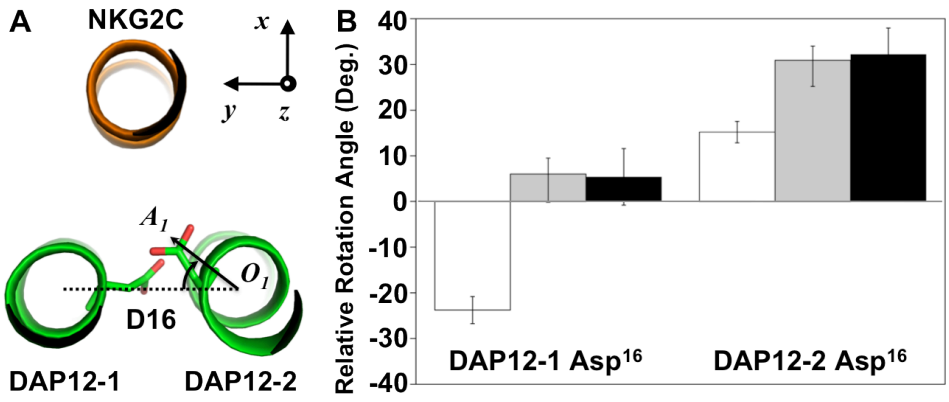


**Figure S2** Relative rotation angles of Asp[16] in DAP12. (A) The top view of one representative structure from the micelle systems. The rotation angles were calculated by aligning DAP12's principal plane to the *y*-*z* plane, keeping the NKG2C above the principal plane, and measuring the angles of the geometrical center of DAP12-2 Asp[16] side-chain oxygen atoms ($A_1$) from the DAP12-2 ($O_1$) relative to the principal plane (similarly for DAP12-1 Asp[16]). If the angle is negative, this Asp[16] is below the DAP12 principal plane and outside the interface. (B) Average DAP12 Asp[16]'s relative rotation angle in different systems (PDB:2L35: white, micelle: gray, bilayer: black).

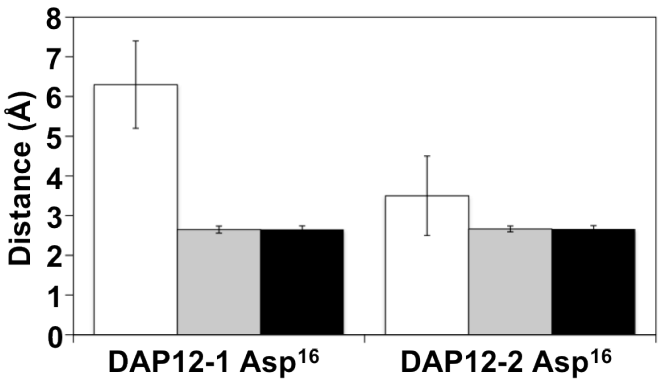


**Figure S3** The distance between the polar groups of DAP12 $Asp^{16}$s and NKG2C $Lys^{52}$ in the different systems (PDB:2L35: white, micelle: gray, bilayer: black). The distance is between the geometrical center of one DAP12 $Asp^{16}$ side-chain oxygens and the NKG2C $Lys^{52}$ side-chain nitrogen.

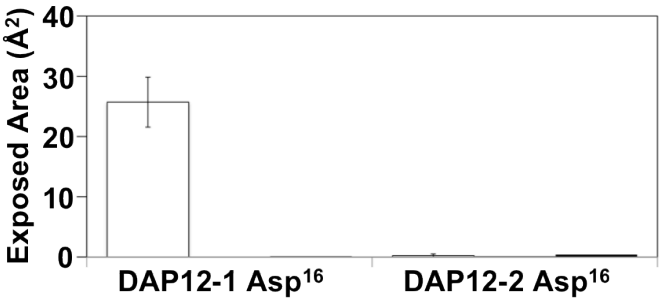


**Figure S4** Water accessible surface area of DAP12 $Asp^{16}$ in the different systems (PDB:2L35: white, micelle: gray, bilayer: black).

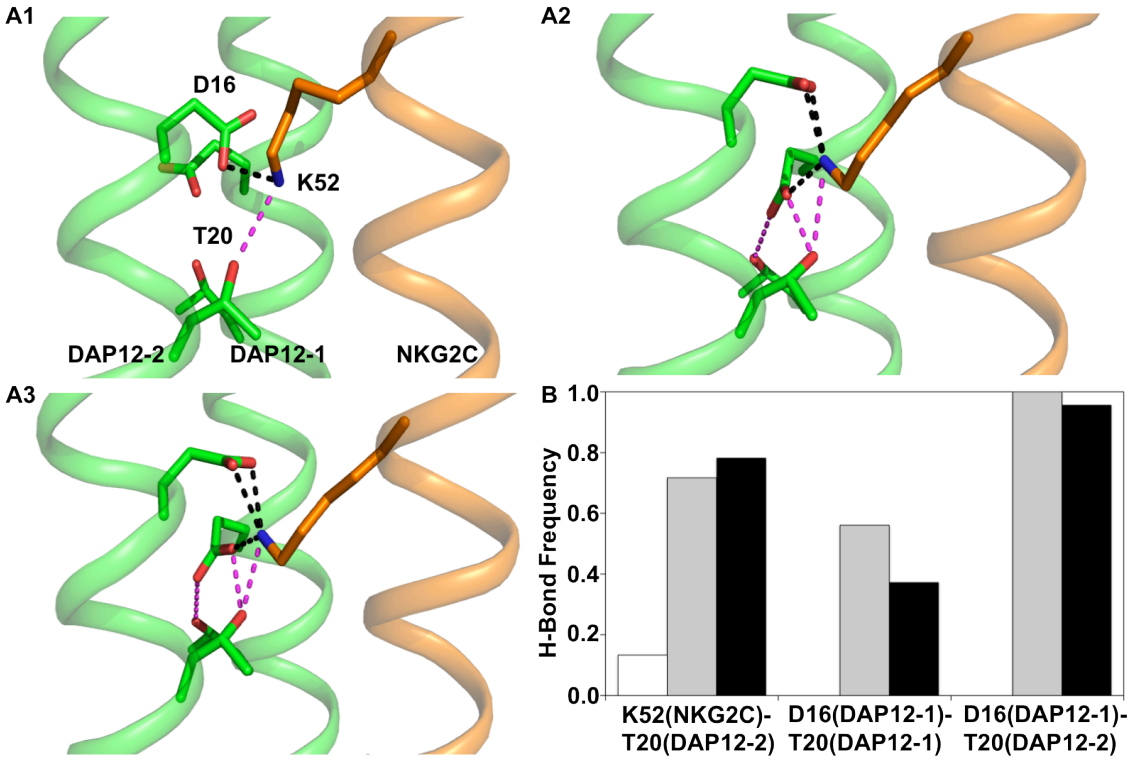


**Figure S5** The interaction network at $Asp^{16}$-$Thr^{20}$-$Lys^{52}$ in (A1) PDB:2L35, (A2) the micelle system, and (A3) the bilayer system, respectively. The H-bond is defined by $d \leq 2.8$ Å and $120° \leq \theta \leq 180°$, where $d$ is the distance between donor and acceptor atoms, and $\theta$ is the H-bond angle. The donors are HZ1/HZ2/HZ3 of NKG2C $Lys^{52}$ side chain, HG1 of DAP12-1/DAP12-2 $Thr^{20}$ side chain, and the acceptors are OG1 of DAP12-2 $Thr^{20}$ side chain, OD1/OD2 of DAP12-1 $Asp^{16}$ side chain (CHARMM atom types). The black dotted line represents the salt bridges. The magnate dotted line shows the all-possible putative H-bonds. In the refined structures, the NKG2C $Lys^{52}$ can form two H-bonds maximally. The frequency of forming those H-bonds in the different systems (PDB:2L35: white, micelle: gray, bilayer: black) are shown in (B).

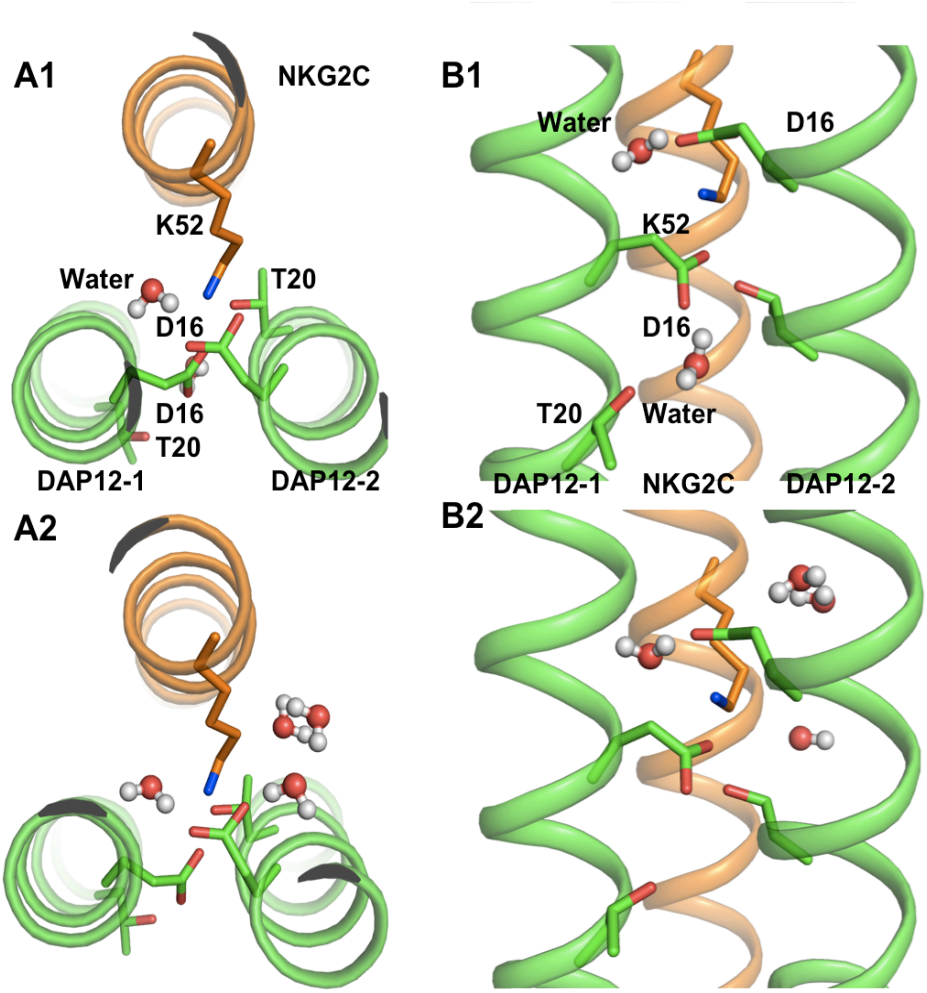


**Figure S6** Top and side views of the DAP12-NKG2C complex showing the water molecules (*spheres*) near the key interfacial residues (*sticks*) in (*A1, B1*) micelle or (*A2, B2*) bilayer systems. All other water molecules and the protein side chains are omitted for clarity.

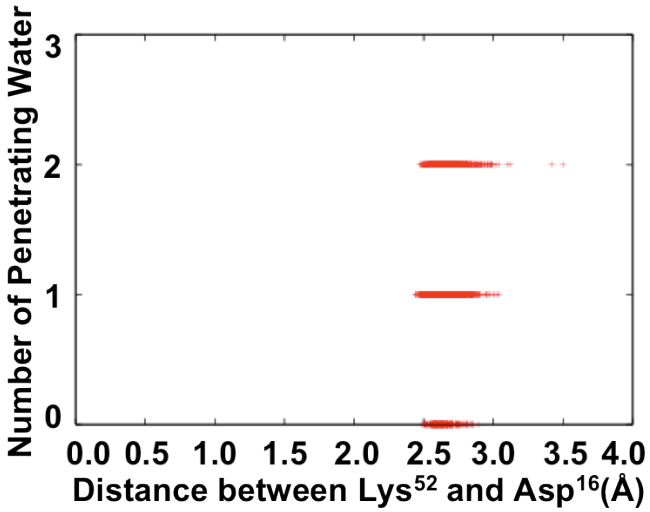


**Figure S7** The relationship between the number of water molecules near NKG2C Lys$^{52}$ and DAP12-1 Asp$^{16}$ and the salt bridge formation between NKG2C Lys$^{52}$ and DAP12-1 Asp$^{16}$. The salt bridge formation is indicated in the *x* axis by the distance between the NKG2C Lys$^{52}$ side-chain nitrogen and DAP12-1 Asp$^{16}$ side-chain oxygens from a MD-refined simulation system that had penetrating water molecules during the simulation. This plot illustrates that the salt bridge is maintained regardless of water molecules near the key polar groups.

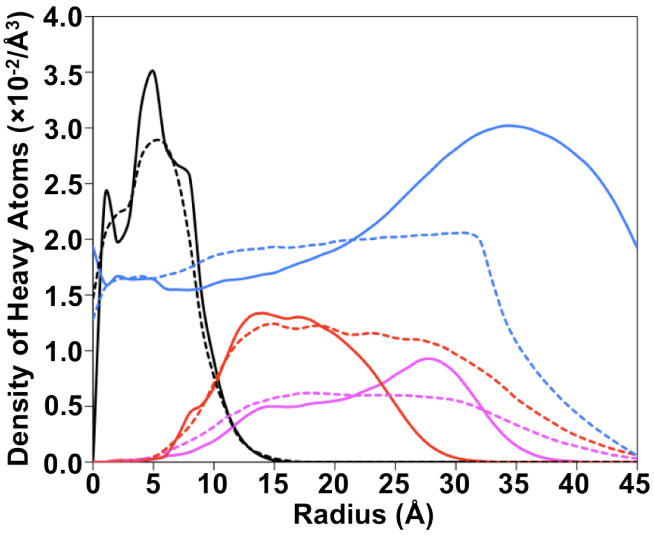


**Figure S8** Cylinder radial distribution of system components (protein: black, detergent/lipid head group: magnate, detergent/lipid carbon tail: red, water: blue) around the DAP12-NKG2C complex principal axis in the micelle (solid lines) and in the bilayer (dotted lines) systems. In the micelle systems, the principal axis of DAP12-NKG2C was aligned to the *z* axis. In the bilayer systems, the *z* axis corresponds to the membrane normal. The component distributions are similar in the micelle and in the bilayer within the radius of 20 Å.