New insights into the microscopic interactions associated with the physical mechanism of action of highly diluted biologics
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Supplementary Information
Supplementary Figure S1: van Hove self correlation function of (a) water in the pure water IFN-γ solution and (b) water in the IFN-γ mixed water – ethanol solution.
Supplementary Figure S2: van Hove self correlation function of hydration shell water of nA6 in (a) water and (b) in the water-ethanol mixture.
**Supplementary Figure S3:** (a) 3-D free energy surface derived from the FCA of the MD trajectory of IFNGR2 from the IFN-γ receptor complex in a mixed water-ethanol solvent. The labels refer to (1) the initial conformational substate that the receptor adopts before the dynamical transition, (1a) the intermediate state that the receptor adopts at the transition, and (2) the conformational substate the receptor adopts after the transition has taken place. The C-α representation of the IFNGR2 structure shows the dominant motion within the representative energy well, where the arrows depict the direction of motion. Red regions have more motion and blue regions are less mobile. (b) The clustering of representative conformations as a function of MD simulation time step. The inset shows a close-up view of the time period occurring right before and right after the transition to conformation 2. (c) The RMSD of hydrogen atom fluctuations of (top panel) water molecules and (bottom panel) of ethanol molecules in the immediate receptor hydration shell.

**Conformational analyses and transition probabilities of IFNGR2 in the IFN-γ complex** The biggest changes that we observe from the FCA analysis of IFNGR2 when transitioning from conformational substate 1 to 1a is (i) the position (angle) of the N-terminal surface loop that is involved with binding IFN-γ and (ii) the C-terminal loop region, that is far more rigid in the intermediate state when compared with the same region in conformation 1 as seen in Supplementary Figure S3a. From our analyses of the MD simulation trajectories, we find that both structural regions are strongly influenced by direct H-bonding with the solvent (water) in the hydration shell. It is likely that the strong H-bonds with the solvent stabilize the conformational intermediate (conformational substate 1a) and as a consequence also modulate the energy barrier that promotes the transition to conformational substate 2 and hence the dynamical transition of the entire receptor complex.

A clustering analysis of the MD simulation of the conformational substates of the receptor has revealed details about the dwell times within the conformational substates as well as the
transition probabilities among them. Using this analysis, we have determined that the majority of
the time (an average of 86.9% of the simulation time) the receptor is in conformation 1. The
receptor fluctuates within this energy basin until the receptor is able to overcome the barrier
separating conformation 1 and conformation 1a. The receptor spends on average only 5.5% of
the MD simulation time in conformation 1a before rapidly transitioning to conformation 2. The
receptor spends again only a short period of time in conformation 2 (3.8% of the MD simulation
time) before transitioning back to conformation 1. After this time period, we see arbitrary
fluctuations between the two major conformational substates (conformation 1 and conformation
2), but on average the receptor spends more time in conformation 1.

In Supplementary Figure S3b, a plot of the clustering of the conformational states as a function
of MD simulation time allows one to clearly discern the “jump” from conformation 1 to
conformation 2. The inset of Supplementary Figure S3b shows the close-up of the time region
preceding the transition from conformation 1 to conformation 2, where one can also distinguish
the transition into the intermediate state (conformation 1a) that is populated briefly before
transitioning to conformation 2.

The influence of the hydration water molecules in the relaxation pathway of IFNγR2 is also
apparent from the plot of the RMSD of the hydrogen atoms in the hydration shell in
Supplementary Figure S3c. Here we find that the dynamics of the water molecule hydrogen
atoms in the hydration shell are directly tied with the relaxation dynamics of the protein. The
change in the RMSD of the hydrogen atoms from the initial population in conformation 1 to the
population when returning to conformation 1 (after the transition from conformation 2) reflects
the more heterogeneous population of interactions after the (dynamical) transition has taken
place. For comparison, we also plot the RMSD of the hydrogen atoms of the ethanol molecules
in the receptor hydration shell. In the latter case, we determine that the ethanol molecules are not
closely coupled with the conformational substate dynamics of the receptor. The changes that we
see in the RMSD of the ethanol H-atoms from before the transition to after the transition reflect
to a greater extent, a more heterogeneous environment of protein – solvent molecule interactions
when compared with the interactions taking place before the transition.
Supplementary Figure S4: The calculated distance (d) dependent absorption coefficient of the protein sidechain fluctuations in the nA6 receptor complex hydration shell from the MD simulation in pure water.
Supplementary Figure S5: Surface filling representation of the nA6 receptor complex in (a) water and (b) in the water-ethanol mixed solvent showing the mobility of the residues in the complex. Regions colored in blue have less mobility and regions in red have more mobility. The arrows also depict the direction in which the residues move in the mobile regions of the antibody complex.
Supplementary Figure S6: (a) The MSD of water hydrogen atoms and (b) the self – ISF of water hydrogen atoms in the nA6 – IFNGR1 dimer complex (magenta) and the IFN-γ complex (cyan) in a mixed ethanol-water solvent. In both figures it is apparent the caging dynamics of the water hydrogen atoms in the IFN-γ complex play a more significant role in the β-relaxation regime preceding the longer time scale α-relaxation. Also, one can deduce that there are two visible boson peaks in the IFN-γ complex at approximately 90 fs and another at 180 fs. The IFNGR1 dimer complex has a single, peak at ~90 fs. The 90 fs peak is associated with the librational dynamics of water hydrogen atoms and the 180 fs with the translational dynamics of the atoms. Together, this suggests that the intermediate relaxation time scale of the hydrogen atom dynamics of the hydration water in IFNGR1 dimer is dominated by librational motions, while the same regime in the hydration water in the IFN-γ complex is governed by a combination of librational and translational dynamics.
Supplementary Figure S7: Error estimate for the block averaging of the RMSD of the 5 distinct MD simulation of the IFN-γ - IFNGR1-IFNGR2 complex in a pure water solvent. The analysis was carried out from the 10 – 50 ns (10,000 – 50,000 ps) using 780,000 points. In the plot, the red dotted line is the average of the individual RMSD measurements and the solid, black line is the error. It is clear from the plot that there is more variation between the measurements near the end of the simulation (around 30 ns) than at the beginning.