

Molecular dynamics simulation of engineered Fc-FcγRIIIa complexes as a framework for anticancer monoclonal antibody development

Jebamani Petrina¹, Nitin Lingayat², Murugan Mukilan³, Ravi Aiswarya Krishna³, Dhamodharan Prabhu^{1*}

¹Centre for Bioinformatics, Department of Biotechnology, Karpagam Academy of Higher Education, Coimbatore, India.

²Department of Paediatrics, Symbiosis Medical College for Women and Hospital, Symbiosis International (Deemed University), Pune, India.

³Department of Biotechnology, Sri Ramakrishna College of Arts and Science, Coimbatore, India.

ARTICLE INFO

Article history:

Received on: June 30, 2026

Accepted on: September 16, 2026

Available Online: XX

Key words:

Fc-FcγRIIIa, cancer immunotherapy, anticancer, lymphoma, monoclonal antibodies, molecular dynamics simulation

ABSTRACT

Therapeutic monoclonal antibodies exert immune-activating functions partly through the interaction between the antibody Fc region and the FcγRIIIa receptor on the effector cells. Increasing or decreasing binding affinity by engineering this interaction is a key approach taken in antibody drug development for cancer, but whether the changes in affinity are due to global transformation of protein conformation or due to localized changes only is unclear. Here, we address this using comparative 200-ns all-atom molecular dynamics simulations of three Fc-FcγRIIIa complexes: the wild type, the affinity-enhanced S239D/I332E double mutant, and the affinity-abolished V263D mutant, analyzed across seven analytical metrics. The affinity-enhanced complex remained structurally convergent, with backbone root mean square deviation stabilizing at 3.8 ± 0.3 Å, the first principal component capturing 51% of total positional variance, and a free-energy landscape confined to a single basin (~ 5 kJ/mol ceiling). The affinity-abolished complex showed progressive structural instability, with RMSD rising to 7.1 ± 1.2 Å in the second half of the simulation and conformational sampling dispersed across multiple free-energy basins (~ 7 kJ/mol ceiling). The wild type occupied a dynamically intermediate regime, with RMSD rising from 4.5 Å to 6–11 Å at approximately 95 ns. These results indicate that binding affinity is consistently associated with global conformational dynamics of the Fc-FcγRIIIa complex. Electrostatic stabilization through three salt bridges drives the enhanced mutant, while hydrophobic core disruption at Val263 drives the abolished mutant. These findings support multi-scale molecular dynamics simulation as a candidate screening approach for engineered therapeutic antibodies in cancer immunotherapy, pending validation with independent replicate simulations and variants of unknown affinity.

1. INTRODUCTION

Antibody-dependent cellular cytotoxicity (ADCC) is one of the principal mechanisms by which therapeutic monoclonal antibodies (mAbs) eliminate tumor cells and infected tissues [1,2]. This activity is mediated through binding of the antibody Fc region to FcγRIIIa (CD16a) on the surface of natural killer cells and macrophages, one of the several Fc receptors that regulate immune effector functions [3,4]. Monoclonal antibody therapies targeting this pathway have transformed the clinical management of hematological malignancies and solid tumors [1], and engineering the Fc-FcγRIIIa interaction remains a priority objective in anticancer drug development globally. Clinically important mAbs such as rituximab and trastuzumab depend on this interaction for their therapeutic effect [1], yet wild-type IgG1 Fc engages FcγRIIIa with only modest affinity ($K_D \sim 0.5$ to $1 \mu\text{M}$)

[5,6]. This limits the efficacy, particularly in patients carrying the lower-affinity F158 allelic variant of the receptor [7]. Engineering this interaction, therefore, has direct clinical consequences in two directions [8,9]. In cancer immunotherapy, enhancing Fc-FcγRIIIa binding amplifies ADCC potency [2,10]. In other settings, such as immune checkpoint inhibition, abolishing FcγR engagement is equally necessary; several approved checkpoint-blocking antibodies are engineered or isotype-selected specifically to prevent FcγR-mediated ADCC/ADCP, which would otherwise deplete the target-expressing immune cells that the antibody is intended to activate [11,12]. The S239D/I332E double mutation represents the most clinically validated affinity-enhancing strategy, conferring greater than 100-fold improvement in FcγRIIIa binding and incorporated into tafasitamab, approved by the US FDA in 2020 for relapsed or refractory diffuse large B-cell lymphoma [10,13,14]. At the opposite end of the affinity spectrum, the V263D substitution was recently identified through betweenness centrality analysis of the residue interaction network as a high-priority silencing mutation, confirmed by ELISA and flow cytometry to abrogate binding to low- and intermediate-affinity FcγRs [15]. These two mutations thus define

*Corresponding Author

Dhamodharan Prabhu, Centre for Bioinformatics, Department of Biotechnology, Karpagam Academy of Higher Education, Coimbatore, India.
E-mail: prabhu.dhamodharan@kahedu.edu.in

the functional boundaries of the Fc-Fc γ RIIIa affinity spectrum and together represent the most therapeutically relevant range of Fc engineering outcomes.

The structural basis of Fc-Fc γ RIIIa recognition has been established by X-ray crystallography, revealing key contacts including the proline sandwich interaction between Pro329 of the Fc CH2-A domain and Trp87/Trp110 of Fc γ RIIIa, and conserved electrostatic contacts between Fc and the receptor D2 domain [16]. Our own computational work has progressively characterized both variants at static and short-timescale resolution: HADDOCK docking confirmed that S239D/I332E introduces three new interfacial salt bridges without disrupting native contacts [17,18]; residue interaction network and 50-ns molecular dynamics (MD) analysis demonstrated enhanced electrostatic stability and hub connectivity in the double mutant [19]; and transition complex theory revealed that a ~60-fold reduction in k_{off} dominates the affinity improvement [20]. Together, these studies establish a detailed picture of the local interfacial consequences of both mutations. However, in addition to these studies, there are no other study that comparably examines the Fc-Fc γ R systems using MD. Kralj *et al.* [21] compared one Fc variant to the wild type in each of the 3 Fc γ Rs—Fc γ RIIa, Fc γ RIIb, and Fc γ RIIIa on shorter simulation timescales. Kutlu *et al.* [22] studied full and truncated Fc-Fc γ RI models on a time scale of 200 ns. Both studies did not characterize the conformational dynamics of a binding affinity spectrum and also did not use a multi-metric analytical framework to distinguish between affinity states.

Despite this, there remains an underlying and fundamental question. Do conformational signatures vary across analytical dimensions in a globally distinguishable manner in cases where the binding affinity is being modulated, or is affinity modulation just a local phenomenon, limited to the immediate binding site? This is of practical significance, as different affinity states might contain different, multi-scale dynamic fingerprints, with these fingerprints being used to calculate fingerprints as a screening criterion for Fc engineering without having to explicitly calculate the binding free energy of every variant. Conversely, if dynamic perturbations are restricted to local interfacial geometry, engineering strategies can focus exclusively on contact-level optimization. Prior MD simulations of this system have been limited to 50 ns or less, and pairwise comparisons of at most two complexes [19], which is insufficient to capture the full conformational landscape or draw systematic conclusions across a binding affinity spectrum. No study has simultaneously characterized complexes spanning the complete affinity range, i.e., enhanced, wild type, and abolished, within a single unified long-timescale framework. Addressing this gap requires a seven-metric analytical framework broad enough to detect convergent or divergent conformational signatures across multiple structural and dynamic dimensions, rather than relying on any single metric.

In this study, we address this gap by simulating three Fc-Fc γ RIIIa complexes spanning the full binding affinity spectrum: the wild type (WT), the S239D/I332E affinity-enhanced double mutant (+mut), and the V263D affinity-abolished mutant (-mut). Each complex is subjected to a single 200 ns all-atom molecular dynamics trajectory and evaluated using seven structural and dynamic parameters: backbone root mean square deviation (RMSD), radius of gyration (Rg), per-residue root mean square fluctuation (RMSF), solvent-accessible surface area, principal component analysis, free-energy landscape, and dynamic cross-correlation analysis. We demonstrate that the three binding affinity states produce distinct, consistently rank-ordered dynamical signatures at every analytical layer: the +mut complex maintains a structurally convergent, electrostatically constrained conformation with a single low-

energy thermodynamic basin, the -mut complex undergoes progressive structural drift driven by hydrophobic scaffold disruption and accesses non-functional conformational states absent from both functional complexes, and the WT occupies a dynamically intermediate regime characterized by a discrete mid-trajectory conformational transition invisible at shorter timescales. These findings indicate that binding affinity is consistently associated with conformational dynamics across the entire complex, not confined to the immediate binding interface, and offer a structural and dynamic explanation that builds on the kinetic insights from our prior work. The multi-scale computational framework described here offers a practical and generalizable method for assessing engineered protein-protein complexes, without the need for explicit free-energy calculations. The rest of this paper follows as below. In Section 2, system preparation, simulation, and analysis procedures are described. Section 3 presents the seven-metric comparative analysis across the three complexes. Section 4 interprets these findings against prior Fc-Fc γ RIIIa studies and addresses the mechanistic basis of affinity modulation. Section 5 summarizes the principal findings and their implications for antibody engineering.

2. MATERIALS AND METHODS

2.1. System Preparation

Three Fc-Fc γ RIIIa complexes were investigated in this study: the WT, the affinity-enhanced S239D/I332E double mutant (+mut), and the affinity-abolished V263D mutant (-mut). The initial three-dimensional structures of these complexes were obtained from previously optimized protein-protein docking models generated using the HADDOCK docking tool [15,17,20], which provided energetically favorable binding conformations validated against the crystal structure of the human IgG1 Fc fragment-Fc γ RIIIa complex (PDB ID: 1E4K) [16,23]. This docking protocol reproduced 27 of 31 experimentally determined binding interface residues at a C α RMSD of 1.24 Å relative to the crystal structure [17]. This model was selected from five candidate active-residue sets tested in the original docking study based on HADDOCK score and cluster size, with the reported model corresponding to the lowest-energy, most populated cluster. The S239D/I332E mutations were introduced computationally into both chains A and B of the Fc fragment as described previously [18]. The V263D mutation was introduced into chain B of the Fc fragment following the protocol described in our previous study [15]. The docked structures were used directly as starting coordinates to ensure consistency in comparative analysis and to preserve the validated binding interfaces. All structures were carefully inspected before the simulation. Missing atoms of the residue side chains were checked using Swiss-Pdb Viewer (SPDBV) (<http://www.expasy.org/spdbv/>) [24], and the structural integrity of each complex was verified. Each system was prepared independently using identical protocols to enable reliable comparison of dynamic behavior across the three binding affinity states. The crystal structure numbering used in Sondermann *et al.* [16] is used to identify residue locations in the Fc fragment throughout the present study. To get a better understanding of the plot, residue indices were used to start from 0.

2.2. Molecular Dynamics Simulation

Molecular dynamics simulations were done using the software package GROMACS 2025.2 [25–27]. All protein interactions were described using the CHARMM36 all-atom force field [28–31]. All complexes were explicitly solvated in a cubic simulation box using the TIP3P water model with a distance of at least 1.0 nm between the protein and the edges of the box to avoid periodic image artifacts. To neutralize each system, counter ions (Na⁺/Cl⁻) were added at a physiological concentration (0.15 mol/l).

The maximum force was converged to less than 1000 kJ mol⁻¹ nm⁻¹ to eliminate steric clashes and unfavorable contacts that were introduced during the system setup, and the energy minimization was performed by the steepest descent algorithm. After minimization, two-stage equilibration was performed on each system. Temperature equilibration was performed under the NVT ensemble for 100 ps at 300 K using the velocity-rescale thermostat ($\tau_T = 0.1$ ps), with the protein backbone position-restrained; this thermostat was retained through NPT equilibration and the 200-ns production run. Pressure equilibration was then performed under the NPT ensemble for 100 ps at 1 bar using the Parrinello-Rahman barostat (isotropic coupling, $\tau_p = 2.0$ ps, compressibility = 4.5×10^{-5} bar⁻¹), with position restraints retained from the NVT phase. Production simulations were carried out for 200 ns per complex with an integration timestep of 2 fs under periodic boundary conditions in all three dimensions, with trajectory coordinates saved every 10 ps (20,000 frames per simulation).

Long-range electrostatics were treated by the particle mesh Ewald method (order 4, 0.16 nm grid spacing), with Coulomb and van der Waals interactions truncated at 1.0 nm (Verlet cutoff scheme, dispersion correction applied to energy and pressure). Neighbor list was updated every 10 steps, and bonds involving hydrogen were constrained with LINCS (order 4). All three systems had the same settings throughout minimization, equilibration, and production. Production trajectories were post-processed prior to analysis to correct for periodic boundary jumps and to reconstruct full molecules. We adopted this trajectory of protein only for subsequent structure and dynamics analyses.

2.3. Structural Stability Analysis

The stability of each complex was evaluated by calculating the RMSD of the complexes using the `gmx rms` command in GROMACS. To demonstrate equilibration behavior and major conformational transitions, RMSD profiles were calculated for 200-ns trajectory. The radius of gyration (R_g) was also determined as a complementary overall structural compactness measurement using the `gmx gyrate` utility. The global compactness of the three complexes, structural expansion or contraction, was determined using the changes in R_g with time.

2.4. Residue-Level Flexibility

The root mean square fluctuation of $C\alpha$ atoms was calculated in R (version 4.1.2) using the `bio3d` package [32]. GROMACS format trajectories (XTC) were converted to DCD format, and R functions were used to read aligned structures. The coordinates of the $C\alpha$ atoms were extracted, and the trajectory was fitted to the reference structure using `fit.xyz()`, with $C\alpha$ atoms being the atoms used for fitting. For the 200 ns trajectory, RMSF values were computed for all residues over the Fc fragment (chains A and B) and FcγRIIIa receptor (chain C), and binding interface residues were investigated for the respective regions of each profile.

2.5. Solvent Accessibility Analysis

The solvent accessible surface area (SASA) of the full Fc–FcγRIIIa complex was determined for the 200 ns trajectory with `gmx sasa`. SASA was calculated at every saved trajectory frame (every 10 ps, yielding 20,000 data points per system). Time-series plots were prepared for each system for a direct comparison.

2.6. Principal Component Analysis

Principal component analysis (PCA) was applied to identify large-scale collective motions in each complex over the 200-ns production run. $C\alpha$ atoms of the entire protein complex, comprising both Fc

chains and FcγRIIIa, were selected for least-squares fitting to remove overall translational and rotational motion, and the covariance matrix of the fitted $C\alpha$ positional fluctuations was then constructed using the `gmx covar` utility in GROMACS. PCA was performed independently for each of the three systems, using its own trajectory, covariance matrix, and eigenvectors. Comparisons across systems are therefore valid for variance concentration and cluster topology, but PC1 and PC2 coordinate values are not defined on a shared basis and should not be compared directly across systems. Eigenvalue decomposition was performed, and the trajectory was projected onto the first two principal components (PC1 and PC2) using `gmx ana eig`. The distribution of conformational states sampled by each complex was visualized in the PC1-PC2 subspace. Color-coded projections from early and late trajectory frames were compared to assess the evolution of conformational sampling over the course of the simulation. Understanding the components of PCA is only reliable as the conformational sampling that has been obtained in the simulation, and the interpretability is necessarily limited by the degree of sampling.

2.7. Free-Energy Landscape

The free-energy landscape (FEL) of each complex was calculated with the `gmx sham` utility, with backbone RMSD and radius of R_g as the two reaction coordinates. The two-dimensional probability distribution of the sampled states in the subspace of RMSD- R_g was converted to Gibbs free energy with the Boltzmann relation

$$\Delta G = -k_B T \ln(P) \quad (1)$$

where k_B is the Boltzmann constant, T is the simulation temperature (300 K), and P is the normalized probability of each bin. Regions of low free energy correspond to thermodynamically stable conformational basins, while regions of high free energy represent rarely sampled or unstable conformations. Differences in the number, depth, spatial extent, and energy ceiling of basins across the three complexes were used to characterize how binding affinity state modulates the thermodynamic accessibility of conformational space. The energy scale in each landscape is defined relative to the most populated bin in that system's own probability distribution, which is set to $\Delta G = 0$, consistent with standard practice for single-trajectory free-energy landscape reconstruction. Because the ceiling values are computed identically across systems from a common Boltzmann relation and reference convention, they are directly comparable to one another; however, as single-trajectory, finite-sampling estimates, they are best used for relative ranking of conformational accessibility across systems rather than as precise thermodynamic quantities. The axis ranges shown in each panel of Figure 6 are scaled to the region of RMSD and R_g space actually sampled by that system, which differs across the three complexes. This visualization scaling is independent of the underlying ΔG values, which use the same zero reference convention and the same units in all three systems and form the basis for the energy ceiling comparison reported in Results and Table 1.

2.8. Dynamic Cross-Correlation Analysis

Correlated motions between all pairs of residues were analyzed using the dynamic cross-correlation matrices (DCCM) using the `bio3d` package in R [32]. The `dccm()` function was applied to the full aligned trajectory using the $C\alpha$ atoms after conversion to DCD format and alignment. Coefficients range from -1 to +1, where values close to +1 indicate fully correlated (synchronized) motion, values close to -1 indicate fully anti-correlated (opposing) motion, and values near zero indicate uncorrelated motion. The DCCM heatmaps were generated

Table 1. Summary of the seven analytical metrics across the three Fc-Fc γ RIIIa complexes.

Metric	Positive mutation	WT	Negative mutation
RMSD (mean / max)	3.8 Å / 5.48 Å	4.5-6.3 Å / 11.05 Å	5.6-7.1 Å / 11.88 Å
RMSF (interface loops)	Dampened	Moderate	Highest
Rg (mean)	3.32 nm, stable	3.38 nm, drifting up	3.30 nm, high-amplitude
SASA (mean)	337.85 nm ² , declining	340.63 nm ² , stable	337.62 nm ² , non-monotonic
PCA (PC1 / PC1-3)	51.0% / 67.6%	26.9% / 55.4%	34.3% / 57.7%
FEL (ceiling, basins)	5 kJ/mol, single basin	5 kJ/mol, multi-basin	7 kJ/mol, multi-basin + satellites
DCCM (relative intensity)	Highest	Intermediate	Lowest

and plotted by `plot.dccm()` with a custom color palette (blue for negative and red for positive correlations). DCCM was computed over the same full 200 ns, 20,000-frame trajectory employed for the RMSD and PCA analyses of each system.

3. RESULTS

3.1. Structural Stability: Root Mean Square Deviation Analysis

The structural stability of the three Fc-Fc γ RIIIa complexes during the 200-ns simulation period was evaluated through backbone RMSD values relative to the initial minimized structure (Fig. 1).

The S239D/I332E mutant (Fig. 1b) exhibited markedly greater conformational stability than the other two systems. RMSD values converged rapidly to approximately 3.8 Å within the first 50 ns (3.78 ± 0.38 Å) and remained stable throughout the trajectory: 3.80 ± 0.24 Å (50–100 ns), 3.82 ± 0.27 Å (100–150 ns), and 3.79 ± 0.25 Å (150–200 ns). The consistently low standard deviation across all windows and a maximum RMSD of 5.48 Å indicate that the S239D and I332E substitutions introduced into both chains A and B of the Fc fragment impose a pronounced restriction on the conformational dynamics of the complex. This stabilization can be attributed to the three salt bridges formed at the interface—Asp239(A)-Lys158(C), Asp239(B)-Lys117(C), and Glu332(A)-Lys158(C)—which were identified in previous structural studies of this mutant [18,19]. They act as geometric constraints that suppress large-scale conformational fluctuations and maintain the relative positioning of the Fc fragment and Fc γ RIIIa in a structurally stable, well-ordered state throughout the simulation.

The WT complex (Fig. 1a) exhibited moderate yet variable structural fluctuation throughout the simulation. Mean backbone RMSD values of 4.53 ± 0.77 Å and 4.81 ± 0.73 Å were observed during the 0–50 ns and 50–100 ns intervals, respectively, broadly comparable to the S239D/I332E mutant values during the same period and indicating initial stability of the docked conformation. However, at approximately 95.5 ns, the RMSD underwent a distinct upward transition, rising to values of 6–11 Å. A notable conformational excursion was recorded between 100 and 150 ns (mean: 6.25 ± 1.62 Å), with instantaneous RMSD reaching up to 11.05 Å, before partially resolving to 5.19 ± 0.65 Å in the final 50 ns. Partial recovery of stability distinguishes the WT from the V263D complex, where the RMSD stayed elevated, and indicates that the WT interface may have affinity-enhancing electrostatic contacts that remain intact even in the absence of such electrostatic interactions. The conformational change captured during the 95–150 ns of the additional simulation time was not observed in our previous simulations of 50 ns of the WT complex [19], underlining the need for longer simulation timescales for this system to capture the full dynamic behavior. The lack of convergence within 200 ns in all the windows indicates that the Fc region is inherently flexible, and that the

WT complex samples a large conformational ensemble that may not be captured within that timeframe. The V263D complex (Fig. 1c) had the highest degree of structural deviation of all three systems. RMSD values were found to rise steadily from 5.61 ± 0.98 Å during the first 50 ns to 5.64 ± 0.73 Å between 50 and 100 ns, suggesting an initial period of continuous drift from the initial conformation. In the second half of the simulation, the deviation became more significant with 7.06 ± 1.18 Å for 100–150 ns, and remained high at 6.93 ± 1.13 Å for the final 50 ns. The RMSD values were the highest, with a maximum of 11.88 Å, for all complexes. The V263D mutant system, on the other hand, did not recover at the end of the trajectory but instead had a gradual drift up with a considerable variance in the second half of the simulation, reflecting the progressive destabilization of the native Fc conformation. The hydrophobic core of Pro238, Val263, Val266, and Leu328 in chain B of the Fc fragment is disrupted by mutation of the aspartate at position 263(B) [15]. The core is usually the structural framework that keeps the Fc region in a conformation to which the Fc γ RIIIa is capable of binding. This anchoring function is destroyed by the disruption, and the binding interface is destabilized, allowing the complex to freely drift away from the initial docked geometry toward another stable equilibrium state. This behavior is fully consistent with the experimental result that the binding affinity of Fc γ R is lost in this variant. Taken together, the RMSD profiles create a clear rank order of conformational stability of -mut, WT, and +mut, which closely resembles the experimental binding phenotype: +mut is the most stable, followed by WT, and then -mut is the least stable (Table 1). The affinity-enhancing double mutant maintains the complex in a well-ordered state throughout the simulation. The WT is more flexible and adopts a wider variety of conformations and has a distinctly different mid-trajectory conformational change, which illustrates the intrinsic flexibility of the unmodified interface. By contrast, the V263D substitution breaks the structure continuously across the simulation due to the loss of receptor binding, which can be attributed to conformational destabilization of the Fc region in this variant. The ranking and temporal behavior (convergence or drift) of the three systems is the main parameter of interest, rather than the RMSD, which represents absolute displacement from the initial structure. Absolute RMSD values were not comparable since all simulations were equilibrated in the same manner.

3.2. Per-Residue Flexibility: Root Mean Square Fluctuation Analysis

In order to assess the residue-level flexibility in the Fc-Fc γ RIIIa complex, the RMSF of the C α atoms was calculated along the 200 ns trajectory for the Fc fragment (Chains A and B, black) and for the Fc γ RIIIa receptor (Chain C, blue) in all three systems. The red dashed vertical line in each panel indicates the boundary between Chain A and Chain B within the concatenated Fc residue index (Fig. 2). Each of the two Fc heavy chains consists of 216 residues, Chain A covering

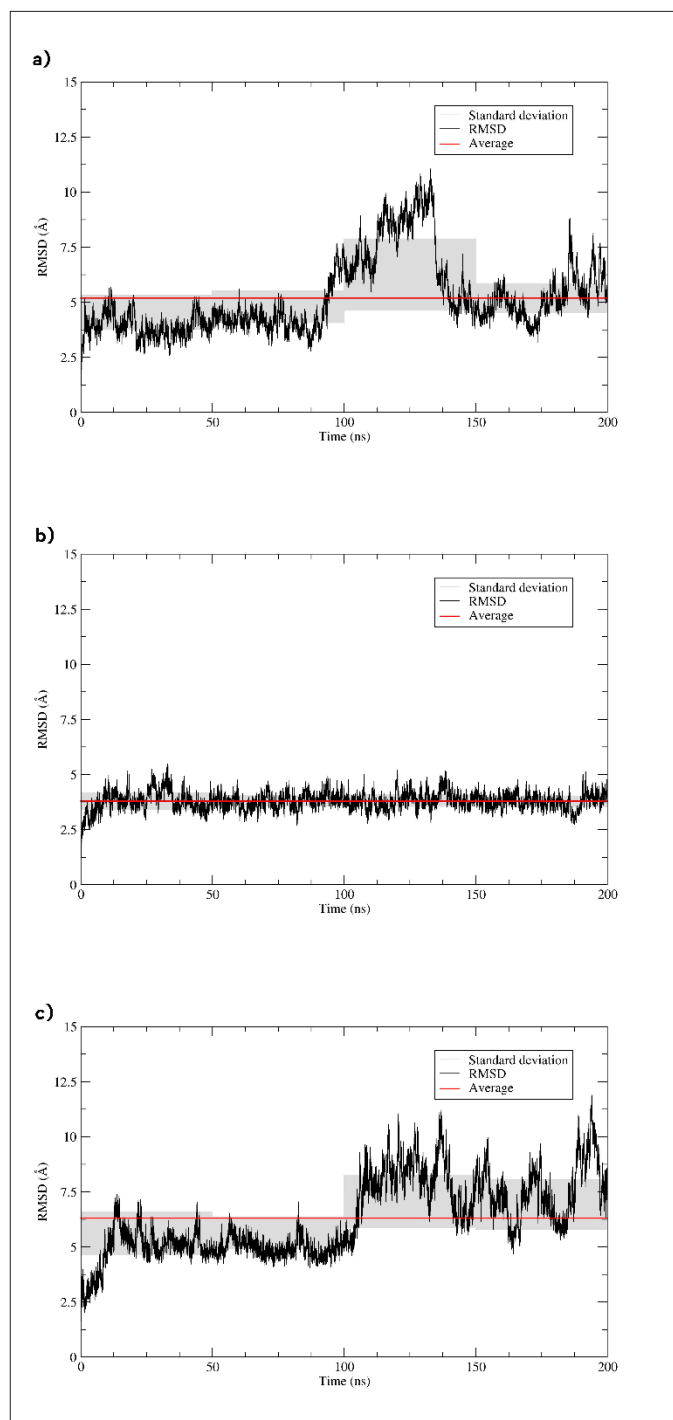


Figure 1. Backbone RMSD profiles of the (a) wild-type, (b) S239D/I332E affinity-enhanced mutant, and (c) V263D affinity-abolished mutant Fc–FcγRIIIa complexes calculated relative to the initial minimized structure over the full 200 ns production trajectory. Black lines represent the instantaneous RMSD, and red horizontal lines indicate the overall trajectory average. The y-axis scale is identical across all three panels to facilitate direct comparison.

residues 1 to 216 and Chain B residues 217 to 432 on the concatenated RMSF index according to EU numbering. Chain C (FcγRIIIa) includes residues 2 to 172 and is plotted on the same axis separately. Analysis of the PDB coordinates showed that the BC loop (Ile85–Trp87) and the FG loop (Arg152–Lys158) of FcγRIIIa are the main receptor contact regions, both of which are located within 5 Å of the Fc fragment [18].

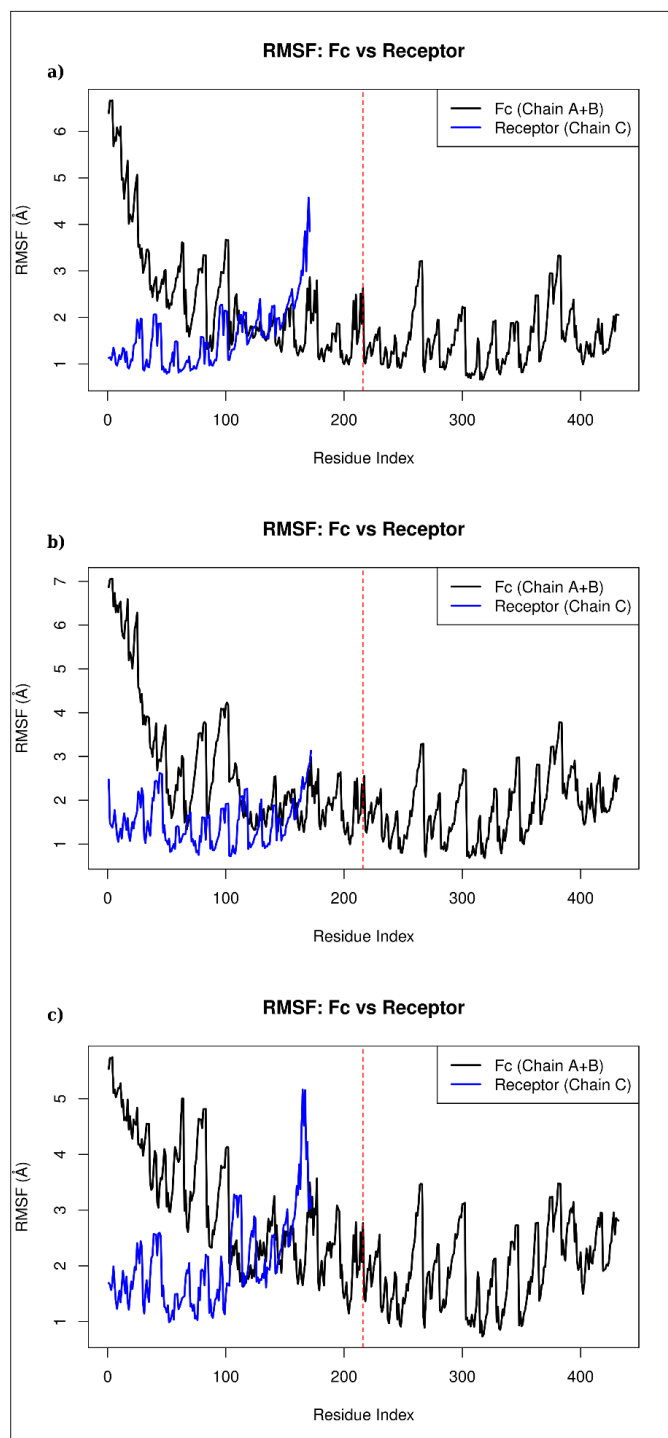


Figure 2. Ca root mean square fluctuation (RMSF) profiles of the Fc fragment and FcγRIIIa receptor in the (a) wild-type, (b) S239D/I332E (+mut), and (c) V263D (-mut) complexes over the 200 ns trajectory. The Fc fragment is shown in black and FcγRIIIa in blue. The red dashed vertical line marks the chain boundary within the concatenated Fc residue index. The figure highlights differences in local flexibility, particularly at the BC and FG loops of FcγRIIIa that form the primary receptor-binding interface.

In the WT complex (Fig. 2a), the Fc fragment showed high flexibility at the N-terminus of Chain A (with an RMSF of about 6.5 Å), which is in agreement with the conformational lability observed in the residues near the hinge region. Fluctuations throughout the rest of Chain A and Chain B were moderate (about 1–3.5 Å), with local

peaks corresponding to loop regions exposed to solvent. The FcγRIIIa receptor (shown in blue) was relatively stable over most of its length (1–2 Å). The residues in the BC loop (Ile85-Trp87) and the FG loop (Arg152–Lys158) displayed moderate fluctuations (about 1.5 to 2.5 Å), indicating that there was partial conformational sampling at the binding interface in the wild type. There is a clear RMSF peak (approximately 4.5 Å) close to the C-terminal end of the receptor trace (at index about 170, i.e., corresponding to residues Gln171–Gly172). This area is not part of the Fc-binding interface and likely reflects flexibility of the terminal chain rather than a functionally significant conformational change.

The S239D/I332E double mutant (Fig. 2b) had a broadly comparable Fc fluctuation profile to the WT, with increased N-terminal Chain A fluctuations (~7 Å) and moderate values of 1–4 Å across both chains. The FcγRIIIa receptor in this system exhibited markedly reduced per-residue fluctuation compared to the WT. The two primary binding interface loops, the BC loop (Ile85-Trp87) and the FG loop (Arg152–Lys158), showed reduced flexibility. The C-terminal peak at index ~170 was substantially diminished, reducing from ~4.5 Å in the WT to ~2.8 Å in the +mut system. Across its full length, the receptor fluctuation profile stayed flat and low, ranging between ~1 and 2.5 Å. The suppression of flexibility in the binding-interface loops is mechanistically significant. It is known that the S239D and I332E substitutions lead to the formation of additional hydrogen bonds and electrostatic contacts with FcγRIIIa. These interactions restrict the conformational freedom of the receptor BC and FG loops. This observed decrease in RMSF would thus explain the mutations that render the receptor in a more constrained conformation that is binding-competent, as seen in these loops. This interface becoming rigid, characteristic of high-affinity complexes, directly supports the increased affinity of this variant.

In contrast, the V263D mutant was also the most flexible on a per-residue basis in both components of the complex (Fig. 2c). The Fc fragment had a lower N-terminal peak (~5.5 Å), but a very high fluctuation profile of about 2–5 Å in large parts of Chains A and B, consistent with the RMSD analysis results of the global structural destabilization. The fluctuation for the FcγRIIIa receptor was higher than for the WT and S239D/I332E mutant, with higher degrees of flexibility for the residues at the interface between the two loops, BC loop (Ile85-Trp87) and FG loop (Arg152–Lys158), and a C-terminal peak at ~170 Å, the highest of all three systems. The increased mobility of the receptor interface loops suggests that the substitution of valine with aspartic acid at position 263 in Chain B does not have a local effect but rather provides disorder in the overall binding interface. This results in destabilization of the BC and FG loops of FcγRIIIa, which directly bind to the Fc fragment. This loss of interfacial complementarity is a plausible structural explanation for the loss of receptor binding affinity in this mutant.

The patterns suggested by the RMSF profiles are consistent: the affinity-enhancing mutant S239D/I332E stabilizes the binding interface between FcγRIIIa and the Fc fragment with a particular preference for the BC and FG loops, whereas the affinity-abolishing mutant V263D enhances the disorder for both the Fc fragment and the receptor interface. These residue-level results suggest that modulation of the flexibility of the interfacial loops is a mechanism by which these mutations have opposing effects on the binding affinity of FcγRIIIa.

3.3. Compactness: Radius of Gyration Analysis

The overall compactness and structural integrity of the three Fc-FcγRIIIa complexes (Fig. 3) were assessed by observing the radius of gyration (Rg) throughout the 200-ns trajectory (Fig. 3).

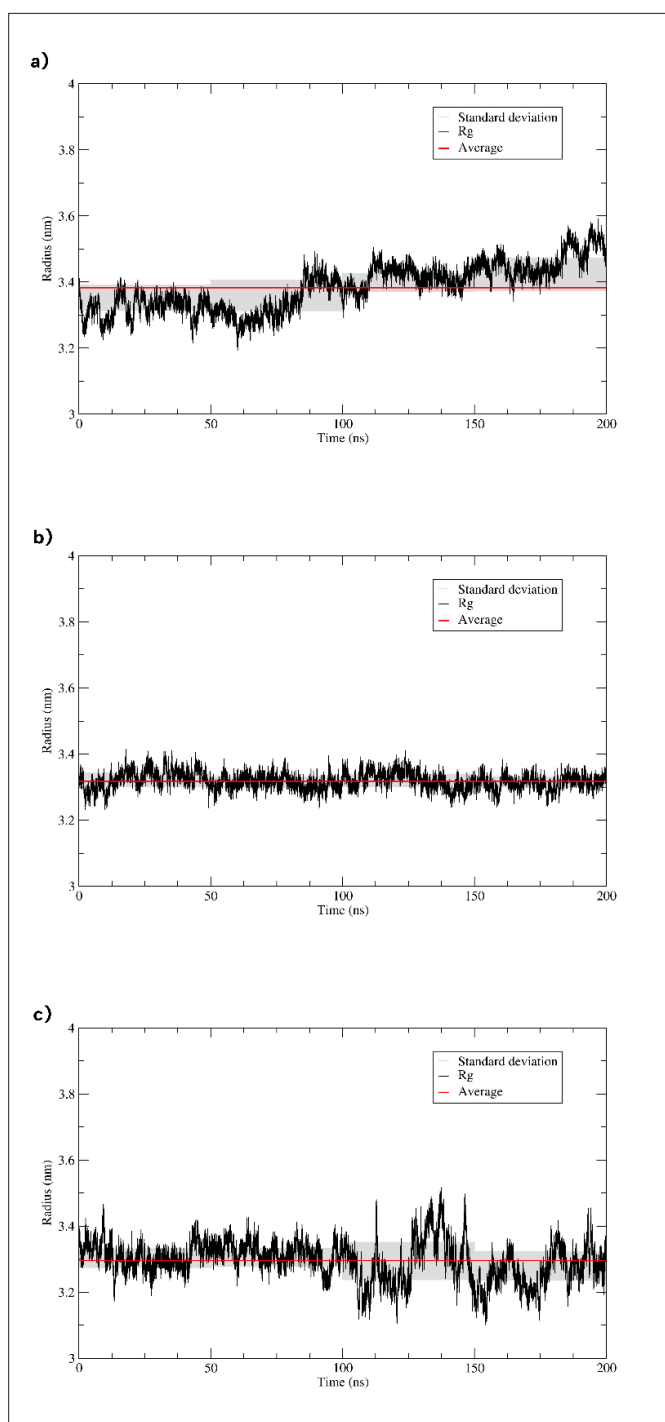


Figure 3. Radius of gyration (Rg) of the (a) wild-type, (b) S239D/I332E (+mut), and (c) V263D (-mut) Fc-FcγRIIIa complexes over the 200 ns simulation period. Black lines indicate instantaneous Rg values, and red horizontal lines denote the trajectory average. The figure compares overall compactness and structural integrity among the three systems, with lower and more stable values indicating greater conformational restraint.

The WT complex (Fig. 3a) exhibited a mean Rg of 3.38 ± 0.05 nm, with a gradual upward increase from approximately 3.35 ± 0.04 nm in the first 50 ns to 3.42 ± 0.05 nm in the final 50 ns. The gradual increase in Rg suggests that the WT complex gradually expands during the simulation, which aligns with the conformational shifts observed in the RMSD analysis and reflects the lack of complete equilibration

of the complex. The deviation of the instantaneous Rg trace (blue line) from the trajectory average (red line) in the second half of the simulation further highlights the greater structural heterogeneity of the WT complex at longer timescales.

The S239D/I332E double mutant (Fig. 3b) had the most compact and conformationally stable behavior of the three systems. The mean Rg obtained for the sample was 3.32 ± 0.02 nm, which was lower than, and much less variable than, that of the WT. The value was identical in all four 50-ns windows, with values of 3.32 ± 0.02 nm, which means that there was no significant structural drift during the course of the simulation. The close overlap between the instantaneous Rg trace and the average value along the trajectory shows that the complex remained well converged and structurally compact. The lower Rg values for the S239D/I332E mutant compared to WT are in agreement with a tighter packing of the complex, as observed by the increase in contacts between the Fc and FcγRIIIa. This compaction is mechanistically consistent with the enhanced binding affinity of this variant.

The V263D mutant (Fig. 3c) showed a unique pattern. This system had a mean Rg of 3.30 ± 0.04 nm (numerically the lowest of the three systems), but with large, high-amplitude fluctuations in the Rg values along the trajectory, ranging from 3.10 to 3.52 nm. The low mean Rg in V263D is not a consequence of the ordered compaction, but rather the result of the irregular contraction and expansion effects in the structure as revealed by the sharp deviations around the trajectory mean Rg, especially after 100 ns. The window of 100–150 ns exhibited the greatest deviation (3.29 ± 0.06 nm), corresponding to the time at which the maximum RMSD elevation occurred in this system. Compared to the S239D/I332E complex, which shows a stable convergence, the compaction seems to be more gradual and disordered, as can be seen from the slight decrease in Rg over the second half of the trajectory (3.31 ± 0.03 nm at 50–100 ns and 3.28 ± 0.04 nm at 150–200 ns). This behavior is consistent with partial collapse of the complex structure caused by disruption of the structural scaffold at Val263, rather than productive interface tightening.

Rg examined across all metrics reinforces the picture established by the RMSD and RMSF data (Table 1). The affinity-enhancing S239D/I332E mutant maintains a compact, stable, and well-converged complex throughout the simulation. The WT size gradually increases, indicating the incomplete equilibration of the structure on the time scale of 200 ns. The most notable result was that the V263D mutant, which is not able to bind to the affinity, showed the lowest mean Rg of the three systems, but this does not mean that this is a sign of productive contact with the affinity. Rather, it is a result of conformationally irregular compaction due to the disruption of the hydrophobic core, a behavior that is consistent with the hydrophobic collapse of the structurally destabilized proteins observed in MD simulations of such proteins, where the drop in Rg is coupled to an increase in RMSD, which is not associated with the recovery of the native state [33,34]. Together, these results highlight the need to look at Rg and RMSD together and not separately, as it is essential to understand the ordered interface tightening in addition to the disordered compaction in engineered protein–protein complexes.

3.4. Total Solvent-Accessible Surface Area Analysis

The total SASA of the Fc–FcγRIIIa complex was calculated for the 200-ns trajectory to investigate changes in solvent exposure and interface burial in the three systems (Fig. 4). The differences between the three systems are subtle in the time-series traces, as total SASA is not very sensitive to subdomain conformational changes that do not

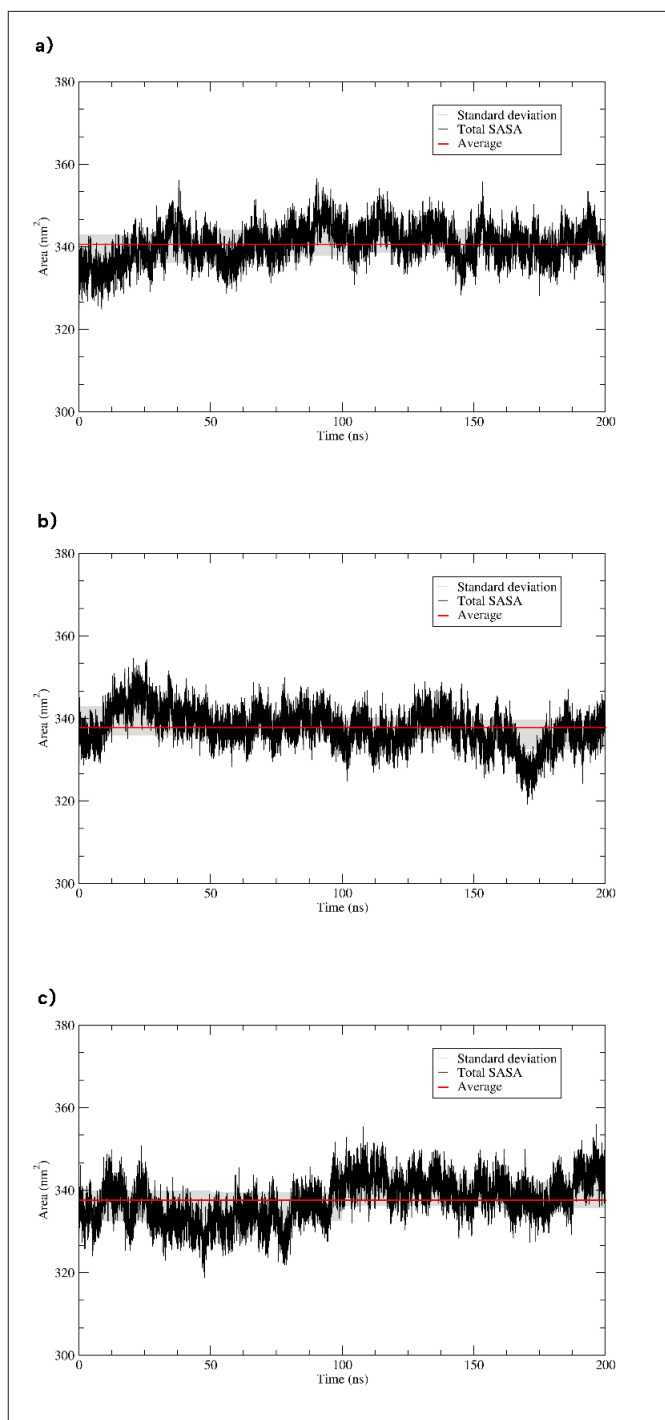


Figure 4. Total solvent-accessible surface area (SASA) of the (a) wild-type, (b) S239D/I332E (+mut), and (c) V263D (-mut) Fc–FcγRIIIa complexes over the 200 ns trajectory. Black lines show instantaneous SASA values, and red horizontal lines indicate the mean SASA for each system. The figure illustrates changes in solvent exposure and interface burial during the simulation.

significantly alter the overall buried surface area. The results below are based on the window-averaged values, not the visual trend in the figures. The WT complex (Fig. 4a) maintained a stable mean SASA of 340.63 ± 2.98 nm² over the full trajectory, with values remaining close to this average across all time windows: 339.54 ± 3.36 nm² (0–50 ns), 340.99 ± 3.02 nm² (50–100 ns), 341.33 ± 2.72 nm² (100–150 ns), and

$340.68 \pm 2.43 \text{ nm}^2$ (150–200 ns). The instantaneous SASA trace shows no significant directional drift from the trajectory mean (red line) and symmetry around it, indicating that the overall exposure of the molecule to the solvent in the WT complex is relatively constant over the length of the simulation. Such stability in total SASA is due to the fact that global exposure to solvent is a relatively insensitive quantity of the overall conformational change of the subdomain which does not significantly change the overall buried surface area. The S239D/I332E double mutant (Fig. 4b) exhibited a mean SASA of $337.85 \pm 3.21 \text{ nm}^2$ modestly but consistently lower than the WT, with a notable progressive decrease across the trajectory: $339.43 \pm 3.41 \text{ nm}^2$ (0–50 ns) declining to $336.12 \pm 3.55 \text{ nm}^2$ (150–200 ns). As SASA decreases over the course of the simulation, this is an indication of progressive burial of solvent-accessible surface. The reduction is in accordance with the progressive tightening of the Fc-FcγRIIIa interface. These S239D and I332E substitutions also provide extra electrostatic and hydrogen-bonding interactions to further support receptor engagement, while the increase in buried surface area of the hydrophobic and polar surface associated with this tighter engagement is seen in the decrease of the SASA values throughout the trajectory. The lower and decreasing SASA of the S239D/I332E mutant system serves as surface-area-level evidence for the interface compaction suggested by the Rg analysis.

The V263D mutant (Fig. 4c) had a mean SASA of $337.62 \pm 3.64 \text{ nm}^2$, which is comparable to the mean SASA of the S239D/I332E system but showed a qualitatively different temporal behavior with the highest standard deviation out of all three systems. Rather than a monotonic trend, the V263D mutant SASA showed a non-monotonic trajectory: an initial low phase ($336.18 \pm 3.55 \text{ nm}^2$ at 0–50 ns and $336.07 \pm 3.40 \text{ nm}^2$ at 50–100 ns) followed by a marked mid-simulation rise ($339.40 \pm 3.17 \text{ nm}^2$ at 100–150 ns) and partial return ($338.84 \pm 3.11 \text{ nm}^2$ at 150–200 ns). The increased RMSD and Rg fluctuations in this system during this 100–150-ns interval are consistent with a pattern of transient surface exposure that does not occur in a stable and ordered fashion. This is because the elevated variance throughout was not due to productive interface reorganization, but rather structural instability.

The SASA data support the conclusions drawn from the preceding analyses (Table 1). The S239D/I332E mutant gradually exposes less solvent-accessible surface area over time, which suggests that the mutant tightens the interface over time and thus engages the receptor more effectively. Solvent exposure is relatively high and stable in the WT, suggesting a weak association/interface. Although the V263D mutant system has a similar mean SASA to the S239D/I332E mutant system, it does so in a transient disordered fashion, rather than being buried at the interface, a pattern fully consistent with its affinity-abolishing phenotype and the structural instability documented by RMSD, RMSF and Rg analyses.

3.5. Principal Component Analysis

Principal component analysis was performed on the full 200 ns trajectory of each complex to characterize the dominant modes of collective motion and to compare the conformational landscapes sampled under each binding affinity state. Each trajectory was projected onto the first two principal components (PC1 and PC2), and these projections are depicted in Figure 5, where each frame is colored from red (early trajectory) to blue (late trajectory) to highlight the temporal evolution of conformational sampling. PC1 and PC2 are not defined together for all the systems but are computed separately as mentioned in Section 2.6. The PC1 and PC2 ranges listed below for each system are thus the local variations and differences among that system's early and late frame clusters. Comparisons between systems are limited to percentage variance and cluster topology (number,

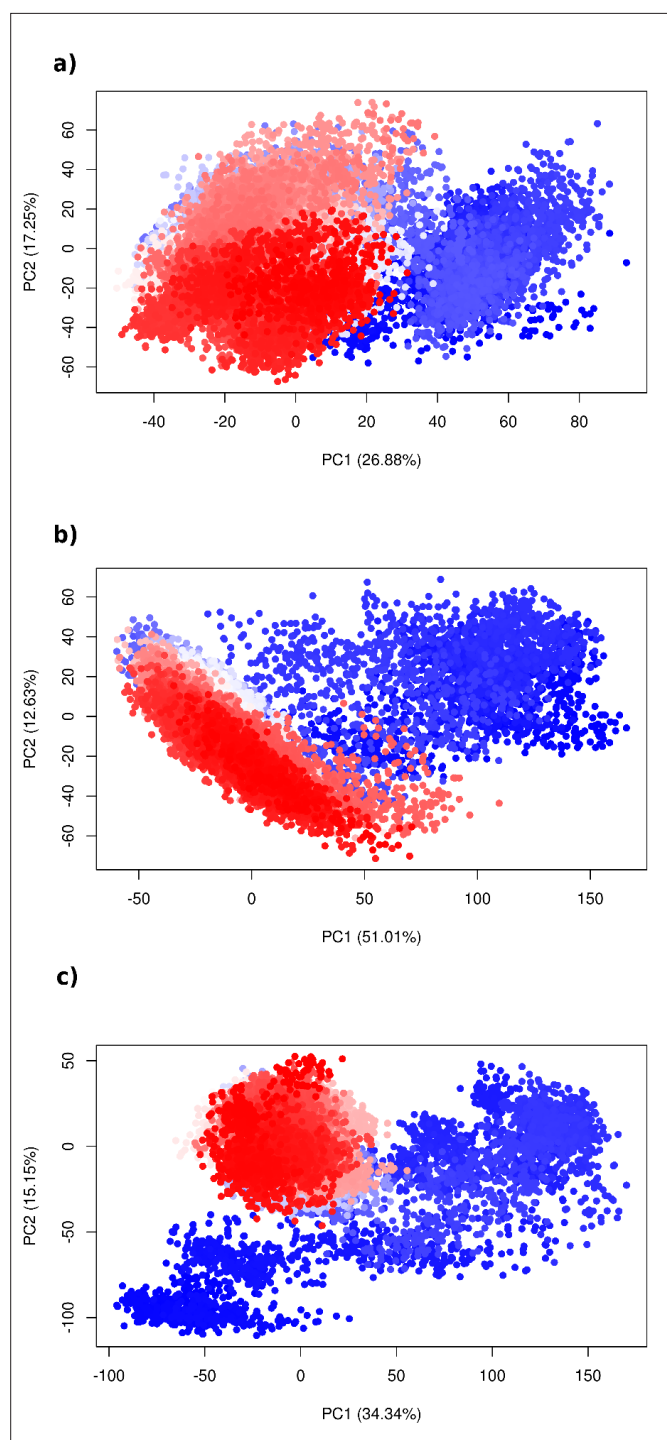


Figure 5. Principal component analysis projections of the (a) wild-type, (b) S239D/I332E affinity-enhanced mutant, and (c) V263D affinity-abolished mutant Fc-FcγRIIIa complexes onto the first two principal components (PC1 and PC2). Individual trajectory frames are colored from red (early frames, 0 ns) to blue (late frames, 200 ns) to illustrate the temporal evolution of conformational sampling. Percentages on each axis indicate the proportion of total positional variance captured by each principal component.

separation, and spatial distinctiveness of clusters) but not to the actual coordinate magnitudes. For the WT complex, PC1 and PC2 explained 26.88% and 17.25% of the total positional variance, respectively, for a total of 44.13% of the total motion. The contributions of the first three components added up to 55.40%, with PC3 adding another 11.27%.

Two clusters were identified in the PC1–PC2 projection that showed a clear separation in space between those for the early and late trajectory frames. The early frames (red) were grouped together and mostly in negative PC1 space (around –40 to +10); the late frames (blue) were mostly in the positive PC1 space (around +20 to +90), with moderate overlap between the early and late frames in the transition zone (PC1 = 0 to +20). The clear temporal separation of clusters along PC1 directly corresponds to the transition in the RMSD profile at ~75–100 ns. This agreement confirms that the WT complex undergoes a discrete and sustained shift in its dominant conformational state during the simulation. The relatively even distribution of variance between PC1 and PC2 indicates that the WT complex samples conformational motions in multiple directions, consistent with a functional interface that is maintained but not rigidly constrained.

The S239D/I332E complex (Fig. 5b) had a very different conformational space. PC1 alone captured 51.01% of the total positional variance—the highest of the three complexes—with PC2 contributing a further 12.63%, giving a combined variance of 63.64%. PC3 accounted for only 3.91% of variance, bringing the cumulative total for the first three components to 67.55% and confirming that collective motion in this complex is concentrated almost entirely within a single dominant mode. The exceptional dominance of PC1 indicates that the collective motion of the S239D/I332E mutant complex is governed by a single well-defined directional mode, reflecting the structural constraint imposed by the three salt bridges formed at the interface by the S239D/I332E mutations. The initial docked conformation was represented by the early trajectory frames (red), which were clustered together in a small, dense area in the negative PC1 range (around –50 to 0). Late frames (blue) show a progressive and directional evolution from the starting geometry of the docked complex toward its equilibrium bound geometry, along the positive PC1 axis up to +160. There was no branching into secondary conformational regions in the transition process from early to late frames, but it took place as a continuous displacement along PC1. This confirms that the S239D/I332E mutant complex follows a single dominant conformational pathway. The conformational space sampled by this complex is therefore highly organized and constrained compared to both the WT and the V263D mutant. This behavior is consistent with the low RMSD and compact Rg observed for this complex and reflects the dynamic stabilization of the interface conferred by the electrostatic mutations.

The V263D complex (Fig. 5c) exhibited the most heterogeneous conformational sampling of the three systems. PC1 and PC2 captured 34.34% and 15.15% of variance, respectively, for a combined total of 49.49%. PC3 contributed a further 8.25%, giving a cumulative total of 57.74% across the first three components. In contrast to the WT, where two temporally separated clusters were evident, and the S239D/I332E mutant, where a single directional transition dominated, the V263D mutant complex displayed multiple scattered cluster regions in the PC1–PC2 subspace. Early frames (red) formed a relatively compact cluster in the upper-left region (PC1 approximately –30 to +10) of the V263D-specific PC1–PC2 subspace, reflecting the same starting docked geometry. However, late frames (blue) were dispersed across two spatially separated regions: a broad diffuse cluster extending along the positive PC1 axis (PC1 approximately +25 to +160, PC2 approximately –20 to +30), and a distinct secondary cluster displaced substantially in the negative PC2 direction (PC1 approximately –50 to +50, PC2 approximately –50 to –100). The emergence of this secondary late-frame cluster, which had no counterpart in the WT or S239D/I332E mutant trajectories, indicates that the V263D mutant complex accesses qualitatively distinct conformational states that are not sampled by the functional complexes. This progressive and multidirectional dispersal of late-trajectory frames is consistent with the continuously rising

RMSD observed for the V263D mutant complex and reflects the loss of the defined conformational constraints that maintain the binding interface in the WT and S239D/I332E mutant systems. By breaking the hydrophobic core of the complex, the disruption of the structural anchoring required for the normally conformationally restricted binding area of the Fc region creates several energetically accessible conformations for the complex, none of which represents a stable bound conformation.

The PCA analysis results across all three complexes show that there is a clear connection between binding affinity state and the nature of conformational sampling (Table 1). The highest affinity mutant complex (S239D/I332E) shows the most organized and directional conformational behavior and highest PC1 variance dominance. The pattern of sampling is two-state for the WT complex, but the pattern is less constrained than in other complexes. The V263D mutant complex, with no measurable binding affinity, displays the most heterogeneous and multidirectional conformational sampling, accessing regions of conformational space unavailable to the functional complexes. This systematic progression provides quantitative evidence that binding affinity state is reflected in the collective dynamic behavior of the complex at the level of conformational landscape topology.

3.6. Free-Energy Landscape

The FEL of each complex was constructed by computing the Gibbs free energy as a function of two structural reaction coordinates: backbone RMSD and radius of Rg, over the full 200 ns trajectory using gmx sham (Fig. 6). As noted in Methods Section 2.7, this landscape reflects the conformational distribution sampled within each trajectory and is not a calculation of the protein–protein binding free energy. This two-dimensional projection provides a complementary view of thermodynamic accessibility that directly links conformational displacement and global compactness to free energy, enabling basin topology and energy ceiling comparisons across the three binding affinity states.

The S239D/I332E complex (Fig. 6b) displayed the most confined and well-defined free-energy landscape of the three systems. For the sampled conformational space, it was constrained to a very small RMSD window (0–0.55 nm), a small Rg window (3.225–3.425 nm), and a narrow low-energy basin (RMSD 0.2–0.25 nm and Rg 3.30–3.35 nm), the tightest among the three complexes. The energy ceiling of this system was about 5 kJ/mol, similar to the WT, but significantly lower than the –mut; the main differences from the WT were the much smaller conformational footprint and the single-basin topology. It can be seen from the steep walls and the uniqueness of this basin that the S239D/I332E mutant complex is thermodynamically restricted to a low-dimensional basin that is structurally homogeneous throughout the simulation. The single basin is consistent with the electrostatic network formed by the three interfacial salt bridges Asp239(A)–Lys158(C), Asp239(B)–Lys117(C), and Glu332(A)–Lys158(C), which limit conformational movements and stabilize the complex in a well-defined, structurally ordered binding geometry. The low energy ceiling and limited range of reaction coordinates are consistent with the increased binding affinity of this variant and serve as thermodynamic support for the convergent RMSD behavior and single-mode conformational sampling found by PCA. In the WT complex (Fig. 6a), a much more fragmented landscape was observed, characterized by an energy ceiling of 5.0 kJ/mol, and with a significantly wider range of sampled states from RMSD ≈ 0–1.0 nm to Rg ≈ 3.20–3.60 nm. The landscape of the WT could be subdivided into several distinct low-energy basins. The primary minimum was found at low RMSD (~0.1 nm) and Rg (~3.27 nm), corresponding to the initial

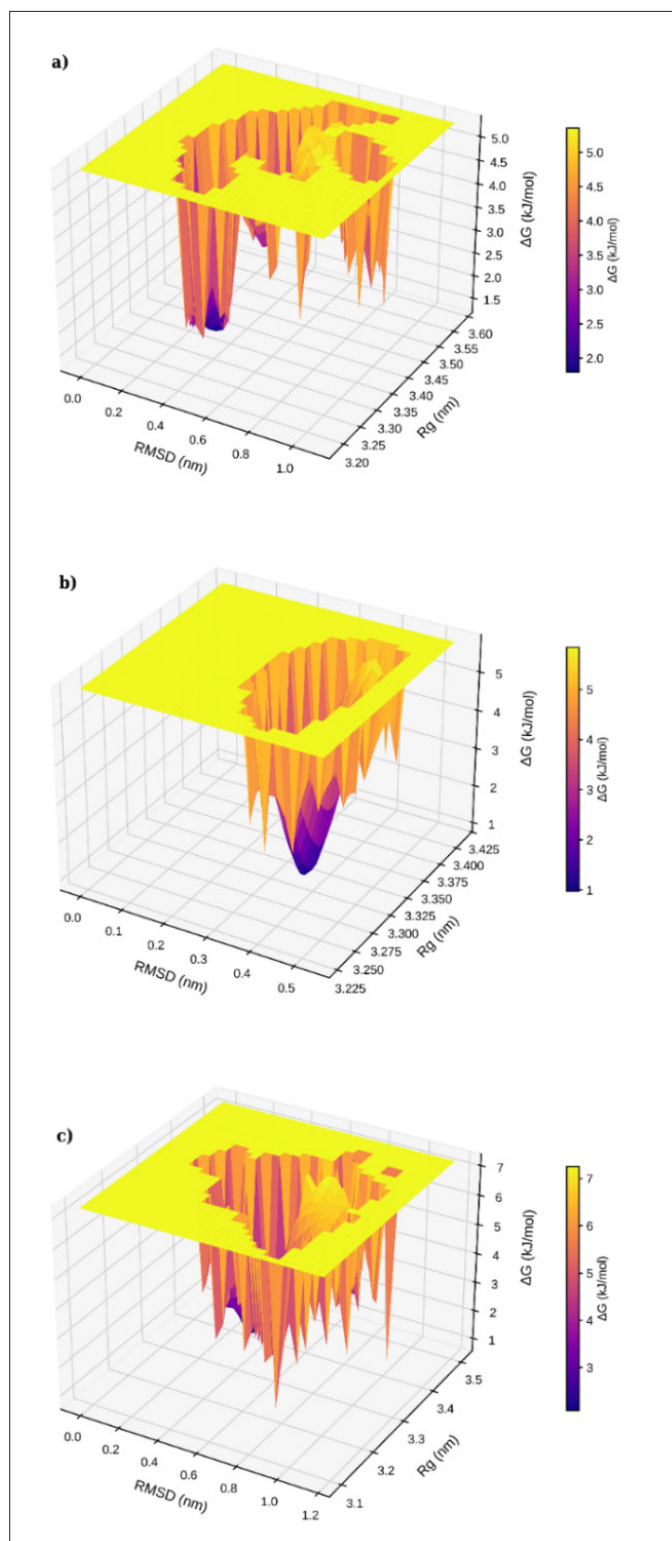


Figure 6. Free-energy landscapes (FEL) of the (a) wild-type, (b) S239D/I332E affinity-enhanced mutant, and (c) V263D affinity-abolished mutant Fc-Fc γ RIIIa complexes, constructed from the full 200 ns production trajectory using backbone RMSD (nm) and radius of gyration, Rg (nm), as reaction coordinates. Darker regions (blue-purple) indicate thermodynamically stable, frequently sampled conformational basins; lighter regions (yellow) correspond to high-energy, rarely sampled states. Axis ranges and color scale limits are set independently for each panel to match the region of RMSD-Rg space sampled by that system. ΔG values across panels use a common zero reference and are directly comparable for the energy ceiling comparisons reported in the text.

docked conformation. At least two additional basins were present at intermediate and elevated RMSD values of approximately 0.3–0.6 nm and 0.8–1.0 nm, respectively. These additional basins correspond to the conformational states accessed following the mid-trajectory transition observed in the RMSD analysis. The multi-basin character of the WT landscape reflects the inherent conformational plasticity of the wild-type Fc-Fc γ RIIIa interface in the absence of additional electrostatic anchoring. The broad range of RMSD and Rg space occupied across these basins is consistent with the two-state conformational behavior identified by PCA. This is further supported by the progressive Rg expansion documented in the gyration analysis, with all three metrics converging on the same interpretation of WT conformational behavior. The separation of basins across the RMSD axis indicates that interconversion between these states is associated with substantial structural displacement, consistent with the irreversibility of the mid-trajectory transition observed in the RMSD profile.

The V263D complex (Fig. 6c) exhibited the most heterogeneous and energetically accessible free-energy landscape of the three systems, with the highest energy ceiling of approximately 7 kJ/mol and the widest RMSD (approximately 0 to 1.2 nm) and Rg (approximately 3.1–3.5 nm) ranges. A primary low-energy basin was present at intermediate RMSD and Rg values but was accompanied by multiple satellite minima distributed across distinct regions of the conformational space, including a spatially isolated basin at low RMSD and a separate cluster at elevated RMSD and lower Rg. These satellite minima, which had no clear counterpart in the +mut landscape and were less prominent in the WT, correspond to the secondary conformational clusters identified by PCA as states inaccessible to the functional complexes. The highest energy ceiling among the three systems implies that the V263D complex readily samples energetically costly conformational states, corresponding with the removal of the hydrophobic scaffold at position Val263(B), which ordinarily limits the conformational flexibility of the binding area. The presence of a main basin together with several satellite minima, which are separated by low-energy barriers between basins, means that the complex is not thermodynamically committed to a single binding geometry; instead, it is distributed among a heterogeneous set of structural states. This thermodynamic heterogeneity thus directly explains why Fc γ R binding is lost in this variant, a result confirmed experimentally [15].

When the FEL analysis is considered as a whole, it reveals a systematic progression in the landscape topology that matches the binding-affinity ranking determined by the earlier analyses (Table 1). The S239D/I332E complex reaches a single, confined low-energy basin having the narrowest conformational footprint and a similar energy ceiling. The WT is found in a multi-basin area that is of intermediate breadth and has a low-energy ceiling. The V263D complex reaches the broadest and most energetically heterogeneous landscape, with a higher energy ceiling and without the satellite minima that are present in the two functional complexes. The transition from a single-basin to a multi-basin landscape topology, with increasing structural disorder, is in agreement with the idea that the binding-affinity state not only determines the local interfacial geometry but also the global thermodynamic accessibility of the conformational space of the Fc-Fc γ RIIIa complex.

3.7. Dynamic Cross-Correlation Analysis

The DCCMs were calculated using the entire 200-ns trajectory of each complex and are presented in Figure 7 along with the surface representations of the respective complexes, with the sites of mutation shown. The DCCM provides data on the pairwise correlated movements of all 603 residues, the axes being divided into the Fc chain

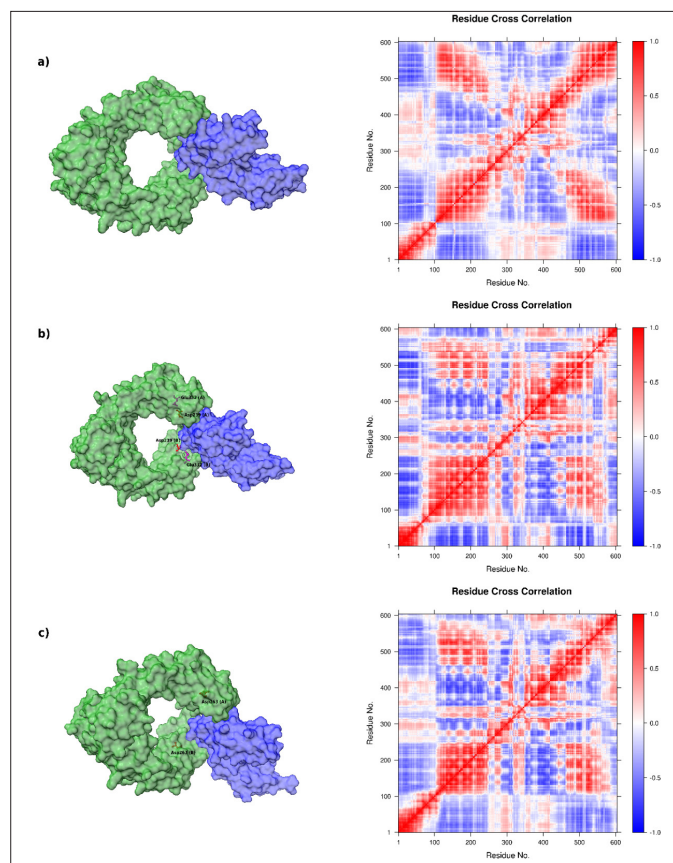


Figure 7. Dynamic cross-correlation matrices (DCCM) and surface representations of (a) the wild-type Fc–FcγRIIIa complex, (b) the S239D/I332E affinity-enhanced mutant complex, and (c) the V263D affinity-abolished mutant complex. In each matrix, residues 1–216 correspond to Fc chain A, residues 217–432 to Fc chain B, and residues 433–603 to FcγRIIIa (chain C). Color scale ranges from –1.0 (fully anti-correlated, blue) to +1.0 (fully correlated, red). Mutation sites are labelled in the surface representations. The Fc fragment is shown in green and FcγRIIIa in blue.

A (residues 1–216), the Fc chain B (residues 217–432) and FcγRIIIa (residues 433–603). The diagonal sections display the correlated motions within each chain, while the off-diagonal sections show the correlated motions between the chains and between the molecules.

In all three systems, there is a strong positive correlation band along the main diagonal, showing that the internal structural unity of both Fc chains and FcγRIIIa is maintained no matter what the binding affinity is. Outside the diagonal, four separate regions exhibiting increased positive correlation can be found in each of the three complexes. Two of these regions are located at the point where the CH2 domain meets the boundary between Chains A and B, around residues 100–260, and at the positions that are symmetric to these. The remaining two regions are associated with correlated motion involving FcγRIIIa and extend approximately from residues 450–600. Together, these four regions give rise to the typical cross-shaped pattern observed in the heatmaps, which reflects the complex's domain structure.

Supplementary Figure 1 provides a schematic showing the location of these four regions relative to the chain boundaries to guide interpretation of the DCCM heatmaps in Figure 7. The regions surrounding these blocks are predominantly blue, indicating widespread anti-correlated motion between structurally distinct regions of the complex.

The three systems exhibit visually distinguishable differences in the intensity, spatial extent, and definition of these correlated regions, and the overall intensity of positive correlation, assessed qualitatively from heatmap appearance, follows the order: S239D/I332E most intense, WT intermediate, and V263D least intense. All four blocks in the S239D/I332E complex (Fig. 7b) are the largest and most intensely red of the three systems, and when considered together, it is evident that they show positive correlation across all inter- and intra-chain blocks of the matrix, which is quite obvious against the background of anti-correlated relationships. The CH2 domain junction block, on the other hand, is rather lengthened compared to the WT, as the increased intra-Fc coherence that is afforded by the salt bridge network is known to anchor both Chains A and B to the receptor. The intra-FcγRIIIa block is also more distinct, indicating that internal coherence of the receptor is also high in the case of an electrostatically constrained interface. The inter-molecular off-diagonal regions between the Fc and FcγRIIIa blocks exhibit a clear positive correlation patch at the Chain A/B–FcγRIIIa interface, which is indicative of organized directional coupling between the two binding partners imposed by the engineered contacts.

In the WT complex (Fig. 7a), the same four regions of correlation are found but with less intensity and spatial extent. The CH2 domain junction block is still a red feature, but it appears to be slightly smaller and not as clearly visible in comparison to S239D/I332E; the inter-molecular positive correlation patches are also more diffuse and less clearly bounded. The overall pattern maintains the cross-shaped diagonal nature, but with more overlap of the blue anti-correlated regions between the correlated blocks, consistent with a less mechanically constrained interface in which the correlated motions exist, but have not been directionally confined by specific electrostatic anchors. The unconstrained opposing motions between the Fc fragment and receptor are widely distributed over the matrix of the WT with a broad blue anti-correlation on the off-diagonal inter-molecular blocks.

V263D complex (Fig. 7c) has the lowest number of correlated regions and the least strength of all. The CH2 domain junction block is visibly contracted owing to the absence of the hydrophobic scaffold integrity at position 263 in Chain B that typically would support this block in a coherent geometry. The intra-FcγRIIIa block is still present, but somewhat decreased in extent, indicating that even receptor-side internal coherence is somewhat lost when the Fc binding partner does not provide a structurally ordered interface. The inter-molecular positive correlation patches are the least well-defined of the three systems, in accordance with the progressive decoupling of Chain B from the receptor after breakage in the hydrophobic core around Val263. The extent of the surrounding blue regions is largely comparable to that of the WT, but the pattern is more diffused, indicating the disappearance of organized directional coupling at the interface.

The DCCM analysis together indicates that the affinity of the Fc–FcγRIIIa binding complex is mirrored in the organization and strength of the correlated motions of the entire complex (Table 1). When the binding is high, the correlated motions are well defined and extensive in space, both intra-chain and inter-molecular, while when the binding is abolished, the correlated motions are contracted and weaker. The intermediate WT pattern is similar to that of a functional but not optimized interface that maintains correlated coupling without the directional mechanical organization that is provided by engineered electrostatic contacts. Table 1 summarizes the headline value for each of the seven metrics across the three systems.

4. DISCUSSION

Whether affinity-modulating mutations alter the global conformational behavior of a protein–protein complex, or act exclusively at the local binding site, has remained an open question in Fc engineering. Comparative 200-ns all-atom molecular dynamics simulations of three Fc-FcγRIIIa complexes spanning the full affinity spectrum address this directly. Across seven metrics, the three complexes produced rank-ordered dynamic profiles that matched their experimentally measured binding phenotypes at every analytical level (Table 1). The affinity-enhanced +mut was structurally convergent and thermodynamically confined to a single low-energy basin. The wild-type occupied a dynamically intermediate regime. The affinity-abolished -mut drifted progressively and sampled conformational states unavailable to either functional complex. These findings indicate a consistent association between binding affinity and the global conformational dynamics of the Fc-FcγRIIIa complex, rather than a pattern confined to local interfacial contact geometry.

Prior computational studies of Fc-FcγR systems have been limited to static structures or short pairwise comparisons. Crystal structures established the geometry of key interfacial contacts [16] and characterized effector-null variants crystallographically [12], but single conformational snapshots cannot represent the dynamic ensemble that governs binding under physiological conditions. Kralj *et al.* [21] compared one Fc variant against wild-type across FcγRIIa, FcγRIIb, and FcγRIIIa, and Kutlu *et al.* [22] examined full and truncated Fc-FcγRI models over 200 ns, but neither study characterized conformational dynamics across a binding affinity spectrum. Our earlier 50-ns pairwise comparison of WT and +mut [19] was the first MD study on the FcγRIIIa complex, yet the timescale was insufficient to capture the conformational transition in the WT that becomes apparent only beyond 95 ns, and an affinity-abolished variant was not included. The present study is, to our knowledge, the first to place three complexes spanning the complete affinity range within a unified long-timescale framework and to demonstrate that rank-ordered conformational signatures are preserved across all analytical dimensions.

The conformational stability of the +mut complex is mechanistically attributable to the three salt bridges formed at the Fc-FcγRIIIa interface: Asp239(A)-Lys158(C), Asp239(B)-Lys117(C), and Glu332(A)-Lys158(C) [18,20]. These electrostatic contacts act as geometric anchors that suppress large-scale relative motion between the Fc fragment and the receptor. Their influence is reflected coherently across all analytical dimensions. Receptor-side BC and FG loop flexibility is dampened relative to both the WT and -mut complexes. Solvent-accessible surface area decreases along the trajectory, indicating the ongoing interface tightening as the complex approaches the equilibrium bound state. A single PCA mode captures 51% of total positional variance, the highest first-mode dominance among the three systems, indicating that collective motion is channeled into one well-defined directional pathway. The free-energy landscape is correspondingly narrow, with a single deep basin and no satellite minima. This single-basin topology is consistent with transition complex theory analysis of the same system [20], which identified an approximately 60-fold reduction in k_{off} as the dominant contributor to the affinity gain. By restricting access to dissociative conformational states, the salt bridge network renders partial unbinding thermodynamically costly. Energy ceilings derived from MD-based free-energy projections reflect the conformational distribution sampled within a single bound-state trajectory, not the full association and dissociation thermodynamic cycle captured by an experimental binding free energy, so quantitative comparison between the two is not warranted. The rank ordering of basin topology across

the three systems is nevertheless internally consistent and mirrors the experimentally determined affinity hierarchy. Biologically, the constrained dynamic regime of the +mut complex sustains durable FcγRIIIa occupancy on NK cell and macrophage surfaces, a geometric prerequisite for efficient ITAM phosphorylation and the superior ADCC potency reported for tafasitamab relative to wild-type IgG1 [14], though this comparison rests on aglycosylated simulation data and its extension to the glycosylated physiological antibody has not been directly tested here. This framework offers a route to evaluate candidate Fc mutations computationally ahead of experimental affinity screening, and could be extended to compare point-mutation strategies such as S239D/I332E against glycoengineering approaches used in other approved antibodies, such as the afucosylation strategy employed in obinutuzumab, to determine whether these represent additive or mechanistically distinct routes to enhanced ADCC potency.

Binding abolition in the -mut complex proceeds through a structurally independent mechanism. The Val263Asp substitution in chain B disrupts the hydrophobic core formed by Pro238, Val263, Val266, and Leu328 [15], removing the structural scaffold that ordinarily holds CH2-B in a receptor-compatible geometry. The result is not just increased flexibility but a qualitative rearrangement of the accessible conformational space. The neg mut free-energy landscape is the broadest of the three systems, with the highest energy ceiling and multiple satellite minima that have no counterpart in the functional complexes. PCA also shows this rearrangement: later trajectory frames of the negative mutant form a separate cluster in negative PC2 space, which is not seen in either +mut or WT, indicating that V263D opens a non-functional conformational pathway rather than simply widening the existing one. It has the lowest mean Rg and the highest RMSD, which may suggest that chain B has become disordered as a result of the disintegration of the hydrophobic core. This change differs from the tightening of the interface seen in +mut, and is distinguished by the different way in which RMSD and Rg respond to the same structural event. The transient increase in solvent-accessible surface area within the 100–150-ns window coincides with peak RMSD and Rg fluctuations, reflecting exposure of buried interface residues during conformational excursions that are not subsequently recovered. This contradicts the monotonic surface burial seen in +mut. These molecular features explain why the Val263Asp substitution abrogates binding to low- and intermediate-affinity FcγRs [15]. The Fc region fails to maintain the stable, receptor-compatible surface presentation required for productive engagement under physiological thermal motion.

These two mutations define structurally independent mechanistic pathways that lead to opposite ends of the affinity spectrum. Electrostatic constraint governs affinity enhancement, whereas hydrophobic scaffold integrity determines the capacity to adopt a receptor-compatible geometry. Since these pathways do not share structural determinants, they offer non-overlapping design strategies for rationally engineering Fc variants with tailored receptor selectivity profiles.

Several limitations of this study should be acknowledged. Each system was analyzed using a single production trajectory. Although the rank-ordered conclusions are internally consistent across seven metrics, independent replicate simulations are necessary to confirm trajectory-specific features such as the precise timing of the wild-type conformational transition. Conducting such replicates with different initial atomic velocities would enable assessment of variance in the transition timing and magnitude to be assessed directly rather than inferred from a single realization. The seven analyses do not represent statistically independent measures. The free-energy landscape

constitutes a two-dimensional projection of RMSD and Rg rather than an independently derived structural observable. Similarly, RMSF, PCA, and DCCM are calculated from the same underlying Cα positional data used for RMSD and Rg. The consistency observed across the seven metrics should be interpreted as agreement among complementary transformations of a single trajectory dataset, rather than as corroboration from independent sources of evidence.

All simulations were performed on aglycosylated complexes, consistent with the experimental system used to validate V263D [15] and with the computational framework applied throughout this series [17–20]. Glycosylation at Asn297 is known to modulate CH2 domain structure and FcγR engagement, and whether the rank-ordered conformational signatures reported here are preserved in glycosylated models remains to be examined [35]. This bears directly on the clinical interpretations drawn above, since tafasitamab and other therapeutic IgG1 antibodies are glycosylated *in vivo*. The starting structures were derived from previously validated docked conformations rather than independently determined crystal structures of each mutant. This docking protocol, using the optimized set of active residues identified in our prior validation study, reproduced 27 of 31 experimentally determined binding residues and 21 of 28 molecular interactions at an RMSD of 1.24 Å relative to the crystal structure, but early-trajectory dynamics may partly reflect relaxation from the initial geometry rather than true equilibrium behavior. Such starting-pose bias could act differently on the three metrics used here. Early-trajectory RMSD could be inflated if a system requires more relaxation from its docked pose than others, independent of intrinsic stability. Early PCA cluster positions could partly reflect proximity to the docked geometry rather than a true early-equilibrium state. The location of the primary FEL basin could likewise be biased toward the starting structure rather than reflecting an independently reached energy minimum.

The V263D-induced destabilization observed here is corroborated by independent evidence: this substitution was identified as the most destabilizing of all possible Val263 substitutions by protein stability prediction, and confirmed by ELISA and flow cytometry to abolish FcγRIIIa binding [15], supporting that the conformational instability captured in this trajectory reflects the intended structural consequence of the mutation rather than an artefact of the docked starting geometry. PCA was performed using Cα fitting on the entire complex rather than the Fc fragment alone, a choice made to preserve inter-molecular conformational relationships between Fc and FcγRIIIa, which are central to the binding-affinity comparisons drawn in this study; fitting on Fc alone would instead isolate intramolecular Fc dynamics at the expense of this receptor-relative information. The sensitivity of the reported PC1 variance values and cluster separations to alternate alignment strategies was not formally tested and remains a candidate for future validation.

Notwithstanding these limitations, the multi-scale analytical framework presented here demonstrates that conformational dynamics encode the affinity state of the Fc–FcγRIIIa complex at every level of description examined in this retrospective analysis. The approach can distinguish and rank-order the three known affinity states studied here without requiring explicit binding free-energy calculations. These results support multi-scale molecular dynamics simulation as a candidate computational screening strategy for anticancer monoclonal antibody development, subject to the prospective validation and replicate-simulation confirmation discussed above.

5. CONCLUSION

This study investigated whether different states of Fc–FcγRIIIa binding affinity lead to globally distinct conformational signatures

or whether affinity modulation is limited to the local structure of the binding interface. Comparative all-atom molecular dynamics simulations lasting 200 ns were carried out on the wild-type form, the affinity-increased S239D/I332E double mutant, and the affinity-lost V263D mutant. The three systems were assessed using seven separate structural and dynamic measures. At each level of analysis, the three complexes showed a consistent and ranked pattern that corresponded to their experimentally determined binding characteristics. The complex with enhanced affinity stayed conformationally converged and was thermodynamically restricted to a single low-energy basin. The complex in which affinity had been lost gradually changed and reached conformational states that were not accessible to either of the functional complexes. The wild-type exhibited a dynamically intermediate behavior, characterized by a clear conformational transition at mid-trajectory. This result is in agreement with the electrostatic stabilization resulting from three interfacial salt bridges in the enhanced mutant and with the disruption of the Val263 hydrophobic core in the mutant with abolished affinity. To our knowledge, this is the first study to include complexes covering the full range of affinities within a single long-timescale, multi-metric framework; it builds upon our earlier static and short-timescale analyses of this system by providing a unified dynamic comparison.

Together, the results show that MD simulations across multiple time and length scales can rank-order Fc–FcγRIIIa affinity states without explicit free-energy calculations, making this a viable computational screening tool for engineered therapeutic antibodies in cancer drug development. When researchers are developing Fc variants, they can use the same seven-metric protocol as an initial computational screening process. Mutations that show a convergent, single-basin dynamic signature should be given priority for further experimental verification, rather than carrying out more expensive binding assays or free-energy calculations. The current analysis is retrospective in nature and demonstrates that the MD-derived metrics can consistently rank the known affinity states; it does not prove that the metrics can predict the affinity of a variant that has not been tested, and this difference should determine how the framework is applied until it has been prospectively validated. The appropriate next step in testing the framework's screening value therefore involves applying it to variants of unknown affinity. Further studies should investigate whether the rank-ordered signatures described here remain valid when the simulations are repeated with independently chosen starting velocities, in the case of glycosylated Fc, and across other FcγR subtypes. The framework could be extended from its current ability to rank known states to one of making quantitative predictions for new variants if it is combined with quantitative binding free-energy methods or machine learning classifiers.

6. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

8. FUNDING

There is no funding to report.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

12. AI DISCLOSURE

The authors used Grammarly (Grammarly, Inc.) solely for grammatical correction and language editing during manuscript preparation. No generative AI tools were used to produce the scientific content, data, analyses, figures, or references. All suggested language edits were reviewed and approved by the authors, who take full responsibility for the final manuscript.

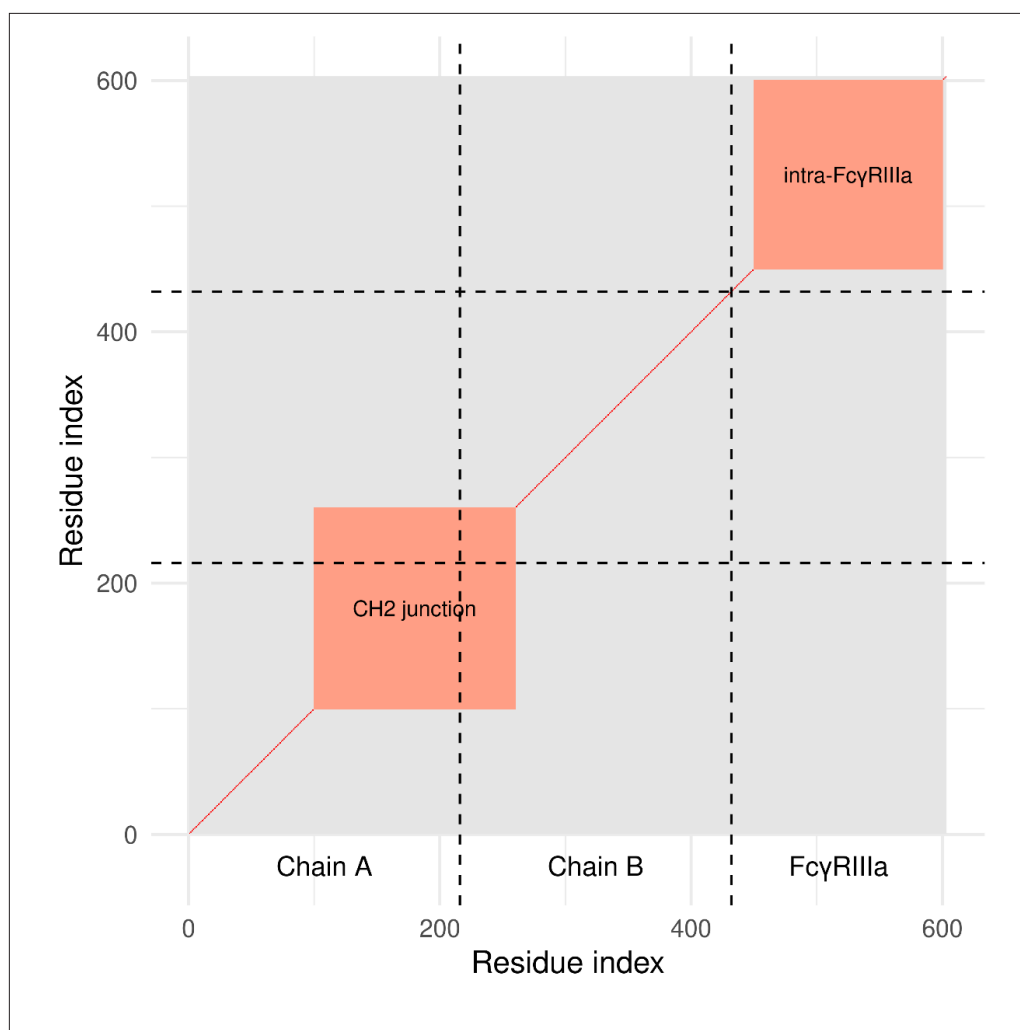
REFERENCES

- Weiner GJ. Building better monoclonal antibody-based therapeutics. *Nat Rev Cancer* 2015;15(6):361–70; doi: <https://doi.org/10.1038/nrc3930>
- Stavenhagen JB, Gorlatov S, Tuailon N, Rankin CT, Li H, Burke S, *et al.* Fc optimization of therapeutic antibodies enhances their ability to kill tumor cells *in vitro* and controls tumor expansion *in vivo* via low-affinity activating Fcγ receptors. *Cancer Res* 2007;67(18):8882–90; doi: <https://doi.org/10.1158/0008-5472.CAN-07-0696>
- Nimmerjahn F, Ravetch JV. Fcγ receptors as regulators of immune responses. *Nat Rev Immunol* 2008;8(1):34–47; doi: <https://doi.org/10.1038/nri2206>
- Raghavan M, Bjorkman PJ. Fc receptors and their interactions with immunoglobulins. *Annu Rev Cell Dev Biol* 1996;12:181–220; doi: <https://doi.org/10.1146/annurev.cellbio.12.1.181>
- Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S, *et al.* Specificity and affinity of human Fcγ receptors and their polymorphic variants for human IgG subclasses. *Blood* 2009;113(16):3716–25; doi: <https://doi.org/10.1182/blood-2008-09-179754>
- Radaev S, Sun P. Recognition of immunoglobulins by Fcγ receptors. *Mol Immunol* 2002;38(14):1073–83; doi: [https://doi.org/10.1016/s0161-5890\(02\)00036-6](https://doi.org/10.1016/s0161-5890(02)00036-6)
- Kremer PG, Barb AW. The weaker-binding Fc γ receptor IIIa F158 allotype retains sensitivity to N-glycan composition and exhibits a destabilized antibody-binding interface. *J Biol Chem* 2022;298(9):102329; doi: <https://doi.org/10.1016/j.jbc.2022.102329>
- Desjarlais JR, Lazar GA. Modulation of antibody effector function. *Exp Cell Res* 2011;317(9):1278–85; doi: <https://doi.org/10.1016/j.yexcr.2011.03.018>
- Wang X, Mathieu M, Brezski RJ. IgG Fc engineering to modulate antibody effector functions. *Protein Cell* 2018;9(1):63–73; doi: <https://doi.org/10.1007/s13238-017-0473-8>
- Jo M, Kwon HS, Lee KH, Lee JC, Jung ST. Engineered aglycosylated full-length IgG Fc variants exhibiting improved FcγRIIIa binding and tumor cell clearance. *MAbs* 2018;10(2):278–89; doi: <https://doi.org/10.1080/19420862.2017.1402995>
- Martineau R, Susini S, Marabelle A. Fc effector function of immune checkpoint blocking antibodies in oncology. *Immunol Rev* 2024;328(1):334–49; doi: <https://doi.org/10.1111/imr.13427>
- Oganesyan V, Gao C, Shirinian L, Wu H, Dall'Acqua WF. Structural characterization of a human Fc fragment engineered for lack of effector functions. *Acta Crystallogr D Biol Crystallogr* 2008;64(Pt 6):700–4; doi: <https://doi.org/10.1107/S0907444908007877>
- Lazar GA, Dang W, Karki S, Vafa O, Peng JS, Hyun L, *et al.* Engineered antibody Fc variants with enhanced effector function. *Proc Natl Acad Sci U S A* 2006;103(11):4005–10; doi: <https://doi.org/10.1073/pnas.0508123103>
- Pirosa MC, Stathis A, Zucca E. Tafasitamab for the treatment of patients with diffuse large B-cell lymphoma. *Hum Vaccin Immunother* 2024;20(1):2309701; doi: <https://doi.org/10.1080/21645515.2024.2309701>
- Jebamani P, M. Jo, S. Park, S. Kim, S.T. Jung, S.G. Lee, *et al.* Design of an Fc mutation to abrogate Fcγ receptor binding based on residue interaction network analysis. *ACS Synth Biol* 2025;14(5):1677–86; doi: <https://doi.org/10.1021/acssynbio.5c00035>
- Sondermann P, Huber R, Oosthuizen V, Jacob U. The 3.2-A crystal structure of the human IgG1 Fc fragment-Fc γRIIIa complex. *Nature* 2000;406(6793):267–73; doi: <https://doi.org/10.1038/35018508>
- Jebamani P, Sokalingam S, Sriramulu DK, Jung ST, Lee SG. Assessment of computational modeling of Fc-Fc receptor binding through protein-protein docking tool. *Biotechnol Bioprocess Eng* 2020;25(5):734–41; doi: <https://doi.org/10.1007/s12257-020-0050-5>
- Jebamani P, Sriramulu DK, Jung ST, Lee SG. Structural study on the impact of S239D/I332E mutations in the binding of Fc and FcγRIIIa. *Biotechnol Bioprocess Eng* 2021;26(6):985–92; doi: <https://doi.org/10.1007/s12257-021-0024-2>
- Jebamani P, Sriramulu DK, Lee SG. Residue interaction network and molecular dynamics simulation study on the binding of S239D/I332E Fc variant with enhanced affinity to FcγRIIIa receptor. *J Mol Graph Model* 2023;118:108327; doi: <https://doi.org/10.1016/j.jmgm.2022.108327>
- Jebamani P, Lee SG. Study on the S239D/I332E mutation effect on the kinetics of Fc–Fc receptor binding through transition complex theory. *Biotechnol Bioprocess Eng* 2024;29(4):759–68; doi: <https://doi.org/10.1007/s12257-024-00104-7>
- Kralj S, M. Hodoscek, B. Podobnik, T. Kunej, U. Bren, D. Janezic, *et al.* Molecular dynamics simulations reveal interactions of an IgG1 antibody with selected Fc receptors. *Front Chem* 2021;9:705931; doi: <https://doi.org/10.3389/fchem.2021.705931>
- Kutlu A, E. Capkin, K. Adacan, M. Yuce. Fc-FcγRIIa Complexes: molecular dynamics simulations shed light on Ectodomain D3's potential role in IgG binding. *ACS Omega* 2024;9(50):49272–82; doi: <https://doi.org/10.1021/acsomega.4c06318>
- Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, *et al.* The protein data bank. *Nucleic Acids Res* 2000;28(1):235–42; doi: <https://doi.org/10.1093/nar/28.1.235>
- Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis* 1997;18(15):2714–23; doi: <https://doi.org/10.1002/elps.1150181505>
- Bekker H, Berendsen HJC, Dijkstra EJ, Achterop S, von Drunen R, van der Spoel D, *et al.* GROMACS: a parallel computer for molecular dynamics simulations. In: de Groot RA, Nadrchal J, editors. *Physics Computing '92*. World Scientific Publishing, Singapore, pp 252–6, 1993.
- Berendsen HJC, Van Der Spoel D, Van Drunen R. GROMACS: a message-passing parallel molecular dynamics implementation. *Comput Phys Commun* 1995;91(1-3):43–56; doi: [https://doi.org/10.1016/0010-4655\(95\)00042-e](https://doi.org/10.1016/0010-4655(95)00042-e)
- Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B, *et al.* GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* 2015;1-2(1-2):19–25; doi: <https://doi.org/10.1016/j.softx.2015.06.001>

28. MacKerell AD, Brooks B, Brooks CL, Nilsson L, Roux B, Won Y, *et al.* CHARMM: the Energy Function and Its Parameterization. In: Encyclopedia of Computational Chemistry. 1998. doi: <https://doi.org/10.1002/0470845015.cfa007>
29. Brooks BR, Brooks CL III, MacKerell AD Jr, Nilsson L, Petrella RJ, Roux B, *et al.* CHARMM: the biomolecular simulation program. *J Comput Chem* 2009;30(10):1545–614; doi: <https://doi.org/10.1002/jcc.21287>
30. Lee J, Cheng X, Swails JM, Yeom MS, Eastman PK, Lemkul JA, *et al.* CHARMM-GUI Input Generator for NAMD, GROMACS, AMBER, OpenMM, and CHARMM/OpenMM Simulations Using the CHARMM36 Additive Force Field. *J Chem Theory Comput* 2016;12(1):405–13; doi: <https://doi.org/10.1021/acs.jctc.5b00935>
31. Best RB, Zhu X, Shim J, Lopes PE, Mittal J, Feig M, *et al.* Optimization of the additive CHARMM all-atom protein force field targeting improved sampling of the backbone phi, psi and side-chain chi(1) and chi(2) dihedral angles. *J Chem Theory Comput* 2012;8(9):3257–73; doi: <https://doi.org/10.1021/ct300400x>
32. Grant BJ, Rodrigues APC, Elsayy KM, McCammon JA, Caves LSD. Bio3d: an R package for the comparative analysis of protein structures. *Bioinformatics* 2006;22(21):2695–6; doi: <https://doi.org/10.1093/bioinformatics/btl461>
33. Wirtz H, Schäfer S, Hoberg C, Reid KM, Leitner DM, Havenith M. Hydrophobic collapse of ubiquitin generates rapid protein-water motions. *Biochemistry* 2018;57(26):3650–7; doi: <https://doi.org/10.1021/acs.biochem.8b00235>
34. Alonso DO, Daggett V. Molecular dynamics simulations of hydrophobic collapse of ubiquitin. *Protein Sci* 1998;7(4):860–74; doi: <https://doi.org/10.1002/pro.5560070404>
35. Van Coillie J, Schulz MA, Bentlage AEH, de Haan N, Ye Z, Geerdes DM, *et al.* Role of N-Glycosylation in FcγRIIIa interaction with IgG. *Front Immunol* 2022;13:987151; doi: <https://doi.org/10.3389/fimmu.2022.987151>

How to cite this article:

Petrina J, Lingayat N, Mukilan M, Krishna RA, Prabhu D. Molecular dynamics simulation of engineered Fc–FcγRIIIa complexes as a framework for anticancer monoclonal antibody development. *J Appl Biol Biotech* 2026. Article in Press. <http://doi.org/10.7324/JABB.2026.326155>



Supplementary Figure 1. Schematic image of the dynamic cross-correlation matrix (DCCM) block structure. The diagonal (red line) reflects intra-chain coherence common to all three chains. The two orange regions mark the approximate location of the CH2 domain junction block (~residues 100–260) and the intra-FcγRIIIa block (~residues 450–600) discussed in the text. Dashed lines mark the boundaries between Fc chain A (residues 1–216), Fc chain B (residues 217–432), and FcγRIIIa (residues 433–603). This panel reflects fixed structural boundaries common to WT, +mut, and -mut, and is provided as a shared reading aid rather than as system-specific data.