



Fc α RI binding at the IgA1 C_H2–C_H3 interface induces long-range conformational changes that are transmitted to the hinge region

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IgA effector functions include proinflammatory immune responses triggered upon clustering of the IgA-specific receptor, Fc α RI, by IgA immune complexes. Fc α RI binds to the IgA1–Fc domain (Fc α) at the C_H2–C_H3 junction and, except for C_H2 L257 and L258, all side-chain contacts are contributed by the C_H3 domain. In this study, we used experimental and computational approaches to elucidate energetic and conformational aspects of Fc α RI binding to IgA. The energetic contribution of each IgA residue in the binding interface was assessed by alanine-scanning mutagenesis and equilibrium surface plasmon resonance (SPR). As expected, hydrophobic residues central to the binding site have strong energetic contributions to the Fc α RI:Fc α interaction. Surprisingly, individual mutation of C_H2 residues L257 and L258, found at the periphery of the Fc α RI binding site, dramatically reduced binding affinity. Comparison of antibody:receptor complexes involving IgA or its precursor IgY revealed a conserved receptor binding site at the C_H2–C_H3 junction (or its equivalent). Given the importance of residues near the C_H2–C_H3 junction, we used coarse-grained Langevin dynamics simulations to understand the functional dynamics in Fc α . Our simulations indicate that Fc α RI binding, either in an asymmetric (1:1) or symmetric (2:1) complex with Fc α , propagated long-range conformational changes across the Fc domains, potentially impacting the hinge and Fab regions. Subsequent SPR experiments confirmed that Fc α RI binding to the Fc α C_H2–C_H3 junction altered the kinetics of HAA lectin binding at the IgA1 hinge. Receptor-induced long-distance conformational transitions have important implications for the interaction of aberrantly glycosylated IgA1 with anti-glycan autoantibodies in IgA nephropathy.

IgA1 antibody | binding energetics | molecular-dynamics simulations | surface plasmon resonance | principal-component analysis

IgA is the second most prevalent antibody isotype in serum and the most abundant isotype in mucosal secretions (1, 2); it performs an important role in preventing and countering pathogenic challenge to the immune system. IgA can be divided into IgA1 and IgA2 subclasses, which differ in the number of glycosylation sites and the length of the hinge region. The IgA1 subclass features a heavily *O*-glycosylated hinge region, with up to six potential *O*-glycans. Aberrant *O*-glycosylation of the IgA1 hinge is a key feature seen in patients with the autoimmune disease IgA nephropathy (IgAN), as it forms a neo-epitope for anti-glycan autoantibodies and leads to deposition of immune complexes in the glomerular mesangium (3).

In the presence of multivalent antigen, IgA initiates signaling via the IgA-specific receptor Fc α RI on immune cells, triggering a range of proinflammatory responses (2, 4, 5). The Fc α RI ectodomain consists of two orthogonal Ig-like domains, D1 and D2 (6, 7). The N-terminal region of Fc α RI D1 contacts IgA at the C_H2–C_H3 (C α 2–C α 3) domain interface (Fig. 1A) (6, 8–13). Analytical

ultracentrifugation, biosensor, and crystallographic studies have shown that two Fc α RI molecules can bind a single IgA antibody (6, 13, 14). The Fc α RI ectodomain can be shed upon activation by the action of ADAM10 and ADAM17, resulting in soluble Fc α RI in serum (15); this soluble receptor form has been implicated in the progression of IgAN (16, 17). However, the role played by soluble Fc α RI in IgAN remains unclear.

Here, we report binding data and computational analyses, providing information on energetic and dynamic aspects of the Fc α RI:IgA1 interaction. We combined alanine-scanning mutagenesis and equilibrium biosensor experiments to complete the first systematic analysis of the energetic contributions of individual IgA residues whose side chains contact Fc α RI, identifying the Fc α energetic hot-spot residues in the binding interface. Comparing these results to other related antibody:receptor pairs revealed a common mode of binding. Using the Fc α RI:Fc α crystal structure, we performed coarse-grained molecular-dynamics

Significance

Antibodies binding to their cognate cellular receptors can trigger important downstream immune responses. We mapped out critical amino acids on the IgA1 antibody that govern binding to its specific receptor, Fc α RI. We found that two of the most important amino acids were located on a different IgA1 domain than the rest of the binding site, separated by a flexible linker. To better understand the interplay between receptor binding and dynamic motions in IgA1, we conducted dynamics simulations on the system. The results indicate that receptor binding perturbs IgA1 conformational dynamics over long distances and can link the receptor binding site to the hinge region of IgA1. We validate this finding experimentally, which has implications for the kidney disease IgA nephropathy.

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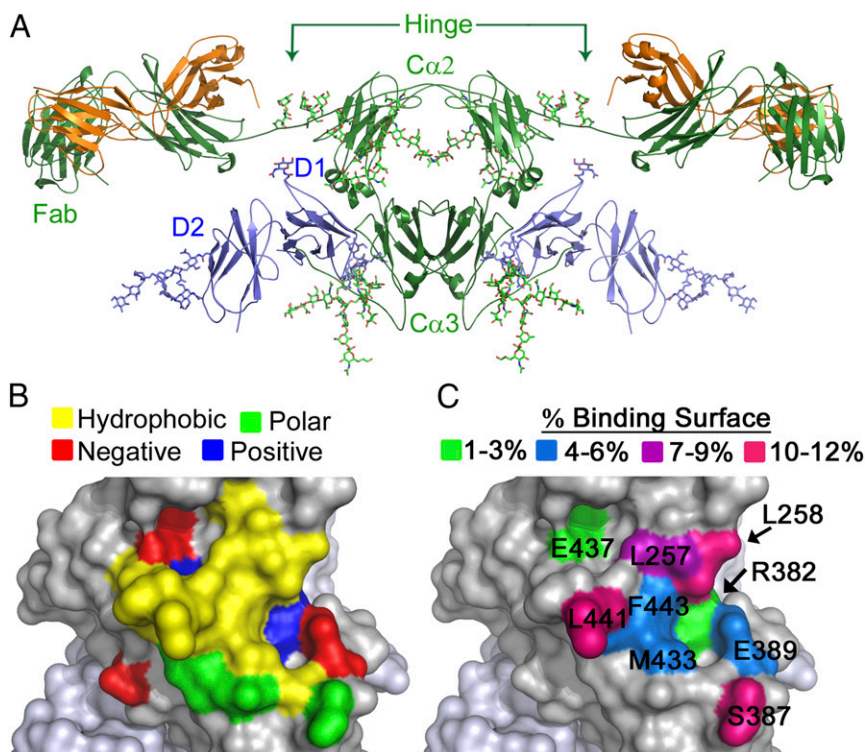


Fig. 1. Fc α RI binds IgA1 at a hydrophobic region of the C α 2–C α 3 junction. (A) Model of the 2:1 complex between Fc α RI (blue) and IgA1 (green/orange) (3, 6) based on the crystal structure of the Fc α RI:Fc α complex (PDB ID code 1OW0) and the solution structure of full-length IgA1 (PDB ID code 1IGA). (B and C) Characteristics of Fc α RI binding site on Fc α : amino acid properties (hydrophobic, yellow; positively charged, blue; negatively charged, red; polar, green) (B); percent contribution of each Fc α residue with side chain contacts to the binding surface (C).

(MD) simulations and principal-component analysis (PCA) to elucidate the role of IgA1–Fc domain motion in receptor binding. We discerned functionally relevant hinge-based dynamics of the IgA1 C α 2 and C α 3 domains based on the compatibility of corresponding principal eigenvectors with the conformational change induced by receptor binding. The analysis predicted that receptor binding at the C α 2–C α 3 interface would induce long-range conformational changes propagating up to the *O*-glycosylated hinge, which was confirmed using biosensor experiments with the hinge-binding lectin HAA. Thus, we propose that long-distance communication in IgA1 is mediated by extensive allosteric networks that couple antigen-binding (Fab) regions and receptor-binding (Fc) regions (18–20). Our results are consistent with experimental and computational studies of widely diverse classes of proteins that revealed that allosteric mechanisms are responsible for inducing large-scale conformational changes (21–24), promoting dynamic coupling (25, 26), or selecting functionally relevant folding pathways (27, 28).

Results

Energetic Analysis of Fc α RI Binding to IgA1. Using the crystal structure of the complex, Fc α residues involved in the Fc α RI:Fc α interaction were identified (6). Nineteen residues, located at the C α 2–C α 3 interface, contact Fc α RI. Ten of these residues have side chains contacting Fc α RI, defined as being within 4 Å of Fc α RI (L257, L258, R382, S387, E389, M433, E437, L441, A442, and F443) (6). Except for L257 and L258, which are located on the AB helix/loop of the C α 2 domain of IgA, all residues are in the C α 3 domain. The Fc α RI binding site on IgA is composed of a central hydrophobic region surrounded by polar and charged residues, an arrangement typical of many protein–protein binding interfaces (Fig. 1B) (6, 29–31). The contribution of each side chain to the buried surface area in the Fc α RI:Fc α complex is shown in Fig. 1C.

Each Fc α residue whose side chain contacts Fc α RI was individually mutated to alanine (with the exception of A442). Binding of Fc α variants to Fc α RI was measured by surface plasmon resonance (SPR) (SI Appendix, Fig. S1). Since two Fc α RI

molecules can bind a single Fc α protein, equilibrium binding data were fitted to a two-site binding model to determine K_{D1} and K_{D2} , equilibrium binding constants corresponding to the first and second binding events (6, 13, 14). Similar to previously published values, wild-type Fc α bound Fc α RI with K_{D1} and K_{D2} values of 46.2 and 223 nM, respectively (Table 1) (13, 14). K_{D1} values were used to compute $\Delta\Delta G$ values for the various mutations. All Fc α mutants had positive $\Delta\Delta G$ values, indicating that each mutation resulted in a less favorable interaction with Fc α RI compared with wild type (Fig. 2A and Table 1). Far-UV circular dichroism experiments demonstrated that all mutant and wild-type Fc α proteins had similar secondary structure content, indicating that decreases in affinity were not due to global structural changes (SI Appendix, Fig. S2 and Table S1).

Mutation of charged or polar Fc α residues located at the periphery of the binding site (R382, S387, and E437) had only mild

Table 1. Equilibrium parameters for Fc α RI binding to Fc α wild type and mutants

Fc α ligand	K_{D1} , nM	K_{D2} , nM	ΔG , kcal/mol	$\Delta\Delta G$, kcal/mol
Wild type	46 ± 2	220 ± 10	–10.00	0.00
R382A	63 ± 3	520 ± 40	–9.82	+0.18
E437A	64 ± 2	450 ± 30	–9.81	+0.19
S387A	73 ± 2	620 ± 30	–9.73	+0.27
E389A	292 ± 6	2,900 ± 100	–8.91	+1.09
L441A	360 ± 10	2,600 ± 100	–8.79	+1.21
L257A	2,390 ± 70	62,000 ± 6,000	–7.67	+2.34
M433A	3,000 ± 2,000	~29,000	–7.52	+2.49
F443A	~6,000	~45,000	–7.14	+2.93
L258A	~15,000	~78,000	–6.56	+3.44

Equilibrium parameters for Fc α proteins were derived from analyses with at least nine different concentrations of injected Fc α RI. K_{D1} values for Fc α F443A and L258A, and K_{D2} values for M433A, F443A, and L258A could not be determined with a high degree of confidence under the experimental conditions. Each binding experiment was carried out in duplicate.

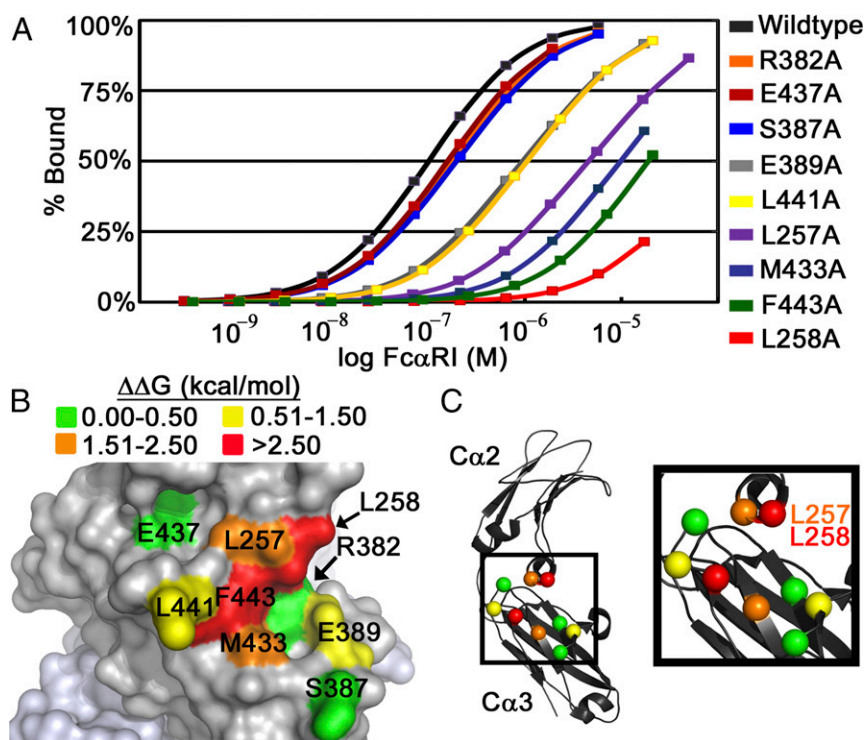


Fig. 2. SPR analysis of Fc α RI binding to Fc α variants identifies energetic hot-spot residues. (A) Coplotted SPR binding isotherms of wild-type Fc α and mutants binding to Fc α RI. Fc α variants were coupled to the SPR chip and soluble Fc α RI was flowed over. Fc α L257A, M433A, F443A, and L258A mutations resulted in the largest decreases in binding affinity. (B) Plotting of the experimentally determined $\Delta\Delta G$ values on Fc α . (C) Location of the C α of all mutated residues, colored according to the $\Delta\Delta G$ values for each alanine mutant.

effects on binding affinity, with $\Delta\Delta G$ values of less than +0.3 kcal/mol compared with wild type (Fig. 2B and Table 1). Two additional mutations, E389A and L441A, had an intermediate effect on binding affinity, with $\Delta\Delta G$ values between +1.0 and +1.2 kcal/mol. In the complex, E389 is sandwiched between Fc α RI residues R52 and R53 and Fc α residue R382, so the E389A mutation likely results in electrostatic repulsion between the arginine side chains. L441 is a hydrophobic residue located in close proximity to the center of the Fc α RI binding site on Fc α . The loss of the hydrophobic side chain in the L441A variant results in a loss of van der Waals interactions with Fc α RI residues Y35, F56, and H85.

Mutation of hydrophobic Fc α residues L257, L258, M433, or F443 resulted in the largest decreases in Fc α RI affinity ($\Delta\Delta G$ values between +2.34 and +3.44 kcal/mol). The side chain of each of these hot-spot residues has a high percentage of its accessible surface area buried in the Fc α RI:Fc α complex (between 90% and 100%; *SI Appendix, Table S2*). However, buried surface area alone is not a reliable indicator of a mutation's effect. In the case of S387, whose surface area is 70% buried in the interface, mutation to alanine resulted in a much smaller $\Delta\Delta G$ value of +0.27 kcal/mol. Experimental $\Delta\Delta G$ values also did not have a strong correlation with the Fc α residues' individual surface area contributions to the total binding interface (Figs. 1C and 2B and *SI Appendix, Table S2*) (32).

The importance of M433A and F443A is expected due to the residues' location in the central hydrophobic region of the protein binding interface (Fig. 1B), which typically contributes significantly to the energetics of complex formation (29, 33–36). Mutation of these hydrophobic residues to alanine would result in a loss of significant van der Waals interactions by disrupting the tight packing of Fc α RI residues Y35, L54, F56, G84, and H85 against the central hydrophobic region of Fc α , with a negative impact on occlusion of bulk solvent at the binding interface.

The dramatic effect of mutating L257 or L258, the only two C α 2 residues with side-chain contacts, indicates the C α 2 domain also plays a very important role in the stability of the Fc α RI:Fc α complex (Fig. 2B and C). In the complex, the side chain of

L257 interacts with Fc α RI residues Y35 and R82 and forms part of a hydrophobic pocket into which the side chain of residue H75 packs; this pocket is responsible for the pH dependence of the Fc α RI:Fc α interaction (14). The L258 side chain interacts with Fc α RI residues Y35, R52, and R53. The importance of L257 and L258 for stable complex formation is further supported by the observation that the IgA1 C α 3 domain alone (expressed in bacteria and refolded from inclusion bodies) showed nearly undetectable binding to Fc α RI by SPR (*SI Appendix, Fig. S3*), confirming that C α 3 alone does not confer stable binding to Fc α RI, despite contributing 80% of the residues whose side chains contact Fc α RI.

Importance of the C α 2 Domain in Related Antibody:Receptor Interactions. Given the importance of the C α 2 L257 and L258 residues in the Fc α RI:Fc α interaction, we compared this complex to a related IgY:receptor pair to ascertain the role of analogous residues. IgY, the predominant serum antibody of lower vertebrates including reptiles, amphibians, and birds (37), is believed to be an ancestor of human IgA (38). The two C-terminal domains of human IgA and chicken IgY share 34% amino acid identity, and the interaction between chicken IgY and the chicken Ig-like receptor, CHIR-AB1, closely resembles the Fc α RI:Fc α interaction (39). Similar to Fc α RI, the gene encoding the CHIR-AB1 receptor is found within the leukocyte receptor cluster and signaling requires the associated Fc γ R coreceptor. Furthermore, CHIR-AB1 binds the Fc domain of chicken IgY (Fc ν) to form a 2:1 complex (40–43). Mutational analyses mapped the IgY contact residues to the C α 3–C α 4 (C α 3–C α 4) junction, which is analogous to the IgA C α 2–C α 3 junction (39, 44). Individual mutation of C α 3 residues 362–364 (LYI), analogous to C α 2 residues 256–258 (LLL), as well as mutation of C α 4 Pro and Arg residues within the PMRF motif (residues 554–557; analogous to C α 3 PLAF residues 440–443), resulted in the greatest decrease in binding of IgY to the CHIR-AB1 receptor. Thus, accessible residues in the C α 2/C α 3 (or equivalent C α 3/C α 4) interfaces involved in receptor interactions are conserved between IgA and IgY (39).

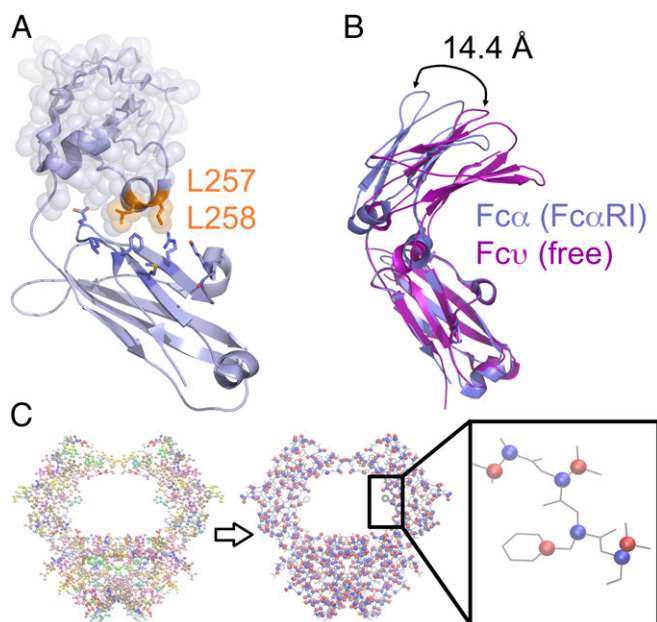


Fig. 3. Comparison of $Fc\alpha$ and $Fc\beta$ crystal structures reveals variability in the C_{H2} domain position, indicating the $C\alpha2$ - $C\alpha3$ junction acts as a hinge point. (A) Structure of the $Fc\alpha$ heavy chain, showing the location of L257 and L258 at the bottom of the $C\alpha2$ domain (highlighted with transparent spheres). (B) Overlay of $Fc\alpha$ from the $Fc\alpha$ RI-bound complex (blue) with unbound IgY $C\alpha3$ - $C\alpha4$ (magenta) revealed a 14.4-Å shift between the top of the $C\alpha2$ and $C\alpha3$ domains. (C) The coarse-grained model of $Fc\alpha$ is shown in a bead representation, with each amino acid represented using two beads. First bead (blue), representing the backbone, is located at C_{α} position, and the second bead (red), representing the side chain, is located at the center of mass of the amino acid's side chain.

The critical contribution of the $C\alpha2$ L257 and L258 residues, and analogous $C\alpha3$ residues, to interactions with their respective receptors is reflective of a conserved binding mode, despite the fact that these residues are located across from the $C\alpha3$ domain boundary where most of the receptor contacts occur (Fig. 3A). A flexible linker exists between the $C\alpha2$ and $C\alpha3$ domains in $Fc\alpha$, which could act as a major hinge point between these two domains. Backbone alignment of $Fc\alpha$ (from the $Fc\alpha$ RI: $Fc\alpha$ structure) with the unbound IgY-Fc fragment (42) showed a 14.4-Å difference in the position of the upper domains (Fig. 3B). Unlike IgA, IgY does not have a disulfide bond linking its $C\alpha3$ domains (analogous to IgA $C\alpha2$ domains), which may account for the observed degree of variation in the position of the upper domains. The variability of the position of $C\alpha2$ or $C\alpha3$ in these structural alignments indicates a substantial degree of flexibility at the $C\alpha2$ - $C\alpha3$ ($C\alpha3$ - $C\alpha4$) junction.

$Fc\alpha$ RI Binding Dampens Intradomain Motions of $Fc\alpha$. To elucidate the effect of $Fc\alpha$ RI binding on $Fc\alpha$ flexibility, we performed Langevin dynamics (LD) simulations of a coarse-grained model of the 2:1 $Fc\alpha$ RI: $Fc\alpha$ complex, a 1:1 $Fc\alpha$ RI: $Fc\alpha$ complex (through removal of the *trans* $Fc\alpha$ RI receptor), and the unliganded $Fc\alpha$ (through removal of both *cis* and *trans* $Fc\alpha$ RI receptors). The initial configurations of these systems were obtained from the crystal structure of the 2:1 $Fc\alpha$ RI: $Fc\alpha$ complex (PDB ID code 1OW0) (6). As indicated in *Materials and Methods*, the coarse-grained procedure describes amino acids by using two virtual particles, C_{α} and side chain (C_{α} -SC), that represent backbone and side-chain atoms (Fig. 3C). Comparison of the B-factor profile of $Fc\alpha$ heavy chains in the 2:1 $Fc\alpha$ RI: $Fc\alpha$ complex with the corresponding experimental values in the crystal structure (*SI Appendix*,

Fig. S4) indicates a similar pattern of structural flexibility, which supports the validity of our LD simulation protocol.

We characterized quantitatively the degree of $Fc\alpha$ flexibility (Fig. 4) in these three systems by computing root-mean-square fluctuations (RMSFs) of protein amino acids in LD trajectories (*Materials and Methods*). Consistent with the stoichiometry of receptor binding, residue fluctuations (Fig. 4A) in the two $Fc\alpha$ chains are symmetric in $Fc\alpha$ and 2:1 $Fc\alpha$ RI- $Fc\alpha$ conformations and asymmetric in 1:1 $Fc\alpha$ RI- $Fc\alpha$ conformations. We note that large flexibility is present in the intersubunit loop regions (Fig. 4); however, subunit structure is largely preserved in our simulations. We propose that the combination of structural stability in the receptor-binding region and conformational flexibility in loop regions at the intersubunit interfaces is important to mediate functional allosteric communication between subunits. As shown in Fig. 4A, comparison of RMSF profiles indicates that the major effect of receptor binding is to reduce flexibility of three $Fc\alpha$ regions, R1 (amino acids 255–260), R2 (380–390), and R3 (430–445). This dampening effect is the direct result of formation of the $Fc\alpha$ RI- $Fc\alpha$ interface as indicated by the nearly identical RMSF differences in these regions in the *cis* $Fc\alpha$ chain in the asymmetric 1:1 and in the symmetric 2:1 complexes compared with the unliganded $Fc\alpha$ (Fig. 4A). As discussed above, these regions contribute to the $Fc\alpha$ RI: $Fc\alpha$ interface and include the hinge formed by C_{H2} and C_{H3} domains. The largest changes in RMSF values in these regions correspond to amino acids L257, L258, G259, S260, S387, Q388, E389, R392, E393, P440, L441, A442, and F443 (Fig. 4). We note that this set includes L257, L258, S387, E389, L441, A442, and F443, which are highlighted as important for $Fc\alpha$ RI binding in our mutagenesis studies.

$Fc\alpha$ RI Binding Results in Weak Perturbation of Fundamental Motions of $Fc\alpha$. Receptor-induced conformational changes in proteins are mediated by allosteric networks that span long distances and may include interdomain and intersubunit interactions. To reveal long-range communication within the $Fc\alpha$ structure activated upon $Fc\alpha$ RI binding, we use PCA, which probes collective motions of distinct $Fc\alpha$ RI: $Fc\alpha$ complexes (*Materials and Methods*). In this approach, the covariance matrix is diagonalized to yield

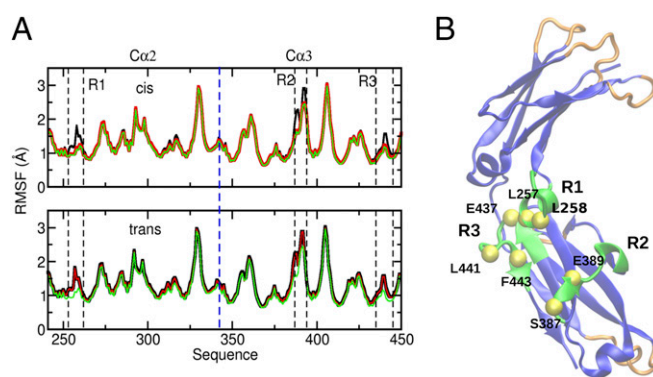


Fig. 4. Backbone flexibility in distinct $Fc\alpha$ complexes. (A) The root-mean-square fluctuations (RMSFs) of C_{α} atoms of $Fc\alpha$ amino acids in unliganded $Fc\alpha$ (black); 1:1 $Fc\alpha$ RI- $Fc\alpha$ (red) and 2:1 $Fc\alpha$ RI- $Fc\alpha$ (green) complexes are shown for the *cis* (*trans*) heavy chain in the *Upper* (*Lower*) panel. In the asymmetric 1:1 $Fc\alpha$ RI- $Fc\alpha$ complex, the receptor is bound to the *cis* $Fc\alpha$ heavy chain. (B) Amino acids (green) corresponding to the R1, R2, and R3 regions in A, located primarily near the linker between the $C\alpha2$ and $C\alpha3$ domains, experience the strongest RMSF dampening upon receptor binding. The yellow spheres with labels indicate the amino acid positions in this set, which are highlighted as important for receptor binding in mutagenesis studies. Six structural regions (orange and green loop in the $C\alpha3$ domain) with the largest flexibility (RMSF > 1.5 Å) primarily include loops involved in the intersubunit interface.

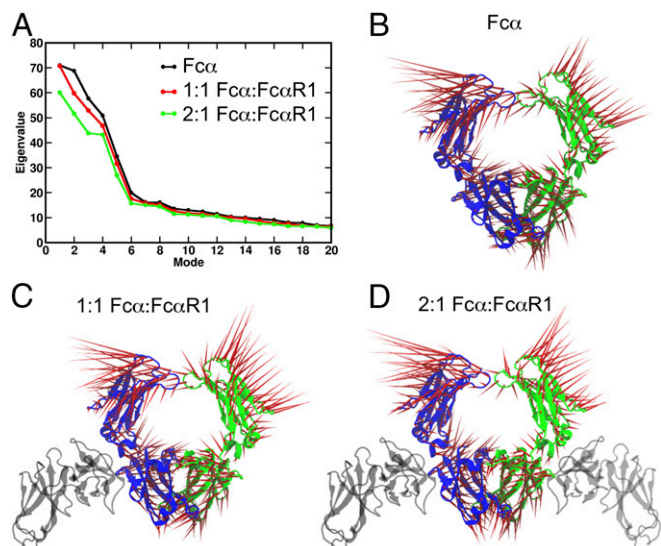


Fig. 5. Principal-component analysis (PCA) of MD trajectories of distinct $Fc\alpha$ complexes. (A) The largest 20 eigenvalues of the PC modes for different $Fc\alpha$ complexes: $Fc\alpha$ (black), 1:1 $Fc\alpha RI-Fc\alpha$ (red), and 2:1 $Fc\alpha RI-Fc\alpha$ (green). (B–D) $Fc\alpha$ motions associated with mode 1 (largest eigenvalue) in the three systems studied. The red vectors illustrate the amplitude and the direction of residue motion for (B) $Fc\alpha$ alone, (C) 1:1 $Fc\alpha RI-Fc\alpha$, and (D) 2:1 $Fc\alpha RI-Fc\alpha$.

the set of eigenvectors that characterize the direction of motion in independent modes and eigenvalues that determine the amplitudes of motions (45). Zero eigenvalues, which correspond to rotations and translations of the entire structure, are excluded from the analysis and nonzero eigenvalues are ranked in order of decreasing value (Fig. 5A). Generally, it is found that collective motions with the largest contribution to the RMSF correspond to a small number of principal component (PC) modes with the largest eigenvalues. In each of the systems studied, we find that the five highest-ranked PC modes have significantly larger eigenvalues than all other modes (Fig. 5A), which indicates that these are the most relevant modes in describing the functional dynamics of the $Fc\alpha$ structure. The common aspect of the eigenvalue profiles for the three systems is consistent with the similar overall pattern of RMSFs. Smaller eigenvalues corresponding to 1:1 and 2:1 $Fc\alpha RI-Fc\alpha$ complexes reflect the dampening effect of $Fc\alpha RI$ binding on $Fc\alpha$ motions. The smaller difference between the $Fc\alpha$ and 1:1 $Fc\alpha RI-Fc\alpha$ profiles indicates that single receptor binding results in a weak perturbation of $Fc\alpha$ motion, while the larger change in eigenvalues upon binding of the second receptor indicates a stronger perturbation in the symmetric complex. To characterize, at the amino acid level, the five significant PC modes that contribute to conformational changes in $Fc\alpha$, we examined in detail the associated motions and directional correlations of amino acid pairs (*Materials and Methods*). $Fc\alpha$ motions associated with the five highest-ranked PC modes involve primarily rigid-body domain motions of the $C\alpha 2$ and $C\alpha 3$ domains around their flexible common joints (Fig. 6, *SI Appendix*, Fig. S5, and *Movies S1–S15*). These movements satisfy constraints imposed by intersubunit interfaces ($C\alpha 2-C\alpha 2$ and $C\alpha 3-C\alpha 3$), in addition to those resulting from intrasubunit hinges ($C\alpha 2-C\alpha 3$). Extensive contacts between $C\alpha 3$ domains of the two chains strongly constrain the relative mobility of these two domains, so that, in all five PC modes, their motions consist largely of rigid-body rotations around the common interface (*SI Appendix*, Fig. S5 and *Movies S1–S15*). Distinct motions of the five PC modes arise primarily from the more flexible $C\alpha 2-C\alpha 2$ interface, which is dominated by disulfide bonds (*Movies S1–S15*). In a given PC mode, associated $Fc\alpha$

motions are similar for all three complexes. For example, mode 1 (Fig. 5B and *Movies S1–S3*) corresponds to torsional motions of the $C\alpha 3$ domains and swing-like motions of $C\alpha 2$ domains. Comparative study of motions and correlations of amino acid pairs further confirms that $C\alpha 2$ and $C\alpha 3$ domains have a higher flexibility to move around their hinge in the unliganded $Fc\alpha$ compared with more restricted movements of these domains in 1:1 and 2:1. Overall, our analysis highlights the importance of the intersubunit ($C\alpha 2-C\alpha 2$) disulfide bond region for effecting $Fc\alpha$ conformational changes. This indicates that perturbations at the intrasubunit junctions, such as those effected by receptor binding, are transmitted primarily to the $C\alpha 2-C\alpha 2$ interface and, therefore, are likely to influence conformational fluctuations at the IgA1 hinge. In addition, the common fundamental motions of the three systems lead us to conclude that $Fc\alpha RI$ binding yields tighter coupling of sites near the $Fc\alpha$ $C\alpha 2-C\alpha 3$ intrasubunit junction without significantly distorting global $Fc\alpha$ motions.

Receptor Binding Activates a Long-Range Allosteric Network in $Fc\alpha$.

To characterize long-range communication between $Fc\alpha$ regions, we computed cross-correlation maps of residue fluctuations along the five highest ranked PC modes for the three distinct $Fc\alpha$ systems (*Materials and Methods*). As illustrated in Fig. 7 and *SI Appendix*, Fig. S5, in each of the five modes, intradomain motions of liganded or unliganded $Fc\alpha$ are strongly correlated, consistent with the rigid-body domain motions noted above. In addition, we find strong correlation between motions of regions of distinct domains, which supports the existence of long-range interactions and coordinated domain movements (Fig. 7). For example, the cross-correlation map of PC modes 1 and 2 indicates strong coupling involving regions of the $C\alpha 2$ and $C\alpha 3$ domains of distinct subunits. In modes 3–5, intersubunit coupling is primarily mediated by strong correlations involving the $C\alpha 2-C\alpha 2$ and $C\alpha 3-C\alpha 3$ interfaces. In addition, strong intrasubunit anticorrelation between $C\alpha 2$ and $C\alpha 3$ domains, observed in modes 1 and 4, highlights hinge-based motions of IgA1. Overall, we surmise that the collective motions of the $Fc\alpha$ molecule are largely determined by strong correlation of intersubunit motions and anticorrelation of intrasubunit motions. These results suggested that allosteric interdomain communications control the global motions of $Fc\alpha$. We also note that the pattern of interdomain coupling in 1:1 and 2:1 complexes is similar to that identified in the unliganded $Fc\alpha$ (*SI Appendix*, Fig. S5), which is consistent with the common fundamental motions of $Fc\alpha$ in the three systems. Nevertheless, the cross-correlation

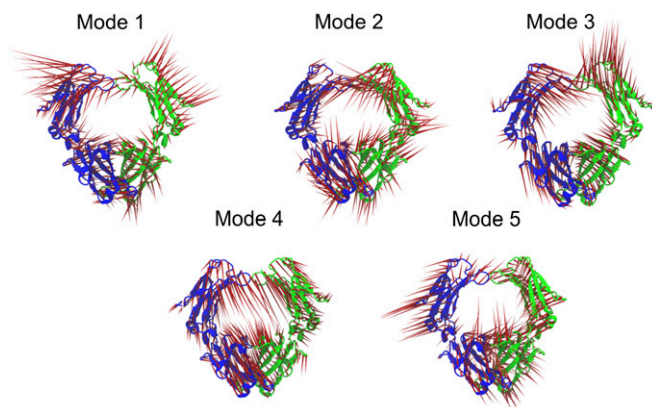


Fig. 6. $Fc\alpha$ motions associated with the highest ranked PC modes. The five panels indicate the mode motions for modes 1–5 for unliganded $Fc\alpha$. The red vectors indicate the direction of residue motion, and the vector length indicates the relative amplitude of the residue motion in each mode.

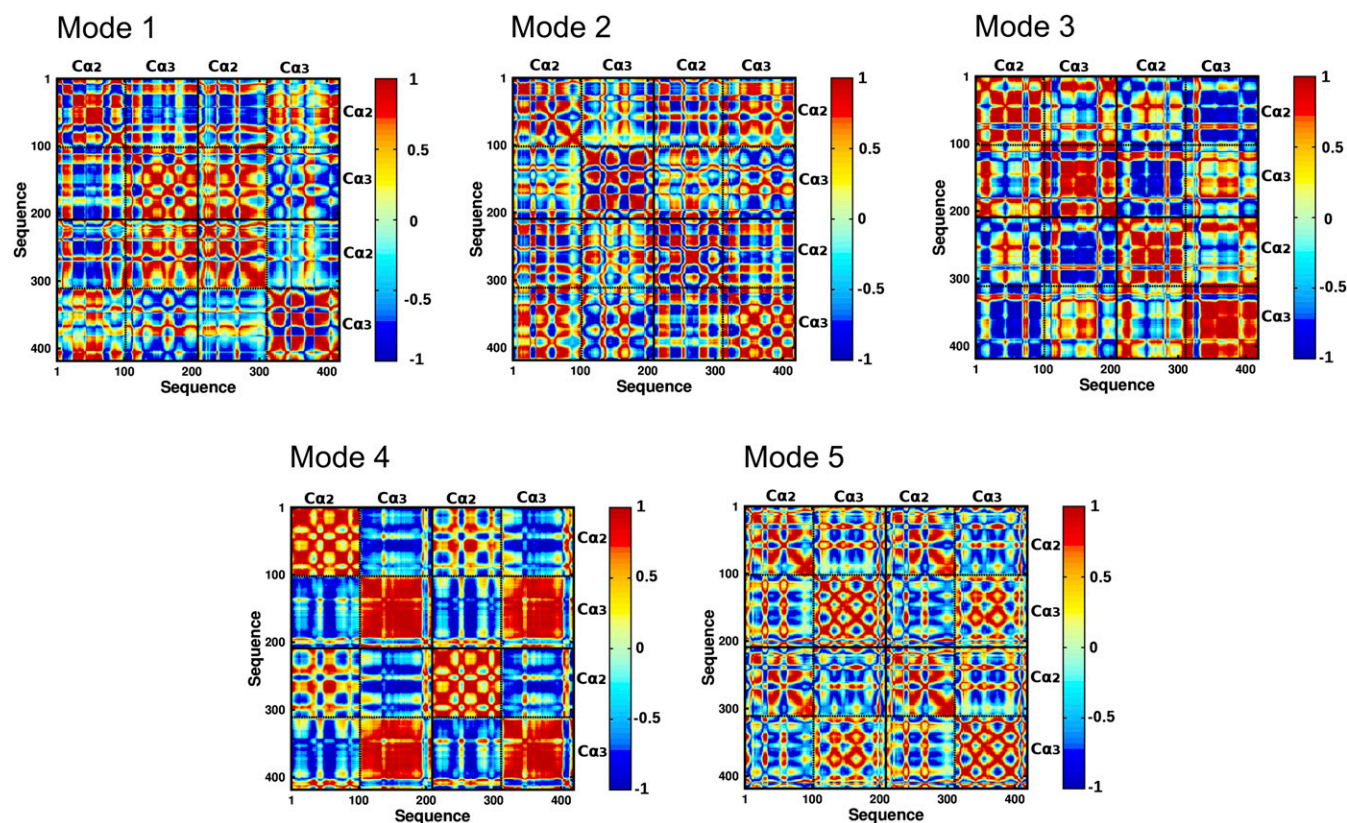


Fig. 7. Maps of directional correlation coefficients of all amino acid pairs in the unliganded $Fc\alpha$ for the five highest ranked PC modes. Correlation of amino acid pairs in modes 1–5. Strong correlation of a given pair of residues is indicated in red, and strong anticorrelation is shown in blue.

maps of the 1:1 complex reveal the allosteric effects of receptor binding to the *cis* subunit on the dynamics of the *trans* subunit.

$Fc\alpha$ Residue Network Mediates Receptor-Induced Intersubunit Communication. To pinpoint the effect of receptor binding on the $Fc\alpha$ allosteric network, we highlight pairs of amino acids within distinct subunits that have significantly modified correlation properties within 1:1 and 2:1 complexes compared with the unliganded $Fc\alpha$. To this end, we consider residue pairs with weakly correlated motions in the unliganded $Fc\alpha$ and strongly correlated/anticorrelated motions in 1:1 and 2:1, as well as pairs that switch from strongly correlated (anticorrelated) motions in the unliganded $Fc\alpha$ to strongly anticorrelated (correlated) motions in $Fc\alpha RI-Fc\alpha$ complexes (*Materials and Methods*). Residue pairs identified according to our criteria for long-range communication are indicated in *SI Appendix, Tables S3 and S4* and their structural location is illustrated in *SI Appendix, Fig. S6* for the five highest ranked PC modes. We find that modes 2 and 3 include the largest populations of these pairs, which suggests that intersubunit coupling associated with modes 2 and 3 provides a large contribution to the propagation of receptor-induced perturbation over long distances. In modes 1, 4, and 5, intersubunit residue correlations are less affected by receptor binding (*SI Appendix, Fig. S5*), indicating that these mode motions are primarily responsible for regulating the other biological roles of $Fc\alpha$ molecule.

We examined in detail the long-distance coupling of each of the nine residues that comprise the receptor site (L257, L258, R382, S387, E389, M433, E437, L441, and F443), which were highlighted by our binding experiments. As shown in Fig. 8 and *SI Appendix, Tables S3 and S4*, long-distance communication involving these nine residues is significantly perturbed by

receptor binding to $Fc\alpha$. We find that $Fc\alpha RI$ binding results in the strongest perturbation of directional correlation of residues L441 and F443 with distant residues. Two regions include large clusters of residues (Fig. 8) that are involved in long-distance communication with the $Fc\alpha RI$ binding site. One of these regions is the $Ca2-Ca3$ junction, consistent with the signaling between the *cis* and *trans* receptor-binding sites. The second region is located at the $Ca2-Ca2$ junction near the IgA1 hinge, which suggests that receptor binding could induce long-range conformational changes in the hinge and Fab regions of IgA1. Notably, our results reveal that asymmetric binding of $Fc\alpha RI$ to the *cis* $Fc\alpha$ subunit, as illustrated by the 1:1 complex, elicits strong long-distance response in the *trans* $Fc\alpha$ subunit (Fig. 8). Overall, we conclude that $Fc\alpha RI$ binding induces tighter coupling of $Fc\alpha$ subunits by altering the underlying allosteric network without strongly perturbing the global fundamental motions.

$Fc\alpha RI$ Binding at the $Ca2-Ca3$ Junction Affects HAA Binding at IgA1 Hinge. We have shown that binding of $Fc\alpha RI$ occurs at a hot spot for dynamic transitions and that $Fc\alpha RI$ binding dampens IgA1 domain motions. Furthermore, the negative cooperativity seen in this study and previous (13, 14) SPR experiments (i.e., K_D2 is 4.8-fold weaker than K_D1) and the PCA analysis together demonstrate the existence of long-range conformational effects across the $Fc\alpha$ dimer, suggesting that receptor binding at the $Ca2-Ca3$ interface could influence dynamics near the $Ca2-Ca2$ interface and the IgA1 hinge regions. Thus, we conducted SPR binding experiments to determine whether $Fc\alpha RI$ binding can affect recognition events at the IgA1 hinge. Each IgA1 hinge region contains six potential *O*-linked glycosylation sites (46–52). The *O*-glycans consist of a core *N*-acetylgalactosamine (GalNAc) linked to a galactose, both of which may be sialylated (48–52).

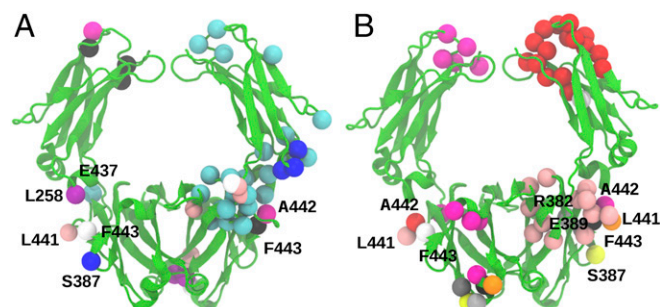


Fig. 8. Long-distance allosteric interactions between intersubunit pairs involving Fc α RI binding sites. (A) Intersubunit residue pairs that switch from weak directional correlation in unliganded Fc α to strong correlation or anticorrelation upon receptor binding. Amino acid pairs, highlighted by distinct colors, include experimentally identified Fc α RI-binding sites L258 (purple, *cis*), S387 (blue, *cis*), E437 (cyan, *cis*), L441 (pink, *cis*), A442 (magenta, *trans*), and F443 (white, *cis*; black, *trans*). (B) Intersubunit residue pairs that switch from strongly correlated (anticorrelated) motions in unliganded Fc α to strongly anticorrelated (correlated) motions in Fc α RI:Fc α complexes. Shown are pairs that include Fc α RI binding sites L441 (pink, *cis*), A442 (red, *cis*), F443 (white, *cis*), R382 (silver, *trans*), S387 (yellow, *trans*), E389 (gray, *trans*), L441 (orange, *trans*), L442 (magenta, *trans*), and F443 (black, *trans*).

This heavy *O*-glycosylation of the IgA1 hinge causes it to be less flexible than IgG hinges and therefore potentially more likely to transmit long-range conformational changes.

Patients with IgAN, a kidney disease characterized by glomerular deposition of IgA1 immune complexes, have aberrantly glycosylated IgA1 that is undergalactosylated compared with control samples (53), resulting in the exposure of GalNAc moieties (54). HAA, a snail lectin, is a hexamer (a dimer of trimers) that specifically recognizes terminal GalNAc residues (55, 56). We have previously shown that HAA is functionally bivalent when binding IgA1 and can simultaneously bind two GalNAc residues on a single IgA1 antibody, presumably one from each hinge (56). If Fc α RI binding at the C α 2–C α 3 junction induces long-range conformational changes that propagate to the IgA1 hinge, this could result in altered recognition of the *O*-glycans on the hinge by HAA due to changes in relative orientation of the GalNAc residues. Therefore, we measured binding of HAA to a myeloma IgA1 protein (IgA1 κ) in the presence or absence of saturating concentrations of Fc α RI to assess any differences in the affinity or kinetics of HAA binding (13).

Equilibrium binding experiments were conducted to evaluate whether the affinity of IgA1 κ for immobilized HAA was affected by the binding of Fc α RI. Due to the low affinity of the IgA1–HAA interaction, full equilibrium binding curves could not be measured, but inspection of SPR sensorgrams from a low-flow rate experiment indicated a potential difference in the kinetics of binding (Fig. 9A). To verify this observation, we repeated binding experiments at 30 μ L/min for kinetic analysis and fitted the data to a bivalent analyte model. The affinity of free IgA1 κ for HAA (222 μ M K_D 1; 4.4 μ M K_D 2) was weaker compared with the Fc α RI:IgA1 κ complex (170 μ M K_D 1; 2.8 μ M K_D 2) (Fig. 9B and Table 2). The higher-affinity interaction of HAA with the Fc α RI:IgA1 complex is solely due to faster association rates for both binding events (30% faster $k_{1,on}$; 54% faster $k_{2,on}$). These faster on-rates are the opposite trend of what would be expected based simply on a diffusion-limited binding event, since the Fc α RI:IgA1 κ complex has \sim 40% larger mass than IgA1 κ alone. Thus, binding of Fc α RI to the C α 2–C α 3 interface of IgA1 induces conformational changes substantial enough to alter the kinetics of HAA binding to a distal site at the IgA1 hinge.

Discussion

We have conducted a systematic analysis of the relative energetic contribution of IgA1 side-chain contacts to the Fc α RI:IgA1 complex, which complements previous mutational studies (10, 11, 57). Mutation of IgA1 hydrophobic residues central to the binding interface greatly reduced binding to Fc α RI, as expected (10, 11). For example, mutation of L441, M433, or F443 at the center of the binding site results in 12%, 25%, or 29% reductions in binding free energy, respectively. Mutation of charged and polar residues forming the outer periphery of the binding site (R382, E437, and S387) had a much milder effect, with reductions in ΔG of only 1.8–2.7%. Mutation of E389 is more deleterious (11% reduction in ΔG), presumably due to its role in mitigating electrostatic repulsion between a cluster of basic residues. It was interesting that mutation of C α 2 AB helix/loop residues L257 or L258 also resulted in a dramatic loss in binding affinity, causing a 23% or 34% loss in binding free energy, respectively. The very low affinity of Fc α RI for a dimeric C α 3-only construct of IgA1 highlights the critical role of the C α 2 domain (*SI Appendix*, Fig. S3). The importance of these residues in the C α 3 energetic hot spot and the C α 2 domain are underlined by their conservation in the related CHIR–ABI:IgY complex. Combined, these data indicate a similar mode of binding and we hypothesize that the C α 2 domain plays a key role in complex formation.

PCA of Fc α motions determined in MD simulations suggests that, despite disulfides tethering the C α 2 domains, free IgA1 exhibits a significant degree of motion of the C α 2 domains relative to the C α 3 domains. Binding of Fc α RI causes a loss in Fc α intradomain and interdomain flexibility. The PC analysis reveals that binding of Fc α RI to only one side of Fc α (forming a 1:1 complex) induces conformational changes across the dimer interface to the opposite heavy chain, explaining the consistent observation of negative cooperativity in SPR binding data for the Fc α RI:Fc α interaction (13, 14). Analysis of RMSFs of Fc α showed that there are three major sites containing dynamically relevant residues, all of which are located at the C α 2–C α 3 junction, including the energetic hot-spot residues. The concentration of the dynamic and energetic hot-spot residues at the C α 2–C α 3 junction accounts for the common mode of binding seen in the interactions of receptors and bacterial immune evasion proteins such as staphylococcal SSL7 (58, 59) with IgA or IgY.

An interesting and exciting prediction from this PC analysis is that Fc α RI binding can induce long-range conformational changes in IgA1. A consensus model of intact IgA1 bound to Fc α RI based on both crystal and solution structures (6, 60) shows that the Fc α RI-binding site is distal from the hinges and Fab regions (Fig. 1A) (3). However, the solution structures of monomeric, dimeric, and secretory IgA1 (60–62) indicate that the C-terminal portion of each hinge comes into close contact with the opposite Fc α heavy chain. The swing-like motions of C α 2 of the Fc α fragment that can occur upon Fc α RI binding as described by the PC analysis (Fig. 5B) might therefore be propagated from the Fc region to the hinge. We confirmed this hypothesis by demonstrating that IgA1 binds with a significantly faster on rate to the GalNAc-specific lectin HAA when Fc α RI is prebound at the IgA1 C α 2–C α 3 interface, indicating that Fc α RI binding induces long-range conformational effects at the IgA1 hinge. HAA is useful as a diagnostic tool for identifying patients with IgAN, since it binds to a similar epitope to that recognized by anti-glycan autoantibodies in IgAN patients (47, 55, 63).

Soluble Fc α RI has been implicated in the pathogenesis of IgAN (16, 17, 64), although its role has been puzzling. We previously showed that alterations in IgA1 *N*-glycans have no effect on Fc α RI binding (13), and the Fc α RI binding site is distal from the *O*-glycosylated hinges (Fig. 1A) (3, 6). The long-range conformational change described here that occurs in IgA1 upon

Snail lectin HAA (lot #101H3871; Sigma-Aldrich) and myeloma patient-derived IgA1 κ (lot #14C06810; Meridian Biosciences) were prepared as described (56). A CM5 chip was immobilized with HAA via random-amine chemistry to final densities of 200, 300, or 400 RU. For equilibrium analysis, 10 μ L of serial threefold dilutions of 9 μ M IgA1 κ in the presence or absence of 18 μ M Fc α RI were injected at 5 μ L/min in degassed TBS-P. For kinetic analysis, threefold serial dilutions of IgA1 κ alone, Fc α RI alone, or the mixtures of Fc α RI: IgA1 κ were injected at 30 μ L/min. Injection of 9 μ M IgA1 κ alone or 18 μ M Fc α RI:9 μ M IgA1 κ mixtures at 30, 50, 75, and 100 μ L/min yielded superimposable binding curves, demonstrating that binding to HAA was not mass transport limited. Data were fitted to a kinetic bivalent analyte model without allowing for bulk refractive index shift. The on rate for the second binding event was converted to molar units using the formula: $k_{2,on} [(M\cdot s)^{-1}] = k_{2,on} [(RU\cdot s)^{-1}] \times 100 \times$ analyte molecular weight in daltons (150,000 Da for IgA1 κ alone; 212,000 for 2:1 Fc α RI:IgA1 κ complex). K_D values were calculated as $K_D1 = 2k_{1,off}/k_{1,on}$ and $K_D2 = k_{2,off}/2k_{2,on}$, where the factors of 2 are statistical correction terms relating the apparent and intrinsic rate constants (14, 73). Additional detailed SPR methods are included in *SI Appendix*.

MD Simulations.

Coarse-grained model of distinct Fc α complexes. Our MD simulations used coarse-grained descriptions of the 2:1 Fc α RI:Fc α complex, the 1:1 Fc α RI:Fc α complex, and the unliganded Fc α dimer of heavy chains. The coarse-graining procedure was performed by representing each amino acid using two virtual particles. One particle, representing the backbone, is located at the C_α position and the other, representing the side chain, is located at the center of mass of the amino acid side chain (SC). The crystal structure (PDB ID code 1OW0) (6) of the 2:1 Fc α RI:Fc α complex was used to obtain the initial configuration of coarse-grained models. The potential energy of the protein is represented by the following equation:

$$V_{tot} = V_{BL} + V_{BA} + V_{DA} + V_{NB}, \quad [2]$$

where V_{BL} is the bond length potential, and V_{BA} and V_{DA} are the bond angle and the dihedral angle potentials, respectively (74). The nonbonded potential (V_{NB}) is calculated using the Lennard–Jones potential:

$$V_{NB} = \sum_{ij} V_{ij}(r_{ij}) = \sum_{ij} 4\epsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^6 \right], \quad [3]$$

where σ_{ij} is the hard-core radius and ϵ_{ij} is the well depth of the interaction between two virtual particles i, j separated by a distance r_{ij} . In this G \ddot{o} -type model, native contacts, identified as amino acid pairs found within a cutoff distance of 8 Å in the crystal structure, were assigned $\epsilon_{ij} = -1.25$ kcal/mol for C_α – C_α as well as for the C_α –SC pairs. Native SC–SC pairs were assigned ϵ_{ij} coefficients based on statistical potentials obtained from table 3 of Kolinski et al. (75). For all native pairs, σ_{ij} is chosen based on the native distance obtained from the crystal structure. Repulsive nonbonded interactions corresponding to nonnative contacts were described by using a Lennard–Jones potential with $\sigma_{ij} = 45.42$ Å and $\epsilon_{ij} = -10^{-12}$ kcal/mol.

Langevin dynamics simulations. The CHARMM program (76) was used to perform Langevin dynamics simulations for the three distinct Fc α configurations at the temperature of 300 K. We used a friction coefficient of 10 ps $^{-1}$ and a time step of 5 fs in our MD simulations. For each Fc α configuration, we obtained 80 trajectories consisting of 2×10^7 steps (representing 100 ns) for a total simulation time of 8 μ s. The effective timescales probed in our simulations are longer by up to several orders of magnitude due to the coarse-grained description of protein amino acids and the absence of explicit solvent representation in our model. Estimates of the real timescales can be obtained by using reduced units (77). The natural unit of time is $\tau = (m\sigma^2/\epsilon)^{1/2} = 3$ ps, where $m = 5 \times 10^{-22}$ g is the average mass of an amino acid, $\sigma = 3.8$ Å is the length of the virtual C_α – C_α

bond, and the energy $\epsilon = 1.25$ kcal/mol. Thus, the time step is 0.002τ , the friction coefficient is $30/\tau$, and the total simulation time is $3.2 \times 10^6\tau$.

MD Analysis.

PCA. We probed the functional dynamics of IgA1 upon receptor binding by comparing the principal collective motions of the Fc α dimer in MD trajectories of the three systems studied. Collective motions in dynamic trajectories were characterized by performing PCA, which consists of diagonalizing the covariance matrix, $C_{ij} = \langle (\vec{r}_i - \langle \vec{r}_i \rangle) \cdot (\vec{r}_j - \langle \vec{r}_j \rangle) \rangle$, to determine the set of eigenvectors and eigenvalues for each simulation type. \vec{r}_i represents the position vector of particle i at a given time, and $\langle \dots \rangle$ is the ensemble average over all of the frames of a trajectory type. In this analysis, amino acid positions were described using the C_α virtual particles. For the PCA, we used 2,000 time frames, separated by 50 ps (16.7 τ), within each trajectory. Rigid-body translations and rotations of the Fc α dimer were removed by aligning conformations corresponding to each frame with the crystal structure. The PCA calculations were performed using the CARMA MD simulation analysis package by Glykos (78).

RMSFs and B factors. Changes in Fc α residue flexibility upon binding to the receptor were probed by computing the RMSFs in each distinct MD simulation. RMSF values were obtained by taking the square root of diagonal elements of the covariance matrix:

$$\text{RMSF}_i = \sqrt{\langle (\vec{r}_i - \langle \vec{r}_i \rangle) \cdot (\vec{r}_i - \langle \vec{r}_i \rangle) \rangle}. \quad [4]$$

To validate the MD simulation protocol, we compared the computational values of B factors, $B_i = (8\pi^2/3)(\text{RMSF}_i)^2$, with the corresponding experimental values. **Directional correlation maps.** The directional correlation coefficient of a residue pair (i, j) in a given PC mode M was calculated by using $\text{Corr}_{ij,M} = \vec{e}_{iM} \cdot \vec{e}_{jM}$, where \vec{e}_{iM} is the unit vector in the direction of the displacement of the i th residue in mode M . These coefficients evaluate the directional similarity of motions of pairs of amino acids. We were able to probe short- and long-range coupling of IgA1 regions using maps of directional correlation coefficients of all amino acid pairs.

Long-range structural perturbation. Residue pairs that mediate long-range structural perturbation across the two Fc α heavy chains were identified based on their weak coupling in the unliganded Fc α dimer and strong coupling in response to Fc α RI binding or switching from strongly correlated (anticorrelated) motions to strongly anticorrelated (correlated) motions. To this end, we determined all residue pairs (i, j), where i and j belong to distinct Fc α heavy chains, which are separated by a minimum distance $d_{ij} > 30$ Å in the crystal structure. Changes from weak to strong coupling were evaluated by identifying pairs with $|\text{Corr}_{ij,M}^{(2:1)}| > 0.9$ in the 2:1 Fc α RI:Fc α complex, $|\text{Corr}_{ij,M}^{(1:1)}| > 0.6$ in the 1:1 Fc α RI:Fc α complex, and $|\text{Corr}_{ij,M}^{(1)}| > 0.2$ in the unliganded Fc α dimer. Changes from strong correlation to strong anticorrelation or vice versa were evaluated by $|\text{Corr}_{ij,M}| > 0.9$ in the 2:1 Fc α RI:Fc α complex and in the unliganded Fc α , with $\text{Corr}_{ij,M}^{(2:1)} \cdot \text{Corr}_{ij,M}^{(1)} < 0$.

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