

Human immunoglobulin E flexes between acutely bent and extended conformations

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Crystallographic and solution studies have shown that IgE molecules are acutely bent in their Fc region. Crystal structures reveal the Cε2 domain pair folded back onto the Cε3–Cε4 domains, but is the molecule exclusively bent or can the Cε2 domains adopt extended conformations and even ‘flip’ from one side of the molecule to the other? We report the crystal structure of IgE–Fc captured in a fully extended, symmetrical conformation and show by molecular dynamics, calorimetry, stopped-flow kinetic, surface plasmon resonance (SPR) and Förster resonance energy transfer (FRET) analyses that the antibody can indeed adopt such extended conformations in solution. This diversity of conformational states available to IgE–Fc offers a new perspective on IgE function in allergen recognition, as part of the B-cell receptor and as a therapeutic target in allergic disease.

Immunoglobulin E (IgE) antibodies play a central role in allergic disease¹. They recognize allergens in two very different contexts, either in a membrane-bound form as part of the B-cell receptor (BCR) or bound to the receptor FcεRI on effector cells such as mast cells and basophils. FcεRI-bound IgE causes long-term sensitization of these cells, and cross-linking by allergen leads to cell degranulation, release of inflammatory mediators and an immediate allergic response. Disruption of the IgE–FcεRI interaction is a validated strategy for therapeutic intervention in allergic diseases including asthma: an anti-IgE monoclonal IgG antibody, omalizumab (Xolair, Novartis Pharmaceuticals), inhibits IgE binding to FcεRI and is effective in the treatment of severe persistent asthma and other allergic diseases².

IgE consists of a dimer of two identical heavy and two identical light chains, but unlike IgG, in which the antigen-binding Fab region is separated from the receptor-binding Fc region by a flexible hinge, IgE contains an additional disulfide-linked pair of domains, (Cε2)₂, forming a (Cε2–Cε3–Cε4)₂ dimer¹. Fluorescence depolarization studies to assess segmental flexibility have shown IgE to be less flexible than IgG^{3–6}, and Förster resonance energy transfer (FRET) studies that determined distances both intramolecular and to the membrane led to a model of a compact, bent structure for IgE both when free in solution and when bound to FcεRI^{6–9}. Although an extended model was also proposed¹⁰, X-ray and neutron-scattering studies in solution confirmed that IgE and IgE–Fc adopt a compact, bent structure^{11,12}. An acutely and asymmetrically bent conformation was subsequently observed in the crystal structure of IgE–Fc (Fig. 1a)¹³. In this bent structure, the (Cε2)₂ domain pair folds back onto the Cε3–Cε4 domains, forming an extensive intramolecular interface (1,520 Å²). The subsequent structure of the complex of IgE–Fc bound

to the extracellular domains of the FcεRI α-chain (sFcεRIα) revealed an even more acute bend upon receptor binding¹⁴, consistent with FRET and fluorescence-depolarization studies that indicated reduced segmental flexibility^{6,15,16}. At this point the existence of an extended conformation of IgE–Fc was all but dismissed.

Although the Cε2 domains are not directly involved in binding FcεRIα, they do contribute to the kinetics of the interaction, decreasing both the association and dissociation rate constants^{14,17}. Interest in their structural and functional roles intensified following the discovery that the Fab fragment of omalizumab binds to a partially unbent conformation of IgE–Fc, as detected in a FRET experiment¹⁶. This first indication that IgE–Fc may not always be bent raises the question of whether the molecule transiently explores more extended conformations, and perhaps even flips between bent structures with the Cε2 domains folded back on opposite sides of the Cε3–Cε4 domains. Trapping of transiently populated conformational states has previously been achieved by antibody binding¹⁸, and so to explore the potential conformational diversity of IgE–Fc, we generated an IgG antibody Fab fragment that binds to IgE–Fc (anti-ε-chain Fab; aeFab) and discovered that it captured an extended conformation.

RESULTS

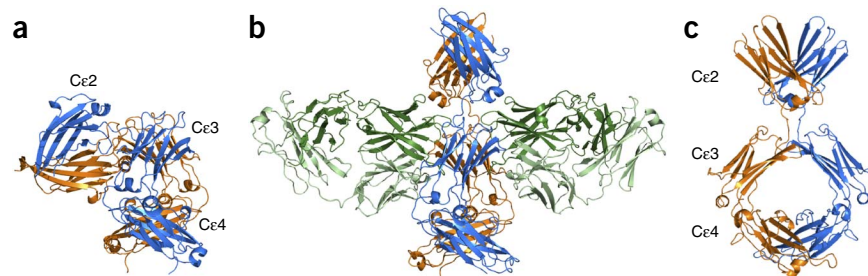
Structure of IgE–Fc bound by two aeFab fragments

The aeFab–IgE–Fc crystal structure was solved at 2.9 Å resolution (Table 1). The IgE–Fc adopts a fully extended conformation, with two aeFab molecules bound one on each side of the almost perfectly symmetrical IgE–Fc (aeFab¹–IgE–Fc–aeFab², with superscripted numbers denoting the two aeFab molecules, Fig. 1b,c). Compared with the structure of IgE–Fc alone, the molecule has undergone a drastic ‘unbending’

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Figure 1 Bent and extended structures adopted by IgE-Fc. (a) The bent structure of free IgE-Fc, with the (Ce2)₂ domain pair making contact with the Ce3-4 domains. IgE-Fc^A is shown in blue and IgE-Fc^B in orange. (b) The structure of IgE-Fc bound symmetrically by two aEFab molecules (shown with heavy chains in dark green and light chains in light green). (c) The extended conformation of IgE-Fc as seen in the complex (rotated 90° relative to b).



of 120° (Fig. 1a, c), completely losing the extensive intramolecular interface between the Ce2 and Ce3-Ce4 domains. This unbending seems to derive largely from movements in the Ce2-Ce3 linker region, in particular residues Pro333, Arg334 and Gly335 (Supplementary Video 1). Although the Ce2 domains show the greatest structural change, the Ce3 domains also undergo considerable movement. Conformational flexibility has been seen in a number of structures of the Fcε3-4 subfragment of IgE-Fc, with the Ce3 domains described as ‘open’ or ‘closed’ (together with a ‘swinging’ of one Ce3 domain relative to the other, Supplementary Fig. 1a)¹⁹. In the bent structure of IgE-Fc alone, one Ce3 is open (chain B) and one is closed (chain A), whereas in the extended conformation of IgE-Fc revealed here, both Ce3 domains adopt an open conformation (Supplementary Fig. 1b). Ce3^A (the Ce3 domain of chain A) thus moves more than Ce3^B (the Ce3 domain of chain B) upon aEFab binding, with a change in the Ce3-Ce4 interdomain angle of 15°. The (Ce4)₂ pair are unchanged upon complex formation. Such is the symmetry of the IgE-Fc in the complex that the local two-fold axes of all three domain pairs are virtually coincident.

As a result of this symmetry, the two aEFab interfaces are structurally equivalent (each ~1,400 Å²), mainly involving contact of the

aEFab heavy and light chains with Ce3, but with a small interaction with Ce2 (Supplementary Fig. 2a). Each aEFab molecule principally contacts a single IgE-Fc chain (aEFab¹ to IgE-Fc^A and aEFab² to IgE-Fc^B), with the exception of a 315 Å² interface with the Ce2-Ce3 linker region (including Ser331 and Asn332) of the other IgE-Fc chain (Supplementary Fig. 2b). The ‘hot spot’ of the aEFab-binding surface on IgE-Fc appears to center on Ce3 residue Arg393, which protrudes into a pocket at the interface of the heavy and light chains of aEFab (Supplementary Fig. 2c), forming a salt bridge (to Asp121 of the heavy chain) and hydrogen bonds (to Glu109 and Asn54 of the light chain; Supplementary Fig. 2d,e). The adjacent Ce3 residues also contribute extensively (Supplementary Fig. 2c).

Comparison of the aEFab¹-IgE-Fc-aEFab² complex with free IgE-Fc shows that the latter presents only one aEFab binding site. When superimposed on the Ce3^A domains, the aEFab-binding hot spot is both unchanged and accessible in bent IgE-Fc, making it a likely point for aEFab engagement. However, the Ce2 domains in the bent structure completely occlude aEFab binding to the other side of the molecule (Ce3^B) and would have to move substantially toward an extended conformation before aEFab access would be possible (Fig. 1a,b).

Molecular-dynamics simulation of IgE-Fc unbending

The crystal structure of the aEFab¹-IgE-Fc-aEFab² complex reveals that an extended conformation of IgE-Fc is feasible. The key question, however, is whether this is just a consequence of aEFab binding, or whether it (or other extended conformations) exists in solution as an intrinsic property of IgE. If the latter, then could the Ce2 domain pair make the transition from one side of the Ce3-Ce4 domains to the other via an extended structure—that is, ‘flip’ between two symmetrically related bent conformations (Supplementary Video 2)? We used metadynamics, an enhanced molecular-dynamics (MD) method^{20–23}, to produce a detailed atomistic simulation of IgE-Fc unbending and its extended conformations. This simulation provides a free-energy surface (potential of mean force) calculated and presented in terms of the two principal components of the molecule’s unbending dynamics (Fig. 2a and Supplementary Fig. 3).

The lowest energy state is a well-defined minimum that corresponds to the bent conformation seen in the free IgE-Fc crystal structure¹³ (Fig. 2, conformation 1). A number of other energy minima may be seen at ~20 kJ/mol above that of the bent state, suggesting possible paths (one of which is indicated, Fig. 2a) leading away from the bent structure toward more extended conformations (Fig. 2a,b, conformations 2 and 3). The dynamic simulation identifies another minimum (Fig. 2a, conformation 4) symmetrically related to conformation 3 and separated by a small energy barrier that corresponds to a linear conformation (indicated in Fig. 2c). As IgE-Fc crosses this low barrier, the Ce2 domain pair passes from one side of the molecule to the other. Having traversed the midpoint ($x = 0$ in Fig. 2a), the Ce2 domains may fold onto the other side of the Ce3-Ce4 domains, completing a full transition from one

Table 1 Data collection and refinement statistics

	aEFab ¹ -IgE-Fc-aEFab ²
Data collection	
Space group	$P2_12_12_1$
Cell dimensions	
a, b, c (Å)	84.59, 100.81, 219.68
Resolution (Å)	2.91 (2.98–2.91)
R_{merge}	0.055 (0.796)
$I / \sigma I$	18.4 (3.0)
Completeness (%)	99.7 (99.2)
Redundancy	5.7 (6.0)
Refinement	
Resolution (Å)	67.01 – 2.91
No. unique reflections	41,987 (3,078)
$R_{\text{work}} / R_{\text{free}}$	0.236 / 0.284
No. atoms	11,712
Protein	11,541
Ligand / ion	122
Water	49
B factors	
Protein	103.8
Sugars	110.9
Water	88.4
r.m.s. deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.566

Values in parentheses are for highest-resolution shell.

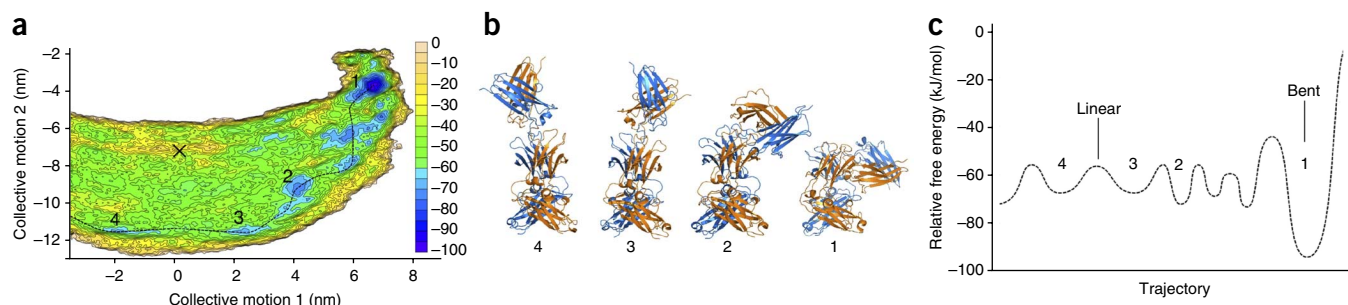


Figure 2 Molecular dynamics simulation of IgE-Fc unbending. **(a)** Free-energy surface representing the IgE-Fc unbending process generated through metadynamics simulation. Axes show the projection along the two lowest-frequency collective motions (extracted from a biased trajectory) for unbending using equations described in Plumed-1.3 documentation⁴⁶. Contours are drawn every 5 kJ/mol and colored accordingly. The simulation covers the transition across the linear conformational states (at $x = 0$) but does not encompass the complete ‘flip’. The conformation seen in the aeFab¹–IgE-Fc–aeFab² complex is indicated (black cross). A possible pathway between energy minima is shown (dotted line). **(b)** Conformations of IgE-Fc corresponding to the energy minima in **a** are numbered accordingly and colored as in **Figure 1**. **(c)** Sketch of the smoothed free-energy profile corresponding to the pathway indicated in **a**; numbers correspond to those in panels **a** and **b**.

bent conformation to the other. (The complete trajectory beyond conformation 4 was not simulated.) The extended conformation seen in the aeFab¹–IgE-Fc–aeFab² complex, indicated by a cross in **Figure 2a**, does not lie directly between conformations 3 and 4. The shift from the proposed pathway corresponds to twisting of (Ce2)₂, presumably stabilized by aeFab binding.

Although comparison of the bent and extended crystal structures identified Pro333, Arg334 and Gly335 in the Ce2–Ce3 linkers as the residues that undergo the most substantial structural rearrangement accompanying (Ce2)₂ domain motions, the simulation revealed additional changes around Gly335 of the A chain and Asn332 of the B chain; smaller movements in the Ce3–Ce4 linker around Ser437 of the A chain are associated with the change in the interdomain angle. The simulation additionally revealed a twisting of (Ce2)₂ relative to the Ce3–Ce4 domains during the unbending process. The mechanism by which domain rearrangements and unbending occur may not simply be rigid-body movements facilitated by linkers that undergo changes from one ordered structure to another (‘mechanical hinges’), but may involve order-disorder transitions as described in other systems^{24–26}. Local disorder or unfolding and re-folding within linkers, or regions of the domains themselves, can facilitate large-scale conformational changes in proteins, providing entropic compensation to reduce high enthalpic barriers^{26,27}. We can now search for evidence of such order-disorder transitions within the wealth of structural information generated by this simulation, but such detailed mechanistic analyses are beyond the scope of the present manuscript.

Regardless of the mechanism, the highest barrier encountered in a complete flip, and thus the rate-determining step, is clearly that involved in leaving the bent conformation; once this is achieved, the barriers to exploring the extended conformations are relatively low. However, the energy difference between the wells corresponding to the bent and extended conformations is such that the fraction of molecules in the extended state will be very small, consistent with the experimental observation that the bent conformation predominates in solution^{9,12,16}.

aeFab binding to IgE-Fc by isothermal titration calorimetry

One way to establish whether IgE-Fc has an intrinsic capacity to unbend in solution is to determine the number of available aeFab binding sites. If IgE-Fc is rigidly and exclusively bent, only one site will ever be accessible, but if it is flexible, then two sites will be accessible. We therefore studied the interaction between aeFab and IgE-Fc by isothermal titration calorimetry (ITC) and compared this with the binding of aeFab to Fcε3-4 which, lacking the Ce2 domains, always has

two accessible sites. The results for both IgE-Fc and Fcε3-4 are similar, showing that both molecules do indeed present two binding sites (**Fig. 3a,b**). The K_d values for aeFab binding to IgE-Fc and Fcε3-4 were found to be 40 nM and 50 nM, respectively (**Fig. 3a,b** and **Table 2**), but in order to distinguish between the binding affinities of the two aeFab molecules, the ITC experiment was then conducted by titrating aeFab into either IgE-Fc or Fcε3-4 (**Fig. 3c,d**). This revealed K_d values for IgE-Fc of 76 nM and 1.5 μM for the first and second binding sites, respectively (with similar values for Fcε3-4; **Table 2**); the second aeFab clearly binds more weakly than the first, and small ΔH values indicate that binding of both aeFab¹ and aeFab² is entropically driven.

Assembly mechanism of aeFab¹–IgE-Fc–aeFab² in solution

The observed 2:1 stoichiometry demonstrates that in solution IgE-Fc can adopt the extended structures needed to expose both aeFab binding sites. The binding of aeFab¹ mediates an allosteric change in IgE-Fc, which may occur through induced fit²⁸, conformational selection²⁹ or a combination of both mechanisms³⁰. In order to investigate the assembly mechanism of the aeFab¹–IgE-Fc–aeFab² complex, we monitored the intrinsic tryptophan fluorescence upon binding in stopped-flow kinetic experiments. When IgE-Fc (or Fcε3-4) was in excess over aeFab, thus restricting the stoichiometry of the aeFab–IgE-Fc (or Fcε3-4) complex to 1:1, a single binding event was observed (**Supplementary Fig. 4a,b**). This has a fast association rate constant and a dissociation rate too slow to measure by this technique (**Table 2**). The observation of monophasic kinetics implies that no conformational change is coupled to formation of aeFab¹–IgE-Fc (although we cannot rule out the possibility of such a change occurring on a timescale faster than that of the experiment).

When repeated with aeFab in excess over IgE-Fc, two-step binding was observed for both IgE-Fc and Fcε3-4 (**Supplementary Fig. 4ab**), consistent with not only aeFab¹ binding faster than aeFab², but also the difference in affinities of the two sites determined by ITC (**Table 2**). Furthermore, a linear concentration dependence for the observed rate constants (k_{obs}) of the binding of both aeFabs to either IgE-Fc or Fcε3-4 suggests that aeFab¹ does not cause a conformational change (such as unbending of IgE-Fc) that is rate-limiting for aeFab² binding (**Supplementary Fig. 4c–f**). In the absence of any evidence for an induced conformational change, our results are most consistent with a conformational selection model, with extended structures selected from a dynamic population of IgE-Fc conformations.

In order to test whether aeFab¹ binding alone traps IgE-Fc in an extended conformation or still allows it to flex between extended and

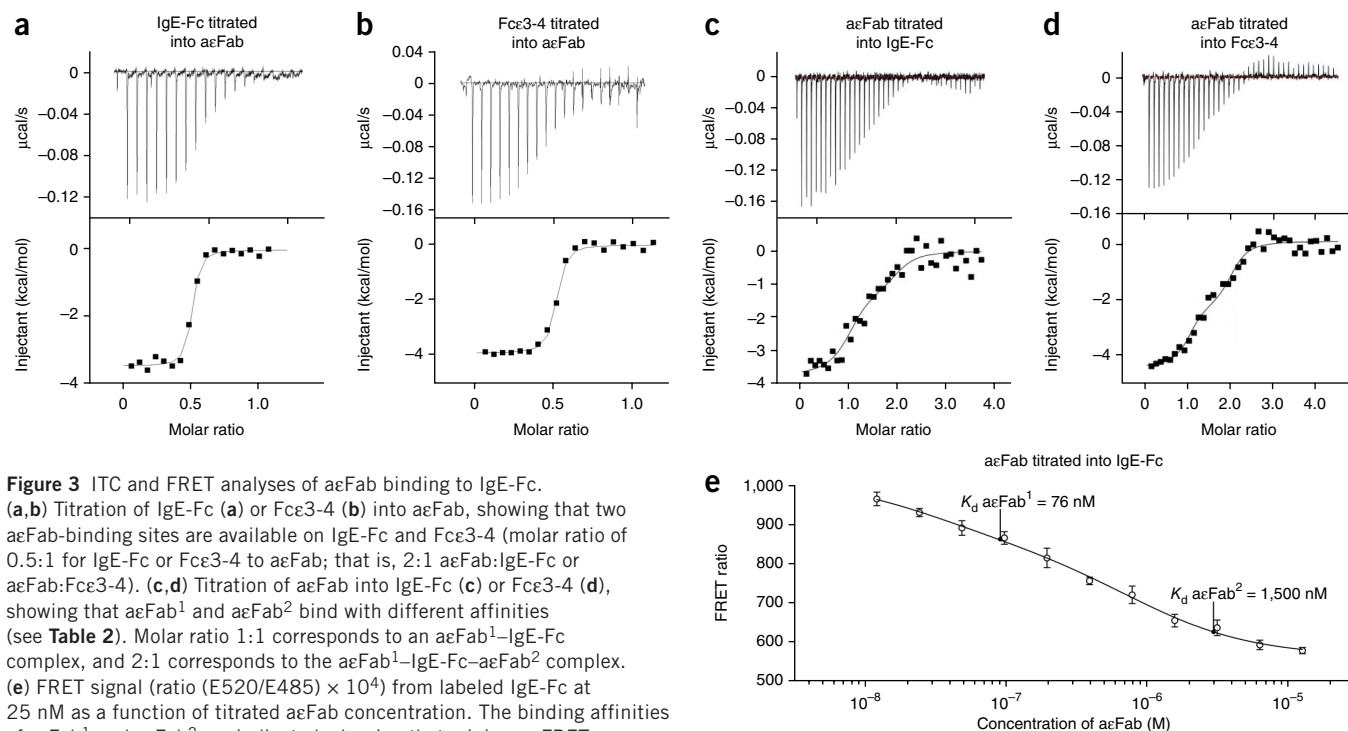


Figure 3 ITC and FRET analyses of aeFab binding to IgE-Fc. (a,b) Titration of IgE-Fc (a) or Fcε3-4 (b) into aeFab, showing that two aeFab-binding sites are available on IgE-Fc and Fcε3-4 (molar ratio of 0.5:1 for IgE-Fc or Fcε3-4 to aeFab; that is, 2:1 aeFab:IgE-Fc or aeFab:Fcε3-4). (c,d) Titration of aeFab into IgE-Fc (c) or Fcε3-4 (d), showing that aeFab¹ and aeFab² bind with different affinities (see Table 2). Molar ratio 1:1 corresponds to an aeFab¹–IgE-Fc complex, and 2:1 corresponds to the aeFab¹–IgE-Fc–aeFab² complex. (e) FRET signal (ratio (E520/E485) × 10⁴) from labeled IgE-Fc at 25 nM as a function of titrated aeFab concentration. The binding affinities of aeFab¹ and aeFab² are indicated, showing that minimum FRET occurs only upon binding of aeFab². ITC and FRET data were fitted to a sequential, two-step binding mechanism, and FRET error bars are s.e.m. (*n* = 6).

bent structures, we carried out intramolecular FRET, with IgE-Fc labeled with donor and acceptor fluorophores in the Cε2 and Cε4 domains, respectively. Upon titration into labeled IgE-Fc, a concentration of aeFab sufficient to occupy the first binding site resulted in a partial decrease of FRET signal (Fig. 3e). This implies that under these conditions the IgE-Fc is not exclusively extended, as would be expected if aeFab¹ binding caused full linearization; nor does IgE-Fc remain bent. Taken together with the stopped-flow data (linear concentration dependence of the kinetics of aeFab² binding and similar behavior of IgE-Fc and Fcε3-4), the FRET data show that IgE-Fc in the aeFab¹–IgE-Fc complex remains conformationally dynamic. It is only upon saturation of the second binding site that no further reduction in the FRET signal occurs (Fig. 3e), corresponding to a fully extended IgE-Fc.

These results are summarized in Figure 4, which depicts a possible mechanism for the formation of the aeFab¹–IgE-Fc–aeFab² complex (see also Supplementary Video 3). Initially, IgE-Fc is predominantly bent in solution (consistent with X-ray and neutron scattering¹² and FRET¹⁶), but it may transiently adopt extended conformations such as those identified in the MD simulation (Fig. 4a). Next, aeFab¹ engages IgE-Fc in the bent or an extended conformation. With aeFab¹ bound, IgE-Fc can still flex between bent and extended conformations (Fig. 4b). Finally, when IgE-Fc is transiently extended, aeFab² engages and completes the aeFab¹–IgE-Fc–aeFab² complex (Fig. 4c).

Effect of aeFab on FcεRI binding

We have demonstrated the existence of a range of conformational states available to IgE-Fc, but how do these different states affect binding of IgE to FcεRIα? Receptor-bound IgE-Fc is even more acutely bent than free IgE-Fc, and interacts with the receptor with high affinity (*K_d* ~ 0.1 nM)¹ through two subsites, one on each Cε3 domain (Fig. 5a)^{14,31}. In the extended conformation of IgE-Fc that results from aeFab binding, both of the FcεRIα-binding subsites are disrupted (Fig. 5b). In subsite 1 on IgE-Fc^A, Arg334 forms a critically important salt bridge with FcεRIα, but this residue is part of the linker that undergoes structural rearrangement upon extension of (Cε2)₂, and is no longer in a position to engage in FcεRIα binding. The second subsite on IgE-Fc^B involves a proline (Pro426 of Cε3^B) sandwiched between two tryptophan residues (Trp87 and Trp110 of FcεRIα), but this proline moves 6 Å away from its receptor-bound conformation in the aeFab complex. Even if receptor engagement with Pro426 of subsite 2 alone occurs, modeling of sFcεRIα in this position on the extended IgE-Fc results in clashes between both Cε3^A and (Cε2)₂ of IgE-Fc with the receptor (Fig. 5c).

Thus, aeFab binding allosterically prevents FcεRIα binding to IgE-Fc by trapping the molecule in an extended state that cannot engage with receptor. But does aeFab¹ alone compete with FcεRIα binding to bent IgE-Fc? We compared the aeFab-bound structure with that of IgE-Fc bound to sFcεRIα. Modeling of a single aeFab

Table 2 ITC and stopped-flow analysis of the interaction between aeFab and IgE-Fc or Fcε3-4

	<i>K_{d1}</i> ITC (μM)	<i>K_{d2}</i> ITC (μM)	<i>k_{on}</i> (M ⁻¹ s ⁻¹)	<i>k_{off}</i> (s ⁻¹)	<i>k_{on2}</i> (M ⁻¹ s ⁻¹)	<i>k_{off2}</i> (s ⁻¹)	Δ <i>H</i> ₁ ITC (kcal/mol)	Δ <i>H</i> ₂ ITC (kcal/mol)
1aeFab–1IgE-Fc	0.040 (±0.02)	–	6.7 (±0.2) × 10 ⁵	n/m	–	–	–3.5 (±0.1)	–
2aeFab–1IgE-Fc	0.076 (±0.07)	1.5 (±1.3)	3.5 (±0.2) × 10 ⁵	3.2 (±0.1)	1.2 (±0.2) × 10 ⁵	n/m	–3.5 (±0.1)	–1.3 (±0.1)
1aeFab–1Fcε3-4	0.050 (±0.02)	–	1.0 (±0.1) × 10 ⁶	n/m	–	–	–3.9 (±0.1)	–
2aeFab–1Fcε3-4	0.034 (±0.03)	0.98 (±0.45)	1.0 (±0.1) × 10 ⁶	0.95 (±0.2)	3.7 (±0.3) × 10 ⁵	n/m	–4.3 (±0.1)	–2.3 (±0.1)

n/m, not measurable (too slow to measure).

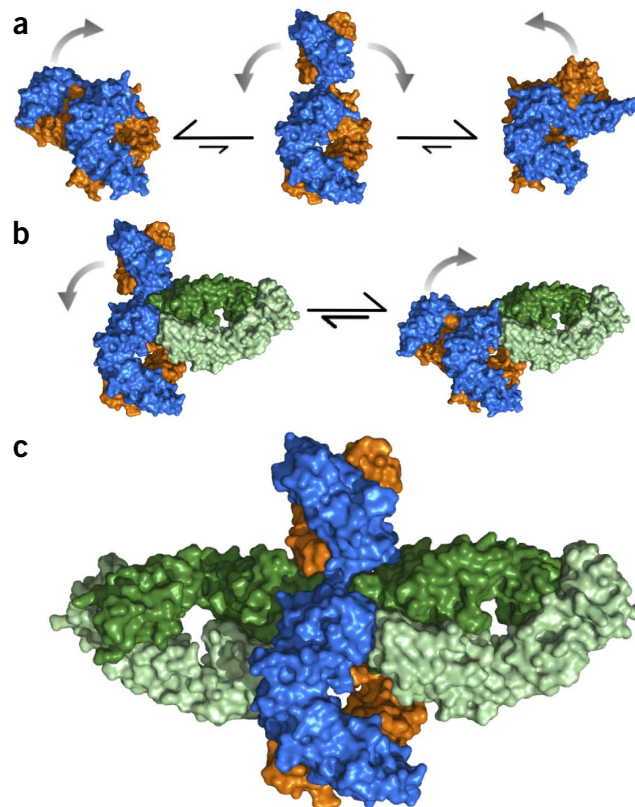
Figure 4 Proposed mechanism of IgE-Fc flexibility and aEFab binding in solution. (a) IgE-Fc is predominantly bent in solution, but is capable of adopting transiently extended conformations through which the (Cε2)₂ domains can flip from one side of the molecule to the other. (b) aEFab¹ engages one of the binding sites of IgE-Fc, restricting its range of flexibility, but IgE-Fc is neither exclusively bent nor exclusively extended. (c) aEFab² engages the extended form of IgE-Fc, capturing the molecule in this otherwise transiently occupied conformation. Colored as in **Figure 1**.

onto the IgE-Fc-sFcεRIα structure shows that although the residues involved in the two interactions are distinct, the aEFab and receptor molecules clash (**Fig. 5d**). Therefore, aEFab¹ and sFcεRIα compete for binding to the bent form of IgE-Fc. This was confirmed using SPR to determine whether aEFab¹ alone is capable of inhibiting sFcεRIα binding to IgE-Fc, or whether aEFab² is required to trap the molecule in an extended conformation before inhibition occurs.

aEFab was first immobilized on an SPR biosensor surface and binding of IgE-Fc was tested over a range of concentrations (**Supplementary Fig. 5a**). The *K_d* of aEFab¹ binding to IgE-Fc was found to be 95 nM (±6 nM), in agreement with ITC results (**Table 2**). Subsequently, binding of aEFab² to form aEFab¹-IgE-Fc-aEFab² was characterized. After immobilization of aEFab on the SPR biosensor surface, IgE-Fc was flowed over the chip to form the aEFab¹-IgE-Fc complex, followed by aEFab² to complete the trimolecular complex (**Supplementary Fig. 5b**). On the basis of an equilibrium analysis, the *K_d* of aEFab² binding was estimated to be 1.4 μM (±0.1 μM), again in good agreement with ITC results. To determine how binding of aEFab¹ to IgE-Fc affects the binding of sFcεRIα, the aEFab¹-IgE-Fc complex was first formed as described above, and before this complex could dissociate, sFcεRIα was passed over the surface in increasing concentrations. No binding of sFcεRIα was observed up to a concentration of 1 μM, confirming that binding of a single aEFab is sufficient to inhibit receptor binding to IgE-Fc (**Supplementary Fig. 5c**). aEFab thus inhibits receptor binding by a steric mechanism when IgE-Fc is bent and also allosterically when IgE-Fc is extended.

Modeling membrane-bound IgE

We previously modeled the conformations accessible to the Fab arms of IgE using the bent, receptor-bound structure of IgE-Fc¹⁶. This



revealed little overlap between the spaces explored by the two Fab arms, one of which was directed away from, the other parallel to, the membrane, consistent with both allergen recognition and receptor cross-linking (**Fig. 6a,b**). However, it is difficult to see how a rigidly bent IgE molecule could function in allergen recognition in the BCR because the Fab arms would be directed toward the membrane (**Fig. 6c**). To test this, we oriented the ensemble of whole IgE models reported previously¹⁶ in a membrane-bound position, with a spacer to represent the extra membrane-proximal domains (EMPD) that separate the C termini of the Cε4 domains from the membrane (**Fig. 6d**). Irrespective of spacer length (the EMPD structure is unknown), the Fab arms only explore space close to the membrane. To determine how an extended conformation of IgE-Fc would present the Fab arms, we implemented the same method of model generation and positioned 2,000 models of IgE relative to the membrane. Extended IgE-Fc

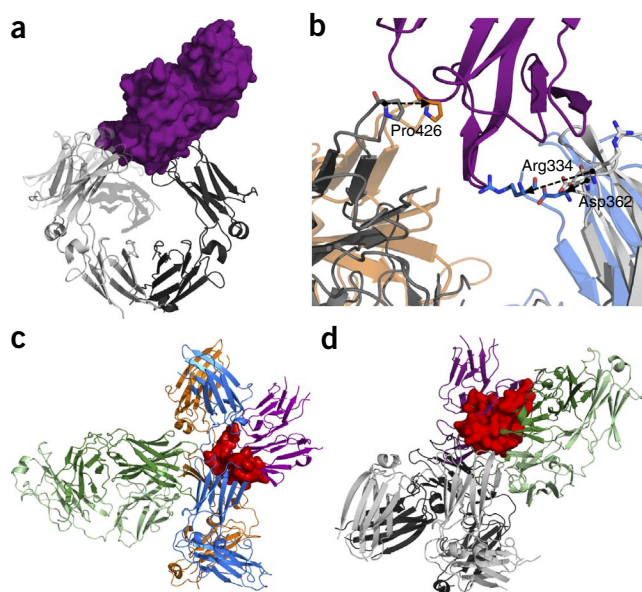


Figure 5 Structural basis for inhibition of IgE-Fc interaction with FcεRIα by aEFab. (a) The structure of IgE-Fc bound to sFcεRIα (PDB 2Y7Q¹⁴). IgE-Fc is shown in gray cartoon representation (IgE-Fc^A in lighter shade, IgE-Fc^B in darker shade); sFcεRIα is shown as purple surface. (b) Detail of the receptor-binding region following overlay of the Cε3 and Cε4 domains of IgE-Fc in receptor-bound (gray) and aEFab-bound (IgE-Fc^A in blue, IgE-Fc^B in orange) conformations. Key residues involved in receptor binding are shifted, as indicated by the arrows. (c) Location of sFcεRIα (purple, cartoon representation) after superposition of the IgE-Fc-sFcεRIα complex onto the aEFab¹-IgE-Fc-aEFab² complex (using the Cε3^B domain and thus maintaining the Pro426 interaction with receptor) and displaying only aEFab². The clashes between receptor and both the Cε3^A and (Cε2)₂ domains are indicated in red. (d) Location of aEFab¹ (cartoon representation, heavy chain in dark green, light chain in light green) after superposition of the aEFab¹-IgE-Fc-aEFab² complex onto the IgE-Fc-sFcεRIα complex (using the Cε4 domains), but displaying only aEFab¹ from the complex. aEFab¹ and sFcεRIα compete for the same region in space, as shown by the overlap region in red.

Figure 6 Modeled structure of the entire IgE molecule in different biological contexts. Panels on the right, the allowed range of locations for each Fab arm is represented by a sphere (blue or yellow for each Fab) placed at the center of the allergen-binding site. (**a,b**) Cartoon (**a**) and schematic depiction (**b**) of acutely and rigidly bent IgE bound to FcεRIα (purple). IgE (chain A in dark and light blue, chain B in dark and light orange) and FcεRIα (purple) are shown. (**c,d**) Cartoon (**c**) and schematic depiction (**d**) of membrane bent IgE as part of the BCR. The extra membrane-proximal domains of mIgE are indicated by a circle (in **c**) and a black spacer bar (in **d**) because their structure is unknown. (**e,f**) Cartoon (**e**) and schematic depiction (**f**) of extended IgE conformations as part of the BCR. Igα and Igβ, the BCR accessory proteins, are shown in green in **c** and **e**.

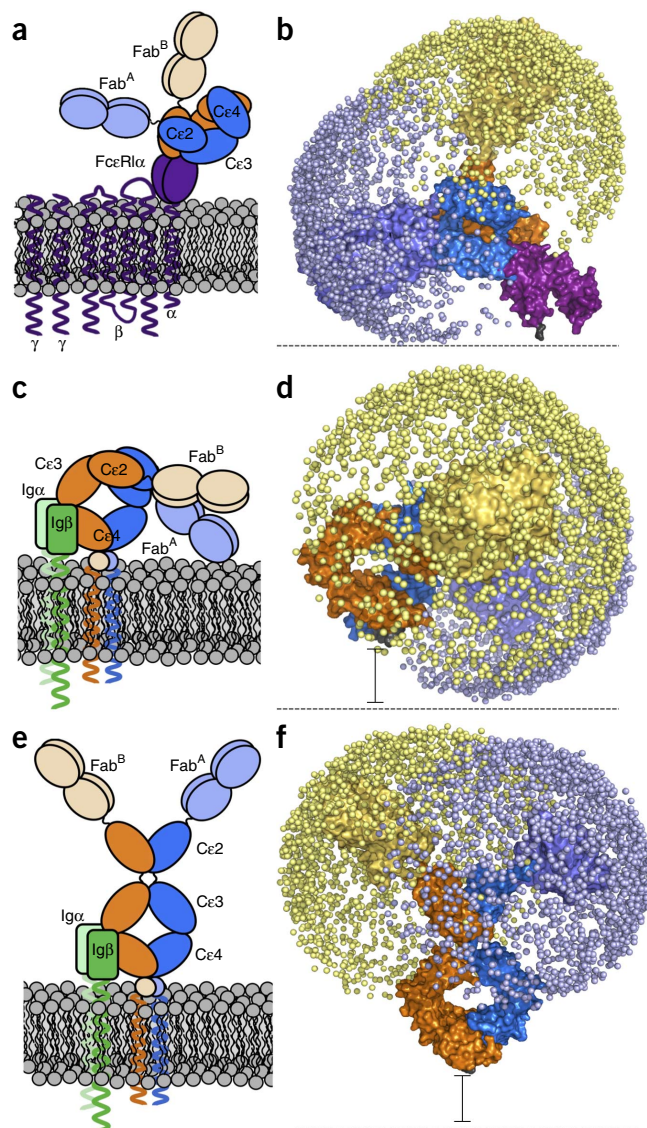
conformations orient the Fab arms away from membrane, optimally for allergen binding (Fig. 6e,f).

DISCUSSION

Ever since the first model of a bent structure for IgE^{6–9}, the determination of the acutely and asymmetrically bent IgE-Fc by X-ray crystallography¹³ and demonstration by X-ray solution scattering and FRET that this structure predominates in solution^{9,11,12,16}, the idea that the (Ce2)₂ domain pair might flip from one side to the other has been only a tantalizing possibility. Fluorescence depolarization analyses of segmental flexibility in IgE and electron microscopy (EM) of immune complexes had shown that the molecule was less flexible than IgG^{3–6,15,32}, and in the crystal structure of its complex with sFcεRIα¹⁴, IgE-Fc was found to be even more acutely bent, consistent with FRET studies in solution¹⁶ and when receptor-bound on cells⁶. Only the binding of omalizumab Fab to IgE-Fc in solution, monitored by FRET, indicated that a partial unbending of IgE-Fc was possible¹⁶. The observation of a fully extended IgE-Fc in the αεFab complex reported here was therefore a considerable surprise. We have now demonstrated by MD simulation and solution biophysical studies that IgE-Fc can indeed unbend and adopt a range of transiently extended conformations, suggesting a possible pathway for the Ce2 domains to flip from one side of the Ce3–Ce4 to the other.

The degree of flexibility described for IgE-Fc here is unlike any reported for other antibody classes. Flexibility between the V and C domains in Fab fragments ('elbow bending') and within the hinge separating Fab and Fc regions in IgG antibodies is well documented, but conformational variation within the Fc has to date been limited to minor changes in the orientation of the Cγ2 domains of IgG³³ or the Ce3 domains of IgE¹⁹. Open and closed conformations for the latter have been reported in structures of IgE-Fc and Fcε3–4 both free and when bound to either sFcεRIα^{14,31} or to sCD23, the soluble IgE-binding domain of the 'low-affinity' receptor on B cells³⁴. The conformational diversity in IgE-Fc reported here is substantially greater than previously seen in Fc structures. In fact, the earlier fluorescence depolarization data on IgE should be reinterpreted in terms of bending at the Ce2–Ce3 linker regions in addition to bending between Fab and Fc.

IgM, an evolutionary precursor of IgE, also contains a domain pair, (Cμ2)₂, rather than a flexible hinge, and is known to undergo substantial conformational changes. Free in solution it adopts planar, star-shaped pentameric or hexameric structures, as determined by small-angle X-ray and neutron scattering and EM studies^{35,36}, but when bound by Fab arms of two or more subunits to an antigenic surface, it adopts 'table-like' structures with the disc of penta- or hexameric Fc regions at 90° to the Fab arms³⁵. However, the location of the bend in the molecule is unknown. Modeling of IgM structures against solution-scattering data placed the bend between



Cμ2 and Cμ3 (ref. 36), as suggested earlier in fluorescence depolarization studies³⁷, whereas later modeling against cryo-EM data was consistent with bending between Cμ1 and Cμ2 (ref. 38). Recent crystallographic analyses of individual IgM-Fc domains leaves the question unresolved³⁹. It is clear, however, that at least in the context of the polymeric structures, the monomeric IgM subunit can adopt either linear or bent conformations, and if the flexibility in IgE is evolutionarily related to that of IgM, bending of the latter would be predicted to occur between Cμ2 and Cμ3. Conformational change in IgM is critical for function, as dislocation upon antigen binding reveals sites for complement binding that are hidden in the planar structures. There is therefore a precedent for an extended conformation of an antibody with the 'additional', hinge-replacing domains that has a clear biological function.

That IgE evolved conformational diversity to allow both a compact bent and an ensemble of extended, flexible structures may be understood in terms of its allergen-binding functions in two distinct contexts. Bound to FcεRI on effector cells (mast cells, basophils or antigen-presenting cells), bent IgE presents its Fab arms appropriately for allergen recognition and cross-linking¹⁶, which triggers the allergic response (Fig. 6a,b). In contrast, as part of the BCR, in which

the role of membrane IgE is to capture allergen and ensure B-cell survival and proliferation, only an extended molecule presents the Fab arms optimally for detection of allergen (**Fig. 6c–f**). One recognized advantage of conformational flexibility is ‘fly casting’: that is, the ability to sample a greater range of conformational space and make ligand (allergen) capture more effective⁴⁰. Conventional depictions of the BCR for all antibody classes show extended Fab arms, and the extended IgE-Fc structure reported here demonstrates that such a conformation is feasible.

In the extensively studied IgM BCR, which contains a single IgM subunit, it has been assumed, in the absence of a crystal structure, that IgM adopts an extended conformation. It has also been proposed that conformational changes occur in the IgM-Fc upon antigen binding as part of the signaling function^{41,42}. Our results for IgE-Fc provide support for the notion of an extended IgM-Fc and suggest one way in which a conformational change might occur. Without knowing the structure of the EMPDs that connect the Cε4 domains to the trans-membrane regions of the ε-chains, or how the accessory Igα and Igβ chains interact with membrane-IgE or membrane-IgM, it remains a matter for speculation how extended structures might be stabilized. A model for the association between Igα and Cμ4 of IgM has been proposed on the basis of the crystal structure of Igβ⁴³, but it is hard to see how interaction with Cμ4 alone could stabilize the extended conformation. Structural studies of the EMPD and the interactions between IgE-Fc and Igα and Igβ are clearly required.

We now consider the nature of the free IgE molecule. The MD analysis shows that the bent conformation exists in a relatively deep energy well (**Fig. 2c**, conformation 1) and that the rate-limiting step to reaching the extended conformations, and then flipping to the alternative bent conformation with the Cε2 domains packed against the other side of the Fcε3-4, is the escape from this well. Once this occurs, the energy barriers between various extended conformations, including the barrier as the IgE-Fc passes through the linear conformation, are much lower (**Fig. 2b,c**, conformations 2–4). Different pathways for unbending may be envisaged, but the most highly populated are expected to have free-energy profiles similar to that sketched in **Figure 2c**. Although kinetic information may not be directly extracted from the simulations presented here, as biasing forces were applied, an estimate for the rate of unbending may be made if we assume that the process of escape from the well that corresponds to the bent conformation can be described by passage over a single free-energy barrier^{26,44}. The resulting rate of 0.15 s⁻¹ (see Online Methods for equation and parameters used^{26,44}) must, however, be considered a very approximate calculation. The free-energy difference between the bent and the various extended states (~8 k_BT, **Fig. 2c**) implies that only a very small fraction of the molecules occupy an extended conformation at any one time. This is in accord with studies of IgE-Fc in solution^{9,12,16}, which indicate that the bent conformation predominates.

Comparison of the structures of IgE-Fc or Fcε3-4 with sFcεRIα and sCD23 has shown that, although the binding sites are spatially distinct, occurring at either end of the Cε3 domains^{14,31,34}, they are allosterically linked such that IgE cannot bind both receptors simultaneously³⁴. This mutual exclusion is vital for controlling IgE function. For example, cross-linking of FcεRI-bound IgE by oligomeric CD23 would trigger the allergic response in the absence of allergen. Flexibility in IgE-Fc, in particular the relative disposition of the Cε3 domains, is key to determining their receptor-binding capacity, and therefore understanding this allostery and conformational flexibility is important for targeting IgE's receptor interactions for therapeutic benefit.

We have shown here that aεFab inhibits FcεRI binding both sterically and allosterically (**Fig. 5**). Because extended IgE-Fc conformations cannot bind FcεRI, it may be possible to exploit the intrinsic capacity of the molecule to unbend and enhance the dissociation of IgE from the receptor. Indeed, the recent demonstration that accelerated dissociation of IgE from FcεRI can occur upon binding of a DARPIn⁴⁵ may be explained in terms of the flexibility that we have identified in IgE-Fc. The reported structure of this DARPIn complexed with a disulfide-constrained Fcε3-4 (ref. 45) (lacking Cε2 domains) leaves its mechanism of action upon IgE unclear. We do know, however, that the binding of the anti-IgE omalizumab Fab causes a partial unbending of IgE-Fc as monitored by FRET¹⁶, which may contribute to its mechanism of action. In any event, the discovery that IgE can flex between bent and transiently occupied extended conformations provides a new framework for understanding IgE function in allergen recognition, and offers a more complete description of the structure of this important therapeutic target.

METHODS

Methods and any associated references are available in the [online version of the paper](#).

Accession codes. Coordinates and structure factors for aεFab¹–IgE-Fc–aεFab² have been deposited in the RCSB Protein Data Bank with accession code 4J4P.

Note: Any Supplementary Information and Source Data files are available in the [online version of the paper](#).

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AUTHOR CONTRIBUTIONS

N.D. and B.P.C. contributed equally to this work. N.D. performed the crystallography and structure analysis. N.D. and J.M.M. conducted SPR experiments, and N.D. and B.J.S. wrote the manuscript. B.C. undertook the molecular dynamics and contributed to writing. A.H.K. was responsible for the ITC and stopped-flow analyses. M.W. generated the aεFab molecule. K.C. performed antibody engineering. H.H. expressed the proteins. A.O. purified the proteins. J.D. and L.K.S. collected intramolecular FRET data using reagents made by M.W.-P.K., and A.J.H. J.M.M., A.J.H. and A.J.B. contributed to writing, data interpretation. B.J.S. designed and supervised the research.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Protein expression and purification. Anti-human IgE-Fc antibody was supplied by UCB and isolated V-region genes sub-cloned in the human IgG₁ Fab format. aeFab was expressed by transient transfection in CHO cells, cultured in CD-CHO medium with the addition of 10 mM glutamine at 37 °C with 8% CO₂ and a rotation of 140 rpm. We resuspended 2×10^8 cells/mL in Earles Balance Salt Solution before adding 400 µg of DNA. Cells were electroporated and resuspended in 100 mL of CD-CHO medium and incubated for 24 h. Incubation continued at 32 °C for 13 days and at 4 days post-transfection sodium butyrate (3 mM final concentration) was added to the culture. On day 14 post-transfection, cell culture supernatants were harvested by centrifugation (400g for 1 h) for purification.

aeFab was purified by Protein G affinity chromatography (GE Healthcare) and bound aeFab eluted in 100 mM glycine-HCl, pH 2.7, and fractions neutralized with 1/25th fraction volume of 2 M Tris-HCl, pH 8.5. aeFab was further purified by size-exclusion chromatography (SEC) on a Superdex S200 column (GE Healthcare) in 25 mM Tris-HCl, 20 mM NaCl, 0.05% (w/v) NaN₃, pH 7.5.

IgE-Fc(N265Q,N371Q) secreted from a stable NS-0 cell line was purified from tissue culture supernatant by cation exchange. Supernatant was buffer-exchanged into 50 mM sodium acetate pH 6.0, 75 mM NaCl and loaded onto a SPHP cation-exchange column (GE Healthcare). IgE-Fc(N265Q,N371Q) was eluted with a $10 \times$ column-volume gradient into 50 mM sodium acetate, pH 6.0, 1 M NaCl. Eluted fractions were pooled, concentrated and further purified by SEC on a Superdex S200 column (GE Healthcare) in PBS, pH 7.4.

aeFab:IgE-Fc complex was prepared by mixing aeFab with IgE-Fc and purification to homogeneity by SEC as described above.

sFcεRIα (Val1-Lys176) was engineered as a C-terminal His₆-tagged protein and transiently expressed in CHO cells as described for the aeFab protein. Harvested tissue culture supernatant was buffer exchanged into 50 mM NaH₂PO₄, pH 7.4, 0.3 M NaCl, 10 mM imidazole and the sFcεRIα-His purified by affinity chromatography on a Ni-NTA column (Qiagen) eluting the bound protein on a linear gradient from 50 mM NaH₂PO₄, pH 7.4, 0.3 M NaCl, 10 mM imidazole into 50 mM NaH₂PO₄, pH 7.4, 0.3 M NaCl, 250 mM imidazole. Pooled fractions were further purified by SEC on a Superdex S200 column (GE Healthcare) in PBS, pH 7.4.

Crystallization. Sitting drop vapor diffusion crystallization experiments were set up with a protein complex concentration of 5 mg/mL in 20 mM NaCl, 25 mM Tris-HCl, pH 7.5, and 0.05% sodium azide. Crystals were grown at 18 °C using 12–22% PEG3350, 0.25 M sodium citrate, and 0.1 M Bis-Tris Propane, pH 7.5–9.0, as precipitant. Drops were microseeded with crystals grown under identical crystallization conditions in earlier trials. Crystals were flash-cooled in liquid nitrogen using 4 M trimethylamine N-oxide as cryoprotectant.

Data collection and structure determination. Diffraction data were collected at beamline I03, Diamond Light Source (Harwell, UK). Xia2 (ref. 47) was used to index, integrate and merge data to 2.9 Å resolution^{48–54}. The phases were solved using Phaser molecular replacement⁵⁵. To generate the aeFab search model, the RCSB PDB protein sequence search engine was used to find 3QH2, from which nonconserved residues were removed using CHAINSAW^{56,57}. IgE-Fc search models were generated by splitting the coordinates from the high-resolution IgE-Fc structure (PDB 2WQR¹⁴) into (Ce2)₂ and (Ce3-4)₂ fragments. Molecules were located in the asymmetric unit sequentially: aeFab¹ was followed by (Ce3-4)₂, aeFab², and finally (Ce2)₂. The structure was initially rebuilt using the autobuild wizard of PHENIX⁵⁸, and then refined using iterative cycles of PHENIX⁵⁸, with 5% of reflections set aside from refinement for calculation of R_{free}. Between refinement cycles, the structure was manually built into 2F_o-F_c and F_o-F_c electron density maps using COOT⁵⁹. Composite omit maps were generated using the autobuild wizard in PHENIX to prevent model bias⁵⁸. Carbohydrate and water molecules were manually built into the structure. MolProbity⁶⁰ and CARP⁶¹ were used to assess protein and carbohydrate geometry, respectively: 93.6% of residues are within the Ramachandran favored region, and 0.2% are outliers. PISA⁶², CONTACT and NCONT as part of the CCP4 program suite⁵⁴ were used for analysis of protein-protein interfaces, and DynDom⁶³ was used to calculate the domain motion involved in the conformational changes. Structure morphs

for movies were calculated using the UCSF CHIMERA package⁶⁴, and videos made using PyMOL (<http://www.pymol.org/>).

Enhanced molecular dynamics. The bent crystal structure (PDB 100V¹³) was used as the starting point for molecular dynamics simulation, after adding hydrogen atoms and removing terminal residues to symmetrize the two chains. Protonation states were predicted with Maestro (Schrödinger LLC). After initial exploratory simulations of unbending, manual checks were made for possible changes to protonation states in the unbent conformations. No changes in protonation states were found that could be justified, and thus the initial states were retained for the final simulation. The AMBER ff99SB-ildn and GLYCAM force fields were used for protein and carbohydrate, respectively. The structure was solvated in a truncated octahedron (dimensions 123 × 123 × 123 Å) such that no protein atom of the extended IgE-Fc conformation was within 8 Å of the edge after pressure equilibration. Monatomic ions were added to a salt concentration of 0.15 M. The final simulation system had 129,459 atoms.

Simulations were carried out with NAMD 2.8 (ref. 65) patched with Plumed-1.3 (ref. 46). Particle mesh Ewald⁶⁶ was used for long-range electrostatics along with 9 Å cut-offs for Coulomb and Lennard-Jones potential functions. A preliminary 500 ns unbiased simulation was used to extract two collective variables (CVs) through principal component analysis (PCA). Only every other α-carbon was included in the PCA CVs. An exploratory metadynamics simulation then used these PCA CVs to explore unbending for 1,200 ns with Gaussians of height 8 kJ/mol and sigma of 0.5 Å added every 4 ps. This exploratory metadynamics run then provided new PCA CVs for a final simulation, hence the final CVs were extracted from a simulation that fully sampled unbending. The final multiple-walker metadynamics simulation converged after 3,755 ns using Gaussians of height 4 kJ/mol added every 4 ps. Convergence of this calculation was gauged by the reduction of the Gaussian heights being added (50–100 times smaller than at the beginning) and a convergence in the relative depths of the lowest free energy wells. Additionally, the converged simulation walkers were able to move through CV space relatively freely, suggesting the suitability of the CVs.

To confirm the results of the metadynamics simulation, we carried out a short (250 ns) unbiased simulation starting from the extended crystal conformation of IgE-Fc (Supplementary Fig. 3d).

An estimation of the rate of transition from a bent to an unbent conformation was made assuming a single rate-limiting barrier according to equation (16) of Whitford *et al.* (ref. 26):

$$1/k_t = 1/C \exp(\Delta G/k_B T) \quad (1)$$

where k_t is the transition rate, C is the “attempt frequency” for barrier crossing and ΔG is the barrier height. A value for C was taken as $10 \mu s^{-1}$ since many protein folding and functional transitions have been found in the range of $\sim 10 \mu s^{-1}$ (ref. 26) and the barrier height was taken as 18 k_BT (Fig. 2c).

Isothermal titration calorimetry. Experiments were carried out using a Microcal iTC200 calorimeter (GE Healthcare) at 20 °C in PBS buffer pH 7.4. Depending on the final ratio required, 25–30 µM of protein were used in the cell with 10–20 times higher concentrations in the syringe. The number and volume of injections were varied as appropriate. Heats of dilution were subtracted from the data before analysis. Analyses were carried out using MicroCal Origin, using a 1:1 binding model when IgE-Fc or Fcε3-4 was titrated into aeFab, or a sequential 2:1 (non-identical) binding model when aeFab was titrated into IgE-Fc or Fcε3-4. The non-identical 2:1 binding model was selected based on prior knowledge of the crystal structure, and observation that the curve clearly deviated from an identical 2:1 binding mode.

Stopped-flow fluorescence. Experiments were carried out using a Chirascan Plus (Applied Photophysics) with a stopped-flow attachment at 20 °C in PBS buffer, pH 7.4, and with pseudo-first order protein concentrations varied as required. Fluorescence was excited at 280 nm (1 nm slit width) and emitted fluorescence above 305 nm detected with a long-band pass. We averaged 6–10 runs for each experiment. Data were collected and analyzed using supplied software according to the manufacturer's instructions. Experimental transients were fitted either to single-exponential (equation (2)); [IgE-Fc] or [Fcε3-4] > [aeFab] and only aeFab¹

binding observable) or double-exponential (equation (3); [aEFab] > [IgE-Fc] or [Fcε3-4] and binding of both aEFab¹ and aEFab² observable) equations:

$$F = \Delta F_1 \exp(-k_{\text{obs}1}t) + F_e \quad (2)$$

$$F = \Delta F_1 \exp(-k_{\text{obs}1}t) + \Delta F_2 \exp(-k_{\text{obs}2}t) + F_e \quad (3)$$

where F is the observed fluorescence, ΔF_n is the fluorescence amplitude change for the n th transient, $k_{\text{obs}n}$ is the pseudo-first order rate constant for the n th step and F_e is the end-point fluorescence. The bimolecular association rate constants for aEFab¹ (k_{+1}) and aEFab² (k_{+2}) binding were determined by fitting the linear concentration dependences of $k_{\text{obs}1}$ and $k_{\text{obs}2}$ to equation (4):

$$k_{\text{obs}n} = k_{+n}[\text{ligand}] + k_{-n} \quad (4)$$

where $k_{\text{obs}n}$ is the pseudo-first order rate constant for the n th transient at the ranges of ligand concentrations used, k_{+n} is the association rate constant for the n th aEFab binding event and k_{-n} is the dissociation rate constant for the n th Fab binding.

FRET. Intramolecular FRET was carried out using IgE-Fc(E289C)-BirA (IgE-Fc with the BirA recognition motif added to the C terminus and biotinylated according to the manufacturer's instructions (Avidity)), labeled with thiol-reactive terbium chelate (Invitrogen) and monovalent streptavidin⁶⁷ labeled with amine-reactive Alexa488 (Invitrogen), each according to the manufacturer's instructions. Terbium-labeled IgE-Fc and Alexa488-labeled streptavidin were mixed in equi-molar ratios (final assay concentration 25 nM) with aEFab titrated to 30 μM in PBS and incubated for 120 min at room temperature. FRET was measured on an Analyst LJJL-HT with excitation 330 nm (80 nm slit width), emission 485 (20 nm slit width) and 520 nm (10 nm slit width). The FRET ratio ($(E_{520}/E_{485}) \times 10^4$) was plotted as a function of aEFab concentration. Data was fit to a sequential, two-step binding mechanism.

Surface plasmon resonance. SPR experiments were carried out on a Biacore T200 instrument (GE Healthcare). Specific binding surfaces were prepared by coupling aEFab through amine coupling. Coupling density was limited to <500 resonance units. IgE-Fc was injected over the sensor chip at a flow rate of 25 μl min⁻¹, for 180 s at concentrations of 78, 156, 313, 625, 1,250, 2,500, and 5,000 nM in running buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 5 mM CaCl₂, and 0.005% (v/v) Surfactant P-20). For sandwich assays, IgE-Fc at 100 nM in running buffer was injected over the sensor chip for 180 s at a flow rate of 25 μl min⁻¹. At 45 s after completion of IgE-Fc injection, aEFab (at concentrations of 0, 78, 156, 313, 625, 1,250, 2,500, and 5,000 nM) or sFcεRIα (at concentrations of 0, 31.2, 62.5, 125, 250, 500, and 1,000 nM) were injected over the chip at a flow rate of 25 μl min⁻¹ for 120 s. Measurements were performed at 25 °C. Double referencing data subtraction methods were performed⁶⁸.

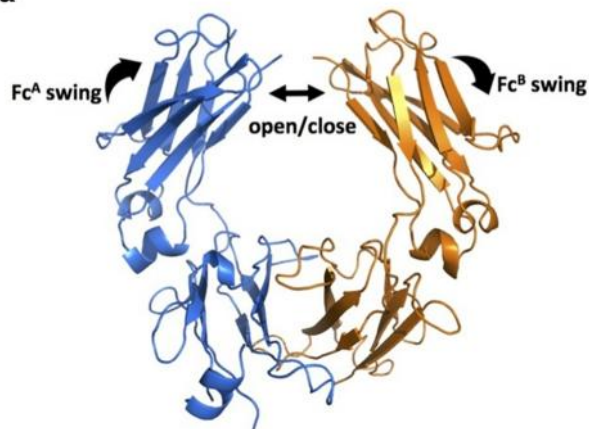
Molecular modeling of whole, extended IgE. FPMOD⁶⁹ was used to model whole, extended IgE molecules. The Fab domains from an IgE-like antibody

(PDB 2R56) were fused onto the extended IgE-Fc structure, and allowed to move rigidly about a flexible linker until 2,000 models had been generated.

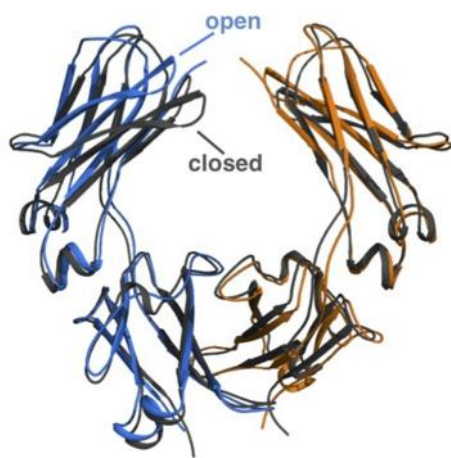
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Supplementary Figure 1: Conformational flexibility of the C ϵ 3 domains.

a

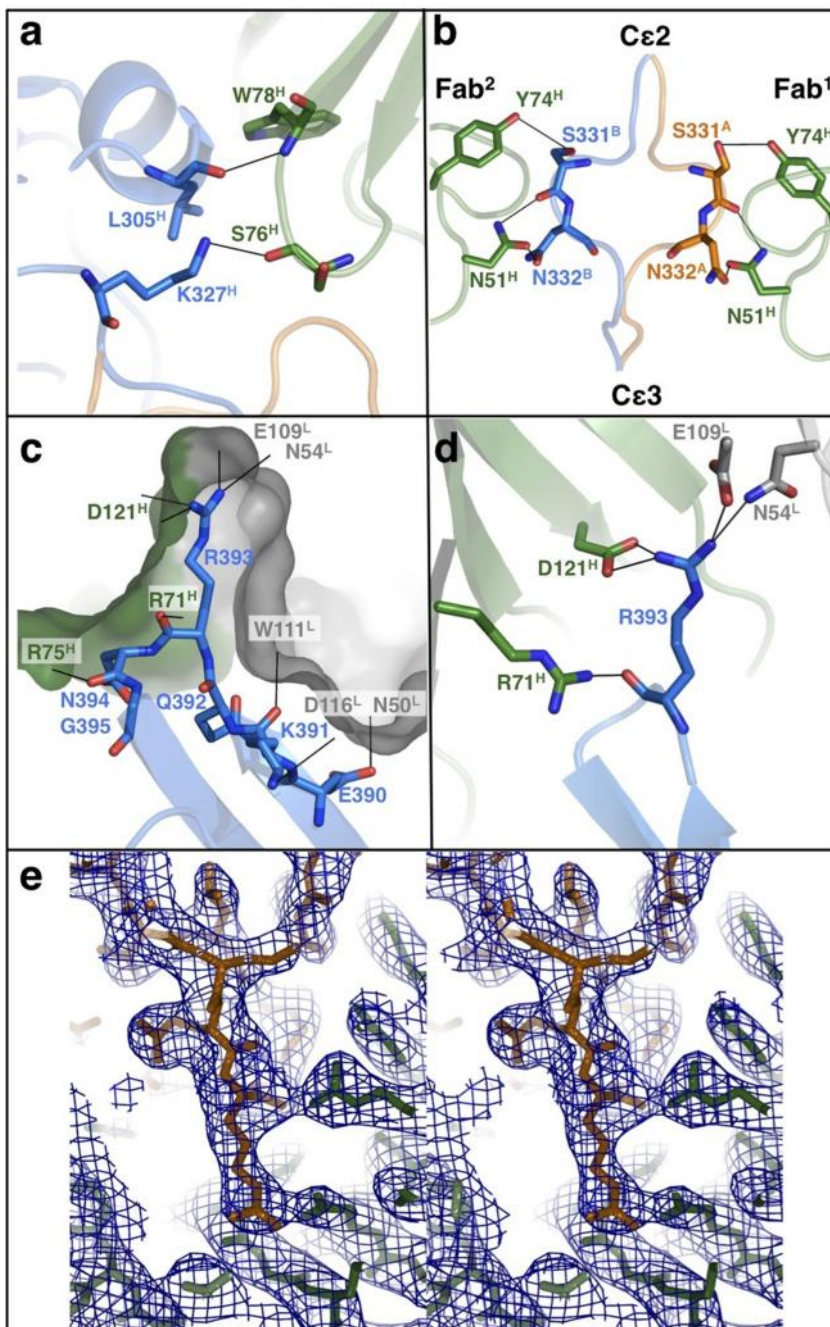


b



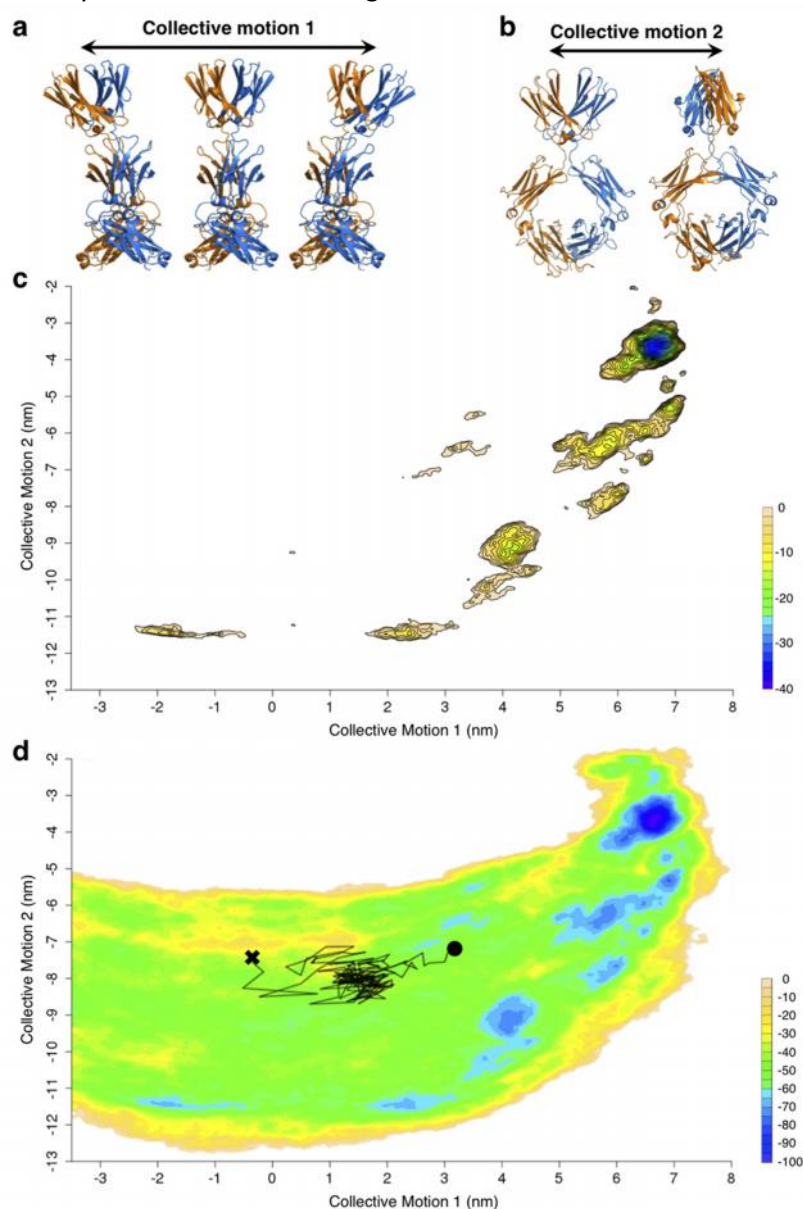
(a) Directions of “open/closed” and “swing” movements between the C ϵ 3 domains are indicated on the free IgE-Fc structure (2WQR, C ϵ 2 domains not shown for clarity). IgE-FcA is shown in blue, IgE-FcB in orange. (b) Conformational change of the C ϵ 3 domains of IgE-Fc upon $\alpha\epsilon$ Fab binding. The C ϵ 3 and C ϵ 4 domains of the extended IgE-Fc structure as seen in the $\alpha\epsilon$ Fab complex (IgE-FcA in blue, IgE-FcB in orange) are overlaid on the C ϵ 3 and C ϵ 4 domains of free IgE-Fc (grey) (C ϵ 2 domains not shown for clarity). In the structure of free IgE-Fc, IgE-FcA is in the closed conformation, and IgE-FcB is in the open conformation, while in the extended IgE-Fc structure, both chains are open. Open (extended IgE-Fc) and closed (free IgE-Fc) forms of IgE-FcA are indicated.

Supplementary Figure 2: Interactions between aεFab and IgE-Fc.



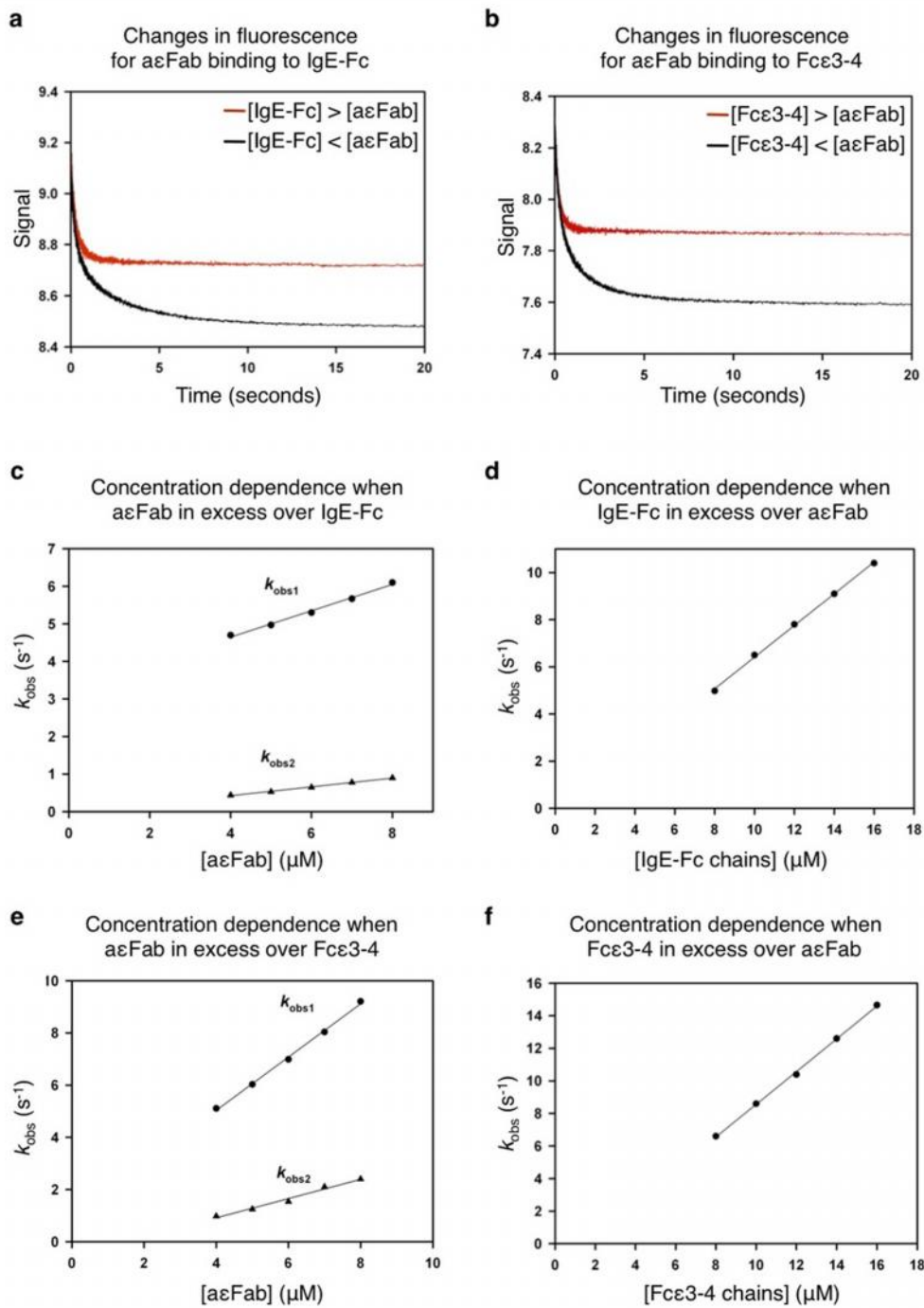
(a) Interactions between aεFab1 heavy chain (green) and the Cε2 domain of IgE-FcA (blue). Hydrogen bonds are indicated by black lines. (b) Contact between IgE-Fc Cε2-Cε3 linker regions and the aεFab molecules. IgE-FcA is shown in blue and IgE-FcB in orange; aεFab1 and aεFab2 are shown in green. The locations of the Cε2 and Cε3 domains are indicated. (c) R393 binding pocket between aεFab heavy (green) and light (grey) chains. Black lines indicate hydrogen bonds formed with aεFab residues. (d) The interactions between R393 and aεFab residues. (e) Stereo image of 2Fo-Fc electron density at 1σ contour level for residues around R393 (shown in orange in the center of the image) at the interface between IgE-FcB (orange) and aεFab2 (green).

Supplementary Figure 3: Representation of the collective motions used as collective variables in the metadynamics simulation of Figure 2.



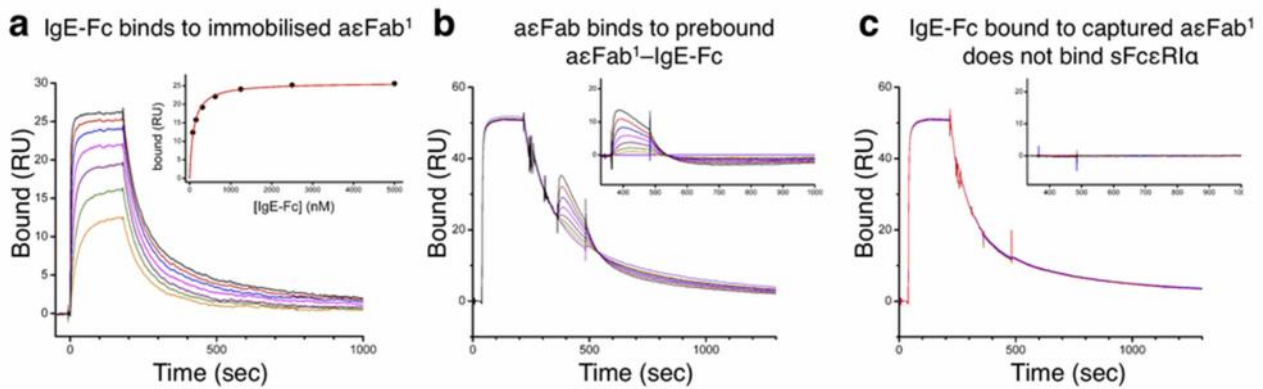
(a) Black arrow indicates collective motion 1 (x-axis in Figure 2), with the middle structure representing $x=0$. (b) Black arrow indicates collective motion 2 (y-axis in Figure 2) with the two structures representing the extremes explored across $x=0$. This motion is principally a twisting of (Cε2)2 relative to the Cε3-Cε4 domains. (c) Free-energy surface representing the IgE-Fc unbending process generated through metadynamics simulation. Axes show the projection along the two lowest frequency collective motions (extracted from a biased trajectory, as for Fig. 2a). This plot shows the features of the surface within 40 kJ/mol of the lowest free-energy minimum, contoured every 2.5 kJ/mol and coloured accordingly. (d) Short unbiased simulation starting from the extended conformation of IgE-Fc (Fig. 1c) in the crystal structure of the complex. The simulation was run for 250 ns and the trajectory is represented by a black line plotted over the free-energy surface (Fig. 2a). The simulation started at $x = -0.4$, $y = 7.6$ (indicated with black cross) and was terminated at $x = 3.1$, $y = -7.3$ (black circle). This trajectory is consistent with the small energy barriers surrounding the extended conformation.

Supplementary Figure 4: Stopped-flow kinetic analysis of $\alpha\epsilon$ Fab binding to IgE-Fc.



Kinetic binding curves showing the change in fluorescence when (a) $\alpha\epsilon$ Fab binds to IgE-Fc and (b) $\alpha\epsilon$ Fab binds to Fc ϵ 3-4. The red traces indicate experiments carried out with IgE-Fc or Fc ϵ 3-4 in excess over $\alpha\epsilon$ Fab, and the black traces are experiments with $\alpha\epsilon$ Fab in excess over IgE-Fc or Fc ϵ 3-4. The kinetic binding parameters demonstrate a linear concentration dependence for $\alpha\epsilon$ Fab binding to IgE-Fc (c and d) and Fc ϵ 3-4 (e and f).

Supplementary Figure 5: SPR analysis of $\alpha\epsilon$ Fab1–IgE-Fc and $\alpha\epsilon$ Fab1–IgE-Fc– $\alpha\epsilon$ Fab2 complex formation, and inhibition of sFc ϵ R1 α binding by $\alpha\epsilon$ Fab1.



(a) SPR sensorgrams of IgE-Fc binding to immobilized $\alpha\epsilon$ Fab. IgE-Fc was injected over the surface at concentrations of 78 (orange), 156 (green), 313 (purple), 625 (magenta), 1250 (blue), 2500 (red), and 5000 nM (black). Data are fit to an equilibrium model of single site binding (inset). (b) $\alpha\epsilon$ Fab binds to pre-bound $\alpha\epsilon$ Fab1–IgE-Fc complex to form $\alpha\epsilon$ Fab1–IgE-Fc– $\alpha\epsilon$ Fab2. $\alpha\epsilon$ Fab was injected over the $\alpha\epsilon$ Fab1–IgE-Fc surface at concentrations of 0 (purple), 78 (orange), 156 (green), 313 (purple), 625 (magenta), 1250 (blue), 2500 (red), and 5000 nM (black). Inset shows $\alpha\epsilon$ Fab2 binding normalised with respect to the buffer only control (purple). (c) When IgE-Fc is bound to $\alpha\epsilon$ Fab1 on the SPR surface, sFc ϵ R1 α is not able to bind. Inset shows sFc ϵ R1 α binding normalised with respect to buffer only control. sFc ϵ R1 α was injected over the $\alpha\epsilon$ Fab1–IgE-Fc surface at concentrations of 0 (orange), 31.3 (green), 62.5 (purple), 125 (magenta), 250 (blue), 500 (red), and 1000 nM (black).

Human immunoglobulin E flexes between acutely bent and extended conformations

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Supplementary Information

Supplementary Figure 1

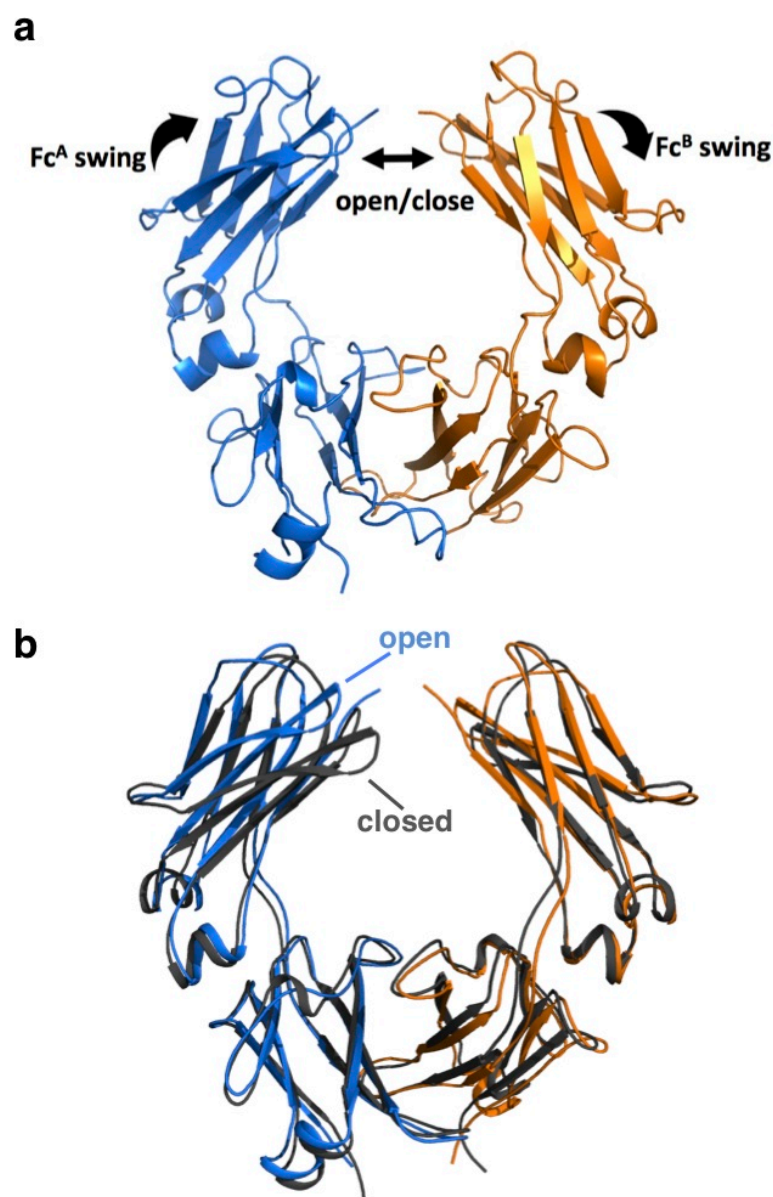


Figure S1 Conformational flexibility of the Cε3 domains. **(a)** Directions of “open/closed” and “swing” movements between the Cε3 domains are indicated on the free IgE-Fc structure (2WQR, Cε2 domains not shown for clarity). IgE-Fc^A is shown in blue, IgE-Fc^B in orange. **(b)** Conformational change of the Cε3 domains of IgE-Fc upon aεFab binding. The Cε3 and Cε4 domains of the extended IgE-Fc structure as seen in the aεFab complex (IgE-Fc^A in blue, IgE-Fc^B in orange) are overlaid on the Cε3 and Cε4 domains of free IgE-Fc (grey) (Cε2 domains not shown for clarity). In the structure of free IgE-Fc, IgE-Fc^A is in the closed conformation, and IgE-Fc^B is in the open conformation, while in the extended IgE-Fc structure, both chains are open. Open (extended IgE-Fc) and closed (free IgE-Fc) forms of IgE-Fc^A are indicated.

Supplementary Figure 2

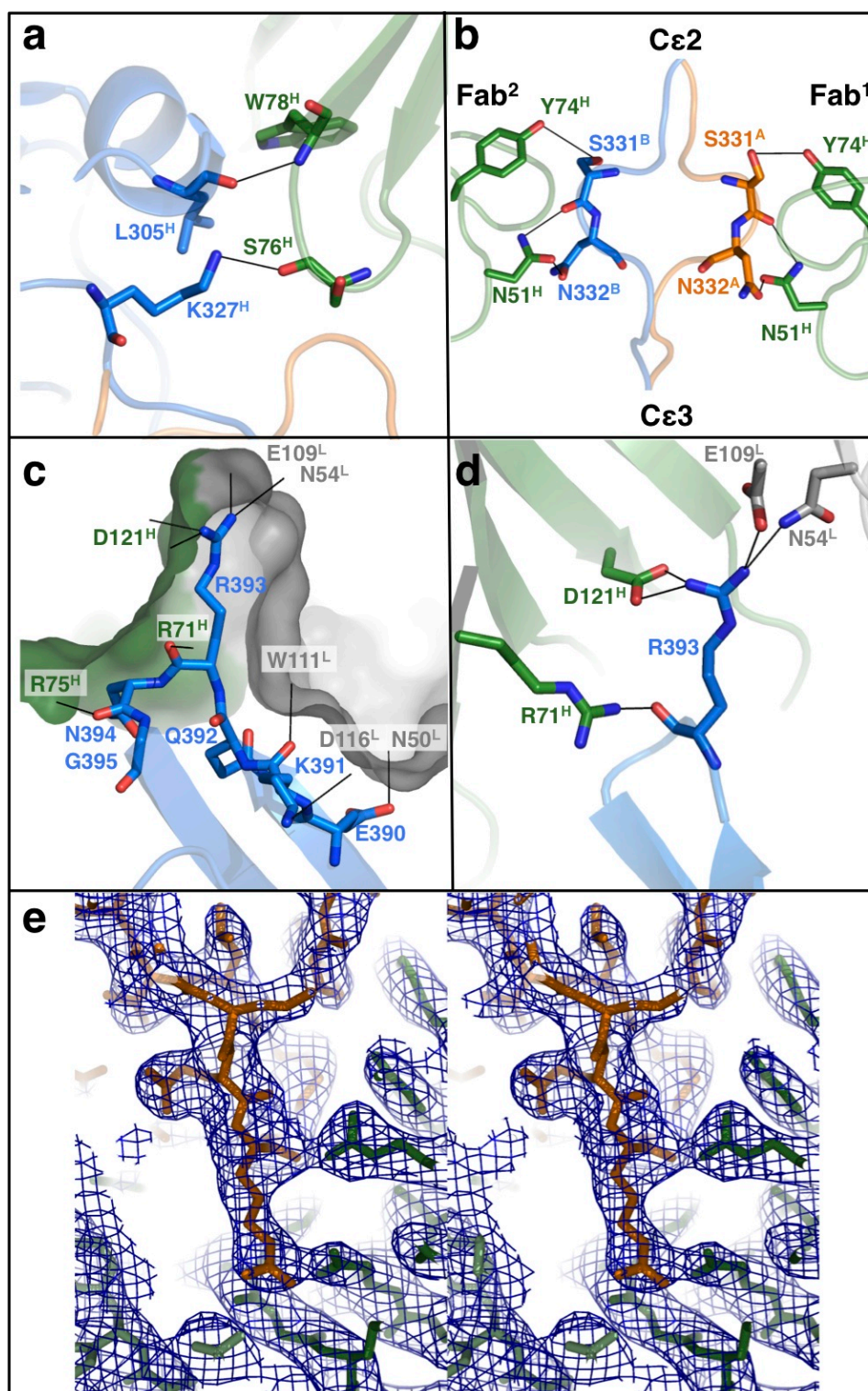


Figure S2 Interactions between aEFab and IgE-Fc. **(a)** Interactions between aEFab¹ heavy chain (green) and the Cε2 domain of IgE-Fc^A (blue). Hydrogen bonds are indicated by black lines. **(b)** Contact between IgE-Fc Cε2-Cε3 linker regions and the aEFab molecules. IgE-Fc^A is shown in blue and IgE-Fc^B in orange; aEFab¹ and aEFab² are shown in green. The locations of the Cε2 and Cε3 domains are indicated. **(c)** R393 binding pocket between aEFab heavy (green) and light (grey) chains. Black lines indicate hydrogen bonds formed with aEFab residues. **(d)** The interactions between R393 and aEFab residues. **(e)** Stereo image of 2F_o-F_c electron density at 1σ contour level for residues around R393 (shown in orange in the center of the image) at the interface between IgE-Fc^B (orange) and aEFab² (green).

Supplementary Figure 3

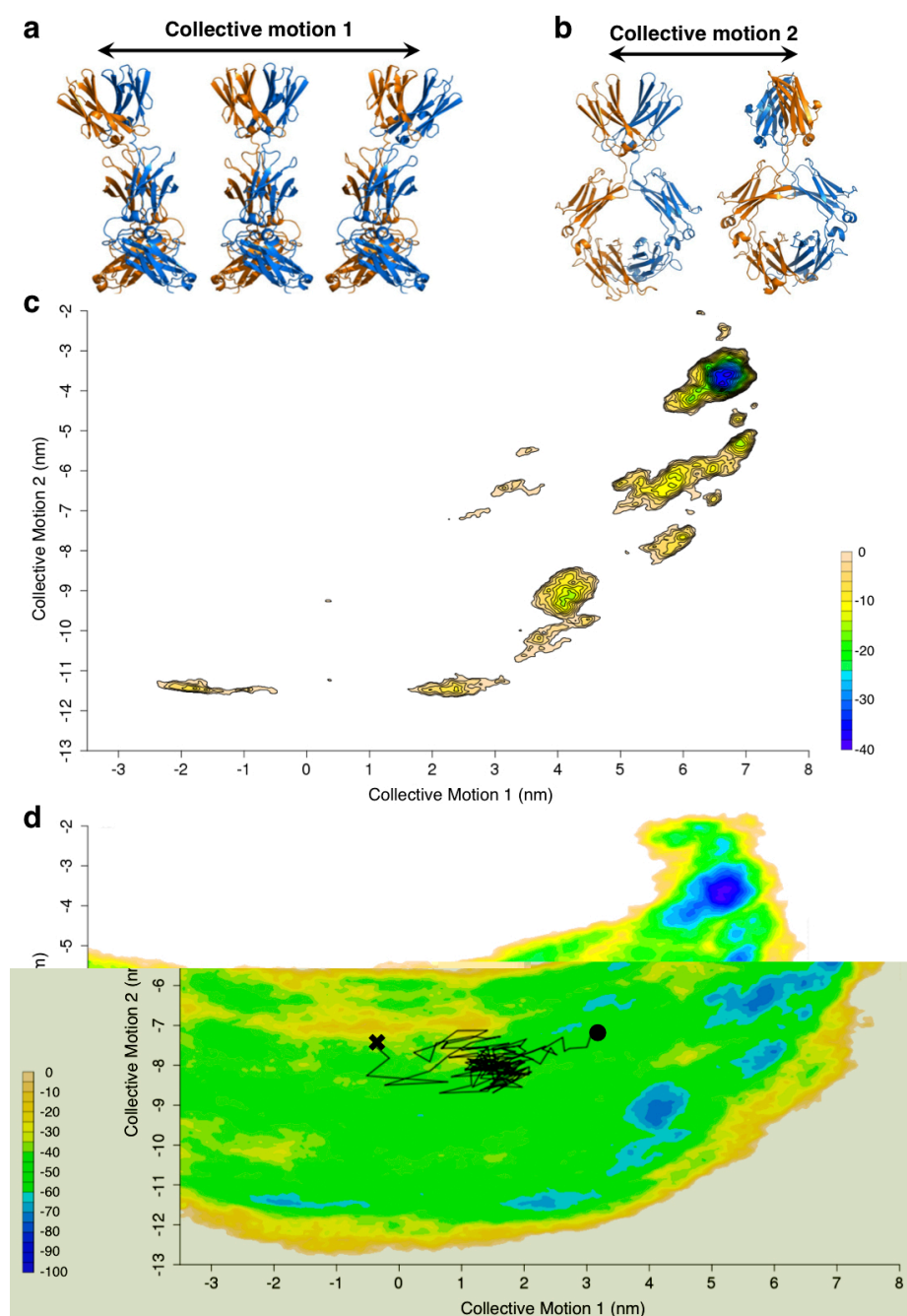


Figure S3 Representation of the collective motions used as collective variables in the metadynamics simulation of Figure 2. **(a)** Black arrow indicates collective motion 1 (x -axis in Figure 2), with the middle structure representing $x=0$. **(b)** Black arrow indicates collective motion 2 (y -axis in Figure 2) with the two structures representing the extremes explored across $x=0$. This motion is principally a twisting of $(C\epsilon 2)_2$ relative to the $C\epsilon 3$ - $C\epsilon 4$ domains. **(c)** Free-energy surface representing the IgE-Fc unbending process generated through metadynamics simulation. Axes show the projection along the two lowest frequency collective motions (extracted from a biased trajectory, as for **Fig. 2a**). This plot shows the features of the surface within 40 kJ/mol of the lowest free-energy minimum, contoured every 2.5 kJ/mol and coloured accordingly. **(d)** Short unbiased simulation starting from the extended conformation of IgE-Fc (**Fig. 1c**) in the crystal structure of the complex. The simulation was run for 250 ns and the trajectory is represented by a black line plotted over the free-energy surface (**Fig. 2a**). The simulation started at $x = -0.4$, $y = 7.6$ (indicated with black cross) and was terminated at $x = 3.1$, $y = -7.3$ (black circle). This trajectory is consistent with the small energy barriers surrounding the extended conformation.

Supplementary Figure 4

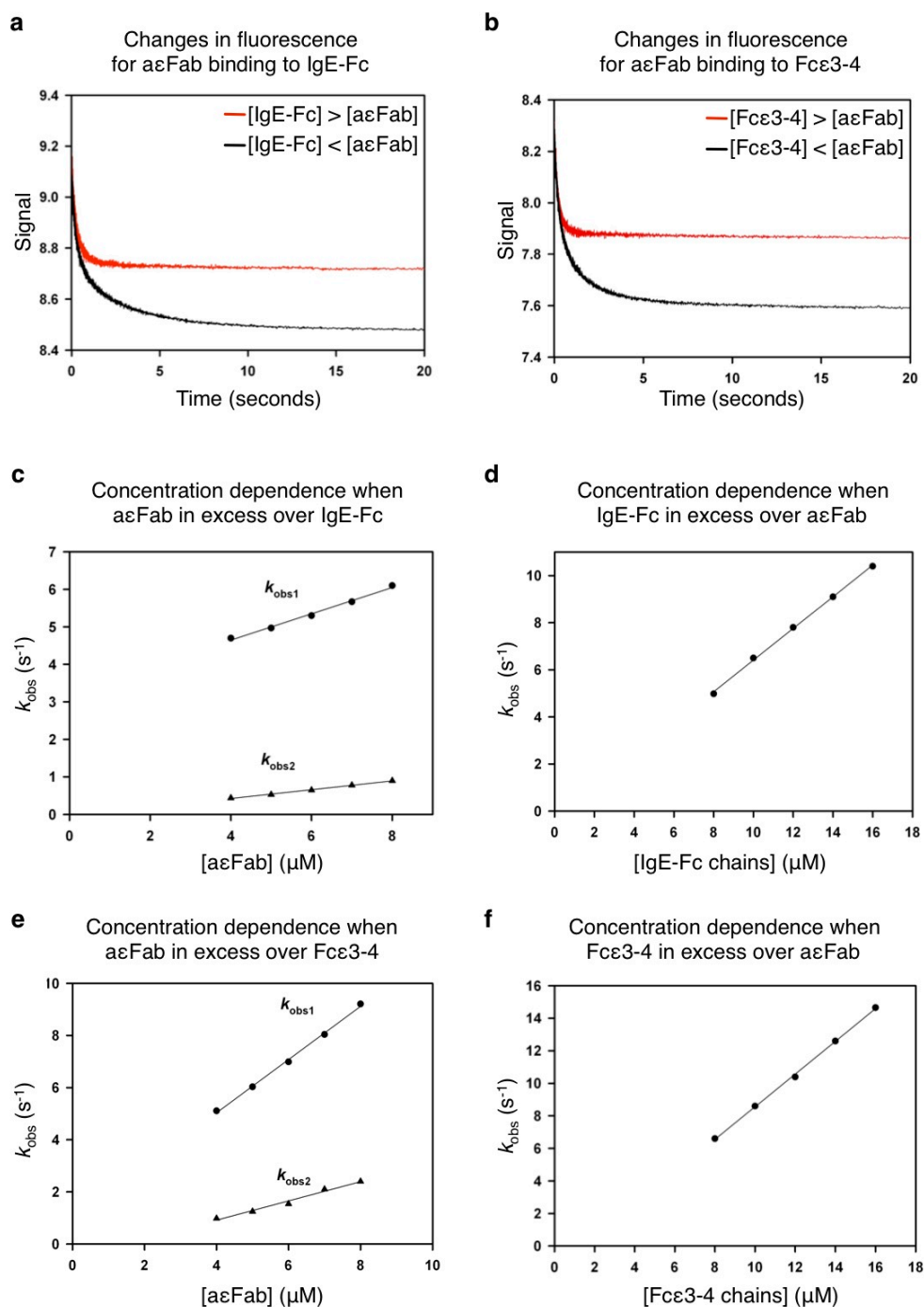


Figure S4 Stopped-flow kinetic analysis of aεFab binding to IgE-Fc. Kinetic binding curves showing the change in fluorescence when (a) aεFab binds to IgE-Fc and (b) aεFab binds to Fcε3-4. The red traces indicate experiments carried out with IgE-Fc or Fcε3-4 in excess over aεFab, and the black traces are experiments with aεFab in excess over IgE-Fc or Fcε3-4. The kinetic binding parameters demonstrate a linear concentration dependence for aεFab binding to IgE-Fc (c and d) and Fcε3-4 (e and f).

Supplementary Figure 5

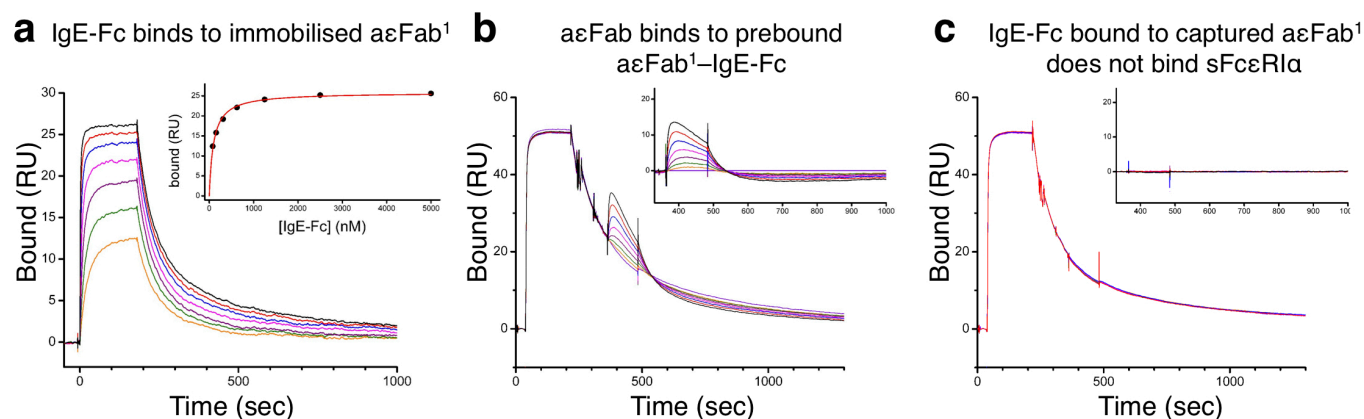


Figure S5 SPR analysis of aεFab¹-IgE-Fc and aεFab¹-IgE-Fc-aεFab² complex formation, and inhibition of sFcεRIα binding by aεFab¹. **(a)** SPR sensorgrams of IgE-Fc binding to immobilized aεFab. IgE-Fc was injected over the surface at concentrations of 78 (orange), 156 (green), 313 (purple), 625 (magenta), 1250 (blue), 2500 (red), and 5000 nM (black). Data are fit to an equilibrium model of single site binding (inset). **(b)** aεFab binds to pre-bound aεFab¹-IgE-Fc complex to form aεFab¹-IgE-Fc-aεFab². aεFab was injected over the aεFab¹-IgE-Fc surface at concentrations of 0 (purple), 78 (orange), 156 (green), 313 (purple), 625 (magenta), 1250 (blue), 2500 (red), and 5000 nM (black). Inset shows aεFab² binding normalised with respect to the buffer only control (purple). **(c)** When IgE-Fc is bound to aεFab¹ on the SPR surface, sFcεRIα is not able to bind. Inset shows sFcεRIα binding normalised with respect to buffer only control. sFcεRIα was injected over the aεFab¹-IgE-Fc surface at concentrations of 0 (orange), 31.3 (green), 62.5 (purple), 125 (magenta), 250 (blue), 500 (red), and 1000 nM (black).

Supplementary Video 1: The structure of IgE-Fc bound symmetrically by two $\alpha\epsilon$ Fab molecules

IgE-Fc^A is shown in blue and IgE-Fc^B in orange; $\alpha\epsilon$ Fab heavy chains are shown in green and light chains in grey. Also shown is the extended conformation of IgE-Fc as seen in the complex, which undergoes an “unbending” of 120° compared to the free structure. The unbending derives largely from hinge movement in the C ϵ 2-C ϵ 3 linker region (residues P333, R334, G335) as shown.

Supplementary Video 2: IgE-Fc may flip between two symmetrically related bent conformations

The existence of an unbent conformation of IgE-Fc in the $\alpha\epsilon$ Fab complex suggests that the molecule may pass through an extended state as it flips between the two bent conformations. This potential motion is shown in two orthogonal orientations, with IgE-Fc^A in blue and IgE-Fc^B in orange.

Supplementary Video 3: Proposed mechanism of IgE-Fc flexibility and $\alpha\epsilon$ Fab binding in solution

IgE-Fc is predominantly bent in solution, but (C ϵ 2)₂ may be capable of flipping from one side of the molecule to the other. $\alpha\epsilon$ Fab¹ engages at either exposed binding site of IgE-Fc, attaching to the C ϵ 3 domain, and limiting the range of accessible conformations. $\alpha\epsilon$ Fab² engages while IgE-Fc transiently occupies the extended conformation, capturing the molecule in a symmetrical state.