

**Human immunoglobulin E flexes between acutely bent
and extended conformations**

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Supplementary Information

Supplementary Figure 1

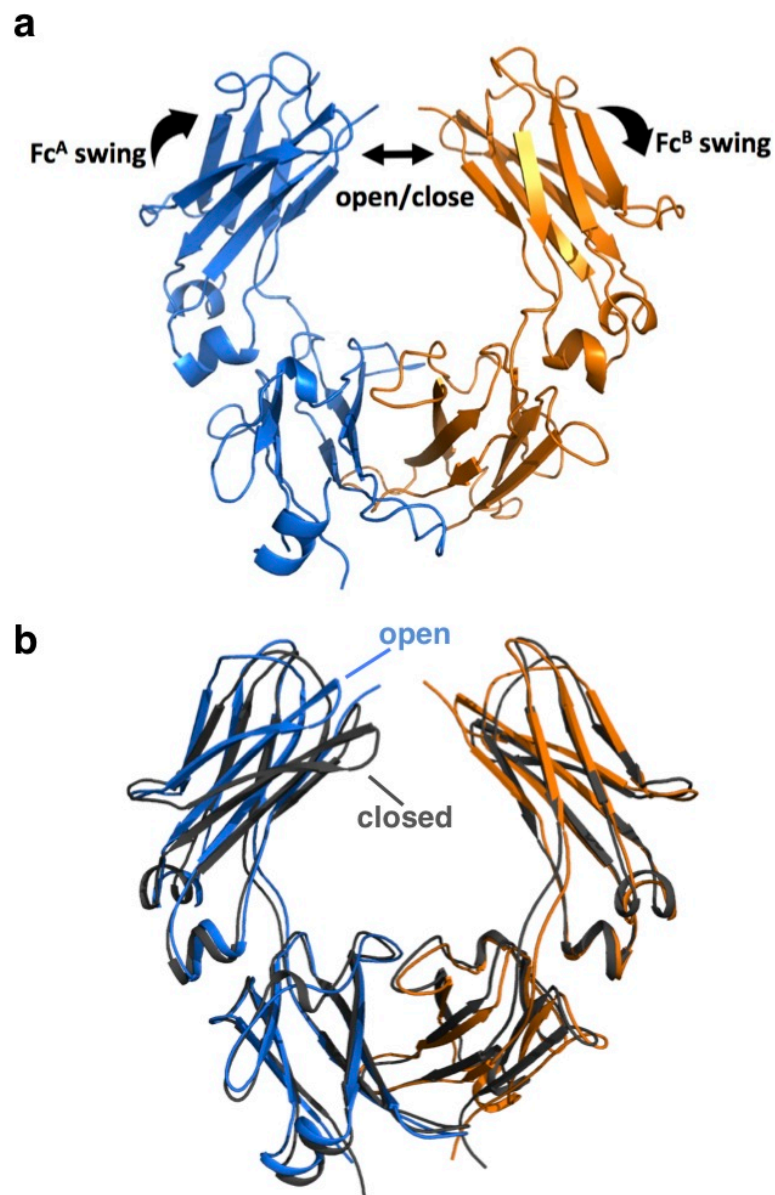


Figure S1 Conformational flexibility of the Cε3 domains. **(a)** Directions of “open/closed” and “swing” movements between the Cε3 domains are indicated on the free IgE-Fc structure (2WQR, Cε2 domains not shown for clarity). IgE-Fc^A is shown in blue, IgE-Fc^B in orange. **(b)** Conformational change 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

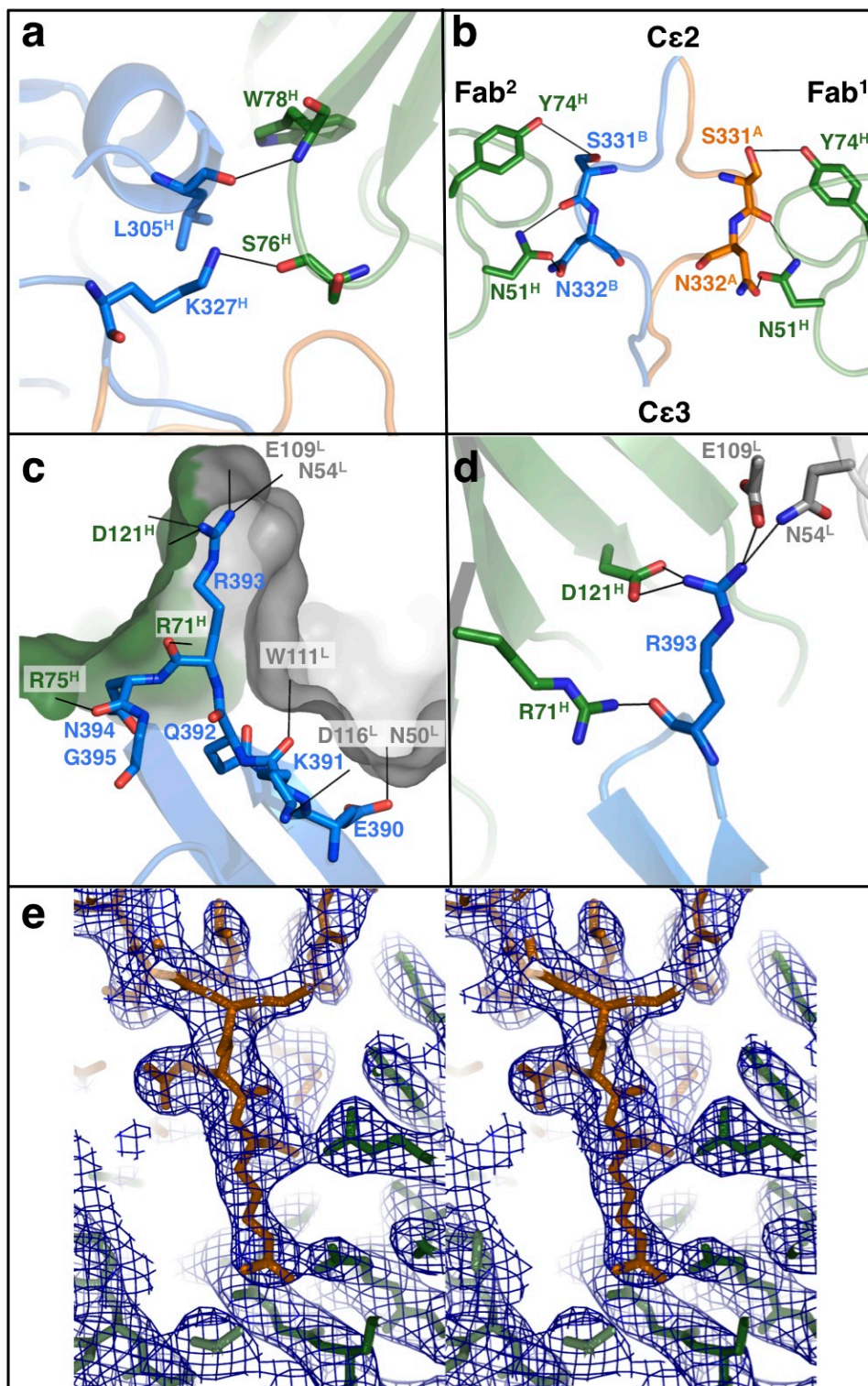


Figure S2 Interactions between aεFab and IgE-Fc. **(a)** Interactions between aεFab¹ heavy chain (green) and the Cε2 domain of IgE-Fc^A (blue). Hydrogen bonds are indicated by black lines. **(b)** Contact between IgE-Fc Cε2-Cε3 linker regions and the aεFab molecules. IgE-Fc^A is shown in blue and IgE-Fc^B 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 $2F_o - F_c$ 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 aεFab² (green).

Supplementary Figure 3

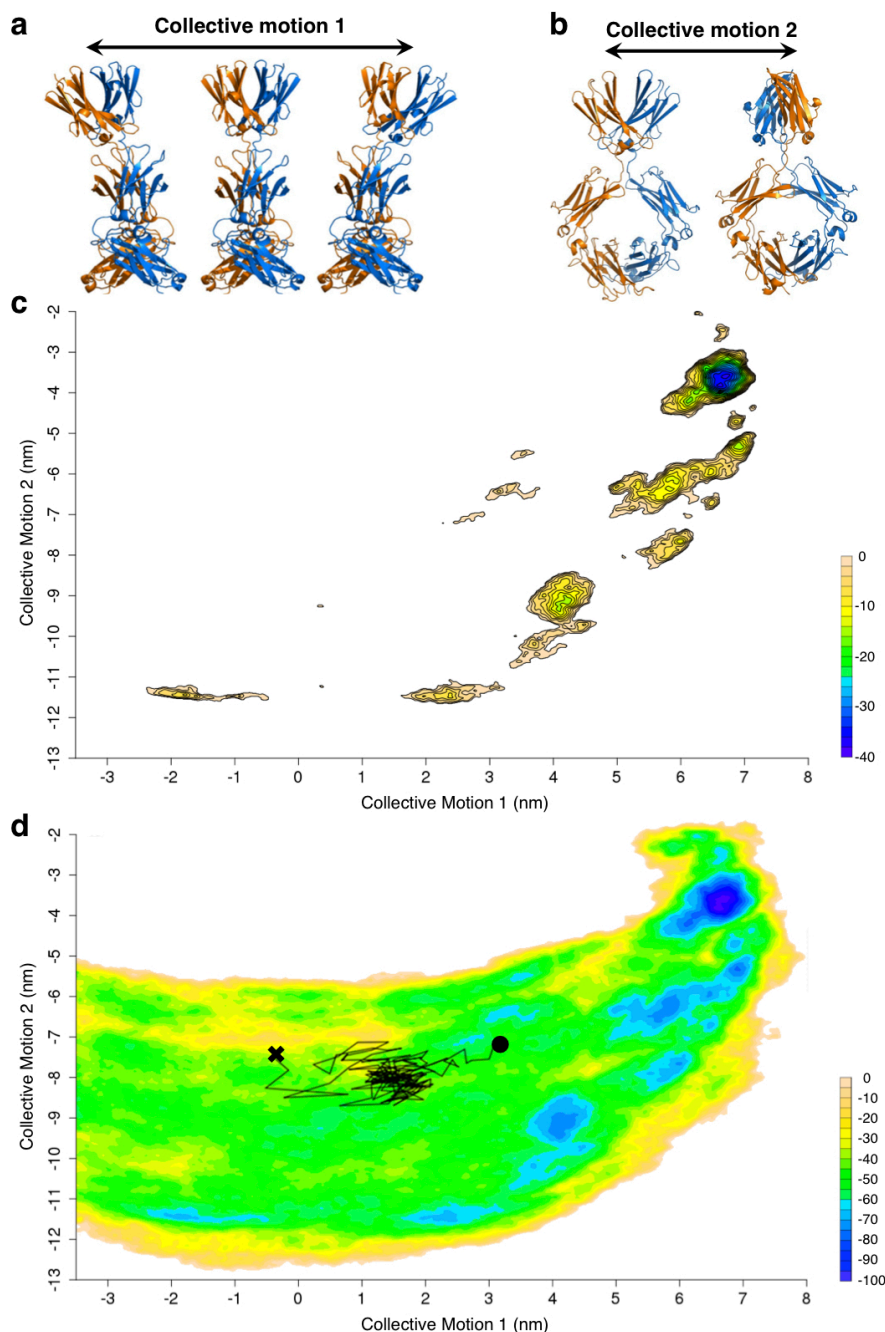


Figure S3 Representation of the collective motions used as collective variables in the metadynamics simulation of Figure 2. **(a)** Black arrow indicates collective motion 1 (x -axis in Figure 2), with the middle structure representing $x=0$. **(b)** Black arrow indicates collective motion 2 (y -axis 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

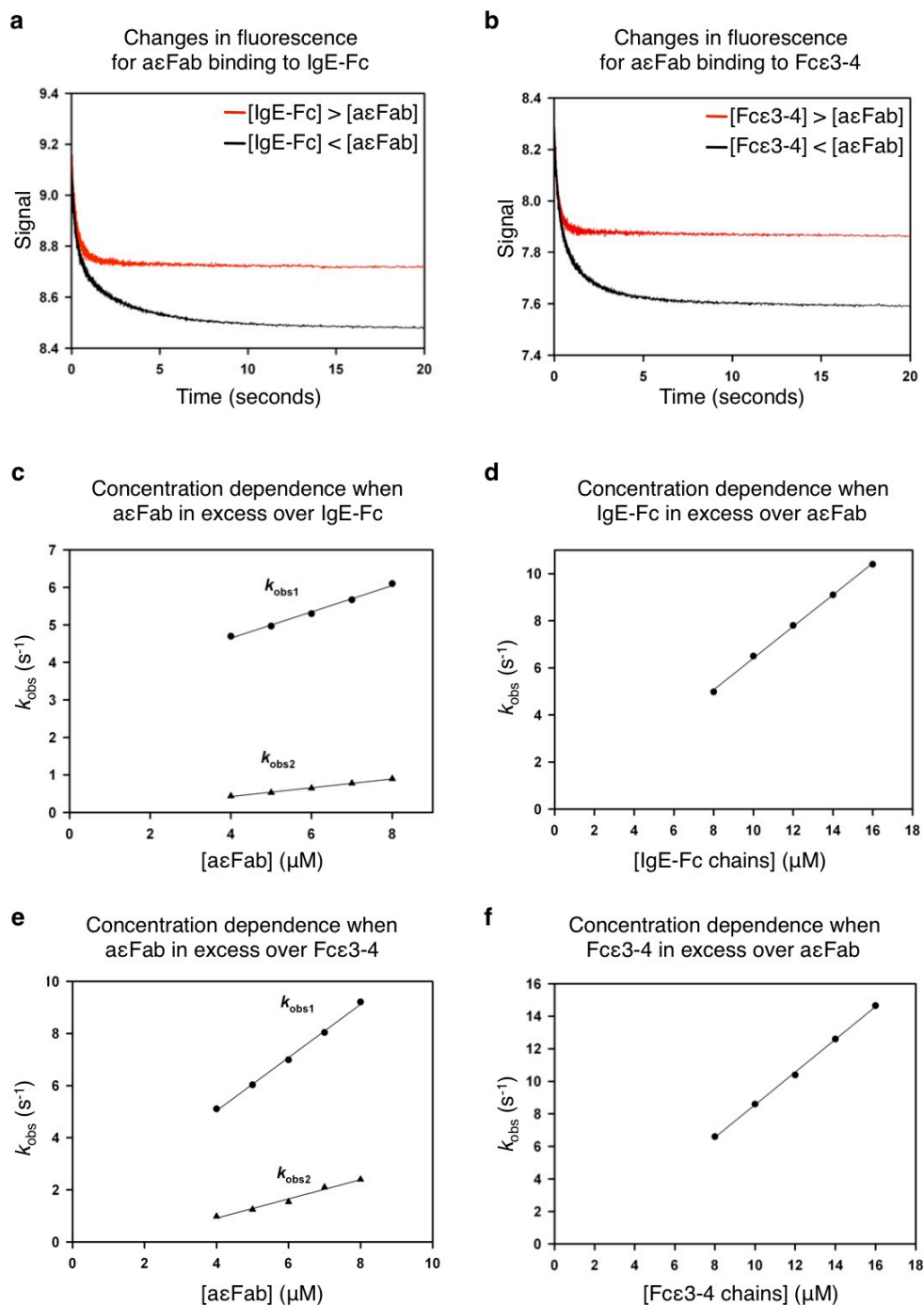


Figure S4 Stopped-flow kinetic analysis of aεFab binding to IgE-Fc. Kinetic binding curves showing the change in fluorescence when (a) aεFab binds to IgE-Fc and (b) aεFab binds to Fcε3-4. The red traces indicate experiments carried out with IgE-Fc or Fcε3-4 in excess over aεFab, and the black traces 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

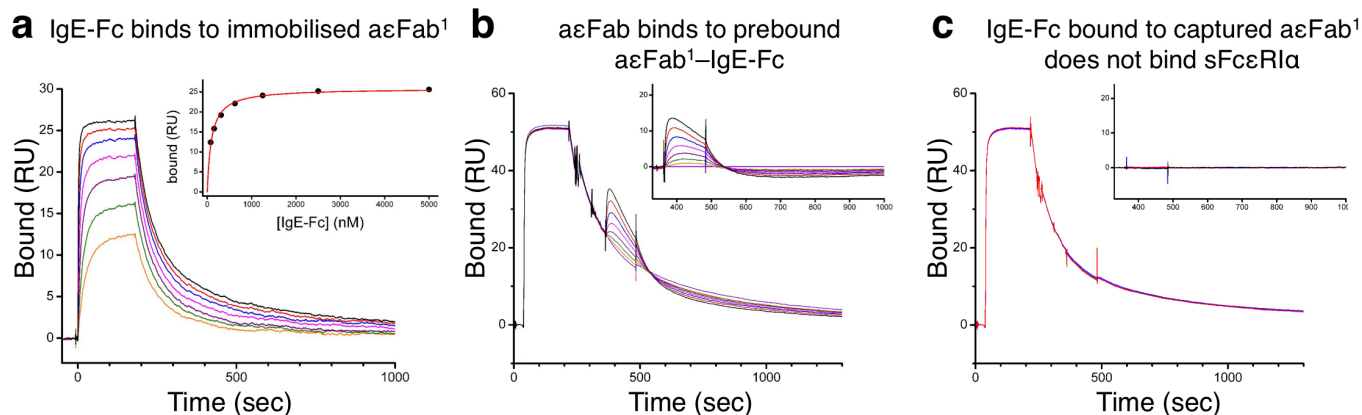


Figure S5 SPR analysis of aεFab¹-IgE-Fc and aεFab¹-IgE-Fc-aεFab² complex formation, and inhibition of sFcεRIα binding by aεFab¹. **(a)** SPR sensorgrams of IgE-Fc binding to immobilized aεFab. IgE-Fc was injected over the surface at concentrations of 78 (orange), 156 (green), 313 (purple), 625 (magenta), 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 α Fab molecules

IgE-Fc^A is shown in blue and IgE-Fc^B in orange; α 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 α 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 α 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. α Fab¹ engages at either exposed binding site of IgE-Fc, attaching to the C ϵ 3 domain, and limiting the range of accessible conformations. α Fab² engages while IgE-Fc transiently occupies the extended conformation, capturing the molecule in a symmetrical state.