Supporting Information

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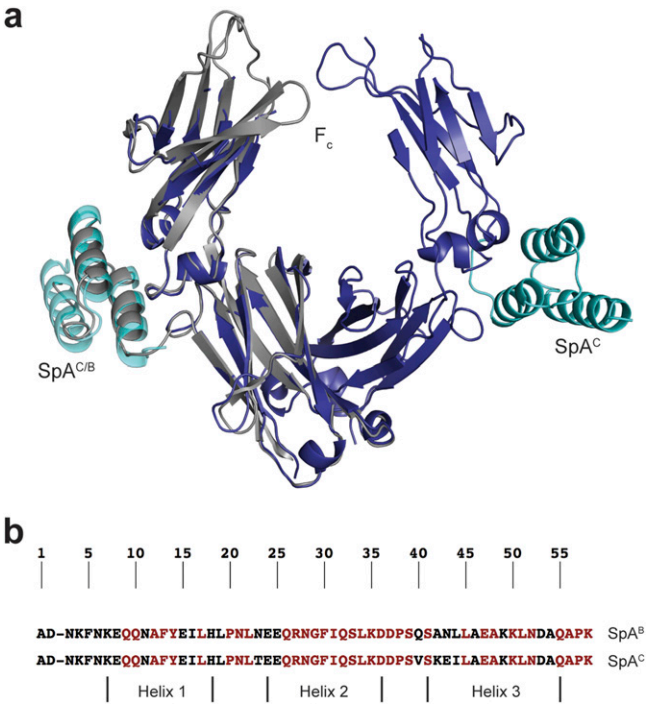

Fig. S1. Comparison between PDB ID codes 1FC2 and 4WWI, our new F_c–SpA^C complex structure. (*A*) SpA^B in 1FC2 binds to the same site on F_c as does SpA^C in the new complex. The F_c portions of both structures superimpose well. According to ref. 30, helix 3 in 1FC2 was folded irregularly and no density was observed, although nonhelical coordinates were still modeled. (*B*) SpA^B and SpA^C contain minimal sequence differences, none of which are interfacial. Red residues are identical in all five SpA protein binding domains.

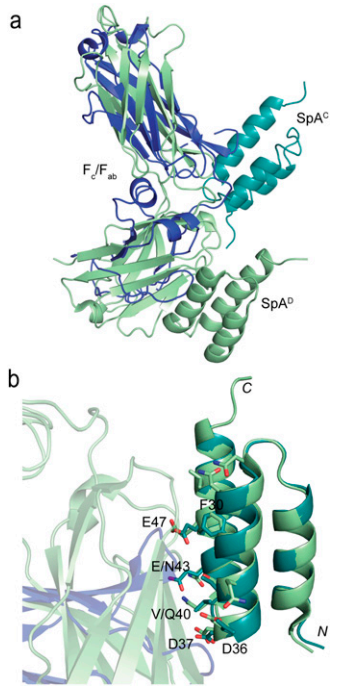

Fig. S2. SpA^C and F_c bind in a secondary mode. (*A*) SpA^C (dark cyan) binds to F_c (blue) in a different location from SpA^D binding to F_{ab} (green; from PDB ID code 1DEE). (*B*) The helix 2/3-binding sites on the SpA domain are nearly homologous, with only a few rotamer differences.

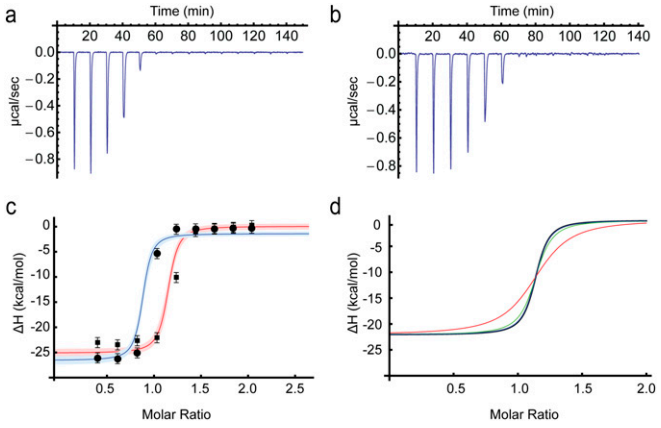

Fig. S3. Isothermal titration calorimetry data of SpA^C–F_c binding. (*A* and *B*) Raw data for (*A*) titration of 5 μM SpA^C with F_c and (*B*) titration of 5 μM F_c with SpA^C. (*C*) Best-fit ITC binding isotherms for titration of F_c into SpA^C (squares, red line) and SpA^C into F_c (circles, blue line). Bands represent 95% confidence intervals of best-fit isotherms. Error bars represent SEM. Best-fit parameter values (95% confidence interval) are K_a, 6.6 (5.6, 9.5) × 10^7 M^{-1}; ΔH, −25.4 (−25.8, −24.6) kcal/mol; n = 1.14 (1.13, 1.15). (*D*) Binding isotherms for titration of SpA^C with F_c were simulated using a two-site–binding model with the affinity, enthalpy, and n values given in *C*. Simulated single-site isotherm (black) and simulated two-site isotherms at various second-site K_d values: 1 μM (red), 10 μM (green), 100 μM (blue).

Table S1. Data collection and refinement statistics for the SpA^C–F_c complex

	SpA^C–F_c complex
Data collection	
Space group	*C*2
Cell dimensions	
a, *b*, *c*, Å	139.0, 88.1, 101.0
α, β, γ, °	90.0, 91.0, 90.0
Wavelength	1.0
Resolution, Å	50.00–2.30 (2.34–2.30)*
R_{sym}	0.17 (1.0)
$I/\sigma I$	29.0 (1.7)
Completeness, %	99.5 (94.8)
Redundancy	14.8 (12.3)
Refinement	
Resolution, Å	41.18–2.31
No. of reflections	50,556
R_{work}/R_{free}	21.2/25.0
No. of atoms	
Protein	6254
Water	165
B factors	
Protein	62.96
Water	76.44
Rms deviations	
Bond lengths, Å	0.004
Bond angles, °	0.817

*Data were collected from a single crystal. Values in parentheses are for the highest-resolution shell.

Table S2. R_ρ for apo SpA^C and SpA^C in complex with F_c

Residue	Apo (4NPD)	Complex (4WWI) chain A	$\frac{R_\rho^{apo}-R_\rho^{cmplx}}{R_\rho^{apo}}$, %	Residue	Apo (4NPD)	Complex (4WWI) chain A	$\frac{R_\rho^{apo}-R_\rho^{cmplx}}{R_\rho^{apo}}$, %
N3	0.59	0.25	58	F30	1.01	0.20	80
K4	0.88	0.80	9	I31*	0.81	0.26	68
F5	0.98	0.45	55	Q32	0.77	0.30	61
N6	0.98	0.12	88	S33	1.00	0.28	72
K7	0.67	0.58	13	L34	0.59	0.07	88
E8	0.70	0.72	−3	K35	0.58	0.29	50
Q9	0.69	0.12	83	D36	0.47	0.06	87
Q10*	0.74	0.16	78	D37	0.98	0.06	94
N11	0.43	0.91	−112	P38	0.52	0.53	−2
F13*	0.79	0.14	82	S39	0.77	0.44	43
Y14*	0.93	0.01	99	V40	0.78	0.50	36
E15	0.65	0.78	−20	S41	0.85	0.27	68
I16	0.85	0.18	79	K42	0.49	0.60	−22
L17*	0.80	0.02	98	E43	0.72	0.28	61
H18*	0.97	0.11	89	I44	0.79	0.45	43
L19	0.88	0.04	95	L45	0.85	0.38	55
P20	0.75	0.33	56	E47	0.94	0.57	39
N21	0.82	0.08	90	K49	0.84	0.35	58
L22	0.95	0.19	80	K50	0.59	0.50	15
T23	0.95	0.43	55	L51	0.87	0.20	77
E24	0.59	0.50	15	N52	0.94	0.39	59
E25	0.59	0.60	−2	D53	0.68	0.23	66
Q26	1.00	0.16	84	Q55	1.00	0.19	81
R27	0.88	0.36	59	P57	0.84	0.15	82
N28	0.60	0.12	80	K58	0.35	0.47	−34

Statistics comparing interfacial and noninterfacial residues

Residue type	Apo mean	Complex mean	$\frac{R_\rho^{apo}-R_\rho^{cmplx}}{R_\rho^{apo}}$, %	
All	0.77 (0.17)[†]	0.32 (0.22)	53 (42)	
Interfacial	0.84 (0.09)	0.12 (0.09)	86 (12)	} $P = 0.00007$[‡]
Noninterfacial	0.76 (0.18)	0.35 (0.22)	48 (42)	

It is difficult to quantitatively estimate the uncertainty in electron-density values; therefore, we have not included uncertainties in R_ρ. Qualitatively, sources of error that contribute to uncertainty in electron density and therefore R_ρ include data collection errors, crystal defects, modeling errors, and refinement artifacts.

*Interfacial residues.

[†]Numbers in parentheses are SDs.

[‡]Based on a Student *t* hypothesis test. The *P* value is the probability that interfacial and noninterfacial residues have the same reductions in conformational heterogeneity upon F_c binding.

Table S3. Comparison of NMR and crystallographic observations of conformational heterogeneity

Residue	Apo (X-ray)*	Apo (NMR)†	^{3}J(Hz)	Chemical shift	Complex (X-ray)*	Complex ρ ratio‡	Note
K4	χ_1: +	χ_1: +	$^3J(C\gamma C') = 2.72 \pm 0.01$		χ_1: +	0.57	
K7	χ_1: +	χ_1: +	$^3J(C\gamma C') = 2.61 \pm 0.01$		χ_1: +	0.45	
I16	χ_1: −	χ_1: −	$^3J(C\gamma_1 C') = 3.95 \pm 0.05$	$C(\delta_1) = 13.7$ ppm	χ_1: −	0.07	From NMR scalar coupling and chemical shift:
	χ_2: −	χ_2: +	$^3J(C\gamma_1 N) = 0.37 \pm 0.21$;		χ_2: −		Population trans 0.80
			$^3J(C\gamma_2 C') = 1.09 \pm 0.01$;				Population gauche− 0.20
			$^3J(C\gamma_2 N) = 2.10 \pm 0.01$				
L17§	χ_1: a	χ_1: +	$^3J(C\gamma C') = 2.97 \pm 0.02$	$C(\delta_1) = 24.7$ ppm	χ_1: −	0.24	
	χ_2: a	χ_2: +	$^3J(C\gamma N) = 0.74 \pm 0.07$	$C(\delta_2) = 23.0$ ppm; $\Delta CS = 1.7$ ppm	χ_2: −		
L19	χ_1: −	χ_1: −	$^3J(C\gamma C') = 3.80 \pm 0.04$;	$C(\delta_1) = 26.6$ ppm	χ_1: −	0.13	
	χ_2: a	χ_2: +	$^3J(C\gamma N) = 0.73 \pm 0.20$	$C(\delta_2) = 22.8$ ppm; $\Delta CS = 3.8$ ppm			
L22	χ_2: −	χ_2: −		$C(\delta_1) = 27.2$ ppm; $C(\delta_2) = 22.2$ ppm; $\Delta CS = 5.0$ ppm	χ_2: −	0.3	
T23	χ_1: −	χ_1: +	$^3J(C\gamma_2 C') = 3.06 \pm 0.01$; $^3J(C\gamma_2 N) = 1.08 \pm 0.01$		χ_1: −	0.1	Crystal contact
I31§	χ_1: −	χ_1: −	$^3J(C\gamma_1 C') = 3.72 \pm 0.03$;	$C(\delta_1) = 12.4$ ppm	χ_1: −	0.13	
	χ_2: m	χ_2: +	$^3J(C\gamma_1 N) = 0.36 \pm 0.20$		χ_2: −		
L34	χ_2: +	χ_2: +		$C(\delta_1) = 25.5$ ppm $C(\delta_2) = 25.4$ ppm; $\Delta CS = 0.1$ ppm	χ_2: +	0.63	
V40	χ_1: −	χ_1: −	$^3J(C\gamma_1 C') = 1.05 \pm 0.01$; $^3J(C\gamma_1 N) = 0.72 \pm 0.01$; $^3J(C\gamma_2 C') = 3.65 \pm 0.01$; $^3J(C\gamma_2 N) = 0.45 \pm 0.02$		χ_1: −	0.10	
I44	χ_1: −	χ_1: −	$^3J(C\gamma_1 C) = 3.60 \pm 0.02$;	$C(\delta_1) = 13.0$ ppm	χ_1: −	0.22	
	χ_2: a	χ_2: +	$^3J(C\gamma_1 N) = 0.52 \pm 0.15$; $^3J(C\delta_1 C\alpha) = 2.77 \pm 0.01$		χ_2: +		
L45	χ_1: −	χ_1: ?		$C(\delta_1) = 23.8$ ppm	χ_1: −	0.26	From NMR chemical shifts:
	χ_2: −	χ_2: −		$C(\delta_2) = 24.5$ ppm; $\Delta CS = -0.7$ ppm	χ_2: −		Population trans 0.43 Population gauche+ 0.57
K49	χ_1: −	χ_1: −	$^3J(C\gamma C') = 3.57 \pm 0.03$		χ_1: −	0.29	
	χ_2: +	χ_2: N			χ_2: +		
L51	χ_1: +	χ_1: ?		$C(\delta_1)$,$C(\delta_2) = 24.1$ ppm; $\Delta CS = 0$ ppm	χ_1: −	0.44	
	χ_2: +	χ_2: +			χ_2: +		

*+, conformational heterogeneity observed in crystal structure; −, no conformational heterogeneity observed; a, anisotropic electron density observed; m, multiple rotamers observed among different apo SpA-domain structures.

†Dynamic side chains as detected by ^{3}J averaging and/or chemical shift deviations from static limits; +, dynamic χ_1 or χ_2; −, static χ_1 or χ_2; N, no observation; ?, ambiguous assignment.

‡Values close to 0 indicate low conformational heterogeneity; values close to 1 indicate high conformational heterogeneity (see text).

§Residues that make contact with F_c.

Table S4. Data collection and refinement statistics for SpA^C Q9W

	SpA^C Q9W
Data collection	
Space group	$P6_5$
Cell dimensions	
a, *b*, *c*, Å	44.5, 44.5, 116.1
α, β, γ, °	90.0, 91.0, 120.0
Wavelength	1.0
Resolution, Å	50.00–1.87 (1.90–1.87)*
R_{sym}	0.091 (0.72)
$I/\sigma I$	123.5 (2.6)
Completeness, %	99.9 (100.0)
Redundancy	17.0 (10.5)
Refinement	
Resolution, Å	36.6–1.87
No. of reflections	9,603
R_{work}/R_{free}	19.9/25.6
No. of atoms	
Protein	921
Water	22
B factors	
Protein	59.3
Water	69.5
Rms deviations	
Bond lengths, Å	0.013
Bond angles, °	1.189

*Data were collected from a single crystal. Values in parentheses are for the highest-resolution shell.

Table S5. Data collection and refinement statistics for SpA^C Q9W in complex with F_c

	SpA^C Q9W–F_c complex
Data collection	
Space group	*C*2
Cell dimensions	
a, *b*, *c*, Å	138.0, 87.2, 103.2
α, β, γ, °	90.0, 91.1, 90.0
Wavelength	1.0
Resolution, Å	50.00–2.28 (2.32–2.28)*
R_{sym}	0.137 (1.0)
$I/\sigma I$	19.9 (1.6)
Completeness, %	100.0 (99.8)
Redundancy	7.9 (6.2)
Refinement	
Resolution, Å	34.8–2.28
No. of reflections	52,860
R_{work}/R_{free}	19.6/24.2
No. of atoms	
Protein	6,197
Water	126
B factors	
Protein	61.91
Water	53.28
Rms deviations	
Bond lengths, Å	0.016
Bond angles, °	1.506

*Data were collected from a single crystal. Values in parentheses are for the highest-resolution shell.