Supporting Information

Gruszka et al. 10.1073/pnas.1119456109

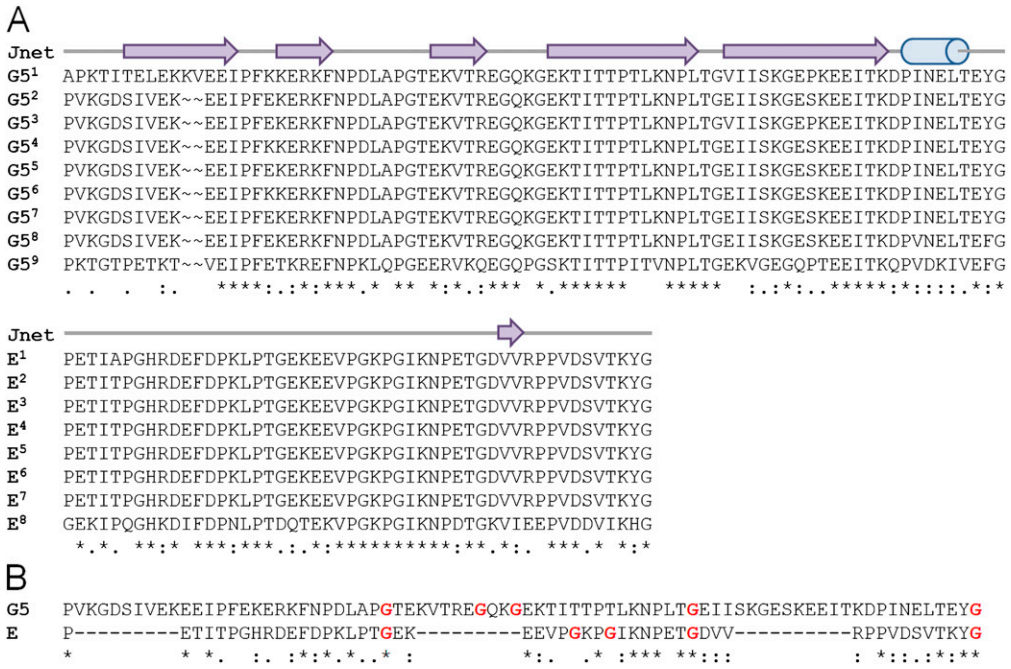

Fig. S1. Sequence alignment of *Staphylococcus aureus* surface protein G (SasG) domains. Conserved residues are indicated with an asterisk. (*A*) Alignment of G5 domains and E segments. The Jnet prediction indicates several regions of β-strand secondary structure for the G5 domain (1), whereas the E segment is predicted to be predominantly disordered (2, 3). (*B*) Alignment of the consensus sequences of G5 and E. The five conserved glycine residues associated with the β–triple helix–β fold are shown in red.

1. Cole C, Barber JD, Barton GJ (2008) The Jpred 3 secondary structure prediction server. *Nucleic Acids Res* 36(Web Server issue):W197-201.
2. Romero P, et al. (2001) Sequence complexity of disordered protein. *Proteins* 42:38–48.
3. Dosztányi Z, Csizmok V, Tompa P, Simon I (2005) IUPred: Web server for the prediction of intrinsically unstructured regions of proteins based on estimated energy content. *Bioinformatics* 21:3433–3434.

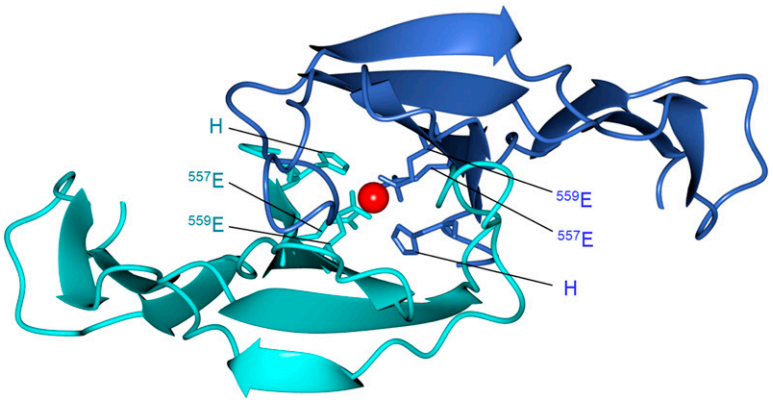

Fig. S2. Crystal structure of a nonnative Zn^{2+}-mediated $G5^2$ dimer. Zn^{2+} (red) is coordinated by a nonnative histidine residue and two native glutamate residues (^{557}E and ^{559}E) from each of the two (cyan and blue) G5 molecules in the dimer.

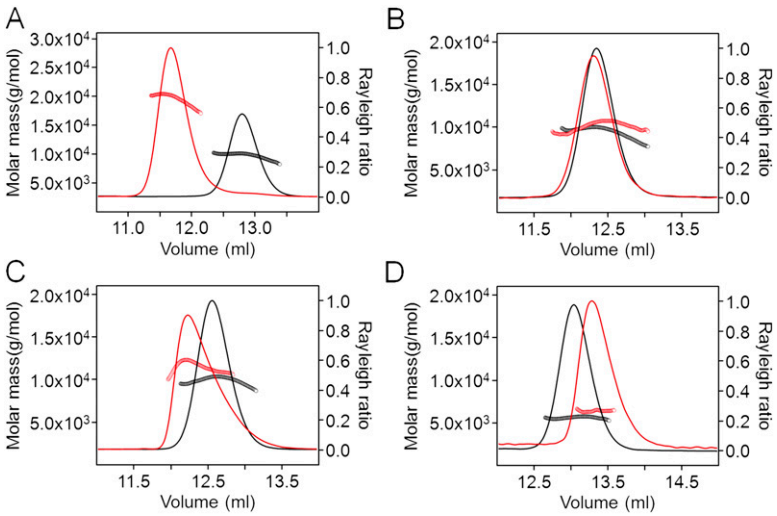

Fig. S3. Size-exclusion chromatography with multiangle laser light scattering analysis of oligomeric state of SasG modules in the presence (red) and absence (black) of Zn^{2+}. (*A*) GPHM-G5^2: native sequence with four additional N-terminal amino acids (i.e., GPHM). (*B*) GPTK-G5^2: native sequence with four additional N-terminal amino acids (i.e., GPTK). (*C*) G5^1: native sequence with four additional N-terminal amino acids (i.e., GPHM). (*D*) E: native sequence with four additional N-terminal amino acids (i.e., GPHM). The exact molar masses and sequences are listed in Table S5.

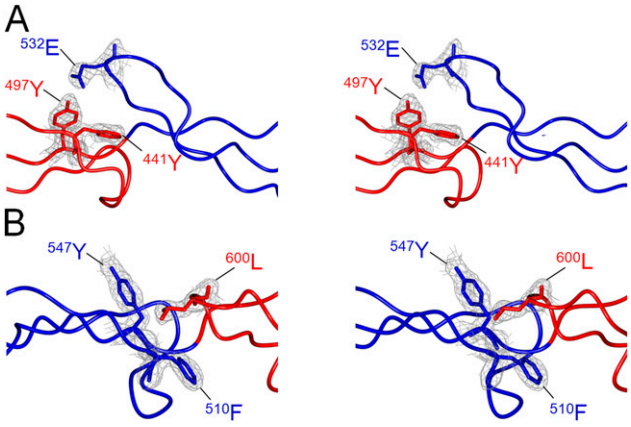

Fig. S4. Stereo images of interdomain interfaces in SasG highlighting the major differences between G5–E (*A*) and E–G5 (*B*). G5 and E domains are shown in red and blue, respectively. Electron density at 1.0 σ is shown for the labeled residues.

Table S1. Thermodynamic parameters of unfolding process calculated for two-state and non–two-state models

	Two-state model			Non–two-state model			
Module	T_m, °C	ΔH_{cal}, kcal/mol	ΔC_p, kcal/mol/°C	T_m, °C	ΔH_{cal}, kcal/mol	ΔH_{VH}, kcal/mol	$\Delta H_{cal}/\Delta H_{VH}$
G5^1	52.80 ± 0.02	59.2 ± 0.1	0.623 ± 0.026	53.31 ± 0.01	58.2 ± 0.1	60.1 ± 0.1	0.97
G5^2	46.94 ± 0.05	58.2 ± 0.1	0.543 ± 0.032	47.61 ± 0.01	58.5 ± 0.1	57.1 ± 0.3	1.02
E-G5^2	53.57 ± 0.04	86.8 ± 0.2	0.598 ± 0.084	53.85 ± 0.02	88.1 ± 0.3	85.5 ± 0.4	1.03
G5^1-E	52.75 ± 0.04	58.0 ± 0.2	—	52.76 ± 0.04	59.6 ± 0.3	56.1 ± 0.4	1.06
G5^2-E	47.13 ± 0.04	57.8 ± 0.2	—	47.13 ± 0.04	57.6 ± 0.4	58.0 ± 0.5	0.99
G5^1-E-G5^2	59.10 ± 0.01	147.0 ± 0.3	1.080 ± 0.085	59.12 ± 0.01	155.0 ± 0.6	140.0 ± 0.7	1.11
E-G5^2-E-G5^3	57.63 ± 0.02	163.0 ± 0.5	1.240 ± 0.159	57.62 ± 0.02	183.0 ± 1.2	146.0 ± 1.2	1.25

All errors quoted are for the fit to the data. Thermograms of folded SasG modules were fitted with the two-state model with ΔC_p. ΔC_p, change in heat capacity upon unfolding; ΔH_{cal}; calorimetric enthalpy; ΔH_{VH}, Van't Hoff enthalpy; T_m, melting temperature.

Table S2. Crystallographic data and refinement statistics for E-G5^2

Statistic	Native E-G5^2	SeMet E-G5^2-L17M-L103M		
Data collection				
Space group	$P2_1$		$P2_1$	
Cell dimensions				
a, *b*, *c*, Å	36.8, 34.7, 55.8	—	54.6, 37.4, 71.8	—
α, β, γ, °	90.0, 99.5, 90.0	—	90.0, 104.6, 90.0	—
		Peak	Inflection	Remote
Wavelength	0.9763	0.9793	0.9797	0.9763
Resolution, Å*	50.00–1.75 (1.86–1.75)	50.00–2.20 (2.28–2.20)	50.00–2.20 (2.28–2.20)	50.00–2.20 (2.28–2.20)
R_{sym}, %*	5.4 (45.2)	9.0 (18.9)	7.5 (18.4)	7.4 (17.9)
$I/\sigma I$*	17.4 (3.0)	12.7 (2.8)	12.2 (2.7)	12.1 (2.9)
Completeness, %*	99.5 (98.7)	85.5 (39.3)	82.4 (32.5)	83.0 (35.7)
Redundancy*	4.0 (4.0)	6.4 (3.9)	3.2 (2.0)	3.2 (2.0)
Refinement				
Resolution, Å	29.37–1.75	—	—	—
No. reflections	15,431	—	—	—
R_{work}/R_{free}	19.7/23.9	—	—	—
No. atoms				
Protein	1,028	—	—	—
Water	216	—	—	—
B-factors				
Protein	29.6	—	—	—
Water	35.5	—	—	—
rmsd				
Bond length, Å	0.011	—	—	—
Bond angle, °	1.6	—	—	—

*Highest resolution shell is shown in parentheses.

Table S3. Crystallographic data and refinement statistics for G5^1-E-G5^2

Statistic	G5^1-E-G5^2
Data collection	
Space group	$P2_1$
Cell dimensions	
a, *b*, *c*, Å	45.5, 84.8, 71.7
α, β, γ, °	90.0, 100.4, 90.0
Resolution, Å*	50.00–1.87 (1.99–1.87)
R_{sym}, %*	5.3 (36.7)
$I/\sigma I$*	14.9 (3.0)
Completeness, %*	98.1 (90.7)
Redundancy*	3.7 (3.7)
Refinement	
Resolution, Å	41.30–1.87
No. reflections	43,210
R_{work}/R_{free}	20.5/26.6
No. atoms	
Protein	3,324
Mg	5
Tris	8
Water	637
B-factors	
Protein	42.6
Mg	34.0
Tris	42.3
Water	43.3
rmsd	
Bond length, Å	0.014
Bond angle, °	1.4

*Highest resolution shell is shown in parentheses.

Table S4. Surface area values for SasG domains and other proteins

Protein	No. of residues	ASA, Å		RSA, %		RSA ratio (nonpolar/all)
		All	Nonpolar	All	Nonpolar	
$G5^1$-E-$G5^2$	214	15,826.1	9,408.9	46.88	48.65	1.04
E-$G5^2$	132	9,470.5	5,537.5	46.29	47.20	1.02
$G5^1$	80	6,222.6	2,428.0	48.47	47.20	0.97
$G5^2$	78	6,122.4	3,542.0	49.53	51.26	1.03
G5 from RpfB	80	6,317.8	3,688.9	50.62	51.79	1.02
E	50	3,774.2	2,352.6	49.66	52.96	1.07
1WJ2*	71	6,255.4	3,865.5	55.76	61.13	1.10
1HMS†	132	7,318.2	4,303.2	35.21	35.86	1.02
1MDC†	132	6,977.5	3,877.2	35.82	35.76	1.00
3CO1†	130	7,779.9	4,505.9	38.90	40.21	1.03
1QX6†	213	10,809.9	5,792.9	32.35	32.59	1.01
1T2U†	210	10,128.7	6,413.2	33.32	36.61	1.10
2IWS†	214	10,644.8	5,900.7	34.39	34.41	1.00

ASA, accessible surface area; RSA, relative accessible surface area. ASA and RSA values, for all atoms and nonpolar side-chains, were calculated using NACCESS (1). RSA for each amino acid was calculated with respect to their ASA in an extended Ala-X-Ala tripeptide, imitating a random coil conformation.

*Other protein with single-layer β-sheets.

†Globular proteins.

1. Hubbard SJ, Thornton J (1993) *NACCESS, Computer Program; Department of Biochemistry and Molecular Biology* (University College, London).

Table S5. Molar masses of SasG constructs in the presence and absence of zinc, determined by SEC-MALLS

Module	Sequence	MW (kg/mol)	SEC-MALLS molar mass (kg/mol)	
			−Zn	+Zn
E	GPHMITPGHRDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRP PVDSVTK	5.29	5.6 ± 0.3	6.3 ± 0.2
$G5^1$	GPHMAPKTITELEKKVEEIPFKKERKFNPDLAPGTEKVTREGQ KGEKTITTPTLKNPLTGVIISKGEPKEEITKDPINELTEYGPET	9.70	10.0 ± 0.7	11.6 ± 1.2
$G5^2$	MYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREGQKG EKTITTPTLKNPLTGEIISKGESKEEITKDPINELTEYGPET	9.36	9.3 ± 0.3	9.2 ± 0.3
$G5^2$-GPHM	GPHMYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREG QKGEKTITTPTLKNPLTGEIISKGESKEEITKDPINELTEYGPET	9.65	9.8 ± 0.6	19.6 ± 1.6
$G5^2$-GPTK	GPTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREG QKGEKTITTPTLKNPLTGEIISKGESKEEITKDPINELTEYGPET	9.62	10.3 ± 1.1	9.7 ± 0.7
B^1	GPHMAPKTITELEKKVEEIPFKKERKFNPDLAPGTEKVTREGQ KGEKTITTPTLKNPLTGVIISKGEPKEEITKDPINELTEYGPETIA PGHRDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRPPVDSVTK	14.5	15.1 ± 0.9	16.2 ± 1.0
B^2	GPHMYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREG QKGEKTITTPTLKNPLTGEIISKGESKEEITKDPINELTEYGPETI APGHRDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRPP VDSVTKYGP	14.8	14.7 ± 0.3	28.4 ± 0.3
E-$G5^2$	GPETIAPGHRDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRP PVDSVTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTR EGQKGEKTITTPTLKNPLTGEIISKGESKEEITKDPINE LTEYGPET	14.4	14.2 ± 0.3	15.3 ± 0.3
E-$G5^2$-HM	GPHMIAPGHRDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRP PVDSVTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTR EGQKGEKTITTPTLKNPLTGEIISKGESKEEITKDPIN ELTEYGPET	14.5	14.5 ± 0.1	17.9 ± 1.4
$G5^1$-E-$G5^2$	GPHMAPKTITELEKKVEEIPFKKERKFNPDLAPGTEKVTREGQ KGEKTITTPTLKNPLTGVIISKGEPKEEITKDPINELTEYGPETIA PGHRDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRPPVDSVT KYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREGQKG EKTITTPTLKNPLTGEIISKGESKEEITKDPINELTEYGPET	23.7	24.3 ± 1.2	26.7 ± 2.9
B^1B^2	GPHMAPKTITELEKKVEEIPFKKERKFNPDLAPGTEKVTREGQ KGEKTITTPTLKNPLTGVIISKGEPKEEITKDPINELTEYGPETIA PGHRDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRPPVDSVT KYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREGQKG EKTITTPTLKNPLTGEIISKGESKEEITKDPINELTEYGPETIAPG HRDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRPPVDSVTK	28.6	29.9 ± 2.7	29.8 ± 1.5
E-$G5^2$-E-$G5^3$	GPHMIAPGHRDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRP PVDSVTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTR EGQKGEKTITTPTLKNPLTGEIISKGESKEEITKDPINELTEYGP ETITPGHRDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRPPV DSVTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREG QKGEKTITTPTLKNPLTGVIISKGEPKEEITKDPINELTEYGPET	28.5	28.5 ± 1.1	32.3 ± 2.3

Nonnative amino acids (from the expression vector after His-tag cleavage) are marked in red. MW, molecular weight; SEC-MALLS, size-exclusion chromatography with multiangle laser light scattering.