Supporting Information

Conrady et al. 10.1073/pnas.1208134110

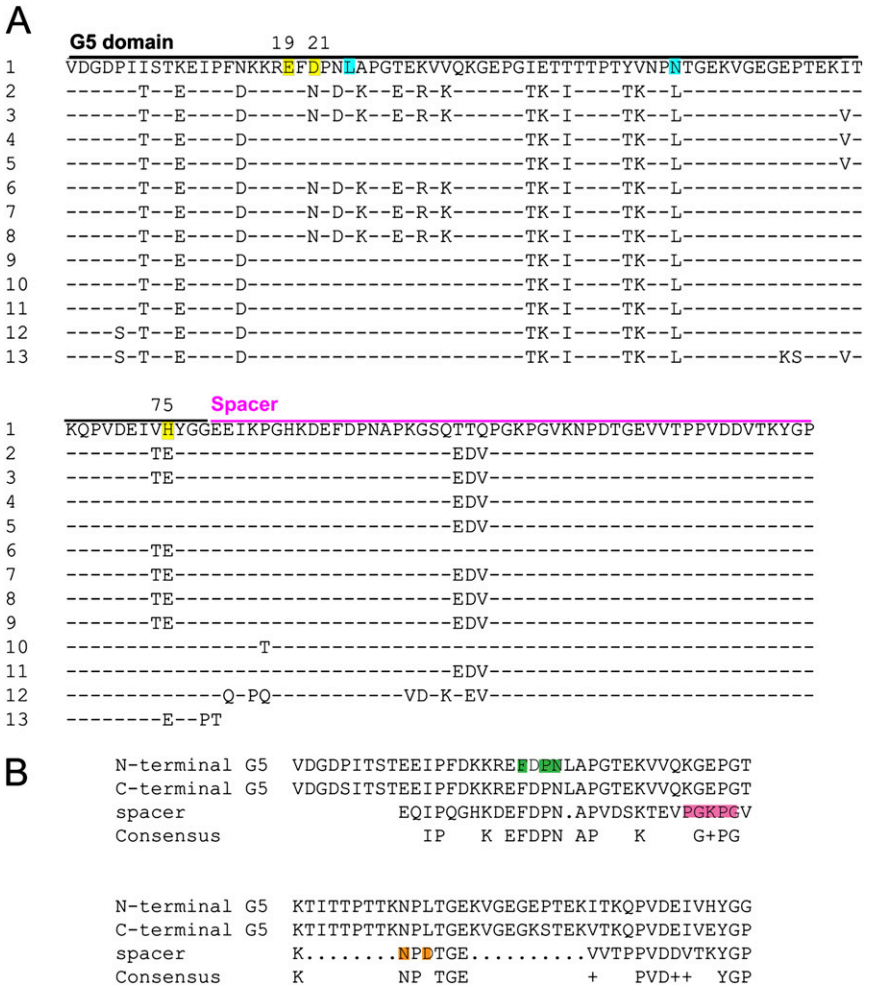

Fig. S1. Alignments of Aap Brpt. (*A*) A sequence alignment of all 13 copies of the B-repeat found in Aap from *Staphylococcus epidermidis* RP62A (GenBank accession no. SERP2398), with the G5 domain indicated by the black line and the spacer motif by the purple line. Residues that are identical to repeat 1 are indicated by a hyphen (-). Zn^{2+}-coordinating residues are highlighted in yellow. Met mutagenesis sites are highlighted in cyan. (*B*) Alignment of the spacer and G5 domains. The alignment of the N- and C- terminal G5 domains with the spacer motif shows regions of high identity separated by sequence gaps indicated by a dot. The PGXPG motif is highlighted in magenta. Note the conservation of residues that contribute to the polar-hydrophobic stack (highlighted in green and orange).

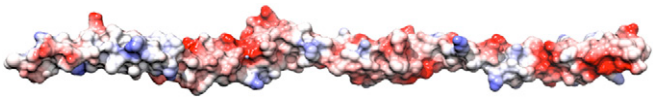

Fig. S2. Hydrophilicity of Brpt1.5. Brpt1.5 is shown colored by charge in surface representation. Electropositive patches are in blue, and electronegative patches are shown in red. To generate this figure the missing side chains were modeled in Coot using the most common rotamer. The orientation of the molecule is identical to that of the upper panel in Fig. 1*C*. The surface is colored according to the Coulombic surface-charge distribution calculated in Chimera (1) [color range of ± 10 kcal/(mol·e)].

1. Pettersen EF, et al. (2004) UCSF Chimera—a visualization system for exploratory research and analysis. *J Comput Chem* 25(13):1605–1612.

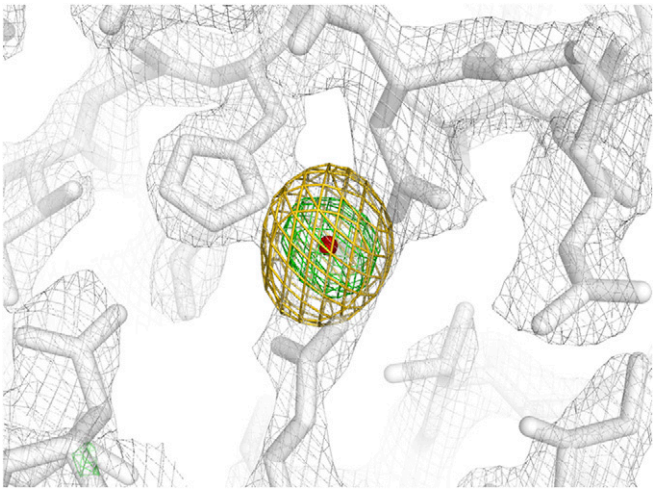

Fig. S3. Zn^{2+} placement in molecular replacement (MR)-phased maps. The MR-phased electron density for the Native1 data set after a single round of rigid-body refinement ($2F_o$-F_c, light gray mesh, 1.5σ), is overlaid with the final refined protein model (white sticks). A peak in the difference map (F_o-F_c, green mesh, 5σ) clearly indicates the Zn^{2+} position (Zn^{2+} was omitted from all MR search models). The position of the anomalous Zn^{2+} peak [F(+)-F(-), gold mesh, 5σ] unambiguously confirms it as the Zn^{2+} site. The Zn^{2+} position in the final refined model is indicated by a red sphere.

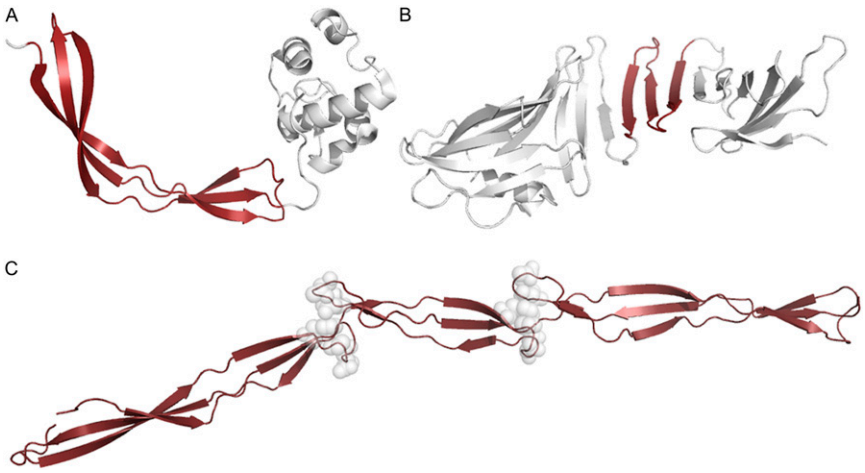

Fig. S4. Structures containing freestanding β-sheets. Globular portions of the structures are colored gray; portions containing freestanding β-sheets (solvent-exposed on both faces) are in red. (*A*) Structure of the G5 domain-containing protein RpfB from *Mycobacterium tuberculosis* [Protein Data Bank (PDB) code 3EO5] (1). RpfB is one of a group of proteins that contain single copies of the G5 domain in tandem with a globular enzymatic domain or domains of unknown function. The functionality of the G5 domain in these proteins is uncharacterized. (*B*) The protein OspA from *Borrelia burgdorferi* (PDB code 1OSP) (2) contains a region of freestanding β-sheet that is continuous with β-sheets in the N- and C-terminal globular domains. (*C*) The 1.5-repeat construct from Aap contains longer stretches of freestanding β-sheet without a canonical globular fold to stabilize either terminus. In the context of proteolytically processed adhesion-mediating Aap, no globular fold is expected to occur; the B-repeat region is found at the N terminus of the protein, with a C-terminal Pro/Gly-rich collagen-like region that is anchored to the cell wall.

1. Ruggiero A, et al. (2009) Crystal structure of the resuscitation-promoting factor (DeltaDUF)RpfB from M. tuberculosis. *J Mol Biol* 385(1):153–162.
2. Li H, Dunn JJ, Luft BJ, Lawson CL (1997) Crystal structure of Lyme disease antigen outer surface protein A complexed with an Fab. *Proc Natl Acad Sci USA* 94(8):3584–3589.

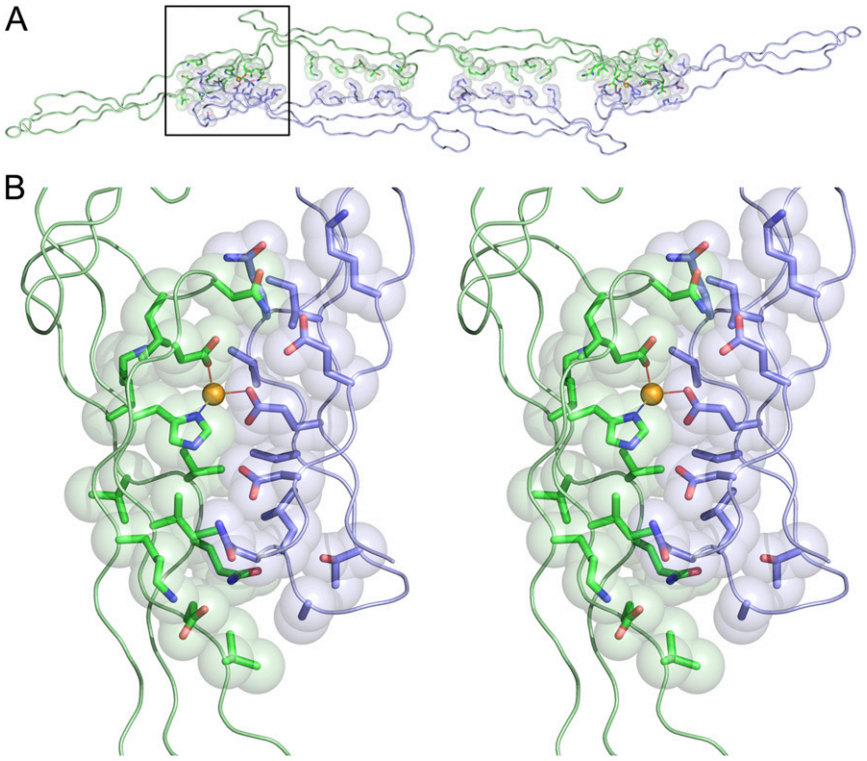

Fig. S5. Dimer interface in the vicinity of the Zn^{2+}-binding site. (*A*) Overview of the Native 1 dimer, in which residues contributing to the dimer interface as defined by the PISA server (www.ebi.ac.uk/msd-srv/prot_int/pistart.html) are shown as sticks with transparent spheres. (*B*) Stereo image of the dimer interface in the vicinity of the Zn^{2+}-binding site (a rotated view of the boxed region in *A*). Zn^{2+} is shown as a gold sphere with lines to illustrate the ligating residues. For clarity, the Zn^{2+} ion is shown with a half-scale radius. The thiocyanate molecule is not visible in this orientation.

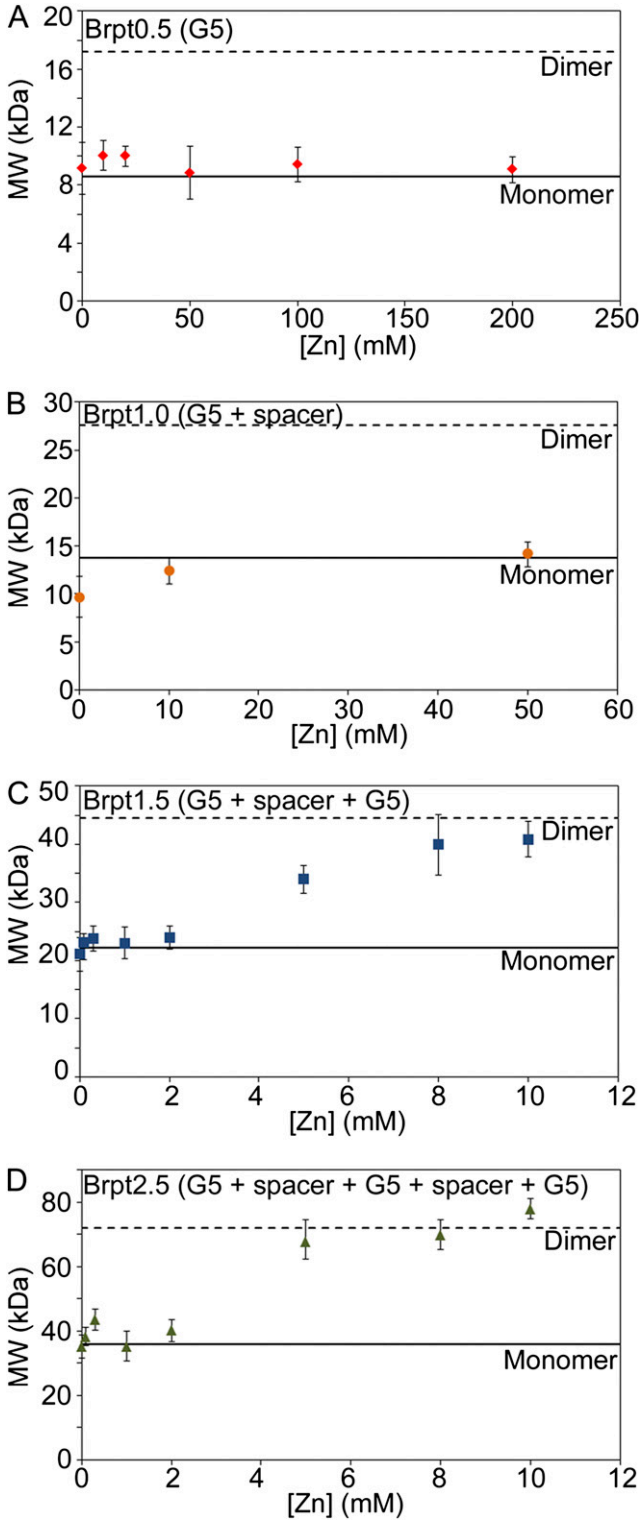

Fig. S6. Zn^{2+}-dependent dimerization of Brpt1.5 constructs. Constructs were investigated using single-speed sedimentation equilibrium experiments in the presence of varying concentrations of Zn^{2+}. Horizontal lines are used to indicate the monomer (solid line) and dimer (dashed line) molecular weights predicted based on the sequences. (*A*) Brpt0.5 (red diamonds) sediments as a monomer in the presence of up to 200 mM Zn^{2+}. (*B*) Brpt1.0 (orange circles) sediments as a monomer in the presence of up to 50 mM Zn^{2+}. (*C*) Brpt1.5 (blue squares) sediments as dimer in the presence of 8–10 mM Zn^{2+}. (*D*) Brpt2.5 (green triangles) sediments as dimer in the presence of 5–10 mM Zn^{2+}.

1. Corrigan RM, Rigby D, Handley P, Foster TJ (2007) The role of Staphylococcus aureus surface protein SasG in adherence and biofilm formation. *Microbiology* 153(Pt 8):2435–2446.

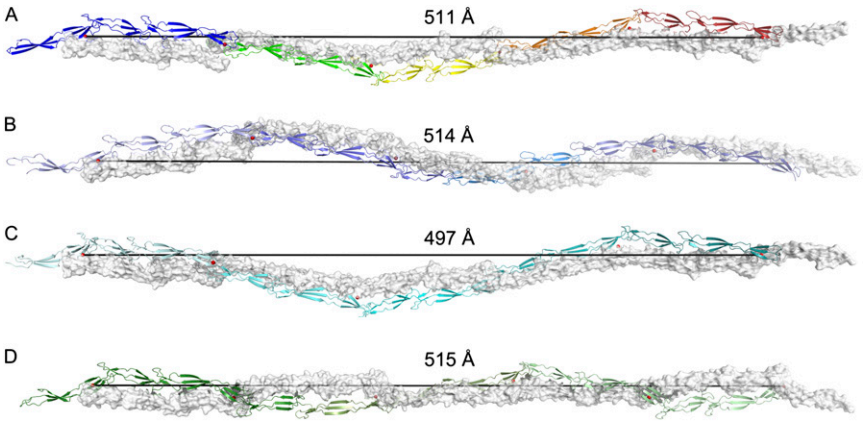

Fig. S7. Models of B-repeat dimers that are 5.5 repeats in length. The appearance of a dimer of minimal biologically active repeat length of five repeats (1) is extrapolated based on sequential structural alignments of N- and C-terminal G5 domains. We observe a twisting cable-type structure in which the subunits donated by one monomer wrap around the antiparallel binding partner. These models are based on the distinct dimers observed in the individual data sets. One putative monomer is shown in white surface representation for all data; the other is shown in cartoon representation. (*A*) The SeMet mutant is shown as a rainbow from blue at the N terminus to red at the C terminus. (*B*) Chain A from the non–Se-labeled mutant structure is shown as a gradient from light blue at the N terminus to dark blue at the C terminus. (*C*) Chain B from the non–Se-labeled mutant is shown colored from light to dark cyan. (*D*) The Native2 protein is shown as a gradient from dark green at the N terminus to light green at the C terminus. The lines shown are measured from the first Zn^{2+}-binding site to the last. Note that the SeMet mutant (in *A*) and chain B from the non-Se mutant (in *C*) share the same Zn^{2+} coordinating residues in the respective crystal structures but differ in predicted 5.5-repeat fibril length.

Table S1. Data collection and refinement statistics

Brpt1.5 construct	SeMet mutant*	Mutant†	Native 1	Native2
Data collection				
APS Beamline	24-IDE	24-IDE	24-IDC	24-IDE
Wavelength (Å)	0.9792	0.9792	1.2822	0.9792
Space group	C2	C2	C2	C2
Unit cell dimensions (Å, °)	a, b, c, β = 89.72, 33.64, 81.33, 121.06	a, b, c, β = 83.10, 34.24, 138.78, 101.31	a, b, c, β = 92.03, 33.56, 83.15, 122.47	a b, c, β = 121.11, 34.62, 49.37, 103.11
Resolution (Å)‡	44.8–2.4 (2.49–2.4)	50–2.5 (2.59–2.5)	46–2.31 (2.58–2.31)	26–1.97 (2.03–1.97)
Observations	212,473	165,754	159,727	172,550
Unique reflections	8,282	13,488	9,395	13,864
Completeness (%)	99.3 (98.9)	99.4 (99.7)	97.6 (96.0)	95.6 (75.9)
Redundancy	5.7 (5.0)	3.6 (3.7)	7.3 (6.9)	5.2 (4.1)
R_{sym} (%)	11.1 (38.7)	7.8 (20.0)	10.2 (27.5)	8.3 (53.7)
I/σI	17.3 (3.7)	37.7 (13.8)	26.3 (7.4)	18.5 (3.0)
SAD phasing				
No. of heavy atom sites	4	N/A	N/A	N/A
Mean figure of merit§	0.36/0.75	N/A	N/A	N/A
Refinement				
R_{work}/R_{free} (%)	19.8/25.5	21.9/27.6	20.3/25.8	20.7/25.7
Atoms (protein/water/ Thiocyanate/Zn^{2+})	1480/63/3/1	2823/108/0/2	1484/78/3/1	1437/132/3/1
rmsd bonds (Å)	0.009	0.01	0.01	0.01
rmsd angles (°)	1.20	1.33	1.24	1.20
Mean B factors (Å^2)				
Protein	64.73	31.25 (A)/34.09 (B)	48.24	46.51
Water	55.66	29.86	47.51	41.01
Thiocyanate	74.9	N/A	46.5	31.82
Zn^{2+}	45.47	39.08	49.2	23.09
Ramachandran distribution¶				
Favored (%)	99.5	99.5	99.5	99.5
Outliers (%)	0	0	0	0
Molprobity score/percentile‖	1.44/99th	1.58/99th	1.14/100th	1.27/99th
Molprobity residues withbad bonds/angles (%)	0/0	0/0	0/0	0/0

SAD, single-wavelength anomalous dispersion.
*SeMet-labeled Brpt1.5-L24M/L51M/L152M/L179M "quadruple mutant."
†Non-Se–labeled Brpt1.5-L24M/L51M/L152M/L179M "quadruple mutant."
‡Numbers in parentheses are for highest resolution shell.
§Figure of merit from phenix AutoSol before/after density modification.
¶As calculated by Molprobity.
‖The 100th percentile is the best among structures solved at comparable resolution; the 0 percentile is the worst.

Table S2. Buried side chains in β-rich proteins

PDB ID code	Description	Length*	β-sheet content† (%)	Residues buried‡ (%)
Eukaryotic β-rich proteins				
1GFL	GFP, *Aequorea victoria*	230	55	33.5
3AYA	Human Galectin-3 CRD	135	66	27.4
3TWY	Rat PKC C2 domain	137	57	24.8
3TZS	Human lipocalin NGAL	174	51	23.0
1BAS	Human basic FGF	125	52	21.6
3L5I	Human GP130, FnIII domains	290	65	20.7
2XYS	Acetylcholine-binding protein, *Apysia californica*	205	59	20.5
3K5R	Mouse T-cadherin EC1-2 domains	218	55	20.2
1HZH-L	Human b12 IgG1, kappa light chain	214	64	19.6
3RB5	*Drosophila* CALX Na/Ca exchanger, CBD1-2 domains	238	64	19.3
1FNF	Human Fibronectin, repeats 7–10	368	69	17.4
2GI7	Human GPVI, Ig-like domains	182	69	17.0
1HZH-H	Human b12 IgG1, heavy chain	443	58	15.8
Average		*228*	*60*	*22*
Bacterial cell-surface proteins				
2ODL	HMW1 secretion domain, *Haemophilus influenzae*	372	74	37.1
1OIO	GAFD (F17C-type) fimbrial adhesin, *Escherichia coli*	178	71	26.4
1R17	SdrG adhesin, *S. epidermidis*	321	58	24.3
1PDK	PapD pilus chaperone, *E. coli*	215	60	20.9
3B2M	Major pilin, *Streptococcus pyogenes*	294	63	20.1
1D2O	CNA collagen-binding domain, *S. aureus*	187	60	18.2
2AXW	DRAD invasin (Ig-like domains), *E. coli*	134	59	14.2
2VYU	Choline-binding protein, *Streptococcus pneumoniae*	300	50	14.0
Average		*250*	*62*	*22*
	Aap Brpt1.5, *S. epidermidis*	206	83	3.9

Proteins at least 125 residues in length with ≥50% β-sheet content and known structure were analyzed for side-chain exposure. All structures analyzed reported a resolution of 2.5 Å or greater (except for 1HZH at 2.7 Å, which is the highest-resolution intact antibody structure available). When multiple chains are found in the PDB model, chain A was used except where noted (i.e., 1HZH).
*Number of amino acids in the PDB file.
†Percent of residues in β-sheets.
‡Percent of residues at least 95% buried, as calculated by the VADAR server (1) (http://vadar.wishartlab.com/).

Table S3. Primers used for site-directed mutagenesis

Mutation	5′ Primer (first listed sequence per mutation) 3′ Primer (second listed sequence per mutation)
Methionine	
L24M	5′-CGAATTTGATCCAAACATGGCGCCAGGTACAGAGAAAGTCG-3′
	5′-CGACTTTCTCTGTACCTGGCGCCATGTTTGGATCAAATTCG-3
L51M	5′-CAACGCCAACAACTAAGAACCCAATGACAGGAGAAAAAGTTGGC-3′
	5′-GCCAACTTTTTCTCCTGTCATTGGGTTCTTAGTTGTTGGCGTTG-3′
Zn^{2+}-binding pocket	
E19A	5′-GATAAGAAACGTGCATTTGATCCAAACTTAGCCCC-3′
	5′-GGGGCTAAGTTTGGATCAAATGCACGTTTCTTATC-3′
D21A	5′-GAAACGTGAATTTGCTCCAAACTTAGCCCC-3′
	5′-GGGGCTAAGTTTGGAGCAAATTCACGTTTC-3′
H75E	5′-GTGGATGAGATTGTTGAGTATGGTGGTGAAC-3′
	5′-GTTCACCACCATACTCAACAATCTCATCCAC-3′
E203A	5′-GTTGACGAAATTGTTGCGTATGGTCCAAC-3′
	5′-GTTGGACCATACGCAACAATTTCGTCAAC-3′
Stability	
F89A	5′-CATAAAGATGAAGCTGATCCAAATGC-3′
	5′-GCATTTGGATCAGCTTCATCTTTATG-3′