Supplementary information for:

# The outer-membrane export signal of *Porphyromonas gingivalis* type IX secretion system (T9SS) is a conserved C-terminal β-sandwich domain

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**Table S1. X-ray data collection and refinement statistics**

| **Protein name** | **rCTD** | **r664i6H** | **r665sXa** | **r665i6H** |
|---|---|---|---|---|
| **PDB entry** | 5HFS | 5AG8 | 5AG9 | - |
| | | **X-ray data collection** | | |
| Space group | $P6_5$ | $P2_1$ | $P2_1$ | $P2_1$ |
| Wavelength (Å) | 1.5419 | 0.9724 | 0.8726 | 0.8726 |
| Unit cell constants *a, b, c* (Å), â (°) | 37.69, 37.69, 184.15, 90.0 | 51.47, 61.74, 55.30, 117.8 | 51.6, 61.8, 54.1, 116.8 | 52.9, 63.2, 56.7, 117.8 |
| Resolution (Å) [a] | 32.7-1.97 (2.17-1.97) | 48.9-1.90(2.0-1.90) | 38.0-2.11(2.22-2.11) | 39.3-2.44(2.57-2.44) |
| Completeness (%) | 100.0 (100.0) | 98.6 (91.9) | 99.9 (100.0) | 95.3 (95.5) |
| No. of measurements / Unique reflections | 58,366 / 10,507 | 86,628 / 23,941 | 65,902 /17,553 | 28,402 / 11,842 |
| $R_{merge}$ | 0.121 (0.411) | 0.049 (0.270) | 0.150 (0.447) | 0.158 (0.447) |
| Average intensity (<I> / s(I)) | 7.0 (2.7) | 20.5 (4.5) | 6.4 (2.9) | 4.9 (2.2) |
| Redundancy | 5.6 (5.3) | 3.6 (3.3) | 3.8 (4.0) | 2.4 (2.4) |
| | | **Refinement** | | |
| Resolution (Å) | ∞-1.97 | ∞-1.90 | ∞-2.10 | |
| No. reflections | 10,402 | 23,171 | 16,650 | |
| $R_{cryst}$ / $R_{free}$ | 0.20/0.24 | 0.16/0.20 | 0.19/0.25 | |

| | | | |
|---|---|---|---|
| No. atoms (protein / ligands / solvent) | 944/6/33 | 2208/37/234 | 2208/25/254 |
| Thermal-displacement parameters (Å$^2$; protein / ligands / solvent) | 39.3/93.2/40.0 | 23.1/49.9/33.1 | 19.3/53.5/28.6 |
| R.m.s.d. bond lengths (Å) | 0.007 | 0.019 | 0.016 |
| R.m.s.d. bond angles (°) | 1.12 | 1.98 | 1.92 |
| Residues in favoured/allowed regions (%) [b] | 95.2/100.0 | 97.2/99.7 | 97.6/99.7 |

[a] Values in parentheses refer to highest resolution shell.

[b] According to MOLPROBITY (Davis *et al.*, 2007, Chen *et al.*, 2010).

$R_{free}$ is the same as $R_{value}$ except that calculated for a set of the data (>500) randomly omitted from refinement.


Chen *et al.* MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr. sect. D* **66,** 12-21 (2010).

Davis *et al.* MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. *Nucl. Acids Res.* **35,** W375-W383 (2007).

Evans. "Scaling and assessment of data quality." *Acta Crystallogr. sect. D* **62,** 72-82 (2006).

García-Castellanos *et al.* Three-dimensional structure of MecI : Molecular basis for transcriptional regulation of staphylococcal methicillin resistance. *J. Biol. Chem.* **278,** 39897-39905 (2003).

Kabsch. XDS. *Acta Crystallogr. sect. D* **66,** 125-132 (2010).

Sztukowska *et al.* The C-terminal domains of the gingipain K polyprotein are necessary for assembly of the active enzyme and expression of associated activities. *Mol. Microbiol.* **54,** 1393-1408 (2004).

Weiss. Global indicators of X-ray quality. *J. Appl. Cryst.* **34,** 130-135 (2001).

**Table S2: SAXS Data Summary**

| | rIgSF-CTD (wild-type) | r664i6H |
|---|---|---|
| Guinier analysis* | | |
| $R_g$ (Å) | 24 ± 1 | 25 ± 1 |
| $qR_g$-range | 0.5 – 1.18 | 0.5 - 1.15 |
| Fidelity | 0.99 | 0.98 |
| *P*(*r*) analysis* | | |
| $R_g$ (Å) | 25.2 ± 0.1 | 26.0 ± 0.2 |
| $d_{max}$ (Å) | 98 | 97 |
| *q*-range ($Å^{-1}$) | 0.02-0.23 | 0.02-0.23 |
| total estimate | 0.68 | 0.67 |
| *MW* from *I*(0) (Da) | 16619 | 15012 |
| Sample details | | |
| Concentration range measured (mg $mL^{-1}$) | 0.2 - 1.7 | 0.14 – 1.4 |
| MW from sequence (Da) | 17030 | 17849 |
| Ratio of *MW* from *I*(0) to *MW* from sequence | 0.98 | 0.84 |
| Ratio of *MW* from DAMMIF to *MW* from sequence | 0.93 | 0.94 |
| Partial specific volume from sequence (*v*, $cm^3\ g^{-1}$) | 0.741 | 0.739 |
| Contrast from sequence and solvent constituents ($\Delta\rho$, $10^{10}\ cm^{-2}$) | 2.790 | 2.814 |
| Model fitting results | | |
| DAMMIF NSD (std dev) | 0.684 (0.031) | 0.654 (0.042) |
| DAMMIF $\chi^2$ range | 1.123 - 1.146 | 1.031 – 1.041 |
| DAMMIF MW (Da) | 15800 | 16700 |
| BUNCH $\chi^2$ | 1.312 | 1.287 |
| EOM $\chi^2$ | 1.04 | 0.97 |

* Parameters reported for averaged *I*(*q*) vs *q* of highest concentration measurement for two repeated dilution series; 10 – 100% dilutions. 100% conc. estimates 1.66 and 1.35 mg/mL for rIgSF-CTD and r664i6H, respectively.

Data collection parameters

| | |
|---|---|
| Instrumentation | Australian Synchrotron SAXS beam-line |
| Beam geometry | 544 μm x 274 μm |

| | |
|---|---|
| *q*-range measured ($Å^{-1}$) | 0.006-0.33 |
| Exposure time (seconds) | 1 s x 24 frames |
| Temperature ($^{o}C$) | 13.5 |

| Software employed for data reduction, analysis and interpretation | |
|---|---|
| SAXS data reduction | ScatterBrain |
| Calculation of expected *MW*, *Δρ* and *v* values | MULCh |
| SAXS data analysis: extrapolation to infinite dilution, Guinier and *P*(*r*) | ATSAS 2.6.0 SAS Data Analysis |
| *ab initio* dummy residue modelling | DAMMIF (via ATSAS on-line) |
| Atomic structure modelling | BUNCH |
| Ensemble modelling | EOM (via ATSAS on-line) |
| 3D graphic model representations | PYMOL |

**Table S3.** Primers used for molecular biology work.

| Primer name | Sequence (5'-3') |
|---|---|
| ***Recombinant protein cloning primers*** | |
| RgpBIgBamHIF | TAGGATCCACACTTGTCCCGACCAAAATGC |
| RgpBCterBamHIF | TAGGATCCACATCTATTGCCGACGTAGCC |
| RgpBCterXhoIR | AACTCGAGAACAGTCTCTTGGCGTAGTGC |
| ***Thrombin cleavage to PreScission cleavage mutagenesis primers*** | |
| RThr2PreFA | CTGGAAGTTCTGTTCCAGGGTCCGACACTTGTCCCGACCAAAATG |
| RThr2PreFB | ACACTTGTCCCGACCAAAATG |
| GThr2PreRA | CGGACCCTGGAACAGAACTTCCAGATCGCTTTTTGGAGGATGGTCGCC |
| GThr2PreRB | ATCGCTTTTTGGAGGATGGTCGCC |
| ***662iXa mutagenesis primers*** | |
| 662iXaFl | ATCGAAGGCCGTGCAGCTACATCTATTGCCGACGTAGCC |
| 662iXaFs | ACATCTATTGCCGACGTAGCC |
| 662iXaRl | AGCTGCACGGCCTTCGATACCTTCCACTTTCACATCCTTTATCAC |
| 662iXaRs | ACCTTCCACTTTCACATCCTTTATCAC |
| ***662i6H mutagenesis primers*** | |
| 662i6HFl | CATCACCATCACCATCACACATCTATTGCCGACGTAGCC |
| 662iXaFs | ACATCTATTGCCGACGTAGCC |
| 662i6HRl | GTGATGGTGATGGTGATGACCTTCCACTTTCACATCCTTTATCAC |
| 662iXaRs | ACCTTCCACTTTCACATCCTTTATCAC |
| ***662sXa mutagenesis primers*** | |
| 662sXaFl | ATCGAAGGCCGTGCAGCTGTAGCCAATGATAAGCCTTAT |
| 662sXaFs | GTAGCCAATGATAAGCCTTAT |
| 662sXaRl | AGCTGCACGGCCTTCGATTTCCACTTTCACATCCTTTATC |
| 662sXaRs | TTCCACTTTCACATCCTTTATC |
| ***664iXa mutagenesis primers*** | |
| 664iXaFl | ATCGAAGGCCGTGCAGCTATTGCCGACGTAGCCAATGATAAGC |
| 664iXaFs | ATTGCCGACGTAGCCAATGATAAGC |
| 664iXaRl | AGCTGCACGGCCTTCGATAGATGTACCTTCCACTTTCACATC |
| 664iXaRs | AGATGTACCTTCCACTTTCACATCCT |
| ***664i6H mutagenesis primers*** | |
| 664i6HFl | CATCACCATCACCATCACATTGCCGACGTAGCCAATGATAAGC |
| 664iXaFs | ATTGCCGACGTAGCCAATGATAAGC |
| 664i6HRl | GTGATGGTGATGGTGATGAGATGTACCTTCCACTTTCACATC |
| 664iXaRs | AGATGTACCTTCCACTTTCACATCCT |
| ***665i6H mutagenesis primers*** | |
| 665i6HFl | CATCACCATCACCATCACGCCGACGTAGCCAATGATA |
| 665i6HFs | GCCGACGTAGCCAATGATA |
| 665i6HRl | GTGATGGTGATGGTGATGAATAGATGTACCTTCCACTTTCAC |
| 665i6HRs | AATAGATGTACCTTCCACTTTCAC |
| ***665sXa mutagenesis primers*** | |
| 665sXaFl | ATCGAAGGCCGTGCAGCTGATAAGCCTTATACTGTAGCTGTATCAGG |
| 665sXaFs | GATAAGCCTTATACTGTAGCTGTATCAGG |
| 665sXaRl | AGCTGCACGGCCTTCGATAGATGTACCTTCCACTTTCACATCC |
| 665sXaRs | AGATGTACCTTCCACTTTCACATCC |
| ***665s6H mutagenesis primers*** | |
| 665s6HFl | CATCACCATCACCATCACGATAAGCCTTATACTGTAGCTGT |
| 665s6HFs | GATAAGCCTTATACTGTAGCTGT |
| 665s6HRl | GTGATGGTGATGGTGATGAGATGTACCTTCCACTTTCAC |
| 665s6HRs | AGATGTACCTTCCACTTTCAC |
| ***RgpB6H mutagenesis primers*** | |

| | |
|---|---|
| 6HRgpBFA | CATCACCATCACCATCACTAATTCACACTGCAATTCTCTAATAAGG |
| DelRgpBFB | TAATTCACACTGCAATTCTCTAATAAGGGC |
| 6HRgpBRA | GTGATGGTGATGGTGATGCTTCACTATAACCTTTTCTGTATACG |
| 6HRgpBRB | CTTCACTATAACCTTTTCTGTATACGTCTTGC |
| ***Cloning primers*** | |
| PG266FrBXbaIF | GCGTCTAGAGTATTCGGACTGCATCT |
| PG266FrBPstIR | ATTACTGCAGCGTCTGAATCGTGCAAA |
| PG266FrANdeIF | TACCACATATGACTGTAGCAGCCATTACAC |
| PG266FrASmaR | ATTAACCCGGGCTCCTATGACTGCTTTGA |
| ermFAMXmaIF | ATTACCCGGGATAGCTTCCGCTATTGC |
| ermFAMXbaIR | GCTCTAGACGAAGCTGTCAGTAGTATACC |
| tetQXmaIF | TATACCCGGGACAACGAATTATCTCCTTAACGT |
| tetQSalIR | ATTTTGTCGACTTTTATTGCCAAGTTCTAATGCT |

**Figure S1.** The structure-based alignment of the CTD of proteins secreted by *P. gingivalis* via T9SS. Conserved hydrophobic residues in β-strands are highlighted in grey. CTDs predicted from MS analysis of culture media are typed in blue with residues after which proteolysis occurred marked in a bold red font (Veith, P. D., *et al.* Protein substrates of a novel secretion system are numerous in the *Bacteroidetes* phylum and have in common a cleavable C-terminal secretion signal, extensive post-translational modification, and cell-surface attachment. *J. Proteome Res*. **12,** 4449-4461 (2013)).

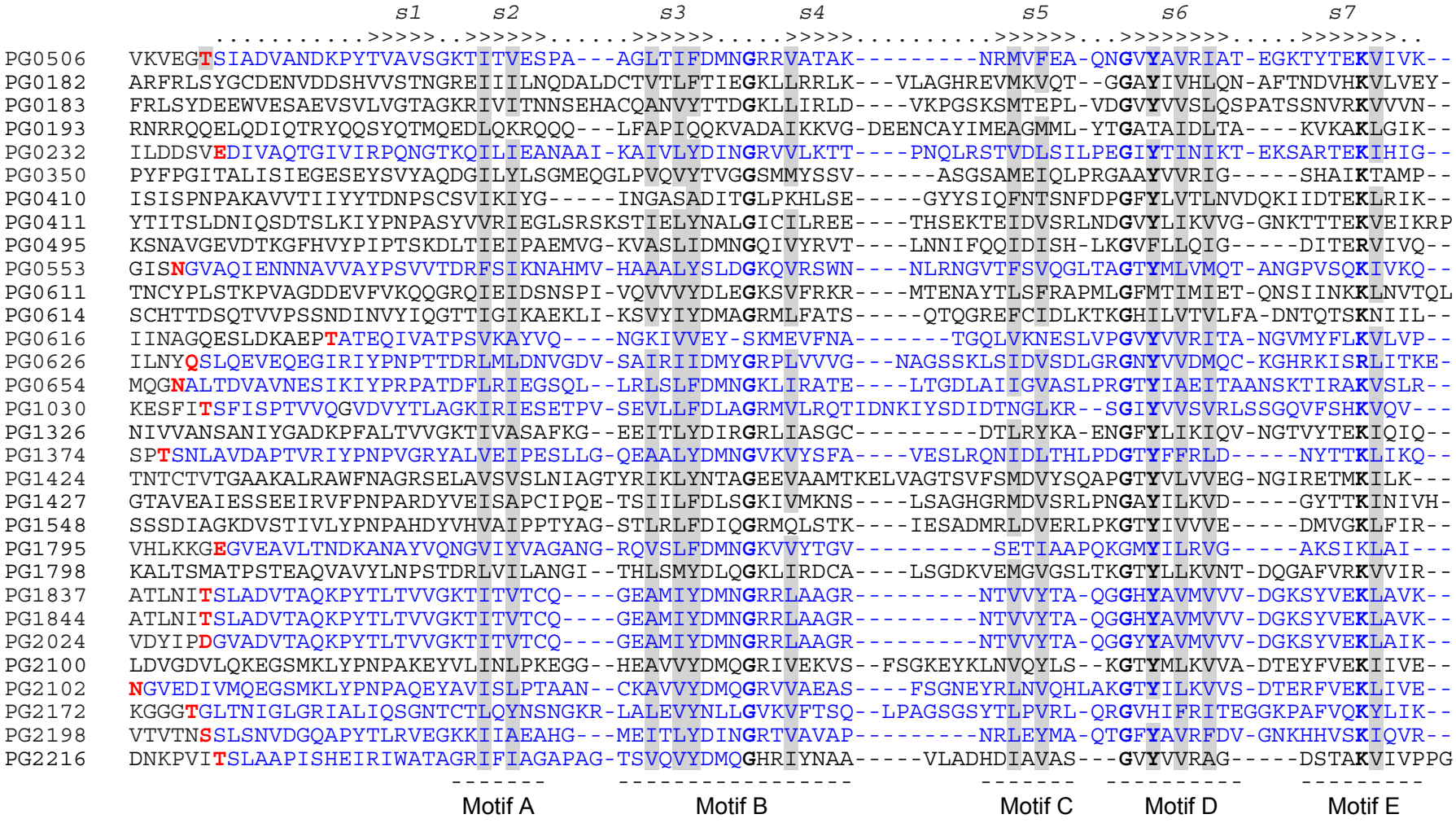
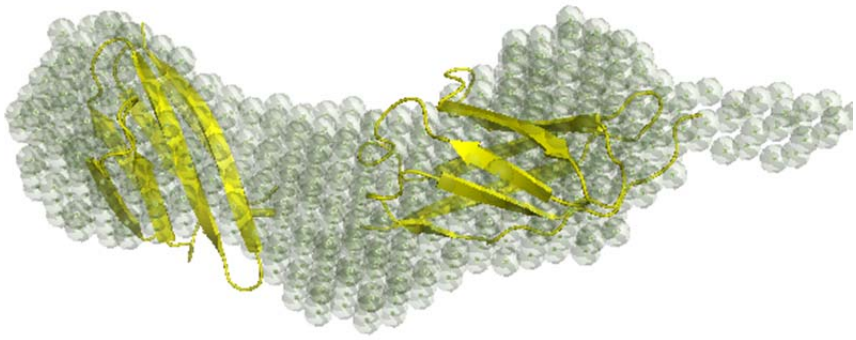


**Figure S2.** Superposition of the *ab initio* bead models and BUNCH atomistic models (cartoon representation of domains only) for the **A.** wild-type rIgSF-CTD and **B.** r664i6H variant**.** These models represent the best-fit average models to the data and show the two domains well separated in space with no significant interaction surface.

**A.**



**B.**

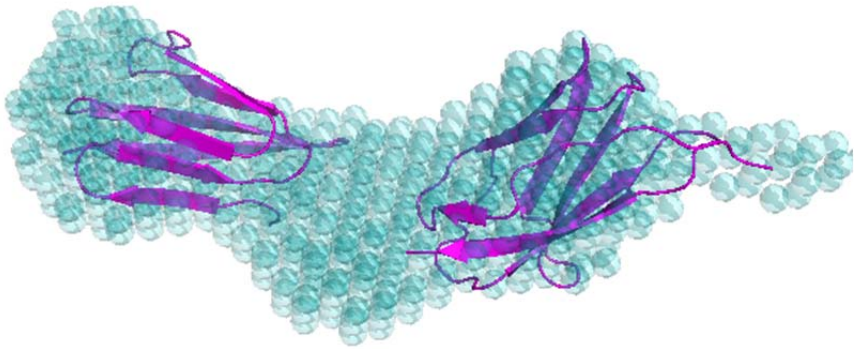


**Figure S3.** $R_g$ (upper panel) and $d_{max}$ (lower panel) distributions for the ensemble optimisation modelling (EOM) of wild-type rIgSF-CTD (black line) and r664i6H (red line) variant.

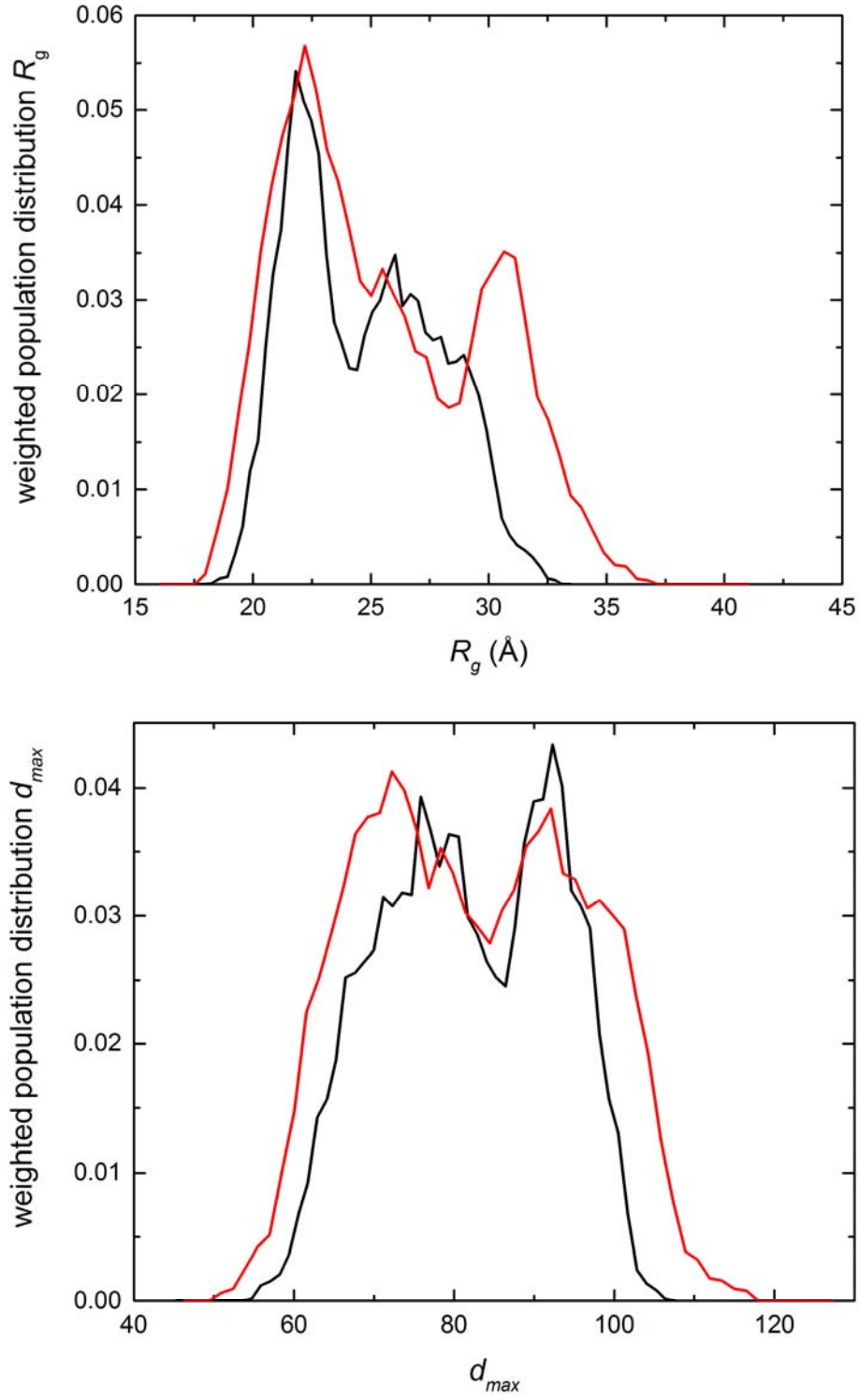


**Figure S4.** Comparison of RgpB phenotype expressed by wild-type *P. gingivalis* W83 and mutant strains with insertion (RgpB662iXa6H) or substitution (RgpB665sXa6H) of hexapeptide (IEGRAA) in the linker between IgSF and CTD domains. The whole culture (c) and spent growth medium (s) were subjected to Western blotting analysis using RgpB specific mAbs.

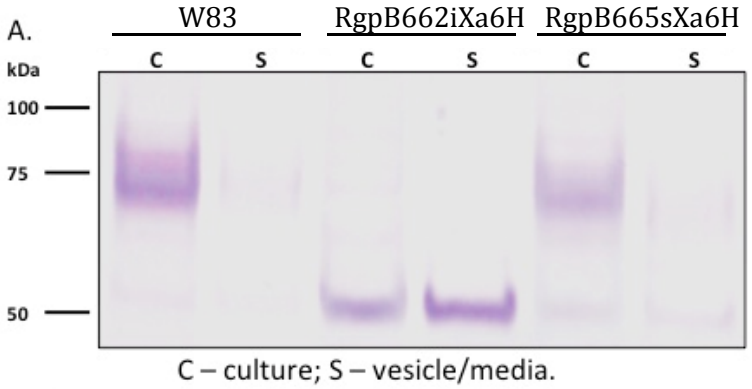


**Figure S5.** Guinier plots for the SAXS data for wild-type rIgSF-CTD ($qR_g$ < 1.18, black symbols) and r664i6H ($qR_g$ < 1.15, red symbols) variant. The solid lines are linear fits to the experimental data which shows the expected good fit for solutions of mono-disperse proteins.