

Supplementary Materials

The docking of the sorting pattern LPNTG against Sp-SrtAS_{370Δ86} led to 100 poses. Four of them were rejected as the LPNTG ligand was outside the catalytic cavity. In the 96 other poses, the LPNTG ligand was within the cavity. To understand the direction that the LPNTG ligand adopts in the catalytic cavity, we divided this cavity into two parts, designated cavity 1 and cavity 2 (Fig. 3). In 24 poses, the LP residues of the ligand were located in cavity 1 that contains the catalytic residue Arg216 whereas the TG residues were orientated towards the catalytic Cys208 (cavity 2) (Fig. 3). Contrarily, in other 22 poses, the ligand adopted the opposite orientation. In the other remaining poses, the ligand was curved in cavity 1. Only the 24 poses that had the LP residues in cavity 1 and the TG residues in cavity 2 were selected for further analysis. More precisely, the distance between the S atom of the Cys208 of Sp-SrtAS_{370Δ86} and the alpha carbon of the threonine residue of the ligand ($d[\text{C}\alpha(\text{Thr})\text{-SH}(\text{Cys208})]$) was measured in order to evaluate the propensity to make a nucleophilic attack, as described in the catalytic mechanism of the sortase. Eight of the 24 poses had a distance $d[\text{C}\alpha(\text{Thr})\text{-SH}(\text{Cys208})]$ below 4 Å. This distance was between 4 Å and 5 Å in 5 other poses. In 11 poses, the distance was higher than 5 Å. The interactions between the ligand and the sortase were analyzed using 2D models (Fig. A2). LP residues of the ligand were shown to interact with hydrophobic residues located in cavity 1 (Met125, Val186, Ala187, Val191, Val193, Ile194, Ile218). Electrostatic interactions were also found with the hydrophilic residues Arg190 and Arg216. TG residues interacted with residues of the cavity 2 through electrostatic and hydrophobic interactions (Ile211, Asp210, Val206, His143, Thr207, Ser141, His142, Cys208, Phe145, Leu113, Ala213 and Leu118). In such binding modes, the localization of the TG pattern was expected to permit the nucleophilic attack by the residue Cys208. The presence of the residues His143 and His142 could help proton transfer. All these results were consistent with those obtained by Race et al. [24], and thus validate our modeling approach.

A similar protocol was applied for the docking of the ligand LPNTG against St-SrtA_{4F44Δ90} and St-SrtA_{ALMD-9Δ90}. For the two systems, 94 and 96 poses were obtained respectively with the LPNTG pattern within the catalytic cavity. As described with Sp-SrtA_{SF370Δ86}, the ligand adopted different orientations towards both catalytic cavities. In the case of the sortase St-SrtA_{4F44Δ90} 6 poses were obtained with the distance $d[\text{Ca}(\text{Thr})\text{-SH}(\text{Cys208})]$ below 4 Å while 5 poses were found in the case of the sortase St-SrtA_{ALMD-9Δ90}. Interactions between the LPNTG motif and the residues constituting the catalytic cavity of St-SrtA_{4F44Δ90} were identical to those observed in the case of Sp-SrtA_{SF370Δ86}. Nevertheless, 4 additional residues (Ala140, Gly114, Pro188 and Asn115) interacting with the ligand may be mentioned (Fig. A2). In the case of St-SrtA_{ALMD-9Δ90}, similar trends were observed. However, in this case only 18 residues constituting the cavity interacted with the ligand versus 21 for Sp-SrtA_{SF370Δ86} and 25 for St-SrtA_{4F44Δ90}. Main residues interacting with the ligand in St-SrtA_{ALMD-9Δ90}:LPNTG complexes were also found in St-SrtA_{4F44Δ90}:LPNTG and Sp-SrtA_{SF370Δ86}:LPNTG complexes (Fig. A2). The residues Val186, Val193, Val194, Val206, Ile218 which are thought to be important for the binding of the LPNTG pattern within the catalytic cavity of the sortase A were not identified as interacting residues in St-SrtA_{ALMD-9Δ90}:LPNTG complexes. Therefore, these analyses did not show any significant difference concerning the binding of the LPNTG motif to Sp-SrtA_{SF370Δ86}:LPNTG, St-SrtA_{ALMD-9Δ90}:LPNTG and St-SrtA_{4F44Δ90}:LPNTG complexes.

Due to the large number of poses obtained with wrong orientation of the LPNTG motif or its folding within the catalytic cavity, further simulations were run with a larger ligand including four additional residues. Moreover, the C-terminal and the N-terminal extremities of the ligand were blocked with an acetyl and a methyl group, respectively [41]. The obtained ligand Ace-QLPNTGEND-Nme was docked against the three systems Sp-SrtA_{SF370Δ86}, St-SrtA_{4F44Δ90} and St-SrtA_{ALMD-9Δ90}. Forty-five poses were automatically retained for the target Sp-

SrtA_{SF370Δ86} versus 34 and 35 poses for the targets St-SrtA_{4F44Δ90} and St-SrtA_{ALMD-9Δ90}. The complexes were analyzed based on the same criteria than previously described (orientation of the ligand towards the catalytic cavity and proximity of the TG residues to the catalytic cystein). In the case of Sp-SrtA_{SF370Δ86}, the ligand was recurved in cavity 1 in 18 poses (binding mode 1). In 23 poses, the moiety Ace-QLPNT was positioned within cavity 1 whereas the rest of the ligand was outside the catalytic cavity (binding mode 2). In 4 poses, the moiety Ace-QLPNT was localized in cavity 1 whereas the other moiety of the ligand was located within cavity 2 (binding mode 3). Among them, two poses had the distance d[Cα(Thr)-SH(Cys208)] below 5 Å (3.31 Å and 4.32 Å). In the case of St-SrtA_{4F44Δ90}, 12 poses adopted the binding mode 1; thirteen poses were similar to binding mode 2; three poses adopted the binding mode 3. In the rest of the poses, the moieties Ace-QLPNT and GEND-Nme were oriented towards cavity 2 and cavity 1, respectively. Similar trends were obtained with the system St-SrtA_{ALMD-9Δ90}. The interactions between the catalytic cavity and the ligand were similar for the three systems and similar to those previously described for the ligand LPNTG. Moreover, the superimposition of the different complexes (Fig. A3) showed no significant difference in the orientation and the conformation of the pattern LPNTG within the cavity.

Table S1 Primers and resulting PCR or Overlapping PCR products used for mutant construction.

Primer	Figure annotation	Sequence (5'→3')	Target	DNA matrix	PCR product (bp)	AT°C in PCR	OL PCR P. L. (bp)	AT°C in OL PCR	Mutant construction
<i>*UpprtMF</i>	Up- <i>XF</i>	AGCCTTGTTGAAAAAGGTG	<i>UpprtM</i>	LMD-9 gDNA	987	55.7			
<i>UpprtMR</i>	Up- <i>XR</i>	CATTTTATCTACTCCTTAAATTGTC							
<i>DownprtMF</i>	Down- <i>XF</i>	ATCATAAGATAAAAGTTGATAATGTTG	<i>DownprtM</i>	LMD-9 gDNA	834	55.7	2556	52.2	LMD-9 _{<i>ΔprtM</i>}
<i>*DownprtMR</i>	Down- <i>XR</i>	AAGGACCTGCACCGATTTC							
<i>prtMeryF</i>	<i>X-eryF</i>	<i>GACAATTTAAGGAGTAGATAAAATGAACAAAAATATAAAATATTCTC</i>	<i>ery</i>	pG+host9	786	58.2			
<i>prtMeryR</i>	<i>X-eryR</i>	<i>CAACATTATCAACTTTATCTTATGATTATTTCTCCCGTTAAATAATAG</i>							
<i>*UpprtSF</i>	Up- <i>XF</i>	ACTCCGAGGTAGCAGACCAA	<i>UpprtS</i>	LMD-9 gDNA	821	56.2			
<i>prtSUpR</i>	Up- <i>XR</i>	TGCAGCTACCGATGGTGC							
<i>SSprtSF</i>	Up- <i>prtS</i> -4F44F	GCACCATCGGTAGCTGCA	<i>prtS_{4F44}</i>	4F44 gDNA	4671	56.2	7530	50	LMD-9 _{<i>prtS4F44</i>}
<i>prtSR</i>	<i>prtS</i> -4F44R	TTATACTTCTCCTTGTGACGTCGTAT							
<i>prtSSpecF</i>	<i>X-specF</i>	<i>ATACGACGTCACAAGGAAGAAGTATAACGTAACGTGACTGGCAAGAG</i>	<i>spec</i>	pSET4s	1188	60.2			
<i>prtSSpecR</i>	<i>X-specR</i>	<i>CACAATGATTAAGCGGTTTTATTATCGCTACGATAACGCCTGTTT</i>							
<i>DownprtSF</i>	Down- <i>XF</i>	ATAATAAAACCGCTTAATCATTGTG	<i>DownprtS</i>	LMD-9 gDNA	920	56.2			
<i>*DownprtSR</i>	Down- <i>XR</i>	TTTCTTGATGACGCAACCAC							
<i>*UpgyraseF</i>	Up- <i>XF</i>	GACAGGTGTGAACGATGACG	<i>UpsrtA</i>	LMD-9 gDNA	870	56.2			
<i>UpgyraseR</i>	Up- <i>XR</i>	TAGTTATCACCACCTTTATTGATCTTC							
<i>gyr-eryF</i>	<i>X-eryF</i>	<i>GAAGATCAATAAAGTGGTGATAACTAATGAACAAAAATATAAAATATTC</i>	<i>ery</i>	pG+host9	781	56.2	2408 (<i>UpsrtA-ery-DownsrtA</i>) / 3571 (<i>UpsrtA-srtA_{4F44}-spec-DownsrtA</i>) / 3571 (<i>UpsrtA-srtA_{LMD-9}-spec-DownsrtA</i>)	50	LMD-9 _{<i>ΔsrtA</i>} LMD-9 _{<i>srtALMD-9</i>} LMD-9 _{<i>srtA4F44</i>}
<i>lyase-eryR</i>	<i>X-eryR</i>	<i>GACCTTACGGCCTTTTCTTATTTCTCCCGTTAAATAATAG</i>							
<i>DownlyaseF</i>	Down- <i>XF</i>	TAAGAAAAGGCCGTAAGGTC	<i>DownsrtA</i>	LMD-9 gDNA	803	56.2			
<i>*DownlyaseR</i>	Down- <i>XR</i>	ATTTGCATAGCCGCTAGTC							
<i>Up-srtA-LMD9F</i>	Up- <i>X-LMD9F</i>	GAAGATCAATAAAGTGGTGATAACTA	<i>srtA_{4F44}</i> / <i>srtA_{LMD-9}</i>	4F44 gDNA / LMD-9 gDNA	788	60.2			
<i>srtA-LMD9R</i>	<i>X-LMD9R</i>	TTAACTTTGAAATTGATTGTATGACT							
<i>SpecF</i>	<i>X-specF</i>	<i>GTCATACAATCAATTTCAAAGTTAACGTGACTGGCAAGAG</i>	<i>Spec</i>	pSET4s	1176	60.2			
<i>SpecR1</i>	<i>X-specR</i>	<i>GACCTTACGGCCTTTTCTTACGCTACGATAACGCCTGTTT</i>							

Table S1 (Continued)

Primer	Figure annotation	Sequence (5'→3')	Target	DNA matrix	PCR product (bp)	AT°C in PCR	OL PCR P. L. (bp)	AT°C in OL PCR	Mutant construction
*Up _{gyraseF}	Up- <i>srtA</i> F	GACAGGTGTGAACGATGACG	Up <i>srtA</i> + <i>srtA</i> _{LMD-9}	LMD-9 <i>srtA</i> LMD-9 <i>gDNA</i>	1545	56.2	3571 (Up <i>srtA</i> + <i>srtA</i> _{LMD-9} - <i>srtA</i> LMD-9 + <i>spec</i> _{<i>srtA</i>} + Down <i>srtA</i>)	50	LMD- 9 <i>srtA</i> :Ile218→Val218
<i>srtA</i> _{V218R}	<i>srtA</i> _{V218R}	CCCTTTGACAACGTACGCTG							
<i>srtA</i> _{V218F}	<i>srtA</i> _{V218F}	CAGCGTACAGTTGTCAAAGGG	<i>srtA</i> _{LMD-9} + <i>spec</i> _{<i>srtA</i>} + Down <i>srtA</i>		2026	56.2			
*Down _{lyaseR}	Down- <i>srtA</i> R	ATTGTCATAGGCCGTAGTC	Down <i>srtA</i>						

AT: annealing temperature; LMD-9/4F44 *gDNA*: genomic DNA of the strain LMD-9 or 4F44; *primers used to amplify recombinant DNA fragments by overlapping (OL) PCR; Italicized nucleotides correspond to homologous tails; *UpprtM*: fragment located upstream of the *prrM* gene; *DownprtM*: fragment located downstream of the *prrM* gene; *ery*: erythromycin resistance gene; *UpprtS*: fragment located upstream of the *prrS* gene; *prrS*_{4F44}: *prrS* allele of the strain 4F44; *spec*: spectinomycin resistance gene; *DownprtS*: fragment located downstream of the *prrS* gene; *UpsrtA*: fragment located upstream of the *srtA* gene; *DownsrtA*: fragment located downstream of the *srtA* gene; *srtA*_{4F44}: *srtA* allele of the strain 4F44; *srtA*_{LMD-9}: *srtA* allele of the strain LMD-9.

Table S2 Primers used for the sequencing of PPIases genes.

Primer couple	Sequences (5'→3')	Gene
ppiA1for LMD-9 \ ppiA1rev	TTGACAATGCACCAATTGAGA \ TAGGAGCAAGCGTAGGGAAG	<i>ppiA</i>
ppiA2for LMD-9 \ ppiA2rev	CTTGACAGGGAGCTATTCACG \ CTTTAATGACTTCGACTGAATTAACA	
ppiA3for \ ppiA3rev	GAAAGGTTTCGCTGTTGAGC \ CCGCTTATCGGGAATATTGT	
pplB1for \ pplB1rev	AAGGATGGTTACGCCCAAAT \ CAGGTTCTAGGTCCAGAATTTCC	<i>pplB</i>
pplB2for \ pplB2rev	TTTTGCTTTTATAGCTTTAAGAAATGA \ TGGCTTTAAGTTCTGGTGTGG	
pplB3for \ pplB3rev	CGTTCAGAATTGATTGATGATG \ AAGCTTCTGCAATTGGTGCT	
pplB4for \ pplB4rev	TCAAGCTTTTCCCTGACCAT \ ACGCTTCTGCCAAAGGTTTA	
pplB5for \ pplB5rev	CGCTTGAGGAAGTTGTTATTGA \ AGGACCATTTCGTTTGC ACT	
prtM1for \ prtM1rev	CCTGATGCTCAGATTACGACAG \ GAGGGCAATGCTTTCCTCTT	<i>prtM</i>
prtM2for \ prtM2rev	TTGCCAAAGACATCATGGAG \ TGTGCAGCTTGGTTAACAGC	
prtM3for \ prtM3rev	TGGTGCTGGTGCTATGTATG \ TGAAGCTCCAGAACCAGTCA	
prtM4for \ prtM4rev	CGAAAGTTGCTAAGGATAACAGC \ GGGTCGTTAACTGGAACCAA	
prtM6for \ prtM6rev	TGCTACAGGATTTGCAGCAG \ CTTGGCAAAGCCAATCATTT	
prtM7for \ prtM7rev	GCTGAACTGGAGGTCTTTGC \ AATGCCCTTTACCTGTTCCA	<i>tig</i>
tig1for \ tig1rev	GAATCCTTGAGCTCCTAGACCA \ GGTTAAATACGGCACGAGGA	
tig2for \ tig2rev	GCATTAGGCTTTCTTCGAAAT \ CCTGCAAGATCAGTAGCTTGA	
tig3for \ tig3rev	TGCAGAAGTTGTTACAAAACCA \ TCAGCTGCAAGGTCATTGAT	
tig4for \ tig4rev	AAATTGCATTTGATGATGCTG \ AAGCCCCATTTGTTATCACC	
tig5for \ tig5rev	TATGTCTAAATCAAGTTTCTGTTGAG \ CGTTTTGGTGCATTTTCATC	

PCR reactions were carried out at an annealing temperature of 55°C.

Table S3 Primers used for the verification of mutant sequences.

Primers couple's	Sequences (5'→3')	sequenced mutant
gyrA2rev \ gyrA2for	CGTGGAGGTCGTGGAGTTC \ TGTCTTAATACCTTTACCACCA	
gyrA1rev \ ERY1 R	CAGTGTGCGTTTCAAAGAGGA \ TTGAGTGTGCAAGAGCAACC	
Ery1F \ Lyase1R	AAAGGGCATTTAACGACGAA \ TAATCAGAAACAATGATAGCGACA	
Sort3F \ Spec2R	TCAAAGGGAACTTGGAATCG \ AGCAAGAAATGGTACCGTGG	LMD-9 Δ srtA
gyrA1rev \ Sort2forR	CAGTGTGCGTTTCAAAGAGGA \ TATCAGAAGCCCCAGCGATA	LMD-9 _{srtA} LMD-9
Sort1rev \ Spec1R	CCCAGATGGGTTCTCAAAAA \ TCCTCCTCACTATTTTGATTAGTACCT	LMD-9 _{srtA4F44}
Spec1F \ Lyase1R	TCAAAATAGTGAGGAGGATATATTTGA \	LMD-9 _{srtA:11c218→Val218}
Spec2F \ Lyase2R	GGGAGAGAATTTTGTAGCAGTTC \ CCTAAAAGATGACCGAGTGTGA	
Lyase1F \ LyasechrLMD-9R	CCAACCTATTCGCAAGGACA \ GCAAAGTTCACCAAAAAGATTGA	
UpUpF \ HEmS02R	TACCAGCCAAATTCATCACG \ AAACCTGCACCCAAAAGAACAG	
Style 1 \ Style 2	CCAACAACAAGGCACTCATTA \ GTTGAGTTGTTTCTACTTCTGT	
Style 3 \ Style 4	ACTAAGGTGGAAGCAACTGTT \ ATTGTCCAGCGACTGGTTGT	
PrtFor \ PrtRev	TACGGTGAATGGTTTAAACG \ AATTACTTTACTACCAACCG	
Style 5 \ Style 6	CAGCTGGTAATGATGGAACAT \ CCAACTTGAAAAATCTGAAAGAA	
Style 7 \ Style 8	CAATCCATCTGTCTTCATTC \ CCGTTGTTAATTGTGTTGAGT	
Style 9 \ Style 10	GTTTATATTTGACTGGCGAAG \ GTCTTGATCGCAGAGTCAG	
Style 11 \ Style 12	GAACCTGTTACAGCACAGC \ GCTGGACGAGGGTTAAATG	
Style 13 \ Style 14	GTTCCAGGTGCAGCAGTACA \ ACCAAATGGTAGCTTAAGAACG	LMD-9 _{prtS4F44}
Style 15 \ Style 16	TCAGTCACCGATGAAACAGG \ ATAGGCAGTTTGAAGTTCTGG	
Style 17 \ Style 18	ACTTGCAAACCAAGTCAAATGTTA \ CGCTGCTTCAACTGCCTGG	
Style 19 \ Style 20	GCAGCGAAAGCCAACTTAGA \ TTCCTTGACGTCGTATGC	
Style 20-1 \ Spec2R	ATTCAGAGCATGTACCGATAG \ CACAATGATTAAGCGGTTTTATTAT	
Spec1F \ HEMS17 R	TCAAAATAGTGAGGAGGATATATTTGA \ TGATCTGAGAACATAAGGGTC	
Spec2F \ Style 20-2	GGGAGAGAATTTTGTAGCAGTTC \ GGTGTTGATGGGCTAGAGGA	
Style 21F \ PrtSDnDnR	GGTGTGATGGGCTAGAGGA \ ACGACGATAACCAAGAATGC	
prtM6F \ prtM6R	TGCTACAGGATTTGCAGCAG \ CTTGGCAAAGCCAAATCATTT	
prtM7F \ prtM7R	GCTGAACCTGGAGGTCTTTGC \ AATGCCCTTTACCTGTTCCA	
prtM2F \ ery 1 R	TTGCCAAAGACATCATGGAG \ TTGAGTGTGCAAGAGCAACC	
ery 1 F \ prtM9R	AAAGGGCATTTAACGACGAA \ CGACCGCCACTTTTCTGTAT	LMD-9 Δ prtM
DownprtMF \ DownprtMR	ATCATAAGATAAAAGTTGATAATGTTG \ AAGGACCTGCACCGATTTTC	
prtM8F \ prtM8R	CCACACGATGTTTGAATGC \ CGGAGAAGACGACGAAGAAC	

PCR reactions were carried out at an annealing temperature of 55°C