The Importance of the Leader Sequence for Directing Lanthionine Formation in Lacticin 481

Supporting Information

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MATERIALS AND METHODS

General PCR Methods for Preparation of LctA Mutants

Mutagenesis via Mega Primer PCR: Mega Primer Generation. PCR amplifications were performed using an automatic thermocycler (PTC 150, MJ Research) with forward mutant primers (0.5 μM) and T7 terminator primer (0.5 μM) obtained from Qiagen or Operon, dNTPs (1 mM) obtained from Promega or Invitrogen, $MgCl_2$ (1.5 mM) from Invitrogen and 10X PCR buffer–$MgCl_2$ (Invitrogen). The appropriate template DNA was isolated from DH5α cells containing the desired plasmid via Qiagen® Plasmid Midi Kit. Taq polymerase, Platinum Taq polymerase, and/or Platinum Pfx polymerase from Invitrogen were used. Thirty cycles were utilized to amplify the DNA. In the first cycle, the denaturation step of 94 ^oC for 1 min was followed by annealing at 50 ^oC for 1 min and extension at 72 ^oC for 2 min. The remaining twenty–nine cycles had a denaturation step of 95 ^oC for 1 min followed by annealing at 55 ^oC for 1 min and extension at 72 ^oC for 45 s. A final extension step at 72 ^oC for 10 min completed the PCR reaction. A 10–μL aliquot of the amplified products and 2 μL of 100 bp DNA ladder (Invitrogen) were run at 144 volts on a 2% agarose (Bio–Rad) gel in 0.5X Tris–borate EDTA (TBE) buffer for 20 min. Successful PCR reactions were concentrated to less than 50 μL using a Labconco Centrivap Concentrator. PCR products were then gel purified on a 2% agarose gel using Qiagen's QIAquick® Gel Extraction kit. The resulting product was utilized as the mega primer in the subsequent PCR.

Mutagenesis via Mega Primer PCR: Mega Primer PCR. Six cycles of linear amplification were performed using an automatic thermocycler with 10 μL of mega primer (concentration unknown), dNTPs (1 mM), 10 μL of template DNA, $MgCl_2$ (1.5 mM), 10 μL of 10X PCR buffer–$MgCl_2$, 2 μL of 50 μM long T7 promoter primer (Operon), and 1 μL of Taq polymerase, Platinum Taq polymerase, and/or Platinum Pfx polymerase. In the first cycle, the denaturation step at 94 ^oC for 5 min was followed by annealing at 68 ^oC for 1 min and extension at 72 ^oC for 2 min. The remaining thirty-five cycles had a denaturation step of 95 ^oC for 1 min followed by annealing at 68 ^oC for 1 min and extension at 72 ^oC for 45 s. A final extension step at 72 ^oC for 10 min completed the PCR reactions. A 10–μL aliquot of the amplified products and 2 μL of 100 bp DNA ladder were run at 144 V on a 2% agarose gel in 0.5X TBE buffer for 20 min.(*1*) Successful PCR reactions were concentrated to less than 50 μL using a Labconco Centrivap Concentrator. PCR products were then gel purified on a 2% agarose gel using Qiagen's QIAquick® Gel Extraction kit.

Construction of Plasmids

pET15b LctA E–8P. The partial *lctA* gene was amplified using the primers LctA E–8P FP: 5'-GAAGTGACAGAAAGTCCGTTGGACCTTATTTTA-3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

pET15b LctA E–8A. The partial *lctA* gene was amplified using the primers LctA E–8A FP: 5'-GAAGTGACAGAAAGTGCATTGGACCTTATTTTA-3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

pET15b LctA L–7E. The partial *lctA* gene was amplified using the primers LctA L–7E FP: 5'-ACAGAAAGTGAAGAAGACCTTATTTTA -3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

pET15b LctA L–7K. The partial *lctA* gene was amplified using the primers LctA L–7K FP: 5'- GAAGTGACAGAAAGTGAAAAAGACCTTATTTTA -3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

pET15b LctA D–6P. The partial *lctA* gene was amplified using the primers LctA D–6P FP: 5'-ACAGAAAGTGAATTGCCTCTTATTTTAGGTGCA-3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

pET15b LctA D–6G. The partial *lctA* gene was amplified using the primers LctA D–6G FP: 5'-ACAGAAAGTGAATTGGGCCTTATTTTAGGTGCA-3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the

template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

pET15b LctA L–5P. The partial *lctA* gene was amplified using the primers LctA L–5P FP: 5'-GAAAGTGAATTGGACCCTATTTTAGGTGCA-3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

pET15b LctA L–5Q. The partial *lctA* gene was amplified using the primers LctA L–5Q FP: 5'-AGTGAATTGGACCAAATTTTAGGTGCA-3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed desired mutation.

pET15b LctA I–4P. The partial *lctA* gene was amplified using the primers LctA I–4P FP: 5'-AAAGTGAATTGGACCTTCCTTTAGGTGCAAAAG-3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

pET15b LctA I–4G. The partial *lctA* gene was amplified using the primers LctA I–4G FP: 5'-AAAGTGAATTGGACCTTGGCTTAGGTGCAAAAG-3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

pET15b LctA G–2V. The partial *lctA* gene was amplified using the primers LctA G–2V FP: 5'-CCTTATTTTAGTGGCAAAAGGCG -3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

pET15b LctA G–2K. The partial *lctA* gene was amplified using the primers LctA G–2K FP: 5'- TTGGACCTTATTTTAAAAGCAAAAGGCGGCAGT -3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

pET15b LctA G–2E. The partial *lctA* gene was amplified using the primers LctA G–2E FP: 5'- TTGGACCTTATTTTAGAAGCAAAAGGCGGCAGT -3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

pET15b LctA A–1D. The partial *lctA* gene was amplified using the primers LctA A–1D FP: 5'-TATTTTAGGTGACAAAGGCGGCA -3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

pET15b LctA A–1K. The partial *lctA* gene was amplified using the primers LctA A–1K FP: 5'- GACCTTATTTTAGGTAAAAAAGGCGGCAGTGGA -3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

pET15b LctA A–1I. The partial *lctA* gene was amplified using the primers LctA A–1I FP: 5'- GACCTTATTTTAGGTATAAAAGGCGGCAGTGGA -3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

pET15b LctA E13A. The partial *lctA* gene was amplified using the primers LctA E13A FP: 5'- CATACAATTTCTCATGCATGTAATATGAATAGC -3' and T7 terminator: 5'-GCTAGTTATTGCTCAGCGG-3' and the plasmid pET15b–LctA was used as the template. The generated mega primer was subsequently used as the terminator primer with the long T7 promoter primer to yield the full *lctA* gene. The PCR product was

digested with *NdeI* and *BamHI* restriction enzymes then ligated into the pET15b vector. DNA sequencing revealed the desired mutation.

Construction of pET15b LctMutA, LctRumA, and MutLctA expression systems

Oligonucleotides corresponding to the full sequence of the MutA propeptide were designed. XhoI and BamHI restriction sites (as sticky ends) flanked this sequence and were irrelevant for this work. The gene corresponding to the leader sequence of LctA was amplified using a forward primer containing the XhoI restriction site followed by 17 bases of *lctA* (5'-CGCTCGAGATGAAAGAACAAAACTC-3') and a reverse primer consisting of bases coding for the LctA leader sequence followed by the first 14 bases coding for the MutA propeptide. (5'-CCTTGCCACCAACGATTCTTTGC-ACCTAAAATAAGGTC-3'). This generated a piece of DNA that contained LctA residues –24 to 1 and a truncated portion of the MutA propeptide. The synthetic oligonucelotides corresponding to the MutA propeptide were annealed and this piece of DNA was subsequently used as template DNA in subsequent PCR reactions. Employing a forward primer consisting of the last set of bases corresponding to the LctA leader sequence followed by the first 14 bases coding for the MutA propeptide (5'-GACCTTATTTTAGGTGCAAAGAATCGTTGGTGGCAAGG-3') and a reverse primer complimentary to the C-terminal portion of the propeptide of MutA flanked by the BamHI recognition site (5'-GCGGGATCCTTAACAGCAAGTGAAAAC -3'), the *mutA* propeptide gene was amplified by PCR. With these two pieces in hand, a subsequent PCR reaction was performed where the two pieces primed to each other at the leader-structural interface and the outside primers containing the restriction sites for the endonucleases were employed for amplification. A similar strategy was undertaken for the construction of LctRumA. The LctA leader (FP: 5'-CGCTCGAGATGAAAGAACAAAACTC-3'; RP: GTTTTAAGAACACCATTACCTGCACCTAAAATAAGGTC-3') and RumA propeptide (FP: 5'-GACCTTATTTTAGGTGCAGGTAATGGTGTTCTTAAAAC-3'; RP: 5'-CGCGGATCCTTAACAACAAGTAAAAAGAAATTGCC-3') were amplified and annealed in the final overlap-extension PCR. The MutLctA peptide was constructed by amplifying the MutA leader sequence (FP: 5'-CCGCTCGAGATGAACAAGTTAAACAGTAACGC-3'; RP: 5'-AACTCCACTGCCGCCTTTACCACCCAAAATAGTATC-3') off genomic DNA isolated from *Streptococcus mutans* T8. The LctA propeptide (FP: 5'-GATACTATTTTGGGTGGTAAAGGCGGCAGTGGAGTT-3'; RP: 5'-CGCGGATCCTTAAGAGCAGCAAGTAAATACAAATTGCCAGC-3') was amplified of pET15b LctA. The pieces were joined in the final overlap-extension PCR. Cut fragments were ligated into pET15b vector digested with XhoI and BamHI. *E. coli* DH5α cells were transformed with ligation mixtures, plated on LB-Ampicillin plates and grown at 37^{o} C for 16 hours. The sequences of purified plasmids were checked and *E.coli* BL21(DE3) cells were transformed with the correct plasmid.

NukA Leader Peptide Oligonucleotide foward: 5'-ATGGAAAACTCTAAAGTTATGAAAGACATCGAAGTTGCAAACCTGCTGGAAGAAGTTCAGGAAGATGAACTGAACGAAGTTCTGGGTGCT-3'

NukA Leader Peptide Oligonucleotide reverse: 5'-AGCACCCAGAACTTCGTTCAGTTCATCTTCCTGAACTTCTTCCAGCAGGTTTGCAACTTCGATGTCTTTCATAACTTTAGAGTTTTCCAT -3'

MutA Structural Peptide Oligonucleotide forward 5'-TCGAGAATCGTTGGTGGCAAGGTGTTGTGCCAACGGTCTCATATGAGTGTCGCATGAATTCATGGCAACATGTTTTCACTTGCTGTTAAG-3'

MutA Structural Peptide Oligonucleotide reverse 5'-GATCCTTAACAGCAAGTGAAAACATGTTGCCATGAATTCATGCGACACTCATATGAGACCGTTGGCACAACACCTTGCCACCAACGATTC-3'

XhoI and BamHI restriction sites are underlined

Long T7 Promoter Primer (for Mutagenesis via Mega Primer PCR: Mega Primer PCR). The *lctA* gene was amplified via a long T7 promoter primer: 5'-CGCGAAATTAATACGACTCACTATAGGGGAATTGTGAG-3'using the appropriate pET15b LctA plasmid and mega primer.

Overexpression and Purification of His_6–LctA and Mutants. All LctA mutants were expressed as N–terminal His_6–tagged peptides. Transformed BL21 (DE3) cells containing the appropriate plasmid were grown in LB containing 100 μg/mL ampicillin at 37 ^oC and were induced with 1 mM IPTG (CalBiochem) when the OD_{600nm} reached 0.6. The cells continued growing at 37 ^oC for an additional 3 h and then were harvested at 4 ^oC for 15 min at 11,900 x *g* in a Beckman JLA10.500 rotor. The cell paste was resuspended in LctA start buffer (20 mM phosphate, pH 7.5, 0.5 mM imidazole, 500 mM NaCl, 20% glycerol) and stored at –80 ^oC until use. The cell paste was thawed and sonicated on ice for approximately 15 min using a Sonics & Materials Inc. Vibra Cell$^{T.M.}$ (35% Amp, 4.0 s on and 9.9 s off). The sample was then centrifuged at 23,700 x *g* for 30 min at 4 ^oC in a Beckman JA20 rotor. The supernatant was discarded and the cell pellet was resuspended in start buffer, sonicated for 5 min, and centrifuged again at 23,700 x *g* for 30 min at 4 ^oC. The supernatant was again removed and the cell pellet was resuspended in LctA buffer 1 (6 M guanidine HCl, 20 mM phosphate, pH 7.5, 0.5 mM imidazole, 500 mM NaCl). After centrifugation, the supernatant was filtered through a 0.45 μm cellulose acetate filter (Corning) and loaded onto a 5 mL His Hi Trap column from Amersham charged with 0.1 M $NiSO_4$ and equilibrated with LctA buffer 1. The resin was washed with 25 mL of LctA buffer 1 and then with 25 mL of LctA buffer 2 (4 M guanidine HCl, 20 mM NaH_2PO_4, pH 7.5, 30 mM imidazole, 300 mM NaCl). The peptide was eluted with LctA elution buffer (20 mM NaH_2PO_4 (or 20 mM Tris), 100 mM NaCl, 1 M imidazole, 4 M guanidine HCl, pH 7.5). The peptide was further purified by RP–HPLC on a Beckman Gold or Waters system using a Vydac C4 analytical column (0.46 cm x 25 cm) or Waters Delta–pak^{TM} C4 15 μm 300 Å 25 x 100 mm PrepPak®

Cartridge, monitoring at 220 nm. The gradient utilized to purify the peptides was 2% B to 100% B over 45 minutes (A: H_2O with 0.1 % TFA; B: 80 % acetonitrile with 0.086 % TFA). The average yield of peptides was 10 mg/L culture and were generally isolated in greater than 90% purity. Peptides were analyzed by MALDI–TOF mass spectrometry on the Voyager–DE–STR (Applied Biosystem) in the Mass Spectrometry Laboratory, School of Chemical Sciences, UIUC.

Chimeric Sequences

LctNukA :
GSSHHHHHHSSGLVPRGSHMKEQNSFNLLQEVTESELDLILGAKKKSGVIPTVSH
DCHMNSFQFVFTCCS

LctMutA:
GSSHHHHHHSSGLVPRGSHMLEMKEQNSFNLLQEVTESELDLILGAKNRWWQG
VVPTVSYECRMNSWQHVFTCC

LctRumA:
GSSHHHHHHSSGLVPRGSHMLEMKEQNSFNLLQEVTESELDLILGAGNGVLKTIS
HECNMNTWQFLFTCC

MutLctA
GSSHHHHHHSSGLVPRGSHMLEMNKLNSNAVVSLNEVSDSELDTILGG
KGGSGVIHTISHECNMNSWQFVFTCCS

Mass spectrometry data

His_6–LctM assay with His_6–LctA E–13*A*. MALDI–TOF MS calcd. 7652 (M), 7580 (M-72), found 7660 (M), 7589 (M-72).

His_6–LctM assay with His_6–LctA E–8*A*. MALDI–TOF MS calcd. 7652 (M), 7580 (M-72), found 7584 (M-72).

His_6–LctM assay with His_6–LctA E–8*P*. MALDI–TOF MS calcd. 7680 (M), 7608 (M-72), found 7684 (M), 7664 (M-18), 7648 (M-32), 7632 (M-54), 7614 (M-72).

His_6–LctM assay with His_6–LctA L–7*A*. MALDI–TOF MS calcd. 7667 (M), 7595 (M-72), found 7637 (M-32), 7619 (M-54), 7601 (M-72).

His_6–LctM assay with His_6–LctA L–7E. MALDI–TOF MS calcd. 7725 (M), 7653 (M-72), found 7668 (M-54), 7651 (M-72).

His_6–LctM assay with His_6–LctA L–7K. MALDI–TOF MS calcd. 7725 (M), 7653 (M-72), found 7728 (M), 7711 (M-18), 7694 (M-32), 7675 (M-54).

His_6–LctM assay with His_6–LctA D–6P. MALDI–TOF MS calcd. 7691 (M), 7620 (M-72), found 7696 (M), 7679 (M-18), 7660 (M-32), 7642(M-54), 7625 (M-72).

His_6–LctM assay with His_6–LctA D–6G. MALDI–TOF MS calcd. 7652 (M), 7580 (M-72), found 7581 (M-72).

His_6–LctM assay with His_6–LctA L–5Q. MALDI–TOF MS calcd. 7724 (M), 7652 (M-72), found 7650 (M-72).

His_6–LctM assay with His_6–LctA L–5P. MALDI–TOF MS calcd. 7694 (M), 7622 (M-72), found 7697 (M), 7680 (M-18), 7662 (M-32), 7644 (M-54), 7626 (M-72).

His_6–LctM assay with His_6–LctA I–4P. MALDI–TOF MS calcd. 7694 (M), 7622 (M-72), found 7627 (M-72).

His_6–LctM assay with His_6–LctA I–4G. MALDI–TOF MS calcd. 7653 (M), 7581 (M-72), found 7582 (M-72).

His_6–LctM assay with His_6–LctA G–2V. MALDI–TOF MS calcd. 7751 (M), 7679 (M-72), found 7680 (M-72).

His_6-LctM Assay with His_6-LctA G–2E. MALDI-TOF MS calcd. 7782 (M), 7710 (M-72), found 7746 (M-36), 7728 (M-54), 7711 (M-72), 7785 (M-72+79).

His_6-LctM Assay with His_6-LctA G–2K. MALDI-TOF MS calcd. 7781 (M), 7709 (M-72), found 7740 (M-36), 7705 (M-72), 7786 (M-72+79).

His_6–LctM assay with His_6–LctA A–1G. MALDI–TOF MS calcd. 7696 (M), 7624 (M-72), found 7702 (M), 7631 (M-72).

His_6–LctM assay with His_6–LctA A–1D. MALDI–TOF MS calcd. 7753 (M), 7681 (M-72), found 7678 (M-72).

His_6-LctM Assay with His_6-LctA A–1I. MALDI-TOF MS calcd. 7752 (M), 7680 (M-72), found 7715 (M-36), 7681 (M-72), 7759 (M-72+79).

His_6-LctM Assay with His_6-LctA A–1K. MALDI-TOF MS calcd. 7767 (M), 7695 (M-72), found 7712 (M-54), 7696 (M-72), 7777 (M-72+79).

His_6-LctM assay with His_6-LctA E13A. MALDI-TOF MS calcd. 7652 (M), 7580 (M-72), found 7584 (M-72).

His_6-LctM assay with pET15b His_6-MutLctA. MALDI-TOF MS calcd. 8081 (M), 8009 (M-72), found 8024 (M-54), 8007 (M-72).

pET15b His_6-LctNukA. MALDI-TOF MS calcd. 7768 (M), found 7772 (M).

His_6-LctM assay with pET15b His_6-LctNukA. MALDI-TOF MS calcd. 7768 (M), 7696 (M-72), found 7703 (M-72).

His_6-LctM and PMBA assay with pET15b His_6-LctNukA. MALDI-TOF MS calcd. 7768 (M), 7696 (M-72), 8016 (M-72+HgAr), 8336 (M-72+2HgAr), 8656 (M-72+3HgAr), found 7772 (M-72).

His_6-LctM C781A/C836A and PMBA assay with pET15b His_6-LctNukA. MALDI-TOF MS calcd. 7768 (M), 7696 (M-72), 8016 (M-72+HgAr), 8336 (M-72+2HgAr), 8656 (M-72+3HgAr), found 8025 (M-72+HgAr).

His_6-LctM assay with pET15b His_6-LctMutA. MALDI-TOF MS calcd. 8556 (M), 8484 (M-72), found 8485 (M-72).

His_6-LctM and PMBA assay with pET15b His_6-LctNukA. MALDI-TOF MS calcd. 8556 (M), 8484 (M-72), 8804 (M-72+HgAr), 9124 (M-72+2HgAr), 9444 (M-72+3HgAr), found 8479 (M-72).

His_6-LctM C781A/C836A and PMBA assay with pET15b His_6-LctNukA. MALDI-TOF MS calcd. 8556 (M), 8484 (M-72), 8804 (M-72+HgAr), 9124 (M-72+2HgAr), 9444 (M-72+3HgAr), found 8800 (M-72+HgAr).

His_6-LctM assay with pET15b His_6-LctRumA. MALDI-TOF MS calcd. 7857 (M), 7785 (M-72), found 7784 (M-72).

His_6-LctM and PMBA assay with pET15b His_6-LctRumA. MALDI-TOF MS calcd. 7857 (M), 7785 (M-72), 8105 (M-72+HgAr), 8425 (M-72+2HgAr), 8745 (M-72+3HgAr), found 7785 (M-72).

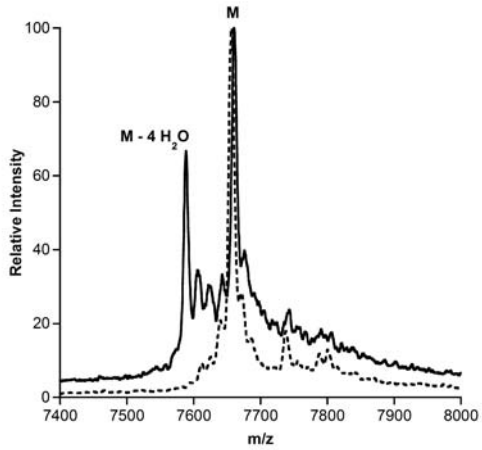
His_6-LctM C781A/C836A and PMBA assay with pET15b His_6-LctRumA. MALDI-TOF MS calcd. 7857 (M), 7785 (M-72), 88105 (M-72+HgAr), 8425 (M-72+2HgAr), 8745 (M-72+3HgAr), found 8106 (M-72+HgAr), 8428 (M-72+2HgAr).

His_6-LctM assay with pET15b His_6-NukA. MALDI-TOF MS calcd. 8435 (M), 8363 (M-72), found 8366 (M-72).

PHMB experiments with the cyclization deficient LctM mutant (LctM-C781A/C836A)
The formation of only one mercury aryl adduct when a cyclization deficient LctM mutant was used with LctNukA suggests that one Cys contains a free thiol whereas the other two

Cys are unreactive. It is assumed that these latter two Cys underwent a non-enzyme catalyzed cyclization reaction. Nonenzymatic reaction of cysteines with Dha is rapid and significantly faster than reaction with Dhb (*2-4*), and therefore we hypothesize that two of the cysteines reacted with the two Dha residues, leaving one Cys with a free thiol.

A E–13A



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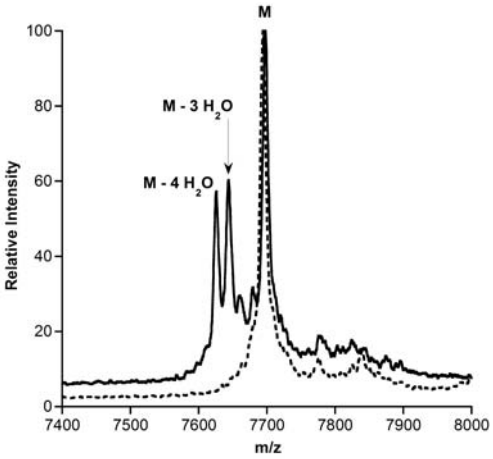
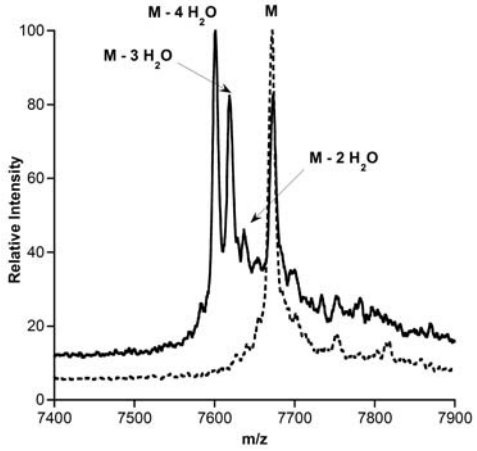
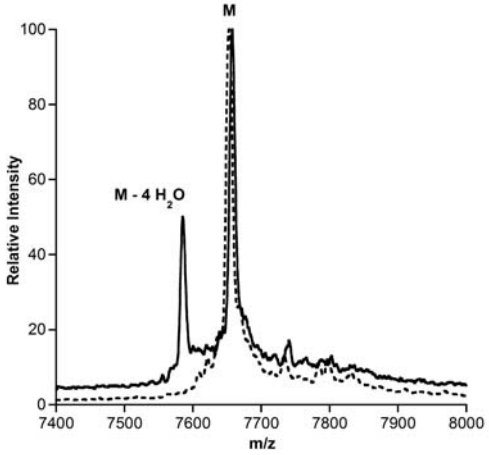


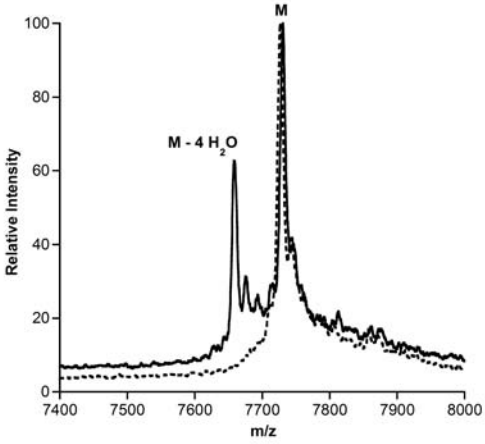
C L–7A



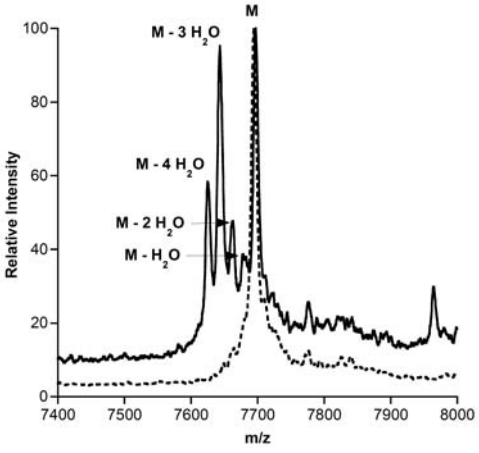
D D–6G



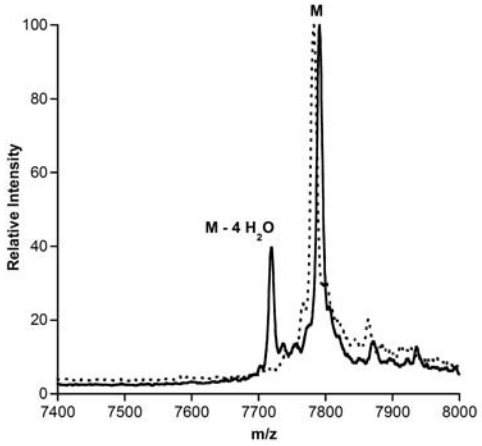
E L–5Q



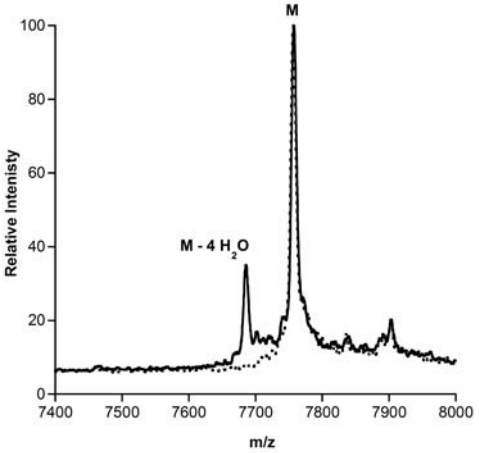
F* I–4P



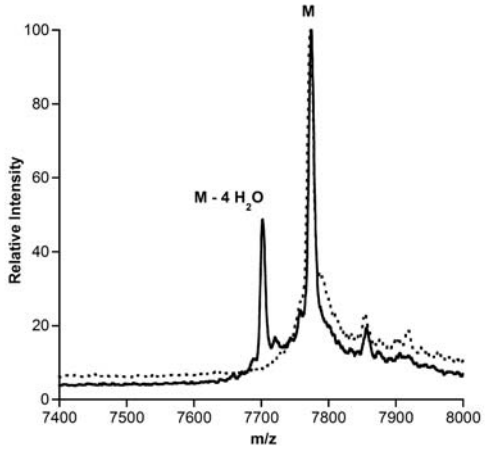
G G–2K



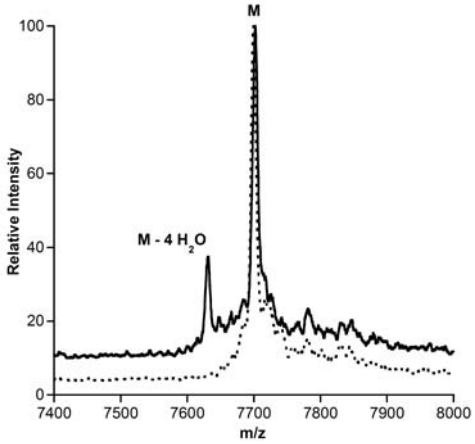
H G–2V



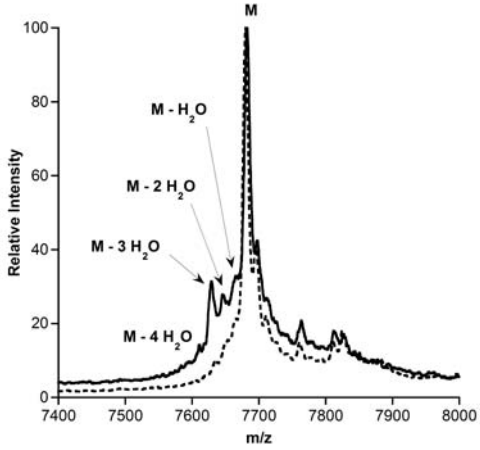
I A–1K



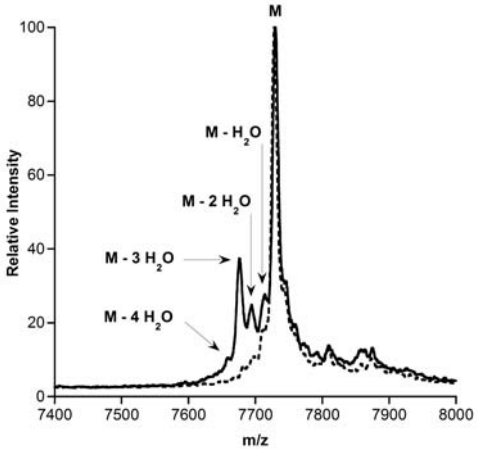
J** A–1G



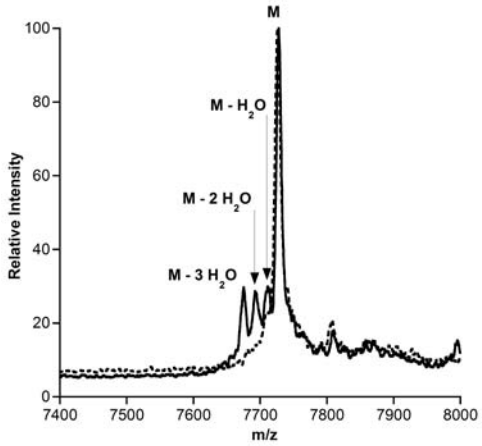
K E–8P



L L–7E



M L–7K



N* D–6P

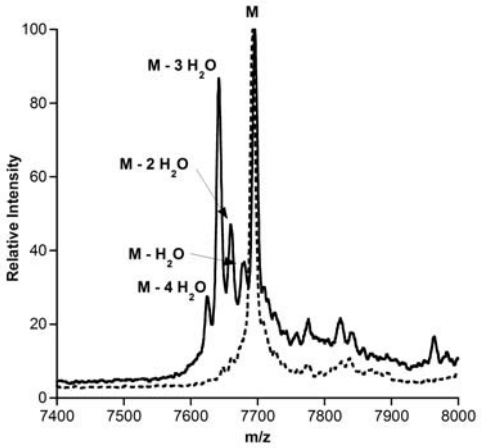
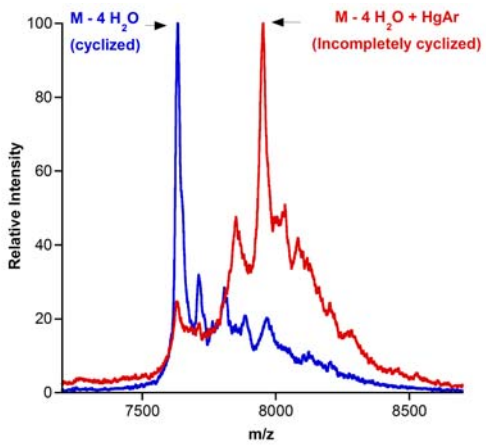
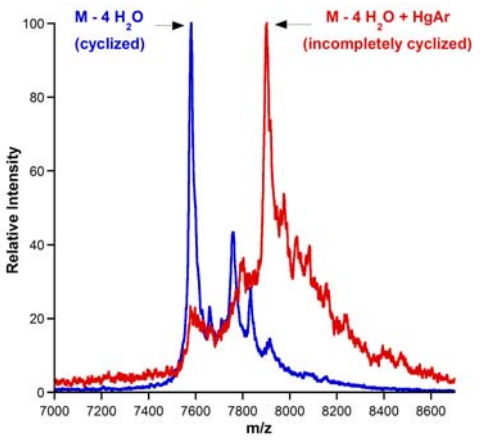


Figure S1. Activity of LctM with LctA leader mutants. MALDI–TOF mass spectra of LctA taken before (dashed) and 10 min after LctM addition (solid). (A) LctA E–13A, (B) LctA L–5P, (C) LctA L–7A, (D) LctA D–6G, (E) LctA L–5Q, (F) LctA I–4P, (G) LctA G–2K, (H) LctA G–2V, (I) LctA A–1K, (J) LctA A–1G, (K) LctA E–8P, (L) LctA L–7E, (M) LctA L–7K, (N) LctA D–6P *The time point shown is 15 min rather 10 min. **Due to the high insolubility of A24G, the time point shown is 20 min rather than 10 min.

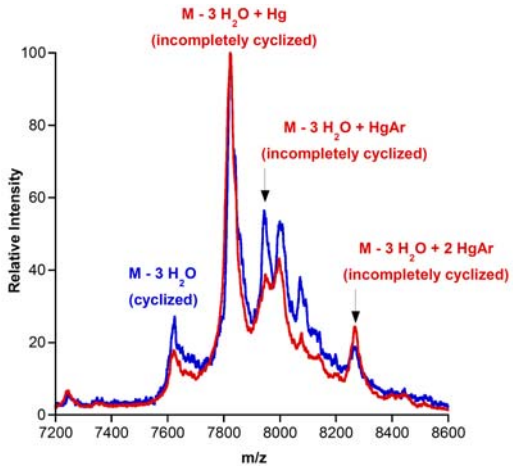
A LctA



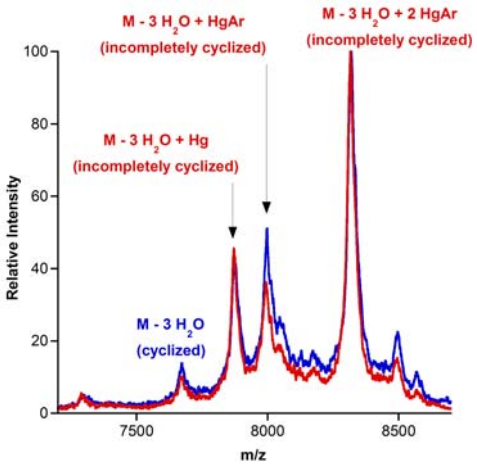
B E–8A



C E–8P



D L–7E



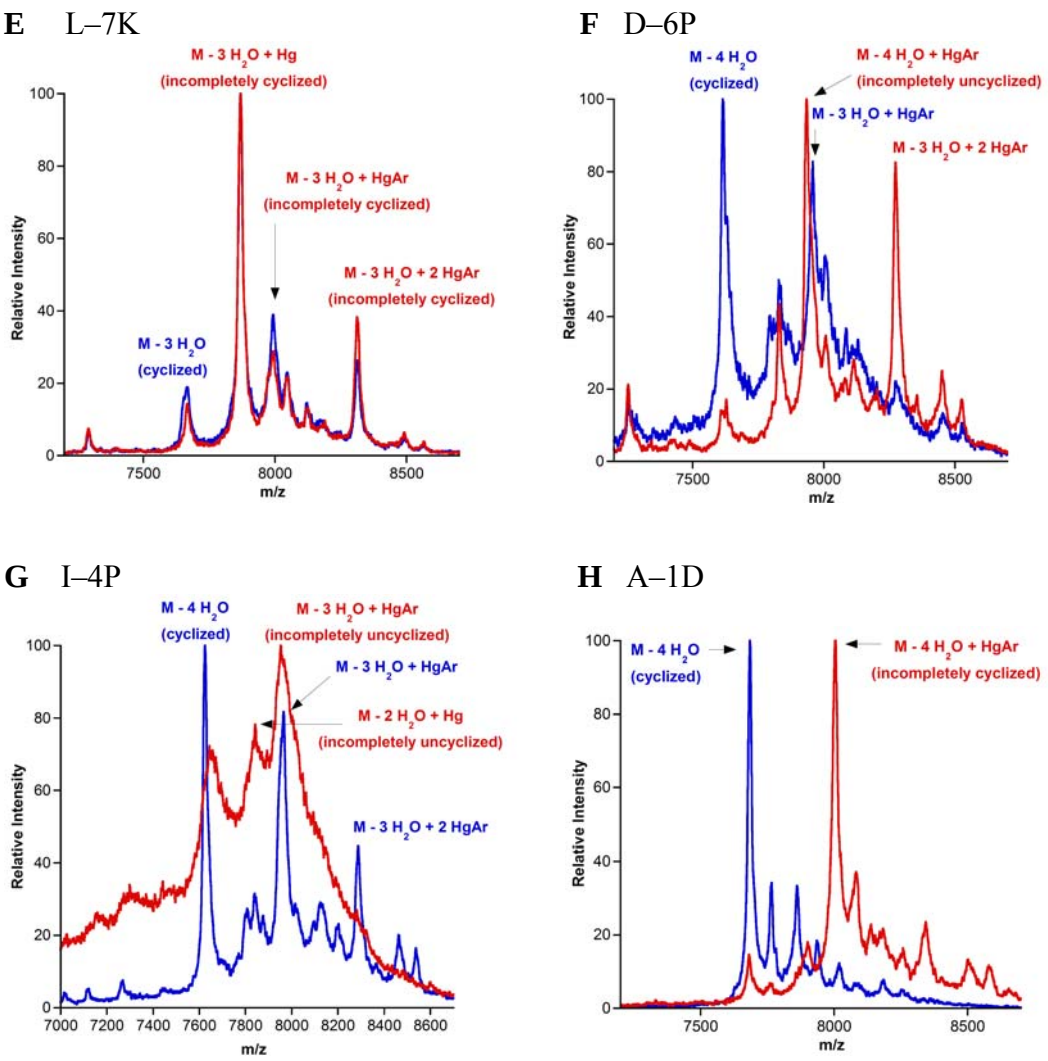
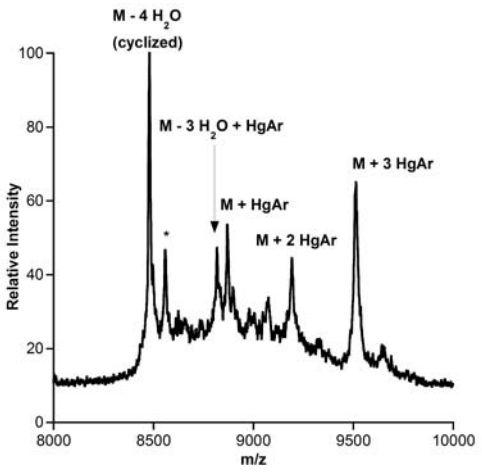


Figure S2. Probing the extent of cyclization of the leader mutants using PHMB. Incubation with LctM followed by PHMB is depicted in blue while assays with a cyclization deficient enzyme, LctM C781A/C836A, and PHMB are displayed in red. (A) LctA (B) LctA E–8A, (C) LctA E–8P, (D) LctA L–7E, (E) LctA L–7K, (F) LctA D–6P**,** (G) LctA I–4P, (H) LctA A–1D.

A



B

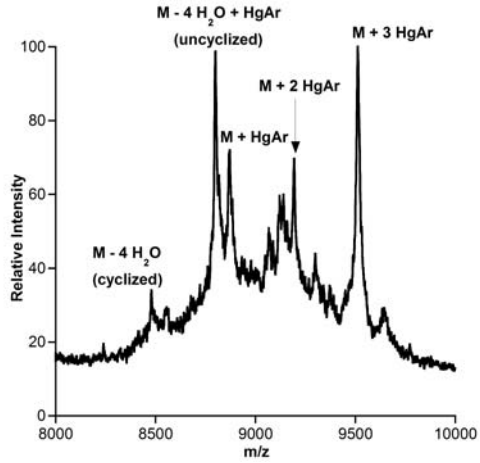
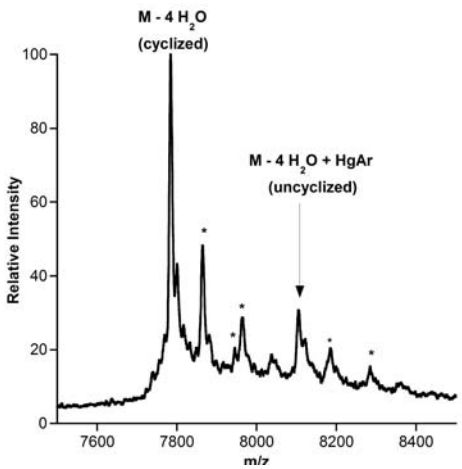


Figure S3. MALDI-MS data of treatment of LctMutA with (A) LctM and PMBA, (B) LctM C781A/C836A and PMBA. The (*) indicates phosphorylated peptides some of which have mercury aryl adducts. Note that in panel A, no peaks are detected for M – 4 H_2O + adduct(s) indicating that upon complete fourfold dehydration, cyclization of all three Cys residues is catalyzed by LctM. Partially dehydrated products on the other hand do have free Cys residue(s) remaining resulting in adduct formation.

A



B

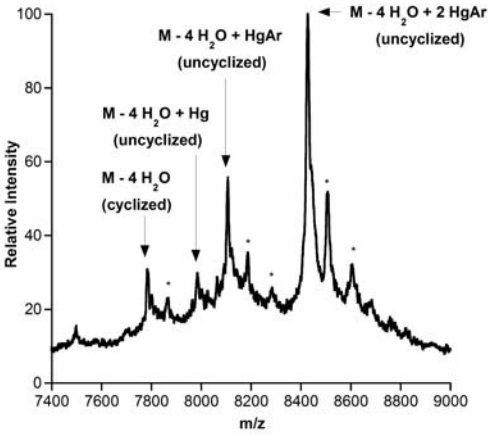
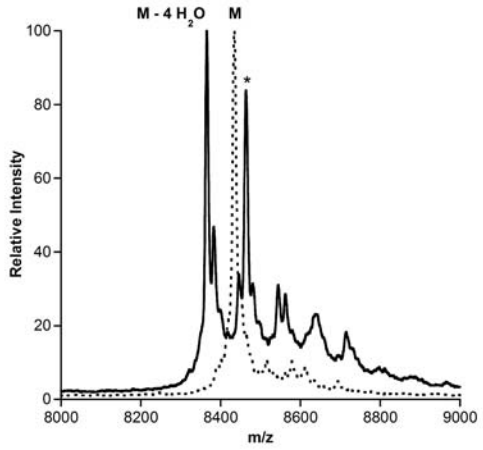


Figure S4. MALDI-MS data of treatment of LctRumA with (A) LctM and PHMB, (B) LctM C781A/C836A and PHMB. The (*) indicates phosphorylated peptides some of which have mercury aryl adducts. The small peak for M – 4 H_2O +HgAr in panel A indicates that some of the fully dehydrated product is not cyclized. However, in comparison with panel B it is clear that a large fraction of the product in panel A is cyclized.

A



B

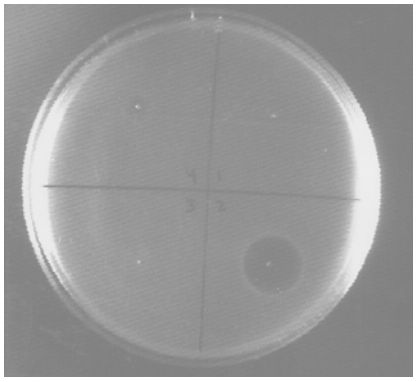
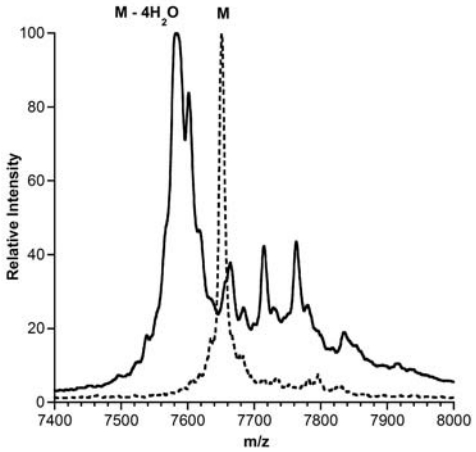


Figure S5. (A) MALDI–TOF mass spectra of NukA taken before (dashed) and after LctM addition (solid). The (*) indicates a phosphorylated peptide (5). (B) Assessment of antibiotic activity against *L. lactis* 117 . Wedge 1, sterile water; Wedge 2, cell free supernatant of lacticin 481 producer *L. Lactis* CNRZ 481; Wedge 3, NukA linear peptide; Wedge 4, NukA treated with LctM and Lys-C.

A



B

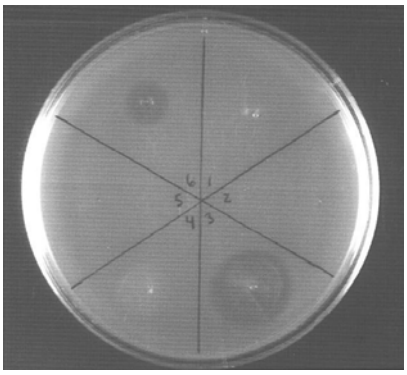


Figure S6. Assay of LctA E13A with LctM. (A) MALDI-TOF mass spectrum of LctA before (dotted) and after (solid) treatment with LctM. (B) Assessment of antibiotic activity against *L. lactis* 117 . Wedge 1, sterile water; Wedge 2, Lys-C buffer; Wedge 3, cell free supernatant of lacticin 481 producer *L. Lactis* CNRZ 481; Wedge 4, LctA E13A peptide; Wedge 5 LctA E13A treated with LctM; Wedge 6, LctA E13A treated with LctM and Lys-C.

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