

Features of the endolysin Lys11 and derivatives used in this study

Relevant features of Lys11 and its derivatives are denoted in the corresponding primary sequences with colored lettering and shading.

Theoretical molecular masses, pI values and net charge at pH 7 (z) of Lys11 and its variants were determined with the Prot pi Protein tool (<https://www.protpi.ch/Calculator/ProteinTool>), using ExPASy as the data source of pKa values.

- **Protein domains**, according to InterPro (<https://www.ebi.ac.uk/interpro/>):

CHAP (IPR007921), here designated **CHAP₁₁**

Amidase (IPR002502), here designated **Ami₁₁**

SH3-like (IPR003646), here designated **CBD₁₁**

eGFP (IPR000786)

- **Linkers** were defined based on an integrated analysis with InterPro (for domain boundaries) and AlphaFold structure prediction of LytO (same as Lys11, <https://alphafold.ebi.ac.uk/entry/Q2FX77>).

- **PGGGSHHHHHH** is the vector-borne C-terminal tail containing the hexahistidine tag.

- “**E**” denotes a Q₂E substitution introduced in the native Lys11 sequence resulting from the use of the restriction enzyme *Nco*I recognition sequence in the cloning procedures.

Endolysin Lys11

>Lys11 (55.21 kDa, pI = 8.49, z = +4.79)

M**EAKLT**KNEFIEWLKTSEGGKQFNVDLWYGFQCFDYANAGWKVLFGLLLKGLGAKDIPFANNFDGLATVYQNTF
D**FLAQ**PGDMVVFGSNYGAGYGHVAWVIEATLDYIIIVYEQNWLGGGWTGIEQPGWGWEKVTRRQHAYDFPMWF
IRPNFKSETAPRSVQSPTQAPKKETAKPQPKAVELKIIKDV**V**KGYDLPKRGSNPKGIVIHNDAGSKGATAEAY
R**NGLV**NAPLSRLEAGIAHSYVSGNTVWQALDESVGWHTANQIGNKYYYGIEVCQSMGADNATFLKNEQATFQ
E**CARLL**KKWGLPANRNTIRLHNEFTSTSCPHRSSLHTGFDPVTRG**LL**PEDKRLQLKDYFIKQIRAYMDGKIP
VATVSNESSASSNTVKPVASAWKRNKYGT**Y**MEESARFTNGNQ**PIT**VRKVGPF**LSCP**VG**YQFQ**PGGYCDYTEV
MLQDGHVWVG**YT**WEGQRY**YLP**IRTWNGSAPPNQILGDLWGEIS**PGGGSHHHHHH**

Lys11 catalytic domain deletion mutants

>CHAP₁₁-CBD₁₁ (31.89 kDa, pI = 5.83, z = -5.72)

M**EAKLT**KNEFIEWLKTSEGGKQFNVDLWYGFQCFDYANAGWKVLFGLLLKGLGAKDIPFANNFDGLATVYQNTF
D**FLAQ**PGDMVVFGSNYGAGYGHVAWVIEATLDYIIIVYEQNWLGGGWTGIEQPGWGWEKVTRRQHAYDFPMWF
IRPN**DGKI**PVATVSNESSASSNTVKPVASAWKRNKYGT**Y**MEESARFTNGNQ**PIT**VRKVGPF**LSCP**VG**YQFQ**
GGYCDYTEV**MLQ**DGHVWVG**YT**WEGQRY**YLP**IRTWNGSAPPNQILGDLWGEIS**PGGGSHHHHHH**

>Ami₁₁-CBD₁₁ (35.15 kDa, pI = 8.93, z = +6.61)

MVELKIIKDV**V**KGYDLPKRGSNPKGIVIHNDAGSKGATAEAYR**NGLV**NAPLSRLEAGIAHSYVSGNTVWQALD
ESQVGWHTANQIGNKYYYGIEVCQSMGADNATFLKNEQATFQ**E**CARLLKKWGLPANRNTIRLHNEFTSTSCPH

RSSVLHTGFDPVTRGLLPEDKRLQLKDYFIKQIRAYMDGKIPVATVSNESSASSNTVKPVASAWKRNKYGTYY
MEESARFTNGNQPIITVRKVGPFLLSCPVGYYQFQPGGYCDYTEVMLQDGHVWVGYTWEQGQRYLLPIRTWNGSAPP
NQILGDLWGEISPGGGSHHHHHH

eGFP fusions to Lys11 domains

>eGFP-Ami₁₁-CBD₁₁ (65.17 kDa, pI = 7.99, z = +3.40)

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPPTLVTTLTLYGVQCFS
RYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNY
NSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDHM
VLLEFVTAAGITLGMDELYKPNFKSETAPRSVQSPTQAPKKETAKPQPKAVELKIIKDVVKGYDLPKRGSNPK
GIVIHNDAGSKGATAEAYRNLVNAPLSRLEAGIAHSYVSGNTVWQALDESQVGWHTANQIGNKYYYGIEVCQ
SMGADNATFLKNEQATFQECARLLKKWGLPANRNTIRLHNEFTSTSCPHRSSVLHTGFDPVTRGLLPEDKRLQ
LKDYFIKQIRAYMDGKIPVATVSNESSASSNTVKPVASAWKRNKYGTYYMEESARFTNGNQPIITVRKVGPFLLS
CPVGYYQFQPGGYCDYTEVMLQDGHVWVGYTWEQGQRYLLPIRTWNGSAPPNQILGDLWGEISPGGGSHHHHHH

>eGFP-Ami₁₁ (51.65 kDa, pI = 8.10, z = +3.33)

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPPTLVTTLTLYGVQCFS
RYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNY
NSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDHM
VLLEFVTAAGITLGMDELYKPNFKSETAPRSVQSPTQAPKKETAKPQPKAVELKIIKDVVKGYDLPKRGSNPK
GIVIHNDAGSKGATAEAYRNLVNAPLSRLEAGIAHSYVSGNTVWQALDESQVGWHTANQIGNKYYYGIEVCQ
SMGADNATFLKNEQATFQECARLLKKWGLPANRNTIRLHNEFTSTSCPHRSSVLHTGFDPVTRGLLPEDKRLQ
LKDYFIKQIRAYMPGGGSHHHHHH

>eGFP-CBD₁₁ (41.50 kDa, pI = 6.01, z = -7.19)

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPPTLVTTLTLYGVQCFS
RYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNY
NSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDHM
VLLEFVTAAGITLGMDELYKD G KIPVATVSNESSASSNTVKPVASAWKRNKYGTYYMEESARFTNGNQPIITVR
KVGPFLLSCPVGYYQFQPGGYCDYTEVMLQDGHVWVGYTWEQGQRYLLPIRTWNGSAPPNQILGDLWGEISPGGG
SHHHHHH