

Supplementary Materials

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1. Supplementary information

1-1. Amino acid sequence of LysM-Q (pI/Mw: 6.99 / 8739.65)

MCTTYTIKSGDTCYAI SQARGISL SDFESWNAGIDC NNLQIGQVVCVSKPSTSTTPSPTPSSSSNGFYPLQ
MRGGHHHHHH

1-2. Amino acid sequence of LysM-muGFP-Q (pI/Mw: 6.04 / 35078.23)

MCTTYTIKSGDTCYAI SQARGISL SDFESWNAGIDC NNLQIGQVVCVSKPSTSTTPSPTPSSSSNGHHHHHH
SKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLT LKFICTTGKLPVPWPTLVTTLT YGVLCFSR
YPDHMKRHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYN
NSHNVYITADKQKNGIKAYFKIRHNVEDGSGVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDH
MVLLEDVTAAGITHGMDELYRGGGGSLLQG

1-3. Amino acid sequence of LysM-TD linker-Q (pI/Mw: 6.04 / 35078.23)

MCTTYTIKSGDTCYAI SQARGISL SDFESWNAGIDC NNLQIGQVVCVSKPSTSTTPGSLVPRGSSPTPSSSS
NGFYPLQMRGGHHHHHH

Brown: LysM2 domain

Blue: Linker sequences derived from PrChia

Purple: Microbial transglutaminase-reactive Gln-containing tag (FQ-tag)

Green: Hexahistidine tag

Dark Green: muGFP

Orange: Microbial transglutaminase-reactive Gln-containing tag (LQ-tag)

Pink: Thrombin linker

2. Supplementary results

2-1. Zeta potential LysM-Q or LysM-lipid

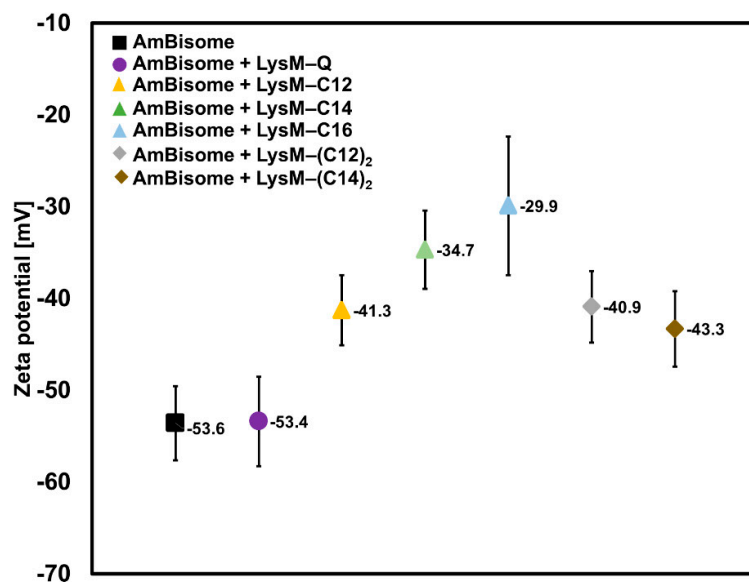


Figure S1. ζ -potential of AmBisome with LysM-Q or LysM-lipid.

2-2. Stability of LysM-lipid-AmBisome for three days at 25°C

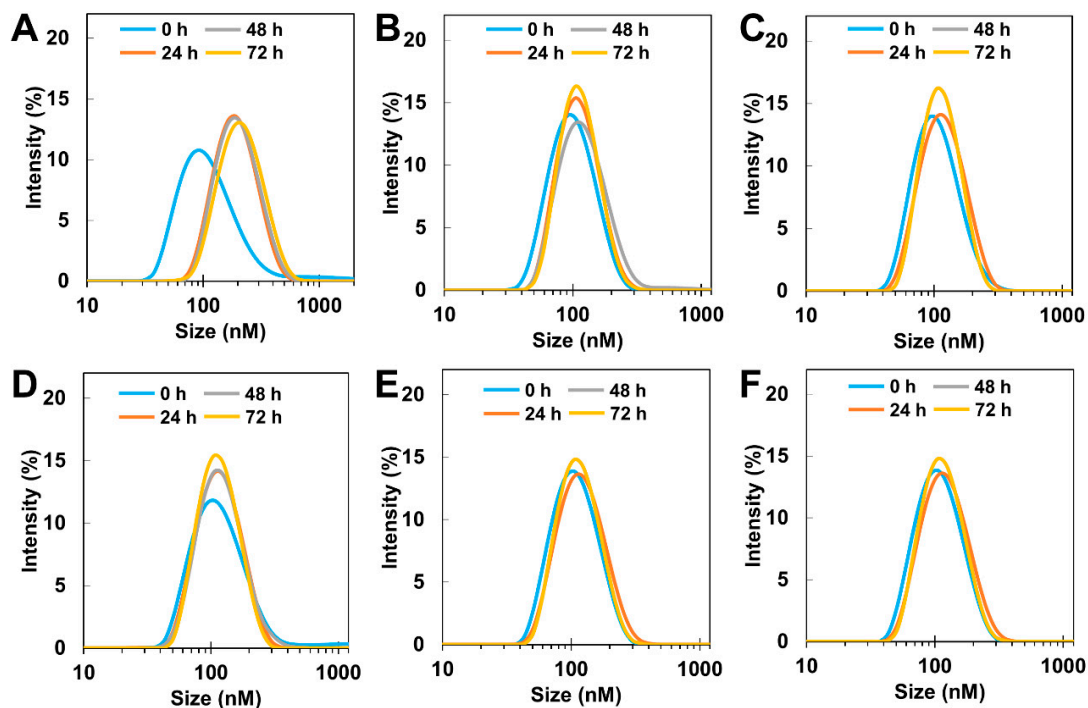


Figure S2. DLS measurements of AmBisome with (A) LysM-Q; (B) LysM-C12; (C) LysM-C14; (D) LysM-C16; (E) LysM-(C12)₂; and (F) LysM-(C14)₂ during three days of incubation at 25 °C.

2-3. CLSM Analysis of Rhod-Lipo with α -chitin

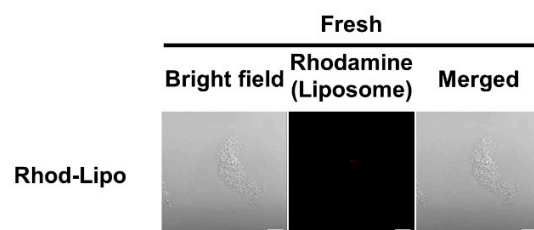


Figure S3. CLSM analysis of Rhod-Lipo (50 μ M) in the presence of 0.5% α -chitin in 20 mM sodium phosphate buffer, pH 7.4, at 25 $^{\circ}$ C (bars: 20 μ m).

2-4. Quantitative results of fluorescent intensity of LysM-lipid with Rhod-Lipo

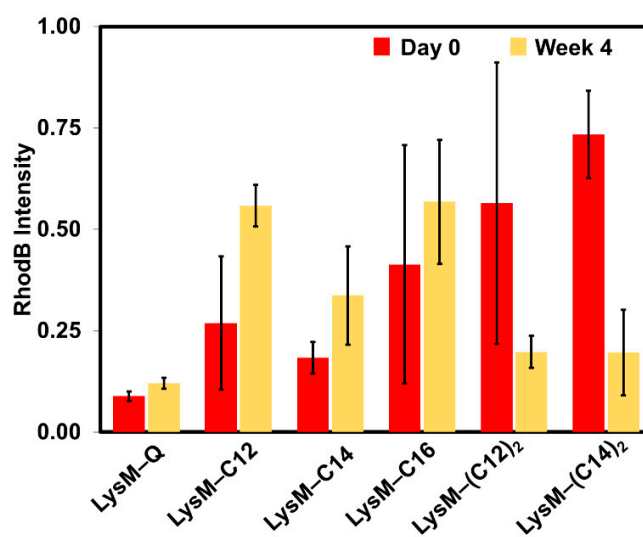


Figure S4. Results of intensity of LysM-lipid labeled with Rhod-Lipo.

2-5. Qualitative results of antifungal activity test for lipid-K

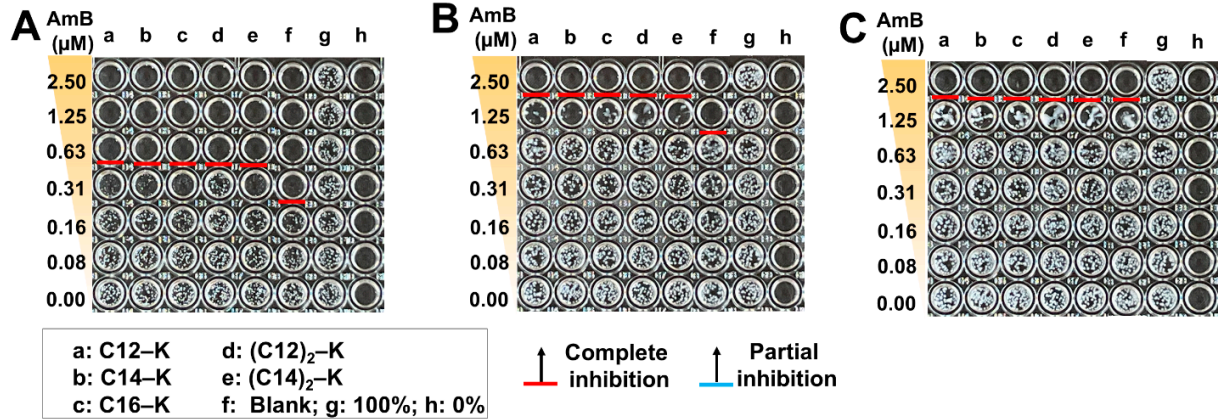


Figure S5. Representative image of a 96-well plate after culturing *C. albicans* in the presence of 0–2.5 μM of AMB with 1 μM of each sample at (A) 24 h, (B) 48 h and (C) 72 h in 20 mM NaPi, pH 7.4, humidity 90% at 35°C.

2-6. Estimation of anchoring ratio of LysM-muGFP-lipid to AmBisome

Table S1. Estimation of the number of LysM-muGFP-lipid or -(lipid)₂ on AmBisome

Samples	Anchoring ratio of LysM-Lipid to AmBisome			Estimation of Number of LysM-lipid on AmBisome
	Total _{LysM-Lipid}	Anch _{LysM-Lipid}	Anchoring ratio	
LysM-muGFP-Q	21.3	1.19	0.0559	457 LysM-muGFP-Q/AmBisome
LysM-muGFP-C12	22.5	2.24	0.0996	814 LysM-muGFP-C12/AmBisome
LysM-muGFP-C14	21.9	3.57	0.163	1332 LysM-muGFP-C14/AmBisome
LysM-muGFP-C16	18.9	3.5	0.1852	1514 LysM-muGFP-C16/AmBisome
LysM-muGFP-(C12) ₂	21.9	6.58	0.3005	2456 LysM-muGFP-(C12) ₂ /AmBisome
LysM-muGFP-(C14) ₂	21.9	6.8	0.3105	2538 LysM-muGFP-(C14) ₂ /AmBisome

Footnote: Total_{LysM-lipid}, the total amount of LysM-muGFP-lipid used in this experiment; Anch_{LysM-lipid}, the amount of anchored LysM-muGFP-lipid.

2-7. Conjugation of Q-Tagged LysM with thrombin linker by MTG

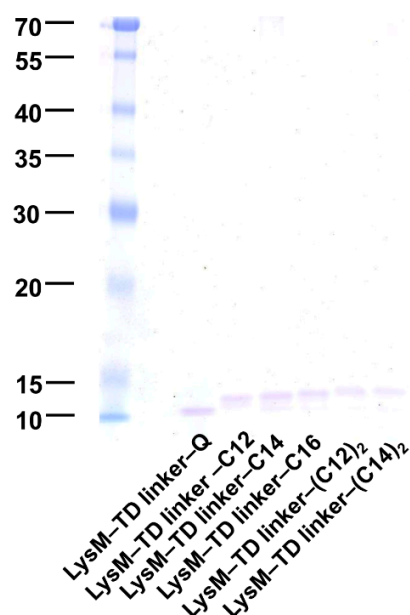


Figure S6. Bioconjugate of the Q-tagged LysMs. (SDS-PAGE analysis results of unmodified LysM-TD linker-Q and LysM-TD linker-Q modified with C12-K, C14-K, C16-K, (C12)₂-K, and (C14)₂-K by MTG. All conjugation reactions were carried out under conditions of 10 μ M LysM-TD linker-Q, 1% DDM, 10 μ M Lipid-K, and 0.1 U/mL MTG in 10 mM Tris-HCl (pH 8.0) at 37 °C for 1 h.

2-8. Zeta potential LysM-TD linker-Q or LysM-TD linker-lipid

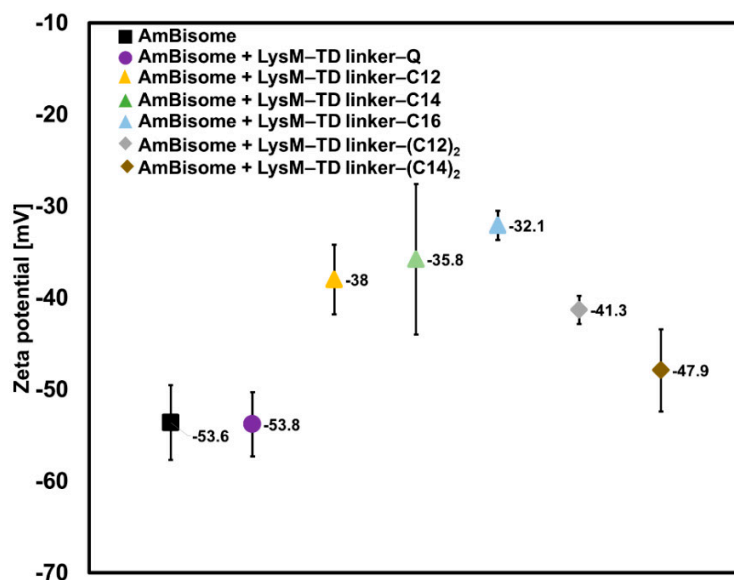


Figure S7. ζ -potential of AmBisome with LysM-TD linker-Q or LysM-TD linker-lipid.

2-9. Qualitative results of antifungal activity test for thrombin

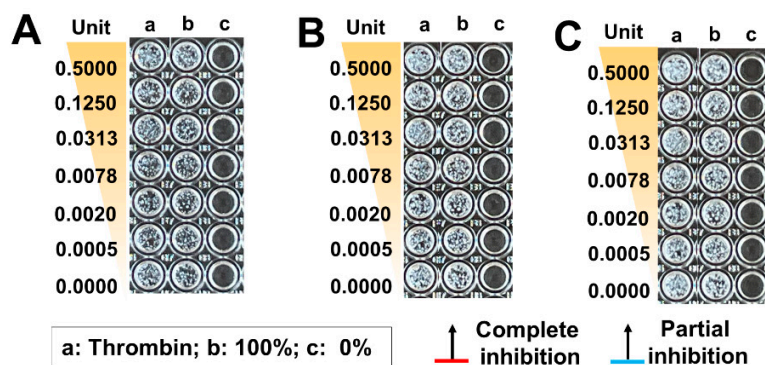


Figure S8. Representative image of a 96-well plate after culturing *C. albicans* in the presence of 0–0.5 Unit of thrombin at (A) 24 h, (B) 48 h and (C) 72 h in 20 mM NaPi, pH 7.4, humidity 90% at 35°C.

2-10. Evaluation of the cleavage of TD linker by thrombin

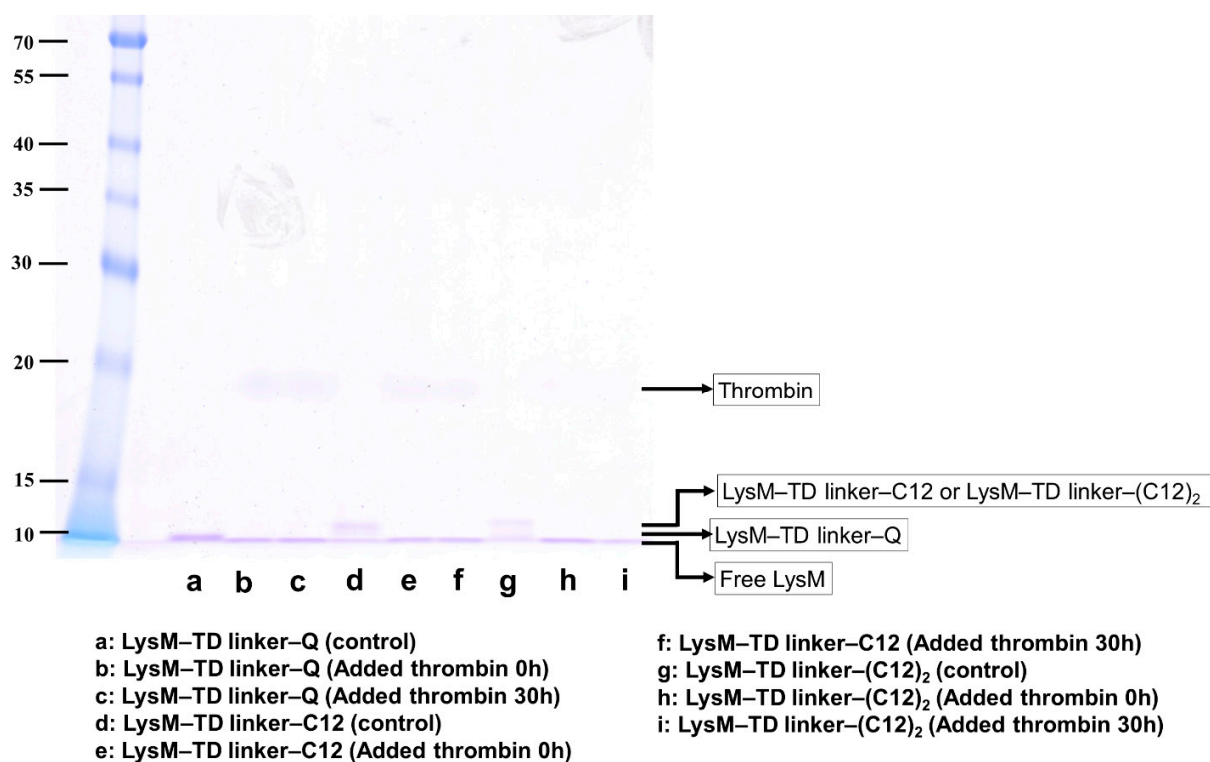


Figure S9. SDS-PAGE analysis of thrombin activity assay after different incubation time of LysM–TD linker–Q, LysM–TD linker–C12 and LysM–TD linker–(C12)₂ at 35°C.