Artificial activation of *Escherichia coli mazEF* and *hipBA* toxin-antitoxin systems by antisense peptide nucleic acids as an antibacterial strategy

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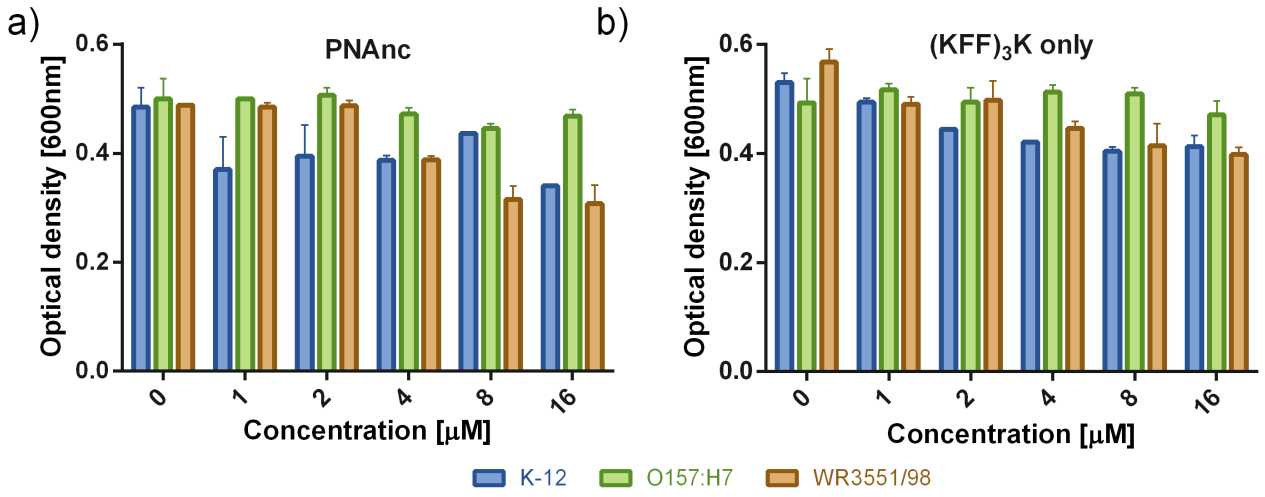
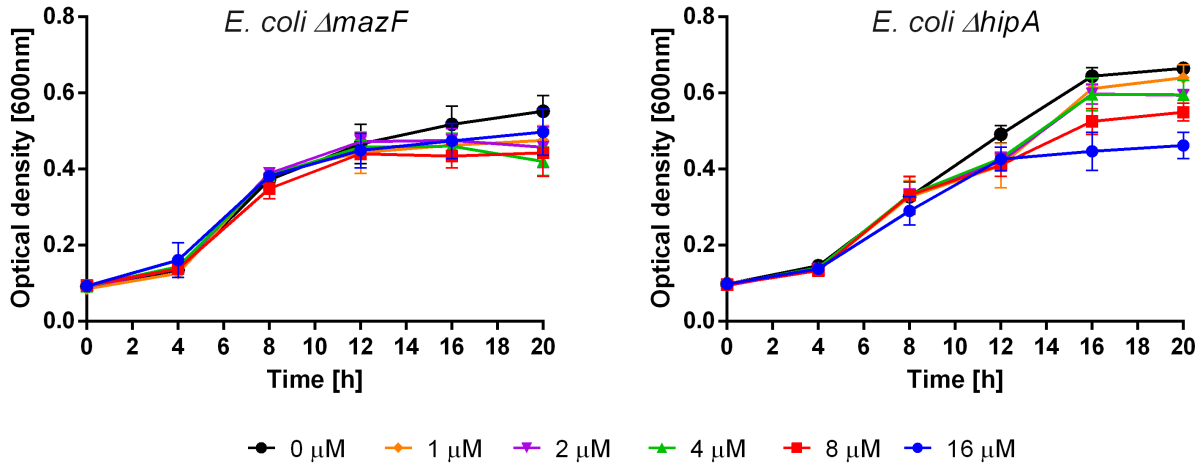
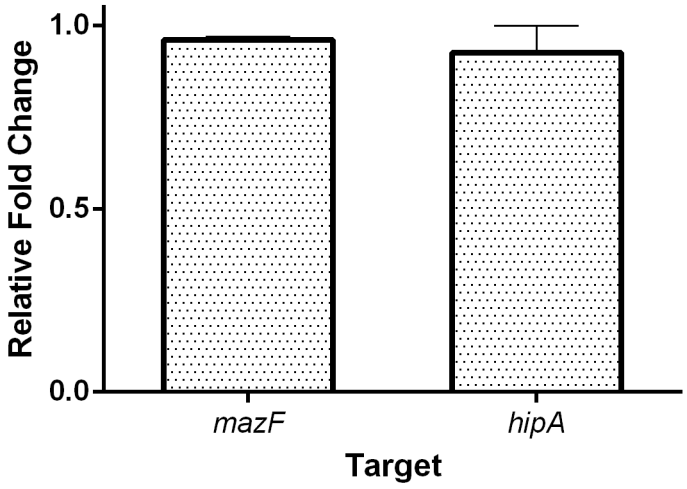


Supplementary Figure 1. No growth inhibition of *E. coli* strains after 20 h incubation with a) PNAnc and b) $(KFF)_3K$ peptide. Error bars represent the standard error of the mean of 3 replicates. The differences between corresponding samples are not significant ($P > 0.05$).



Supplementary Figure 2. No growth inhibition of *E. coli* strains lacking toxin genes after 20 h incubation with the corresponding PNAs. Error bars represent the standard error of the mean. The differences between 0 µM and 16 µM at 20 h for *E. coli ΔmazF* are not significant ($P > 0.05$) and for *E. coli ΔhipA* are significant ($P < 0.01$).



Supplementary Figure 3. The effect of treatment with PNAs on the level of toxin mRNA transcripts in *E. coli* K-12. The graph shows the relative fold change in expression compared to untreated control samples. Error bars represent the mean ± SEM, n=2.

Supplementary Table 1. Bacterial strains used in this study.

Strains	Relevant characteristics	Reference
E. coli K-12 MG1655	$F^- \lambda^-$ wild-type strain	(Guyer et al., 1981)
E. coli 0157:H7 ST2-8624	(Δ*stx2::cat gfp)*	(Łoś et al., 2008)
E. coli WR3551/98	$bla_{CTX\text{-}M\text{-}15}$ ESBL+	(Baraniak, 2002)
E. coli DH5αλpir	F- *ΔlacU169 (ΦlacZΔM15), recA1, endA1, hsdR17, thi-1, gyrA96, relA1, λpir+*	(Platt et al., 2000)
E. coli β2163	MG1655::*ΔdapA::(erm-pir)RP4-2-Tc::Mu [*Km^R*]*	(Demarre et al., 2005)
E. coli K-12 MG1655 *ΔmazF*	MG1655 (*ΔmazF::kan)*	This study
E. coli JW1500-2 *ΔhipA*	*ΔhipA728::kan*	(Baba et al., 2006)

Supplementary Table 2. Oligonucleotide primers used in this study.

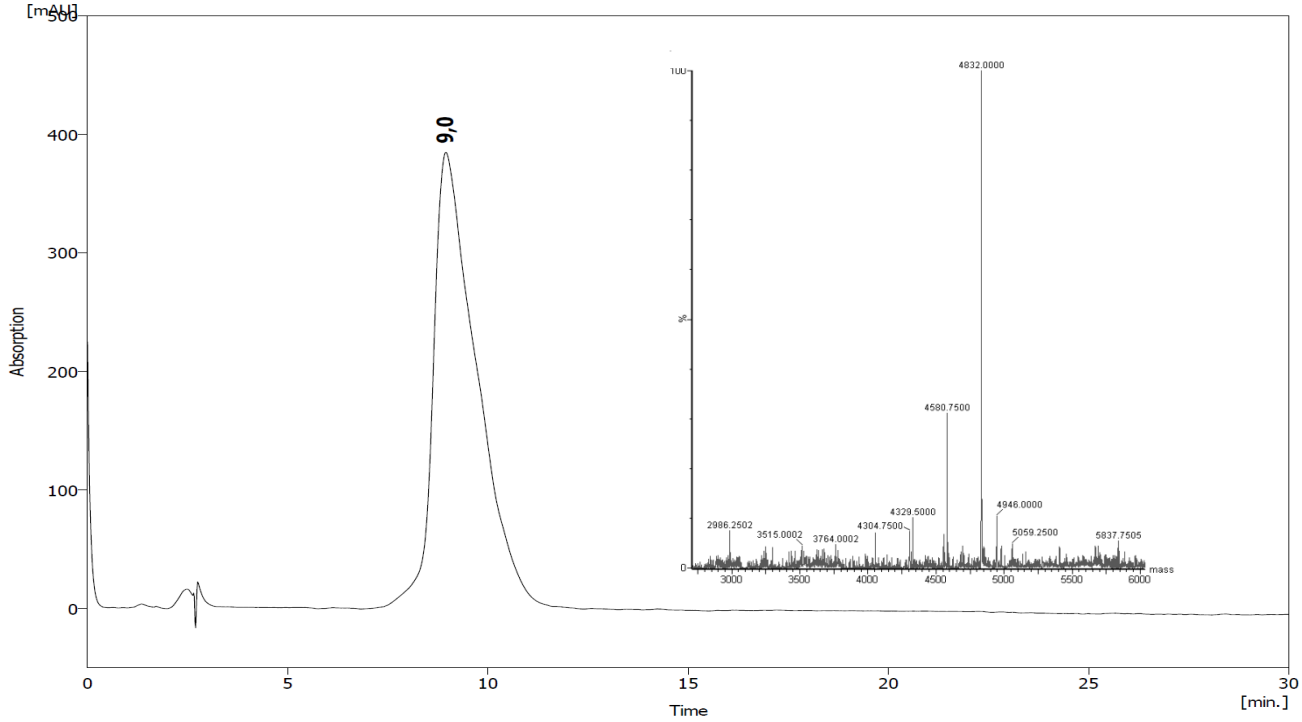
Name	Sequence (5' → 3')	Application
pds132X	CCCATGTCAGCCGTTAAGTG	construction of a cassette for deletion of *mazF*
pds132Y	ATCGCGCGGGTTTGTTACTG	
mazF1	CAGTAACAAACCCGCGCGATCTACTCCGCCG GATATTCGC	
mazF2	AGAGATTTTGAGACACAACGGATCGCCCATA TCGGGTACG	
mazF3	TAATTGGTTGTAACACTGGCAGAGGAGCAAC GAAGAAAGG	
mazF4	CACTTAACGGCTGACATGGGCAAGCGTCGTC CAATCGAAG	
KML	CGTTGTGTCTCAAAATCTCT	
KMR	GCCAGTGTTACAACCAATTA	
LmazFspr	CAAGCGTCGTCCAATCGAAG	confirmation of *mazF* deletion
RmazFspr	GGCGCGGTATTTCAGTACAC	
mazE_F1	AAGCGTTGGGGAAATTCACC	RT-qPCR for *mazE* transcript
mazE_R1	AATTTGCCATCCACCAGGTC	
mazF_F1	ACAAAAGGTAGCGAGCAAGC	RT-qPCR for *mazF* transcript
mazF_R1	GCGTTGTACAAGGAACACACAG	
thyA_F1	GGCCAGTGTATGGTAAACAG	RT-qPCR for *thyA* transcript
thyA_R1	TATCCAGTTCGCCTACGTTC	
hipB_F1	TTAAGCAGGCGACGATTTCC	RT-qPCR for *hipB* transcript
hipB_R1	TTCTGGCGAGGCATTTTTCG	
hipA_F1	TGGCGAAAGAACTTGGGTTG	RT-qPCR for *hipA* transcript
hipA_R1	AACGCCTGTCAAAACGTTCG	
gltX_F1	CGTTTTGATCGCTACAACGC	RT-qPCR for *gltX* transcript
gltX_R1	TTTCGCCATTTGCTCTTCGC	
gyrA_F1	TGGAAGTTGACGCCAAAACC	RT-qPCR for *gyrA* transcript (reference)
gyrA_F2	ATGCCTTCCACGCGTTTTTC	
mazEF-For	GCAGGCGCTGCATAATAGTG	amplification of *mazE* gene for DNA sequencing
mazEF-Rev	TGCGCCAGAACGCATTGTTG	

hipBA-For	GCTGTCTCGCCCTATTTCTG	amplification of *hipB* gene for DNA sequencing
hipBA-Rev	CGCCGTGAAGATGAATGGTC	
gltX-For	TAGAAACGTGCGCGTAAACC	amplification of *gltX* gene for DNA sequencing
gltX-Rev	ACGGCGGAGACTACATAAAG	
thyA-For	TGCTGCGCCATCATATGCAC	amplification of *thyA* gene for DNA sequencing
thyA-Rev	CGCACCACTCATTCCGTTTG	

Supplementary Table 3. Retention times (tR) and molecular masses of the synthesized conjugates. [a]The product was analyzed by RP-HPLC. To increase water solubility, each PNA has a lysine at the C-terminus.

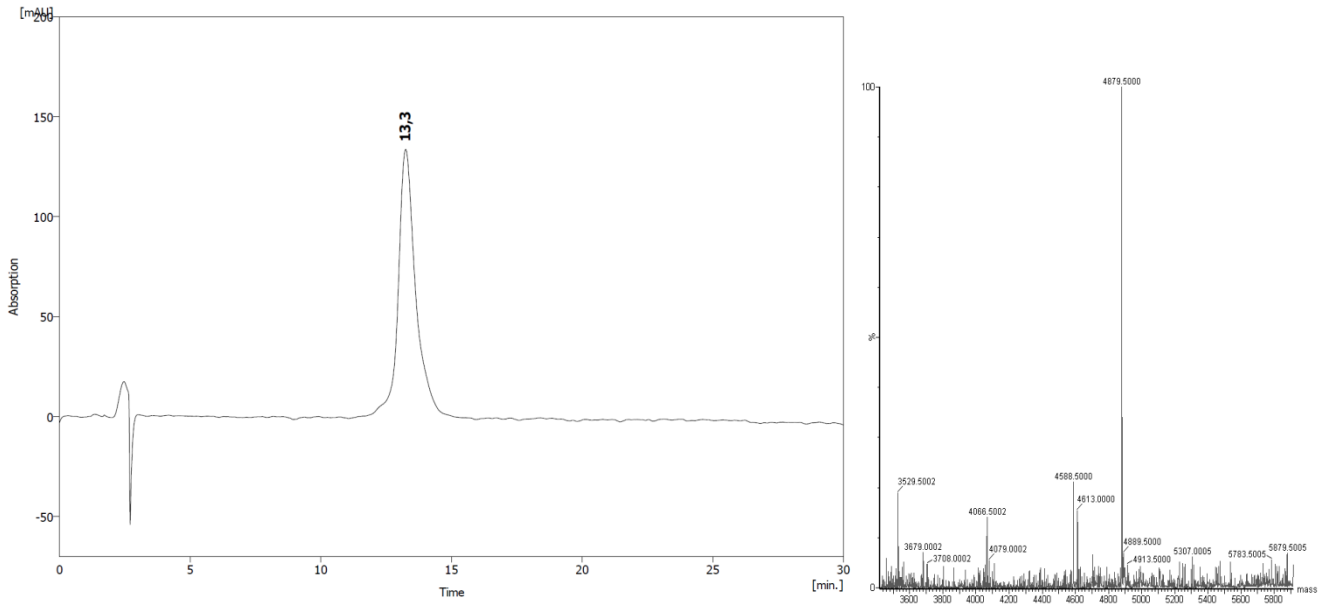
					Molecular mass [g/mol]	
Nr	Compound	Short name	HPLC tR[a] [min]	HPLC method	**Calculated**	**Detected**
1	$(KFF)_3$K-AEEA-PNA anti-*mazE*-K	anti-*mazE* PNA	9.0	26-29% ACN 30 min	4833.17	4832
2	$(KFF)_3$K-AEEA-PNA anti-*thyA*-K	anti-*thyA* PNA	13.3	25-32% ACN 30 min	4880.17	4879.5
3	$(KFF)_3$K-AEEA-PNA anti-*hipB*-K	anti-*hipB* PNA	24.9	0-50% ACN 30 min	4646.87	4646.25
4	$(KFF)_3$K-AEEA-PNA anti-*gltX*-K	anti-*gltX* PNA	20.7	0-50% ACN 30 min	4401.67	4401.5
5	$(KFF)_3$K-AEEA-PNAnc-K	PNAnc	12.7	25-35% ACN 30 min	4880.17	4879.5

(KFF)$_3$K-AEEA-PNA anti-*mazE*-K



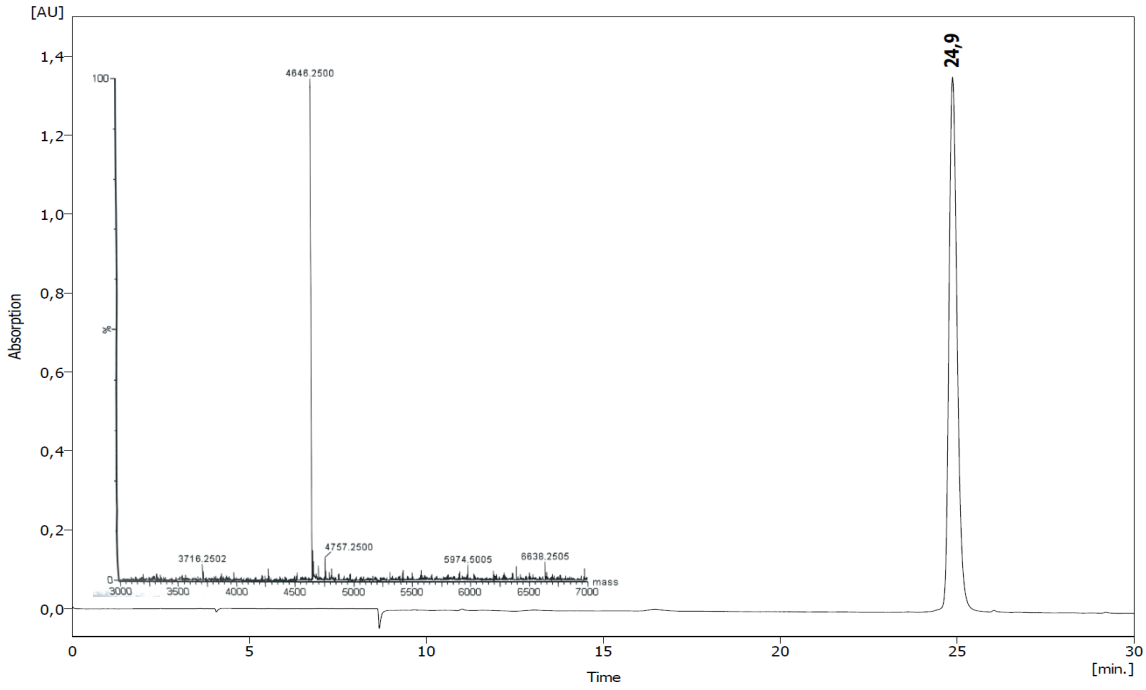
Supplementary Figure 4. Mass spectrum and HPLC chromatogram for (KFF)$_3$K-AEEA-PNA anti-*mazE*-K

(KFF)$_3$K-AEEA-PNA anti-*thyA*-K



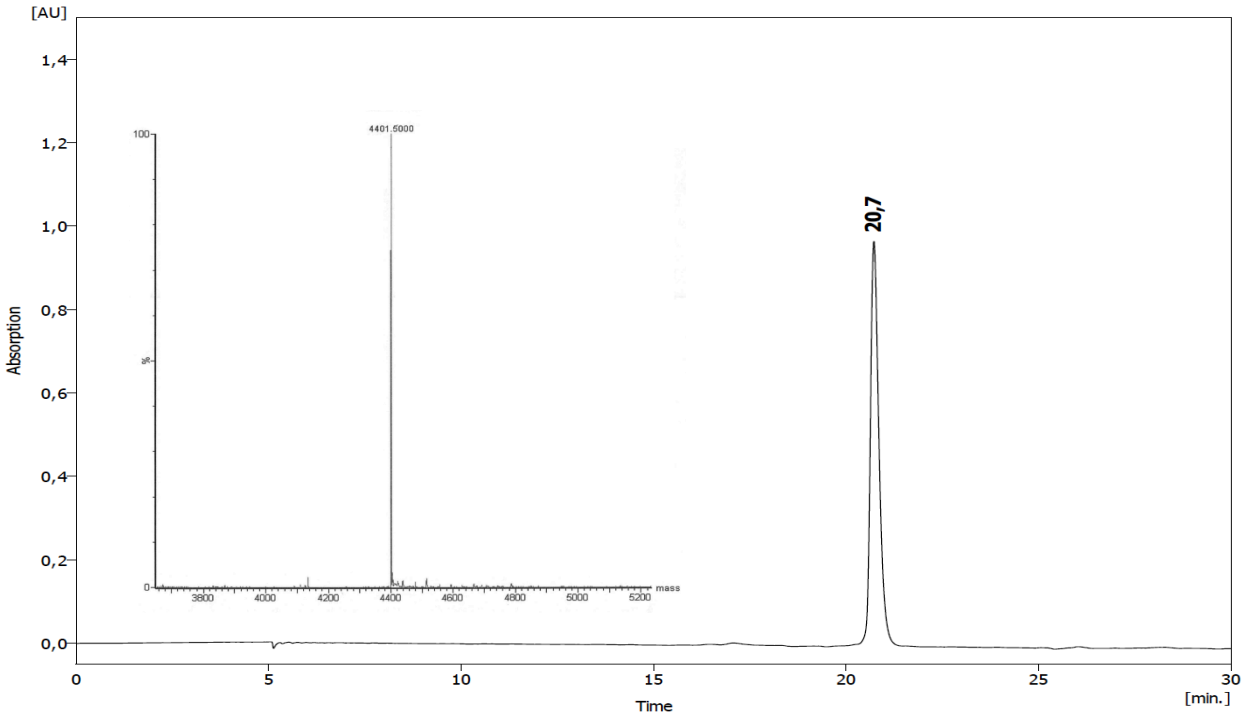
Supplementary Figure 5. Mass spectrum and HPLC chromatogram for (KFF)$_3$K-AEEA-PNA anti-*thyA*-K

(KFF)$_3$K-AEEA-PNA anti-*hipB*-K



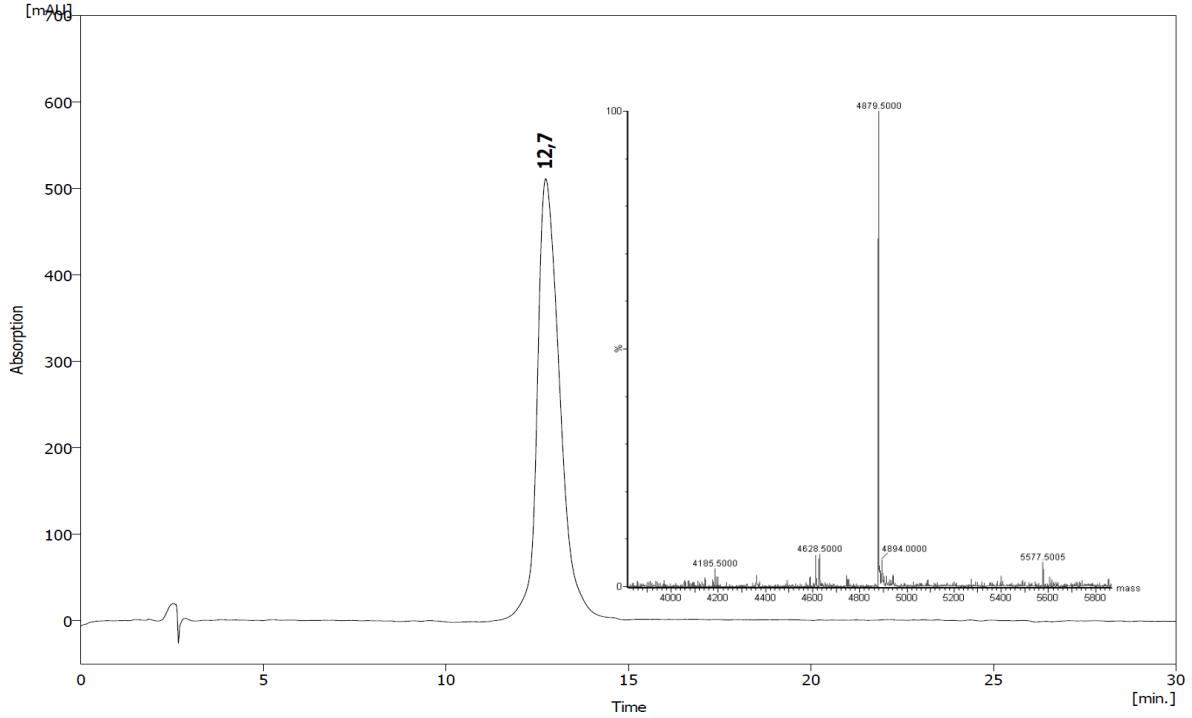
Supplementary Figure 6. Mass spectrum and HPLC chromatogram for (KFF)$_3$K-AEEA-PNA anti-*hipB*-K

(KFF)$_3$K-AEEA-PNA anti-*gltX*-K



Supplementary Figure 7. Mass spectrum and HPLC chromatogram for (KFF)$_3$K-AEEA-PNA anti-*gltX*-K

$(KFF)_3K$-AEEA-PNAnc-K



Supplementary Figure 8. Mass spectrum and HPLC chromatogram for $(KFF)_3K$-AEEA-PNAnc-K

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