

In vitro TR/TR in presence of Myticalin A5 (*E. coli* system)

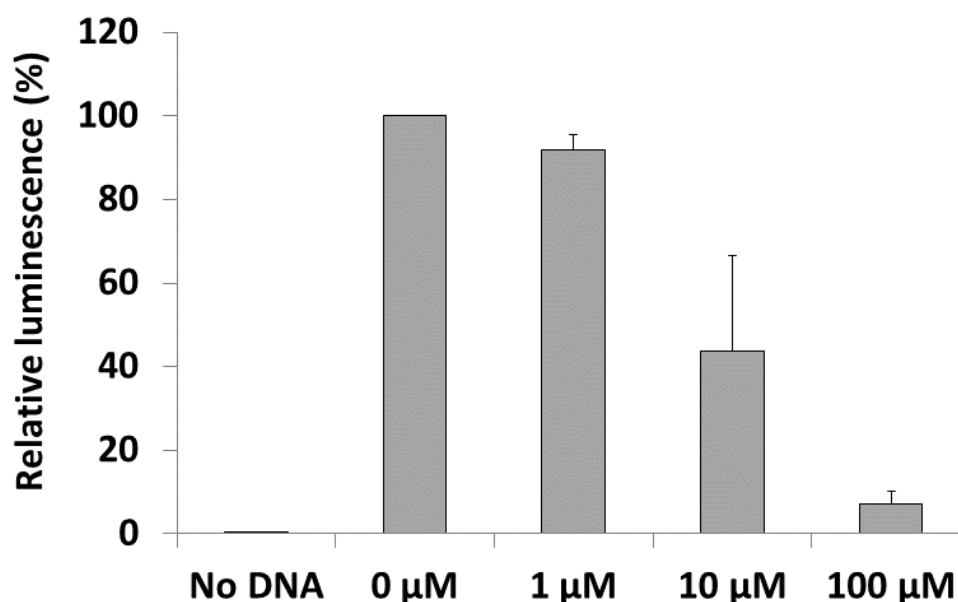


Figure S1. *in vitro* coupled transcription/translation in presence of Myticalin A5 in an *Escherichia coli* system. The positive control received water instead of the peptide. The negative control received water instead of the peptide and the DNA template encoding the luciferase. The results are the average of three independent experiments.

Table S1: Presence/absence patterns of myticalin genes in 20 mussel specimens collected in the Gulf of Trieste, Italy. ✓: present; ✗: absent; M: presence of multiple amplification bands.

Myticalin	sample number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A3/A4/A5/A8/A10	M	M	✓	M	M	✓	✓	✓	M	M	M	✓	M	✓	✓	✓	M	M	M	✓
B1	✓	✓	✗	✗	✗	✓	✗	✗	✗	✗	✓	✗	✗	✓	✗	✗	✗	✗	✗	✗
C2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
C6	✗	✓	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓	✓
C8	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
D1/D2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
D3/D4/D5	M	✓	✓	M	M	M	M	M	✗	✗	✗	✗	✓	✗	✗	M	M	M	✓	✓

Table S2. List of transcriptomes screened for the presence of myticalins.

<i>Mytilus</i> spp.	
Species	SRA datasets
<i>Mytilus californianus</i>	SRX565219-20
<i>Mytilus chilensis</i>	SRX1850488-9
<i>Mytilus coruscus</i>	SRR2895130-2, SRX792025, SRX791940
<i>Mytilus edulis</i>	SRX565221-4, ERR1414442-3, SRR1560431
<i>Mytilus galloprovincialis</i>	SRX126947-50; SRX565225-30; SRX1246876-7; SRX1240182; SRX389462-6; SRX389338; SRX386628
<i>Mytilus trossulus</i>	SRX565231-2
Other mytiloids	
Species	SRA datasets
<i>Bathymodiolus azoricus</i>	DRR048281
<i>Bathymodiolus platifrons</i>	SRX1933530; SRX1933483; SRX610901-2; SRX436365
<i>Bathymodiolus manusensis</i>	SRX2481526-32
<i>Geukensia demissa</i>	kindly provided by prof. PA Fields
<i>Lithophaga lithophaga</i>	SRX1940727
<i>Modiolus modiolus</i>	SRX2357782
<i>Modiolus kurilensis</i>	SRX2481530-2
<i>Modiolus philippinarum</i>	SRX1934914-8
<i>Mytilisepta virgata</i>	SRX2497505; SRX2515148; SRX2515152; SRX2515284; SRX2515292
<i>Perna viridis</i>	SRX643367; SRX643380-5; SRX1873938-47
<i>Perumytilus purpuratus</i>	SRX2210805
Other bivalves	
Species	SRA datasets
<i>Anadara trapezia</i>	SRX323049
<i>Arctica islandica</i>	SRX687761
<i>Argopecten irradians</i>	SRX470082
<i>Astarte sulcata</i>	SRX644667
<i>Atrina rigida</i>	SRX687763
<i>Azumapecten farreri</i>	SRX404364; SRX218546
<i>Cardites antiquata</i>	SRX687773
<i>Cerastoderma edule</i>	SRX687776
<i>Corbicula fluminea</i>	SRX209446
<i>Crassostrea angulata</i>	SRX481252-4
<i>Crassostrea corteziensis</i>	SRX641340; SRX471689
<i>Crassostrea hongkongensis</i>	SRX365659-60
<i>Crassostrea virginica</i>	SRX551498-516; SRX118365
<i>Cycladicama cumingii</i>	SRX687783
<i>Cyrenoida floridana</i>	SRX687777
<i>Diplodonta</i> sp. VG-2014	SRX701840

<i>Donacilla cornea</i>	SRX687785
<i>Elliptio complanata</i>	TSA: GAHW00000000.1
<i>Ennucula tenuis</i>	SRX091980
<i>Eucrassatrella cumingii</i>	SRX687772
<i>Galeomma turtoni</i>	SRX687768
<i>Glossus humanus</i>	SRX687780
<i>Hiatella arctica</i>	SRX687781
<i>Lampsilis cardium</i>	SRX687767
<i>Lamychaena hians</i>	SRX687779
<i>Lasaea adansoni</i>	SRX687778
<i>Laternula elliptica</i>	SRX1614611; SRX807593-8
<i>Lyonsia floridana</i>	SRX687774
<i>Margaritifera margaritifera</i>	SRX687769
<i>Mercenaria campechiensis</i>	SRX644681
<i>Meretrix meretrix</i>	SRX666741-4
<i>Mizuhopecten yessoensis</i>	SRX220583; SRX221165
<i>Mya arenaria</i>	SRX687786
<i>Myochama anomioides</i>	SRX644684
<i>Neotrigonia margaritacea</i>	SRX644685
<i>Ostrea chilensis</i>	SRX565248-9
<i>Ostrea edulis</i>	SRX565250-9
<i>Ostrea lurida</i>	SRX175407
<i>Ostreola stentina</i>	SRX565260-1
<i>Pecten maximus</i>	SRX497464
<i>Phacoides pectinatus</i>	SRX687782
<i>Pinctada martensi</i>	SRX268302
<i>Placopecten magellanicus</i>	SRX687766
<i>Polymesoda caroliniana</i>	SRX687784
<i>Pyganodon grandis</i>	SRX310669-84
<i>Ruditapes decussatus</i>	SRX348803-8
<i>Ruditapes philippinarum</i>	SRX076440-63
<i>Saccostrea glomerata</i>	SRX1884692-727; SRX1838009-12; SRX1797576-85; SRX964403-13; SRX878217; SRX845376
<i>Solemya velum</i>	SRX091478
<i>Sphaerium nucleus</i>	SRX687787
<i>Uniomerus tetralasmus</i>	SRX310669-97
<i>Villosa lienosa</i>	SRX101836-9
<i>Yoldia limatula</i>	SRX687760

Table S3. In silico prediction of mytilin biological activities.

	sequence of mature peptide	length	isoelectric point	CAMP SVM	CAMP Random Forest	CAMP Discriminant Analysis	CAMP Neural Artificial Network	DBA predictor	iAMP-2L	ANTIBP2 score	ANTIBP2 classification	AMPA	Class AMP - SVM	ClassAMP - SVM (classification)	ClassAMP - RF (classification)	ADP top hit	ADP details	AVP pred	dPA BBs	cell PD	Pro%	Arg%	Trp%	Tyr%	Thr%	
A1	IGWPRFRPRLPRTPRYPYPR YPTWPTYPRYPSWPRYA-NH2	37	11.29	0.99	0.705	0.849	NO	NO	YES - antibacterial	0.291	Insect - lebecin	83%	94%	antiviral	85%	antibacterial	42.85%	cathelicidin PR39	1	NO	CPP	29%	21%	8%	16%	8%
A2	IGWPRFRPRLPRTPRYPYPR YPRWPTYPRYPSWPRYA-NH2	37	11.51	0.997	0.719	0.903	NO	NO	YES - antibacterial	0.304	Insect - lebecin	89%	94%	antiviral	87%	antibacterial	45.23%	cathelicidin PR39	1	NO	CPP	29%	24%	8%	16%	5%
A3	YGWPRMPRIPRKPYPYPR YPRWPRHPTIYA-NH2	32	11.57	0.975	0.512	0.878	NO	YES	YES - antibacterial	0.413	Insect - lebecin	95%	94%	antiviral	94%	antibacterial	42.42%	A3-APO	1	NO	CPP	28%	25%	6%	15%	3%
A4	YSWPRMPRIPRKPYPYPR YPRYPRWPRHPTIYA-NH2	35	11.51	0.992	0.558	0.697	NO	NO	YES - antibacterial	0.305	Insect - lebecin	94%	93%	antiviral	93%	antibacterial	42.22%	Arasin-2	1	NO	CPP	28%	25%	5%	17%	2%
A5	YSWPRMPRIPRKPYPYPR YPRWPRWPRQPTIYA-NH2	35	11.72	0.982	0.541	0.424	NO	NO	YES - antibacterial	0.399	Insect - lebecin	94%	99%	antibacterial	95%	antibacterial	43.47%	Arasin-2	1	NO	CPP	28%	25%	8%	14%	2%
A6	YSWPRMPRIPRKPYPYPR WPRHPTIYA-NH2	29	11.63	0.977	0.694	0.732	NO	NO	YES - antibacterial	0.428	Insect - lebecin	89%	95%	antifungal	96%	antibacterial	44.82%	A3-APO	1	NO	CPP	27%	24%	6%	17%	3%
A7	YSWPRMPRIPRKPYPYPR YPRWPRHPTIYA-NH2	32	11.56	0.993	0.702	0.742	NO	NO	YES - antibacterial	0.357	Insect - lebecin	92%	94%	antifungal	95%	antibacterial	42.42%	A3-APO	1	NO	CPP	28%	25%	6%	15%	3%
A8	YSWPRMPRIPRKPYPYPR YPRWPRHPTIYA-NH2	32	11.56	0.973	0.515	0.69	NO	NO	YES - antibacterial	0.391	Insect - lebecin	92%	95%	antibacterial	95%	antibacterial	42.42%	A3-APO	1	NO	CPP	28%	25%	6%	15%	3%
A9	YSWPRIPRIPRKPYPYPR YPRYPRWPRHPTIYA-NH2	35	11.51	0.997	0.705	0.837	NO	NO	YES - antibacterial	0.309	Insect - apidaecin	93%	94%	antiviral	93%	antibacterial	43.18%	cathelicidin OaBac5	1	NO	CPP	28%	25%	5%	17%	2%
B1	LRWTPPTSPYPRYTRSRGSR WSR-NH2	23	12.01	0.201	0.621	0.977	NO	NO	YES - antibacterial, antifungal	-0.445	/	100%	95%	antiviral	68%	antibacterial	40%	A3-APO	0	NO	CPP	17%	26%	8%	8%	13%
C1	GRRRKYRYWRRGDRYWR GVTIQERSKSTLNTED	35	11.44	0.697	0.466	0.962	NO	NO	YES - antibacterial	0.189	Mammals - cathelicidin	100%	89%	antifungal	68%	antibacterial	35%	rhesus macaque myeloid alpha-defensin-6	1	YES	CPP	0%	28%	5%	8%	8%
C2	GRRRRRYRYWRRGLTIQGR SSTTITGD	27	12.1	0.619	0.508	0.978	NO	NO	YES - antibacterial, antifungal	-0.119	/	100%	92%	antiviral	71%	antibacterial	37.50%	chicken cathelicidin 2	1	NO	CPP	0%	33%	3%	7%	14%
C3	RRRRRYRYWRRGLTIQGRSK SPLNTGD	28	12.1	0.65	0.467	0.91	NO	NO	YES - antibacterial, antifungal	0.168	Mammals - cathelicidin	100%	93%	antiviral	79%	antibacterial	37.83%	rhesus macaque myeloid alpha-defensin-6	1	NO	CPP	3%	32%	3%	7%	7%
C4	RRRRRYRYWRRGVTIQGRS KYSTLNTGE	28	11.91	0.711	0.458	0.878	NO	NO	NO	0.091	Mammals - cathelicidin	100%	93%	antiviral	65%	antibacterial	36.83%	rhesus macaque myeloid alpha-defensin-6	1	YES	CPP	0%	32%	3%	10%	10%
C5	RRRRWPRRVTRRIRIPRYLTL NTH	24	12.6	0.997	0.67	0.996	YES	NO	YES - antibacterial, antifungal	-0.138	/	100%	98%	antiviral	97%	antibacterial	39.28%	WLBUE2	1	YES	CPP	8%	41%	4%	4%	12%
C6	RRRRFRVIRIRIRPKYLTLIN TE	24	12.37	0.997	0.637	0.993	YES	NO	YES - antibacterial, antifungal	-0.043	/	100%	93%	antiviral	82%	antibacterial	41.37%	chicken cathelicidin 2	1	YES	CPP	4%	41%	0%	4%	8%
C7	RRRRWRRIRRGISIRLPKFAT LNTTE	25	12.54	0.981	0.719	0.984	YES	NO	YES - antibacterial, antifungal	-0.007	/	100%	97%	antiviral	89%	antibacterial	39.28%	NRC-3	1	YES	CPP	4%	36%	4%	0%	8%
C8	RRRRWRRIRRGISHRLPKFA TLNSD	25	12.54	0.935	0.593	0.983	YES	YES	YES - antibacterial, antifungal	-0.089	/	100%	95%	antiviral	90%	antibacterial	35.71%	NRC-3	1	YES	CPP	4%	36%	4%	0%	4%
C9	RRRRRYRYWRRGLTIQGRP KSLPLNTGD	28	12.1	0.722	0.495	0.903	NO	NO	YES - antibacterial, antifungal	0.015	Mammals - cathelicidin	100%	94%	antiviral	81%	antibacterial	36.84%	BG-CATH37	2	YES	CPP	7%	32%	3%	7%	7%
C10	GRRRRRYRYWRRGYRSWRR GVTIQERSKSTLNTED	35	11.77	0.602	0.501	0.984	NO	NO	YES - antibacterial	0.039	Mammals - cathelicidin	100%	89%	antifungal	74%	antibacterial	36.58%	rhesus macaque myeloid alpha-defensin-6	1	NO	CPP	0%	31%	5%	8%	8%
D1	WGRRWRIRIPSPRLRPWP RPYPWPWSATINTDQ	36	12.3	0.509	0.681	0.455	NO	NO	YES - antibacterial	0.346	Frog - other	0%	99%	antifungal	94%	antibacterial	41.60%	cathelicidin OaBac5	1	NO	CPP	25%	25%	11%	2%	5%
D2	WGRRWRIRIPRPPWPWP PKWWSATINTDQ	32	12.48	0.738	0.699	0.726	NO	NO	YES - antibacterial	0.146	Frog - other	99%	99%	antifungal	96%	antibacterial	39.02%	CRS4C-2	1	NO	CPP	21%	25%	12%	0%	6%
D3	WPRFPKPKPTYSGPTYGPT YPRPTLPRPTWRRSATIGT DH	42	11.4	0.321	0.405	0.013	NO	NO	YES - antibacterial	0.007	Frog - other	0%	99%	antifungal	81%	antibacterial	38.29%	Gm pro-rich pept 2	1	NO	CPP	26%	14%	4%	7%	16%
D4	WPRFPKPKPTYSGPTYGPT TWPRPTWRRSATIGT TEH	42	11.38	0.345	0.419	0.008	NO	NO	YES - antibacterial	0.117	Frog - other	0%	99%	antifungal	74%	antibacterial	37.25%	cathelicidin OaBac5	1	NO	CPP	28%	14%	9%	4%	16%

