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SVEUČILIŠTA U SPLITU



Targeted amplicon sequencing for obtaining novel antimicrobial peptide sequences



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What are antimicrobial peptides...

Gene encode molecules with

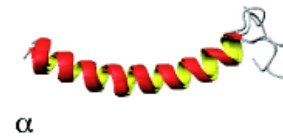
- Direct **antimicrobial** activity
- High molecular **diversity**
- Mostly **cationic**

- Helical AMPs are among the **most abundant and studied**

A. Frog aurein 1.2



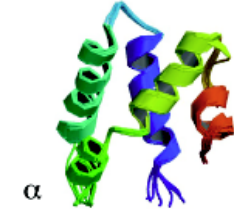
B. Human cathelicidin LL-37



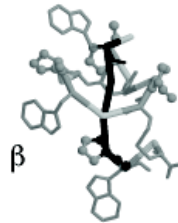
C. Frog distinctin



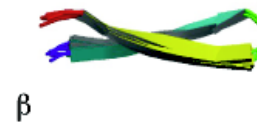
D. Worm caenopore 5



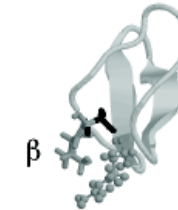
E. Lasso: BI-32169



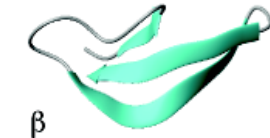
F. Rhesus theta defensin 1 (RTD-1)



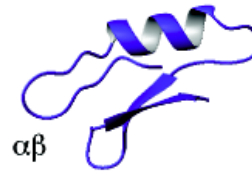
G. Plant kalata B1



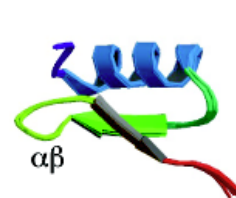
H. Human alpha defensin 1 (HNP1)



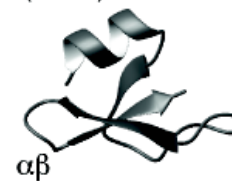
I. Fungal plectasin



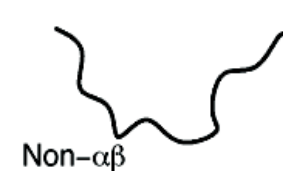
J. Mussel MGD-1



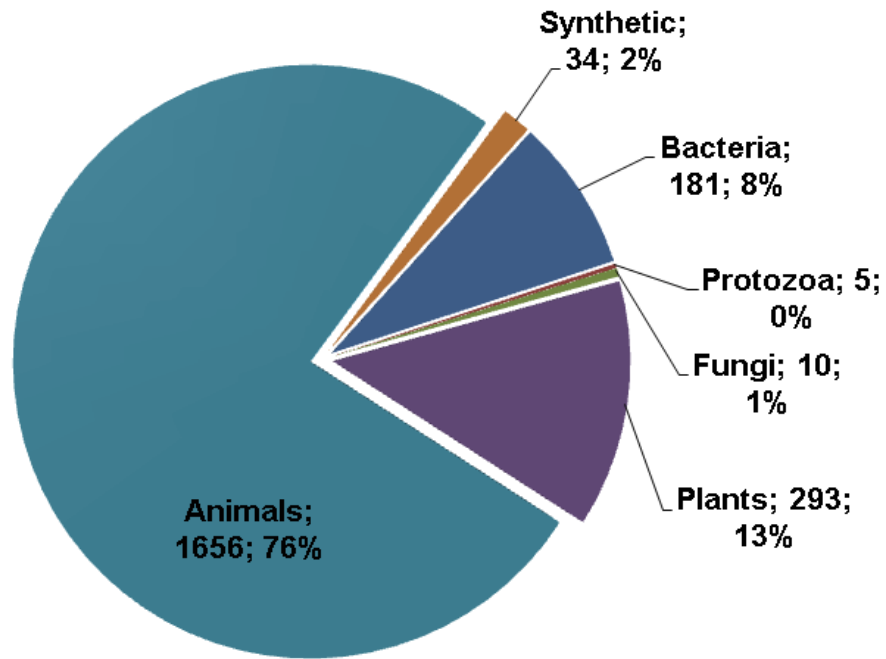
K. Human beta defensin 1 (hBD-1)



L. Bovine indolicidin

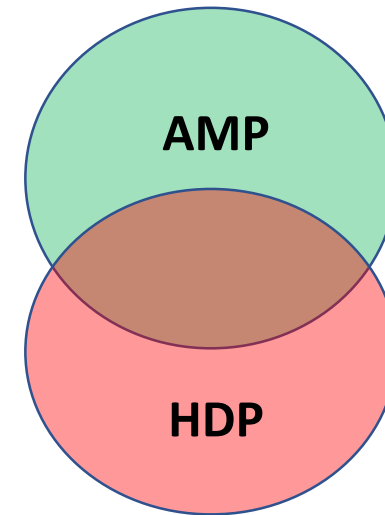


...and where can we find them?



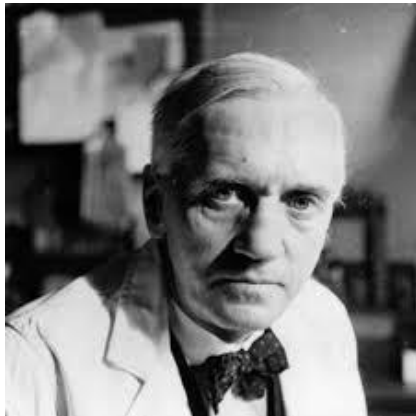
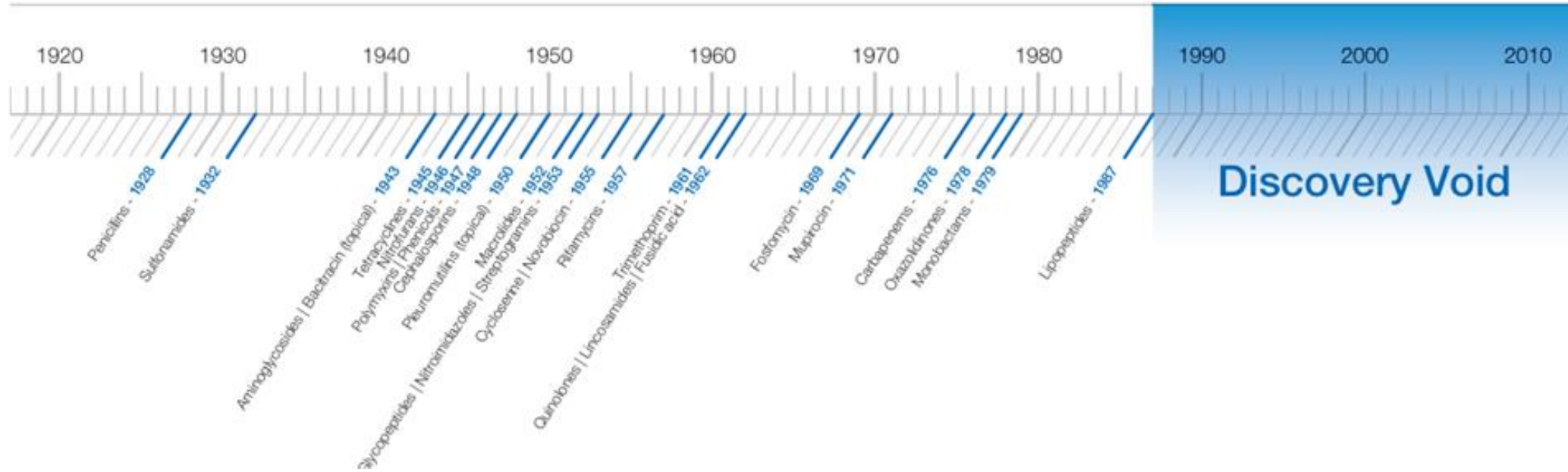
HDP = host defense peptides

- Endogenous peptides
- Co-evolved with microbes
- Innate and adaptive defenses



„...the term CHDPs has now been adopted to encompass the antimicrobial, immunological and other biological functions of these molecules...”

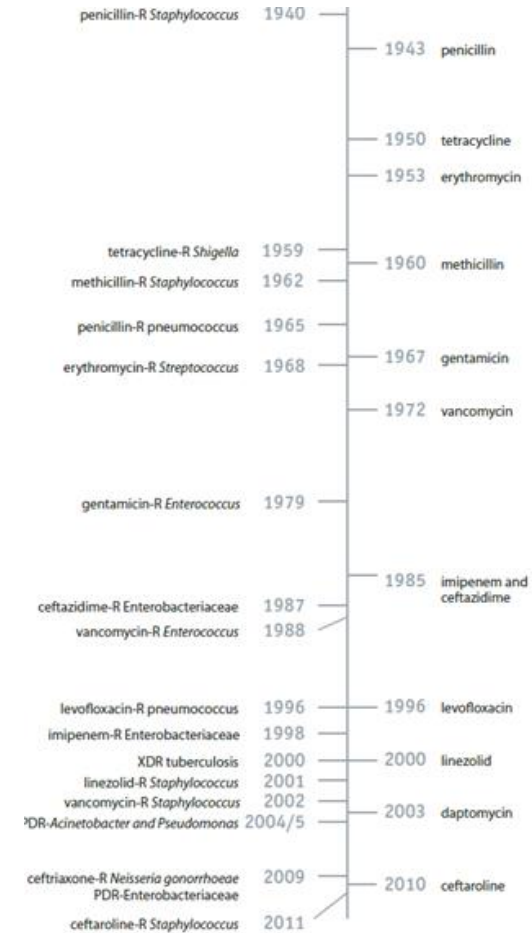
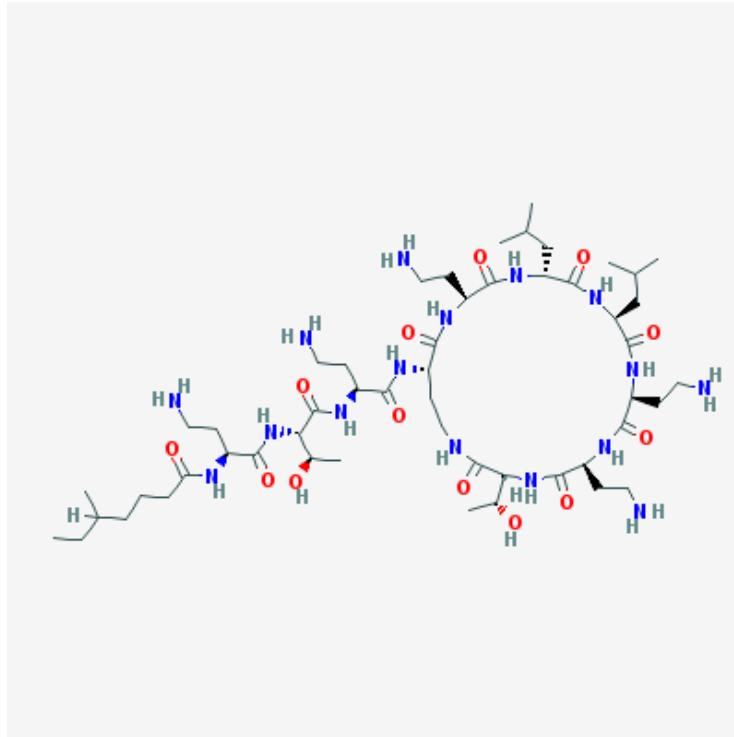
Development of antibiotics...



Taken from: World Economic Forum, Global Risk Report 2013

...and bacterial resistance

- Bacterial resistance was first observed in the 1940s
- MRSA was isolated 2 years after introducing methicillin



Taken from: <http://pubchem.ncbi.nlm.nih.gov/compound/5311054#section=Top>

Taken from CDC report: 'Antimicrobial Resistance Threats in the United States', 2013

How to fight „superbugs” (multiresistant bacteria)?



Possible alternative – frog AMPs



„Classical approach“:

- Treat the frogs with electric shocks or norepinephrine (noradrenaline)
- Extract and purify by precipitation and chromatographic techniques
- Activity testing of fractions to identify the active principles

Targeted amplicon sequencing

Quick look at the AMP structure:

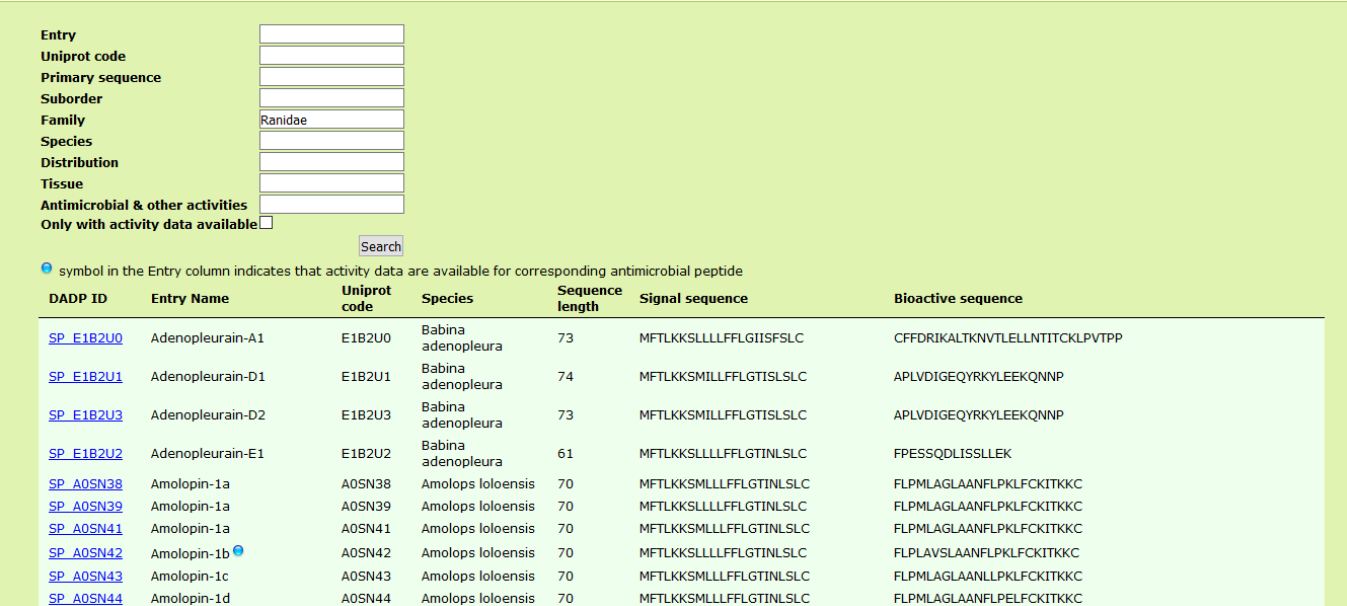


Targeted amplicon sequencing

- 1) Obtain *single individuals* from the frog species of interest for which signal sequences are available in DADP database (e.g. *Ranidae* family)

Ranidae:

- *Pelophylax kl. esculentus* (Linnaeus, 1758)
- *Pelophylax ridibundus* (Pallas, 1771)
- *Rana arvalis* (Nilsson, 1842)
- *Rana dalmatina* (Fitzinger in: Bonaparte, 1838)
- *Rana temporaria* (Linnaeus, 1758)



Entry

Uniprot code

Primary sequence

Suborder

Family

Species

Distribution

Tissue

Antimicrobial & other activities

Only with activity data available

• symbol in the Entry column indicates that activity data are available for corresponding antimicrobial peptide

DADP ID	Entry Name	Uniprot code	Species	Sequence length	Signal sequence	Bioactive sequence
SP_E1B2U0	Adenopleurain-A1	E1B2U0	Babina adenopleura	73	MFTLKKSLLLLFFLGIISFSLC	CFFDRIKALTKNVLELLNTITCKLPVTPP
SP_E1B2U1	Adenopleurain-D1	E1B2U1	Babina adenopleura	74	MFTLKKSMLLFFLGTISLSLC	APLVDIGEYQRKYLEEKQNNP
SP_E1B2U3	Adenopleurain-D2	E1B2U3	Babina adenopleura	73	MFTLKKSMLLFFLGTISLSLC	APLVDIGEYQRKYLEEKQNNP
SP_E1B2U2	Adenopleurain-E1	E1B2U2	Babina adenopleura	61	MFTLKKSLLLLFFLGTINLSLC	FPESQDLISSLLEK
SP_A0SN38	Amolopin-1a	A0SN38	Amolops loloensis	70	MFTLKKSMLLFFLGTINLSLC	FLPMLAGLAANFLPKLFCKITKCC
SP_A0SN39	Amolopin-1a	A0SN39	Amolops loloensis	70	MFTLKKSLLLLFFLGTINLSLC	FLPMLAGLAANFLPKLFCKITKCC
SP_A0SN41	Amolopin-1a	A0SN41	Amolops loloensis	70	MFTLKKSMLLFFLGTINLSLC	FLPMLAGLAANFLPKLFCKITKCC
SP_A0SN42	Amolopin-1b	A0SN42	Amolops loloensis	70	MFTLKKSLLLLFFLGTINLSLC	FLPLAVSLAANFLPKLFCKITKCC
SP_A0SN43	Amolopin-1c	A0SN43	Amolops loloensis	70	MFTLKKSMLLFFLGTINLSLC	FLPMLAGLAANFLPKLFCKITKCC
SP_A0SN44	Amolopin-1d	A0SN44	Amolops loloensis	70	MFTLKKSMLLFFLGTINLSLC	FLPMLAGLAANFLPELFCCKITKCC

Targeted amplicon sequencing

2) Assemble transcriptomes of those species available in SRA (*Sequence Read Archive*) database which are closely related to the target species (e.g. pertaining to the same family)

NCBI Resources How To Sign in to NCBI

SRA (anura) AND "Ranidae"[orgn__txid8397] Search

Access Public (165) Source RNA (165) Clear all Show additional filters

Summary 20 per page Filters: Manage Filters

View results as an expanded interactive table using the RunSelector. Send results to Run selector

Search results Items: 1 to 20 of 165

Filters activated: RNA. Clear all to show 263 items.

1. [ILLUMINA MiSeq paired end sequencing of SAMD00035464](#)
1 ILLUMINA (Illumina MiSeq) run: 25.6M spots, 8.7G bases, 4.1Gb downloads
Accession: DRX036565
2. [454 GS FLX+ sequencing of SSUB004586 - RC_Liver4T](#)
1 LS454 (454 GS FLX+) run: 549,116 spots, 401.7M bases, 1Gb downloads
Accession: DRX036564
3. [454 GS FLX+ sequencing of SSUB004586 - RC_Liver4C](#)
1 LS454 (454 GS FLX+) run: 630,472 spots, 454.7M bases, 1.1Gb downloads
Accession: DRX036563
4. [454 GS FLX+ sequencing of SSUB004586 - RC_Liver28T](#)
1 LS454 (454 GS FLX+) run: 577,824 spots, 389.7M bases, 1Gb downloads
Accession: DRX036562
5. [454 GS FLX+ sequencing of SSUB004586 - RC_Liver28C](#)
1 LS454 (454 GS FLX+) run: 627,209 spots, 439.7M bases, 1.2Gb downloads
Accession: DRX036561
6. [RNA-Seq of Rana catesbeiana ventral skin](#)
1 ILLUMINA (Illumina HiSeq 3000) run: 49.1M spots, 9.8G bases, 3.8Gb downloads
Accession: SRX2989059
7. [RNA-Seq of Rana catesbeiana ventral skin](#)
1 ILLUMINA (Illumina HiSeq 3000) run: 74.1M spots, 14.8G bases, 5.6Gb downloads
Accession: SRX2989058

Results by taxon Taxonomic Groups (List) Ranidae (165) Rana (157) Pelophylax (6) Odorrana (2)

Search in related databases Database Access public controlled all BioSample 241 241 BioProject 26 26 GEO Datasets 8 8

Find related data Database: [Select] Find items

Search details ("Anura"[Organism] OR anura[All Fields]) AND "Ranidae"[orgn] AND "biomol_rna"[Properties]

Search See more...

Full Send to: Related information BioProject BioSample Taxonomy

Recent activity Turn Off Clear

Q (anura) AND "Ranidae"[orgn] AND ("biomol_rna"[Properties]) (165) SRA

Q (anura) AND "Ranidae"[orgn] (263) SRA

Q anura (4776) SRA See more...

SRX2989057: RNA-Seq of Rana catesbeiana: ventral skin
1 ILLUMINA (Illumina HiSeq 3000) run: 35M spots, 7G bases, 2.7Gb downloads

Design: Total RNA was extracted from TRIzol-stored sample using Direct-zol RNA Kit by Zymo Research. RNA libraries were prepared using NEBNext Library Prep Kit and Poly(A) selection strategy. RNA libraries were sequenced on an Illumina HiSeq 3000 System.

Submitted by: University of California, Davis

Study: Transcriptome responses of two frog species exposed to Batrachochytrium dendrobatidis
[PRJNA392411](#) • [SRP111327](#) • [All experiments](#) • [All runs](#)
[show Abstract](#)

Sample: SampleF04
[SAMN07299208](#) • [SRS2340980](#) • [All experiments](#) • [All runs](#)
Organism: [Rana catesbeiana](#)

Library:
Name: AB-46-Bd2014Study
Instrument: Illumina HiSeq 3000
Strategy: RNA-Seq
Source: TRANSCRIPTOMIC
Selection: cDNA
Layout: PAIRED

Runs: 1 run, 35M spots, 7G bases, 2.7Gb

Run	# of Spots	# of Bases	Size	Published
SRR5810413	35,015,973	7G	2.7Gb	2017-07-07

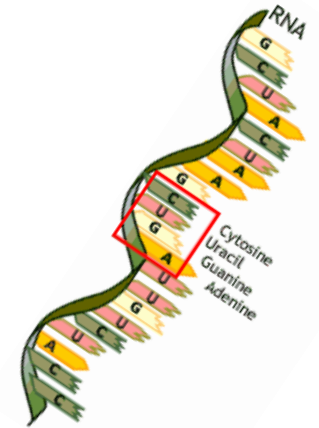


Targeted amplicon sequencing

- 3) Derive a consensus signal sequences based on the HMM profile
- 4) Translate transcripts to all six possible reading frames
- 5) Screen translated transcripts with consensus signal sequences and obtain *1st batch of potential AMPs*
- 6) Based on the signal peptide regions of acquired AMPs design degenerate primers (reverse primer designed on the poly-A tail of mRNA)

Targeted amplicon sequencing

7) Isolate RNA from skin tissue



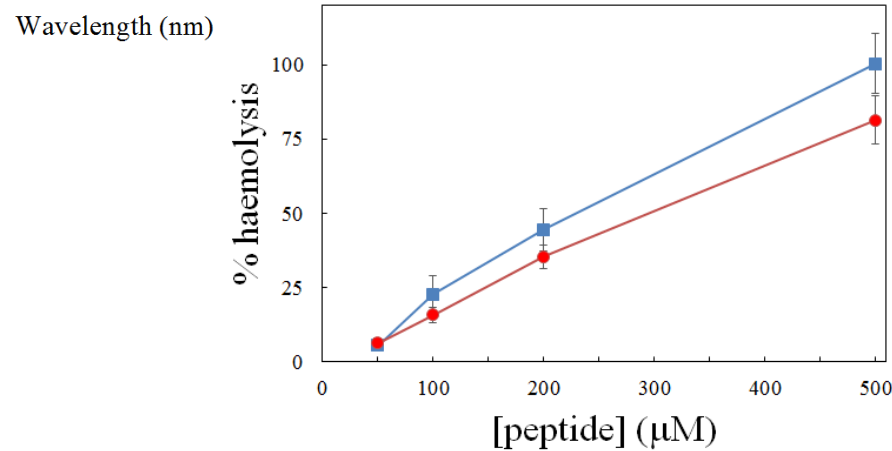
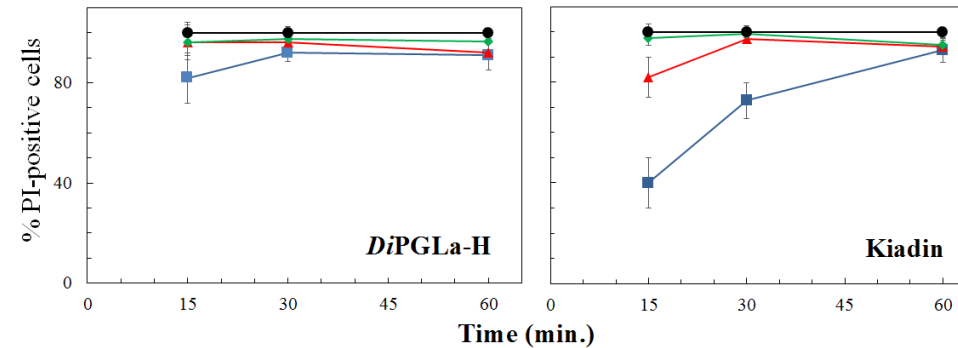
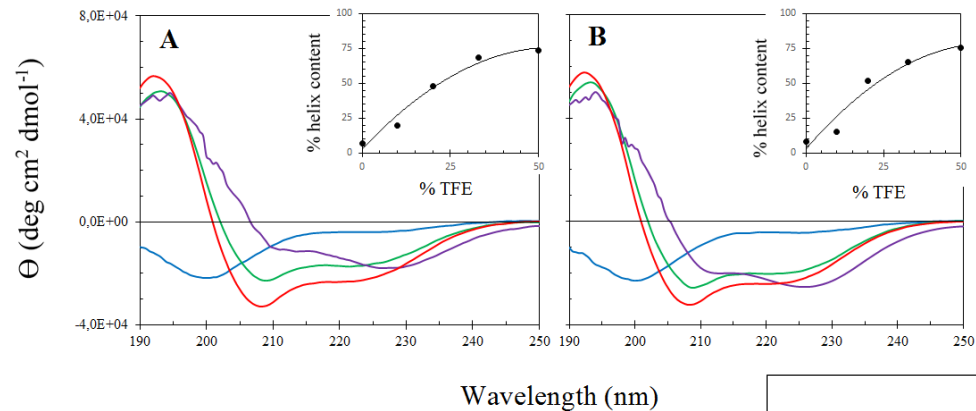
8) Synthesize cDNA library and perform PCR amplification

<https://www.nobelprize.org>

9) Size-select resulting amplicons and proceed to ion-semiconductor sequencing \longrightarrow assemble reads into contigs \longrightarrow translate to amino acid sequence \longrightarrow *2nd batch of potential AMPs*

Future steps...

10) Choose the most suitable peptides for synthesis and proceed to extensive characterization \longrightarrow structure, microbiological activity, mode of action, toxicity etc.



BBA-Biomembranes 2017; 1859 (2): 228-237, doi: 10.1016/j.bbamem.2016

AARC 2017, Trieste, Italy, 28-30 August 2017

THANK YOU FOR YOUR ATTENTION !!!