

XXVIII Symposium on Bioinformatics and Computer-Aided Drug Discovery
24-26 May 2022, Moscow, Russia

AMYLOIDOGENIC PEPTIDES: NEW CLASS OF ANTIMICROBIAL PEPTIDES WITH THE NOVEL MECHANISM OF ACTIVITY

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The beginning of the golden age of antibiotics

The Nobel Prize in Physiology or Medicine 1945

"for the discovery of penicillin and its curative effect in various infectious diseases"



Photo from the Nobel Foundation archive.

**Sir Alexander
Fleming**

Prize share: 1/3



Photo from the Nobel Foundation archive.

Ernst Boris Chain

Prize share: 1/3

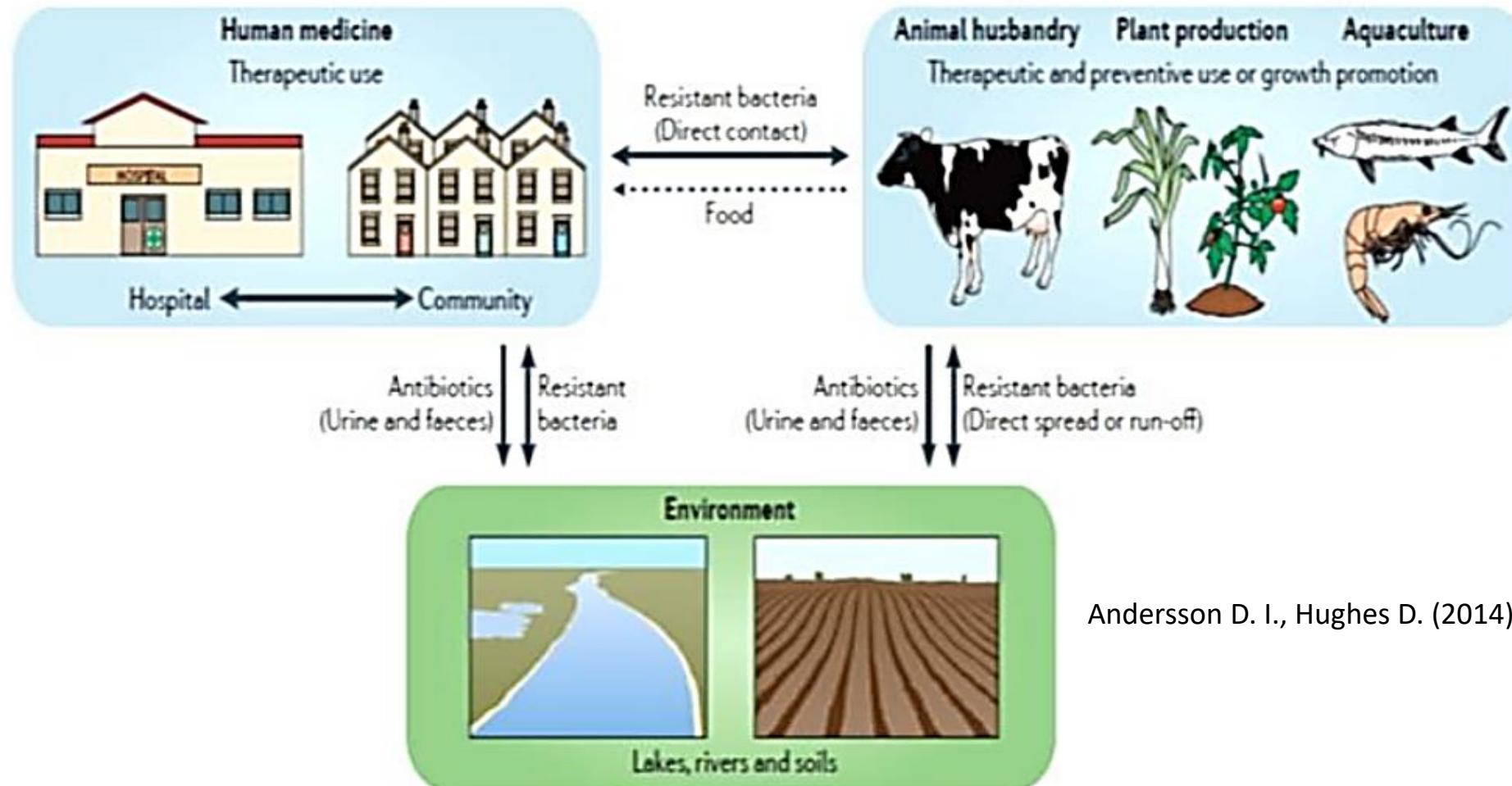


Photo from the Nobel Foundation archive.

**Sir Howard Walter
Florey**

Prize share: 1/3

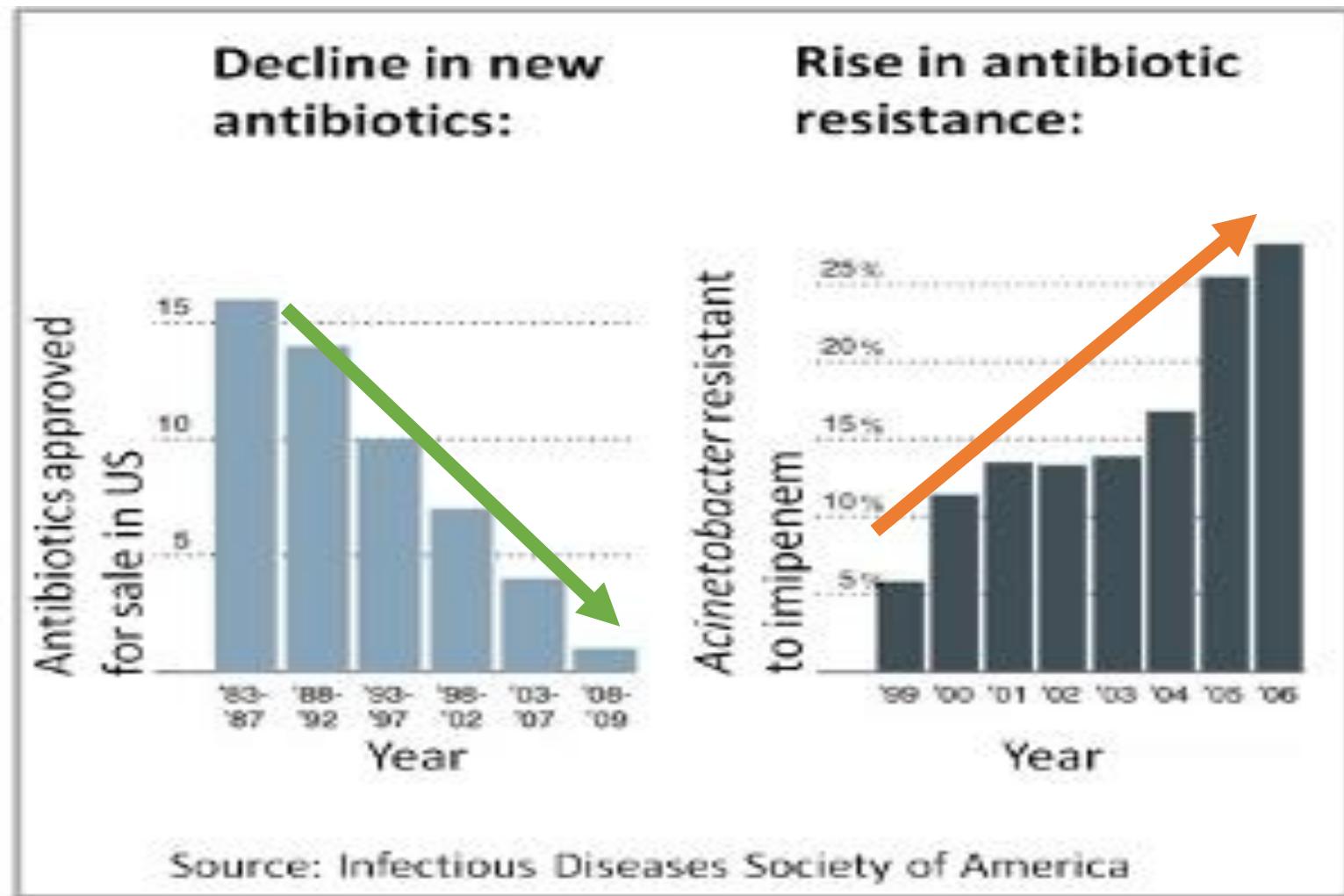
Widespread use of antibiotics



Andersson D. I., Hughes D. (2014). 10.1038/nrmicro3270

The widespread use of antibiotics in medicine, animal husbandry, aquaculture, plant growing forced the microworld to adapt, which led not only to antibiotic resistance, but also the spread of this phenomenon among various taxa of microorganisms.

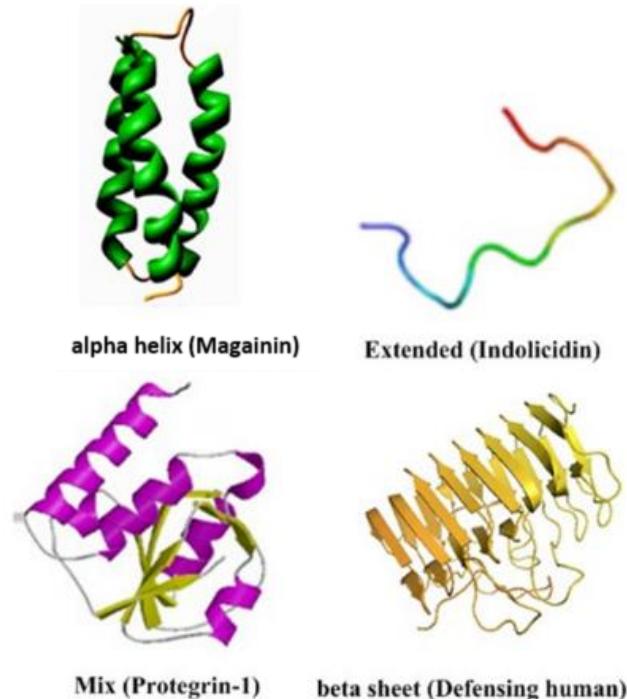
The problem of antibiotic resistance



Antimicrobial peptides

Biological action:

- Antimicrobial
- Immunomodulatory
- Antibiofilm
- Anticancer
- Antiviral

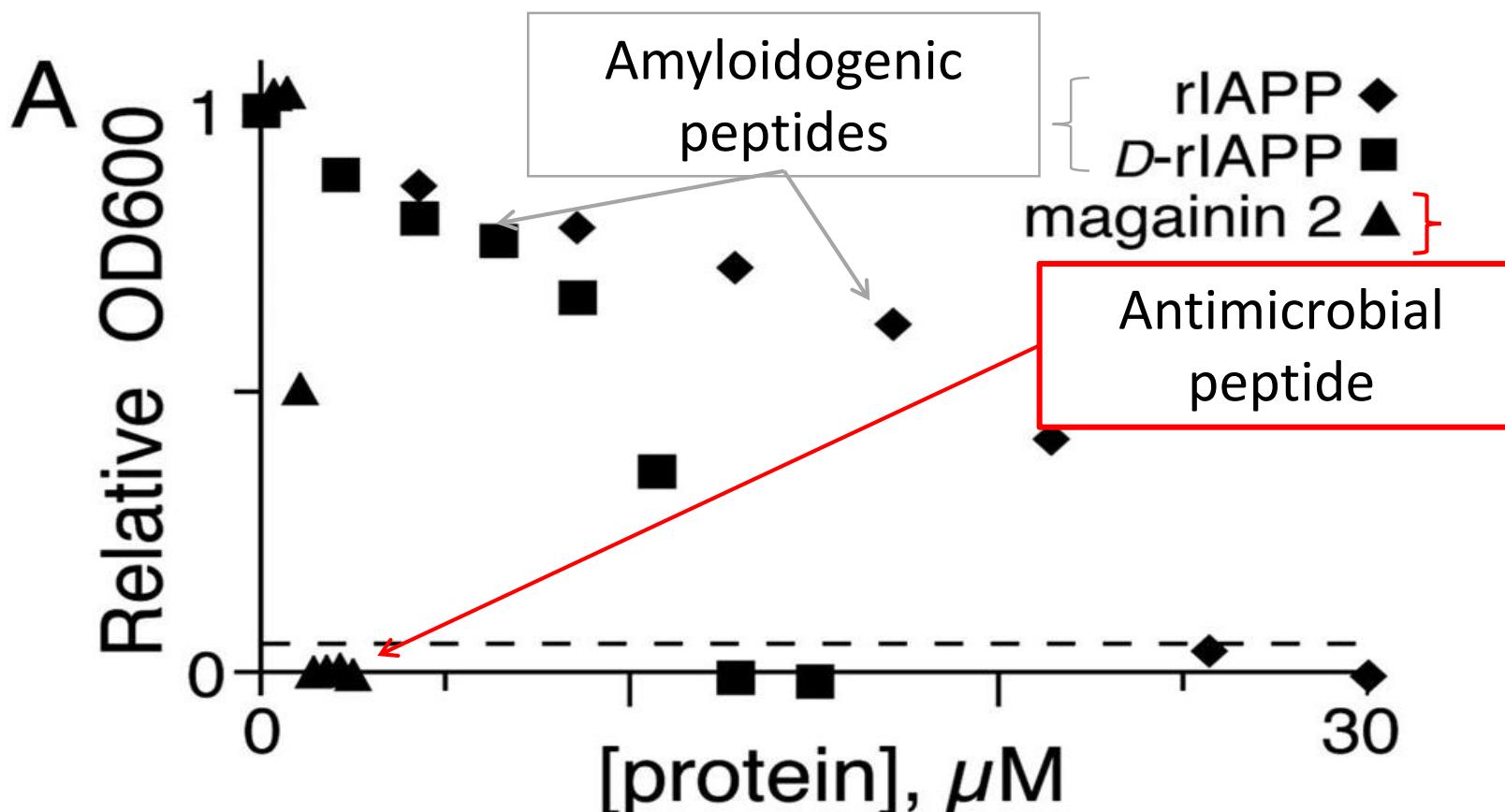


Structural classes of antimicrobial peptides

Seyfi R. et al. International Journal of Peptide Research and Therapeutics.
– 2020. – Vol. 26. – №. 3. – pp. 1451-1463.

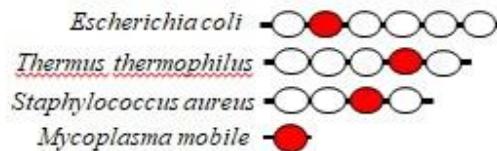
The ability of amyloidogenic peptides to exhibit antimicrobial activity

Dependence of the optical density of the cell culture of *Paracoccus denitrificans* on the concentration of peptides

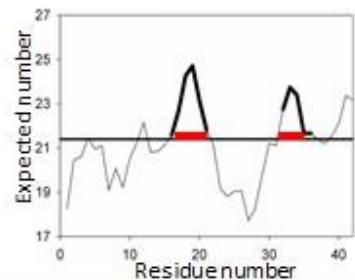


Creation of new antibacterial peptides based on targeted protein aggregation

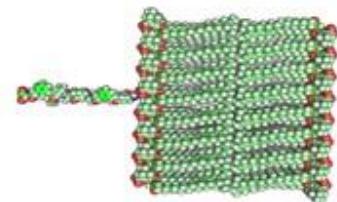
Theoretical methods



Search for unique domains in the bacterial proteins

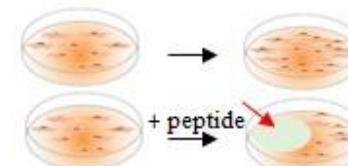


Bioinformatics search for amyloidogenic regions

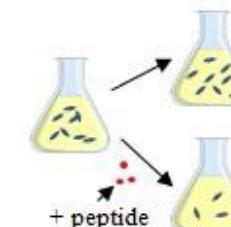


Molecular dynamics modeling of the penetration of peptides through the lipid layer

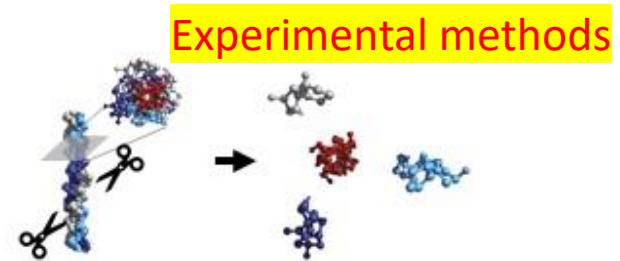
Design and synthesis of the amyloidogenic/antibacterial peptides



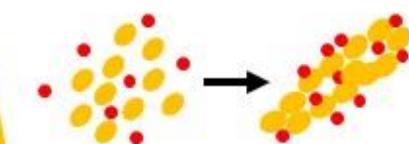
Toxicity testing of peptide against fibroblasts



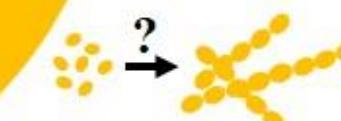
Determine the antimicrobial activities of peptide



Identification of protein regions in the backbone of amyloid fibrils by mass spectrometry analysis

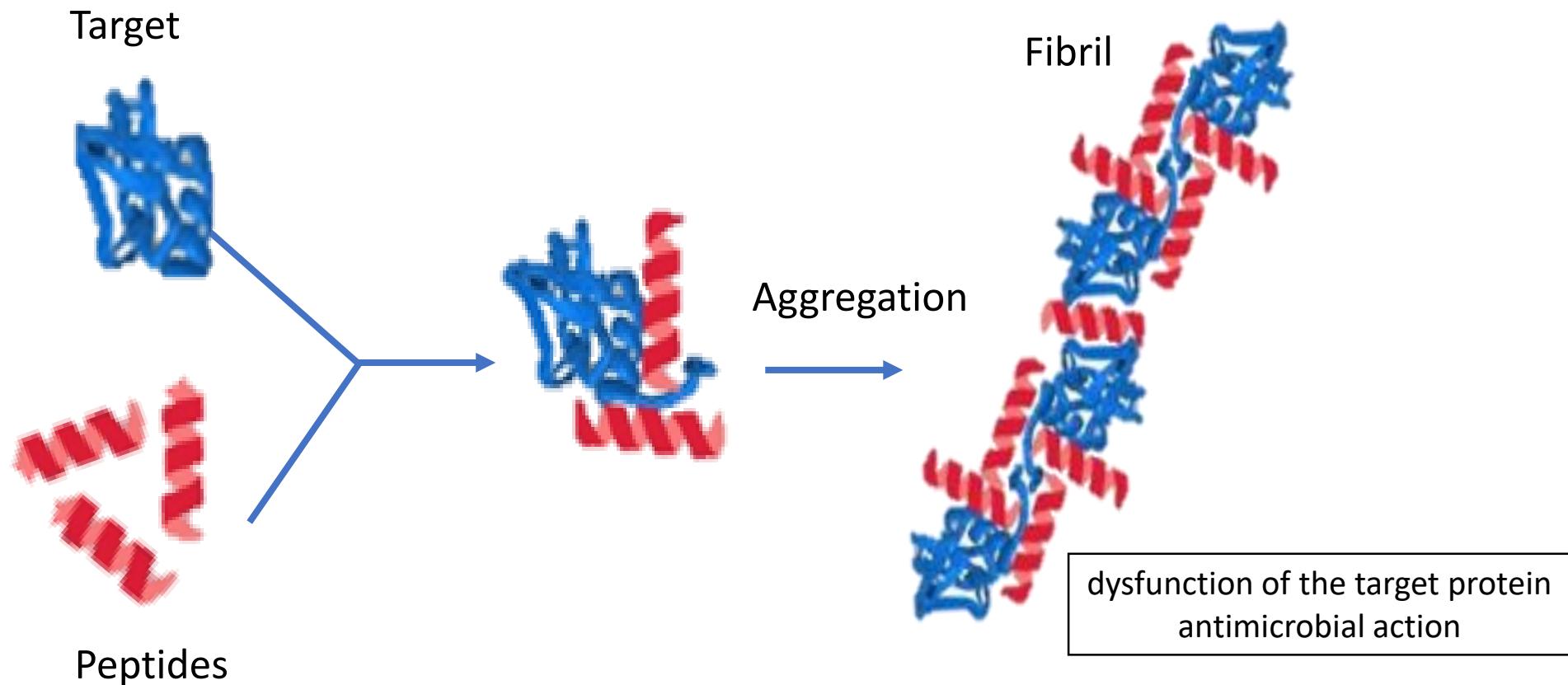


Co-aggregation of amyloidogenic peptide and protein-target

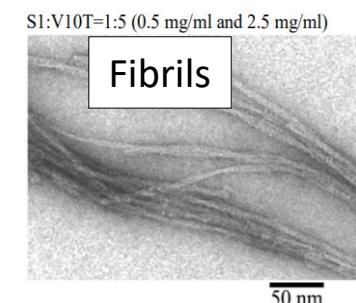
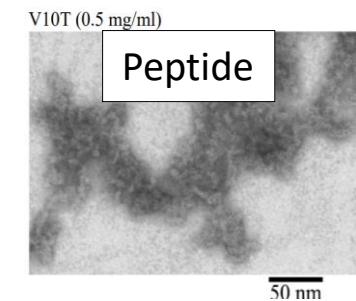
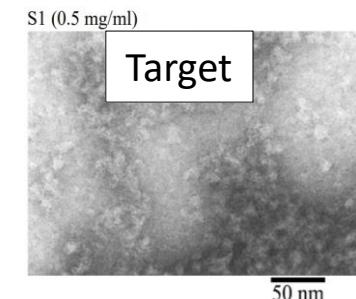


Studies of the process of fibril formation by AMPs

A model of directed co-aggregation of an amyloidogenic peptide based on the S1 ribosomal protein and the protein itself



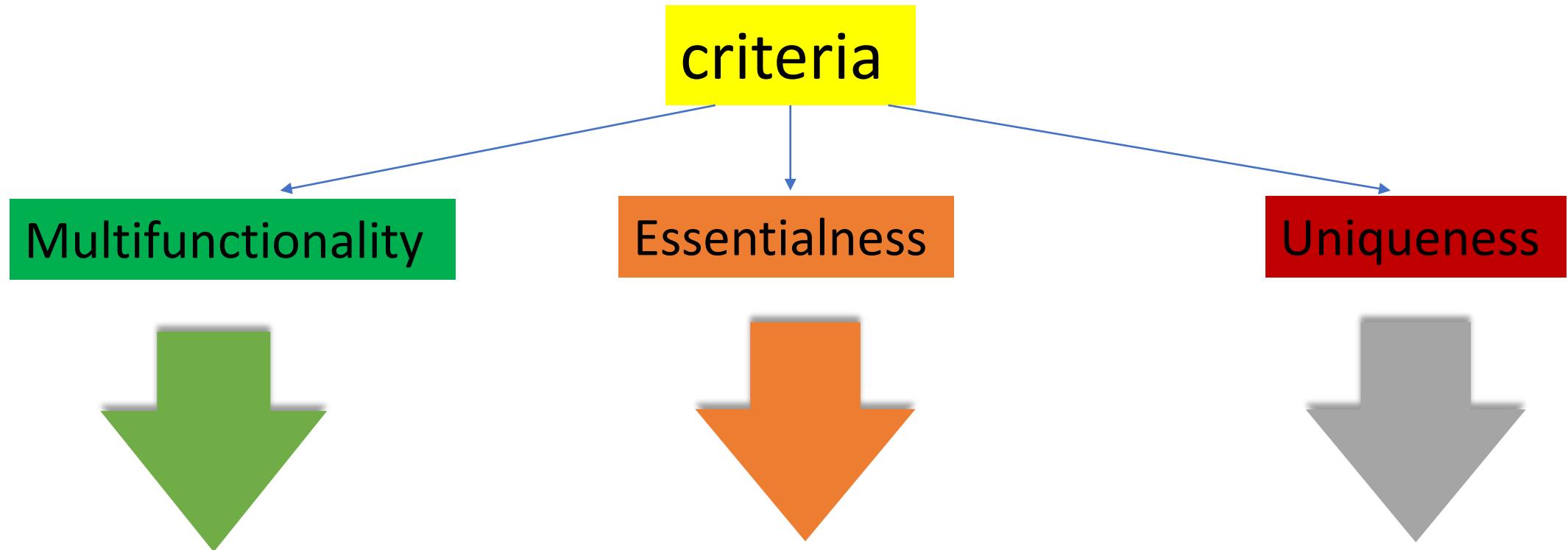
Experimental data



**Antimicrobial and Amyloidogenic Activity of Peptides
Synthesized on the Basis of the Ribosomal S1 Protein
from *Thermus Thermophilus***

by Stanislav R. Kurpe¹, Sergei Yu. Grishin¹, Alexey K. Surin^{1,2,3}, Olga M. Selivanova¹,
Roman S. Fadeev⁴, Ulyana F. Dzhus¹, Elena Yu. Gorbunova², Leila G. Mustaeva²,
Vyacheslav N. Azev² and Oxana V. Galzitskaya^{1,4,*}

Choosing the target protein



Ribosomal S1 protein

Participates in translation
Initiation and regulation

S1 knockout leads
to cell death

Only present in the
bacterial cell

Number of structural S1 domains in bacteria changes strictly within a limited range from one to six

A

Source: *Mycoplasma hominis*
Source phylum: Tenericutes
UniProt ID: D1J8E8
Length: 111 a.a.



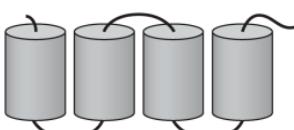
Source: *Listeria aquatica*
Source phylum: Firmicutes
UniProt ID: W7BIQ4
Length: 170 a.a.



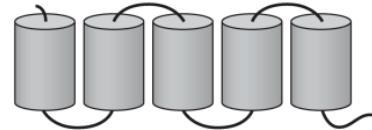
Source: *Microcystis aeruginosa*
Source phylum: Cyanobacteria
UniProt ID: L7E4P2
Length: 330 a.a.



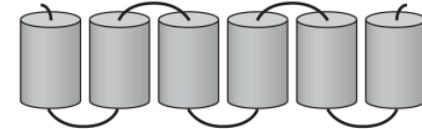
Source: *Bifidobacterium bifidum*
Source phylum: Actinobacteria
UniProt ID: E4V933
Length: 490 a.a.



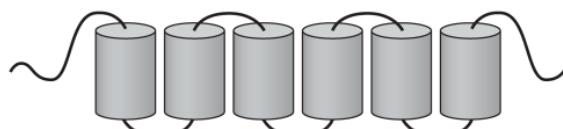
Source: *Thermus thermophilus*
Source phylum: Deinococcus-Thermus
UniProt ID: Q83YV9
Length: 536 a.a.



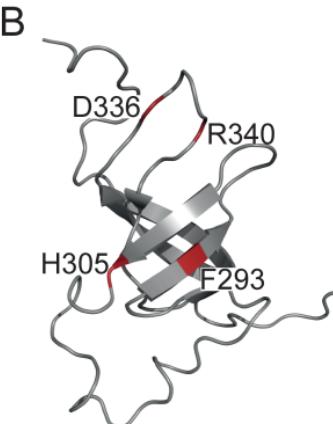
Source: *Escherichia coli*
Source phylum: Proteobacteria
UniProt ID: P0AG67
Length: 557 a.a.



Source: *Salinibacter ruber*
Source phylum: Bacteroidetes
UniProt ID: D5HA65
Length: 876 a.a.

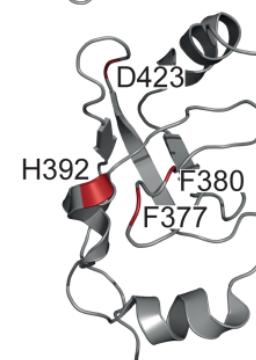


B



4 domain,
2KHI

C



5 domain,
5XQ5

D

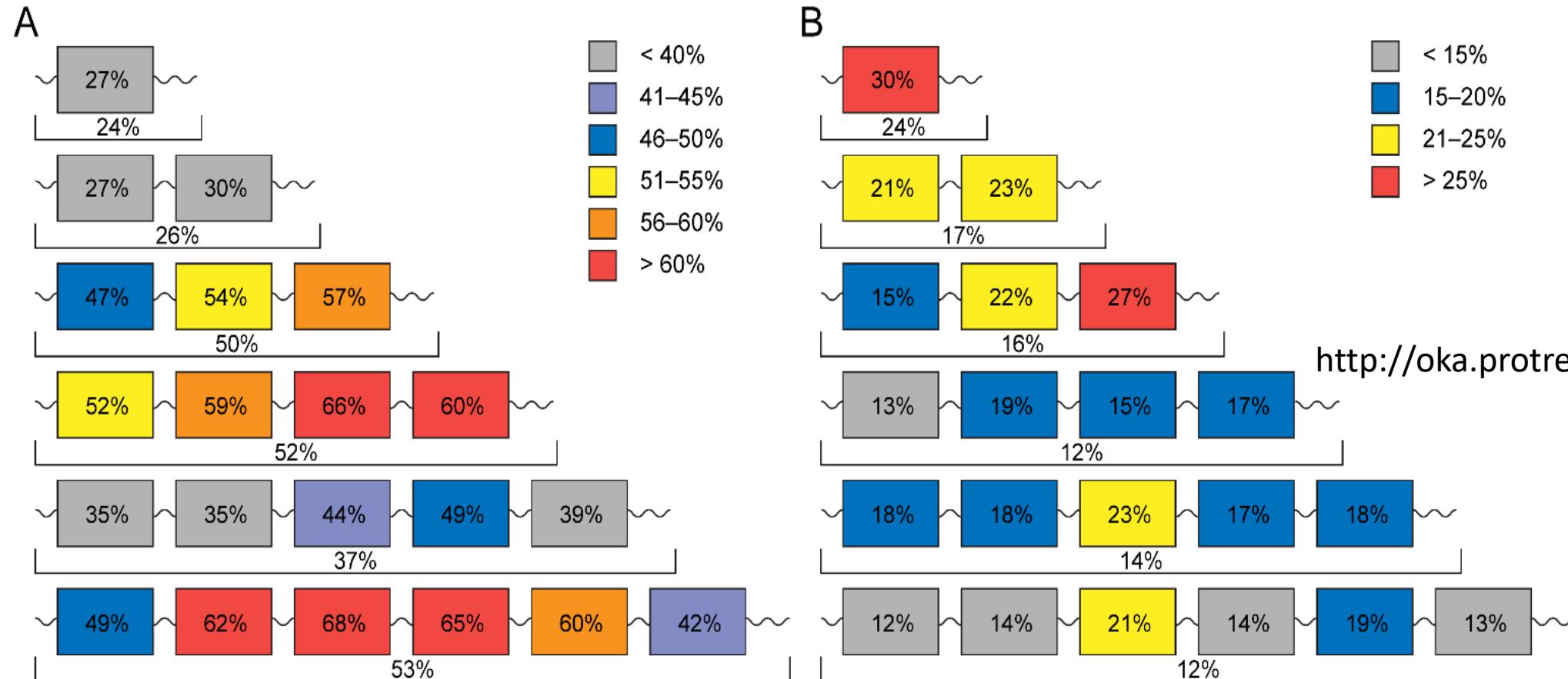


6 domain,
2KHJ

(A) Number of structural S1 domains in different bacteria (according to the SMART database). (B), (C), (D) NMR structures of the fourth (2KHI), fifth (5XQ5) and sixth (2KHJ) S1 domains from *E.coli*.

Analysis of amyloidogenicity of the 1453 sequences of S1 proteins and its domains

Average percentage of identity (A) and amyloidogenic regions (B) predicted by the FoldAmyloid program

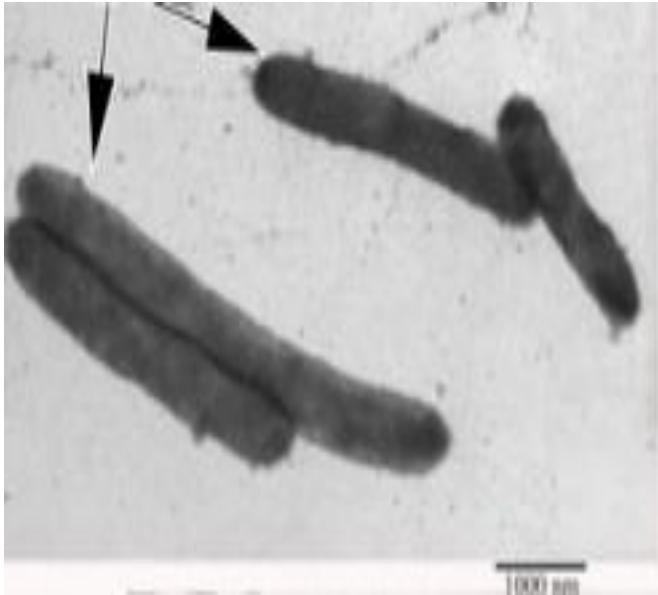


<http://oka.protres.ru:4200/>

Sequence and evolutionary analysis of bacterial ribosomal S1 proteins. *Proteins*. doi:10.1002/prot.26084

<http://bioinfo.protres.ru/fold-amylloid/> Bioinformatics. 2010 Feb 1;26(3):326-32.

Thermus thermophilus as a model organism for molecular biotechnology research



EM image of cells *T. thermophilus*

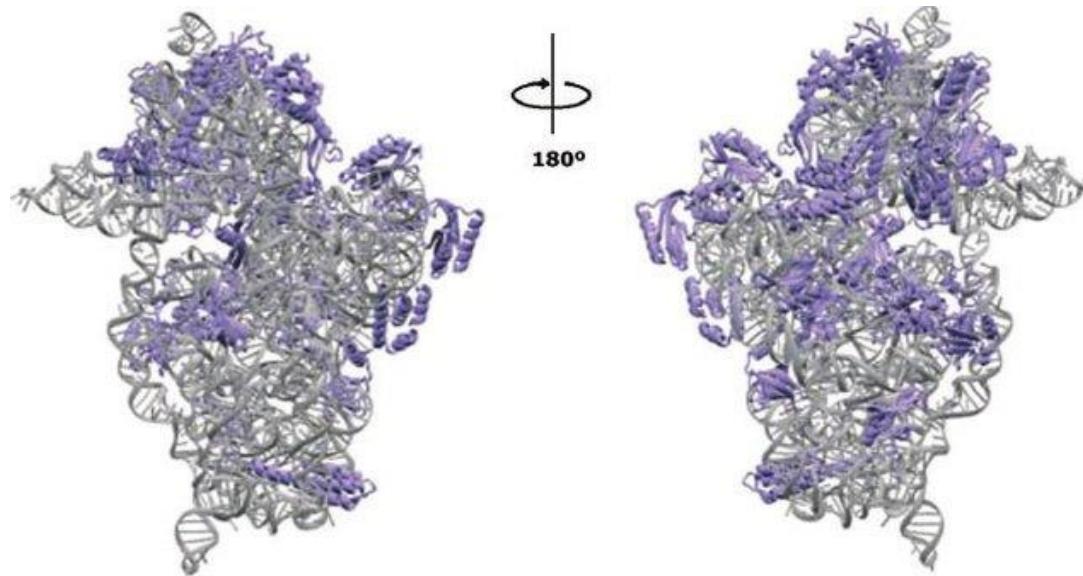


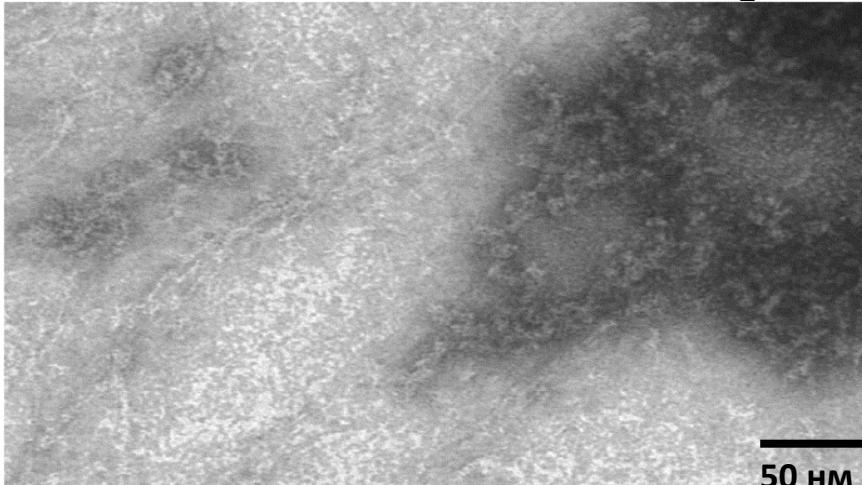
Figure 50S ribosome subunit
T. thermophilus

(<https://www.researchgate.net/publication/41058475>,
2009)

T. thermophilus, E. coli are model organisms

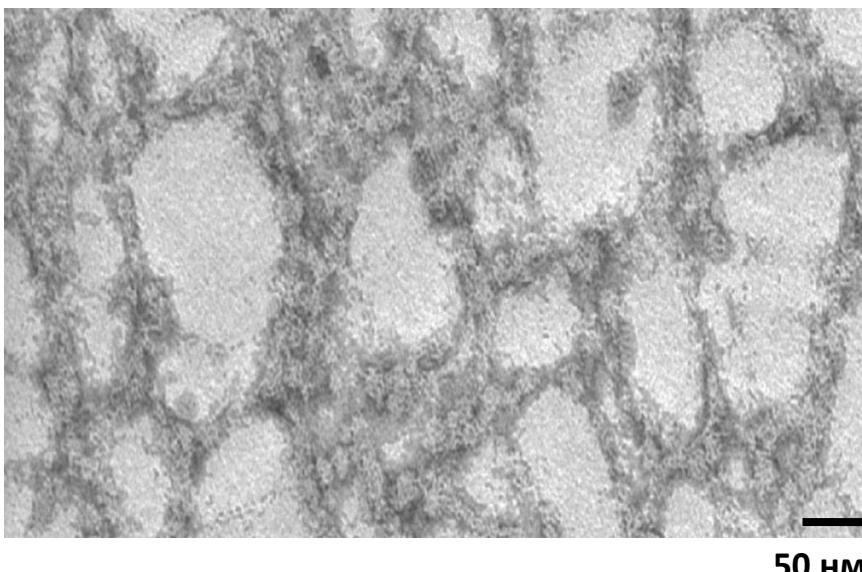
Pseudomonas aeruginosa, Staphylococcus aureus are pathogenic organisms

Aggregation of S1 ribosomal proteins, problems with crystallization



EM images of S1 protein
aggregates from
T. thermophilus (bS1)

Grishin et al. Biochemistry (Mosc). 2020
Mar;85(3):344-354.



EM images of S1 protein
aggregates from
P. aeruginosa (bPaS1)

Grishin et al., Int J Mol Sci. 2021 Jul
7;22(14):7291.

Purpose: Development of amyloidogenic and antibacterial peptides based on the S1 protein from *T. thermophilus*, *E. coli*, *P. aeruginosa*, *S. aureus*

Tasks:

1. Search for amyloidogenic regions of the S1 protein.
2. Synthesize peptides based on the identified amyloidogenic sites.
3. Check the amyloidogenic and antibacterial properties of the obtained peptides.
4. Determine the minimum inhibitory concentration (MIC).
5. Determine the response of the cell proteome to treatment with AMP.
6. Determine the toxicity of peptides for eukaryotic cells.

S1 from *E. coli*

1 MTES**FAQLFE**ESLKTIELTRPG**IVRGVVVAIDKDVLVDAG**LKSESAIPAEQFKNAQGEL 60

61 EI**QVGDEVDAVEDGFGETLLSRE**KAKR**HEAWITLE**KAYEEA**ETVVGVINGKVKGFE** 120

121 **TVELNGIRAFLPGSLVD**VRPVRDTLHLEGKELE**FKVIKLDQKRNNVVVSRRAVIESNSA** 180

181 ERDQLLENLQE**GMEVKGIVKNLTDY****GAFVDLGGVDGLLHITDMAWKR**VKHPSIEVN**VGDE** 240

241 **ITVKVLKF**D**RERTRVSLGLKQLGEDPWV****AIAKR**YPE**GTKLTGRVTNL****TDYGCFCVEIEEGV** 300

301 **EGLVHVSE**MDWTNKNIHP**SKVVNVGDVVEVMVLD**I**DEERRISLGLK**QCKNNNPWQQFAET 360

361 HNK**GDRVEGKIKS****ITDFGIFIGLDGG**IDGL**VHLSDISWNV**AGEEAVREYKKGD**EIAAVVL** 420

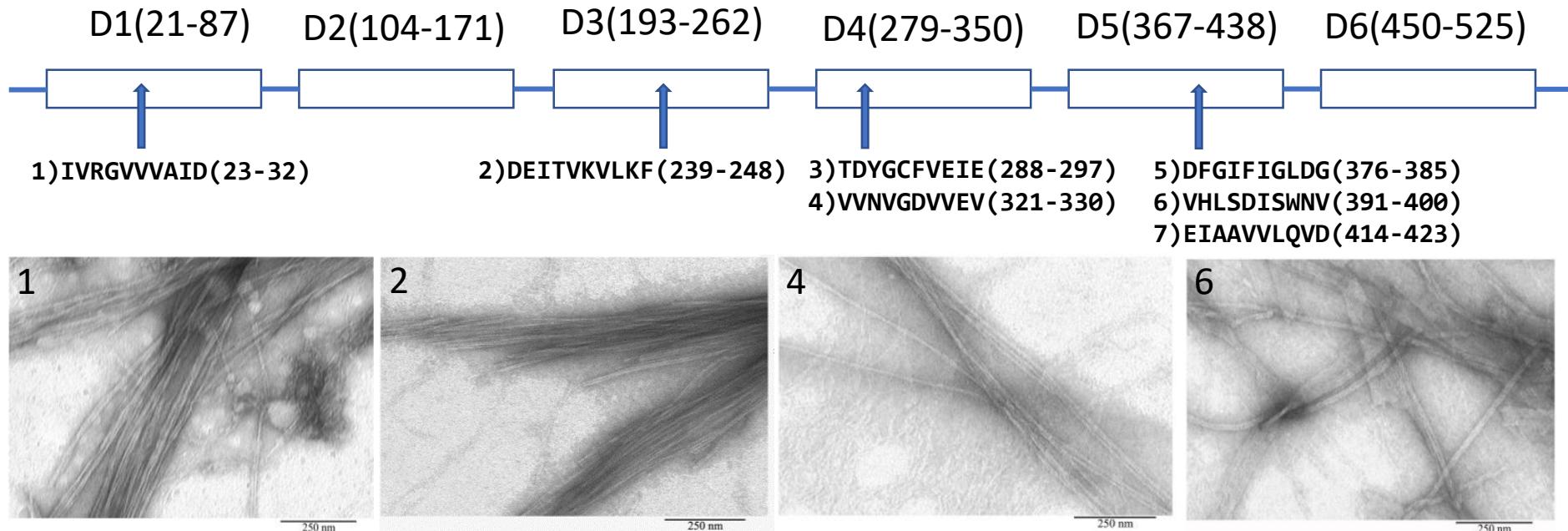
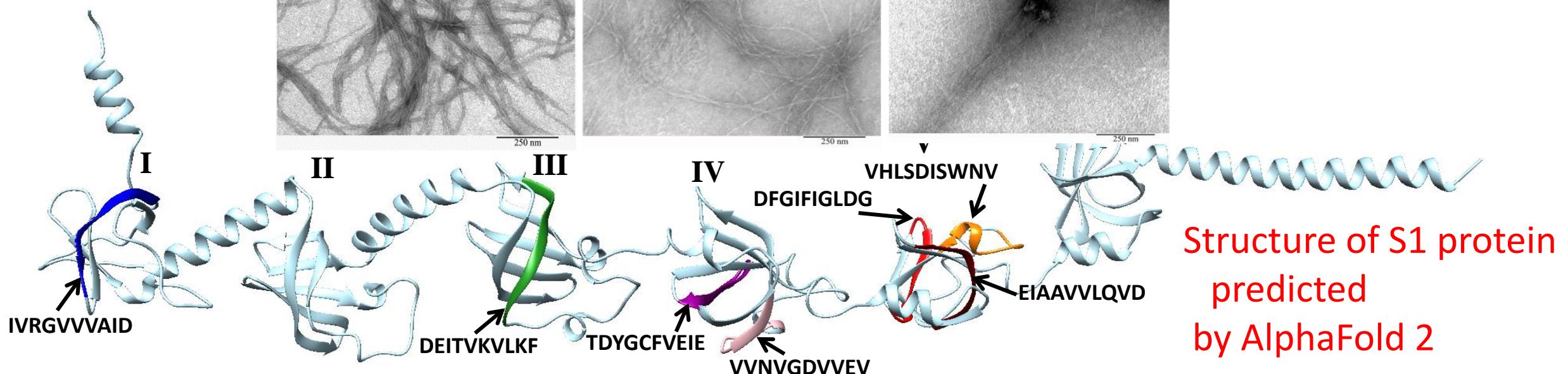
421 **QVDAERERISLGVK**QLAEDPF**NNWVA**LNKK**GAIVNGKVTAVDAKGATVELADGVEGYLRA** 480

481 SEASRDRV**EDATLV**LNVGDDVEAKFTGVDRKNRAISLSVR**AKDEADEKDAIATVNQEDA** 540

541 NFSNNAMAEAFKAAKGE

Amyloidogenic regions
FoldAmyloid
Waltz
AGGRESCAN
PASTA 2.0

1 S1 motif: 1) IVRGVVVAID (23-32)
2 S1 motif
3 домен: 2) DEITVKVLKF (239-248)
4 домен: 3) TDYGCFCVEIE (288-297) и 4) VVNVDGVVEV (321-330)
5 домен: 5) DFGIFIGLDG (376-385), 6) VHLSDISWNV (391-400) и 7) EIAAVVLQVD (414-423)
6 домен

A**S1 from *E. coli*****B *E. coli***

S1 from *T. thermophilus*

1 MEDKATQTPEQTFSMEAALQETEARLEKRVRP**GQILTGKVVLVGSEGVAVDIGAKTEGI**I 60

61 **PFNQLTTKPLSEEELRNLLSPGDEVKVQVLRVDPETGOILLSRK**KIEAQEKWDRIQELYE 120

121 K**GEPVTVTIKERVKGGVAELDGIOQFMPASQLDLRRVPNLDEFVGQOVLAKIIEFHRRK** 180

181 GRVILSRRAVLEEEQKKAREAFLKSLEP**GQVVEGTVVEVTDFGVFVNLD**PVDGLVHRSEI 240

241 **TWGRFNHPREVIQKGQKV**KARVLSVDPEKERVN**LSIKALI**PDPWTTVAEKYPV**GT**RVRGK 300

301 **VVGLTQFGAFVEVEPGLEGЛИHISEL**SWTKRPKHPSEVVKEGDEVE**AVVLR**LDPEERRLS 360

361 L**GLKQTQPDPWQQLTEKYPPGT**VLKGKV**VTDFGVFVEI**E**PGIEGLVH**VSELDHKRVEN 420

421 PAALFKKG**DEM****EVVVLNID**PVEQRVSLSR**KRLLPPPL**PQEEERPRRSGKERARRKGAP 480

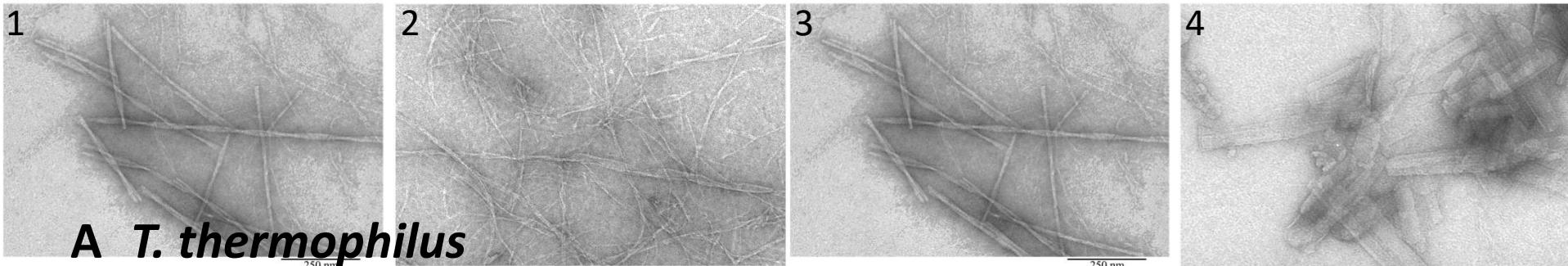
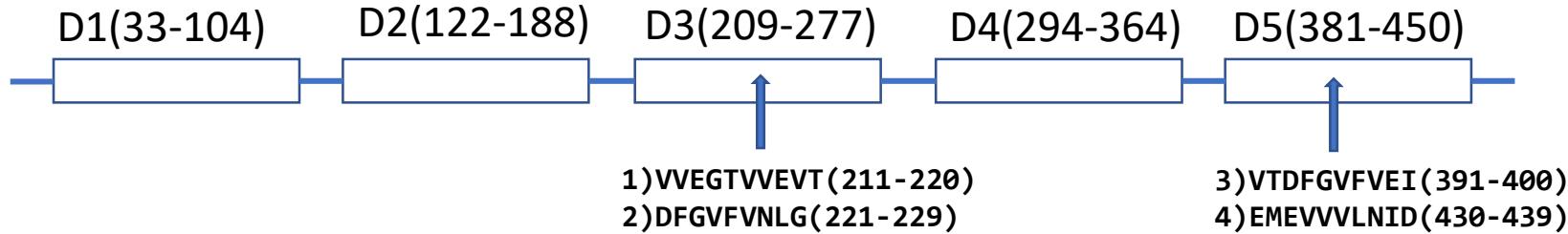
481 RREDRREYEYG**AVAЕYNLYDA**SSVPTTTATVKLG**DLYGDILLA**SLGLEEEAEEKSRG

Amyloidogenic regions:
FoldAmyloid
Waltz
AGGRESCAN
PASTA 2.0

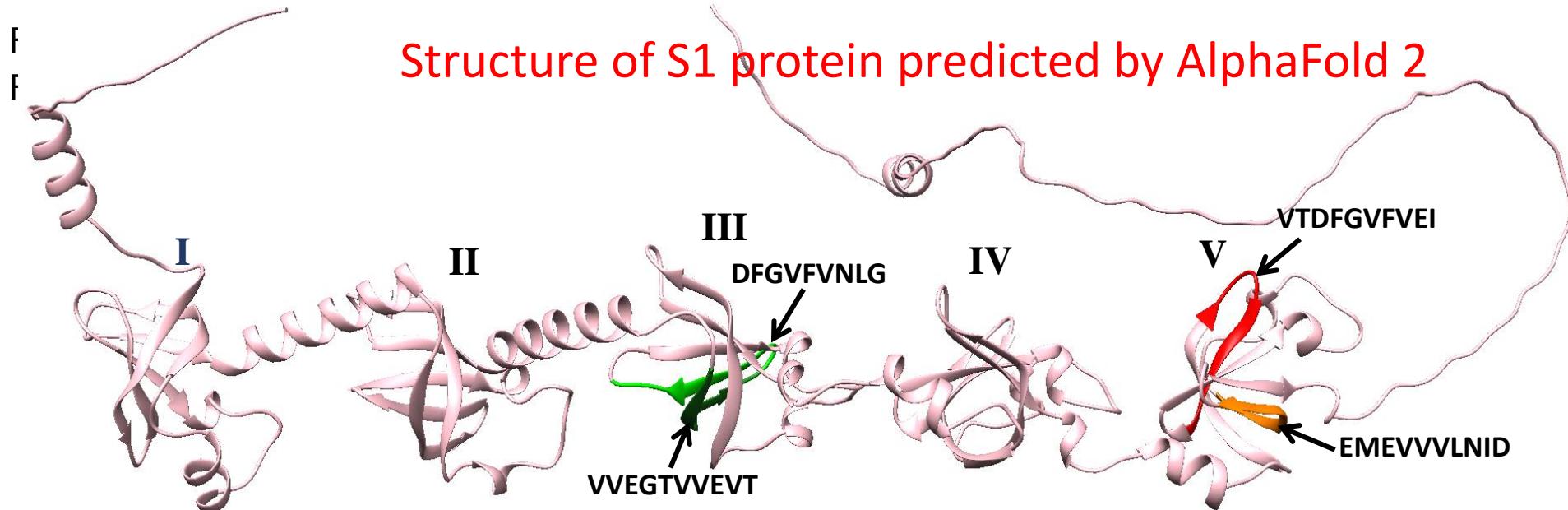
1 domain
2 domain
3 domain: 1) VVEGTVVEVT (211-220) и 2) DFGVFVNLD (221-229)
4 domain
5 domain: 3) VTDFGVFVEI (391-400) и 4) EMEVVVLNID (430-439)

B

S1 from *T. thermophilus*

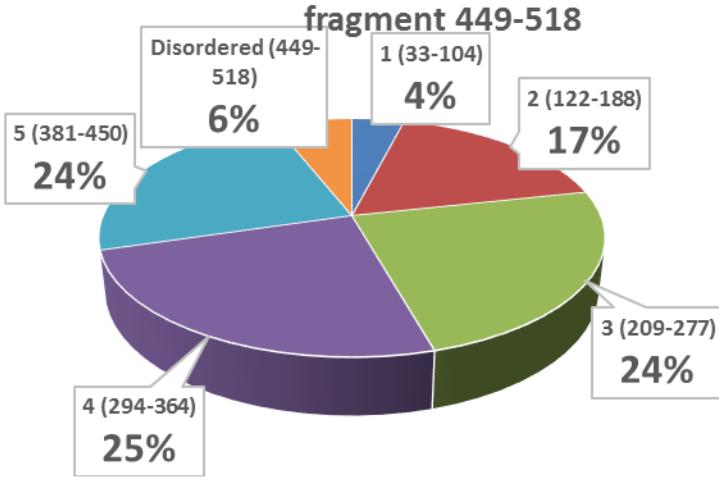


A *T. thermophilus*

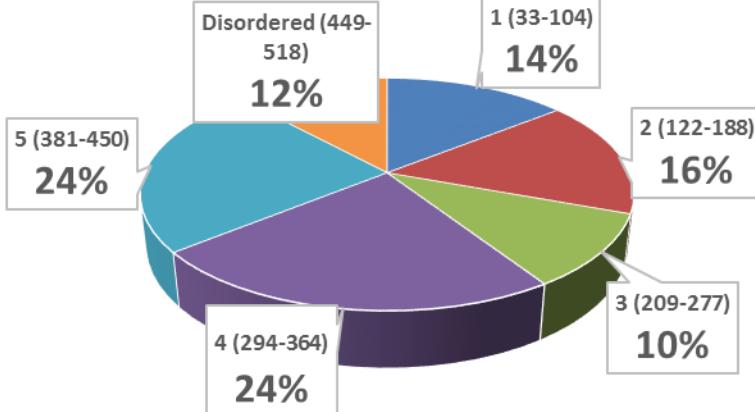


Results of HPLC-MS analysis products of the hydrolysis of S1 protein from *Thermus Thermophilus*

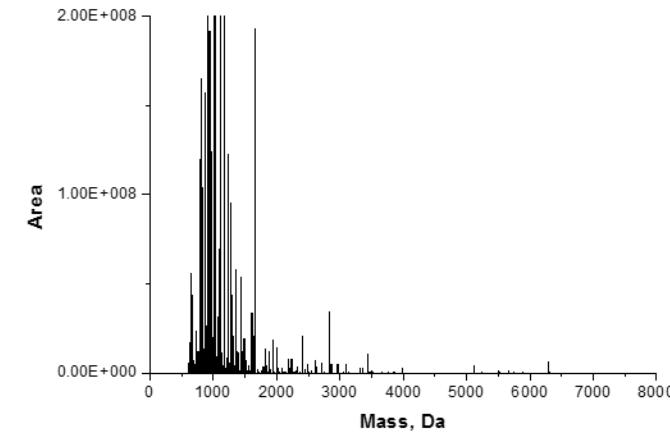
The proportion of the number of peptides of the S1 protein (non aggregated) by domain + fragment 449-518



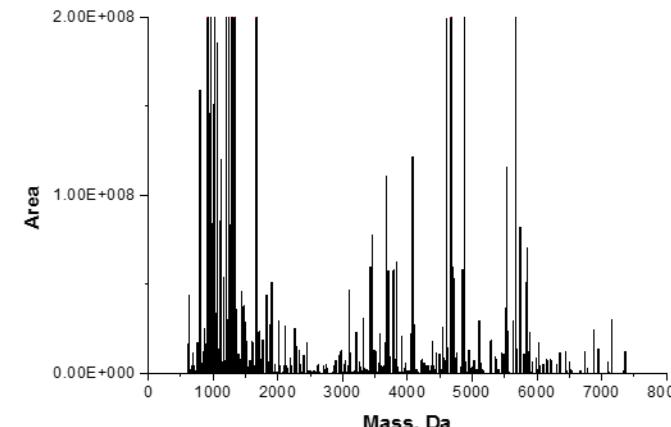
The proportion of the number of peptides of S1 aggregates by domain + fragment 449-518



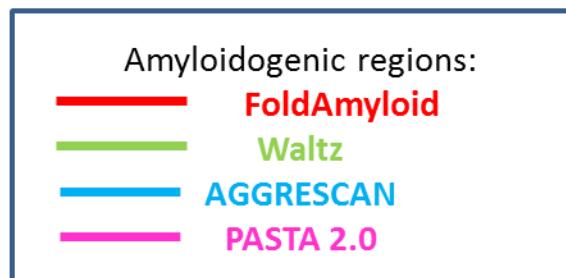
The Peptide Mapping for products of the hydrolysis of S1 protein (non aggregated)



The Peptide Mapping for products of the hydrolysis of S1 aggregates



Peptides from products of limited proteolysis of associates S1 Thermus:



F.SMEAALQE TEAR.L **14-25**

R.VRPGQ **ILTGKVVLVGVSEGVA**VDIGAKTEGI **IPF.** N **30-62**

F.**N**QLTTKPLSEEELRNLLSPGDEVK**VQVLR**VDPETG**QILLS**RKKIEAQEKWDR.I **63-114**

R.RVPNLDEFVGQ**QVLA**KIIEFHRRKG RVL SR.R **157-187**

R.VKGGV**VAELDGIQG**FMPASQL.D **133-153**

K.SLEPGQ**VVEGT**VVEVTDF**FGVFVN**LGPVDGLVHRSE**ITWGR**FNHPREVIQKGQK.V **205-257** 211-220, 221-229

K.SLEPGQ**VVEGT**VVEVTDF**FGVFVN**LGPVDGLVHRSE**ITWGR**FNHPR.E **205-249**

R.VRGKVVGLT**QFGAF**VEVEPGLEG LIHIS ELSWT KRP KHPSEVVKEGDEVEAVVL.R **297-350**

R.VRGKVVGLT**QFGAF**VEVEPGLEG LIHIS ELSW.T **297-328**

K.**ALI**PDPWT TVAE KYPVGT**VRGKVVGLT****QFGAF**VEVEPGLEG LIHIS ELSW.T **278-328**

K.**ALI**PDPWT TVAE KYPVGT**VRGKVVGLT****QFGAF**VEVEPGLEG LIHIS ELSWT KRP KHPSEVVK.E **278-340**

F.KKGDEM**EVVVLNID**PVEQRVSLSRKRLLPPLPQEEERPR.R **426-465**

K.RVENPAALFKKGDEM**EVVVLNID**PVEQRVSLSRKRLLPPLPQEEERPR.R **417-465** 430-439

K.QTQPDPWQQLTEKYPPGT**VLKGKV**TGVTD**FGVF**VEIEPGIEGLVHVS ELDHK.R **365-416**

391-400

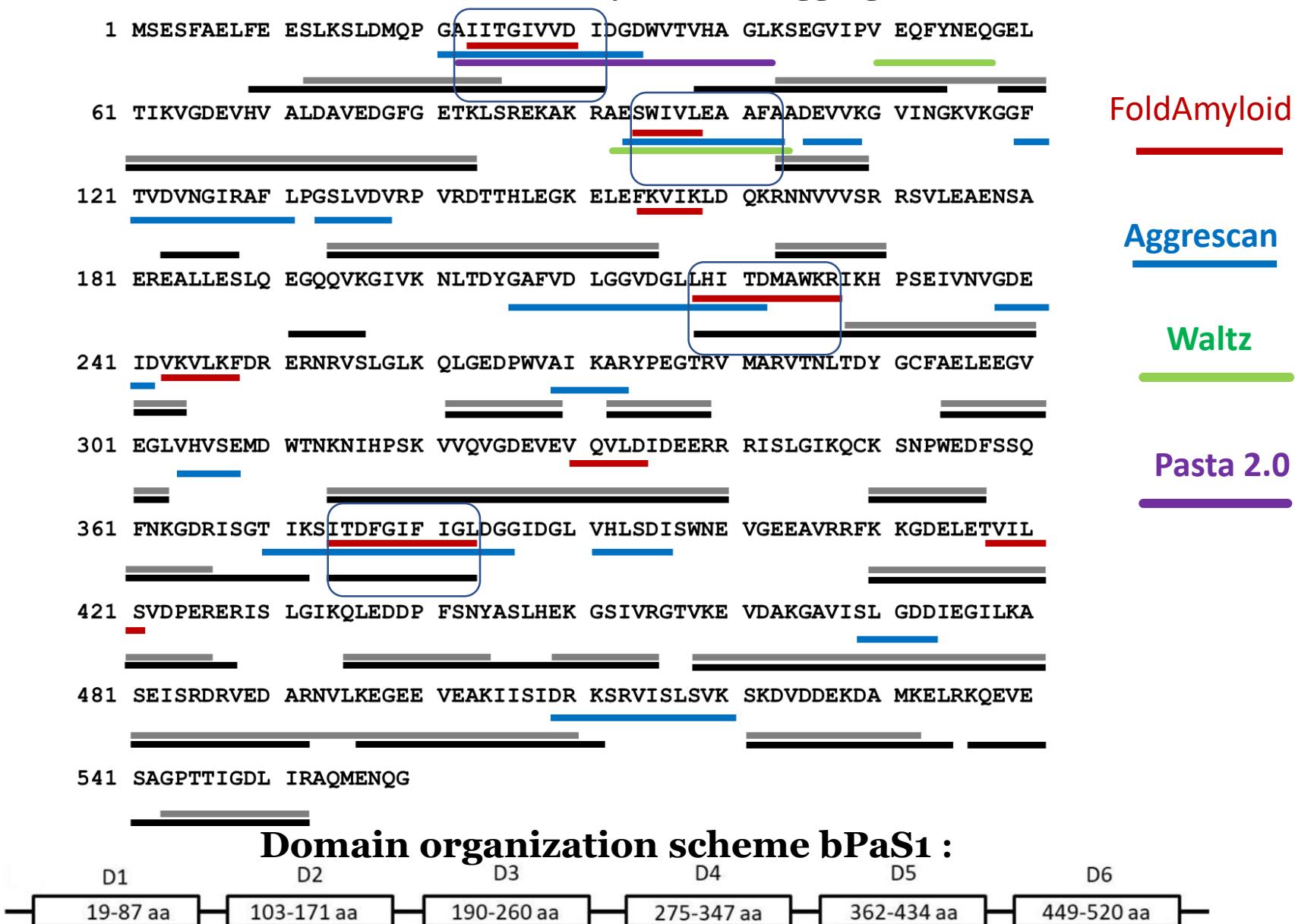
Y.**DASSVPTTTATVK.**L **500-512**

R.REDRREYEYG**AVA**EYNLYDASSVPTTTATVKLG**DLYGDILLA**SLGLEEEAEK.S **482-533**

R.KGAPRREDRREYEYG**AVA**EYNLYDASSVPTTTATVKLG**DLYGDILLA**SLGLEEEAEK.S **477-533**

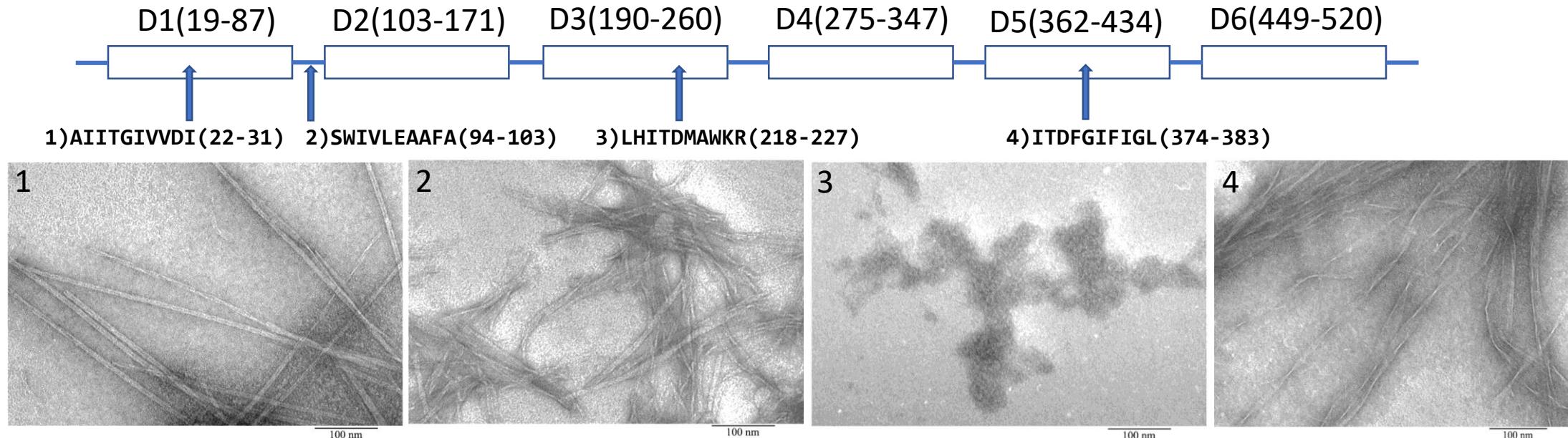
Search results for amyloidogenic sites bPaS1

- Results of HPLC-MS analysis of bPaS1 aggregates
- Results of HPLC-MS analysis of "unaggregated" bPaS1

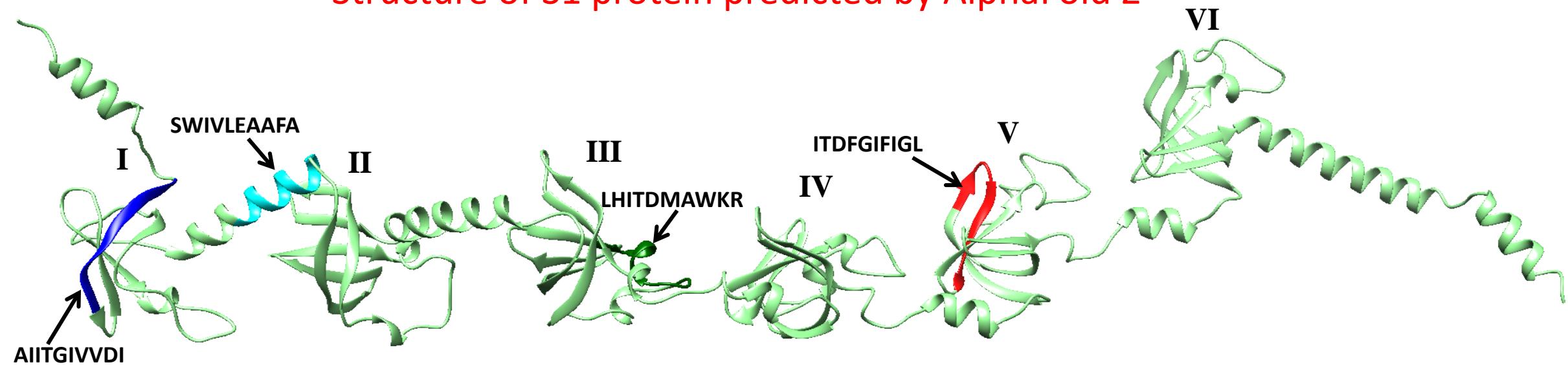


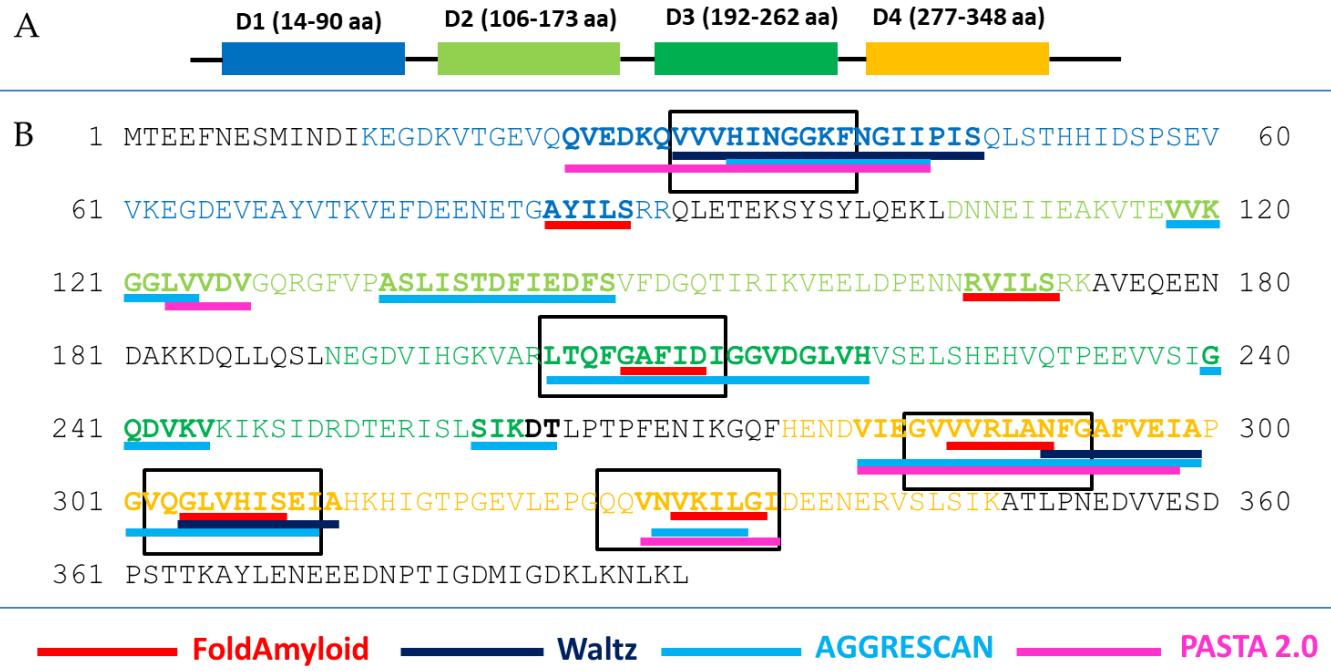
C

S1 from *P. aeruginosa*

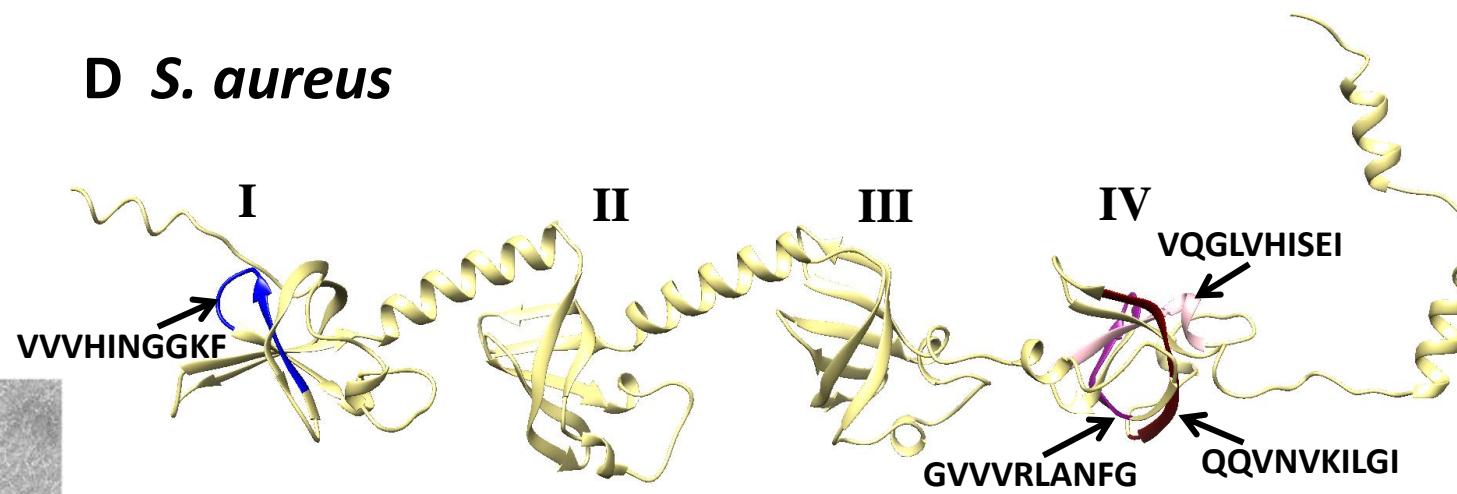
C *P. aeruginosa*

Structure of S1 protein predicted by AlphaFold 2





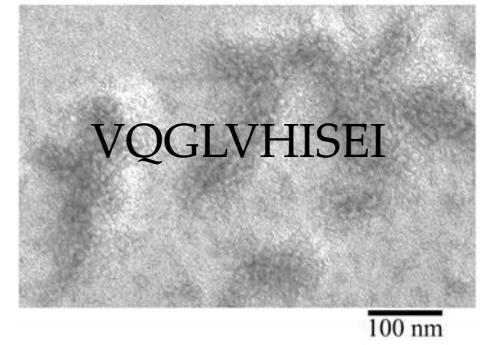
D *S. aureus*



VVVHINGGKF

100

Structure of S1 protein predicted by AlphaFold 2



Development and design of peptides based on the ribosomal S1 protein *T. thermophilus*

Peptide	Sequence	Prediction of antimicrobial activity (CAMPR3)		
		Support vector machine (SVM)	Random Tree Method (RF)	Discriminant Analysis (DA)
D9G	DFGVFVN LG	0.00	0.41	0.03
E10D	EMEVVV LNI D	0.10	0.45	0.01
<u>V10I</u>	VTDFGVF VEI	0.80	0.44	0.03
V10T	VVEGTVVE VT	0.08	0.50	0.00

Development and design of peptides based on the ribosomal S1 protein *T. thermophilus*

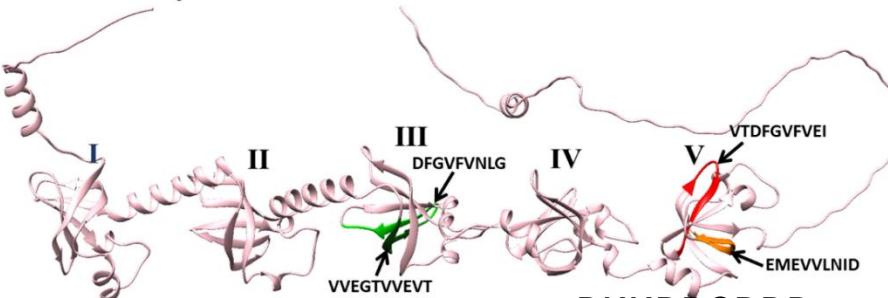
Peptide	Sequence	Prediction of antimicrobial activity (CAMPR3)		
		Support vector machine (SVM)	Random Tree Method (RF)	Discriminant Analysis (DA)
R23I	RKKRRQRRR GGAGVTDFGVFVEI	0.03	0.51	0.99
R23T	RKKRRQRRR GGAGVVEGTVVVEVT	0.01	0.47	0.59

Fragment of the HIV-1 TAT peptide

- 1. Increases antimicrobial activity
- 2. Promotes penetration into the eukaryotic cell
- 3. Does not interfere with the formation of aggregates of proteins and fibrils

Structures of S1 proteins predicted by AlphaFold 2

A *T. thermophilus*

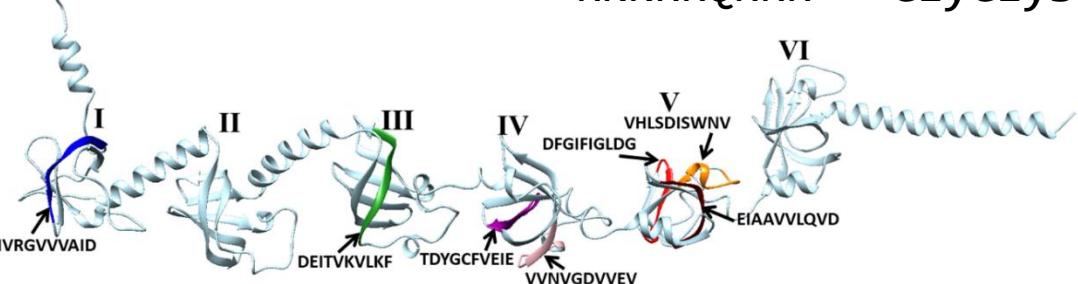


Construction of hybrid peptides

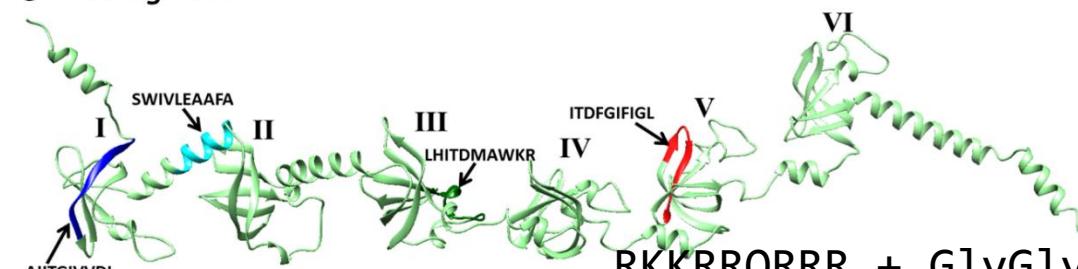
RKKRRQRRR + GlyGlySarGly + **VVEGTWVEVT**
RKKRRQRRR + GlyGlySarGly + **VTDFGVFVEI**

R23T
R23I

B *E. coli*



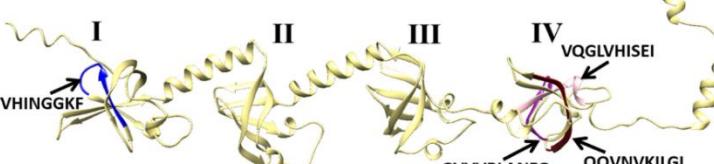
C *P. aeruginosa*



RKKRRQRRR + GlyGlySarGly + **LHITDMAWKR**
RKKRRQRRR + GlyGlySarGly + **ITDFGIFIGL**

R23R
R23L

D *S. aureus*



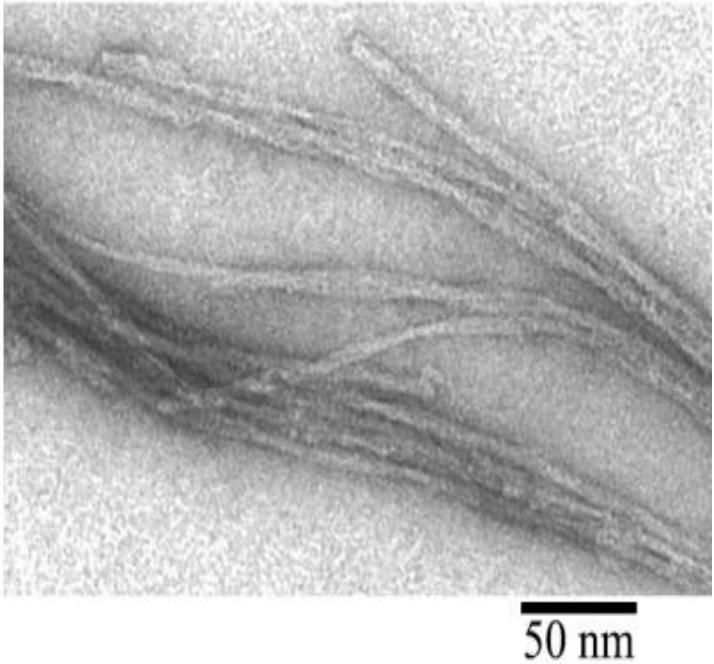
RKKRRQRRRGGSarGVVVI-Asi-GGKF
RKKRRQRRRGGSarGLTQFGAFIDI
RKKRRQRRRGGSarGVQGLVHISEI

R23F
R23DI
R23EI

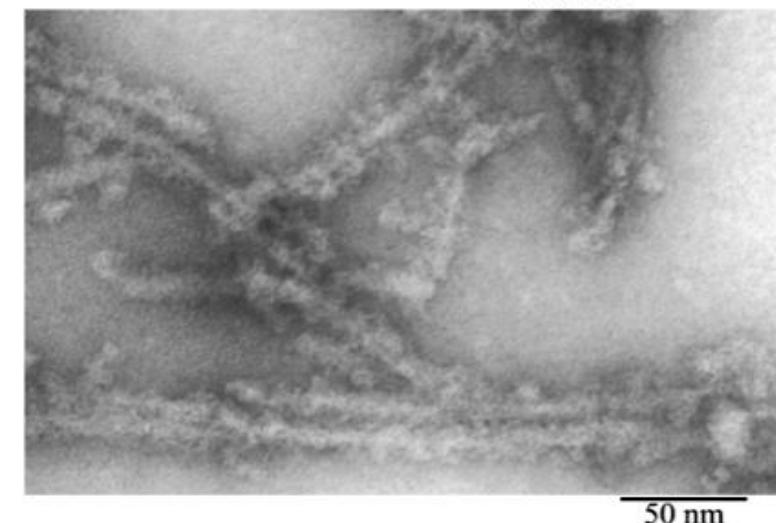
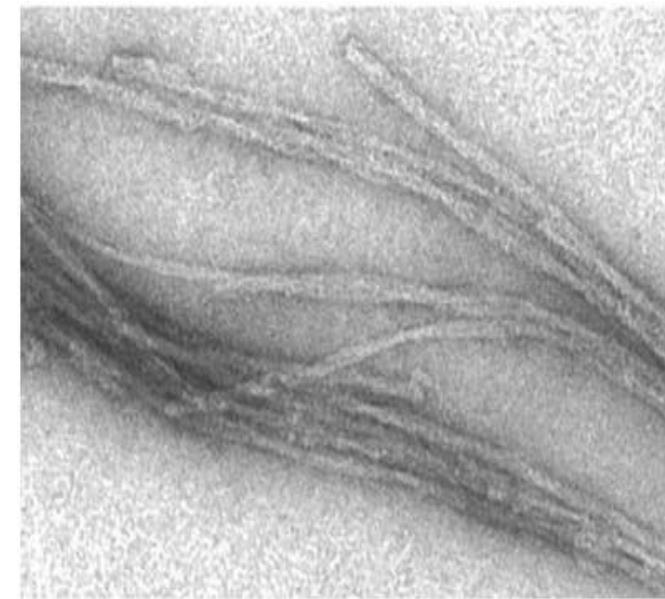
Co-aggregation of amyloidogenic peptides and ribosomal S1 protein

S1:R23T=1:5 (0.5 mg/ml and 2.5 mg/ml)

S1:V10T=1:5 (0.5 mg/ml and 2.5 mg/ml)



It was similarly verified that coaggregation of the V10T peptide and the S1 protein at 5:1 ratio led to the formation of fibrils.



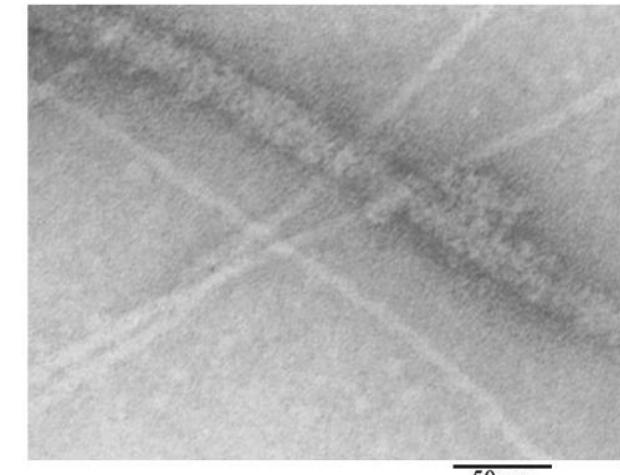
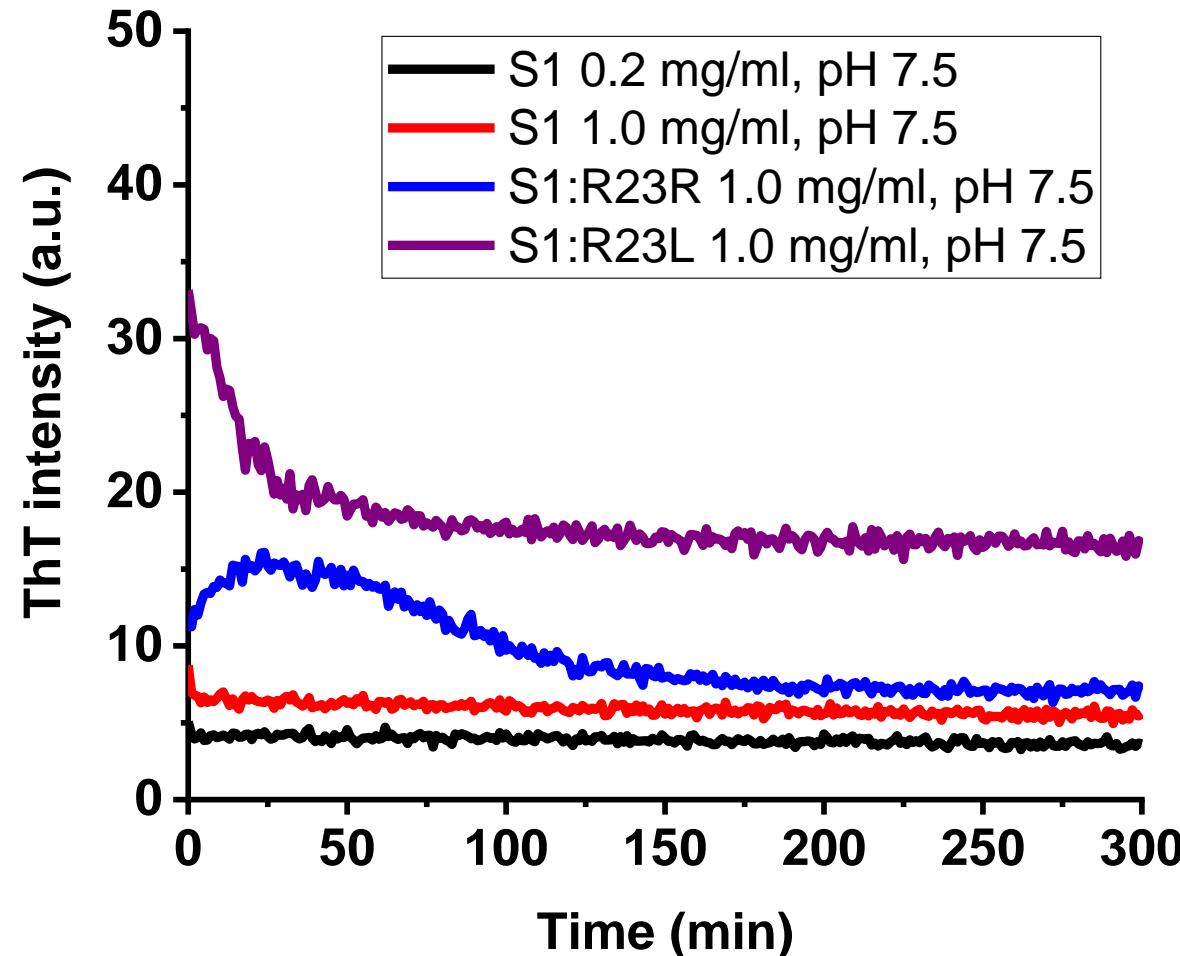
S1:R23I=1:5
(0.5 mg/ml
and 2.5
mg/ml) from
T.
thermophilus

Kinetics of S1 coaggregation with peptides R23R and R23L

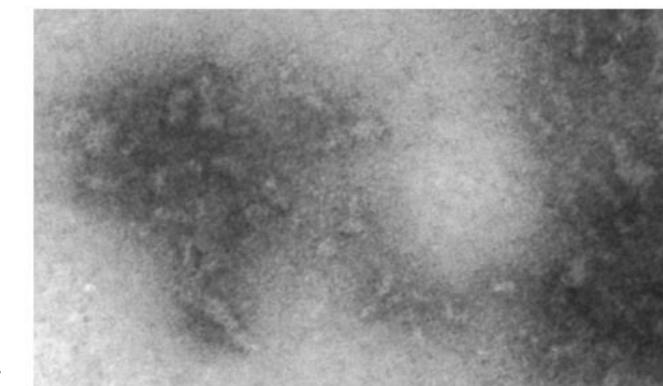
Fluorescence results with ThT, incubation at 37 °C

ThT intensity for peptides and S1 *P.aeruginosa*

S1:R23R=1:5 (0.2 mg/ml and 1.0 mg/ml) from *P. aeruginosa*



S1:R23L=1:5 (0.2 mg/ml and 1.0 mg/ml) from *P. aeruginosa*



During co-aggregation of the S1 protein and R23R from *P. aeruginosa*, both aggregates of different sizes and fibrils of different diameters are observed. In addition, film-like polymers can sometimes be observed.

Antibacterial properties of peptides synthesized based on the predicted amyloidogenic sites of S1

$$\Theta = 1 - \frac{A(\text{Experiment})}{A(\text{Control})} \quad (1)$$

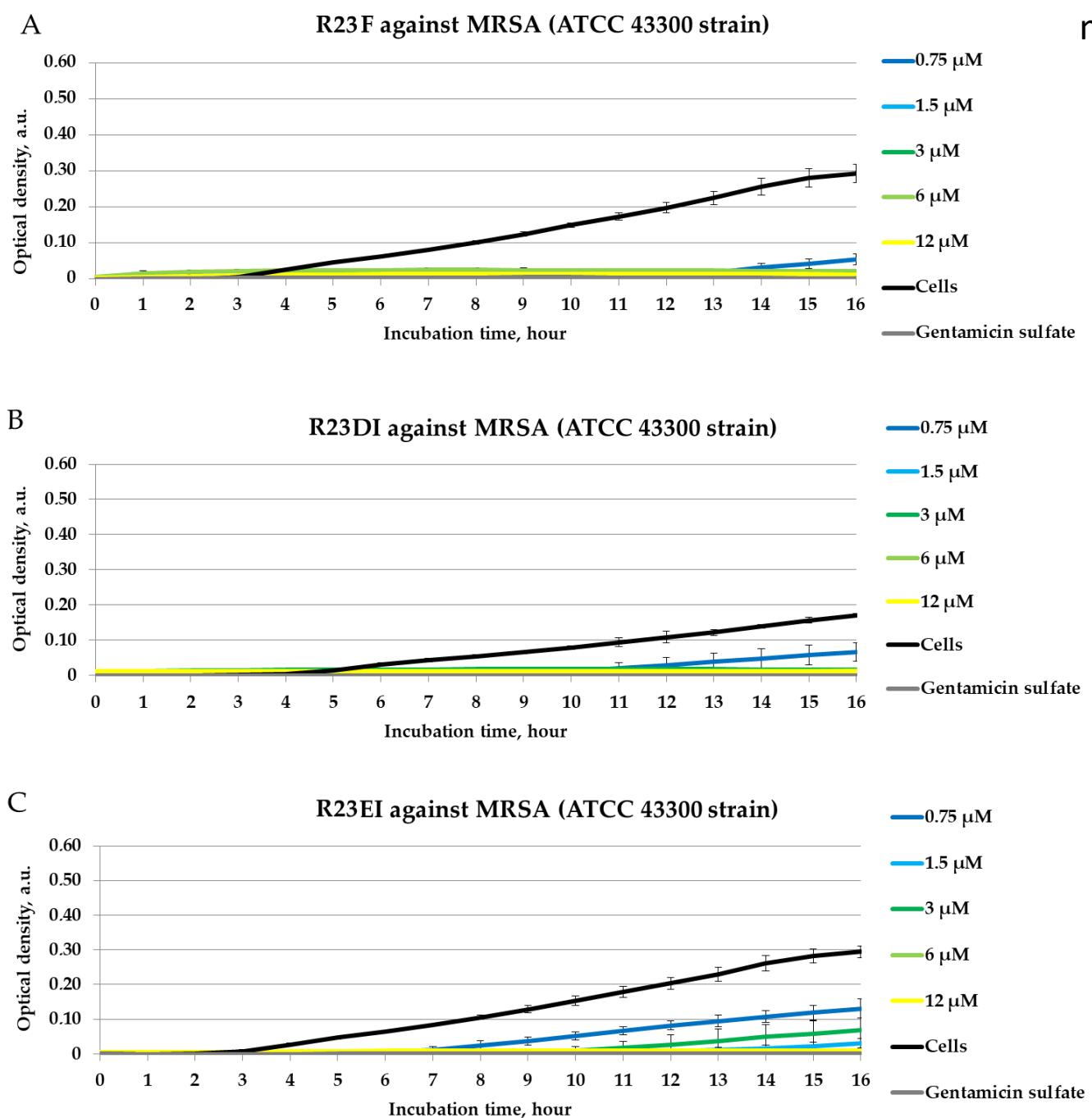
The evaluation of the antibacterial effect (E) was carried out according to formula (1), where A is the light absorption of the liquid culture of *T. thermophilus* after 24 hours of incubation.

An E value greater than 0.5 indicates an antibacterial effect.

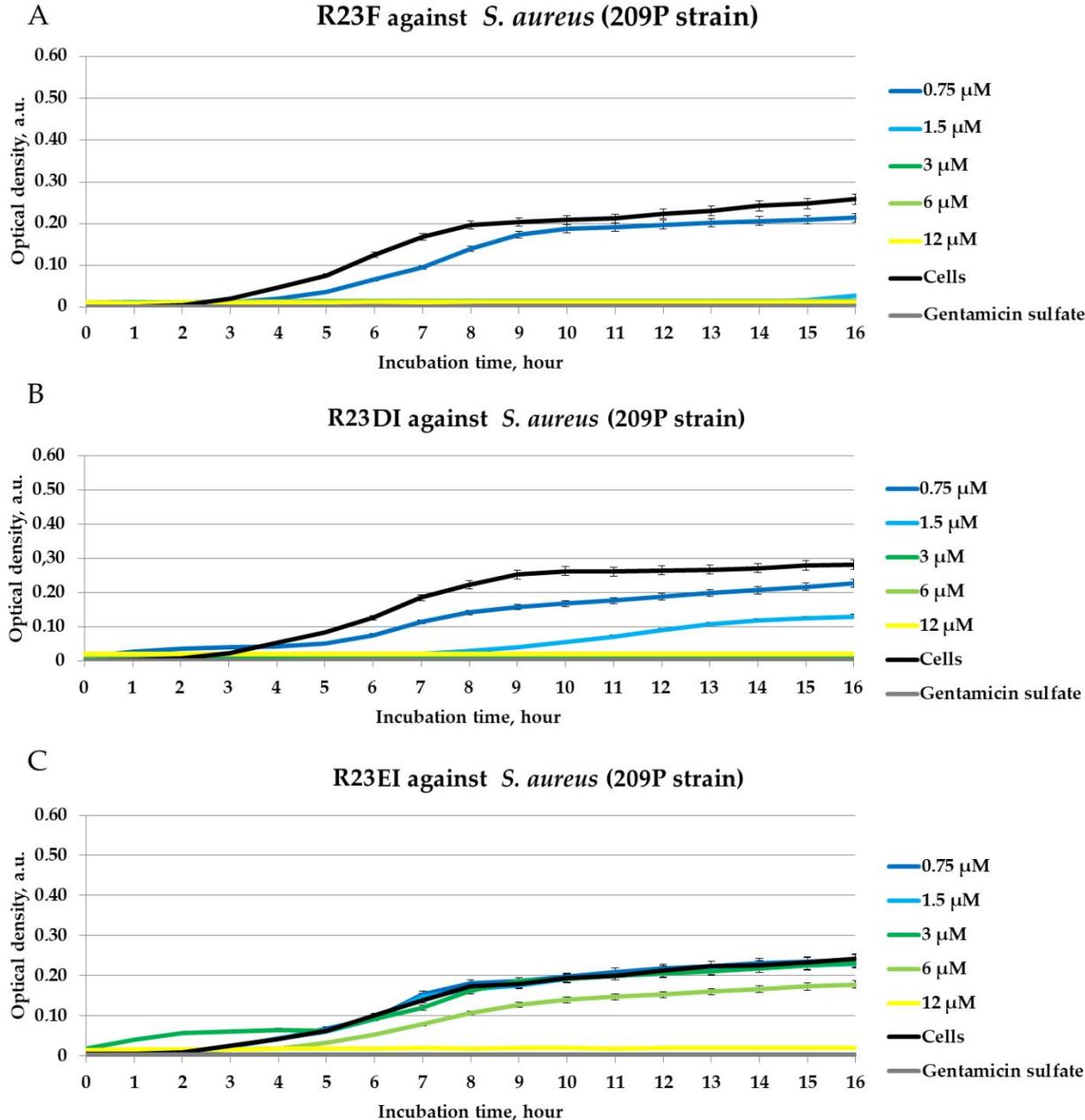
Sequence peptide	Peptide concentration and presence (+) or absence (-) antibacterial effect					
	1 μg/ml	10 μg/ml	50 μg/ml	100 μg/ml	500 μg/ml	1000 μg/ml
DFGVFVN LG	—	—	—	—	—	—
EMEVVVLN ID	—	—	—	—	—	—
VTDFGVFVEI	—	—	—	—	—	+
VVEGTVVEVT	—	—	—	—	—	—
RKKRRQRRRGGSarG VTDFGVFVEI	—	—	+	+	+	—
RKKRRQRRRGGSarG VVEGTVVEVT	—	—	—	—	+	+

Inhibitory activity of peptides

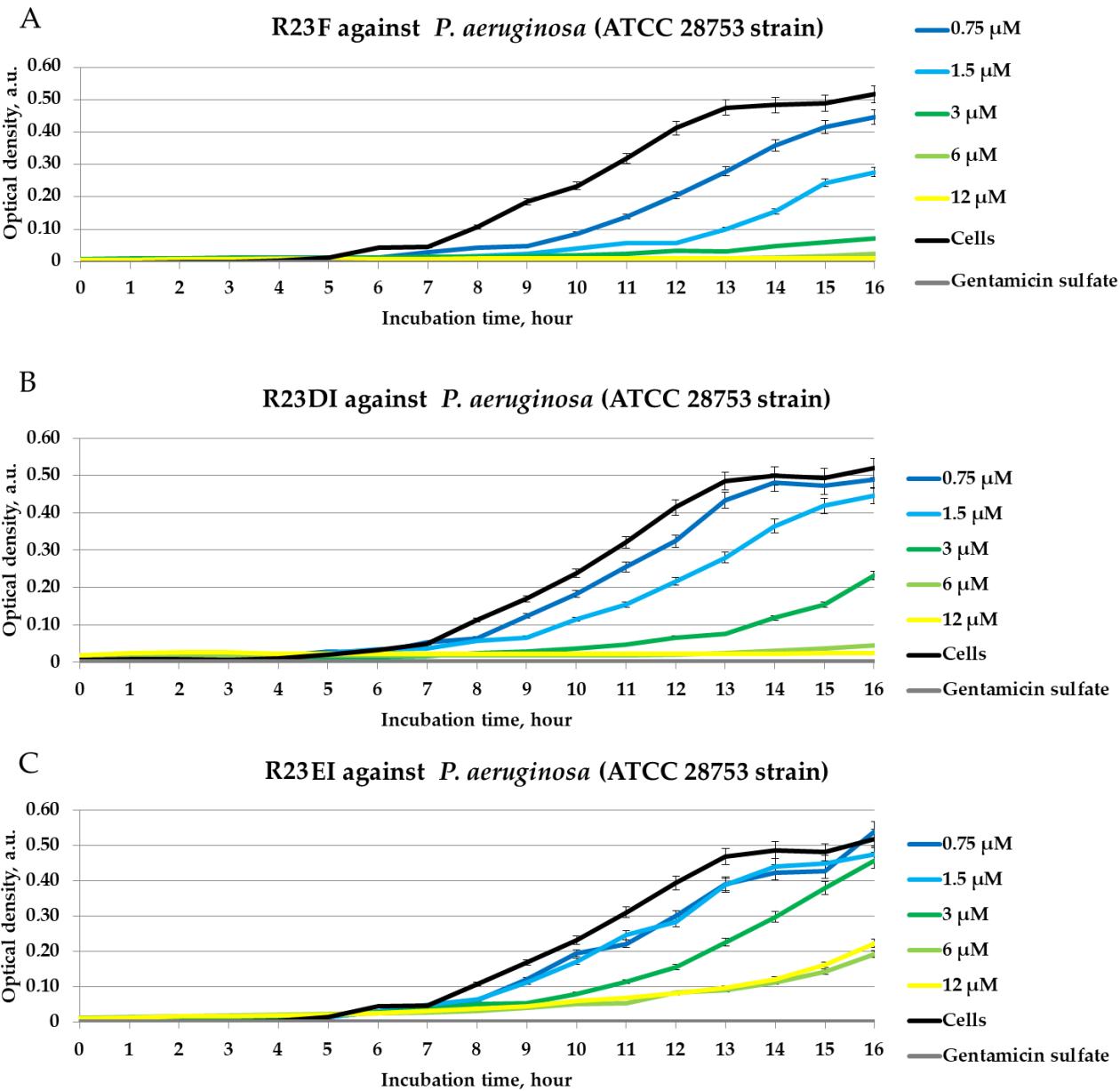
methicillin-resistant *S. aureus*



Inhibitory activity of peptides



Inhibitory activity of peptides

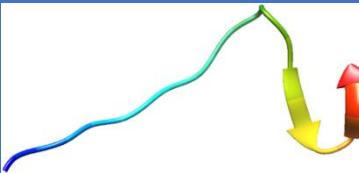
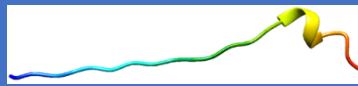


Amyloidogenic AMP peptides based on S1 ribosomal protein *P. aeruginosa*

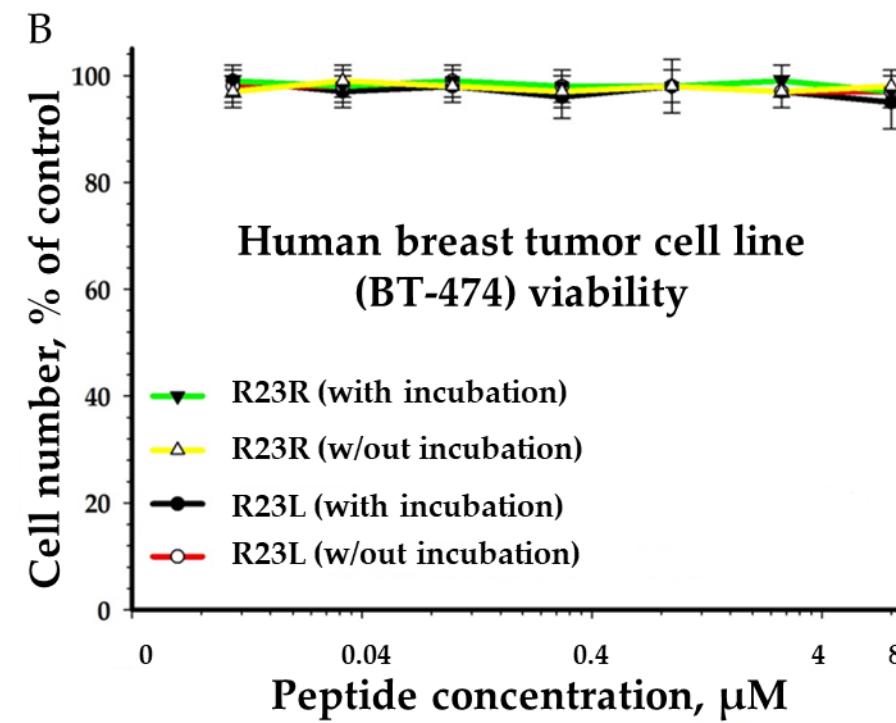
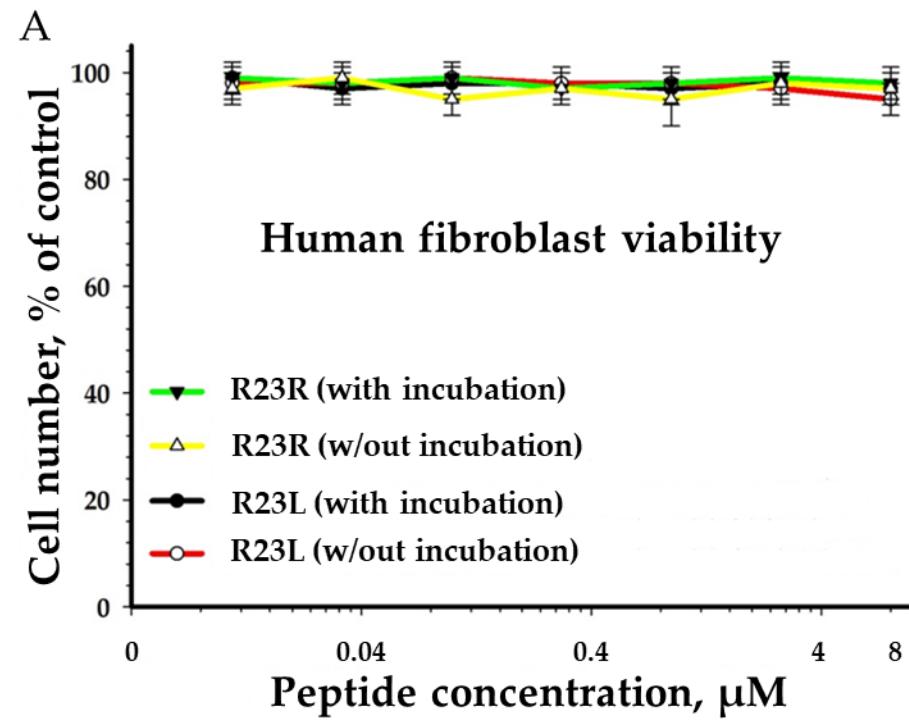
Sequence of hybrid peptides and folding patterns predicted by AlphaFold	Strain of the pathogenic microorganism	MIC for the tested hybrid peptide (μM)
Based on the sequence S1 protein from <i>P. aeruginosa</i>		
RKKRRQRRRGGGITDFGIFIGL 	MRSA strain ATCC 43300 (resistant to ampicillin) S. aureus strain 209 (resistant to aztreonam) P. aeruginosa (strain ATCC 28753) (resistant to sulfamethoxazole)	12 >12 12
RKKRRQRRRGGSarGLHITD-Nle-AWKR 	P. aeruginosa (strain ATCC 28753) (resistant to sulfamethoxazole) P. aeruginosa (strain PA 103) (resistant to levomycin)	12 >12
RKKRRQRRRGGSarGITDFGIFIGL 	P. aeruginosa (strain ATCC 28753) (resistant to sulfamethoxazole) P. aeruginosa (strain PA 103) (resistant to levomycin)	12 >12

Grishin SY, Domnin PA, Kravchenko SV, Azev VN, Mustaeva LG, Gorbunova EY, Kobyakova MI, Surin AK, Makarova MA, Kurpe SR, Fadeev RS, Vasilchenko AS, Firstova VV, Ermolaeva SA, Galzitskaya OV. Int J Mol Sci.;22(18):9776. (2021)

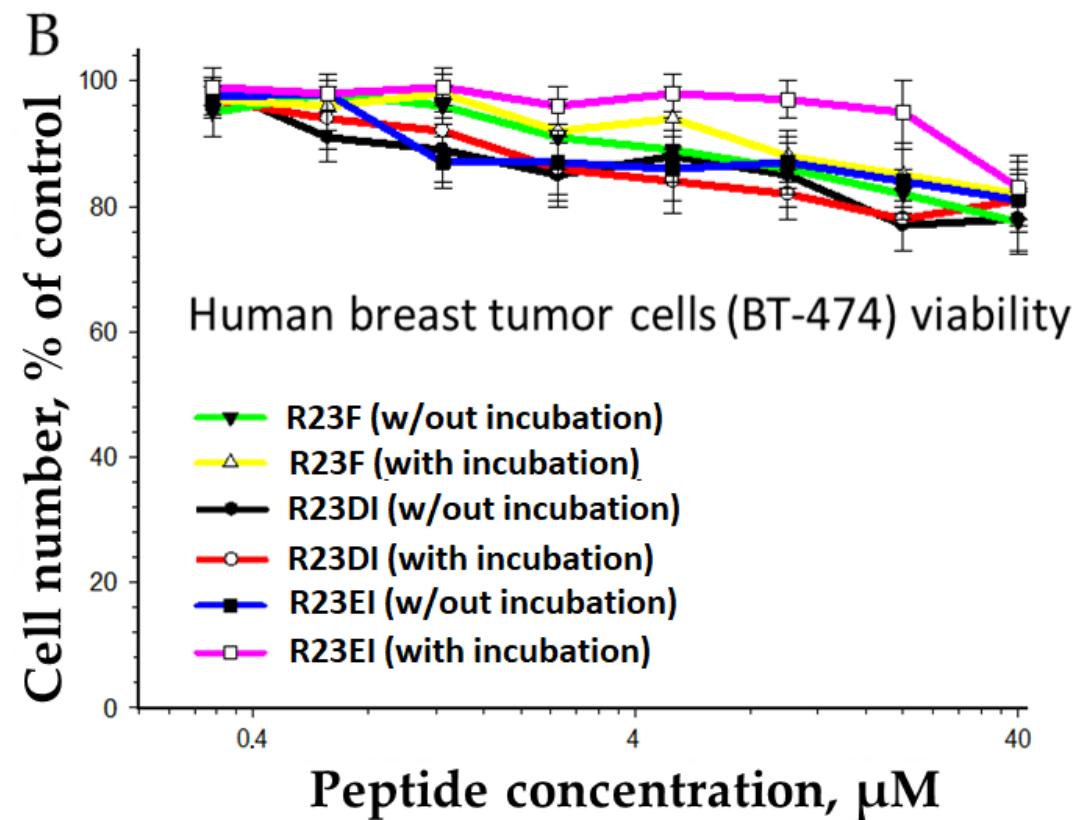
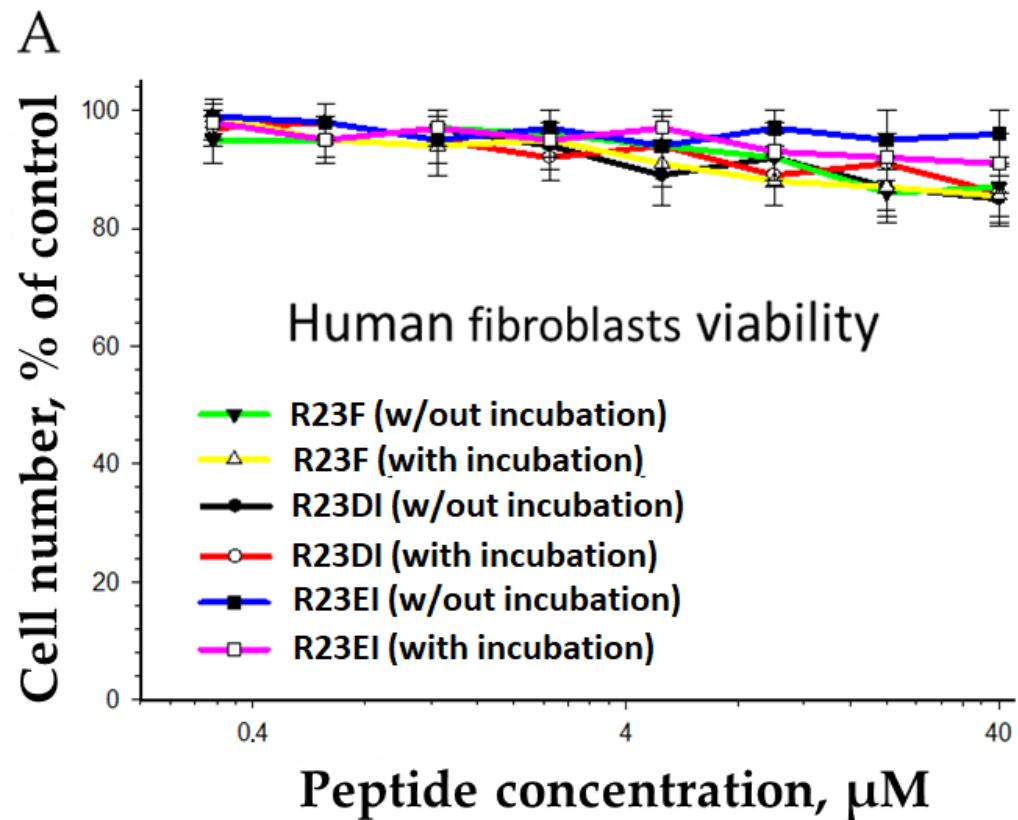
Amyloidogenic AMP peptides based on S1 ribosomal protein *S. aureus*

Based on the sequence S1 protein from <i>S. aureus</i>			
RKKRRQRRRGGSarGVVH-I-Asi-GGKF 	MRSA strain ATCC 43300 (resistant only to ampicillin) S. aureus strain 209 (resistant to aztreonam) P. aeruginosa strain ATCC 28753 (resistant to sulfamethoxazole)	3 3 12	
RKKRRQRRRGGSarGLTQFGAFIDI 	MRSA strain ATCC 43300 (resistant only to ampicillin) S. aureus strain 209 (resistant to aztreonam) P. aeruginosa strain ATCC 28753 (resistant to sulfamethoxazole)	3 6 12	
RKKRRQRRRGGSarGVQGLVHISEI 	MRSA strain ATCC 43300 (resistant only to ampicillin) S. aureus strain 209 (resistant to aztreonam) P. aeruginosa strain ATCC 28753 (resistant to sulfamethoxazole)	6 12 >12	

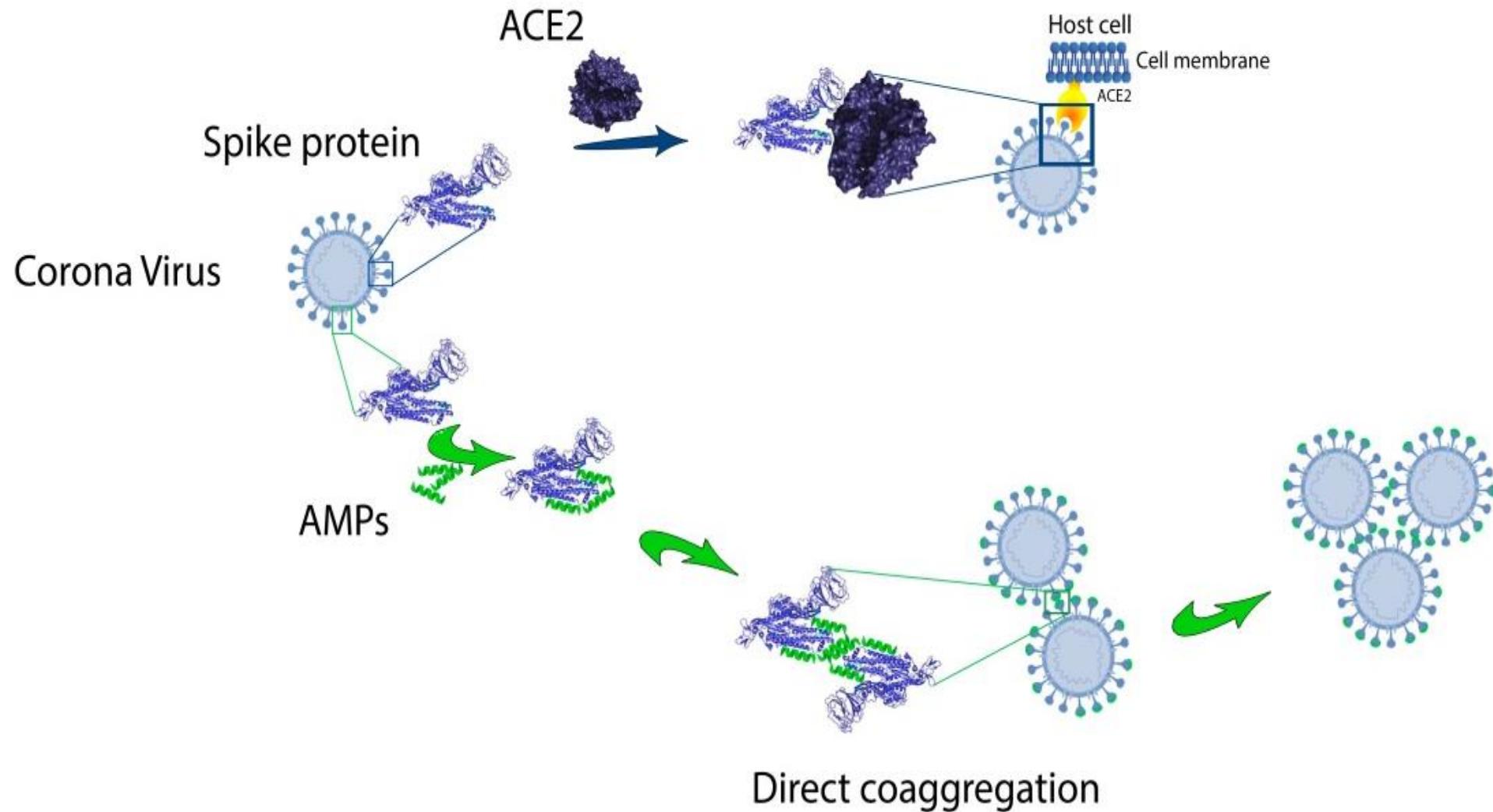
Effects of peptide treatment on survival the human fibroblasts (A) and breast tumor cell line BT-474 (B). Error bars show standard errors



Effect of peptide treatment on the survival of human fibroblasts (**A**) and the breast tumor cell line BT-474 (**B**) without preliminary incubation (18 h, 37 °C, in DMEM with 10% FBS) and after 72 h of co-incubation with peptide. Each of the experiments was carried out at least three times ($n \geq 3$)



Hypothetical mechanism of targeted co-aggregation against CoV



Kurpe S.R., Grishin S.Yu., Surin A.K., Panfilov A.V., Slizen M.V., Chowdhury S.D., **Galzitskaya O.V.** Antimicrobial and Amyloidogenic Activity of Peptides. Can Antimicrobial Peptides Be Used Against SARS-CoV-2? INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 21, 9552. [https://doi.org/10.3390/ijms21249552 \(2020\)](https://doi.org/10.3390/ijms21249552)

Conclusion

Theoretically predicted and experimentally confirmed the ability to manifest aggregation (amyloidogenic) and antibacterial properties of the studied peptides.

New antibacterial peptides have been created that effectively suppress the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, pathogenic bacteria that cause hospital infections.

Acknowledgments

Grishin SY, Kravchenko SV, Domnin PA, Azev VN, Mustaeva LG,
Gorbunova EY, Kurpe SR, Kobyakova MI, Surin AK, Suvorina MY,
Makarova MA, Dzhus UF, Glukhov AS, Selivanova OM, Balobanov
VA, Panfilov AV, Glyakina AV, Deruysheva EI, Machulin AV., Kochetov
AP, Slizen MV, Fadeev RS, Vasilchenko AS, Firstova VV, Ermolaeva SA

The work was supported by the grants from the
Russian Science Foundation 18-14-00321.



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