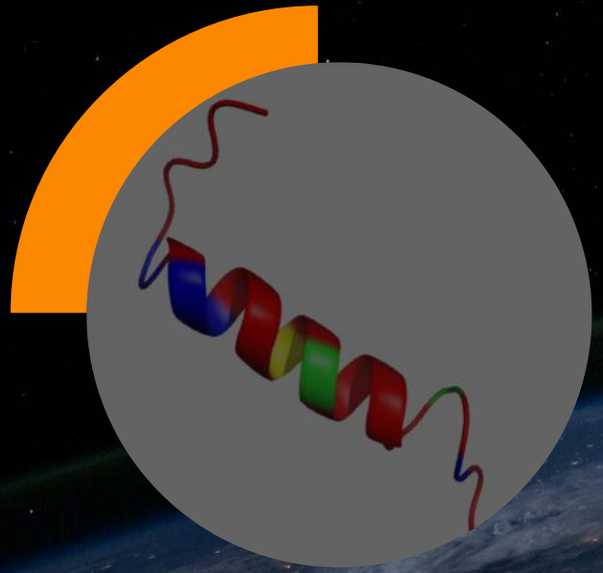
BIOSAXS – A METHOD TO ACCELERATE AND DE-RISK ANTIMICROBIAL DRUG DEVELOPMENT

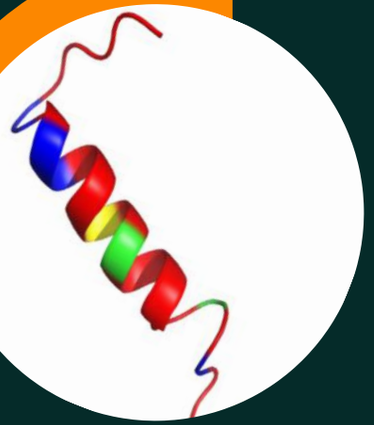


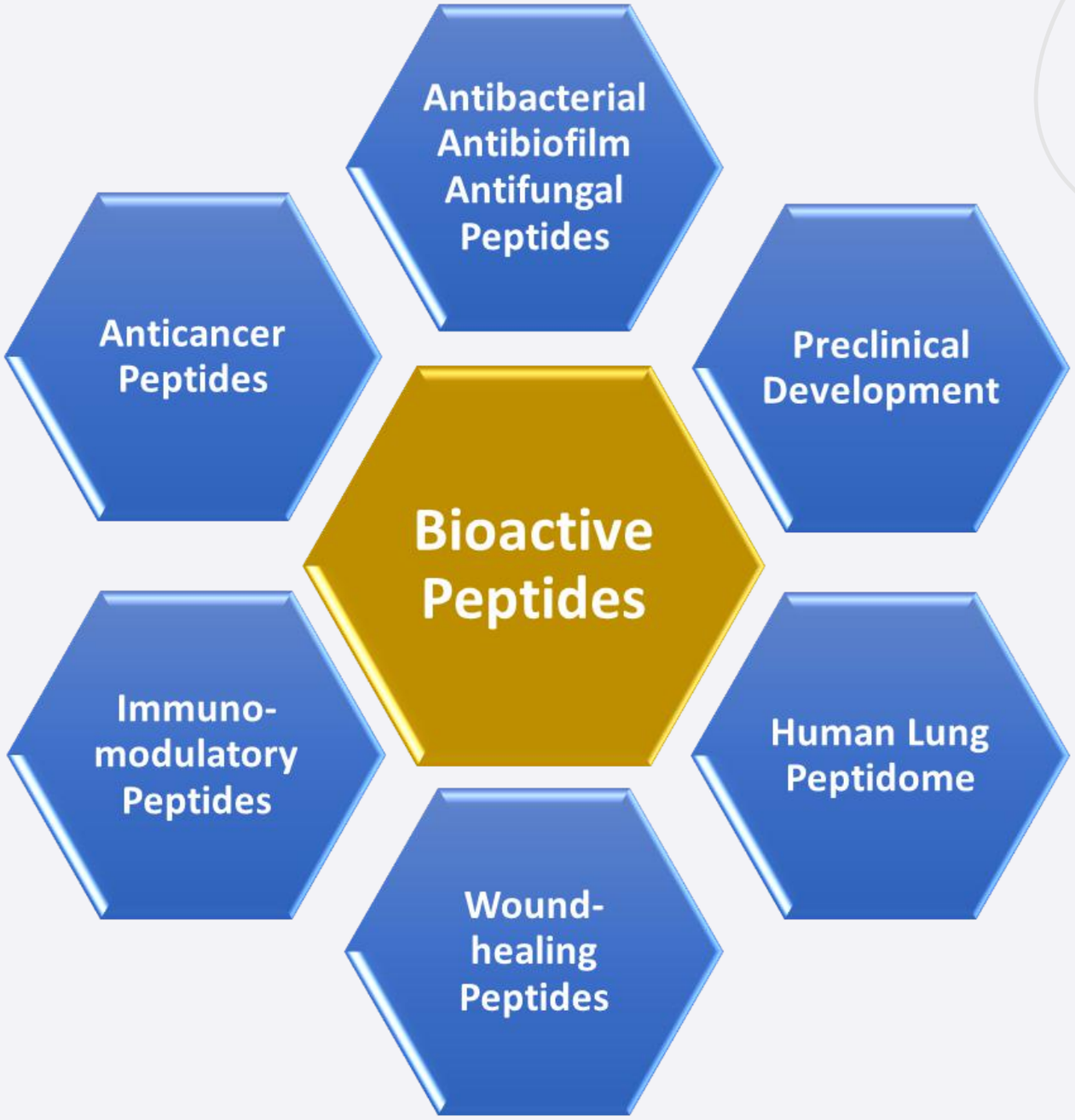
PRESENTED BY KAI HILPERT
READER IN INFECTION AND IMMUNOLOGY
FOUNDER AND DIRECTOR OF TIKA DIAGNOSTICS

CONTENT

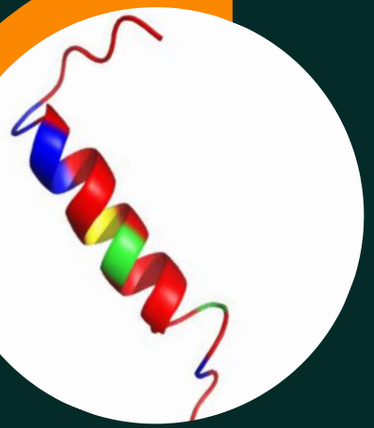
- Overview of our research
- Motivation
- Cationic antimicrobial peptides
- SPPS - on cellulose
- BioSAXS and bacteria
- Take home message
- Thanks and questions

OVERVIEW




Antibacterial Antibiofilm Antifungal Peptides
Anticancer Peptides
Preclinical Development
Bioactive Peptides
Immuno-modulatory Peptides
Human Lung Peptidome
Wound-healing Peptides

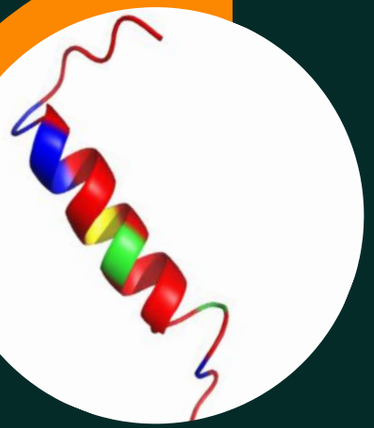
OVERVIEW



TB Diagnostics

- 2013 Discovery of growth-stimulating peptides
- 2014 TiKa Diagnostics LTD
- 2014-2022 £ 1.5 million funding
- 2017 ISO 13485 for research and production of peptides
- 2017 First sales
- 2019 Successfully finished 1. clinical trial
- 2023 Estimated finish of 2. clinical trial

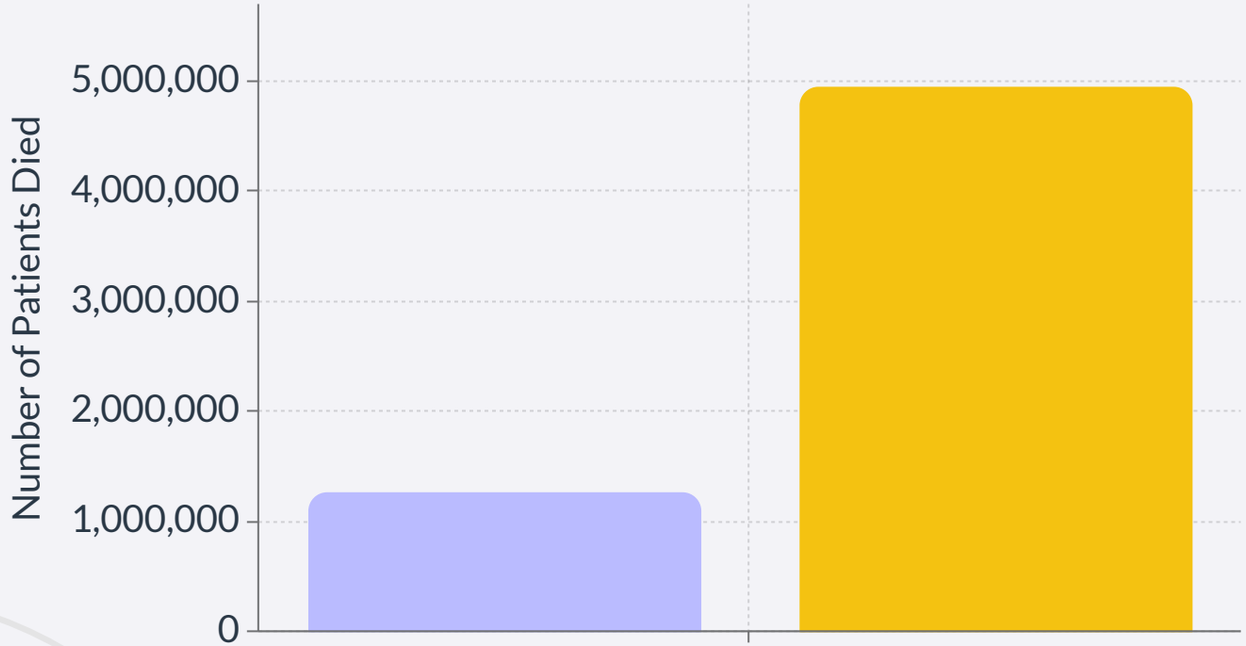
OVERVIEW




Diagnostic kit for
Mycobacterium avium paratuberculosis

MOTIVATION

2019 - Across 88 Pathogen–Drug Combinations



doi:10.1016/S0140-6736(21)02724-0

CATIONIC ANTIMICROBIAL PEPTIDES

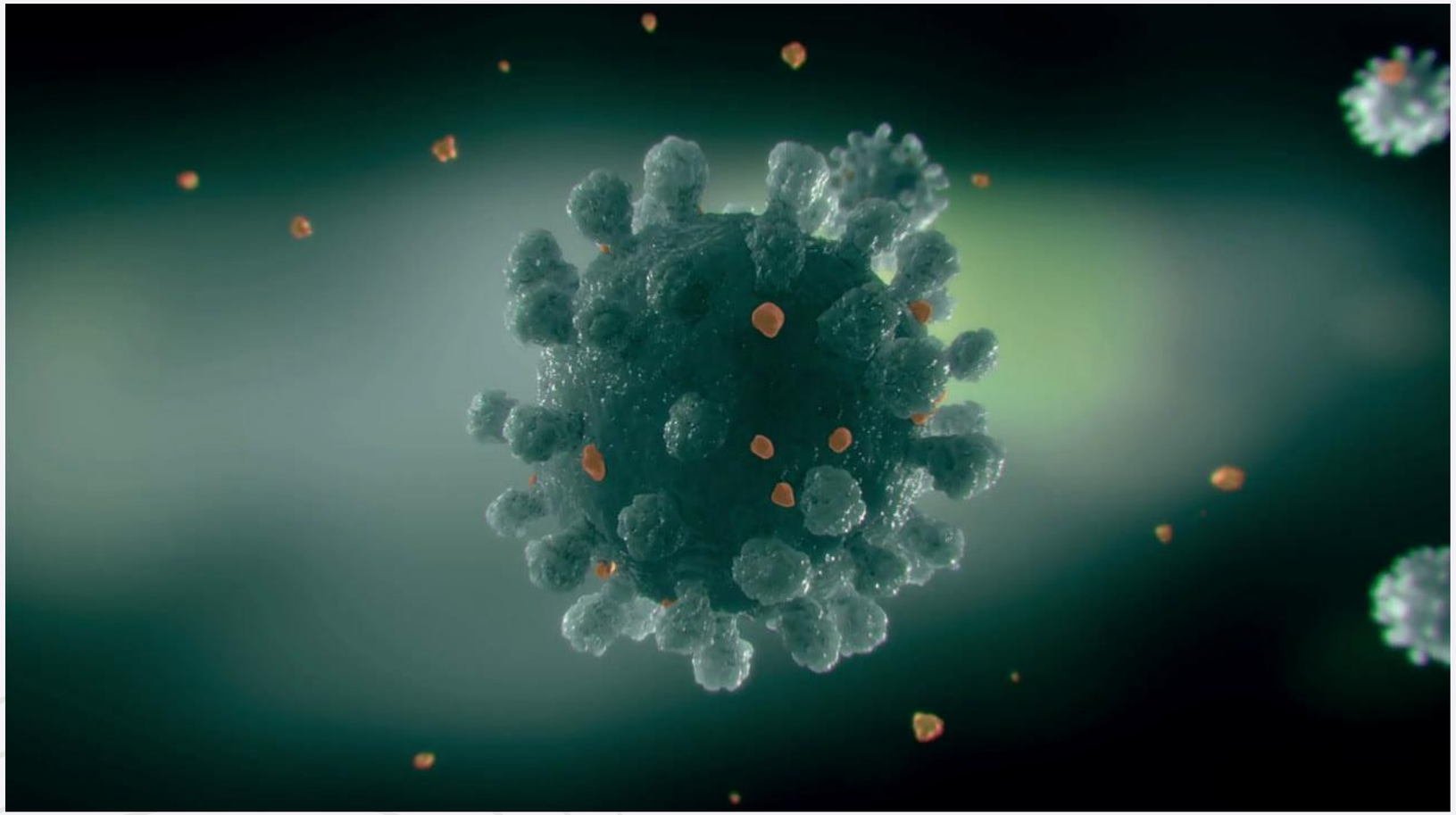
- In bacteria, fungi, insects, tunicates, amphibians, crustaceans, birds, fish and mammals
- About 3,000 natural AMPs known
- About 15,000 artificial AMPs
- Part of the innate immunity in higher organism
- 12 to 80 aa, cationic, amphiphilic
- Different structures

MULTIPLE FUNCTIONS OF AMPs

MULTIPLE FUNCTIONS OF AMPs

I. Antibacterial
II. Antifungal

MULTIPLE FUNCTIONS OF AMPs

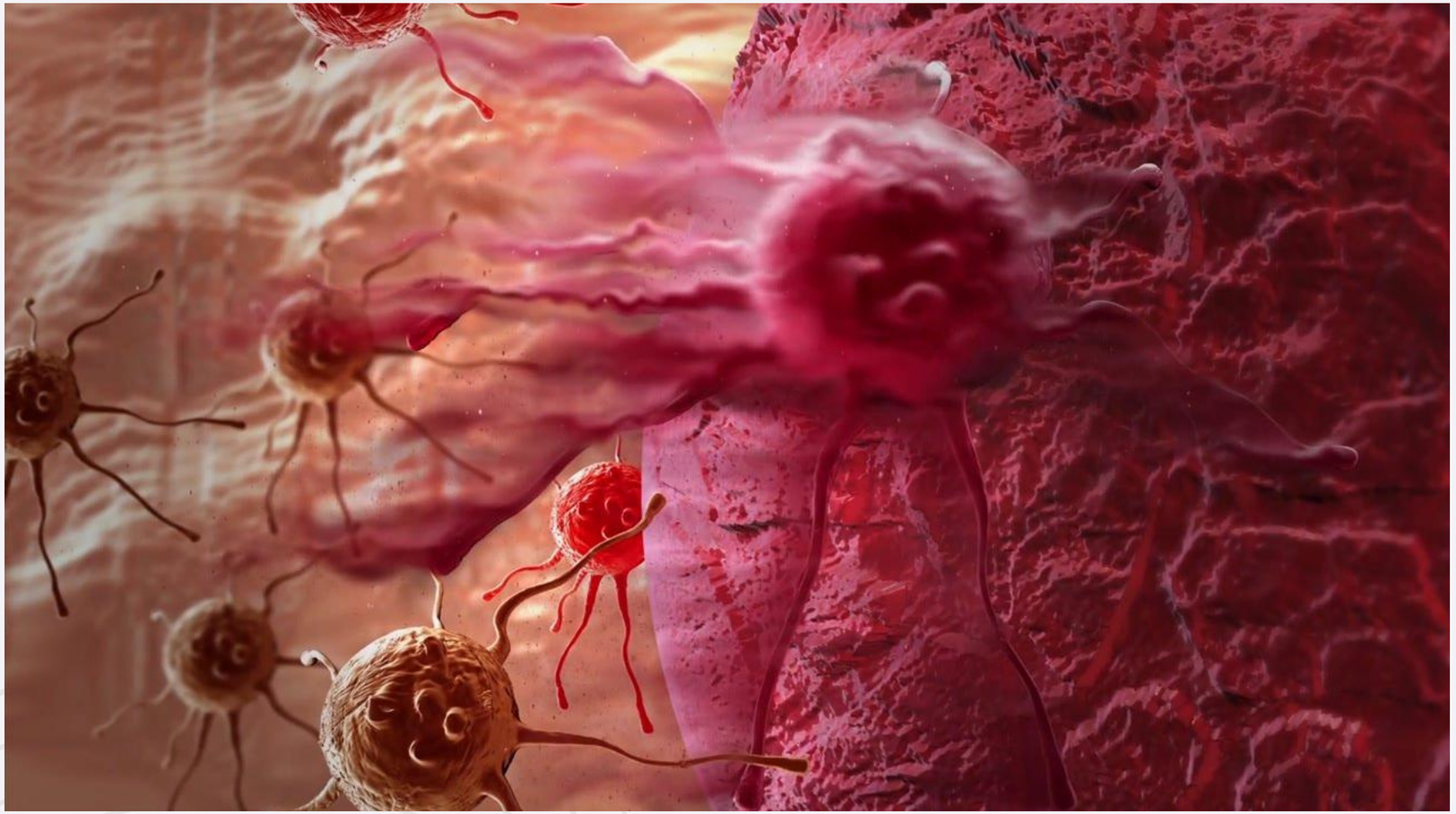


I. Antibacterial
II. Antifungal
III. Antiviral

MULTIPLE FUNCTIONS OF AMPs

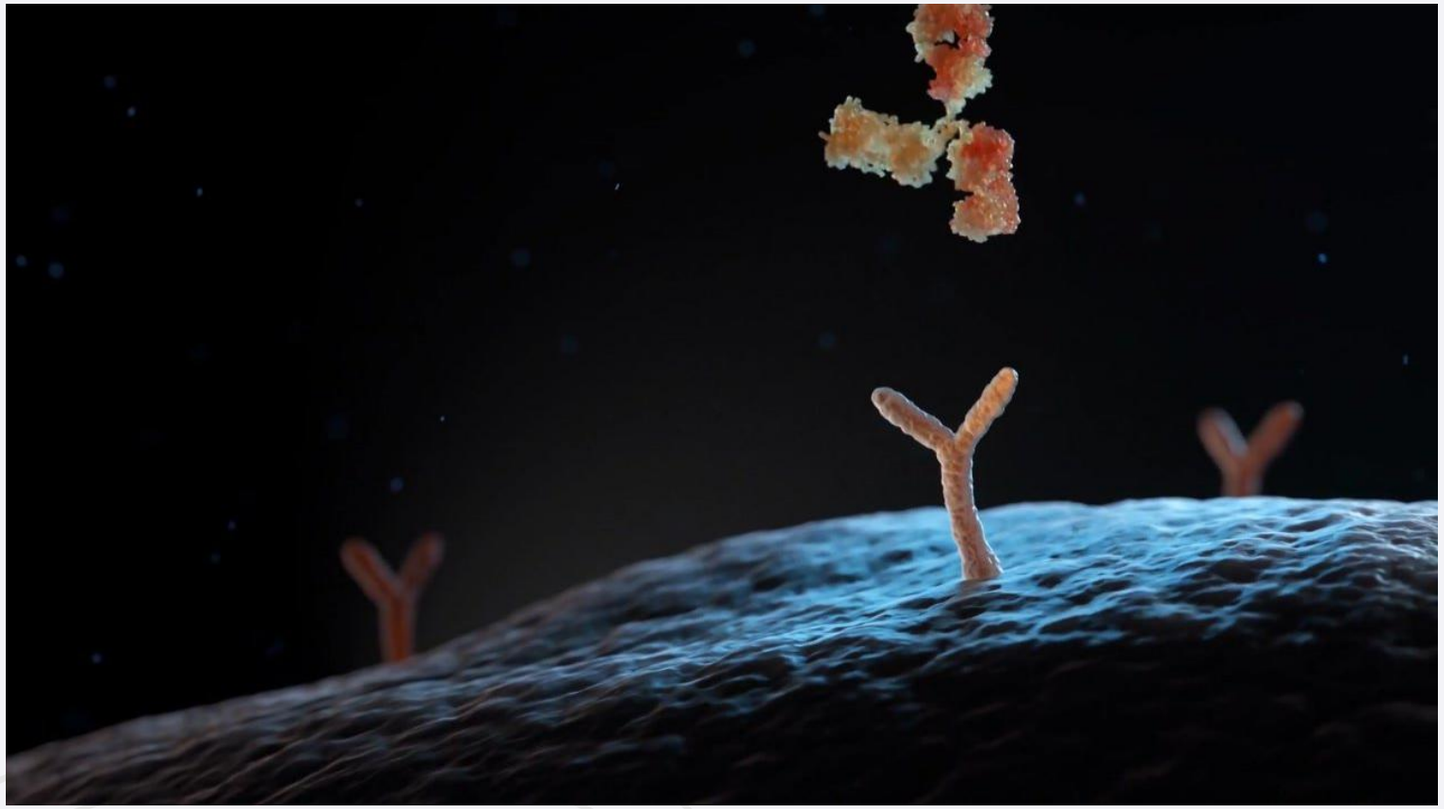
I. Antibacterial
II. Antifungal
III. Antiviral
IV. Antiparasitical

MULTIPLE FUNCTIONS OF AMPs

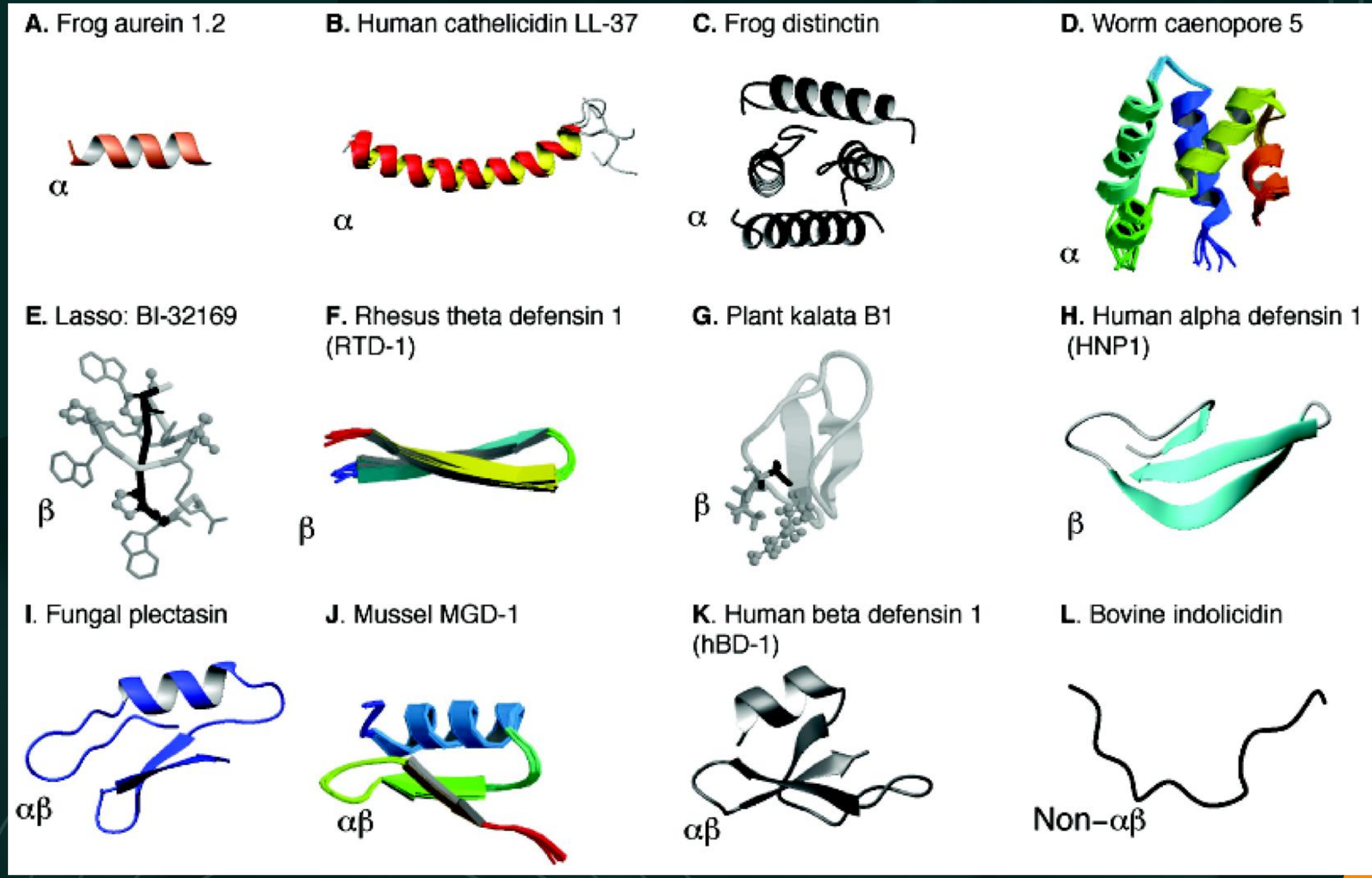


I. Antibacterial
II. Antifungal
III. Antiviral
IV. Antiparasitical
V. Anticancer

MULTIPLE FUNCTIONS OF AMPs

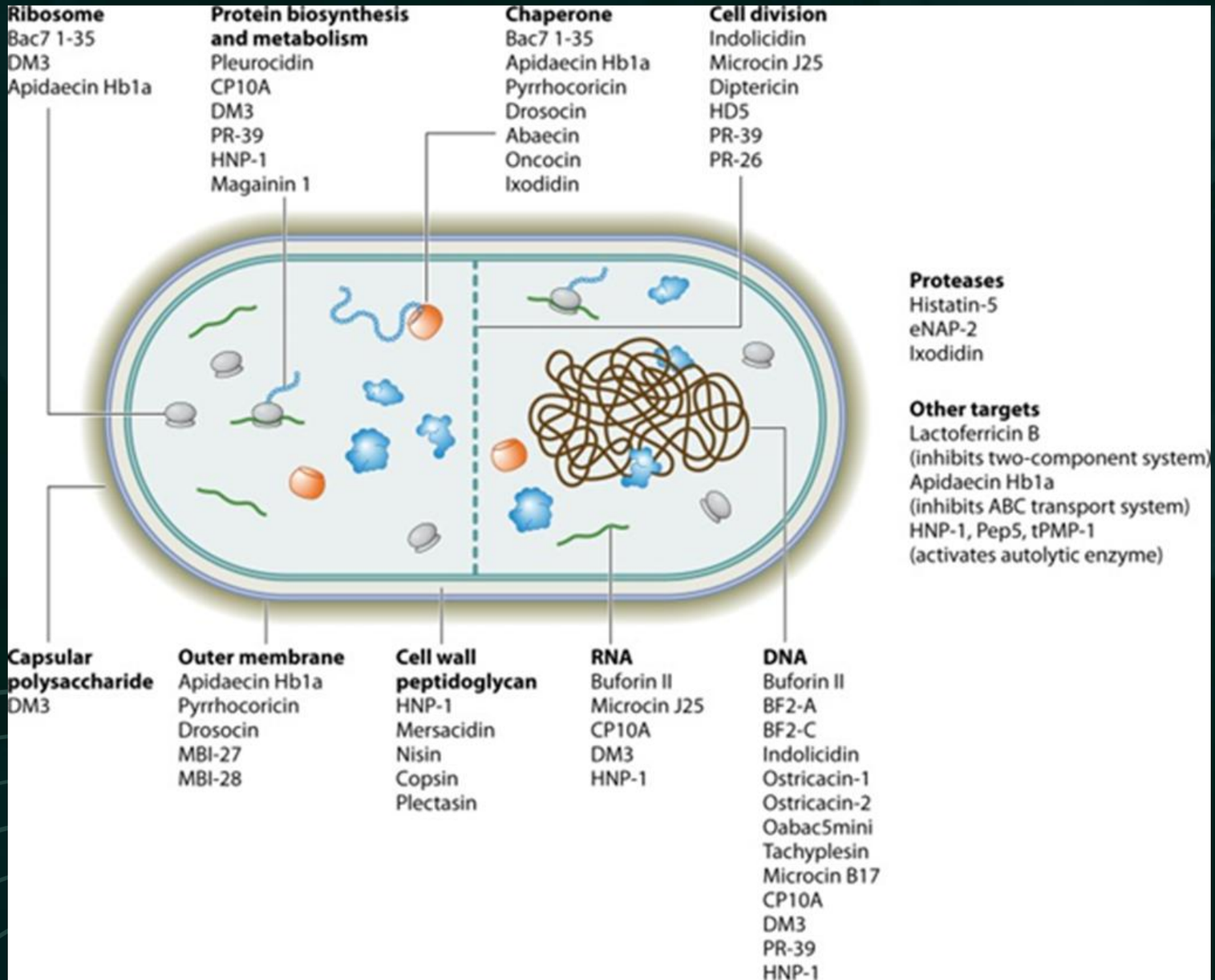


I. Antibacterial
II. Antifungal
III. Antiviral
IV. Antiparasitical
V. Anticancer
VI. Immuno-modulatory
VII. Chemotactic



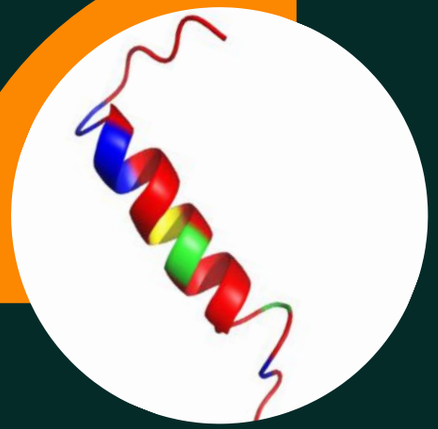
doi.org/10.3390/ph6060728

TARGETS OF ANTIMICROBIAL PEPTIDES

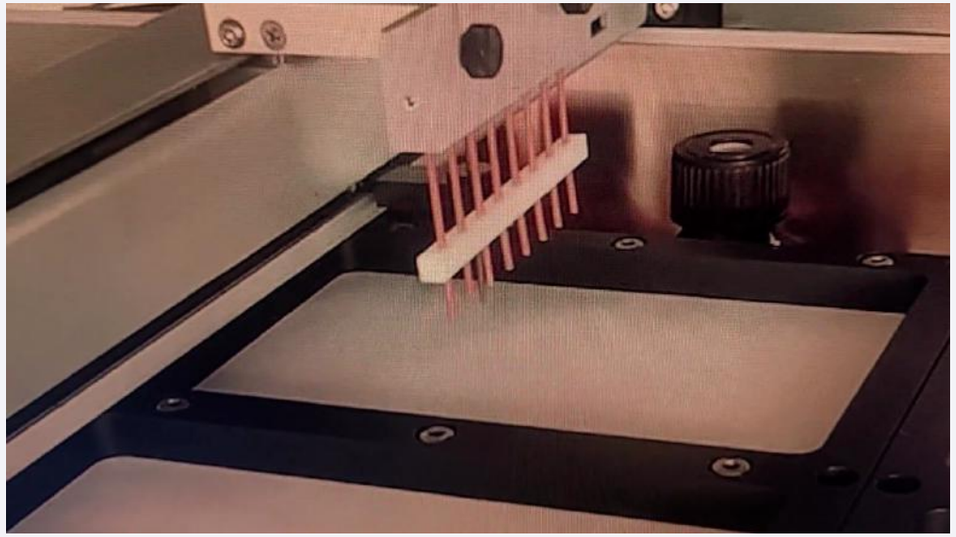


DOI: 10.1128/AAC.02340-16

HISTORY

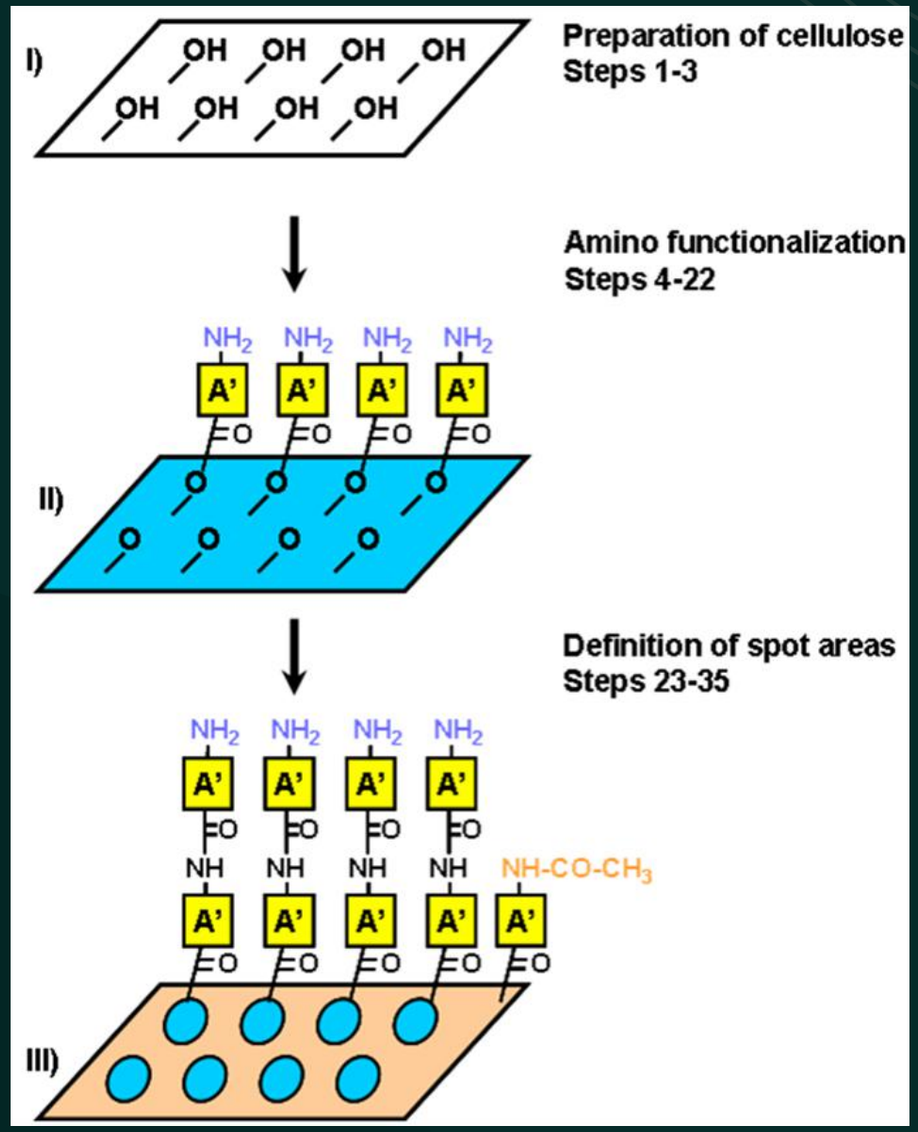


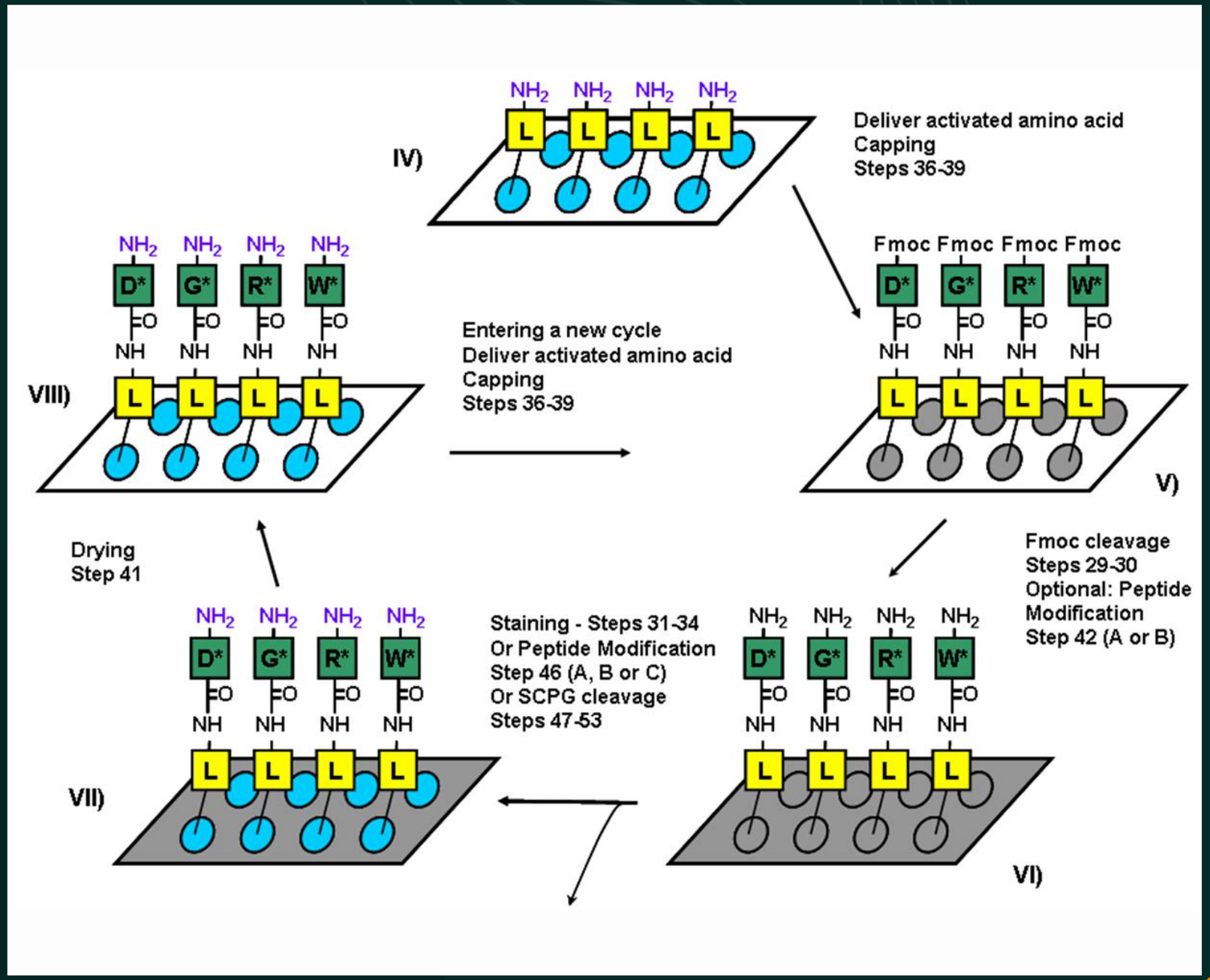
SPPS - ON CELLULOSE

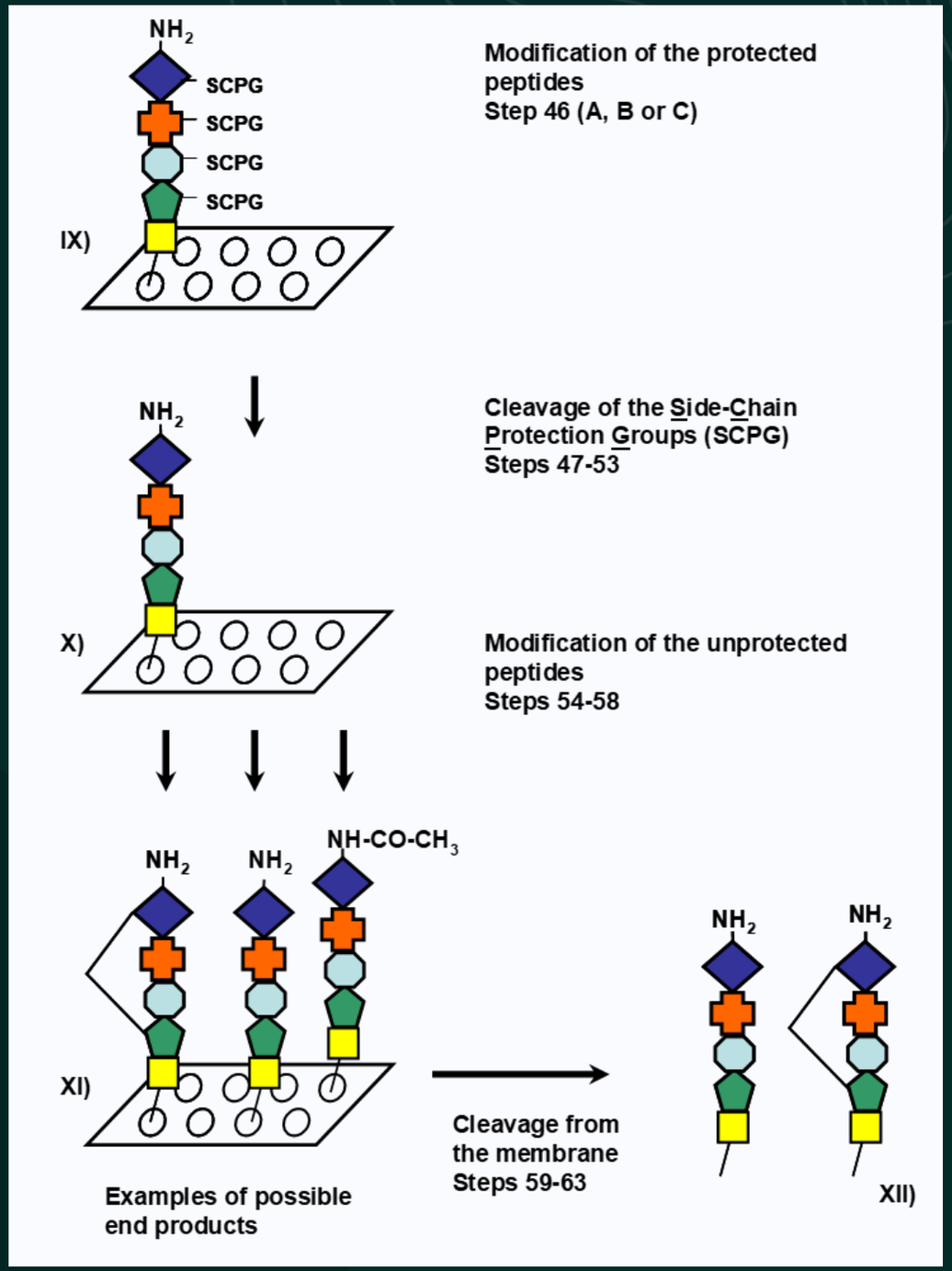


Ronald Frank

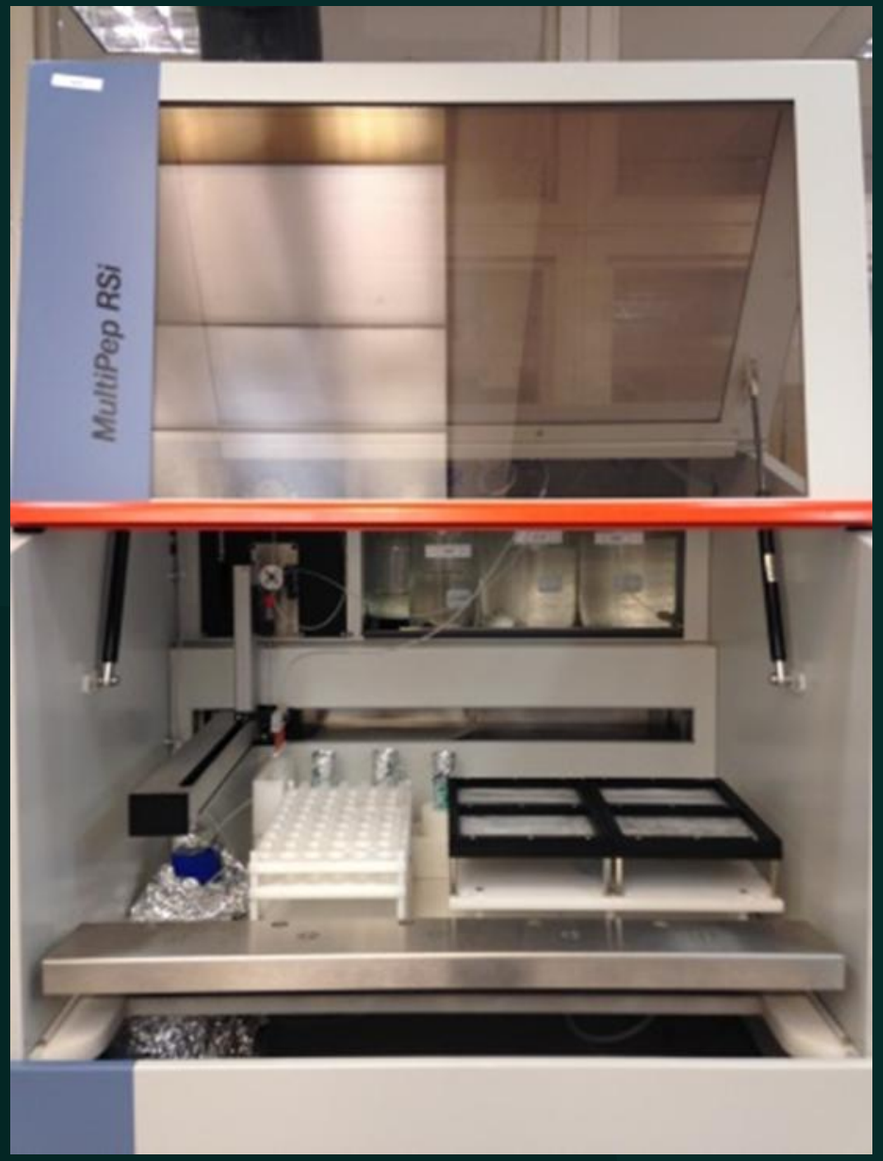
TECHNOLGY

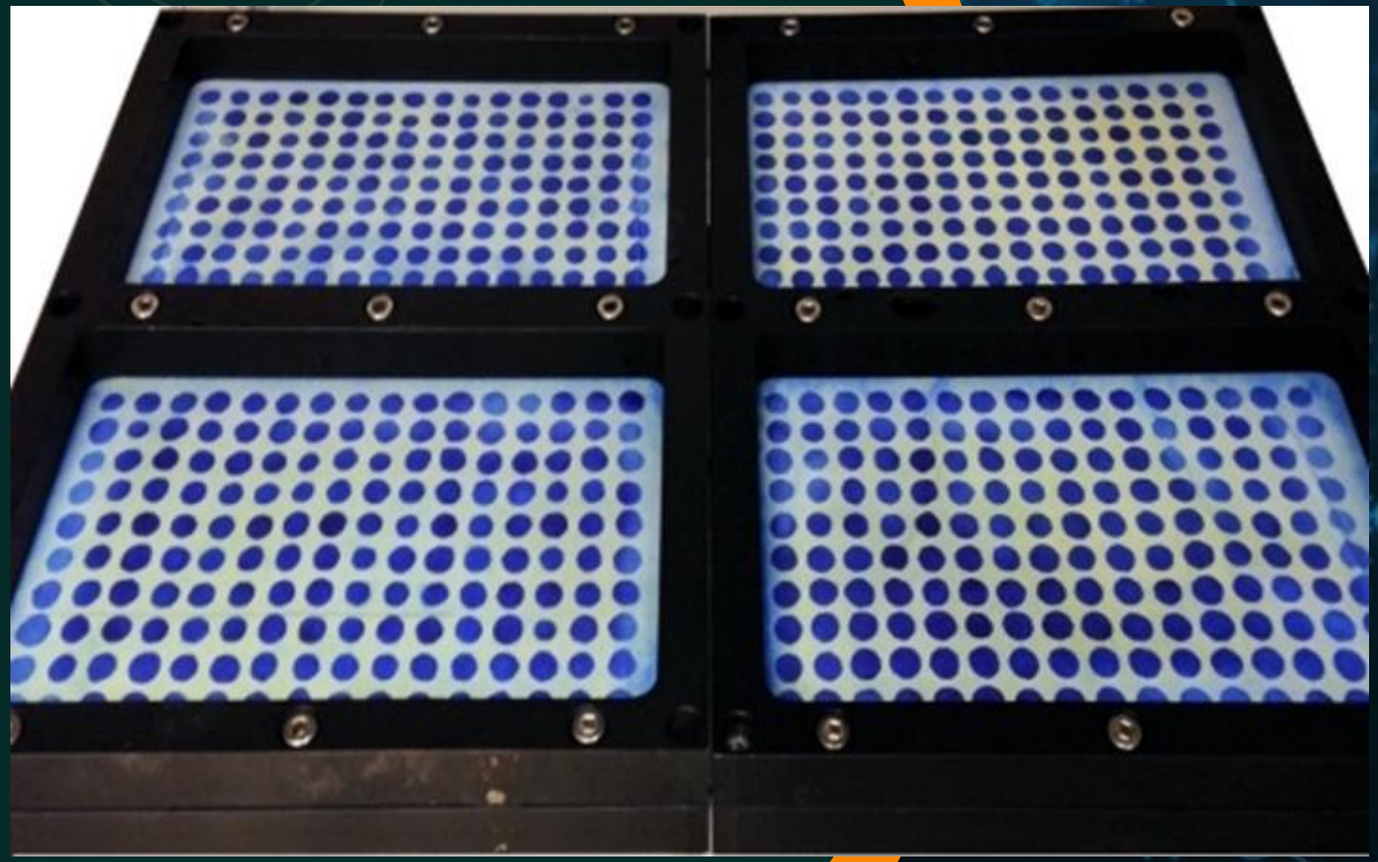

I)
OH OH OH OH
OH OH OH OH
Preparation of cellulose
Steps 1-3
Amino functionalization
Steps 4-22
NH2 NH2 NH2 NH2
A' A' A' A'
O O O O
II)
Definition of spot areas
Steps 23-35
NH2 NH2 NH2 NH2
A' A' A' A'
O O O O
NH NH NH NH
NH-CO-CH3
A' A' A' A' A'
O O O O O
III)


IV)
Deliver activated amino acid
Capping
Steps 36-39
V)
Fmoc cleavage
Steps 29-30
Optional: Peptide
Modification
Step 42 (A or B)
VI)
Staining - Steps 31-34
Or Peptide Modification
Step 46 (A, B or C)
Or SCPG cleavage
Steps 47-53
VII)
Drying
Step 41
VIII)
Entering a new cycle
Deliver activated amino acid
Capping
Steps 36-39

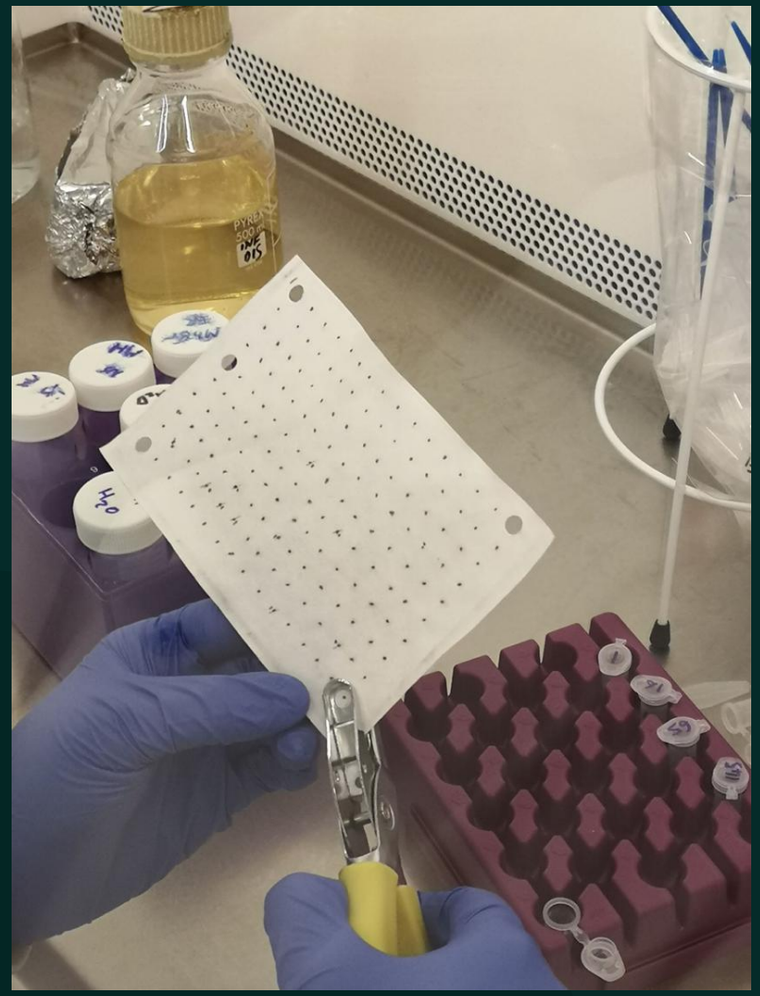

NH2
SCPG
SCPG
SCPG
SCPG
IX)
Modification of the protected peptides
Step 46 (A, B or C)
NH2
X)
Cleavage of the Side-Chain Protection Groups (SCPG)
Steps 47-53
Modification of the unprotected peptides
Steps 54-58
NH2
NH2
NH-CO-CH3
XI)
Examples of possible end products
Cleavage from the membrane
Steps 59-63
NH2
NH2
XII)

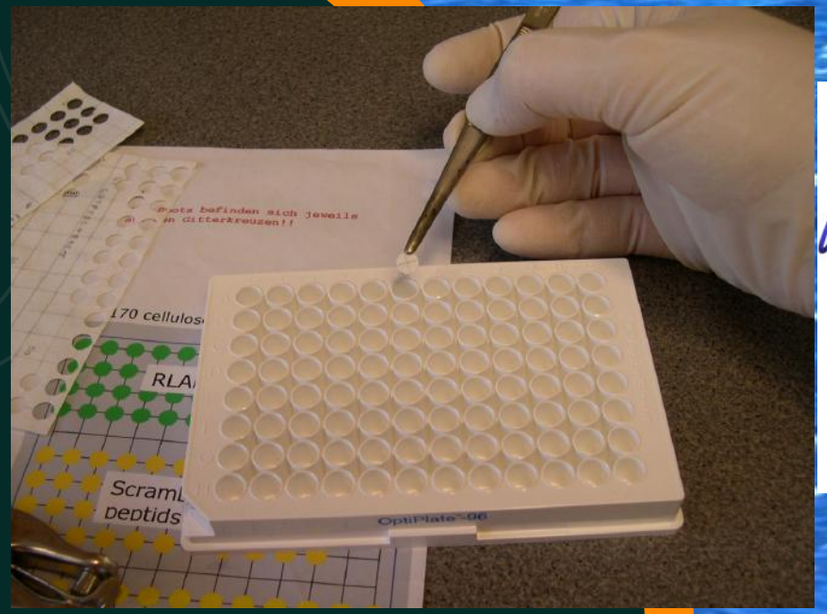
TECHNOLGY

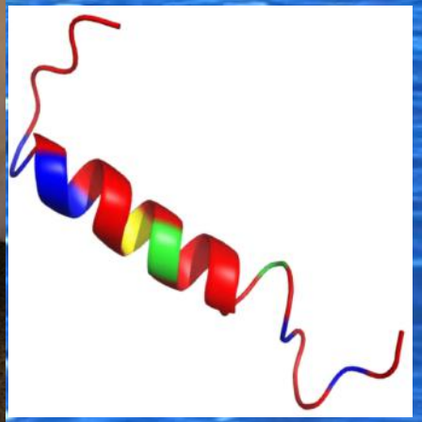


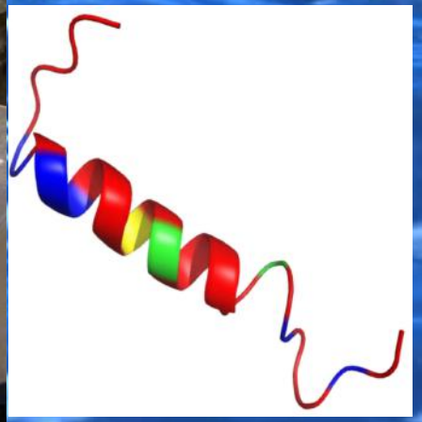


TECHNOLOGY









TECHNOLOGY

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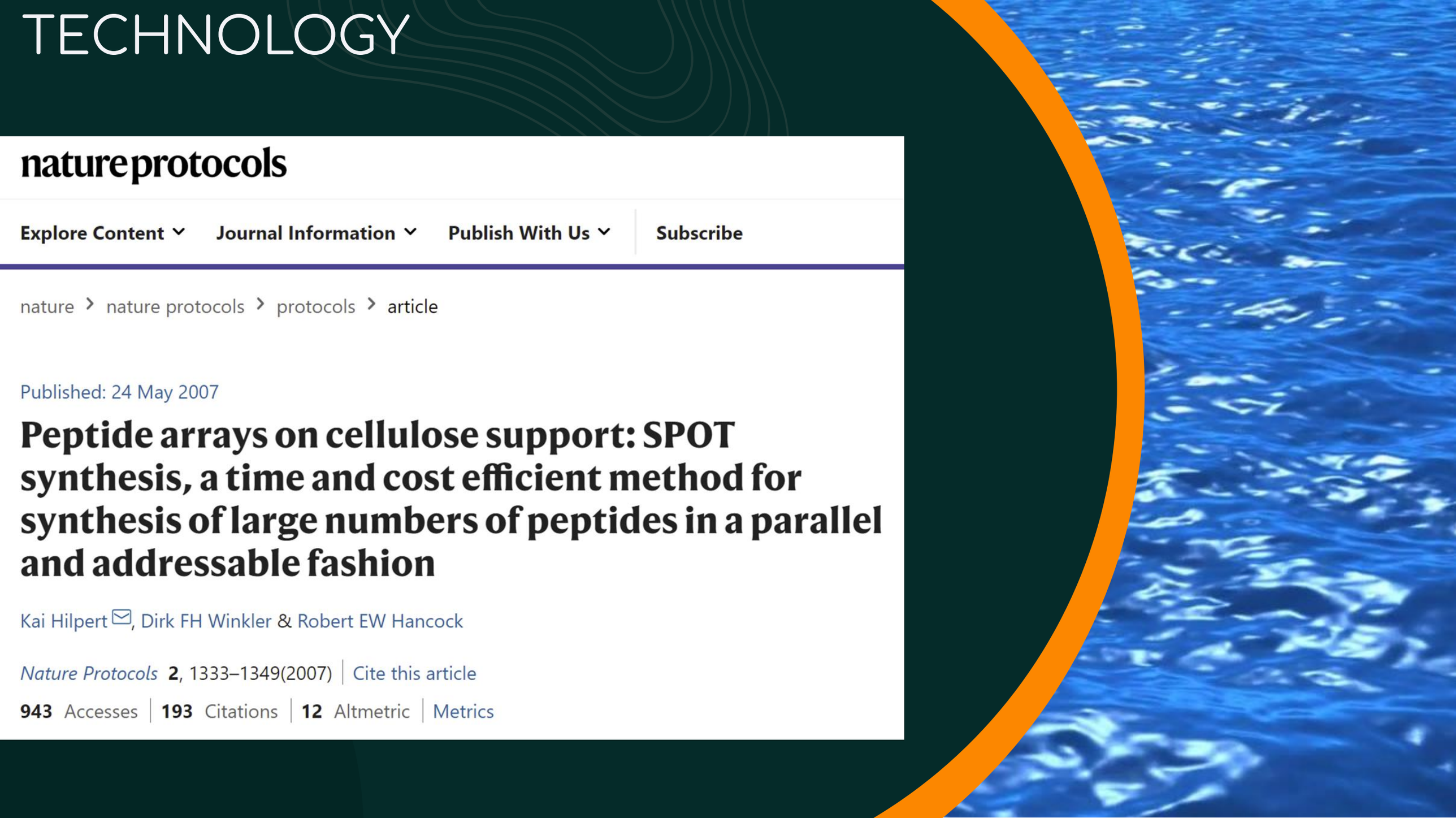
Published: 24 May 2007

Peptide arrays on cellulose support: SPOT synthesis, a time and cost efficient method for synthesis of large numbers of peptides in a parallel and addressable fashion

Kai Hilpert ✉, Dirk FH Winkler & Robert EW Hancock

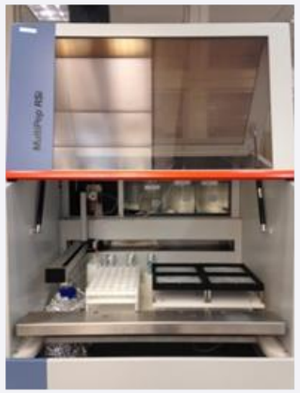
Nature Protocols **2**, 1333–1349(2007) | Cite this article

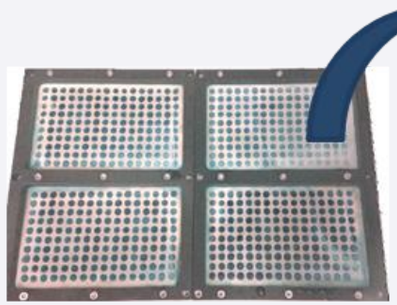
943 Accesses | **193** Citations | **12** Altmetric | Metrics



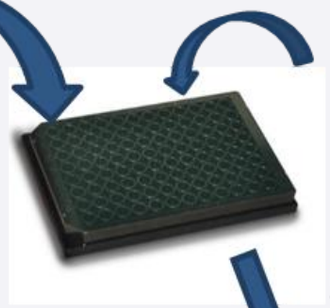
ANTIMICROBIAL SCREEN

430 natural peptides



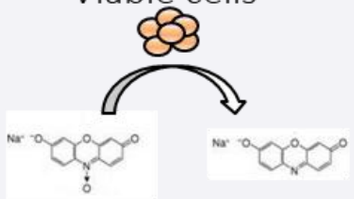


MRSA/VRE/P. aeruginosa,
C. albicans



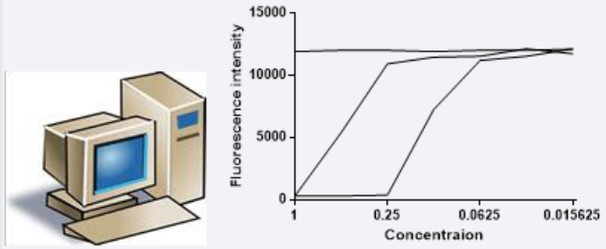
5 hour incubation

Viable cells



Resazurin (weakly fluorescent)

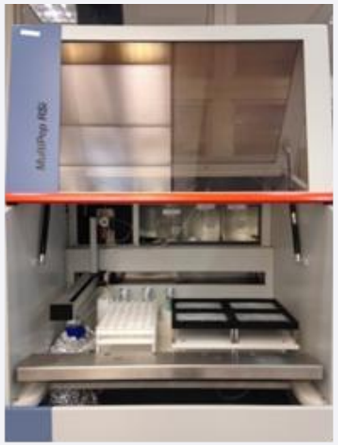
Resorufin (highly fluorescent)

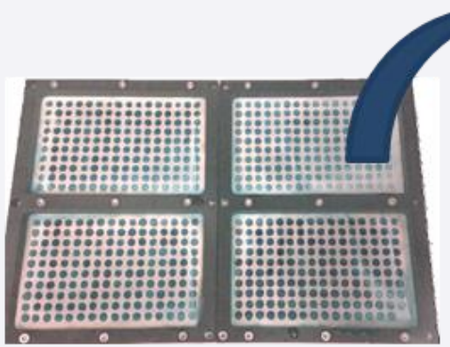


Data analysis and IC75 calculation using GaitCAD

HEMOLYTIC SCREEN

430 natural peptides

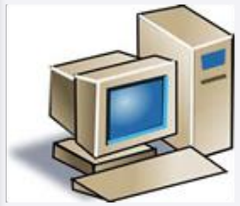


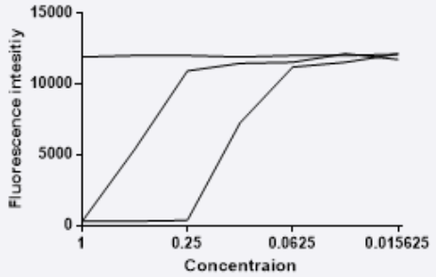


Human red blood cells

1 hour incubation

Haemoglobin of damaged cells absorbs at 540nm





Data analysis and IC75 calculation using GaitCAD

SOURCE

DBAASP v3.0

Database of antimicrobial activity and structure of peptides

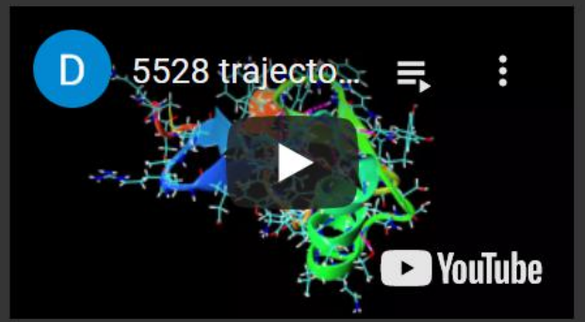
Home Search Tools Statistics About Terms And Conditions API Help Feedback

DBAASP peptides with reported activities against: SARS-CoV-2

DBAASP peptides with reported activities against enveloped positive-sense RNA viruses: HIV, HCV, Coronaviruses, PRRSV

News

Monomer:	18405
Multimer:	246
Multi Peptide:	227
News Records with the Data on Synergistic Activities:	567



See all MD models

Overview

Database of Antimicrobial Activity and Structure of Peptides (DBAASP) is the manually-curated database. It has been developed to provide the scientific community with the information and analytical resources for designing antimicrobial compounds with a high therapeutic index.

APPLICATIONS

nature biotechnology

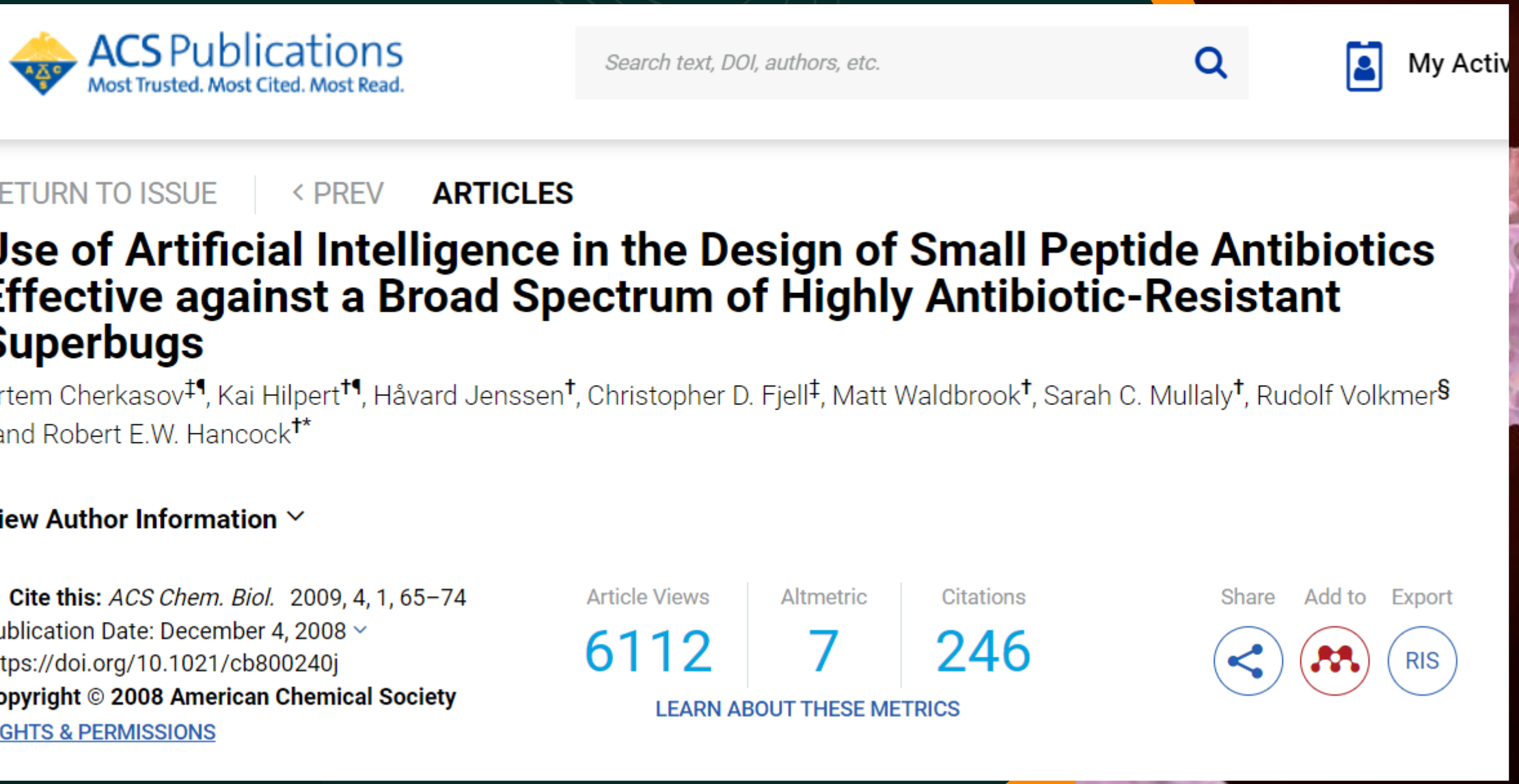
Published: 24 July 2005

High-throughput generation of small antibacterial peptides with improved activity

Kai Hilpert, Rudolf Volkmer-Engert, Tess Walter & Robert E W Hancock

Nature Biotechnology **23**, 1008–1012 (2005) | Cite this article

APPLICATIONS



APPLICATIONS

RETURN TO ISSUE | < PREV **ARTICLE** NEXT >

Peptide Inhibitors of Bacterial Protein Synthesis with Broad Spectrum and SbmA-Independent Bactericidal Activity against Clinical Pathogens

Mario Mardirossian, Riccardo Sola, Bertrand Beckert, Erica Valencic, Dominic W. P. Collis, Jure Borišek, Federica Armas, Adriana Di Stasi, Jan Buchmann, Egor A. Syroegin, Yury S. Polikanov, Alessandra Magistrato, Kai Hilpert, Daniel N. Wilson , and Marco Scocchi*

Cite this: *J. Med. Chem.* 2020, 63, 17, 9590–9602
Publication Date: July 29, 2020
https://doi.org/10.1021/acs.jmedchem.0c00665

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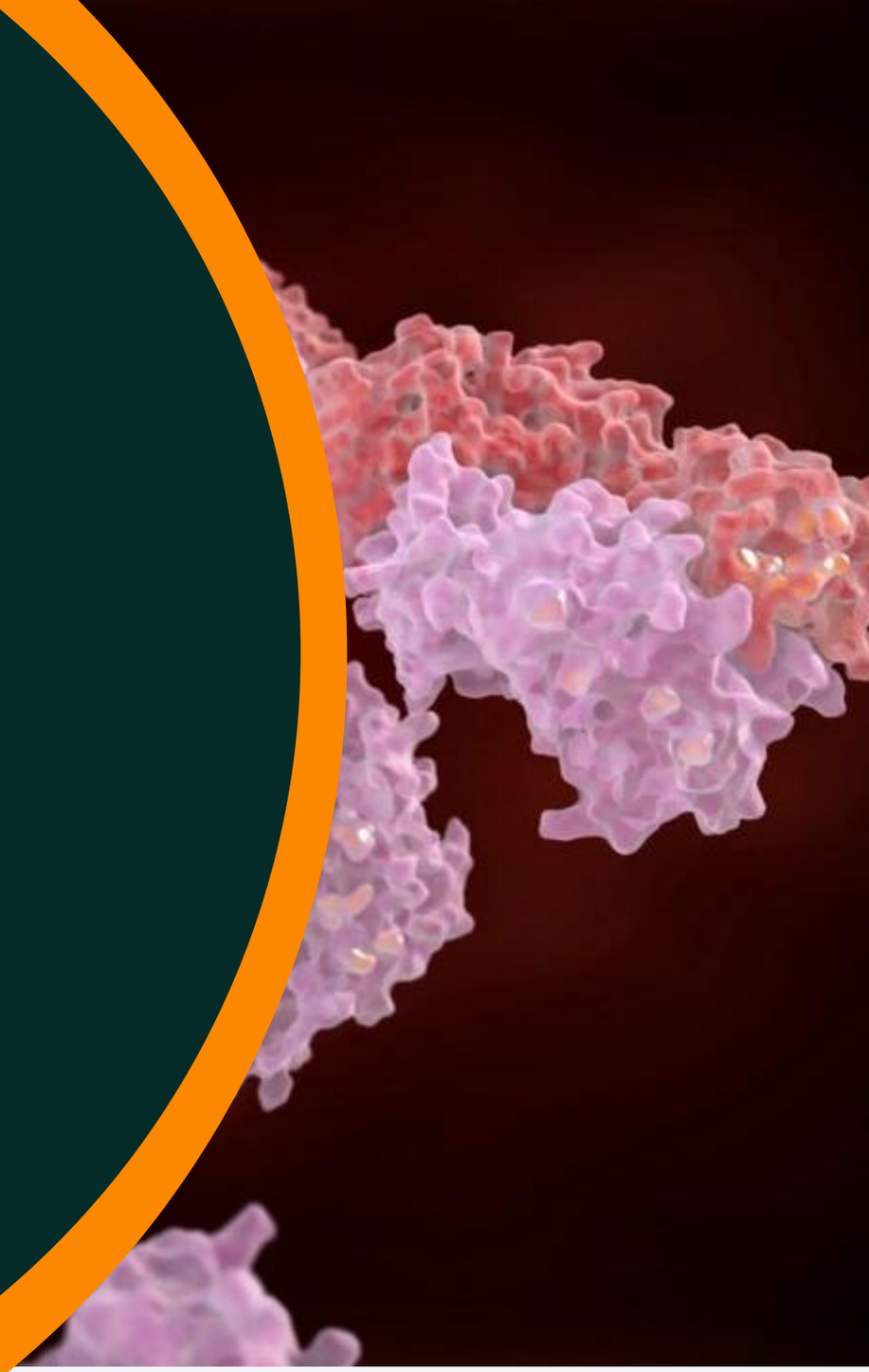
Journal of Medicinal Chemistry

CHALLENGE

Discovered hundreds of peptides with high therapeutic potential

Peptide with a new mode of action best

Mode of action studies expensive and time consuming



SOLUTION

BIOSAXS ???

Pioneering work

Measurements are very fast (2 sec)

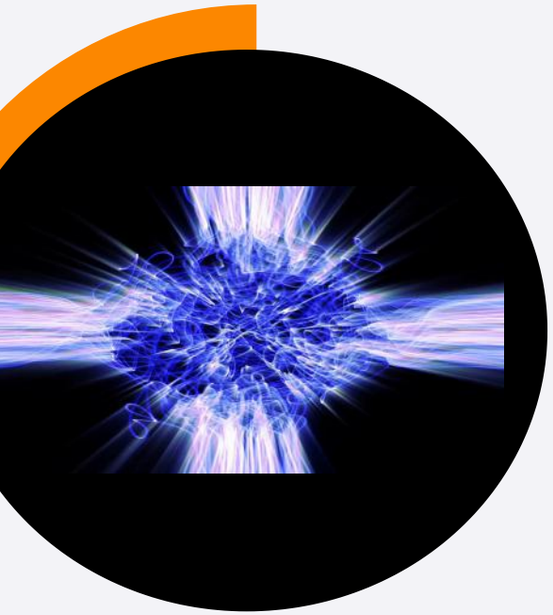
Robust statistic

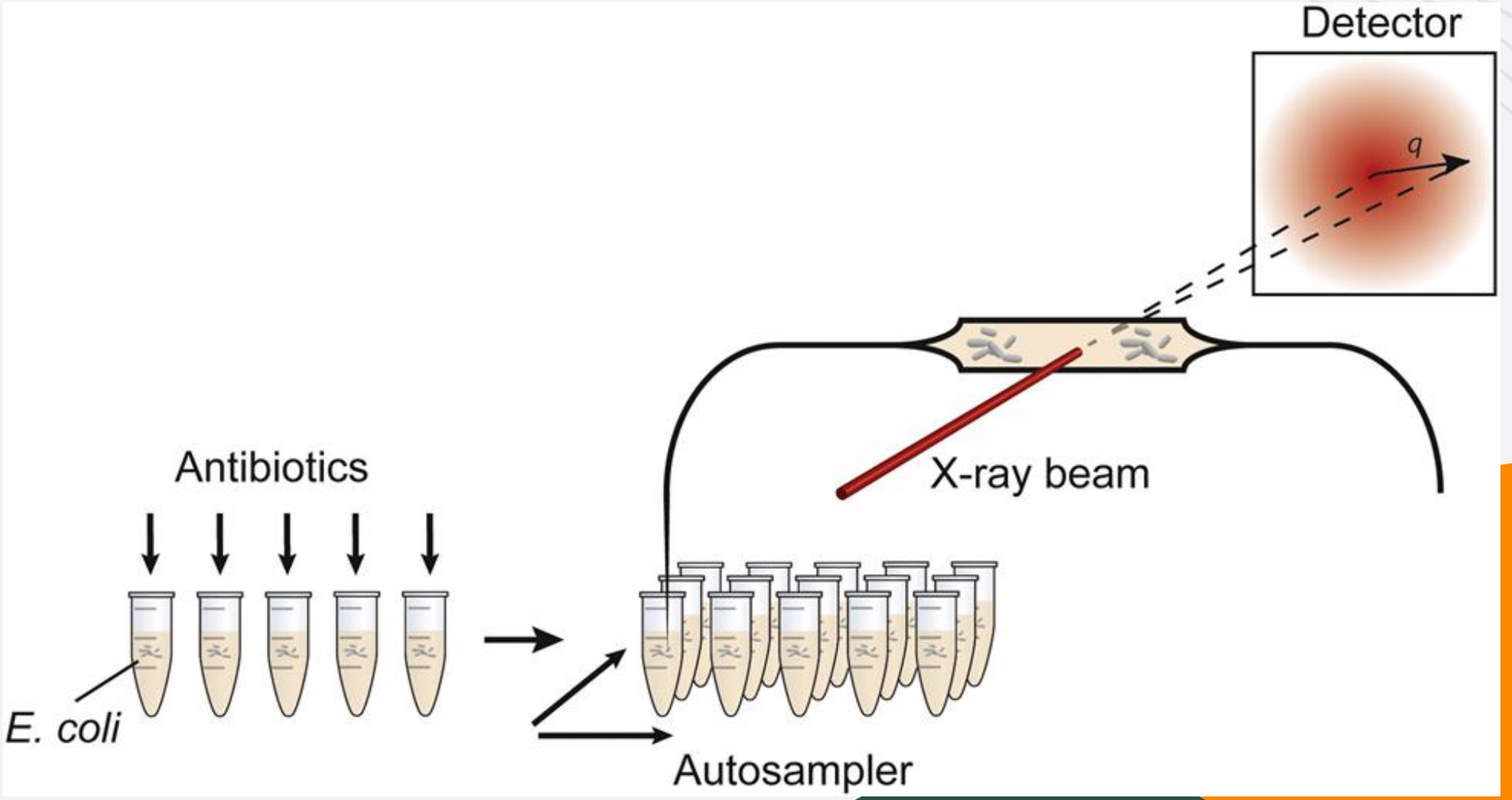
Hundreds of compounds can be screened

Data visualisation via PCA

PROOF OF PRINCIPLE

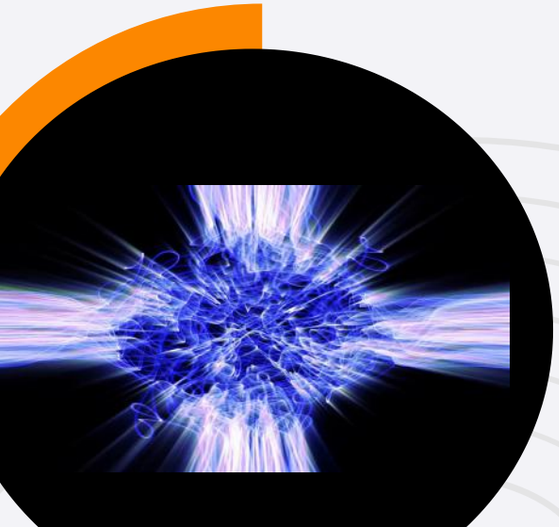
PROOF OF PRINCIPLE

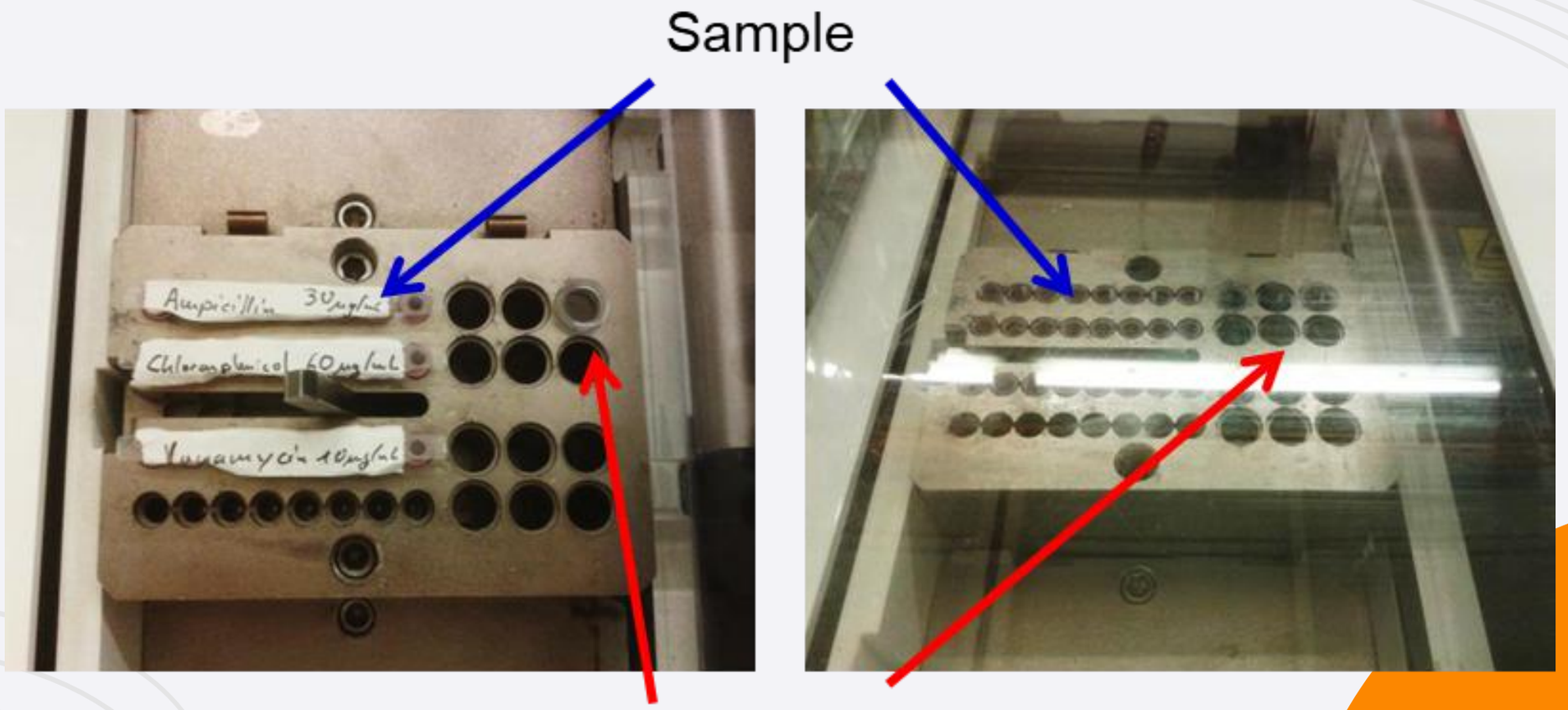




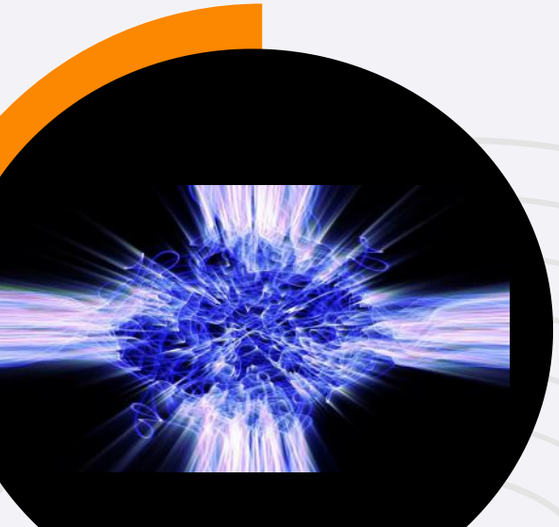
BioSAXS Beamline P12, PETRA III, Hamburg

BioSAXS Beamline P12, PETRA III, Hamburg





PROOF OF PRINCIPLE



ELSEVIER

Biochimica et Biophysica Acta (BBA) - Biomembranes

Volume 1858, Issue 5, May 2016, Pages 918-925

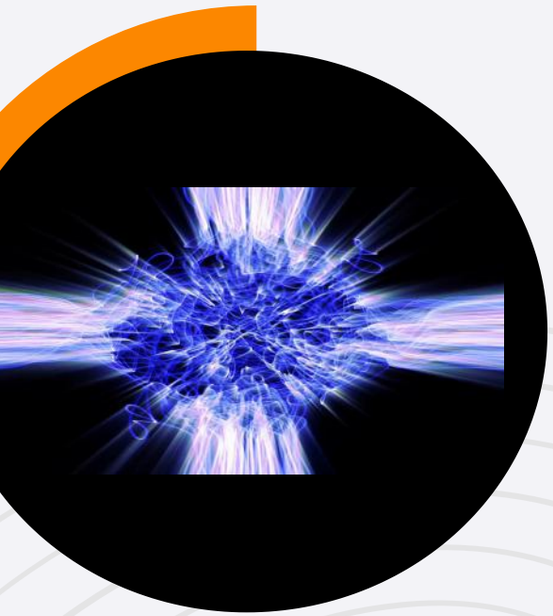
Small angle X-ray scattering as a high-throughput method to classify antimicrobial modes of action ☆

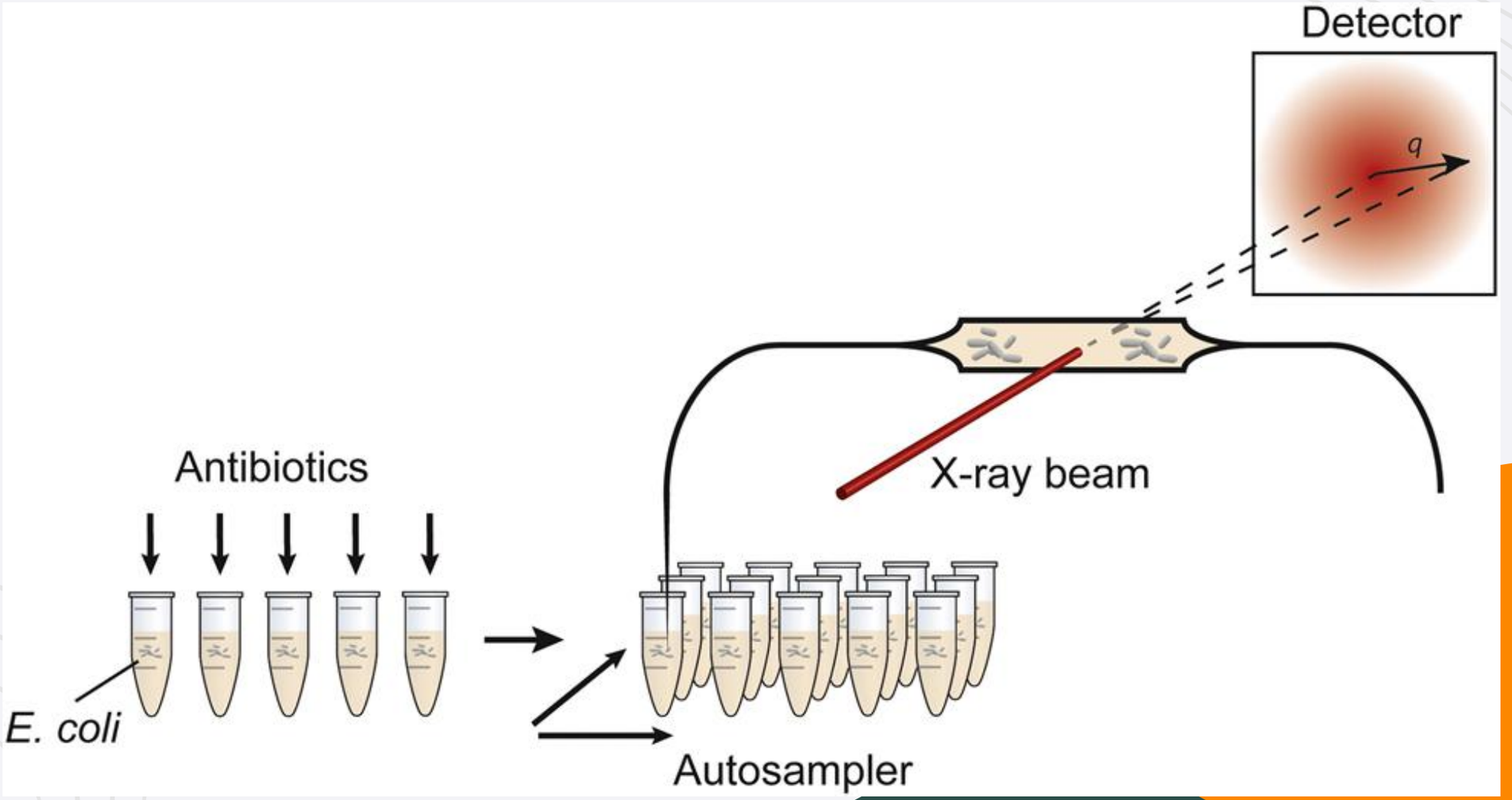
A.R. von Gundlach [a], V.M. Garamus [b], T. Gorniak [a], H.A. Davies [c], M. Reischl [d], R. Mikut [d], K. Hilpert [e] [1], A. Rosenhahn [a, 1]

PROOF OF PRINCIPLE

- Cell wall synthesis-inhibiting beta-lactams (ampicillin, cefepime, piperacillin),
- Cell wall- and cell membrane disrupting lipopeptide (polymyxin B),
- Gyrase inhibiting quinolone (ciprofloxacin),
- RNA synthesis inhibitor (rifampicin),
- Preventing the association of a new tRNA (tetracycline),
- Blocking peptidyl transferase (chloramphenicol),
- Aminoglycosides, binding to 30S ribosome (gentamycin, kanamycin),
- Peptide with unknown mode of action (AMP)

PROOF OF PRINCIPLE





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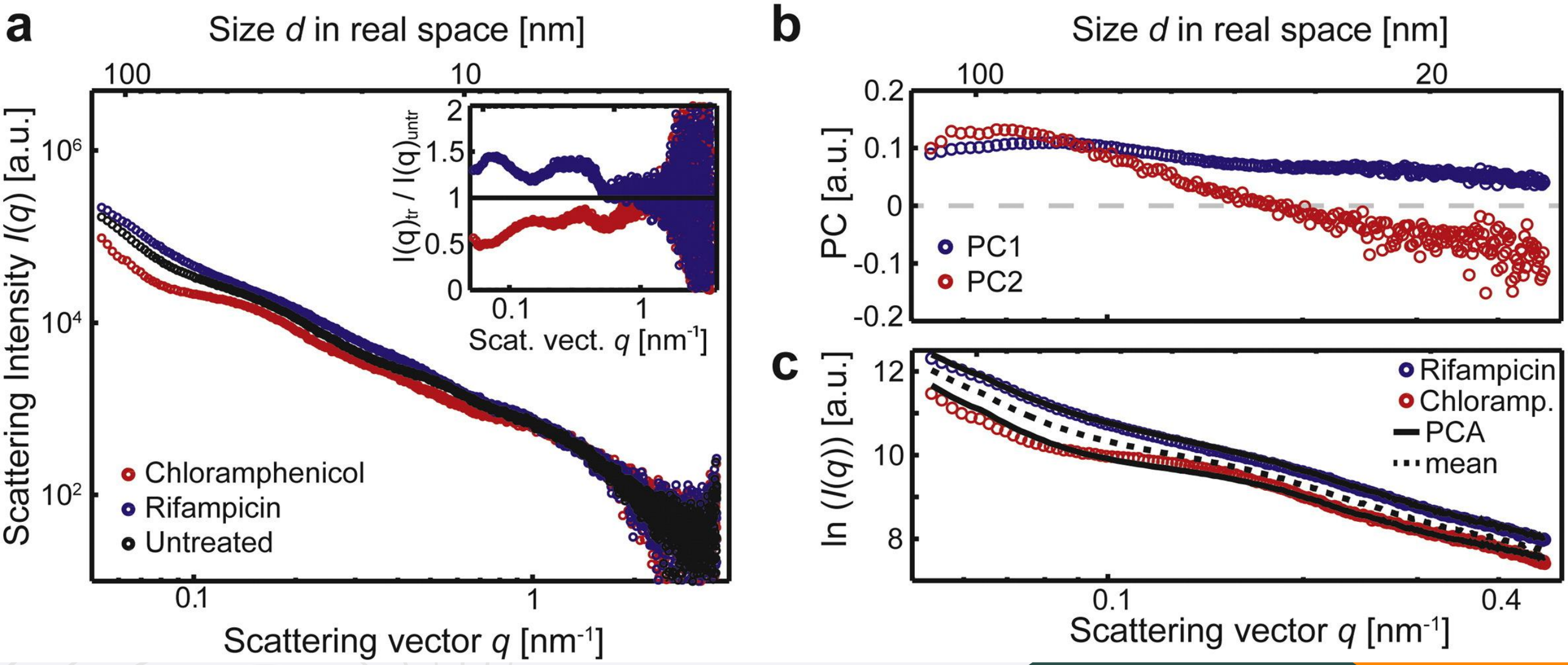

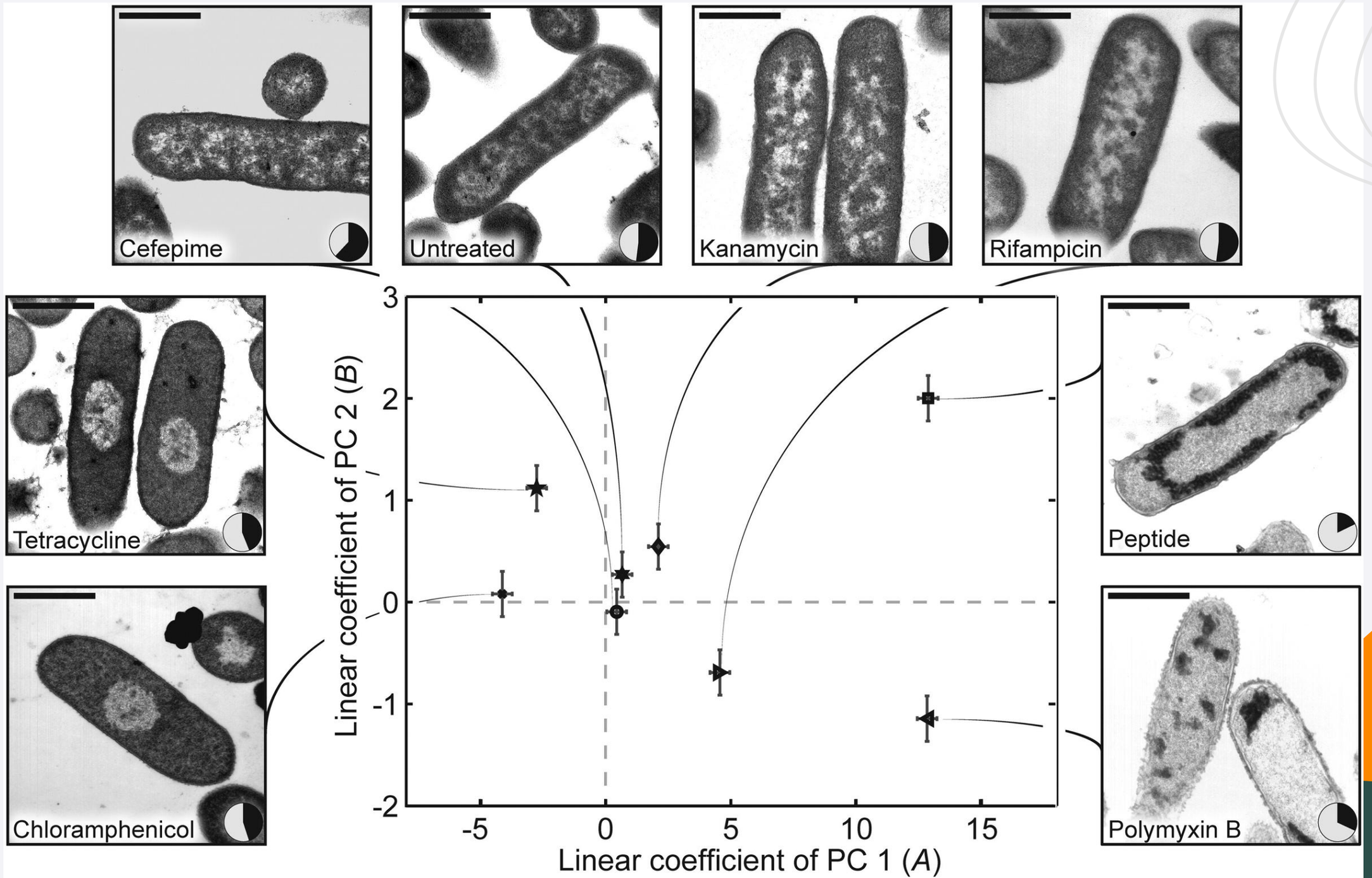
Cefepime
Untreated
Kanamycin
Rifampicin
Tetracycline
Peptide
Chloramphenicol
Polymyxin B
Linear coefficient of PC 2 (B)
Linear coefficient of PC 1 (A)
3
2
1
0
-1
-2
-5
0
5
10
15

PROOF OF PRINCIPLE II

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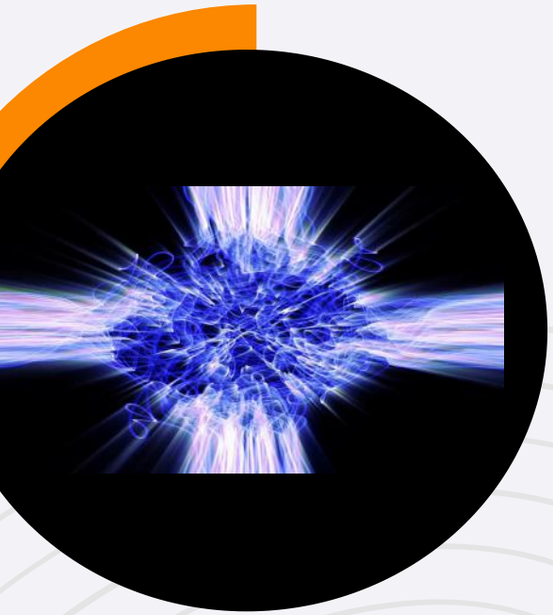
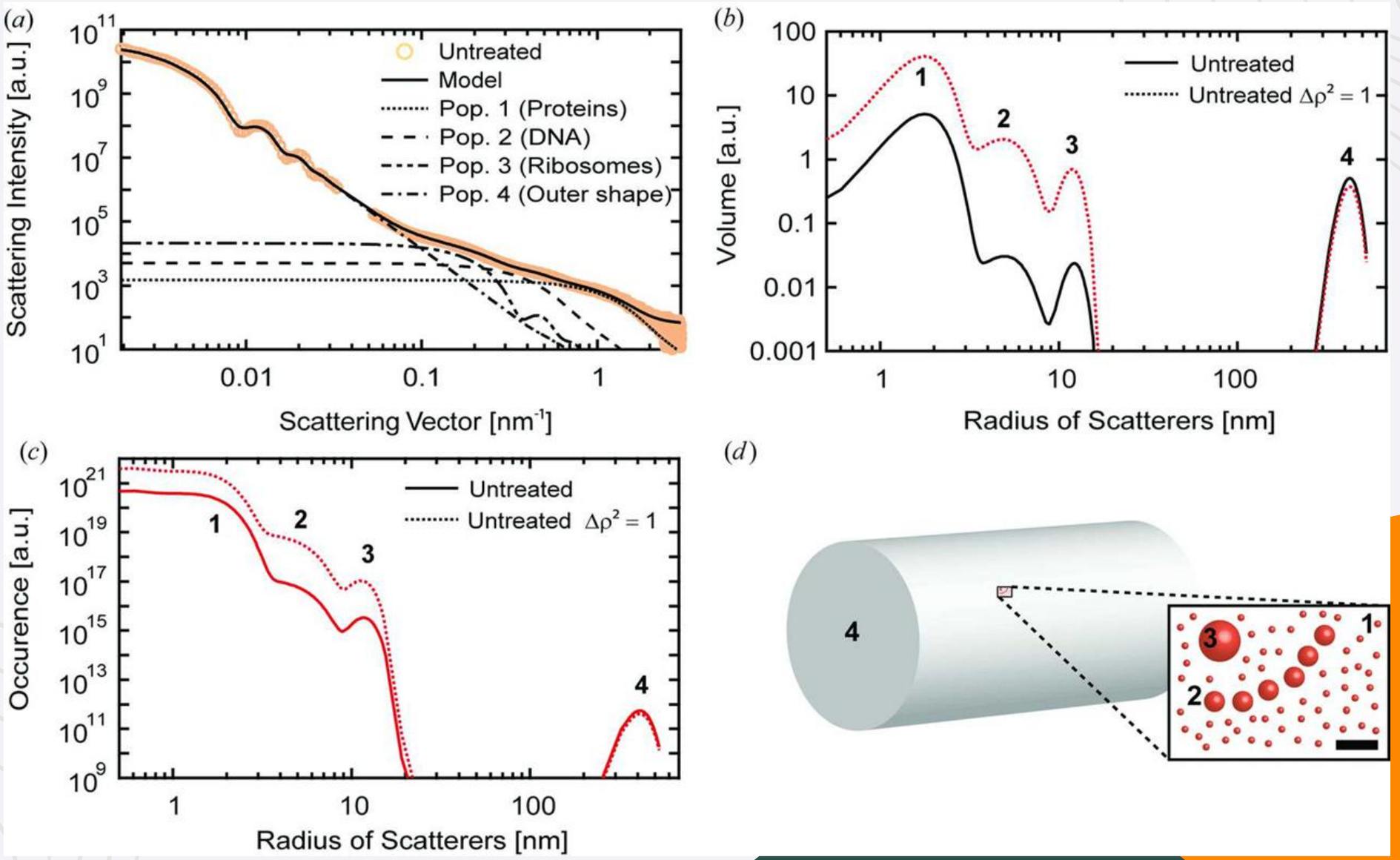
Use of small-angle X-ray scattering to resolve intracellular structure changes of *Escherichia coli* cells induced by antibiotic treatment[1]

A. R. von Gundlach,[a*] V. M. Garamus,[b] T. M. Willey,[c] J. Ilavsky,[d] K. Hilpert[e‡] and A. Rosenhahn[a‡]

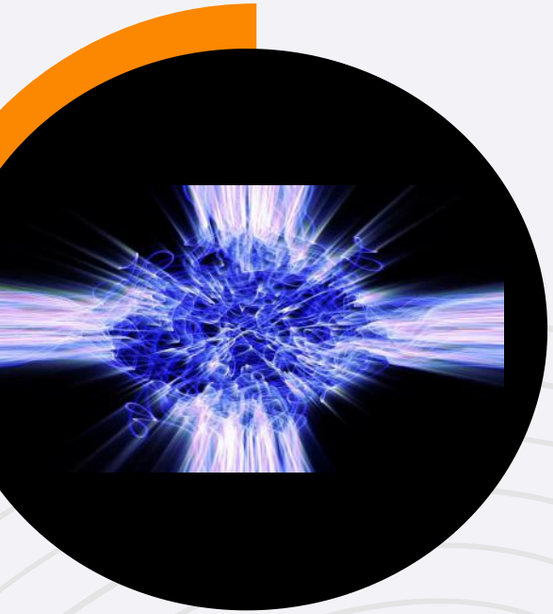
PROOF OF PRINCIPLE II

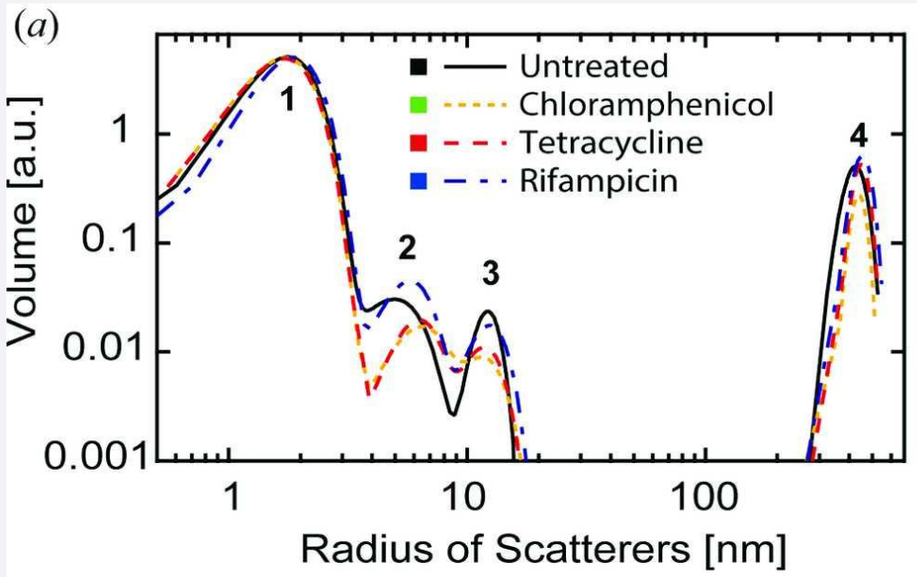


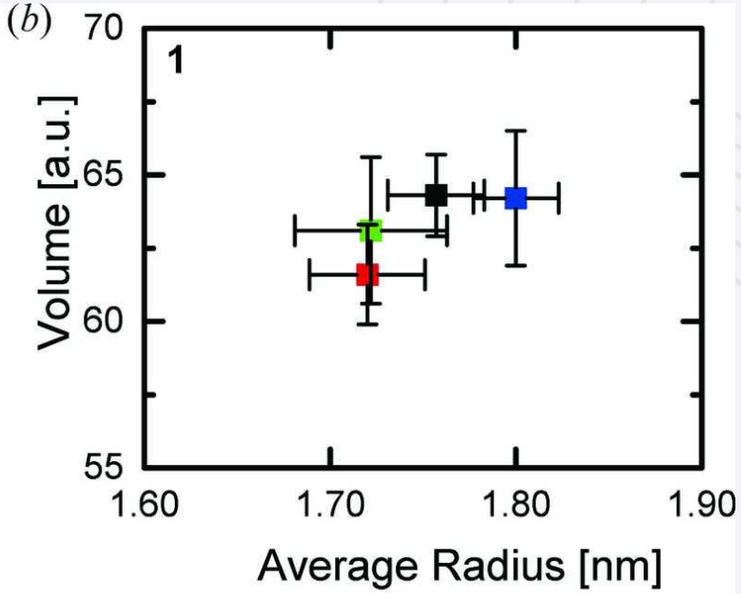


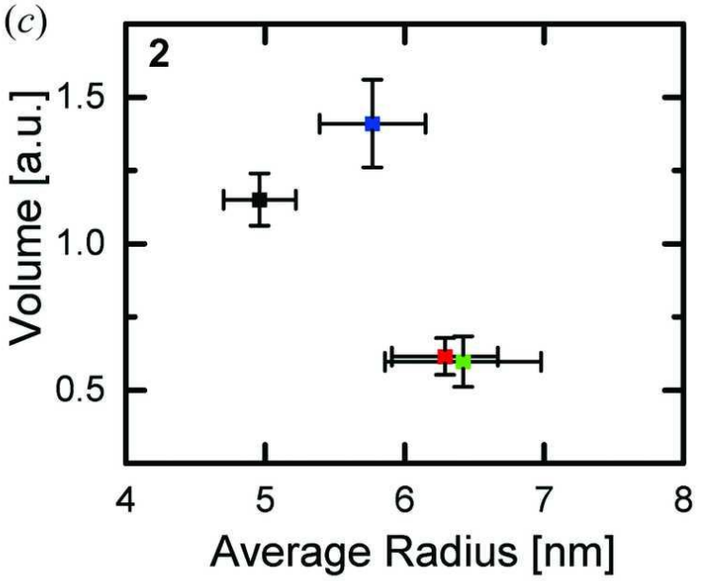
PROOF OF PRINCIPLE II

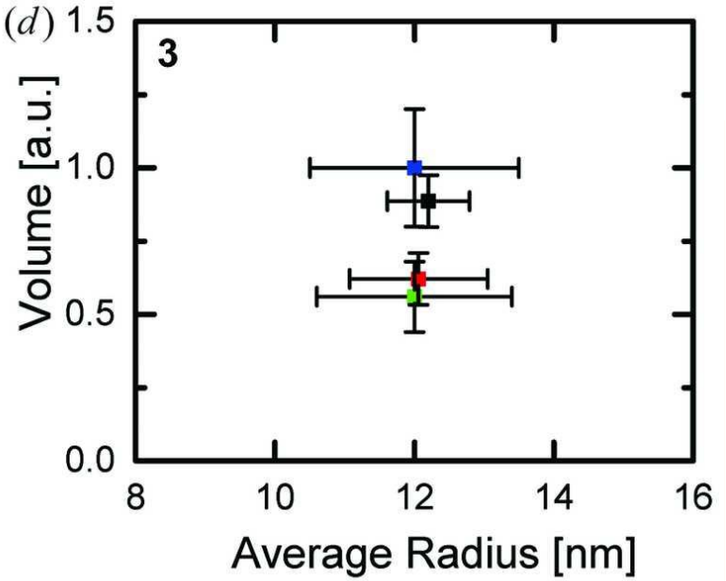
b) Proteins
c) DNA-fibres with histones
d) ribosoms



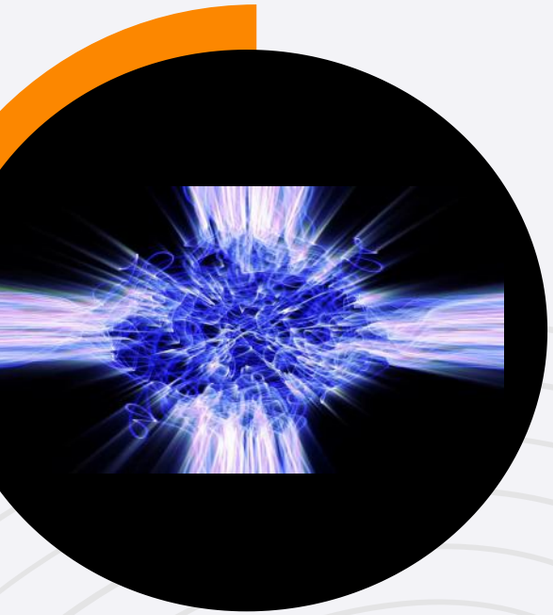




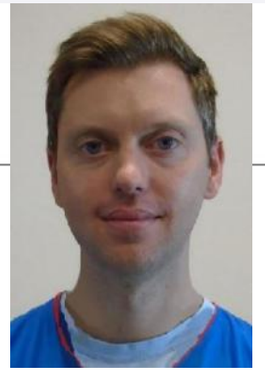




EXPANSION OF POSSIBILITIES



frontiers in Pharmacology

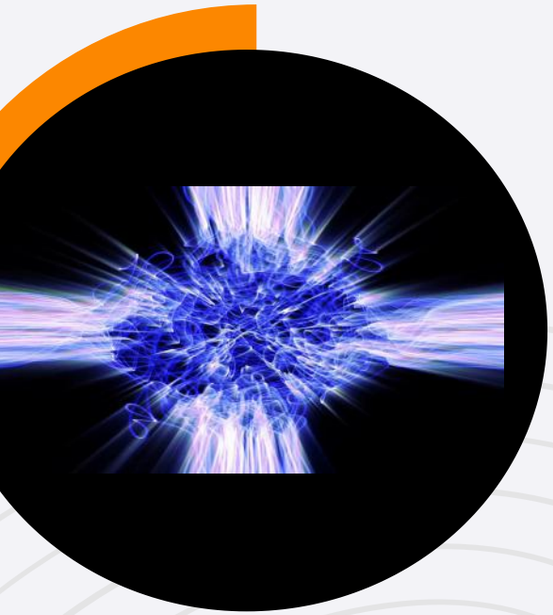


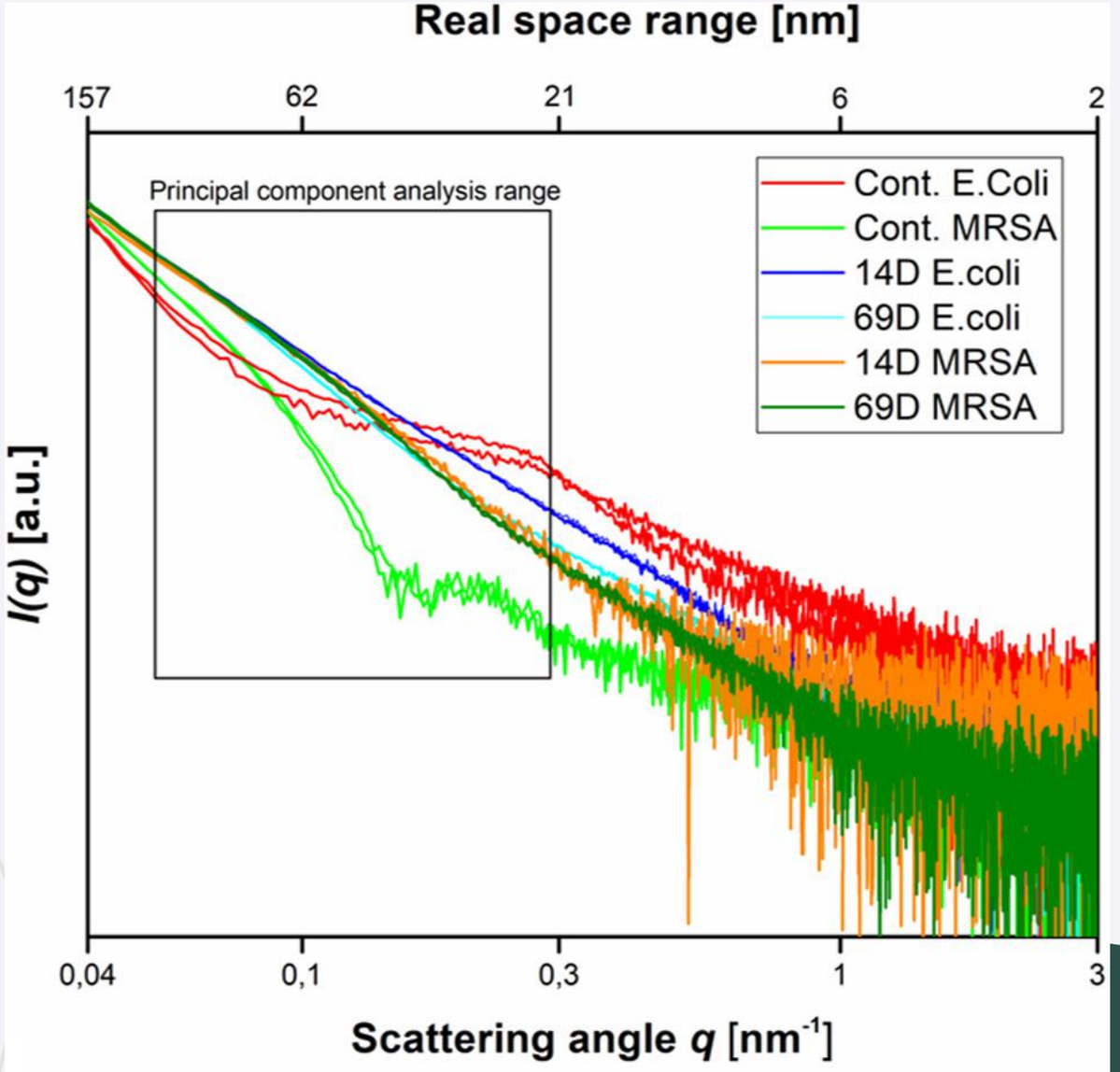
BioSAXS Measurements Reveal That Two Antimicrobial Peptides Induce Similar Molecular Changes in Gram-Negative and Gram-Positive Bacteria

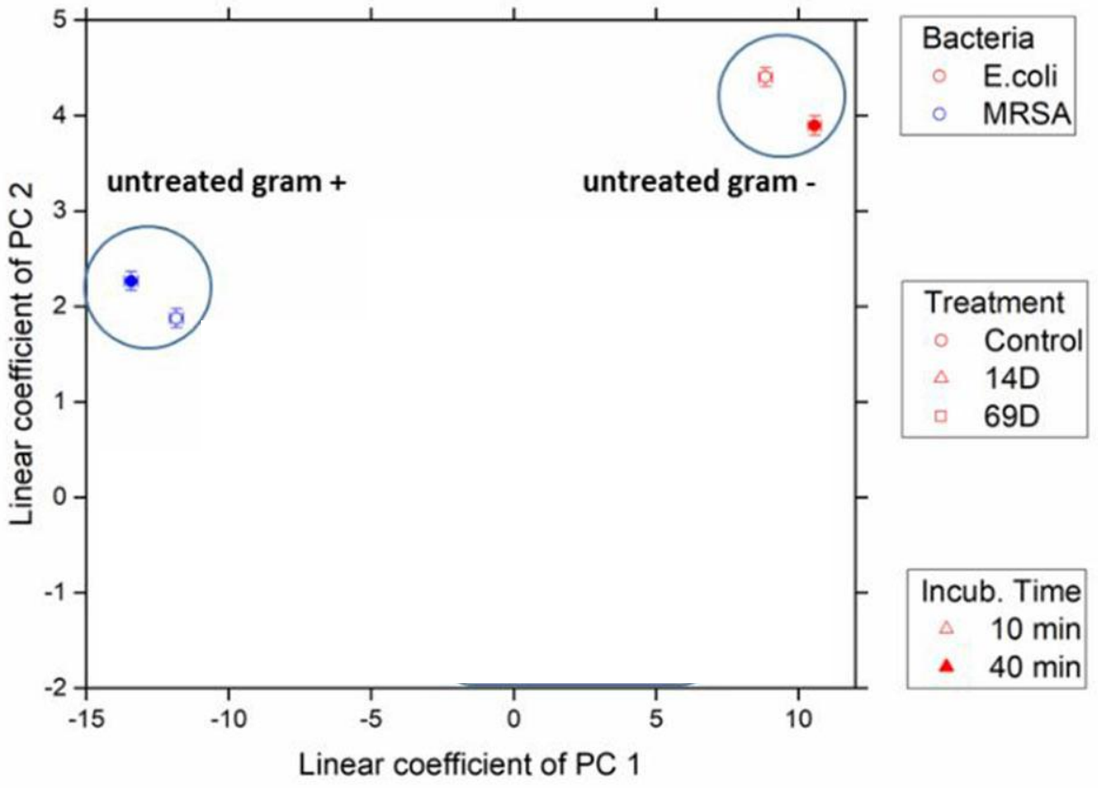
Andreas von Gundlach[1], Martin P. Ashby[2], Jurnorain Gani[2], Paula Matilde Lopez-Perez[3], Alan Roy Cookson[4], Sharon Ann Huws[5], Christoph Rumancev[1], Vasil M. Garamus[6], Ralf Mikut[7], Axel Rosenhahn[1†] and Kai Hilpert[2†]*

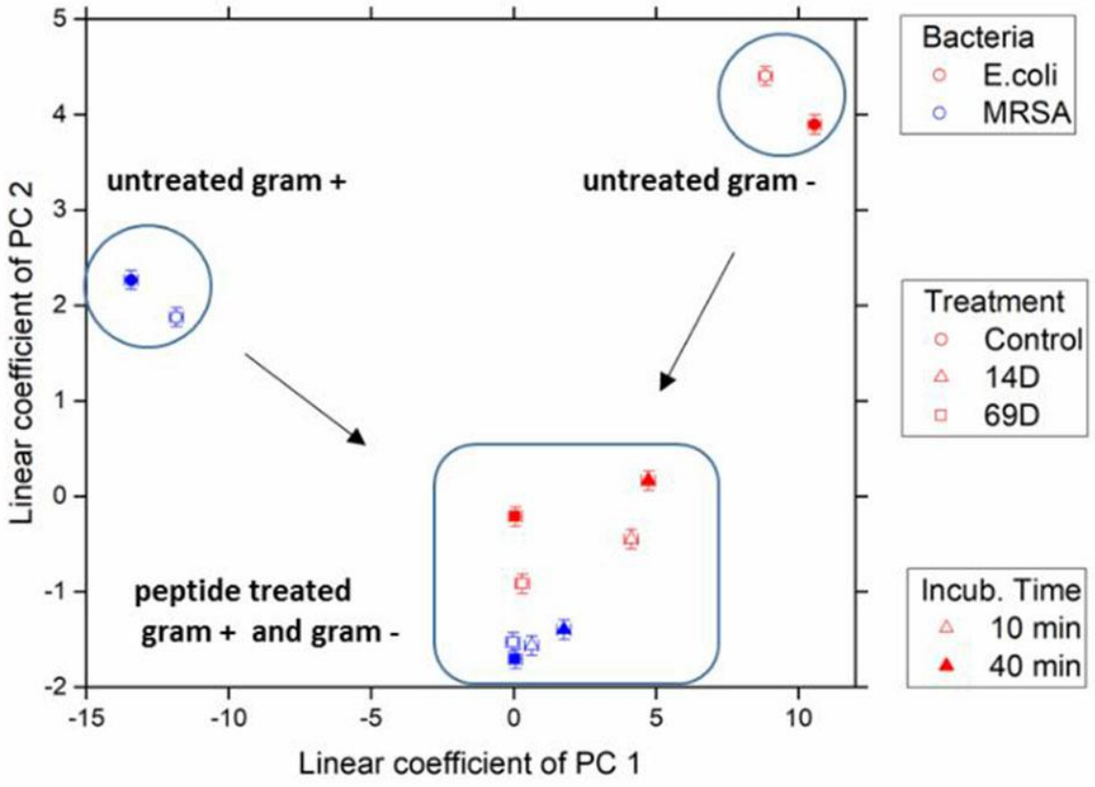
OPEN ACCESS

SCATTERING CURVES






untreated gram +
untreated gram -
Linear coefficient of PC 2
Linear coefficient of PC 1
5
4
3
2
1
0
-1
-2
-15
-10
-5
0
5
10
Bacteria
E.coli
MRSA
Treatment
Control
14D
69D
Incub. Time
10 min
40 min


untreated gram +
untreated gram -
peptide treated
gram + and gram -
Linear coefficient of PC 2
Linear coefficient of PC 1
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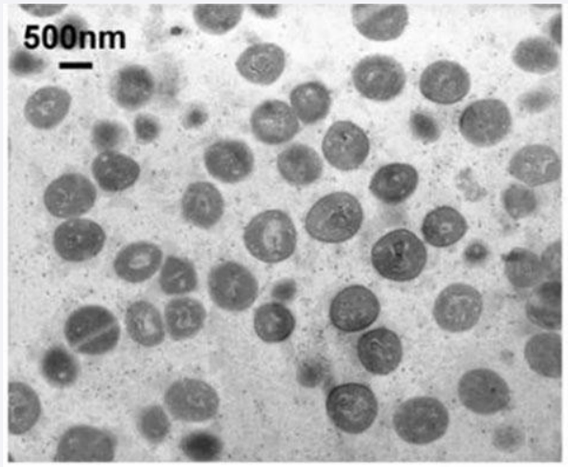
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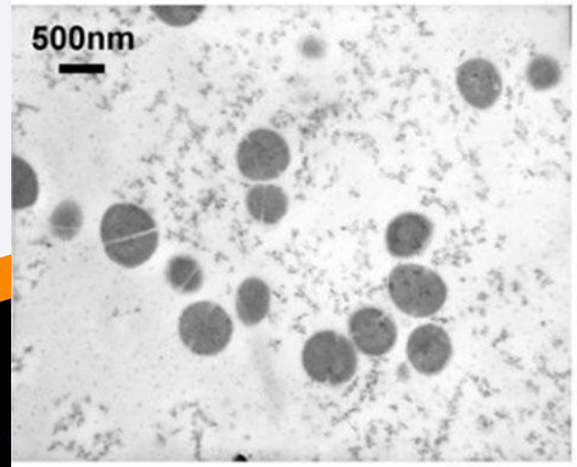
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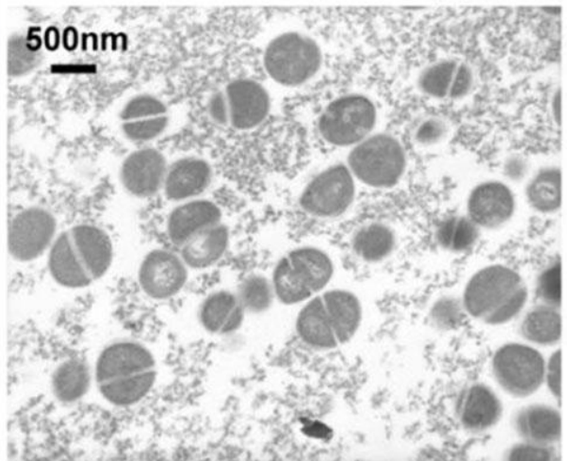
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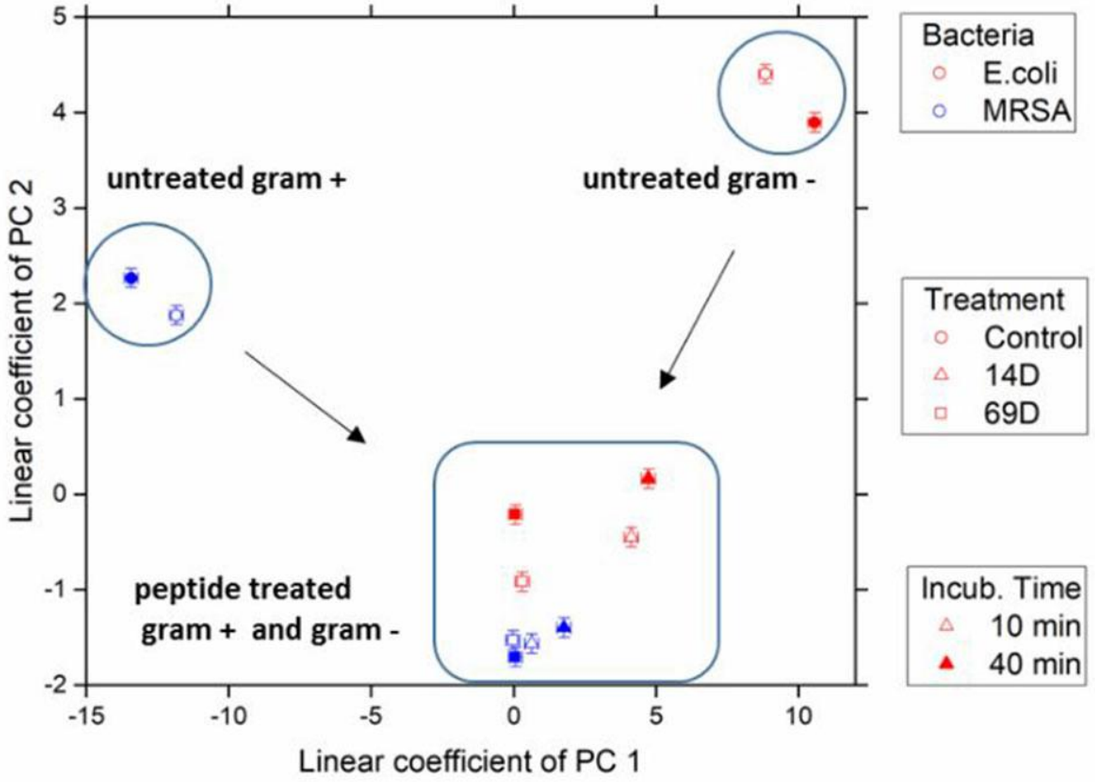
untreated gram +
untreated gram -
peptide treated
gram + and gram -
Linear coefficient of PC 2
Linear coefficient of PC 1
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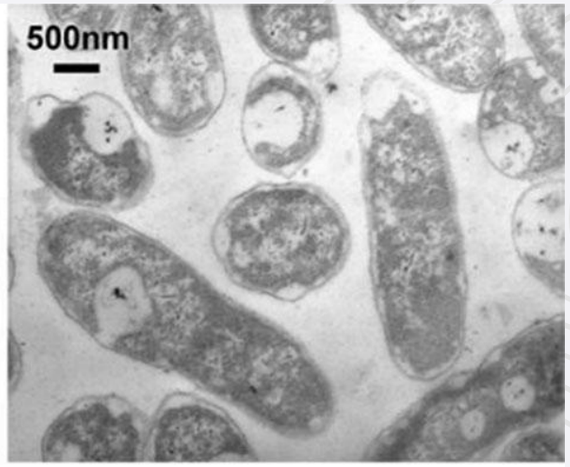
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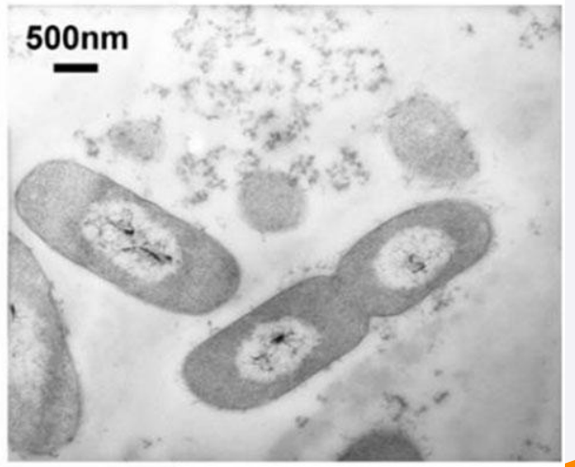
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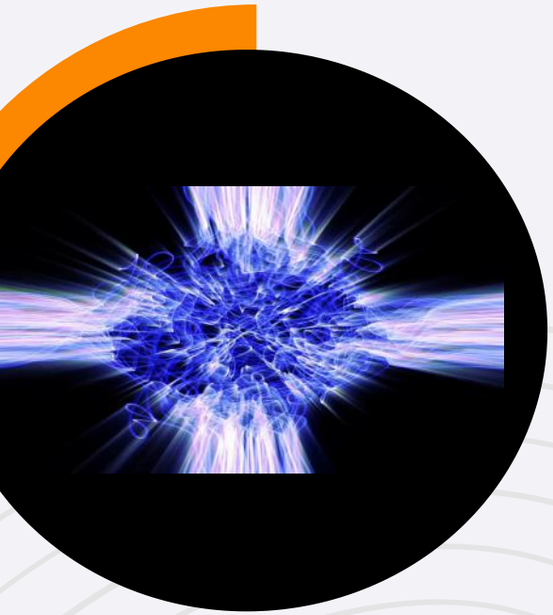
500nm

FIRST APPLICATION



frontiers in Pharmacology | Pharmacology of Infectious Diseases

SECTION ABOUT ARTICLES RESEARCH TOPICS FOR AUTHORS EDITORIAL BOARD ARTICLE ALERTS

‹ Articles

ORIGINAL RESEARCH article

Front. Pharmacol., 13 December 2021 | https://doi.org/10.3389/fphar.2021.769739

Rational Designed Hybrid Peptides Show up to a 6-Fold Increase in Antimicrobial Activity and Demonstrate Different Ultrastructural Changes as the Parental Peptides Measured by BioSAXS

Kai Hilpert[1]*, Jurnorain Gani[1], Christoph Rumancev[2], Nathan Simpson[1], Paula Matilde Lopez-Perez[3], Vasil M. Garamus[4], Andreas Robert von Gundlach[2], Petar Markov[5], Marco Scocchi[6], Ralf Mikut[7]* and Axel Rosenhahn[2]

Journal of
Medicinal Chemistry

Subscriber access provided by UNIVERSITA STUDI TRIESTE

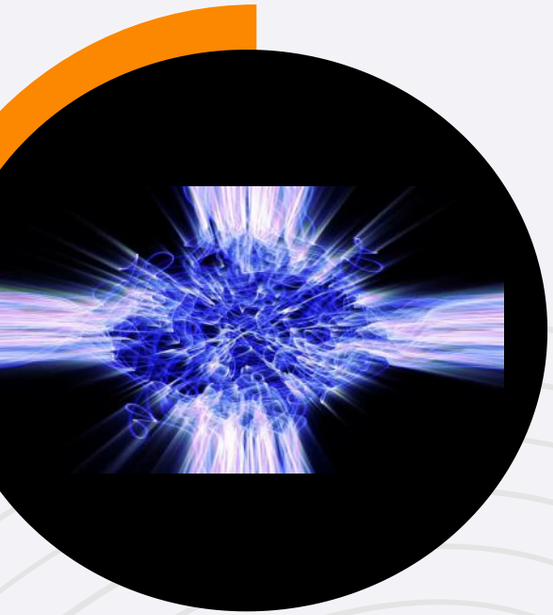
Article

Peptide inhibitors of bacterial protein synthesis with broad spectrum and SbmA-independent bactericidal activity against clinical pathogens.

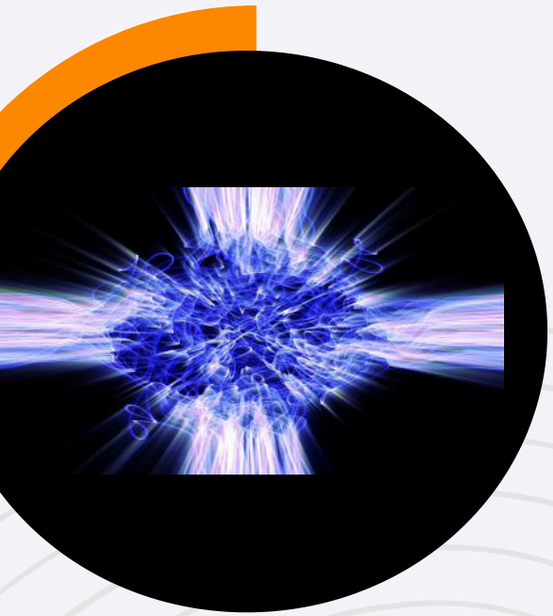
Mario Mardirossian, Riccardo Sola, Bertrand Beckert, Erica Valencic, Dominic W. P. Collis, Jure Borišek, Federica Armas, Adriana Di Stasi, Jan Buchmann, Egor A. Syroegin, Yury Polikanov, Alessandra Magistrato, Kai Hilpert, Daniel N. Wilson, and Marco Scocchi

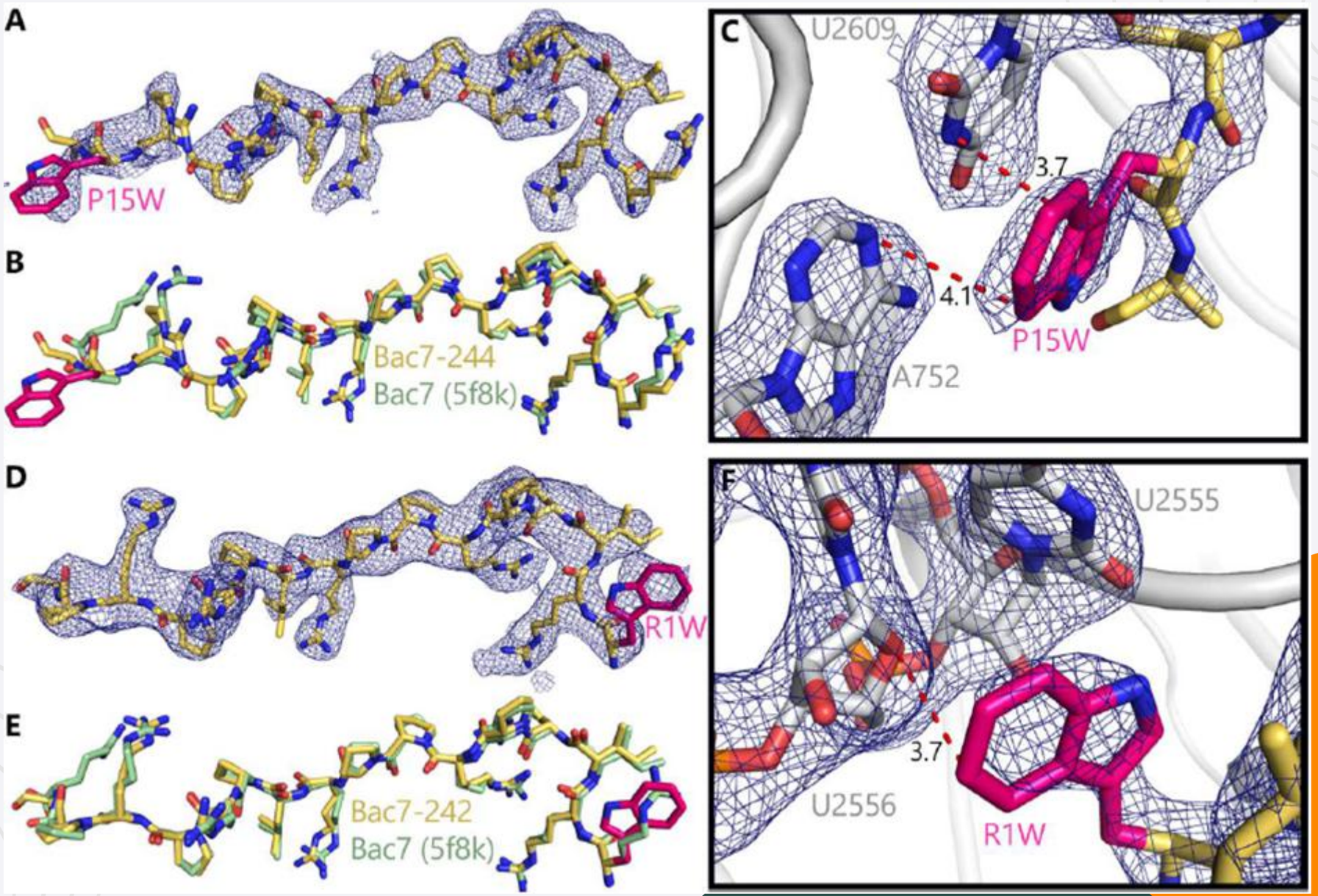
J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.0c00665 • Publication Date (Web): 29 Jul 2020

B7[a]	MIC (μM)									
	wt[b]	scr[c]	G	A[d]	S	P	R	E	F	W
R_1	8	64	8	4	16	8	-	64	4	2
R_2			16	16	16	64	-	64	16	8
I_3			16	16	16	16	8	64	16	4
R_4			16	16	32	16	-	>64	16	8
P_5			8	8	16	-	8	32	8	4
R_6			32	16	32	32	-	>64	16	8
P_7			16	16	16	-	8	32	8	4
P_8			16	16	16	-	16	64	16	4
R_9			64	64	32	16	-	>64	16	4
L_{10}			64	64	32	64	32	>64	32	16
P_{11}			32	32	32	-	16	64	32	8
R_{12}			32	32	16	32	-	>64	16	8
P_{13}			16	16	16	-	8	64	8	2
R_{14}			32	32	32	32	-	64	8	4
P_{15}			16	8	16	-	16	32	8	4
R_{16}			16	16	16	32	-	64	8	8



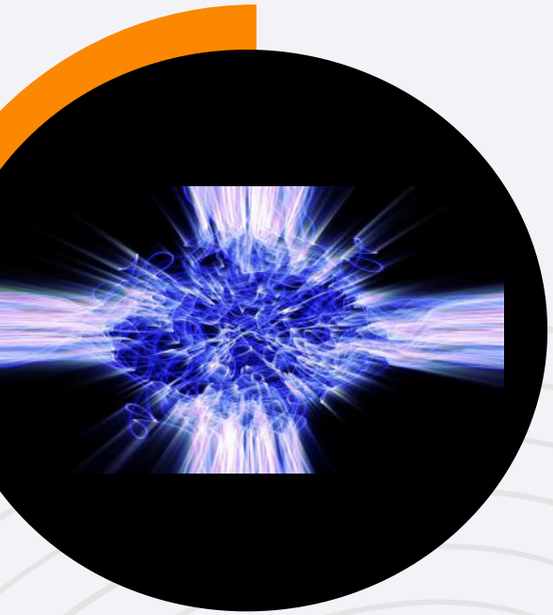
RIBOSOM BINDING

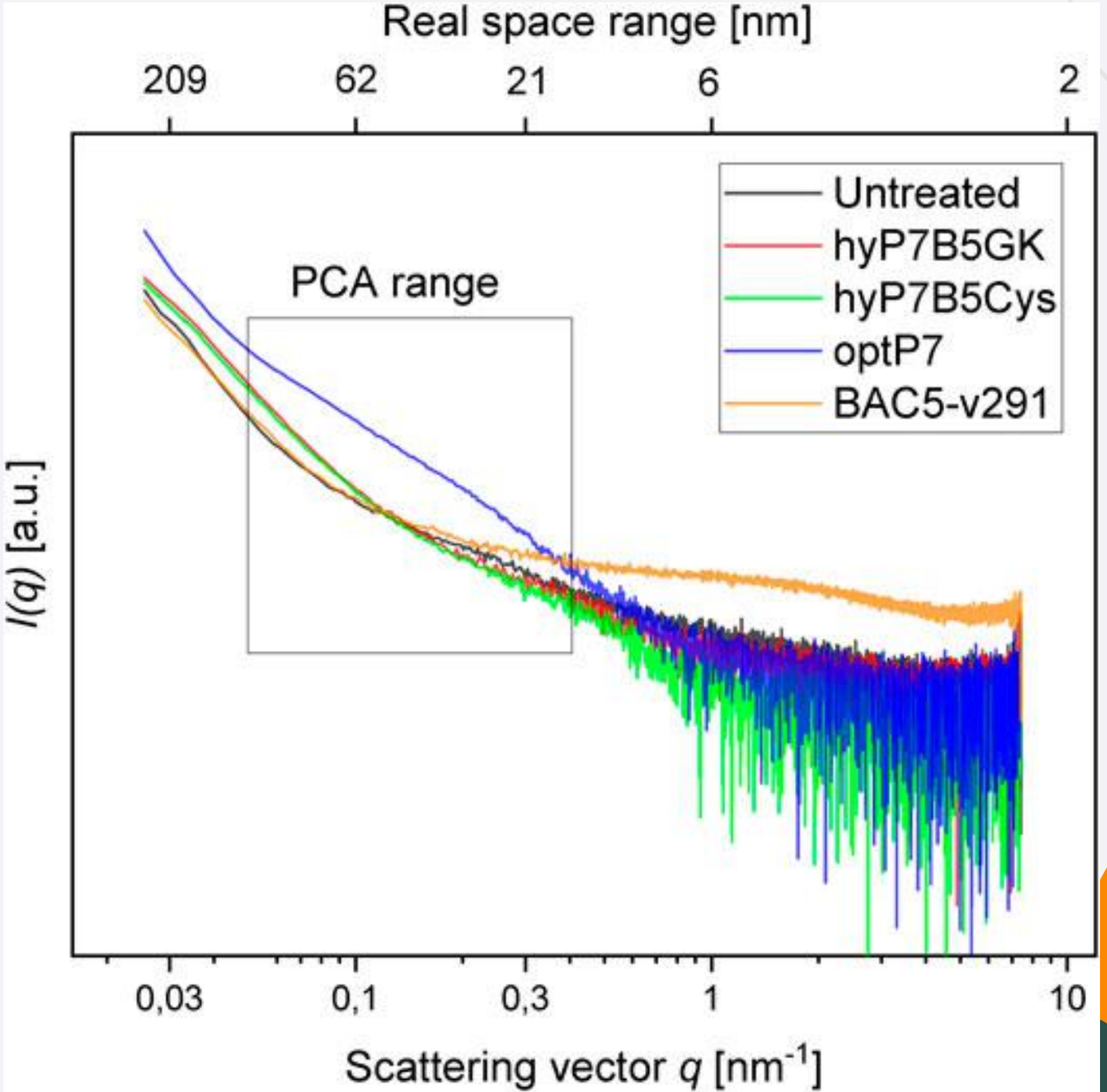


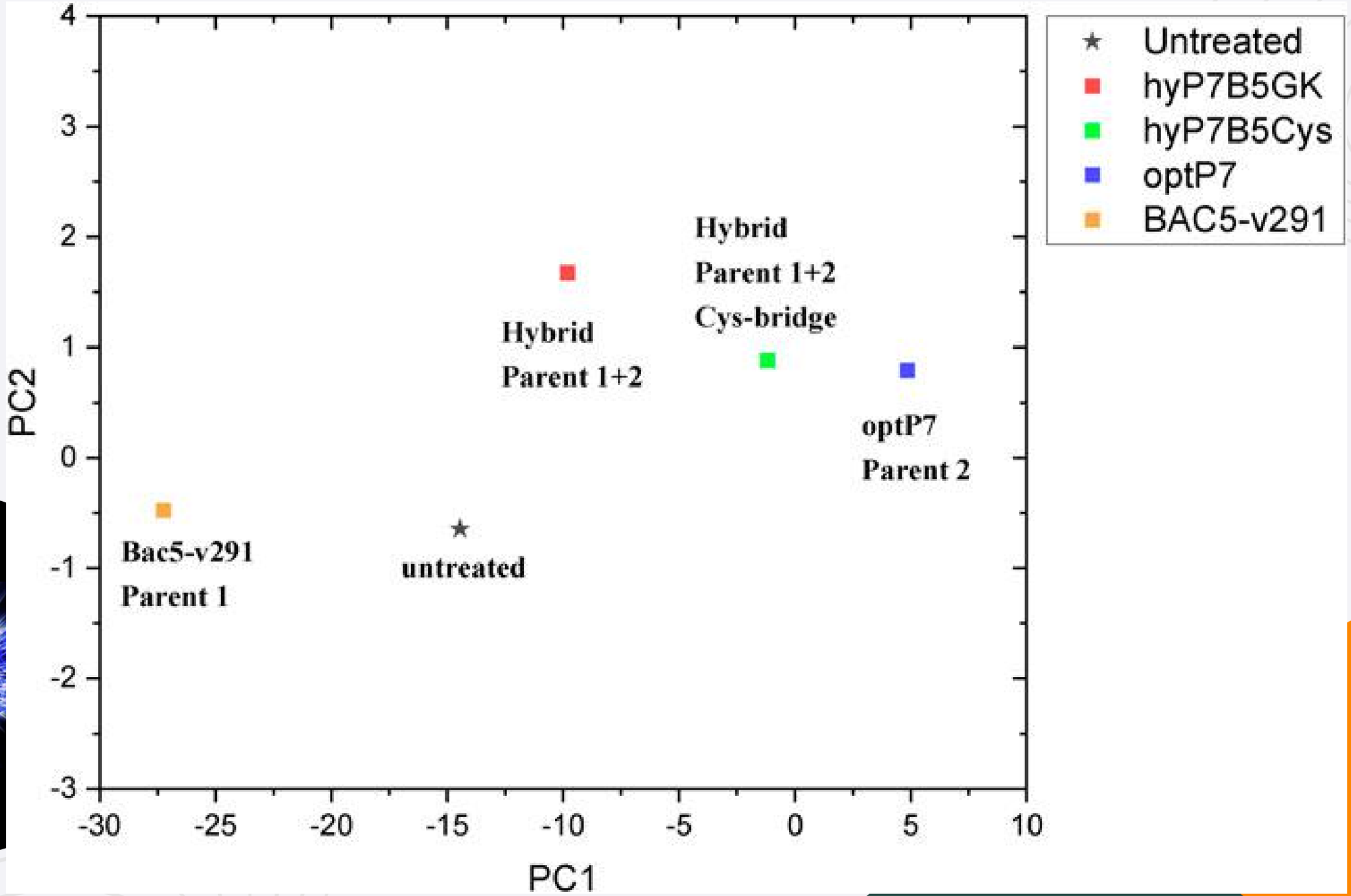


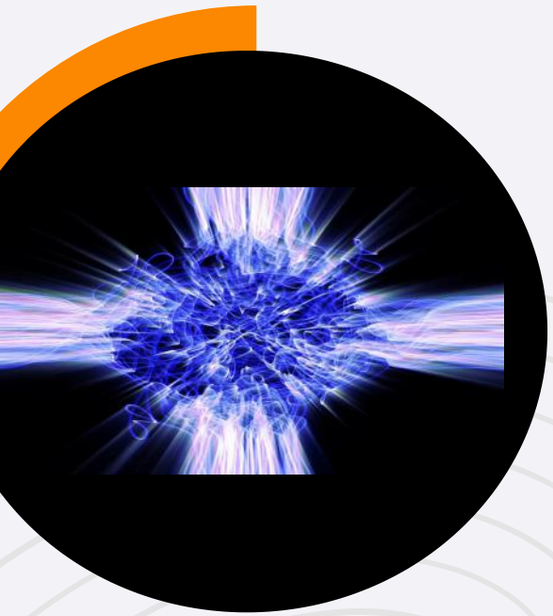
Name	Description	Sequence*	MRSA	E. coli	PA	HC50	Therapeutic window HC50/ MIC(MRSA)
optP1	Optimized 9mer	KIILRIRWR	1.5	1	6	205	137
optP7	Optimized 9mer	KRRVRWIIW	6	1.5	3	>195	>32.5
consP1	Consensus sequence derived from multiple alignment	VRKPPYLPRPRPRPL	>139	35	>139	>139	n.a
hyP1CoG1	Hybrid peptide with Gly linker, optP1 C-terminal	KIILRIRWRGGGVRKPPYLPRPRPRPL	2.5	1	1	>157	>62.8
hyP1CoG2	Hybrid peptide with Gly linker, optP1 C-terminal	VRKPPYLPRPRPRPLGGGKIILRIRWR	2.5	1	2.5	>157	>62.8
hyP7CoG1	Hybrid peptide with Gly linker, optP7 C-terminal	VRKPPYLPRPRPRPLGGGKRRVRWIIW	5	1	5	>154	>30.8
hyP7CoG2	Hybrid peptide with Gly linker, optP7 N-terminal	KRRVRWIIWGGGVRKPPYLPRPRPRPL	5	2.5	2.5	>154	>30.8
Bac5-v291	Optimized Bac5(1–17) variant 291	RWRRPIRRRPIRPPFWR	27	1.7	27	>278	>10.3
hyP7B5G	Hybrid peptide with Gly linker, optP7 C-terminal	RWRRPIRRRPIRPPFWRGGGKRRVRWIIW	2	2	2	102	51
hyP7B5K	Hybrid peptide with Lys linker, optP7 C-terminal	RWRRPIRRRPIRPPFWRKKKKRRVRWIIW	2	2	2	82	41
hyP7B5GK	Hybrid peptide with Gly-Lys linker, optP7 C-terminal	RWRRPIRRRPIRPPFWRKGKGKGKRRVRWIIW	1	1	2	>120	>120
hyP7B5Cys	Hybrid peptide with disulfide bridge, designed to be cleaved in the cytosol, optP7 C-terminal	RWRRPIRRRPIRPPFWRKGKC-S-S-CKGKRRVRWIIW	1	0.6	2	102	>102

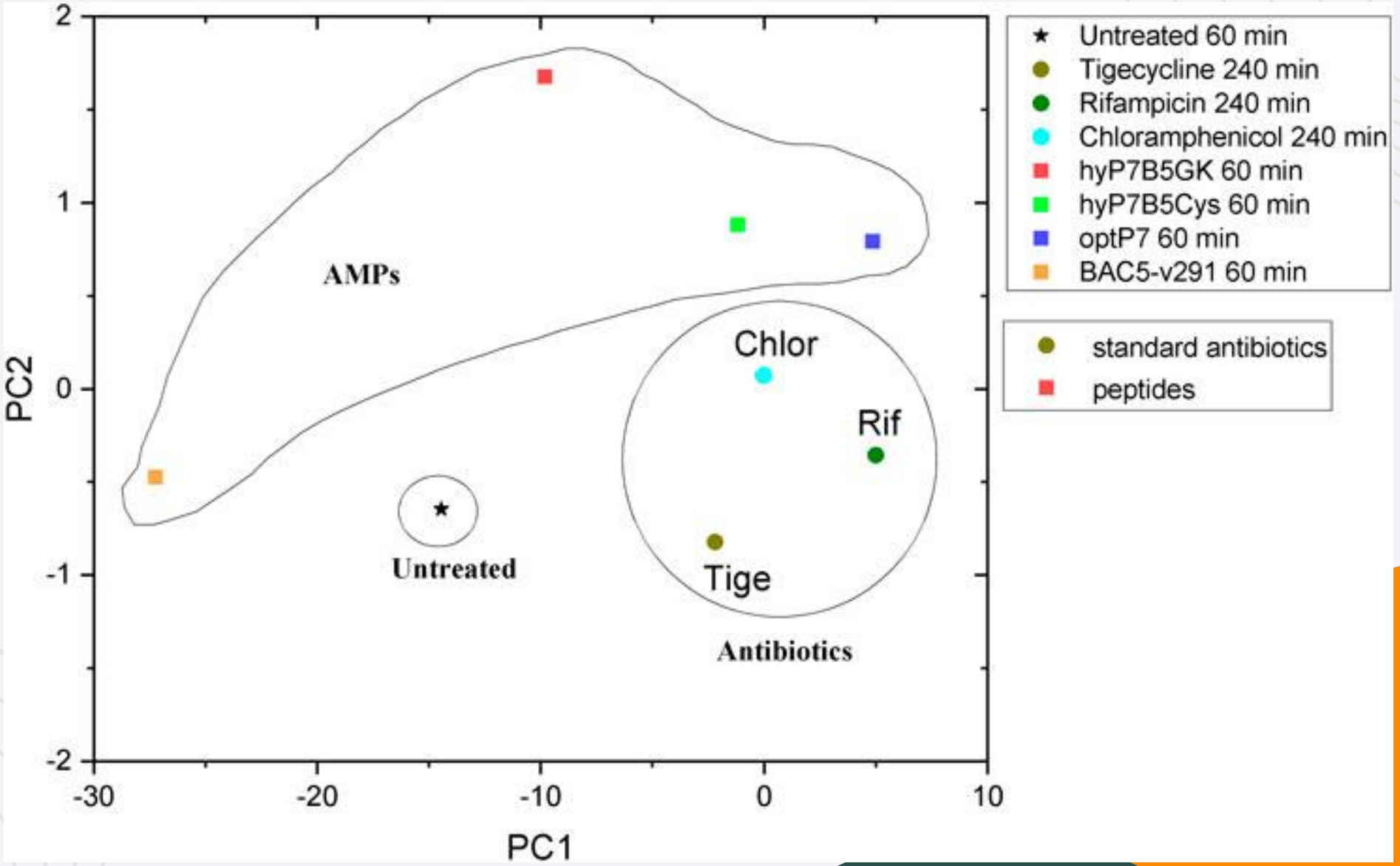
SCATTERING CURVES






Untreated
hyP7B5GK
hyP7B5Cys
optP7
BAC5-v291
Hybrid
Parent 1+2
Hybrid
Parent 1+2
Cys-bridge
optP7
Parent 2
Bac5-v291
Parent 1
untreated
PC2
PC1
4
3
2
1
0
-1
-2
-3
-30
-25
-20
-15
-10
-5
0
5
10

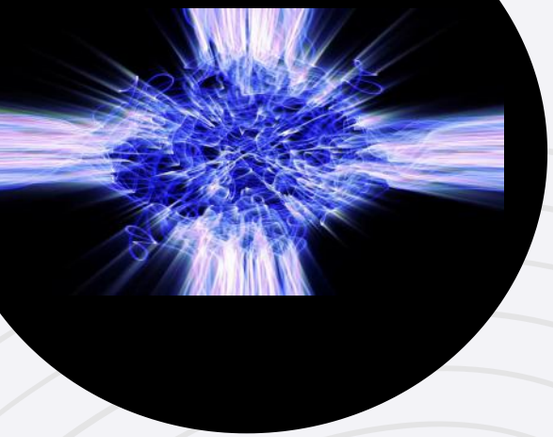



2
1
0
-1
-2
PC2
-30
-20
-10
0
10
PC1
AMPs
Untreated
Chlor
Rif
Tige
Antibiotics
Untreated 60 min
Tigecycline 240 min
Rifampicin 240 min
Chloramphenicol 240 min
hyP7B5GK 60 min
hyP7B5Cys 60 min
optP7 60 min
BAC5-v291 60 min
standard antibiotics
peptides

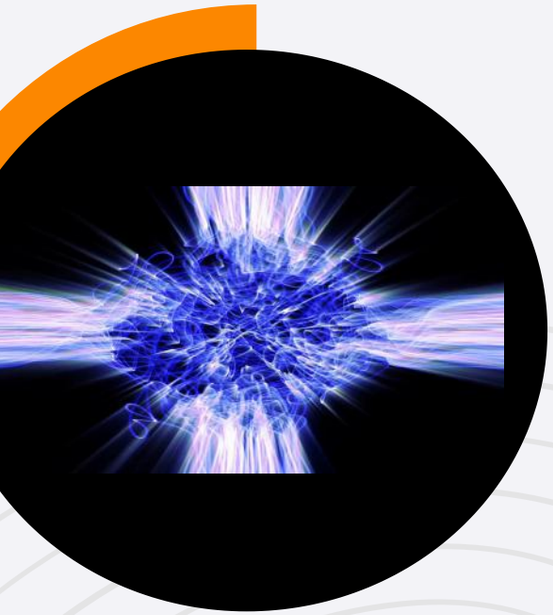
ANTIFUNGAL PEPTIDES

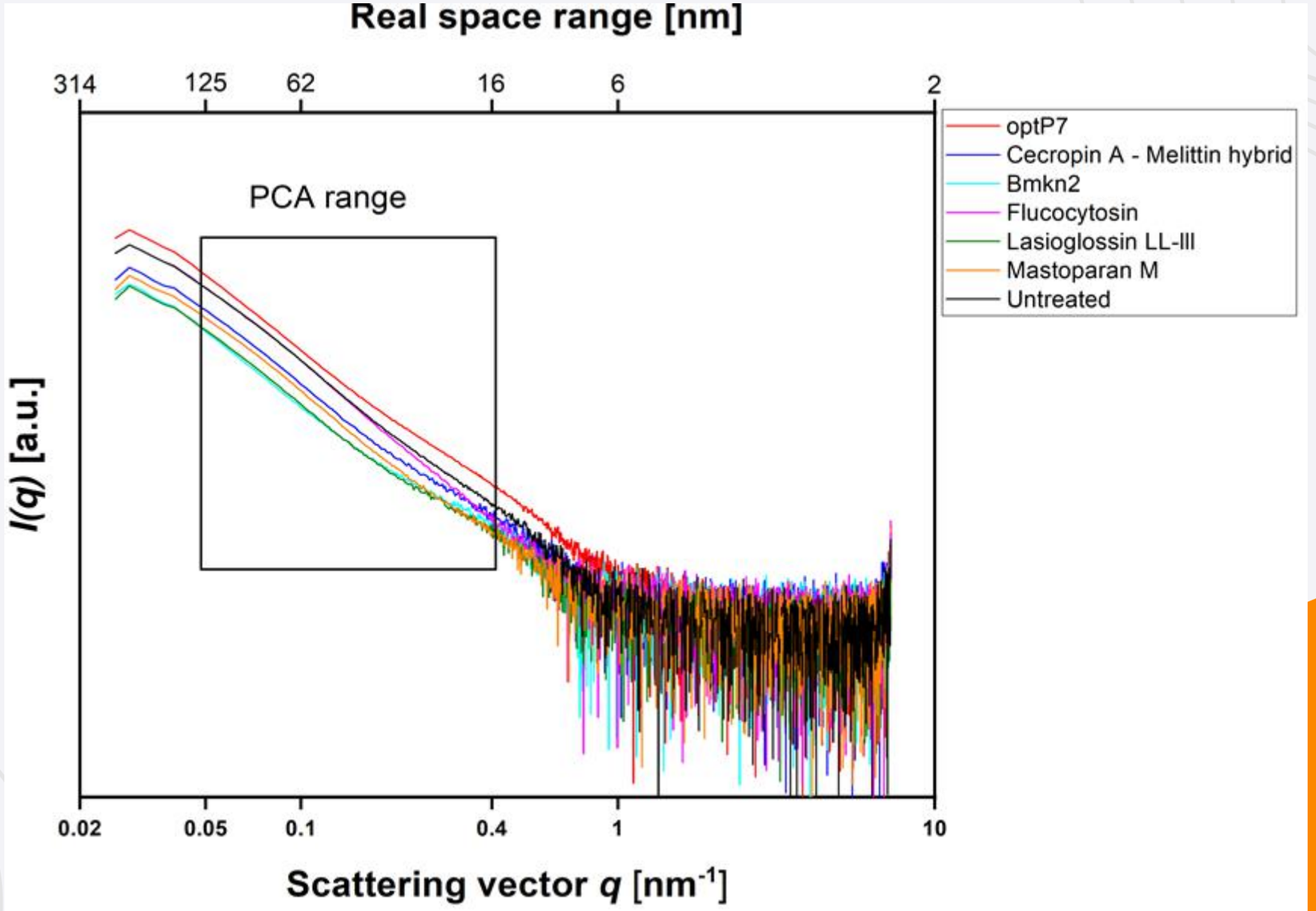
BioSAXS Can Discriminate Modes Of Action Of Antifungal Substances In Yeast – An Exploratory Study

Kai Hilpert[1*], Christoph Rumancev[2], Paula Matilde Lopez-Perez[3], Dominic W. P. Collis[3], Jurnorain Gani[1]. Vasil M. Garamus[4]. Ralf Mikut[5]. Axel Rosenhahn[2*]

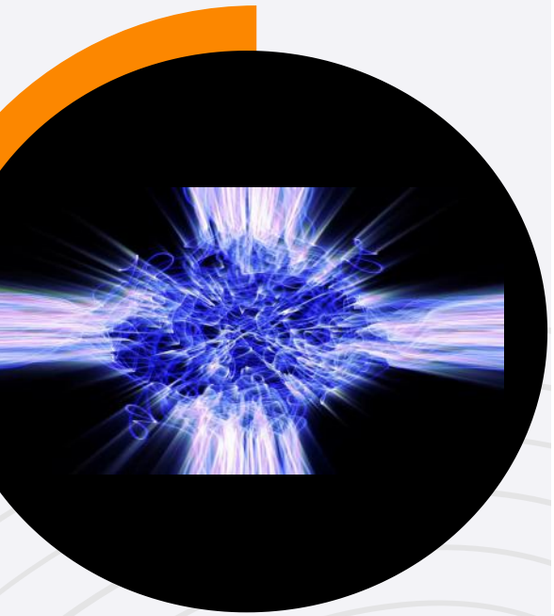


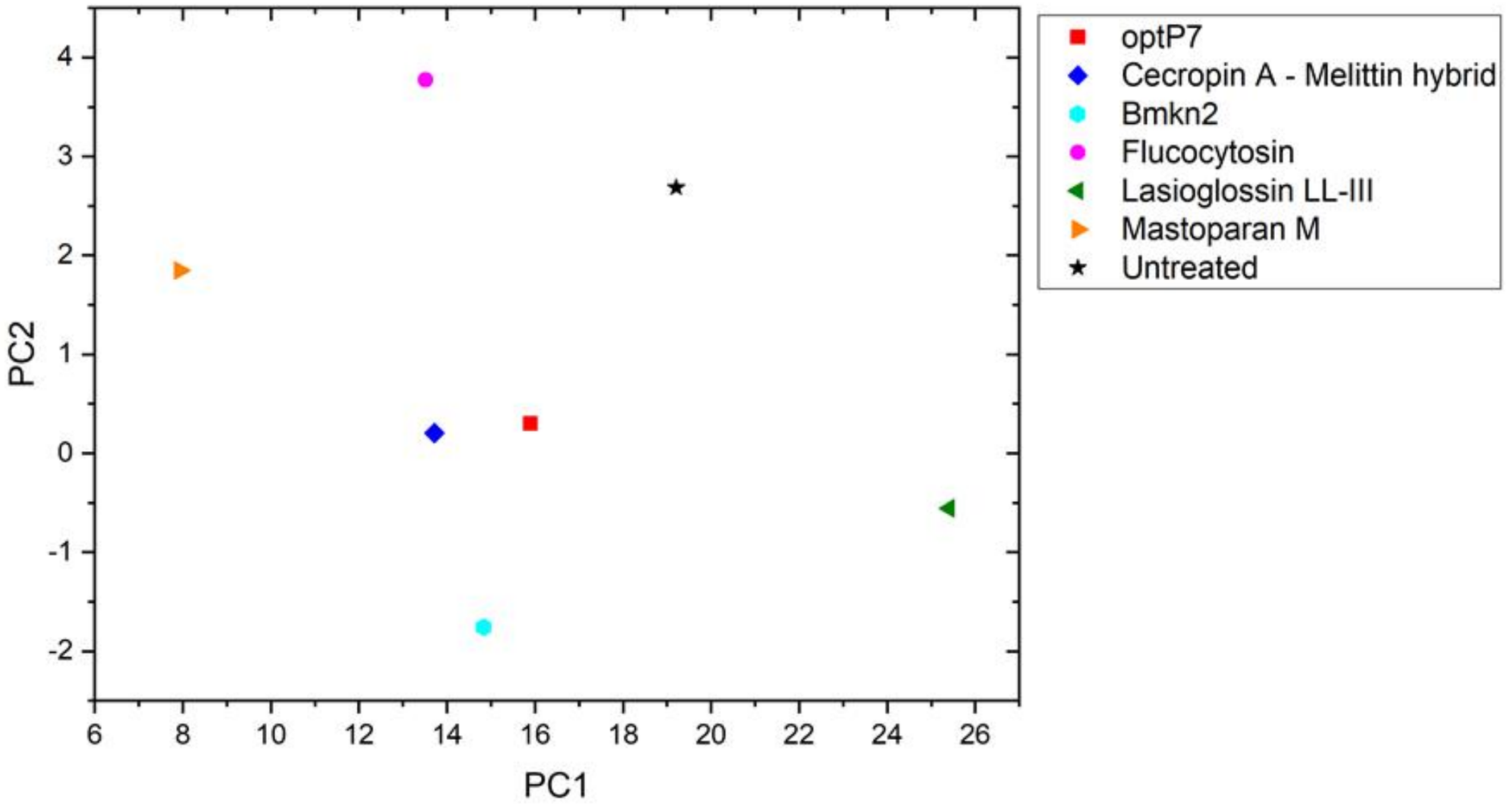
SCATTERING CURVES



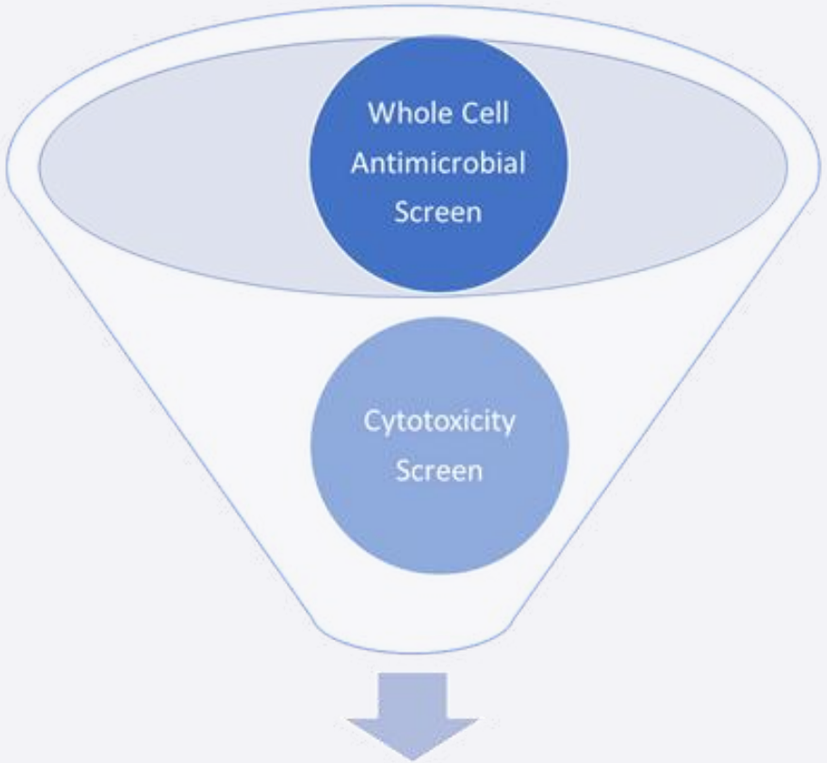


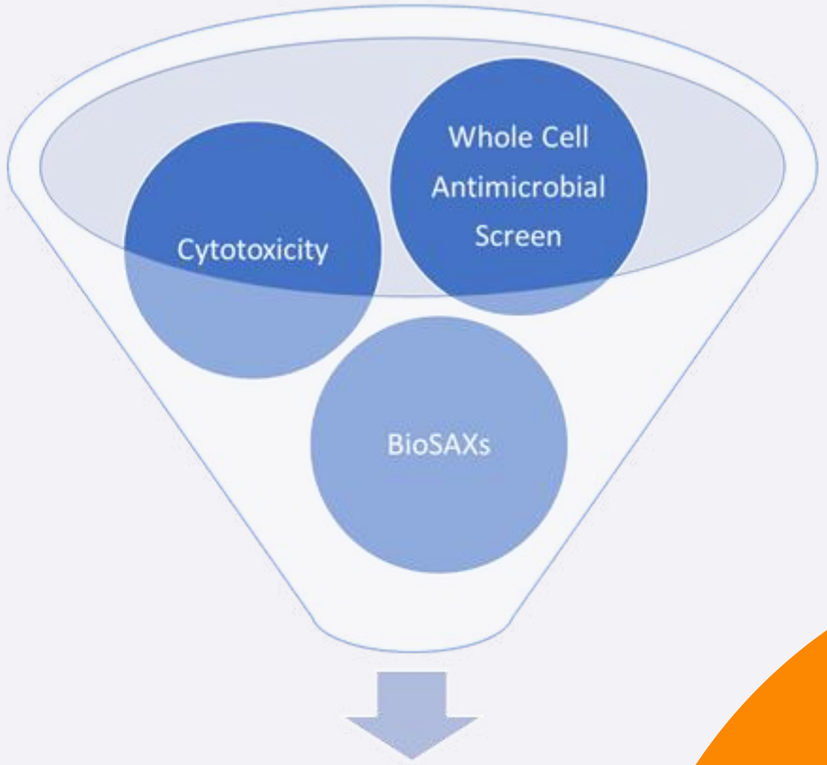
ANTIFUNGAL PEPTIDES





TAKE HOME MESSAGE





THANK YOU

- Peptide Synthesis
  - Rudolf Volkmer
  - Sven Hofmann
  - Martin Ashby
  - Jurnorain Gani
  - Paula Lopez
- MRSA optimization
  - Martin Ashby
- *P. aeruginosa* optimization
  - Jurnorain Gani
- Bioinformatic
  - Ralf Mikut
  - Artem Cherkasov
  - Chris F. Fjell
  - Ben Thomas

BioSAXS

Andreas von Gundlach

Christoph Rumancev

Axel Rosenhahn

Vasil M. Garamus

Ralf Mikut

Jurnorain Gani

Martin Ahsby

THANK YOU


UKRI
Innovate
UK


SBRI
HEALTHCARE


St George's
University of London


DESY.


EMBL

THANK YOU


THANKS!
THANKS!
THANKS!
THANKS!
THANKS!
THANKS!
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THANKS!
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