Engineered bacteriophages to target amyloid-β in the brain

Ivone M. Martins

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- Health

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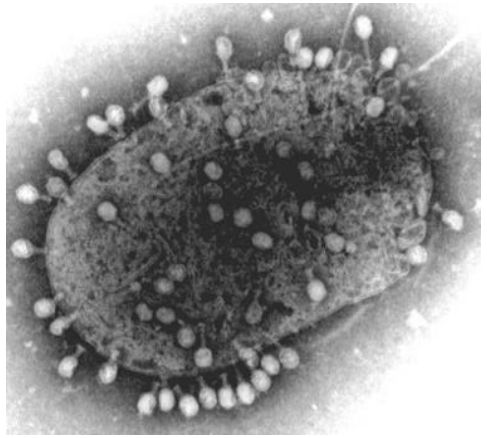
Bacteriophages (Phages)

From the Greek *phagein: to eat* or *to devour*

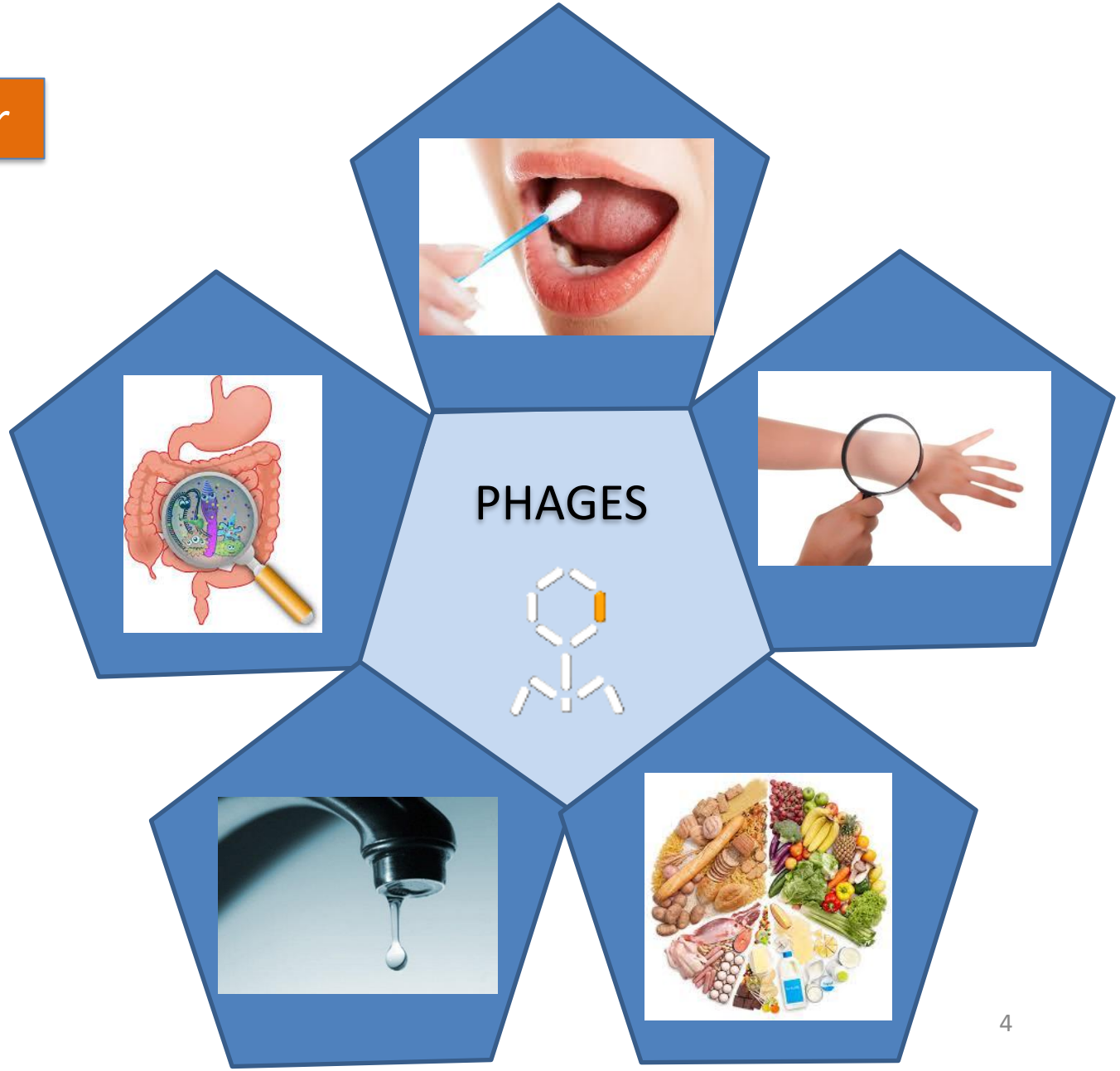
Virus that only infect bacterial cells

The oldest and most abundant organisms on Earth



Cornell Integrated Microscopy Center

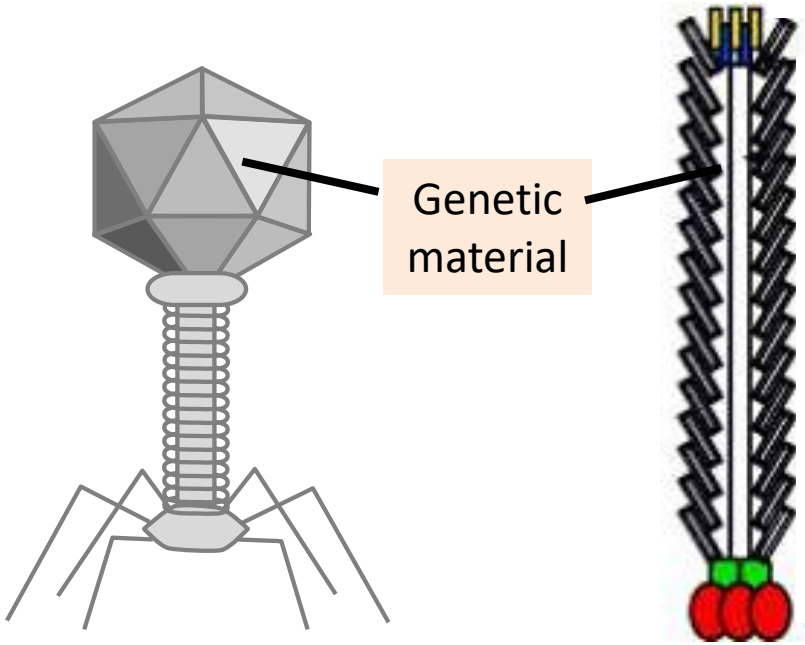
Natural components of the human microbiome



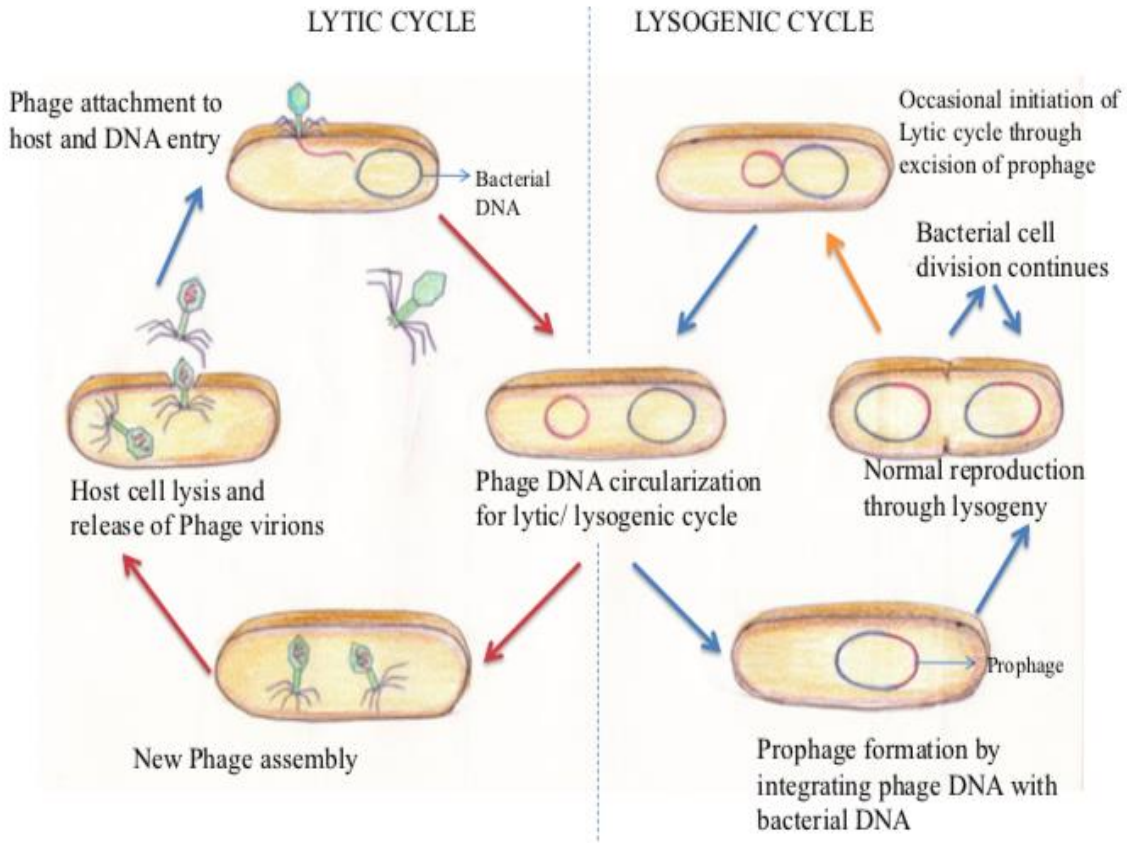
Phages – structure and infection process

Very diverse in size (ranges from 24 to 200 nm), morphology and genomic organization (RNA or DNA, linear or circular)

Icosahedral shape | **Filamentous shape**



Life Cycle



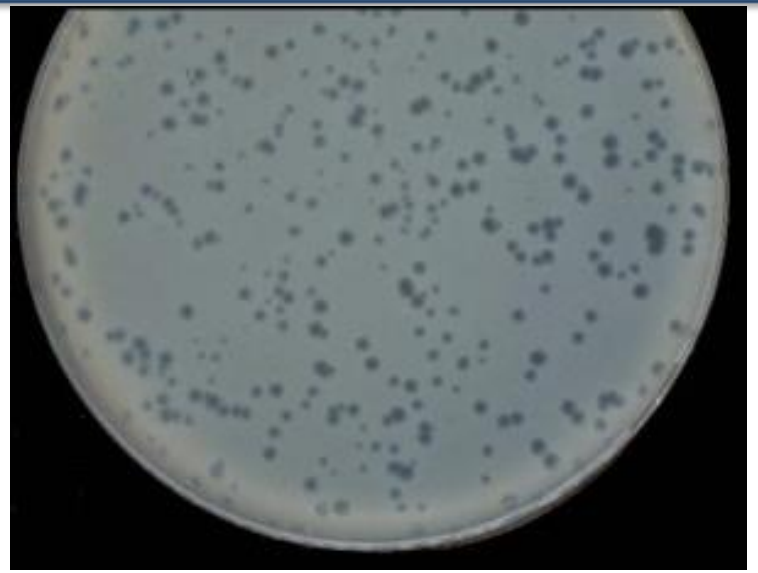
Kasman LM, Whitten RA. Bacteriophages. StatPearls Publishing; 2018

Phages: historical overview

Early 1900s

Frederick Twort | Félix d'Herelle

Growth inhibition and cell lysis in cultures of bacteria



Clinical practice to treat bacterial infections

Dysentery Cholera

Penicillin and other Antibiotics

Phage therapy was eclipsed

Phages: historical overview

Multidrug-resistant bacteria

https://scfh.ru/en/papers/bacteriophages-100-years-in-the-service-of-mankind/

Nature Reviews Microbiology | AOP, published online 9 November 2015; doi:10.1038/nrmicro3564

PERSPECTIVES

TIMELINE

A century of the phage: past, present and future

George P. C. Salmond and Peter C. Fineran

Abstract | Viruses that infect bacteria (bacteriophages; also known as phages) were discovered 100 years ago. Since then, phage research has transformed fundamental and translational biosciences. For example, phages were crucial in establishing the central dogma of molecular biology — information is sequentially passed from DNA to RNA to proteins — and they have been shown to have major roles in ecosystems, and help drive bacterial evolution and virulence. Furthermore, phage research has provided many techniques and reagents that underpin modern biology — from sequencing and genome engineering to the recent discovery and exploitation of CRISPR–Cas phage resistance systems. In this Timeline, we discuss a century of phage research and its impact on basic and applied biology.

molecular biology research. In this Timeline, we highlight the impact of phages in the first 100 years since their discovery in terms of the origins of molecular biology, our knowledge of ecology and evolution, and their biotechnological exploitation. We encourage readers to try to imagine what the modern world would look like if phages did not exist; we are clearly indebted to the most abundant biological entities on Earth.

The origins of molecular biology

Key questions in biology addressed. In the early twentieth century, the nature of the gene was a central biological question. Physicists, including Leo Szilard, Salvador Luria and Max Delbrück as well as other researchers (the 'phage group'), began tackling this and other fundamental biological questions by working with phages as biological models[5]. Delbrück urged researchers to

Phages as therapeutic agents

MICROBIOLOGY

Phage therapy gets revitalized

The rise of antibiotic resistance rekindles interest in a century-old virus treatment

BY SARA REARDON

For decades, patients behind the Iron Curtain were denied access to some of the best antibiotics developed in the

PERSPECTIVE

For reprint orders, please contac

Phages targetin
approach to ph

Andrzej Górski[*1,2], Krystyna Dąb
Borysowski[2], Ryszard Międzybr

ABSTRACT While the true effic
clinical trials, it continues to offer r
have failed. Novel developments
future clinical trials would evaluat
conclusions regarding the true val
develop and establish a bank of phages specific to most threatening pathogens ar
with homing peptides enabling their localization in infected tissues in densities
efficient and stable eradication of infection.

frontiers in Microbiology

OPEN ACCESS

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Queen Astrid Military Hospital, Belgium

Bacteriophage Procurement for Therapeutic Purposes

Beata Weber-Dąbrowska[1,2], Ewa Jończyk-Matysiak[1], Maciej Żaczek[1], Małgorzata Łobocka[3,4], Marzanna Łusiak-Szelachowska[1] and Andrzej Górski[1,2,5]*

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Bacteriophages (phages), discovered 100 years ago, are able to infect and destroy only bacterial cells. In the current crisis of antibiotic efficacy, phage therapy is considered as a supplementary or even alternative therapeutic approach. Evolution of multidrug-resistant and pandrug-resistant bacterial strains poses a real threat, so it is extremely important to have the possibility to isolate new phages for therapeutic purposes. Our phage laboratory and therapy center has extensive experience with phage isolation, characterization, and therapeutic application. In this article we present current progress in bacteriophages isolation and use for therapeutic purposes, our experience in this field and its practical implications for phage therapy. We attempt to summarize the state of the art: properties of phages, the methods for their isolation, criteria of phage selection for therapeutic purposes and limitations of their use. Perspectives for the use of genetically engineered phages to specifically target bacterial virulence-associated genes are also briefly presented.

AMERICAN SOCIETY FOR MICROBIOLOGY | Micro Biolo

PERSPECTIVE

Genetically En
Decade

REVIEW
published: 12 August 2016
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ANTIBIOTICS OUTLOOK

The age of the phage

It's time to use viruses that kill bacteria again, say **Shigenobu Matsuzaki, umpei Uchiyama, Iyo Takemura-Uchiyama and Masanori Daibata.**

ankorva,[c] Joana Azeredo,[c] Timothy K. Lu[a,b]

nce, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA[a]; Department of Biological bridge, Massachusetts, USA[b]; Centre of Biological Engineering, University of Minho, Braga, Portugal[c]

microb Chemother 2014; **69**: 2326–2336
).1093/jac/dku173 Advance Access publication 28 May 2014

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obial
Chemotherapy

Viruses versus bacteria—novel approaches to phage therapy nst multidrug-resistant pathogens

HLA ISSN 2059-2302

ke Viertel, Klaus Ritter and Hans-Peter Horz*

promising therapeutic

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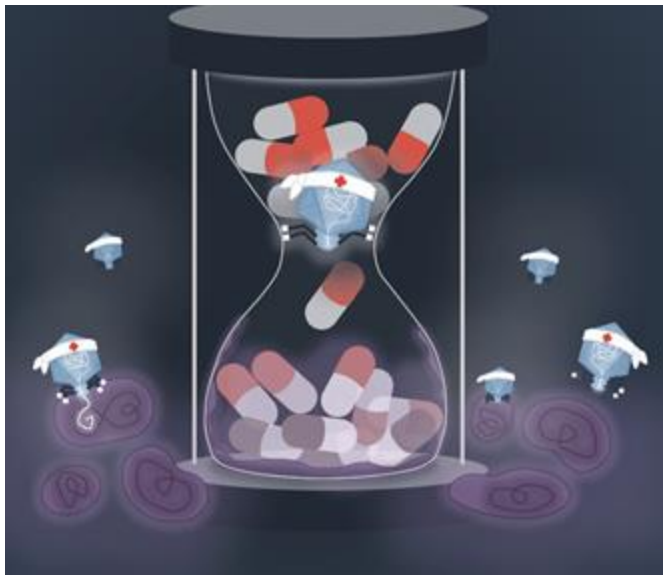
redators of bacteria, are becoming increasingly
utical applications. After their discovery almost
rticularly instrumental in the comprehension of
tics processes. The more recent emergence of
s novel therapeutic strategies, and phages are being
l antibacterial tools. Furthermore, phages are also
production, gene/drug carriers, bacterial detection
and typing. These new alternative approaches using phages are of major interest and have allowed unexpected developments, from the decipherment of fundamental biological processes to potential clinical applications.

on of phages to treat bacterial infections) has a tradition dating back almost
rapy slowed down in the West when antibiotics were discovered. With the
l by multidrug-resistant bacteria and scarce prospects of newly introduced
urrently being reconsidered as alternative therapeutics. Conventional phage
or treatment and recent human clinical trials have revealed encouraging
odern approaches to phages as therapeutics have been made *in vitro* and
phages and antibiotics has resulted in significant reductions in the number
d phages have overcome many of the problems of conventional phage ther-
or reversed the resistance of drug-resistant bacteria. The use of enzymes
sin, as therapeutic agents has been efficient in the elimination of Gram-posi-
s novel strategies for phage-related therapies and describes our current
s within the human microbiome. Our aim is to provide an overview of the
ogical concepts, thereby encouraging further research on this topic, with
s therapeutic or preventative medicines in daily clinical practice.

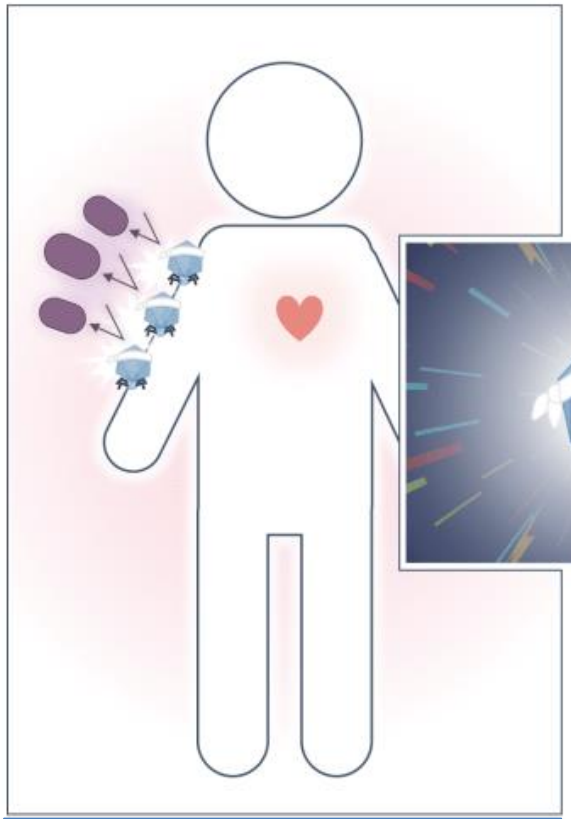
nan virome, engineered phage, endolysin

Fédération de Médecine Translationnelle de Strasbourg (FMTS)
Université de Strasbourg

Phages *vs* Antibiotics

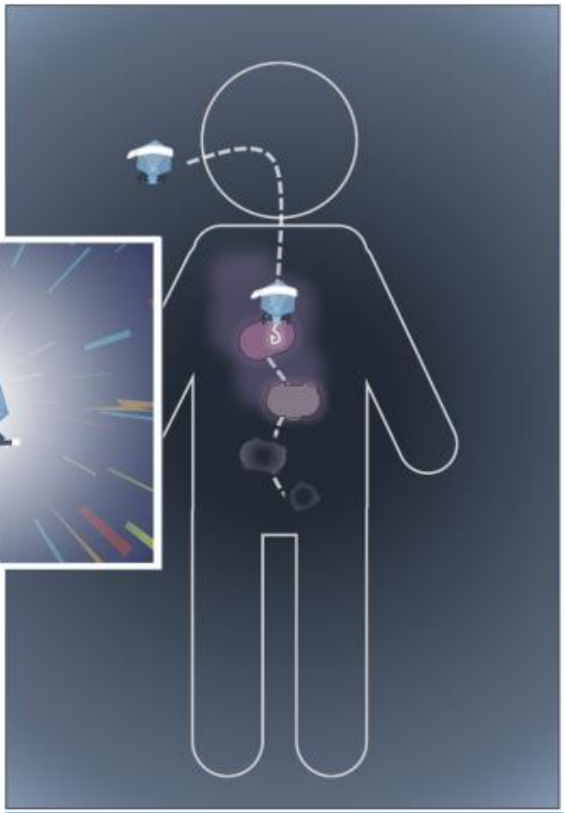


- **Bacterium-specific**
- **No side effects**
- **Capacity to kill multidrug-resistant bacteria**



Prevention

[Phagoburn](#) (EU)



Treatment

[ListshieldTM](#) (US)

http://sitn.hms.harvard.edu/flash/2018/bacteriophage-solution-antibiotics-problem/

Phages can save your life!!

https://tritonmag.com/phage/

Tom Patterson

Acinetobacter baumanii infection

↓

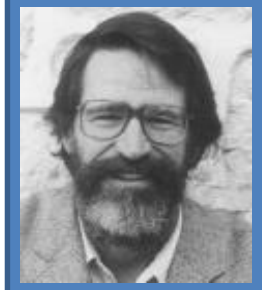
Multidrug-resistant strain

↓

Phage therapy with *A. baumanii* phage cocktail purified from sewage

Phages – other applications

Phage Display - Display of foreign peptides on the surface of a phage particle



Smith, G. P. (1985) Filamentous fusion phage: novel expression vectors that display cloned antigens on the *virion* surface, *Science 228*, 1315-1317.

E. coli phages used to produce viable modified virus with a foreign protein on its surface

Molecular recognition / selection technique

Direct physical link between genotype and phenotype

↓

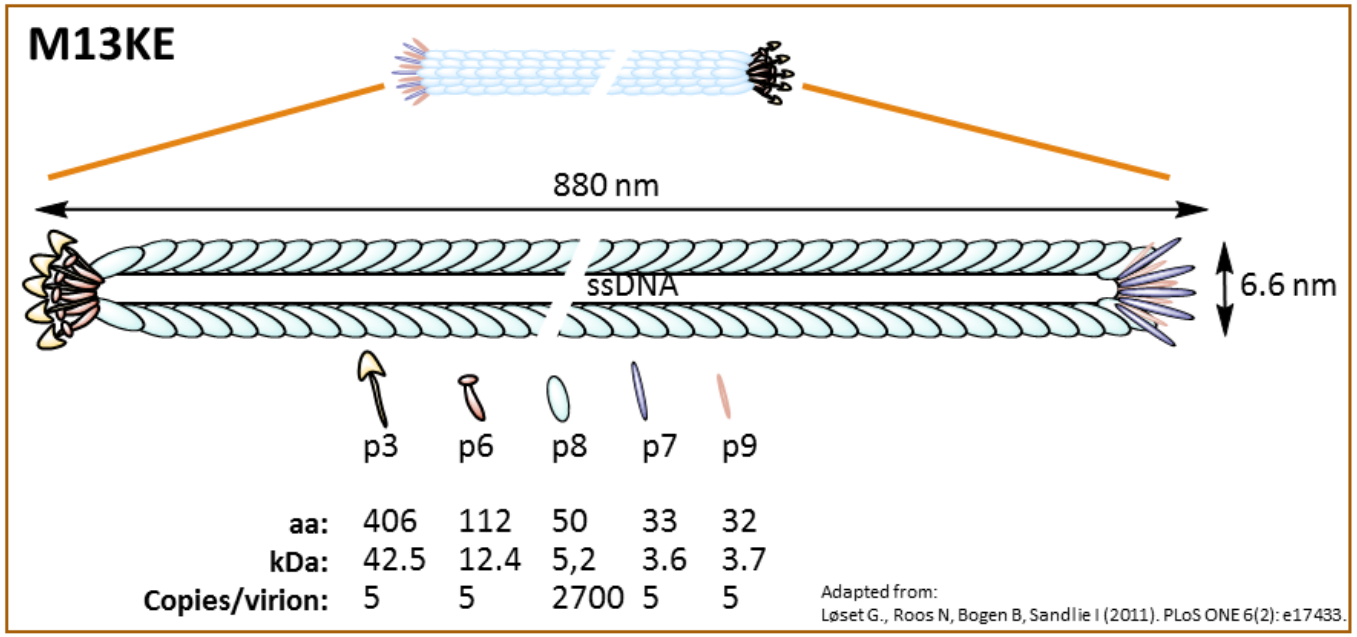
DNA sequences are cloned into the genome of phage, resulting in the expression of the foreign peptide or protein in fusion with one of the coat proteins of the phage particle

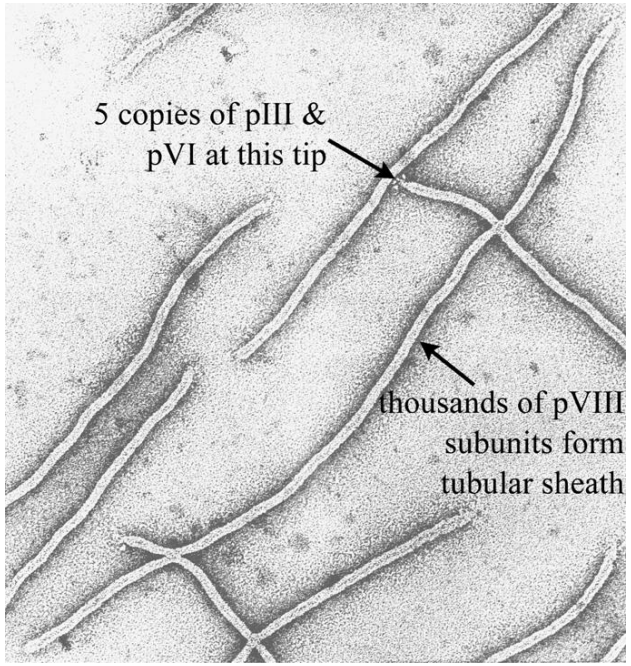
↓

The capsid protein serve only as an anchor for the displayed peptide not interfering with its structure

The filamentous phage M13

Tube-shape that packages the DNA (non lytic phage)





Electron micrograph of filamentous phage.
V.A. Petrenko / Microelectronics Journal 39 (2008) 202–207

By genetic engineering a foreign peptide can be cloned on phage genome with consequent display on the correspondent coat protein of the phage.

By chemical functionalization a given molecule (imaging agent; drug) can be displayed on the p8 coat protein of the phage.

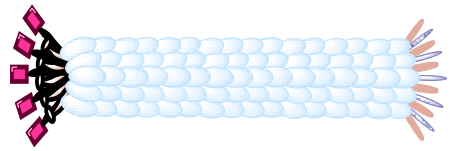
The filamentous phage M13

Single display

Polypeptides are normally displayed as fusions to the major coat protein 8 (p8) or the minor coat protein 3 (p3)

p8 display

p3 display



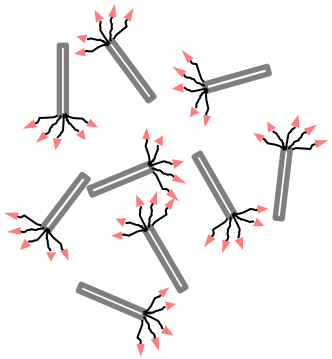
Doble display

The double display is also used when there is the need to use more that one protein; a drug or an imaging agent

p8/p3 display

Triple display

Phage display: traditional biopanning



Phage display library

Target exposure: cells + library

Amplified phages are used for another round

After 3-4 rounds, individual random clones are sequenced

Several sequences

1st Round	QLTMRLQ
	YGRETST
	SAESRHY
2nd Round	RPLPYPD
	LASPNGH
	GAASTRY
3rd Round	MANPGRA
	MQPSLVH
	MTTYPLS
	MTTTMQL
	MAVDPLR
4th Round	MQLPPDT
	MQTPLAP
	MSLPLLP
	MTAPPAR
	MGNPINH

Consensus sequences are shown in orange

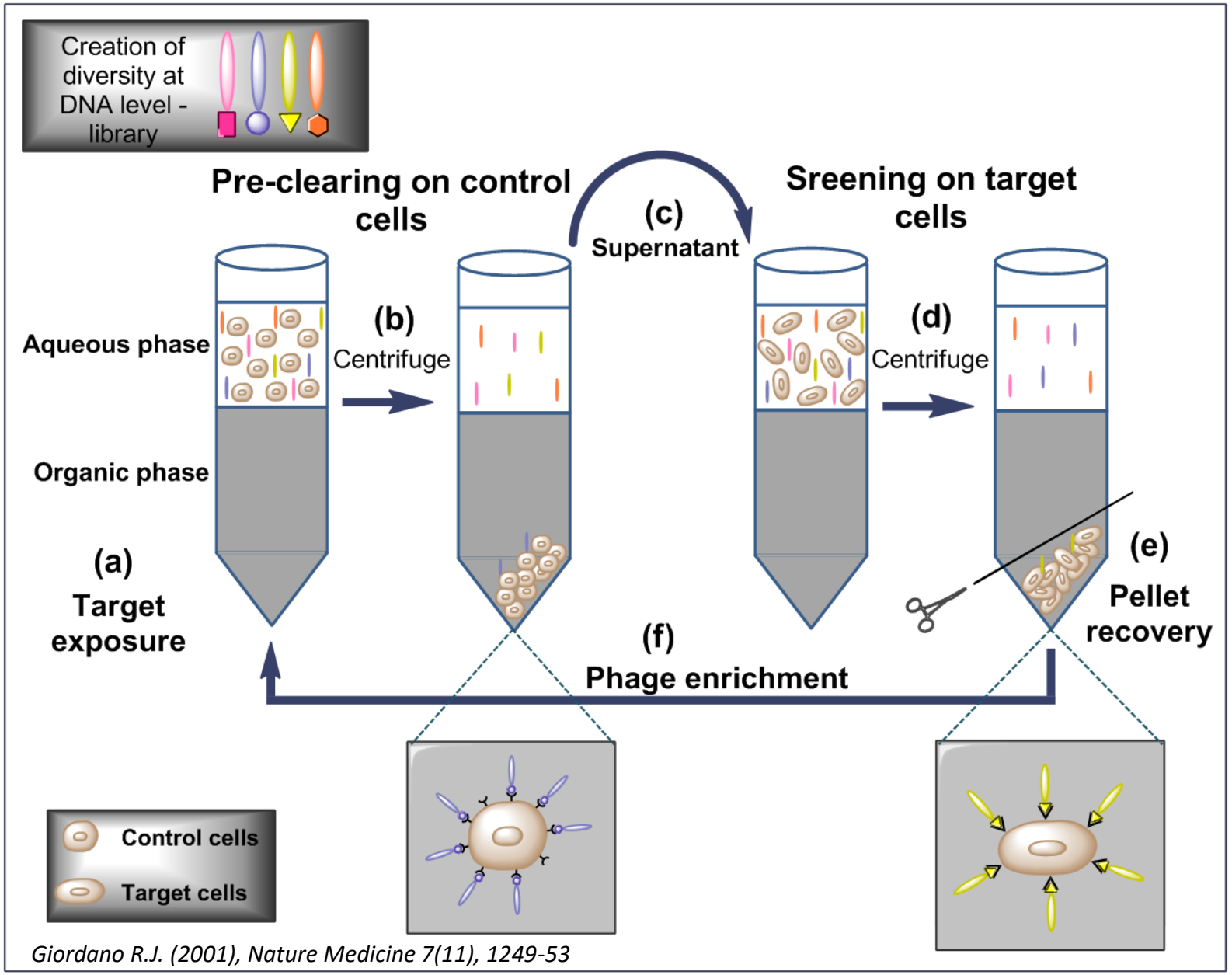
Phage amplification in *E. coli*

Biopanning round

Removal of unbound phages

Recovery of bound phages - elution

BRASIL: Biopanning and Rapid Analysis of Selective Interactive Ligands



Giordano R.J. (2001), Nature Medicine 7(11), 1249-53

Phage display applications – recognition and more

Functionalized synthetic substrates (2D surfaces) for stem cell expansion

Functionalized hydrogels (3D environments) for recreating/manipulating stem cell niches

Cell targeting gene therapy vectors

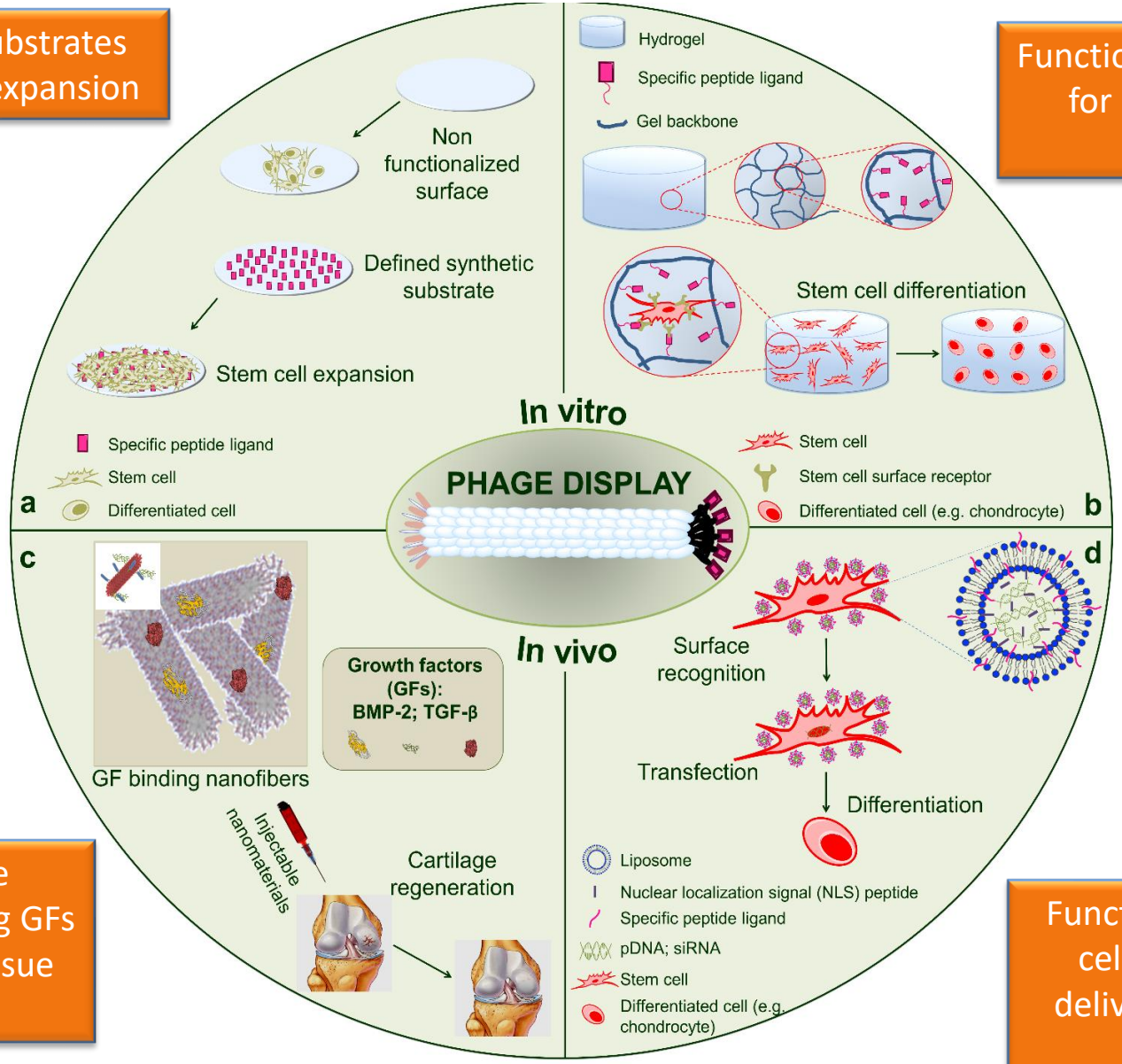
Multifunctional probes for tissue imaging

Creation of immunogens and nanovaccines

Study of protein-ligand interactions

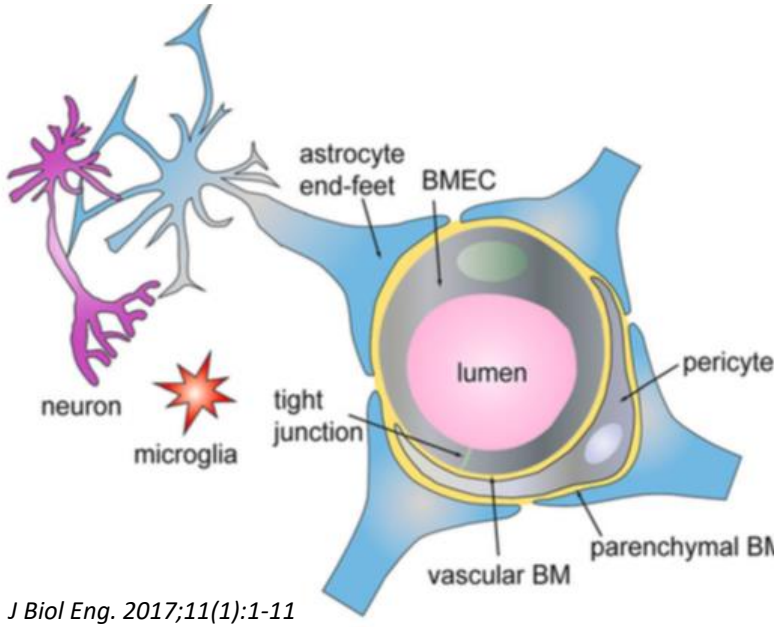
Functionalized injectable biomaterials for sequestering GFs to promote endogenous tissue repair

Functionalized nanocarriers for cell reprogramming (target delivery of genetic material to specific cells)



Martins I.M. (2016), ACS Chemical Biology 11, 2962-80

Phages in Neurosciences



J Biol Eng. 2017;11(1):1-11

Ability of the phages to penetrate into brain tissue

Blood Brain Barrier

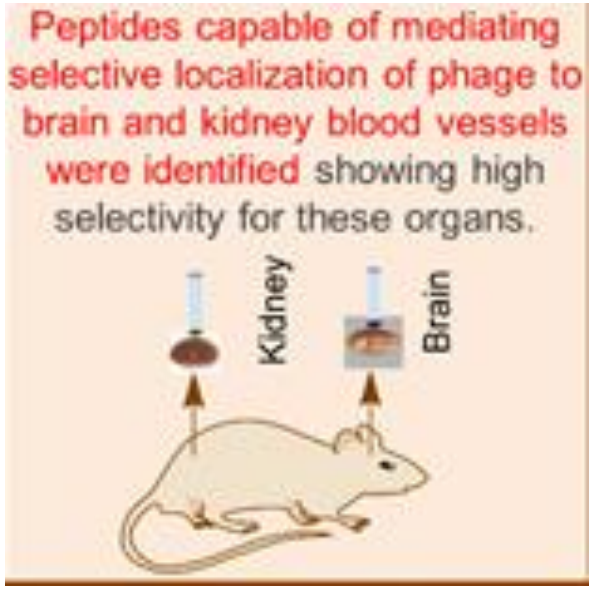
Brain homeostasis

Organ targeting *in vivo* using phage display peptide libraries

Renata Pasqualini & Erkki Ruoslahti

La Jolla Cancer Research Center, The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, California 92037, USA

PREFERENTIAL homing of tumour cells[1,2] and leukocytes[3,4] to specific organs indicates that tissues carry unique marker molecules accessible to circulating cells. Organ-selective address molecules on endothelial surfaces have been identified for lymphocyte homing to various lymphoid organs and to tissues undergoing inflammation[5–8], and an endothelial marker responsible for tumour homing to the lungs has also been identified[9]. Here we report a new approach to studying organ-selective targeting based on *in vivo* screening of random peptide sequences. Peptides capable of mediating selective localization of phage to brain and kidney blood vessels were identified, and showed up to 13-fold selectivity for these organs. One of the peptides displayed by the brain-localizing phage was synthesized and shown to specifically inhibit the localization of the homologous phage into the brain. When coated onto glutaraldehyde-fixed red blood cells, the peptide caused selective localization of intravenously injected cells into the brain. These peptide sequences represent the first step towards identifying selective endothelial markers, which may be useful in targeting cells, drugs and genes into selected tissues.



Phages in Neurosciences

M13 show low immunogenicity and biodegradability when injected in murine bloodstream

M13 is able to bind to and remodel multiple types of misfolded protein aggregates *in vitro*

Systemic combinatorial peptide selection yields a non-canonical iron-mimicry mechanism for targeting tumors in a mouse model of human glioblastoma

Fernanda I. Staquicini,[1] Michael G. Ozawa,[1] Catherine A. Moya,[1] Wouter H.P. Driess
E. Magda Barbu,[1] Hiroyuki Nishimori,[2] Suren Soghomonyan,[3] Leo G. Flores 2nd,[3] Xiaowe
Vincenzo Paolillo,[3] Mian M. Alauddin,[3] James P. Basilion,[5] Frank B. Furnari,[2] Oliver Bo
Frederick F. Lang,[6] Kenneth D. Aldape,[7] Gregory N. Fuller,[7] Magnus Höök,[4] Juri G. Gel
Richard L. Sidman,[8] Webster K. Cavenee,[2] Renata Pasqualini,[1,3] and Wadih Arap[1]

[1]David H. Koch Center, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA.
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The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA. [4]Texas A&M University System Health Science Cente
Houston, Texas, USA. [5]Center for Molecular Imaging Research and National Foundation for Cancer Research (NFCR)
Center for Molecular Analysis and Imaging, Department of Radiology, Massachusetts General Hospital, Boston, Massachusetts, US
[6]Department of Neurosurgery and [7]Department of Pathology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas,
[8]Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA.

The management of CNS tumors is limited by the blood-brain barrier (BBB), a vascular interface that the passage of most molecules from the blood into the brain. Here we show that phage particles with certain ligand motifs selected in vivo from a combinatorial peptide library can cross the BB normal and pathological conditions. Specifically, we demonstrated that phage clones displaying mimic peptide were able to target a protein complex of transferrin and transferrin receptor (TfR) t non-canonical allosteric binding mechanism and that this functional protein complex mediated t of the corresponding viral particles into the normal mouse brain. We also showed that, in an ort mouse model of human glioblastoma, a combination of TfR overexpression plus extended vascula ability and ligand retention resulted in remarkable brain tumor targeting of chimeric adeno-associat phage particles displaying the iron-mimic peptide and carrying a gene of interest. As a proof of cor delivered the HSV thymidine kinase gene for molecular-genetic imaging and targeted therapy of intr xenografted tumors. Finally, we established that these experimental findings might be clinically rel determining through human tissue microarrays that many primary astrocytic tumors strongly exp Together, our combinatorial selection system and results may provide a translational avenue for the detection and treatment of brain tumors.

Journal of Alzheimer's Disease 15 (2008) 193–198
IOS Press

Filamentous Bacteriophage as a Novel Therapeutic Tool for Alzheimer's Disease Treatment

Beka Solomon*
Department of Molecular Microbiology & Biotechnology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel-Aviv, Israel

Abstract. Antibodies towards the N-terminal region of the amyloid-β peptide (AβP) bind to Aβ fibrils, leading to their disaggregation. We developed an immunization procedure using filamentous phages displaying the only four amino acids EFRH encompassing amino acids 3–6 of the 42 residues of AβP, found to be the main regulatory site for Aβ formation. Phages displaying EFRH epitope are effective in eliciting humoral response against AβP which, in turn, relieves amyloid burden in brains of amyloid-β protein precursor transgenic mice, improving their ability to perform cognitive tasks. In order to overcome the low permeability of the blood brain barrier for targeting 'anti-aggregating' monoclonal antibodies (mAbs) to Aβ plaques in the brain, we applied antibody engineering methods to minimize the size of mAbs while maintaining their biological activity. Single-chain antibodies displayed on the surface of filamentous phage showed the ability to enter the central nervous system (CNS). The genetically engineered filamentous bacteriophage proved to be an efficient, nontoxic viral delivery vector to the brain, offering an obvious advantage over other mammalian vectors. The feasibility of these novel strategies for production and targeting of anti-aggregating antibodies against Aβ plaques to disease affected regions in the CNS may have clinical potential for treatment of Alzheimer's disease.

Parkinson disease

Journal of Applied Microbiology 2005, 98, 7–13 doi:10.1111/j.1365-2672.2004.02422.x

A REVIEW

Bacteriophage penetration in vertebrates

K. Dabrowska[1], K. Switala-Jelen[1], A. Opolski[1], B. Weber-Dabrowska[1] and A. Gorski[1,2]
[1]Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland, and [2]The Medical University of Warsaw, Warsaw, Poland

2004/0311: received 19 March 2004, revised 5 July 2004 and accepted 5 July 2004

A Bacteriophage Capsid Protein Provides a General Amyloid Interaction Motif (GAIM) That Binds and Remodels Misfolded Protein Assemblies

193

Haim Tsubery[1], Ming Y. Proschitsky[1], Eva Asp[1],
Gilead[1], Myra Gartner[1], Jonathan P. Waltho[2,3],
sa M. Hounslow[3], Daniel A. Kirschner[4], Hideyo Inouye[4],
son Wright[1], Beka Solomon[5] and Richard A. Fisher[1]

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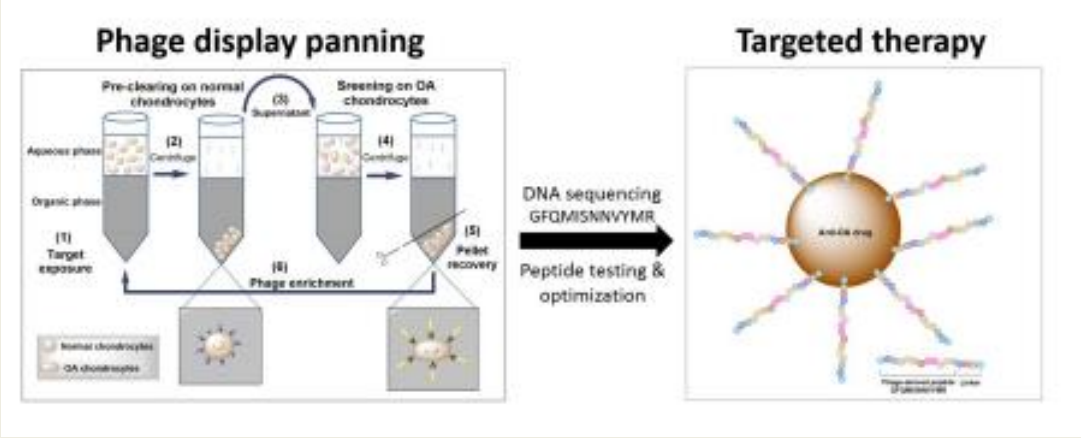
n Krishnan and Richard A. Fisher: rkrishnan@neurophage.com;

014.04.015

as, characterized by a canonical amyloid fold, play a central role in the erative diseases. Agents that bind and sequester neurotoxic intermediates of e assembly or promote the destabilization of such protein aggregates are in w that the gene 3 protein (g3p) of filamentous bacteriophage mediates potent yloid fold. We have characterized the amyloid binding and conformational n array of techniques, including X-ray fiber diffraction and NMR. The mechanism appears to reflect its physiological role during infection of *Escherichia coli*, which o-sensitive interdomain unfolding and *cis–trans* prolyl isomerization of g3p. In for g3p, TolA-C, competitively interferes with Aβ binding to g3p. NMR studies fibers is predominantly through middle and C-terminal residues of the Aβ subunit, ractions. A recombinant bivalent g3p molecule, an immunoglobulin Fc (Ig) l g3p domains, (1) potently binds Aβ fibers (fAβ) (K_D = 9.4 nM); (2); blocks M) and (3) dissociates fAβ (EC_{50} = 40–100 nM). The binding of g3p to s is generic, and amyloid-targeted activities can be demonstrated using other Taken together, our studies show that g3p(N1N2) acts as a general amyloid

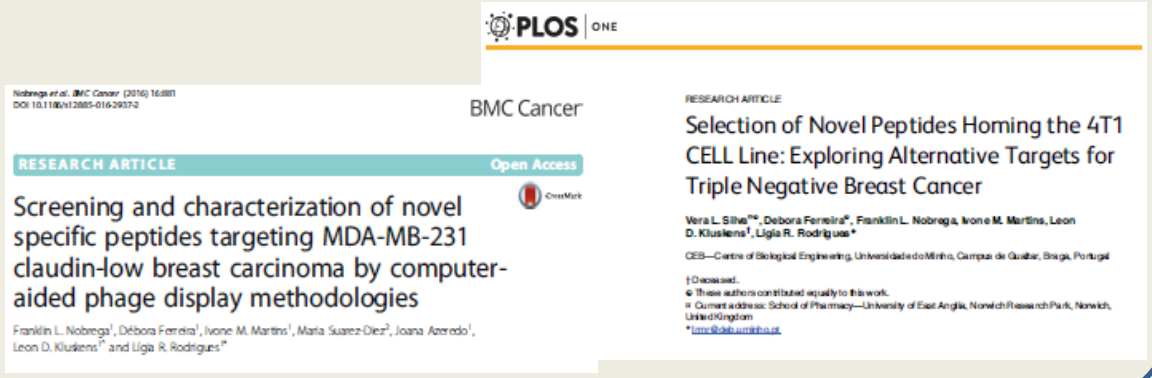
Phages to target amyloid-β in the brain

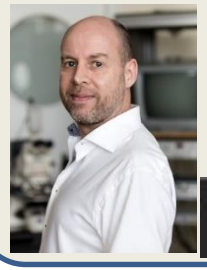
EPITHOPE: Identification of peptide sequences targeting osteoarthritic chondrocyte cells



LIPID´NP´PHAGE: Design of multifunctional phage-based nanocarriers for specific drug delivery

Design of novel BREAST and COLON cancer tumor ´targeted´ multifunctional nanoparticles





Professor Helmut Kessels

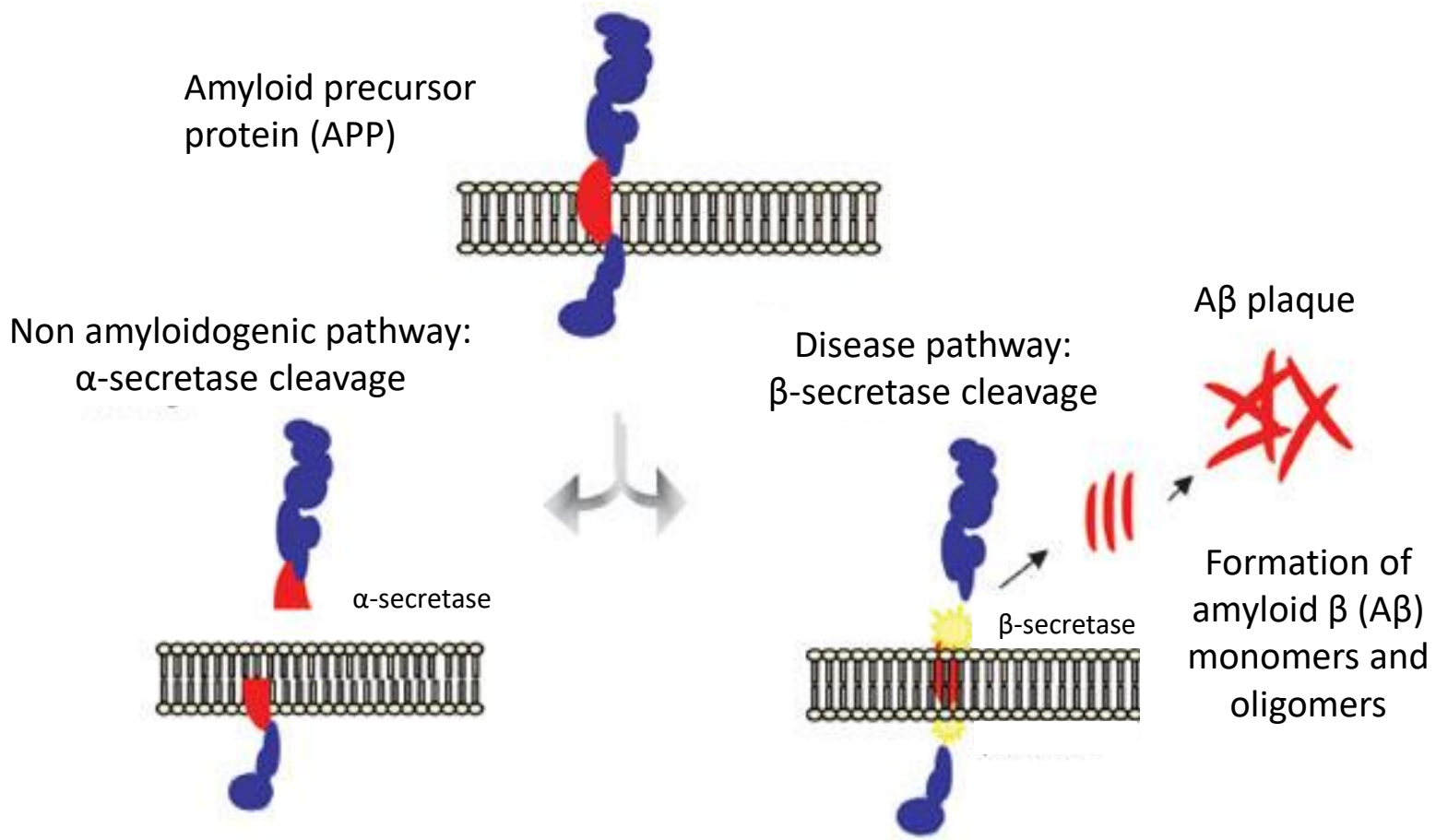
UNIVERSITY OF AMSTERDAM

!!PHAGES IN THE BRAIN!!
Diagnose/Treat Alzheimer´s Disease

Alzheimer´s Disease (AD)

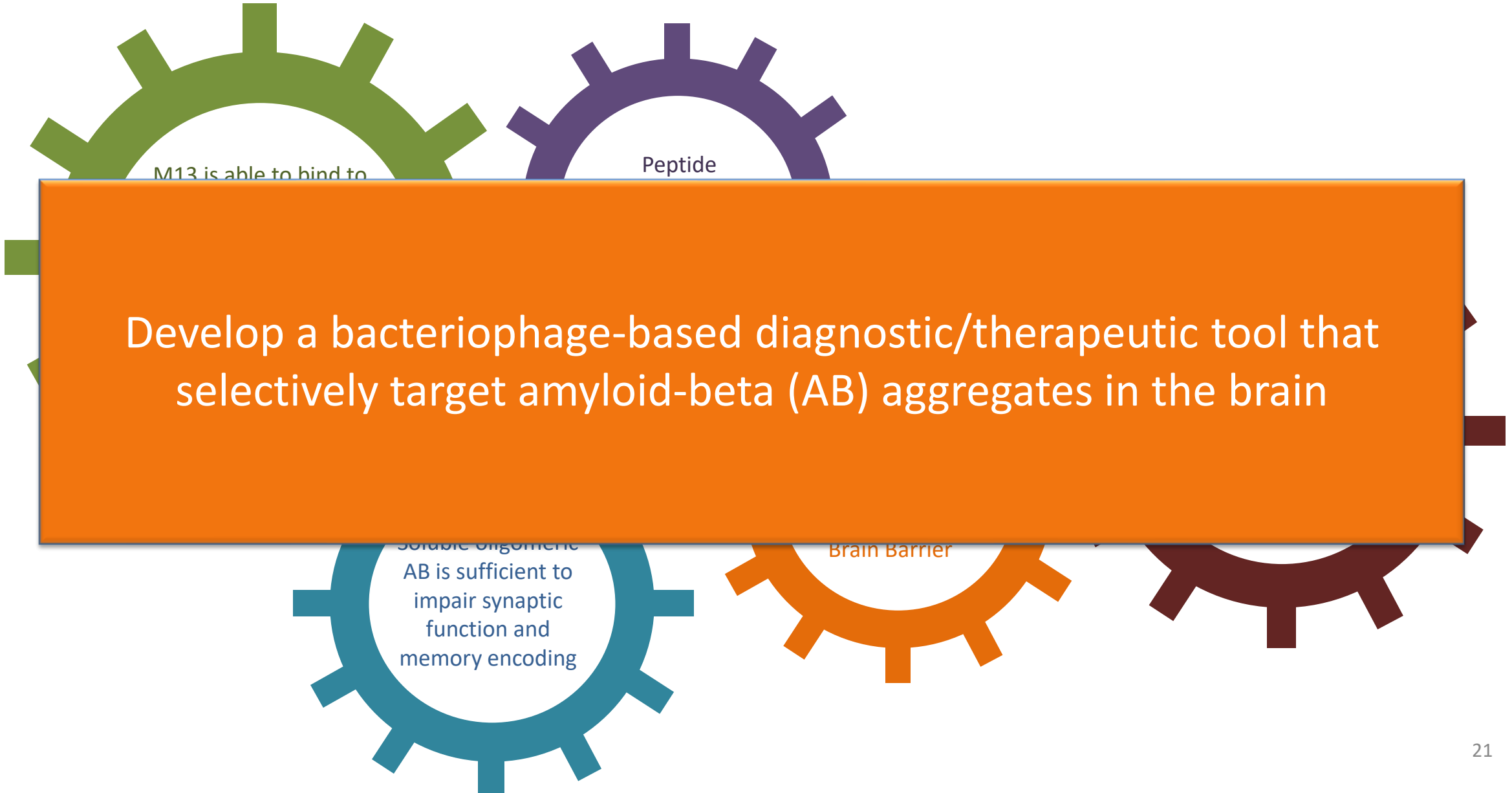
Characterized by an increased deposition of plaques, which consist of amyloid-beta (AB)

AB in soluble oligomeric form is sufficient to impair synaptic function and memory encoding



De Paula V. (2009), Dementia & Neuropsychologia 3(3), 188-194

Motivation



Develop a bacteriophage-based diagnostic/therapeutic tool that selectively target amyloid-beta (AB) aggregates in the brain

Specific aims

1. Engineer AB-specific phages

2. Assess *in vitro* the interation of AB-specific phages and AB

3. Assess *ex vivo* the efficiency of AB-specific phages to recognize and target mouse and human AD tissue

4. Assess *ex vivo* the efficiency of AB-specific phages to cross the BBB

5. Assess *in vivo* the efficiency of AB-specific phages to cross the BBB and be detected in the brain of an AD mouse-model

6. Assess *in vivo* if AB-specific phages labelled with a NIR-fluorochrome, are traceable and detectable in the brain of an AD mouse-model

7. Understanding the AB-specific phages intervention, by themselves or with the aid of therapeutic drugs, in the AB-driven synaptic and memory deficits

Engineer AB-specific phages: M13 genetic manipulation

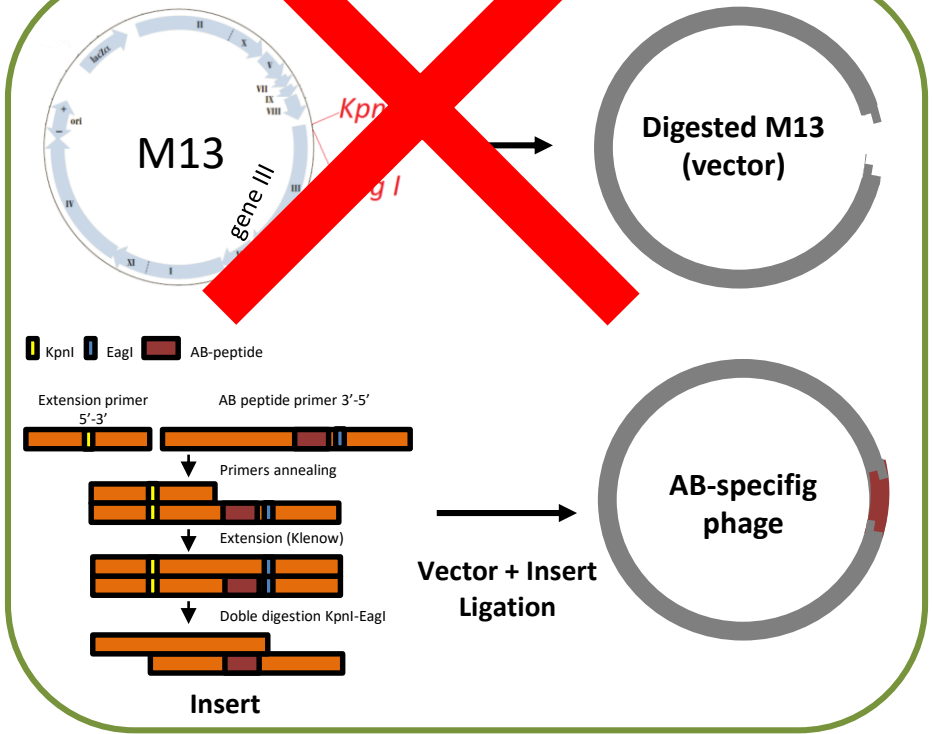
Aβ Sequences direct cloning

Phage display library synthesis

Noren, Methods, 2001

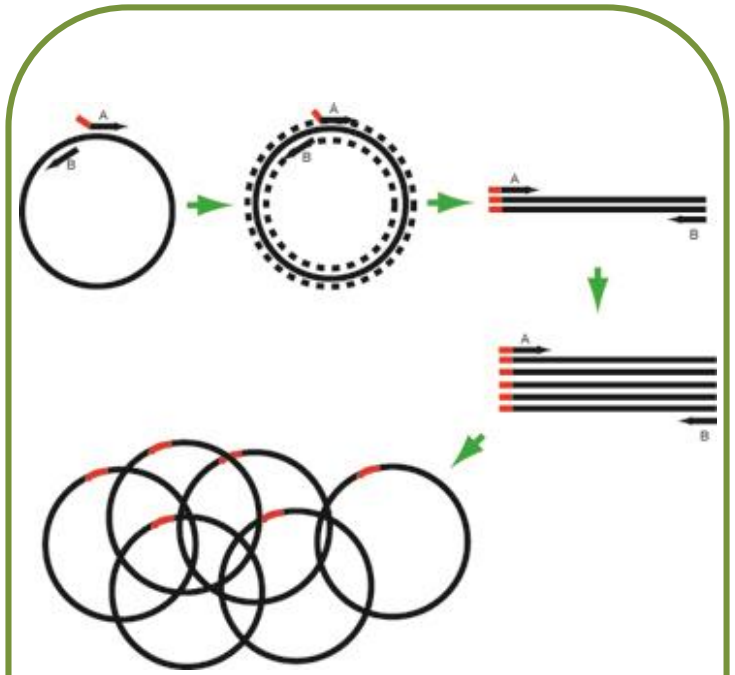
Complementarity primer method

Rangel, Nature Protoc… 2012



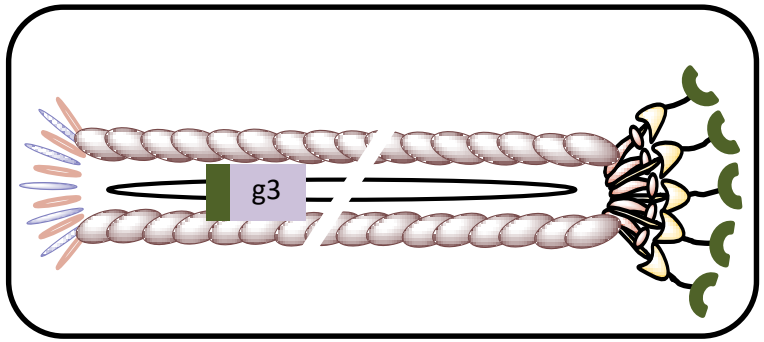
Round PCR

http://openwetware.org/wiki/'Round-the-horn_site-directed_mutagenesis", 2016



A – Primer with the AB sequence
B – Phosphorylated primer

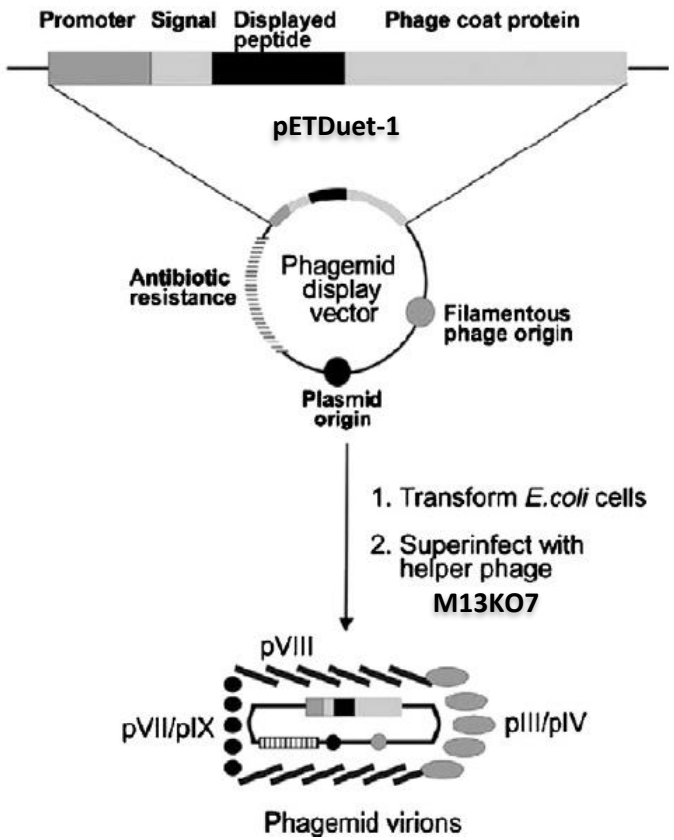
AB1: AB36-39 (VGGV)



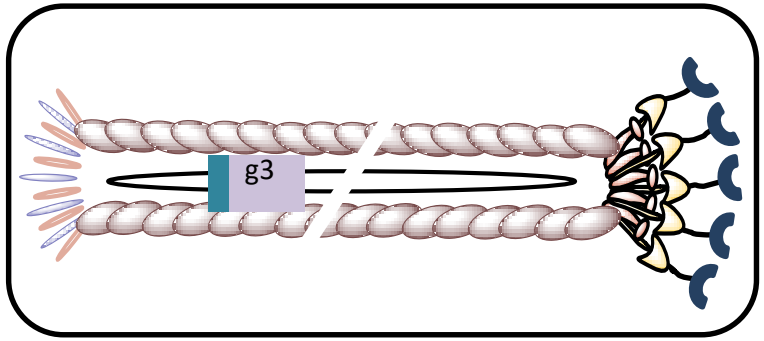
Engineer AB-specific phages: M13 genetic manipulation

Phagemid cloning system

Qi et al. 2012. J Mol Biol 417

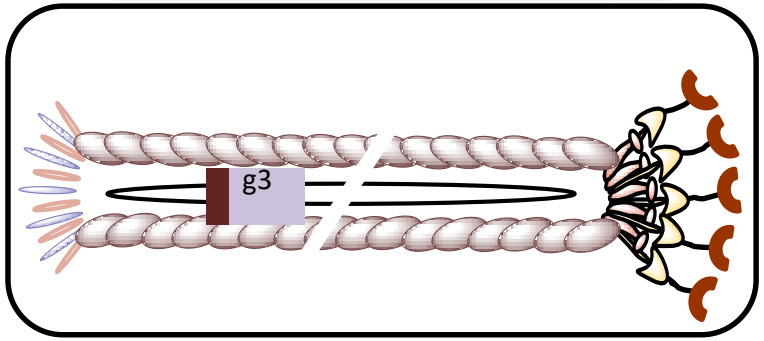


AB2: AB30-39 (AIIGLMVGGV)



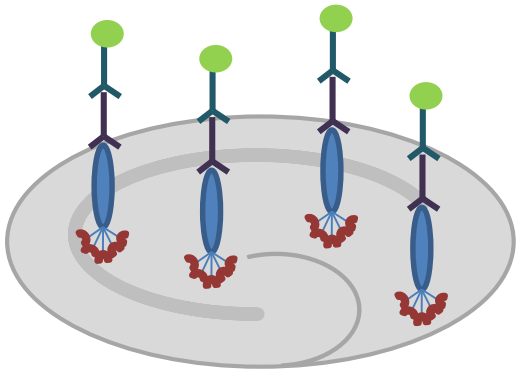
Specifically recognize AB fibrils

AB3: AB33-42 (GLMVGGVVIA)



Specifically recognize AB amyloid fibrils and oligomers

AB1-specific phage is able to recognize and bind to AD-tissue

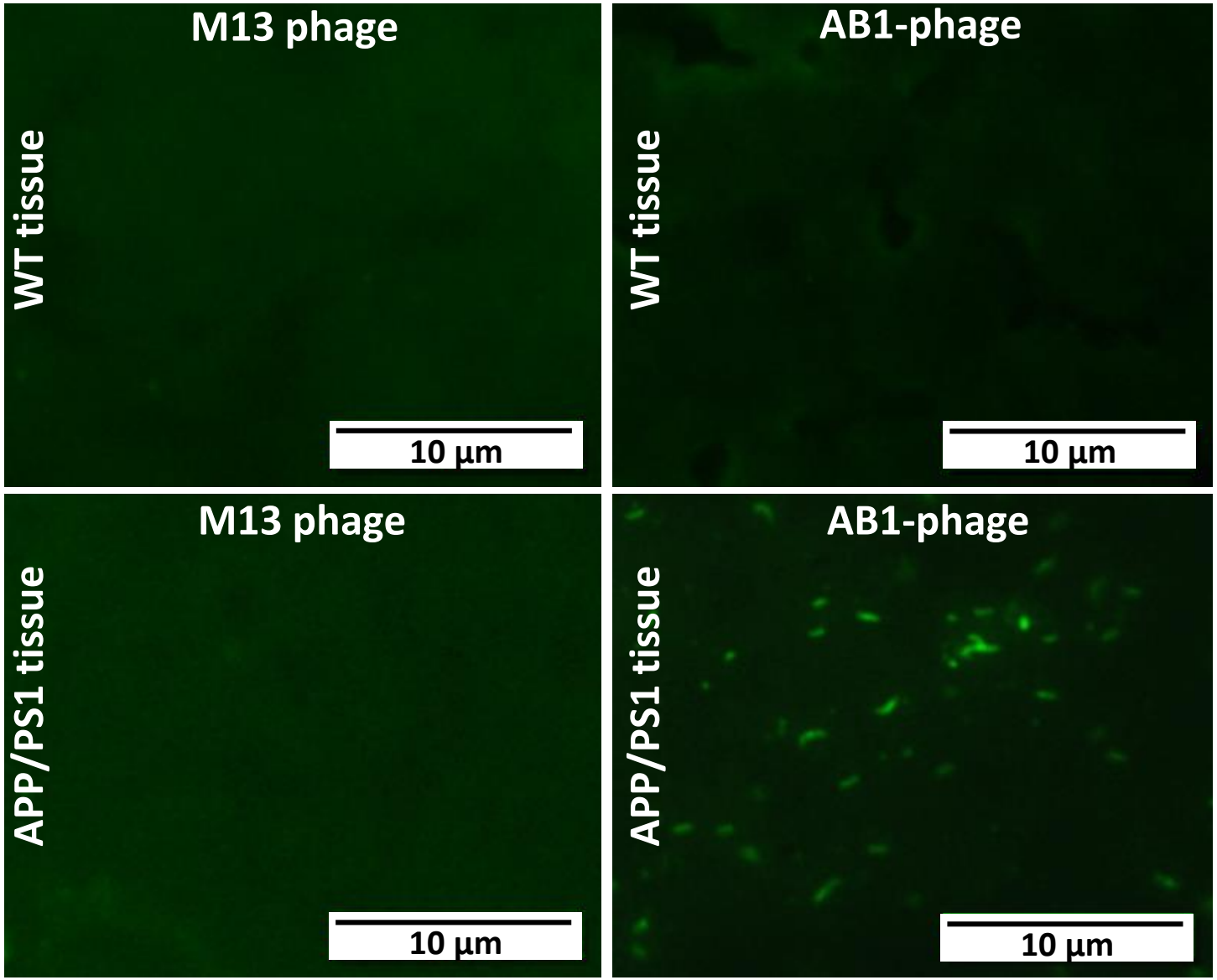


Hippocampal AD-brain tissue
APPswe/PS1dE9

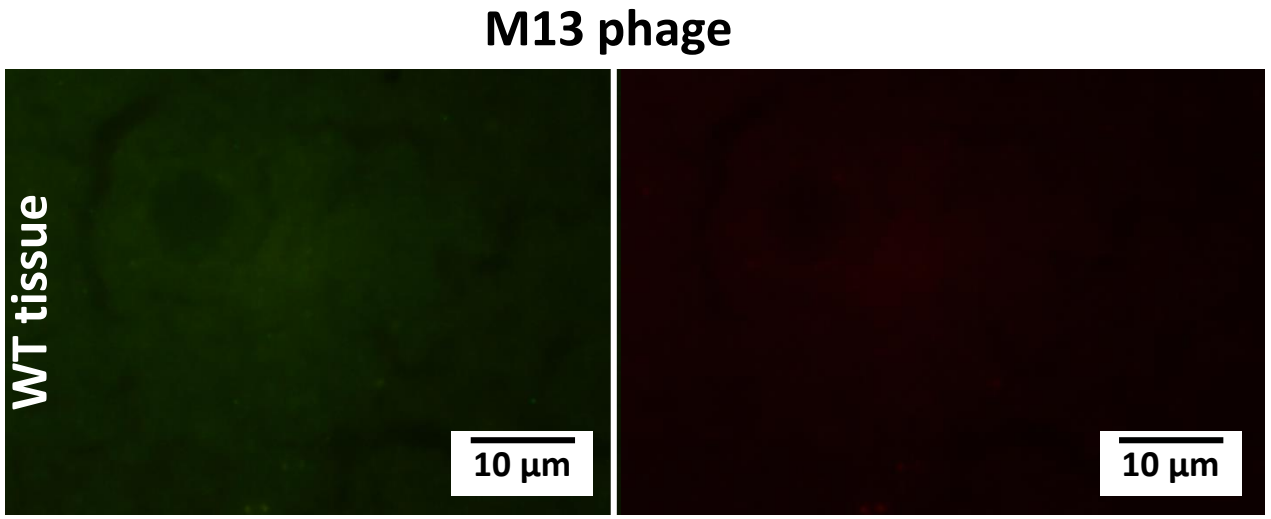
AB-specific phage

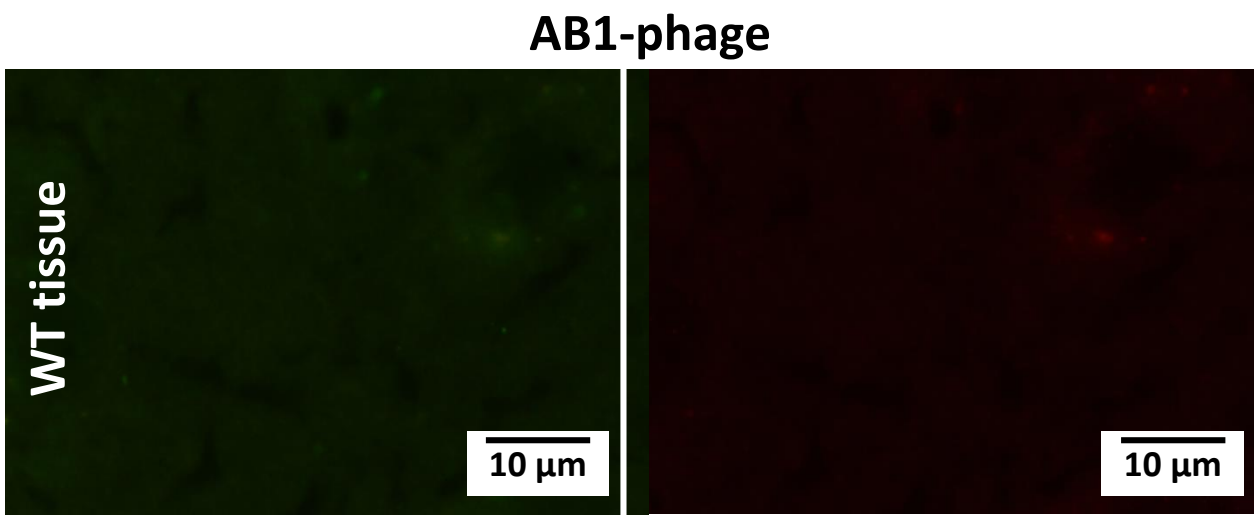
Anti-M13 primary antibody

FITC-labeled secondary antibody

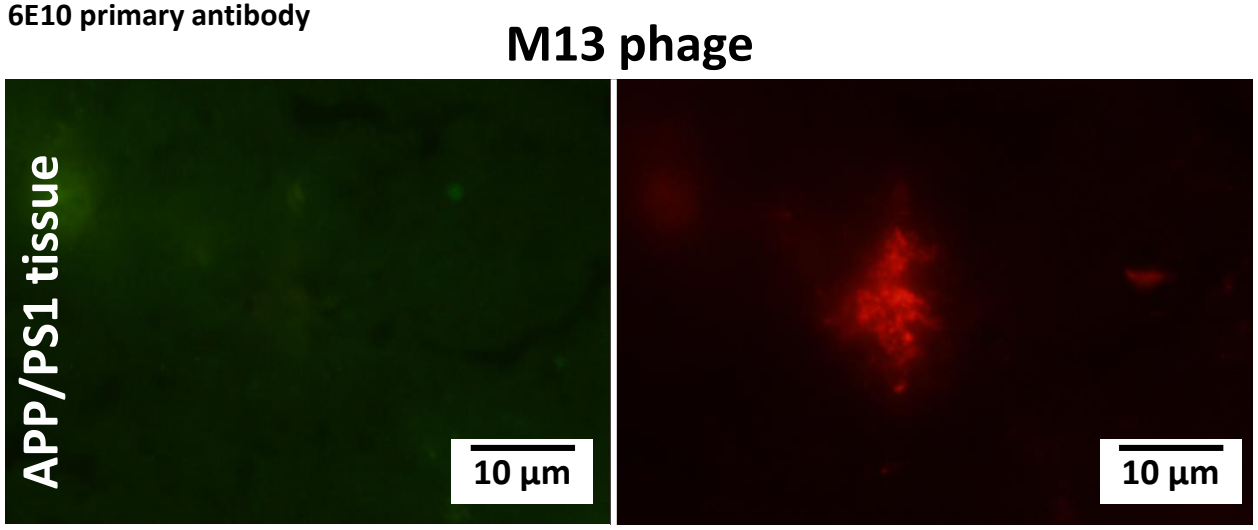


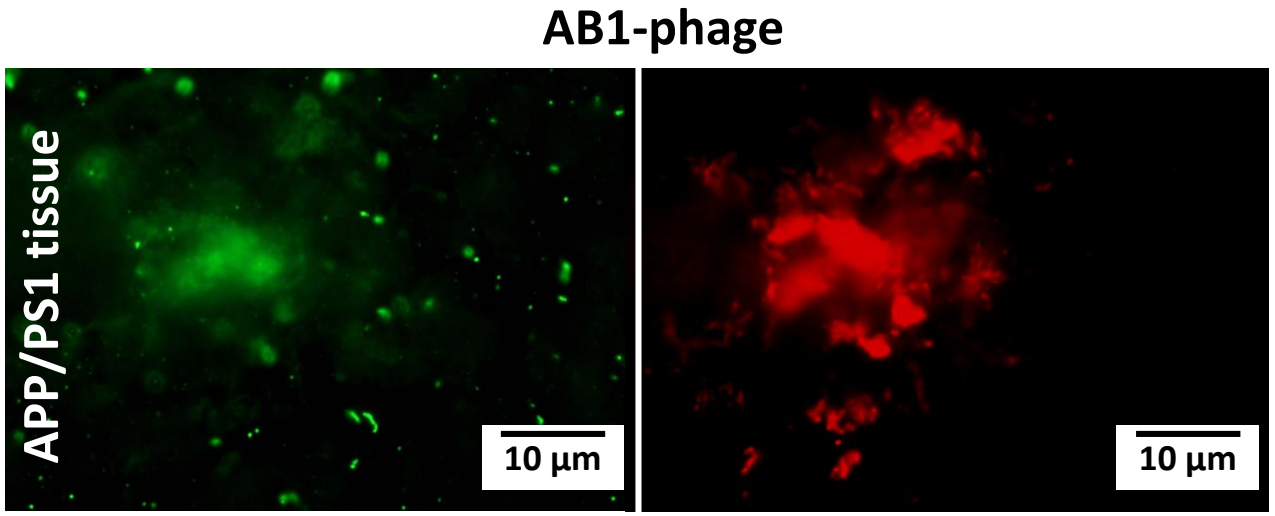
AB1-specific phage is able to recognize and bind to AD-tissue





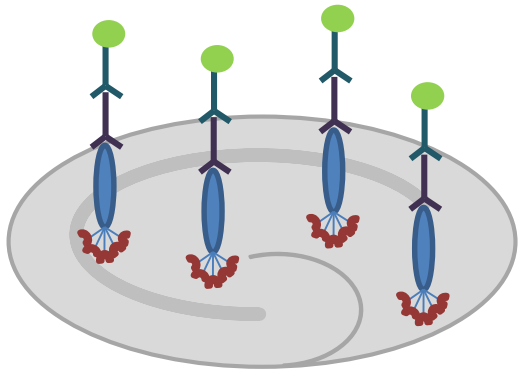
Anti-M13 primary antibody
6E10 primary antibody



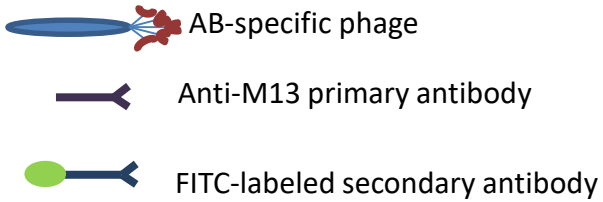


Ongoing work

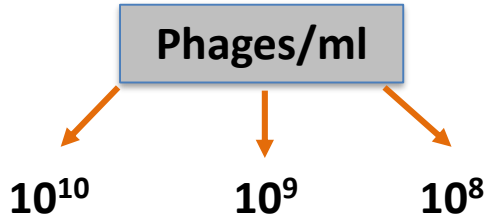
AB detection *ex vivo* on AD-tissue (mouse and human)



Cortex AD-brain tissue



AB-phages and AB interaction and modulation



Thioflavin T assay

AB fibrils disagregation assays

FTIR

Dot blot

AFM

Assessment of M13 passage across the BBB

HBMEC monolayer

Two chambers system

Transport studies

Evaluation of HBMEC line integrity

Cellular uptake studies

Future work

Optimal dose/time-point assessment

Phage administration; several time points and dose

AD-model

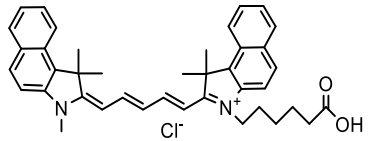
Blood samples

Brain tissue

Determine phage titer

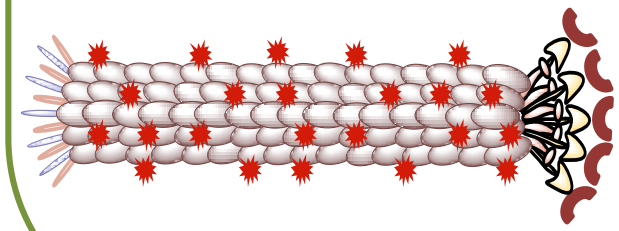
Brain slices for immunohistochemistry

AB-phages p8 chemical functionalization



Cy5.5

EDC/NHS chemistry



AB-phages tracking/biodistribution

NIR-labeled AB-specific phages administration (optimal dose assessed previously)

Fluorescence Molecular Tomography (FMT) monitoring: AB-phages biodistribution

Sacrifice animals at the optimal time-points after phage administration

Test if the AB-specific phages can intervene in the AB-driven synaptic and memory deficits

Can we prevent AB-mediated loss of synapses?

Spine loss in the hippocampus.

Can we prevent AB-mediated memory impairment in live brain?

Memory loss – contextual fear conditioning.

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BioISI Biosystems and Integrative Sciences Institute


Alexandra Brito

LISBOA

FACULDADE DE FARMÁCIA

Engineering of specific bacteriophages for early diagnosis of Alzheimer's disease

THANK YOU FOR YOUR ATTENTION!

Ivone M. Martins

BioISI Research Seminar
Faculdade de Ciências – Universidade de Lisboa
June 2018

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University of Minho
School of Engineering

CENTRE OF BIOLOGICAL ENGINEERING