Caffè Scientifico, 8 Maggio 2019, Sala Cinese

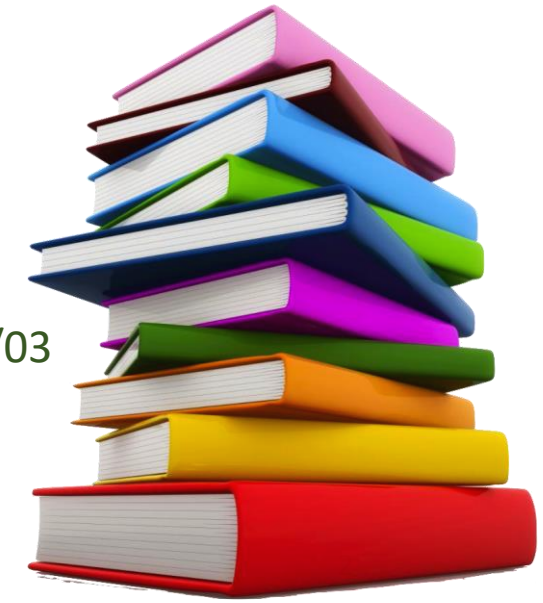
Peptidi: trasportatori, antibatterici ed antivirali

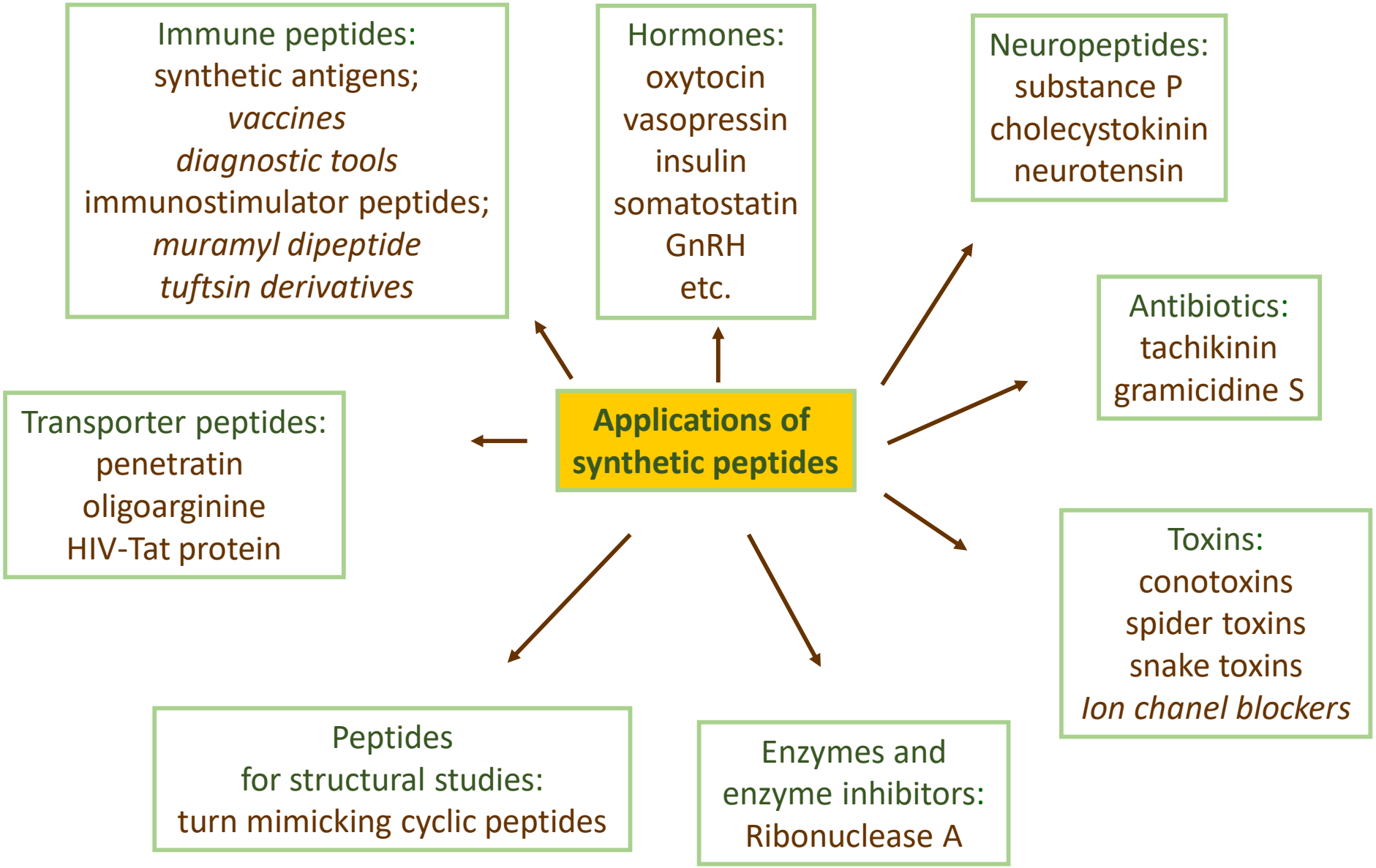
Dr. Annarita Falanga

Università degli Studi di Napoli Federico II

Education

- **2004** Degree in Scienze e Tecnologie Alimentari at Department of Agraria della Federico II
- **2008** PhD in Experimental and Clinical Microbiology, II University of Naples, Department of Experimental Medicine,Italy
- **2007-2008** Collaboration contract Centro Regionale di Competenza
- **2008-2010** Scholarship at consorzio interuniversitario di ricerca in chimica dei metalli nei sistemi biologici
- **2010** Collaboration contract at Centro Regionale di Competenza
- **2011-2012** Collaboration contract at Dip. of Scienze Biologiche dell'Università degli Studi di Napoli Federico II
- **2014·2020 National Scientific Habilitation 03/B1 CHIM03**
- **2015-2017** Research grant at Dip.of Pharmacia of Università degli Studi di Napoli Federico II
- **2017-2018** Teaching assignment in Chimica Generale ed Inorganica at Department of Agraria CHIM/03
- **2018** RTDb SSD CHIM-03 (chimica generale ed inorganica)




Applications of synthetic peptides
Immune peptides:
synthetic antigens;
vaccines
diagnostic tools
immunostimulator peptides;
muramyl dipeptide
tuftsin derivatives
Hormones:
oxytocin
vasopressin
insulin
somatostatin
GnRH
etc.
Neuropeptides:
substance P
cholecystokinin
neurotensin
Antibiotics:
tachikinin
gramicidine S
Toxins:
conotoxins
spider toxins
snake toxins
Ion chanel blockers
Enzymes and
enzyme inhibitors:
Ribonuclease A
Peptides
for structural studies:
turn mimicking cyclic peptides
Transporter peptides:
penetratin
oligoarginine
HIV-Tat protein

Why chemists are needed?

Gene expression is very popular, relatively easy and cheap method:
it is good for long linear peptides or proteins containing L-amino acids.

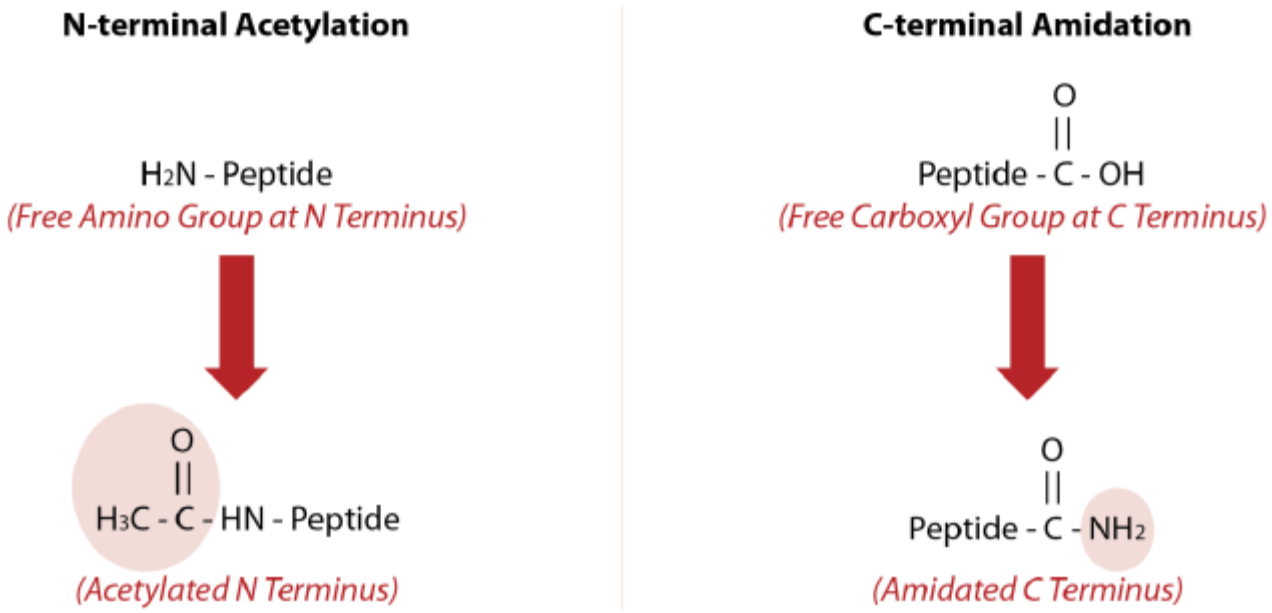
However:

- no D-amino acids
- no unnatural amino acids
- no post translation (Hyp, Pyr, glyco- and phosphopeptides)
- no branches
- no cyclic peptides
- no fluorescent or isotop labeling

***Peptides as drugs*: there are not too many, because of the price and their fast biodegradation**.

"Peptides have and will continue to be important sources of lead compounds in many drug discovery programs. However, due to their generally poor pharmacokinetic properties and hydrolytic instability, natural peptide structures are usually substituted with mimics of the actual peptide constuction."

N-terminal Acetylation and C-terminal amidation



Chemically synthetized peptides may have free amino and carboxyl termini.

N-terminal acetylation and C-terminal amidation reduce overall charge of the peptide. This can increase the stability of the peptide and its resemblance to the native protein.

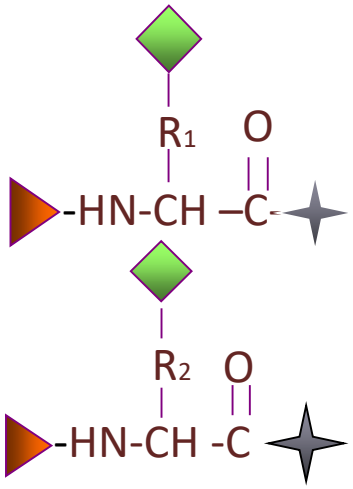
TFA

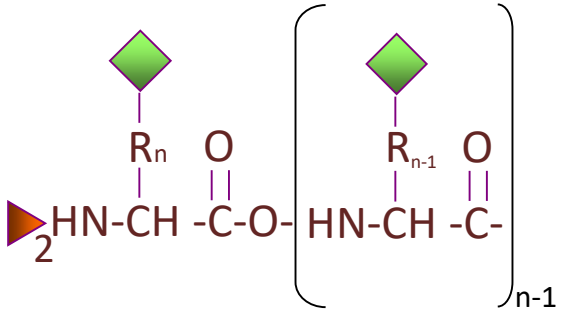
SOLID PHASE PEPTIDE SYNTHESIS

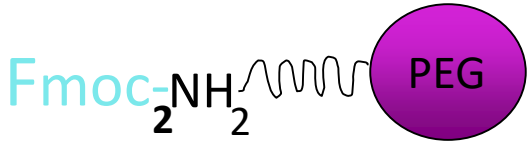
Bruce Merrifield published in 1963

Nobel Prize in Chemistry in 1984

The idea:







Gruppo attivante funzione COOH.

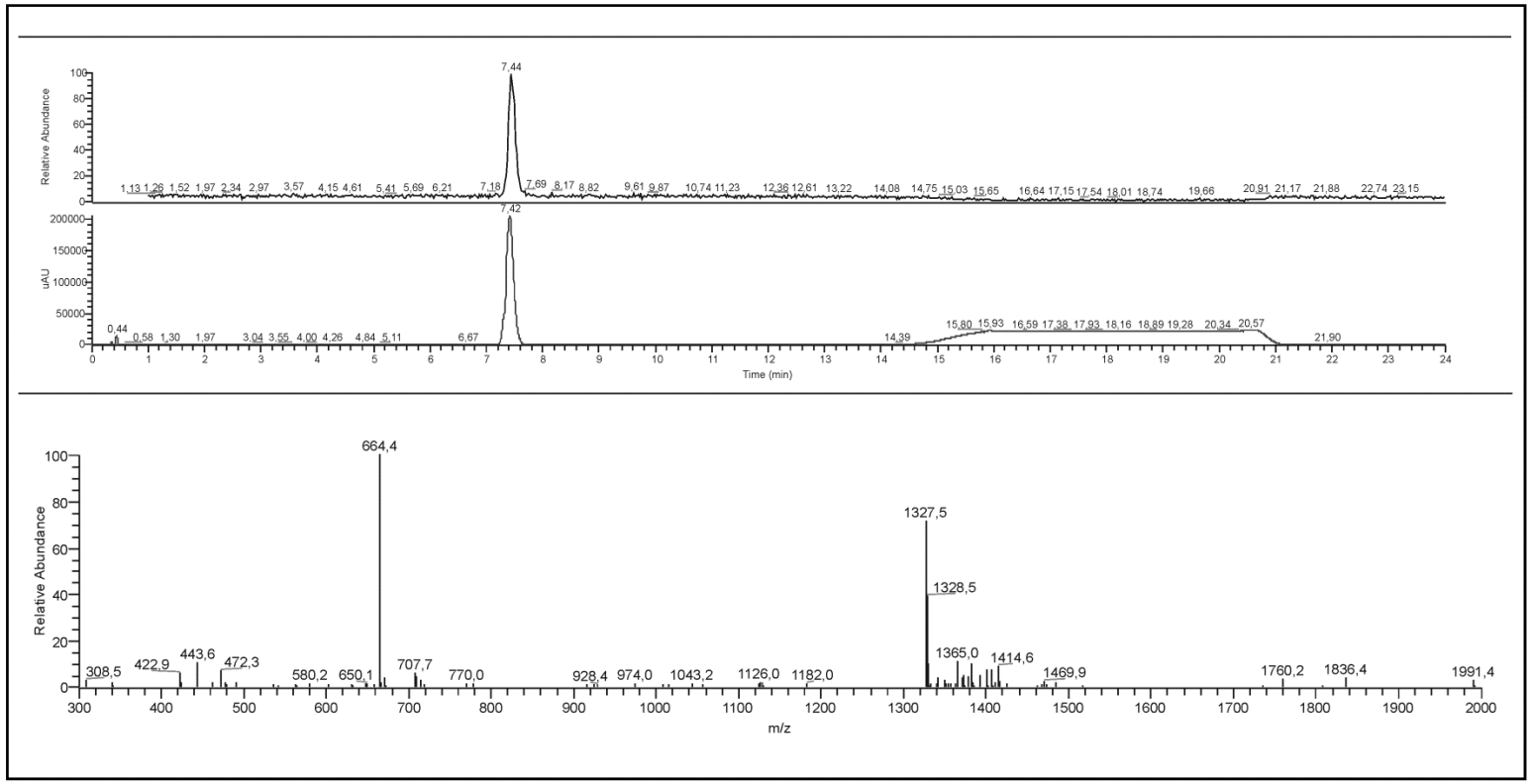
Gruppo protettore funzione NH_2

Gruppo protettore catena laterale R.

Supporto polimerico.

Purification and Characterization

- HPLC, Mass Spectroscopy



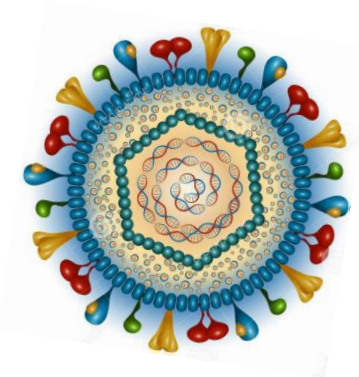
- Circular Dichroism, Fluorescence,UV, Electrophoresis, Surface Plasmon Resonance

What did I do?

Membrane interaction studies of Nanosystems and Peptides

Enveloped virus: HSV-1

- Chemical characterization of the mechanism of membrane fusion
- Rational design of inhibitors targeting viral entry



Cell Penetrating Peptides (CPPs)

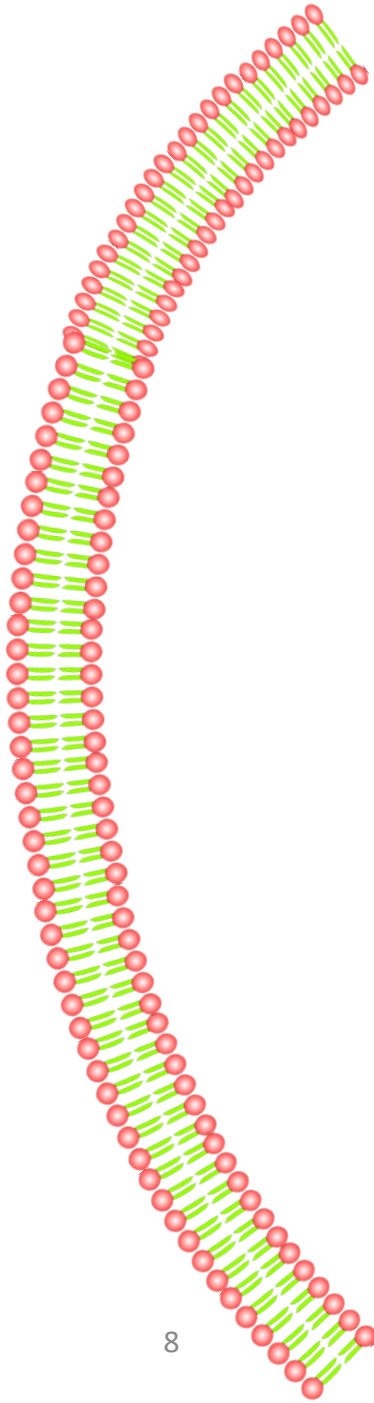
- Chemical characterization of the mechanism of membrane permeabilization by membranotropic peptides
- Applications to the delivery of nanoparticles across the Blood Brain Barrier
- Applications to the delivery of nanosystems

C P P

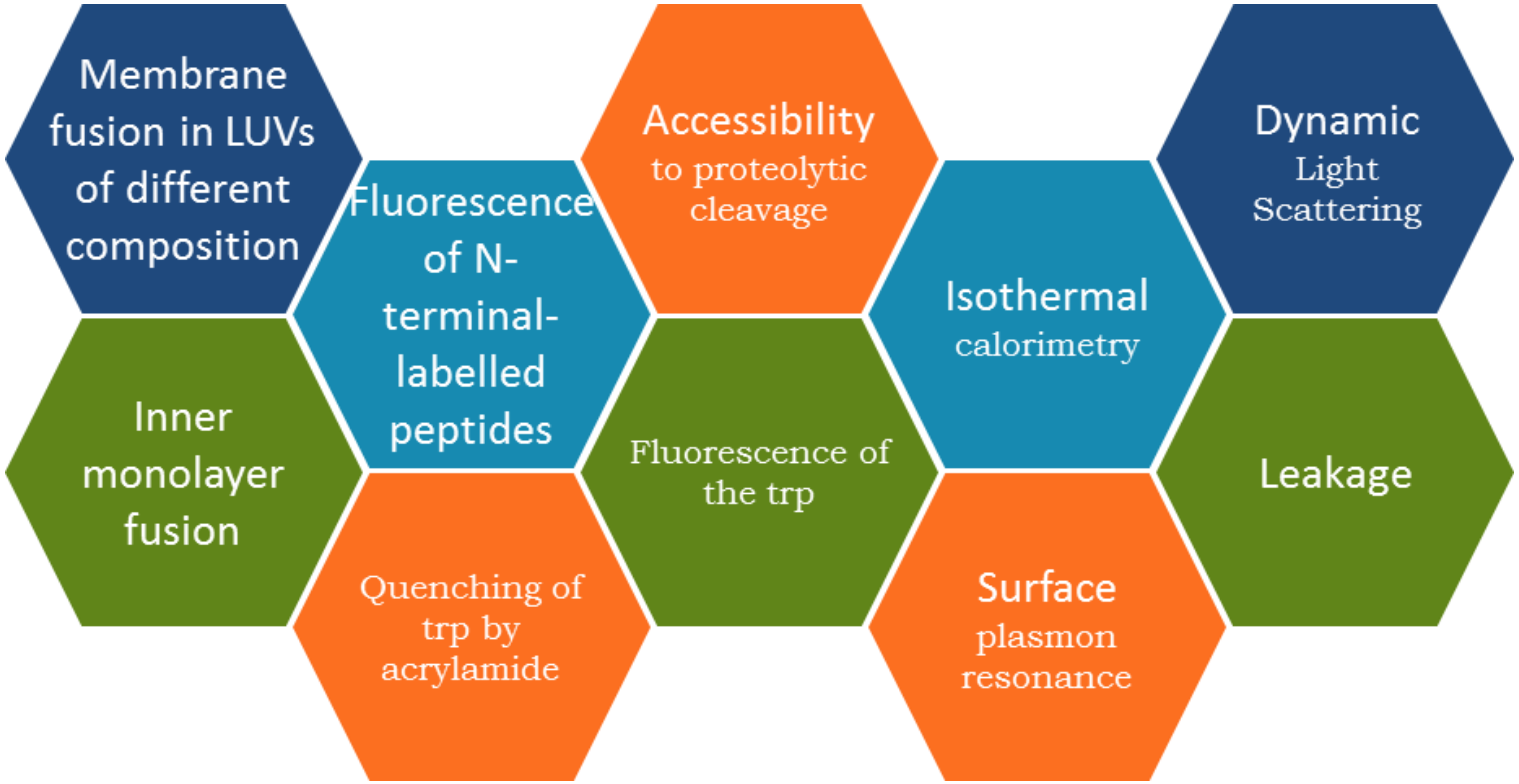
Antimicrobial Peptides (AMPs)

- Chemical characterization of the mechanism of membrane permeabilization
- Rational design of novel antimicrobial peptides

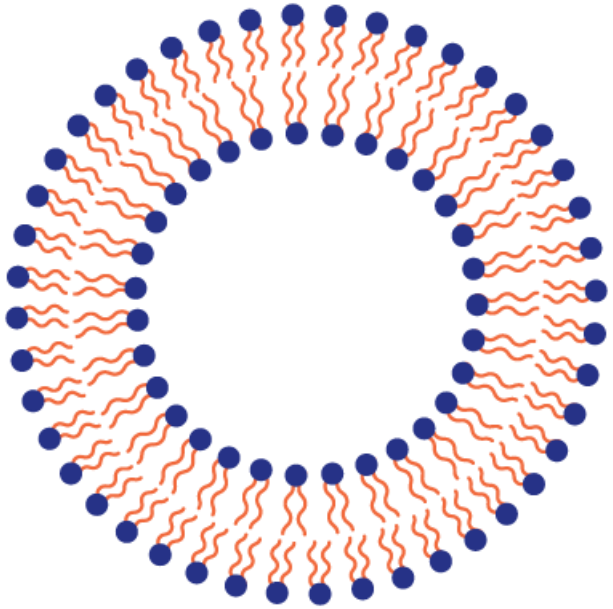
A M P



How did I study the membrane interactions?



Model system: liposomes



Galdiero et al. J. Biol. Chem. 2008;
Falanga et al Scientific Report 2017;
Falanga et al. Scientific Report 2018

Section 1

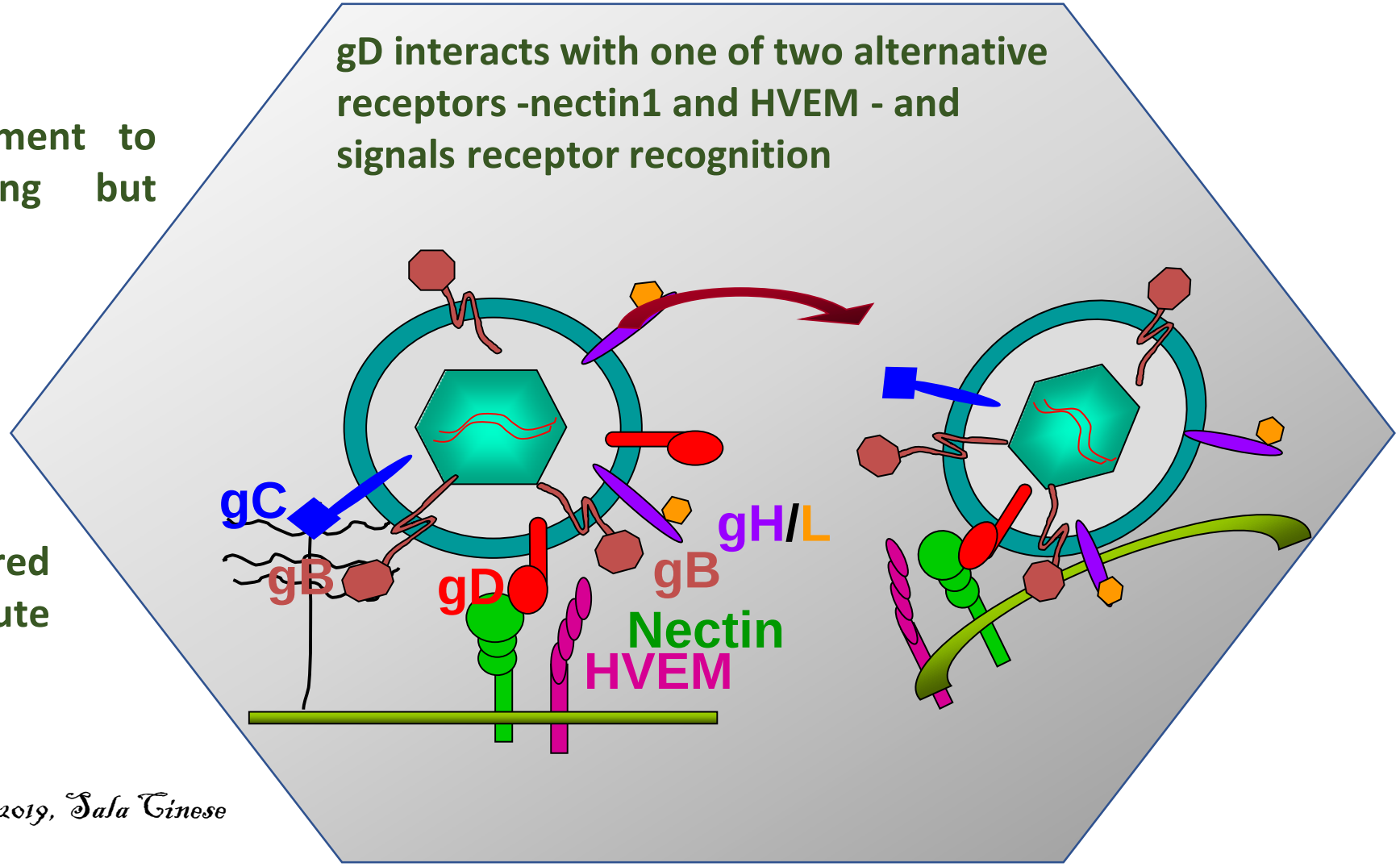
Enveloped virus: HSV-1

- Chemical characterization of the mechanism of membrane fusion
- Rational design of inhibitors targeting viral entry

Herpes simplex virus type I: a prototype virus with multipartite fusion machinery

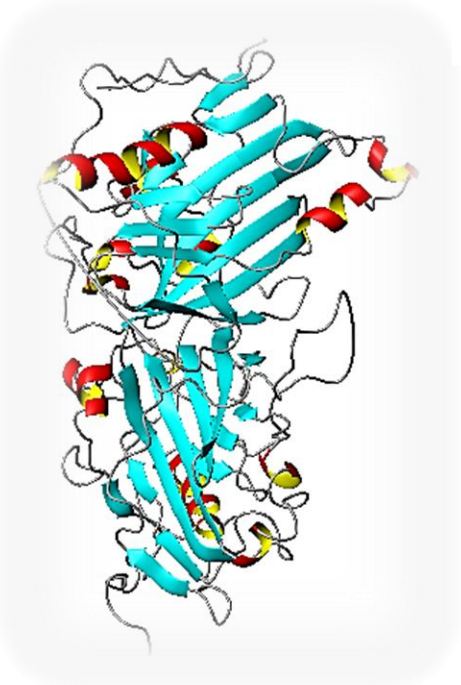
gC and gB mediate attachment to heparan sulphate enhancing but dispensable step

gD interacts with one of two alternative receptors -nectin1 and HVEM - and signals receptor recognition



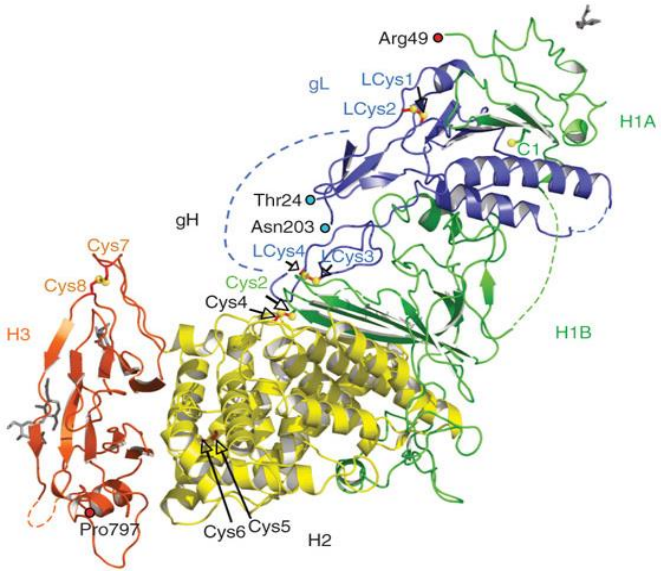
gB, gH, gL are required downstream of gD to execute fusion

Three-dimensional structures of essential glycoproteins



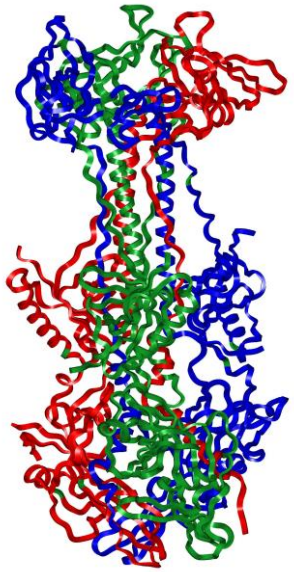
gD

Krummenacher et al.
The EMBO Journal, 2005



gH/gL

Tirumala K Chowdary et al.
Nature structural and molecular biology, 2010



gB

Heldwein et al. Science, 2006

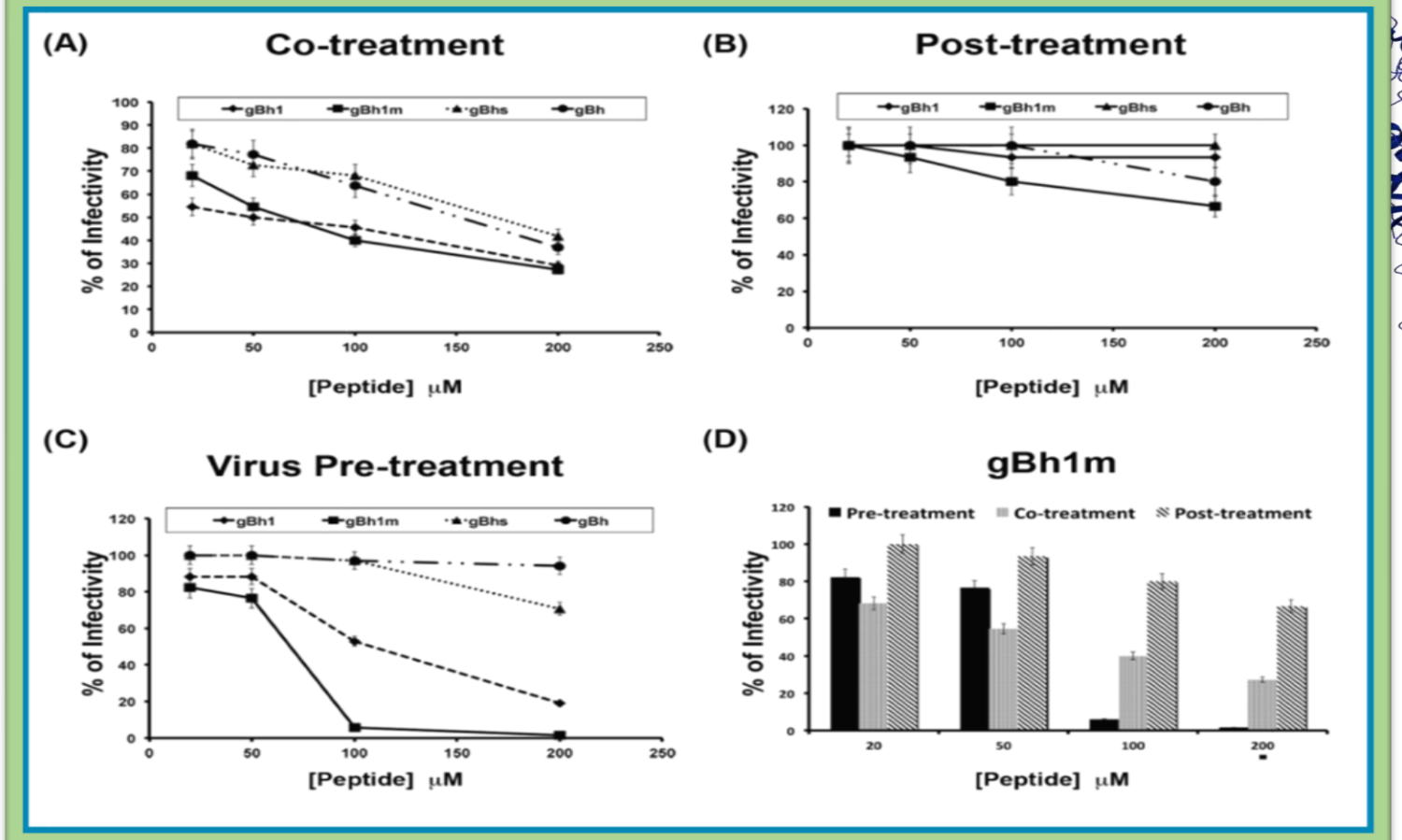
Journal of Medicinal Chemistry
Article
pubs.acs.org/jmc

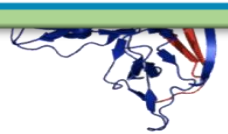
Conformational Modifications of gB from Herpes Simplex Virus Type 1 Analyzed by Synthetic Peptides

Marco Cantisani,[†,‡,∥,▽] Annarita Falanga,[†,§,▽] Novella Incoronato,[⊥] Luigi Russo,[⊥] Alfonso De Simone,[#] Giancarlo Morelli,[†,‡,□] Rita Berisio,[□] Massimiliano Galdiero,[*,‡,⊥] and Stefania Galdiero[*,†,‡,§,□]

International Journal of Nanomedicine
Dovepress
open access to scientific and medical research
Open Access Full Text Article
ORIGINAL RESEARCH

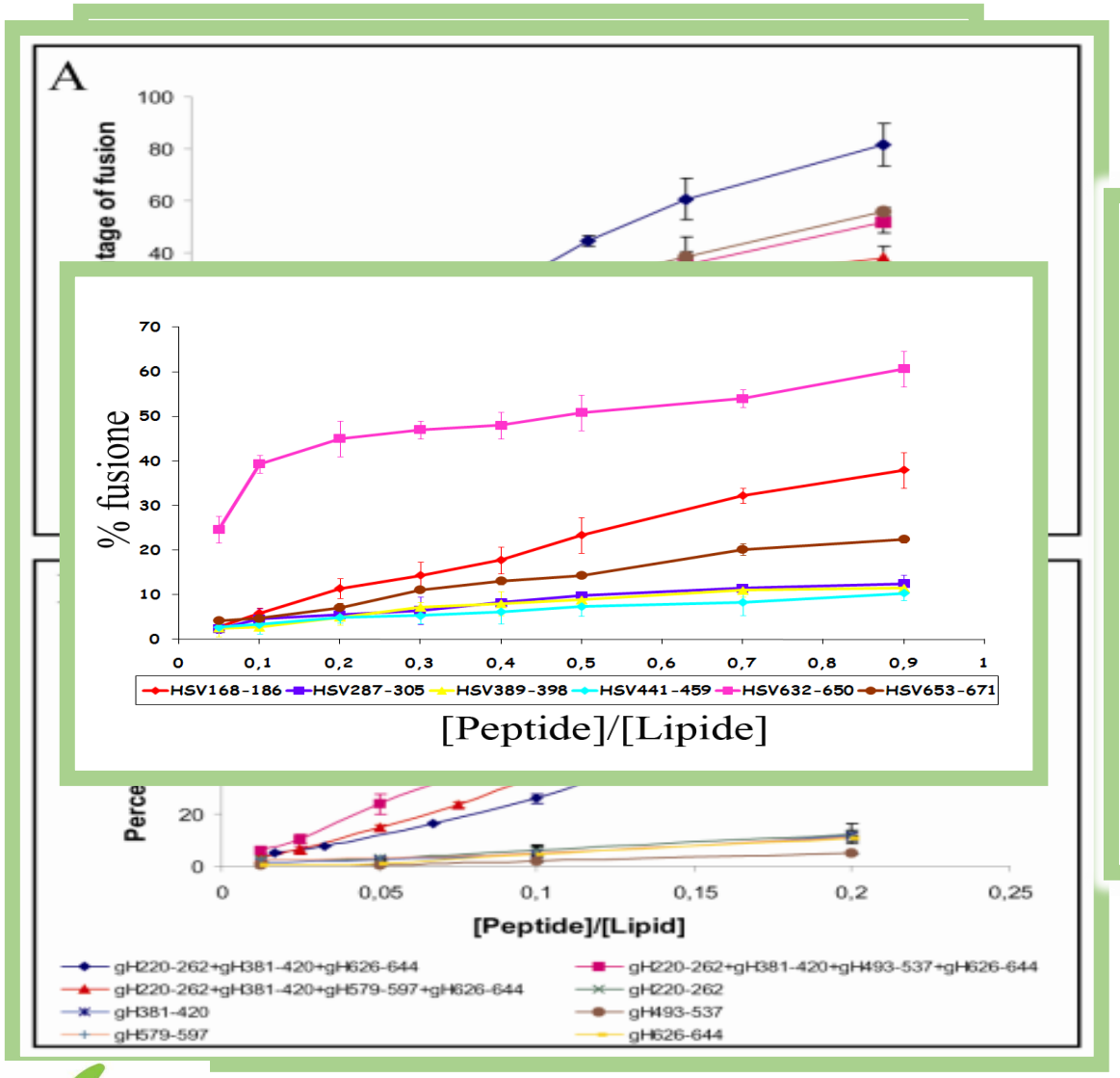
Dendrimers functionalized with membrane-interacting peptides for viral inhibition

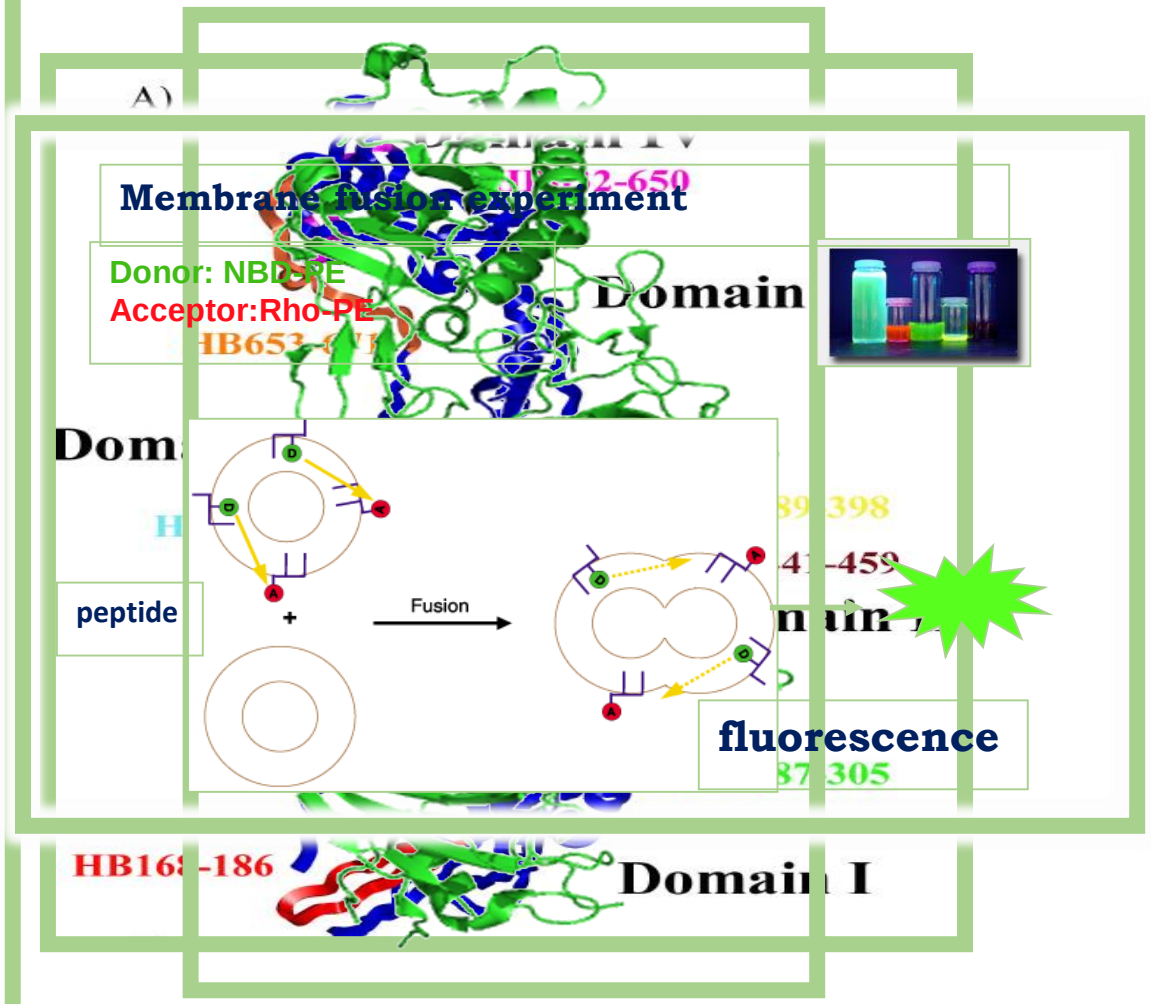




Caffè Scientifico, 8 Maggio 2019, Sala Cinese
Dr Annarita Falanga

Structural characterization of glycoproteins gH and gB

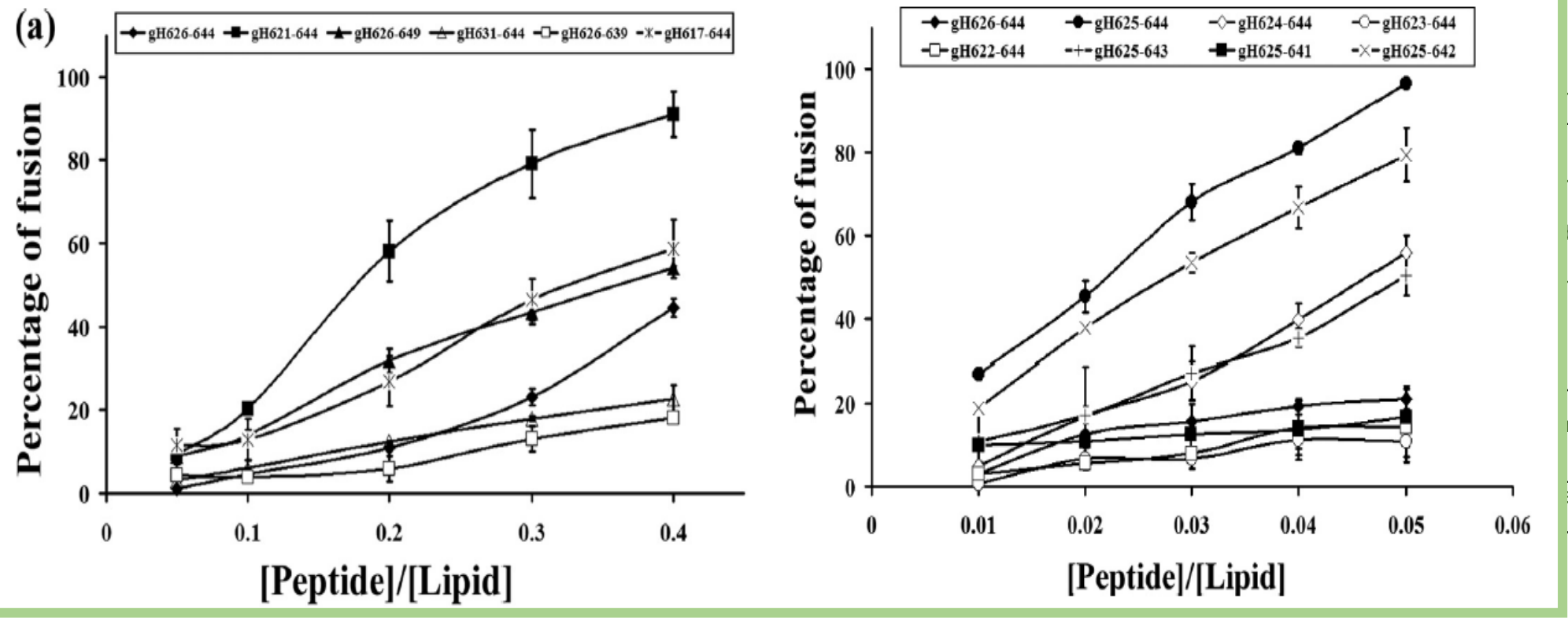




Galdiero et al. J. Biol. Chem. 2005;
Galdiero et al. ChemBioChem. 2007, 2008;
Falanga et al. Plos One 2012

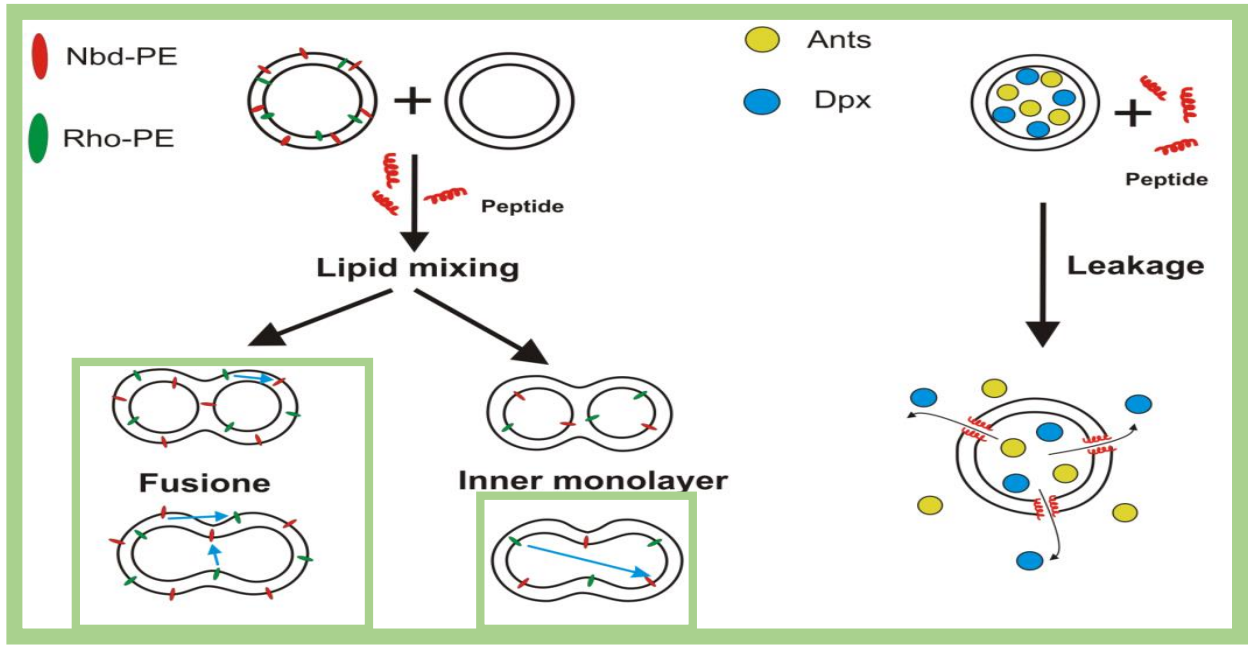
Determination of the sequence with the highest fusion activity: gH625

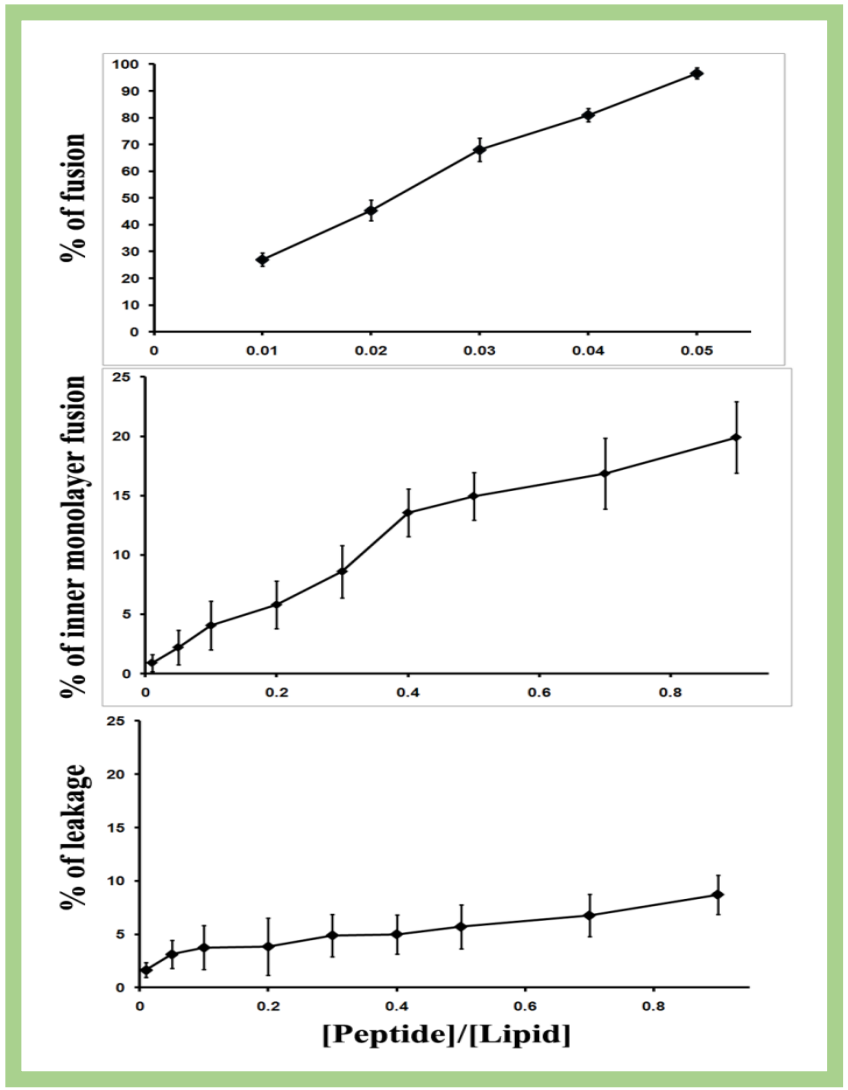
Fusion experiment performed at 37° C



Galdiero et al. J. Biol. Chem. 2008;
Galdiero et al. J. Biol. Chem. 2010
Falanga et al. J. Plose one. 2012;

Fluorescence assays

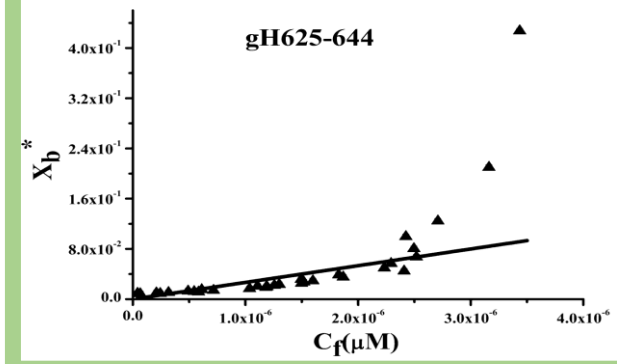




Mechanism of membrane interaction

- The peptide gH625 is able to penetrate inside liposomes made of PC/Chol
- The binding constant is approximatively 10^4

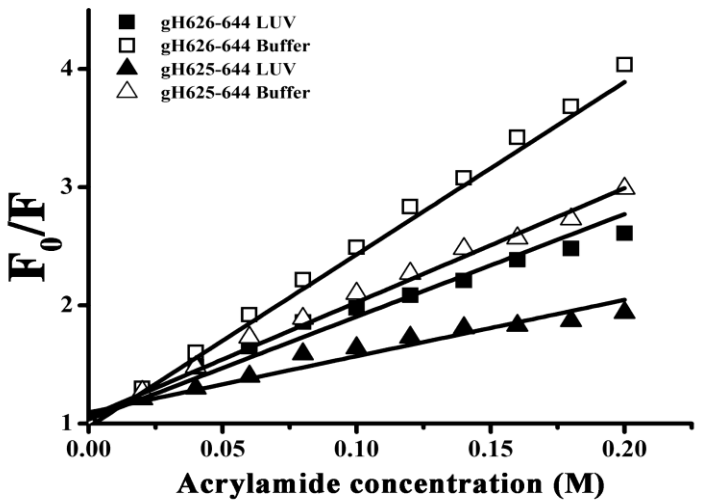
Binding curves



Partition coefficient for the binding of gH625 with PC/Chol

	gH625
Kp	$(2.7 \pm 0.2)10^4$

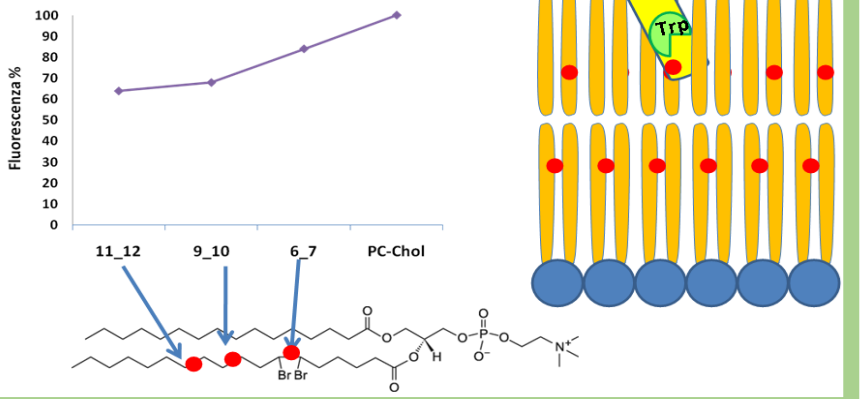
Quenching of tryptophan by acrilamide



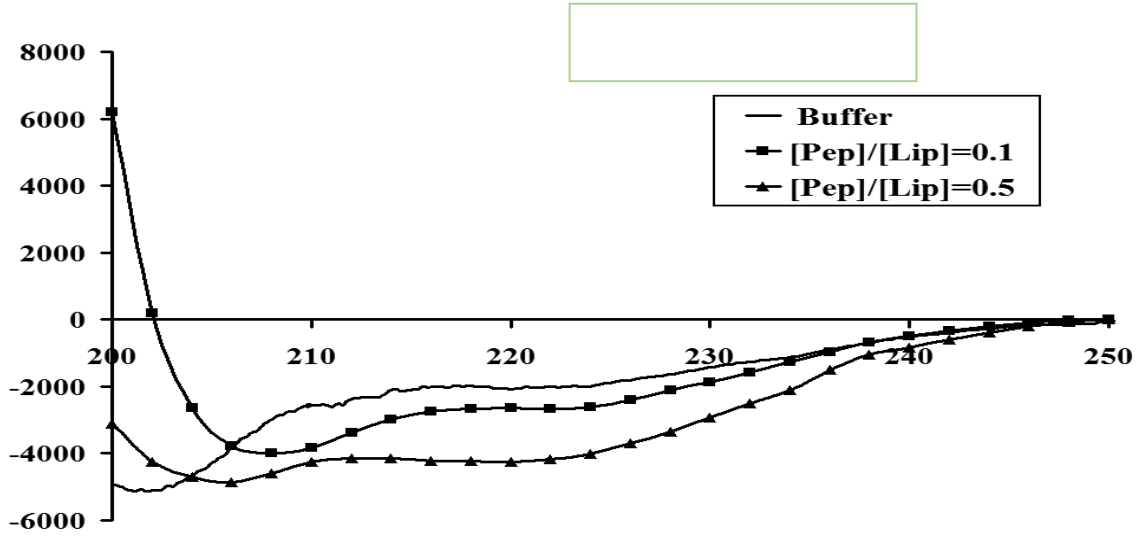
Galdiero et al. J. Biol. Chem. 2008; 2010
Falanga et al Scientific Report 2018

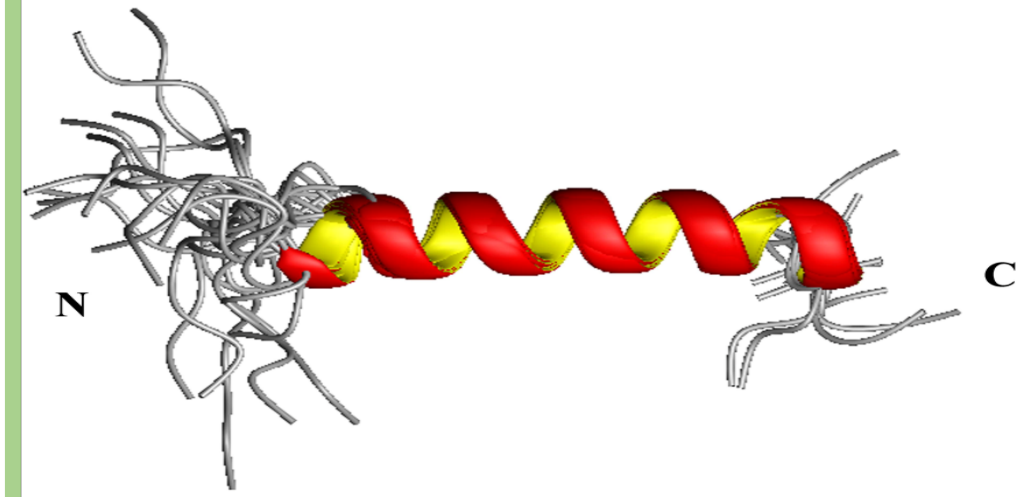
Mechanism of membrane interaction

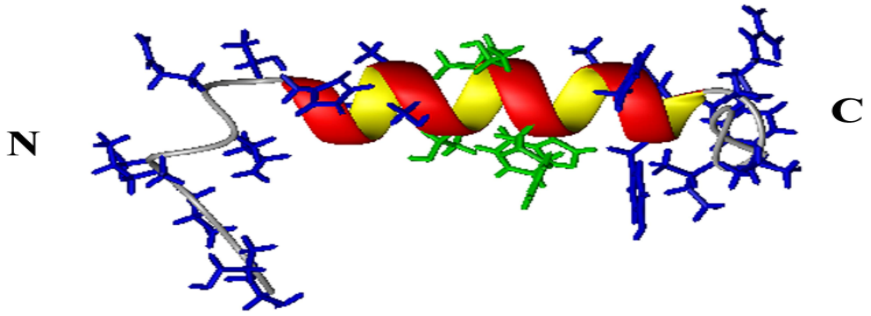
Fluorescence %



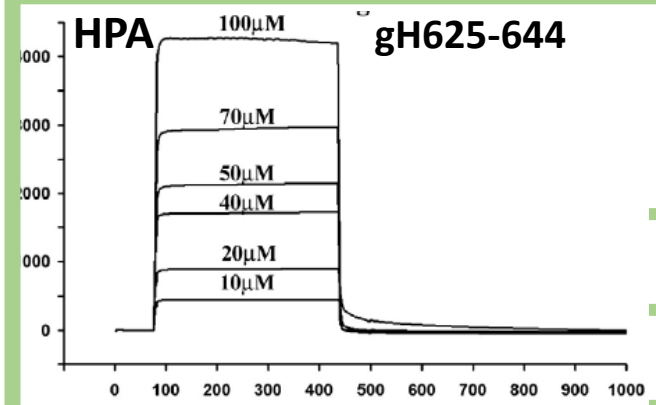
CD Spectra

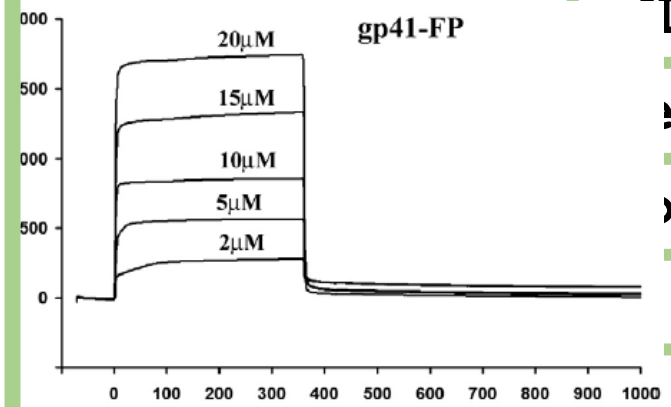


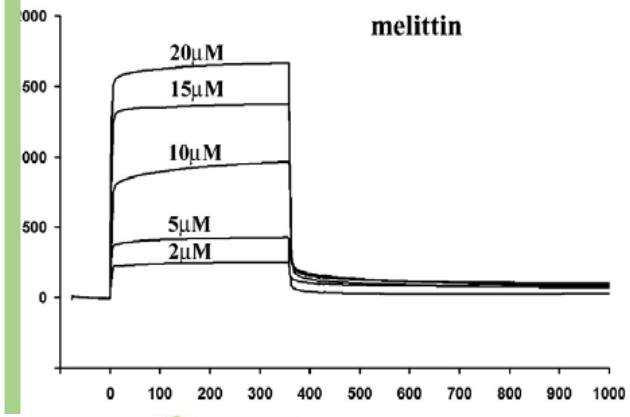




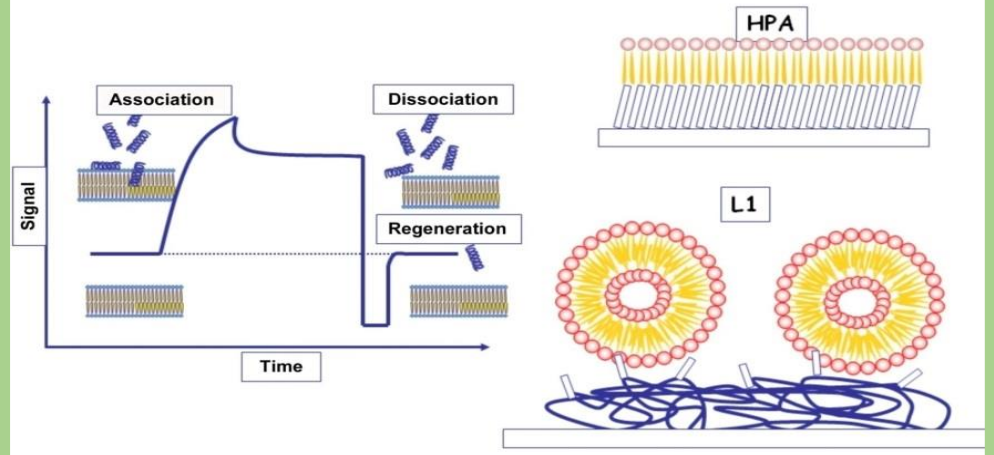
Surface Plasmon Resonance

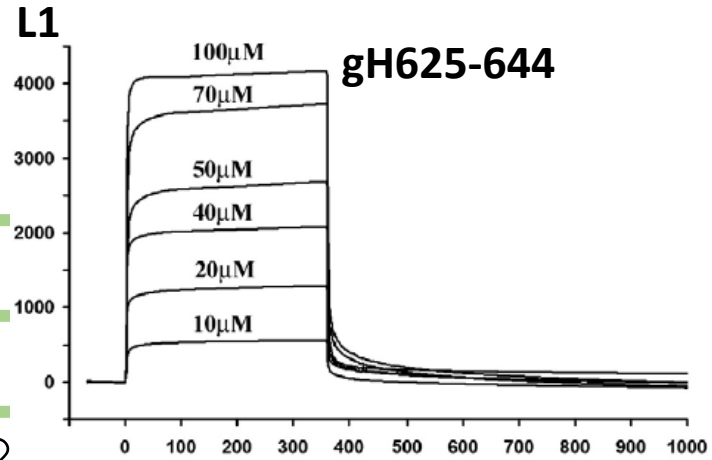


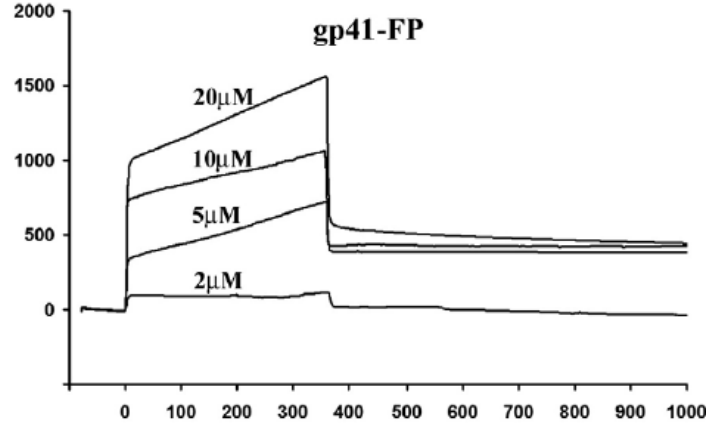


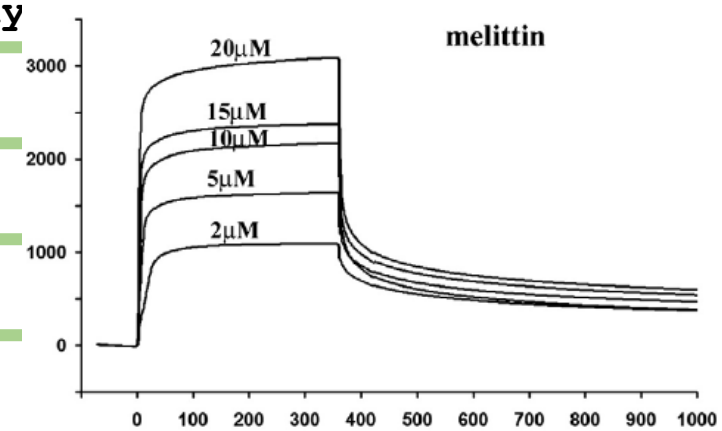


	HPA	L1
625	$1.00\ 10^5$	7.
elit		
41-		
625		
littin	36	
41-FP	0.8	







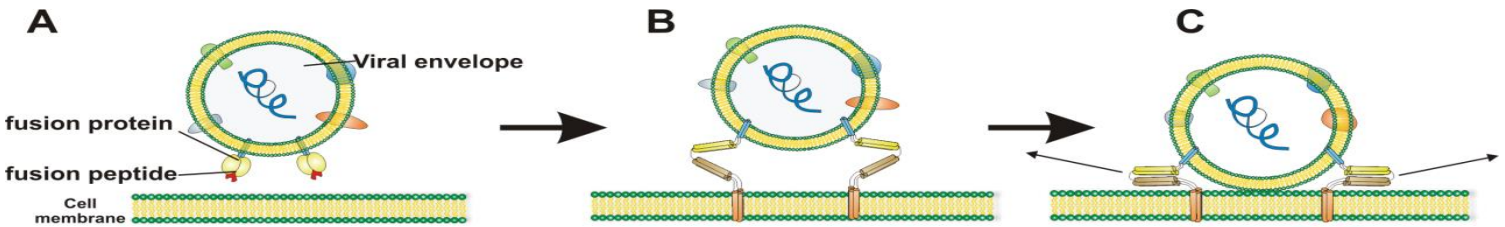


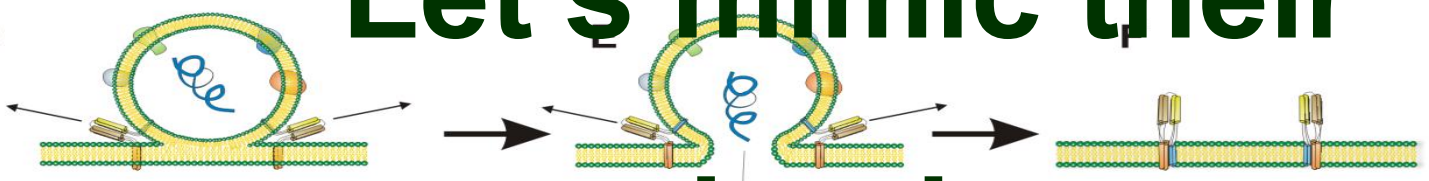
Galdiero et al. J. Biol. Chem. 2008;
Galdiero et al. BBA 2010

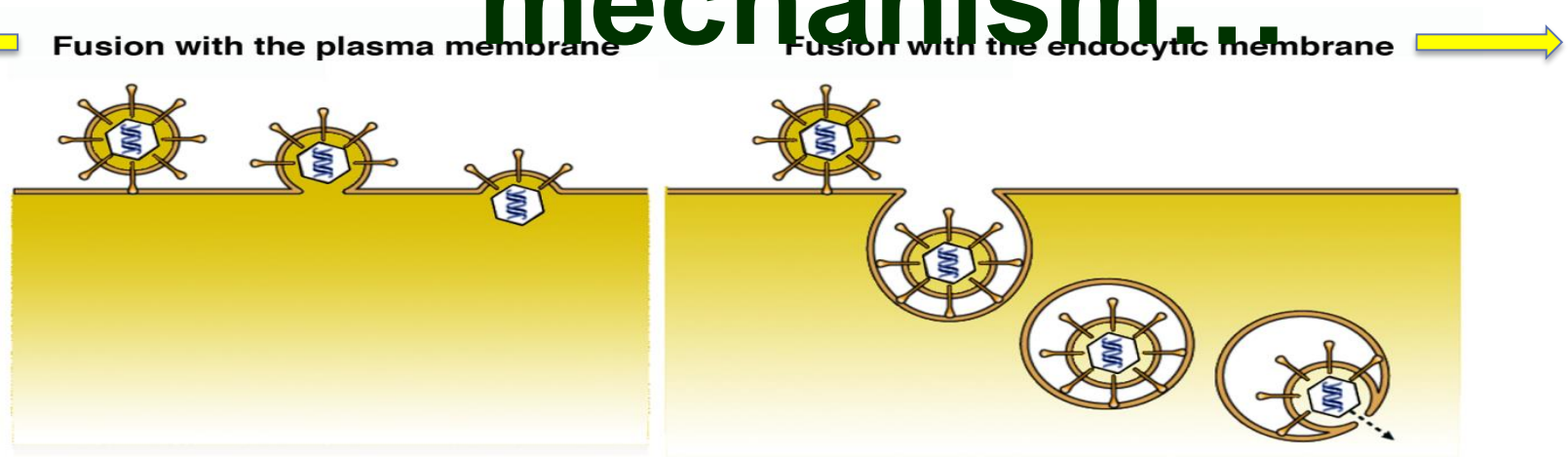
Results

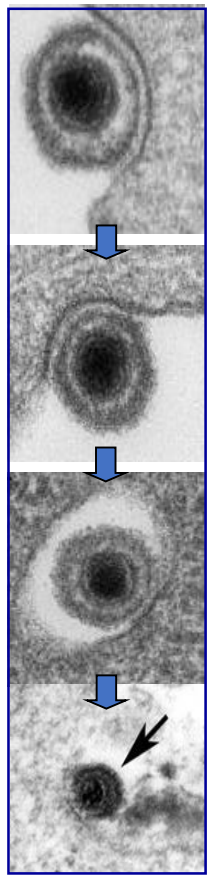
- N-terminal insertion
- Deeply inserted inside the bilayer
- No pores
- Oblique orientation
- No leakage
- Binding constants

Let’s mimic their mechanism…







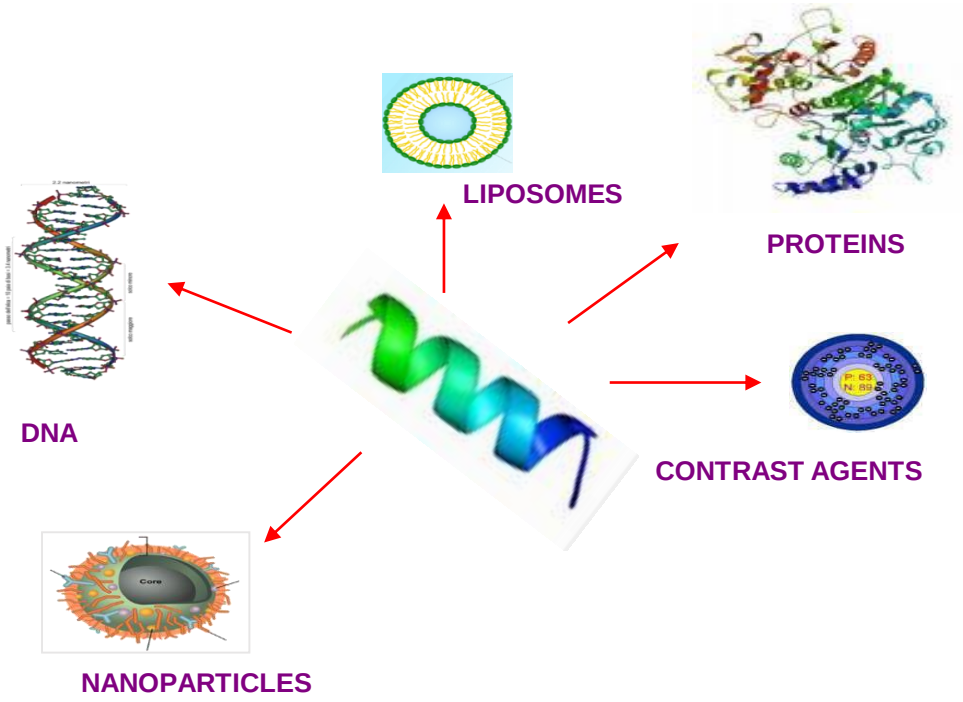


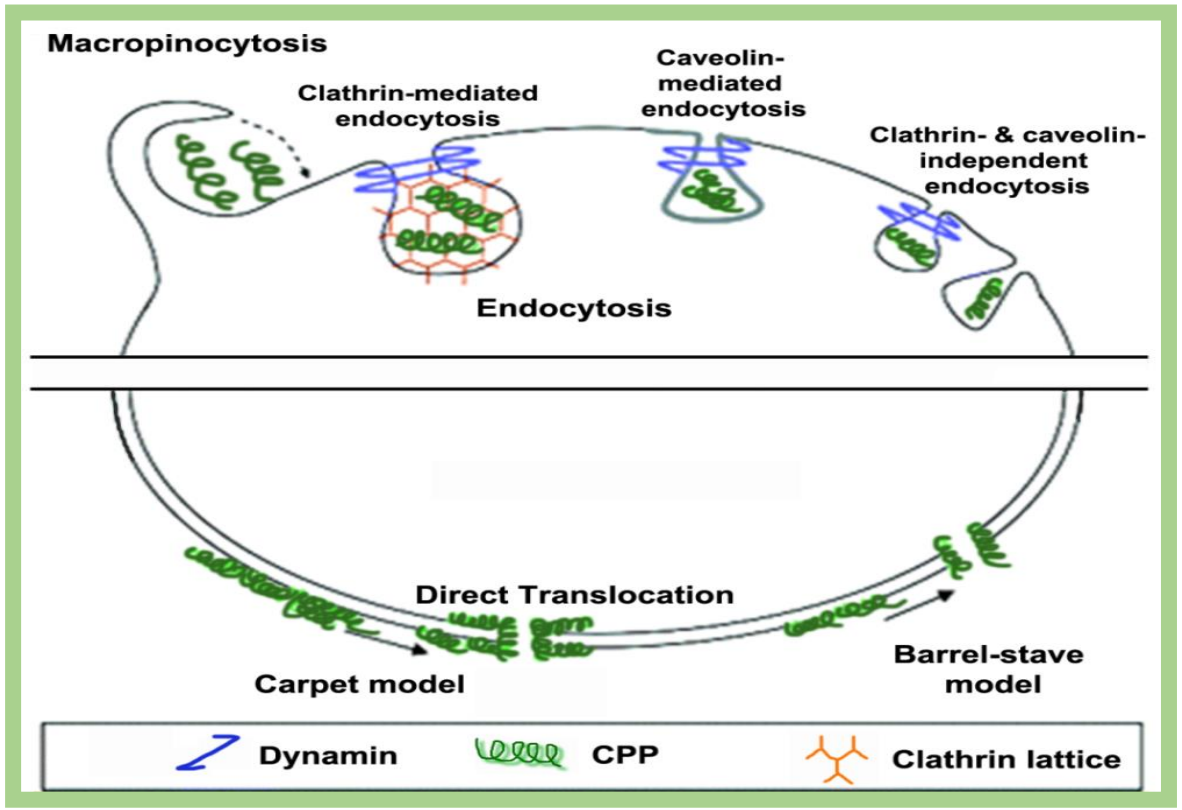
Section 2

Cell Penetrating Peptides (CPPs)

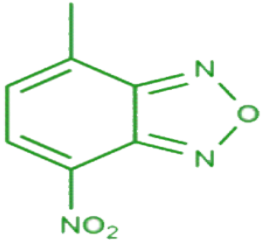
- Chemical characterization of the mechanism of membrane permeabilization by membranotropic peptides
- Applications to the delivery of nanosystems
- Applications to the delivery of nanoparticles across the Blood Brain Barrier

Cell Penetrating Peptides (CPPs)

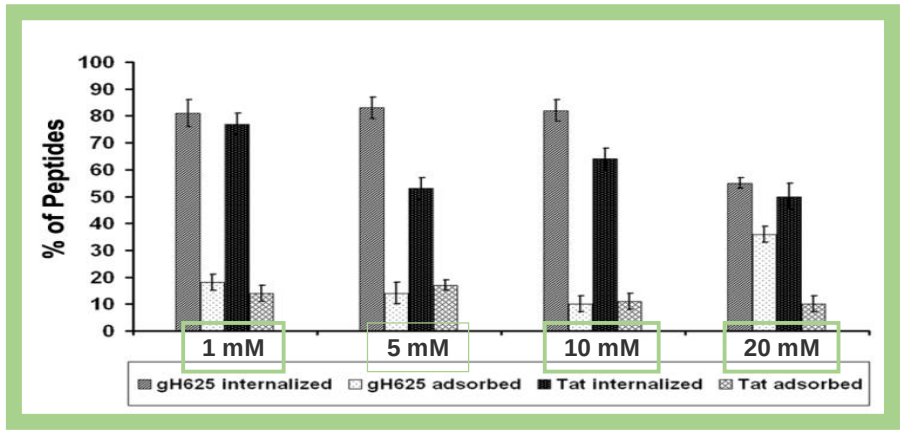




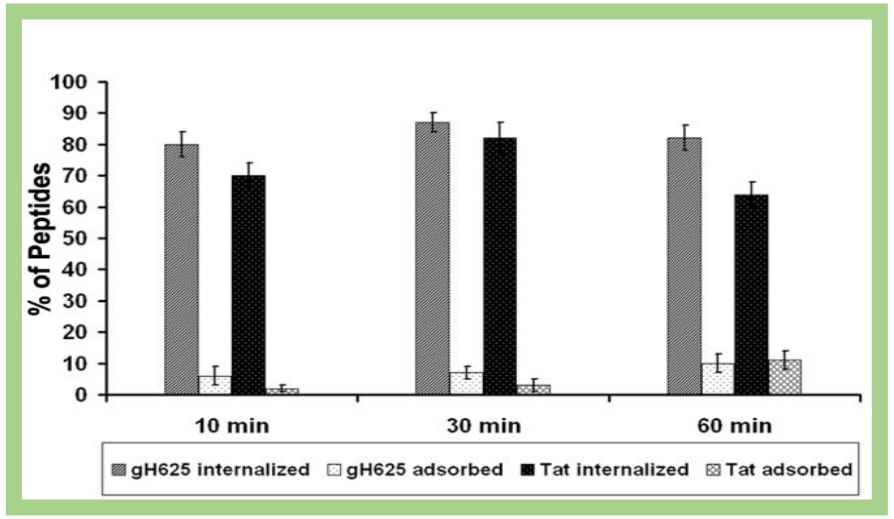
- CPPs are among the most promising strategies
- They are short peptides of less than 30 aminoacids either derived from protein domains or from synthetic sequences
- They are usually amphipathic and have a net positive charge
- Tat 48-60 Hiv-1 Transactivator (Tat) GRKKRRQRRRPPQ

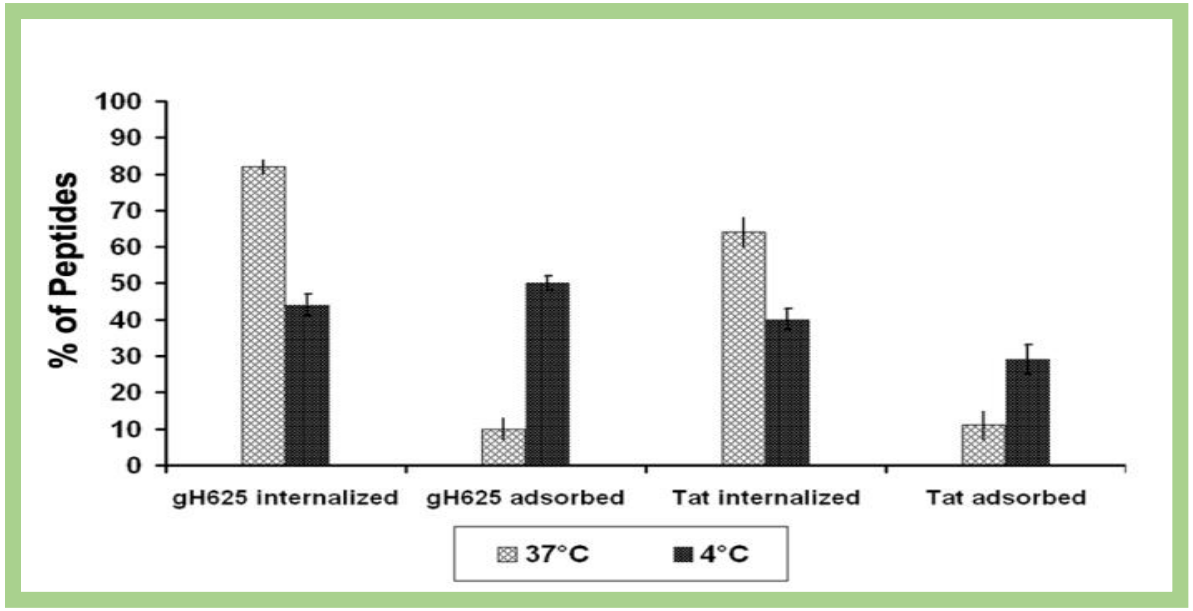


Determination of NBD-gH625 cellular uptake



Internalized peptides quantification is determined by comparing the fluorescence intensity before and after addition of sodium dithionite on HeLa cells

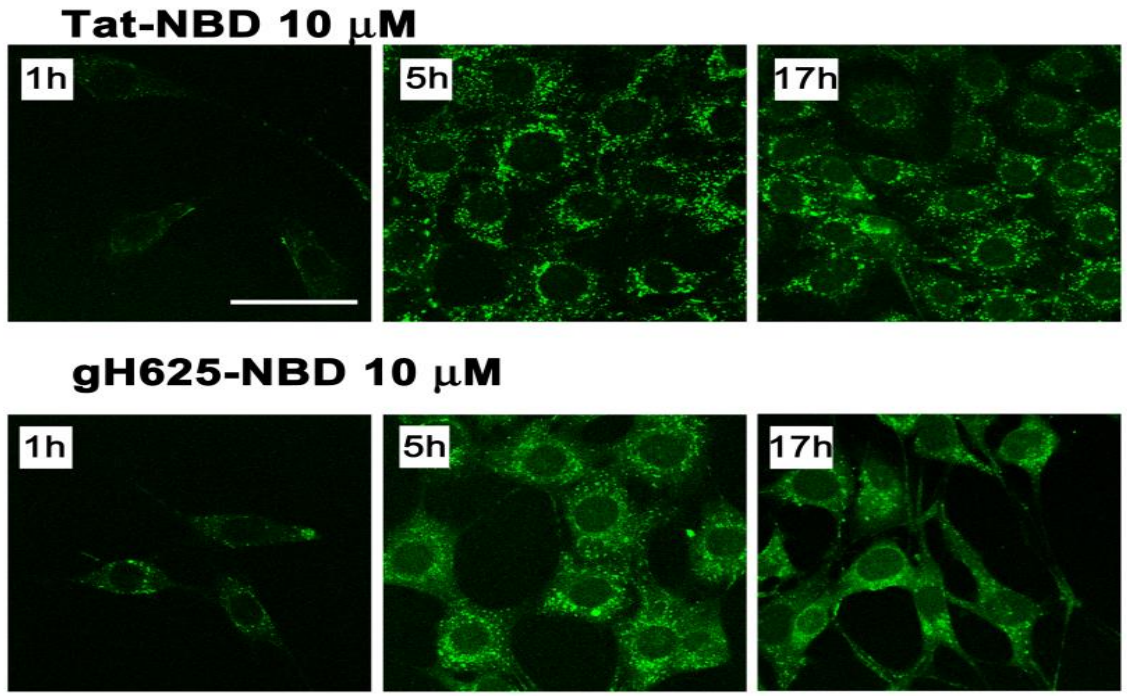




Falanga et al. Nanomedicine 2011

Confocal microscopy studies on live cells

Intracellular uptake on live cells

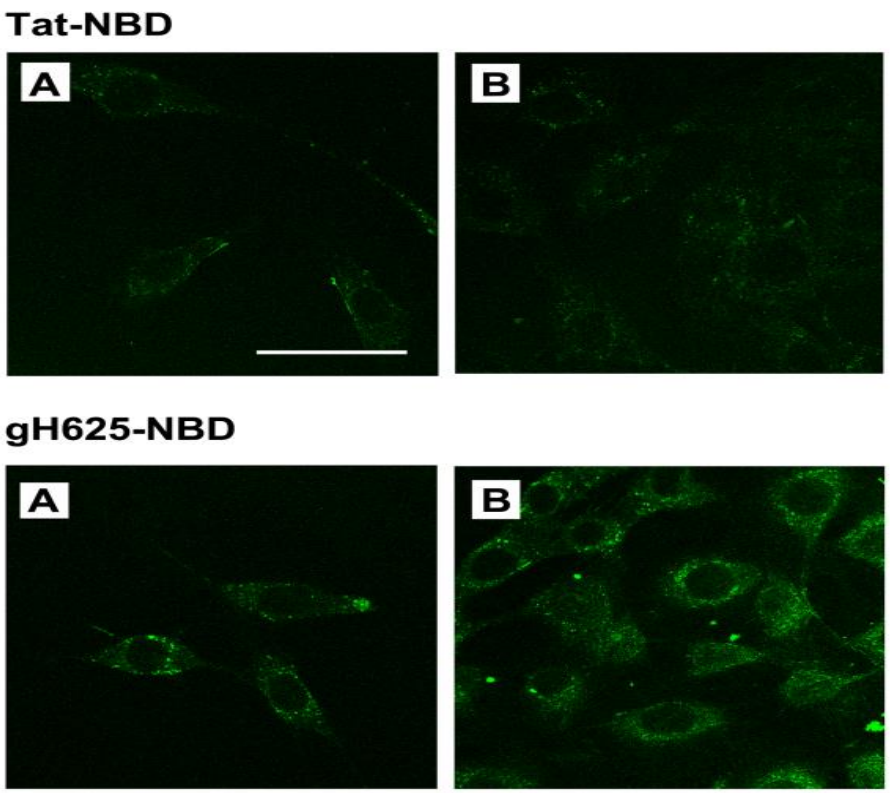


Endocytosis Inhibition

Cells incubated for 1h with NBD-peptides:

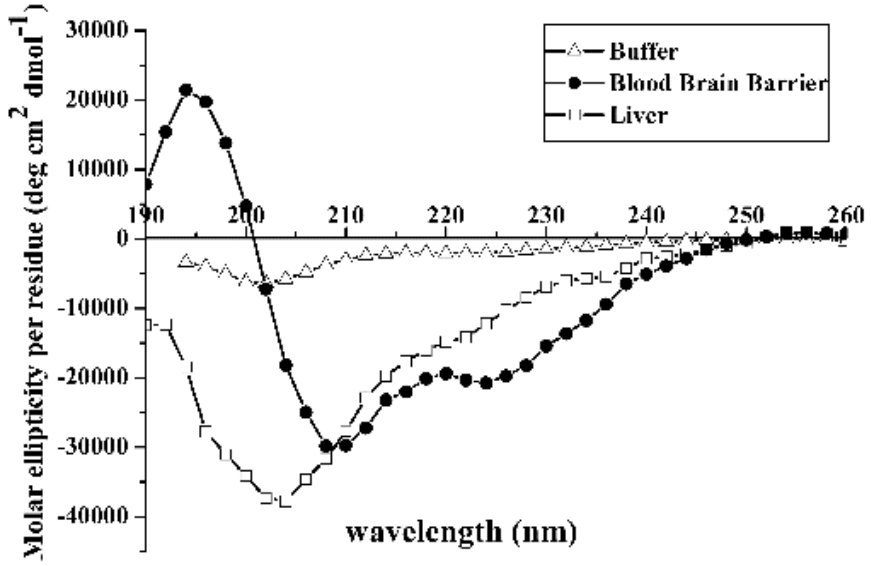
A) non-treated cells

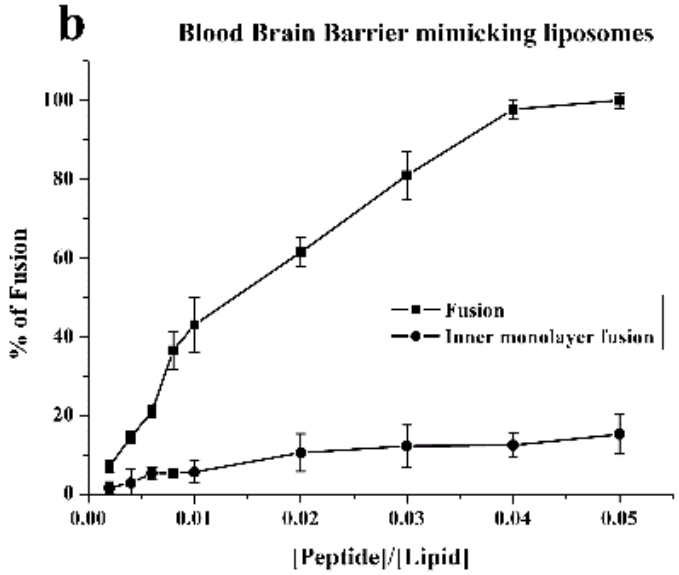
B) 40 μM NaN_3 (oxidative phosphorylation inhibitor) treated cells

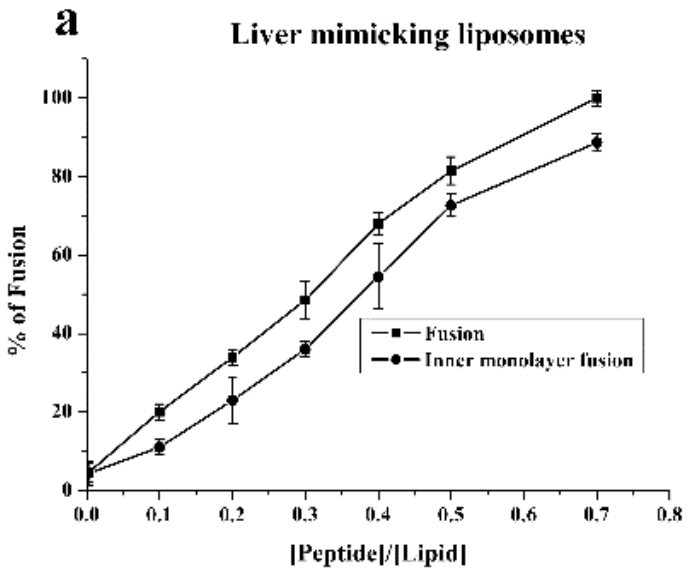


Mechanism of uptake by liver and brain

Characterization of the mechanism and implications for delivery (Falanga, Scientific Reports 2018: in press)







Partition coefficients (Kp) and Stern-Volmer (Ksv) quenching constants.

	Buffer	BBB	Liver
K_p	-	$(4.24\pm0.06)\ 10^4$	$(1.55\pm0.08)\ 10^3$
$K_{sv}(M^{-1})$	10.21±0.15	2.05±0.08	3.04±0.06

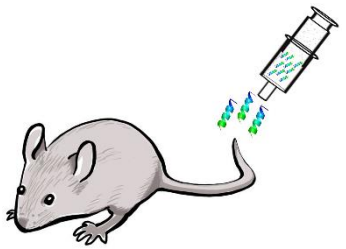
Membrane fluidity evaluation exploiting the generalized polarization (GP) value

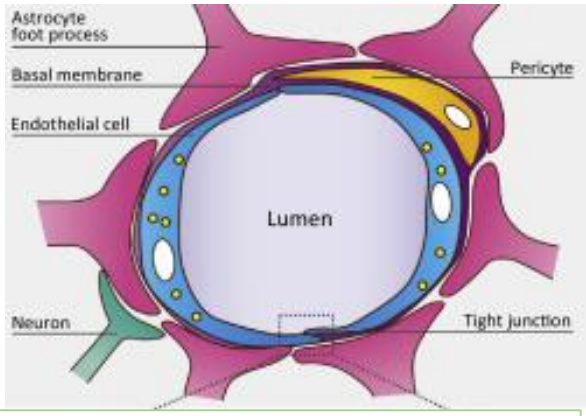
	No peptide 25°C	No peptide 37°C	+ gH625 37°C
Liver	0.44	0.40	0.49
BBB	0.20	0.19	0.15

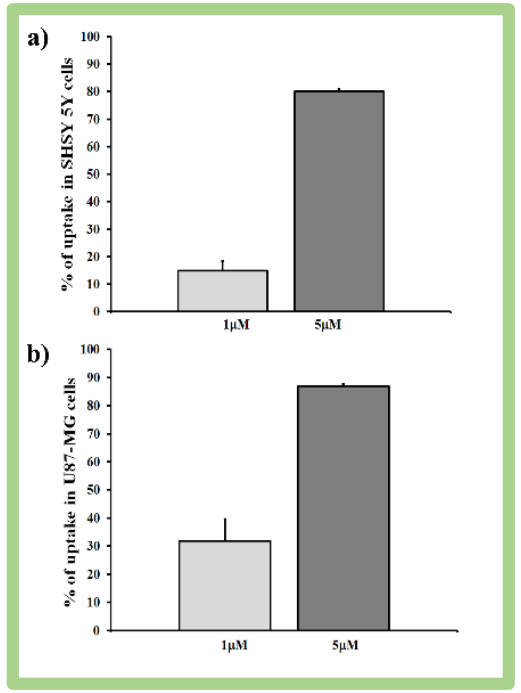
Crossing of the Blood Brain Barrier in rats

International Journal of Nanomedicine
Dovepress
open access to scientific and medical research
Open Access Full Text Article
ORIGINAL RESEARCH

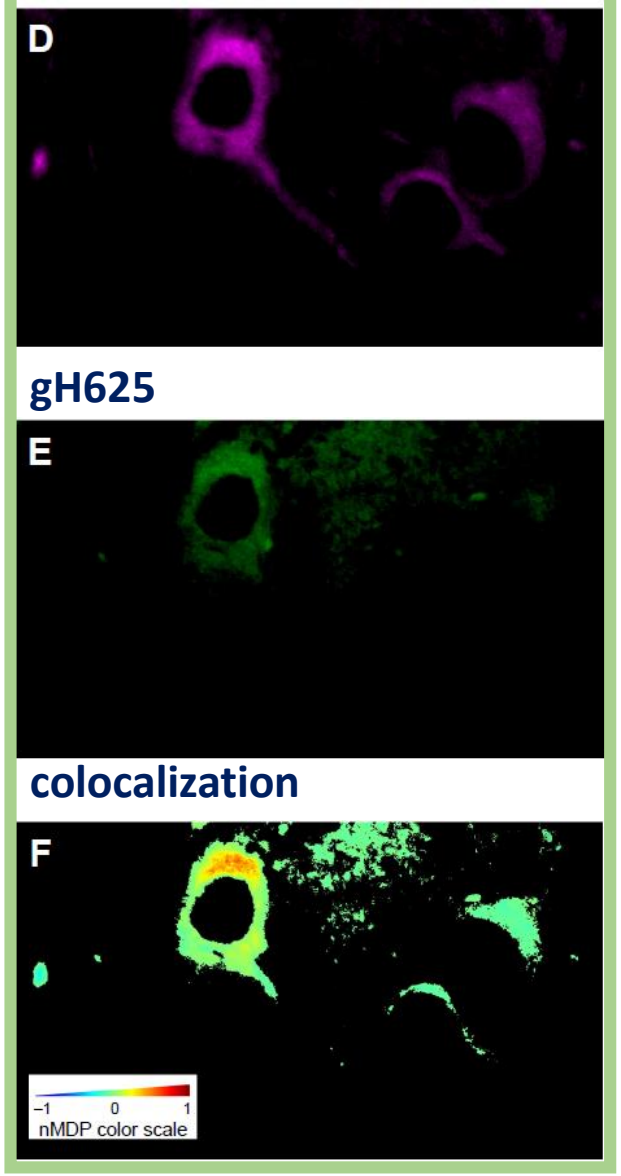
Peptide gH625 enters into neuron and astrocyte cell lines and crosses the blood–brain barrier in rats



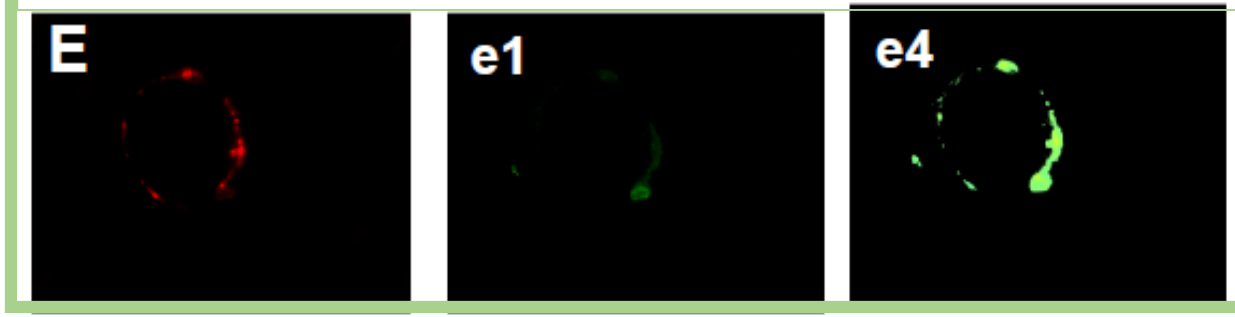




Staining of:



Staining of:

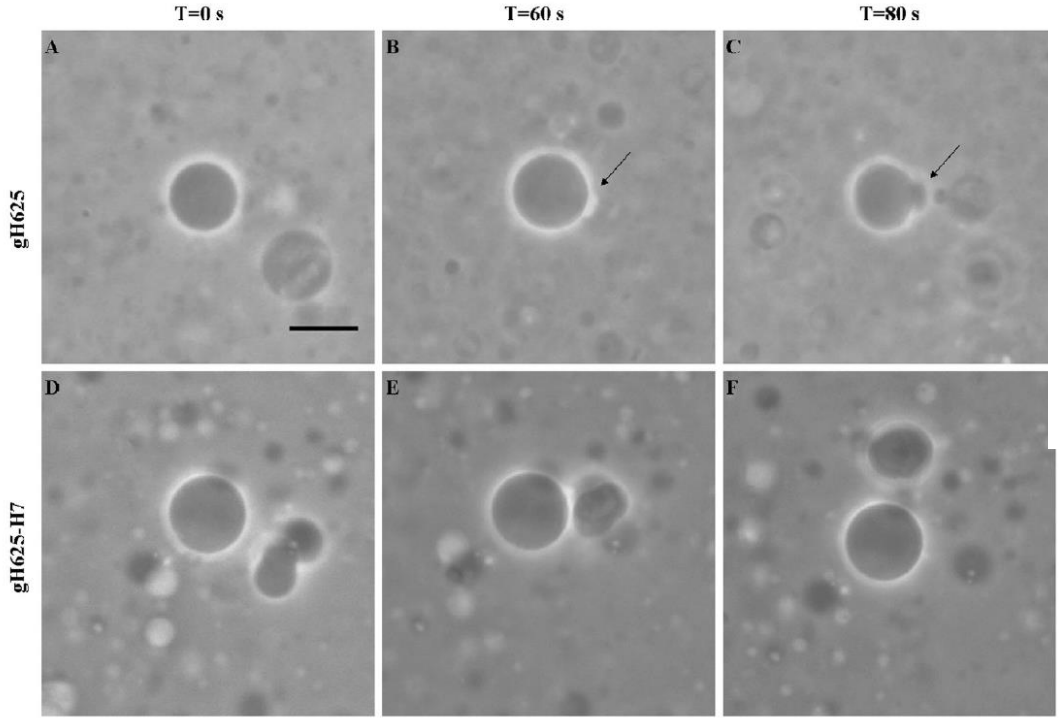


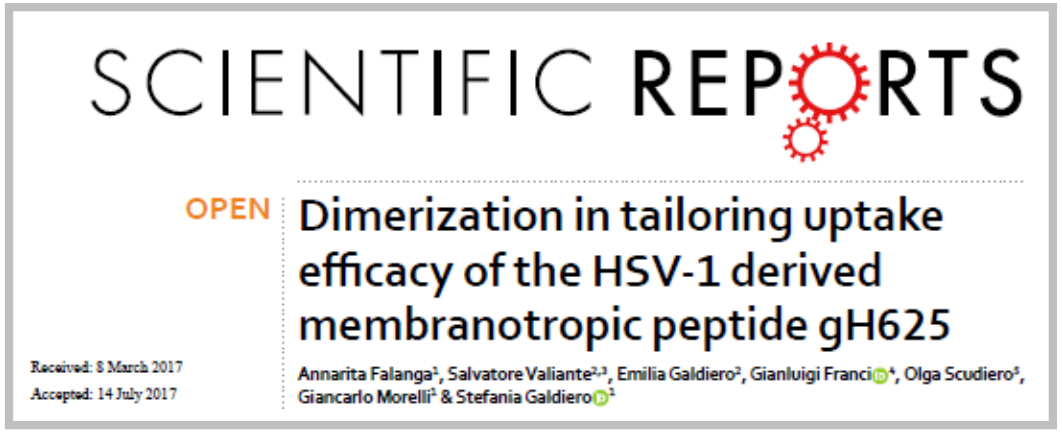
Caffè Scientifico, 8 Maggio 2019, Sala Cinese
Dr Annarita Falanga

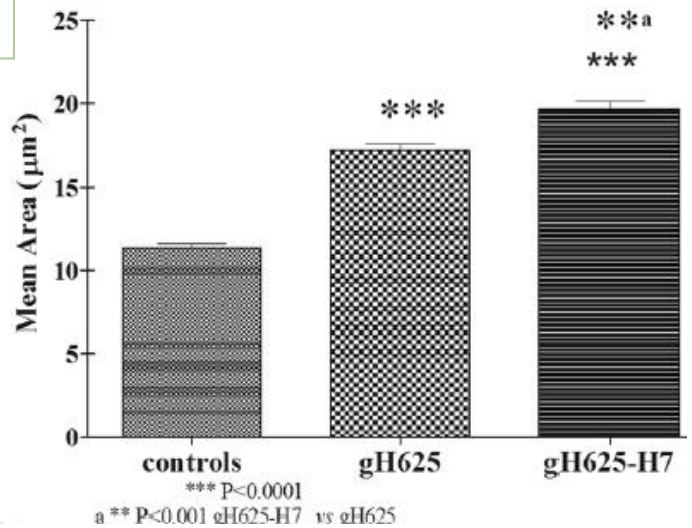
Progetto F.A.R.O. (Finanziamento per l'avvio di Ricerche Originali) III tornata. Titolo: Meccanismi innovativi di trasporto attraverso la barriera ematoencefalica: peptidi e nanoparticelle. Componente: Annarita Falanga

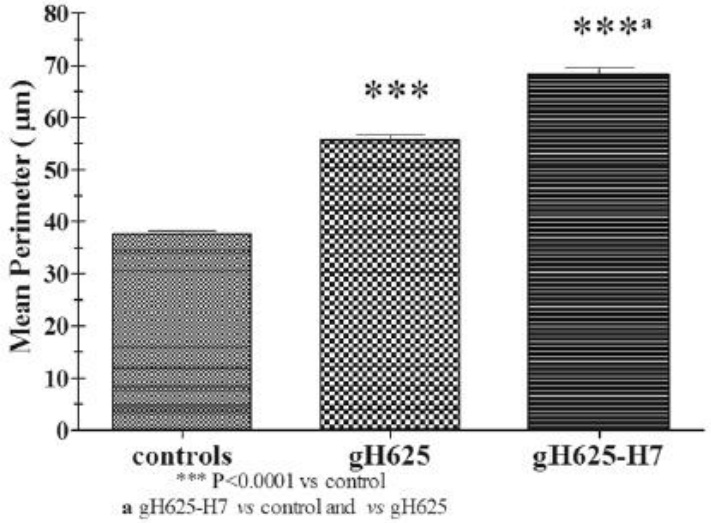
Multivalency

Peptide sequences		
	Sequence	
gH625	NH_2-HGLASTLTRWAHYNALIRAF-$CONH_2$	monomer
gH625-H7	NH2-HGLASTLTRWAHYNALIRAFGCGKKKK-CONH2 I NH2-HGLASTLTRWAHYNALIRAFGCGKKKK-CONH2	dimer



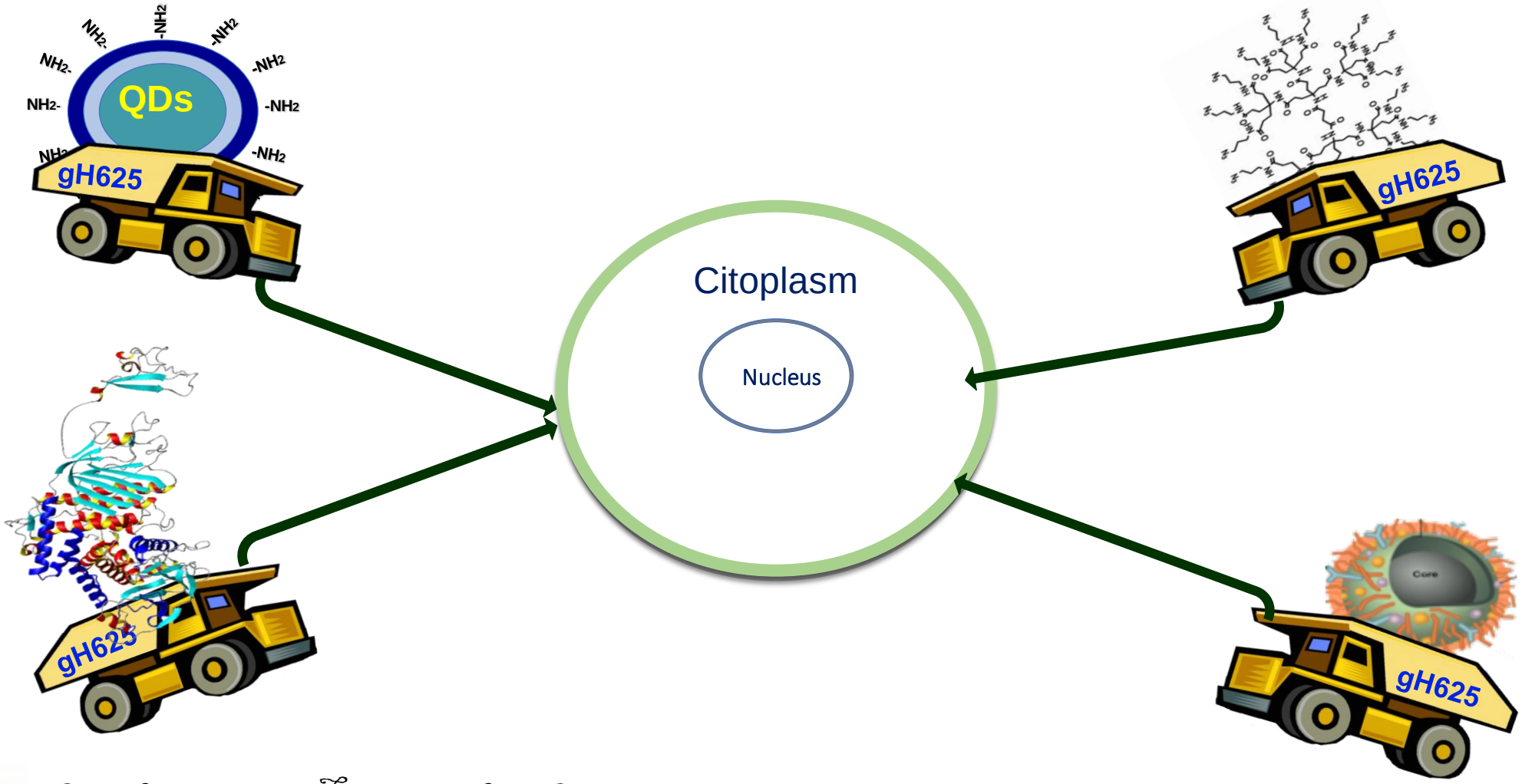






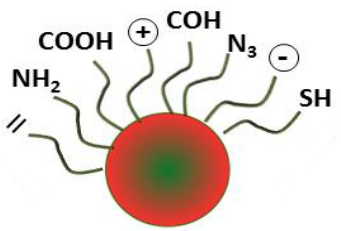
Caffè Scientifico, 8 Maggio 2019, Sala Cinese
Dr Annarita Falanga

Is gH625 able to enter inside cells? and to deliver a cargo intracellularly?

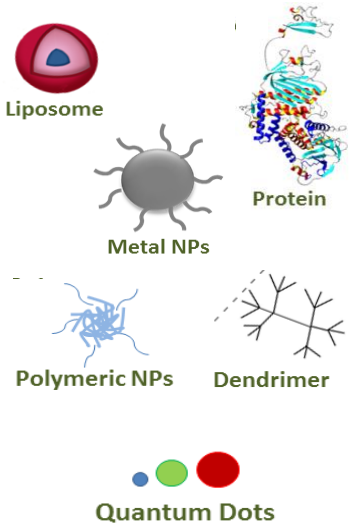


Toolbox for theranostics

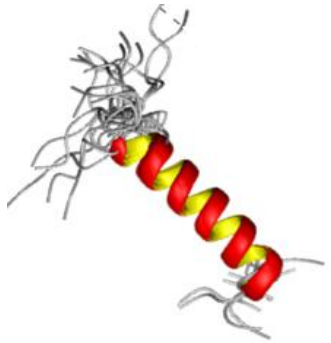
Surface chemistry



Composition



Delivery peptide



Targeting peptides

EGBP:
NH_2-FPMFNHWEQWPPG-$CONH_2$

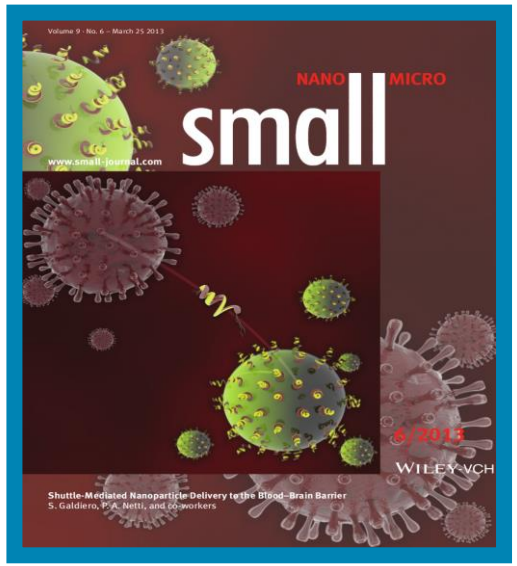
Drug

Doxorubicin

Mitoxantrone

Acyclovir

siRNA



Falanga, Nanomedicine 2011
Guarnieri, Small 2013
Falanga, Plose One 2014

Section 3

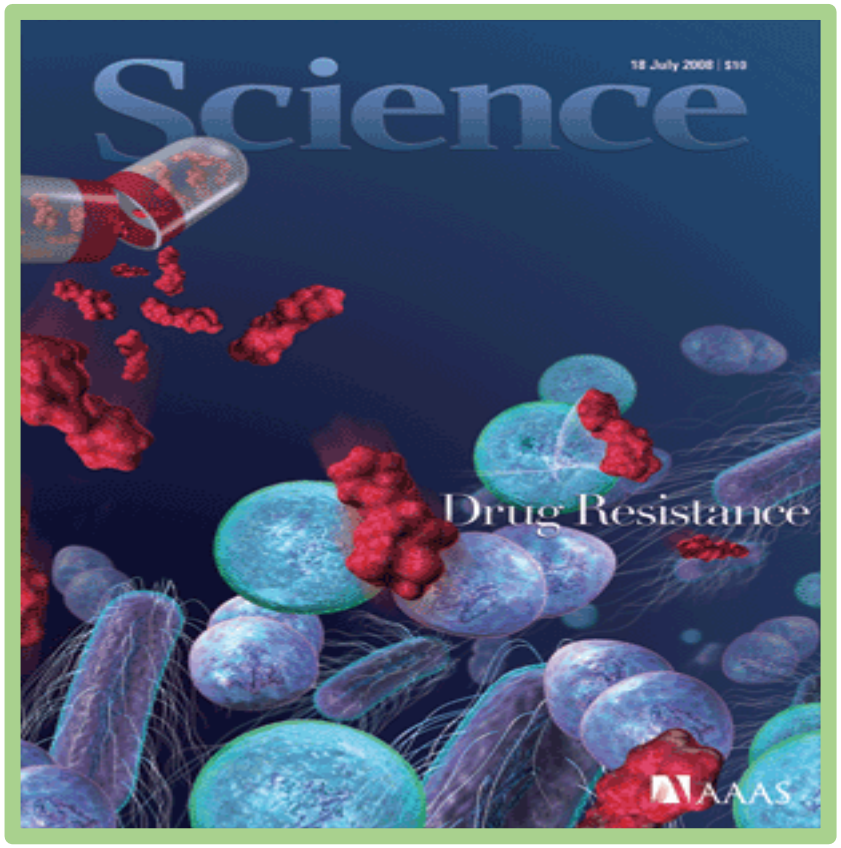
Antimicrobial Peptides (AMPs)

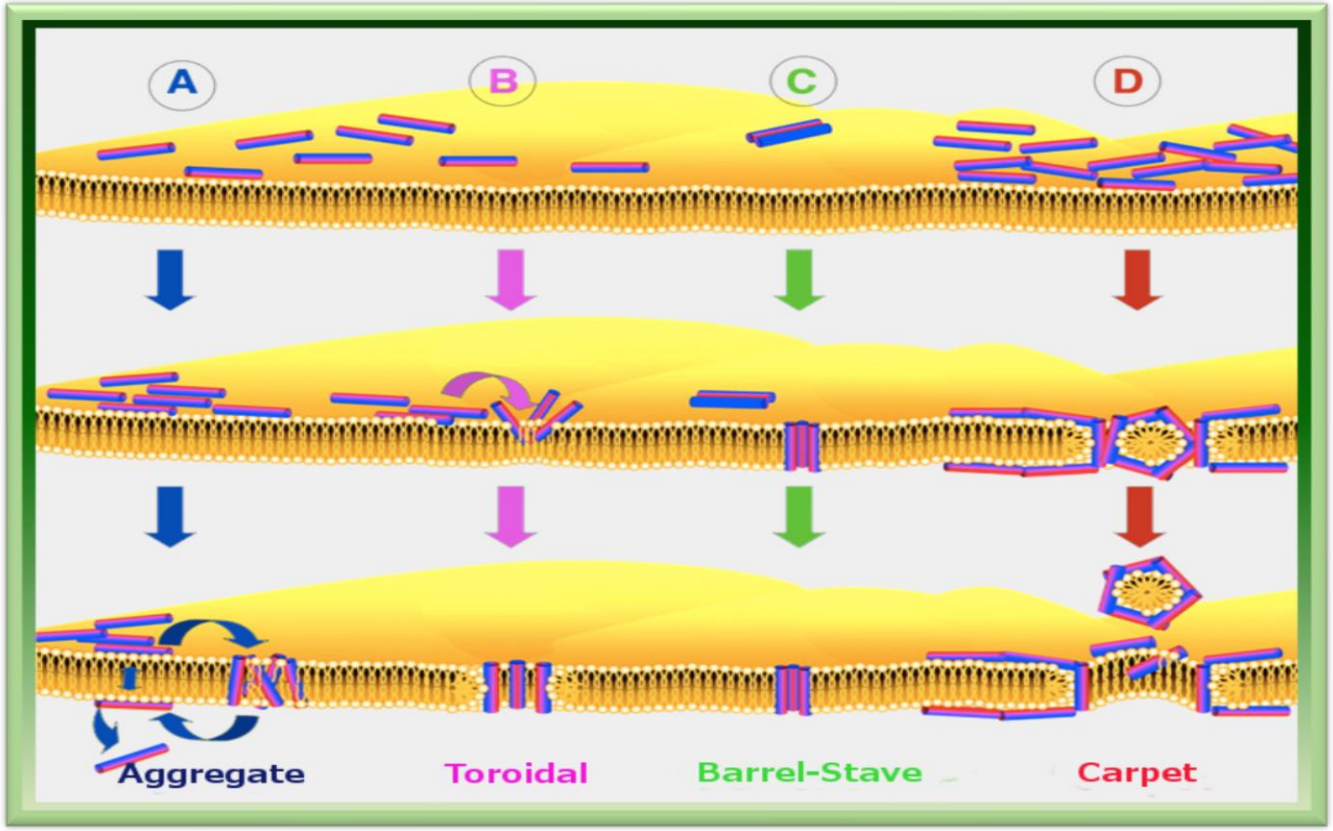
- Chemical characterization of the mechanism of membrane permeabilization
- Rational design of novel antimicrobial peptides

Current Medicinal Chemistry, 2015, *22*, 1665-1677

Antimicrobial Peptides as an Opportunity Against Bacterial Diseases

Stefania Galdiero[1,*], Annarita Falanga[1], Rita Berisio[2], Paolo Grieco[1], Giancarlo Morelli[1] and Massimiliano Galdiero[3]





AAC
Journals.ASM.org

Structural Insights into and Activity Analysis of the Antimicrobial Peptide Myxinidin

Marco Cantisani,[a,b,c] Emiliana Finamore,[d] Eleonora Mignogna,[d] Annarita Falanga,[a,b] Giovanni Francesco Nicoletti,[e] Carlo Pedone,[a,b] Giancarlo Morelli,[a,b] Marilisa Leone,[b] Massimiliano Galdiero,[d] Stefania Galdiero[a,b]

Peptide	Sequence
myxinidin	NH_2-GIHDILKYGKPS-$CONH_2$
WMR	NH2-WGIRRILKYGKRS-CONH2

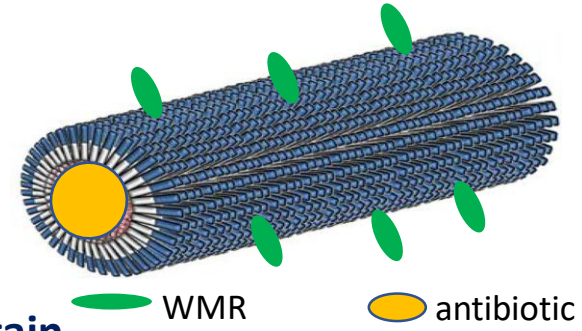
Interaction of the peptides myxinidin and WMR with lipid membranes (E. coli and P. aeruginosa)

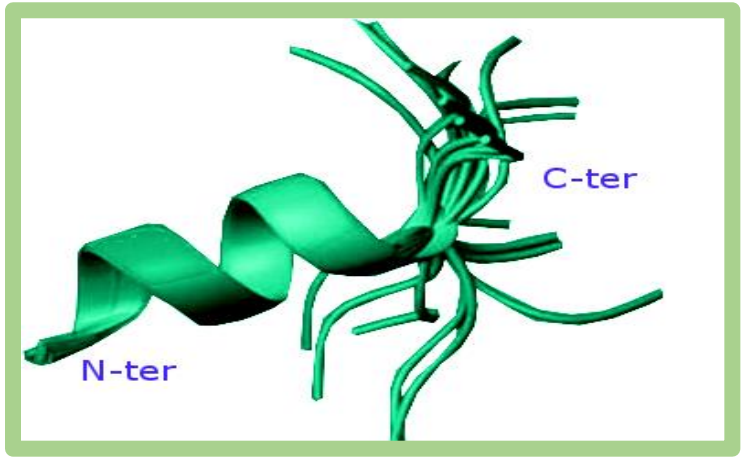
↓

- Lipid fusion and leakage
- Kinetic constants
- Bilayer structure
- Thermodinamic parameters

↓

Design and synthesis of self-assembling peptides to gain access to the intracellular melieu





Biomacromolecules

Enhancing the Potency of Antimicrobial Peptides through Molecular Engineering and Self-Assembly

Lucia Lombardi[†‡], Yejiao Shi[‡], Annarita Falanga[§∥], Emilia Galdiero[⊥], Elisabetta de Alteriis[⊥], Gianluigi Franci[#], Igor Chourpa[¶], Helena S. Azevedo[‡] , and Stefania Galdiero*[†§]

2019

CAC

Table 1 - Sequence and molecular weight (Mw) of the designed peptides

Name	Sequence	Mw (g/mol)
WMR	NH2-WGIRRILKYGKRS-CONH2	1632.88
WMR1	NH2-WGIRRILKYGKRSAAAAAA-CONH2	2059.45
WMR2	NH2-WGIRRILKYGKRSAAAAAAK(C19)-CONH2	2466.79
P1	NH2-WKRSAAAAAAK(C19)-CONH2	1410.14
P2	NH2-GDDSAAAAAAK(C19)-CONH2	1226.94

Table 2 - CAC (µM) and zeta potential (mV) values determined for peptides and complexes

	WMR1	WMR2	P1	P2	P1+WMR2	P2+WMR2
CAC	-	5	16	19	6	4
zeta potential		56.5	48.5	-37.9	37	34.8

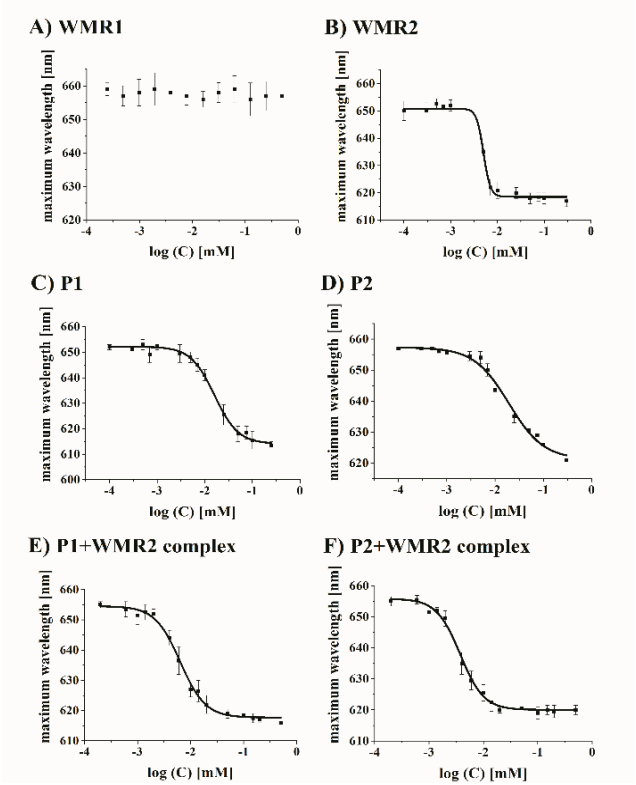
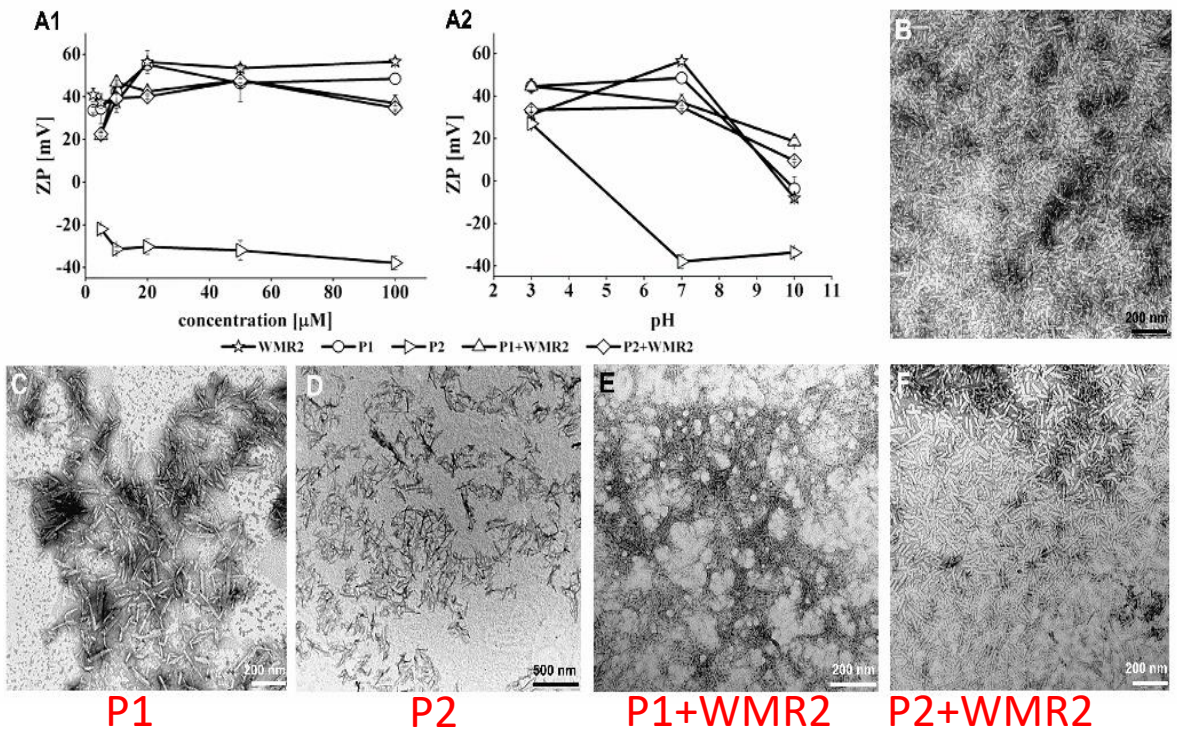


Figure 2 - The wavelength corresponding to the maximum fluorescence emission of Nile red was plotted as a function of concentration of the peptide WMR1 **(A)**, WMR2 **(B)**, P1 **(C)**, P2 **(D)**, and the complex P1+WMR2 **(E)** and P2+WMR2 **(F)** to determine their CAC. The measurements were repeated three times.

Characterization

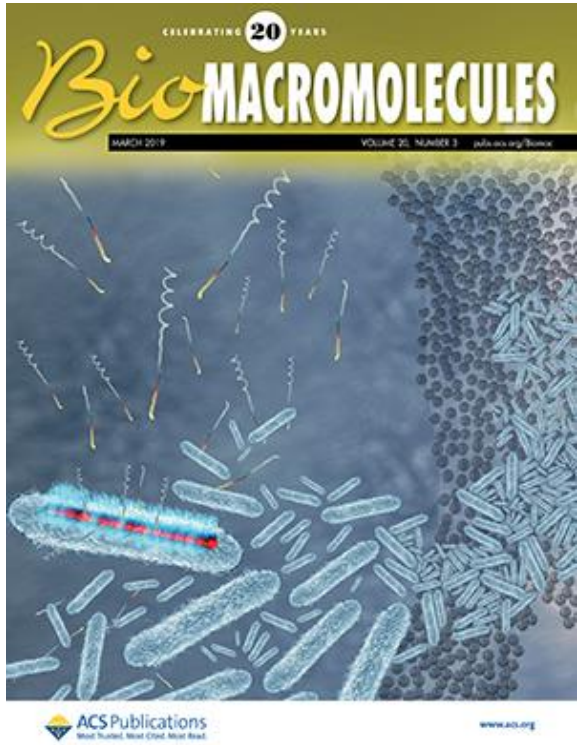
TEM

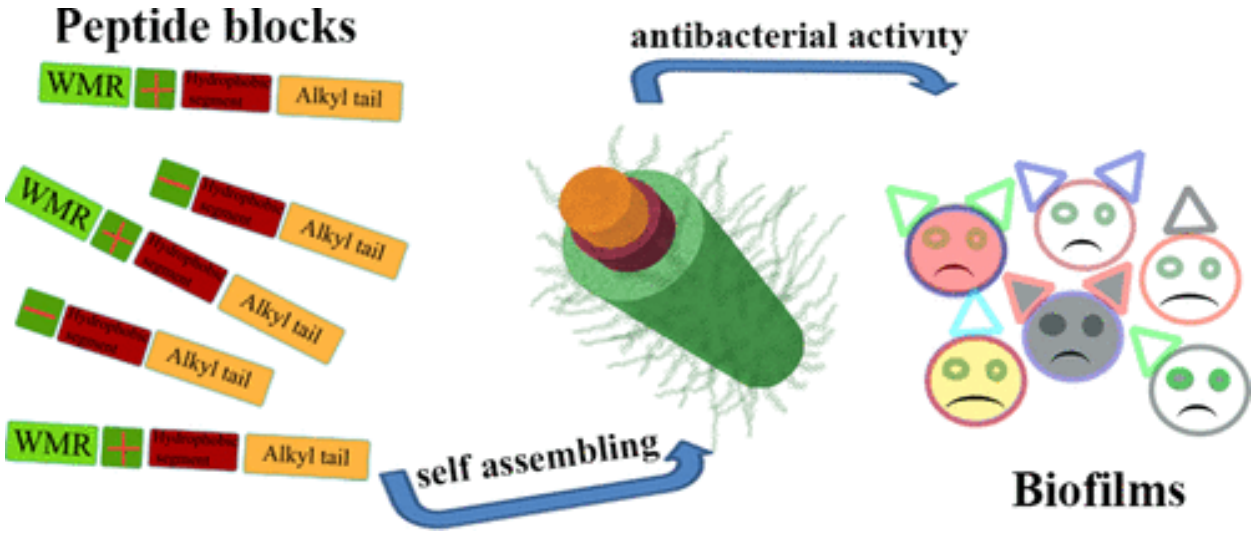


WMR2

P1 P2 P1+WMR2 P2+WMR2

- Circular dichroism
- Raman spectroscopy
- Anti-biofilm activity
- Biocompatibility





Mini-β-Defensin

International Journal of Nanomedicine

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Design and activity of a cyclic mini-β-defensin analog: a novel antimicrobial tool

molecules

Review

Cyclic Peptides as Novel Therapeutic Microbicides: Engineering of Human Defensin Mimetics

Annarita Falanga [1,†], Ersilia Nigro [2,3,†], Margherita Gabriella De Biasi [1], Aurora Daniele [2,3], Giancarlo Morelli [1], Stefania Galdiero [1,*] and Olga Scudiero [3,4,*]

Table 1 Sequences of the hBD1, AMC, and hBD3 peptides

Peptide	Sequence	Charge
hBD1	DHYNCVSSGGQCLYSACPIFTKIQGTCYRGKAKCCK	+4
AMC	CPIFTKIQGTC---------GG----------RRKK	+5
hBD3	GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK	+11

- Highly stable
- Low toxicity
- Preserve Antibacteria and antiviral properties

Caffè Scientifico, 22 Maggio 2019, Sala Cinese

Il luna park della sensoristica ambientale: acrobazie, magie, stupore…sorseggiando un buon caffè

Dr. Nunzio Romano

Università degli Studi di Napoli Federico II

T
H
A
N
K

Y
O
U

THANK YOU