Institut of Biomolecules Max Mousseron
Montpellier, FRANCE

THERAPEUTIC PEPTIDES

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2018

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THERAPEUTIC PEPTIDES : Overview

I. Introduction

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II. A peptide as drug ?

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II.4. Protein mimics to inhibit protein/protein interactions

III. Peptides and conjugates as tools ?

III.1. Cell-targeting peptides

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I. INTRODUCTION

I.1. What is a peptide? Back to basics

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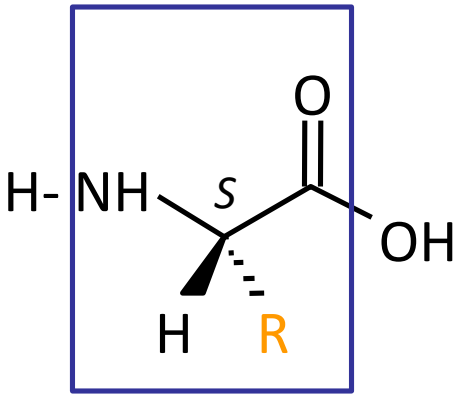
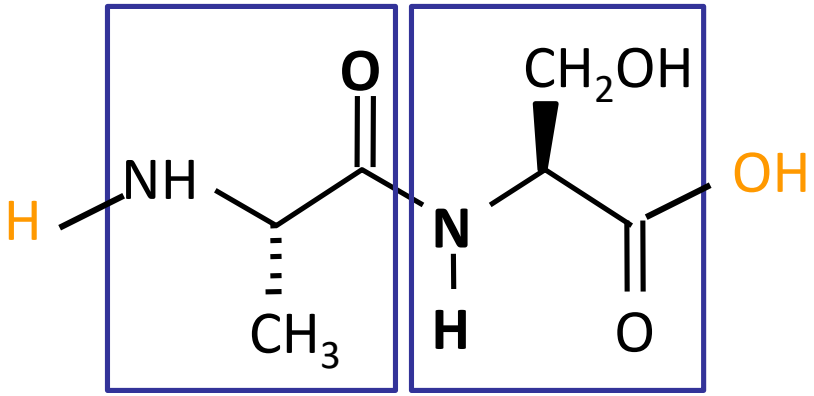
Definition: peptides

From grec ‘pepsis’ : digestion , oligomer constituted from amino acids
For peptide chemists Peptide = Protein < 50 amino acids



R = side chain

H-Ala-Ser-OH or H-AS-OH



N-terminus → C-terminus

Biosynthesis direction, writing the CONH bond and numbering the amino acids

Amino acid residue
1-letter code : A
3-letters code : Ala
Amino acid :
Ex if R=CH_3 H-Ala-OH

Chemical synthesis direction C-ter to N-ter

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2012 Draft Guidance for Industry on Biosimilars

FDA distinguish **proteins** from **peptides** based solely on size.

"**chemically synthesized polypeptide**" means any alpha amino acid polymer that

(1) is made entirely by chemical synthesis and

(2) is **less than 100 amino acids** in size.

A chemically synthesized polypeptide, as defined, is not a **"biological product"** and will be regulated as a drug.

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2 main classes of peptides

Ribosomal peptides

- synthesized mRNA translation
- modifed by proteolytic enzymes from propeptides (longer peptides chains) to yield their active form.
- subjected to multiple post-translational modifications(phosphorylation, hydroxylation, palmitoylation, glycosylation, disulfide bon formation…)

Non-ribosomal peptides

- synthesized by non-ribosomal peptide synthetases independant of mRNA, very often produced by microorganisms (bacteria, fungi)
- High structural diversity: linear, cyclic, branched

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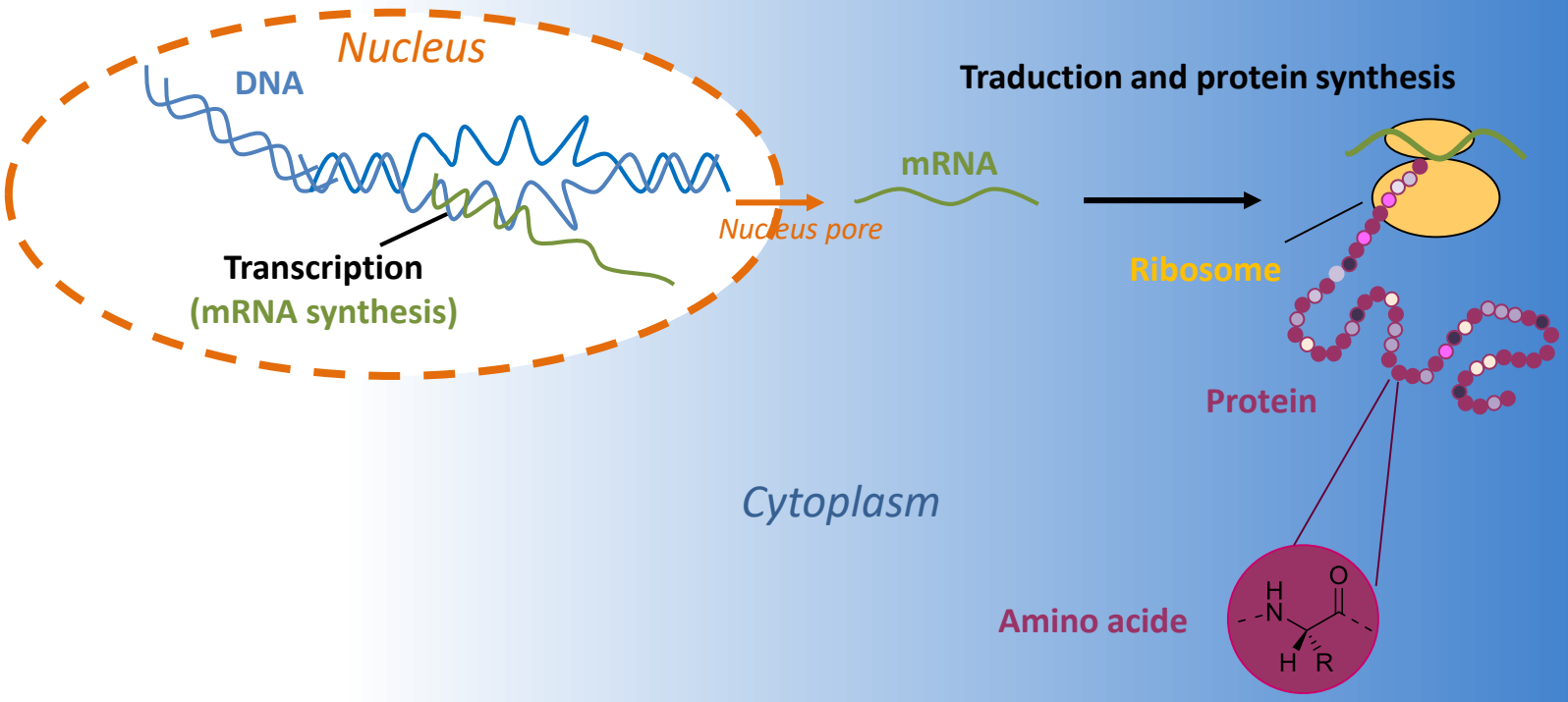
Biosynthesis of ribosomal peptides

Ribosomal synthesis of peptide proceeds under genetic control, from N-terminus to C-terminus using proteinogenic α-amino acids.



Peptides and proteins play a major and central role in nearly the physiological processes of living cell. They present a remarkable range of biological properties

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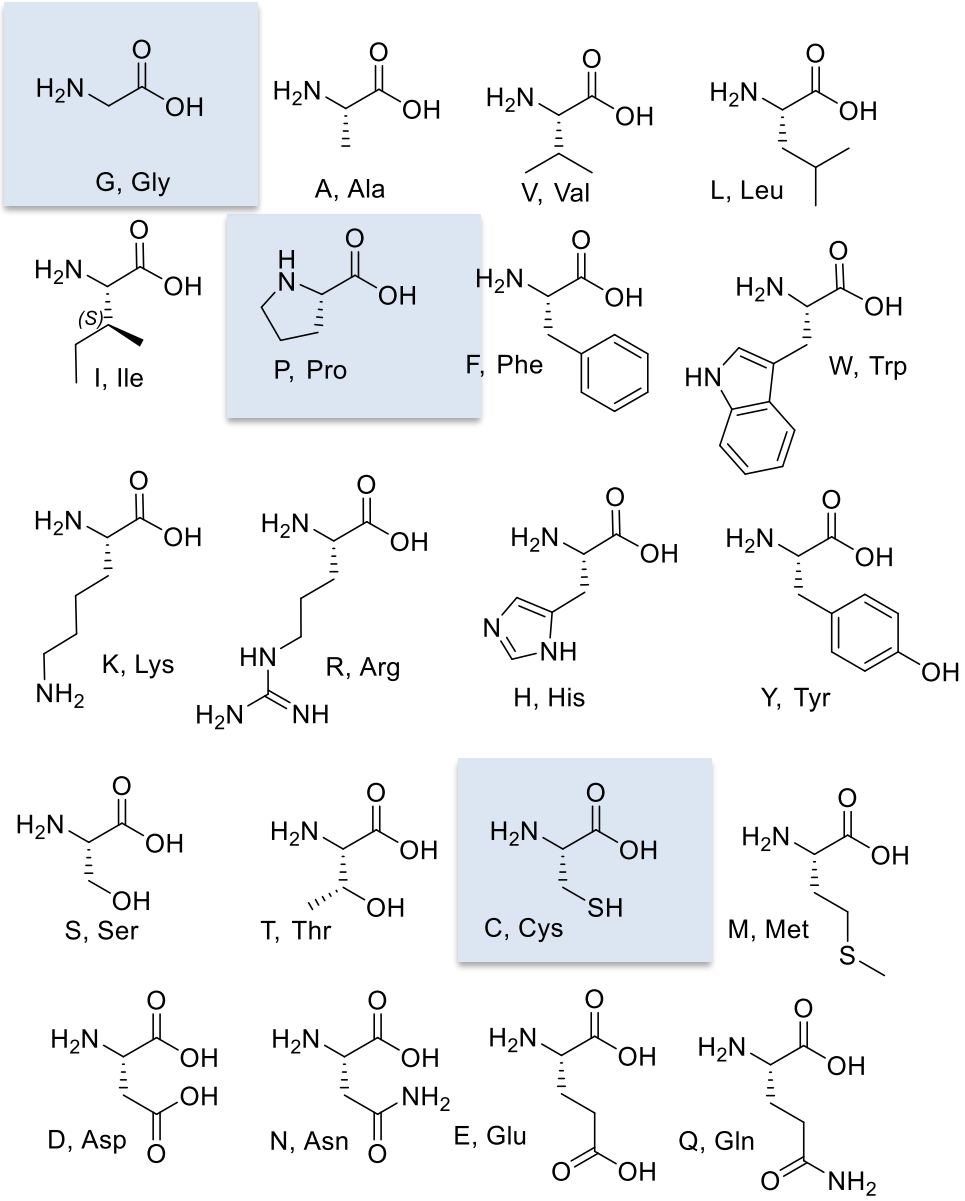
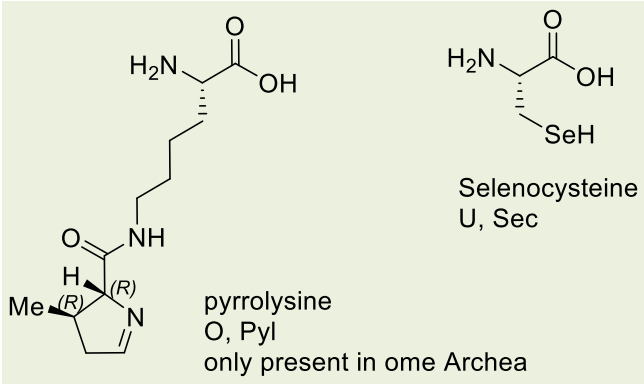
Proteinogenic amino acids



Proteinogenic : 22 amino acids directly coded and inserted in the peptide chain (e.g. coded by a stop codon UGA in the ribosome for Sec) not synthesized post translationally.



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Fundamental roles of proteins and peptides

-the biological scaffold/frame of the cells (collagen, hemoglobin)
-regulators : hormones and receptors
-catalyzers and effectors : enzymes
-immunologic : antibodies
-Venoms, toxins, antibacterials
-Targeting systems, transport and delivery

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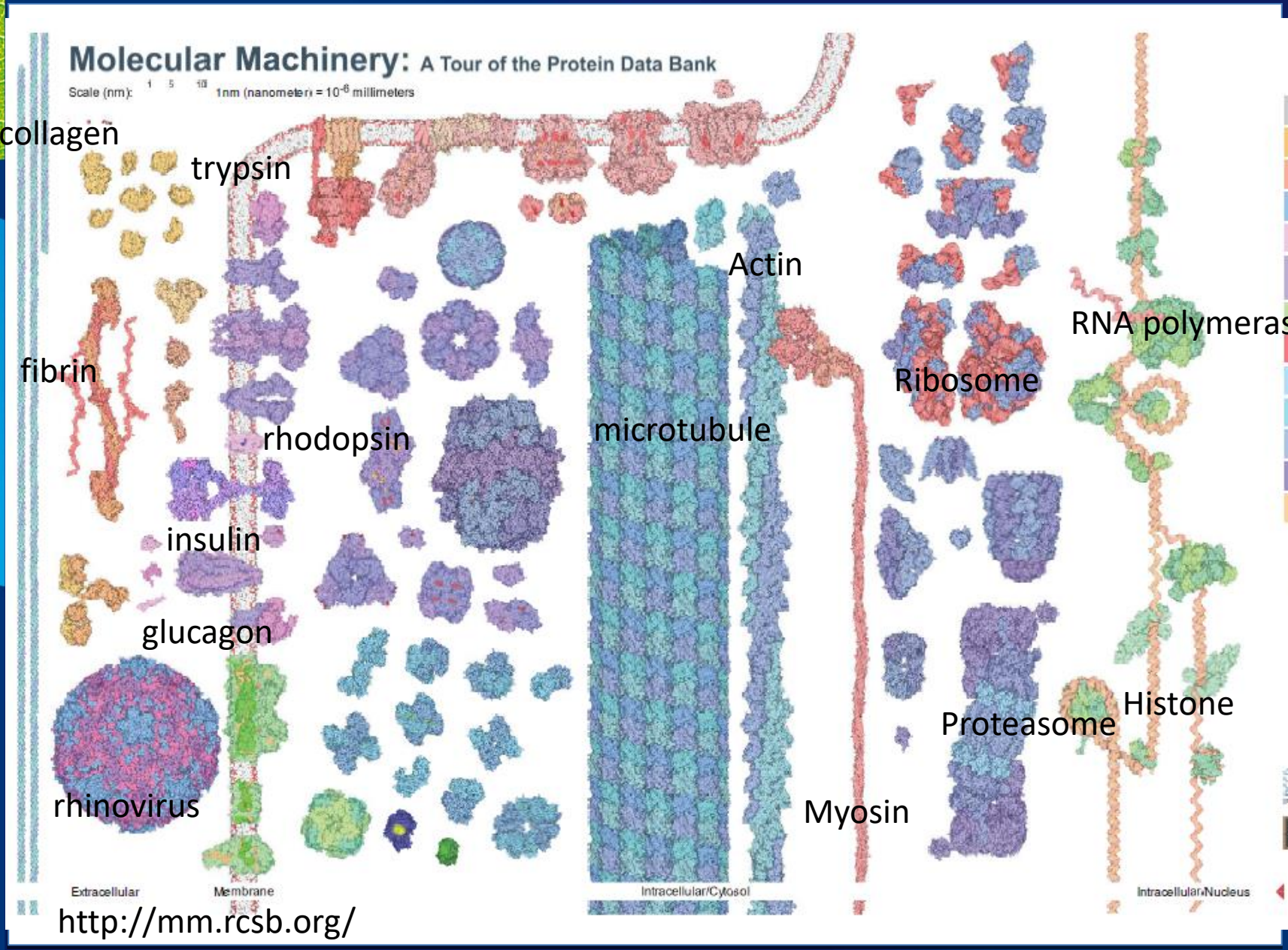
Fundamental roles of proteins and peptides



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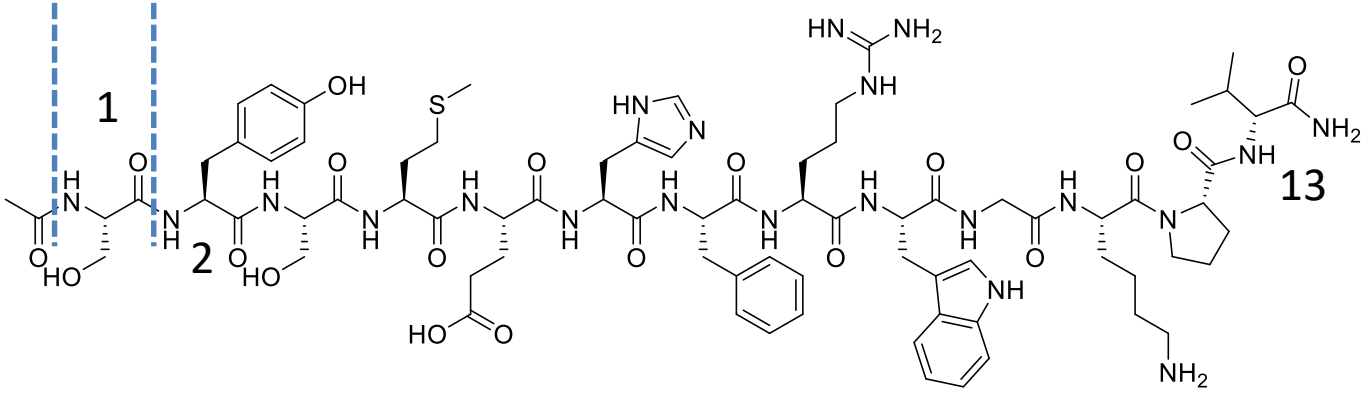
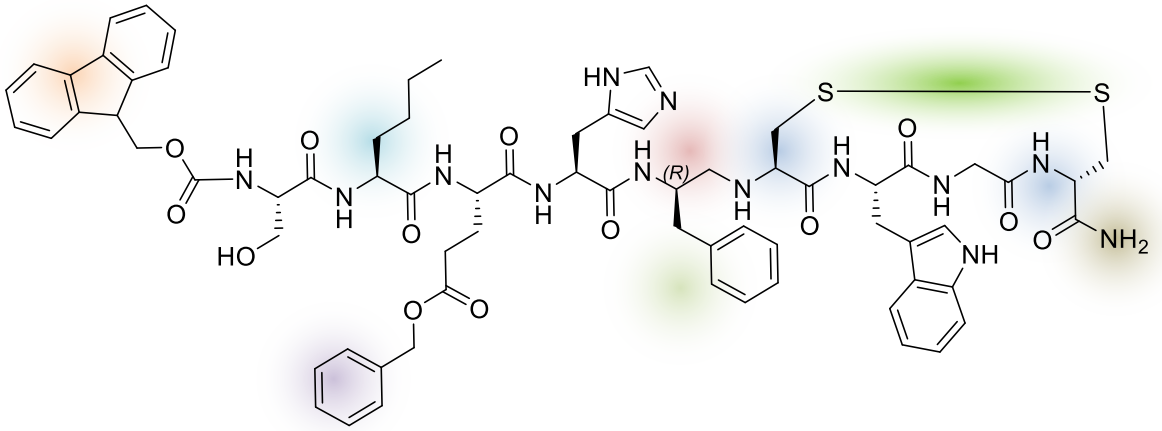
Numbering, writing, encoding

α-MSH: Ac-Ser^{1}Tyr^{2}Ser^{3}Met^{4}Glu^{5}His^{6}Phe^{7}Arg^{8}Trp^{9}Gly^{10}Lys^{11}Pro^{12}Val^{13}-NH_2



Cyclo8/11 Fmoc[Nle^{4},Glu(Obzl)^{5},(D)Phe ^{7}–ψ(CH_2NH) Cys ^{8},Cys^{11}-NH_2]α-MSH(3-11)



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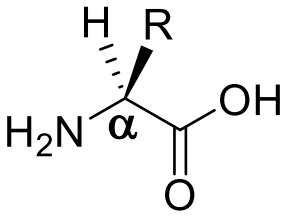
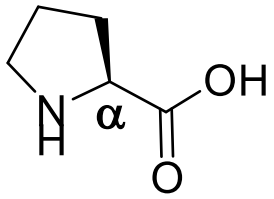
Stereochemistry



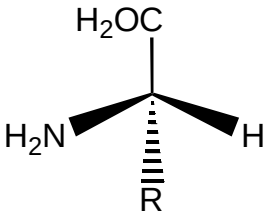


Proline

α-amino acides (amine and carboxylic acid functions are carried by the same carbon (α)

Chiral compounds, optically actives

L-Configuration (analogy with L glyceraldehyde)



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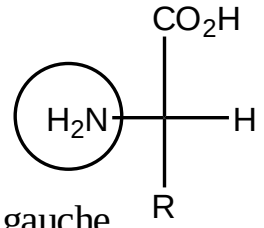
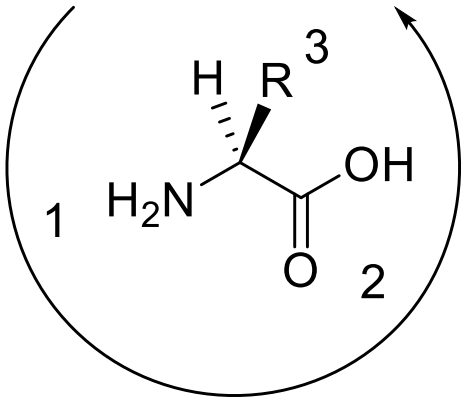
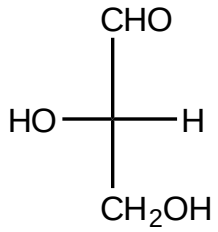


gauche



S stereochemistry

glyceraldéhyde



***Fisher representation**
Carbonated chain presented verticaly with more oxidized atom up

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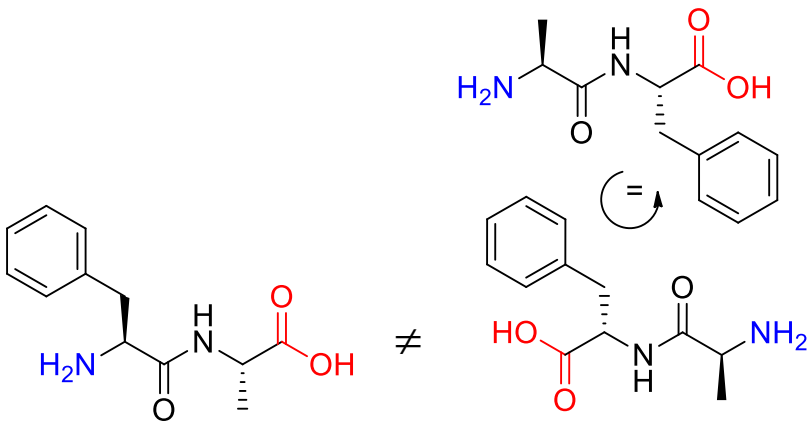
Writing direction is important

HPheAlaOH

HAlaPheOH *(retro peptide de HPheAlaOH)*



H(D)Ala(D)PheOH *(inverso peptide de HAlaPheOH)*

(retro inversopeptide de HPheAlaOH)

Retro-inverso petpide : same relative position of side chains BUT N-ter and C-ter are exchanged, NHCO instead of CONH

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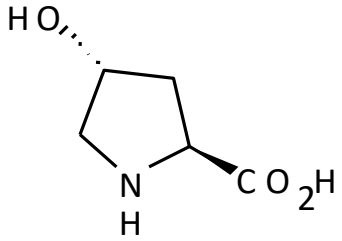
Non proteogenic amino acids

Modified after incorporation in peptide chains

hydroxyproline from collagen, by oxidation of proline



- β-amino acides : β-alanine (NH_2-CH_2-CH_2-CO_2H), vitamin precursor

- γ-amino acides : GABA (NH_2-CH_2- CH_2-CH_2-CO_2H), neurotransmitter

-D-amino acids : components of some antibiotics

-Sarcosine : component of antimicrobial peptides CH_3-NH-CH_2-CO_2H

- α-amino butyric acid : α, α' disubstituted H_2N-C(CH_3)$_2$-CO_2H

-Ornithine, Norvaline, Norleucine, dehydroalanine..

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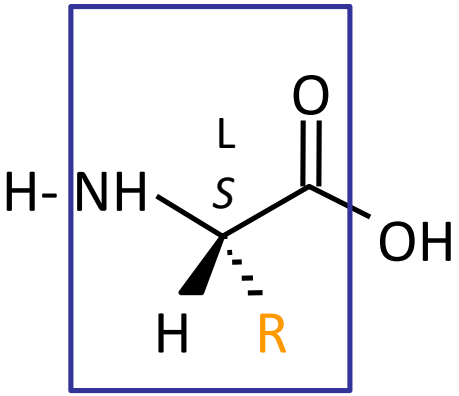
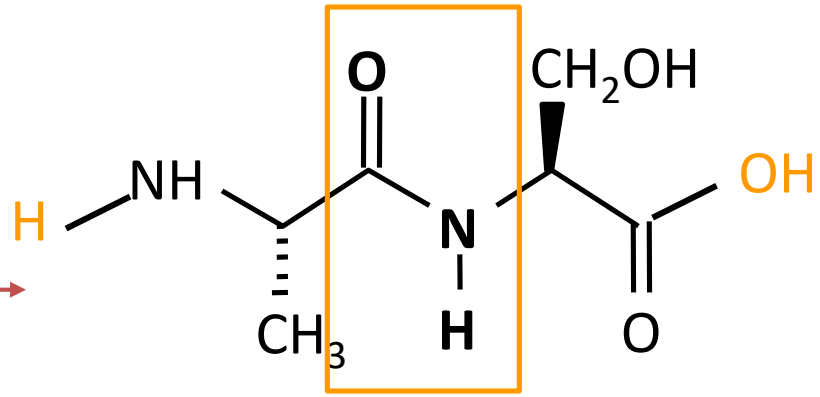
Definition: peptides



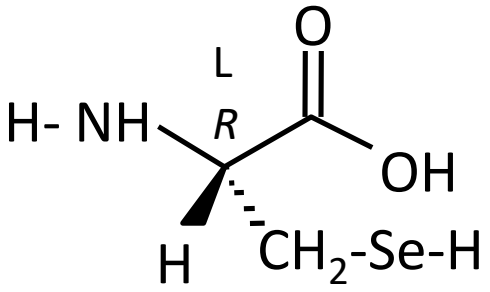


R = side chain

Amide bond= peptide bond

Amino acid residue
1-letter code : A
3-letters code : Ala
Amino acid :
Ex if R=CH_3 H-Ala-OH

U: selenocystein



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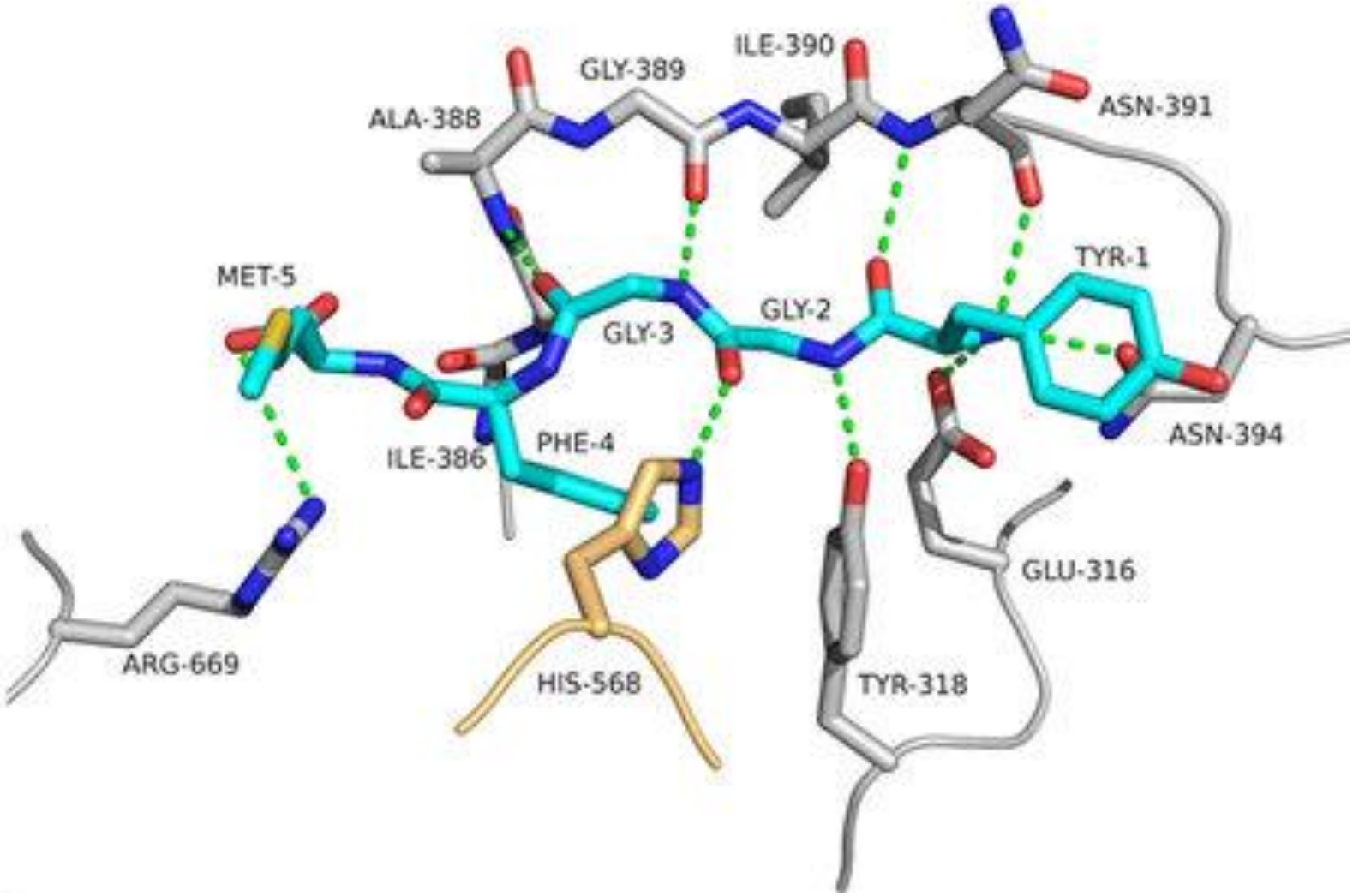
Sterochemistry is related to bioactivity : very important to control

Interactions of Met-enkephaline with human enzyme dipeptidylpeptidase III



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How to synthesize peptides and proteins?

By purification (optotherapy)

From natural extracts (ex : bovine collagen, growth hormone (in the 80's) from human pituitary gland)

By molecular biology

Recombinant proteins: e.g. insulin, growth hormone = somatotropine 191 amino acids

By chemical synthesis

solution

Solid support

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Opotherapy

	Source		
Insulin	Porcine pancreas	1920	
ACTH / corticotropin	Porcine pituitary	1952	
Calcitonin / Salcitonin recombinant	Salmon ultimobranchial gland	1983	

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How to synthesize peptides and proteins?

Expression systems:

By molecular biology

Recombinant proteins:

- bacteria *(E. coli)* *prokaryotic cells without nucleus*
- yeasts *(Saccharomyces. cerevisiae, Pichia pastoris)* *eukaryotic cells with nucleus*
- baculovirus infecting insect cells
- Eukaryotic cells (CHO, HEK, COS, etc.)

30L biomass reactor fermentation. This production capacity makes it possible to meet the majority of production needs for therapeutic proteins and recombinant vaccines for clinical phases I and II (max 1g)

Limitations: Post translational modifications not permitted, non natural amino acids

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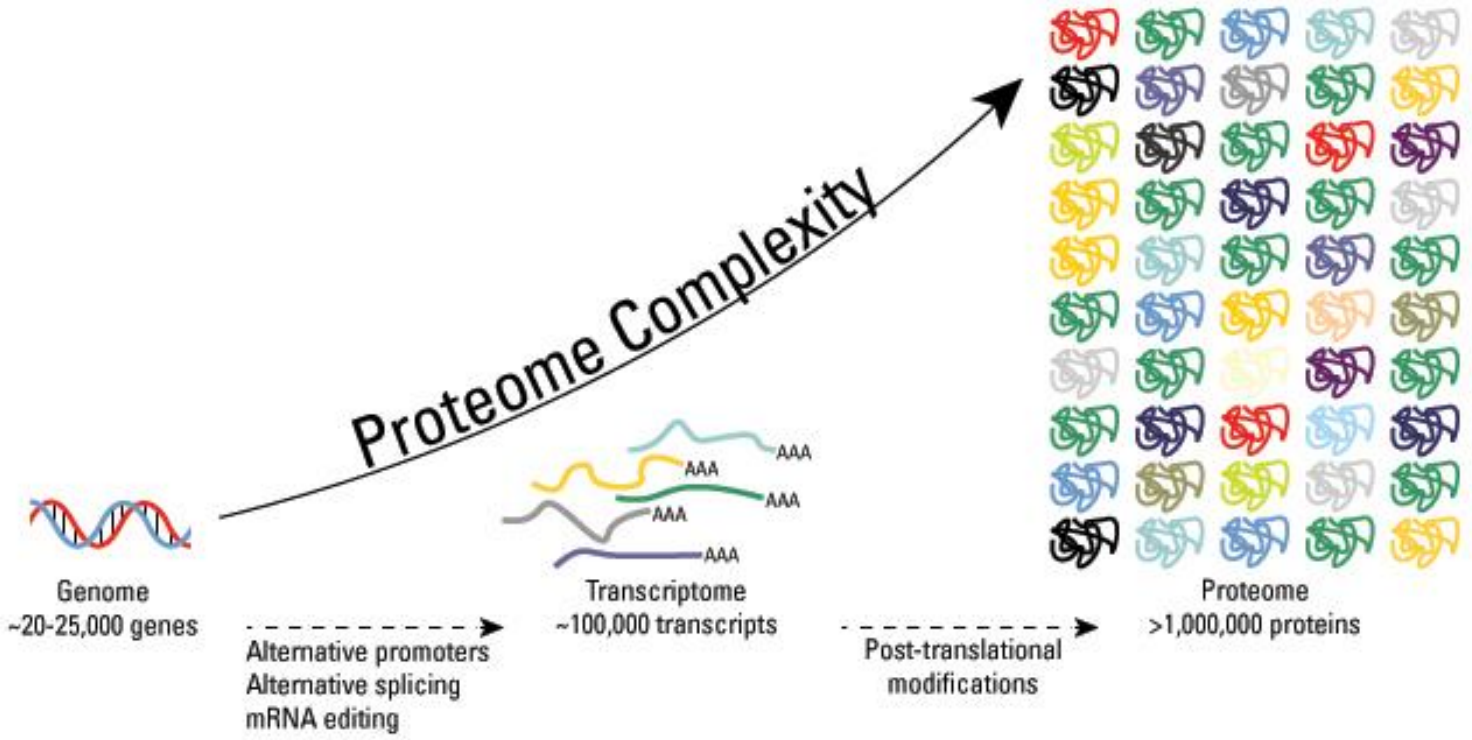
Post translationnal modifications



https://www.thermofisher.com/fr/fr/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-post-translational-modification.html

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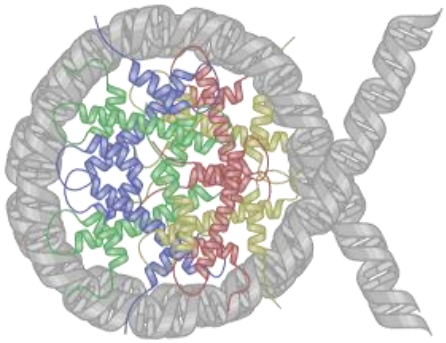
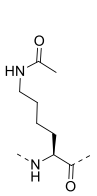
Post translationnal modifications

Effectuées dans le reticulum endoplasmique ou l'appareil de Golgi

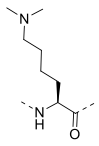
- S-S bridge formation
- Acetylation (Lys, promotes transcription by neutralizing histone charges: lower affinity for DNA)
- Methylation (Arg, Lys, observed in histones, modulation of gene expression)
- N-glycosylation (Asn) N-acetylglucosamine in the endoplasmic reticulum: future membrane glycoproteins: point of attachment
- O-glycosylation (Ser, Thr, Tyr) N-acetylgalactosamine: future proteoglycans of ECM.
- C-glycosylation (Trp)
- N-Myristoylation, palmitoylation, prenylation. Attachment to the intracellular part of the plasma membrane
- Hydroxylation (Lys, Pro): collagen
- Lipoylation (Lys) cofactor red-ox
- Sulfation (Tyr), phosphorylation (Ser, Thr, Tyr) (increase H bonds and modulate Prot / prot interactions
- C-ter amidation, citrullination of arginine (inflammation or myelin), deamidation of Asn or Gln in Glu, isoasp or aspartimide: degradation of proteins



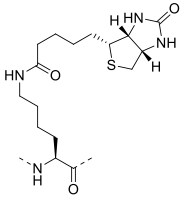
Nucleosome: Histone+ADN



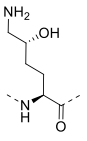
Lys(Ac)



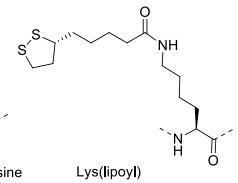
Lys(Me_2)



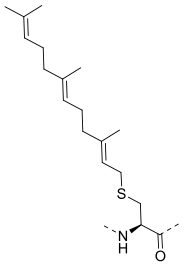
Lys(Biot)



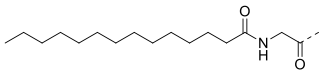
5R hydroxylysine



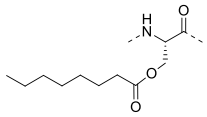
Lys(lipoyl)



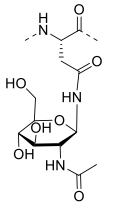
Cys(Farnesyl)



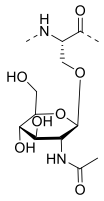
Myristyl-Gly--



Ser(Octanoyl)

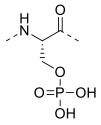


Asn(N-GlcNAc)

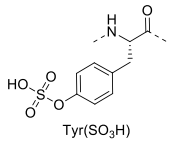


Ser(O-GalNAc)

(2S,4R)-4-hydroxyproline



Ser(PO_3H_2)



Tyr(SO_3H)

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I.2. Naturally occurring bioactive peptides

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Naturally occurring peptides

First peptide discovered Insulin (Macleod & Banting, 1923)
Synthesized only in 1964 Katsoyannis PG et al. JACS **1964**, 86, 930–932.

First peptide synthesized: Oxytocin (Vincent Du Vigneaud, 1962)

Today, more than **7000 natural bioactive peptides** have been identified with crucial roles in physiological mechanisms as:

Hormones : chemical communication and coordination: secreted by **neuroendocrine cells (release in the blood)** -> circulation to stimulate a response on another organ.

Neuropeptides: hormones **but which are secreted and used in the CNS**. Unlike neurotransmitters, they are not recycled.

Growth and differentiation factors,
Ion channel ligands , anti-infectious, transporters of substances through membranes

Review Keld Fosgerau, Torsten Hoffmann
Peptide therapeutics: current status and future directions.

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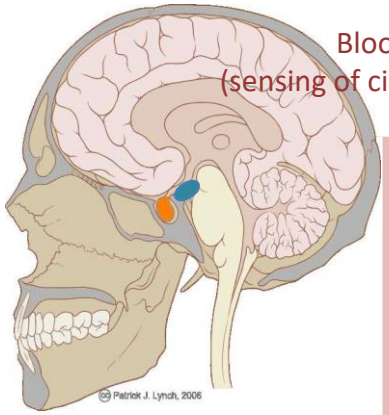
Peptide hormones

1. Hypothalamus-pituitary axis



Blood composition*
(sensing of circulating concnetrations)

Upper brain centers

Spinal cord

Hypothalamus (*window in BBB) circadian rhythms

CRH
Corticotropin releasing hormone

GnRH ou LHRH
Gonadoliberine

GHIH
Somatostatin

1st level

Anterior pituitary

Posterior pituitary

Oxytocin

ACTH FSH LH TSH GH Vasopressin (ADH)

2d level

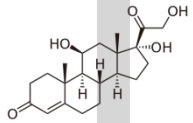
Adrenal Cortex

Testicles ovaries

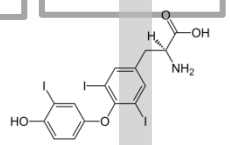
Tyroïd

Liver

Hormones Adrenocorticales
Glucocorticoides, androgenes, steroides



Androgenes
oestrogenes



T3 T4

Somatomedins (IGF-2)

3d level

Reproductive system
bones
liver
muscles
tissues
Arterioles
Water reabsorptin
Uterus
contractions

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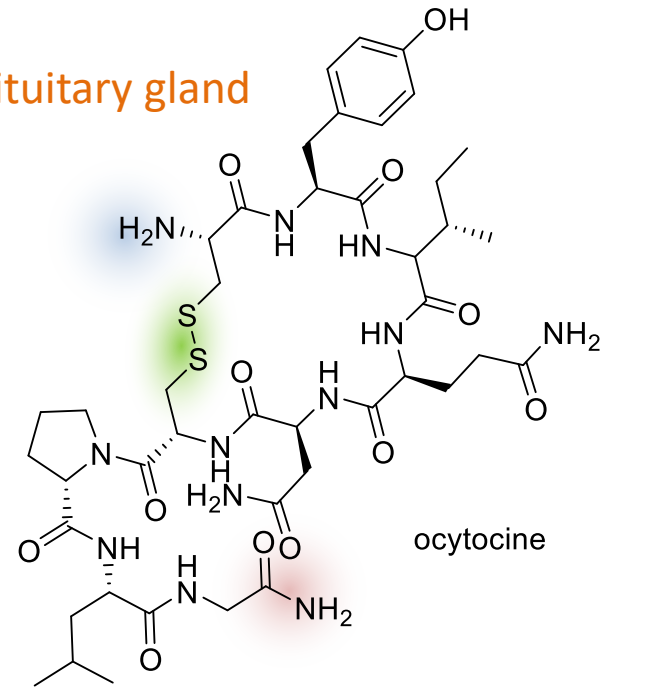
Oxytocin

Oxytocin
9 AA
C ter amide, 1 SS bridge
Secreted by the posterior pituitary gland



Main activities: often described as 'happiness hormone', compassion, attachment, mother-child relationship
Acts on the mammary glands and smooth muscles of the uterus to speed up labor of childbirth.

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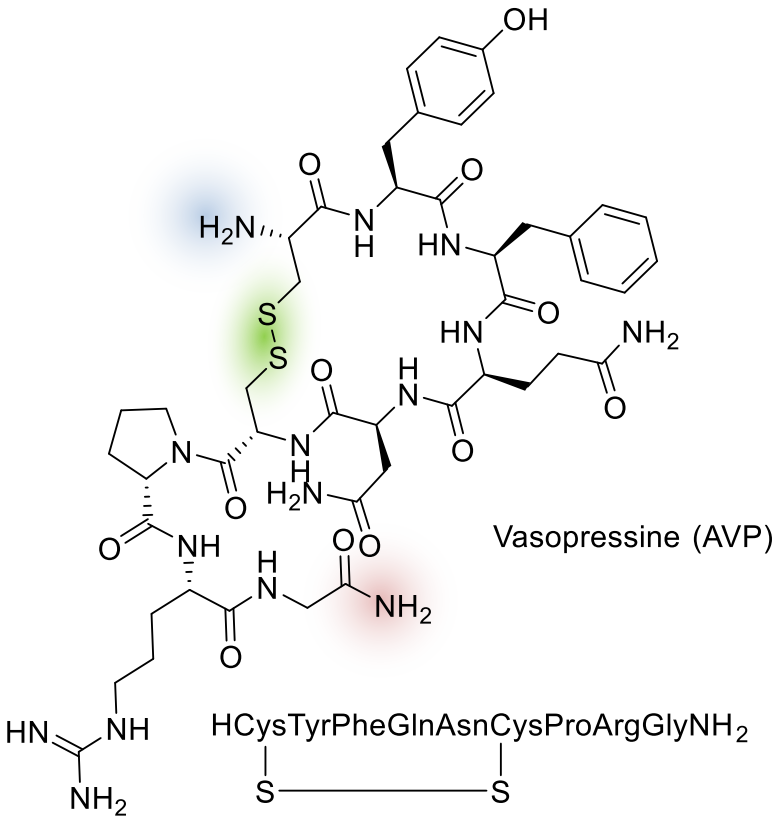
Vasopressin

Arginine vasopressin (AVP) in humans or ADH (*Antidiuretic hormon)*
9AA, Cter amide, one SS bridge

Secreted by the posterior pituitary, stimulated by the reduction of blood plasma volume.
Very close to oxytocin (crosstalk possible)



Vasopressine (AVP)

Main activities: antidiuretic, induces the re-adsorption of water.
Vasoconstrictor.

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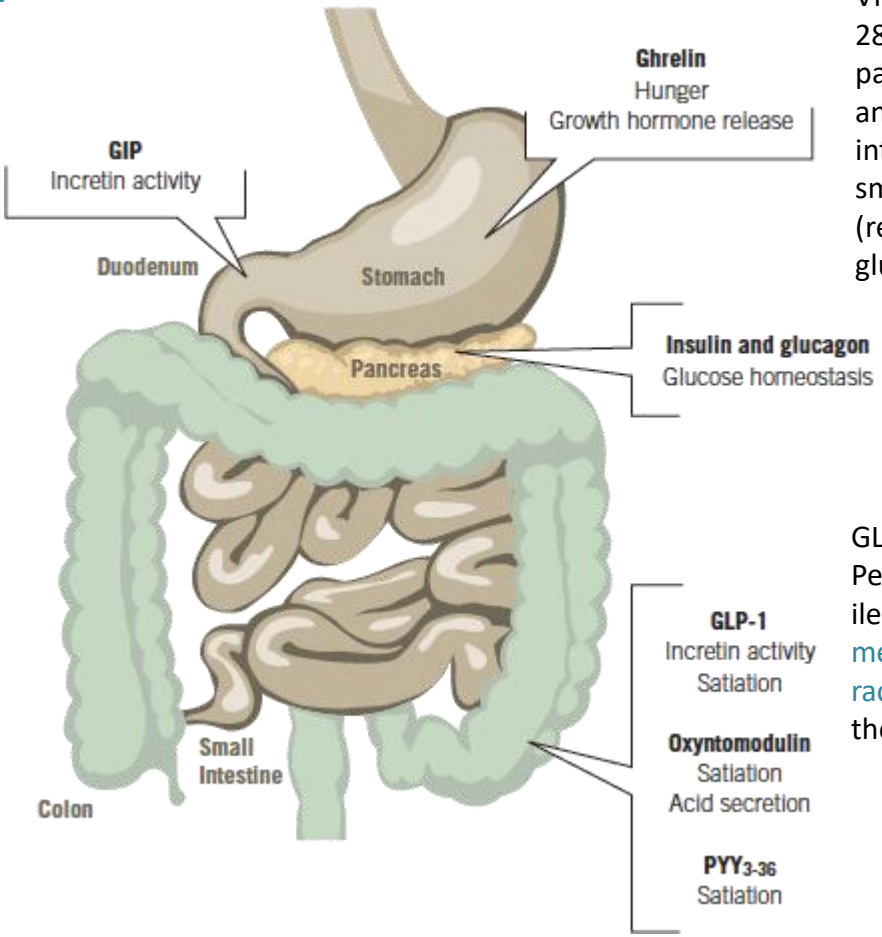
Peptide hormones

2. Gastroenteropancreatic axis: more than 100 identified bioactive peptides regulating digestion

Many operate as neuropeptides, are also produced in the CNS, have receptors and central action.

GIP (Gastric inhibitory peptide, 42AA) produced in the duodenum. Incretine: induces the production of insulin by the pancreas and helps to lower the level of sugar in the blood (insulinotropic)



VIP (Vasointestinal peptide 28AA) produced in the pancreas, stomach, intestines and hypothalamus. Induces intestinal motility, relaxation of smooth mucles, glycogenolysis (release of sugars) and increase glucose level in blood.

GLP-1 (Glucagon-like Peptide1, 30AA) secreted in ileon (small intestin) and medulla oblongata bulbe rachidien. Incretine. Action in the CNS: anorexigenic effect

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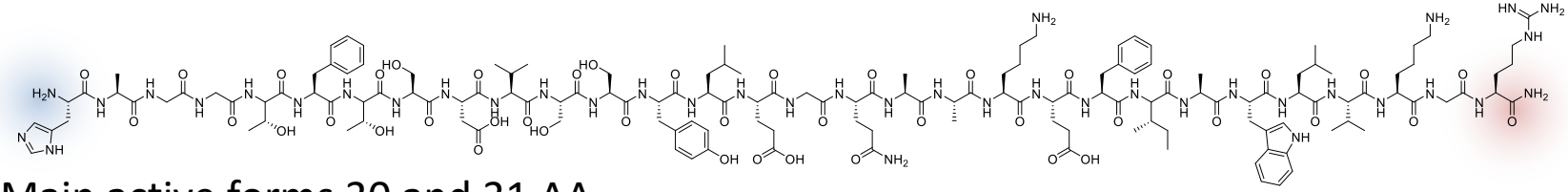
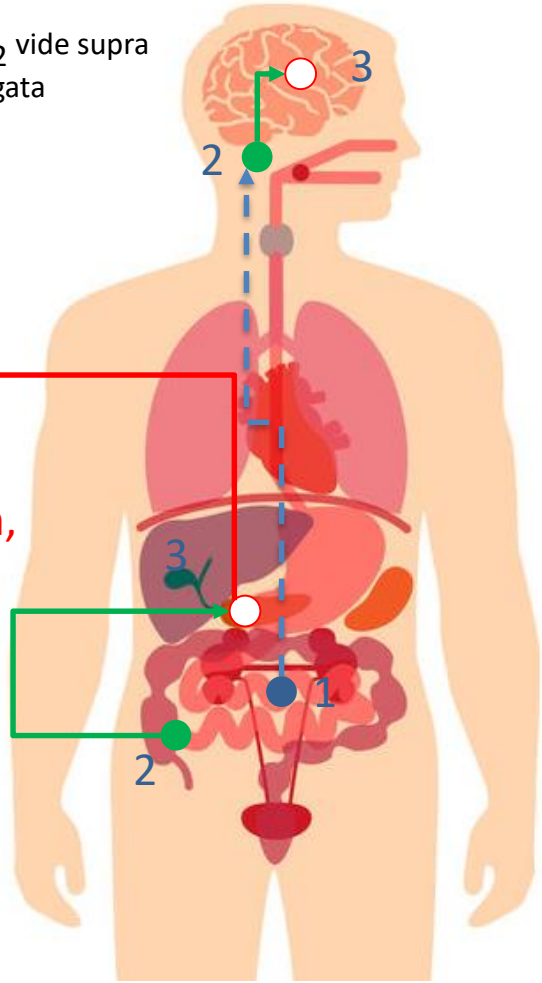
Glucagon-like peptide 1 GLP1: anorexigen and antidiabetic



Main active forms 30 and 31 AA

GLP1 7-Gly^{37} et 7-Arg ^{36} NH_2 vide supra

Secreted by intestine and medulla oblongata



3) Activation of GLP1R receptors of neurons : anorexigene effecg

2) Production of GLP1 by Medulla Oblongata

3) Activation of GLP1R of pancreas: insulin secretion, inhibition of glucagon production

Sugar level decrease

2) Production of GLP1 By Ileon

1) High sugar levels: glucose absorbed by intestine: Signal sent to CNS and production by Ileon

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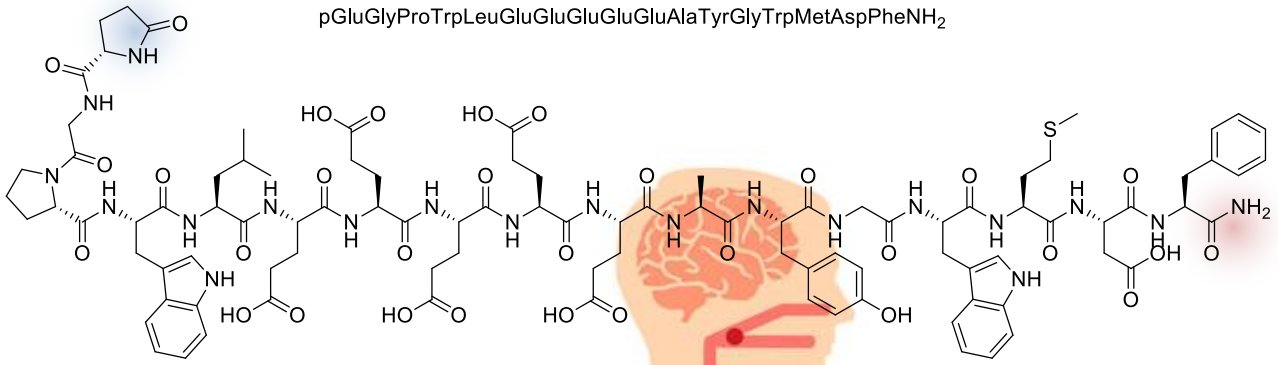
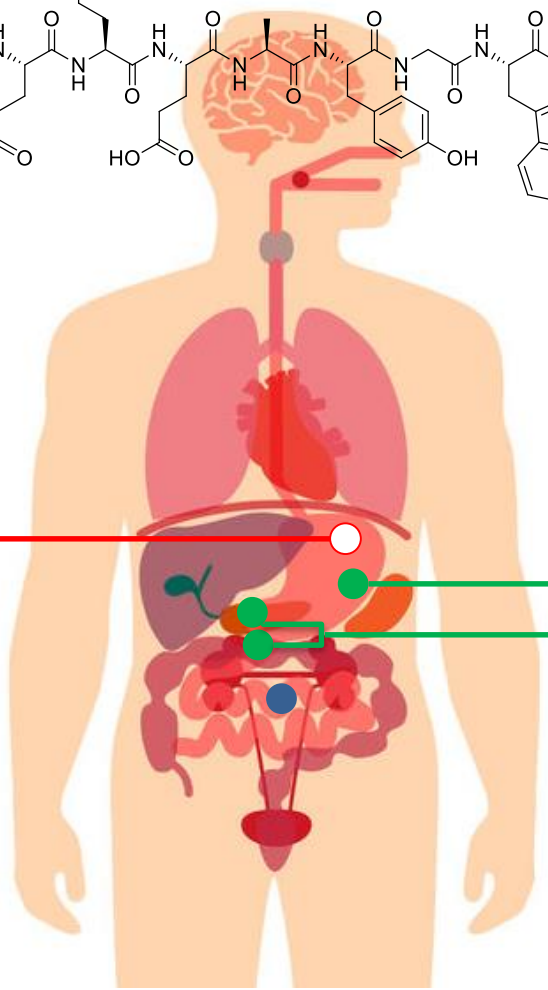
Gastrin

Principales formes actives 34, 17 et 14 AA, mêmes 5AA C ter que CCK





2) Production de gastrine
Par l'estomac, le pancreas le duodenum

3) Sécrétion d'HCl (CCK_B-R)

1) L'estomac se remplit

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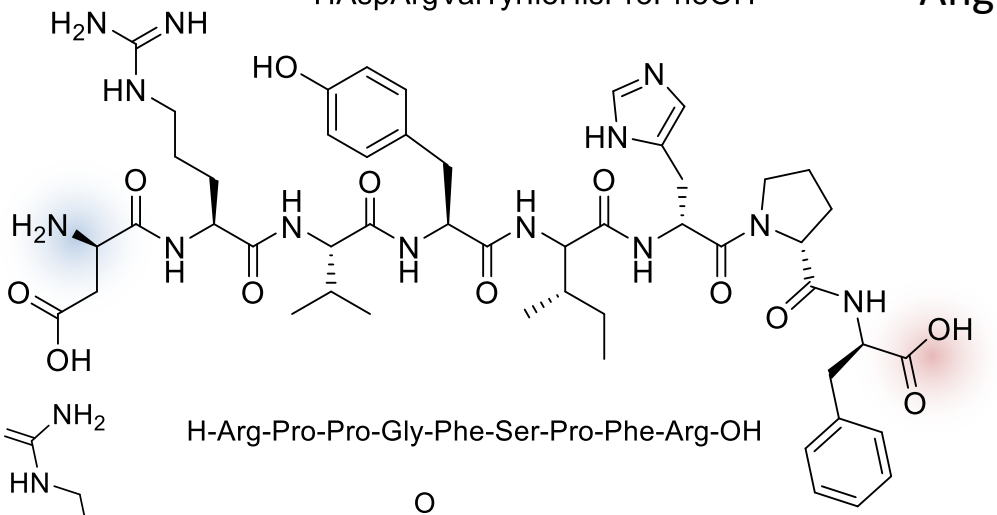
Peptide hormones

3. Cardiovascular System

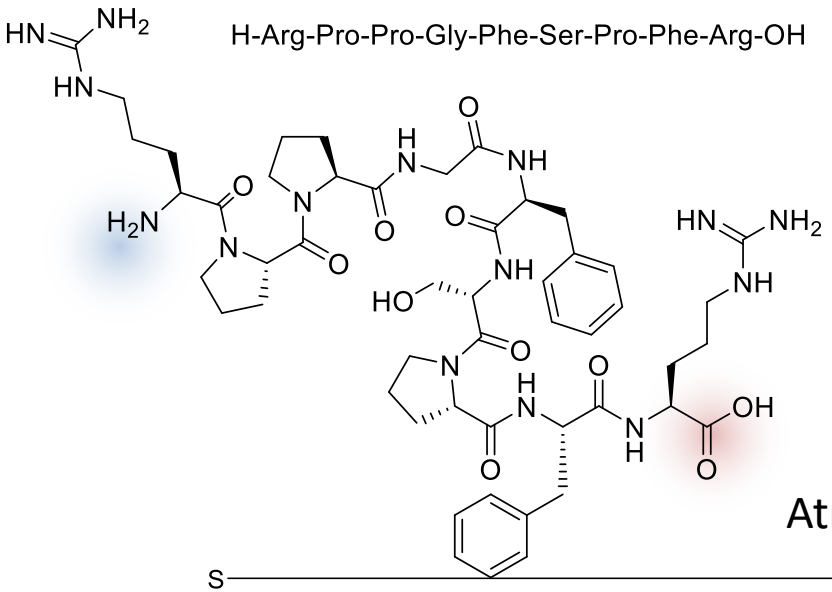
HAspArgValTyrIleHisProPheOH

Angiotensin II 8 AA

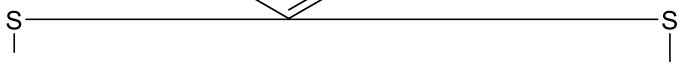


H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH

Bradykinin 9 AA



Atrial Natriuretic Peptide 28 AA



HSerLeuArgArgSerSerCysPheGlyGlyArgMetAspArgIleGlyAlaGlnSerGlyLeuGlyCysAsnSerPheArgTyrOH

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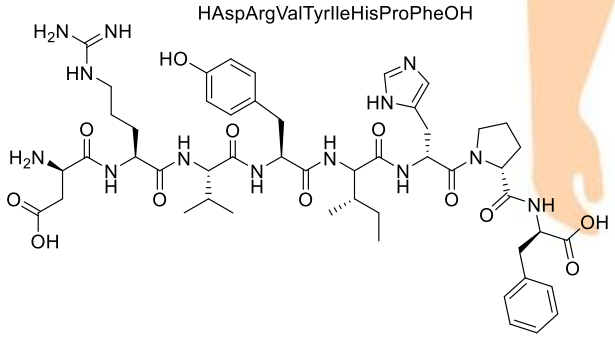
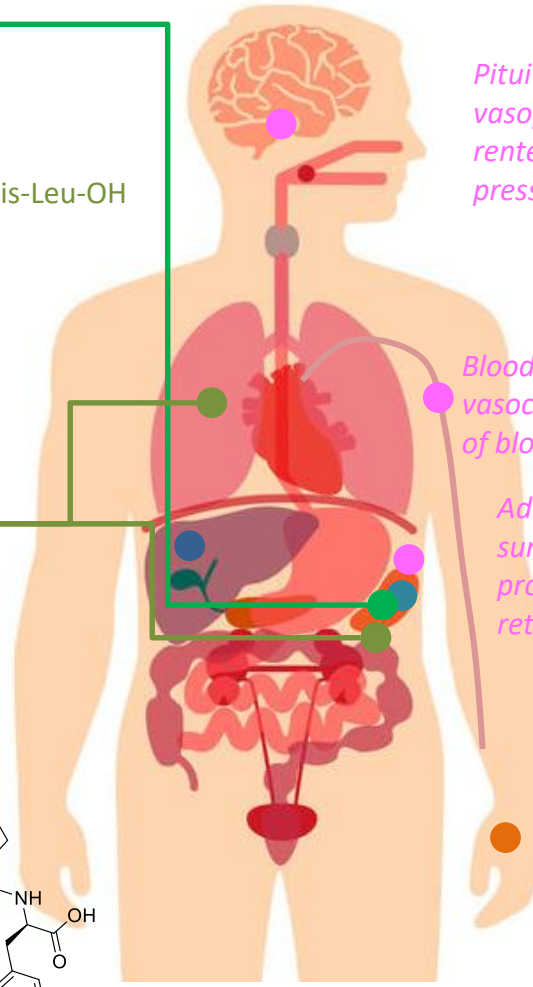
Angiotensin

3) Cleavage of angiotensinogene in agiontensine I by renin: 10AA

H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-OH

4) Cleavage of angiotensine I in agiotensine II : 8AA by ACE (lungs and kidneys)





5) Angio II induces

Pituitary gland: production of vasopressin and water rentetion, increase of blood pressure

Blood vessels: vasoconstriction and increase of blood pressure

Adrenal glands (glandes surrenales): aldosterone production and water retention

1) Decrease of blood pressure detected by liver: production of angiotensinogene 485 AA)

2) Decrease of blood pressure: production on Renin enzyme by kidneys

6) Increase of blood pressure inhibits Renin production: negative feedback

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Neuropeptides

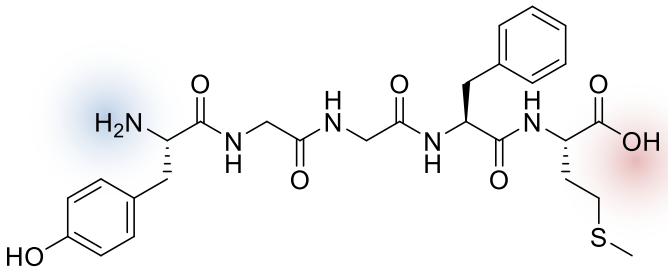
4. Central Nervous System:

Neuropeptides: on the contrary to hormones, they are expressed and released in neurons (and NOT in blood circulation), modulate neuronal communication *via* membrane receptors. 2 important classes :

Opioids peptides targeting opioid receptors mu (μ), delta (δ) and kappa (κ)

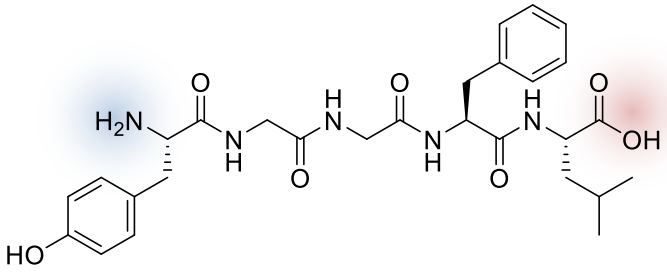
Met-Enkephalin
recepteurs
δ-opiod
analgesie

HTyrGlyGlyPheMetOH



Leu enkephaline

HTyrGlyGlyPheLeuOH



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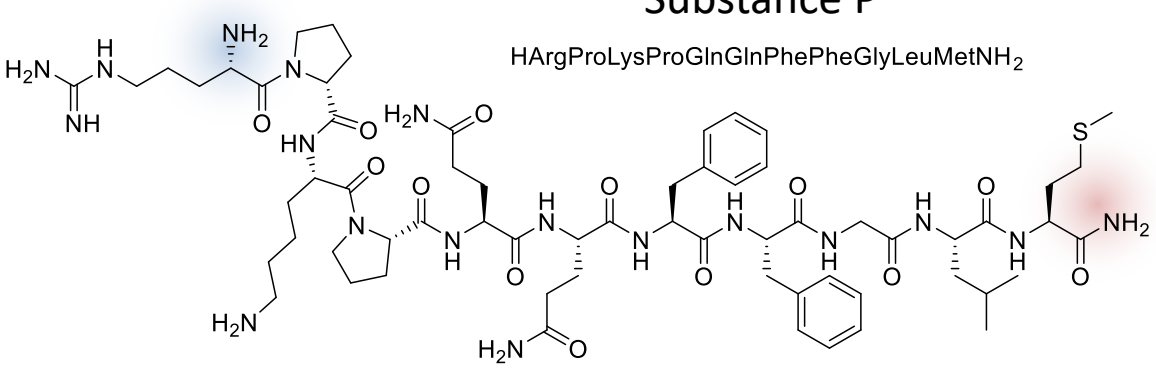
Neuropeptides

Tachykinines

Target NK receptors excite neurons, evoke behavioral responses, are potent vasodilators, and contract (directly or indirectly) many smooth muscles

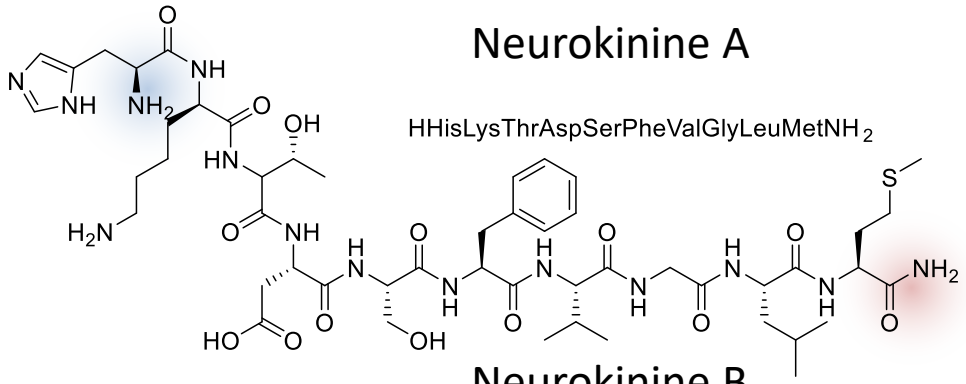
Substance P

HArgProLysProGlnGlnPhePheGlyLeuMetNH$_2$



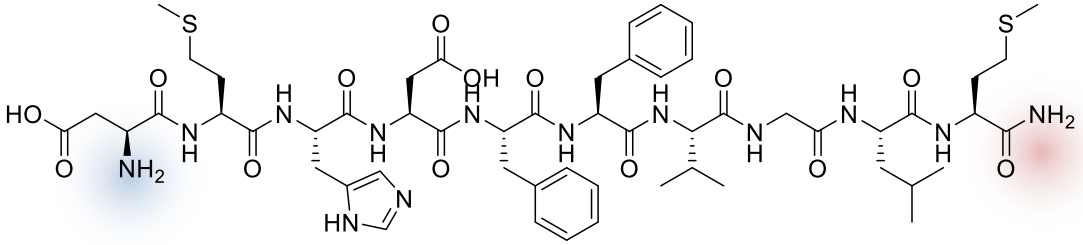
Neurokinine A

HHisLysThrAspSerPheValGlyLeuMetNH$_2$



Neurokinine B

HAspMetHisAspPhePheValGlyLeuMetNH$_2$



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Peptide hormones

5. other peptide hormone

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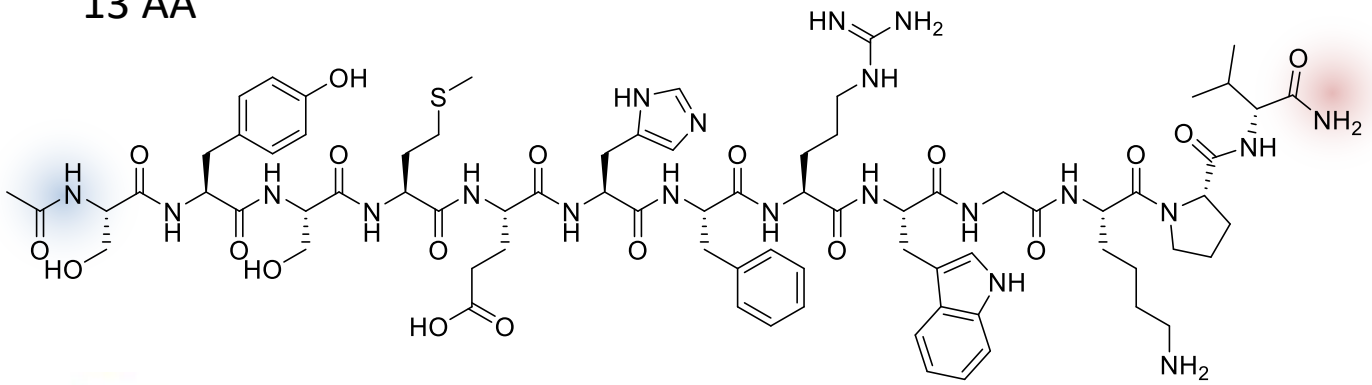
α-MSH

Ac-SerTyrSerMetGluHisPheArgTrpGlyLysProVal-NH_2

13 AA



Produced in the skin by melanocytes upon UV stimulation.
Inthe brain: stimulate apetite and sexual behavior

highly conserved in animals (ex: xenope)

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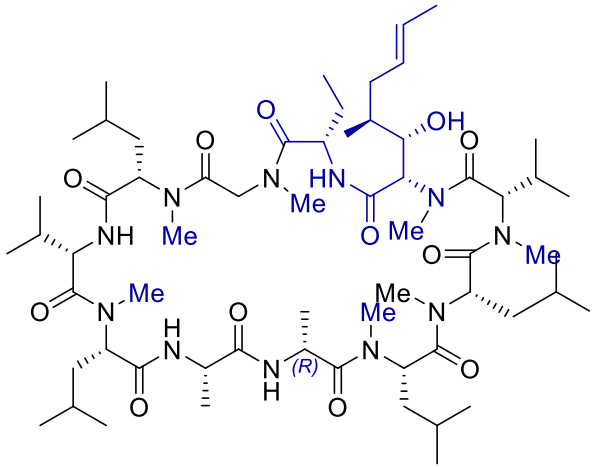
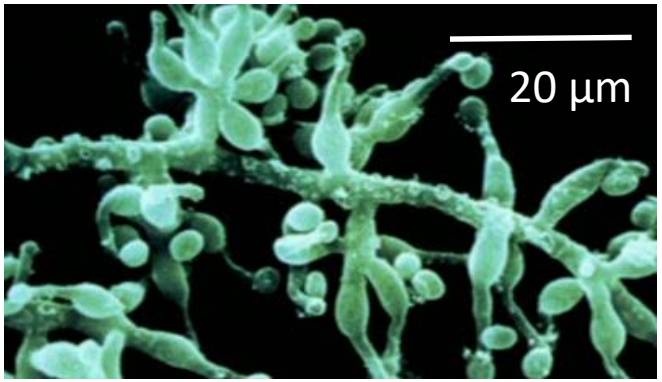
Cyclosporin: immunosuppressant

11 AA, Immunosuppressant, used to treat auto immune diseases and graft rejection
Synthetized by a non ribosomalpeptide synthethase.
Isolated from a microscopic fungi (1976), *Tolypocladium inflatum* from soil samples.
Inhibits an enzyme (cyclophiline) et blocks lyphocytes T





c[MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-DAla-MeLeu]

Abu= aminobutyric acid
MeBmt = Butenyl-methyl-L-threonine
Sar= sarcosine (i.e. N-Methyl Glycine)

cyc[MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-Dala-MeLeu]

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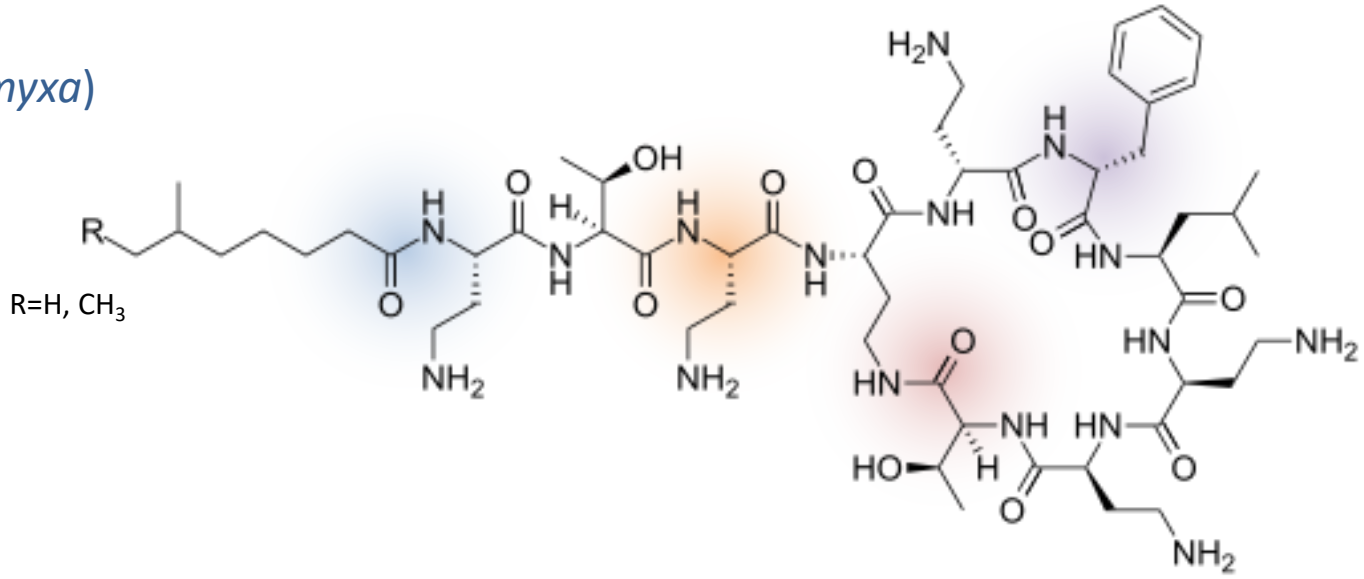
Antibacterial Peptides

Mainly produced by bacteria and fungi. They kill microorgnisms or inhibit their growth

Polymyxin B
(*Bacillus polymyxa*)



Affect the integrity of membrane. Interact specifically with liposaccharides which consituted gram negatif bacteria membranes
Administrated parenterally (injection), or by inhalation
N terminal acylated by lipid chain, cyclisation C ter with diaminobutyric acid, (Dab) and DPhe

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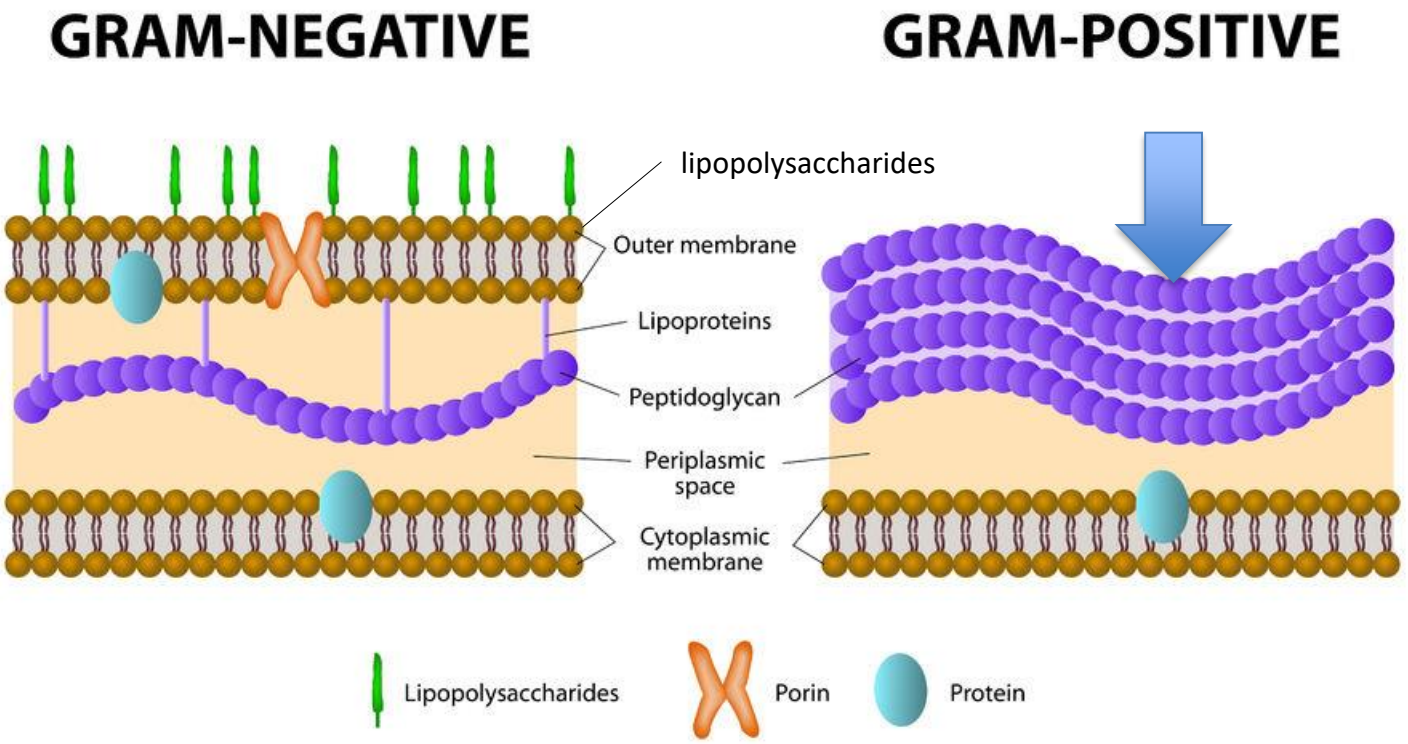
Bacteria membranes



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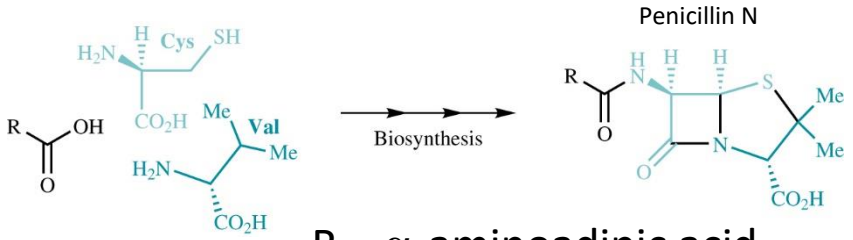
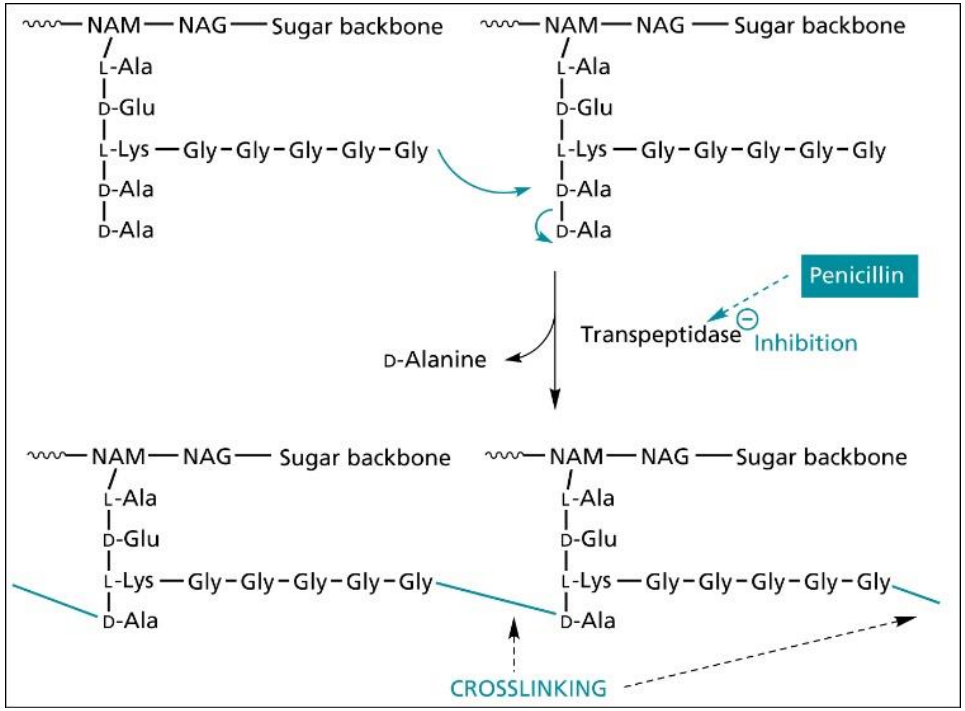
Antibacterial Peptides

Penicillins, Cephalosporins (pseudodipeptides)

Efficients against Gram +, and somme Gram – bacteria, inhibition of synthesis of inter-peptidoglycanes links in the bacteria membrane





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Peptides venoms and poisons

Defense against aggression or for predation : Isolated from snakes, frogs, spiders, scorptions, bees, anemones…

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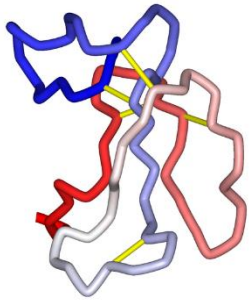
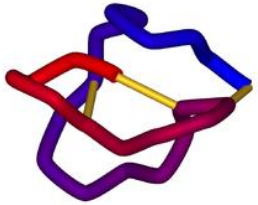
Peptides venoms and poisons

multiple SS bonds (Cystein knots) that blocks the 3D structure and makes them resistant to proteases.

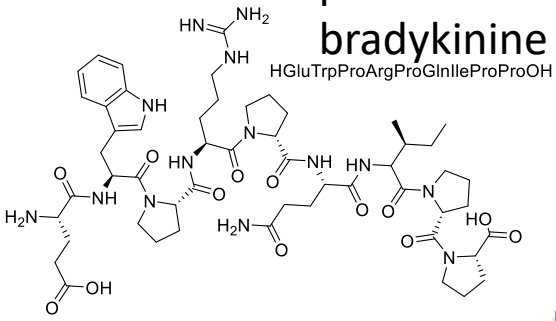


α-bungarotoxin (74-mer isolated from venom snake *Bungarus*)



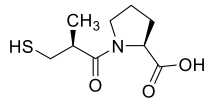
ω-conotoxin (isolated from cone snail *Conus magus*)
> Ziconotide (Prialt®)

Inhibition of ACE
antihypertensive,
potentialise les effets de la bradykinine



9AA

téprotide



captopril

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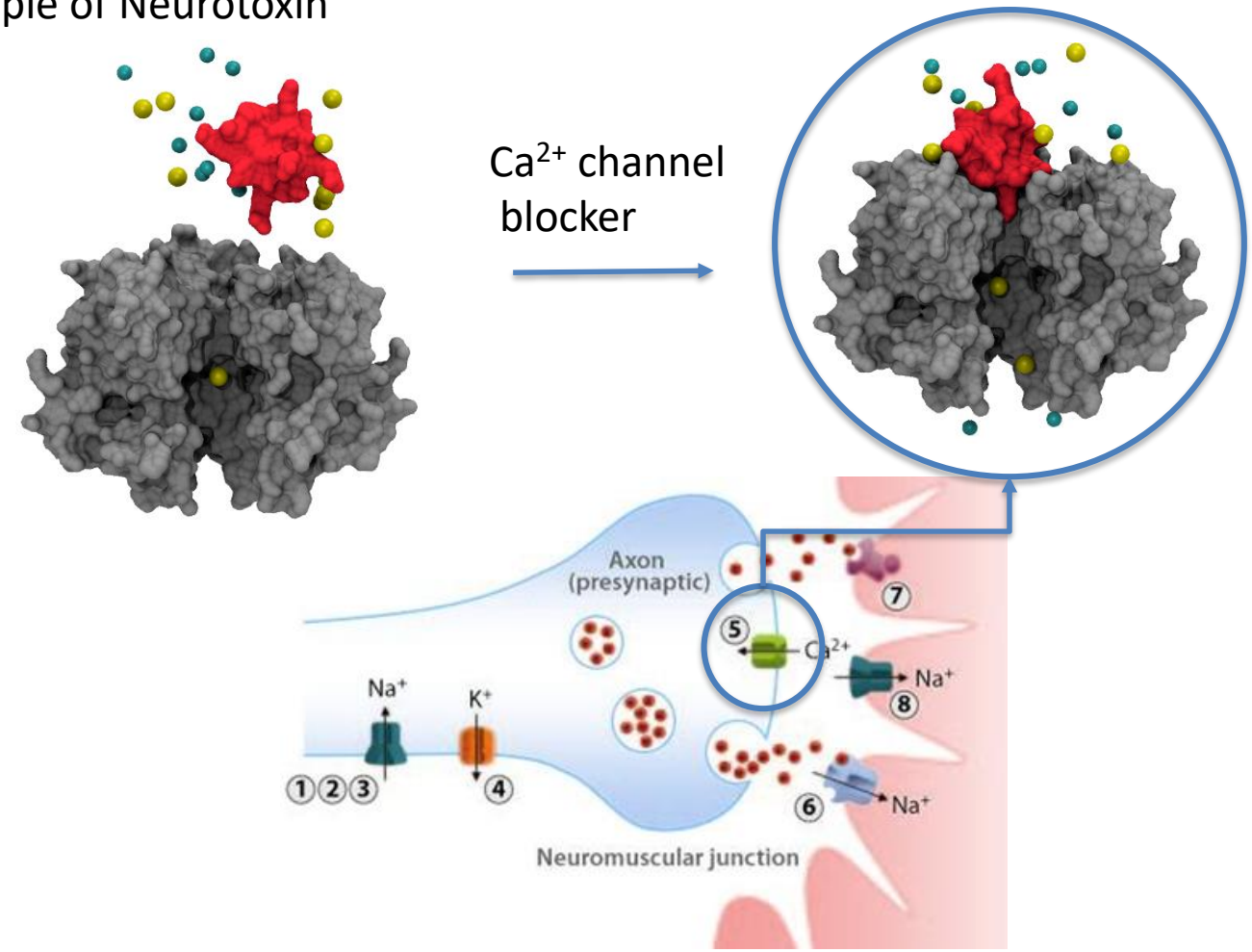
Molecular targets

Hemotoxins target the blood coagulation
Neurotoxins target the neuromuscular junction

Example of Neurotoxin



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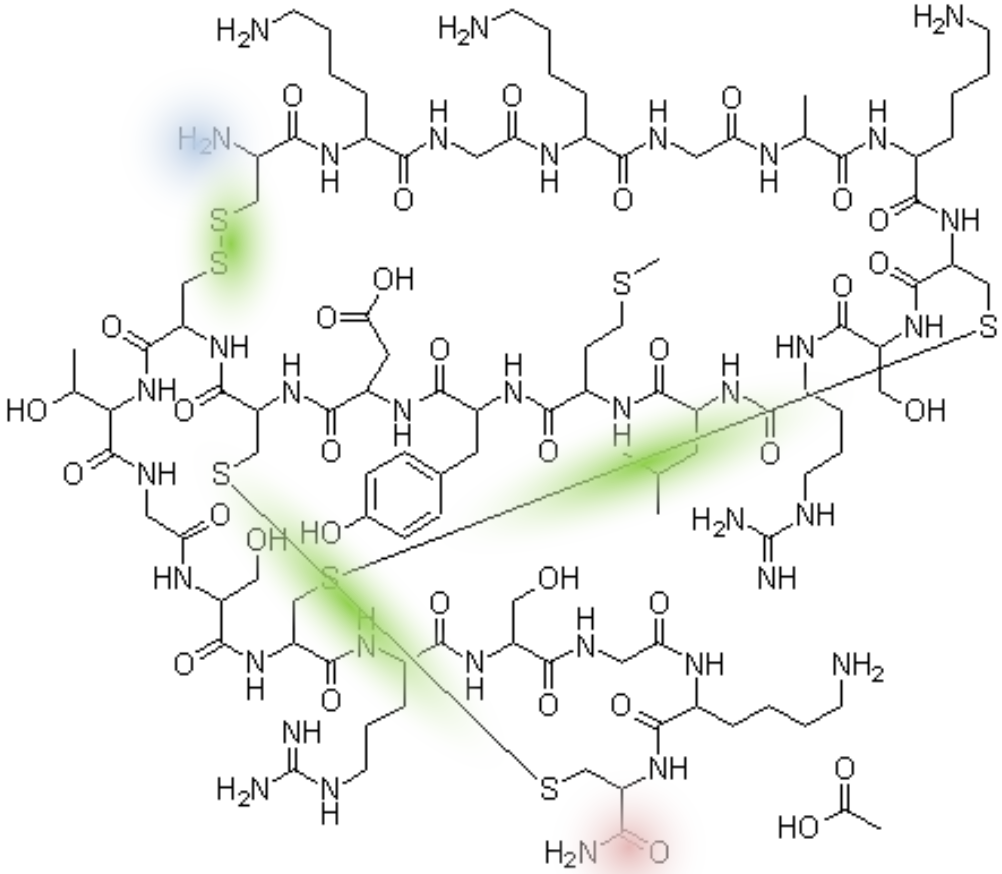
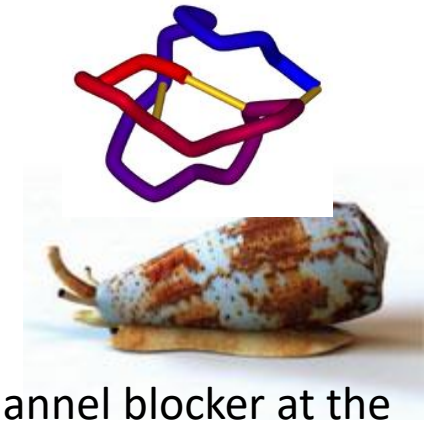
Ziconotide

ω-Conotoxine MVII A isolated from sea snail Conus Magus



H-Cys-Lys-Gly-Lys-Gly-Ala-Lys-Cys-Ser-Arg-Leu-Met-Tyr-Asp-Cys-Cys-
Thr-Gly-Ser-Cys-Arg-Ser-Gly-Lys-Cys-NH_2

Ziconotide
Prialt 2004



Ion channel blocker at the neuromuscular junction. It provokes the inhibition of pro-nociceptive compounds (glutamate, substance P, calcitonin gene related peptide) which are responsible of pain feeling.

Ziconotide: intrathecal injection in spinal cord

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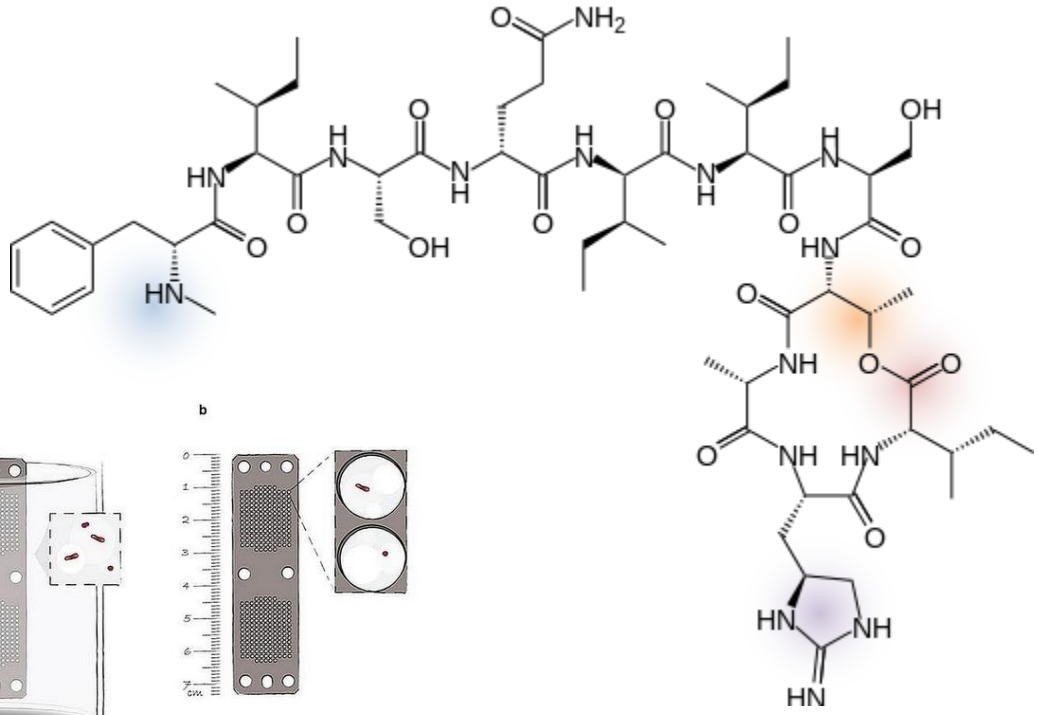
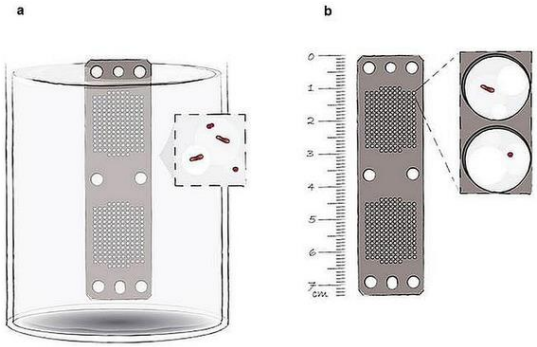
Teixobactine

Produced in the soil by bacteria (Novobiotic) *Eleftheria terrae*.
System allowing soil nutrients to enter and isolating the bacteria
N-Methylated, Cter cyclized via an ester linkage with threonine (cyclized by lactone = depsipeptide) Non-natural analogous Arg amino acid: enduracididine





L. L. Ling, et al., Nature **2015**, 517, 455–459.

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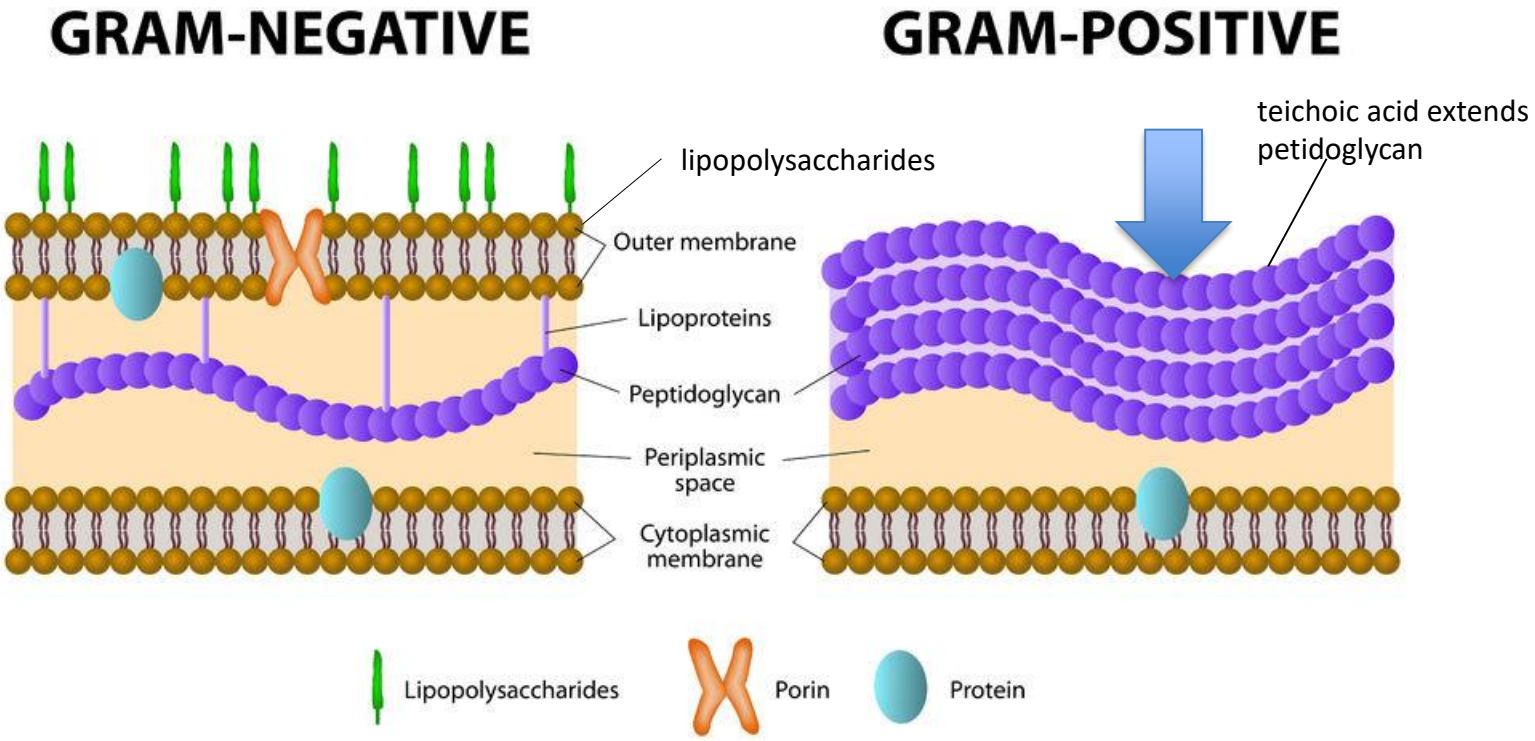
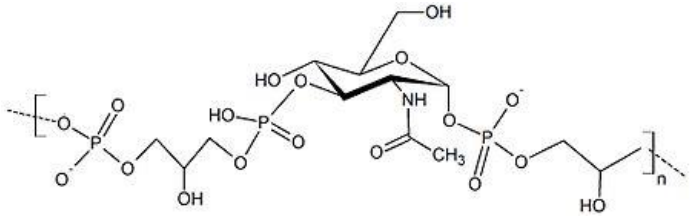
Bacteria membranes



Teixobactin inhibits the synthesis of peptidoglycan (idem penicillin that inhibits an enzyme) binding to teichoic acid and lipids, substrates of enzymes that synthesize peptidoglycan.

Active against gram-positive bacteria.



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II. PEPTIDES AS DRUGS?

II.1. Pro and Cons

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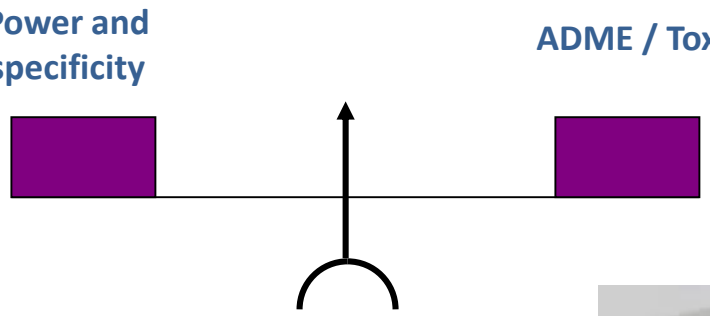
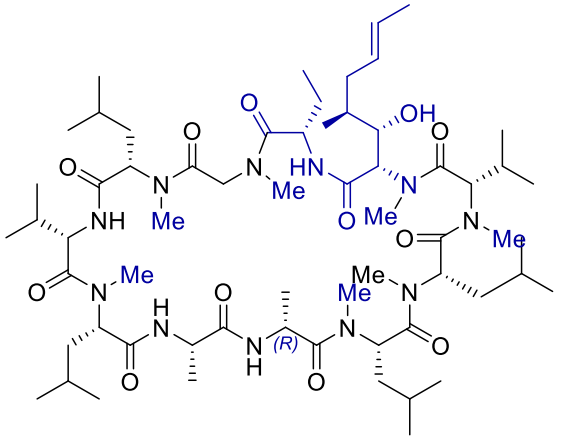
Drugs ?

"A chemical substance used for the treatment, care, prevention, diagnosis of diseases or otherwise used to improve physical or mental well-being."



Peptides DO NOT comply to Lipinski rules but ….display unique behaviors and properties. Example : cyclosporine…can be taken by oral administration !



- **MW< 500 g/mol**
- **clog P < 5**
- **No more than 5 H-bond donnors**
- **No more than 10 H-bonds acceptors**

CA Lipinski, ***Adv. Drug Del. Rev.*** **1997,** *23*, ***3***.

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nature
International weekly journal of science

14 September 1978 Vol 275 No 5676 pp81-162

The decline and fall of protein chemistry?

from Alan D. B. Malcolm

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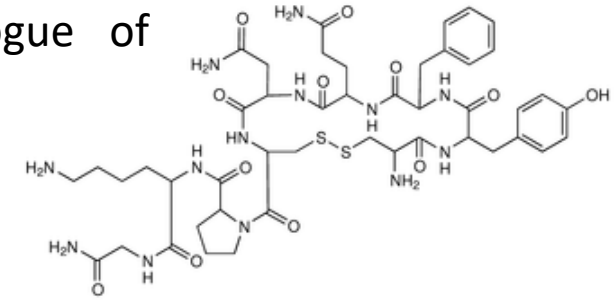
Peptide drugs: the market

Market opens in 1970 with **Lypressin** (analogue of vasopressine, hypertensive)



❖ Today more than **60 peptides drugs are marketed**

❖ 140 peptides are in clinical trials and more than 600 in preclinical development

❖ **Market** : 2018 US$ 25.4 billion

❖ **Blockbusters**

Drug Disc. Today, **2015,** *20*, 122-128.

US$ 7.9 billion (2013)

Analogue insuline

US$ 2.3 (2011)

Leuprorelin : agoniste (hyperstimulation) des recepteurs GnRH-R
Traitement des cancers hormonaux dépendants, puberté précoce.

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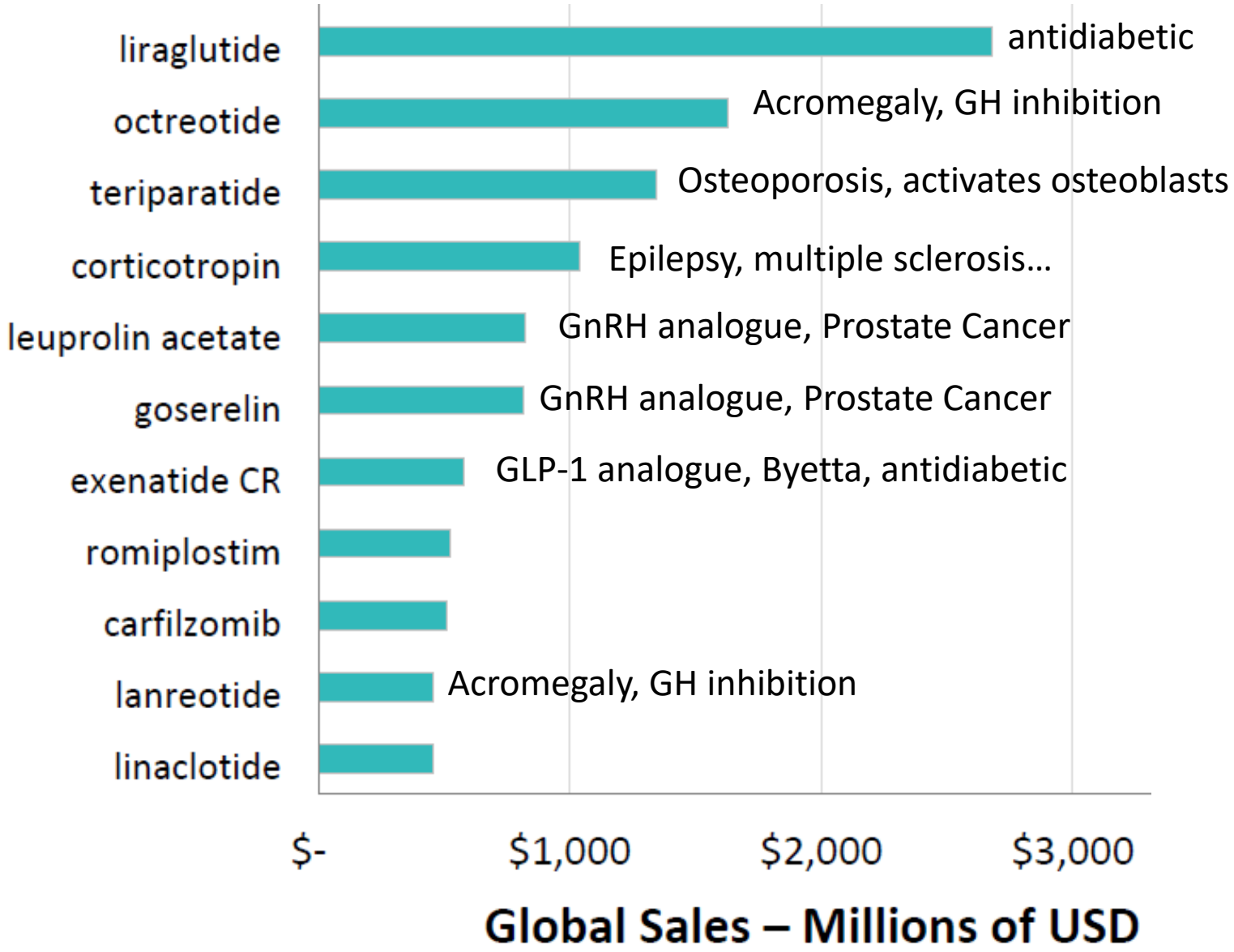
Peptide Top Sellers, 2015



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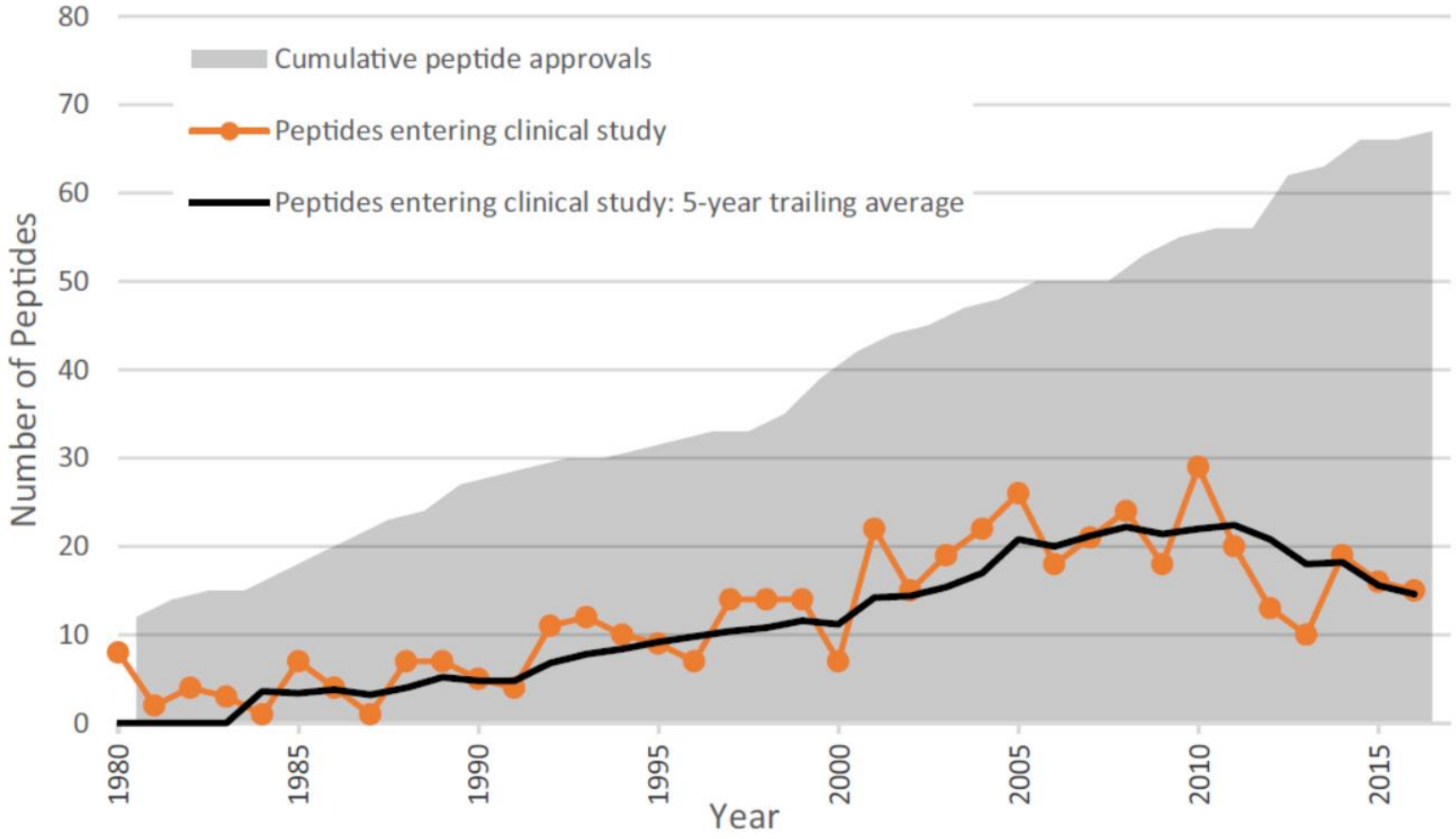

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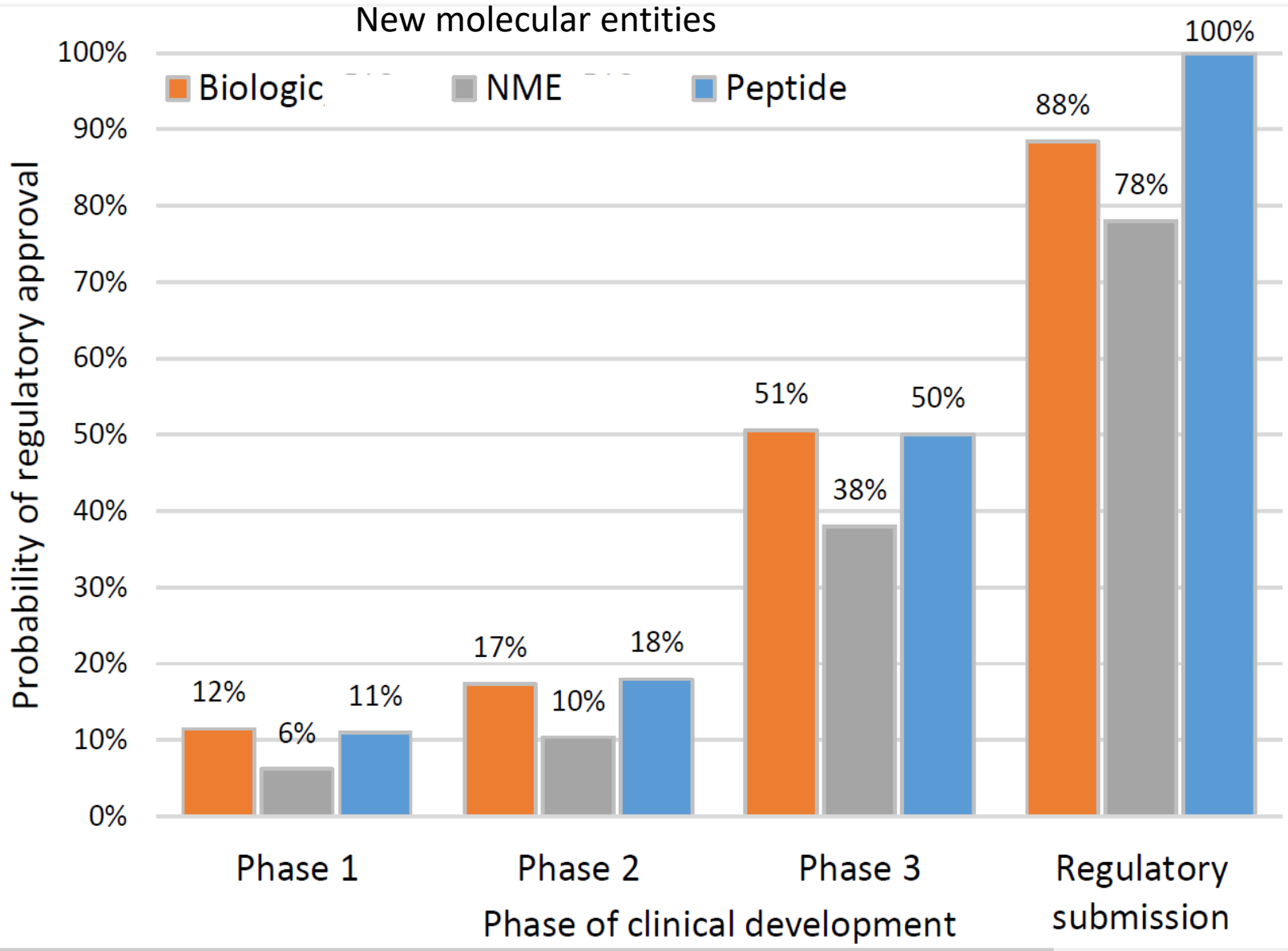
Attrition rate



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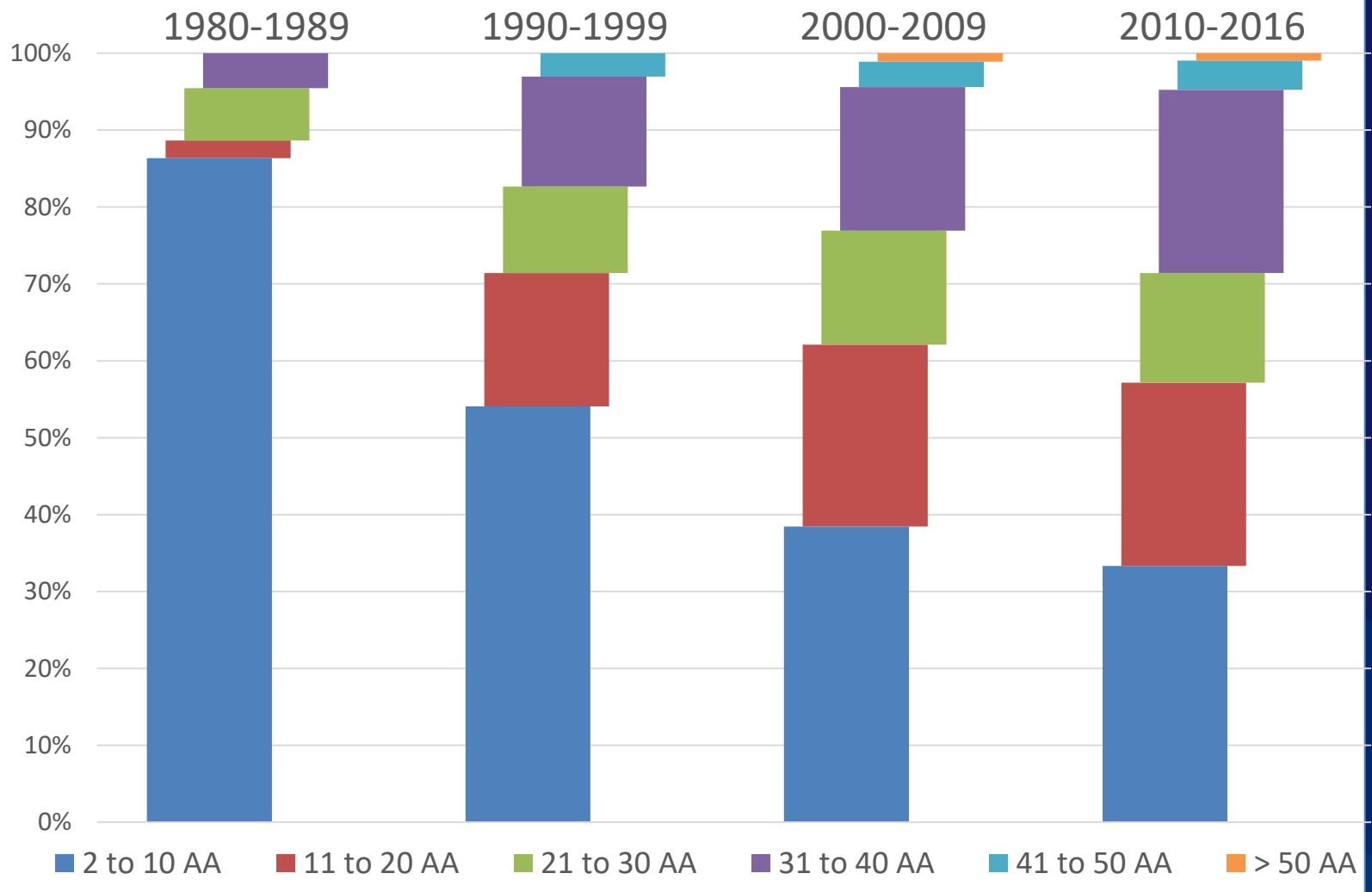
Length of peptides entering clinical development



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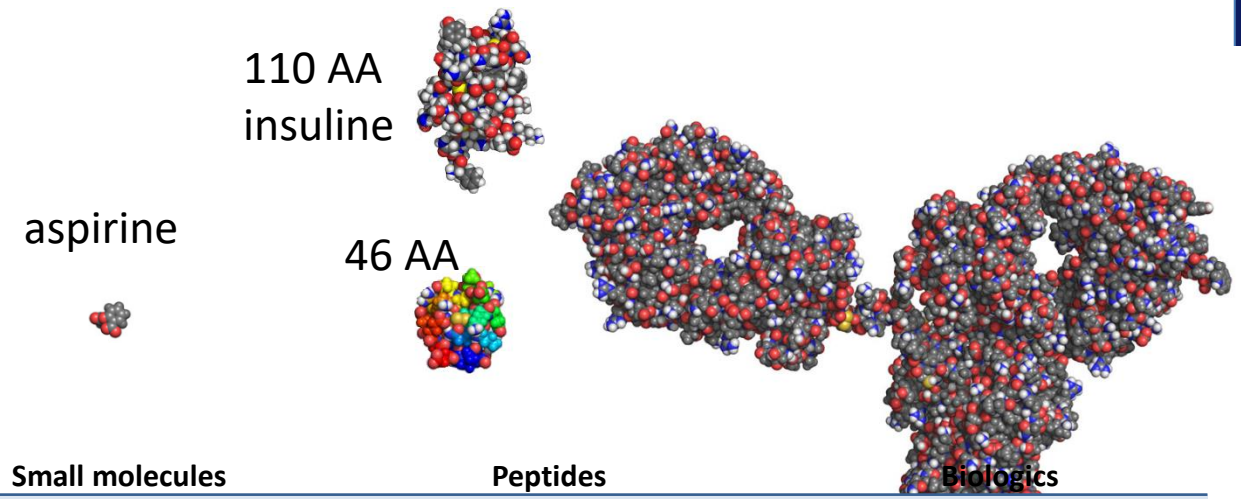
Peptides: the best of two worlds



	Small molecules	Peptides	Biologics
Size	<1KDa	1-3 kDa	>10 kDa (mAb 150 kDa)
Immunogenicity	No	No (adjuvant is needed if you need to make vaccines)	Potential
Selectivity	Weak to Very good	Very good And can be optimized	Very good
Optimisation d'un lead	Difficulté moyenne	Facile et rapide	n.a.
Taux d'attrition	high	weak	weak
Production	Chemical synthesis	Chemical synthesis or recombinant (and NCL potential)	Recombinant, complexe
Access to intracellular targets (cell-penetration)	Good	possible and can be optimized	Faible
Delivery	All routes including per os	Parenteral route mainly: i.v., s.c. but non-parenteral possible	Parenteral route : i.v., s.c.
Action	mainly **antagonistes**	mainly **agonistes** (but new antagonists (PPI inhibitors) in dvlpt	mainly **antagonistes** (antibodies)
Cost	weak	Weak to average but decreases	High

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Limitations of peptide drugs

•**Weak biodisponibility**: they are degraded by peptidases (endo, exo) before reaching blood stream : half-life of several minutres. Can be optimized, new adminsitration modes

enzyme	type	Catalytic residue/site	Where?	Cleavage site?
pepsine	endopeptidase	Asp	stomac (pH 1,8-4,4) pancreas->duodenum	Xxx-Aromatic
trypsine	endopeptidase	Ser	pH 6 pancreas->duodenum	Lys/Arg-Xxx
chymotrypsine	endopeptidase	Ser	pH 6	hydrophobic/aromatic-Xxx
carboxypeptidase pancreatic A1	exopeptidase	Metalloprotaase Zn	pancreas->duodenum pH 6	Xxx-AaaOH
thrombine	endopeptidase	Ser	blood	Lys/Arg-Xxx
plasmine	endopeptidase	Ser	blood	Lys/Arg-Xxx

•**Quikly cleared, kidney clearance and hepatic clearance**

•~~Often several activities and several targets: considered as not very specific because they are non-selective of a single target. This is not a generality and Optimizable!~~

•~~Production cost~~. This is no longer the case : 1$/AA/g

•Sensibles à l'oxydation, hydrolyse : Optimisable, la plupart sont très stables

•**Weak solubility** (DMSO) mais puissants donc faible concentration

•**Difficulties to cross membranes** compared to small hydrophobic molecules

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Advantages of Peptide drugs

- Many leads: Discovery of new active peptides (natural products, xxxics, fractionation of proteins ...) and easy and fast optimization (good starting point in comparison of small molecules
- Low immunogenicity
- Efficacy especially on extracellular, polar targets and shallow binding pockets (many GPCR ligands)
- Few nonspecific drug / drug interactions and off target
- No accumulation in the tissues
- Very good selectivity for the target (or easily optimized)
- Many technologies and generic strategies associated with peptides: combinatorial libraries, phage display, conjugations, CPP, vectors, etc.
- Customizable
- Low toxicity, good tolerance
- Metabolites easy to predict: amino acids
- Shorter development time
- Well-known synthesis protocols
- Very good efficiency

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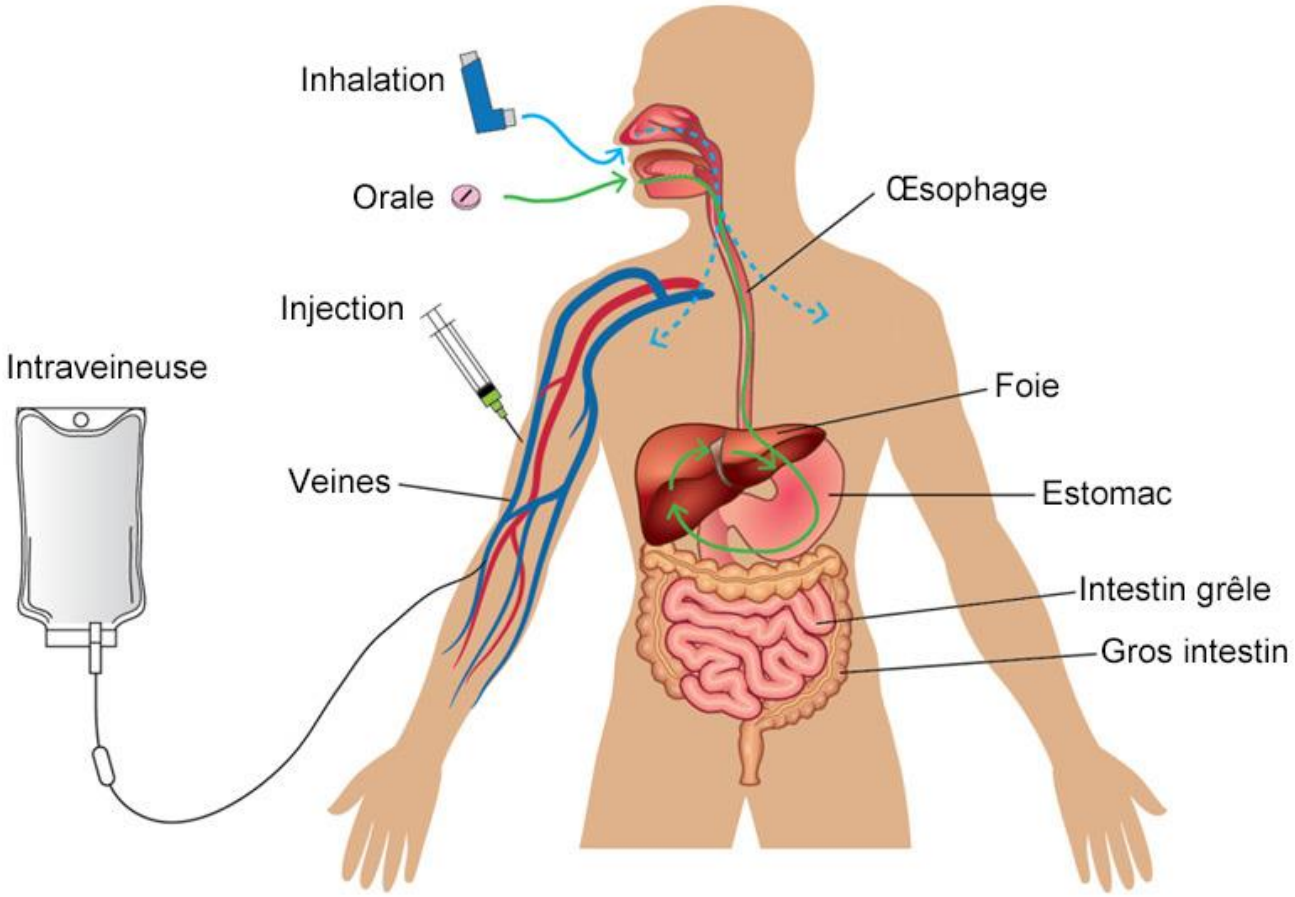
Alternative mode of administration



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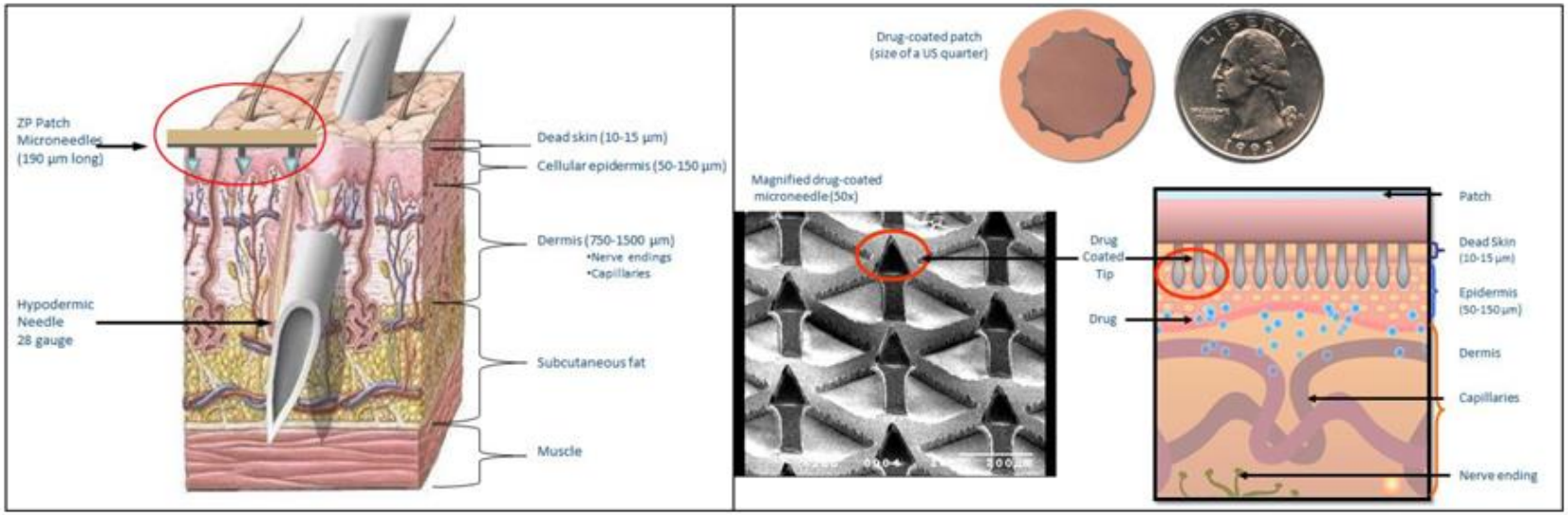
Transdermal patches
Zosano Pharma (CA, USA) - Macroflux



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Oral administration possible?

Strategies to improve the oral bio-distribution without chemical modification of the active ingredient

- Use of gastro-resistant capsules
- Use of enzyme inhibitors
- Use of Absorption Enhancers and Transient Permeability Enhancer
- Use of muco-adhesive polymers
- Encapsulation systems - nano particles

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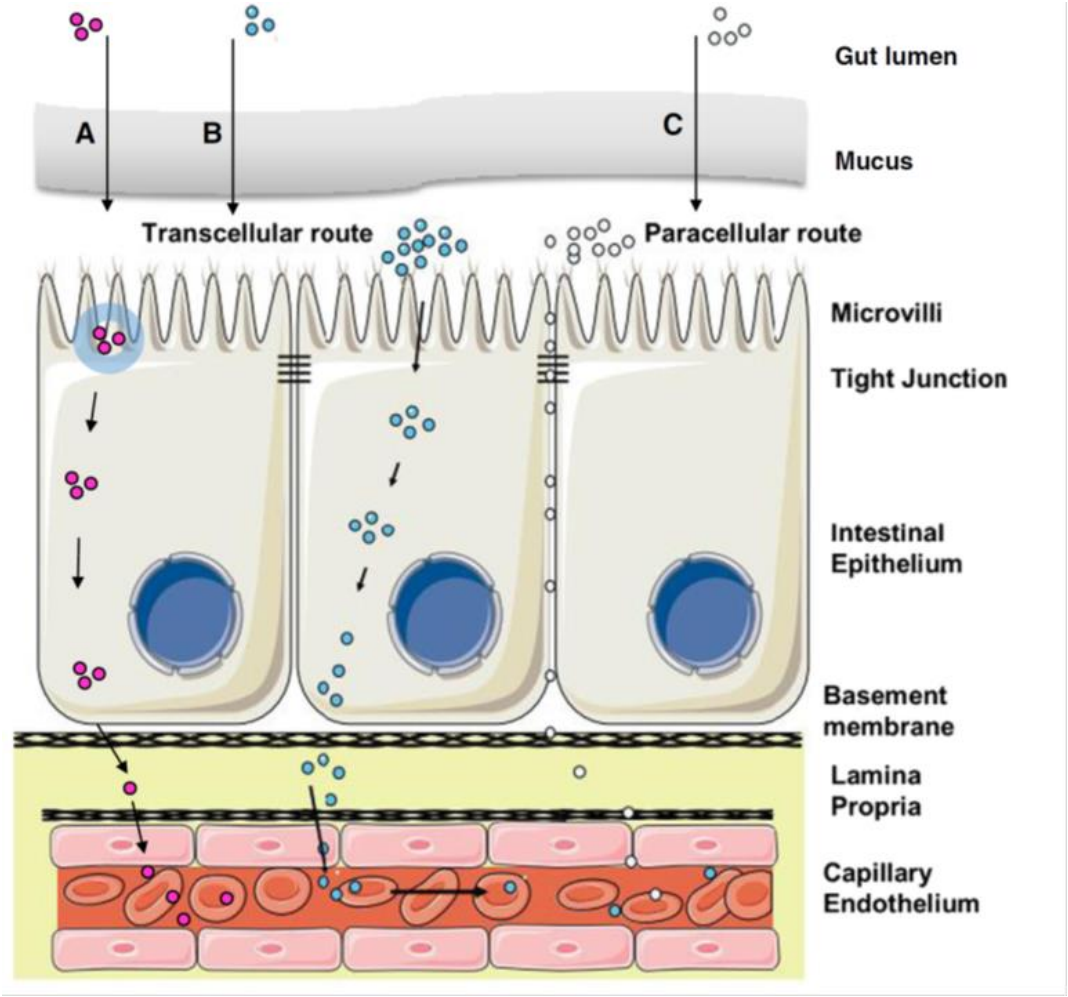
Small intestine - absorption and crossing

Small molecule drugs: small, non-polar, neutral molecules

Peptides : larges molecules, often polar and charged



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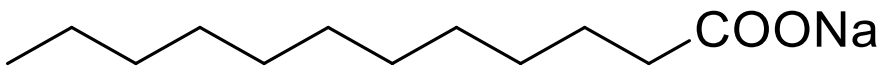
Chiasma Ltd - Mycapssa®

http://www.chiasmapharma.com/tpe

Transient Permeability Enhancer (TPE®)

1) Protection from enzymatic degradation
2) Temporary expansion of thight junctions: para cellualr transport

Penetration enhancer: lauric acid sodium carboxylate



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Increasing stability by chemical modification

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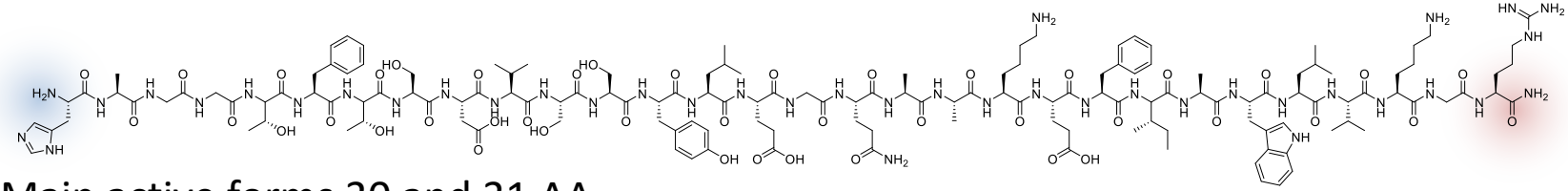
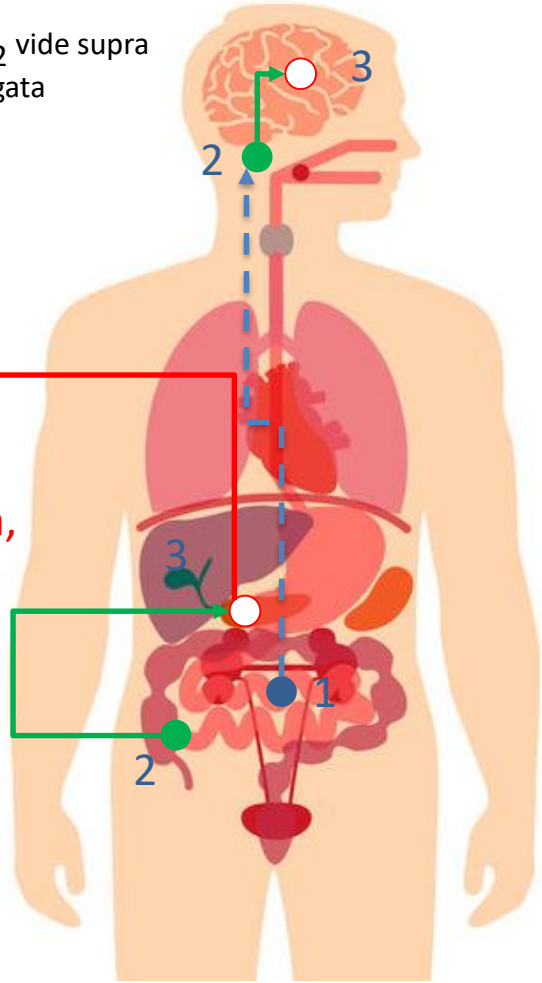
Glucagon-like peptide 1 GLP1: anorexigen and antidiabetic



Main active forms 30 and 31 AA

GLP1 7-Gly^{37} et 7-Arg ^{36} NH_2 vide supra

Secreted by intestine and medulla oblongata



3) Activation of GLP1R receptors of neurons : anorexigene effects

2) Production of GLP1 by Medulla Oblongata

3) Activation of GLP1R of pancreas: insulin secretion, inhibition of glucagon production
Sugar level decrease

2) Production of GLP1 By Ileon

1) High sugar levels: glucose absorbed by intestine: Signal sent to CNS and production by Ileon

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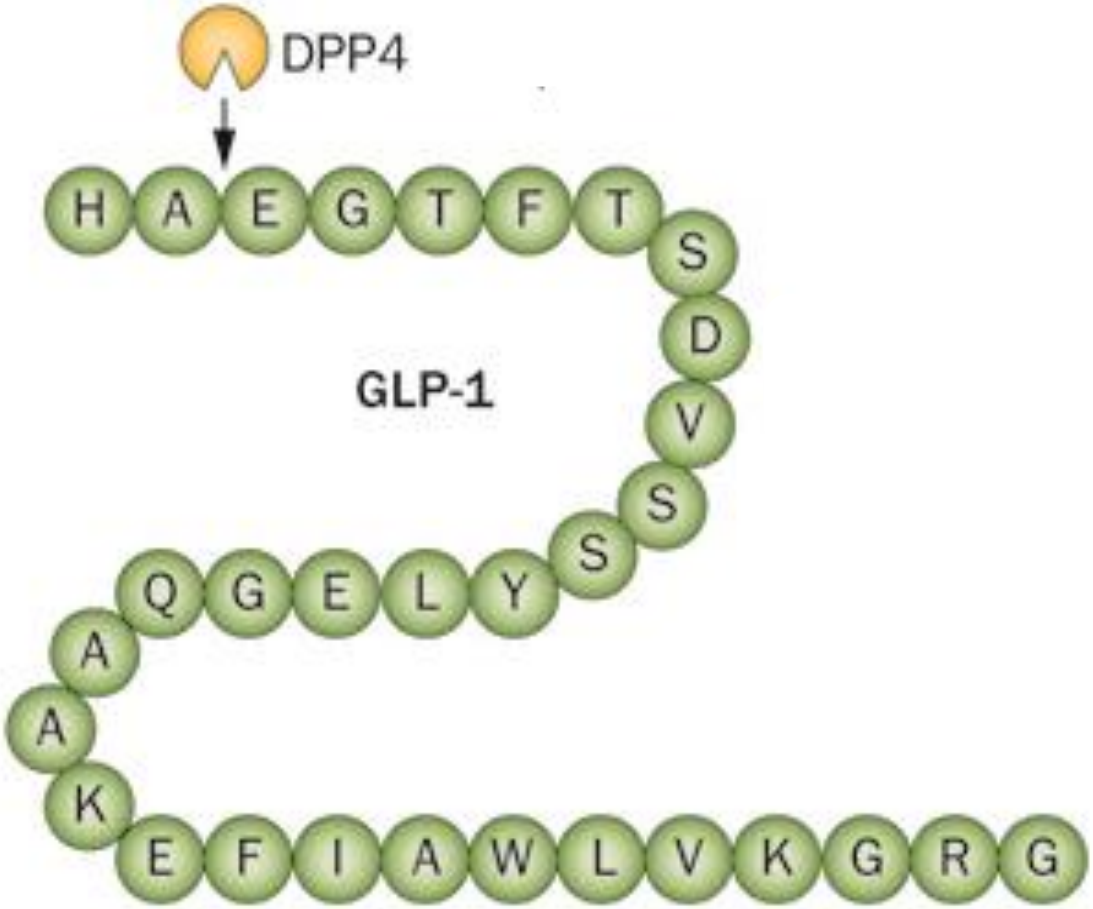
GLP-1 Structure and stability

$t_{1/2}$ = 2 min (**degradation** by dipeptidyl peptidase 4 DPP-4 and glomerular filtration in kidney)



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GLP-1 Structure and stability

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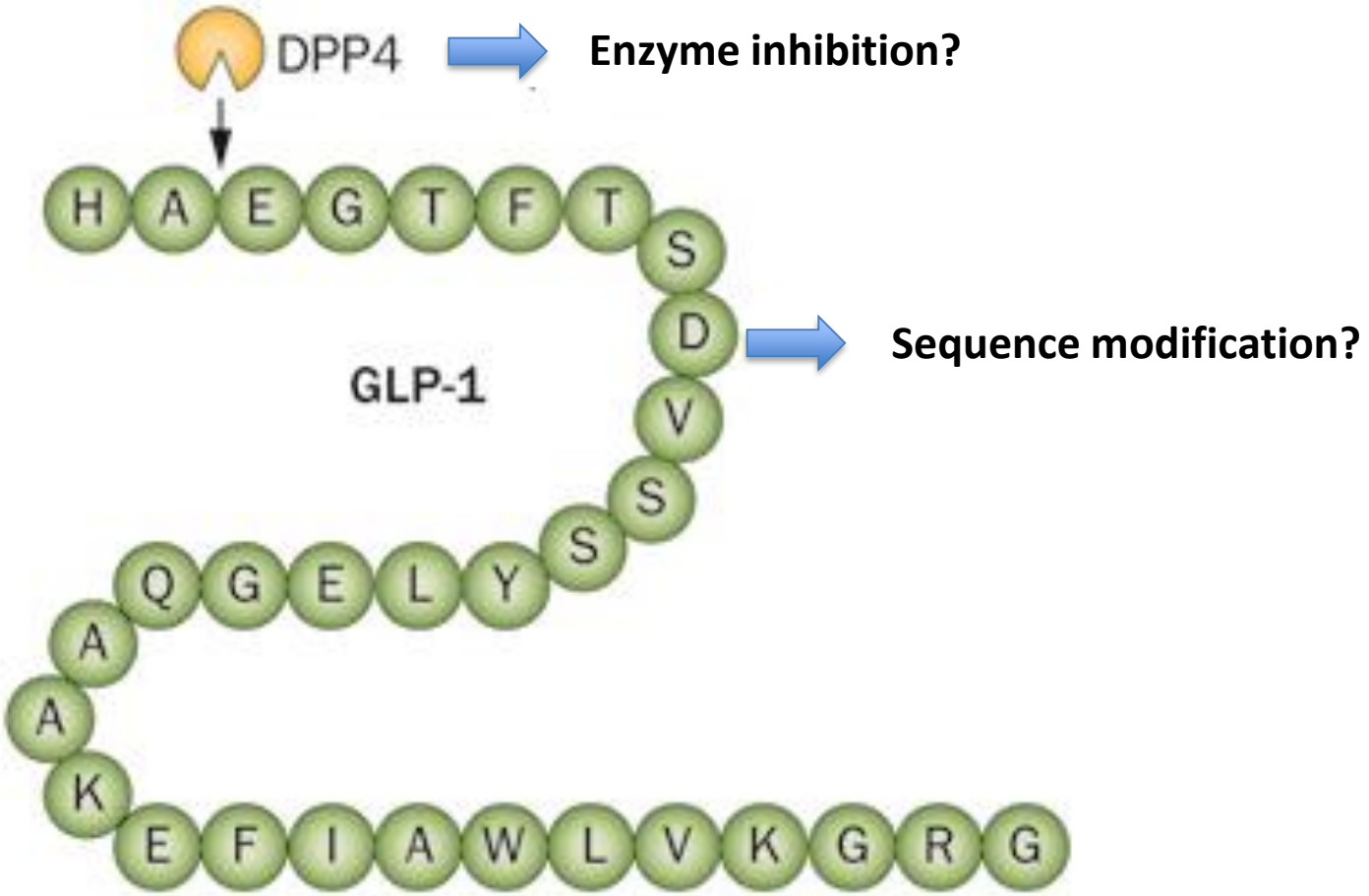

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1995 Then comes this guy

Exendin4: **HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS**
GLP-1: **HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG**

Heloderma suspectum, « Gila monster », venomous lizard from North America
Symptoms from a bite include pain, oedema and weakness associated with a drop in blood pressure.

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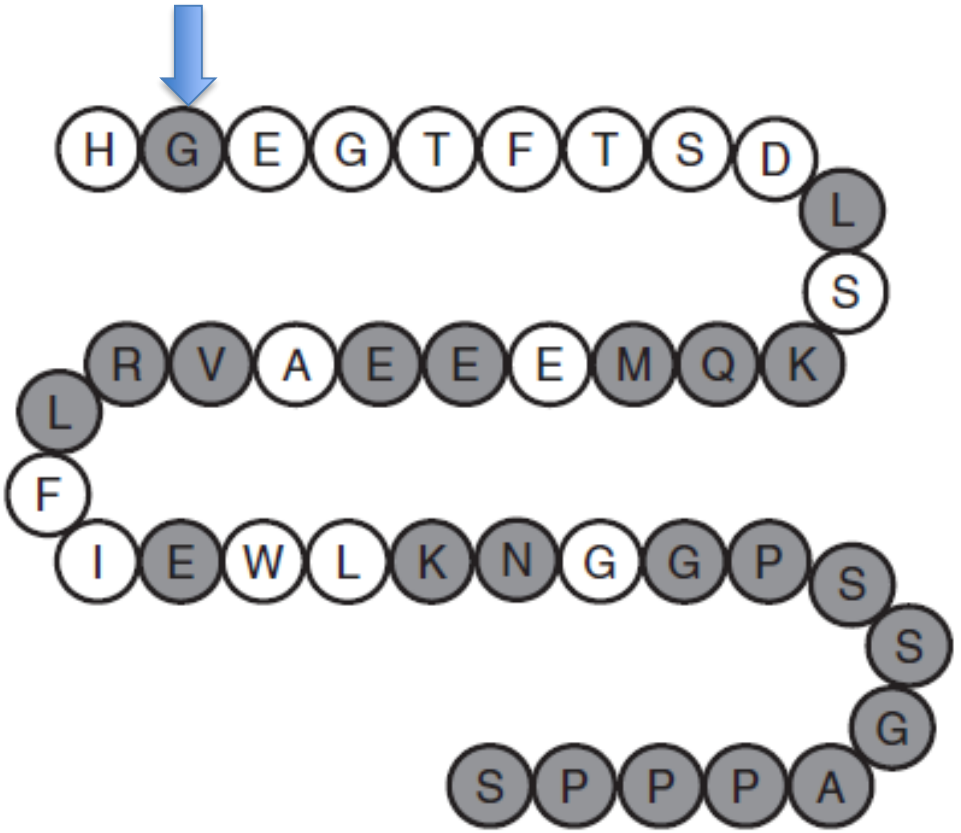
Exenatide-4/ Exenatide marketed in 2005 (Byetta™)

$t_{1/2}$ = 2-4h / 2 injections s.c. per day



- Weight loss- 2-3kg / no hypoglycemy
- Nausea, vomiting and diarrhea (moderated side effects) ~40–60% of patients

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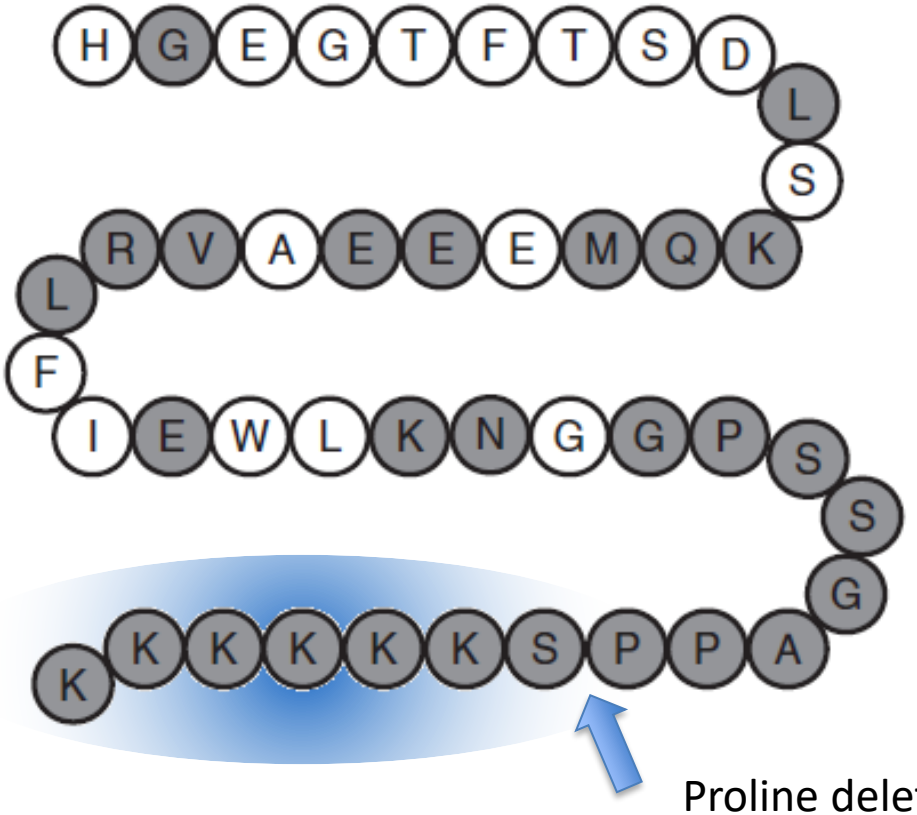
Lixisenatide (Sanofi) – 2013

$t_{1/2}$ = 3-5h / 1 injections s.c. per day



- Weight loss- 1-3kg / no hypoglycemy
- Less side effects

Proline deletion and K6 addition:
Better biding to GLP-1 Receptor and improved circulation time

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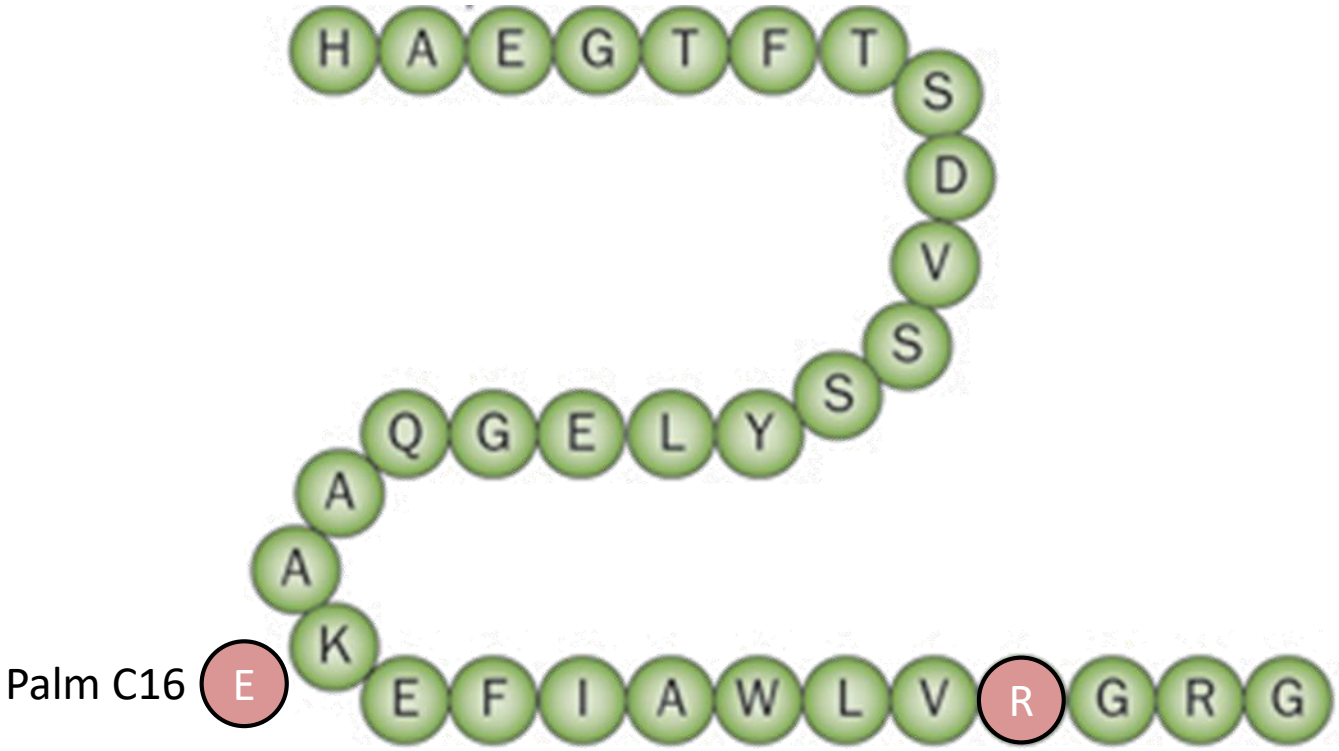
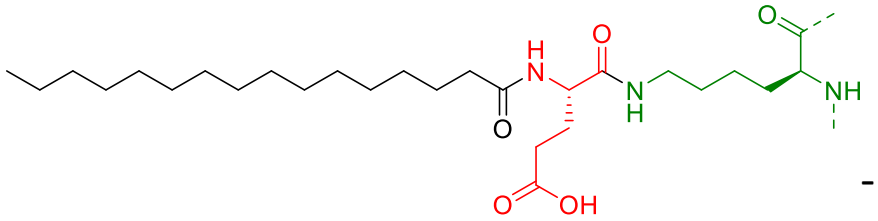
Liraglutide – Novo Nordisk 2009

$t_{1/2}$ = 12-15h / 1 injections s.c. per day





- Weight loss- 1-3kg / no hypoglycemy
- Almost no side effects

Keeps associated with albumin after s.c. injection

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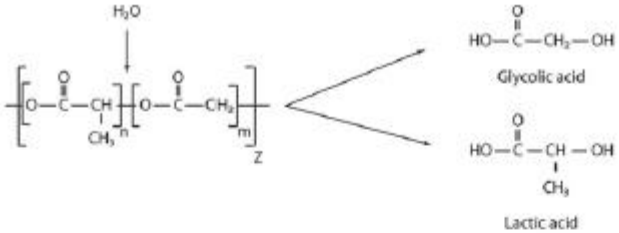
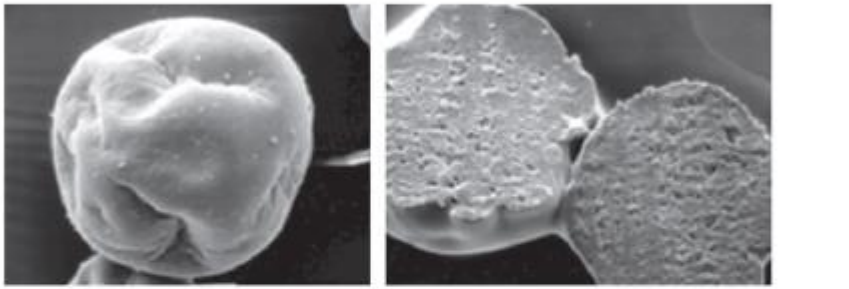
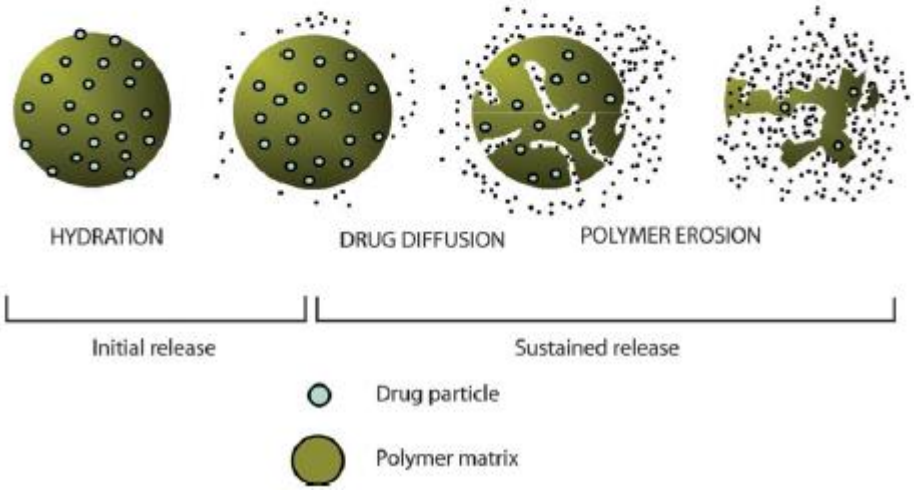
Byetta -> Once-weekly Bydureon
Medisorb microsphere technology (Alkermes) 2011

Microspheres of poly-(d,l-lactide-co-glycolide) degradable polymer







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II.4. Peptides as protein mimics to inhibit protein/protein interactions

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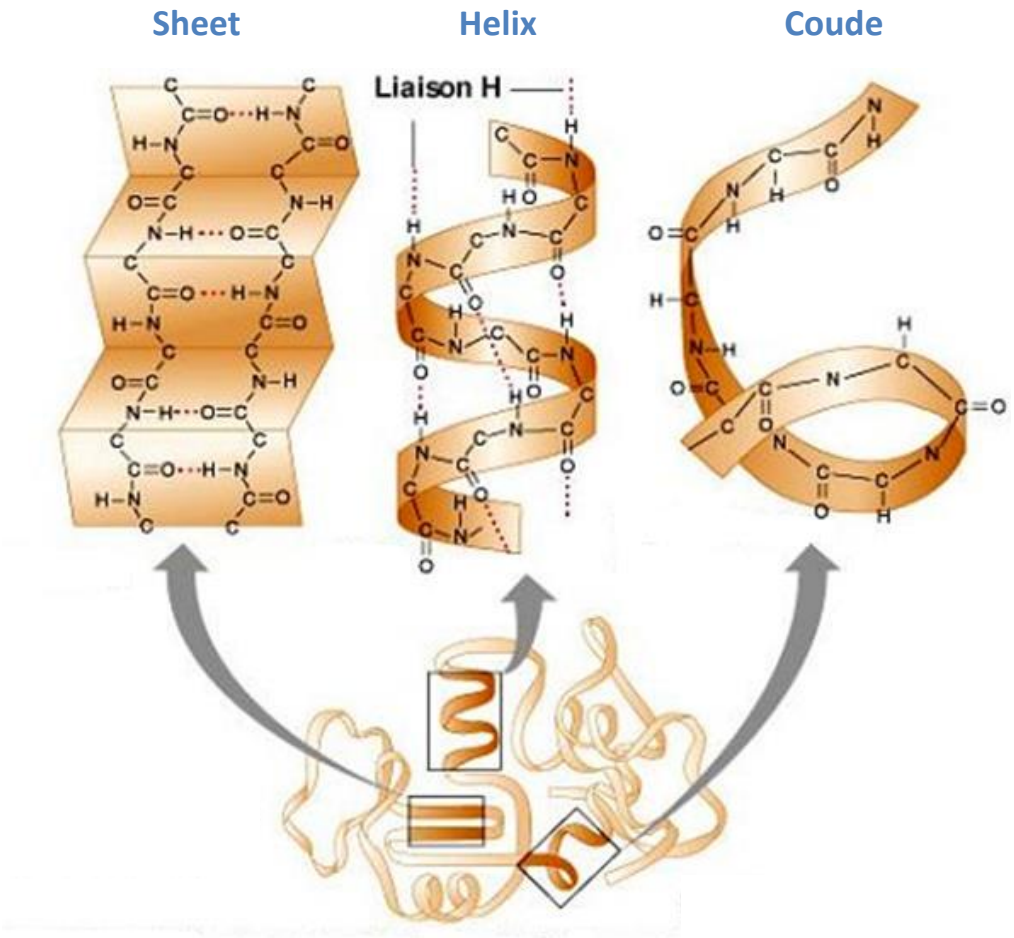
Secondary structures of proteins

3 main types of structural features



Implication in numerous biological processes via specific interactions (proteins/proteins; proteins/DAN, protaines/RNA….)

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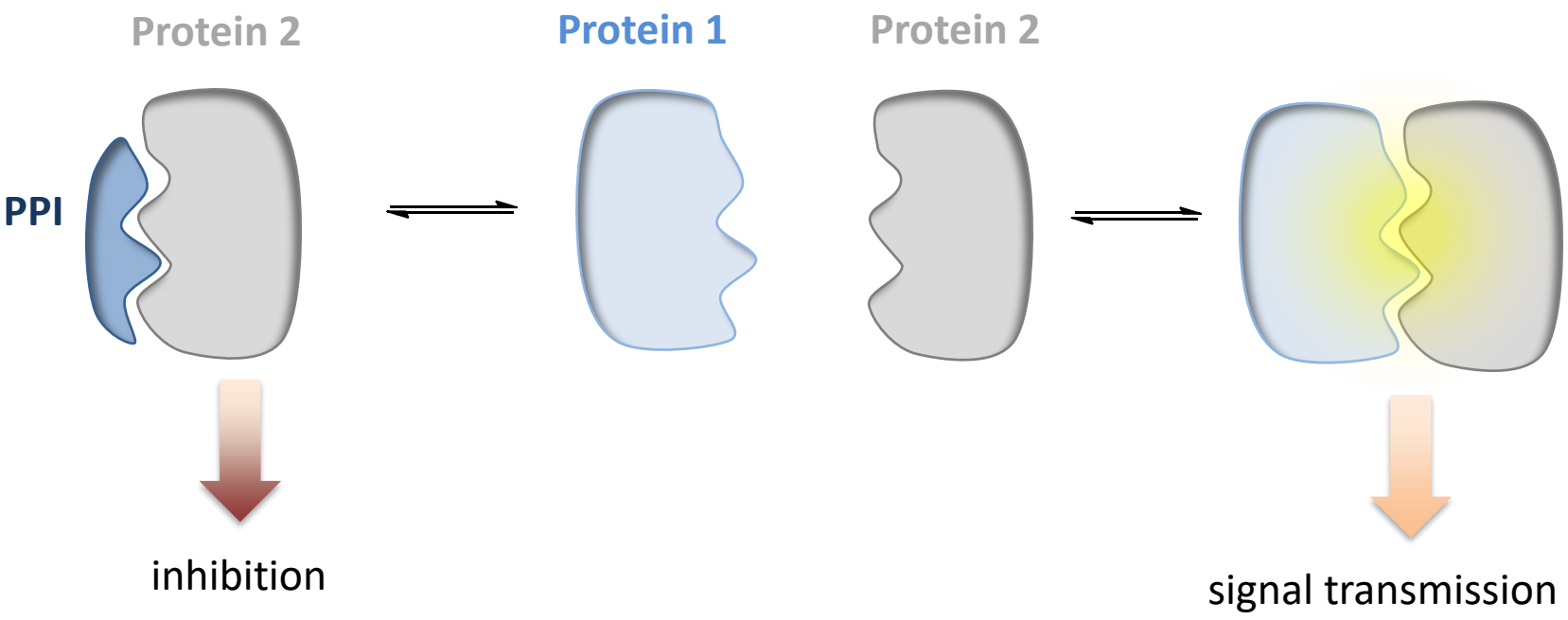
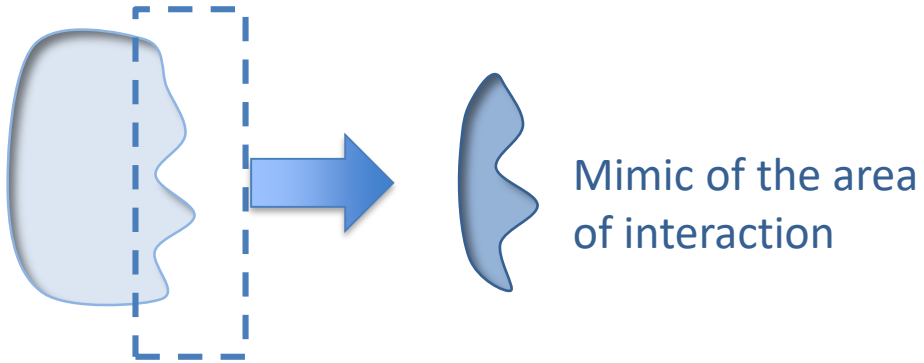
Protein-Protein interaction inhibitor (PPI)



Rational design of PPI



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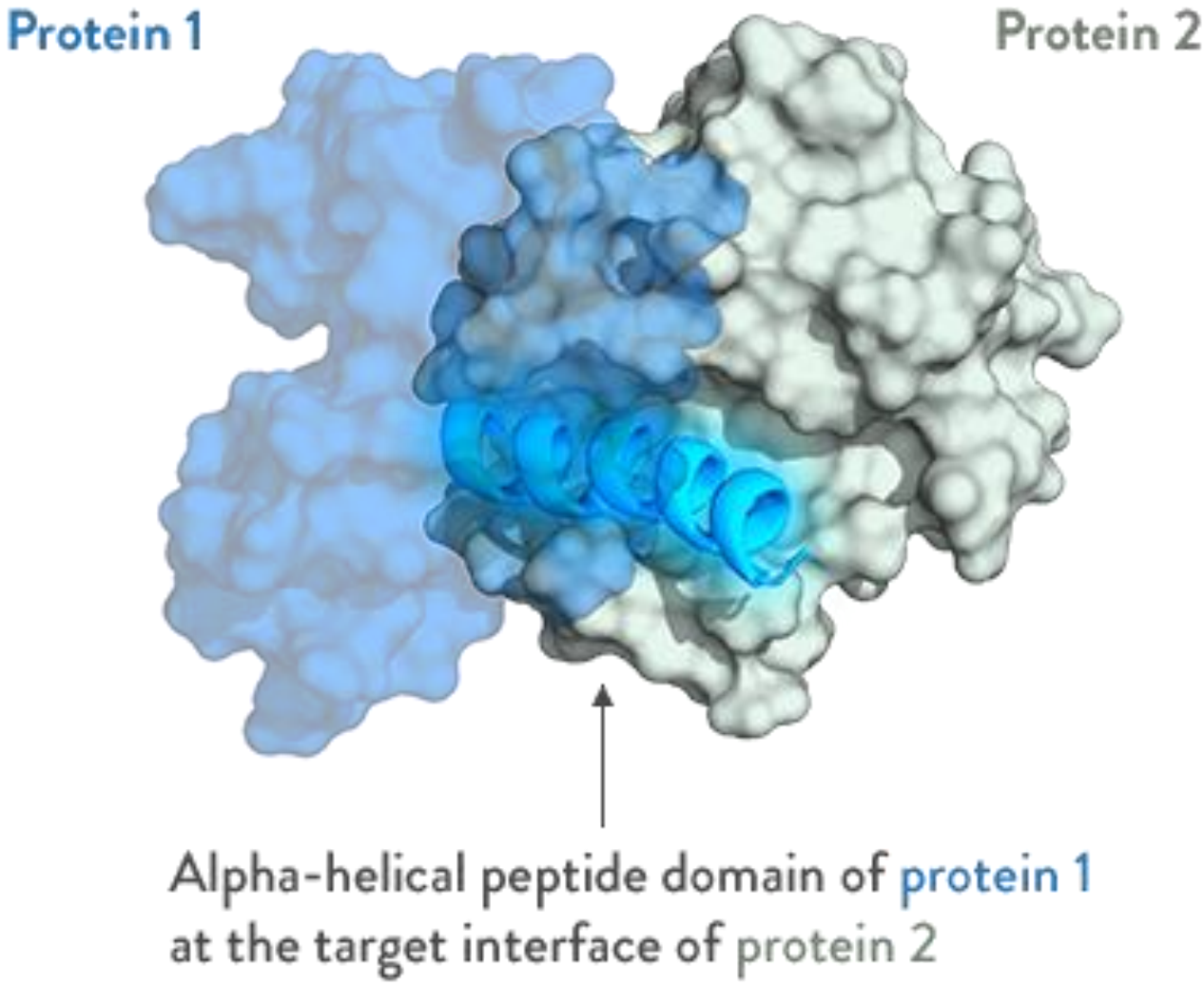
Helixes are important domains found in PPI



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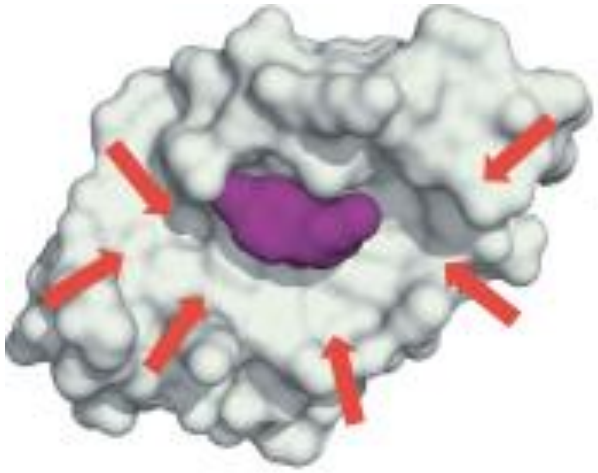
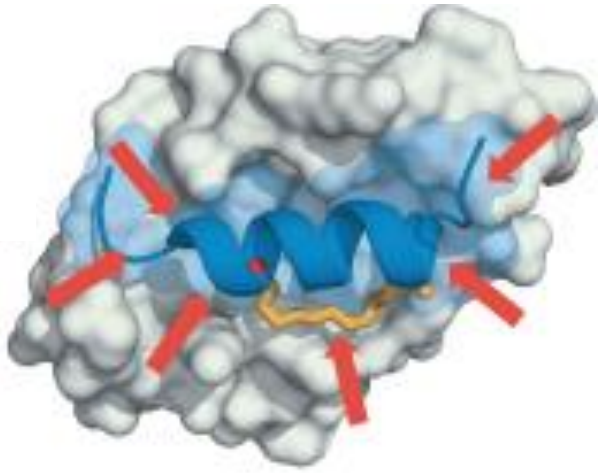
Protein-Protein interaction inhibitor

Mimic of the area of interaction

?





Small molecule
Limited contact with interaction surface

Structured peptide (e.g. helix)
Perfect mimic of the protein
Binds entire interaction surface

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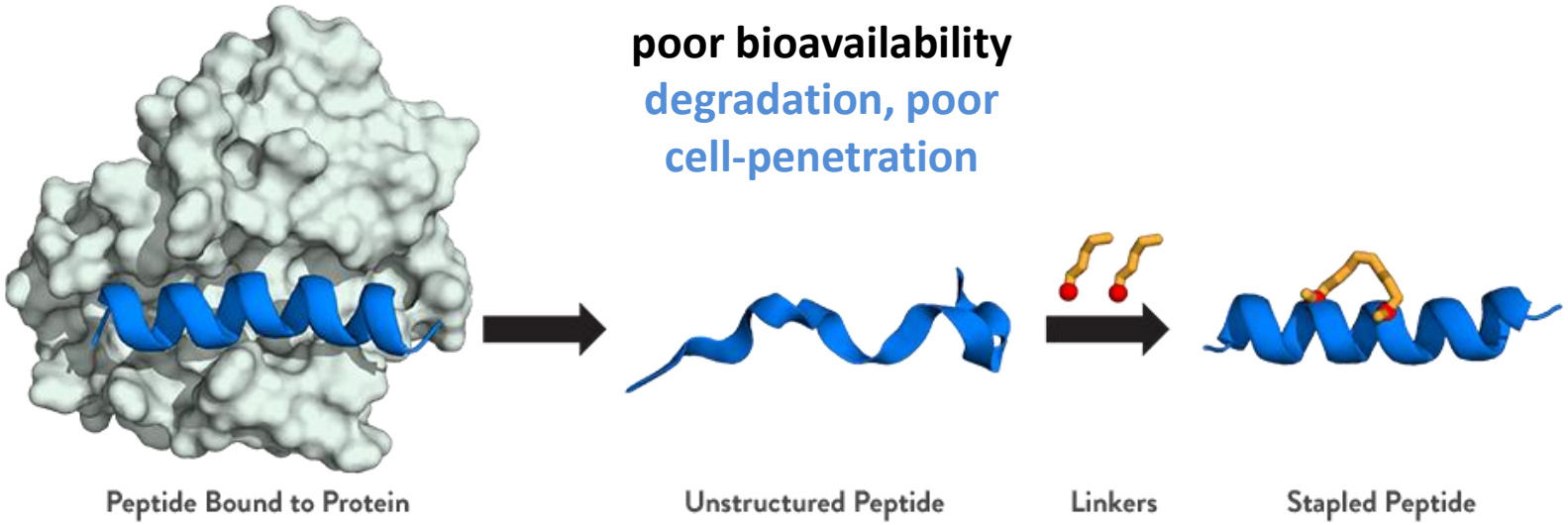
Principle of Stapled Peptides



The peptide sequence adopts a helical structure in the context of the protein

Once 'removed' from the proteic context (i.e. synthesized as linear peptide) **it loses its helical structure and its affinity** for the protein partner

Stapled peptide Restore structre and enhance stability and cell penetration

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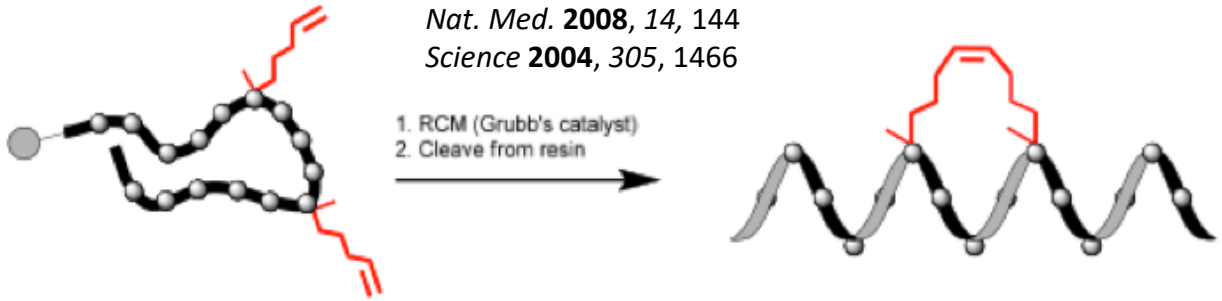
First example of stapled peptides

Verdine Laboratory

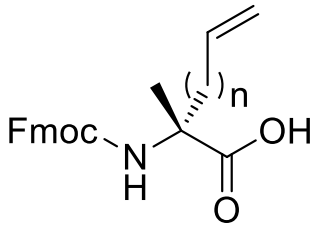
Department of Chemistry and Chemical Biology
Harvard University

AILERON THERAPEUTICS

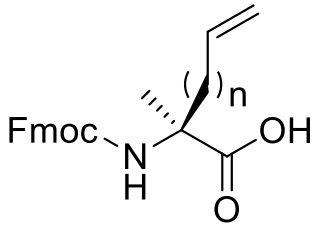
Nature **2009**, *462*, 182
Nat. Med. **2008**, *14*, 144
Science **2004**, *305*, 1466



Introduction of unatural amino acids to staple the peptide by ring closure metathesis



Fmoc-R5-OH (n= 2)
Fmoc-R8-OH (n= 5)



Fmoc-S5-OH (n= 2)
Fmoc-S8-OH (n= 5)

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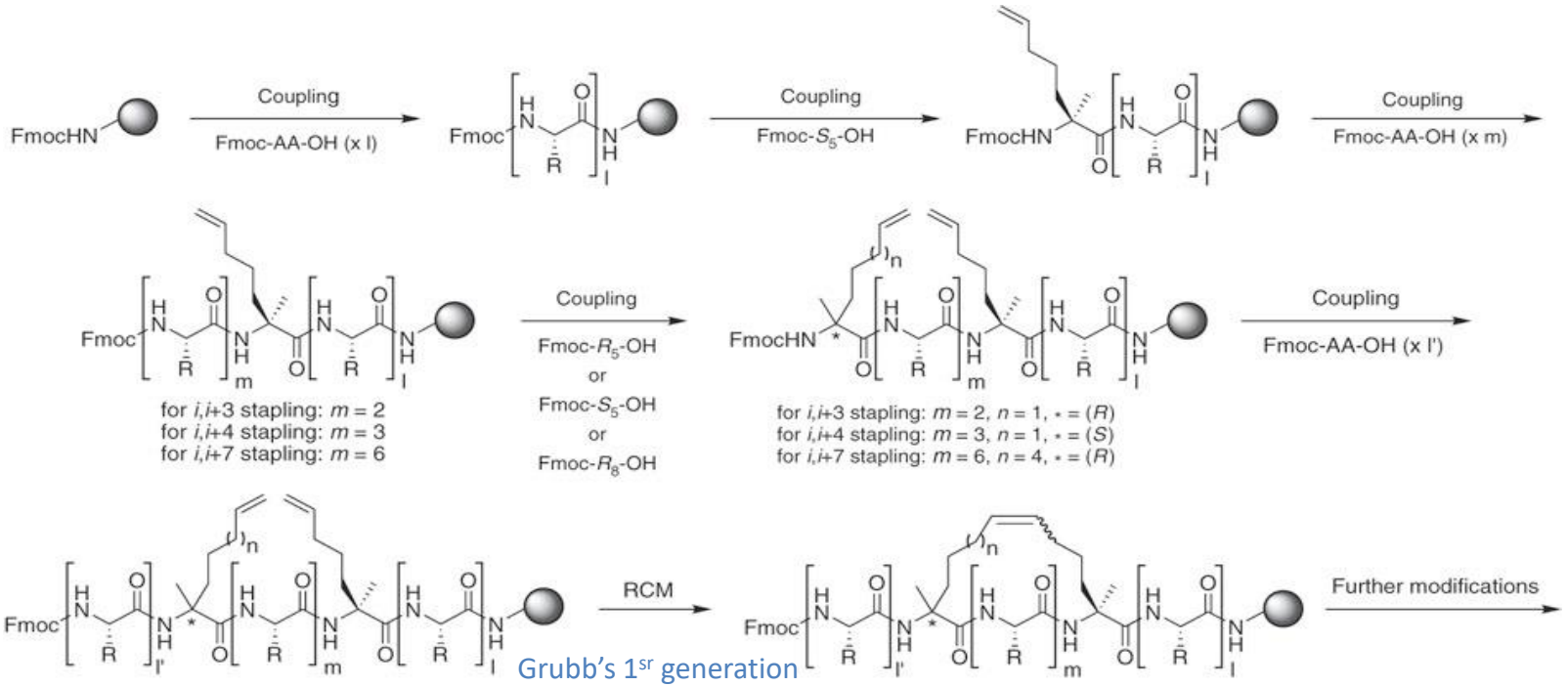
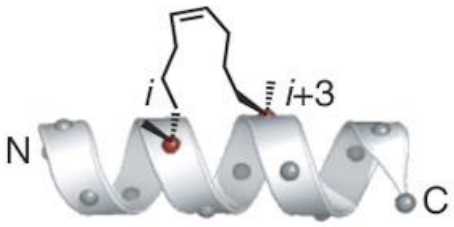
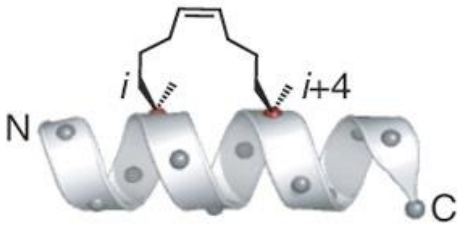
Synthesis of Stapled Peptides



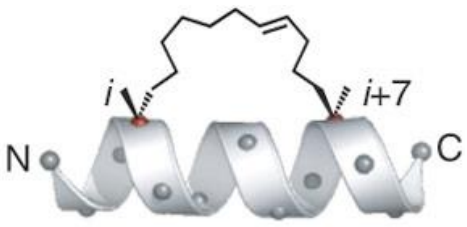
Grubb's 1[sr] generation catalyst



$R_{i,i+3}S(8)$ staple



$S_{i,i+4}S(8)$ staple



$R_{i,i+7}S(11)$ staple

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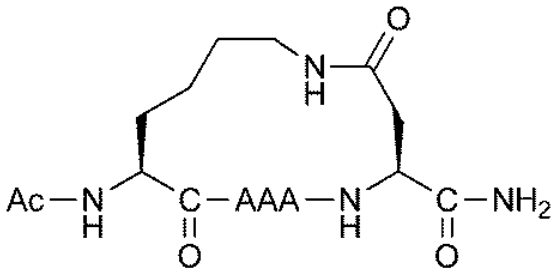
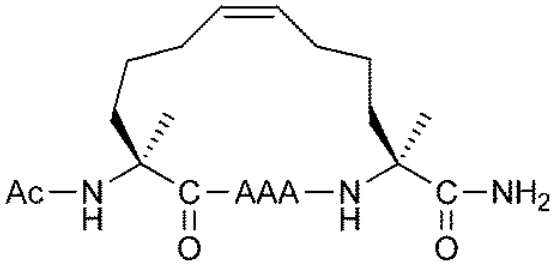
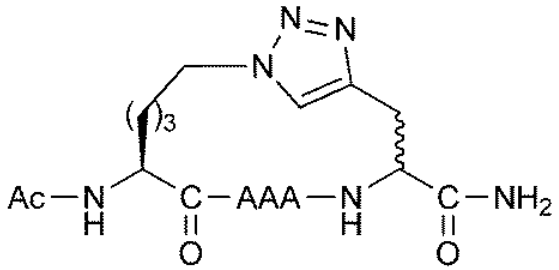
Autres exemples de peptides agrafés



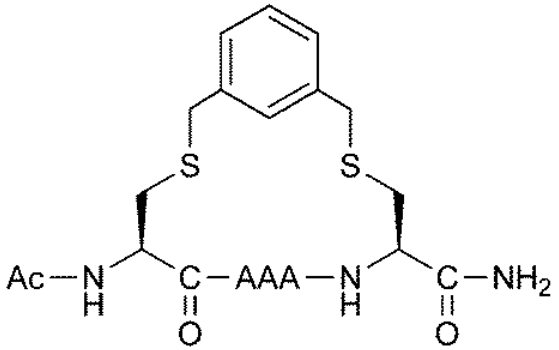
lactam



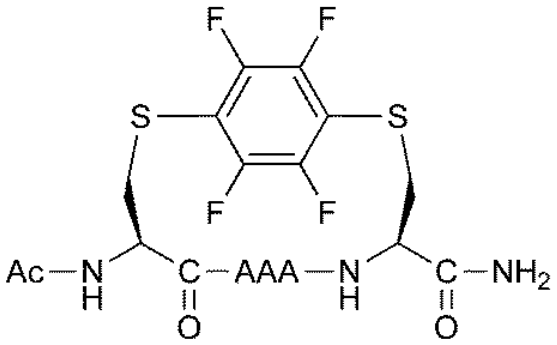
hydrocarbon



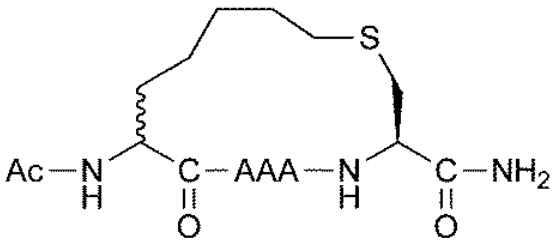
CuAAC



bis-thioether



perfluorobenzene



thioether

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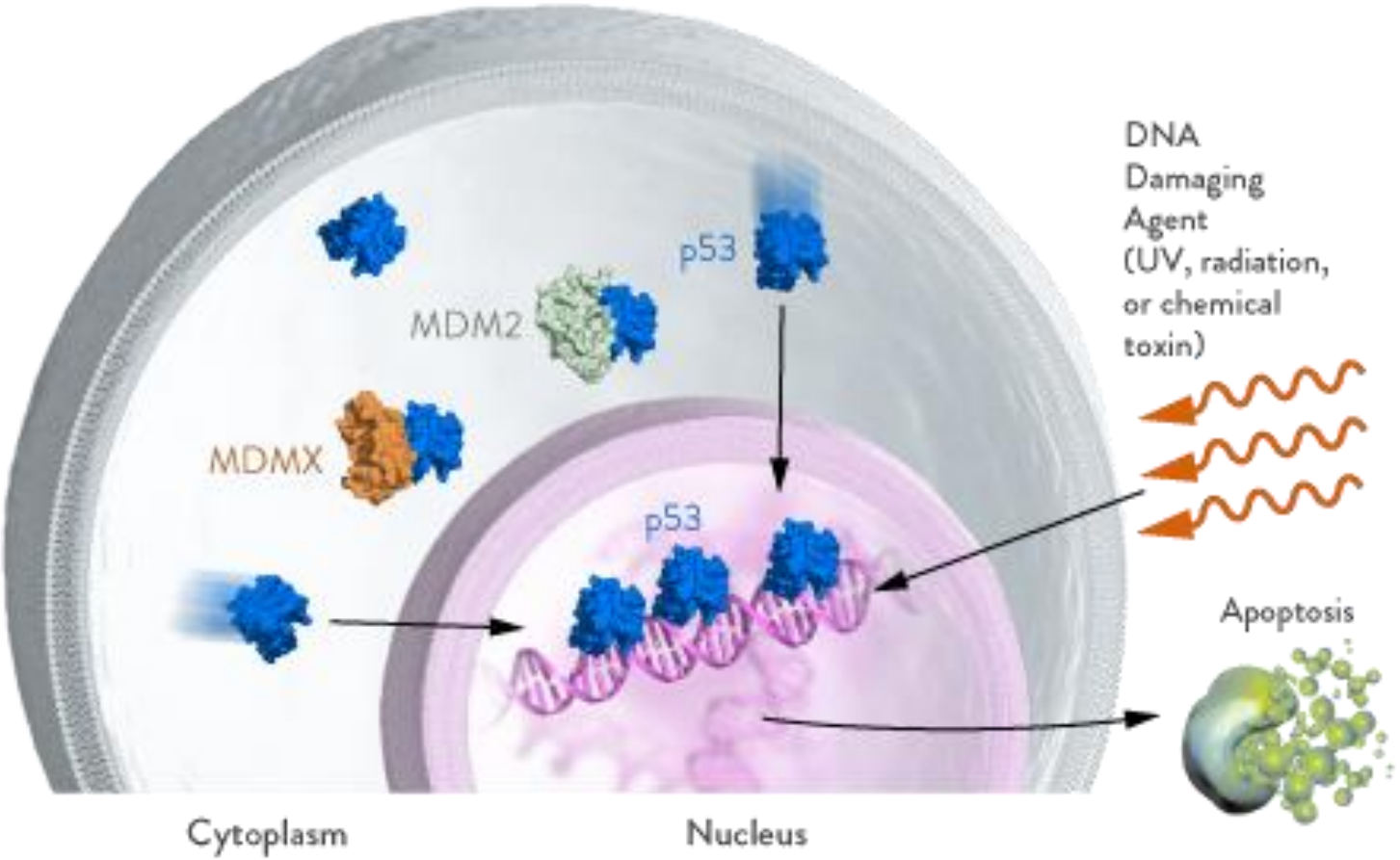
P53 ACTIVATION IN NORMAL CELLS



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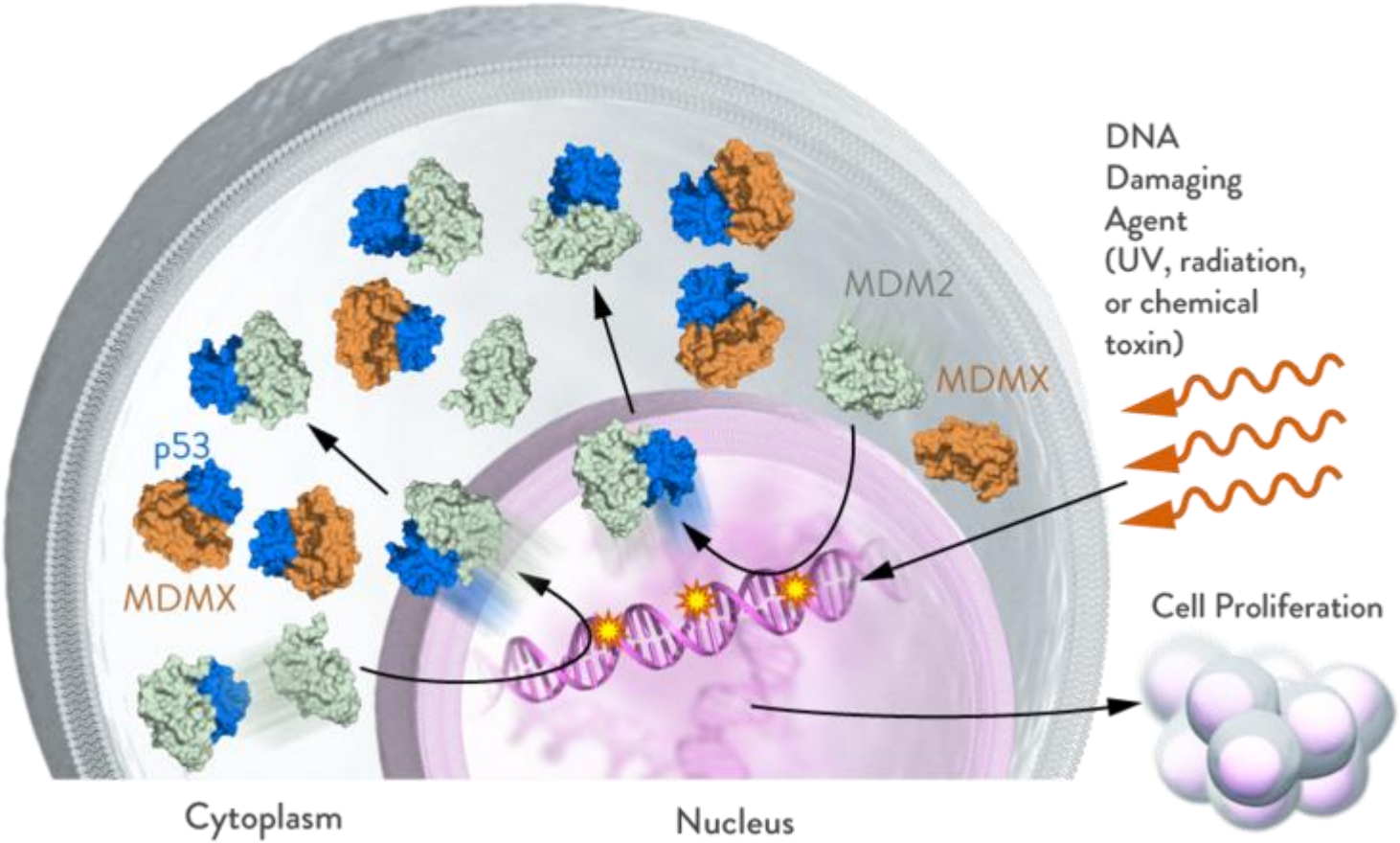
P53 SUPPRESSION IN CANCER CELLS



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Optimization of the sequence

PNAS

PNAS PLUS

Stapled α–helical peptide drug development: A potent dual inhibitor of MDM2 and MDMX for p53-dependent cancer therapy

Yong S. Chang[a,1,2], Bradford Graves[b,1], Vincent Guerlavais[a], Christian Tovar[b], Kathryn Packman[b], Kwong-Him To[b], Karen A. Olson[a], Kamala Kesavan[a], Pranoti Gangurde[a], Aditi Mukherjee[a], Theresa Baker[a], Krzysztof Darlak[a], Carl Elkin[a], Zoran Filipovic[b], Farooq Z. Qureshi[b], Hongliang Cai[a], Pamela Berry[b], Eric Feyfant[a], Xiangguo E. Shi[a], James Horstick[a], D. Allen Annis[a], Anthony M. Manning[a], Nader Fotouhi[b], Huw Nash[a], Lyubomir T. Vassilev[b,2], and Tomi K. Sawyer[a,2]

[a]Aileron Therapeutics, Inc., Cambridge, MA 02139; and [b]Roche Research Center, Hoffmann-La Roche, Inc., Nutley, NJ 07110

Edited* by Robert H. Grubbs, California Institute of Technology, Pasadena, CA, and approved July 12, 2013 (received for review February 17, 2013)

Proc. Natl. Acad. Sci. U. S. A. **2013**, *110, E3445–E3454*

AILERON THERAPEUTICS

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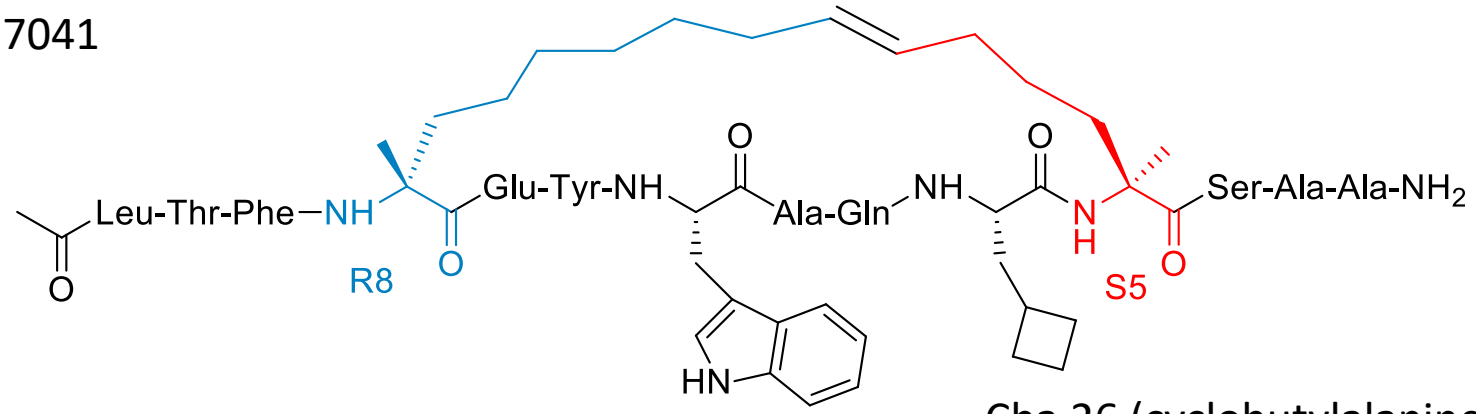
Optimization of the sequence

ATSP#		14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30		Ki MDM2 (nM)	Ki DMX (nM)
3848	Ac-				Leu	Thr	Phe	Glu	His	Tyr	Trp	Ala	Gln	Leu	Thr	Ser			-NH2	14.6	47.4
3900	Ac-				Leu	Thr	Phe	R8	His	Tyr	Trp	Ala	Gln	Leu	S5	Ser			-NH2	1.0	18.3
4641	Ac-				Leu	Thr	Phe	R8	Ala	Tyr	Trp	Ala	Gln	Leu	S5	Ser			-NH2	4.9	34.3
6935	Ac-				Leu	Thr	Phe	R8	Glu	Tyr	Trp	Ala	Gln	Leu	S5	Ser			-NH2	1.2	8
7041	Ac-				Leu	Thr	Phe	R8	Glu	Tyr	Trp	Ala	Gln	Cba	S5	Ser	Ala	Ala	-NH2	0.9	6.8
7342	Ac-				Leu	Thr	Ala	R8	Glu	Tyr	Trp	Ala	Gln	Cba	S5	Ser	Ala	Ala	-NH2	536	>1000

3848 is a linear sequence found by phage display
7041 vs **3900** : 10-fold increased cell potency
- **improved cell penetration** efficiency due, in part, to its greater amphipathic α−helical nature (related to the C-terminal Ala29-Ala30 extension)
- **improved solubility** His21 to Glu21 modification

7041



Cba 26 (cyclobutylalanine)

Y. S. Chang, B. Graves, V. Guerlavais, C. Tovar, K. Packman, K.-H. To, K. A. Olson, K. Kesavan, P. Gangurde, A. Mukherjee, et al., *Proceedings of the National Academy of Sciences* **2013**, 110, E3445–E3454.

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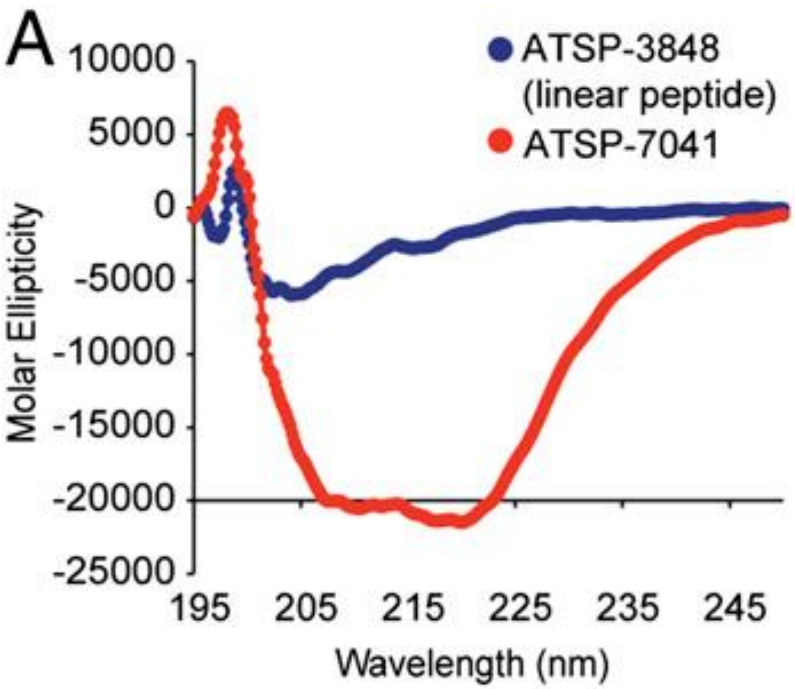
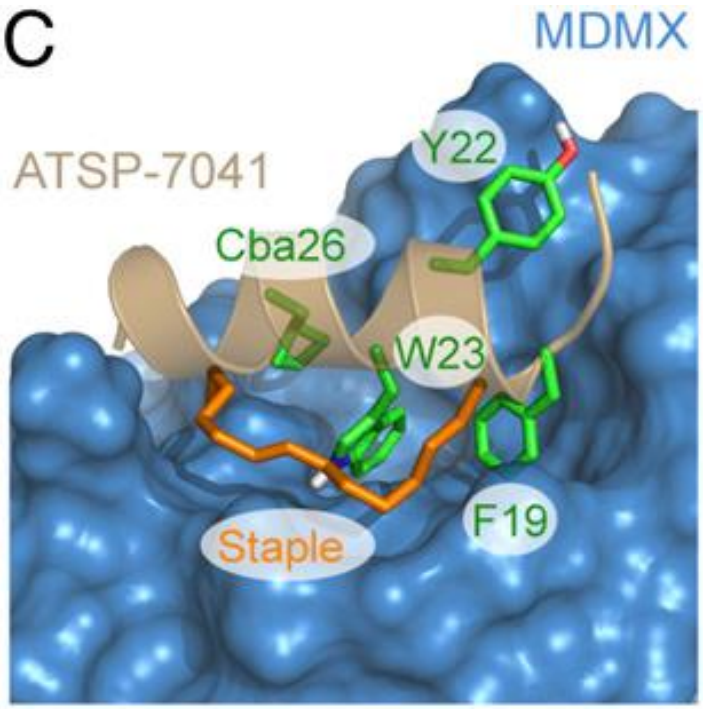
Optimization of the sequence





Circular Dichroïsm : signatures of secondary structures

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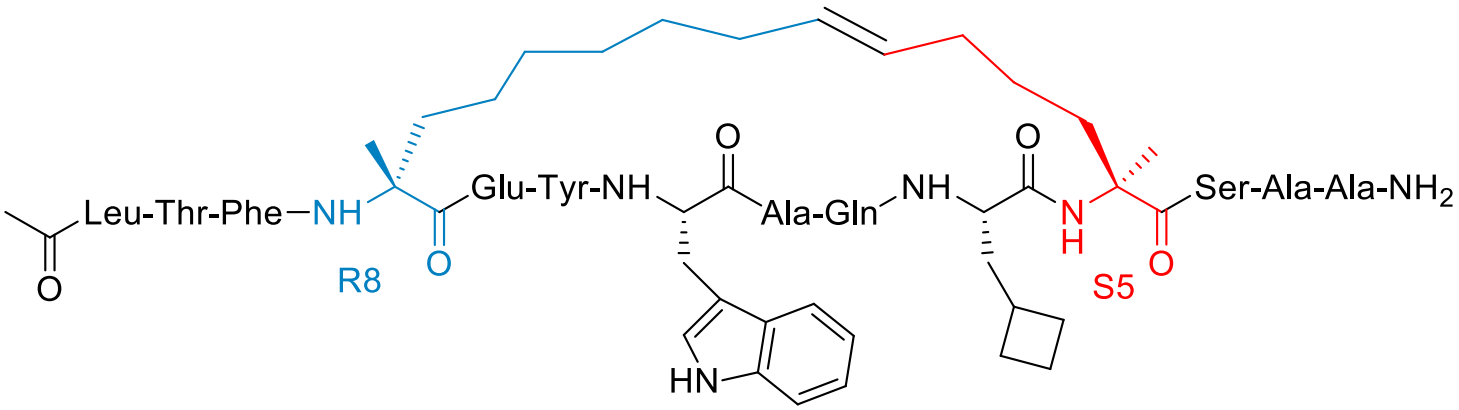
ALRN-6924 a stapled peptide in clinical trials



Oral presentation at the 2017 American Society of Clinical Oncology (ASCO) Annual Meeting held in Chicago, June 2 – 6, **2017**.

- Phase 1 trial for the treatment of advanced solid tumors or lymphomas
- Phase 2a trial for the treatment of peripheral T-cell lymphoma (PTCL)
- Phase 1 trial for the treatment of acute myeloid leukemia (AML), and advanced myelodysplastic syndrome (MDS), as a monotherapy
- Phase 1b trial for the treatment of AML/MDS in combination with cytosine arabinoside (Ara-C)

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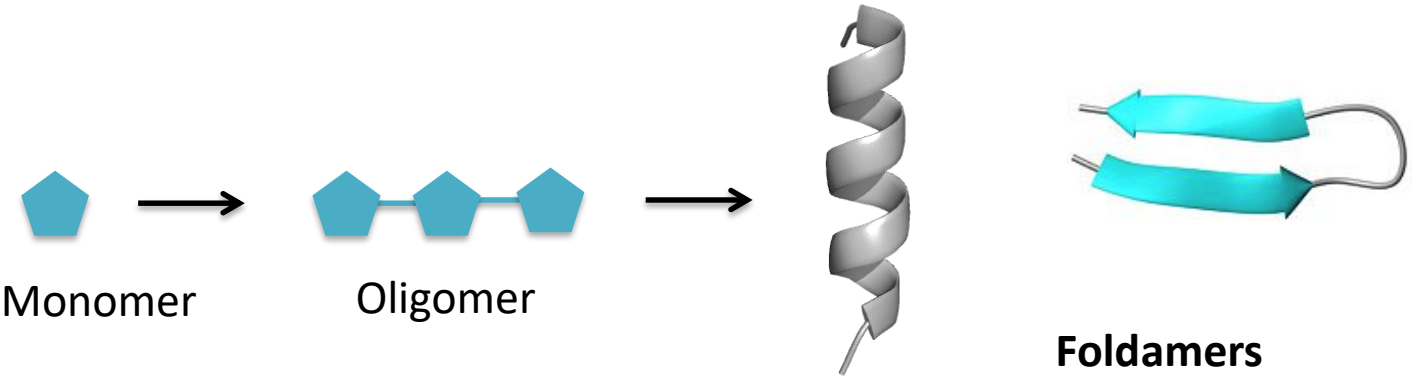
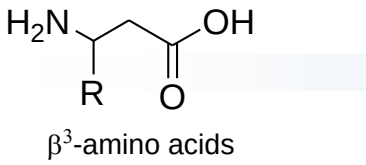
Another way to mimic helixes: foldamers

Definition:
Artificial oligomers able to 'fold' in a stable, well defined ad predictable secondary structure.

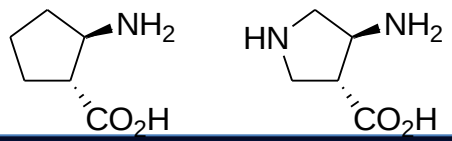
- Whereas natural peptides are composed of α amino acids, foldamers may include of may be exclusively composed of unatural aminoacids (e.g. **β-peptides**, δ-peptides, γ-peptides)
- Compared to peptides, they display a high proteolytic stability
- They may adopt a stable secondary structure with few monomers (blocs)
- Some foldamers can be different from polyamides backbone -NHCO- (e.g. **oligoureas NHCONH**)





β³-amino acids

Control of secondary structure



Gellman *Acc. Chem. Res.* **1998**
Moore et al. *Chem. Rev.* **2001**
Seebach et al, *Chem Biodivers* **2004**
Hill, D. J. et coll. *Chem. Rev.* **2001**

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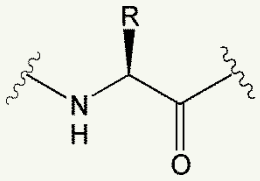
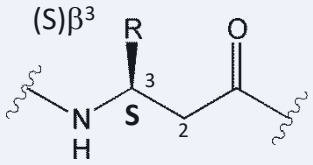
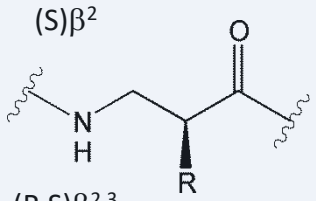
Examples of monomer blocks

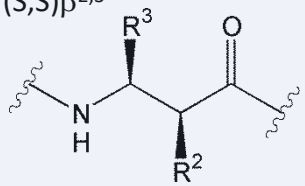
α-amino acids

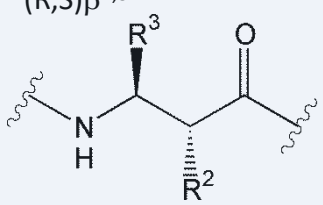


β-amino acids acyclic

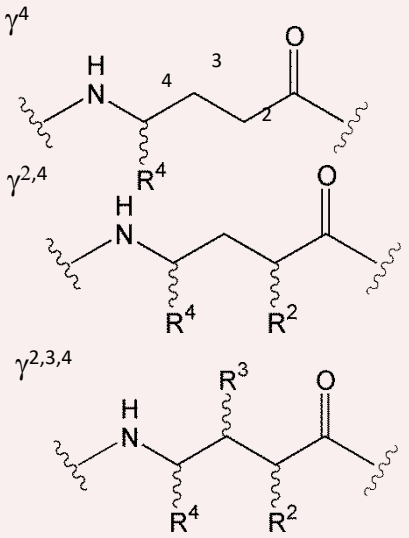




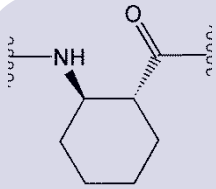




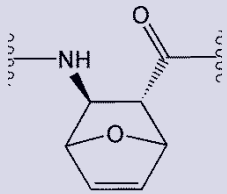
γ-amino acids acyclic



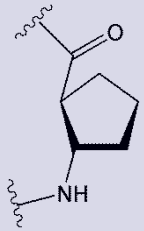
β-amino acids cyclic



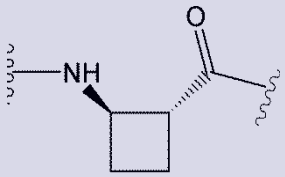
trans-ACHC



trans-oxanorbornène



trans-ACPC



trans-ACBC

Homo-oligomers
Hetero-oligomers
Hybrid-oligomers

Foldamers

and ε-amino acids, hydrazino, β-thio-amino acids, α-aminoxydes…

Peptoïdes

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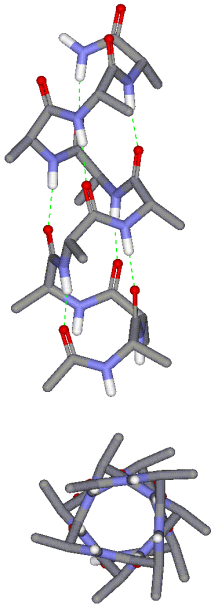
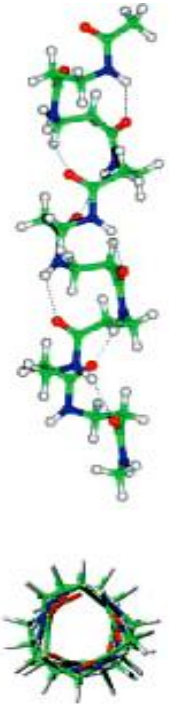
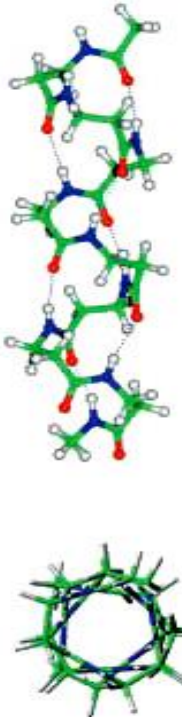
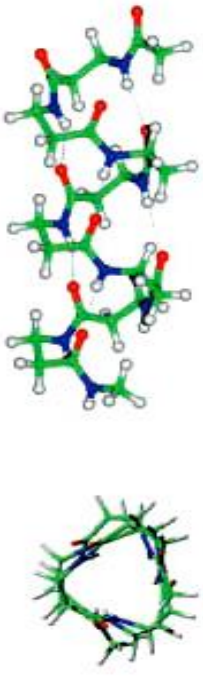
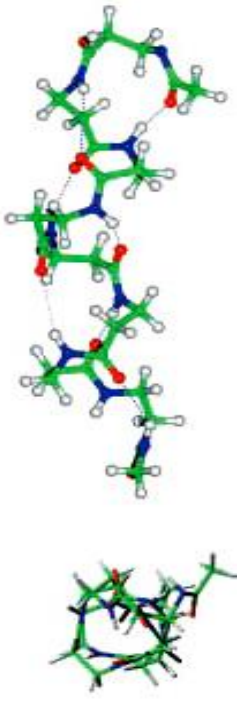
A diversity of helixes











13-helix

α-helix composed of α amino acids

10-helix

cyclic (4) β-aa

12-helix

$\beta^{2,3}$ -aa
cyclic (5) β-aa
αγ

14-helix

β^2 or β^3-aa
cyclic (6) β-aa

12/10-helix

β^2 /β^3-aa
αγ

Seebach, D. *et coll. Angew. Chem. Int. Ed.* **2003**
Fleet, G. W. *et coll. Tetrahedron Lett.* **2001**
Gellman, S. H. *et coll. Nature* **1997**
Seebach, D. *et coll. Helv. Chim. Acta* **1996**
Seebach, D. *et coll. Helv. Chim. Acta* **2002**

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Mimicking gp41 sub unit with a foldamer

Structural and biological mimicry of protein surface recognition by α/β-peptide foldamers

W. Seth Horne[a], Lisa M. Johnson[a], Thomas J. Ketas[b], Per Johan Klasse[b], Min Lu[c], John P. Moore[b], and Samuel H. Gellman[a,1]

[a]Department of Chemistry, University of Wisconsin, 1101 University Avenue, Madison, WI 53706; [b]Department of Microbiology and Immunology, Weill Medical College of Cornell University, New York, NY 10021; and [c]Department of Biochemistry, Weill Medical College of Cornell University, New York, NY 10021

Edited by David Baker, University of Washington, Seattle, WA, and approved July 2, 2009 (received for review March 10, 2009)

Proc. Natl. Acad. Sci. U. S. A. **2009**, *106*, 14751–14756.

Enfuvirtide

2012

LONGEVITY BIOTECH™

Preclinical trials

2016: Diabetes LBT-6030 is a dual agonist targeting the validated Glucagon-like peptide-1 (GLP-1) receptor in addition to Gastric inhibitory polypeptide receptor (VIP)

2016: Neuroinflammation with an agonist of Vasoactive Intestinal polypeptide receptor VPAC 2 (VIP receptor family) inducing neuroprotection on dopamine-producing neurons.

2016: Fusion inhibitor

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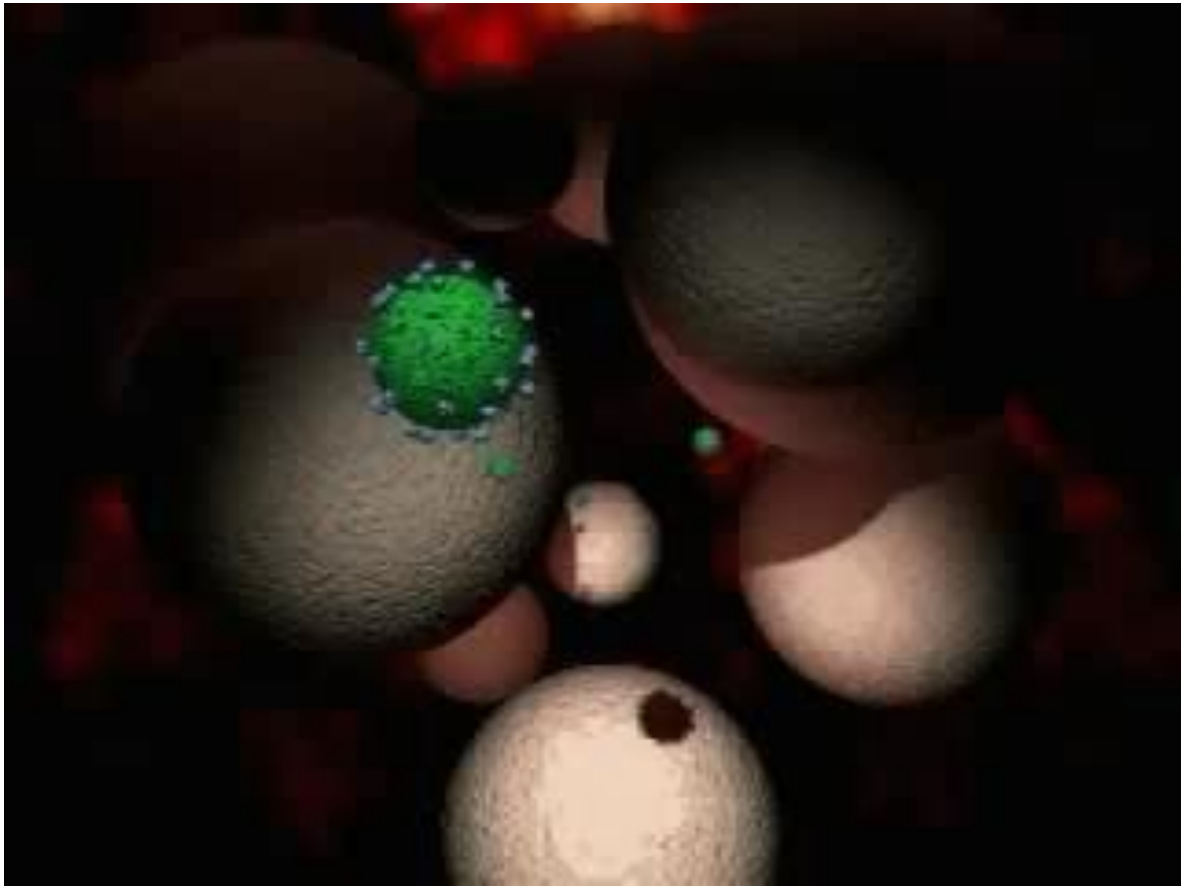

CHR C-terminal heptad repeat (HR2) and NHR (HR1) of gp41 associate through antiparallel 6-helix bundle leading to juxtaposition of host cell and viral membranes.

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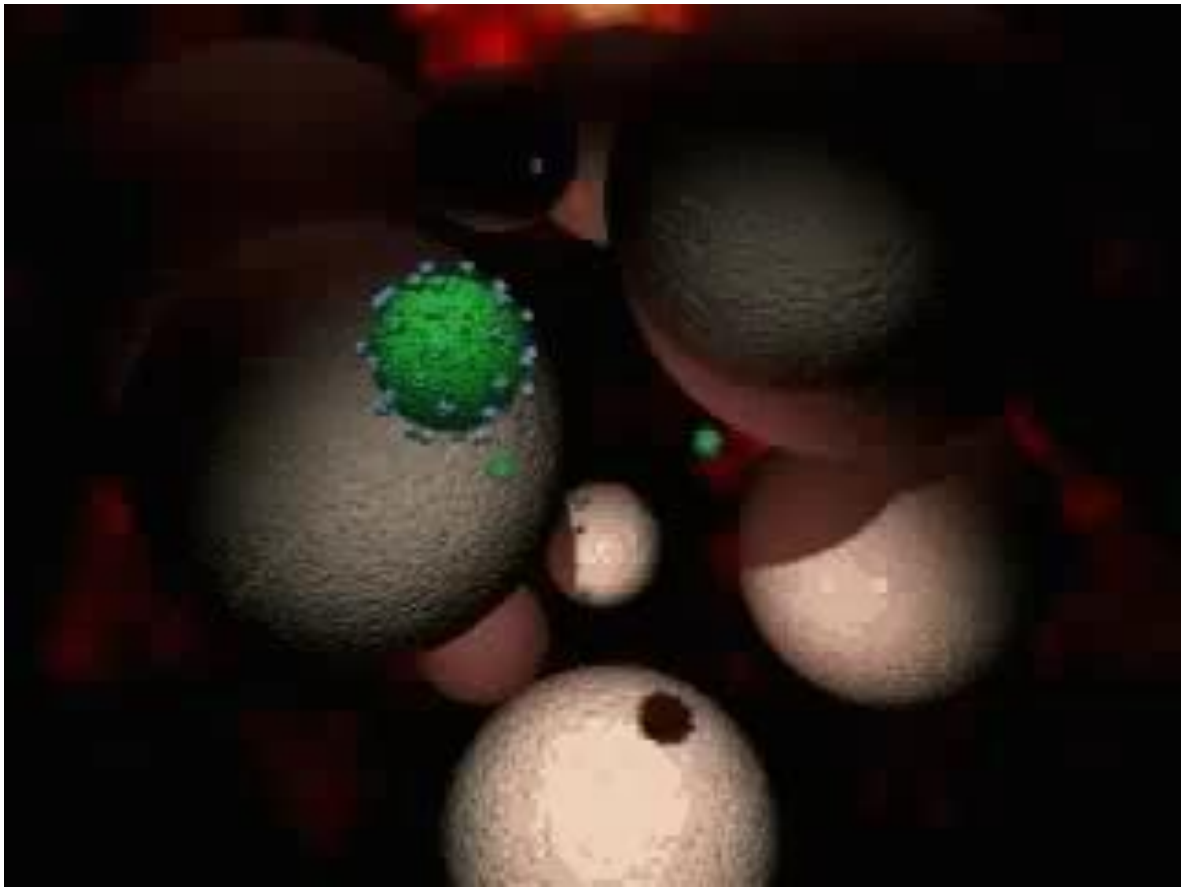

CHR C-terminal heptad repeat (HR2) and NHR (HR1) of gp41 associate through antiparallel 6-helix bundle leading to juxtaposition of host cell and viral membranes. The drug enfuvirtide (**T-20**) is a 36-residue peptide derived from the CHR region which inhibits the HR1-HR2 association

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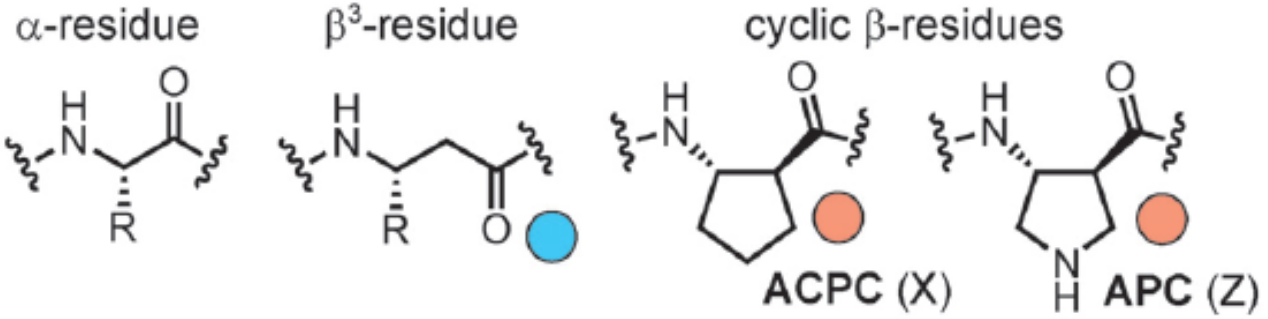
Optimization of the sequence (C-ter Heptad Repeat peptide 3)



(3) Lead alpha peptide enhaced helical propensity compared to T-20 (Enfuvitide)

Ac-TTWEAWDRAIAEYAARIEALIRAAQEQQEKNEAALREL-NH_2 **(3)**

Ac-TTWEAWDRAIAEYAARIEALIRAAQEQQEKNEAALREL-NH_2 **(4)**

Ac-TTWEAWDRAIAEYAARIEALIRAAQEQQEKNEAALREL-NH_2 **(5)**

Ac-TTWEAWDRAIAEYAARIEALIRAAQEQQEKNEAALREL-NH_2 **(6)**

Ac-TTWEAWDRAIAEYAXRIEXLIRAAQEQQEKNEXALREL-NH_2 **(7)**

Ac-TTWEAWDRAIAEYAXRIEXLIZAAQEQQEKNEXALZEL-NH_2 **(8)**

Ac-TTWEXWDRAIAEYAXRIEXLIRAAQEQQEKNEXALREL-NH_2 **(9)**

Ac-TTWEXWDZAIAEYAXRIEXLIZAAQEQQEKNEXALZEL-NH_2 **(10)**

Ac-AEYAXRIEXLIZAAQEQQEKNEXALZEL-NH_2 **(11)**

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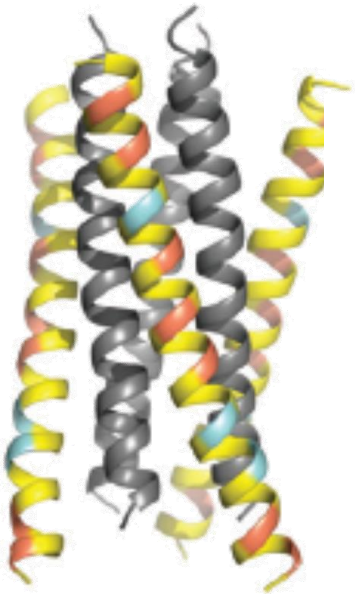

1+10

Oligomer	gp41–5 binding affinity by FP* K_i, nM	NHR + CHR stability by CD† $T_{m,app}$, °C	Stability to Proteinase K‡ $t_{1/2}$, min	Cell-cell fusion inhibition§ IC_{50}, nM
3	< 0.2	77	0.7	9 ± 3
4	3,800	-‖	14	390 ± 40
10	9	55	200	5 ± 2

Ac-TTWEAWDRAIAEYAARIEALIRAAQEQQEKNEAALREL-NH_2 **(3)**

Ac-TTWEAWDRAIAEYAARIEALIRAAQEQQEKNEAALREL-NH_2 **(4)**

Ac-TTWEXWDZAIAEYAXRIEXLIZAAQEQQEKNEXALZEL-NH_2 **(10)**

In grey: helix 1, NHR
In yellow: foldamer CHR mimic

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2012

Preclinical trials

- 2016: Diabetes LBT-6030 is a dual agonist targeting the validated Glucagon-like peptide-1 (GLP-1) receptor in addition to Gastric inhibitory polypeptide receptor (VIP)
- 2016: Neuroinflammation with an agonist of Vasoactive Intestinal polypeptide receptor VPAC 2 (VIP receptor family) inducing neuroprotection on dopamine-producing neurons.
- 2016: Fusion inhibitor

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III. Peptides and conjugates as tools

III.2. Cell Penetrating Peptides (CPPs)

Some reviews on the subject

- Bashyal, S., Noh, G., Keum, T., Choi, Y. W. & Lee, S. Cell penetrating peptides as an innovative approach for drug delivery; then, present and the future. *J. Pharm. Investig.* **46,** 205–220 (2016)
- Copolovici, D. M., Langel, K., Eriste, E. & Langel, Ü. Cell-Penetrating Peptides: Design, Synthesis, and Applications. *ACS Nano* **8,** 1972–1994 (2014).
- Munyendo, W. L., Lv, H., Benza-Ingoula, H., Baraza, L. D. & Zhou, J. Cell Penetrating Peptides in the Delivery of Biopharmaceuticals. *Biomolecules* **2,** 187–202 (2012).
- Koren, E. & Torchilin, V. P. Cell-penetrating peptides: breaking through to the other side. *Trends Mol. Med.* **18,** 385–393 (2012)

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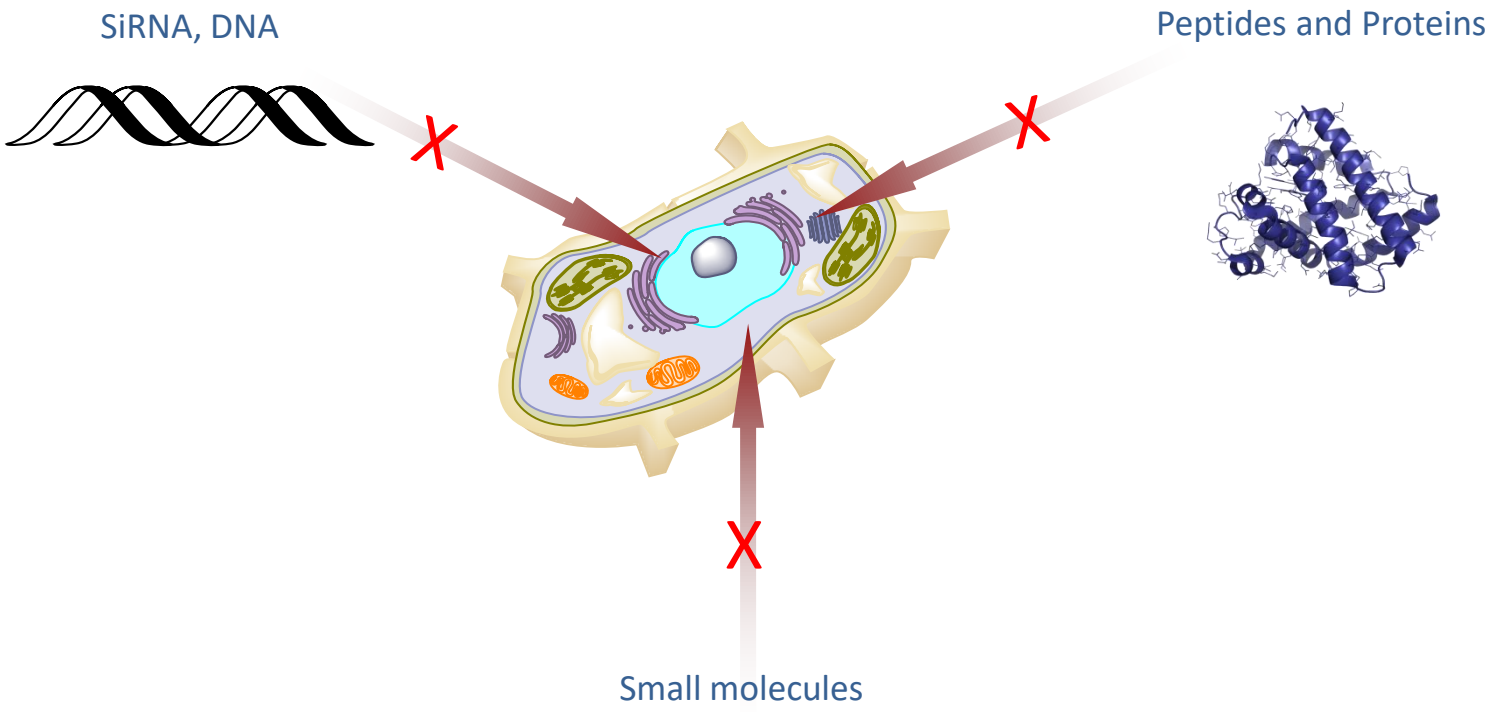
It’s about crossing membranes

Drugs targeting GPCR are a notable exceptions but a lot of **drugs have to cross cell membranes** because they have **an intracellular target**.
Moreover, they have to be delivered to a specific compartment (lysosome, nucleus…)



Strategy: associating the drug with a carrier in a noncovalent or covalent way **(bioconjugate)**

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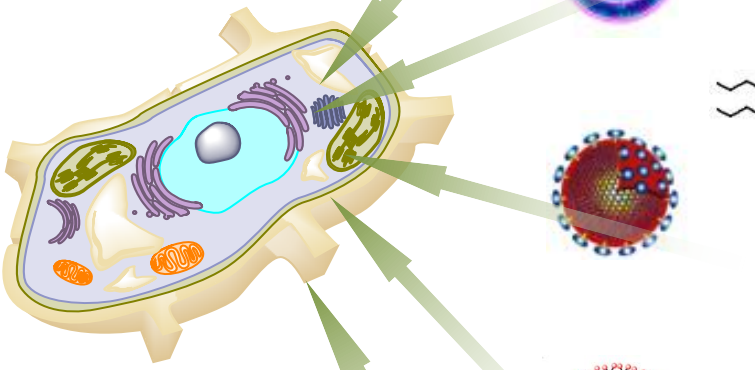
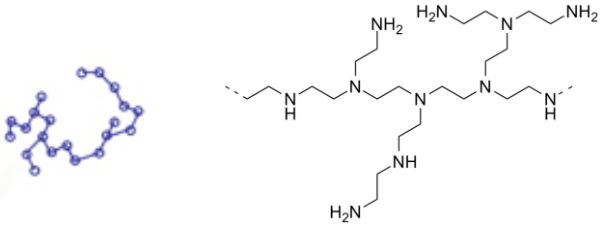
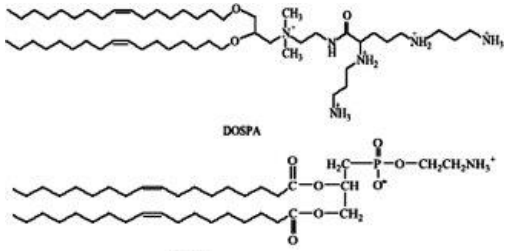
Nanometric vectors

Most of the time, non covalent association with the cargo (i.e.the drug)



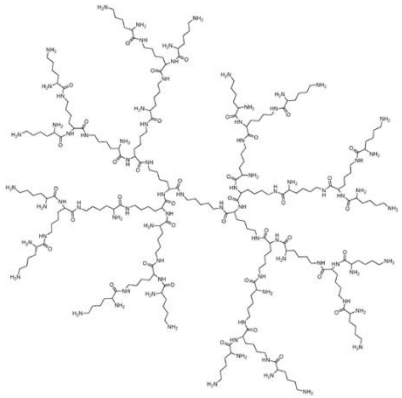


Cationic polymers
(PEI, chitosan, polyLysine)

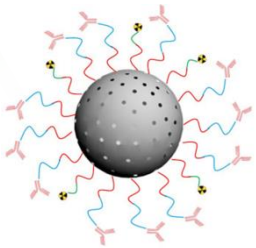


Liposomes (lipofectamine,
Cationic lipids)

Viruses



Dendrimers
(e.g. Lysine dendrimers)



Nanoparticles (e.g. mesoporous SiNPs)

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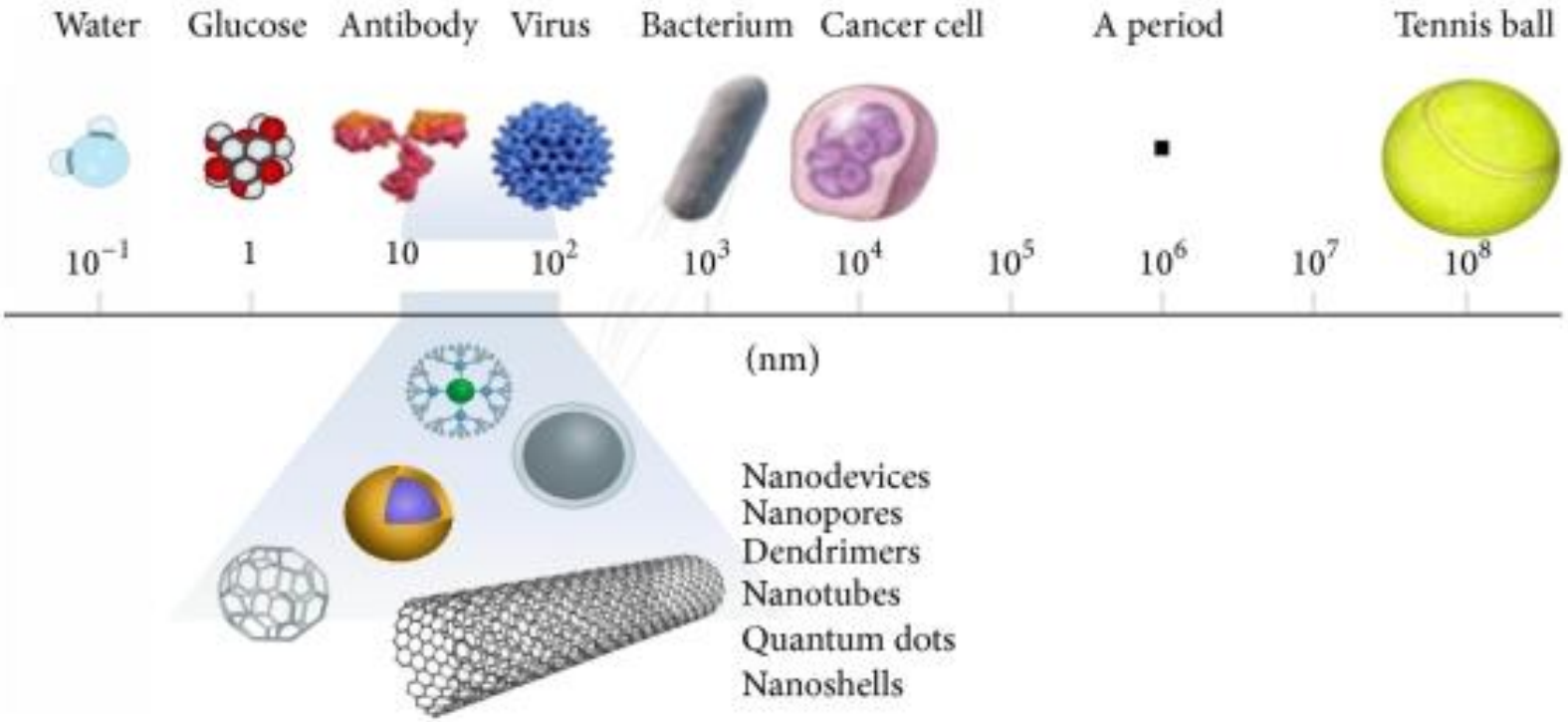
Nanometric vectors



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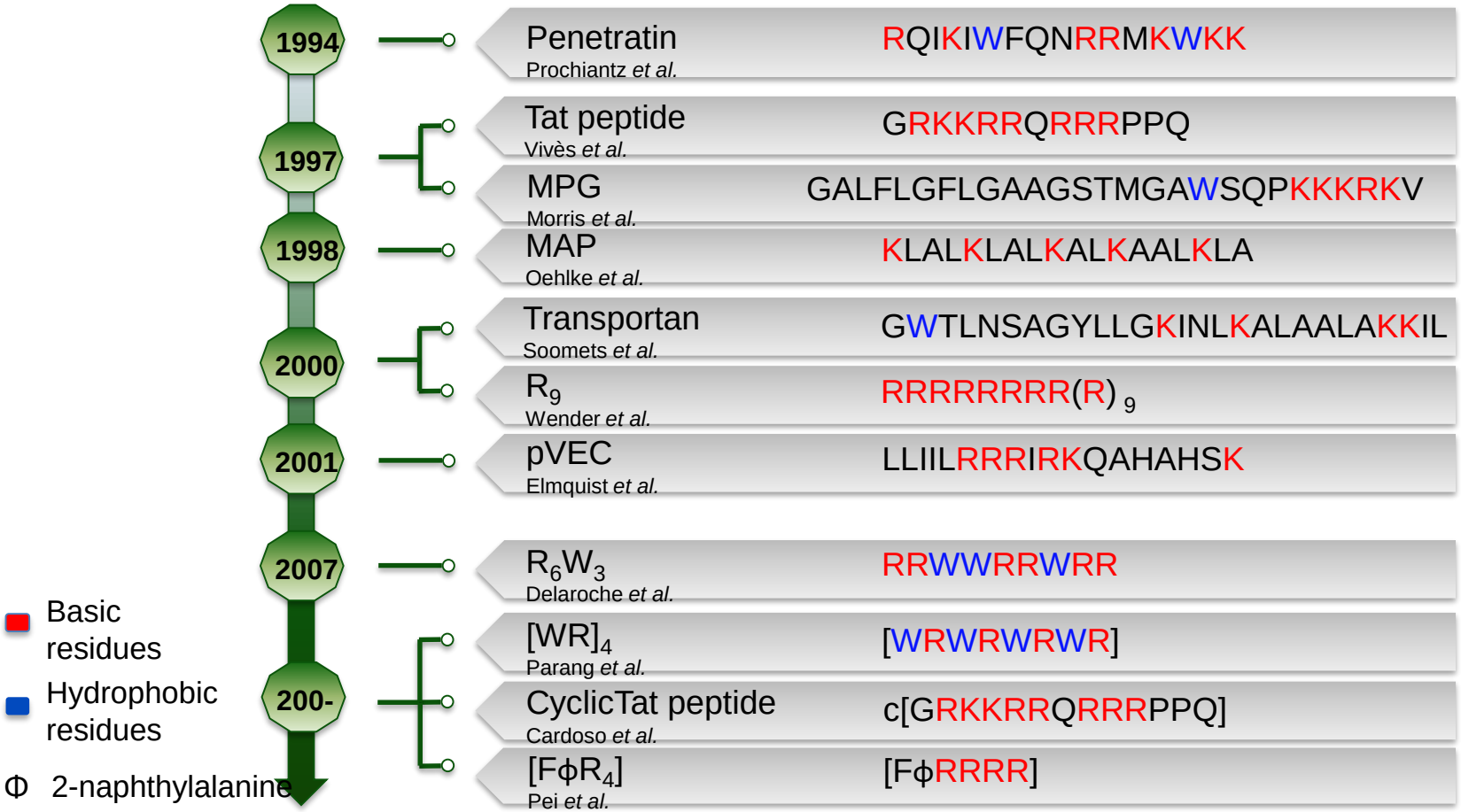
Cell penetrating peptides

25 years of history

10-30 residues

Most of the time, covalent association with the cargo (i.e.the drug) through a linker which can be cleaved to assure the delivery of the cargo



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Peptides de pénétration intracellulaire

CPP- séquences < 30 AA
Riche en aminoacides basiques

Peptides amphipatiques

Pénétratine (Antp) : RQIKIWFQNRRMKWKK, Prochiantz et al, 1994

MPG: GALFLGFLGAAGSTMGA
Pep1: KETWWETWWTEWSQPKKKRKV

} Complexes non-covalents avec oligonucléotides et protéines/peptides (interactions électrostatiques ou hydrophobes)

Peptides cationiques

Tat peptide : TAT (48-60) : GRKKRRQRRRPPQ, Lebleu et al, 1997
polyArg: R_n, Futaki et al. ; Wender et al, 2000

Peptides hydrophobes

VTVLALGALAGVGVG, Hawiger 1995 et AAVLLPVLLAAP, 1996
PFVYLI et al, Futaki 2009

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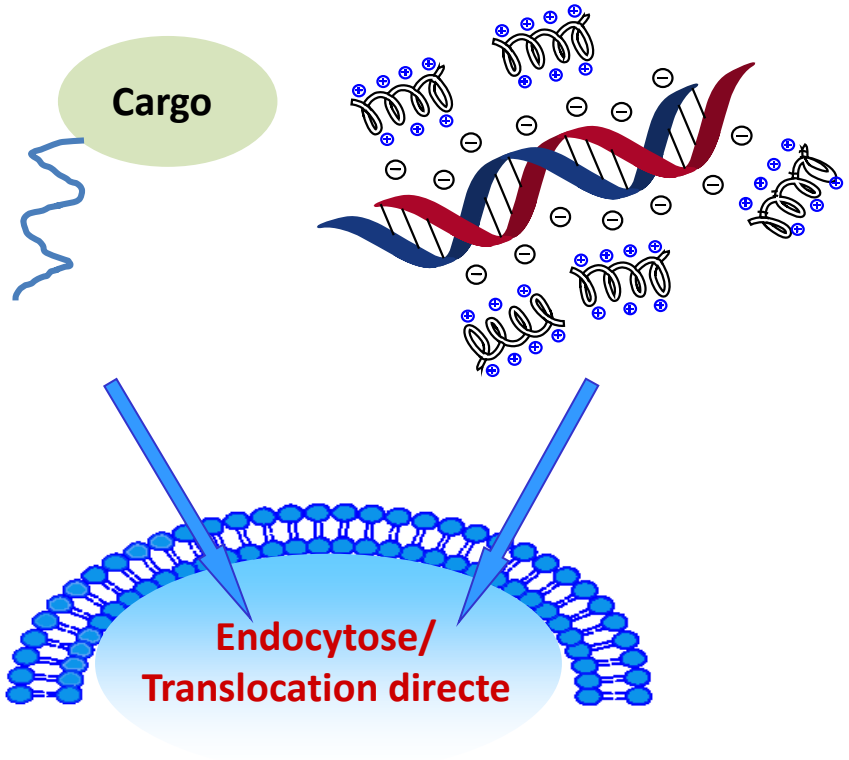
Cargo/CPP

Stratégie covalente **Stratégie non covalente**



Cargo	CPP
Oligonucleotides (DNA, plasmide DNA, phosphorothioates, siRNA)	Antp, Tat, oligomers, MPG, Pep1, oligolysines
PNA	Antp, transportan, Tat
Biological active peptides	Polyarginine, Tat, Antp, HSV-1 VP22
Proteins	Tat, Antp, HSV-VP22, poly-ornitine, poly-lysine, poly-arginine
Enzymes	HSV-VP22, Tat, poly-arginine, poly-lysine
Nanoparticules	Tat
Small molecules	penetratine, poly-lysine, Antp, Tat, MAP, transportan,

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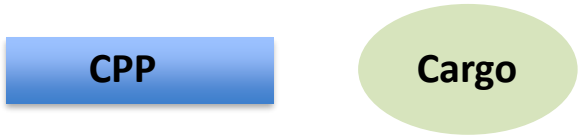
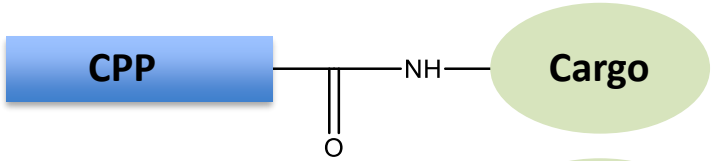
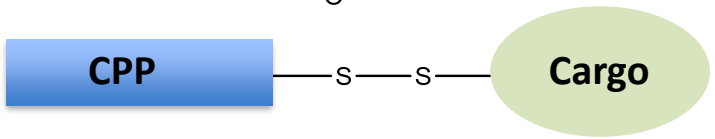
Stratégies covalente et non-covalente



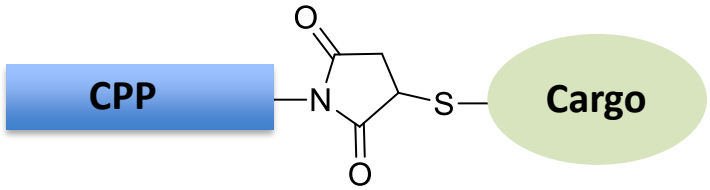
Exemples de liens covalents utilisés pour lier le cargo au CPP



Liaison peptidique

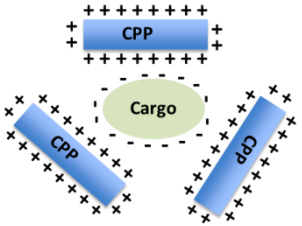


Liaison S-S



Liaison S-maléimide

Stratégie non covalente



Interaction électrostatique
(charges dépendant)

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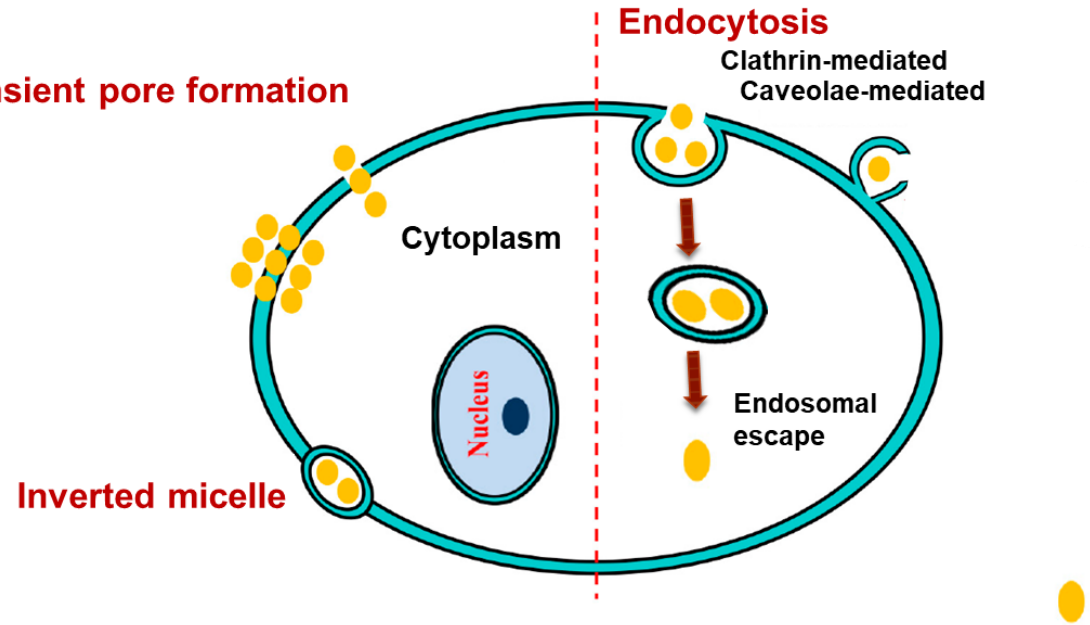
Direct translocation : entry through transient membrane perturbation

Endocytosis : entry by vesicles created at the cell membrane



The energy dependence of the internalization mechanism is unique because all endocytotic pathways are inhibited at low temperature. Consequently, at low temperature, internalization likely reflects a direct translocation mechanism.

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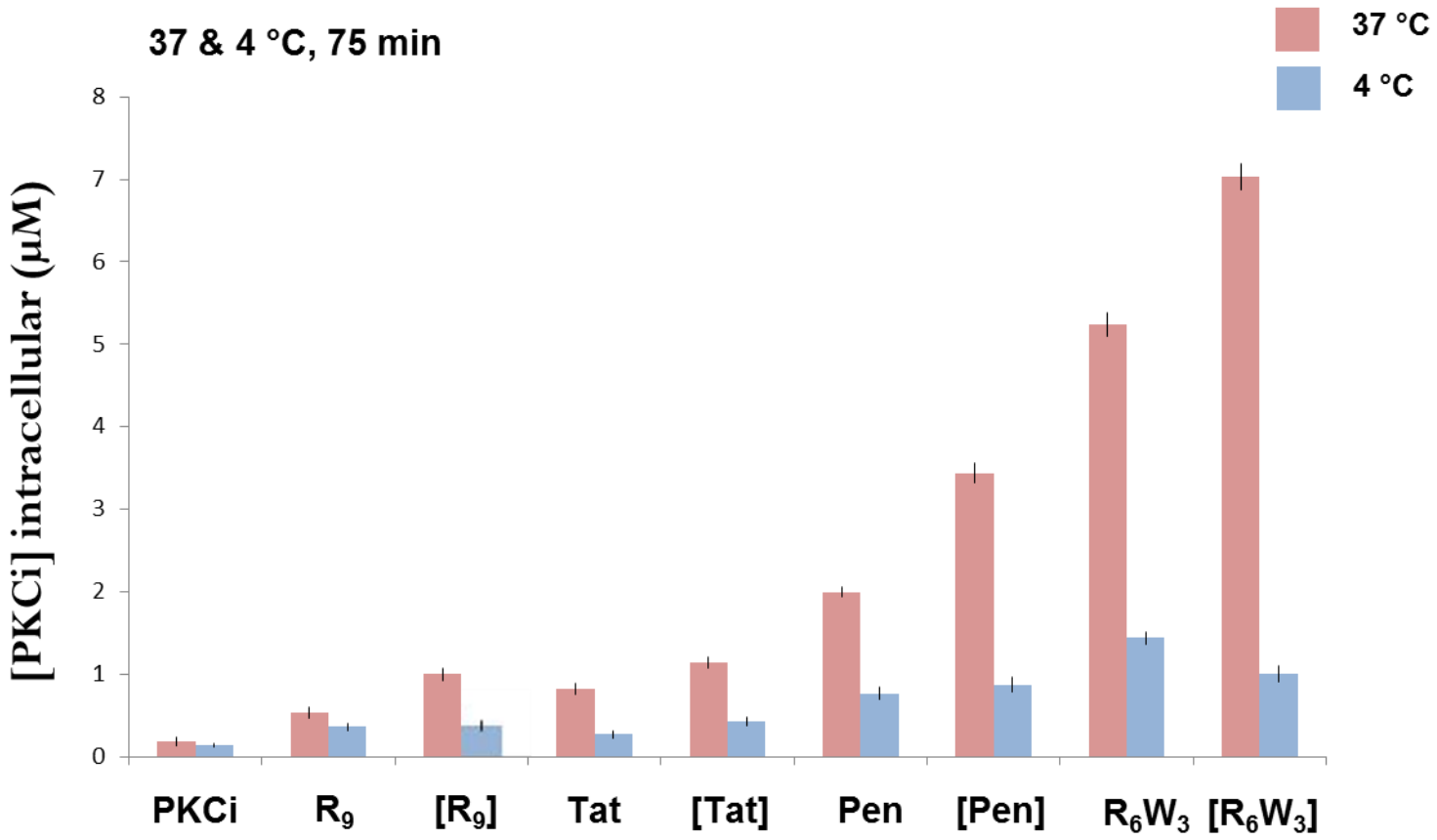

- The uptake efficiency after cyclisation is due to a better entry by endocytosis (main pathway of entry)

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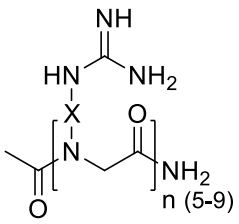
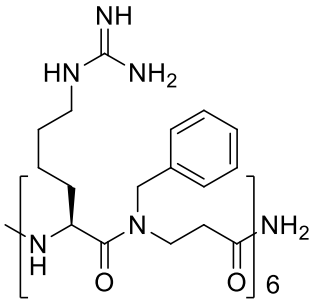
Dérivés des CPPs cationiques
Augmenter la biodisponibilité

✓**Peptoïdes**

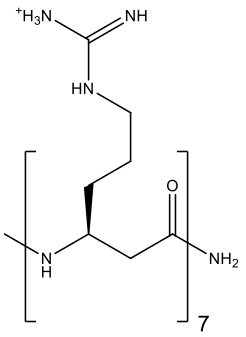


Wender et al, PNAS 2000



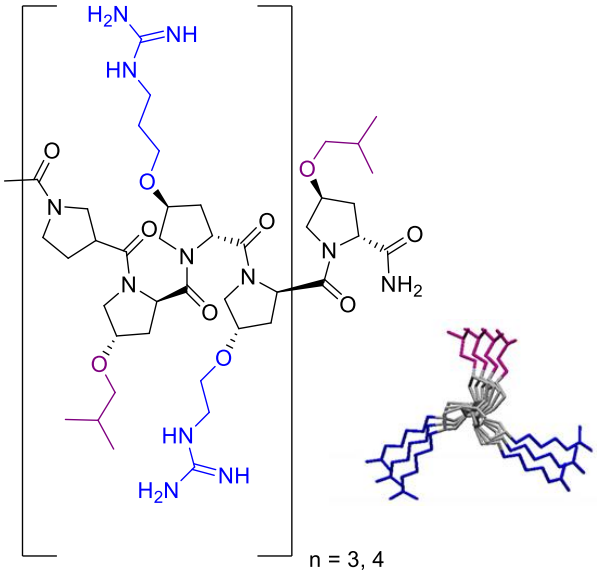
Chimeric peptoid
Foged et al, *BBA* - **2008**

✓ **β-peptides**



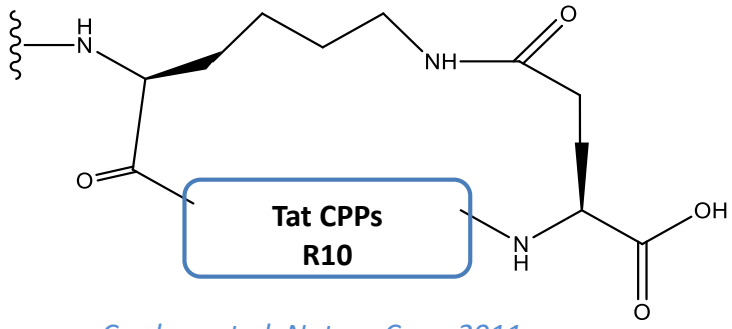
Seebach, ChemBioChem 2002
*Gellman, JACS 2001 (*analogue of Tat 47-57*)*
Guichard, ACIE 2015 (oligourées, Delivery of DNA)

✓**Pro-based scaffolds**



Chmielewski, JACS 2005;
Giralt, JACS 2005

✓**Peptides cycliques**



Cardoso et al, Nature Com, 2011;
ACIE 2015 (Délivrance de la protéine GFP)
Marsault,Bioconjugate Chem, 2015

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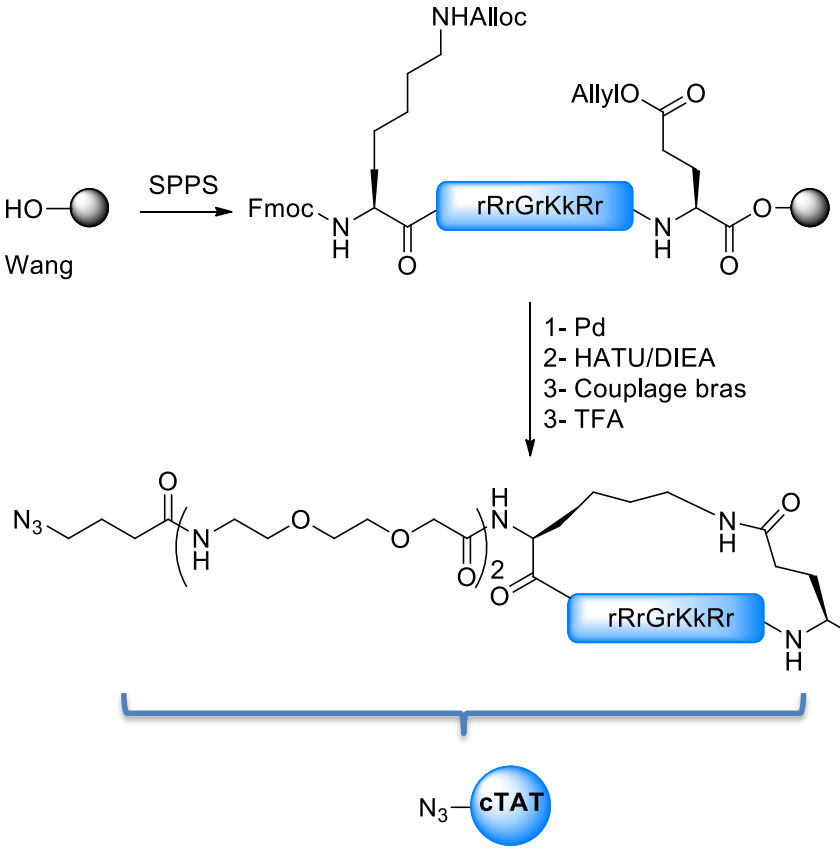
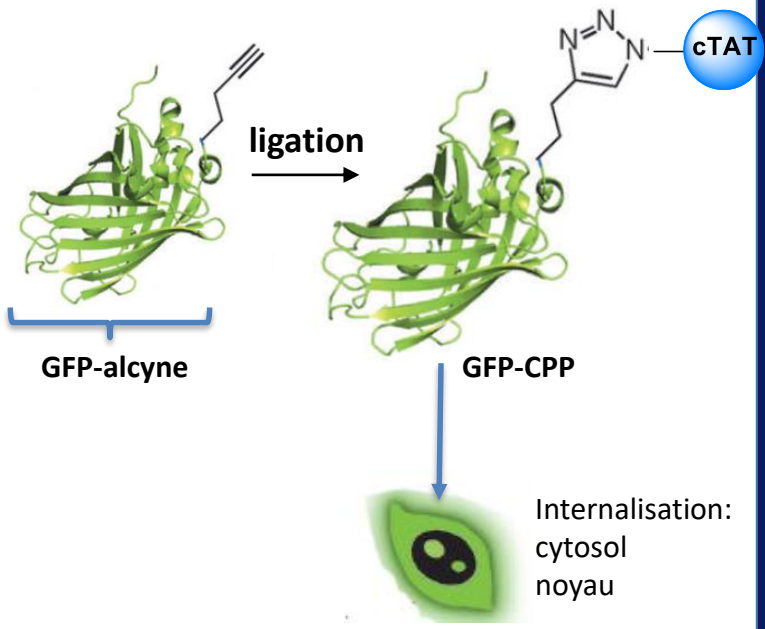
Exemples de conjugués

Conjugué CPP (Tat cyclique)-protéine





Conjugué TAT48-57-inhibiteur kinase c-Jun (JNK)

H-GRKKRRQRRR-**Inhibiteur JNK** (peptide amide 22 mer)

Traitement: perte audition
Phase clinique III

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III.1. Cell targeting peptides

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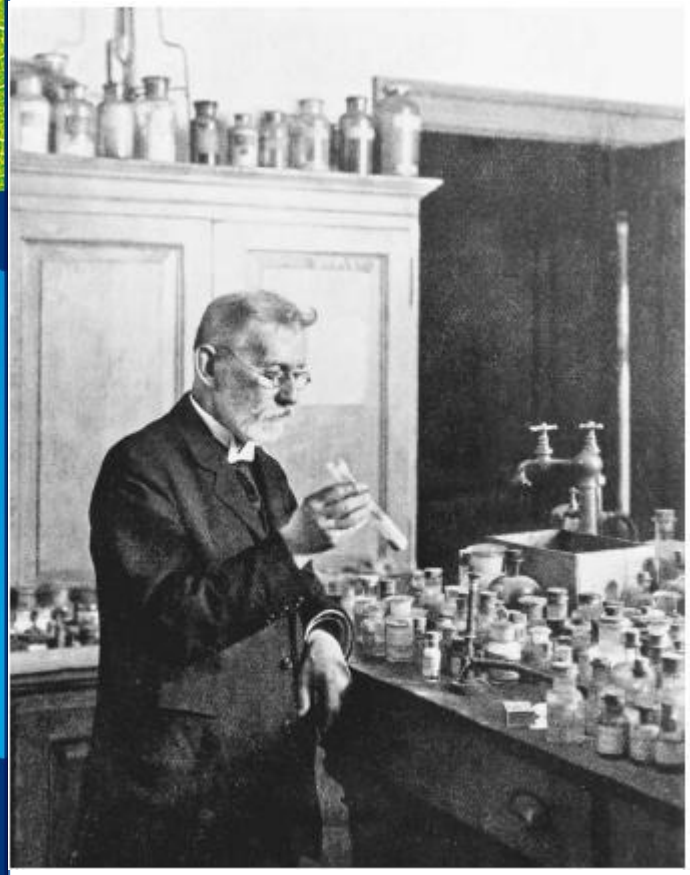
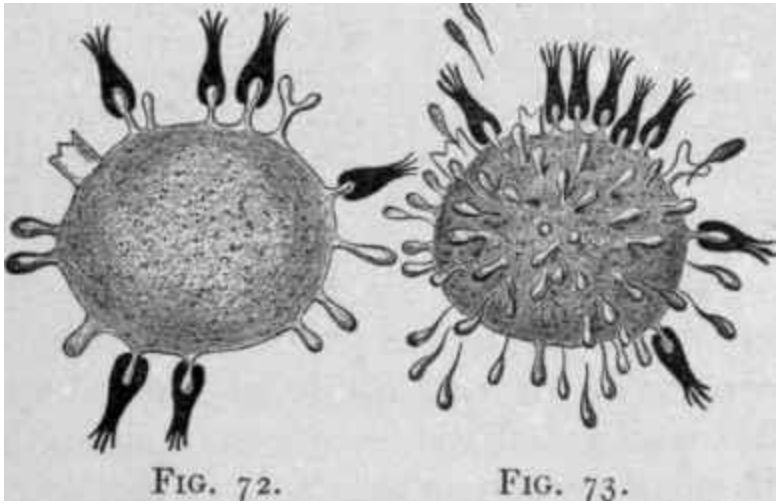
Targeting: an old story



Paul Ehrlich (1854-1915) german physicist
Nobel price 1882 *in recognition of his work on immunity"*



Father of modern chemotherapy (Salvarsan, the first real 'chemical drug' , against syphilis)
Hypothetizes that what he called *« 'side chains of cells' (i.e. membrane _ bound biomolecules) can be recognized »*
Introduced the concept of **'Magic Bullet'** : *« a specific agent that kills only one organism/cell »*

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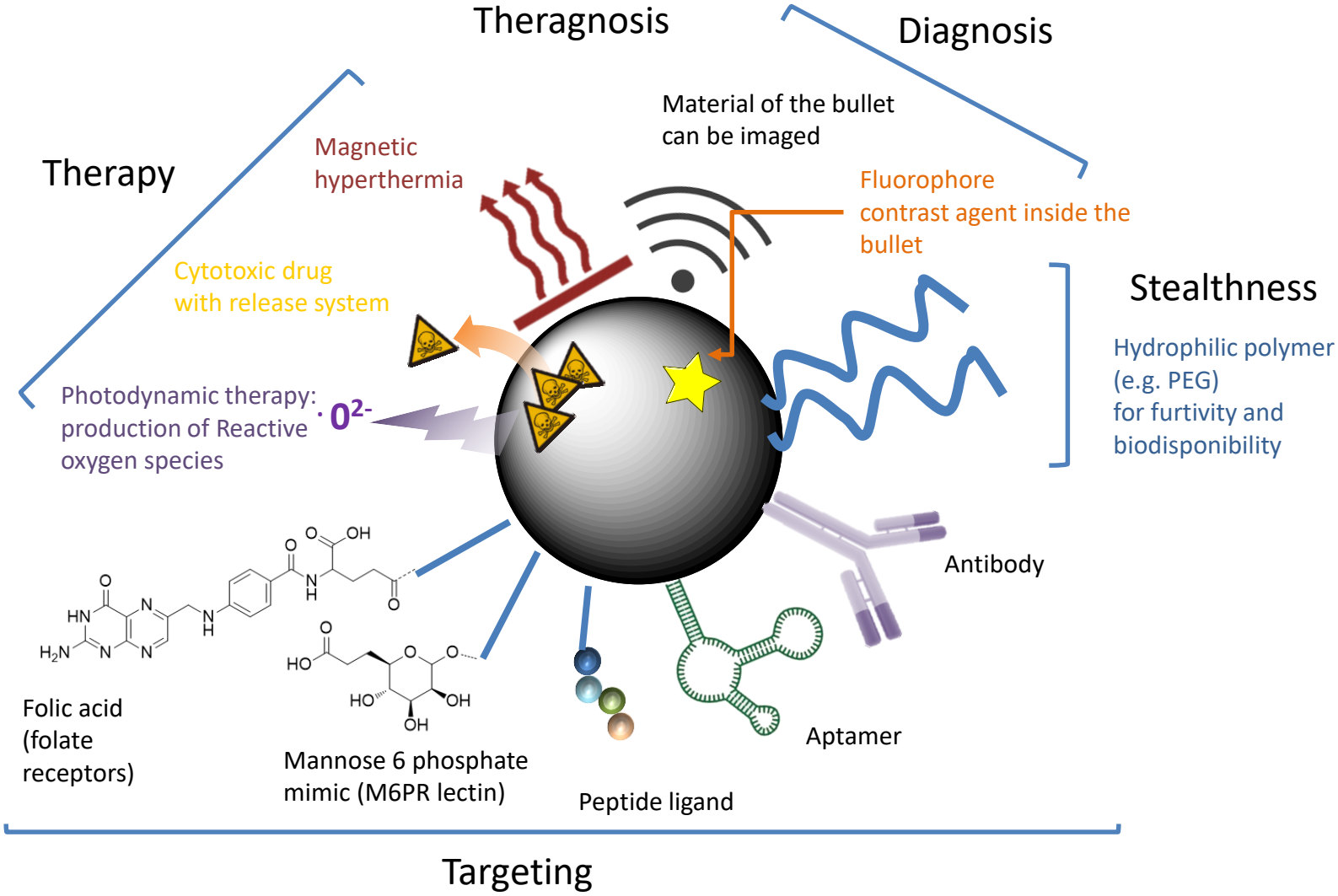
The updated ‘magic bullet’ concept



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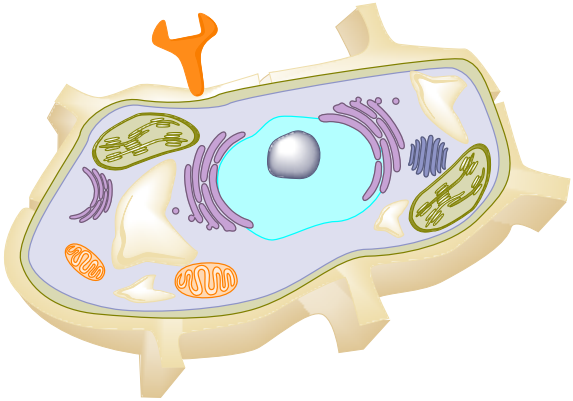
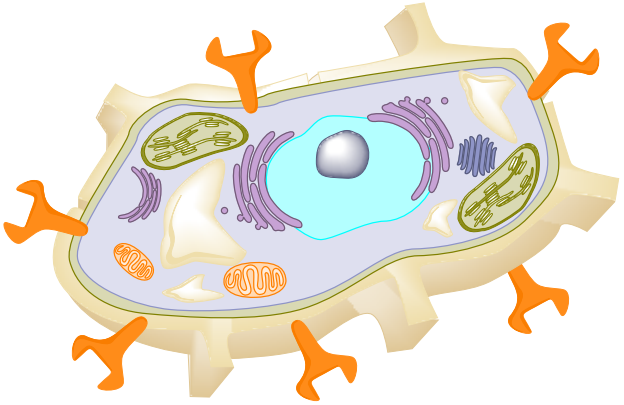
How to target cancer cells?

Normal cell



Cancer cell



over-express particular receptor 'X'

(e.g. involved in proliferation, survival, migration, adhesion, or normally found in early stages of embryo development)

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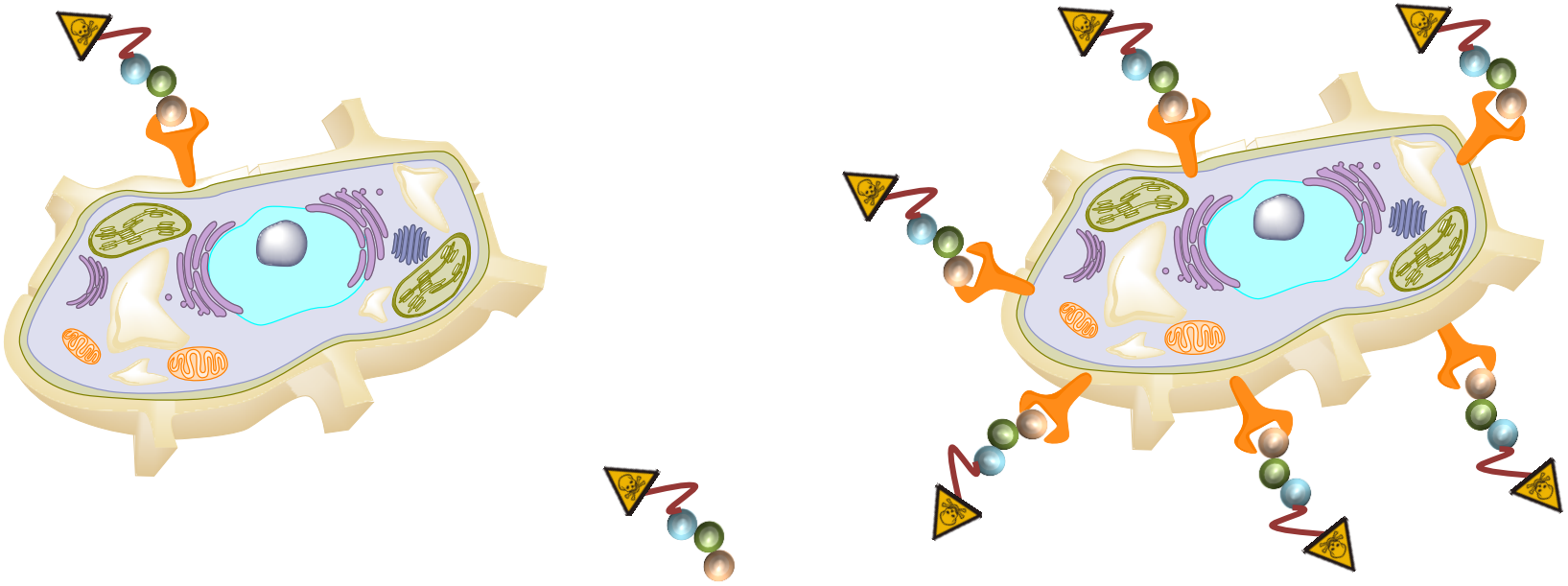
How to target cancer cells?

Normal cell

Cancer cell



Selective Ligand of the receptor X conjugated (via a linker) to a cytotoxic drug (and/or an imaging agent)
Agonist: prefered for drug conjugates
Antagonists: prefered for imaging conjugates

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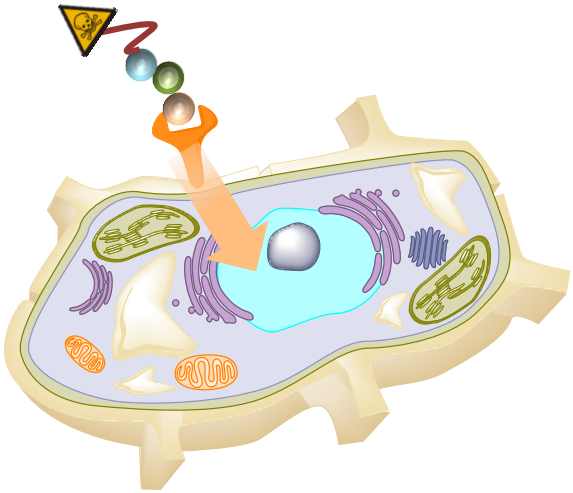
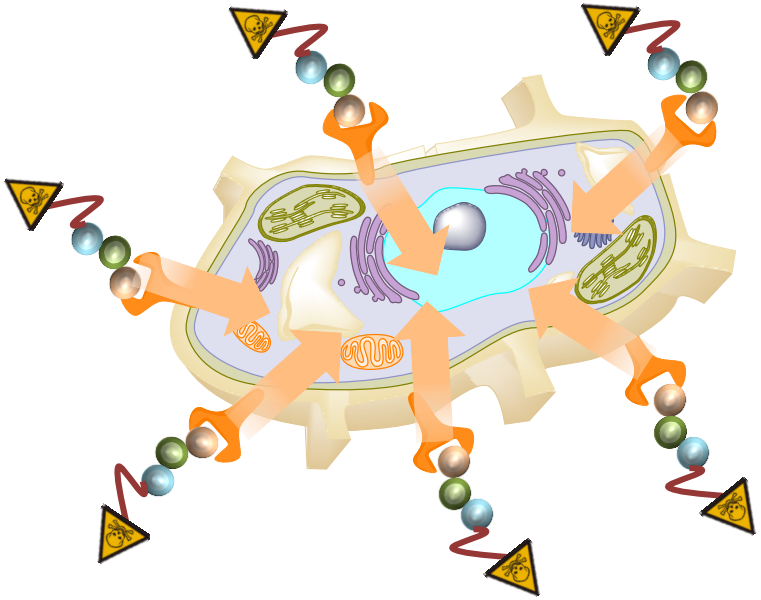
How to target cancer cells?

Normal cell



Cancer cell



Agonist: receptor mediated endocytosis

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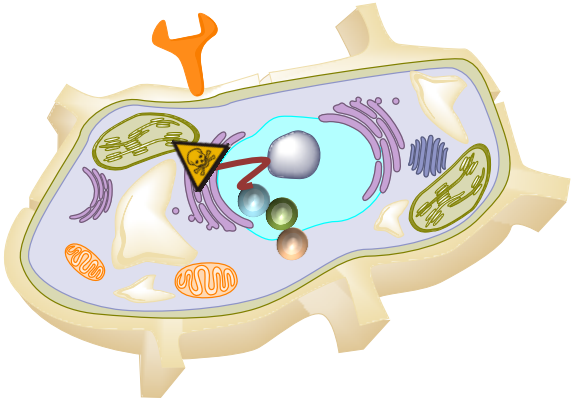
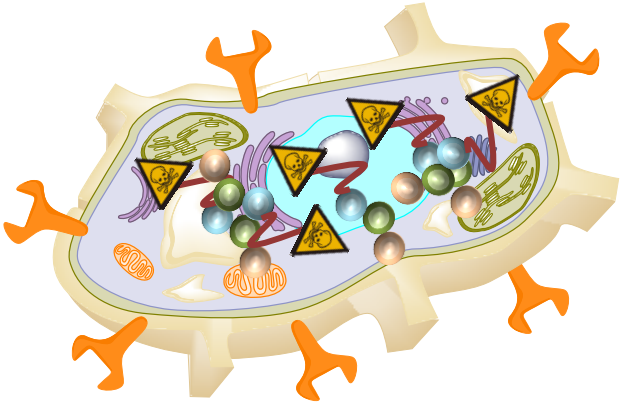
Normal cell



Cancer cell



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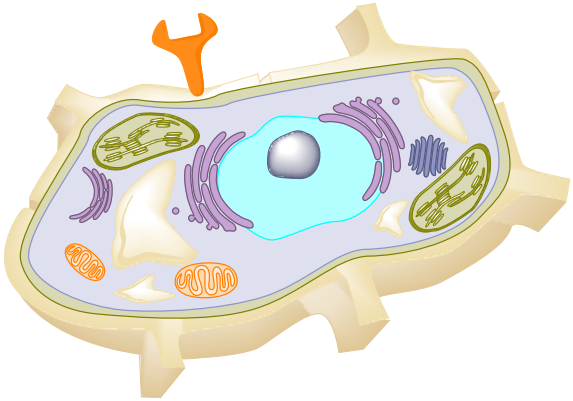
Normal cell



Cancer cell

Apoptosis

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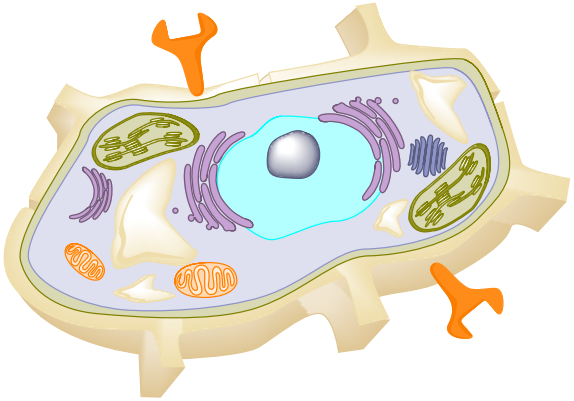
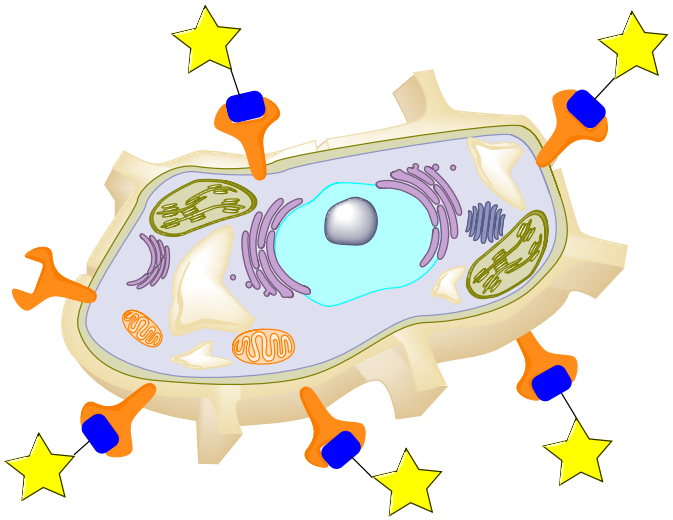
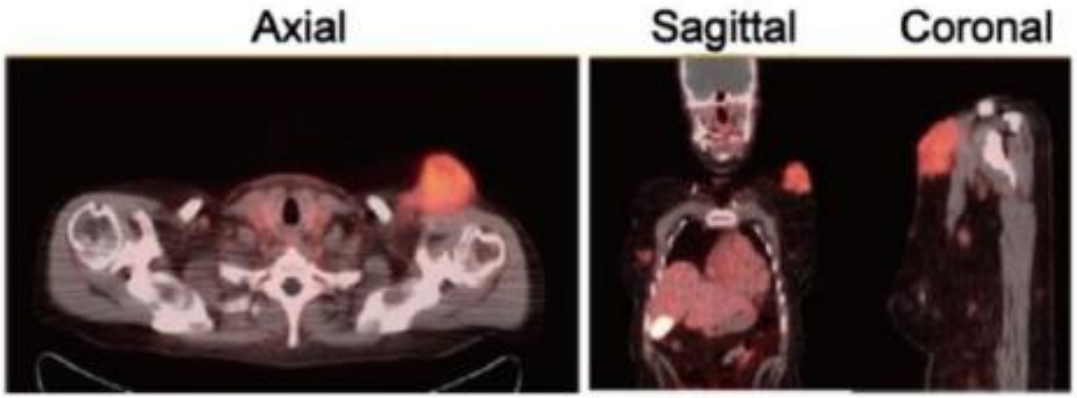
Ligand peptidique plus agent de contraste

Cellule saine



Cellule cancéreuse





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Overexpressed membrane receptors- Integrins

24 types of Integrin receptors
A short tripeptide retains the binding properties of integrins on their receptor: ArgGlyAsp (RGD)

Over expression of integrin receptors in cancer cells :

- Angiogenesis
- Proliferation
- Migration
- Invasion
- Remodelling of the extra cellular matrix

metastasis

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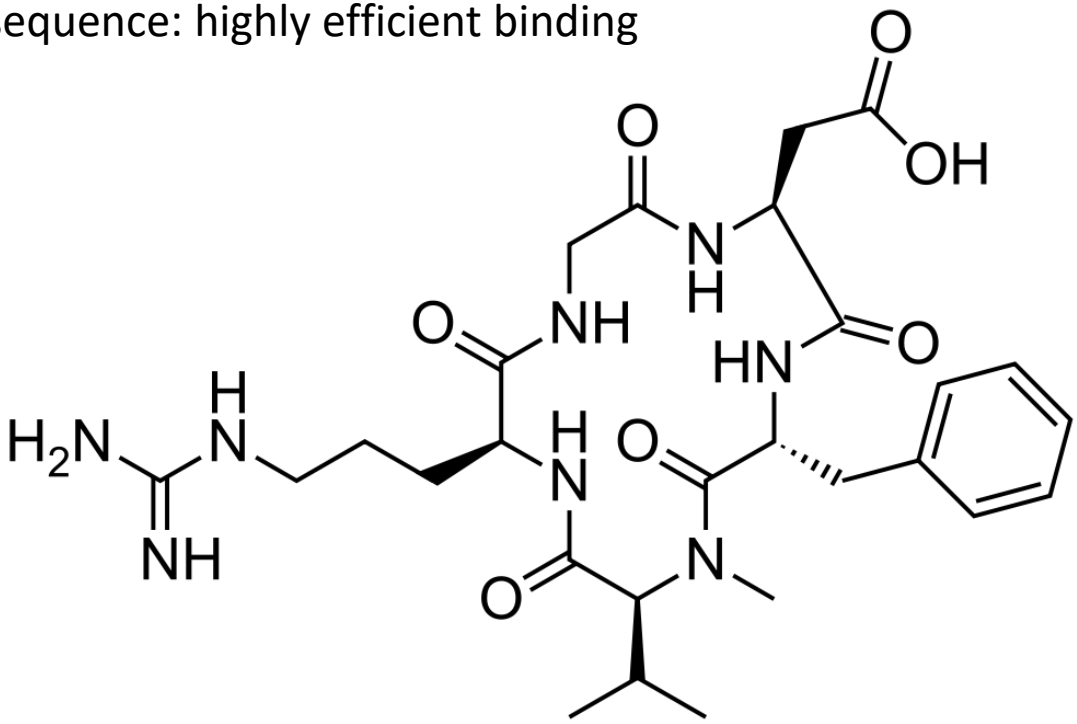
Cilengitide - EMD 121974 (Merck)

Cyclic RGD sequence: highly efficient binding



Antagoniste of αv-integrins
Anti-angiogenic for glioblastome treatment
High doses: 2g– 2 times a week
EORTC phase III – 500 people –cilengitide + standard therapy

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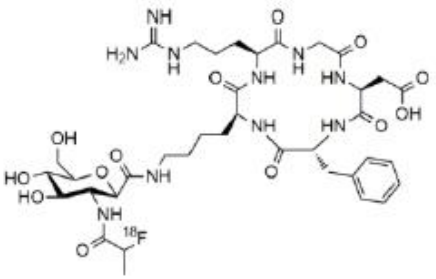
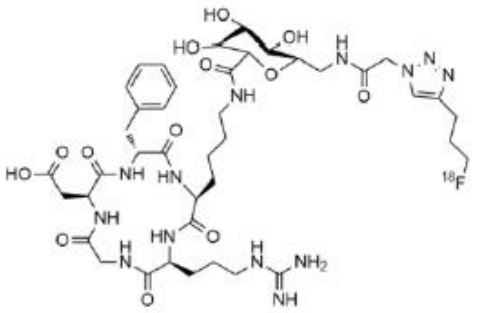
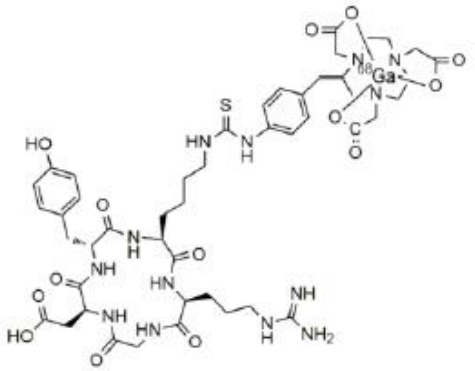
Positron Emission Tomography PET-RGD



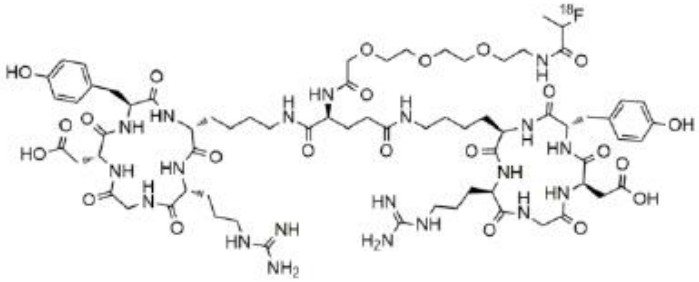
[^{18}F]Galacto-RGD



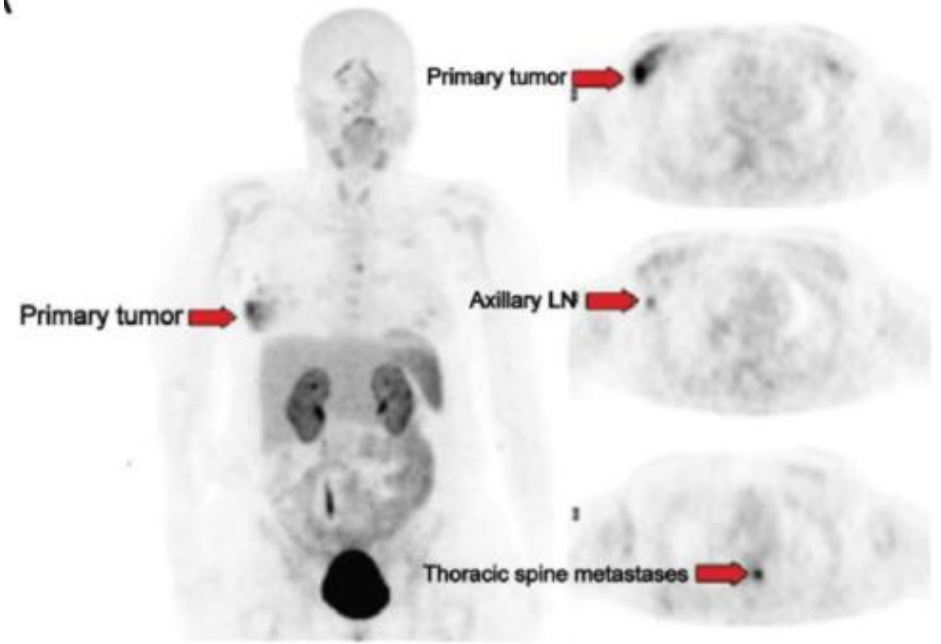
[^{18}F]RGD-K5



[^{68}Ga]NOTA-RGD



[^{18}F]FPPRGD2



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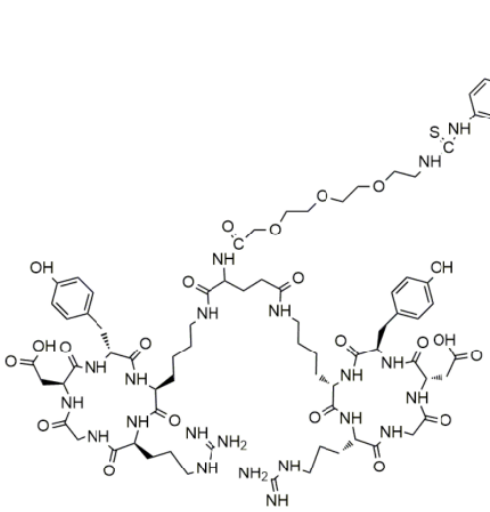
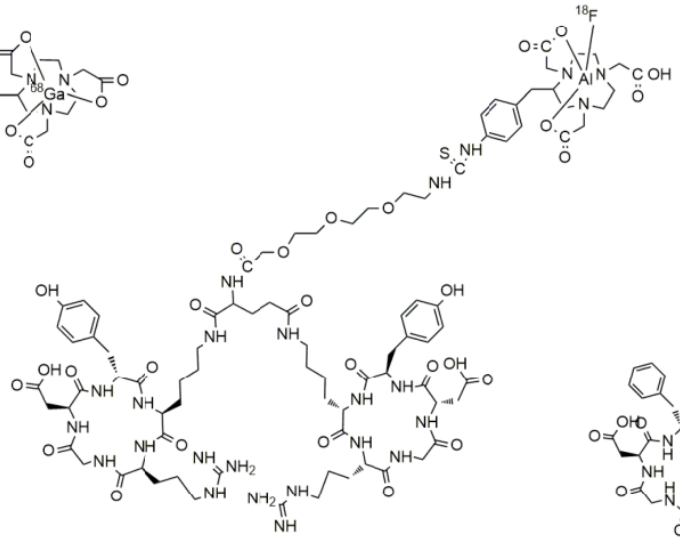
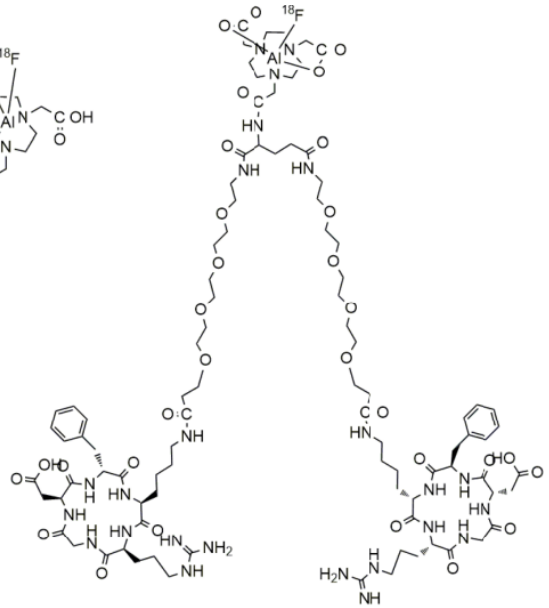
Tomographie par émission de positons - RGD



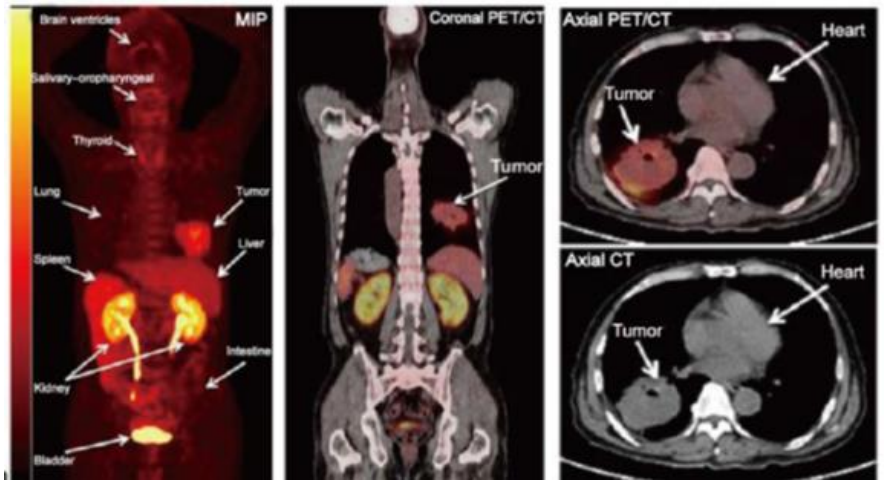
[^{68}Ga]NOTA-PRGD2



[^{18}F]Alfatide



[^{18}F]Alfatide II



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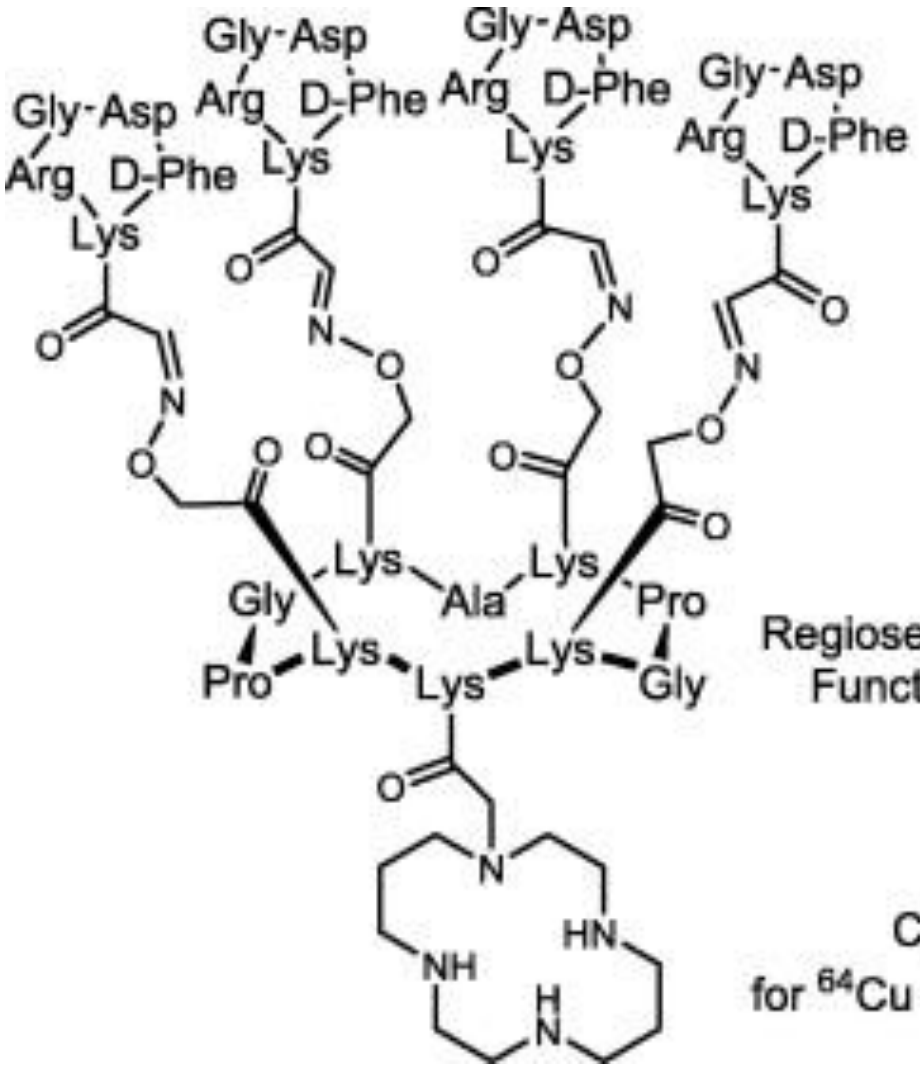
Système RAFT- RGD



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CD 13- aminopeptidase N

CD 13 – aminopeptidase N

Métalloprotéinase membranaire surexprimée par les cellules lors de l'angiogenèse

Ciblage de la vascularisation des tumeurs solides

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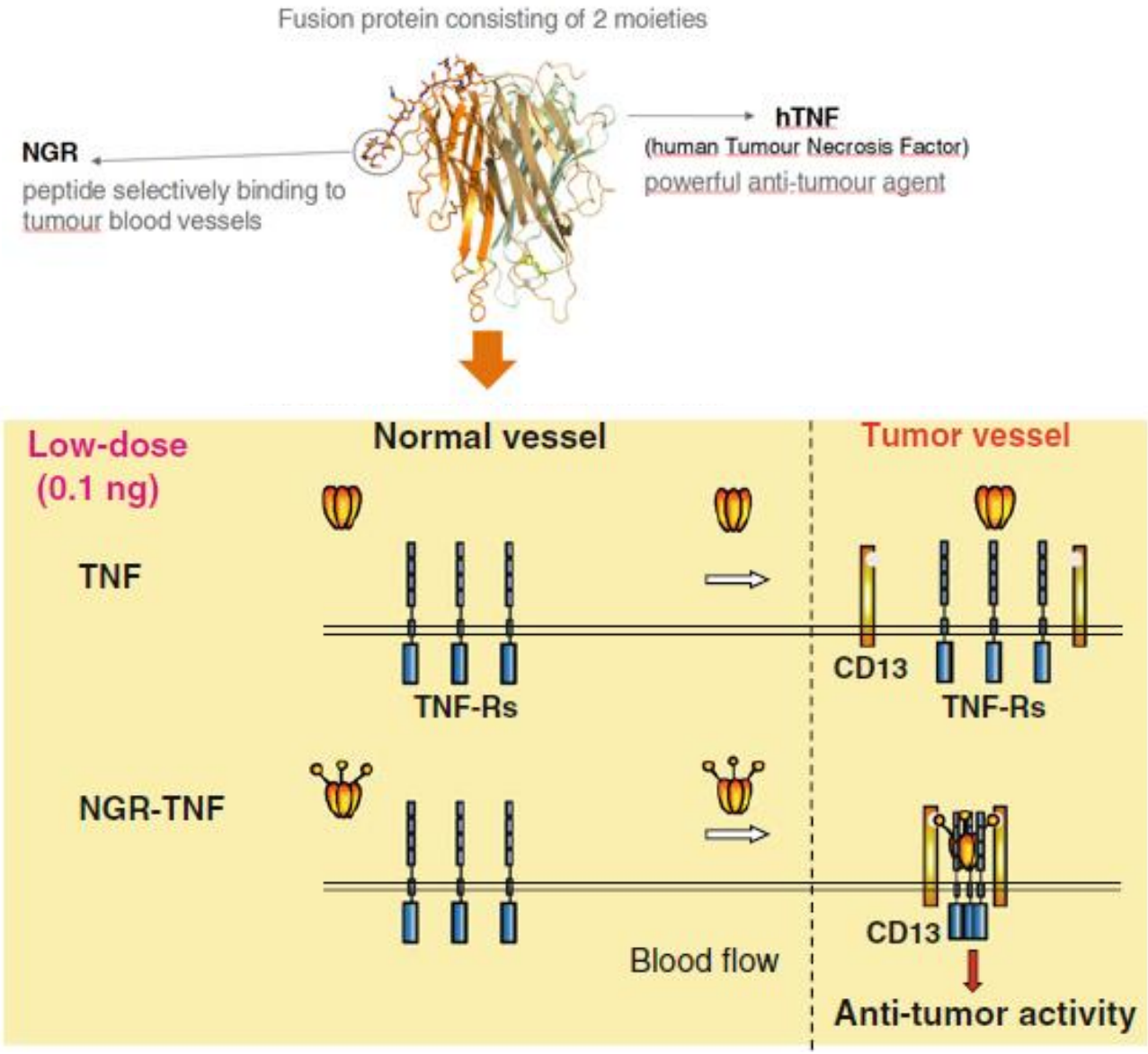
Molmed NGR-hTNF



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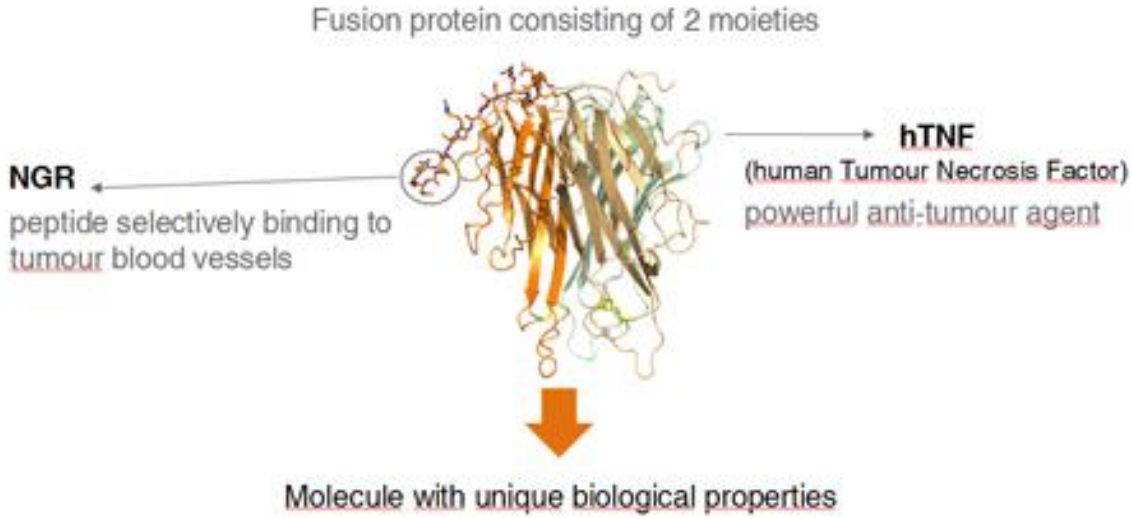
Molmed NGR-hTNF



Effet anti-cancéreux

Perméabilisation vasculaire – améliore l'efficacité des chimiothérapies déjà existantes

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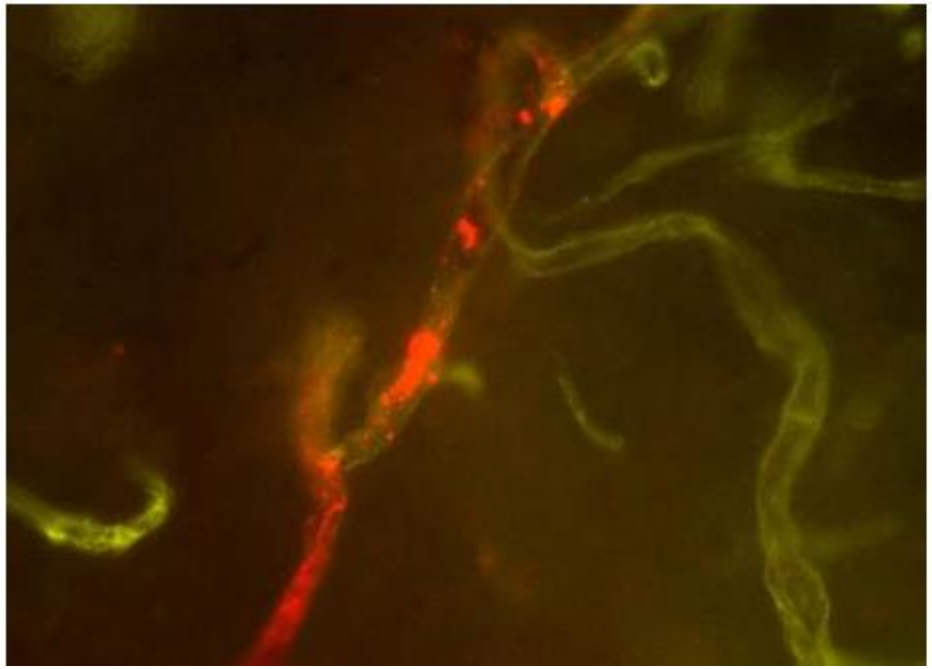
Molmed NGR-hTNF

MOLMED

Home Company Clinical Trials Pipeline Technological platforms GMP Solutions Partnering Investors Media

NGR-hTNF



- TK
- NGR-hTNF
  - COLORECTAL CANCER
  - LIVER CANCER
  - NON-SMALL CELL LUNG CANCER - NSCLC
  - SMALL CELL LUNG CANCER - SCLC
  - OVARIAN CANCER
  - SOFT TISSUE SARCOMAS
  - PLEURAL MESOTHELIOMA
  - SOLID TUMOURS
  - FAQ

NGR-hTNF main results

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Zoptarelin doxorubicin (AEZS-108) - AEterna Zentaris

- Luteinizing hormone releasing hormone - L'hormone lutéinisante

Agonistes/ antagonistes - Traitement de cancers hormono-dépendants

LHRH – surexprimé par les cellules des cancers :

- prostate
- sein
- ovarien
- de l'endomètre

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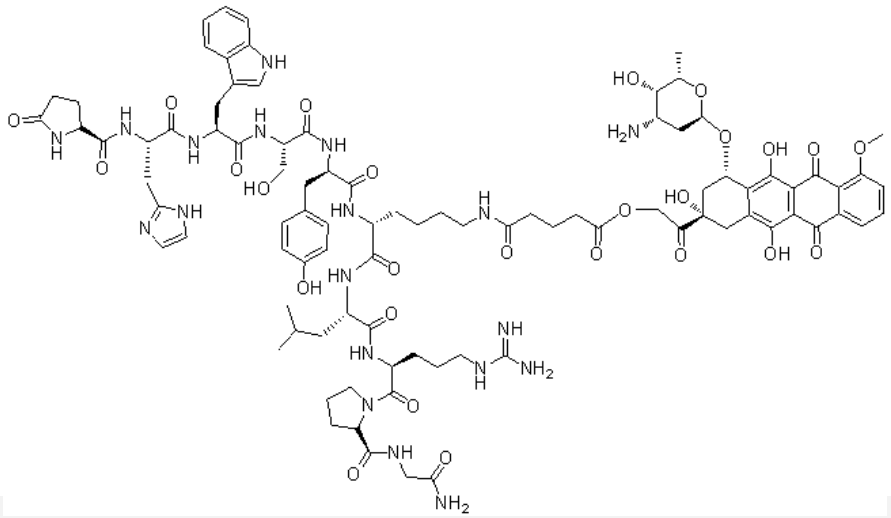
Zoptarelin doxorubicin (AEZS-108) - AEterna Zentaris



PRECLINICAL	PHASE 1	PHASE 2	PHASE 3
AEZS-120 Prostate cancer[3] *Product sheet*		**ZOPTREX™ (ZOPTARELIN DOXORUBICIN)** Ovarian cancer[1] Completed *Product sheet*	**MACRILEN™ (MACIMORELIN)** Evaluation of Adult Growth Hormone Deficiency (AGHD) *Product sheet*
ERK INHIBITORS Oncology (4) *Product sheet*		**ZOPTREX™ (ZOPTARELIN DOXORUBICIN)** Prostate cancer[2] *Product sheet*	**ZOPTREX™ (ZOPTARELIN DOXORUBICIN)** Endometrial cancer *Product sheet*

Doxorubicin

Statut de médicament orphelin

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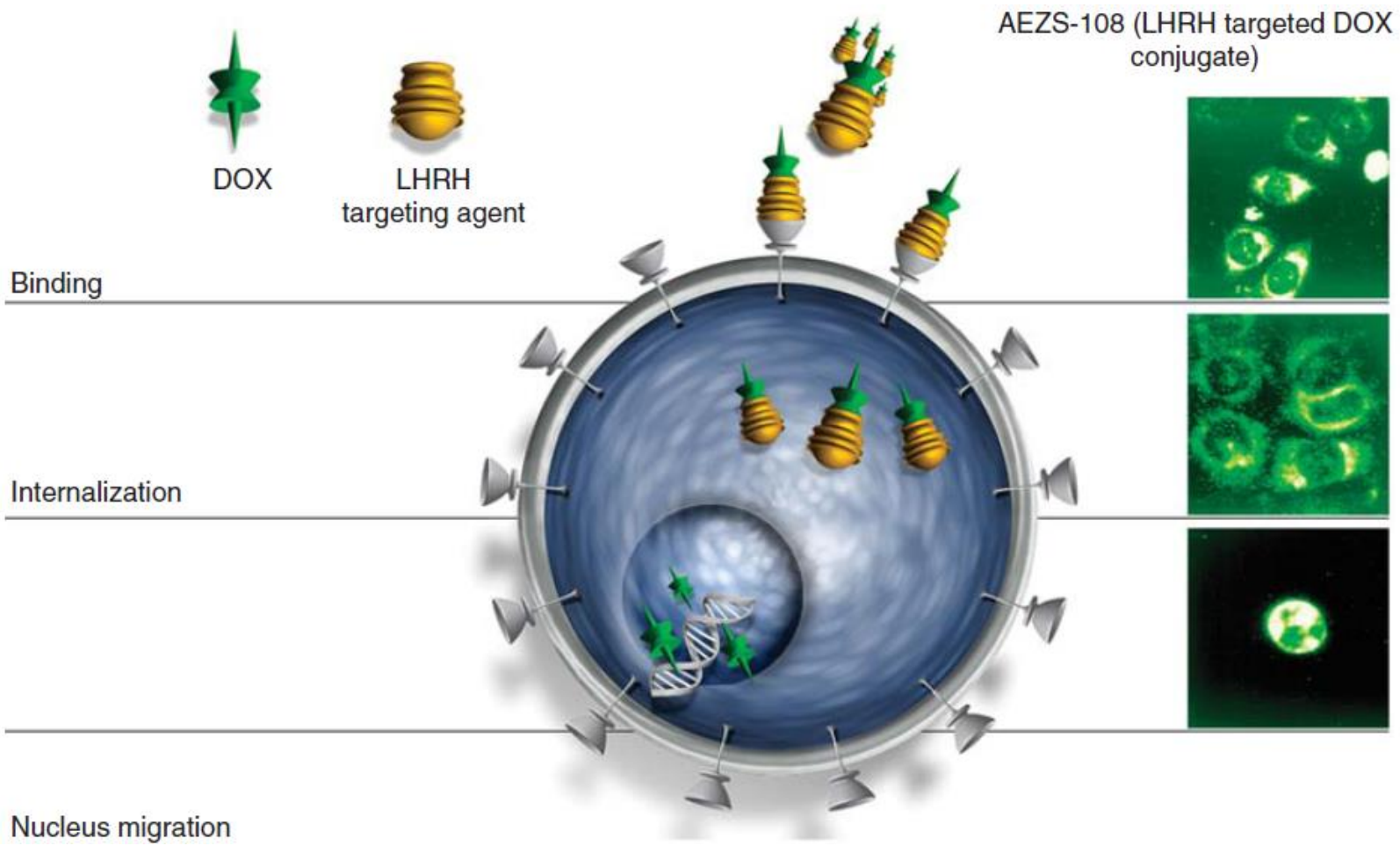
Zoptarelin doxorubicin (AEZS-108) - AEterna Zentaris



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Zoptarelin doxorubicin (AEZS-108) - AEterna Zentaris

Résultats de Phase II – traitement du cancer endométrial avancé ou récurrent / 50 patientes

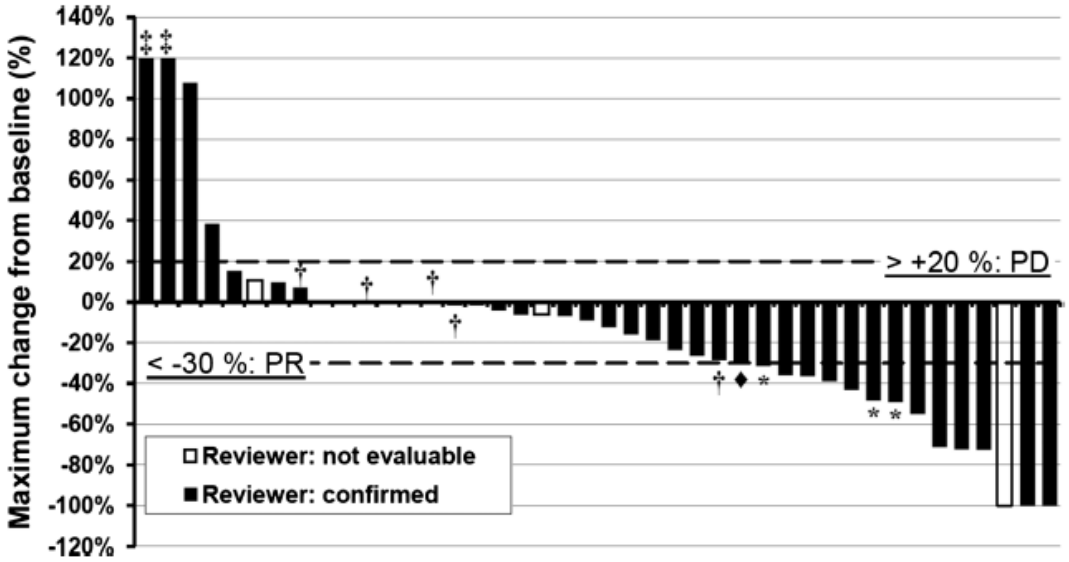

FIGURE 1. The maximal percent change of target lesion. *Three PRs not confirmed at a subsequent time point. †Progressive disease based on occurrence of new lesions. ‡Symptomatic deterioration or death due to malignancy before cycle 2, with maximum change arbitrarily assigned as 120%. Note: In nonevaluable cases, the complete disappearance of a lesion was not accepted as a CR because the lesion size at baseline did not meet the Response Evaluation Criteria in Solid Tumors requirements. One (noncancer death before cycle 2) excluded from the plot because no tumor size assessment was available.

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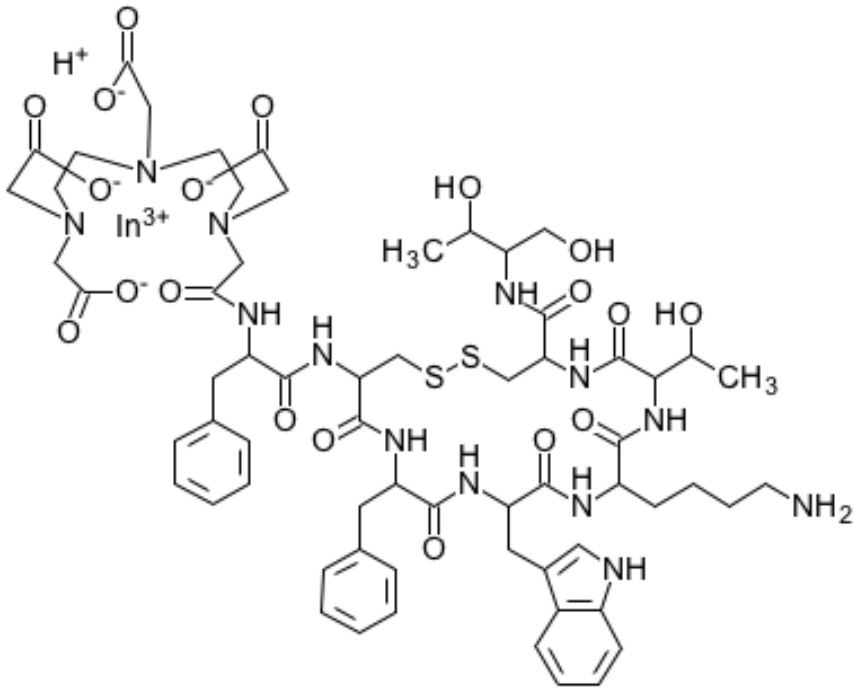
Récepteur de la somatostatine (SSTR)

SSTR RCPG - Surexprimé par plusieurs types de cancer

Octreoscan – Détection des tumeurs pancréatiques neuroendocrines avec une sensibilité de 75-100%



D02108

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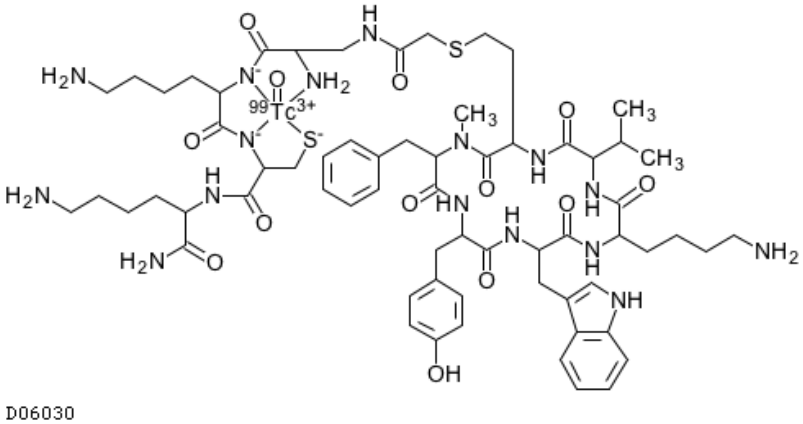
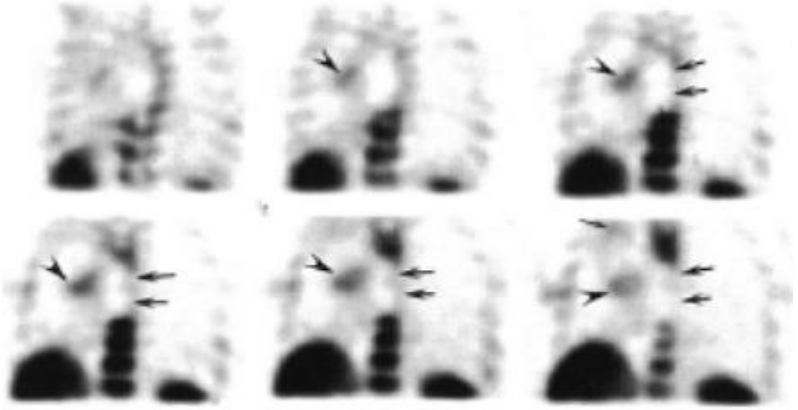
Récepteur de la somatostatine (SSTR)

SSTR RCPG - Surexprimé par plusieurs types de cancer

Depreotide – Détection du cancer du poumon





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Screening of homing peptides

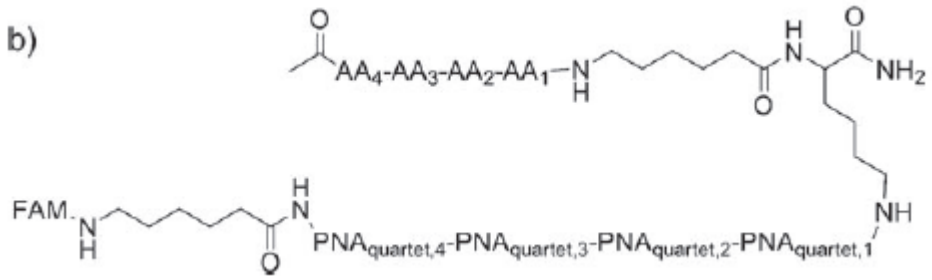
Angewandte International Edition Chemie

Homing Peptides

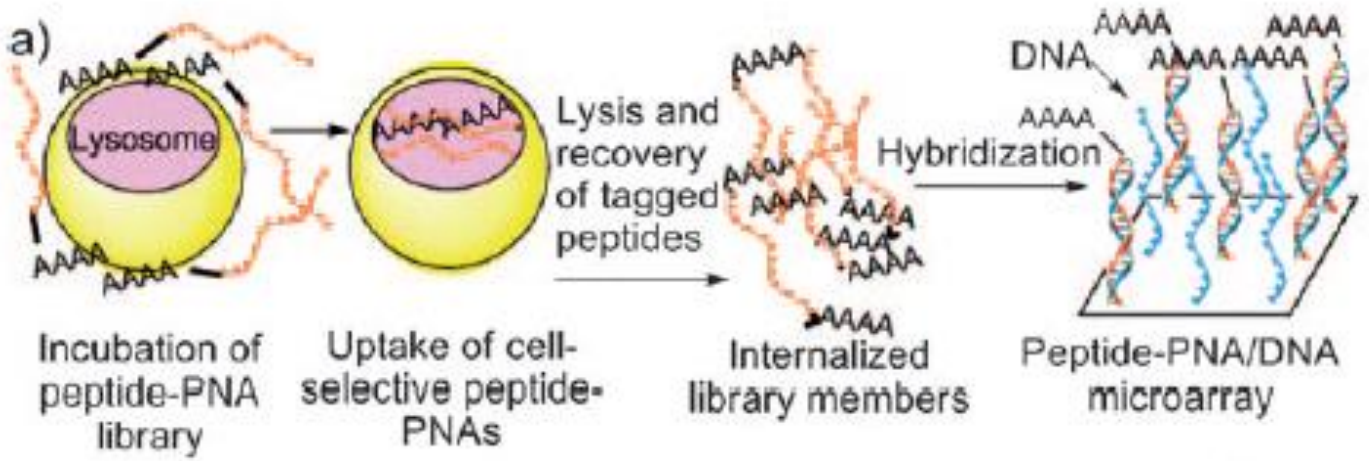
DOI: 10.1002/anie.201101804

Screening of a Combinatorial Homing Peptide Library for Selective Cellular Delivery** 2011

*Nina Svensen, Juan José Díaz-Mochón, Kevin Dhaliwal, Songsak Planonth, Michael Dewar, J. Douglas Armstrong, and Mark Bradley**



6 aminoacides différentes: $6^{4=}$ 1296 tetrapeptides



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Exemple: chimiothèque codée en solution

Identification Consensus sequence Glu-Llp-Glu-Glu

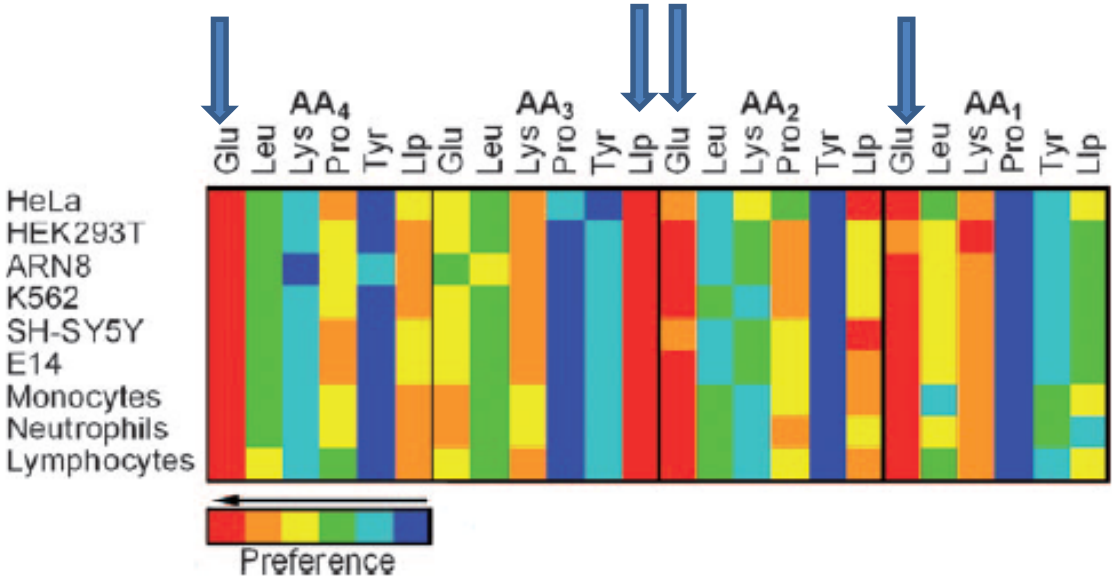

Figure 2. DNA microarray analysis of the extracted members of the peptide–PNA Library 1, which identifies a generic consensus sequence of highly cell-penetrating peptides. Each microarray consisted of four subarrays of 44 000 features each, with 33 replicates of each oligonucleotide complementary to each member of the library as well as 1232 noncoding negative controls. The heat map shows the cell-penetrating peptide preferences extracted from the scatter plots.

Utile aussi pour la détermination de peptides sélectifs d'un type cellulaire

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III.3. Crossing the blood brain barrier

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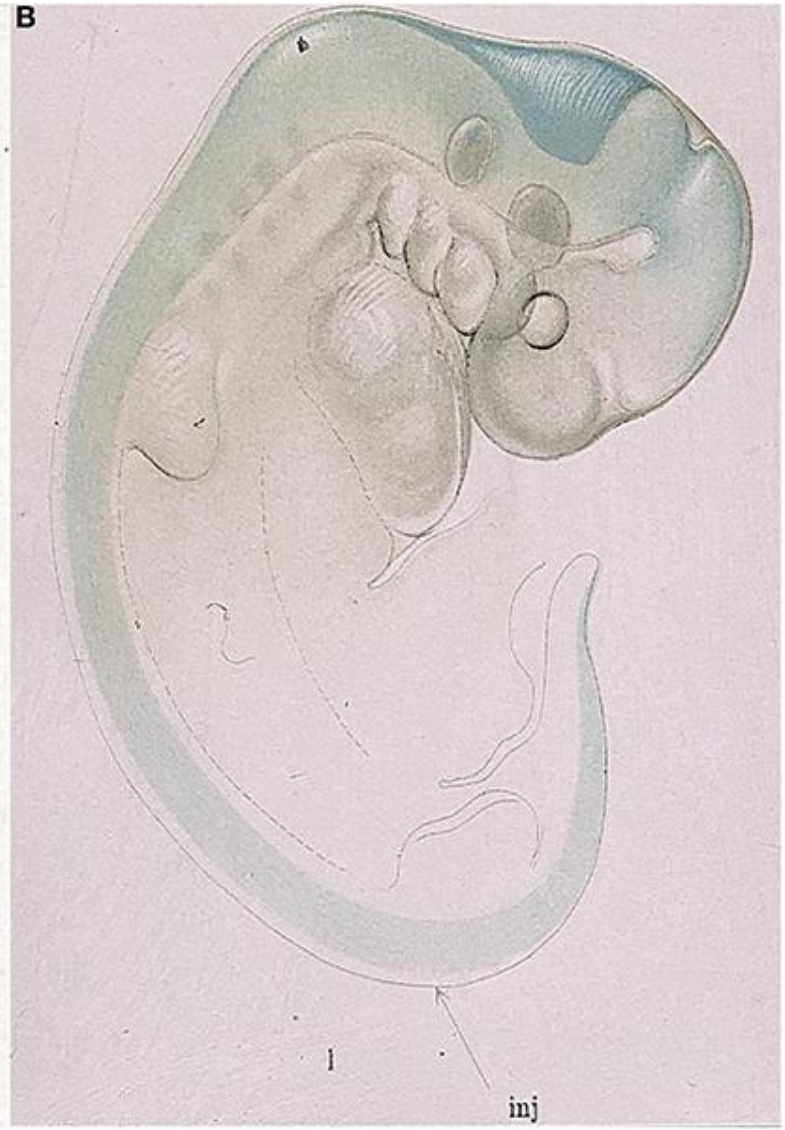
Guinea Pig embryo
Parenterally injection of dye

Wislocki, G. B. **1920**. Experimental studies on fetal absorption. I. The vitality stained fetus. *Contrib. Embryol. Carnegie Inst*. 11, 45–60.

Pig embryo
Intra spinal canal injection



Weed, L. H. **1917**. The development of the cerebrospinal fluid spaces in pig and in man. *Contrib. Embryol. Carnegie Inst*. 5, 3–116.

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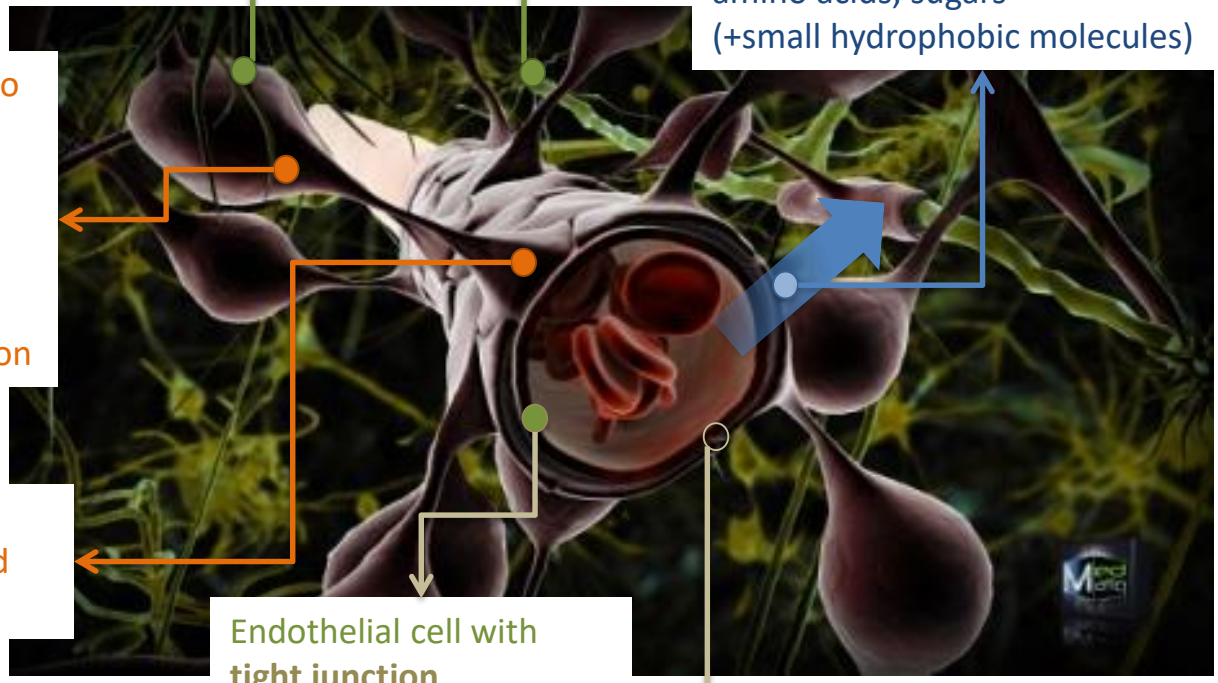
The Blood-Brain-Barrier (BBB) hematoencephalic barrier

Neurons

Crossing is possible for water, urea, glycerol, O2, CO2, amino acids, sugars (+small hydrophobic molecules)

Astrocytes: Glial cell (no electrocial impulse) provinding support for neurons, regualtes homeostasis , nutrimetns and myelin production for insulation



Astrocyte end feet connected fo blood vessel

Endothelial cell with **tight junction**

Barrier constituted by endothelial cells assembled through tight junctions (600km of vessels and capillaries , 20 m^2). BBB separates the circulating blood from the brain and extracellular fluid in the central nervous system (brain+ spinal cord)

98% of drugs do not cross the BBB

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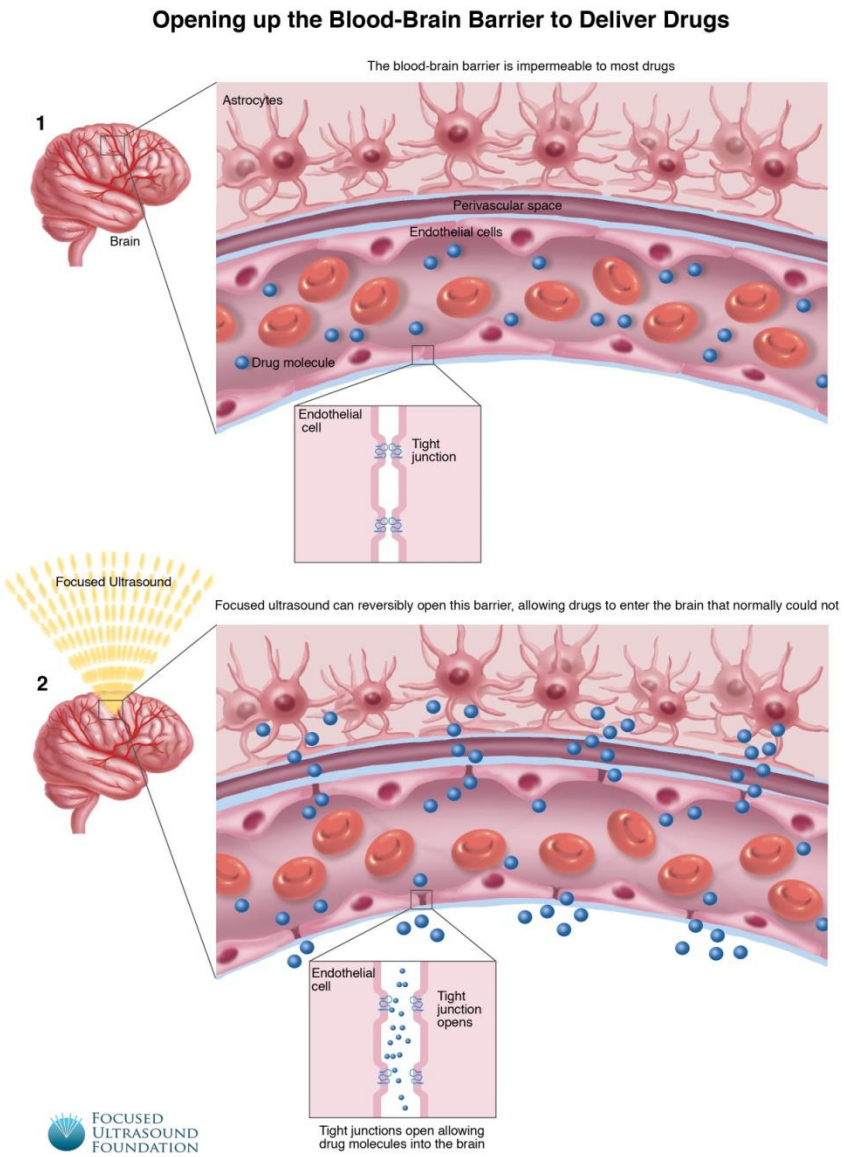
How to cross BBB? : simply enlarging the junctions



HIFU: High intensity focused ultrasound:
microbubles + drug
Vibration induce opeinign of thight junctions
CarThera https://youtu.be/f3uC5foRFlg

Vasodilatator (e.g. bradykinine)

Inhibiteurs de glycoproteine-P to limit the efflux. Br J Pharmacol. 2012 Jun;166(4):1333-43. doi: 10.1111/j.1476-5381.2012.01858.x. **Inhibition of P-glycoprotein enhances transport of imipramine across the blood-brain barrier: microdialysis studies in conscious freely moving rats.**

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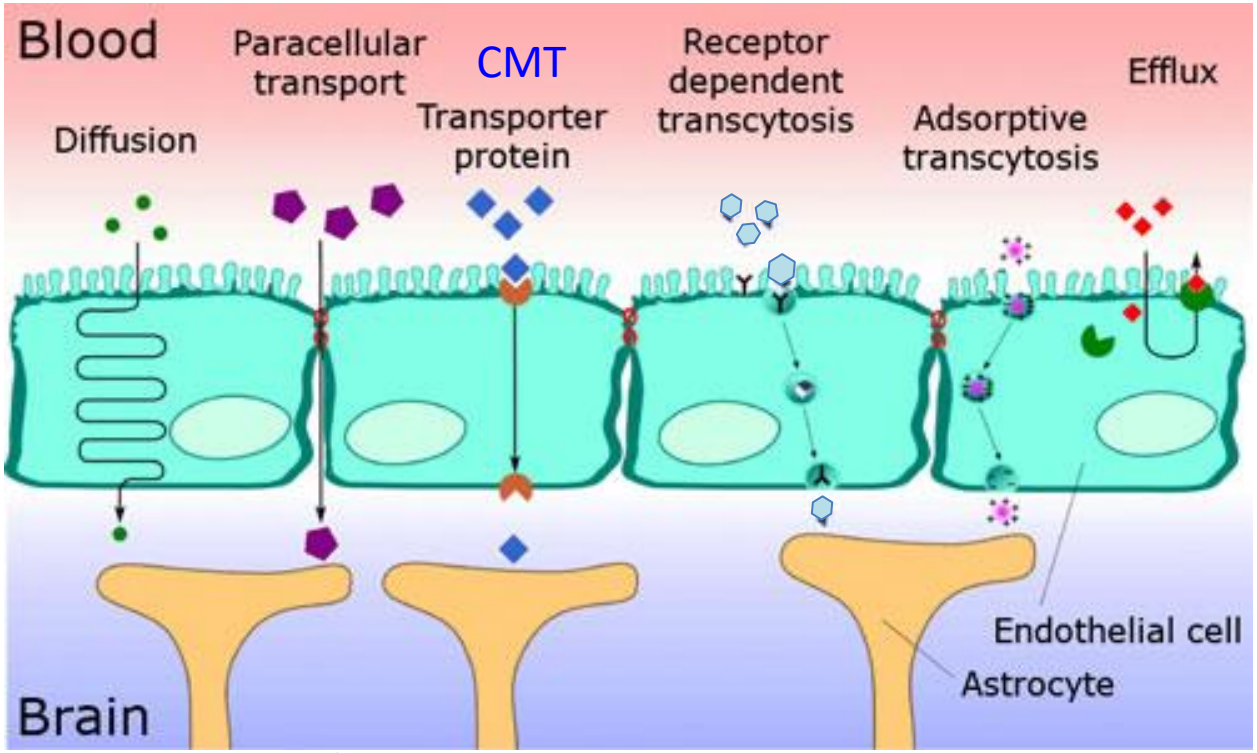
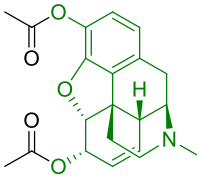
Modes of crossing BBB

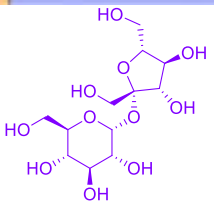


Carrier-mediated transport ou CMT : Example GLUT1 glucose transporters, MCT1 lactate transporters, LAT1 large neutral amino-acid transporters and CNT2 adenosine transporters, L-amino acid transporter (LAT) for dopa . OK for small molecules

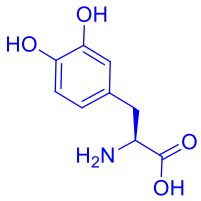
P-glycoprotein Transporter protein efflux pump that moves molecuels from the cytoplam to the lumen and limits the efficiency of drugs



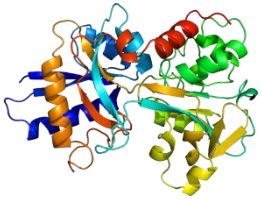
Heroin



sucrose



DOPA



transferrin

Insulin, leptin…

Cationic CPPs and liposomes, NPs

Ethanol
Heroïn (more hydrophobic)
>Morphin

Adsorptive transcytose: *Curr Pharm Biotechnol.* **2012** , 13(12):2340-8. Adsorptive-mediated brain delivery systems.

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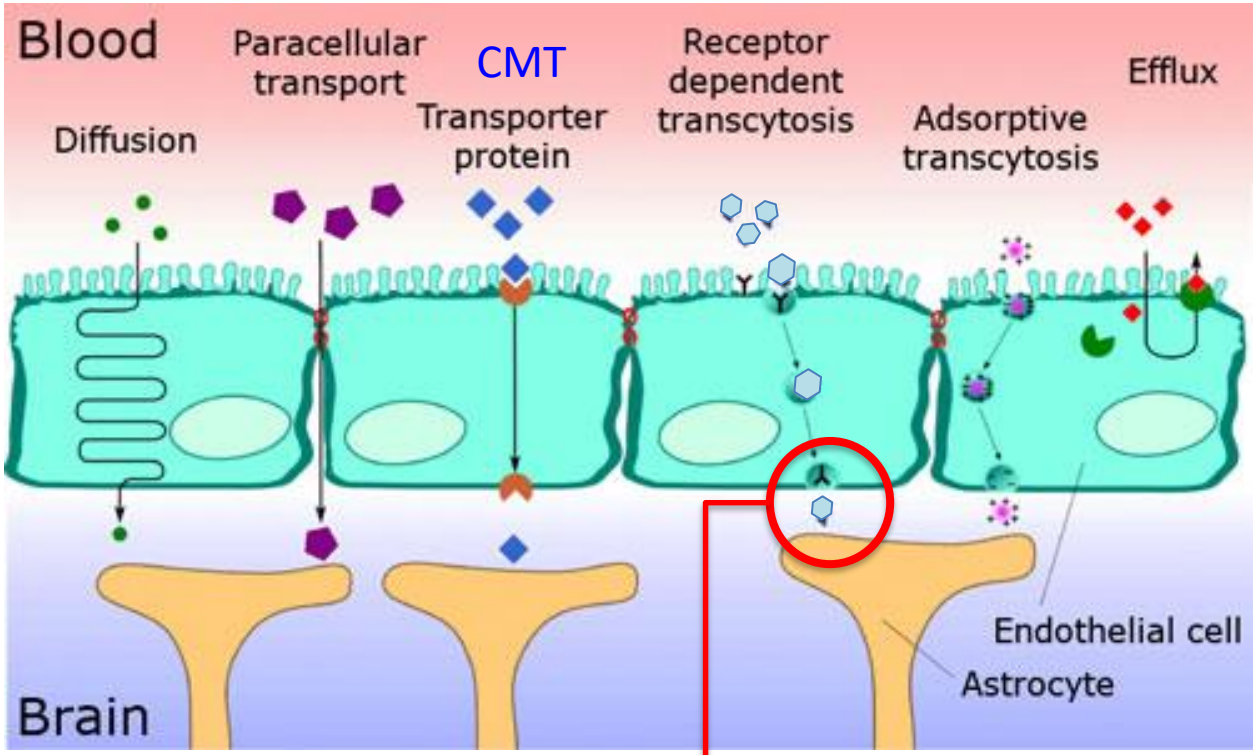
Focus on receptor-mediated trancytosis



In endosomes, the pH decrease (pH 6-6.5) which leads to the dissociation of receptor-ligand .

Endosome should be repositioned at the basolateral membrane

Affinity of the ligand should not be too high to be able to dissociate

OR a system **of cleavable linker** should be designed.

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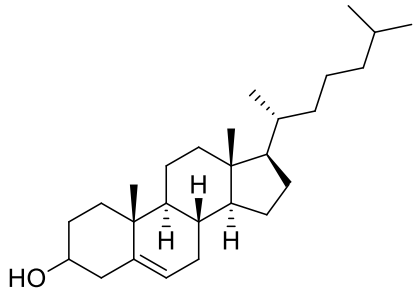
Using a ligand of Low Density Lipoprotein Receptor LDL-R

LDL transports cholesterol and other lipids in the brain via a specific receptor:
LDL-R and receptor dependant transcytosys.



A peptide ligand of LDL-R was identified by phage display (cyclic 15-mer).

Ac-AspSerGlyLeuCysMetProArgLeuArgGlyCysAspProArg-NH_2

S — S

It was optimized in 8-mer

Ac-(D)CysMetProArgLeuArgGlyCys-NH_2

S — S

=

Can be conjugated with a cargo (dye, drug)
at N or C-ter

VECT-HORUS
BREAKING BARRIERS

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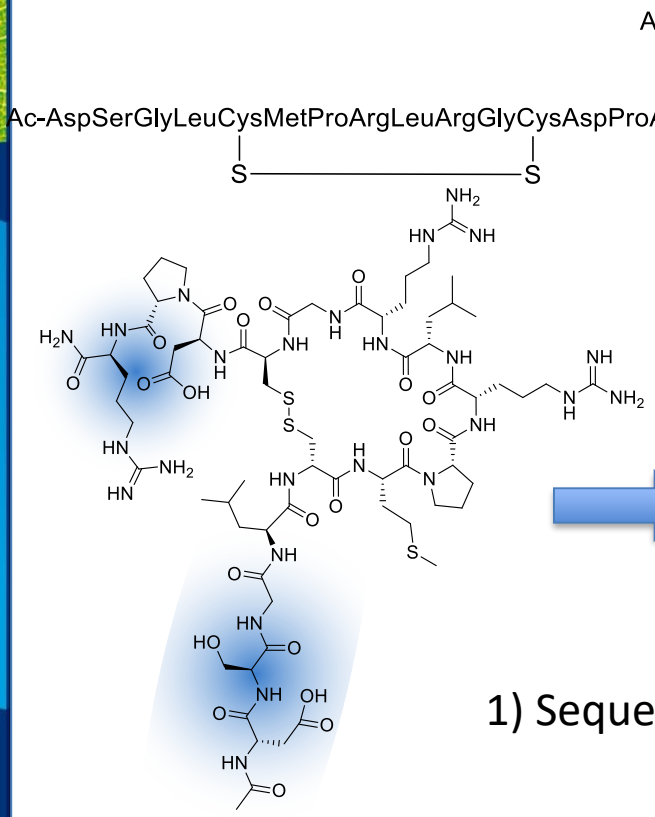
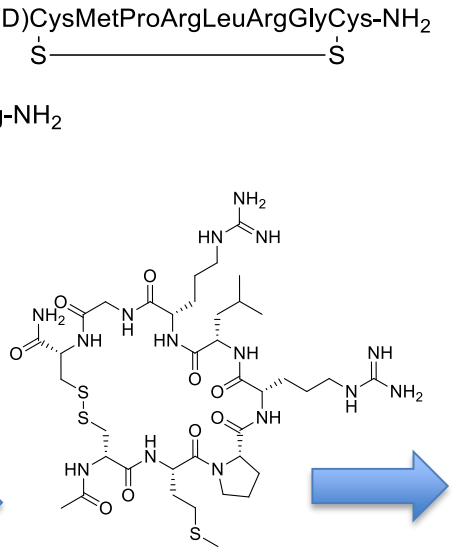
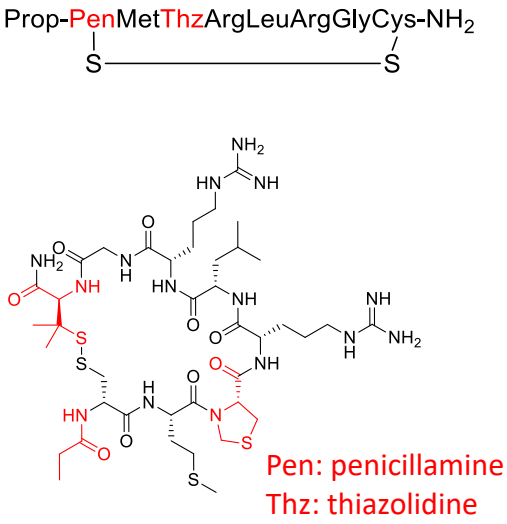
Malcor et al. *J Med Chem*. **2012** 8, 55, 2227-2241
Jacquot et al. *Mol. Pharmaceutics* **2016,** 13, 12, 4094-4105

Sequence Optimization







1) Sequence reduction

KD= 76 nM
in vitro t1/2 blood= 3 h

2) Non proteogenic AA introduction

KD = 18 nM
in vitro t1/2 blood= ~4.3 hrs

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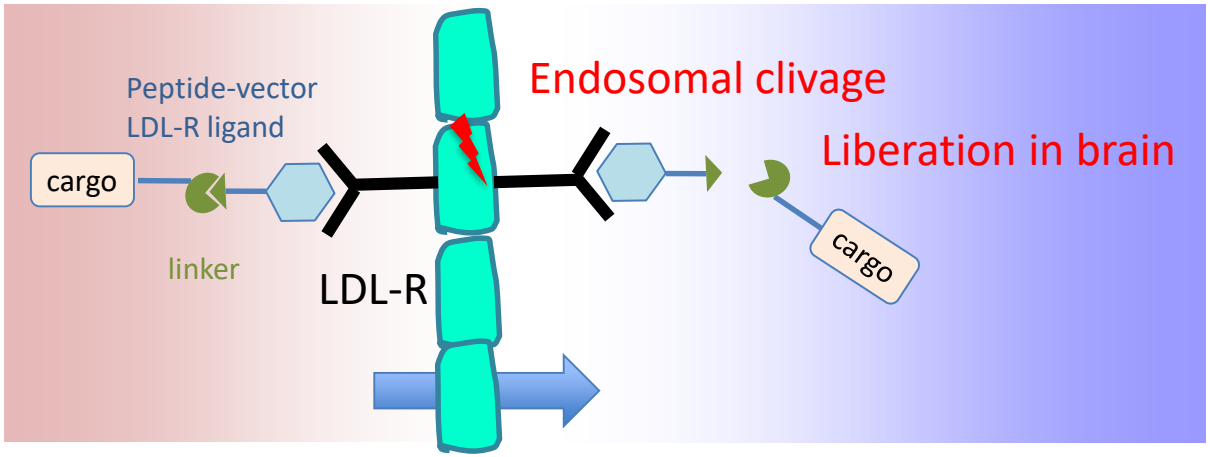
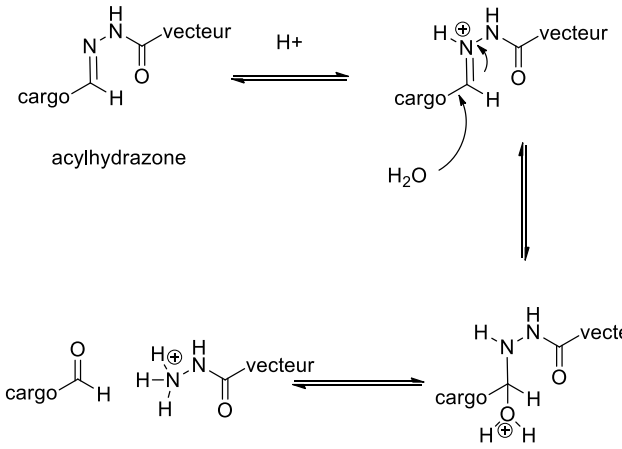
Bioconjugate Cargo-LDL peptide ligand



biological cleavage

Conditions	linker	compartiment	Cancer ?
Glutathion	R-**S-S**-R’	Cytoplasme	/
Cathepsine B	R-GFL**G-NH**-R’ R-Val-**Cit-NH**-R’	Lysosome (endosome+enzymes)	+++
PSA *(prostate-specific antigen) Kallikrein*	R-HSSKL**Q-NH**-R’	Cytoplasme (prostate)	+++
Caspase 3	R-DEV**D-NH**-R’	Cytoplasme	/

chemical cleavage



lien hydrazone pH-sensitive (cancer pH6, endosome pH 6.5)

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III.4. Peptides as controlled delivery systems

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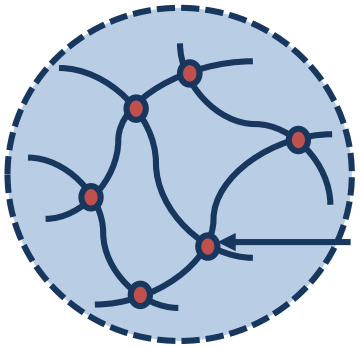
Types of Hydrogels

Definition : Three-dimensional network made of water-swollen and cross-linked hydrophilic polymers or macromolecular assemblies

Weak interactions

Physical hydrogel



Covalent bonds

Chemical hydrogel

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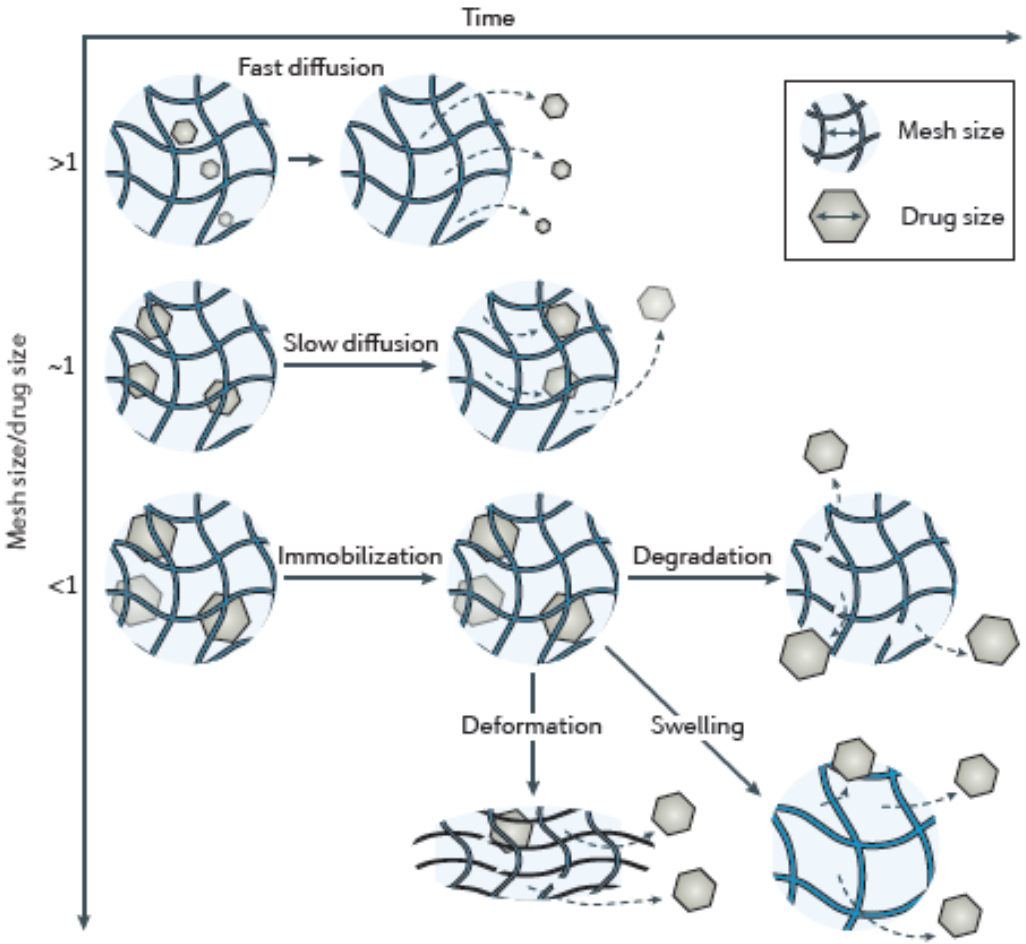
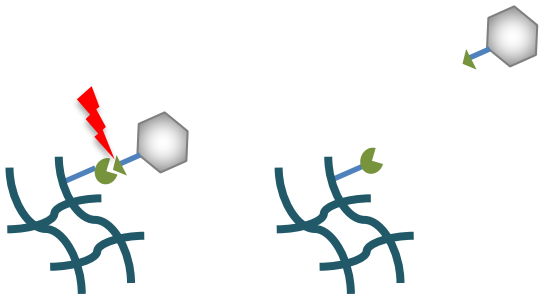
Hydrogels as (drug) delivery platforms

Hydrogels provide **spatial** and **temporal** control over the release of therapeutic agents such as small-molecule drugs, but also macromolecular drugs and cells.

(i) Non-covalent binding of the drug



(ii) Covalent binding of the drug
With cleavable linker



Li et al. *Nature Reviews Materials* **2017**

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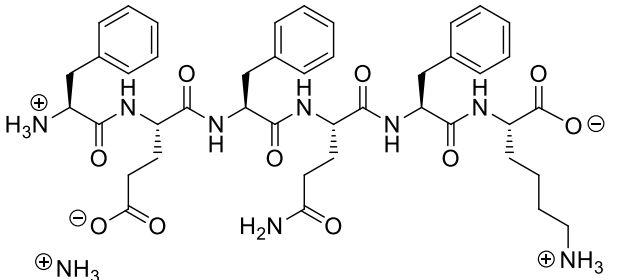
Peptides self assemble and form hydrogels

Peptide self-assembly in antiparallel beta-sheets

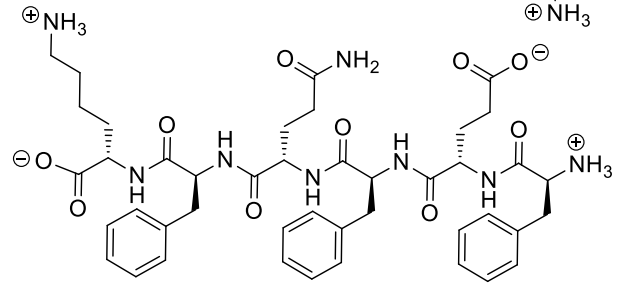
N-ter

C-ter



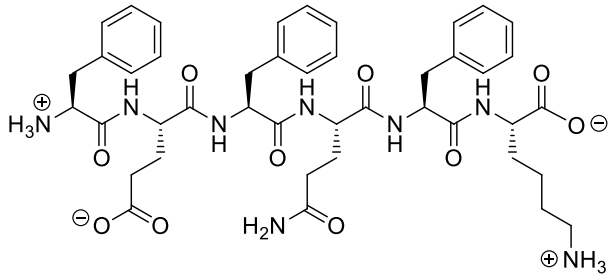
C-ter

N-ter



N-ter

C-ter



Dasgupta, A. *et al*. *RSC Advances* **2013**, *3* (24), 9117.
Martin, C. et al *Materials Today Chemistry*, **2017**

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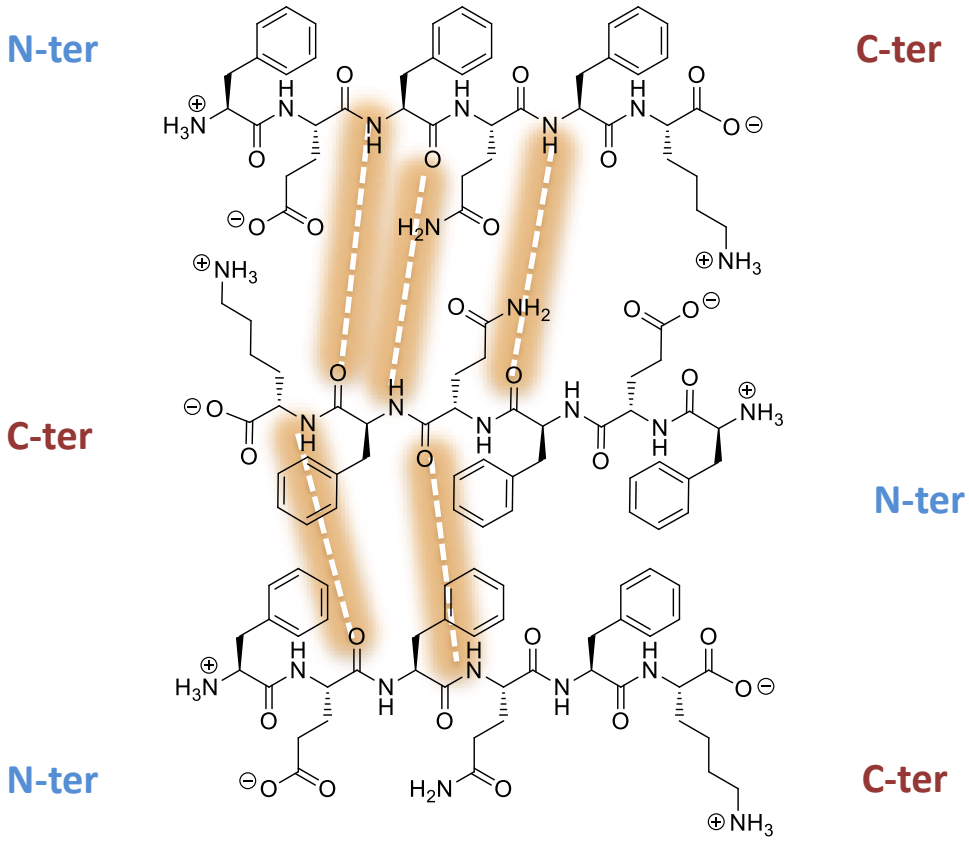
Peptides self assemble and form hydrogels

Peptide self-assembly in antiparallel beta-sheets



Dasgupta, A. *et al*. *RSC Advances* **2013**, *3* (24), 9117.
Martin, C. et al *Materials Today Chemistry*, **2017**

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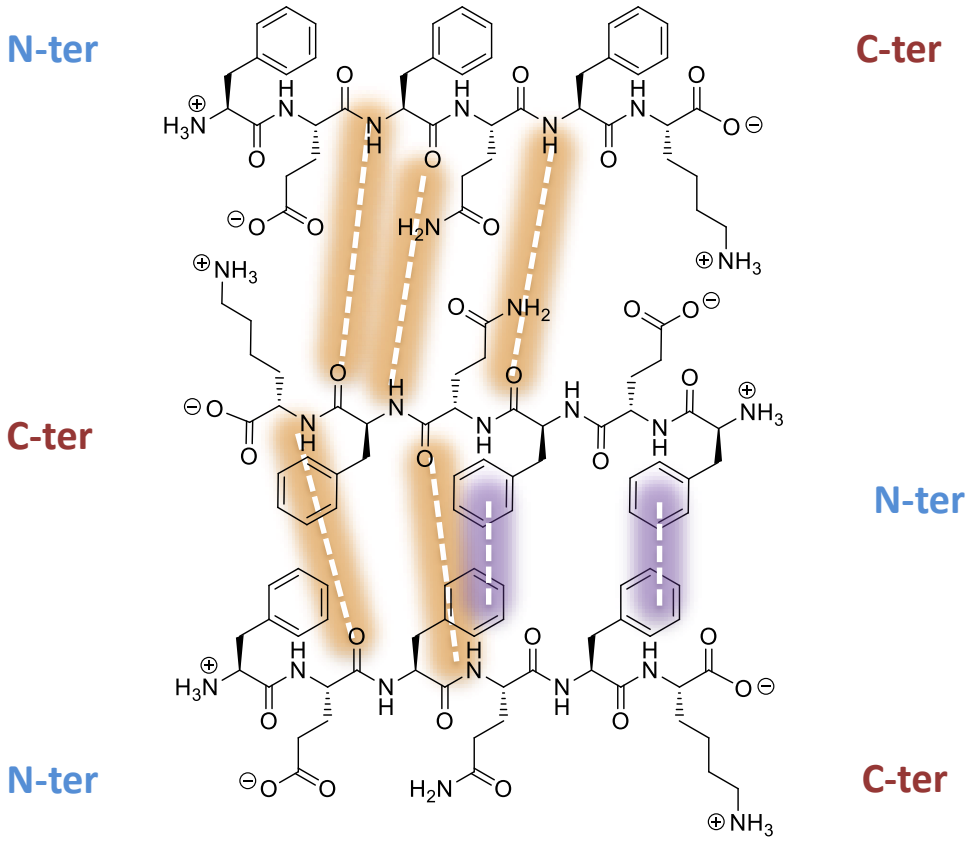

Dasgupta, A. *et al*. *RSC Advances* **2013**, *3* (24), 9117.
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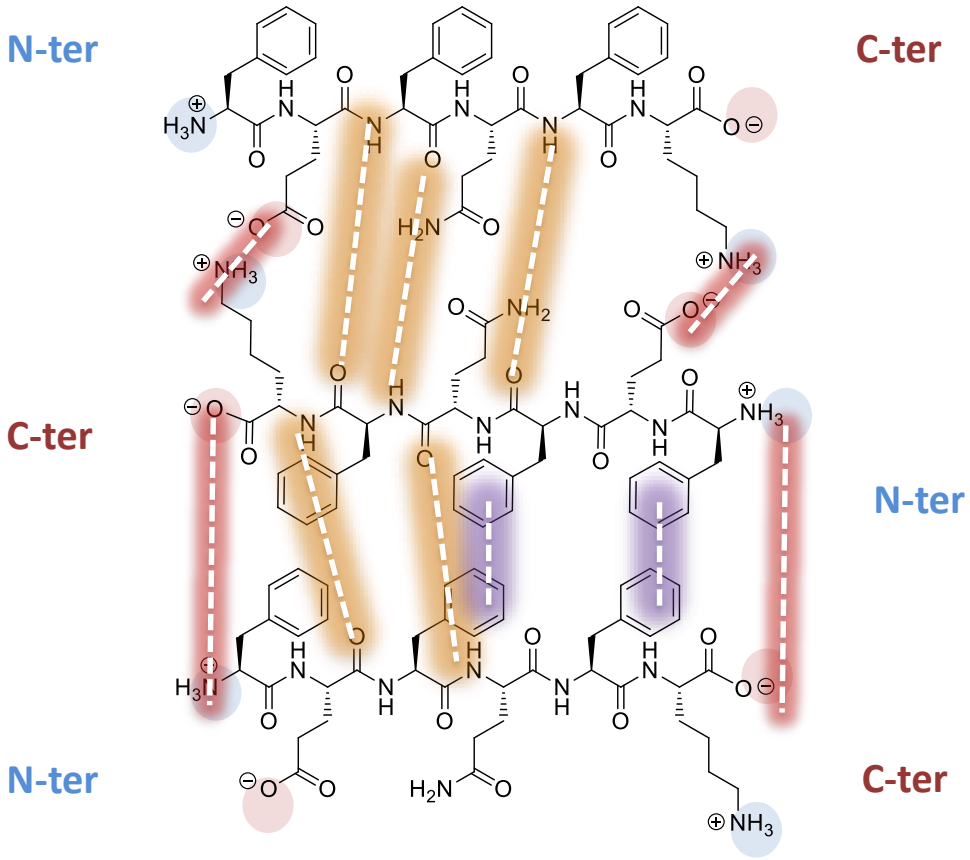

Dasgupta, A. *et al*. *RSC Advances* **2013**, *3* (24), 9117.
Martin, C. et al *Materials Today Chemistry*, **2017**

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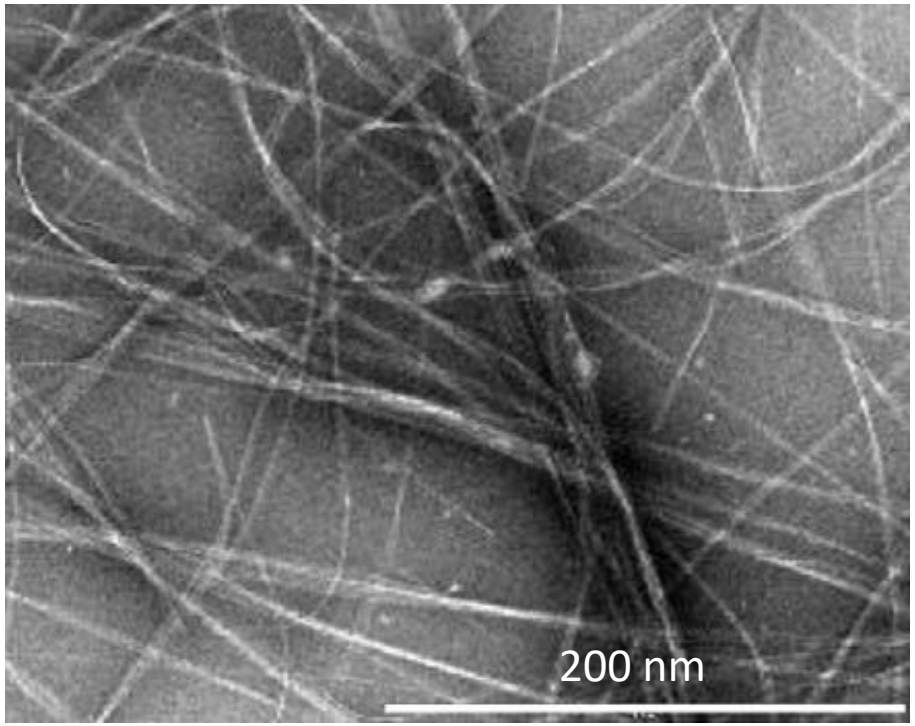
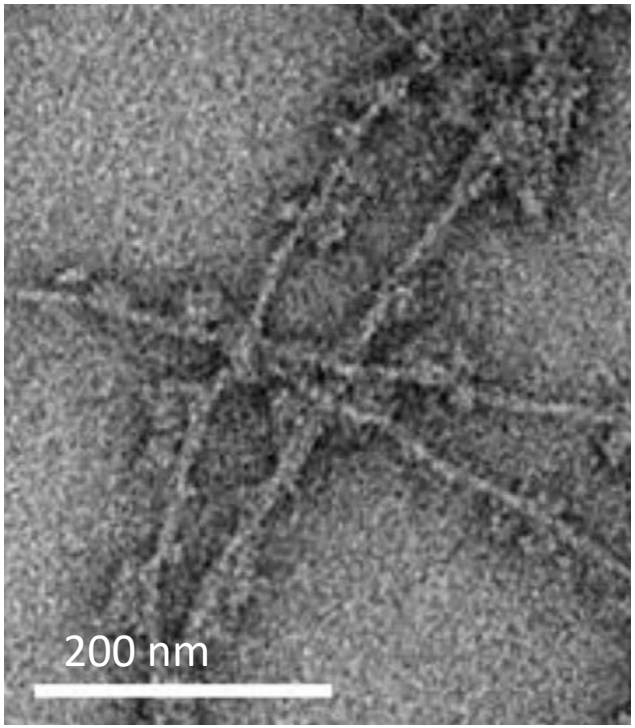
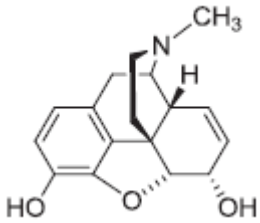
TEM images of hydrogel network

Hydrogel network



Morphine-loaded network





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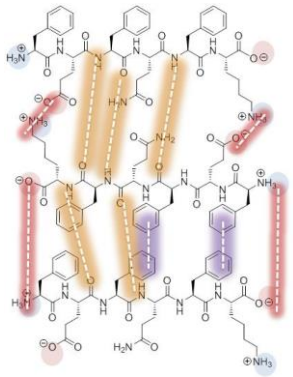
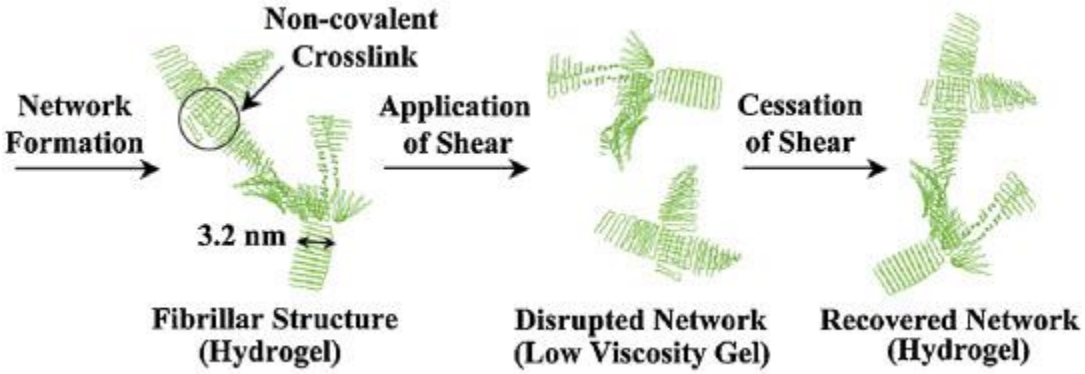
Physical hydrogels with weak interaction enable shear-thinning properties

Betasheet and reversible supra molecular assembly





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III.5. Peptides probes and cleavable linkers

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A lot of peptides and proteins are modified by enzymes
Kinases -> phosphorylation of Ser, Thr or Tyr
Proteases -> cleavage of specific sequences.

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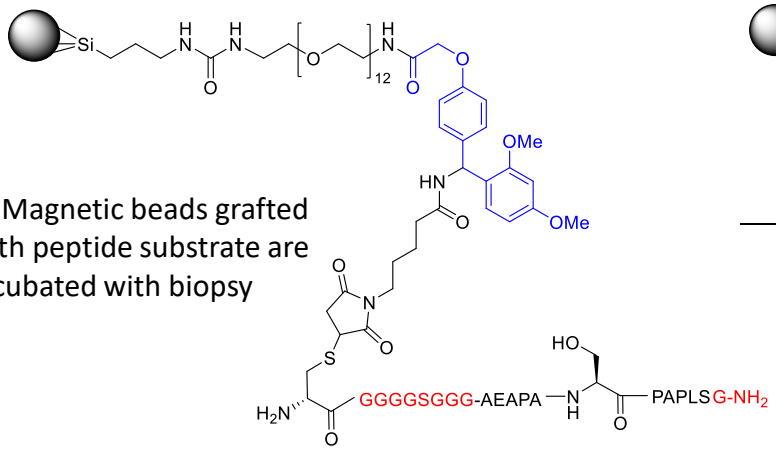
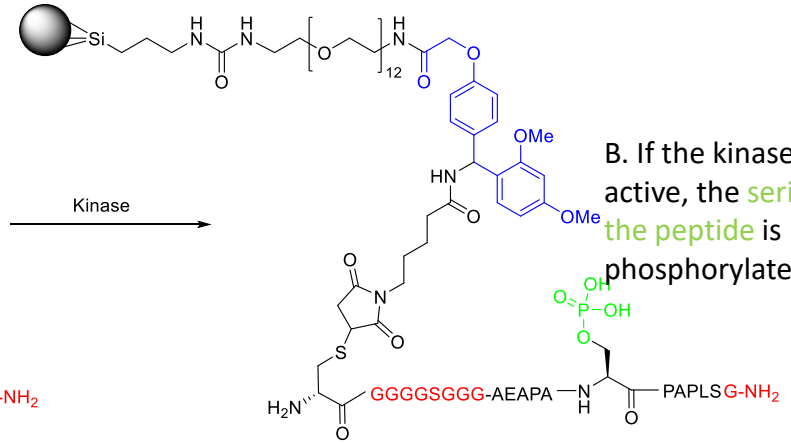
Peptide kinase substrates to monitor activity in complex samples

Some kinases are overexpressed in cancer (can be the target of drugs)
Knowing the kinase activity profile is of high importance for diagnosis and treatment

A. Magnetic beads grafted with peptide substrate are incubated with biopsy



Kinase



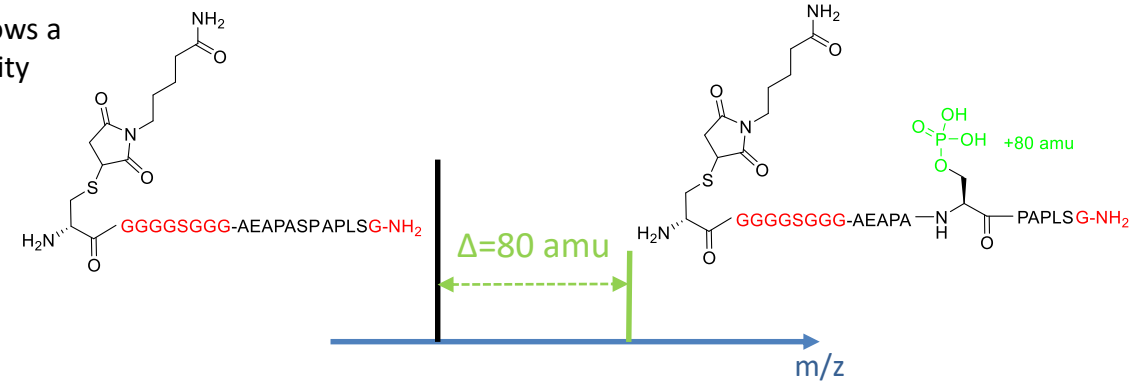
B. If the kinase is active, the serine of the peptide is phosphorylated

TFA acidic cleavage

C. Beads are removed with a magnet and washed

D. Linker is cleaved to release the peptide substrate from the beads

E. MALDI-Tof MS analysis allows a quantification of kinase activity



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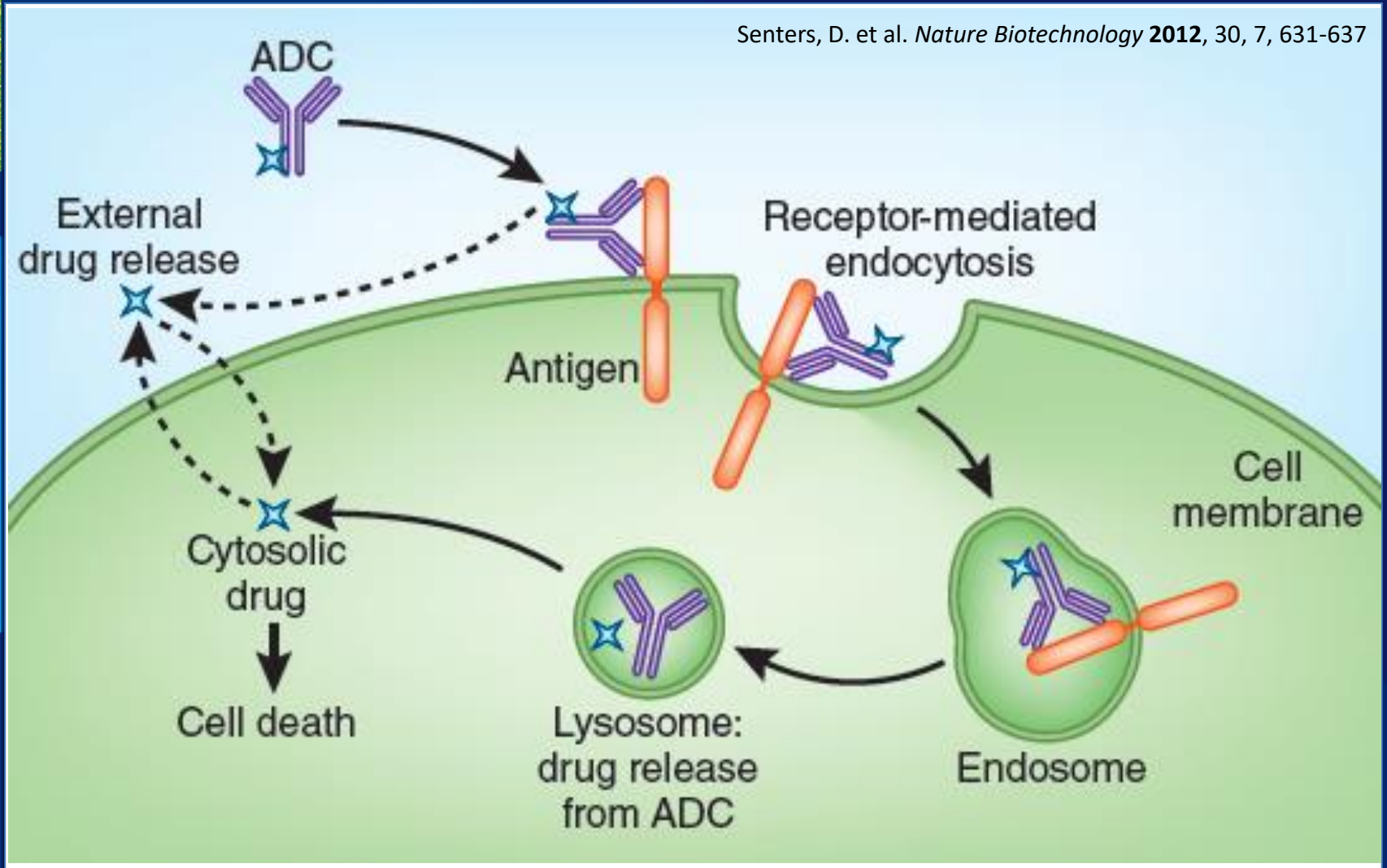
Mechanisms of drug delivery mediated by Antibody-drug conjugates (ADC)



Shortcomings: conjugate immunogenicity, low drug potency, antigen expression on normal tissues and **instability of the linkers** that joined the drugs to the mAbs.

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PERSPECTIVE

nature biotechnology

The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma

Peter D Senter & Eric L Sievers

Brentuximab vedotin (Adcetris), ADC FDA approved on November 9, 2017 for the treatment of cutaneous anaplastic large cell lymphoma (pcALCL)

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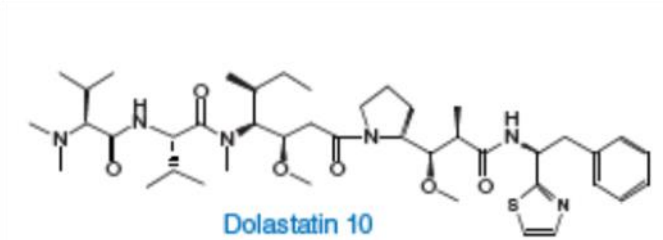
Selection of the drug : MMAE

Isolation/identification

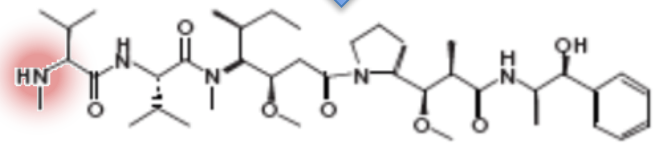
Sea hare, mollusc (*Dolabela auricularia Indian Ocean)*



Dolastatin 10
Potent toxic antomitotic agent

Pettit, G.R. et al, *J. Am. Chem. Soc.* 109, 6883–6885 (**1987**).

Optimisation (combichem)



MMAE

monomethyl auristatin E, more hydrophilic, more stable and displaying a conjugation point

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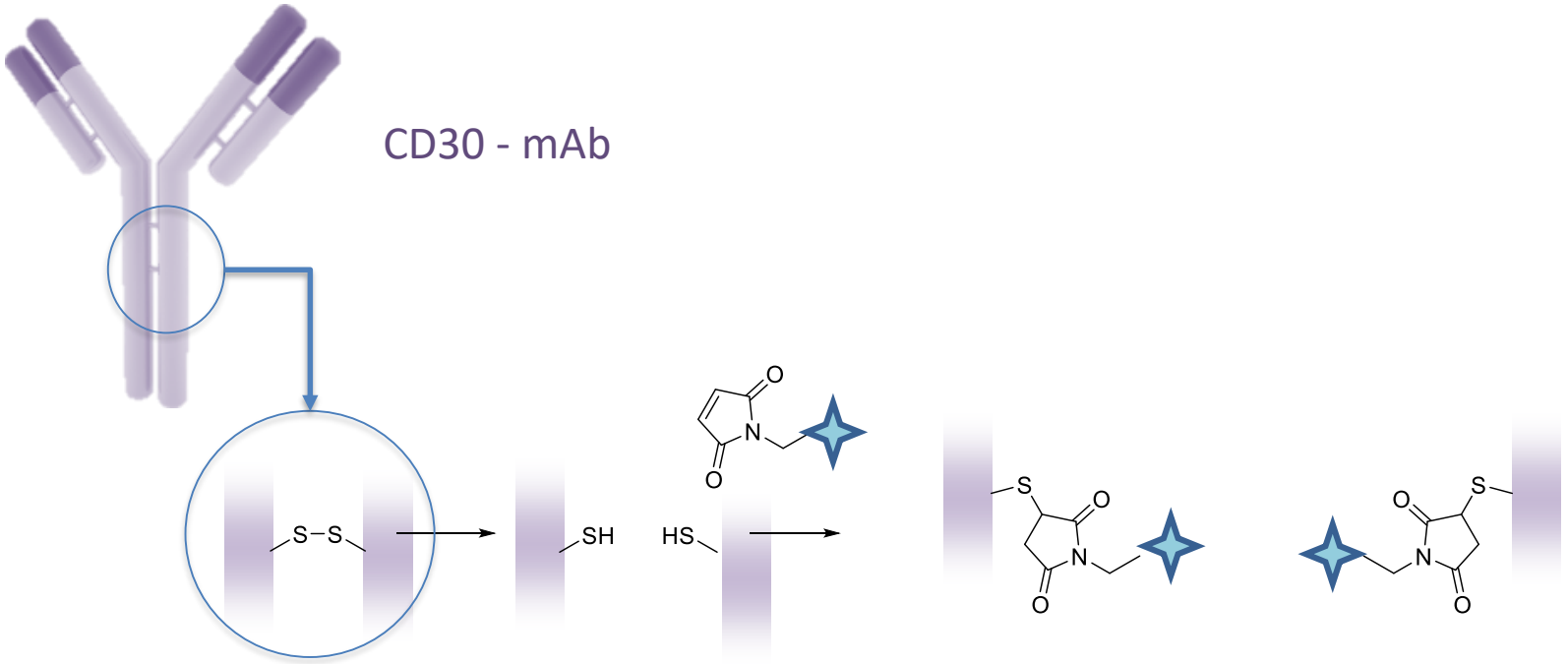
Selection of the mAb and the conjugation

CD30 is a tumor necrosis factor receptor (TNFR) superfamily member, which stimulates apoptosis via TNFR-associated factor 2 degradation.

CD30 antigen is highly expressed in Hodgkin lymphoma of cutaneous anaplastic large cell lymphoma (ALCL). Cross-reactivity of CD30 on normal tissues is very low, with some expression on activated but not resting, T and B cells.

Unconjugated anti-CD30 mAbs (MDX-060) has been tested in a phase 1/2 trial in patients with Hodgkin lymphoma, ALCL . It was well tolerated and provided some evidence of clinical activity.



Partial reduction

Maleimide conjugation
(random ≈4 modifications)

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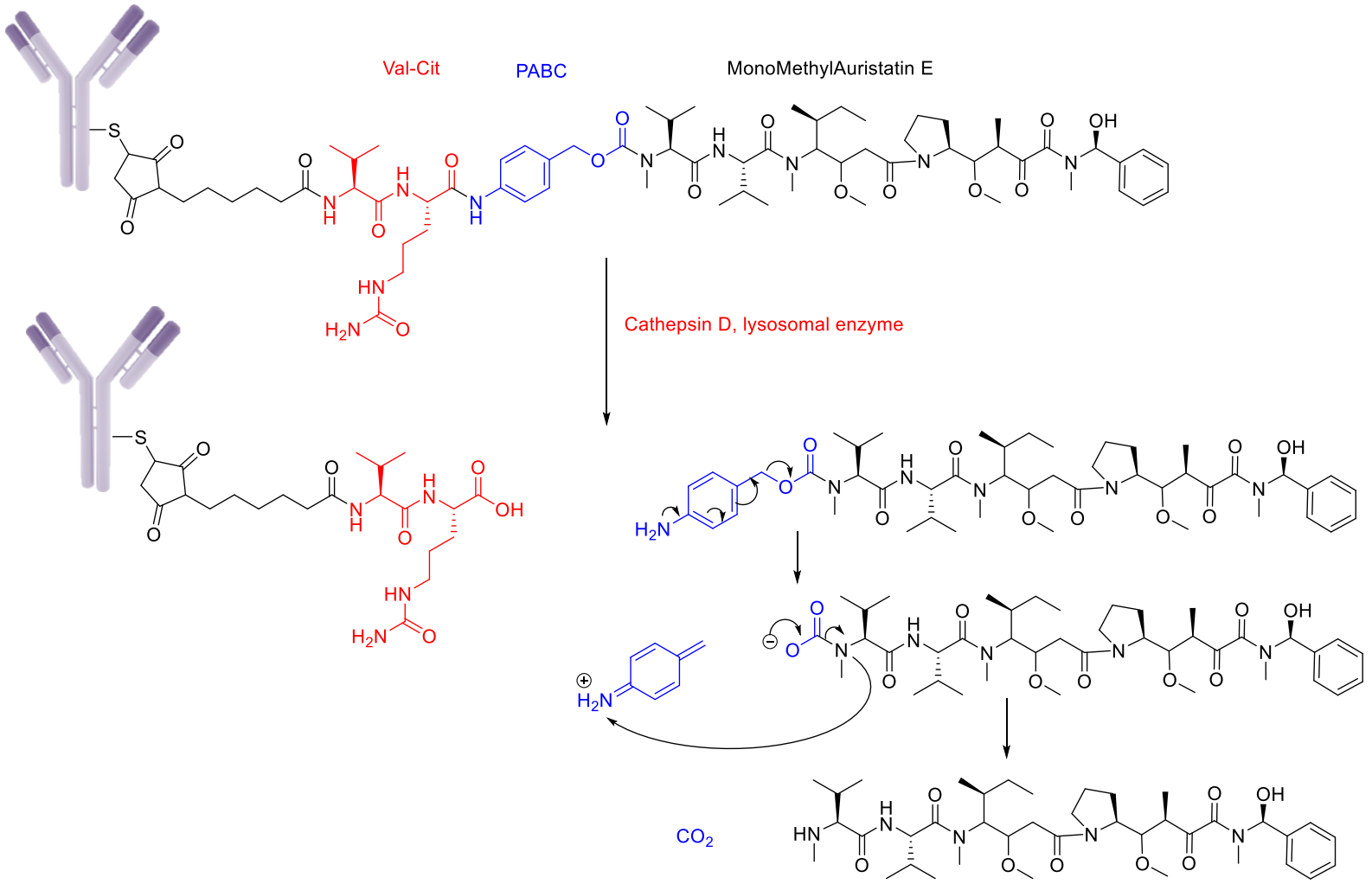
Mechanism of drug release via Val-Cit and PABC linker



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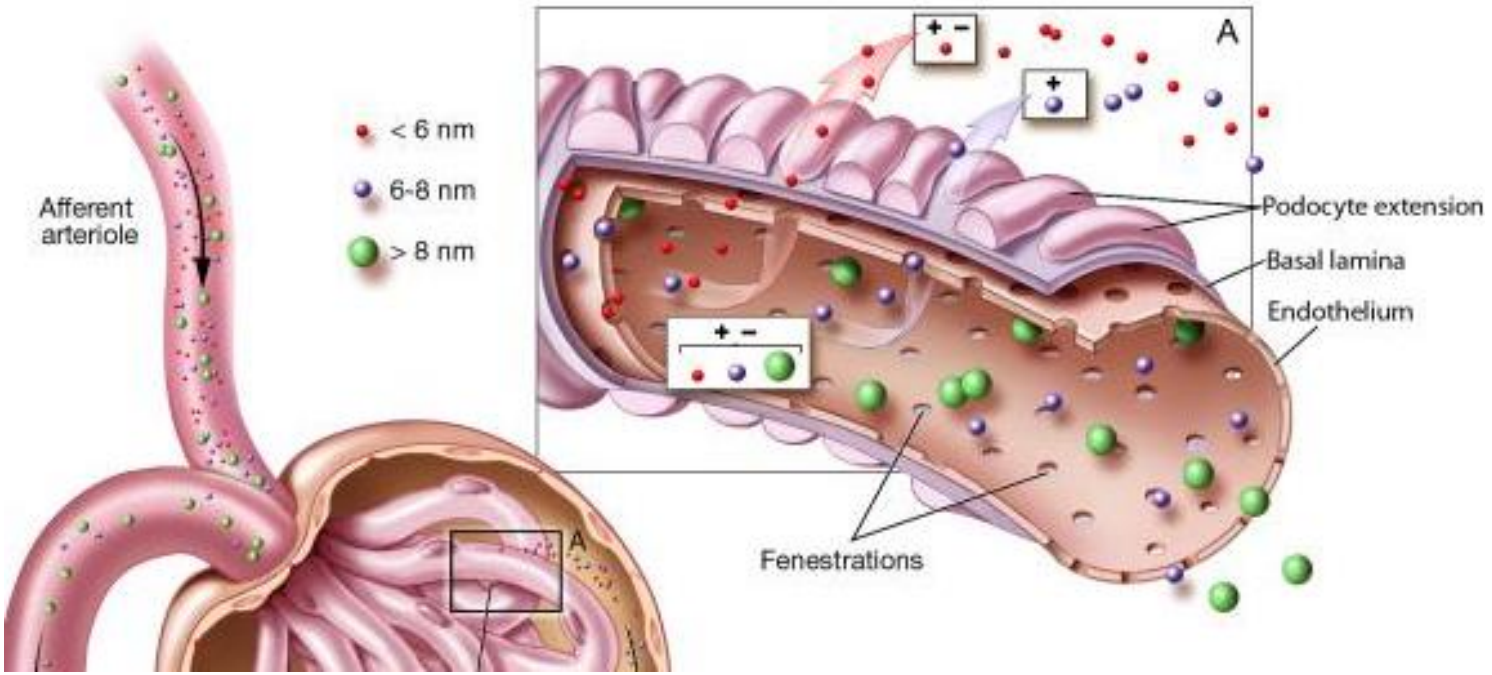
Multifunctional nanoparticles for cancer targeting

A matter of size: Renal clearance > 8 nm



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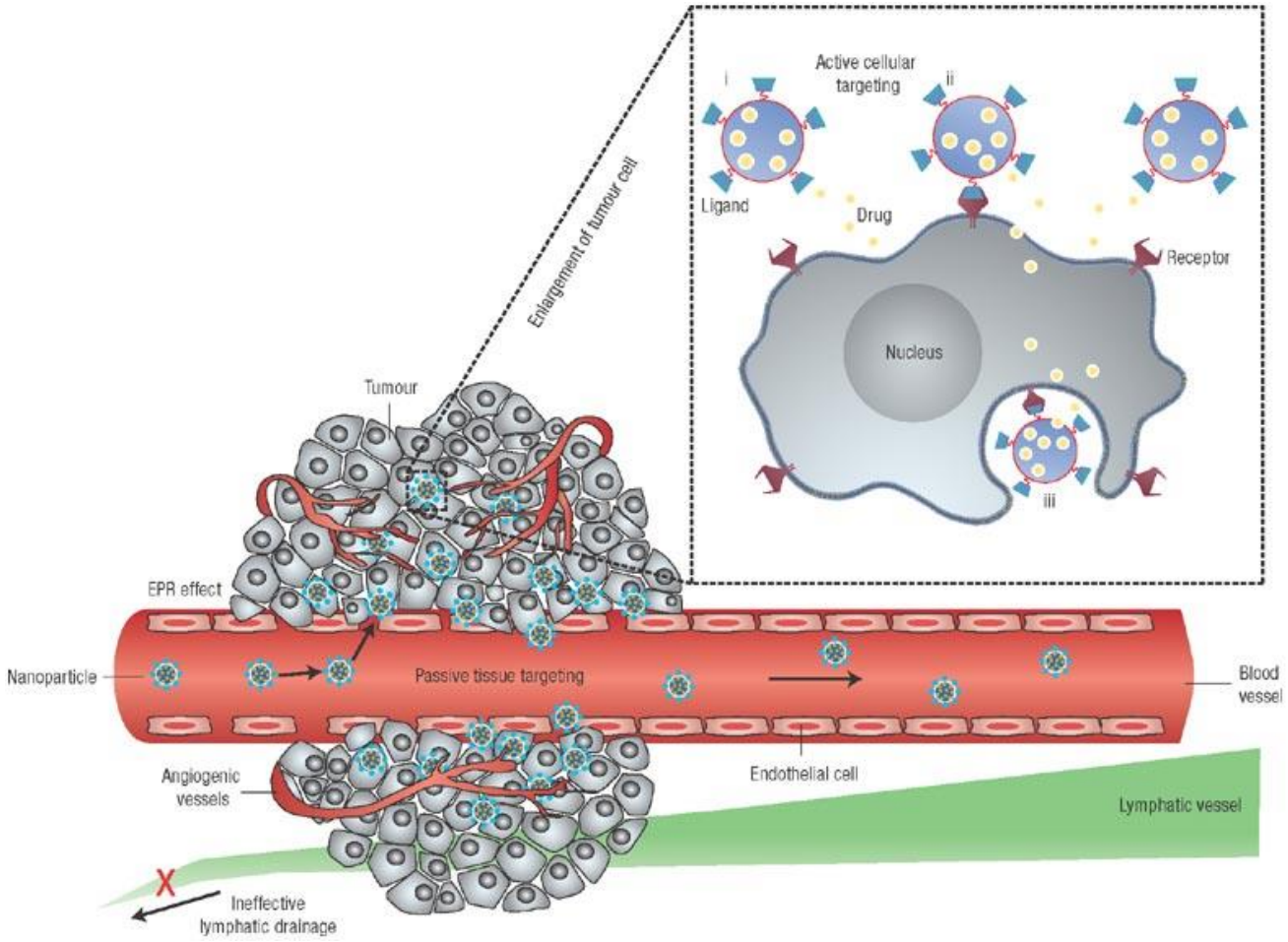
Multifunctional nanoparticles for cancer targeting

A matter of size: EPR effect: Enhanced Permeation and Retention effect

Neovascularization induces pathological leaky vasculature with wider fenestrations between endothelial cells. Nanoparticle (50-200 nm) may accumulate in tumor. Ineffective lymphatic drainage enhance the phenomenon.



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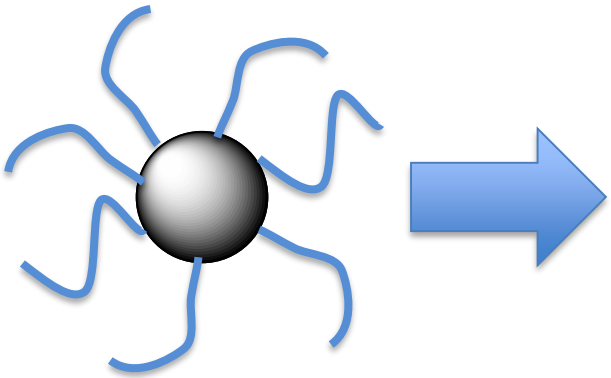
A matter of stealthness

In blood circulation, nanoparticles are opsonized (i.e. binding of antiobodies and protein of the complement) mainly produced in the liver that induce their uptake by macrophages.



Covering nanoparticles with hydrophilic neutral corona (e.g. PEG) decrease opsonization and increase their stay in the blood circulation.

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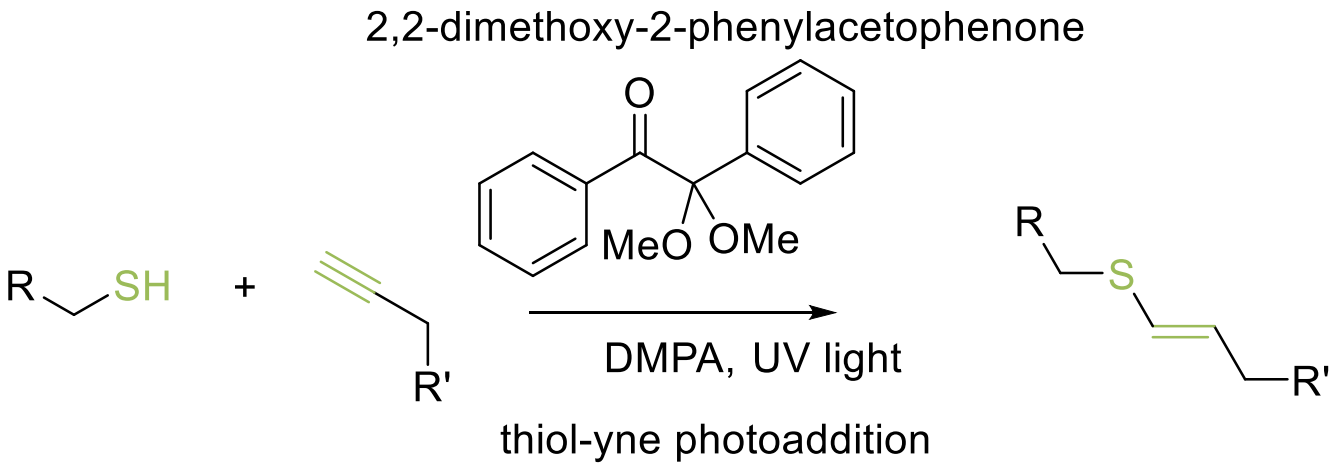
Synthesizing a multifunctional polymer particle



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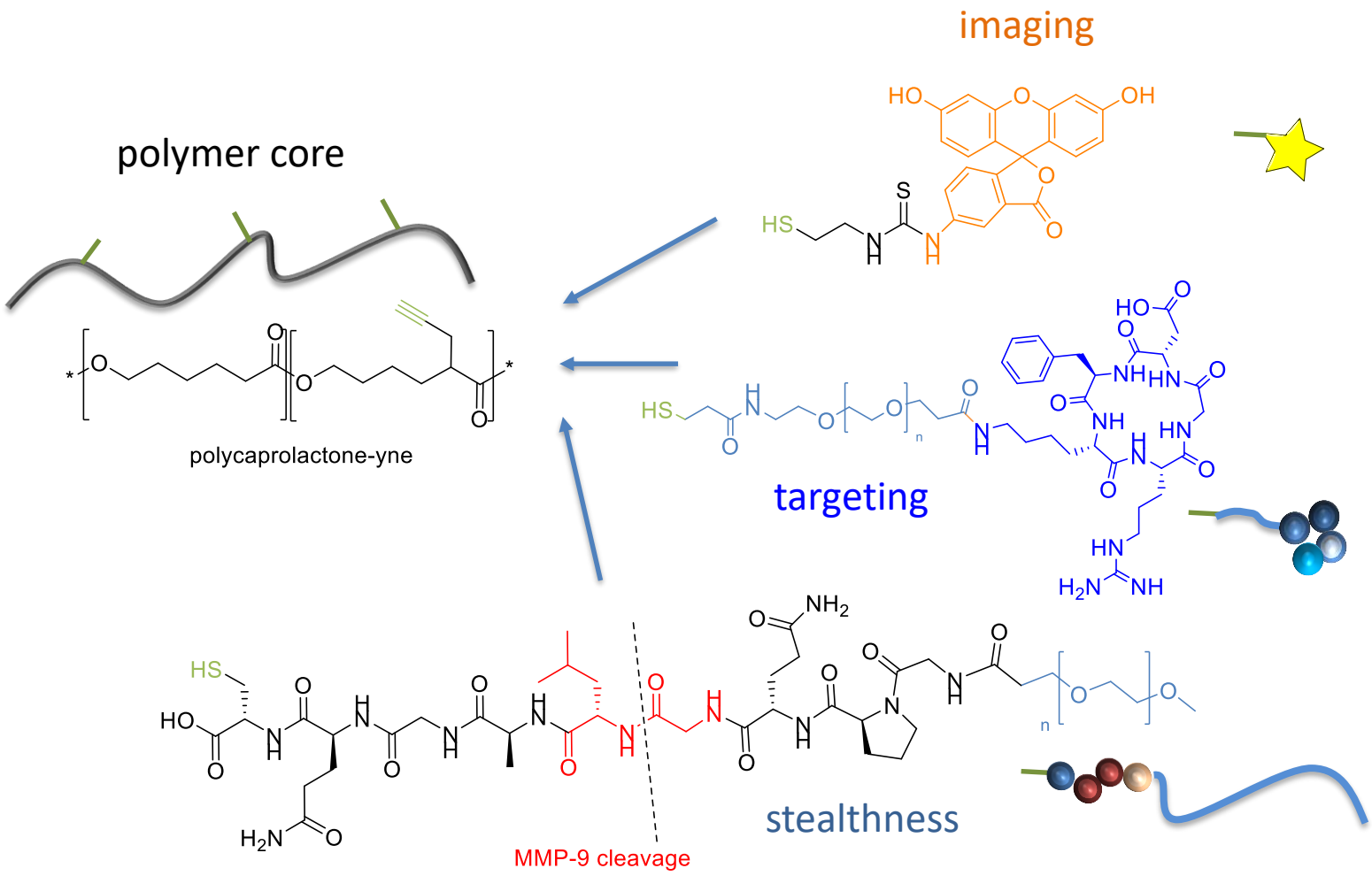
Synthesizing a multifunctional polymer particle



A cytotoxic drug can be mixed with the polymer

A. A. Samad et al., *Macromolecular Rapid Communications* **2018**, *39*

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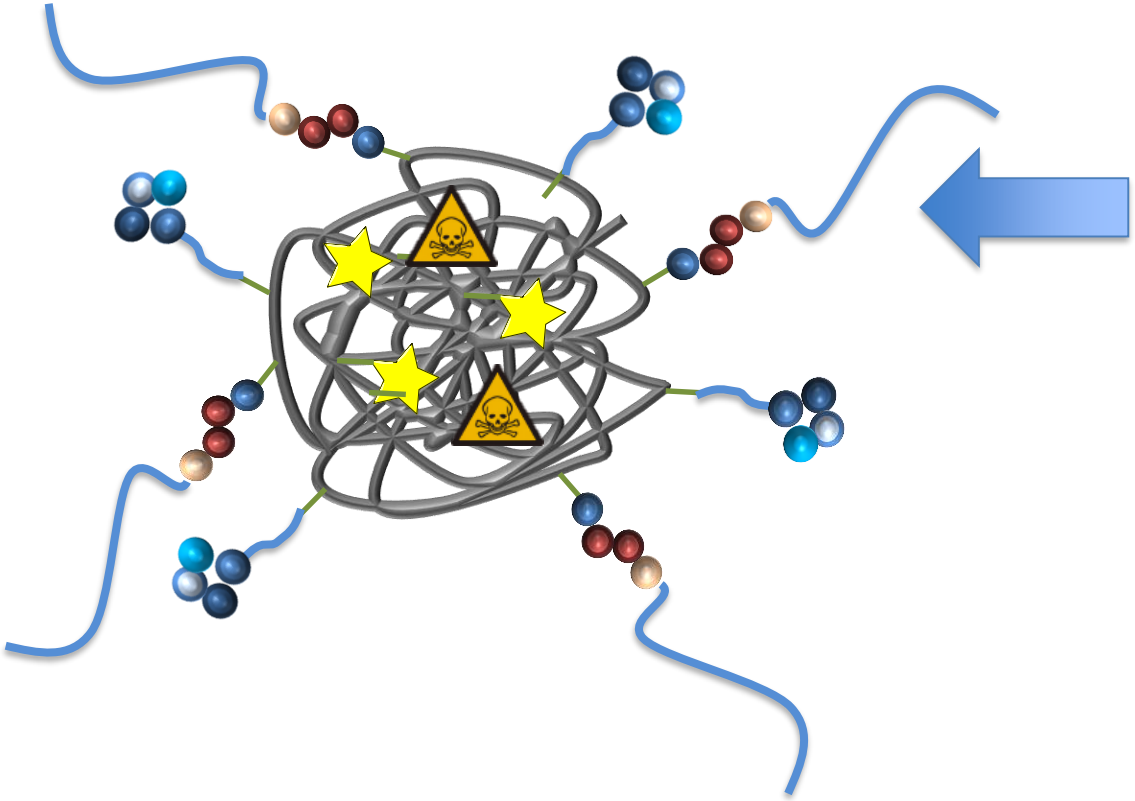

PEG chains enhance stealthness, increase circulation time and minimize macrophage uptake in the liver

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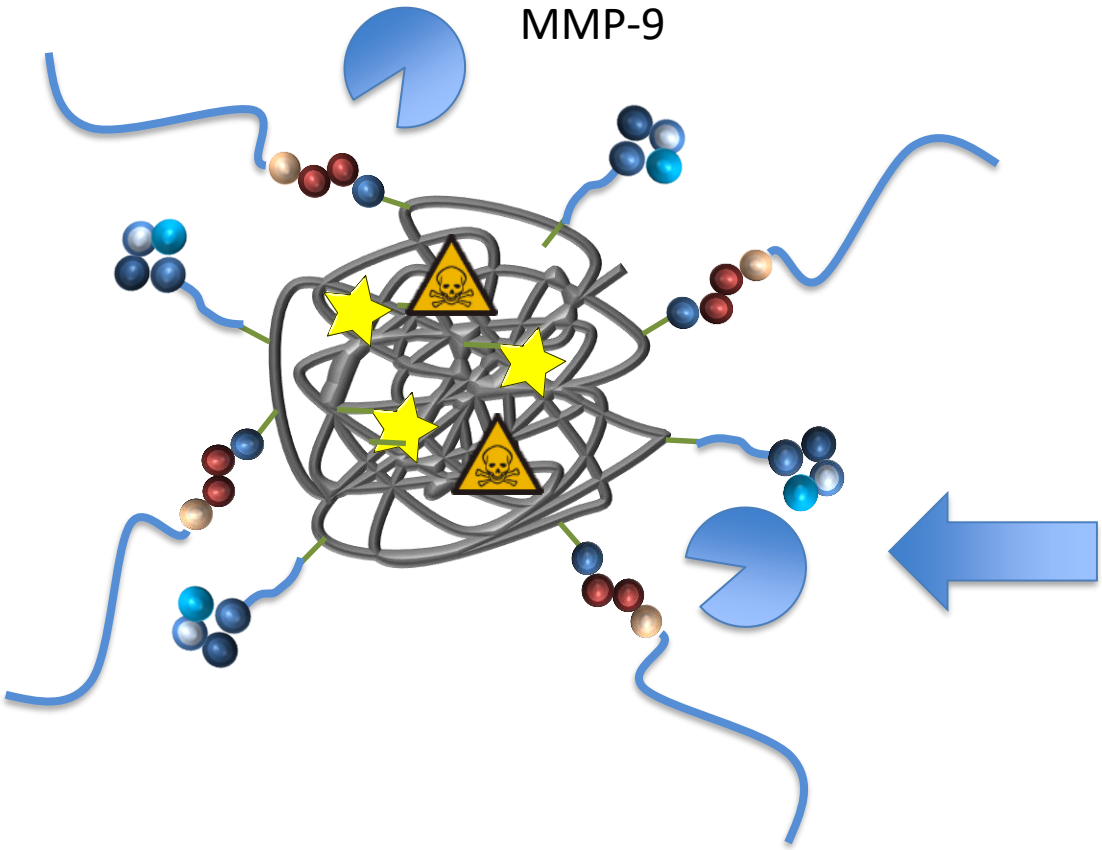

After accumulation in the tumor thanks to EPR effect, matrix metalloproteases (i.e. MMP-9) cleave the PEG chains

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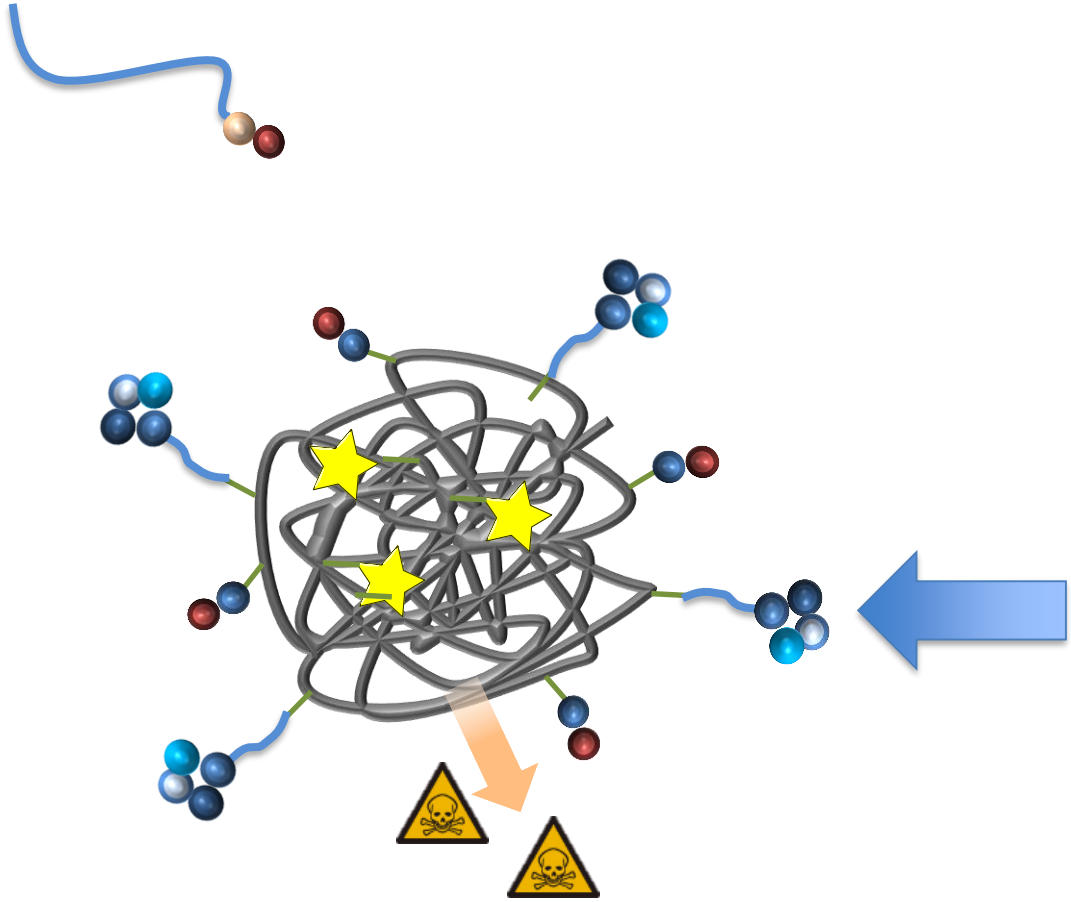

Receptor-mediated endocytosis is favored and cytotoxic can be delivered in the cancer cells

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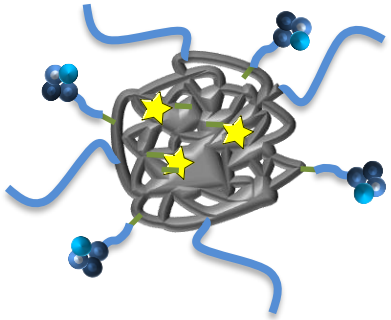
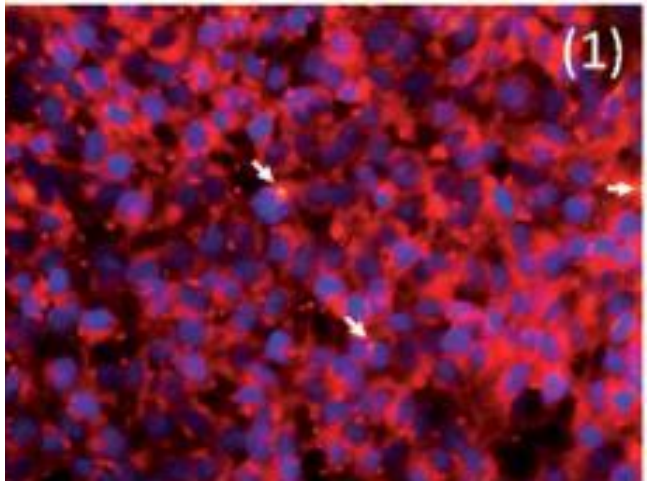
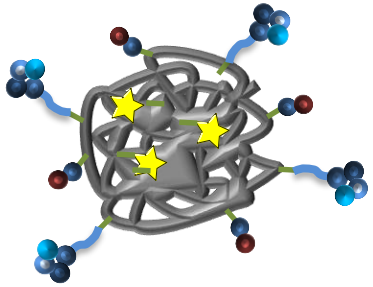
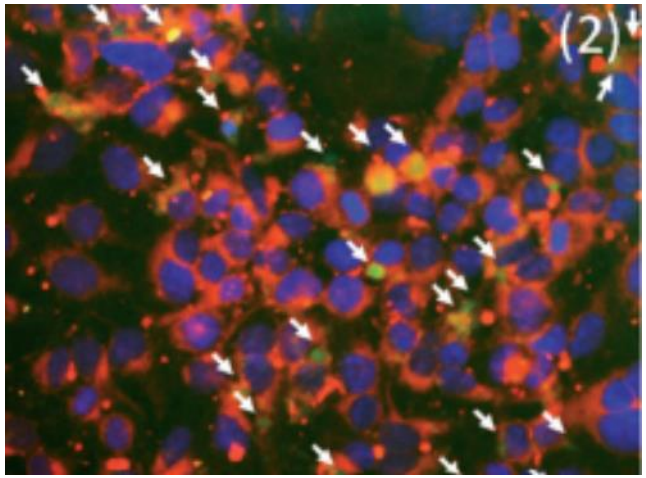


Stealthness agent (PEG) is not removed:
Poor internalization





Stealthness agent (PEG) is removed:
Good internalization



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