Drug targeting and delivery systems

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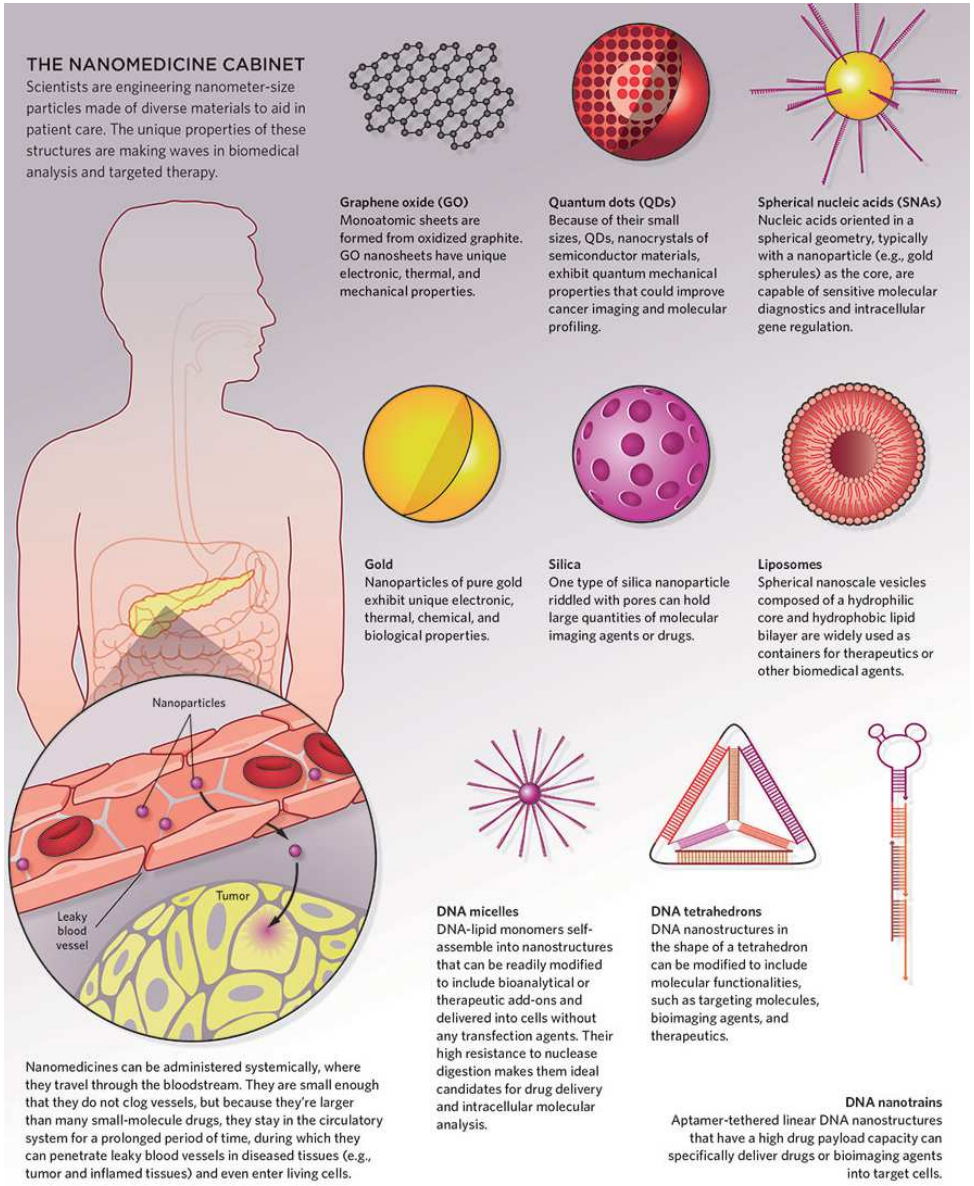
PhD Course
Eötvös Loránd University

Nanomedicine, drug targeting



Nanomedicine, From bioimaging to drug delivery and therapeutics, nanotechnology is poised to change the way doctors practice medicine., Guizhi Zhu, Lei Mei, and Weihong Tan; The Scientist, August 2014, Cover Story (https://www.the-scientist.com/?articles.view/articleNo/40598/title/Nanomedicine/)

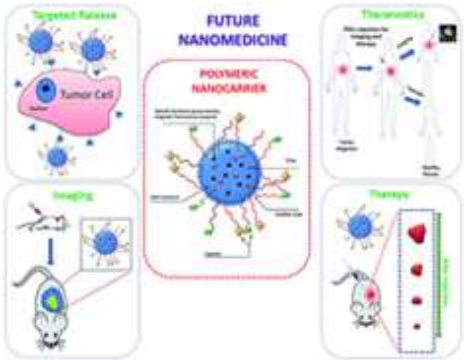
In a 1959 lecture at Caltech famously dubbed

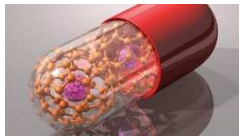
"There's Plenty of Room at the Bottom,"

American physicist and Nobel laureate–to-be **Richard Feynman** discussed the idea of manipulating structures at the atomic level.

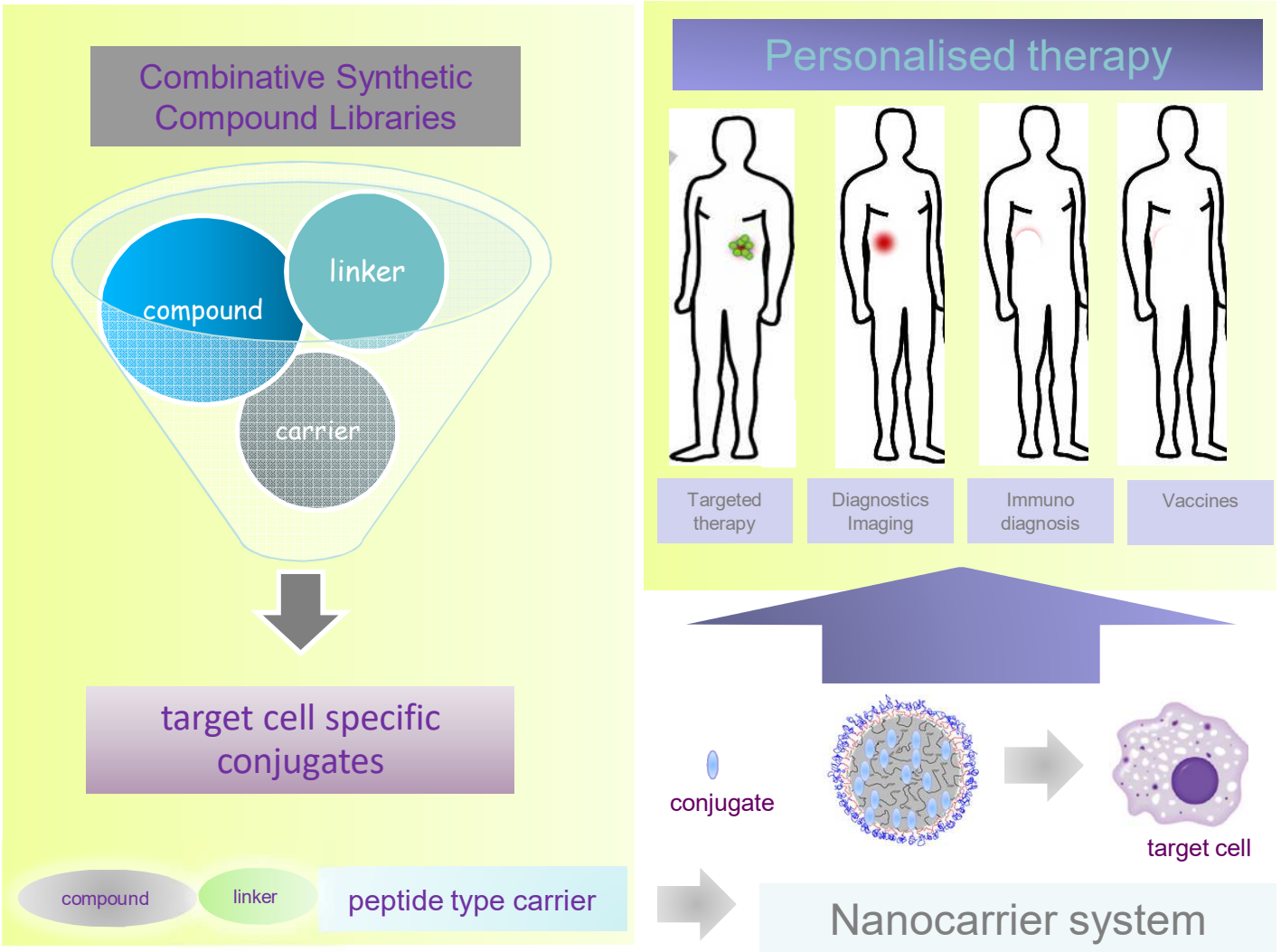
Although the applications he discussed were theoretical at the time, his insights prophesied the discovery of many new properties at the nanometer scale that are not observed in materials at larger scales, paving the way for the ever-expanding field of nanomedicine.

These days, the use of nanosize materials, comparable in dimension to some proteins, DNA, RNA, and oligosaccharides, is making waves in diverse biomedical fields, including biosensing, imaging, drug delivery, and even surgery.

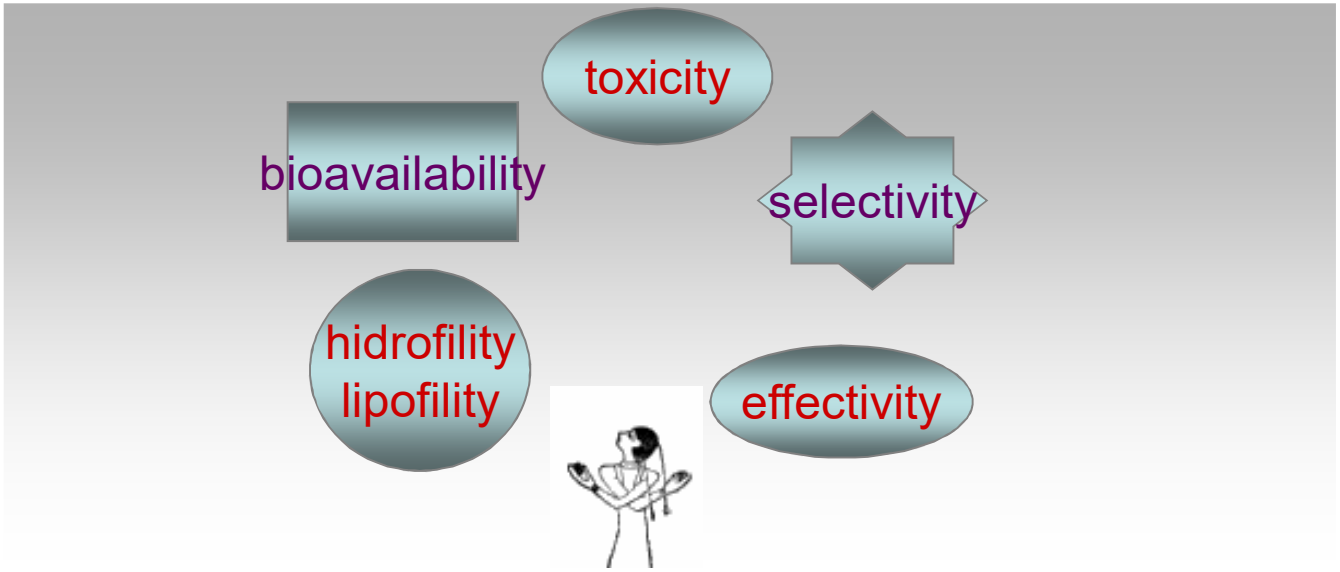




Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös Loránd University



Drug targeting, drug delivery systems (DDS)



- **the interaction of drug compouns with cells**
- cell membrane related biological events at action sites (concentration dependent manner)

- **selective and effective localization of the pharmacologically-active moiety**
- pre-identified target(s) in therapeutic concentration

- **restricting access to non-target(s) „normal" cells**
- minimizing toxic effects, maximizing the therapeutic effect/index

Drug delivery systems (DDS), reasons and aims

The systemic drug administration, even biodistribution of pharmaceuticals throughout the body.
The lack of drug specific affinity towards a pathological site.

- drug instability, solubility (hydrophil/lipophil)
- low absorption
- short half-life, large volume of distribution
- low specificity and therapeutic index

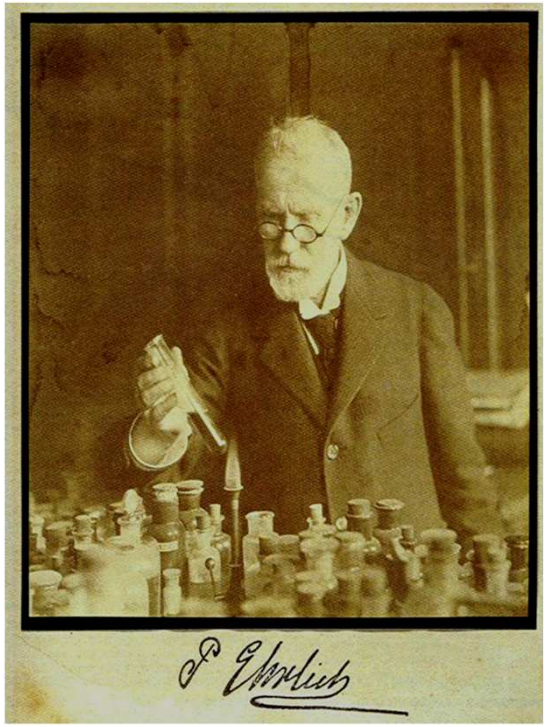
The necessity of a large total dose of a drug. Non-specific toxicity and other adverse side effects.
Drug targeting may resolve some of these problems.

- controlling the distribution, incorporating in a carrier system
- altering the structure of the drug at molecular level
- controlling the input of the drug into bioenvironment
- „programmed” and desirable biodistribution

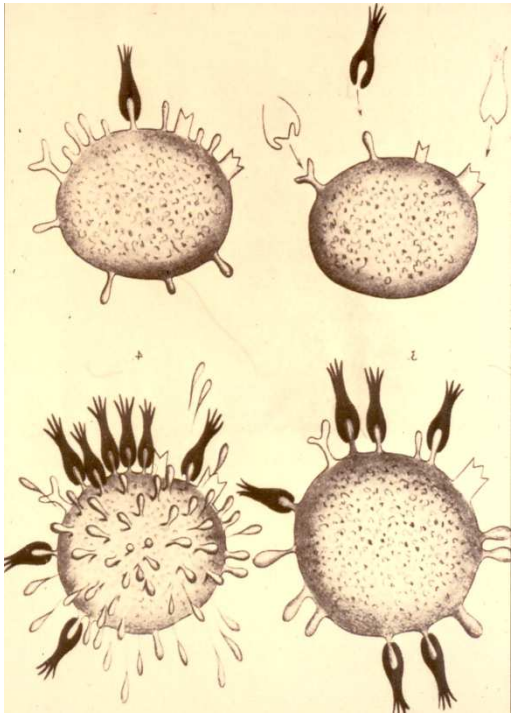
„Magic Bullet” Concept: Drugs Would Be Targeted By Virtue Of Groups Having Affinity For Specific Cells.

The MAGIC BULLET theory: specific delivery of active agents

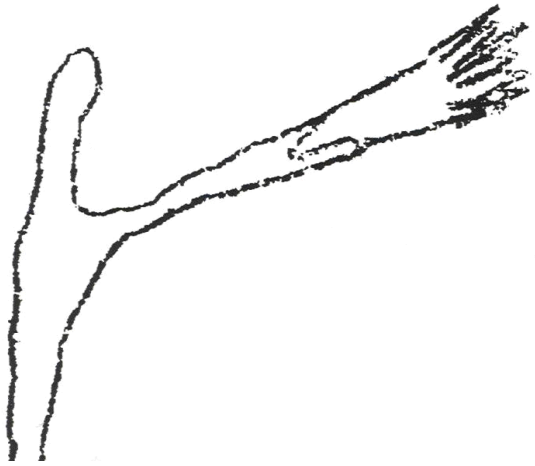
Paul Ehrlich
1854-1915



Nobel Prize in Medicine (1908)



1900: „side chain theory"



- enhanced cellular uptake
- increased drug selectivity
- enhanced bioavailability
- prevents multidrug resistance of the cells

Drug delivery, excipients

„excipere" (to receive; the excipient receives the active substance.)
an excipient contained in a dosage form is something other than the active substance

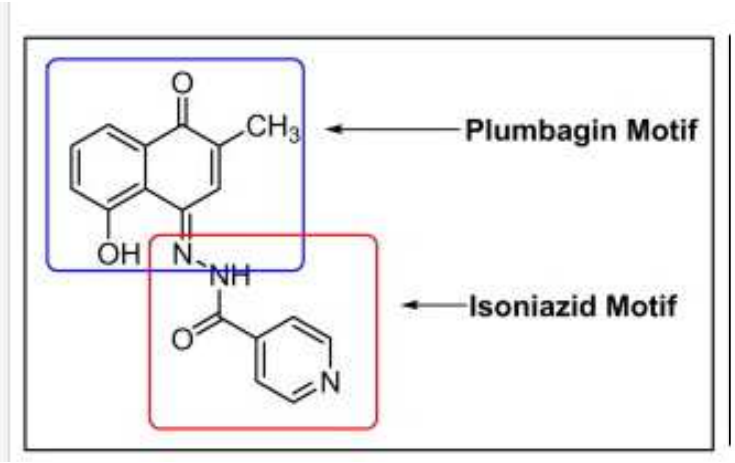
WHO – (Technical Report Series, No. 961, Annex 10 (Procedure for prequalification of pharmaceutical products)

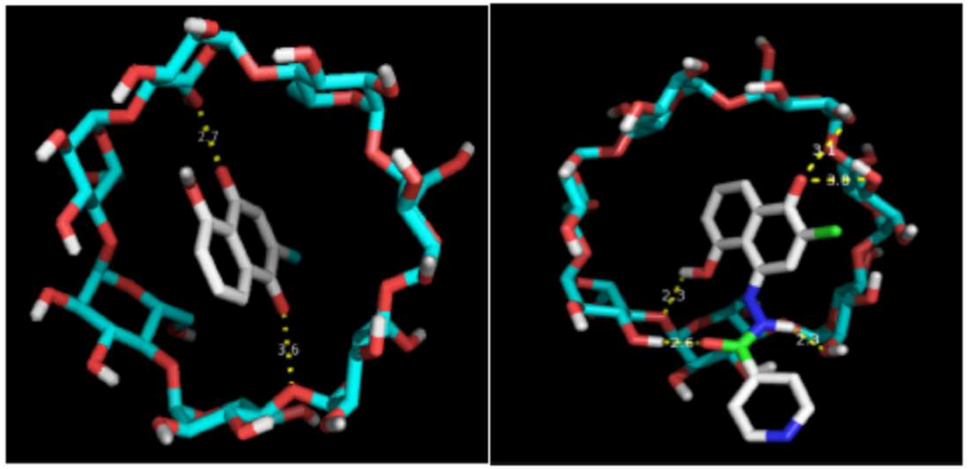
active pharmaceutical ingredient (API)
"a substance used in a finished pharmaceutical product, intended to furnish pharmacological activity or to otherwise have direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease, or to have direct effect in restoring, correcting or modifying physiological functions in human beings."

The European Pharmacopoeia (Ph. Eur.):
"An excipient is any component, other than the active substance(s), present in a medicinal product or used in the manufacture of the product. The intended function of an excipient is to act as the carrier (vehicle or basis) or as a component of the carrier of the active substance(s) and, in so doing, to contribute to product attributes such as stability, biopharmaceutical profile, appearance and patient acceptability and to the ease with which the product can be manufactured. Usually, more than one excipient is used in the formulation of a medicinal product."

https://www.europeanpharmaceuticalreview.com/article/18434/the-central-role-of-excipients-in-drug-formulation-2/
https://www.americanpharmaceuticalreview.com/Featured-Articles/36925-Application-Challenges-and-Examples-of-New-Excipients-in-Advanced-Drug-Delivery-Systems/

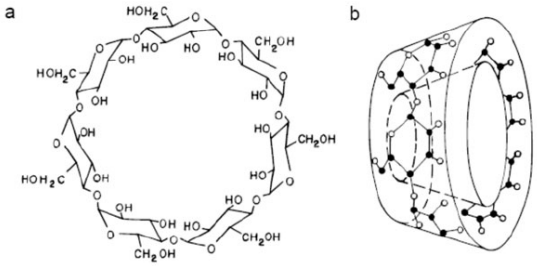
Cyclodextrins as drug carriers, excipients

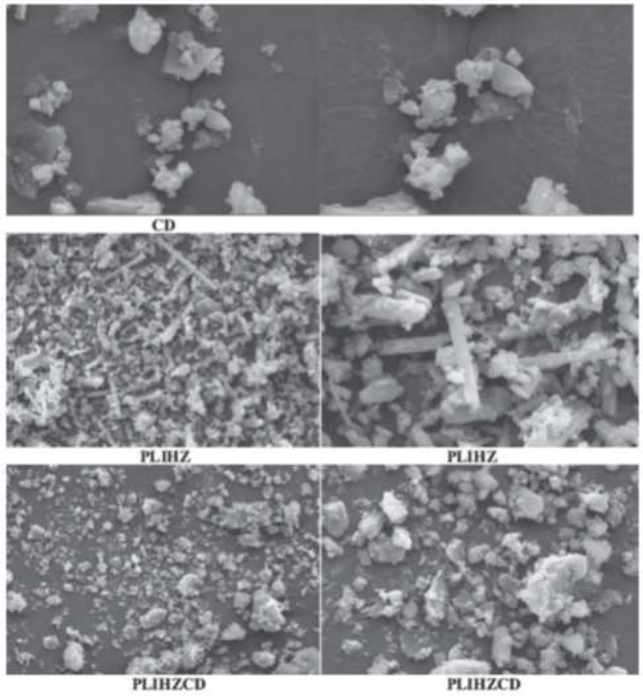




Plumbagin-β-cyclodextrin (**PLCD**) (1:1)

PLIHZCD (1:1)





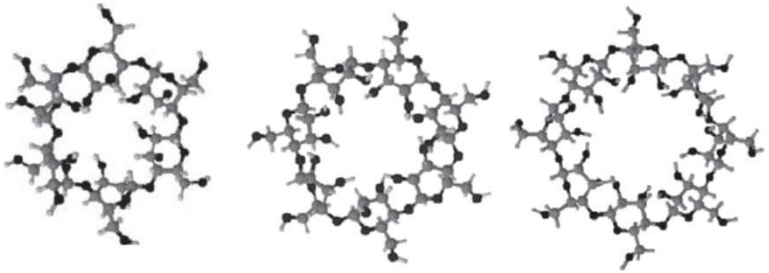
maintains INH activity, avoids resistance
(inhibition of enzymatic activity)

application of cylodextrin carriers:

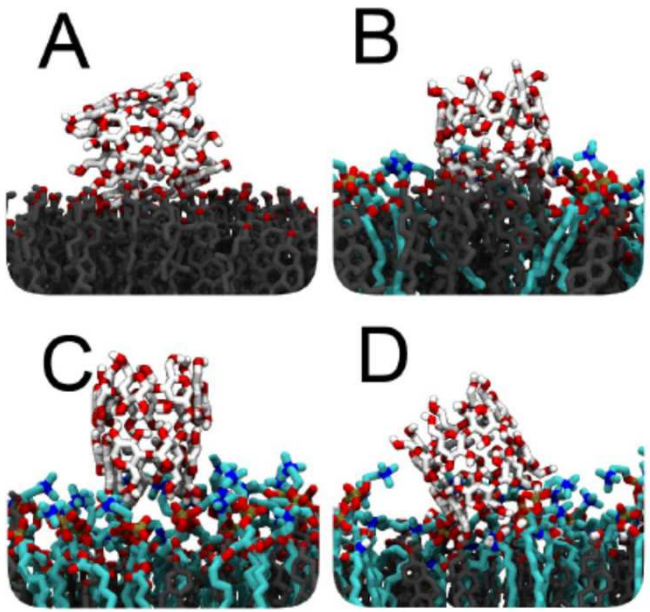
Clofazimine
Clarithromycin
Nitroimidazopirán (PA-824, 100 mg/ttkg)
Moxifloxacin

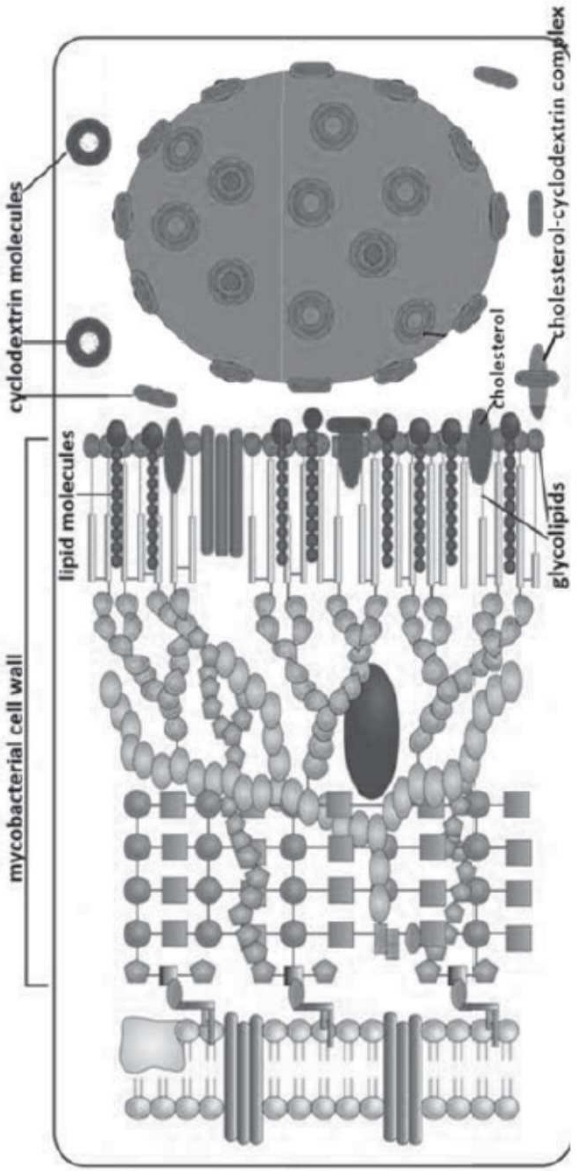
Dandawate P. et al., Bioorg Med Chem Lett. (2014) 24(21), 5070-5.

Mycobacteriaceae cell wall and cyclodextrin constructs



Parameters	α-cyclodextrin	β-cyclodextrin	γ-cyclodextrin
Number of glucopyranose fragments	6	7	8
Molecular weight	973	1135	1297
Internal cavity diameter (nm)	0.47–0.53	0.60-0,66	0.75-0.83
External diameter (nm)	1.46±0.04	1.54±0.04	1.75±0.04
Torus height (nm)	0.79±0.01	0.79±0.01	0.79±0.01
Cavity volume approx. (mL/mol)	104	157	256
$[\alpha]_D$ at 25°C	150±0.5	162.5±0.5	177.4±0.5
Water solubility (g/100 mL at 25°C)	14.5	1.85	23.2





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C. A. Lopez et al., Scientific Reports 3 (2013) Article number: 2071 doi:10.1038/srep02071
D. Castagne et al., J. Pharm. Pharmaceutical Sci. (2010) 13 (3) 362-377.
V. Boldescu and G. Duca, Chem. J. Moldova (2014) 9 (1) 8-13.

Special terms target, vehicle, carrier, ligand

TARGET

A cell or group of cells in minority, identified to be in the need of treatment.

VEHICLE is the carrier, composed of one or more components, for the active substance(s)

CARRIERS/MARKERS

Carrier is the entity essentially required for effective transportation of loaded drugs. They are vectors, which sequester, retain drug and transport or deliver it into the vicinity of the target cells.

LIGANDS

The ligands confer recognition and specificity upon carrier/vector and lend them to approach the respective target and deliver the drug (antibodies, polypeptides, endogenous hormones etc.).

Important properties influencing drug targeting

DRUG

- concentration (at action site)
- location and distribution
- molecular weight
- physio-chemical properties
- drug and carrier interactions

CARRIER/MARKER

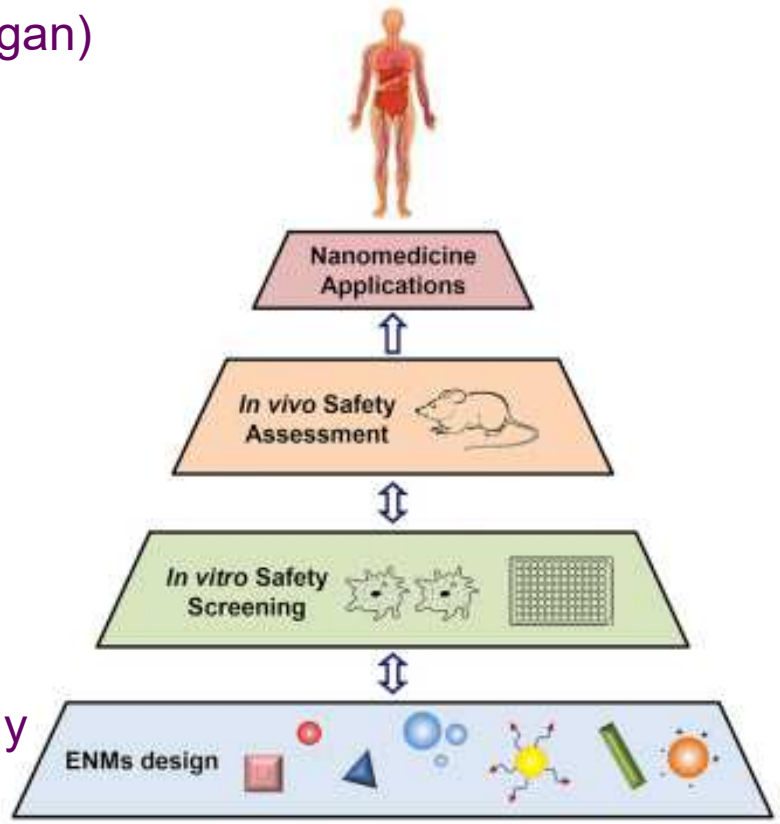
- carrier type (amount of excipients)
- surface characteristics, size, density,
- release characteristics

***IN VIVO* ENVIROMENT**

- pH, polarity, ionic strength, enzymes etc.

Ideal properties of drug delivery systems

- nontoxic, biocompatible
- physico-chemical stability (*in vivo, in vitro*)
- restrict drug distribution to target cells (tissue, organ)
- uniform capillary distribution
- controllable and predictable rate of drug release
- drug release not affect the drug action
- therapeutic amount of drug release
- minimal drug leakage during transit
- biodegradable or readily eliminated from the body
- preparation simple, cost effective



Mirshafiee, Vahid et al. Facilitating Translational Nanomedicine via Predictive Safety Assessment
Molecular Therapy, 25, 7 , 1522 – 1530.

Properties of the carrier I.

Entity required for successful transportation of the loaded active compound. Vehicles, vectors which, retain and transport active compounds; deliver it within or in the vicinity of target employing their inherent characteristic or acquired through structural modification.

- it must be able to cross anatomical barriers
  (tumour chemotherapy: tumour vasculature)
- it must be recognized specifically and selectively by the target cells
- it must maintain the specificity of the surface ligands

The linkage of the drug and the directing unit (ligand) should be stable in plasma, interstitial and other biofluids. Carrier should be non-toxic, non- immunogenic and biodegradable particulate or macromolecule.

The biomodules used as carrier should not be ubiquitous
(existing or being everywhere at the same time)
After recognition and internalization, the carrier system should release the drug moiety inside the target organs, tissues or cells.

Properties of the carrier II.

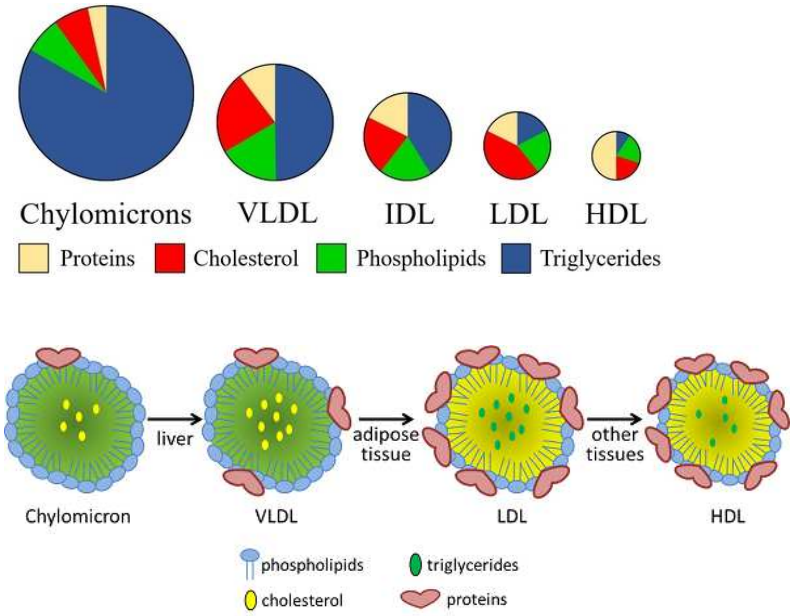
Based on the nature of their origin

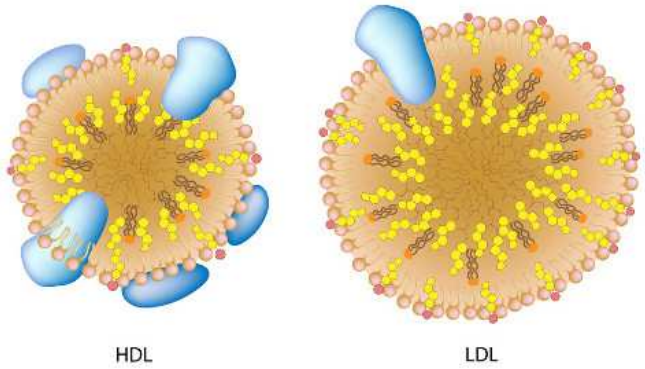
Endogenous carriers

LDL, HDL chylomicrons, serum albumin, erythrocytes

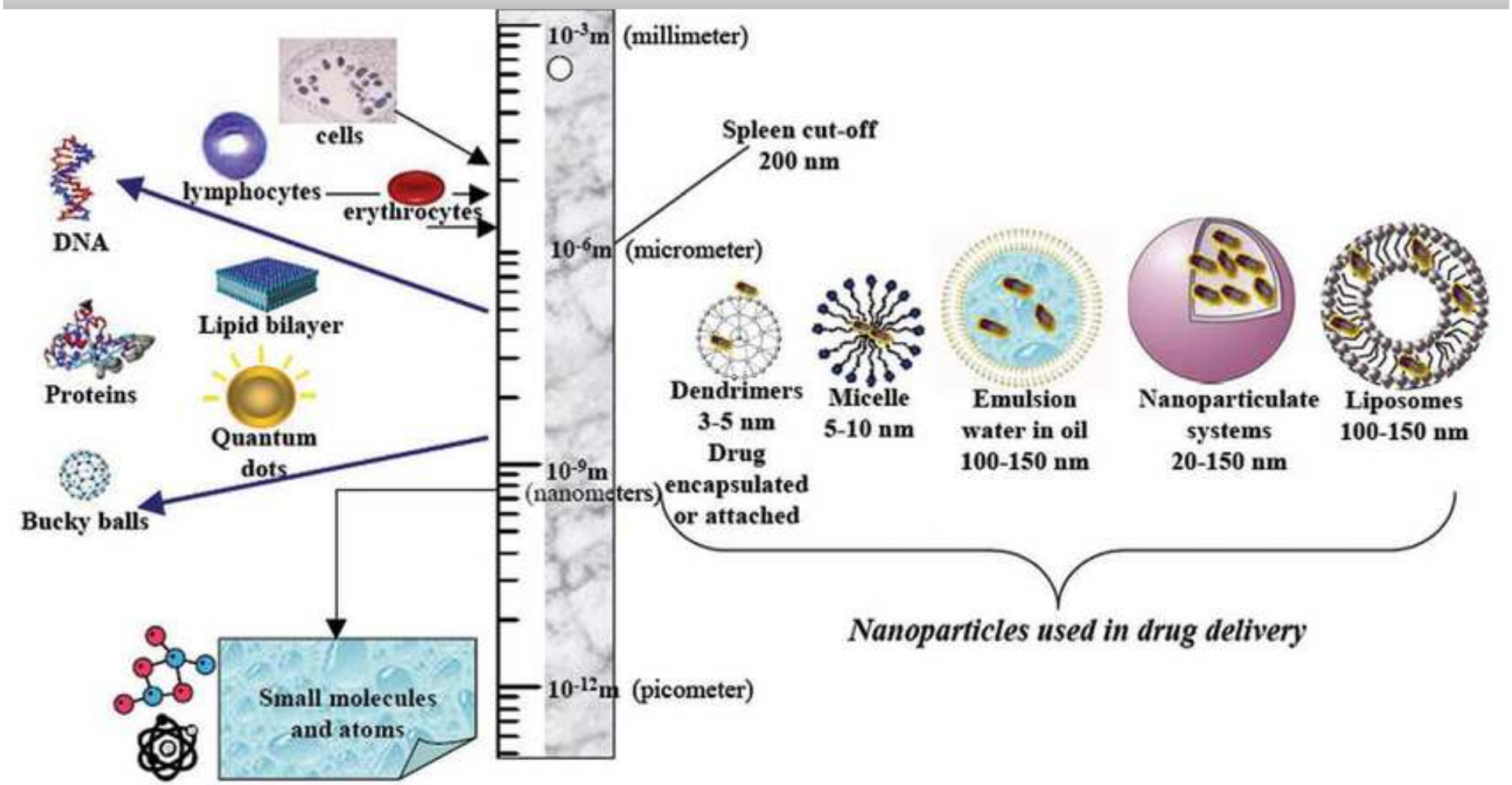
Exogenous carriers

microparticulates, soluble polymeric and biodegradable polymeric drug carriers





Properties of the carrier III.



Pharmaceutical carriers

Targeted drug delivery systems

A special form of drug delivery system, the pharmacologically active compound is selectively targeted or delivered only to its site of action or absorption and not to the non-target organs, tissues or cells.

- administration protocols simplified
- drug quantity greatly reduced (as cost of therapy)
- drug concentration sharply increased
- no negative effects on non-target compartments

Carriers for targeted drug delivery systems

Colloidal carriers

Vesicular systems

liposomes, niosomes, pharmacosomes, virosomes, immunoliposomes etc.

Microparticulate systems

microparticles, nanoparticles, magnetic microspheres, albumin microspheres, nanocapsules etc.

Cellular carriers

erythrocytes, serum albumin, antibodies, platelets, leucocytes, nucleic acids etc.

Supramolecular delivery systems

micelles (reverse, mixed, polymeric), liquid crystals,
lipoproteins (chylomicrons, VLDL, LDL)

Polymer based systems

signal sensitive, muco-adhesive, biodegradable, soluble synthetic polymeric carriers

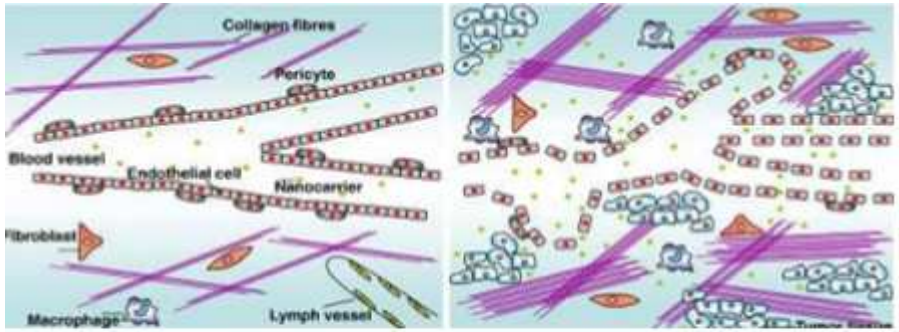
Macromolecular carriers

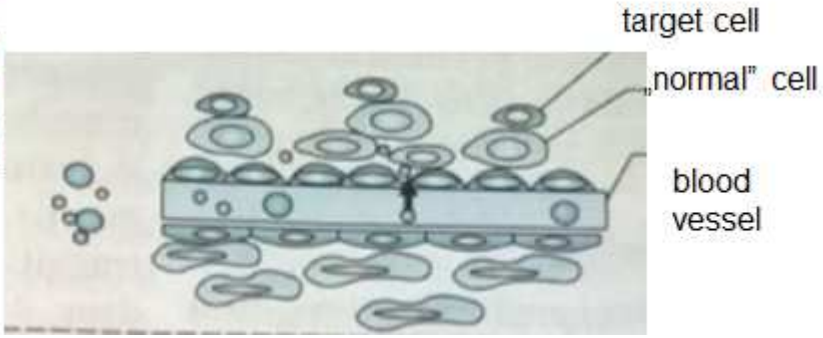
proteins, glycoproteins, artificial viral envelopes (AVE), glycosylated water soluble polymers (poly-L-lysine), Mabs, Fab fragments, antibody-enzyme complexes, bispecific Abs, toxins, immunotoxins, lectins and polysaccharides etc.

Level of drug targeting: strategies and approaches I.

Passive Targeting

- „natural route" of biodistribution; (reticulo-endothelial system (RES))
- capture of the colloidal type carriers by macrophages
- offers opportunities for the antimicrobial compounds, some tumors





Inverse Targeting

- avoidance of passive uptake of colloidal carriers by the RES
- suppressing the function of RES by pre- junction of a large amount of blank colloidal carriers or macromolecules like dextran sulphate
- modification and defined manipulation of the size, surface charge, composition, surface rigidity and hydrophilicity characteristics of carriers for desirable biofate

Level of drug targeting: strategies and approaches II.

Active Targeting

involves the modification or functionalization of the drug carriers so the contents are delivered exclusively to the site corresponding to which the carrier is architected
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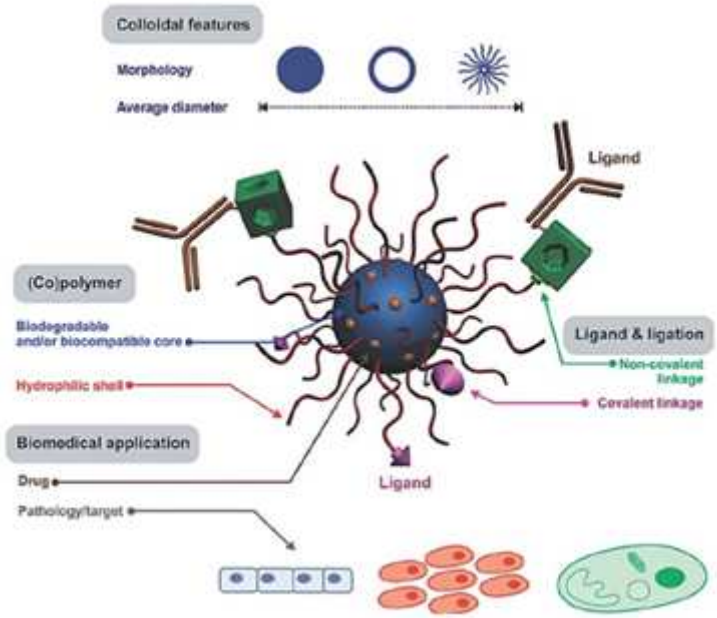
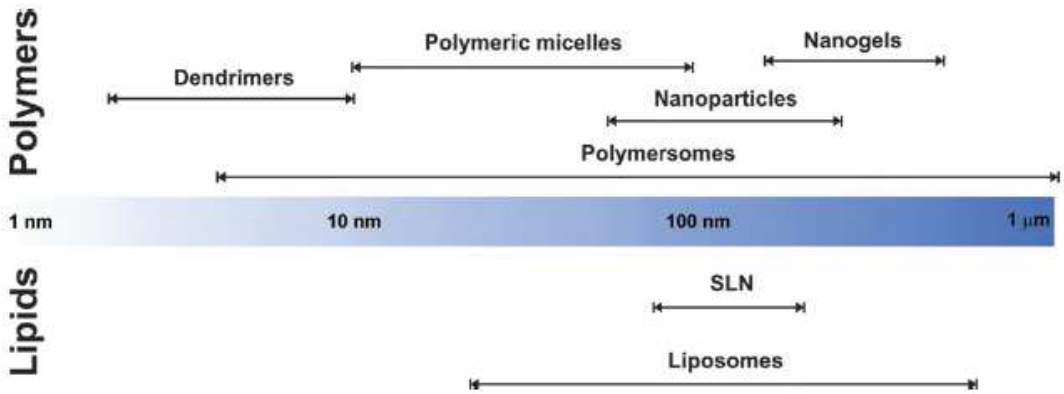
The construct are delivered exclusively to the site corresponding to which the carrier is architected.





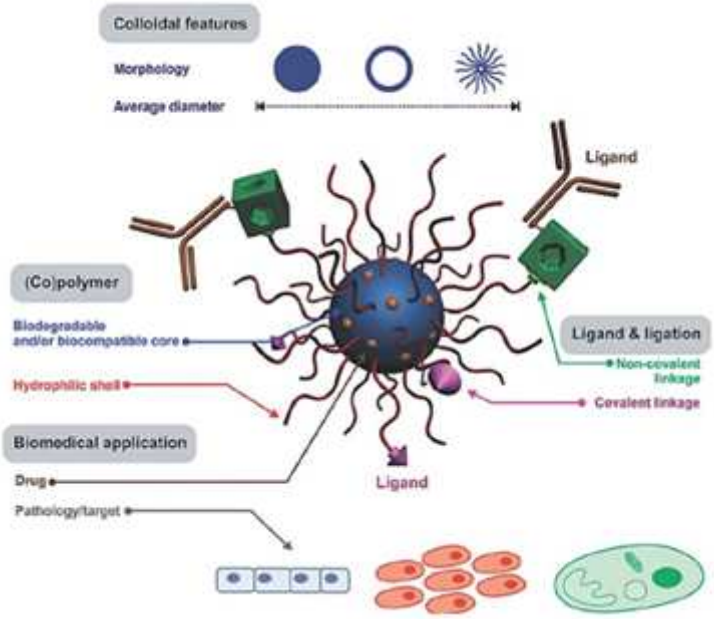
B. Daglar, E. Ozgur, M. E. Corman, L. Uzund and G. B. Demirel, Polymeric nanocarriers for expected nanomedicine: current challenges and future prospects RSC Adv., 2014, 4, 48639-48659.
Nicolas J, Mura S, Brambilla D, Mackiewicz N, Couvreur P. Design, functionalization strategies and biomedical applications of targeted biodegradable/biocompatible polymer-based nanocarriers for drug delivery. Chem Soc Rev. 2013;42(3):1147-235. doi: 10.1039/c2cs35265f. Review.

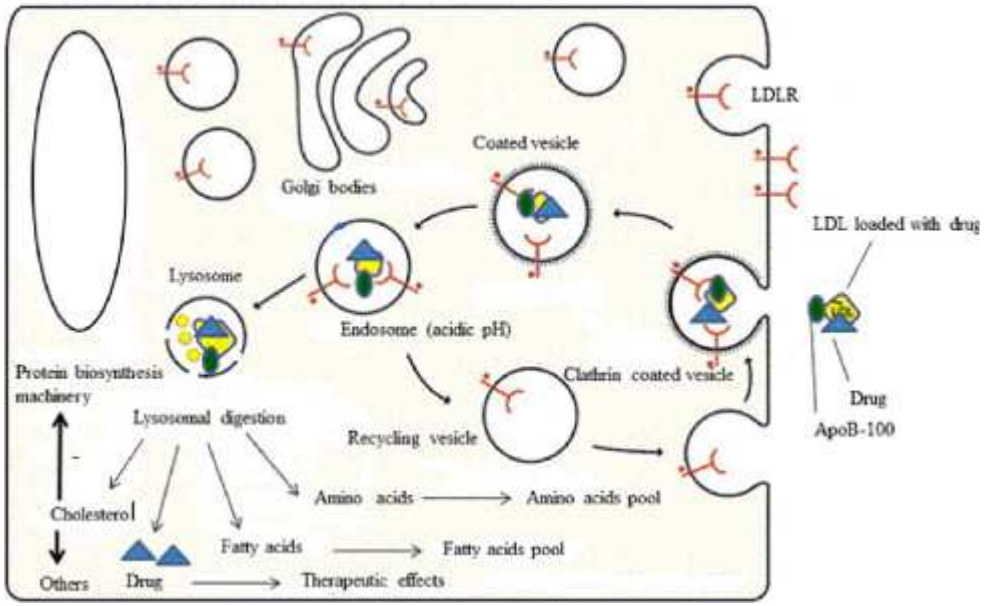
Ligand associated active targeting

- ligands are carrier surface group(s)
- selectively direct the carrier to the pre-specified site(s)
- housing the appropriate receptor units to serve as ‘homing device’ to the carrier/drug

Most of the carrier systems are colloidal and can be specifically functionalized using various biologically-relevant molecular ligands (antibodies, polypeptides, oligosaccharides, viral proteins & fusogenic residues etc.).

The ligands confer recognition and specificity upon drug carrier, endow them with an ability to approach the respective target selectivity and deliver the drug.





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Harisa GI, Alanazi FK. Low density lipoprotein bionanoparticles: From cholesterol transport to delivery of anti-cancer drugs. Saudi Pharm J. 2014;22(6):504-15.
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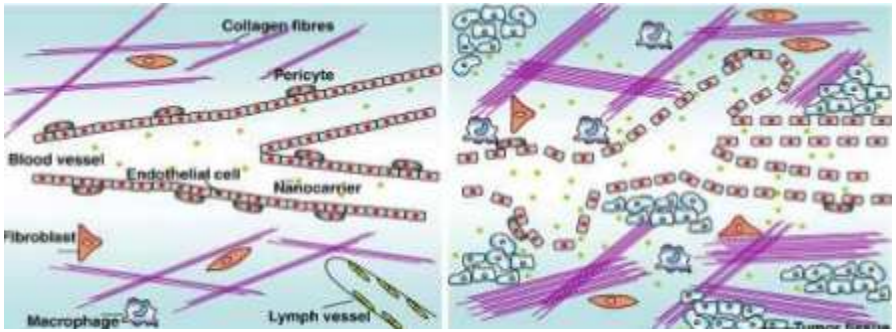
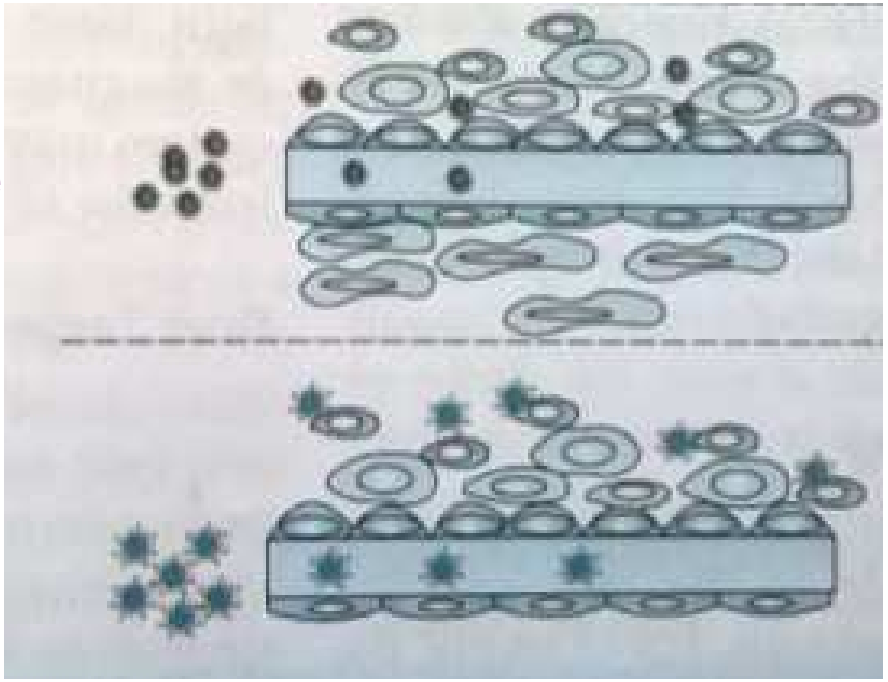
Level of drug targeting: strategies and approaches II.

Passive



Reverse

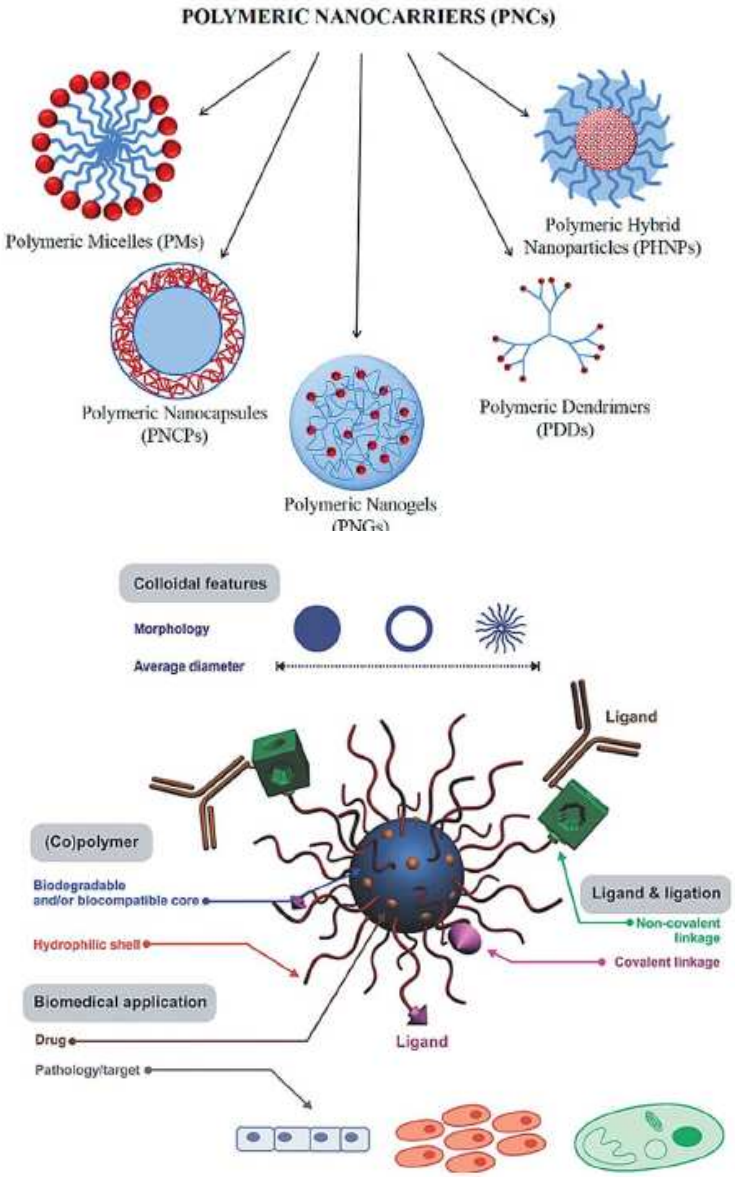


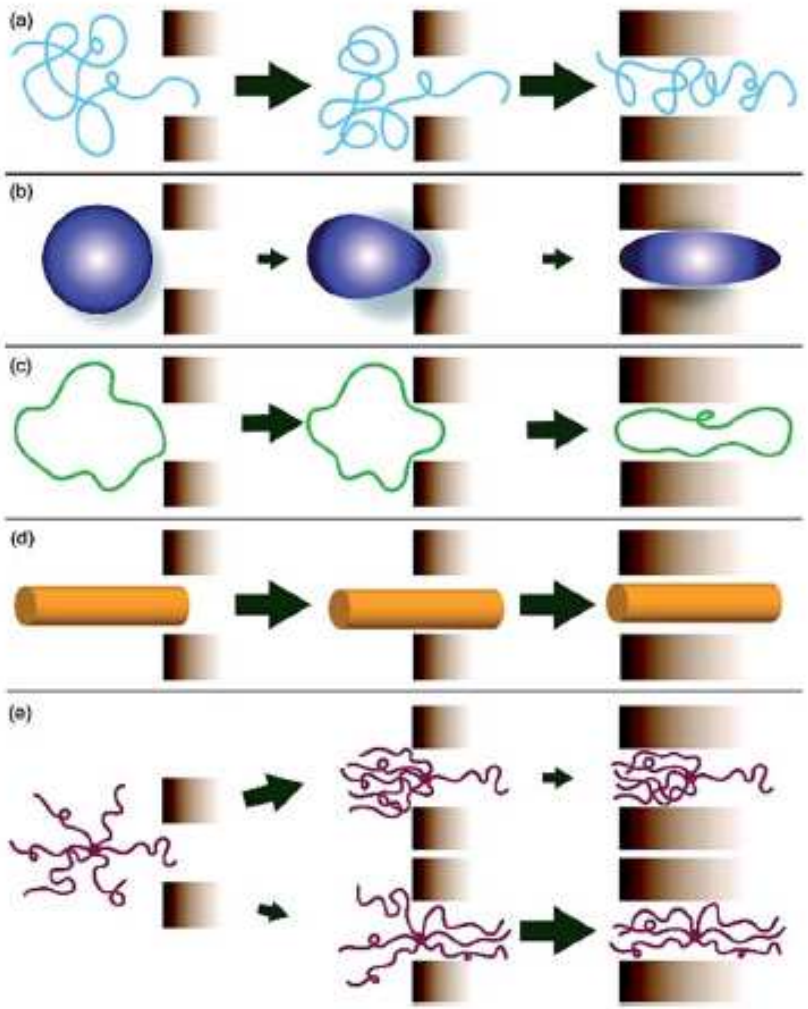
long circulating carriers, higher uptake rate

Active

targeted carrier, maximum selectivity, specificity

Vesicular and polimeric delivery systems





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Physical targeting

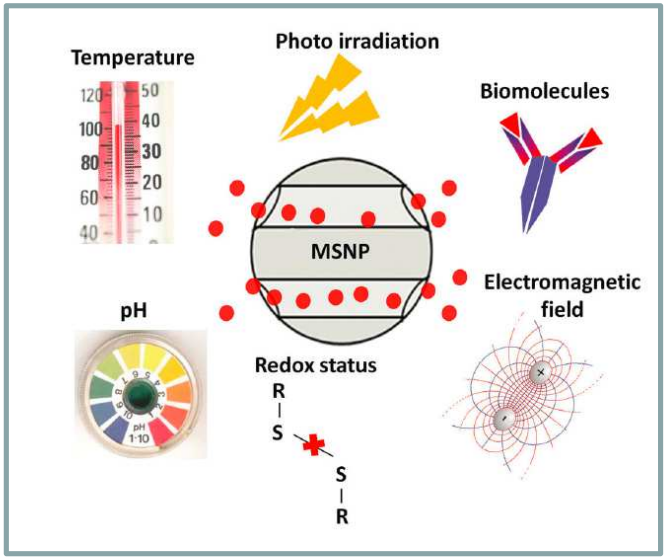
Characteristics of environment changes:
(pH, temperature, light intensity, electric field, ionic strength etc.)

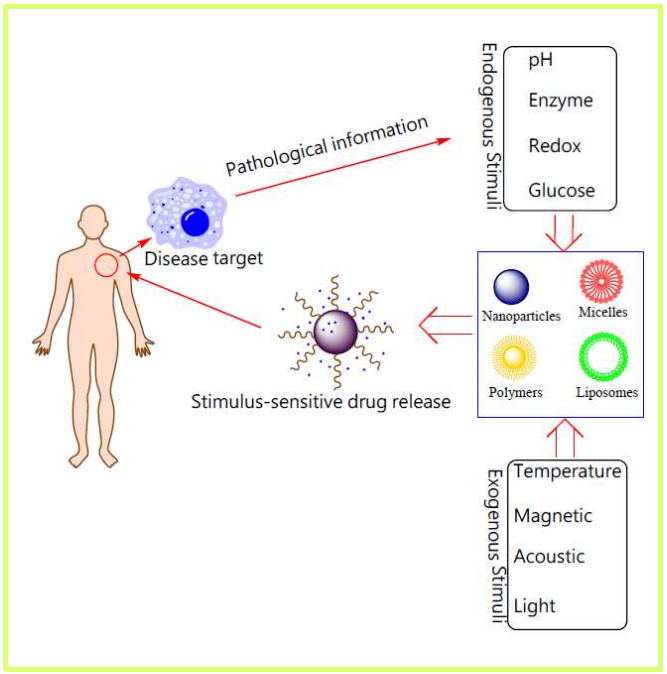
Physical Targeting	Formulation System	Mechanism for Drug Delivery
Heat	Liposome	Change in Permeability
Magnetic Modulation	Magnetically Responsive Microspheres Containing Iron oxide	Magnetic Field can retard fluid Flow of particles
Ultrasound	Polymers	Change in Permeability
Electrical Pulse	Gels	Change in Permeability
Light	Photo responsive Hydro gels Containing Azo-Derivatives	Change in Diffusion Channels, Activated by Specific Wavelength

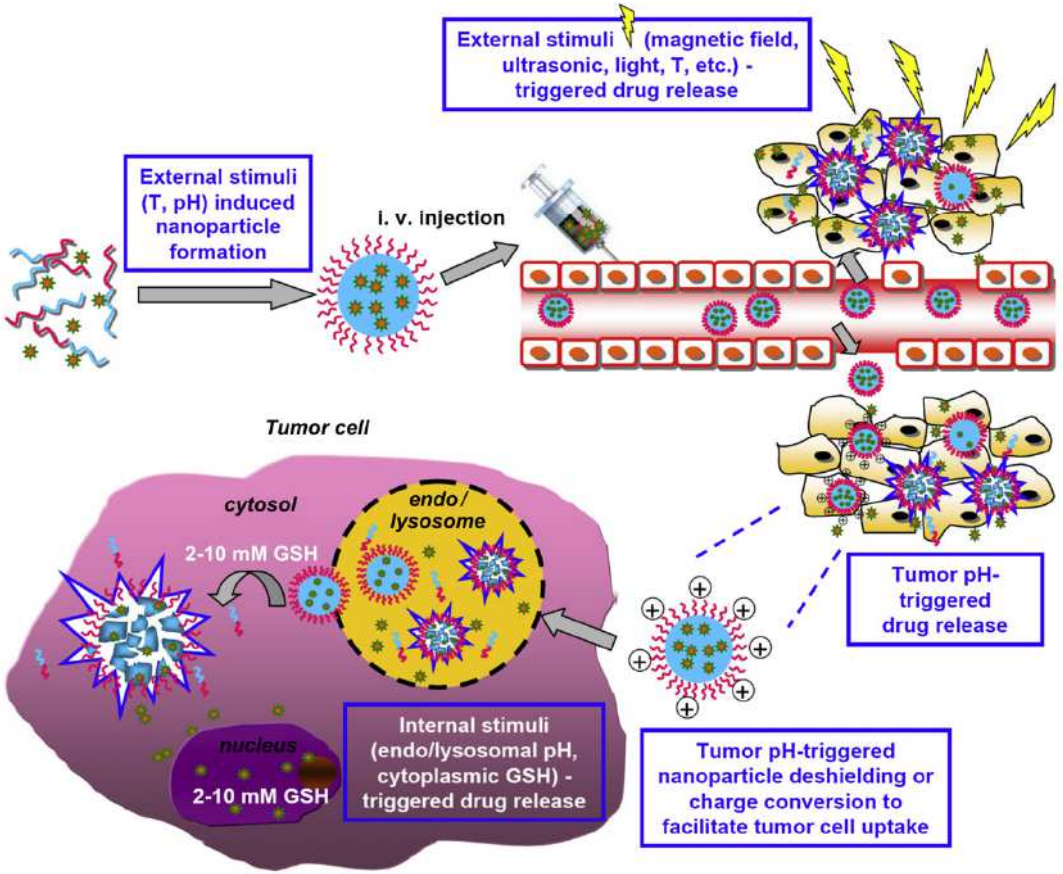
This approach exceptional for tumor targeting, cytosolic delivery of entrapped drug or genetic material.

Liu D, Yang F, Xiong F, Gu N. The Smart Drug Delivery System and Its Clinical Potential. Theranostics. 2016, 7;6(9):1306-23. Review.

Physical targeting





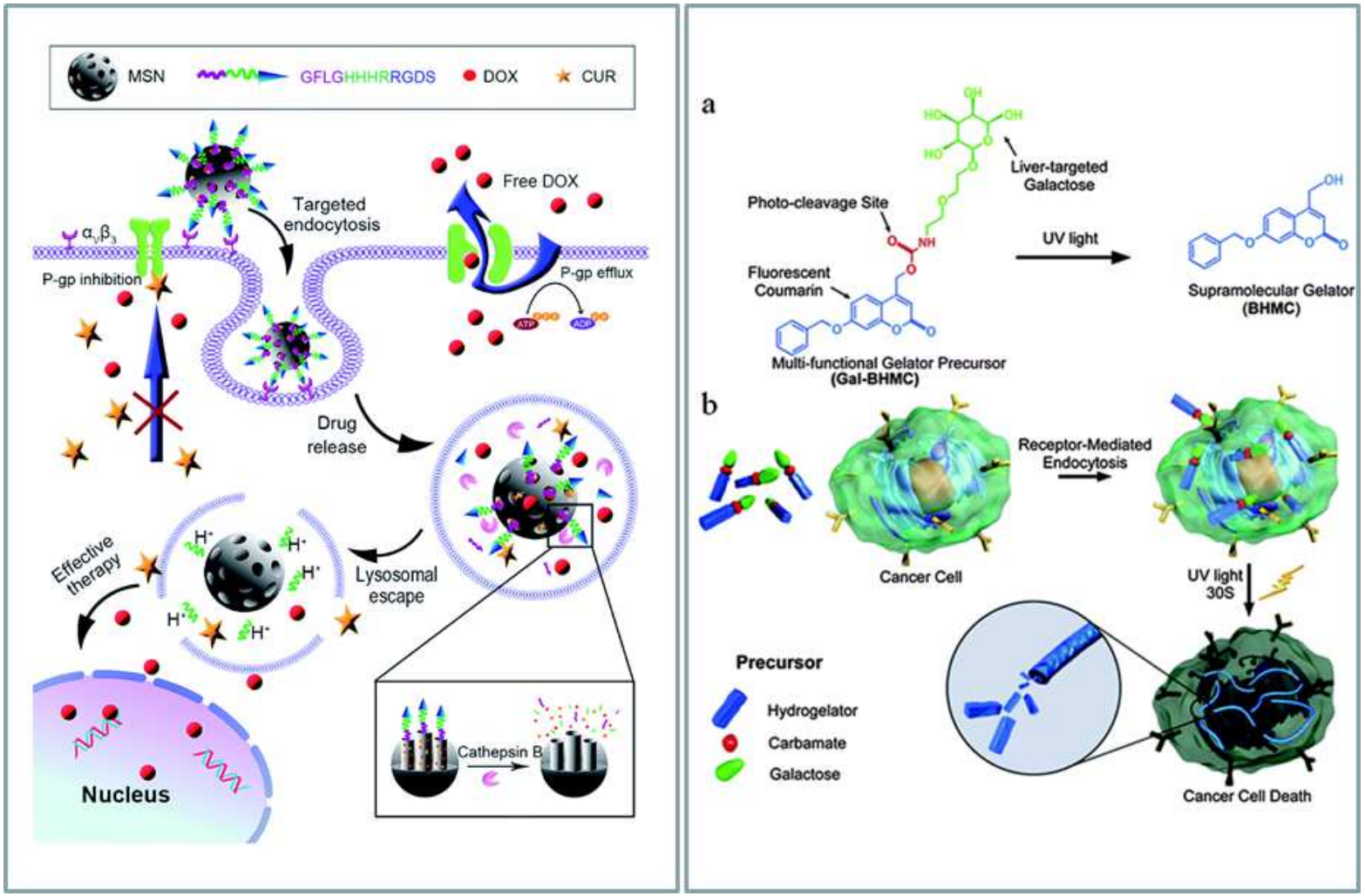


Liu D, Yang F, Xiong F, Gu N. The Smart Drug Delivery System and Its Clinical Potential. Theranostics. 2016, 7;6(9):1306-23. Review.

Dual, double and combination targeting

carrier molecule - therapeutic activity; increased therapeutic effect; synergistic effect

spatial control	targeting to specific organs, tissues, cells or even sub cellular compartment
temporal control	the rate of drug delivery to target site



Lipid-based nanovesicles for targeting

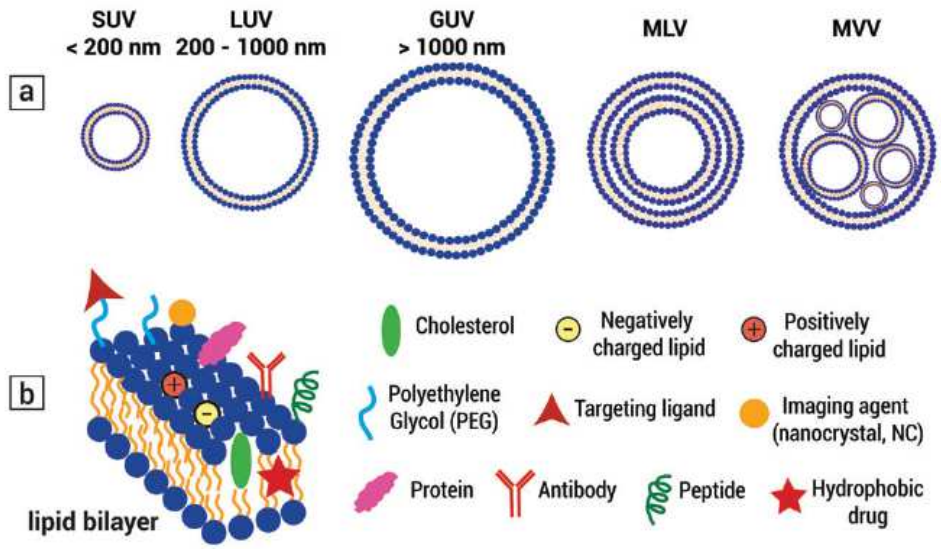


Fig. 1 Schematic representation of lipid-based vesicles. (a) Classification of vesicles regarding their size and lamellarity; (b) structure of the vesicle bilayer (left side) and examples of (bio)-actives to be physically encapsulated or chemically conjugated.

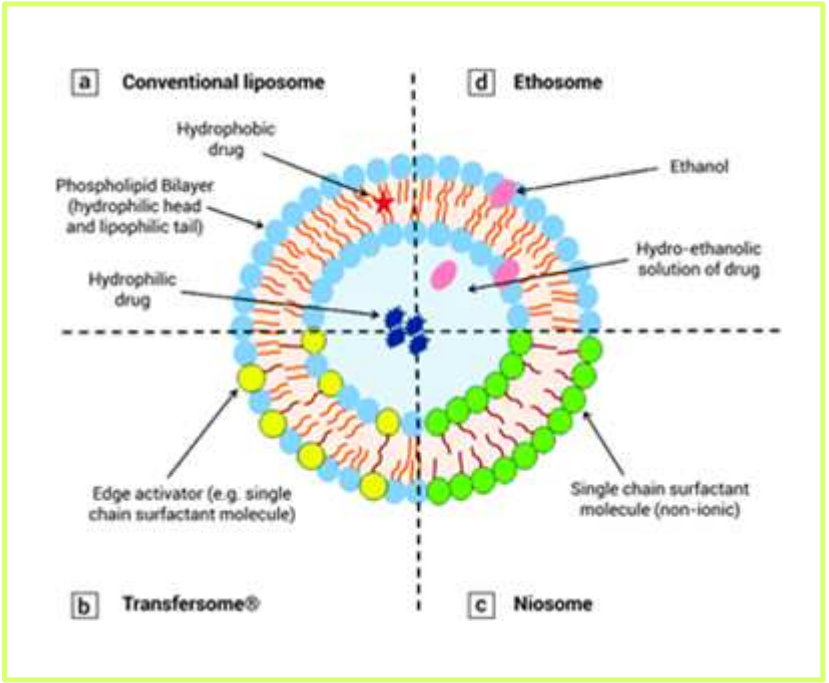
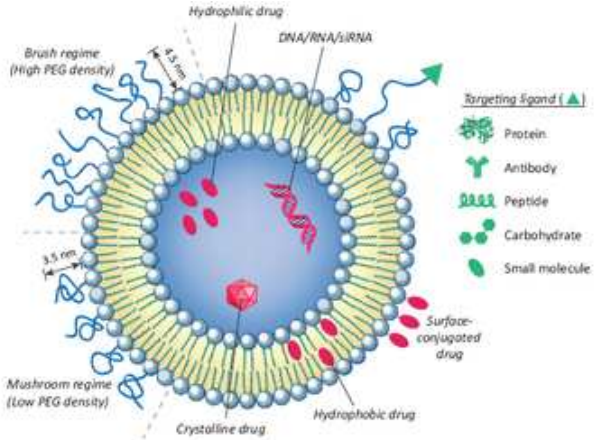


Table 1 Examples of non-liposomal L-NVs and their main characteristics

System	Composition	Size lamellarity	Stability	Example of the preclinical study[ref.]
Niosomes	Chol/non-ionic surfactants	Nano and sub-micron range Unilamellar & multilamellar	Short-term stability (few months)	*In vitro*[70,79,85,88] *In vivo*[70]
Transfersomes	Phospholipids/surfactants	Nano and sub-micron range Unilamellar & multilamellar	Short-term stability (few months)	*In vitro*[42,89–91] *In vivo*[42,71,89]
Ethosomes	Phospholipids/alcohols	Nano and sub-micron range Unilamellar & multilamellar	Short-term stability (few months)	*In vitro*[69,92–94] *In vivo*[69]
Sphingosomes	Chol/Sphingolipids	Nano and sub-micron range Unilamellar & multilamellar	Long-term stability (several months)	*In vitro*[95] *In vivo*[95,96]
Ufasomes	Fatty acids/surfactants	Nano and sub-micron range Unilamellar & multilamellar	Short-term stability (few months)	*In vitro*[97–99] *In vivo*[99]
Pharmacosomes	Phospholipids/drugs	Nano and micron range Unilamellar & multilamellar	Short-term stability (few months)	*In vitro*[100,101] *In vivo*[101]
Virosomes	Phospholipids/viral envelope proteins	Nano and sub-micron range Unilamellar & multilamellar	Short-term stability (few months)	*In vitro*[73,102–104] *In vivo*[73,102–105]
Quatsomes	Chol/cationic surfactants	Nano range Unilamellar	Long-term stability (several years)	*In vitro*[74]

Grimaldi N, Andrade F, Segovia N, Ferrer-Tasies L, Sala S, Veciana J, Ventosa N. Lipid-based nanovesicles for nanomedicine. Chem Soc Rev. 2016 Nov 21;45(23):6520-6545. Review.
https://liposomes.weebly.com/the-basics.html
Hong CA, Nam YS. Functional Nanostructures for Effective Delivery of Small Interfering RNA Therapeutics. Theranostics 2014; 4(12):1211-1232. doi:10.7150/thno.8491. http://www.thno.org/v04p1211.ht

Lipid-based nanovesicles evolution



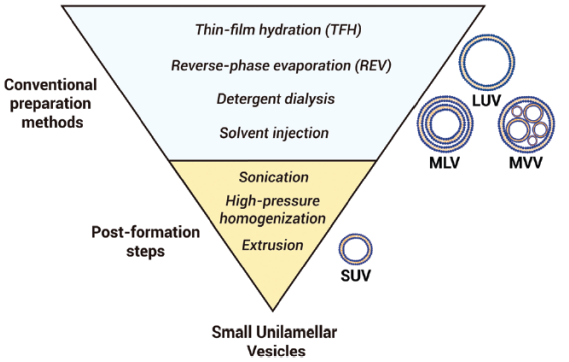
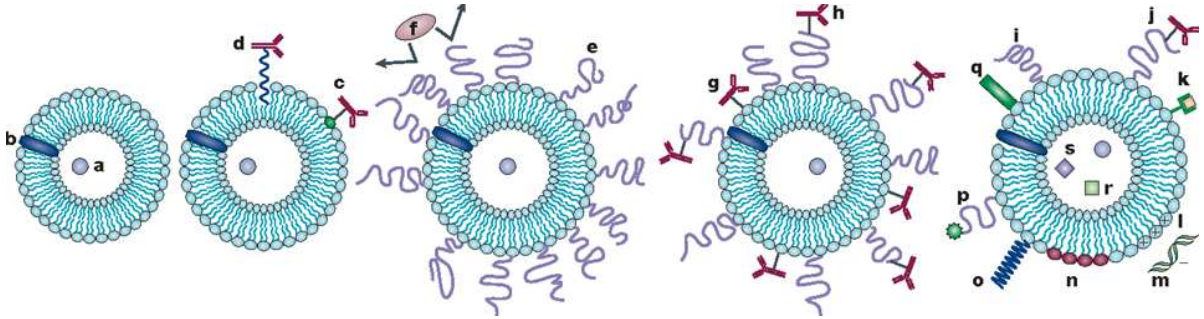


Fig. 3 Schematic representation of the most common conventional synthetic approaches for L-NV production.

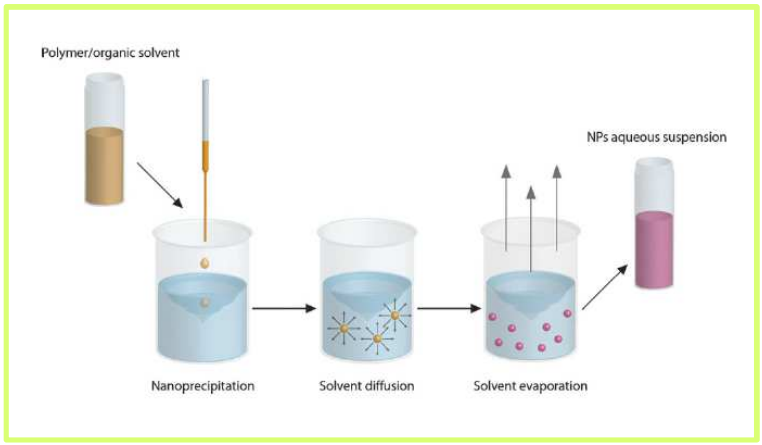


Passive Targeting:
intramuscularly or subcutaneously administered and allowed to passively circulate to a target
PEGylated (grafted with polyethylene glycol), increase their circulation time

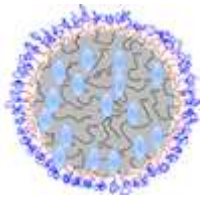
Active Targeting:
drugs that require rapid delivery, targeted liposomes, contain specific ligands or receptors
recognize and bind to certain proteins on the surface of target cells
(PEG interferes with protein binding, phosphatidylethanolamine and antibodies,
retaining long circulation time)

Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov. 2005;4(2):145-60. Review.
Grimaldi N, Andrade F, Segovia N, Ferrer-Tasies L, Sala S, Veciana J, Ventosa N. Lipid-based nanovesicles for nanomedicine. Chem Soc Rev. 2016, 21;45(23):6520-6545. Review.

Vesicular, polimeric delivery systems, nanoparticles

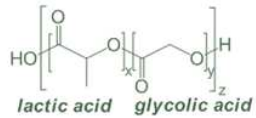


nanoprecipitation

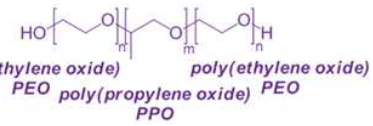


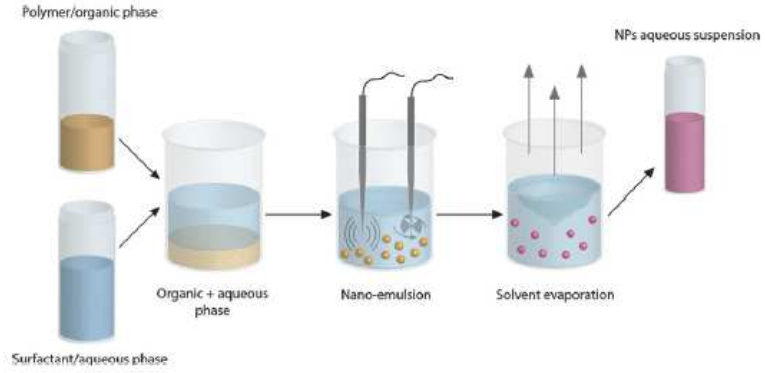
PLGA copolymer

drug

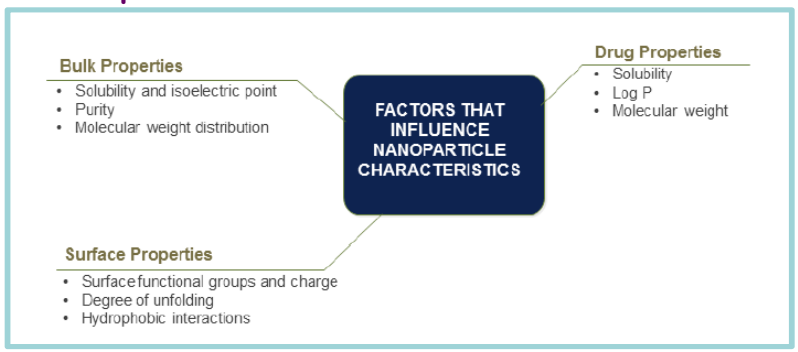


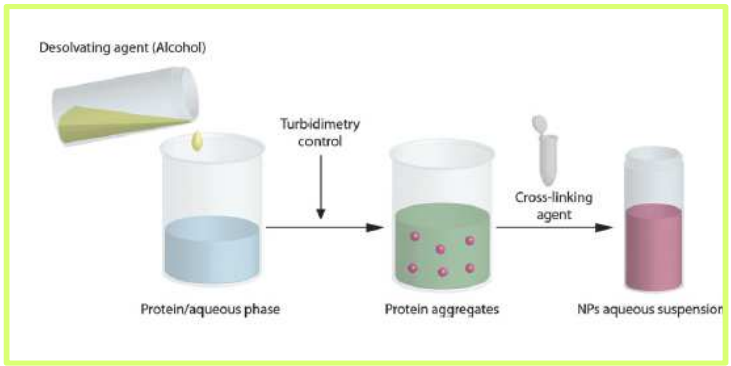
Pluronic block-copolymer



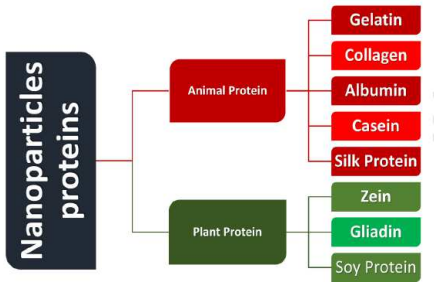


emulsion-solvent evaporation



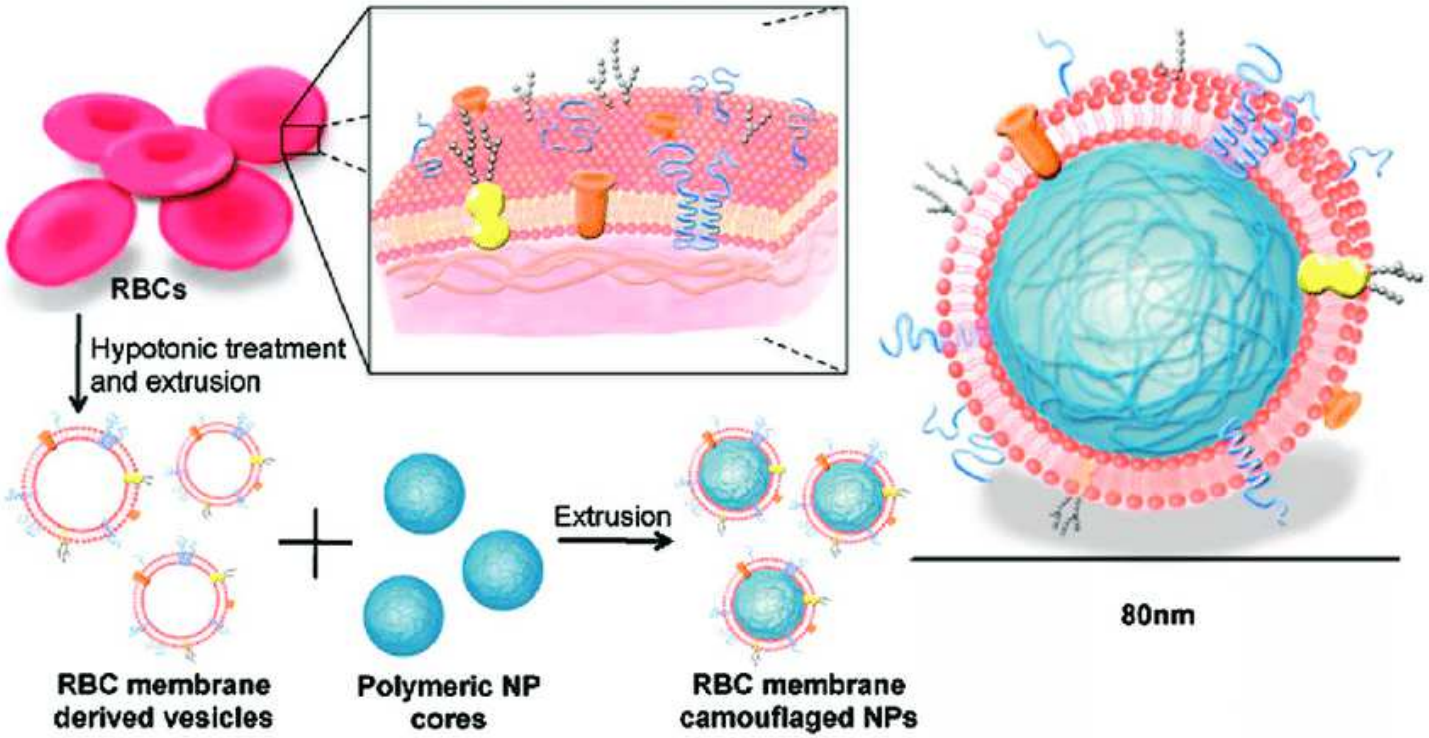


desolvation process



Nicolas J, Mura S, Brambilla D, Mackiewicz N, Couvreur P. Design, functionalization strategies and biomedical applications of targeted biodegradable/biocompatible polymer-based nanocarriers for drug delivery. Chem Soc Rev. 2013;42(3):1147-235. doi: 10.1039/c2cs35265f. Review.
Tarhini M, Greige-Gerges H, Elaissari A. Protein-based nanoparticles: From preparation to encapsulation of active molecules. Int J Pharm. 2017;522(1-2):172-197. doi: 10.1016/j.ijpharm.2017.01.067.

Preparation for RBC-membrane-coated PLGA NPs.



RBC membrane-coated NPs extrusion-based fusion proces between the osmotic shock-derive RBC membranes and the nano-size PLGA NPs.

Cell Sources	Substrates	Reference	Cell Source	Substrates	Reference
Red Blood Cell membranes	PLGA-NPs	[39,41,45,61]	White Blood Cell membranes	Silica NPs	[50,62]
				ATPEs-Si	[63]
	Au-NPs	[64]		PLGA-NPs	[65]
	Gelatins	[66]		Janus NP	[67]
	Yb^3+, Er^3+ and etc.	[68,69]	Platelet membranes	PLGA-NPs	[43,70]
	Iron oxides	[71]	Cancer cell membranes	PLGA-NPs and upcon-version NPs	[42,72]
	Janus particle	[73]	Exosomes	PLGA-NPs	[74]
	Si particles	[62]	Stem cell membranes	gelatin nanogels	[75]
Bacterial membranes (*E. coli*)	Au-NPs	[76,77]		superparamagnetic iron oxide nano-particles (SPIO NPs)	[78]

Jang HS. The Diverse Range of Possible Cell Membrane Interactions with Substrates: Drug Delivery, Interfaces and Mobility. Molecules. 2017, 11;22(12). pii: E2197. doi: 10.3390/molecules22122197. Review.

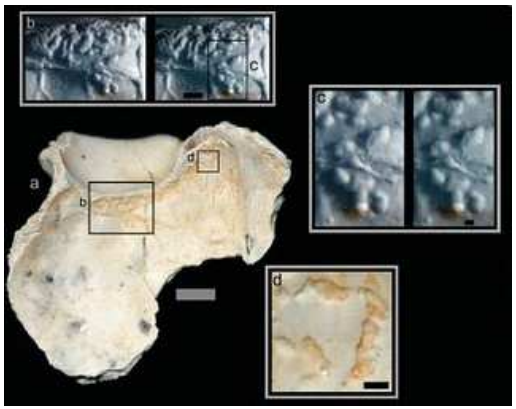
Homo sapiens versus Mycobacterium tuberculosis, milestones

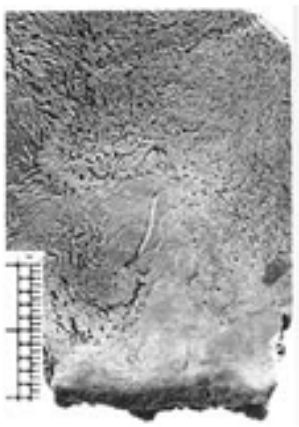
Mycobacterium genus

over one hundred species, slow and rapid growers (SGM, RGM)

pathogen species, slow growers

- Hominidae (*H. erectus*, ca. 500 000 BC, fossils)
- first evidence of the disease (ca. 5000 BC, Neolithic period)
- extrapulmonary tuberculous spondylitis – Pott's disease (ca. 3000-2400, mummies)





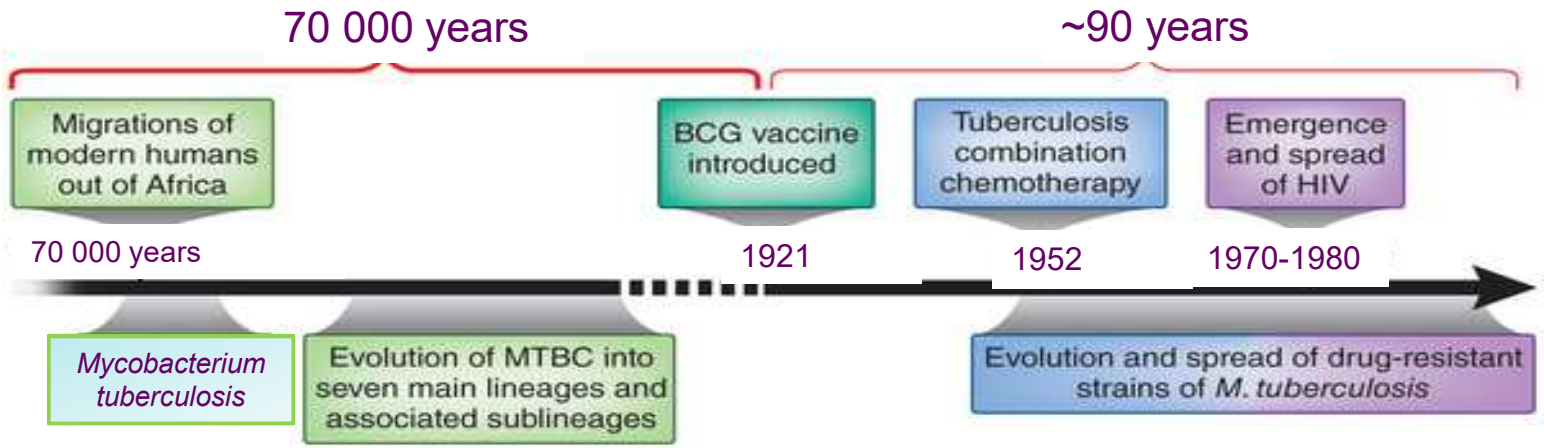
Wirth T, Hildebrand F, Allix-Béguec C, Wölbeling F, Kubica T, Kremer K, van Soolingen D, Rüsch-Gerdes S, Locht C, Brisse S, Meyer A, Supply P, Niemann S.Origin, spread and demography of the Mycobacterium tuberculosis complex, PLoS Pathog. 2008, 19;4(9):e1000160.

Zink AR, Sola C, Reischl U, Grabner W, Rastogi N, Wolf H, Nerlich AG. Characterization of Mycobacterium tuberculosis complex DNAs from Egyptian mummies by spoligotyping. J Clin Microbiol. 2003, 41(1):359-67.

Ziskind B, Halioua B. Tuberculosis in ancient Egypt, Rev Mal Respir. 2007, 24(10):1277-83.

Kappelman J, Alcicek MC, Kazanci N, Schultz M,Ozkul M, etal. (2008) First Homo erectus from Turkey and implications for migrations into temperate Eurasia. Am J Phys Anthropol 135:110.

Homo sapiens versus Mycobacteriaceae: evolution

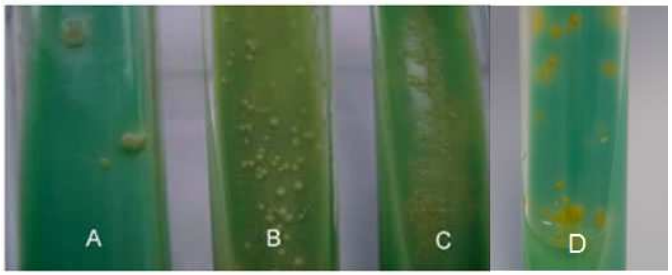


M. tuberculosis complex

M. bovis
M. microti
M. canetti
M. africanum

Atypical *Mycobacterium*
„Runyon categories"

Photochromogens	Scotochoromogens	Non-chormogens	Rapid growers
M. kansasii	*M. xenopi*	*M. avium*	*M. abscessus*
M. marinum	*M. scrofulaceum*	*M. intracellulare*	*M. chelonae*
			M. fortuitum



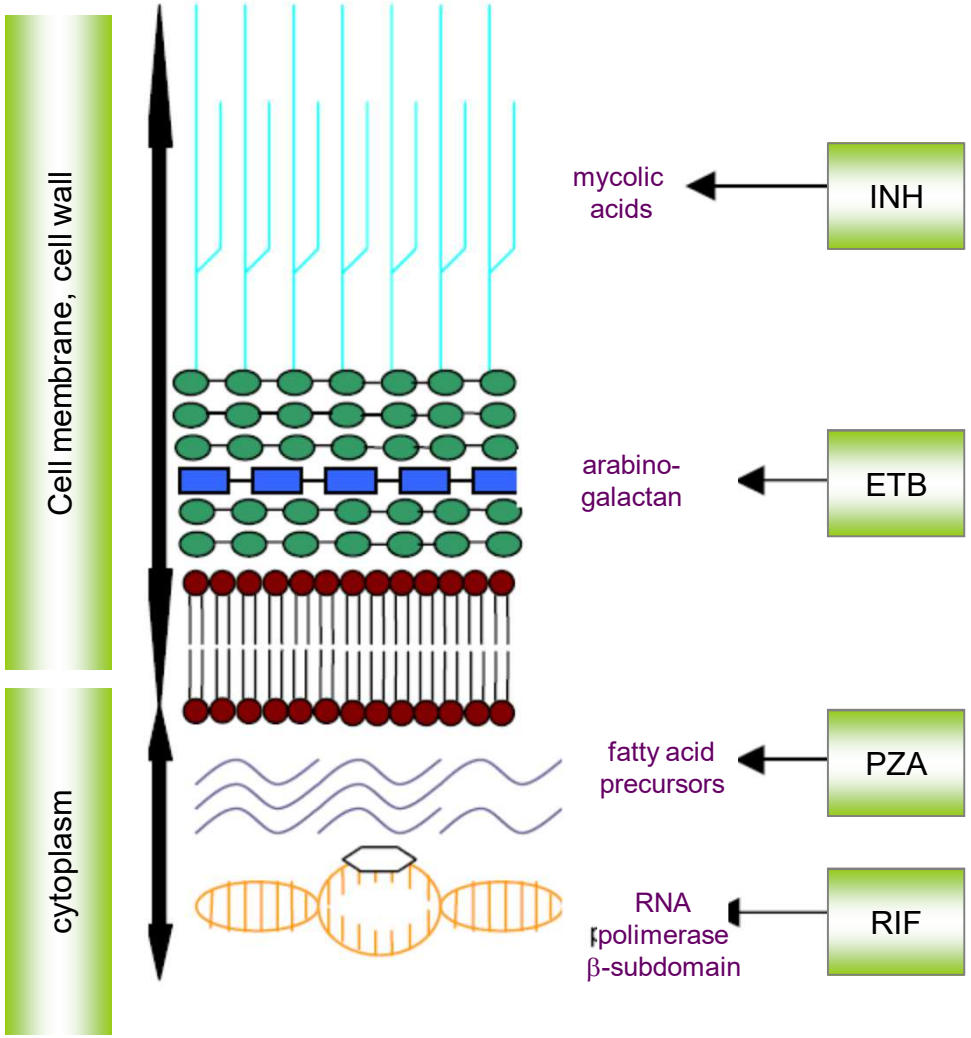
A	*M. tuberculosis*
B	*M. bovis*
C	*M. avium*
D	*M. kansasii*

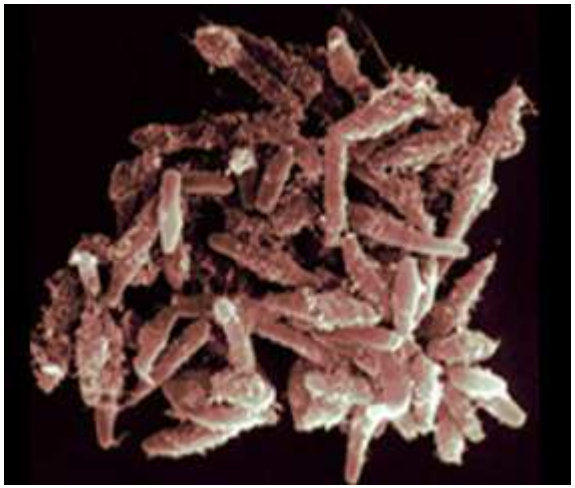
D. F. Warner; V. Mizrahi, Nature Genetics (2013) 45, 1107–1108.
Runyon EH (1959). "Anonymous mycobacteria in pulmonary disease". The Medical clinics of North America. 43 (1): 273–90. PMID 13612432

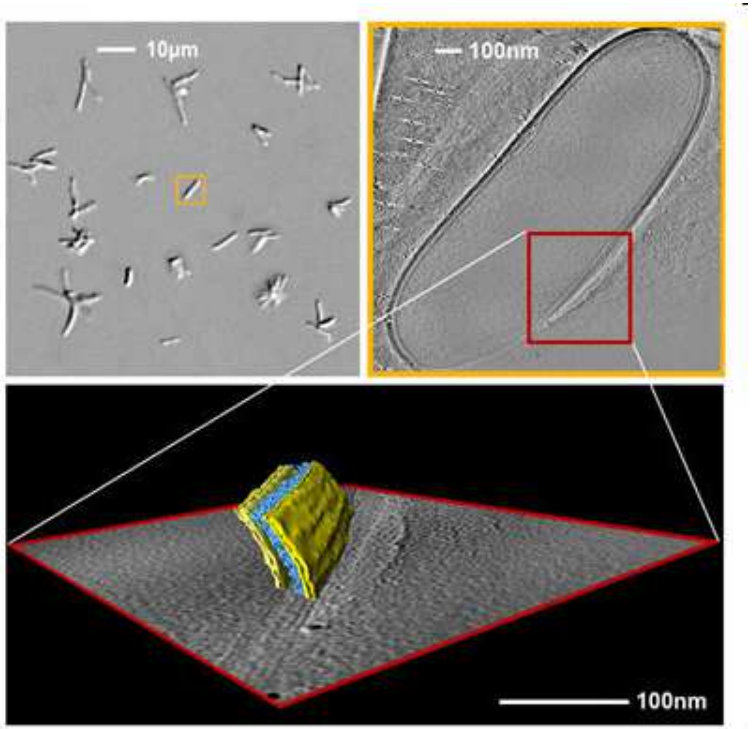
Mycobacterium tuberculosis „survival kit”

250 enzyme,
E. coli

„metabolome”
50 enzyme

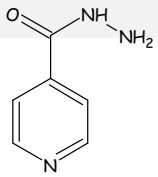






du Toit LC, Pillay V, Danckwerts MP. Tuberculosis chemotherapy: current drug delivery approaches. Respir Res. 2006; 7, 118. Review.

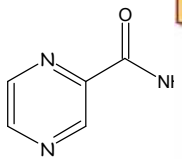
Antitubercular drugs



INH (isoniazid, isonicotinic acid hydrazide, 1912, 1952)

- frontline drug, min. 6 months therapy
- bactericide
- prodrug (KatG (catalase/peroxidase): activation,
  NAT: inactivation)
- inhibits the formation of myciloic acid cell wall

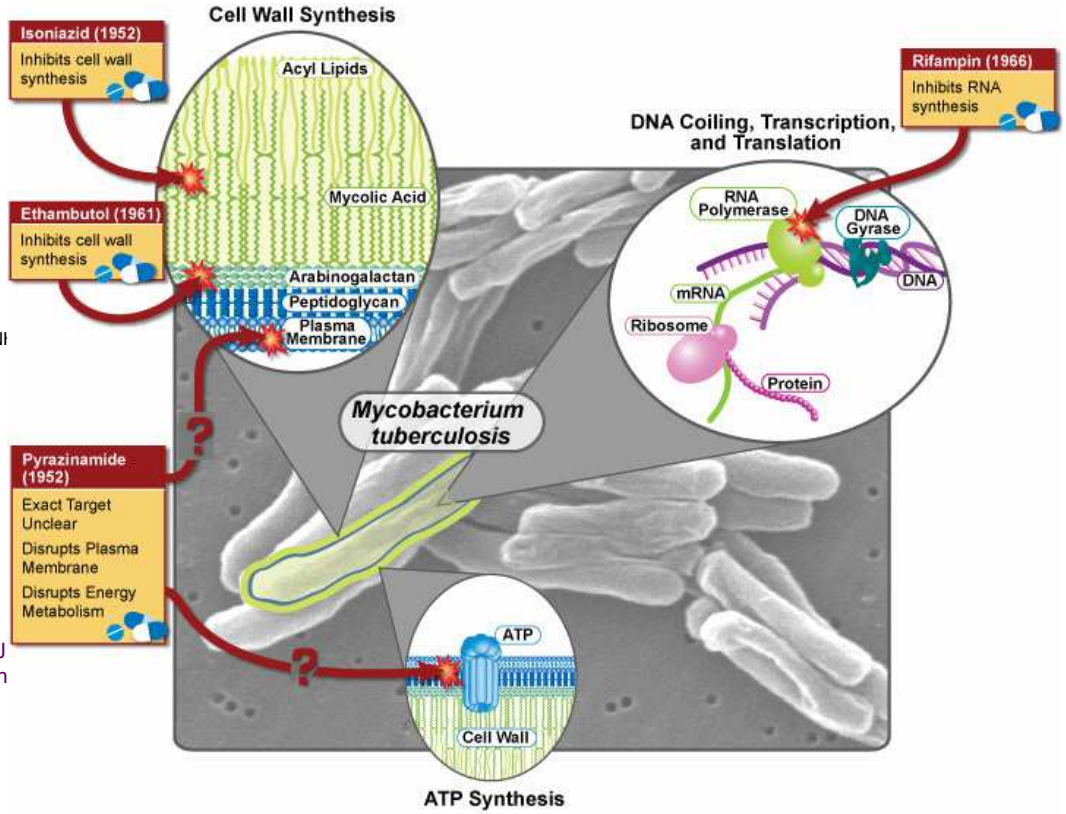
Bernstein, J., W. A. Lott, B. A. Steinberg, and H. L. Yale. 1952. Chemotherapy of experimental tuberculosis. V. Isonicotinic acid hydrazide (nydrazid) and related compounds. Amer. Rev. Tuberc. 65:357-364.



PZA (pyrazinamide, 1936, 1952)

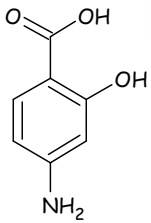
- frontline drug
- bactericide
- prodrug (bacterial nicotinamidase/pyrazinamidase
  -> pyrazinoic acid)
- active at acidic pH, effective against dormant bacteria

Kushner, S., H. Dalalian, J. L. Sanjurjo, F. L. Bach, Jr., S. R. Safir, V. K. Smith, J and J. H. Williams. 1952. Experimental chemotherapy of tuberculosis. II. The syn of pyrazinamides and related compounds. J. Am. Chem. Soc. 74:3617–3621.



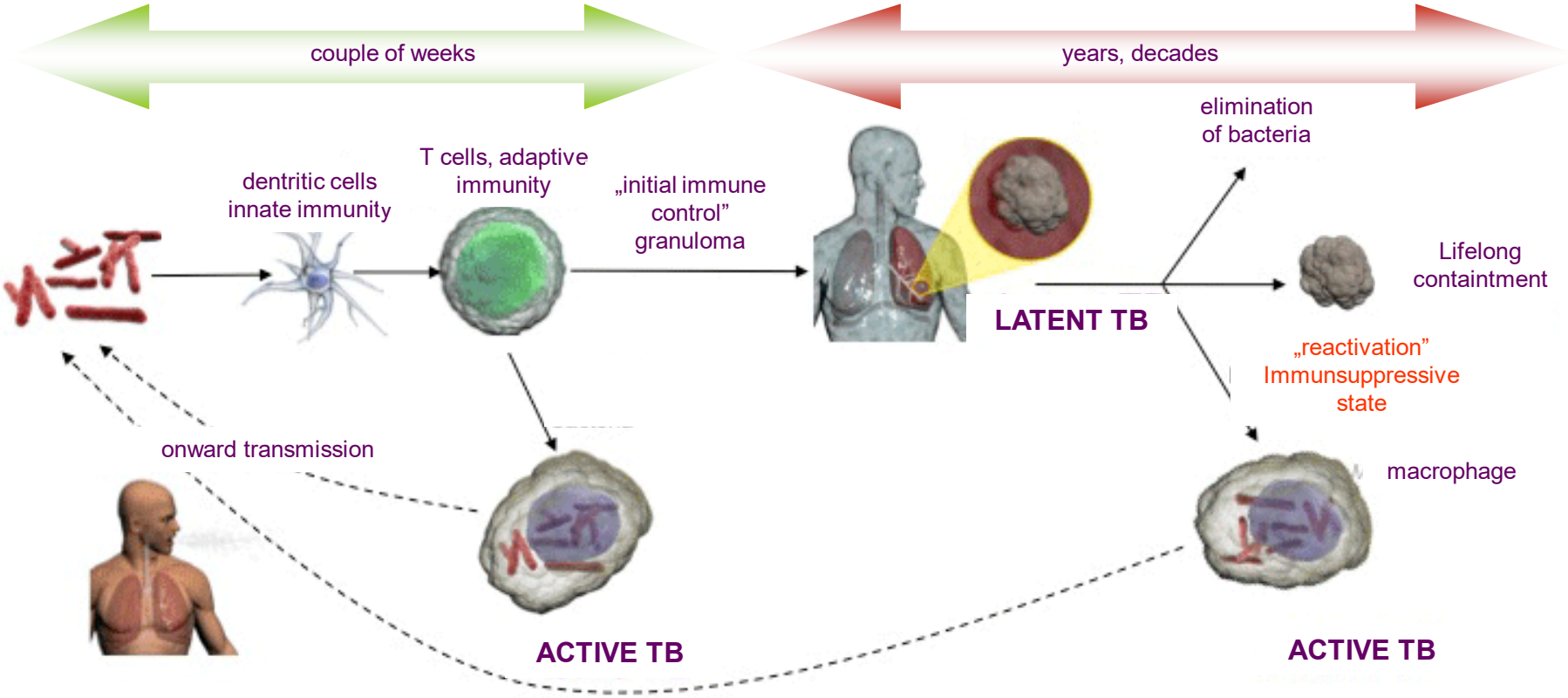
PAS (para-aminosalicylic acid, 1946)

- second-line antibiotic, MDR-TB
- bacteriostatic
- 150-200mg / kg / day (!)
- hepatotoxicity, cardiotoxcity



Lehmann, J. 1946 Chemotherapy of tuberculosis. The bacteriostatic action of paminosalicylic acid (PAS) and closely related compounds upon the tubercle bacillus, together with animal experiments and clinical trials with PAS. Svensk. Läkartidn.,43, 2029-2041.

Natural history of *Mycobacterium tuberculosis* infection



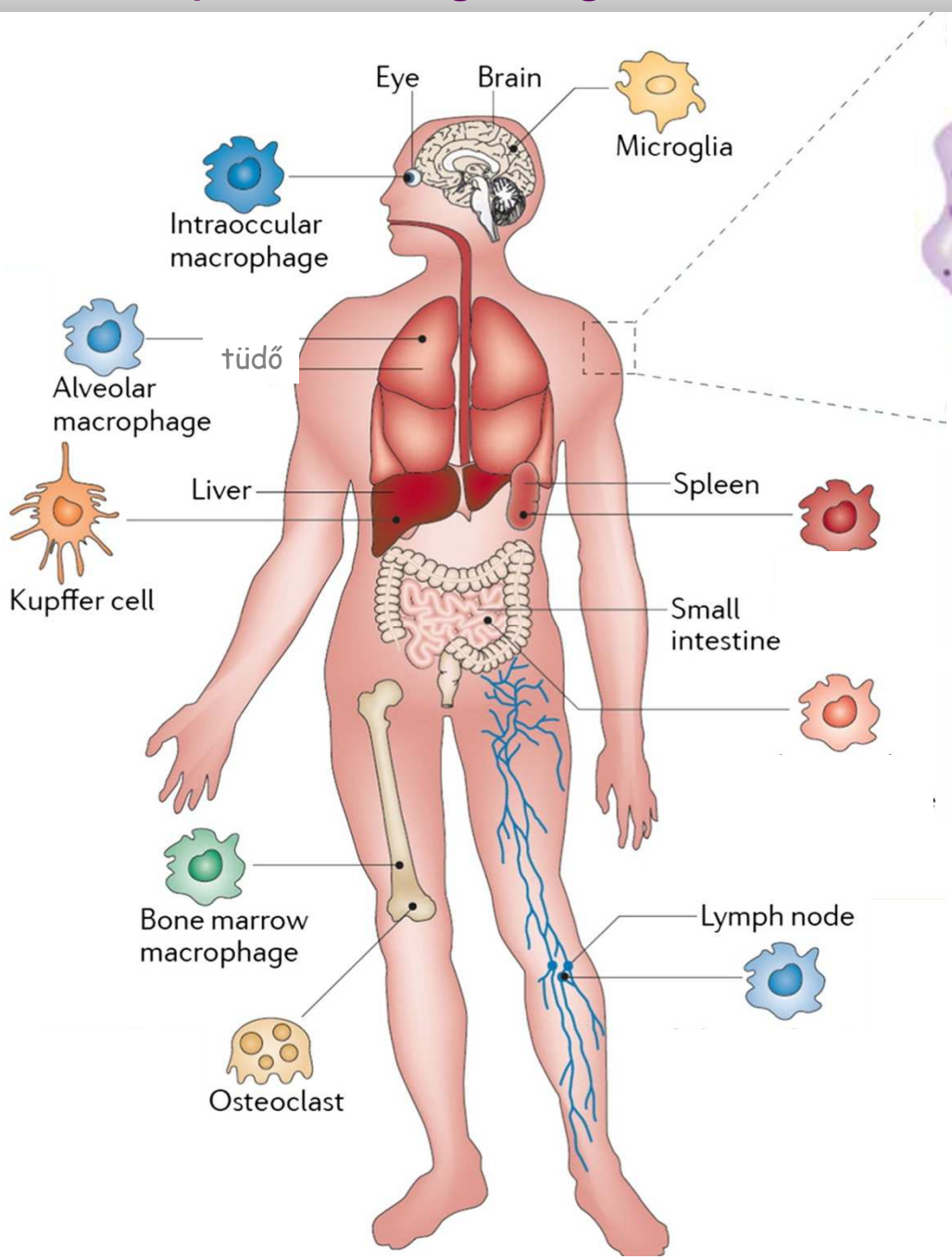
aerob, intracellular pathogen

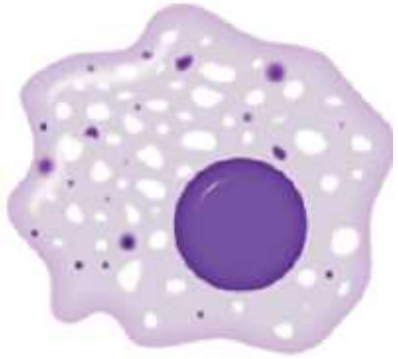
host cells: mainly alveolar macrophages

reactivation: in autoimmune and tumor patients,
HIV and hepatitis infected individuals etc.

Handbook of Tuberculosis: Clinics, Diagnostics, Therapy, and Epidemiology,
Ed.,S. H. E. Kaufmann, 2008, Wiley, ISBN: 978-3-527-31888-9
Pusztai, R., Mycobacteriaceae p. 175-182. Orvosi mikrobiológia, Szerk. Gergely Lajos. 2003, Budapest
http://www.oxfordimmunotec.com/T-SPOT.TB_Overview_North_America
David G. Russell, Mycobacterium tuberculosis: here today, and here tomorrow, Nature Reviews Molecular Cell Biology 2, 569-586, 2001

Host cell specific targeting





- mannosyl/fucosyl receptor
- galactosyl receptor
- scavenger receptor
- lectin receptor
- integrin receptor
- Fc receptor
- MHC
- **tuftsin receptor/NP-1**

Taylor, P. R. et al, *Annu. Rev. Immunol.* (2005) 23: 901–44
Becker, M.et al, *Eur J Immunol.* (2006) 36: 950-60
Basu, Biochem. Pharmacol., (1995) 40:1941-1946
H. Soyez et al. 1996, Adv. Drug Delivery Rew. 21: 81-86

Aims and strategies

new antitubercular compounds

chemical modification of existing compounds

***in silico* identified drug candidates**

in vitro antimycobacterial activity

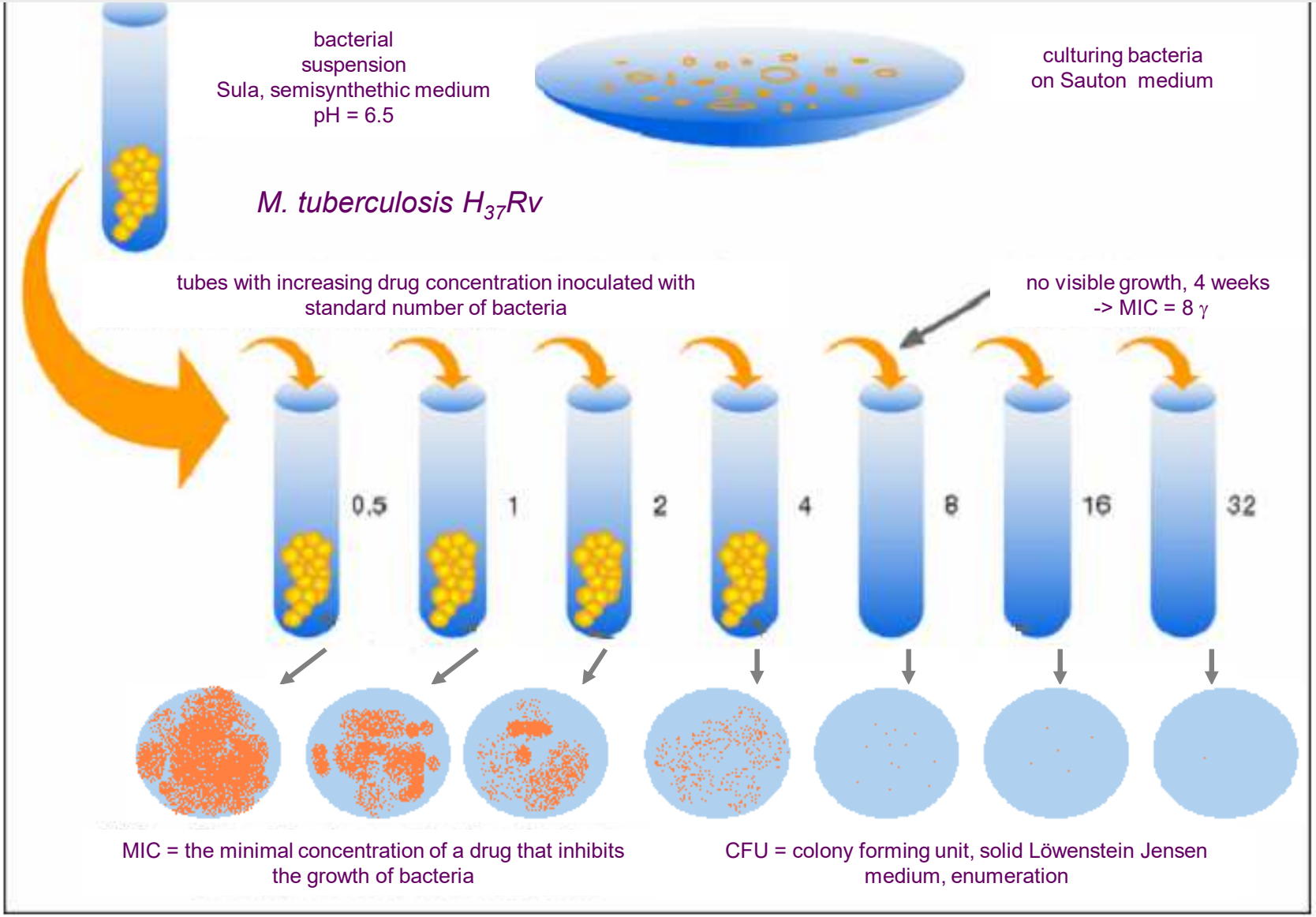
structure – activity relationship

chemical tailoring, optimization

host cell specificity, enhancing cellular uptake and bioavailability

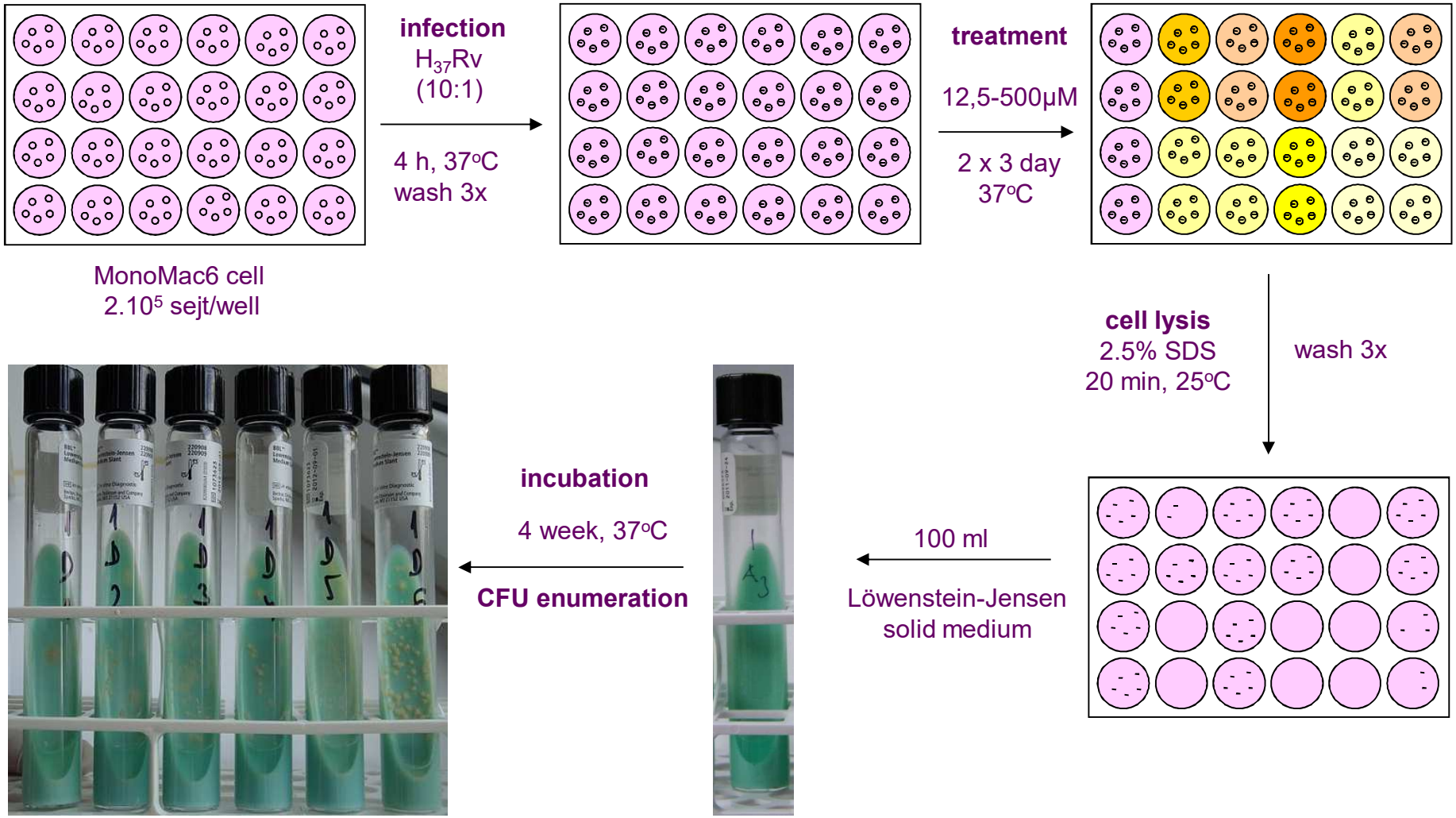
peptide carriers (with or without recognition unit)
RES specific nanoparticles

Determination of Minimal Inhibitory concentration (MIC) and Colony Forming Unit (CFU), *in vitro* extracellular model



Jensen, K. A. Zentralb. Bakteriol Parasitenkd infektionskr hyg Agt I Orig, 1932. 125: p. 222.
Löwenstein, E. Zentralb. Bakteriol Parasitenkd infektionskr hyg Abt I orig, 1931. 120: p. 127.
Sula, L. Bull World Health Organ, 1963. 29(5): p. 607-625.
Sula, L. Bull World Health Organ, 1963. 29(5): p. 589-606.

Evaluation on intracellular bacteria, *M. tuberculosis* infected MonoMac-6 cells



Horváti K, Bacsa B, Szabó N, Dávid S, Mező G, Grolmusz V, Vértessy B, Hudecz F, Bősze S. Enhanced cellular uptake of a new, in silico identified antitubercular candidate by peptide conjugation. Bioconjug Chem. 2012 May 16;23(5):900-7.

In silico method for identification new drug candidates

New docking algorithms, FRIGATE

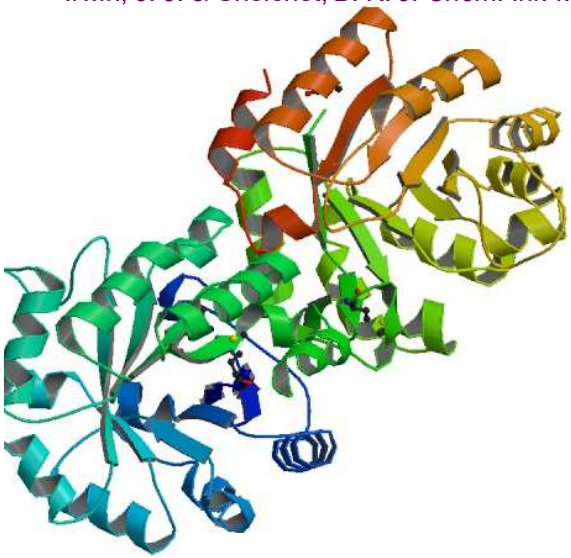
Zinc database
(Zinc – http://zinc.docking.org/)

C. Scheich et al. Discovery of novel MDR-*Mycobacterium tuberculosis* inhibitor by new FRIGATE computational screen. PLoS ONE **2011** 6(12): e28428.

Irwin, J. J. & Shoichet, B. K. *J. Chem. Inf. Model.* **2005.** 45:177-82

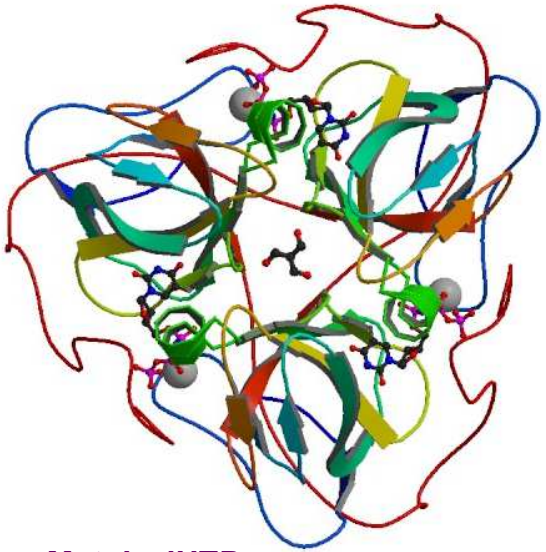
3D structure, RS-PDB
(crucial for the maintance of integrity and survival)

Szabadka, Z., Grolmusz, V., *Conf Proc IEEE Eng Med Biol Soc*. **2006**. 1:5755-8



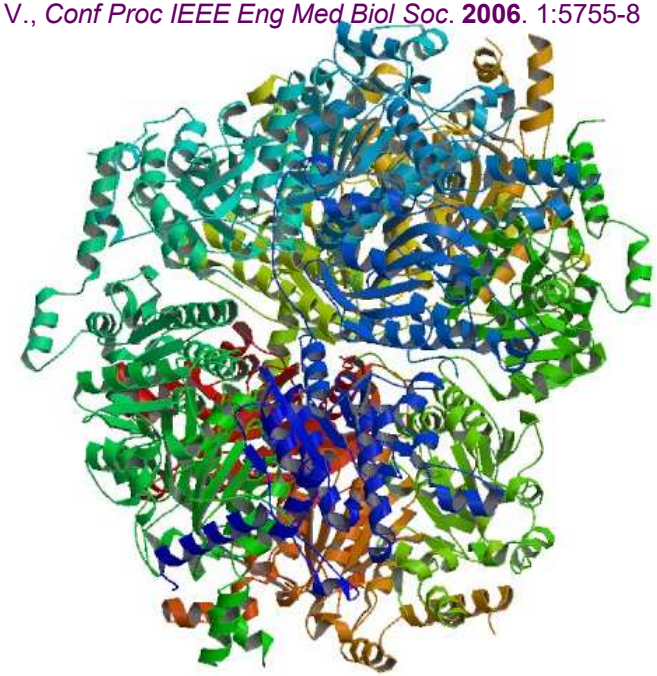
PriA (hisA)
(involved in histidine and triptophan biosynthesis)
EC 5.3.1.16; Rv1603;
PDB code: 1qo2

Lang, D. et al. *Science* **2000.** 289: 1546.



***M. tub*. dUTPase**
(involved in thymidylate biosynthesis and preventive DNA repair mechanism)
EC 3.6.1.23; Rv2697c;
PDB code: 2py4

Varga, B. et al. *Biochem Bioph Res Co* **2009.** 373: 8.



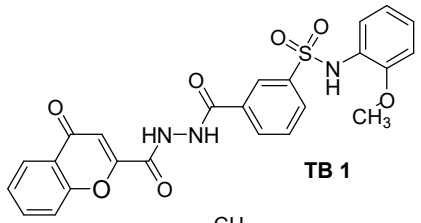
AccD5 (2bzr)
(key enzyme in the catabolic pathway of odd-chain fatty acids, isoleucine, threonine, methionine, and valine) EC 6.4.1.3; Rv3280;
PDB code: 2bzr

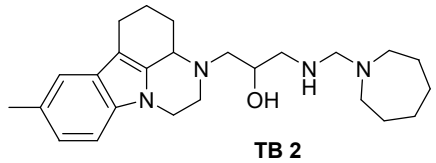
Holton, S.J. et al. *FEBS Lett* **2006**. 580: 6898.

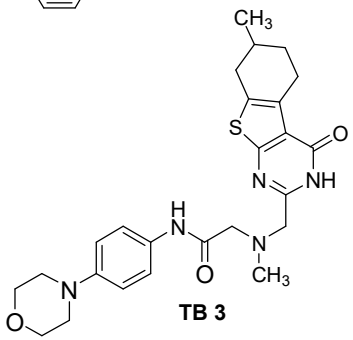
First hits on dUTPase

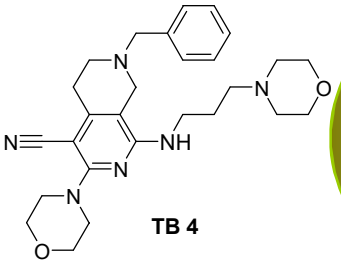
compound	MIC [a] (μg/mL)	MIC (μM)
control	no inhibition	
isoniazid (INH)	0.16	1.2
norfloxacin	5	15
TB1	25	51
TB2	5	12
TB3	15	31
TB4	30	63
TB5	20	46
TB6	45	100
TB7	25	53
TB8	1	3.7
TB9	45	93
TB10	45	110

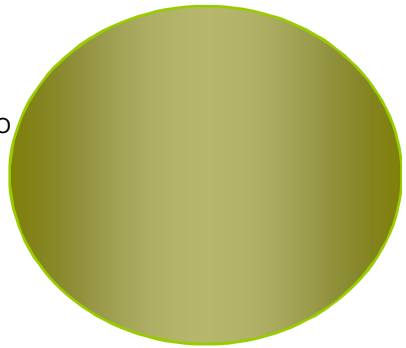
[a] MIC (Minimal Inhibitory Concentration) was determined on *M. tuberculosis* $H_{37}Rv$ strain in Sula semisynthetic media, pH 6.5 (4 weeks)
To confirm the sterility CFU (Colony Forming Unit) was determined on Löwenstein-Jensen solid media (4 weeks)

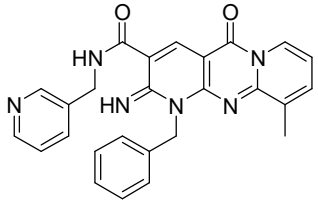

TB 1

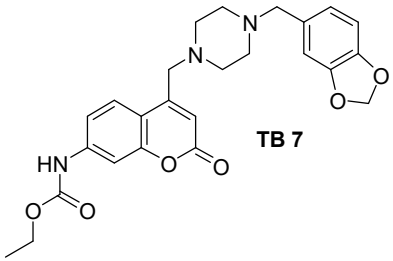

TB 2

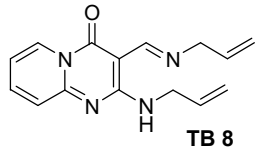

TB 3

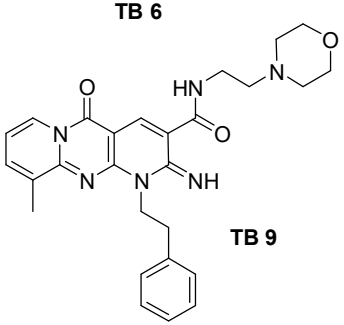

TB 4

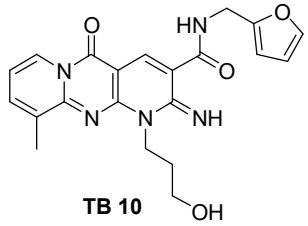



TB 6


TB 7

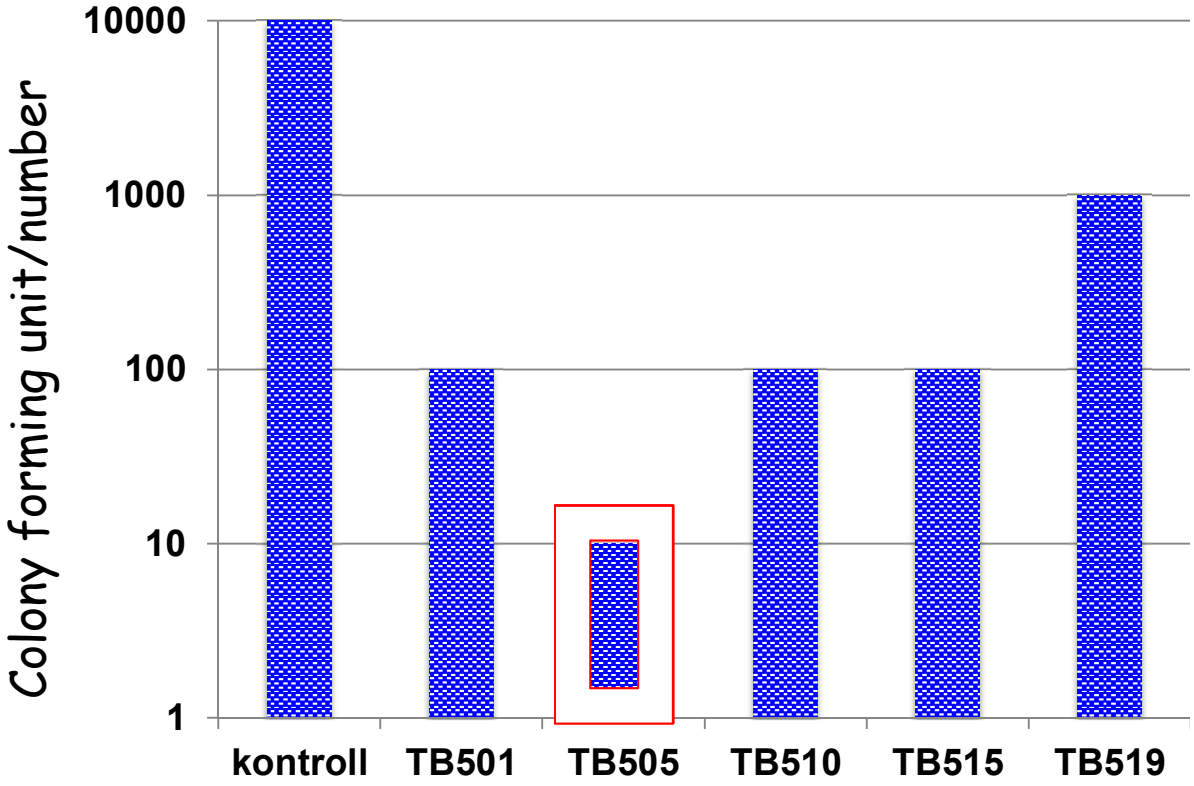

TB 8


TB 9


TB 10

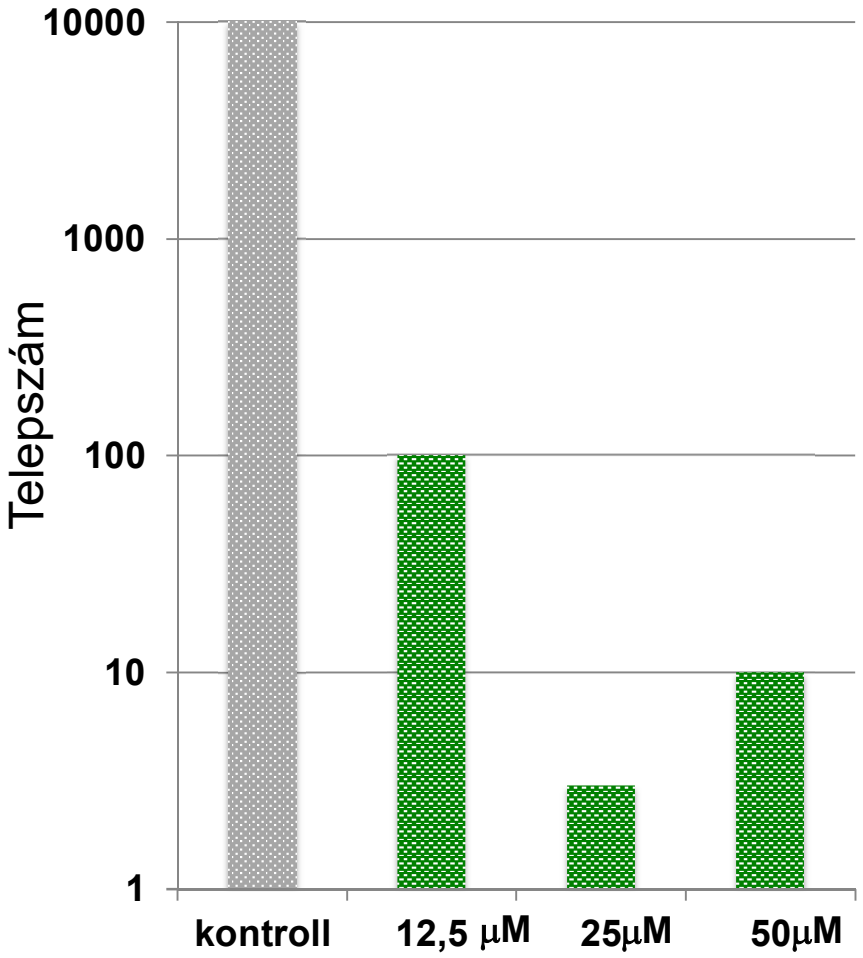
Horváti K, Bacsa B, Szabó N, Dávid S, Mező G, Grolmusz V, Vértessy B, Hudecz F, Bősze S. Enhanced cellular uptake of a new, in silico identified antitubercular candidate by peptide conjugation. Bioconjug Chem. 2012 May 16;23(5):900-7.

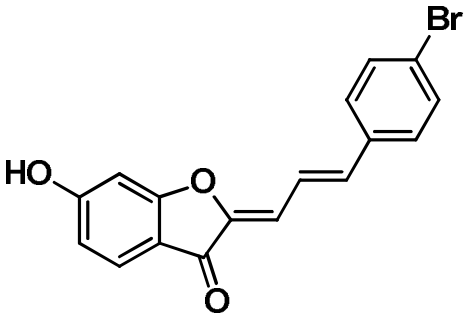
Antimycobacterial activity on *M. tuberculosis* H_{37}Rv infected MonoMac6 cell, inhibition of intracellular bacteria, 2 treatments

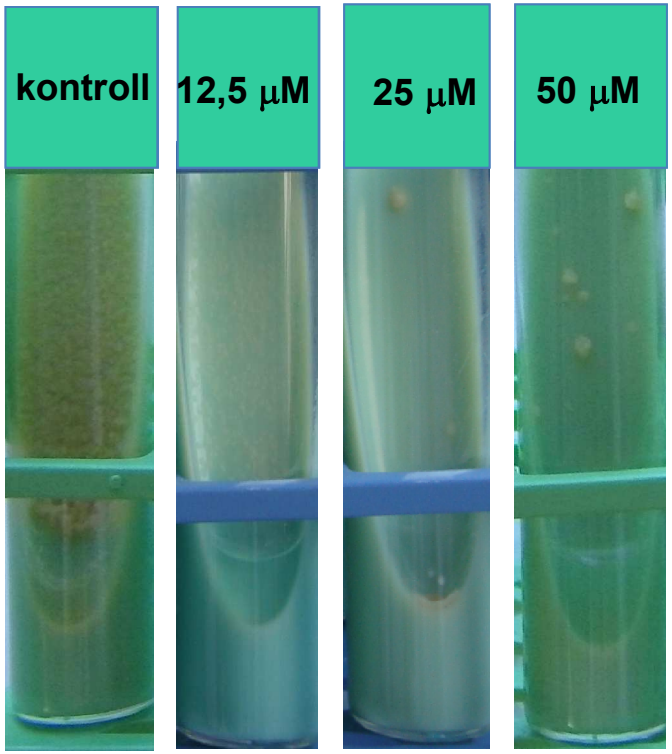


treatment : 50 μM

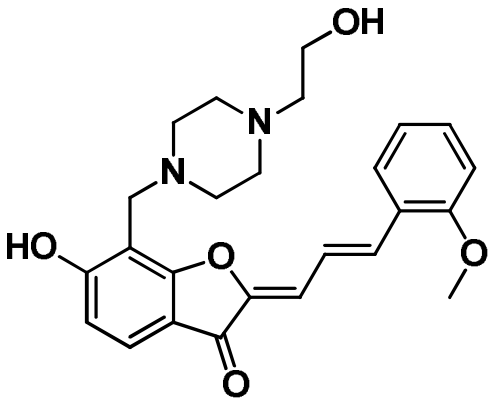
Antimycobacterial activity on *M. tuberculosis* $H_{37}Rv$ infected MonoMac6 cell, inhibition of intracellular bacteria, 2 treatments, TB505 compound





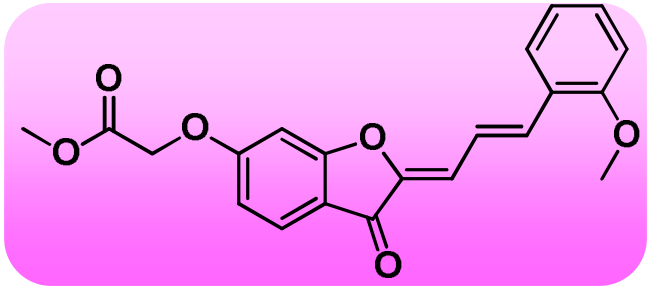


TB 5 family, *in vitro* selectivity



TB 501

MIC 20γ (48μM)*
IC_{50} 46μM**



TB 514

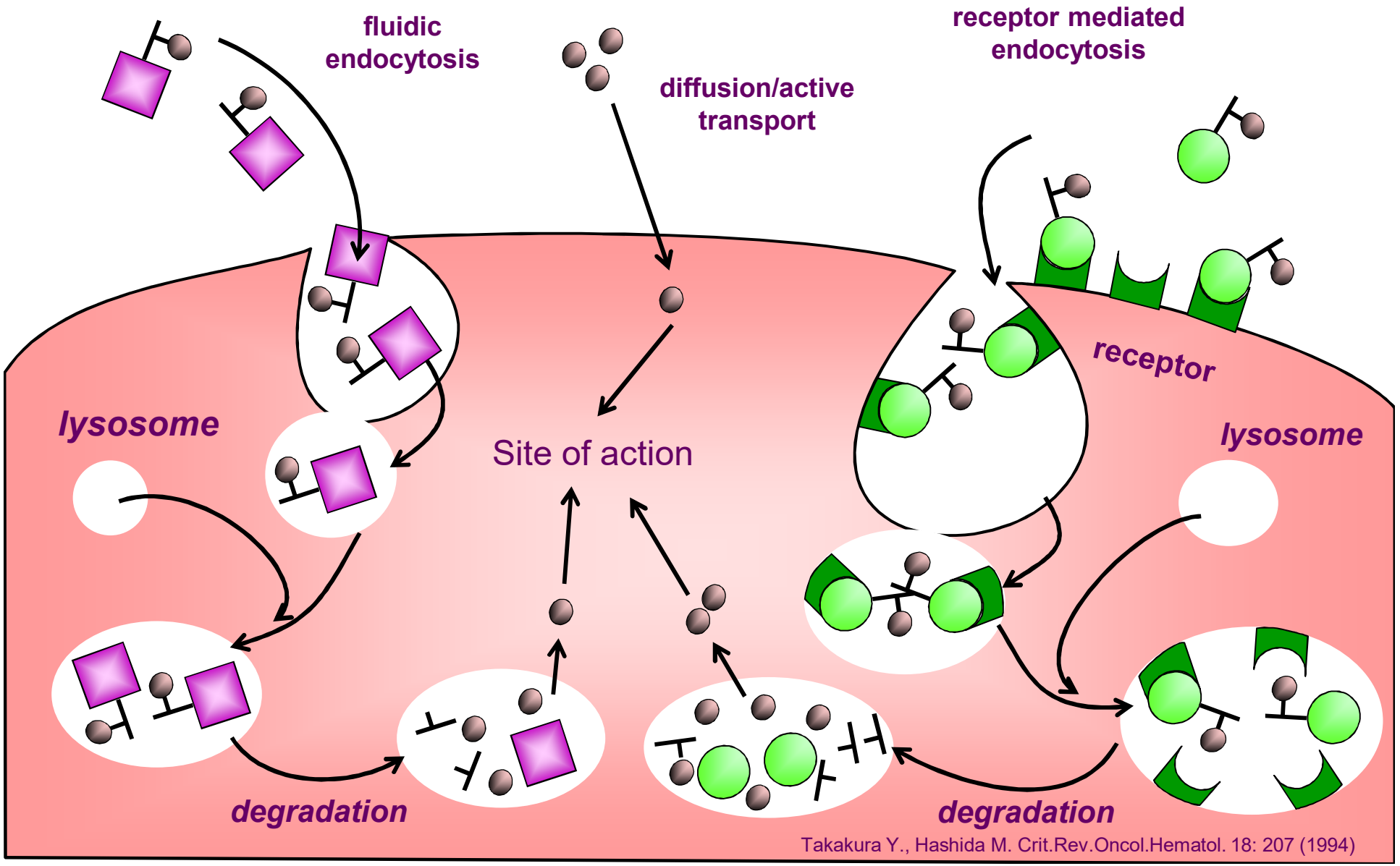
MIC 5γ (14μM)*
MDR A8 MIC <0,5γ (1,4μM)
M.kansasii MIC 4γ (11μM)

IC_{50} 209μM**
IC_{50} >250μM
(PBMC toxicitás)

***In vitro* intracellular inhibition - 25 μM**

* H_{37}RV *M. tuberculosis* strain, ** cytostatic activity, HepG2 cells

Internalisation pathways of bioactive molecules



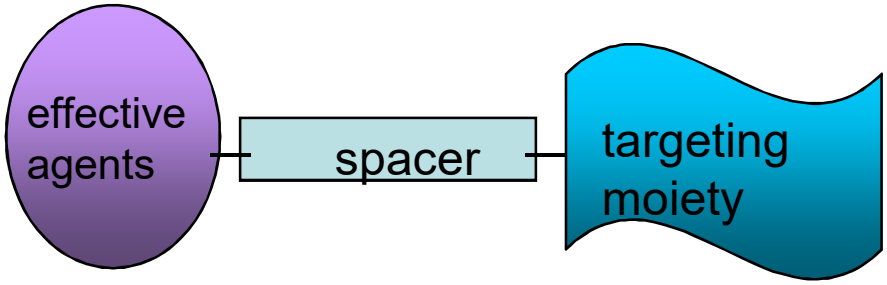
Takakura Y., Hashida M. Crit.Rev.Oncol.Hematol. 18: 207 (1994)

Peptide based drug targeting/delivery

Recognition unit

YES

NO



Peptide
- VEGF/NRP-1 (tufsin)

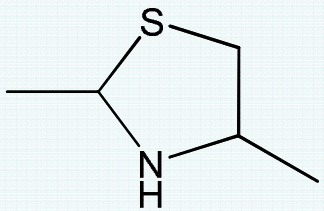
Peptide
CPP, antimicrobial

-Polyamino acids
-Branched chain polypeptides

Chemical linkage

amide	oxime	tioether	disulfide
-NH-CO-	-CH=N-O-	$-CH_2-S-CH_2-$	$-CH_2-S-S-CH_2-$

thiazolidine



hydrazone

-NH-N=CH-

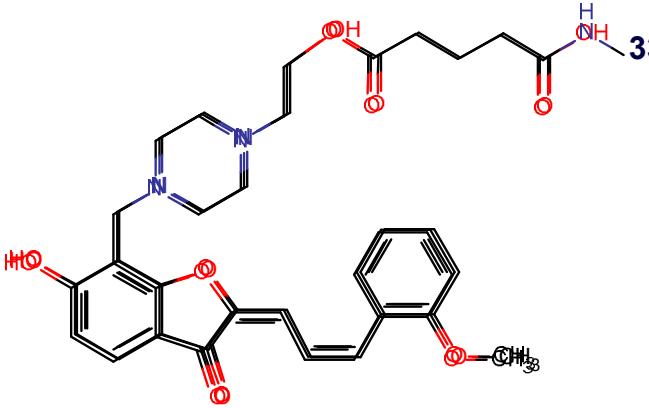
hydrazine

$-NH-NH-CH_2-$

- mannosyl-fucosyl receptors
- galactosyl receptors
- scavenger receptors
- **tuftsin receptor/NP-1**

Taylor, P. R. et al, *Annu. Rev. Immunol.* (2005) 23: 901–44
Becker, M.et al, *Eur J Immunol.* (2006) 36: 950-60
Basu, Biochem. Pharmacol., (1995) 40:1941-1946
H. Soyez et al. 1996, Adv. Drug Delivery Rew. 21: 81-86

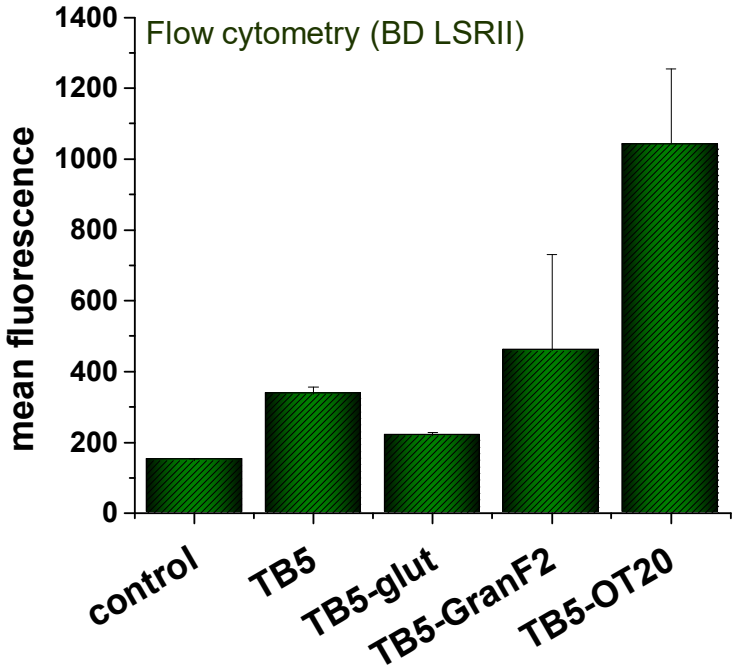
Peptide carrier to target intracellular bacteria

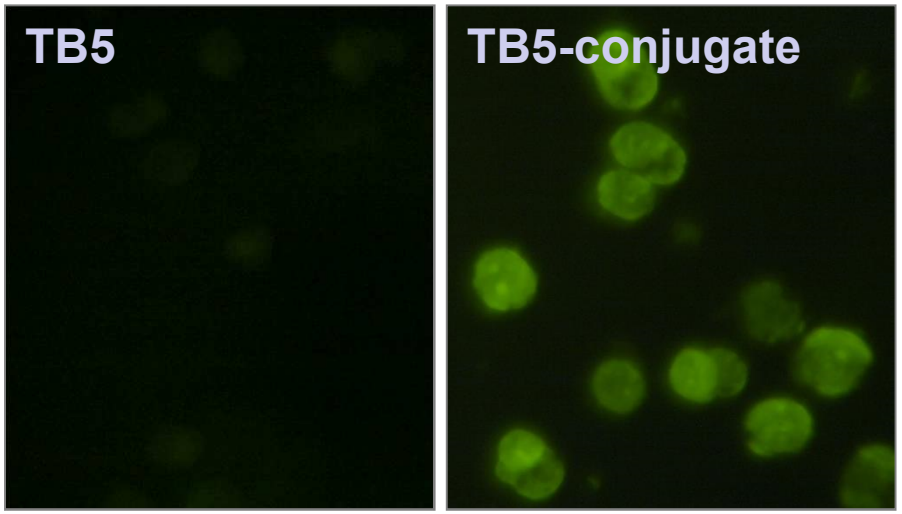


TB5-glut

^{33}VCRTGRSRWRDVCRNFMRRYQSR^{55} (GranF2)

TKPKGTKPKGTKPKGTKPKG (tuftsin tetramer, OT20)

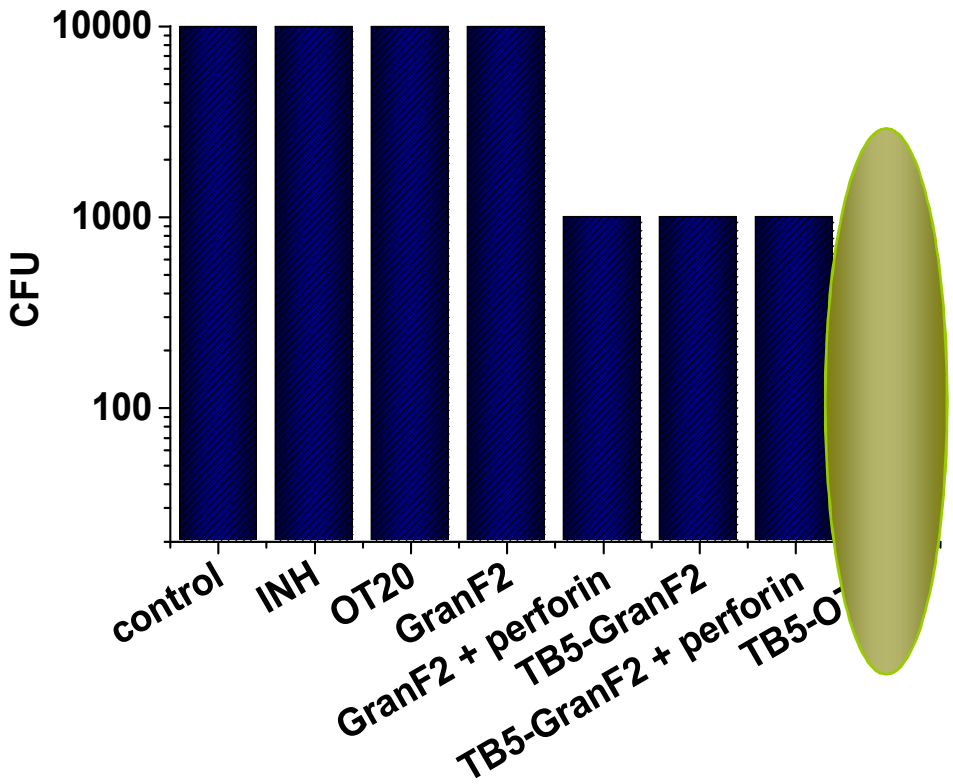


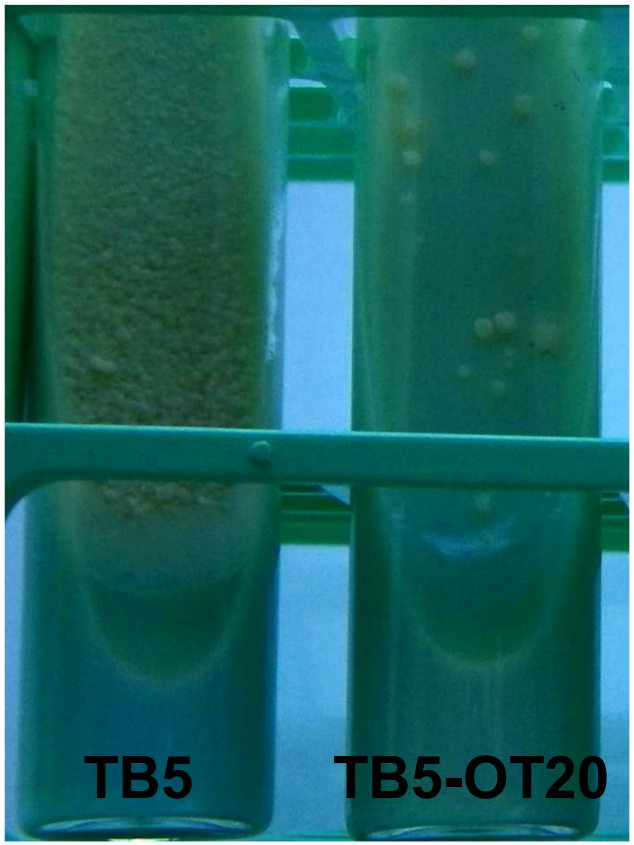


Cellular uptake of TB5 and TB5-conjugates by human monocytes, host cell model (MM6)

Horváti K, Bacsa B, Szabó N, Dávid S, Mező G, Grolmusz V, Vértessy B, Hudecz F, Bősze S. Enhanced cellular uptake of a new, in silico identified antitubercular candidate by peptide conjugation. Bioconjug Chem. 2012 May 16;23(5):900-7.

Inhibition of intracellular bacteria by peptide conjugates

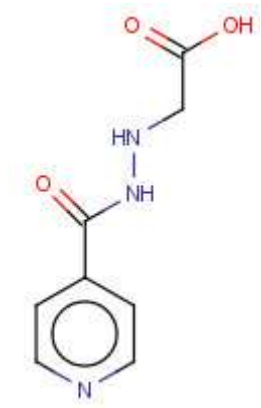




MM6 cells were infected with *Mtb* H_{37}Rv and treated with compounds at 50 mM final concentration. As control, untreated cells were used. In the case of GranF2 peptide, perforin peptide was added at 100 mM concentration. After SDS lysis, the colony forming units (CFU) of *Mtb* was enumerated on LJ solid media.

Horváti K, Bacsa B, Szabó N, Dávid S, Mező G, Grolmusz V, Vértessy B, Hudecz F, Bősze S. Enhanced cellular uptake of a new, in silico identified antitubercular candidate by peptide conjugation. Bioconjug Chem. 2012 May 16;23(5):900-7.

Synthesis of the lipotuftsin conjugates



izonicotinoylhydrazinoacetic acid

pal-TK(Fmoc)PK(Fmoc)G-

2% DBU/2 % piperidin/DMF

pal-TKPKG-

6 ekv. INH-derivatives/
DIC/HOBt/DMF 1 h

pal-TK(*INH*)PK(*INH*)G-

HF/p-crezol 1,5 h

pal-TK(*INH*)PK(*INH*)G

$Boc\text{-}NH_2\text{-}O\text{-}CH_2\text{-}COOH$
Boc-Aoa

6 ekv. Boc-Aoa/DIC/HOBt/DMF, 1 h
33% TFA/DCM (2+20 min)
10% DIEA/DCM (4x1 min)

pal-TK(*Aoa*)PK(*Aoa*)G-

HF/p-crezol 1.5 h

pal-TK(*Aoa*)PK(*Aoa*)G

1,9 ekv. TB820
0,2 M NaOAc pH=5.03, 15 h

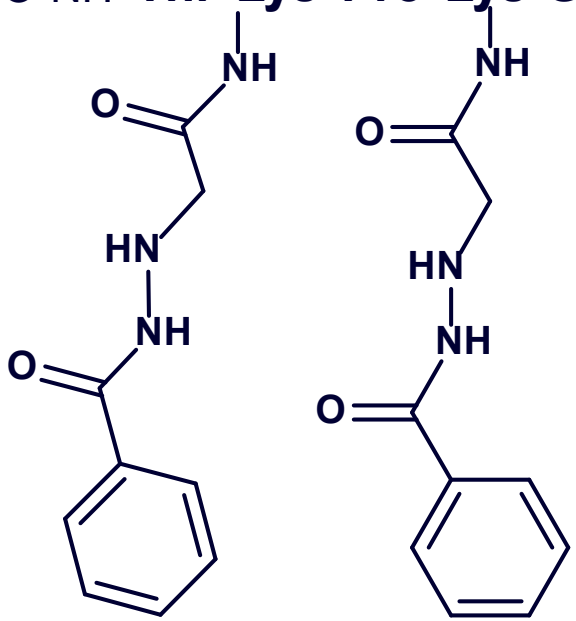
pal-TK(*TB820*)PK(*TB820*)G

= MBHA resin
pal = palmytic acid $(CH_3\text{-}(CH_2)_{14}\text{-}COOH)$

Horváti K, Mezo G, Szabó N, Hudecz F, Bosze S. Peptide conjugates of therapeutically used antitubercular isoniazid-design, synthesis and antimycobacterial effect. J Pept Sci. 2009 15(5):385-91.
Horváti K, Bacsa B, Kiss E, Gyulai G, Fodor K, Balka G, Rusvai M, Szabó E, Hudecz F, Bősze S.Nanoparticle encapsulated lipopeptide conjugate of antitubercular drug isoniazid: in vitro intracellular activity and in vivo efficacy in a Guinea pig model of tuberculosis. Bioconjug Chem. 2014 25 (12):2260-8.

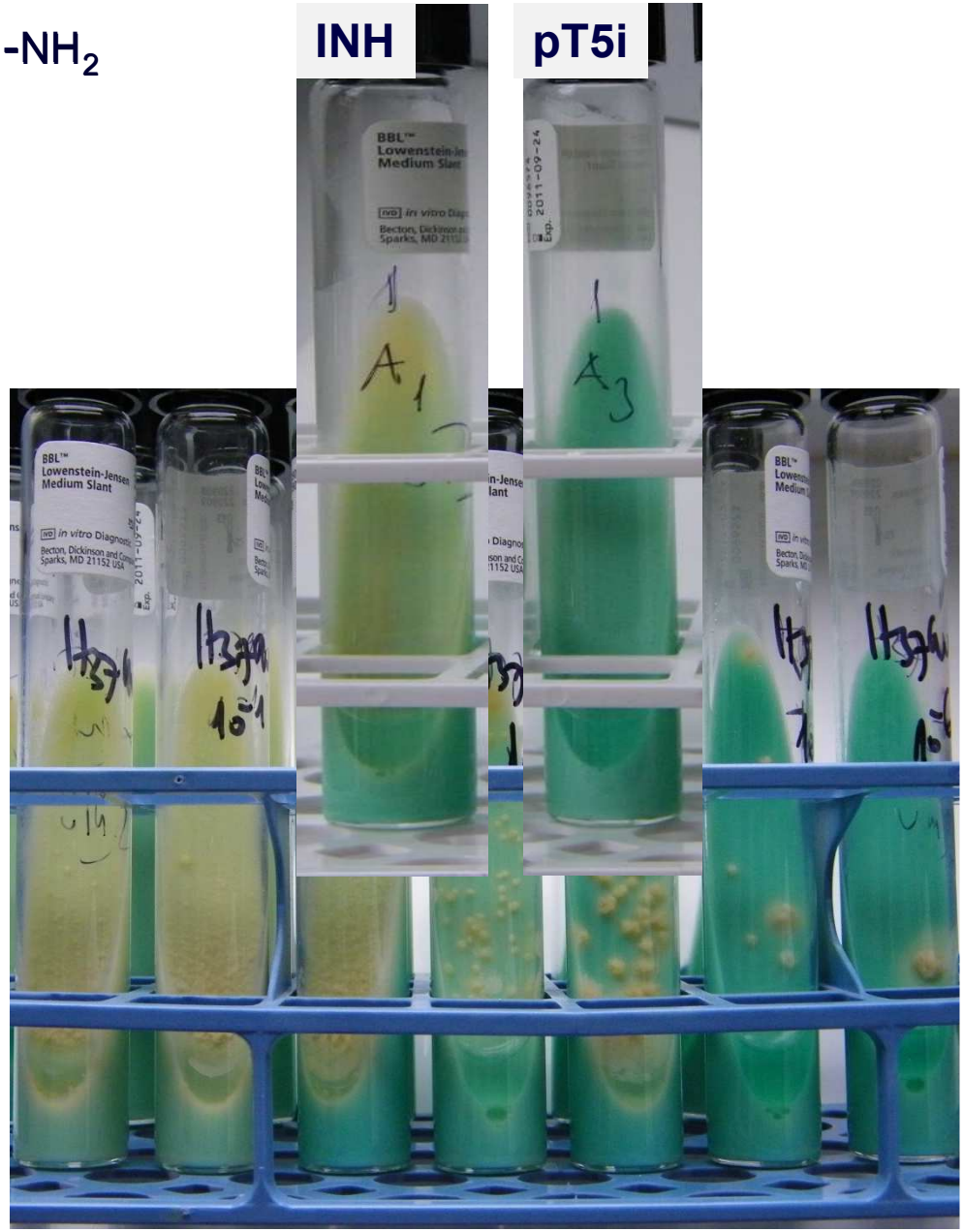
Inhibition of intracellular bacteria by INH-lipopeptide conjugate

CH_3-$(CH_2)_{14}$-CO-NH-**Thr-Lys-Pro-Lys-Gly**-NH_2



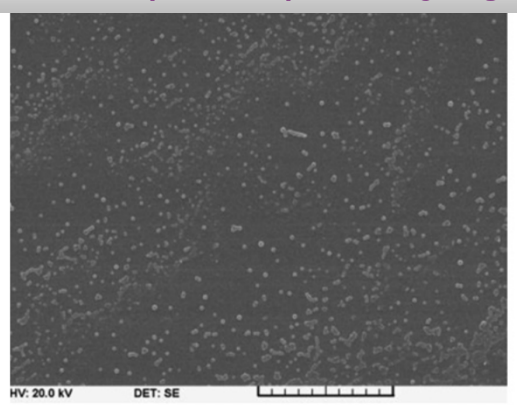
	CFU
control	+++
INH	+++
pT5i	0

100µg/ml INH or pT5i conjugate
+++ : confluent colonies; ++ : innumerable colonies, but not confluent; + : 50-100 colonies; 0 : no colonies were observed

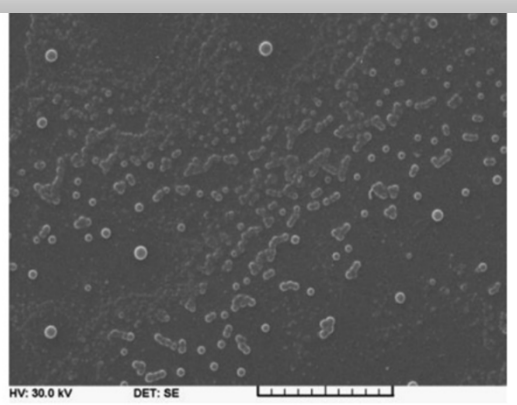


Horváti K, Mezo G, Szabó N, Hudecz F, Bosze S. J Pept Sci. 2009 15(5):385-91.
Horváti K, Bacsa B, Kiss E, Gyulai G, Fodor K, Balka G, Rusvai M, Szabó E, Hudecz F, Bősze, Bioconjug Chem. 2014 25 (12):2260-8.

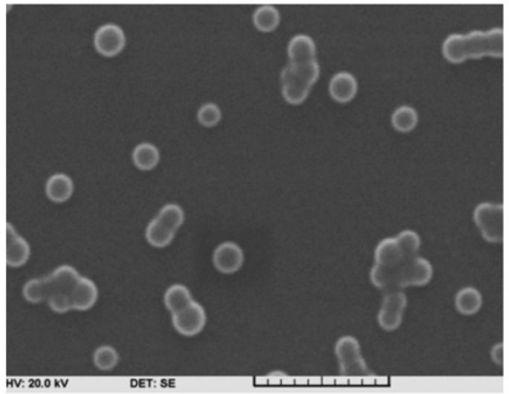
INH and lipotuftsin (pT5i) conjugate loaded PLGA nanoparticles

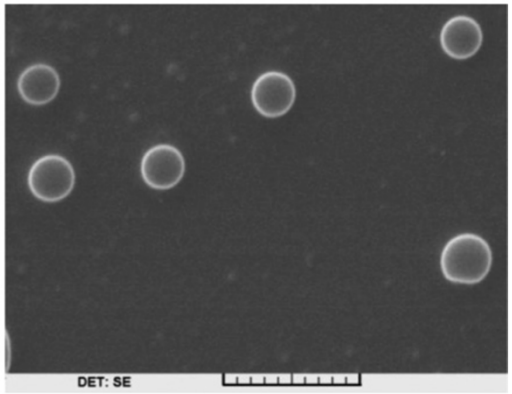


PLGA-INH



PLGA-pT5i

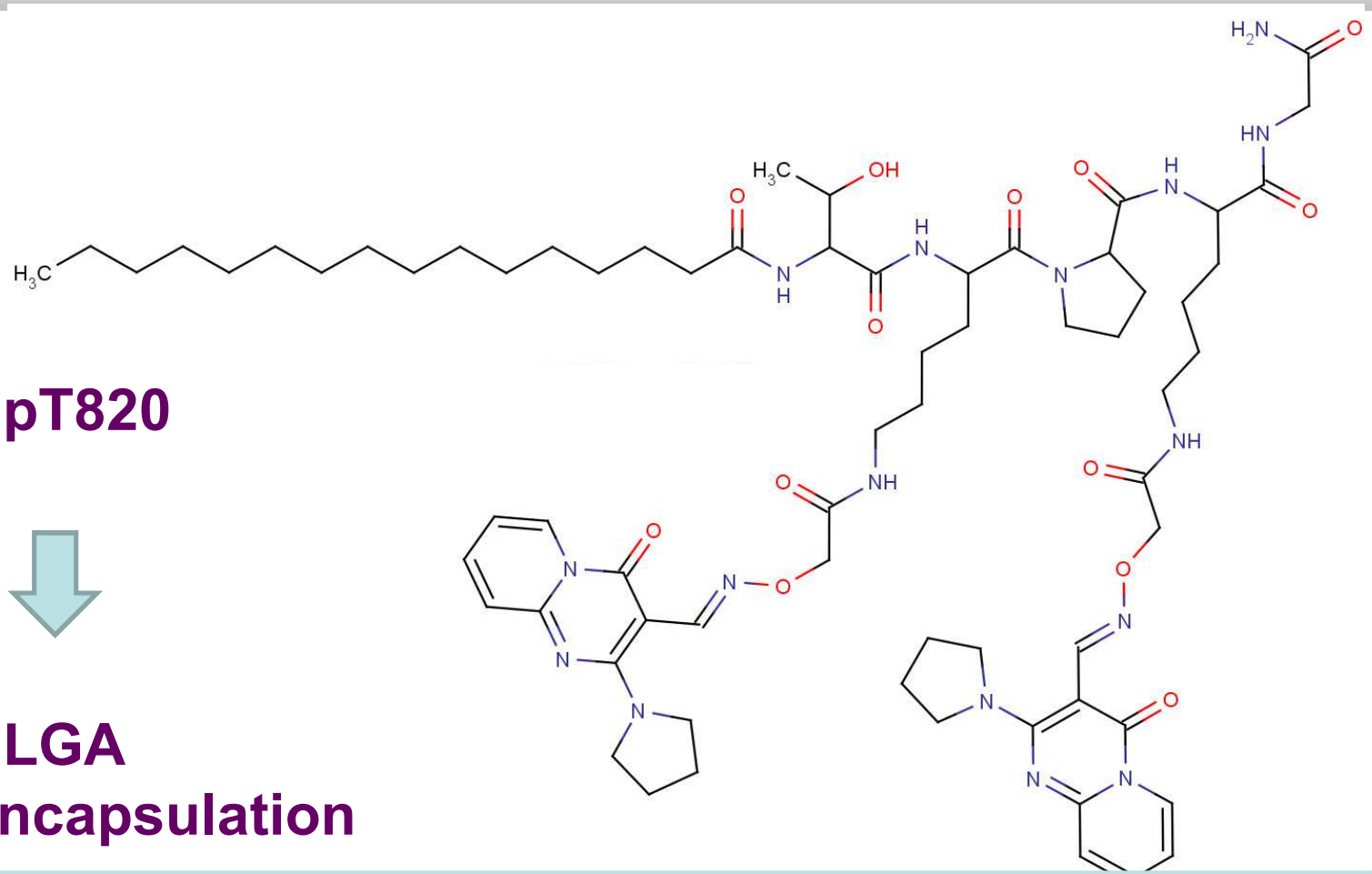




compound	ESI-MS M_{mo}	HPLC R_t (min)	drug content %	Log P	encapsulation efficiency %	SEM particle size nm	polydisp.
INH	137.1	5.0	-	-1.12	-	-	-
pT5i	1120.6	26.0	-	-0.20	-	-	-
PLGA-pT5i	-	-	43 ± 6	-	92 ± 7	185 ± 52	0.08 ± 0.02
PLGA-INH	-	-	0.5 ± 0.4	-	10 ± 5	305 ± 114	0.03 ± 0.01

Cs. B. Pénzes et al. *Colloids and Surfaces A*. 143:142-148 (2012); É. Kiss et al. *J. Dispers. Sci. Tech*. 32(12):1728-1734 (2011)

Palmitoylated-tuftsin conjugate of TB820 compound



pT820

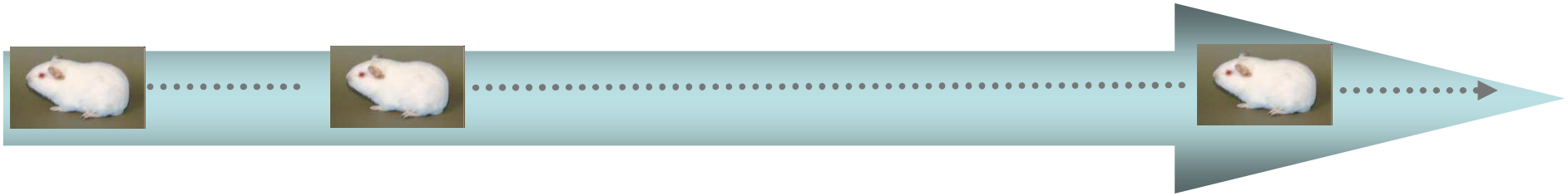
PLGA encapsulation

compound	ESI-MS M_{mo}	MIC *Mtb* $H_{37}Rv$ (µg/ml)	MIC *Mtb* $H_{37}Rv$ (µM)	drug content %	encapsulation efficiency %
TB820	243.1	1	4.1	-	-
pT820	1362.8	5	3.7	-	-
PLGA-pT820	-			20.2	83

Horváti K, Bacsa B, Szabó N, Fodor K, Balka G, Rusvai M, Kiss É, Mező G, Grolmusz V, Vértessy B, Hudecz F, Bősze S. Antimycobacterial activity of peptide conjugate of pyridopyrimidine derivative against Mycobacterium tuberculosis in a series of in vitro and in vivo models. Tuberculosis (Edinb). 2015, 95 Suppl 1:S207-11.

In vivo antitubercular effect of PLGA-pT820

- inbred, female guinea pigs (6 weeks old)
- intramuscular infection with *Mtb* $H_{37}Rv$
- 3 weeks of incubation before treatment



- oral administration, twice a week, 12 weeks
- 50 mg/kg bw, suspension in 1 mL sterile water)
- 6 animals/group

- diagnostic autopsy, histophatology
- CFU determination from tissue homogenates

approved by the Hungarian Scientific Ethical Committee on Animal Experimentation (No: 22.1/3720/003/2009).

lung

spleen

liver

kidney

heart

limph nodes

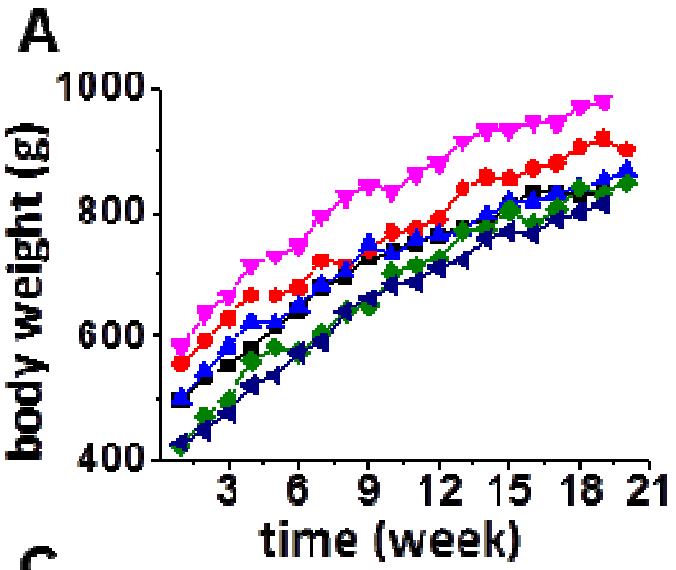
Horváti K, Bacsa B, Szabó N, Fodor K, Balka G, Rusvai M, Kiss É, Mező G, Grolmusz V, Vértessy B, Hudecz F, Bősze S. Antimycobacterial activity of peptide conjugate of pyridopyrimidine derivative against Mycobacterium tuberculosis in a series of in vitro and in vivo models. Tuberculosis (Edinb). 2015, 95 Suppl 1:S207-11.

Horváti K, Bacsa B, Kiss E, Gyulai G, Fodor K, Balka G, Rusvai M, Szabó E, Hudecz F, Bősze S.Nanoparticle encapsulated lipopeptide conjugate of antitubercular drug isoniazid: in vitro intracellular activity and in vivo efficacy in a Guinea pig model of tuberculosis. Bioconjug Chem. 2014 25 (12):2260-8.

In vivo effect of PLGA-pT820 conjugate

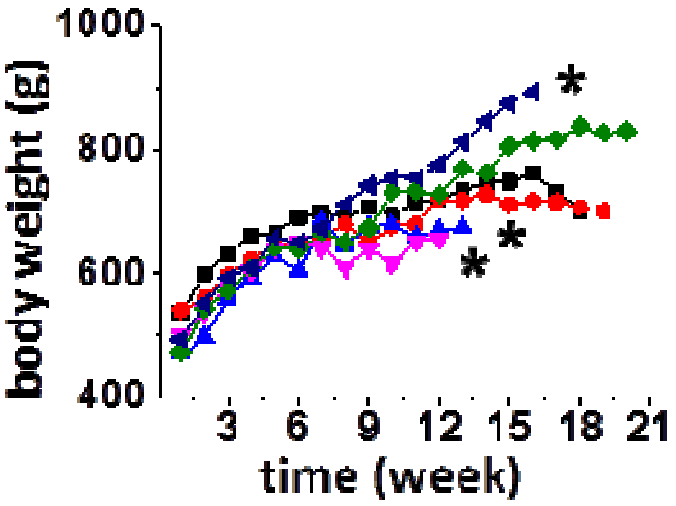
PLGA-pT820

untreated



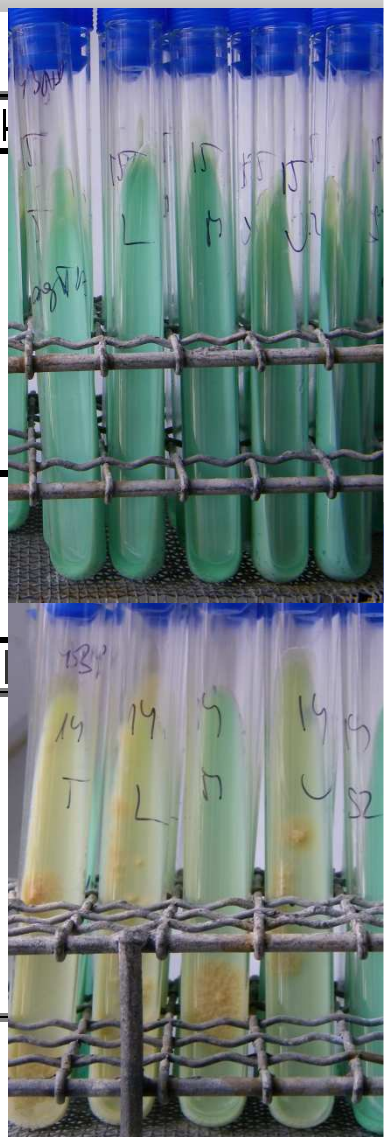
B

animal	lung	spleen	liver
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	-	-	-
6	-	-	-



D

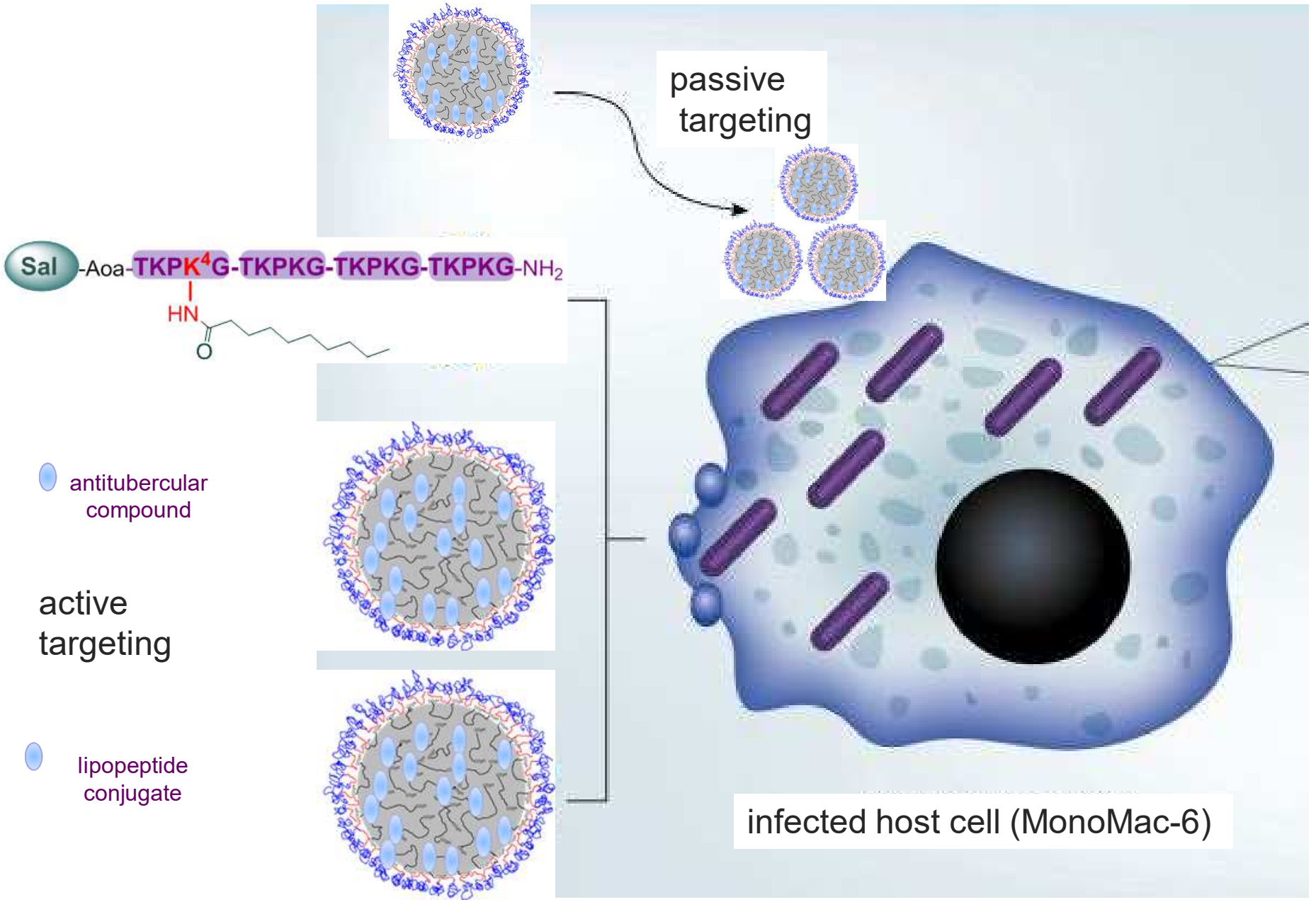
animal	lung	spleen	liver
1	-	++	-
2	-	+	-
3	+	++	+
4	++	+++	++
5	+	+++	+
6	-	+++	++



PLGA-pT820 treated animals gained weight steadily (**A**) and no mycobacterial colonies were observed in the tissue homogenates (**B**). In panel **C**, the weight gain of infected but untreated control animals is presented (* indicates the death of an animal). To prove TB infection, CFU was determined from the plated tissue homogenates (**D**)
(+++ : confluent colonies; ++ : innumerable colonies, but not confluent; + : 50-100 colonies; - : no colonies were observed).

Horváti K, Bacsa B, Szabó N, Fodor K, Balka G, Rusvai M, Kiss É, Mező G, Grolmusz V, Vértessy B, Hudecz F, Bősze S. Antimycobacterial activity of peptide conjugate of pyridopyrimidine derivative against Mycobacterium tuberculosis in a series of in vitro and in vivo models. Tuberculosis (Edinb). 2015, 95 Suppl 1:S207-11.

PLGA nanoparticles for passive and active targeting

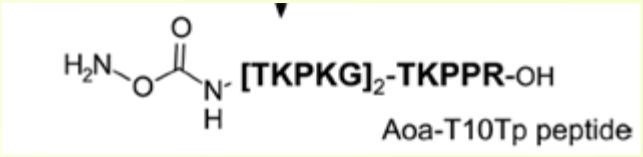


Pluronic F127 modification with receptor specific peptide

Bioconjugate Chemistry
Communication
Cite This: Bioconjugate Chem. XXXX, XXX, XXX−XXX
pubs.acs.org/bc

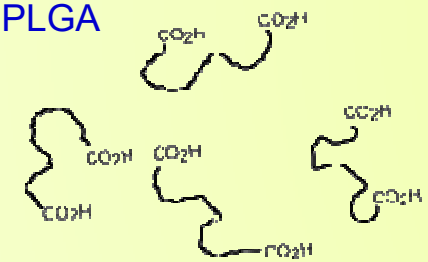
Surface Layer Modification of Poly(D,L-lactic-*co*-glycolic acid) Nanoparticles with Targeting Peptide: A Convenient Synthetic Route for Pluronic F127−Tuftsin Conjugate

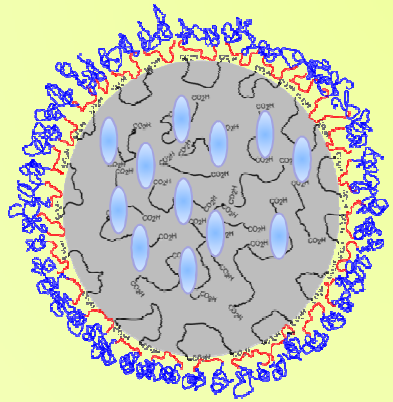
Kata Horváti,[†] Gergő Gyulai,[‡] Antal Csámpai,[∥] János Rohonczy,[§] Éva Kiss,[§] and Szilvia Bősze*[,†]



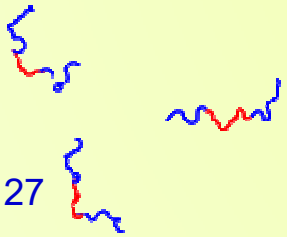
active compound
(TB515)

PLGA

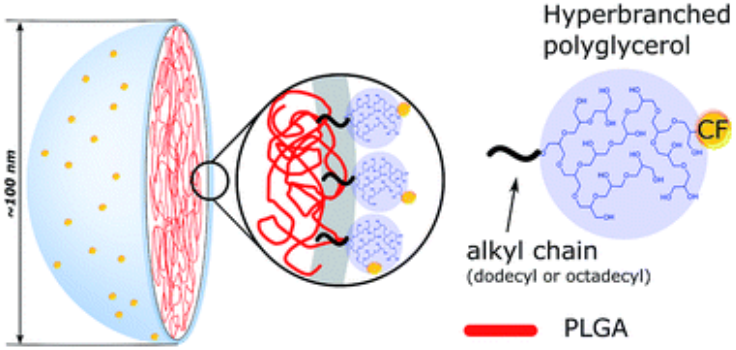




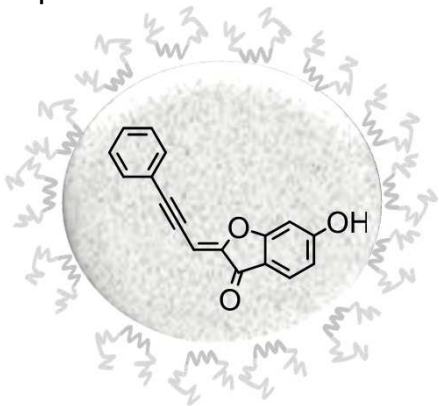
Pluronic F127



PLGA: poly(lactic-co-glycolic acid) (1:1, M: 50 000-75 000)
Pluronic® (PEO-PPO-PEO, copolymer, M: 12 000)

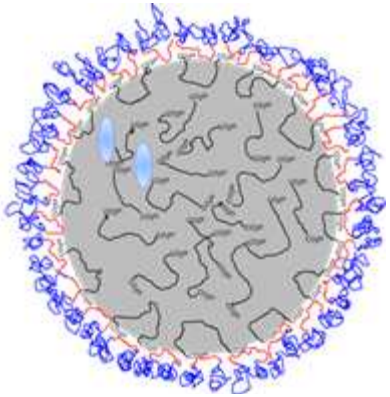


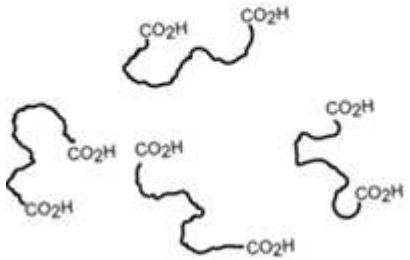
PLGA nanoparticle with Tuftsin-Pluronic coating

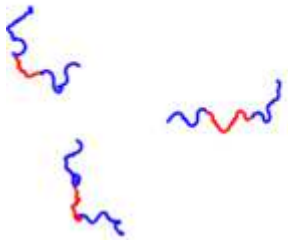


intracellular killing activity of drug loaded, peptide conjugated PLGA NPs on *Mycobacterium tuberculosis*

Pluronic F127 modification with receptor specific peptide







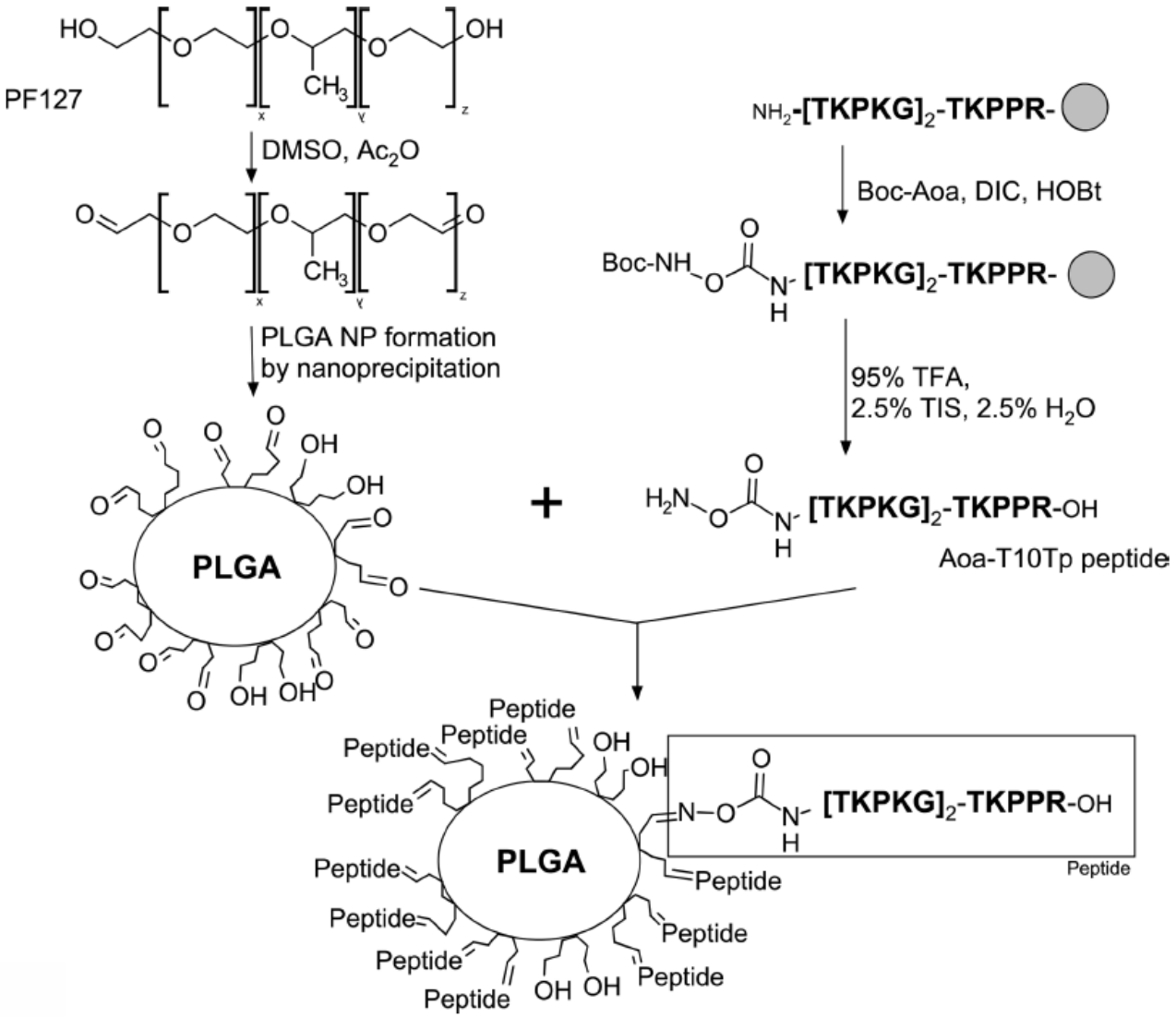
F127

M_w=12.6*10³

$HO-[CH_2CH_2O]_{100}-[CH_2CHO]_{65}-[CH_2CH_2O]_{100}-H$

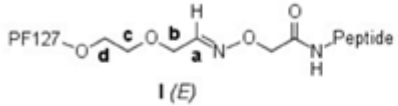
poly(ethylene oxide) - poly(propylene oxide)-poly(ethylene oxide)

Hydrophylic Hydrophobic Hydrophylic

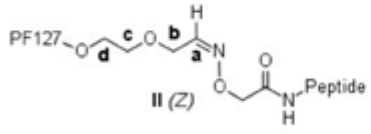


Horváti K, Gyulai G, Csámpai A, Rohonczy J, Kiss É, Bősze S. Surface Layer Modification of Poly(d,l-lactic- co-glycolic acid) Nanoparticles with Targeting Peptide: A Convenient Synthetic Route for Pluronic F127-Tuftsin Conjugate. Bioconjug Chem. 2018, doi: 10.1021/acs.bioconjchem.8b00156.

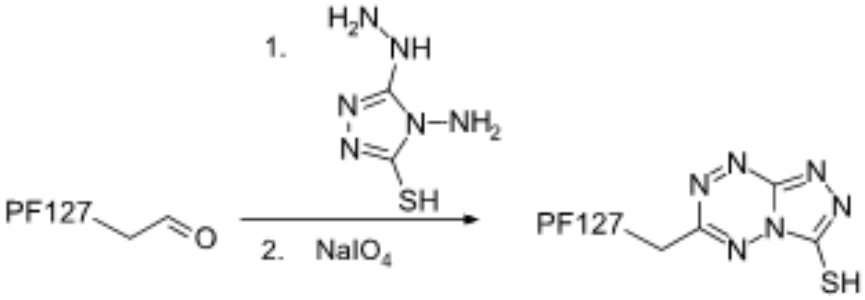
Characterisation of the peptide decorated Pluronic F127

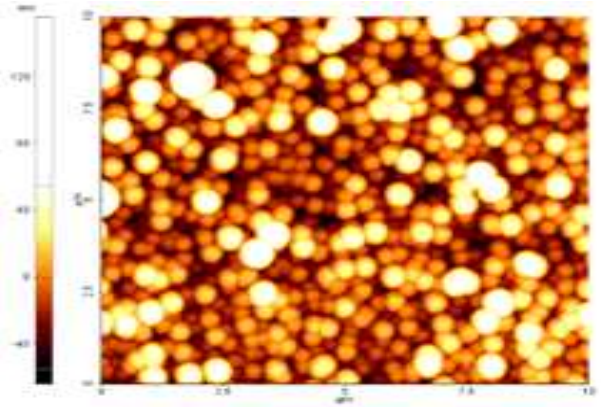


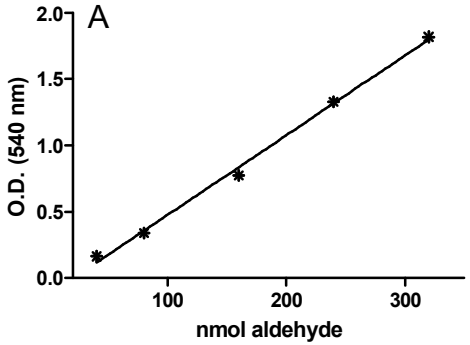
δH_a: 7.58 ppm (t, J = 6.4 Hz)
δH_b: 3.99 ppm (partly overlapped by the signals of skeletal protons of the conjugate)
δH_c and δH_d: 4.61 ppm (s)
δC_a: 150.2 ppm
δC_b: 67.1 ppm
δC_c and δC_d: 74.9 ppm



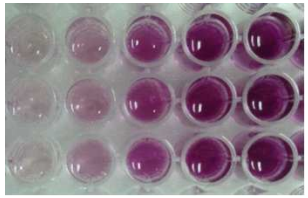
δH_a: 7.43 ppm (t, J = 6.4 Hz)
δH_b: 3.95 ppm (partly overlapped by the signals of skeletal protons of the conjugate)
δH_c and δH_d: 4.61 ppm (s)
δC_a: 147.3 ppm
δC_b: 67.1 ppm
δC_c and δC_d: 74.9 ppm

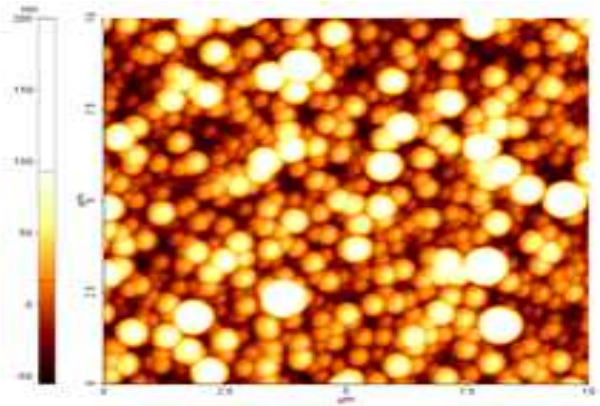






B

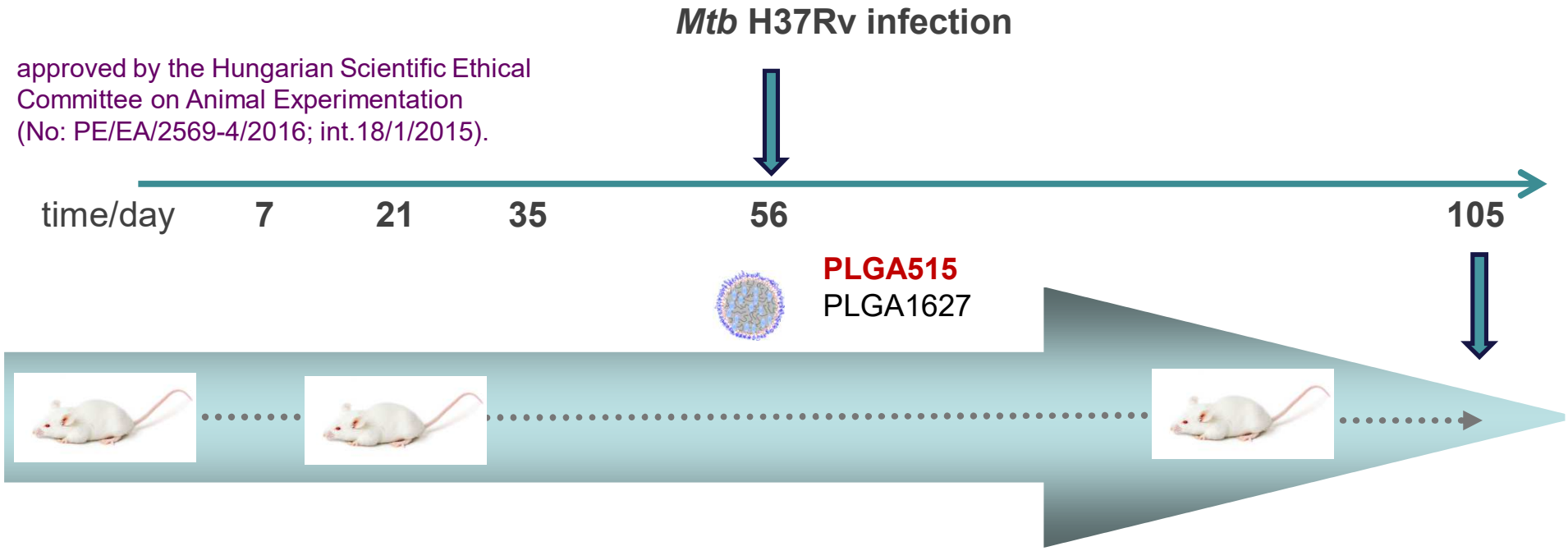


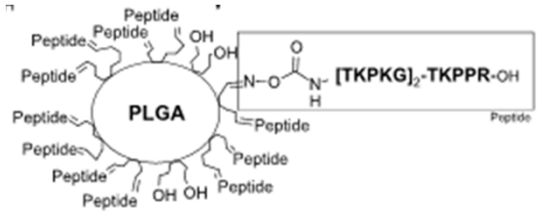


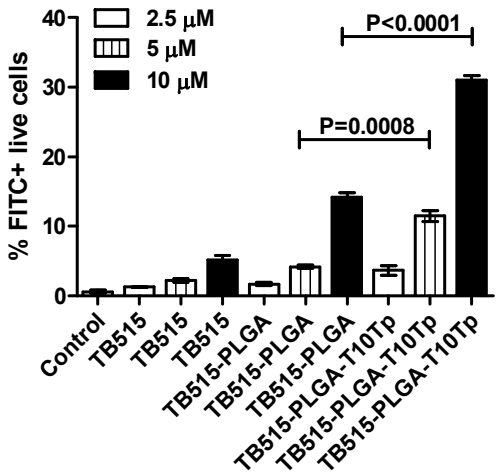
Horváti K, Gyulai G, Csámpai A, Rohonczy J, Kiss É, Bősze S. Bioconjug Chem. 2018, doi: 10.1021/acs.bioconjchem.8b00156., Supplementary

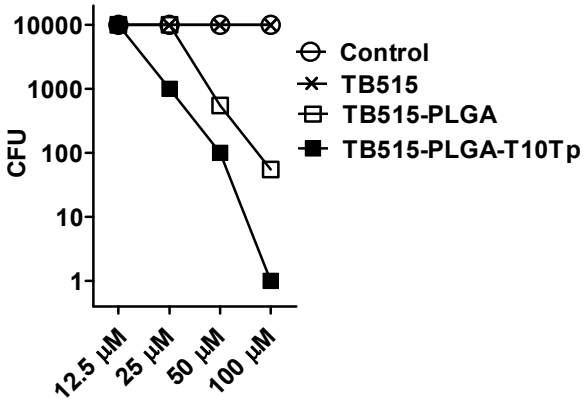
In vitro and *in vivo* evaluation tuftsin decoratid PLGA

approved by the Hungarian Scientific Ethical Committee on Animal Experimentation (No: PE/EA/2569-4/2016; int.18/1/2015).









É. Kiss et al., J. Dispersion Sci. Tech. (2012) 32(12), 1728-1734;
K. Horváti et al., Tuberculosis (Edinb). 2015, 95 Suppl 1:S207-11.;

É. Kiss et al., Colloids Surfaces A (2014) 458, 178-186;
K. Horváti et al., Bioconj. Chem. (2014) 25 (12), 2260-2268
Anselmo AC, Mitragotri S. J Control Release. (2014) 190, 531-41.
K. Horváti et al. Bioconjugate Chem. accepted 2018

Infected murine model: lung homogenates, CFU enumeration

INH
per os

K1	CFU
	0
	1
	1
	0
	0

INH-Dvar4
Sc

K2	
	16
	0
	38
	2
	13

PLGA 1627
per os

K3	
	18
	1
	9
	6
	2

PLGA 515
per os

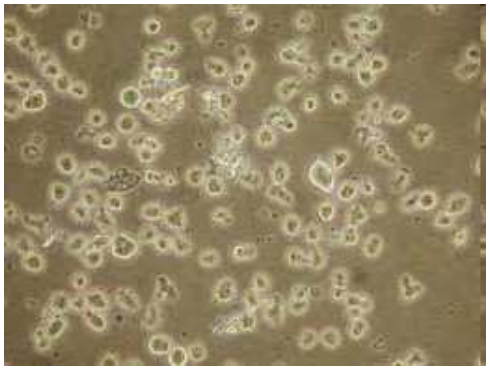
K4	
	8
	22
	9
	3
	1

control

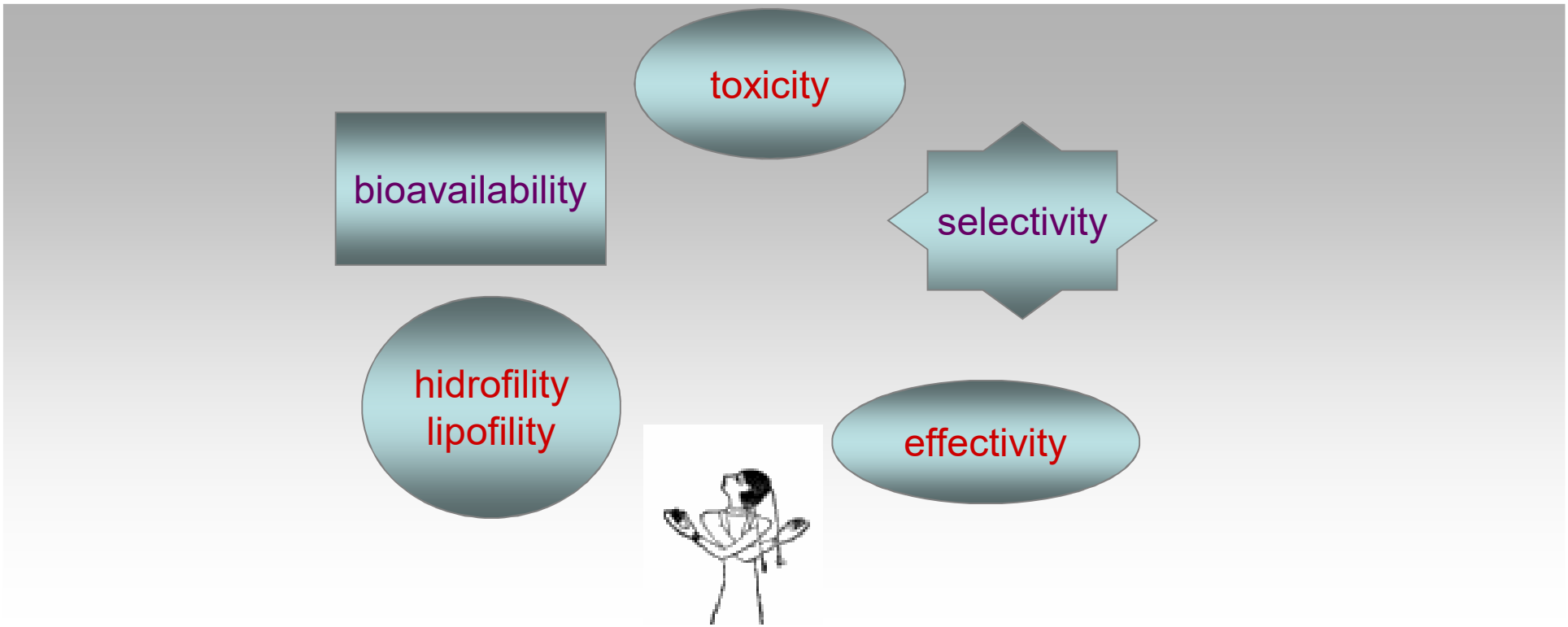
K5	
	1
	22
	47
	2

Enhance of cellular uptake and bioavailabity, *in vitro* és *in vivo* modells

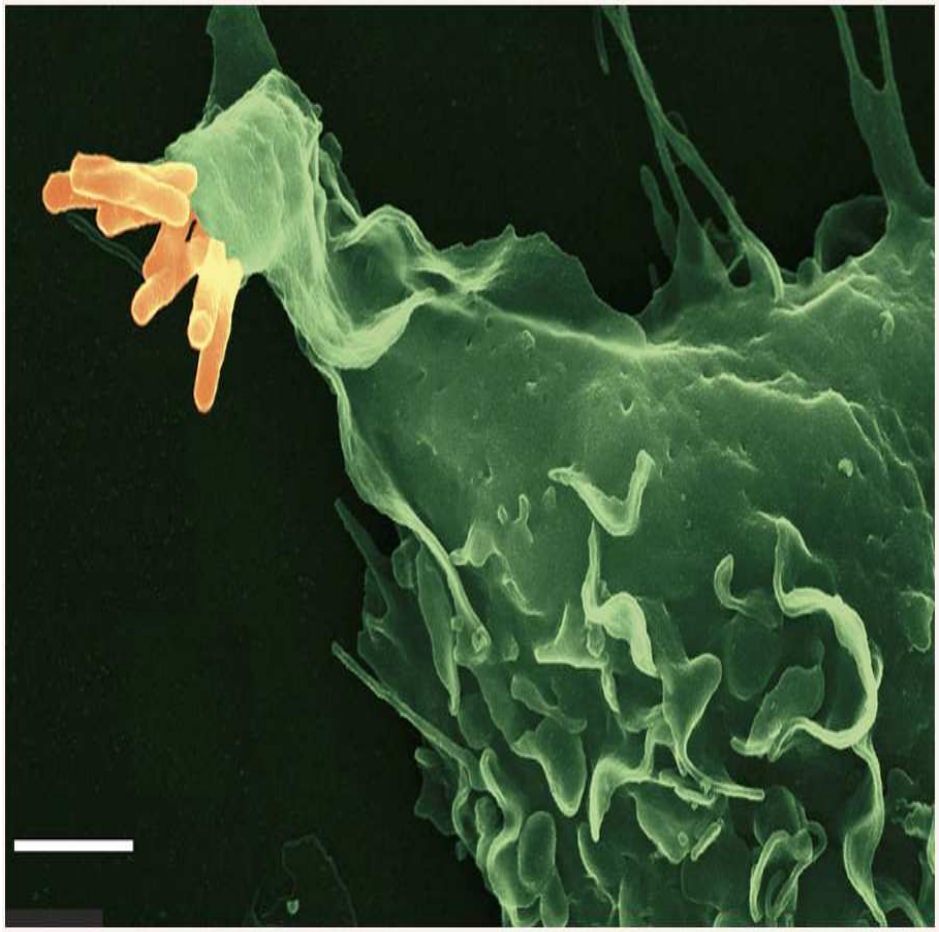
peptide and lipopeptide conjugates



nanoparticules PLGA



Thank you for your attention!



Kaufmann SHE, Nature 453: 295; (MTB-TLR2-MF)

Strelitzia (bird of Paradise plant)

tbinter

http://www.tb-inter.org/

Uratim Ltd.	*in silico* methods
Ubichem Ltd.	Synthesis of compounds, chemical optimisation
Institute of Enzimology Research Centre for Natural Sciences	enzyme activity studies, dUTPase characteriasation, *in silico* methods
MTA-ELTE Research Group of Peptide Chemistry	design of antitubercular agents, design, synthesis and chemical characterisation *in vitro, in vivo* evaluationn
Laboratory of Interfaces and Nanostructures	penetration studies, nanoparticles

Ubichem Research URATIM

University Eötvös Loránd

Hungarian Academy of Sciences

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István Csonka

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National Center for Epidemiology

Hungarian Research Fund, OTKA 104275, 104928
National Research and Development Programme (NKFP_07_1-TB_INTER-HU)
IGA NT 13346 (2012)
European Social Fund and Czech Republic Project No. CZ.1.07/2.3.00/20.0235
Social Renewal Operational Programme (TAMOP-4.2.2.B-10/1), Szent István University"

Chemical modification of existing drugs, chemical tailoring

European Journal of Medicinal Chemistry 46 (2011) 4937–4945

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

New fluorine-containing hydrazones active against MDR-tuberculosis

Eva Vavříková [a], Slovenko Polanc [b], Marijan Kočevar [b], Kata Horváti [c], Szilvia Bősze [c], Jiřina Stolaříková [d], Kateřina Vávrová [a], Jarmila Vinšová [a,*]

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[c] Eötvös Loránd University, Research Group of Peptide Chemistry, Hungarian Academy of Science, Pázmány Péter Sétány 1/A, Budapest, H-1117, Hungary
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European Journal of Medicinal Chemistry 46 (2011) 5902–5909

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

New series of isoniazid hydrazones linked with electron-withdrawing substituents

Eva Vavříková [a], Slovenko Polanc [b], Marijan Kočevar [b], Janez Košmrlj [b], Kata Horváti [c], Szilvia Bősze [c], Jiřina Stolaříková [d], Aleš Imramovský [e], Jarmila Vinšová [a,*]

[a] Charles University, Faculty of Pharmacy, Department of Inorganic and Organic Chemistry, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
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[e] University of Pardubice, Faculty of Chemical Technology, Studentská 573, 532 10 Pardubice, Czech Republic

European Journal of Medicinal Chemistry 109 (2011) 692–704

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Combating highly resistant emerging pathogen *Mycobacterium abscessus* and *Mycobacterium tuberculosis* with novel salicylanilide esters and carbamates

Zsuzsa Baranyai [a], Martin Krátký [b], Jarmila Vinšová [b], Nóra Szabó [c], Zsuzsanna Senoner [c], Kata Horváti [a], Jiřina Stolaříková [d], Sándor Dávid [a,c], Szilvia Bősze [a,*]

European Journal of Medicinal Chemistry 45 (2010) 6106–6113

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

New amino acid esters of salicylanilides active against MDR-TB and other microbes

Martin Krátký [a], Jarmila Vinšová [a,*], Vladimír Buchta [b,c], Kata Horvati [d], Szilvia Bösze [d], Jiřina Stolaříková [e]

[a] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
[b] Department of Clinical Microbiology, Faculty of Medicine and University Hospital, Charles University, Sokolská 581, 500 12 Hradec Králové, Czech Republic
[c] Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
[d] Research Group of Peptide Chemistry, Eötvös Lórand University, Hungarian Academy of Science, Pázmány Péter Sétány 1/A, Budapest, H-1117, Hungary
[e] Laboratory for TBC, Regional Institute of Public Health in Ostrava, Partyzánské náměstí 7, 702 00 Ostrava, Czech Republic

Bioorganic & Medicinal Chemistry 23 (2015) 868–875

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and in vitro biological evaluation of 2-(phenylcarbamoyl)phenyl 4-substituted benzoates

Martin Krátký [a], Szilvia Bősze [b], Zsuzsa Baranyai [b], Ildikó Szabó [b], Jiřina Stolaříková [c], Georgios Paraskevopoulos [a], Jarmila Vinšová [a,*]

[a] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
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[c] Laboratory for Mycobacterial Diagnostics and Tuberculosis, Regional Institute of Public Health in Ostrava, Partyzánské náměstí 7, 702 00 Ostrava, Czech Republic

Bioorganic & Medicinal Chemistry Letters 27 (2017) 5185–5189

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evolution of hydrazones derived from 4-(trifluoromethyl)benzohydrazide

Martin Krátký [a,*], Szilvia Bősze [b], Zsuzsa Baranyai [b], Jiřina Stolaříková [c], Jarmila Vinšová [a]

Peptide based delivery of antituberculars

Tuberculosis 95 (2015) S207–S211

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

Antimycobacterial activity of peptide conjugate of pyridopyrimidine derivative against *Mycobacterium tuberculosis* in a series of *in vitro* and *in vivo* models

Kata Horváti [a], Bernadett Bacsa [a], Nóra Szabó [b], Kinga Fodor [c], Gyula Balka [d], Miklós Rusvai [d], Éva Kiss [e], Gábor Mező [a], Vince Grolmusz [f], Beáta Vértessy [g], Ferenc Hudecz [a, h], Szilvia Bősze [a, *]

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[b] Laboratory of Bacteriology, Korányi National Institute for Tuberculosis and Respiratory Medicine, Budapest, Hungary
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[d] Department of Pathology and Forensic Veterinary Medicine, Faculty of Veterinary Science, Szent István University, Budapest, Hungary
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[f] Protein Information Technology Group, Eötvös L. University, Budapest, Hungary
[g] Institute of Enzimology, Hungarian Academy of Science, Budapest, Hungary
[h] Department of Organic Chemistry, Eötvös L. University, Budapest, Hungary

Bioconjugate Chemistry

Article

pubs.acs.org/bc

Enhanced Cellular Uptake of a New, *in Silico* Identified Antitubercular Candidate by Peptide Conjugation

Kata Horváti,[†] Bernadett Bacsa,[†] Nóra Szabó,[‡] Sándor Dávid,[†,‡] Gábor Mező,[†] Vince Grolmusz,[§,#] Beáta Vértessy,[∥,⊥] Ferenc Hudecz,[†,○] and Szilvia Bősze*[,†]

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[‡]Laboratory of Bacteriology, Korányi National Institute for Tuberculosis and Respiratory Medicine, Budapest, Hungary
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[#]Uratim Ltd., Budapest, Hungary
[○]Department of Organic Chemistry, Eötvös L. University, Budapest, Hungary

Bioconjugate Chemistry

Article

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Nanoparticle Encapsulated Lipopeptide Conjugate of Antitubercular Drug Isoniazid: In Vitro Intracellular Activity and in Vivo Efficacy in a Guinea Pig Model of Tuberculosis

Kata Horváti,[†] Bernadett Bacsa,[†] Éva Kiss,[‡] Gergő Gyulai,[‡] Kinga Fodor,[§] Gyula Balka,[∥] Miklós Rusvai,[∥] Eleonóra Szabó,[⊥] Ferenc Hudecz,[†,#] and Szilvia Bősze*[,†]

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[§]Department of State Veterinary Medicine and Agricultural Economics and [∥]Department of Pathology and Forensic Veterinary Medicine, Faculty of Veterinary Science, Szent István University, Budapest, 1078 Hungary
[⊥]Laboratory of Bacteriology, Korányi National Institute for Tuberculosis and Respiratory Medicine, Budapest, 1122 Hungary

Colloids and Surfaces A: Physicochem. Eng. Aspects 458 (2014) 178–186

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

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Tuneable surface modification of PLGA nanoparticles carrying new antitubercular drug candidate

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Nanoencapsulation of Antitubercular Drug Isoniazid and Its Lipopeptide Conjugate

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Peptide conjugates of therapeutically used antitubercular isoniazid – design, synthesis and antimycobacterial effect

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Bioconjugate Chemistry

Communication

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Surface Layer Modification of Poly(D,L-lactic-*co*-glycolic acid) Nanoparticles with Targeting Peptide: A Convenient Synthetic Route for Pluronic F127–Tuftsin Conjugate

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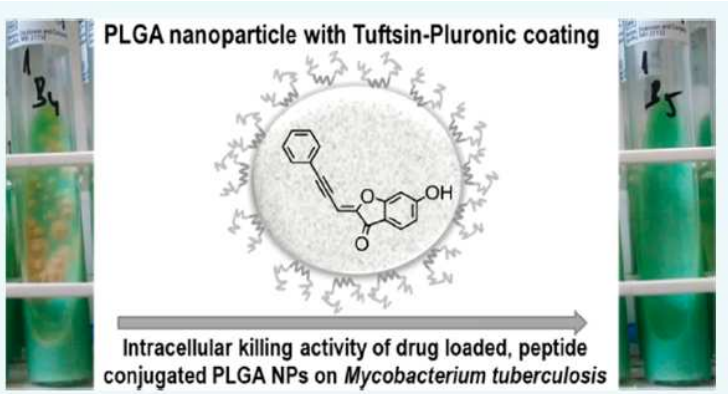

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Comparative analysis of new peptide conjugates of antitubercular drug candidates—Model membrane and *in vitro* studies

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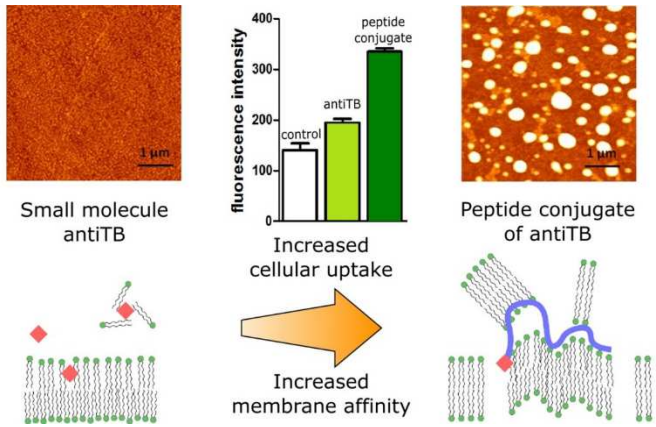

MTA-ELTE Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös L. University, Budapest

Target cell specific delivery
Bioconjugates

Antitimicrobial
and antitumor compounds

Epitope mapping of immundominant proteins
Lokalisation of B and T cell epitopes
Synthetic antigens
Diagnostic tools

Neuropeptides

Development
Antitumor and antimicrobial compounds

Methods

Synthesis
Bioactive peptides
Bioconjugates
Fluorescent peptides

Chemical characterisation
Liquid chromatography
Elementar analysis
Mass spectrometry
Amino acid analysis
Electrophoresis

Functional studies (*in vitro*)
Cytotoxicity, cytostatic effect
Cellular uptake and mechanisms
Stability studies
RT-PCR
Fluorescent and confocal microscopy

MTA-ELTE Research Group of Peptide Chenistry, host cell specific delivery of peptide based carriers

In Vitro Cytotoxicity, Chemotactic Effect, and Cellular Uptake of Branched Polypeptides with Poly[L-Lys] Backbone by J774 Murine Macrophage Cell Line

Rita Szabó,[†] Gábor Mezö,[†] Éva Pállinger,[‡] Péter Kovács,[§] László Köhidai,[§] Szilvia Bösze,[†] and Ferenc Hudecz*,[†,||]

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Uptake of Branched Polypeptides with Poly[L-Lys] Backbone by Bone-Marrow Culture-Derived Murine Macrophages: The Role of the Class A Scavenger Receptor

Rita Szabó,[†,⊥] Leanne Peiser,[||,⊥] Annette Plüddemann,[‡,⊥] Szilvia Bösze,[†] Sigrid Heinsbroek,[‡] Siamon Gordon,[‡] and Ferenc Hudecz*,[†,§]

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Methotrexate Conjugate with Branched Polypeptide Influences *Leishmania donovani* Infection in Vitro and in Experimental Animals[†]

György Kóczán,[‡] Asoke C. Ghose,[§] Ananda Mookerjee,[§] and Ferenc Hudecz*,[‡]

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Carrier Design: New Generation of Polycationic Branched Polypeptides Containing OH Groups with Prolonged Blood Survival and Diminished in Vitro Cytotoxicity

Ferenc Hudecz,*,[†] Malcolm V. Pimm,[‡] Éva Rajnavölgyi,[§] Gábor Mezö,[†] Angels Fabra,[||] Dezsö Gaál,[⊥] Attila L.Kovács,[#] Attila Horváth,[§] and Mária Szekerke[†]

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Thomas Huckle Weller Immunology and Parasitology Session, Classroom No. 2, 11.00 – 12.30

POP-3

RITA OLÁHNÉ SZABÓ[1],MÓNIKA SEBESTYÉN[1], GYÖRGY KÓCZÁN[1], LÁSZLÓ KŐHIDAI[2] AND FERENC HUDECZ[1, 3]

DRUG TARGETING STRATEGY WITH POLYEPTIDE BASED METHOTREXATE CONJUGATES AGAINST *LEISHMANIA* INFECTION

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