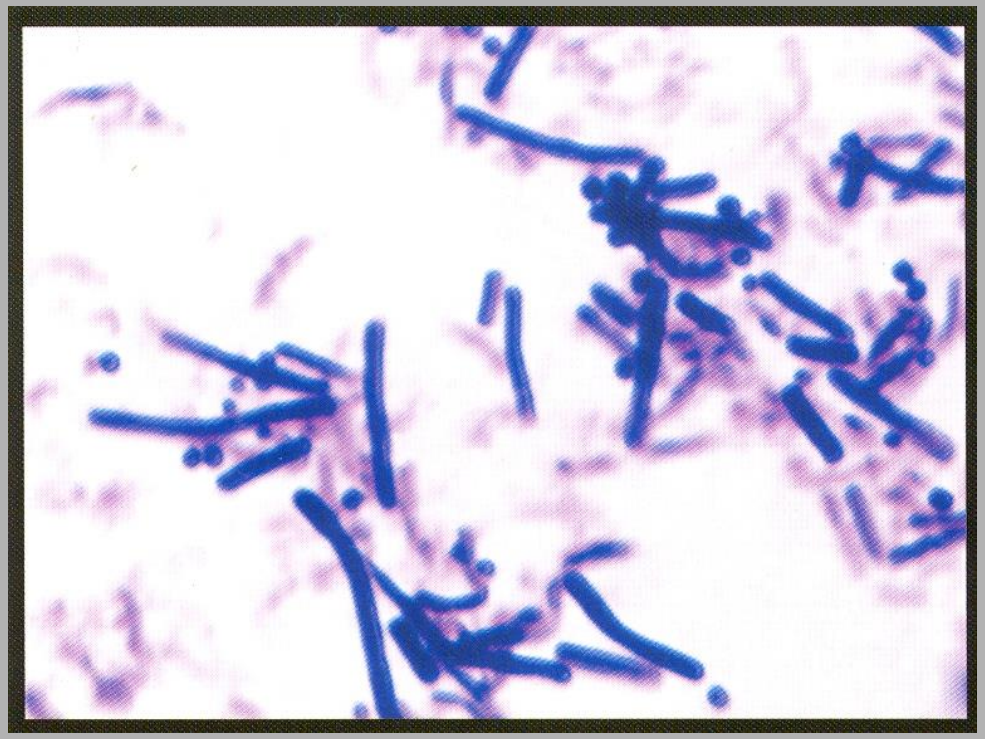
Recombinant vaccines

Jaroslav Turánek

Historické, sociální a medicínské aspekty infekčních chorob

„Historie je svědkem času, světlem pravdy, esencí paměti, učitelkou života, poslem z minulých věků“

Marcus Tullius Cicero



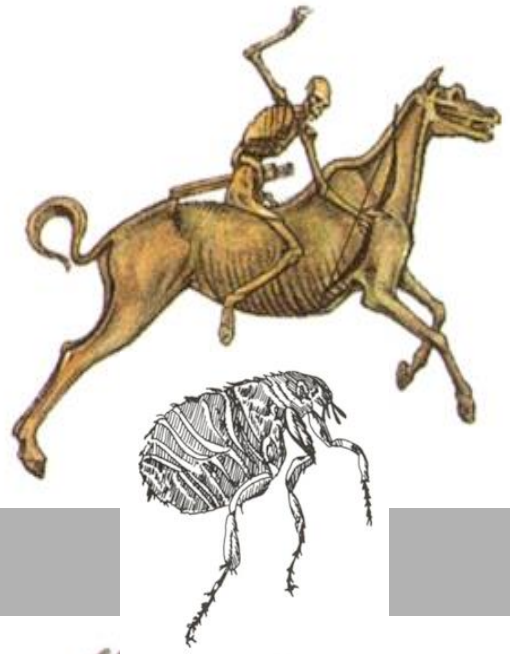
„V neustálém cyklu proměn malé se stává velkým a velké nepatrným“

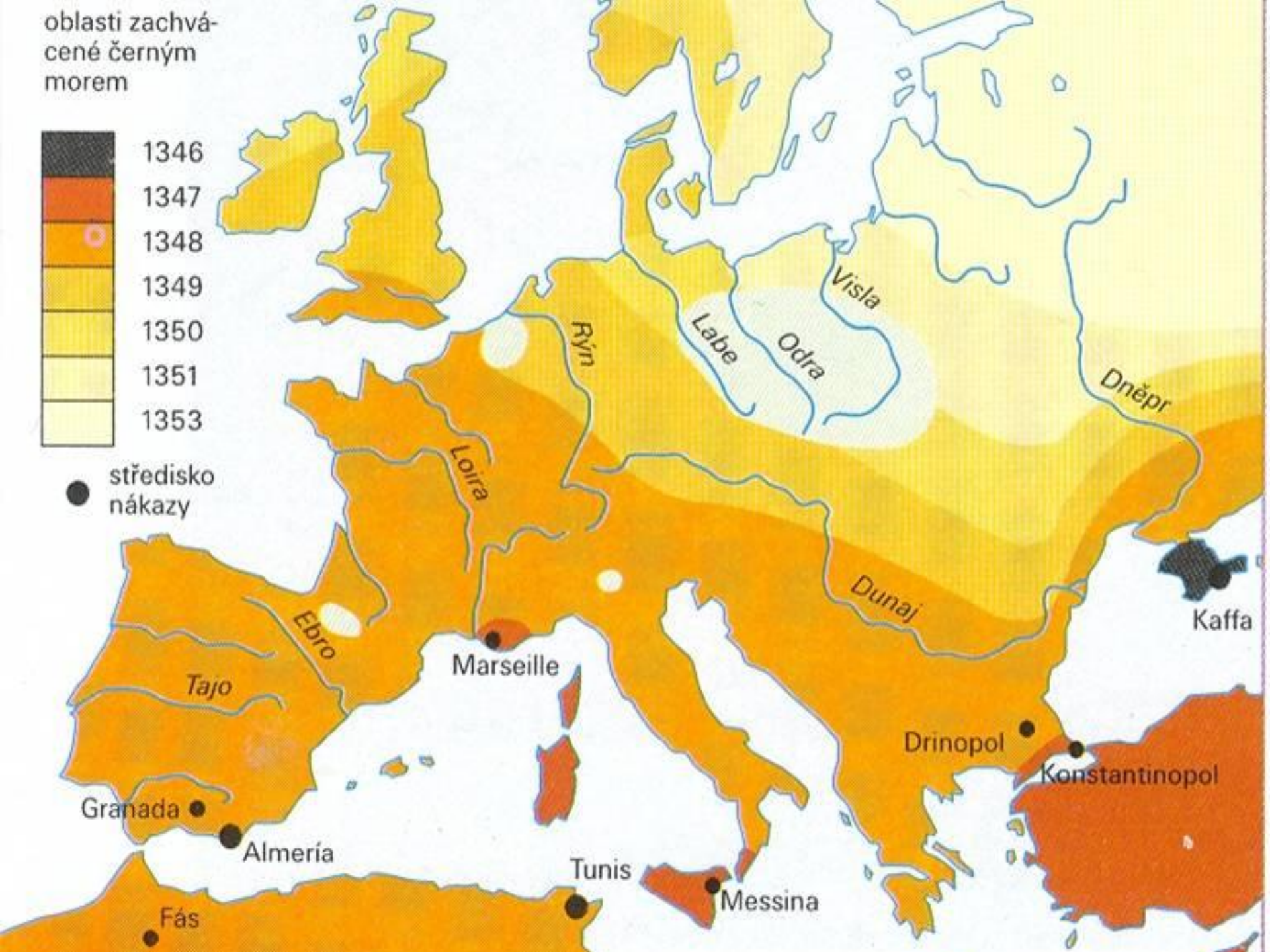
Lao‘c

Středověk

Černá smrt – Mor 1346-1353

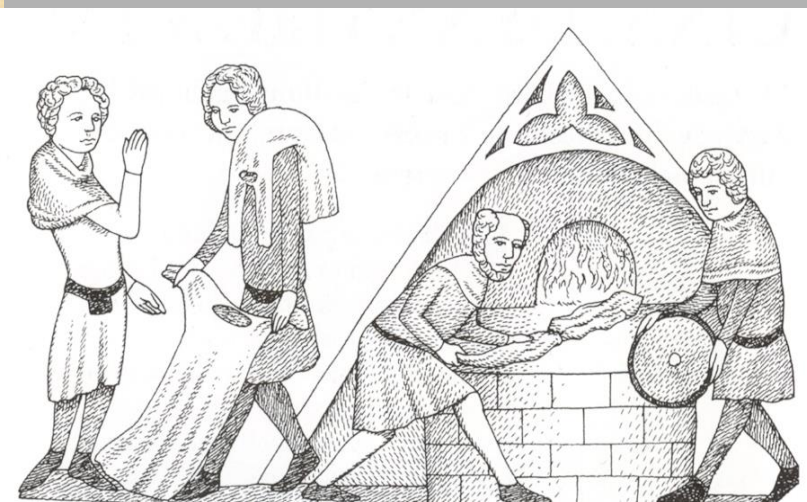
Yersinia pestis




oblasti zachvácené černým morem
1346
1347
1348
1349
1350
1351
1353
středisko nákazy
Ryn
Labe
Odra
Visla
Dněpr
Loira
Ebro
Tajo
Dunaj
Marseille
Kaffa
Drinopol
Konstantinopol
Granada
Almería
Tunis
Messina
Fás

Epidemie – epidemiologická opatření

Vyneste své mrtvé !!!

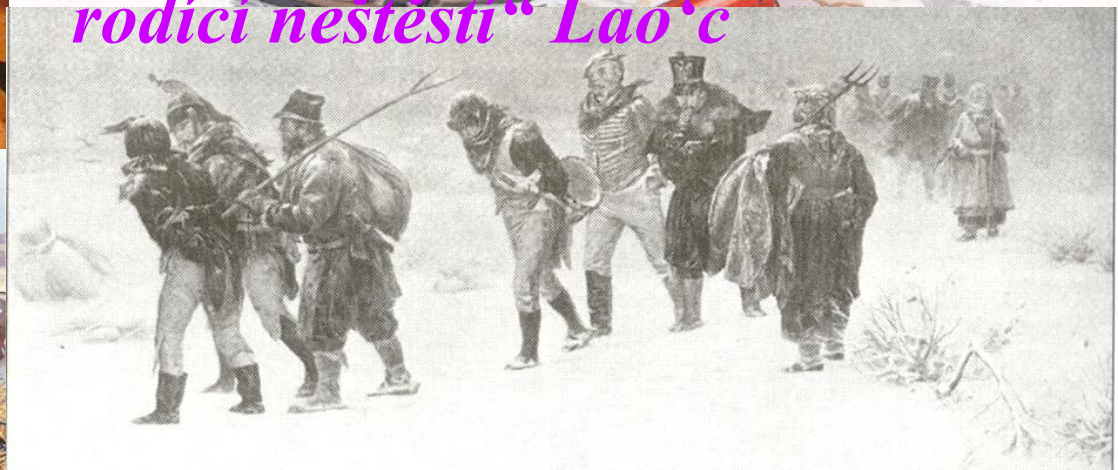


Třicetiletá válka
1618-1648

„Štěstí vyrůstá z úcty a neštěstí z násilí“ Lao‘c

Napoleonské války 1797 -1815
Generálové Mráz, Hlad a Tyfus

„Dobré vojsko je prostředek rodící neštěstí“ Lao‘c



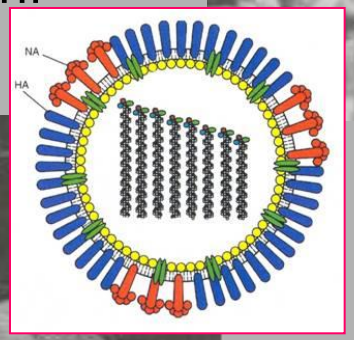
Chřipkové epidemie ve 20. století

vysoká koncentrace mladých lidí

stresové faktory

reservoár patogenu – prasata, drůbež, koně

migrace a promíchávání obyvatelstva

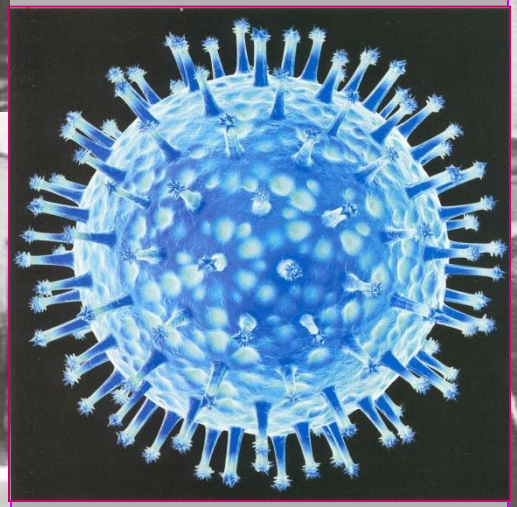


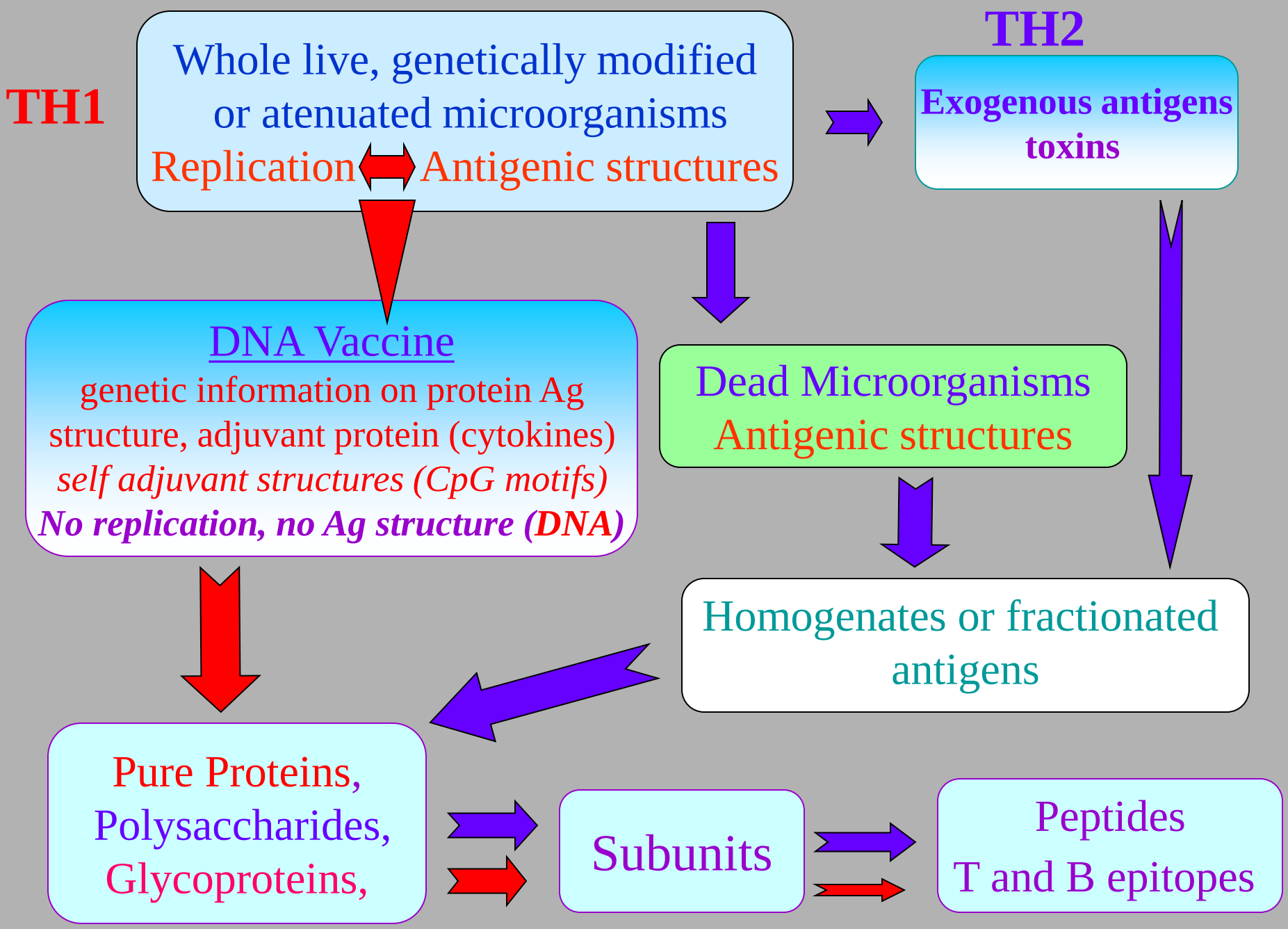
zranění

koncentrace nemocných v polních lazaretech

zajatecké tábory

Podmínky pro vznik pandemie španělské chřipky 1918-1919




TH1
Whole live, genetically modified
or atenuated microorganisms
Replication
Antigenic structures
TH2
Exogenous antigens
toxins
DNA Vaccine
genetic information on protein Ag
structure, adjuvant protein (cytokines)
self adjuvant structures (CpG motifs)
No replication, no Ag structure (DNA)
Dead Microorganisms
Antigenic structures
Homogenates or fractionated
antigens
Pure Proteins,
Polysaccharides,
Glycoproteins,
Subunits
Peptides
T and B epitopes

Vaccinology

Biology – Medicine

Immunology, Immunopathology, Cell Biology, Virology, Bacteriology, Parasitology, Cancer Biology, Molecular Biology…

Chemistry: -Bio -Physical -Polymer -Organic

Colloids, Proteins, Lipids, Peptides, DNA, Polymers, Polysaccharides, …

Industrial technologies

Fermentation, Purification of Biopolymers, Organic Synthesis, Production of Colloid Systems, Sterilisation, Lyophilisation, …

Economy – Government - Health Policy - Anti-vaccination Movement

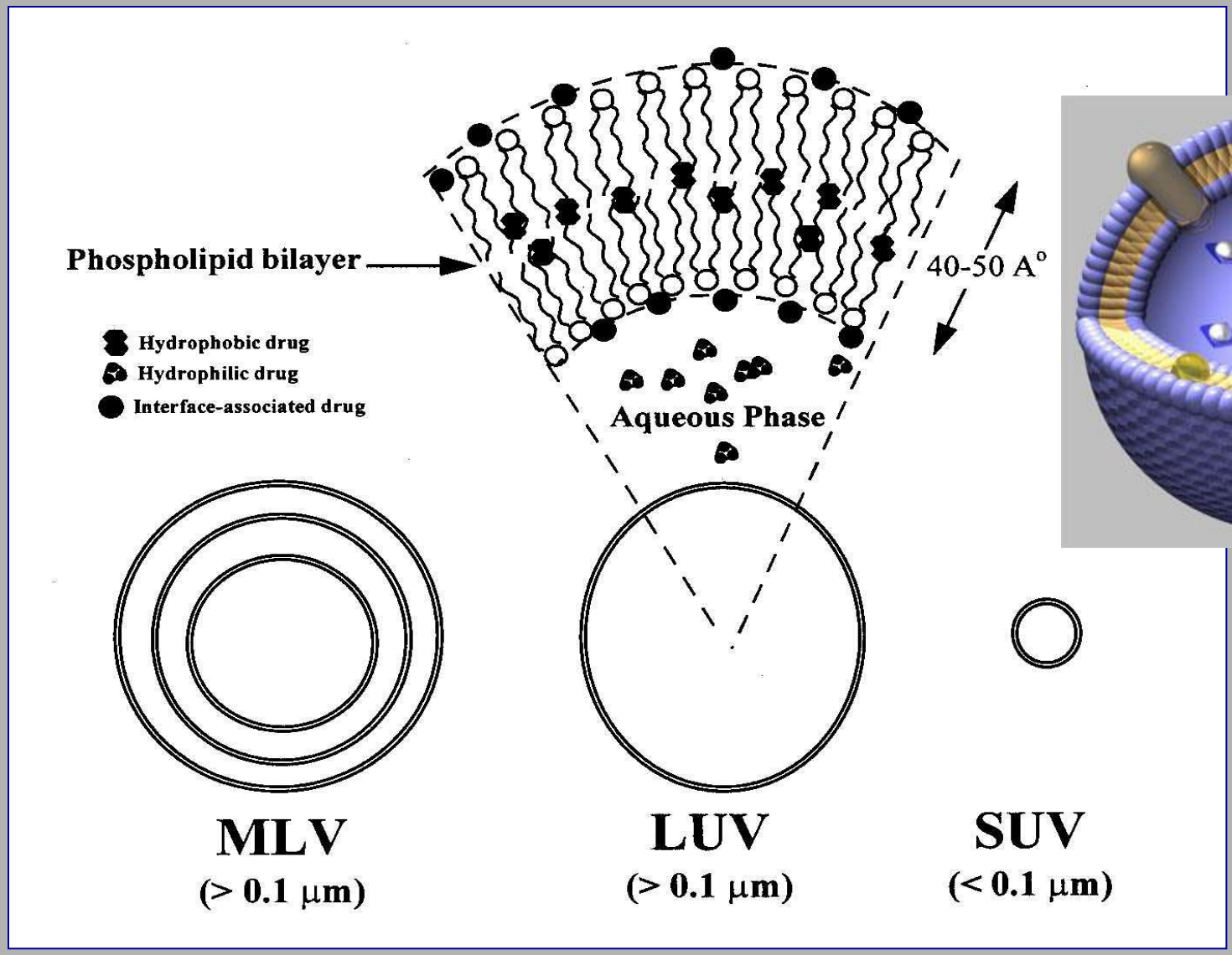
Reverse vaccinology

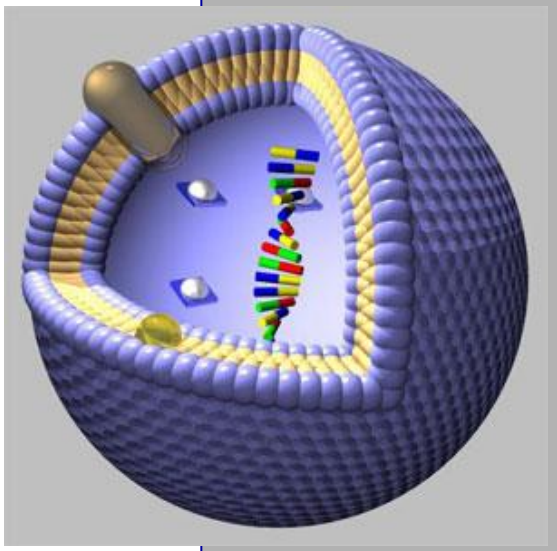
Production of recombinant protein/antigen
Synthesis of peptide antigen/epitop

A need for adjuvans and delivery systems owing to low immunogenity

Liposome based recombinant vaccines

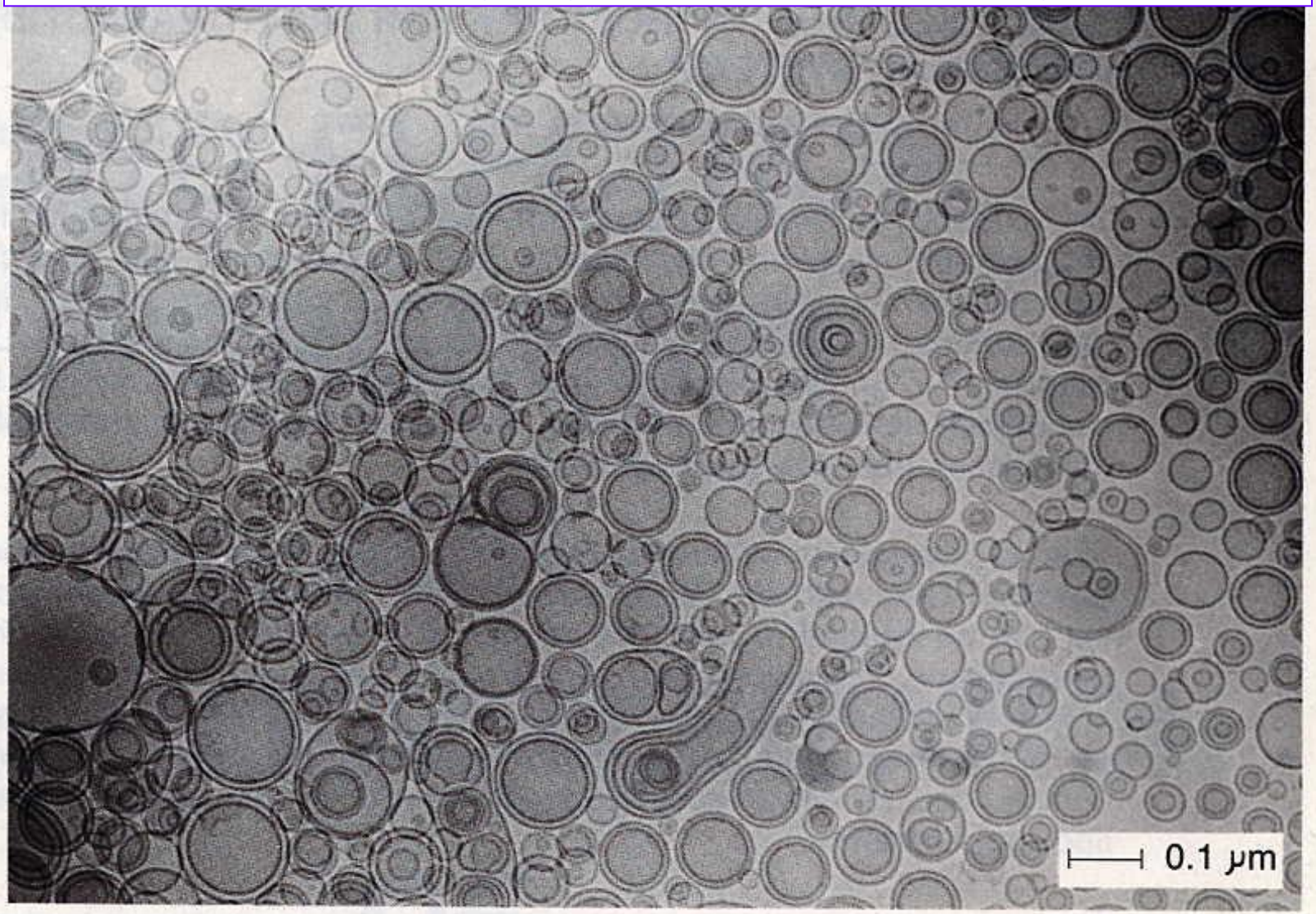
Classification of liposomes





Morphology of liposomes

Viewed by cryoelectron microscopy



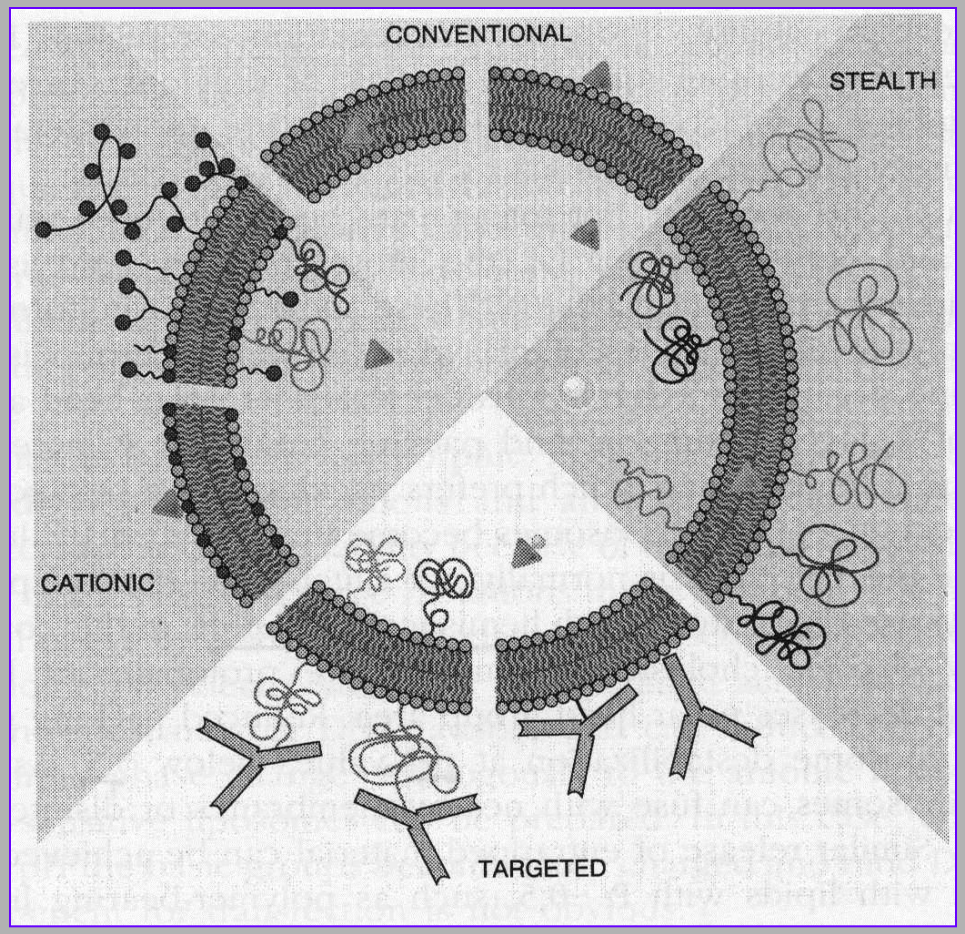
Classes of liposomes based on their functionality

Conventional liposomes *nonspecific interaction with melieu*

Stealth liposomes *sterically shielded , low non-specific interactions, long circulating*

Targeted liposomes *specific interaction via coupled ligand*

Polymorphic-*Cationic* *change their phase upon inter-action with specific agents, pH or temperature sensitive*



Liposome-based products

Antimicrobial drugs Amikacin (MiKasome) Amphotericin B (AmBisome) Econazole, Nystatin

Anticancer drugs Doxorubicin (Doxil), Daunorubicin (DaunoXome),Vincristine (VincaXome), CisPlatin, Paclitaxel

Vaccines Newcastle disease, Avian rheovirus, Hepatitis A, Trivalent influenza (Epaxal-Berna)

Dermatological preparations

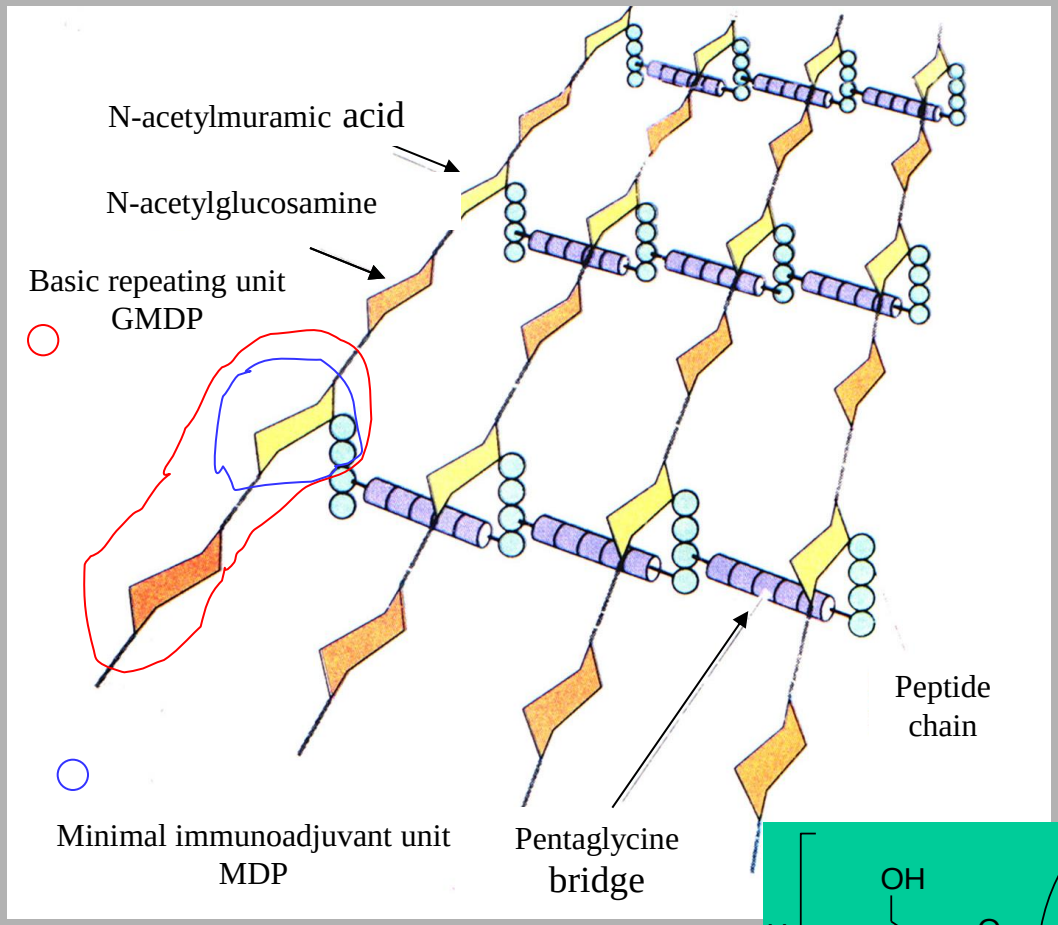
Adjuvans and recobinant vaccines

Reverse vaccinology

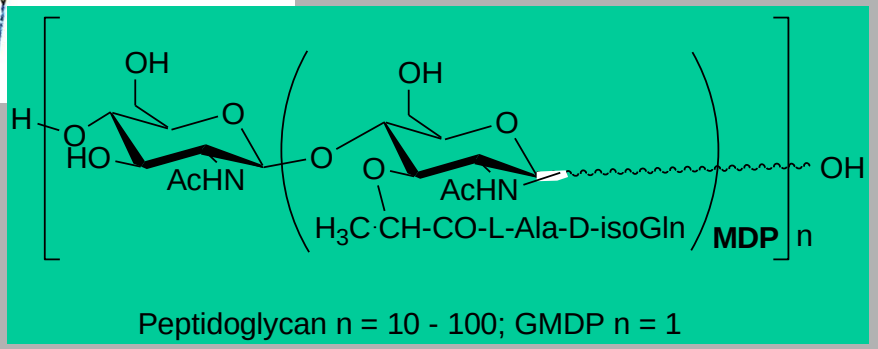
Production of recombinant protein/antigen
Synthesis of peptide antigen/epitop

A need for adjuvans and delivery systems owing to low immunogenity

Muramyl glycopeptides

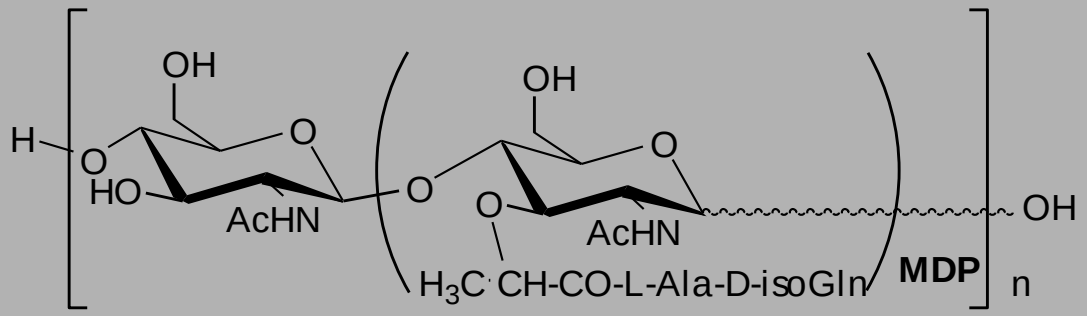


Block scheme of the peptidoglycan of bacterial cell wall

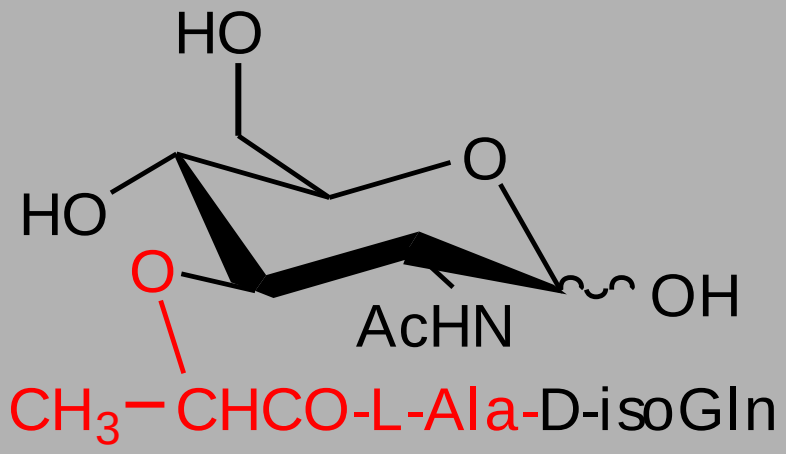


Peptidoglycan n = 10 - 100; GMDP n = 1

Muramyldipeptide

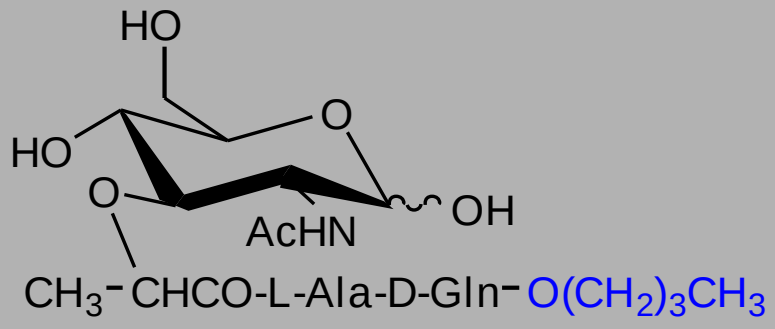


Peptidoglycan (n = 10 - 100)
MDP ("Muramyldipeptide", i.e. minimal immunoadjuvant unit)
GMDP (n = 1; "Glucosaminylmuramyldipeptide", i.e. basic repeating unit)

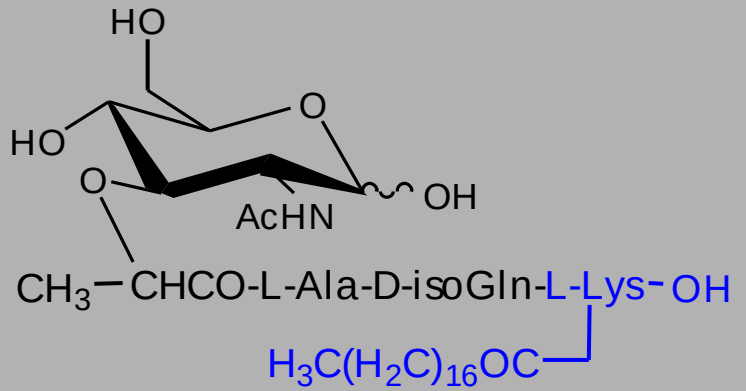


MDP

Analogs of MDP and GMDP developed as potential immunotherapeutics by pharmaceutical industry

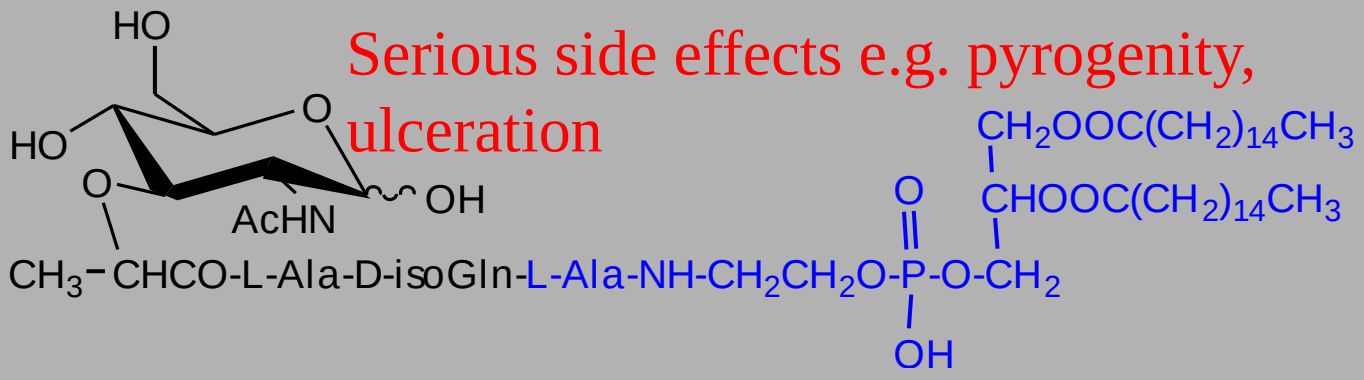


Murabutid (Inst. Choay, France)



Romurtid (Muroctasin)
(Daiichi Pharmaceutical Co., Japan)

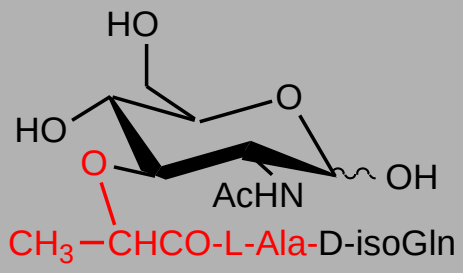
Serious side effects e.g. pyrogenity, ulceration



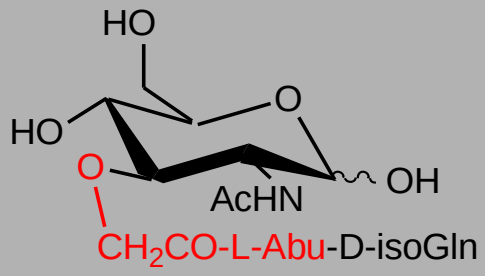
MTP-PE NovaArtis (Ciba-Geigy,Switzerland)

Analogs of muramylglycopeptides developed on IOCB in collaboration with VRI

Transformations of MDP and GMDP molecules to norAbu-MDP and norAbu-GMDP analogs resp. led to decrease or elimination the pyrogenicity and enhancement immunoadjuvant activity

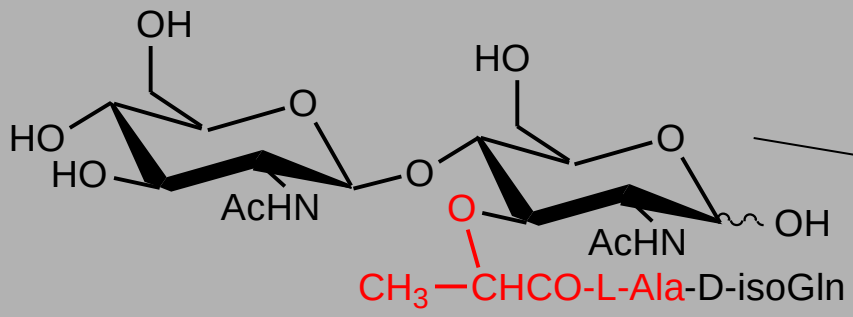


MDP



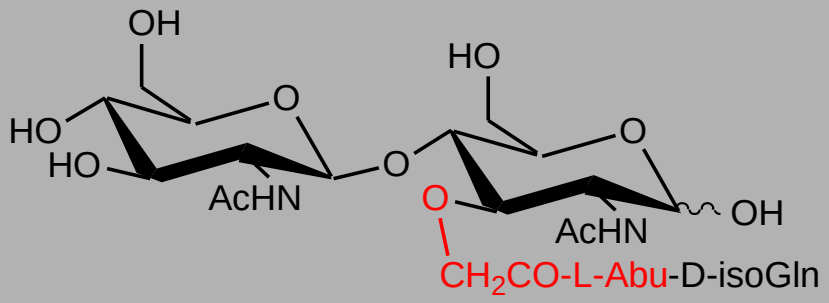
norAbu-MDP

Less pyrogenic and higher immunoadjuvant than MDP



GMDP

Less pyrogenic and higher immunoadjuvant than MDP



norAbu-GMDP

Nonpyrogenic and higher immunoadjuvant than GMDP

Registrated by Russian company Peptek

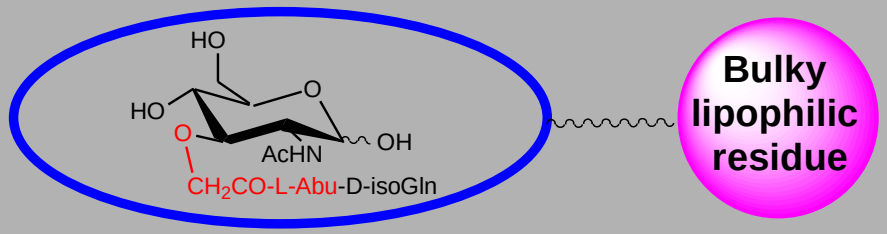
Adjuvans based on lipophilic analogues of norAbuMDP norAbuGMDP

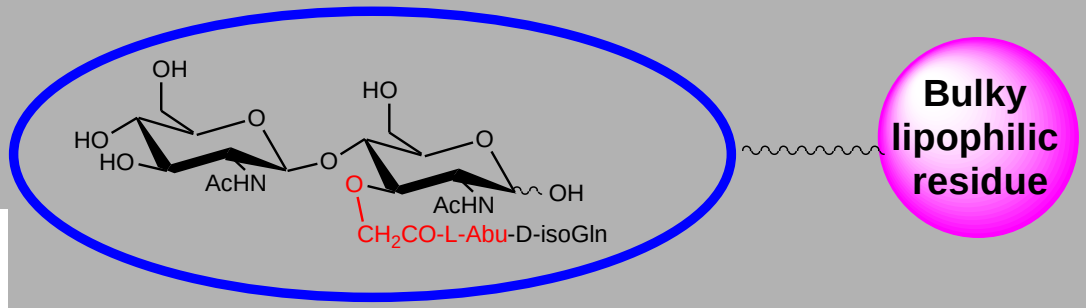
Activity domain

interaction with intracellular receptors

Accesory domain

Incorporation into lipid structures



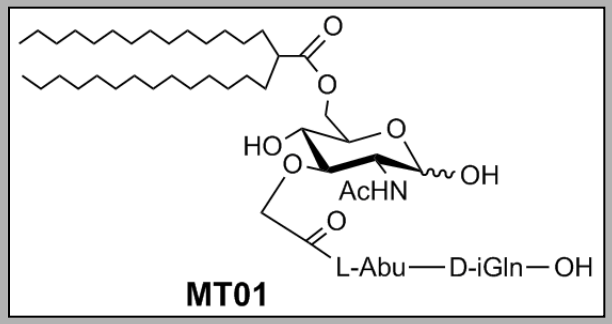


Mendel Therapeutics

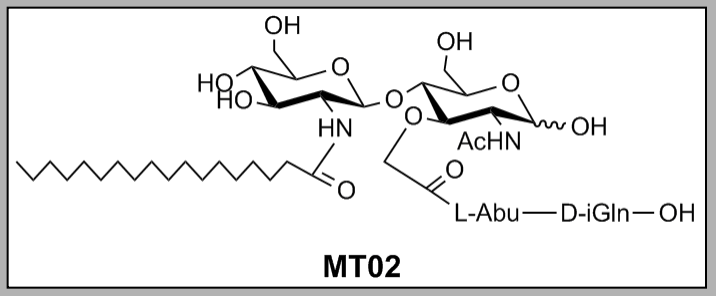
Ledvina M., Ježek J., Hříbalová V., Turánek J.: Liphilic analogues of N-acetyl-normuramyl-L-a-aminobutanoyl-D-isoglutamine withs immunostimulatory activity, Czech Pat. CZ 296 720, **2006**.

Ledvina M., Turánek J., Miller A.D., Hipler K: Adjuvants, International Appl.(PCT. Appl.) No. 0804989.2, 2009.

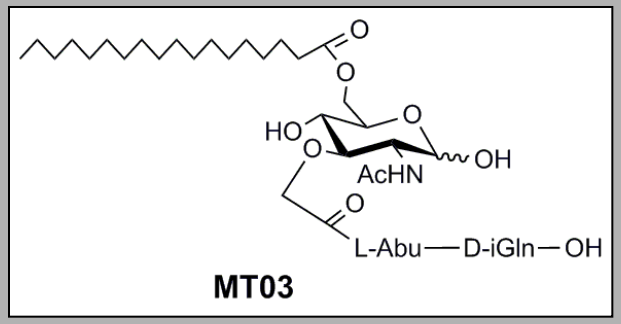
Lipophilic derivatives of norAbuMDP/GMDP



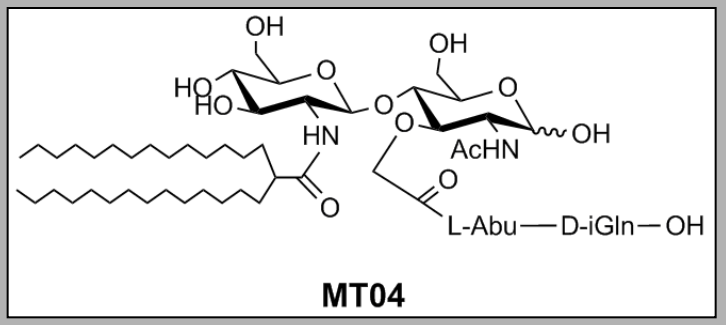
MT01



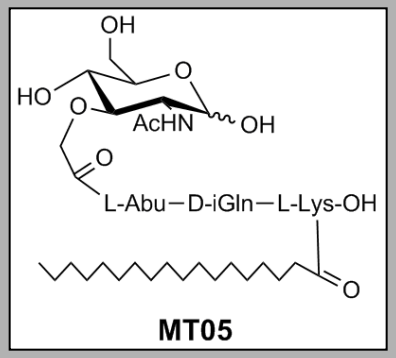
MT02



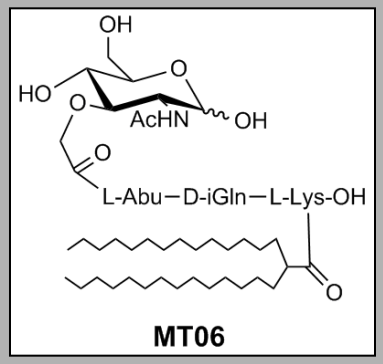
MT03



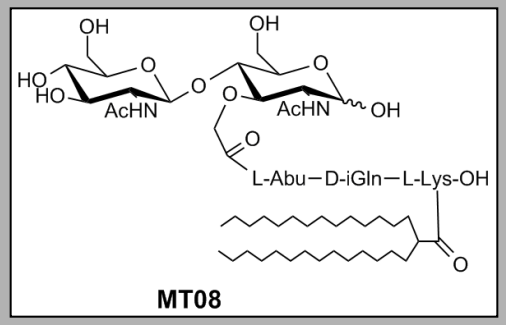
MT04



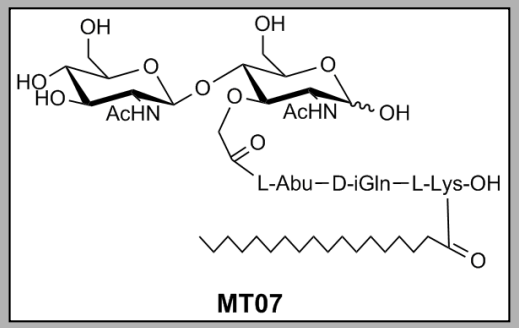
MT05



MT06



MT08



MT07

All Compounds are Non-Pyrogenic

	Tested compounds									
	Lipo-somes	liposomal MDP	free MDP	MT01	23	24	32	33	40	41
+ΔTmax °C	0.2	2.7	2.9	0.5	0.7	0.7	0.5	0.4	0.6	0.8

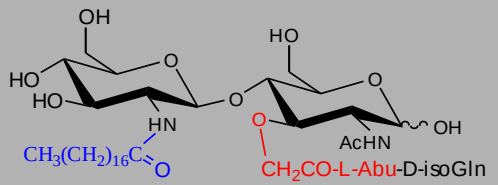
Data: Rabbit

Dose: 100nmol/kg (ca 0.1mg/kg) NMGP s.c.

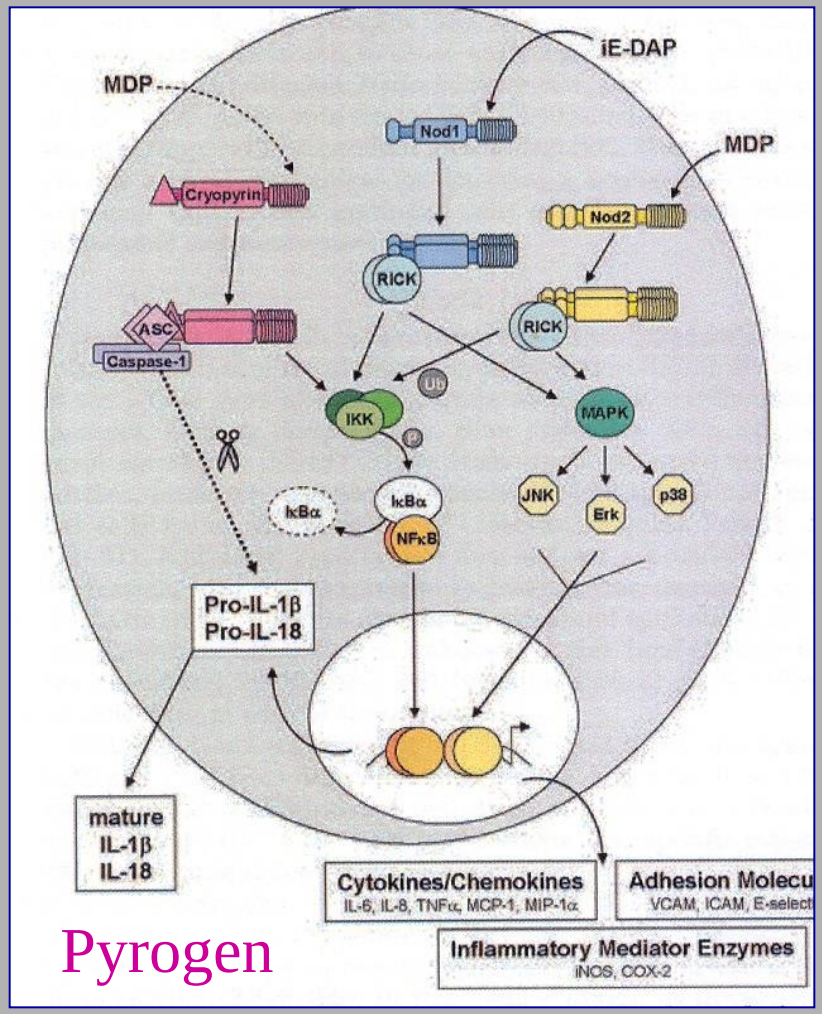
Temperature was measured in 1, 2, 3, 4, 6 and 24 hour
20 intervals.
The preparation passes the test if the Sum $+\Delta T_{max} < 1.1$ °C
(3 rabbits per group)

Structural subunits of peptidoglycan and their intracellular receptors

Hypothesis - ***Low affinity of Nor-abu-MDP/GMDP for Cryopyrin could be responsible for low pyrogenicity of some derivatives of MDP***



N-L18-norAbuGMDP



Pyrogen

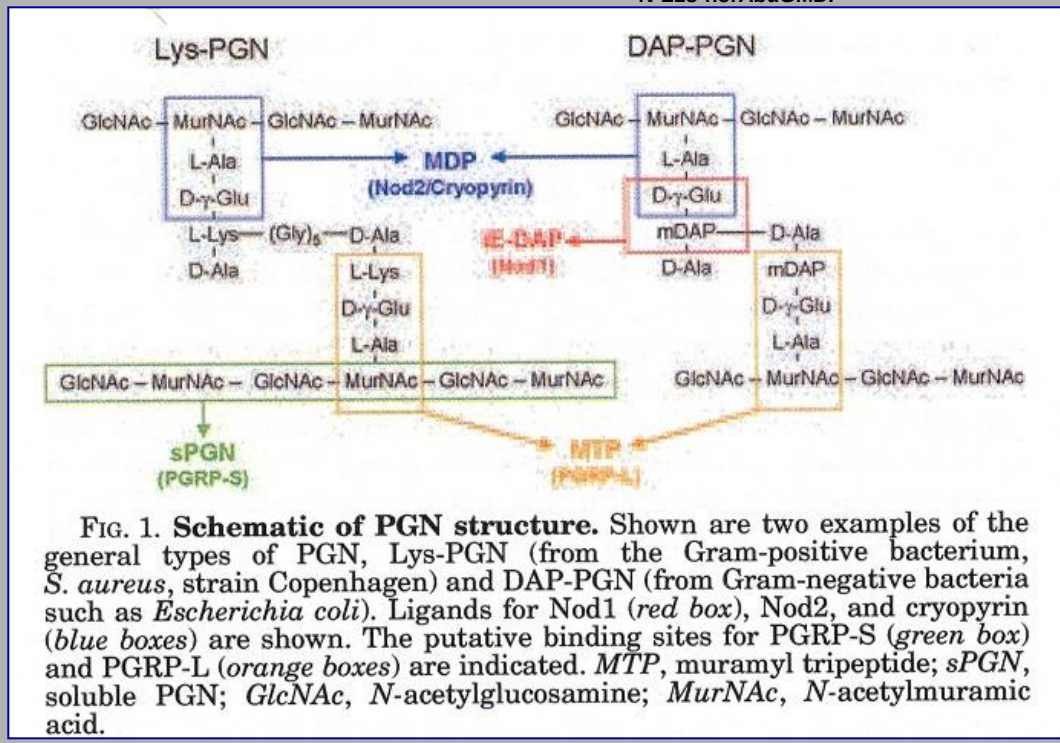
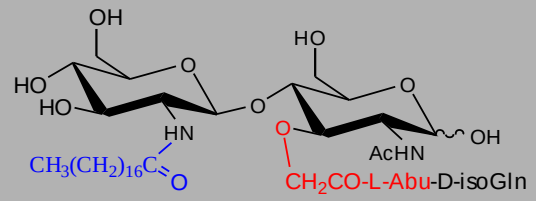


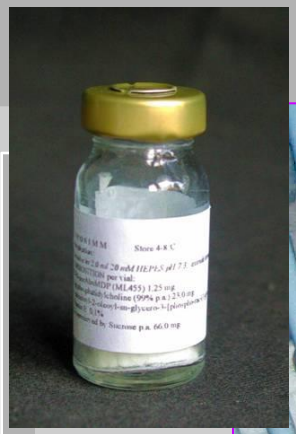
FIG. 1. **Schematic of PGN structure.** Shown are two examples of the general types of PGN, Lys-PGN (from the Gram-positive bacterium, *S. aureus*, strain Copenhagen) and DAP-PGN (from Gram-negative bacteria such as *Escherichia coli*). Ligands for Nod1 (*red box*), Nod2, and cryopyrin (*blue boxes*) are shown. The putative binding sites for PGRP-S (*green box*) and PGRP-L (*orange boxes*) are indicated. *MTP*, muramyl tripeptide; *sPGN*, soluble PGN; *GlcNAc*, *N*-acetylglucosamine; *MurNAc*, *N*-acetylmuramic acid.

Antimicrobial/anticancer peptides

Stimulation of innate immunity of newborn kids against *Cryptosporidium parvum*

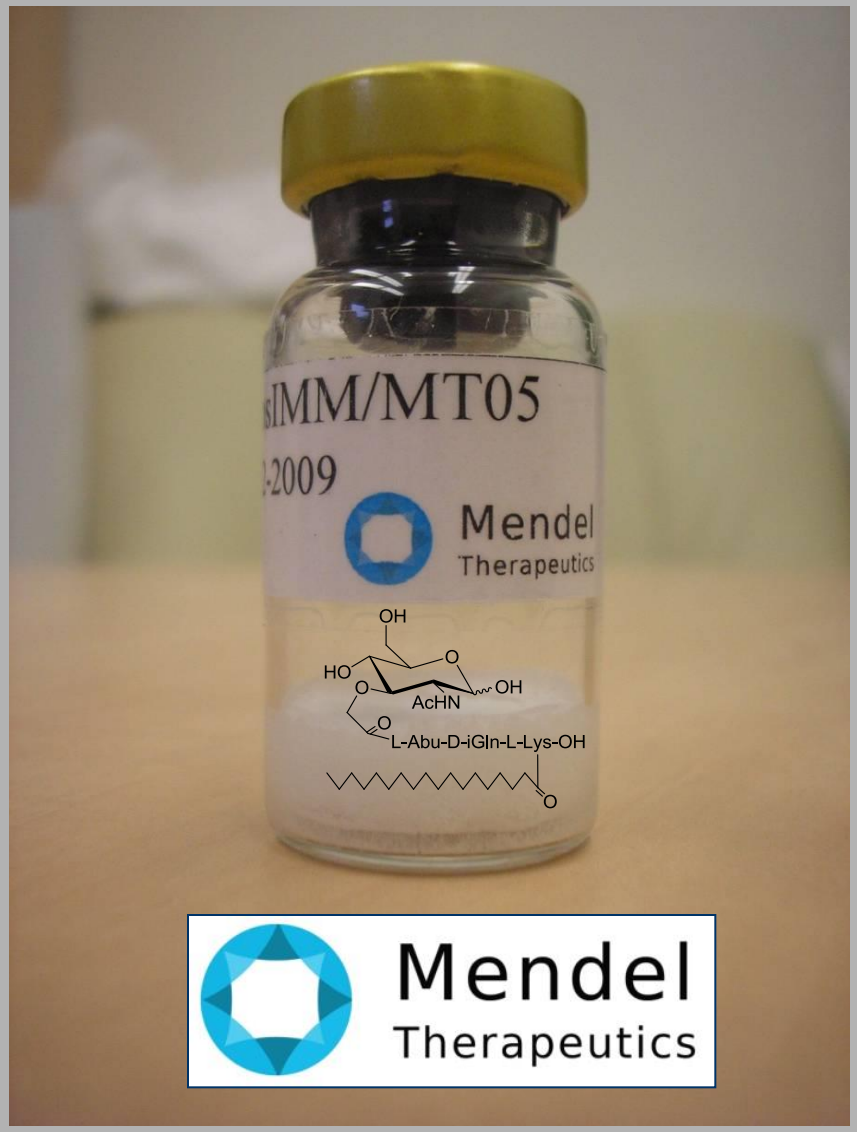


N-L18-norAbuGMDP



J. Turánek et. al. Stimulation of innate immunity of newborn kids against ***Cryptosporidium parvum*** by application of immunomodulator ß-D-GlcNstearoyl-(1→ 4)-norMurNAc-L-Abu-D-isoGln entrapped in liposomes. ***Parasitology*** *2005* **131: 601-608**

Lyophilised liposomal formulation of immunomodulator NorAbuMDP LiposIMM/MT05



Liposomal vaccines

Biodegradable and nontoxic carriers for preparation of **corpuscular antigens**

Simple incorporation or covalent attachment of peptide (T and B epitops) and protein antigens to the membrane or internal water phase

Preservation of natural conformation of membrane protein antigens

Liposomal vaccines

Simultaneous incorporation of antigens and adjuvatns in one structure (MPLA, lipoproteins, glucans, MDP analogues, cytokines)

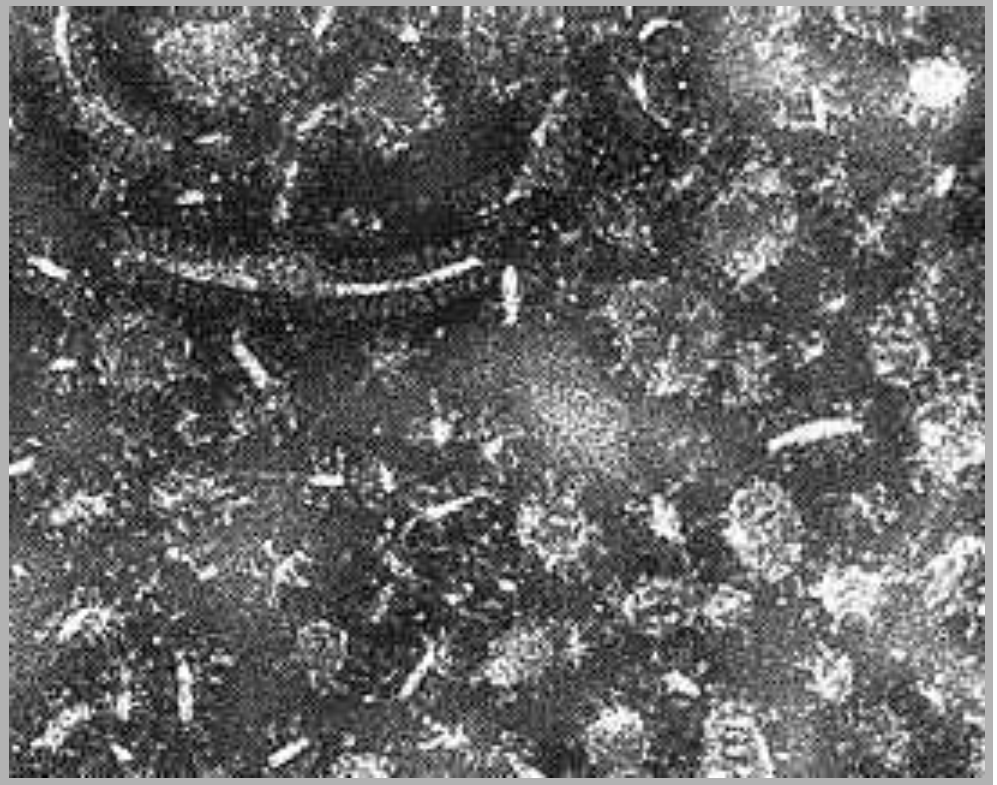
Systemic, **intradermal** and **mucosal** application

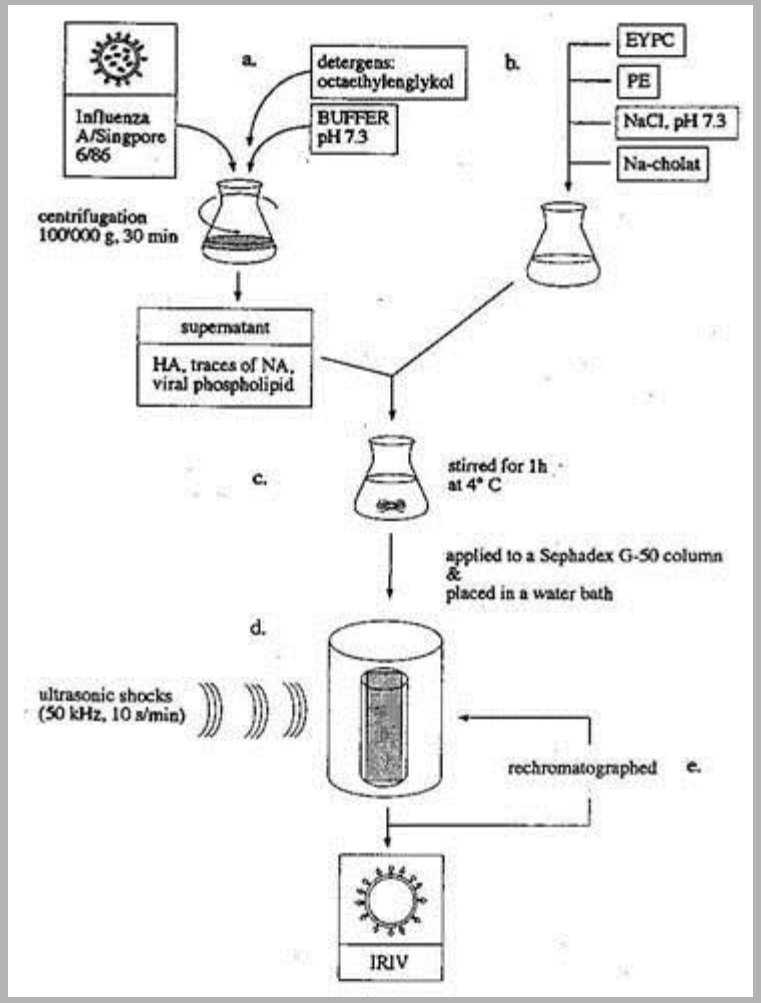
Oral delivery

Control of the MHC I or MHC II antigen presentation is possible (TH1/2 immune)

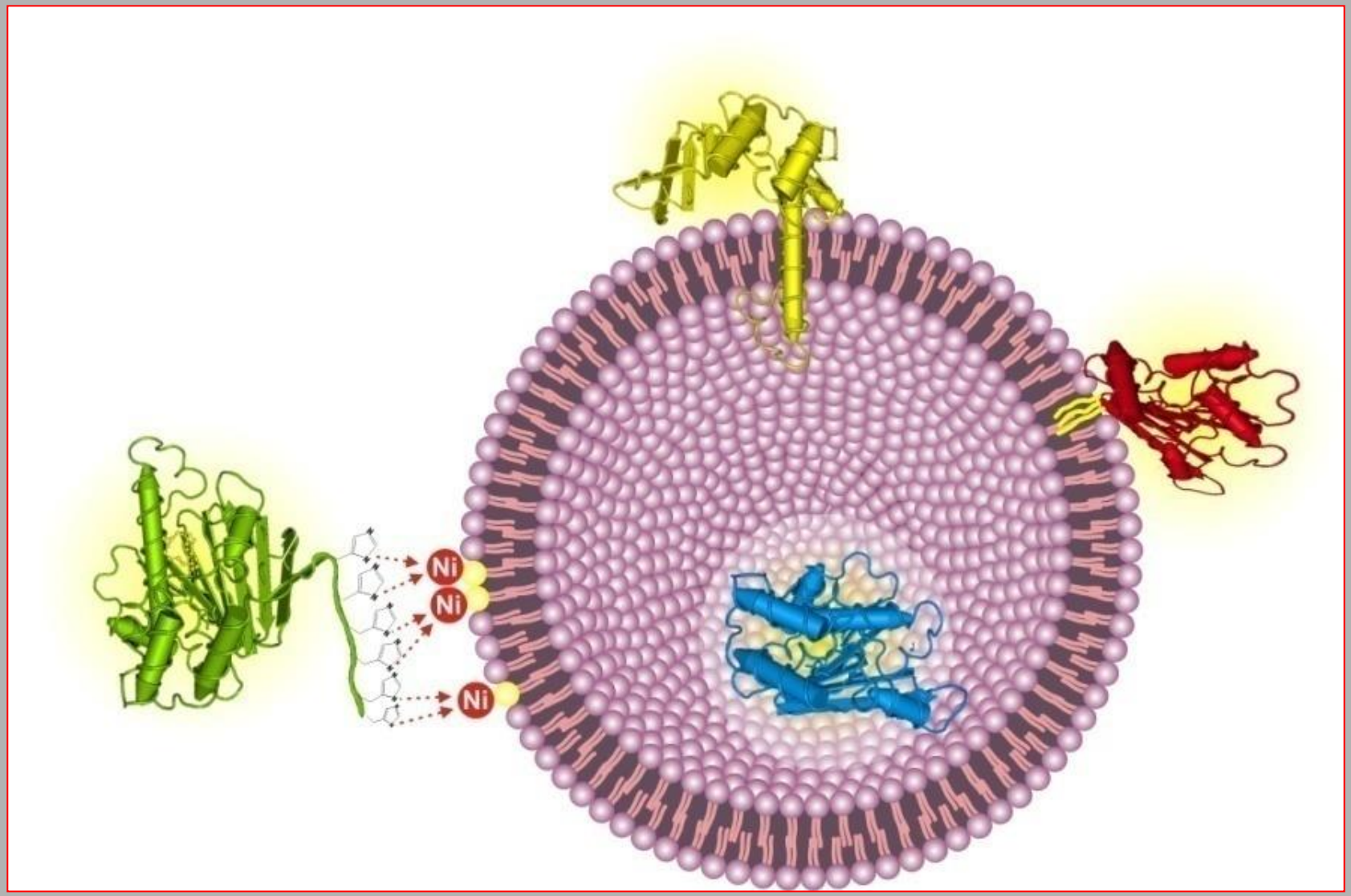
Preparation of IRIV vaccine
Epaxal-Berna

TEM micrograph

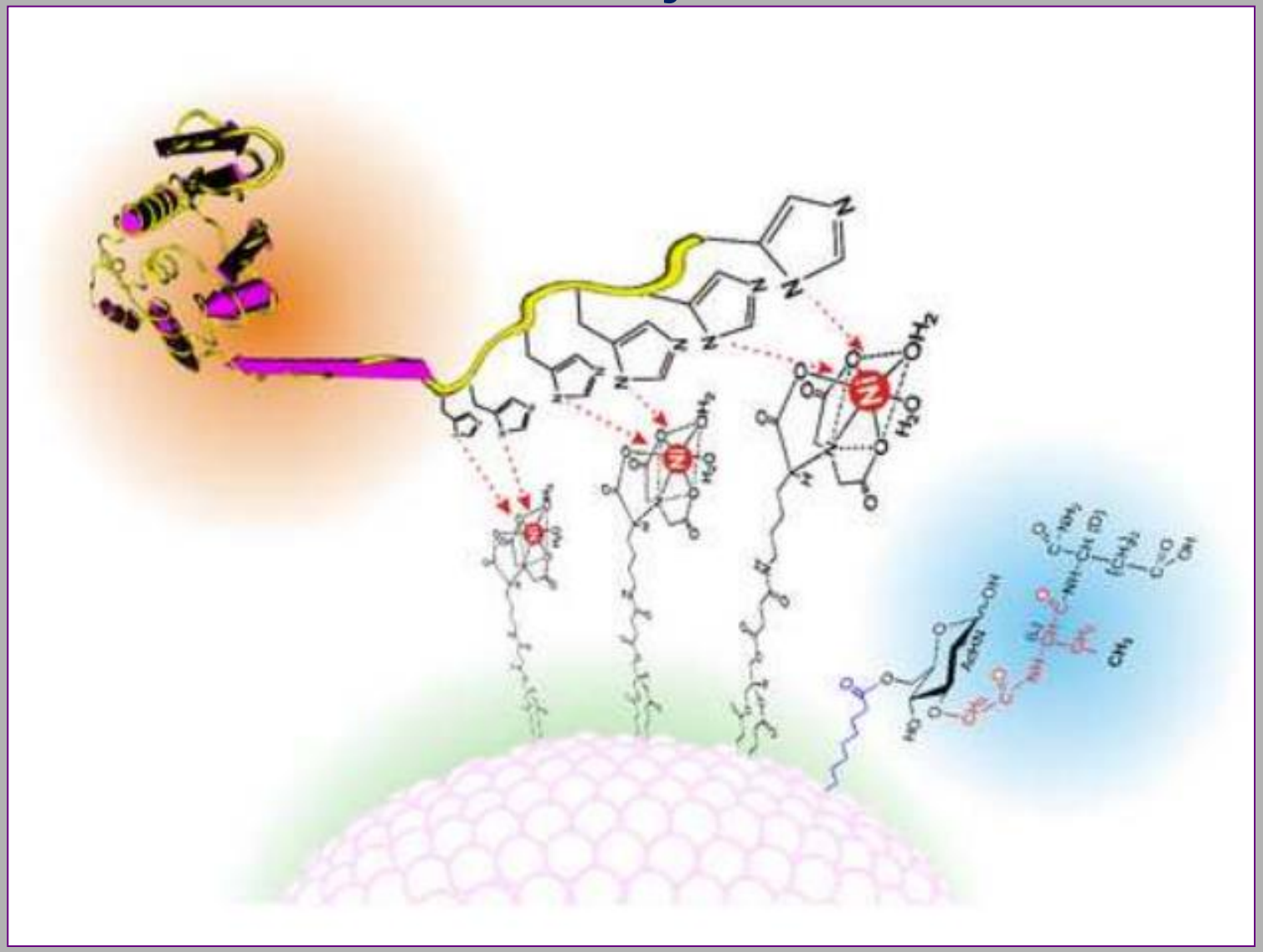




Association of protein antigen with liposome



Liposome based self-assembling immunogenic nanosystems



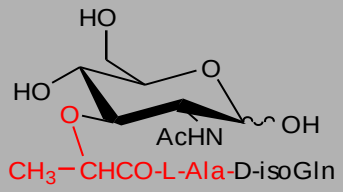
Adjuvans based on lipophilic analogues of norAbuMDP norAbuGMDP

Activity domain

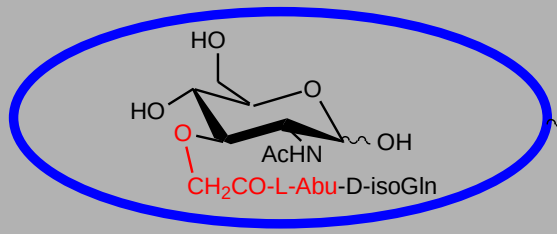
interaction with intracellular receptors

Accesory domain

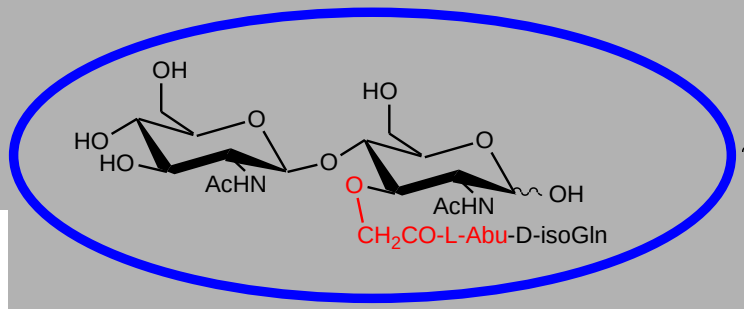
Incorporation into lipid structures



MDP



Bulky lipophilic residue



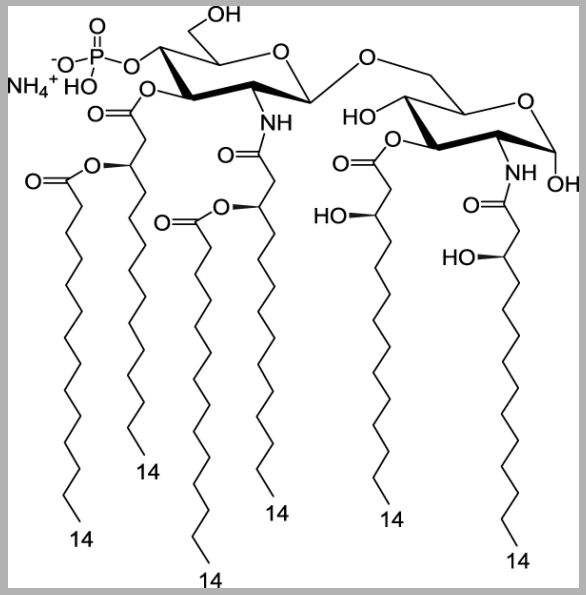
Bulky lipophilic residue

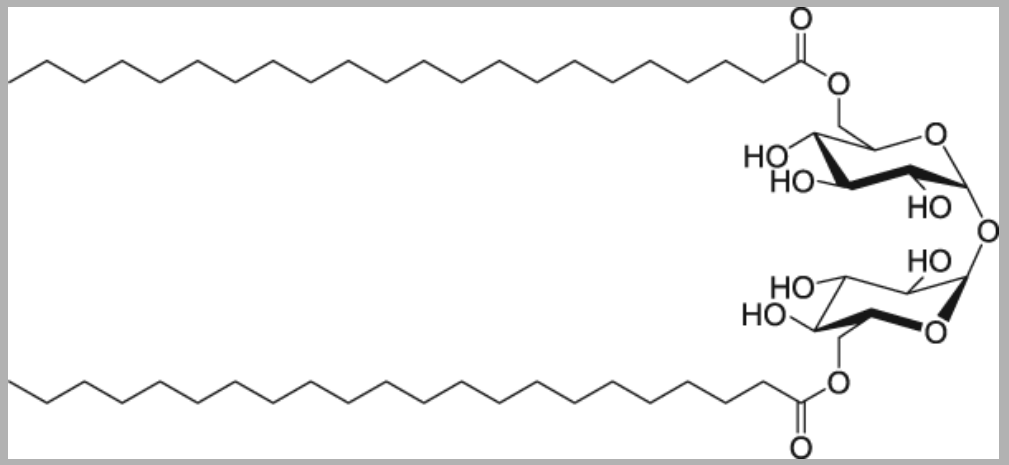
Mendel Therapeutics

Ledvina M., Ježek J., Hříbalová V., Turánek J.: Liphilic analogues of N-acetyl-normuramyl-L-a-aminobutanoyl-D-isoglutamine withs immunostimulatory activity, Czech Pat. CZ 296 720, **2006**.

Ledvina M., Turánek J., Miller A.D., Hipler K: Adjuvants, International Appl.(PCT. Appl.) No. 0804989.2, 2009. **EU and USA patent application** September 2010

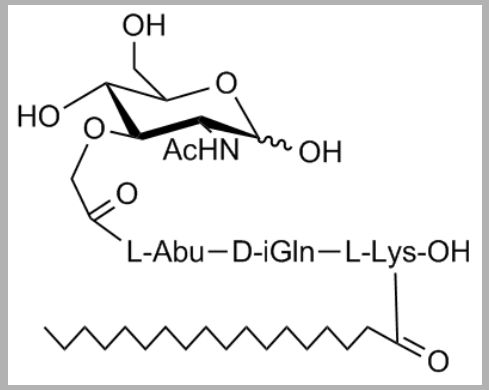
New lipophilic adjuvants for liposomal vaccines



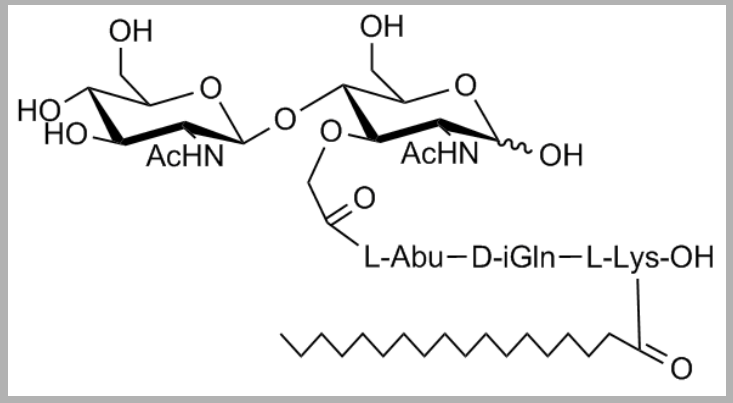


Monophosphoryl Lipid A (MPL A)

D-(+)-trehalose 6,6'-dibehenate (TDB)

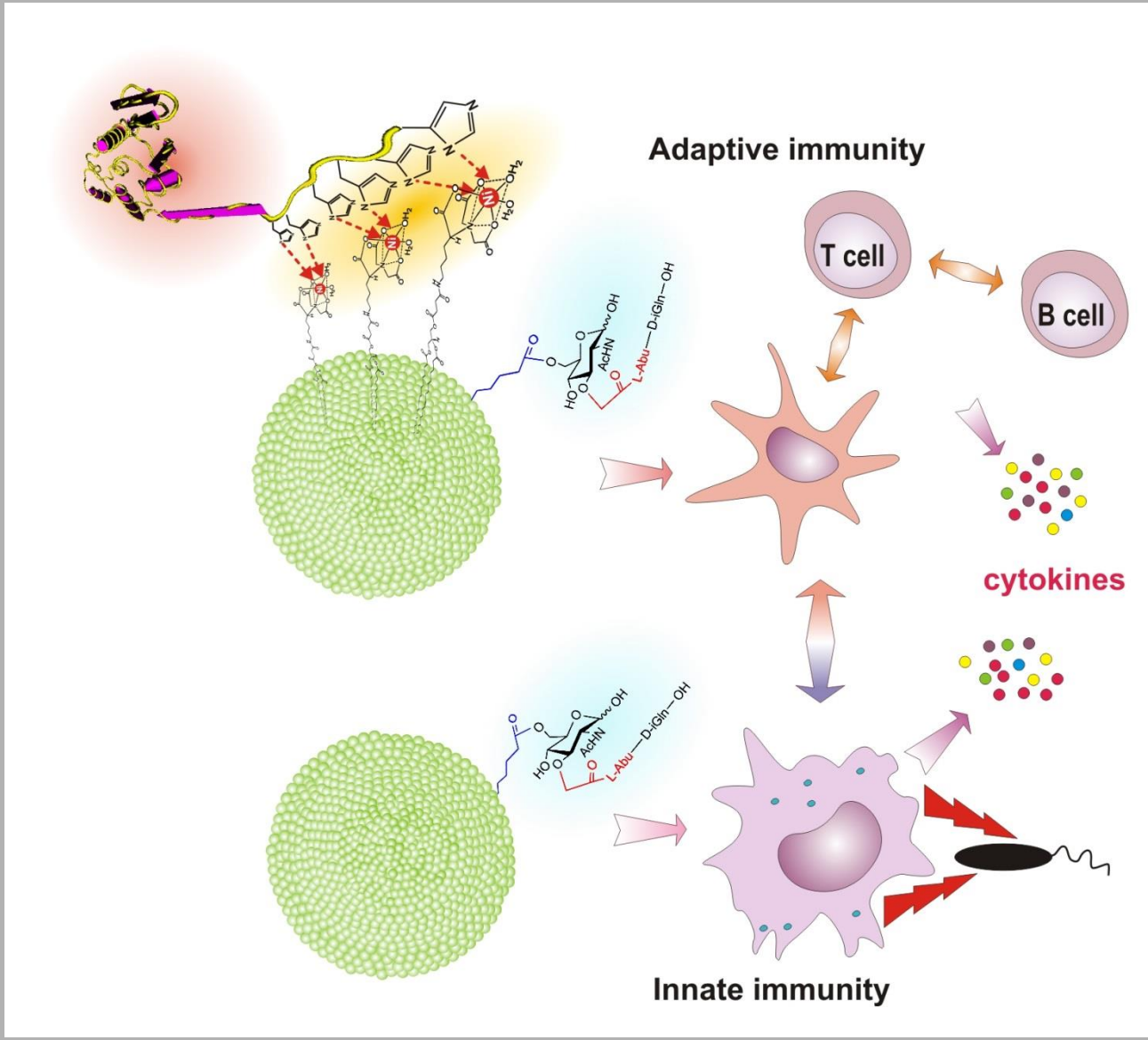


nor-AbuMDP



nor-AbuGMDP

Role of molecular adjuvants

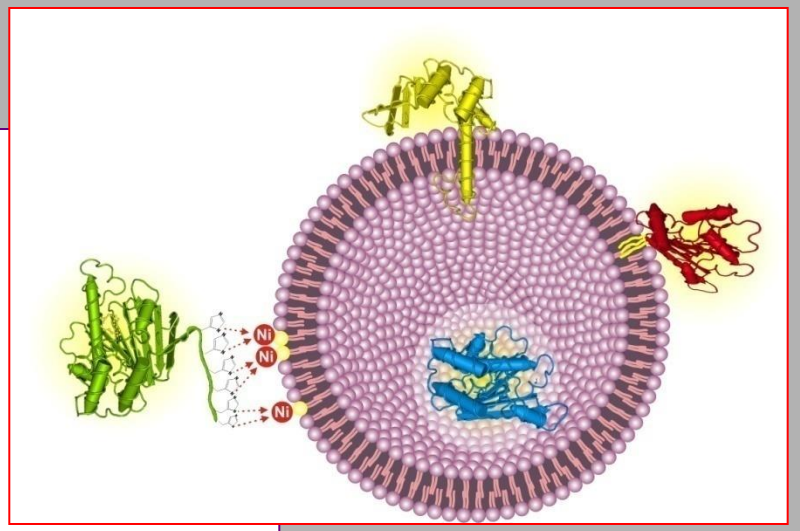


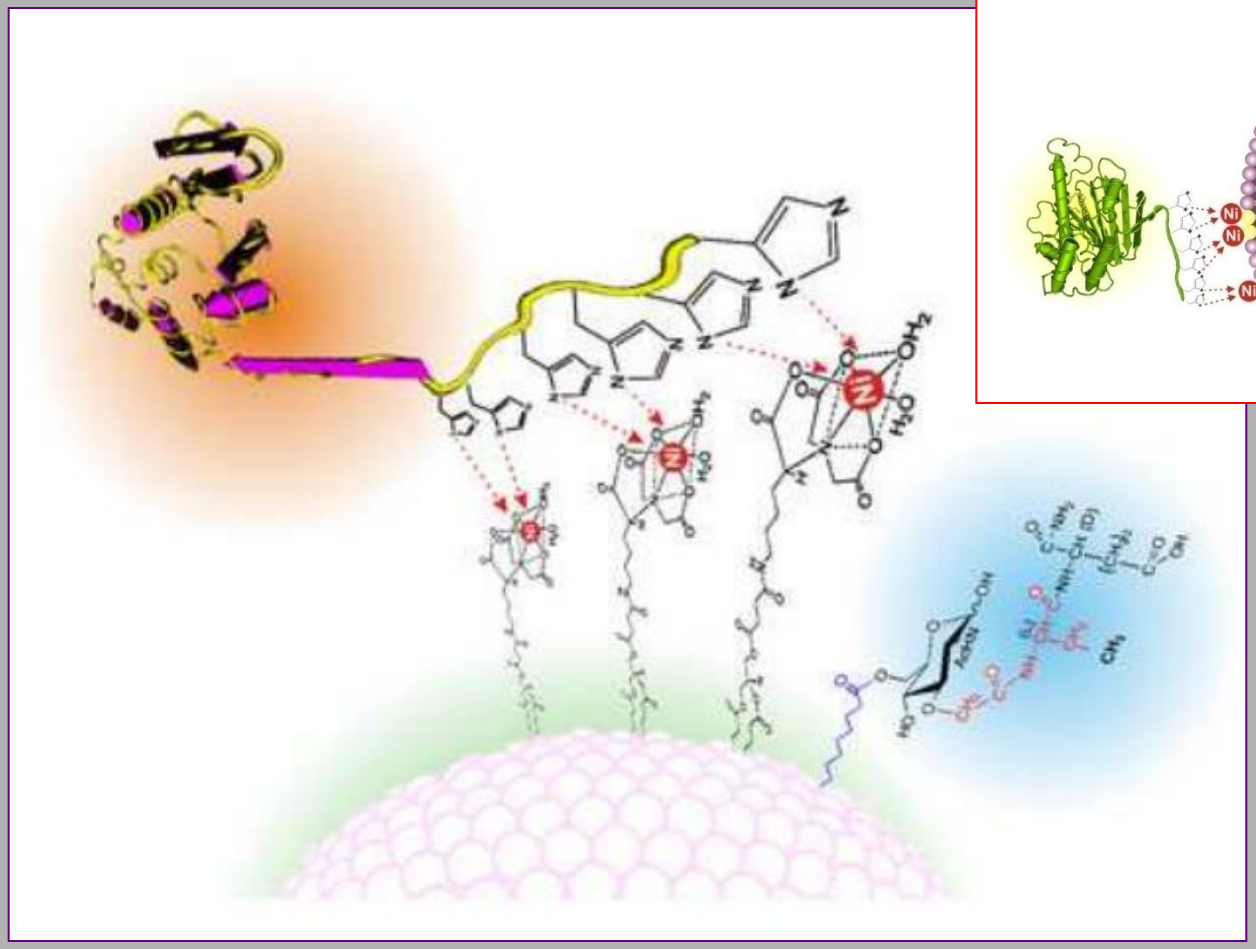
Liposome based self-assembling immunogenic nanosystems

Surface modification- targeting

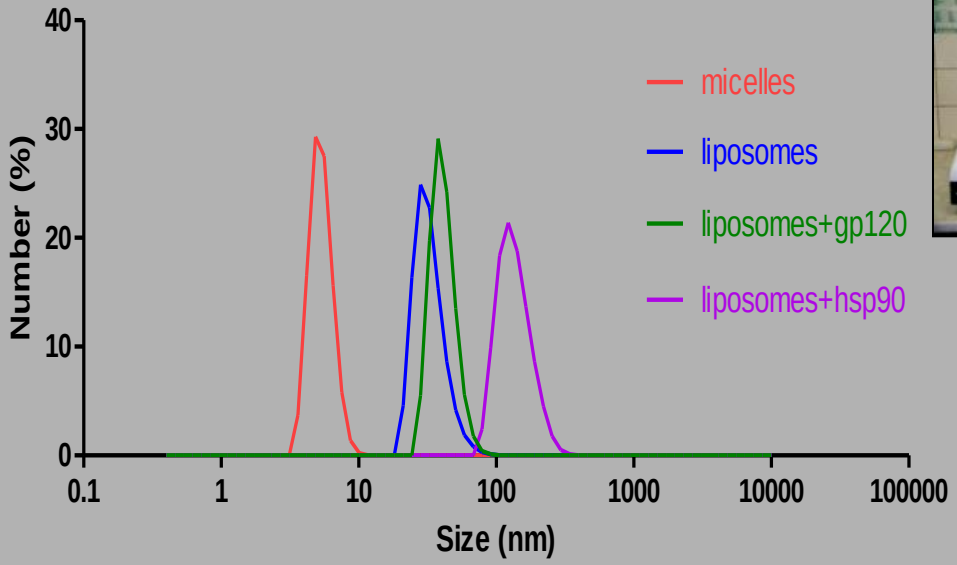
Hyaluronic acid, chitosan, cationic lipids, glycolypids, dendrimers, functionalised lipids – covalent and noncovalent binding of antigens

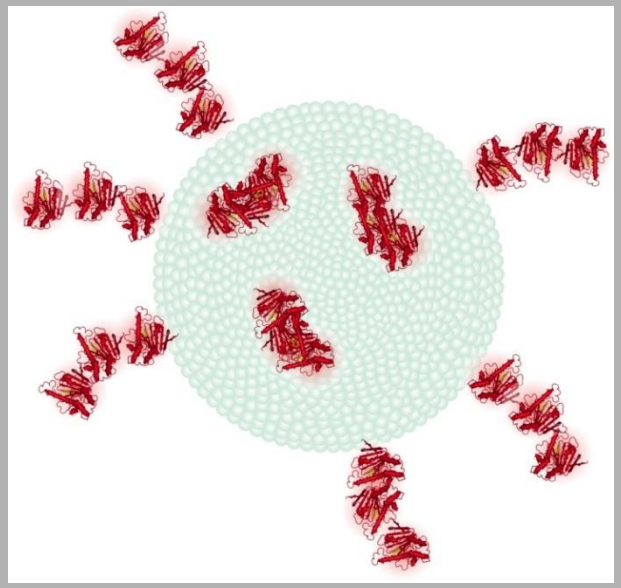
Synthetic and structuraly defined adjuvans
(CpG, MPL-A, lipophilic MDP analogues)

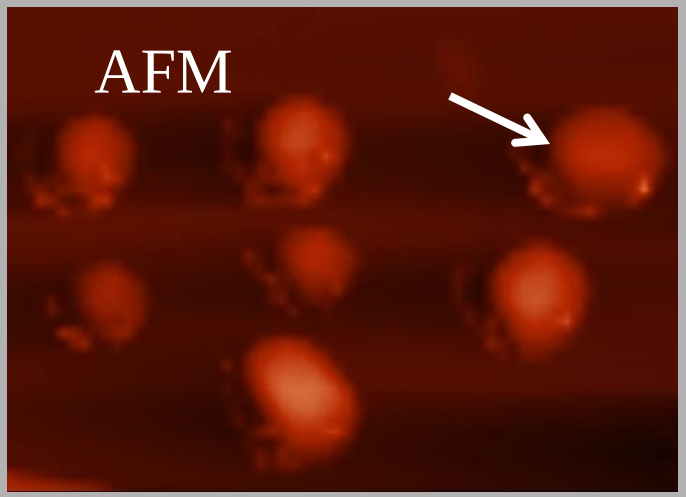


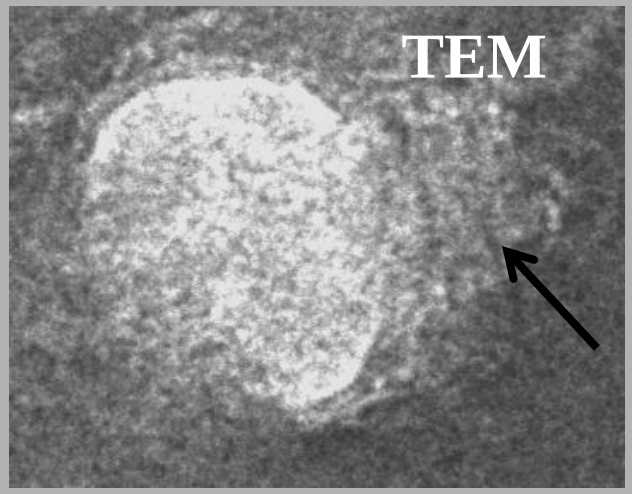


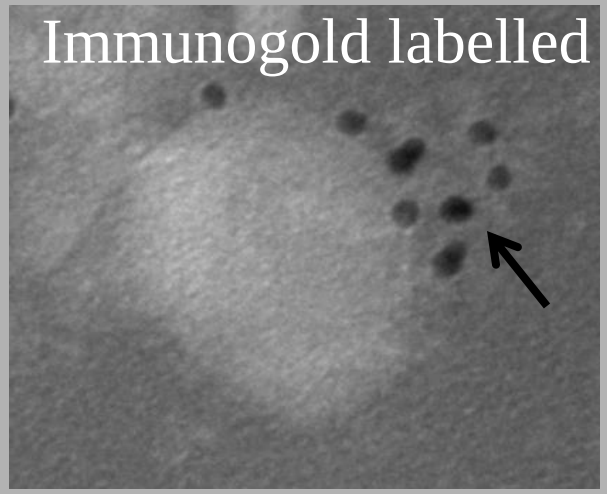
AF microscopy and TEM pictures of rHSP90 metallochelating liposomes





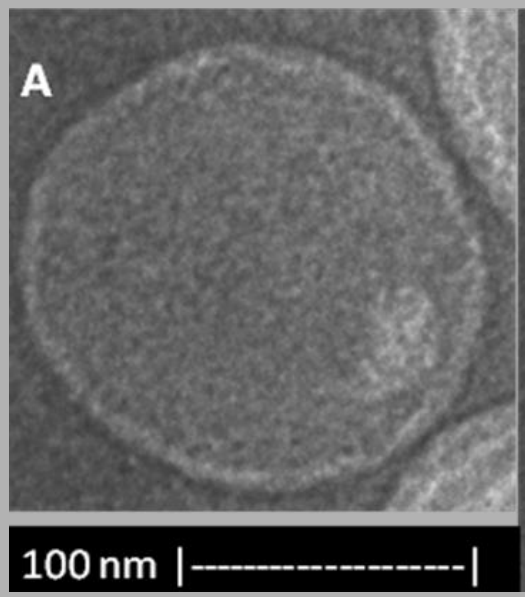


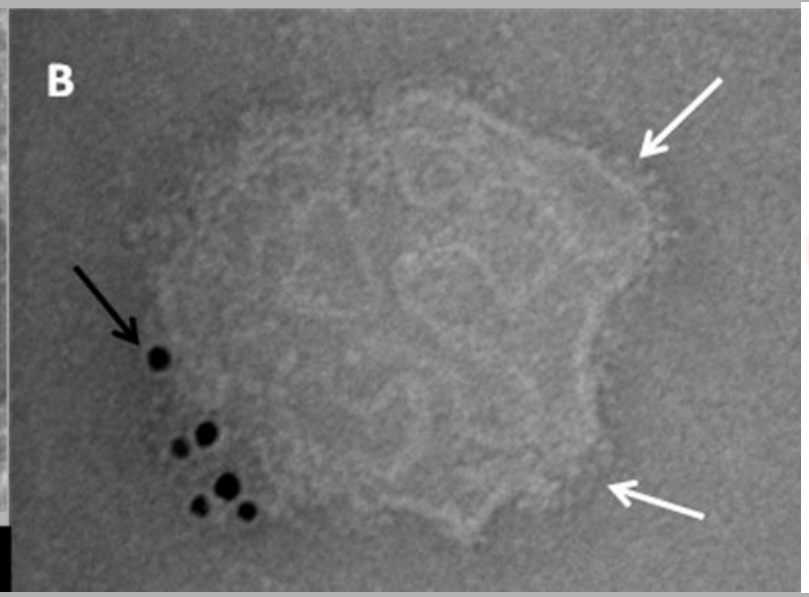


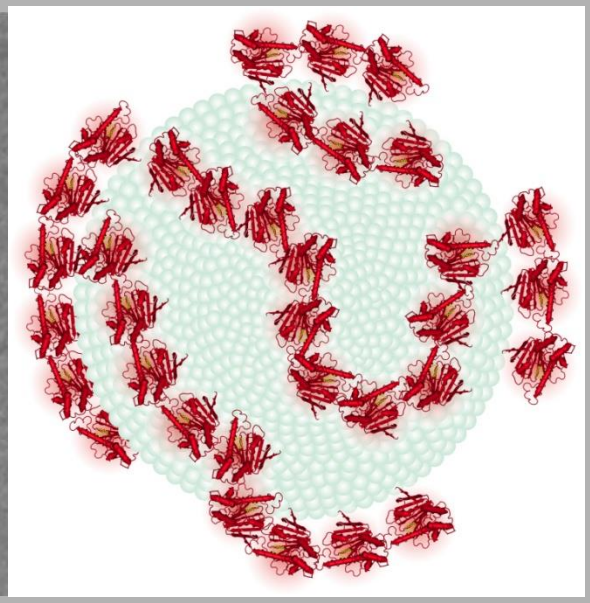


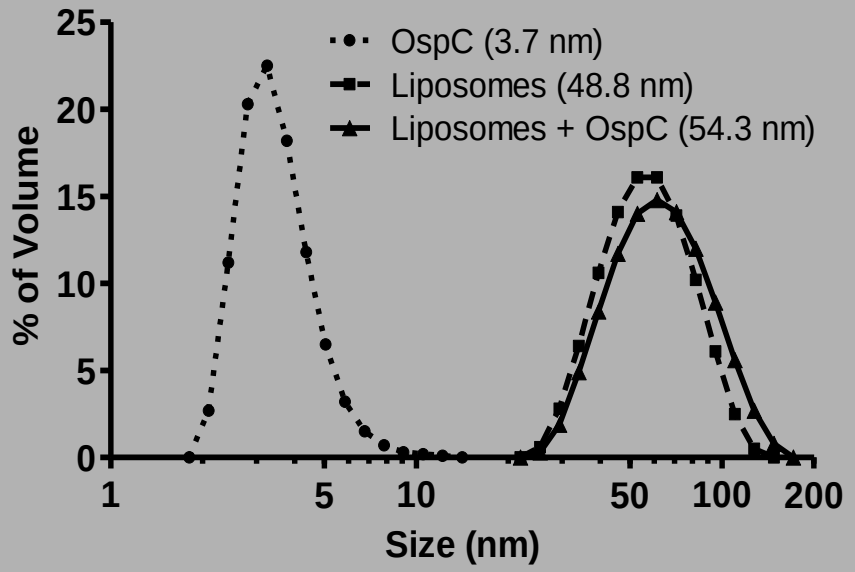
Mašek et al. J. Control. Release, 2011, 151 (2), 193-201.

TEM of liposomal bound rOsp C

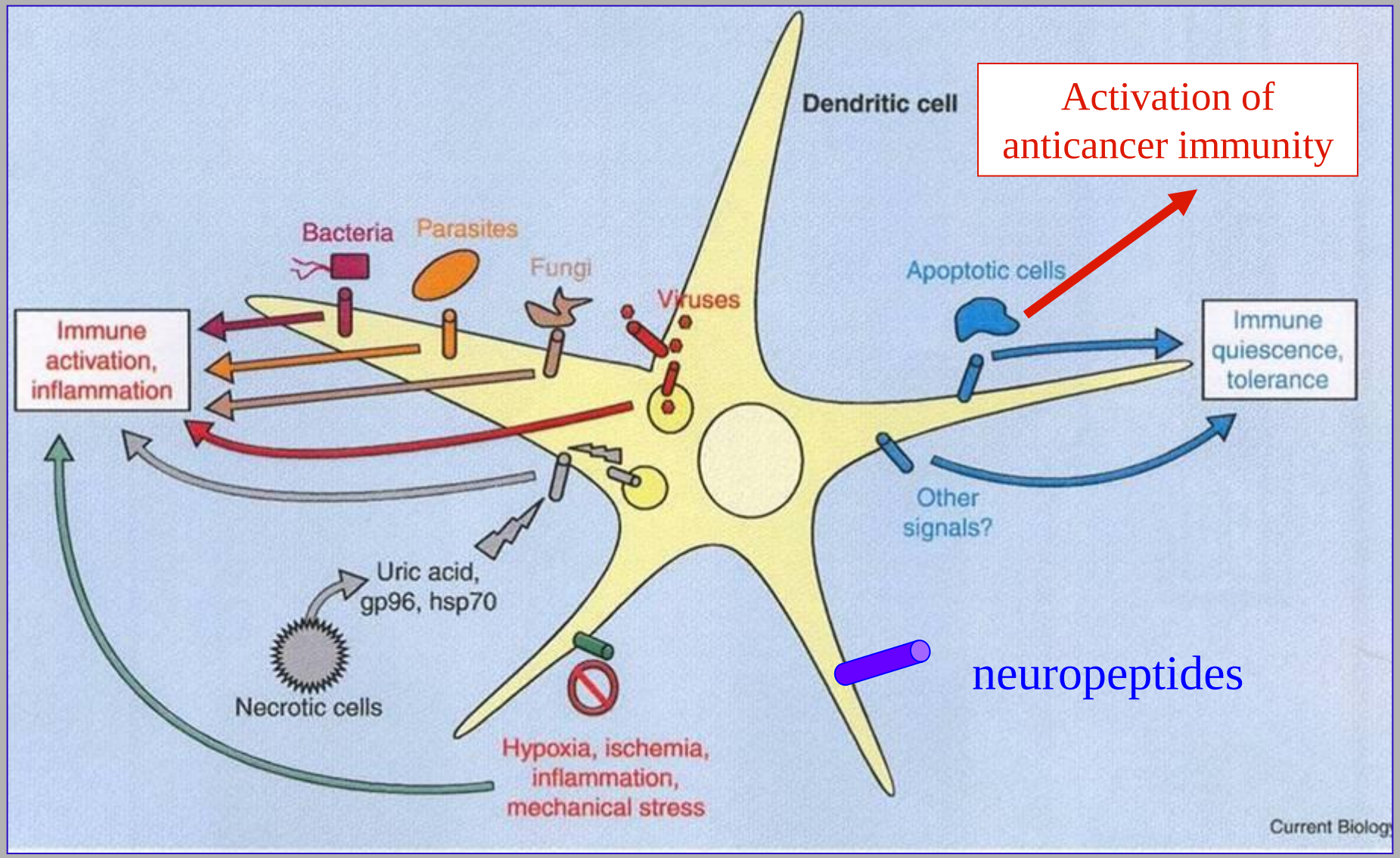




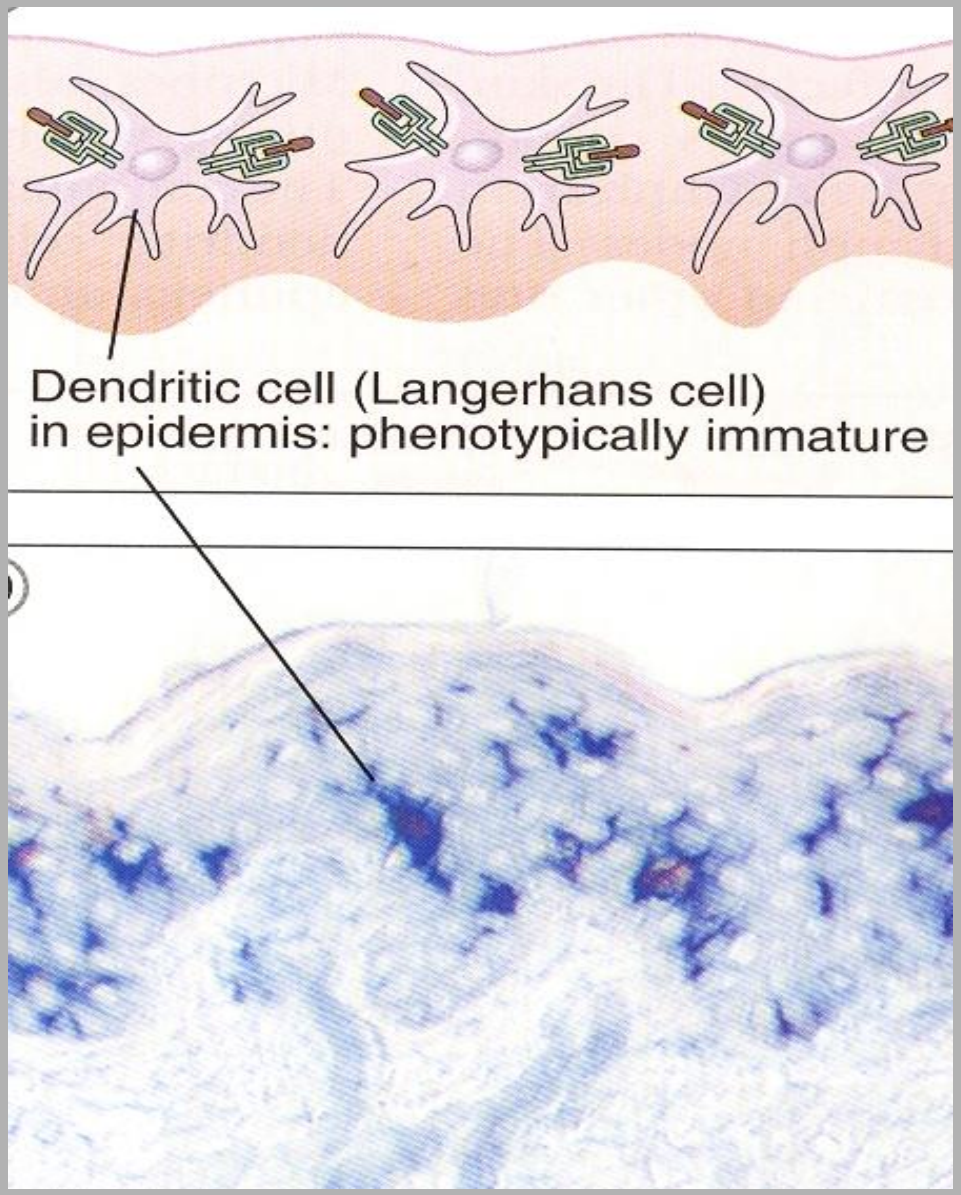




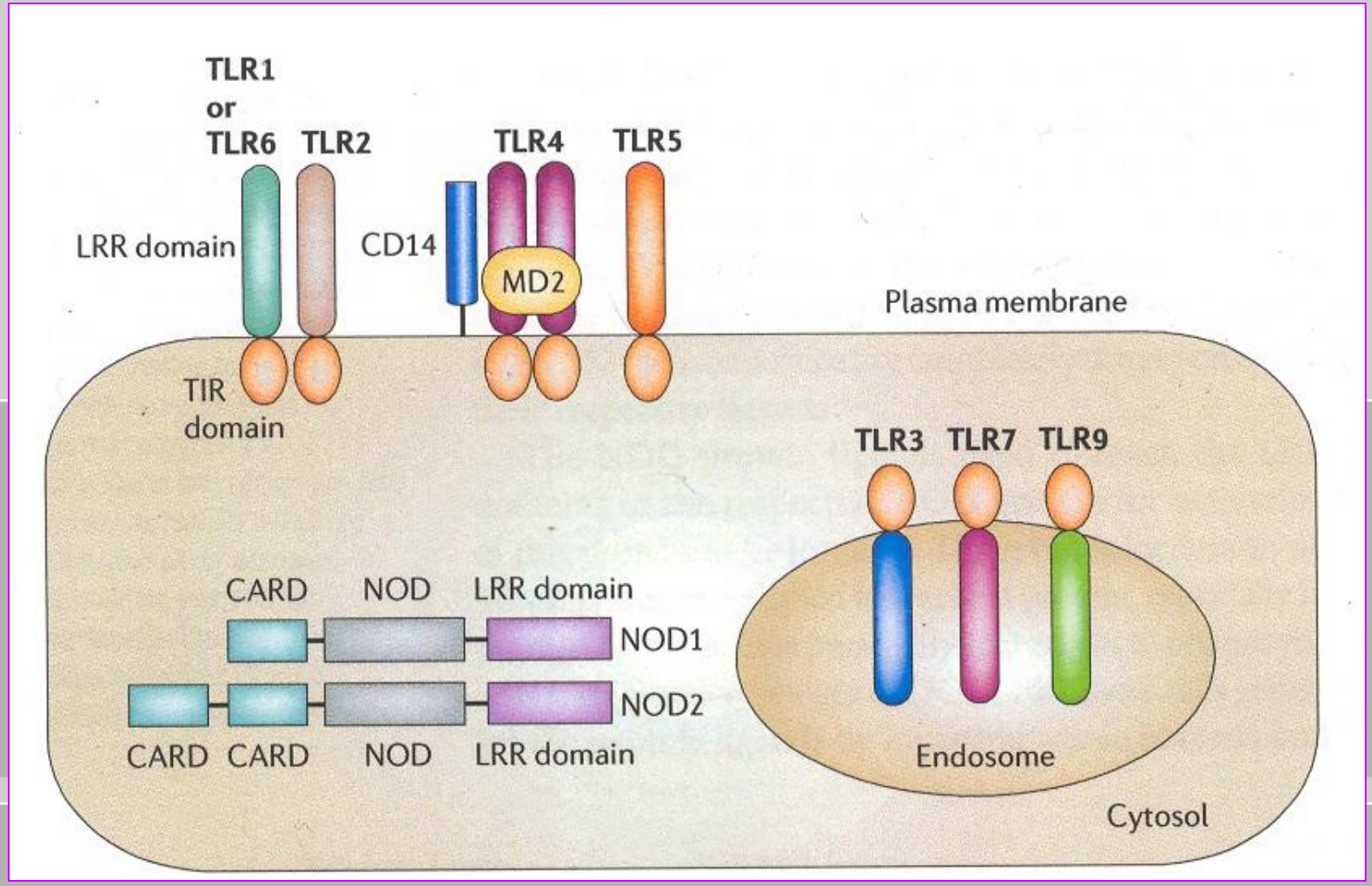
Dendritic cell– presentation of antigen, regulation and direction of immune response



Intradermal immunisation

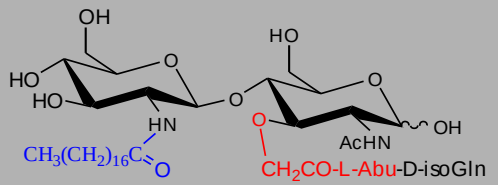


Structure and cellular location of TLRs and NOD1 and NOD2

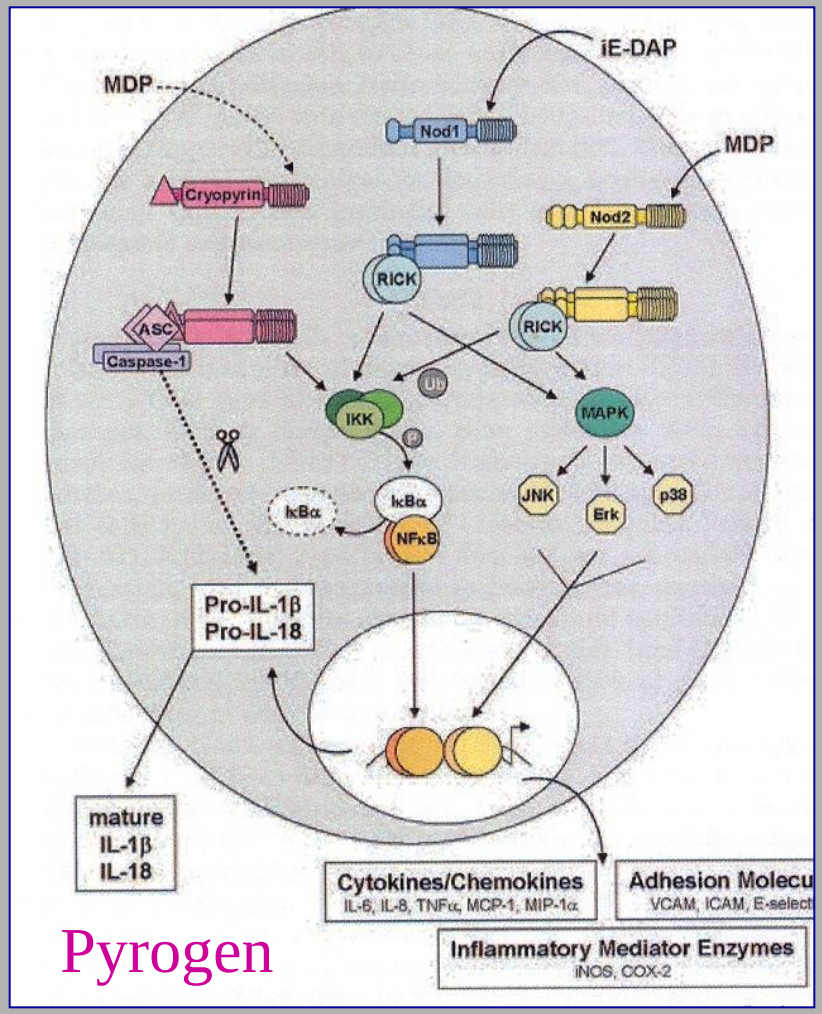


Structural subunits of peptidoglycan and their intracellular receptors

Hypothesis - ***Low affinity of Nor-abu-MDP/GMDP for Cryopyrin could be responsible for low pyrogenicity of some derivatives of MDP***



N-L18-norAbuGMDP



Pyrogen

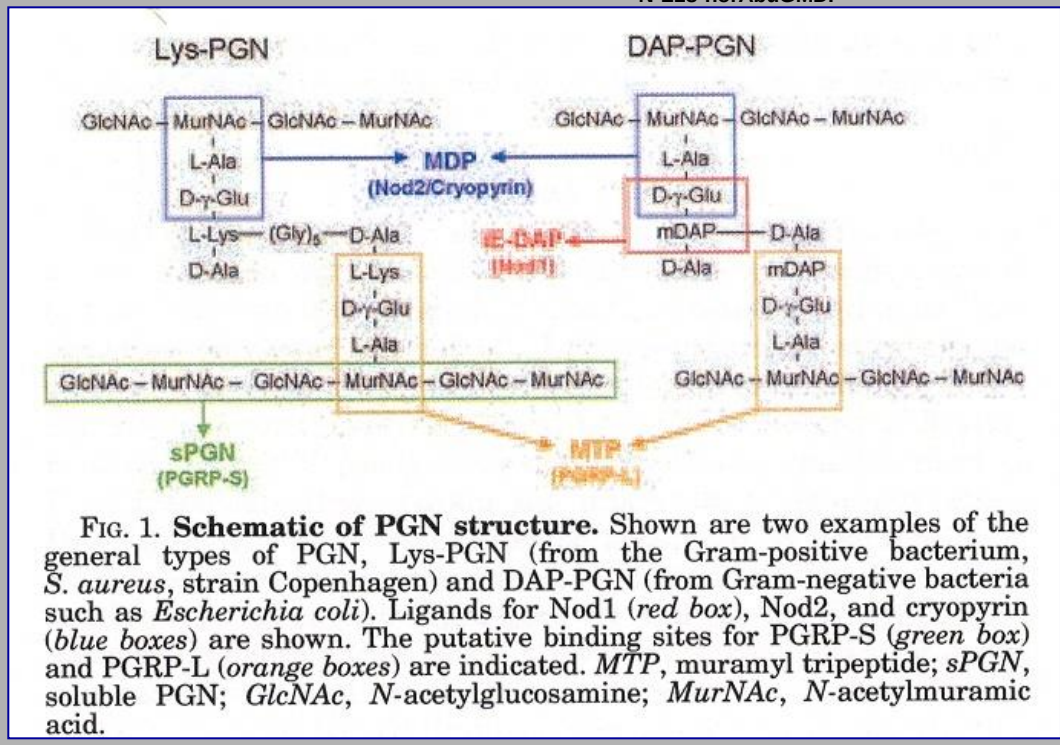


FIG. 1. **Schematic of PGN structure.** Shown are two examples of the general types of PGN, Lys-PGN (from the Gram-positive bacterium, *S. aureus*, strain Copenhagen) and DAP-PGN (from Gram-negative bacteria such as *Escherichia coli*). Ligands for Nod1 (*red box*), Nod2, and cryopyrin (*blue boxes*) are shown. The putative binding sites for PGRP-S (*green box*) and PGRP-L (*orange boxes*) are indicated. *MTP*, muramyl tripeptide; *sPGN*, soluble PGN; *GlcNAc*, *N*-acetylglucosamine; *MurNAc*, *N*-acetylmuramic acid.

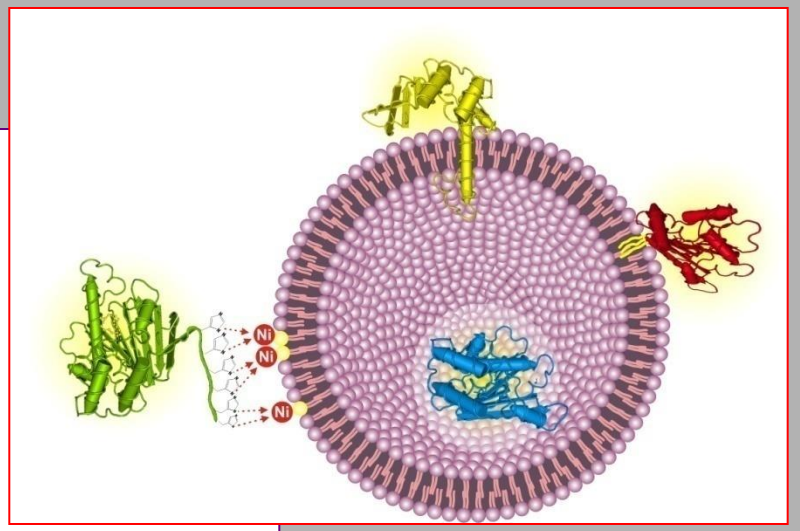
Antimicrobial/anticancer peptides

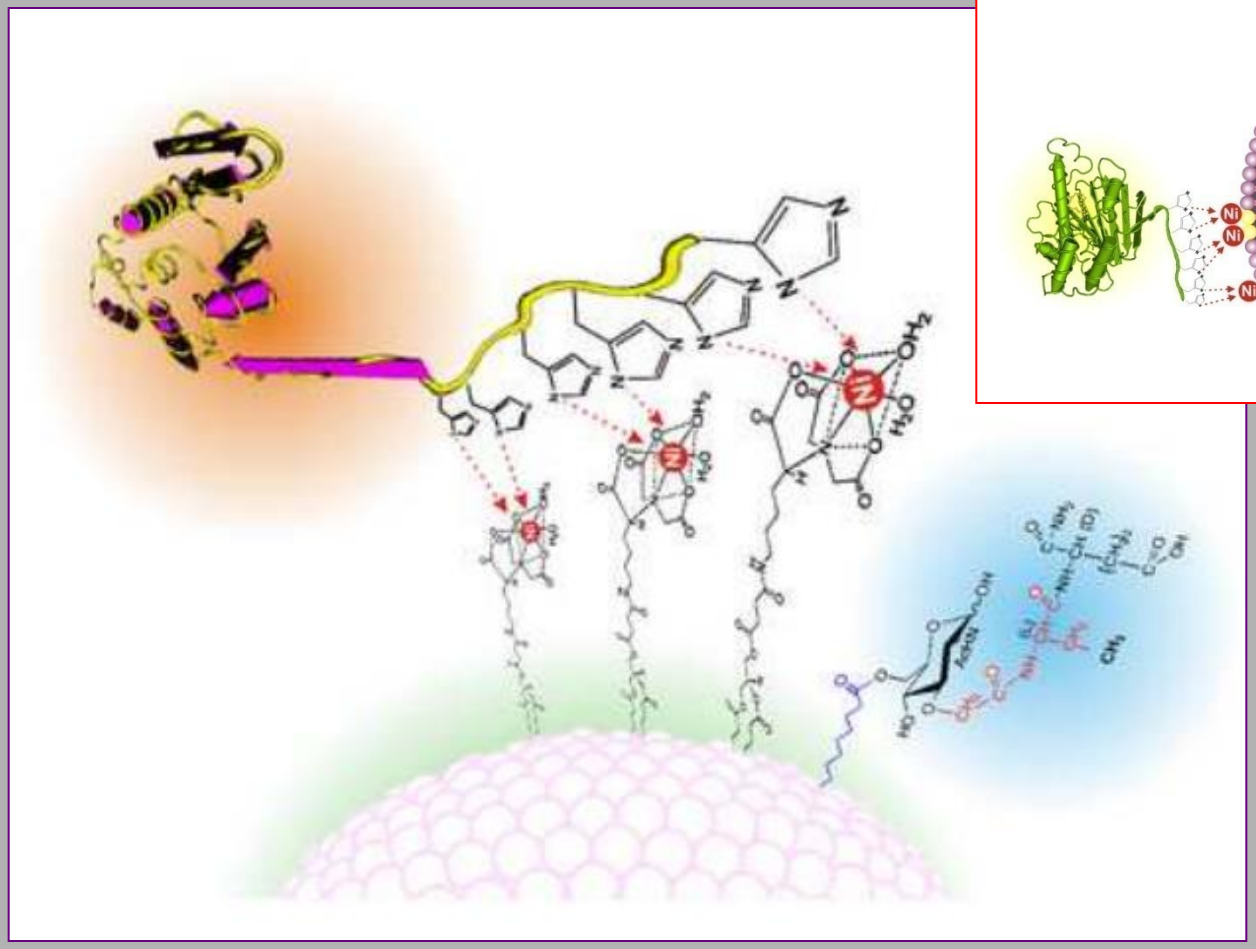
Liposome based self-assembling immunogenic nanosystems

Surface modification- targeting

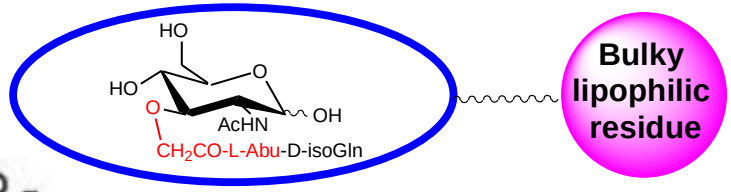
Hyaluronic acid, chitosan, cationic lipids, glycolypids, dendrimers, functionalised lipids – covalent and noncovalent binding of antigens

Synthetic and structuraly defined adjuvans
(CpG, MPL-A, lipophilic MDP analogues)

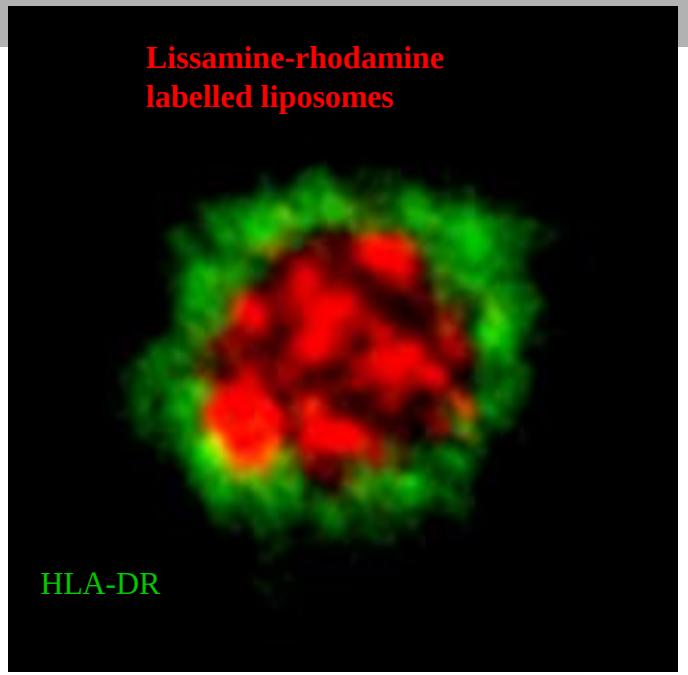




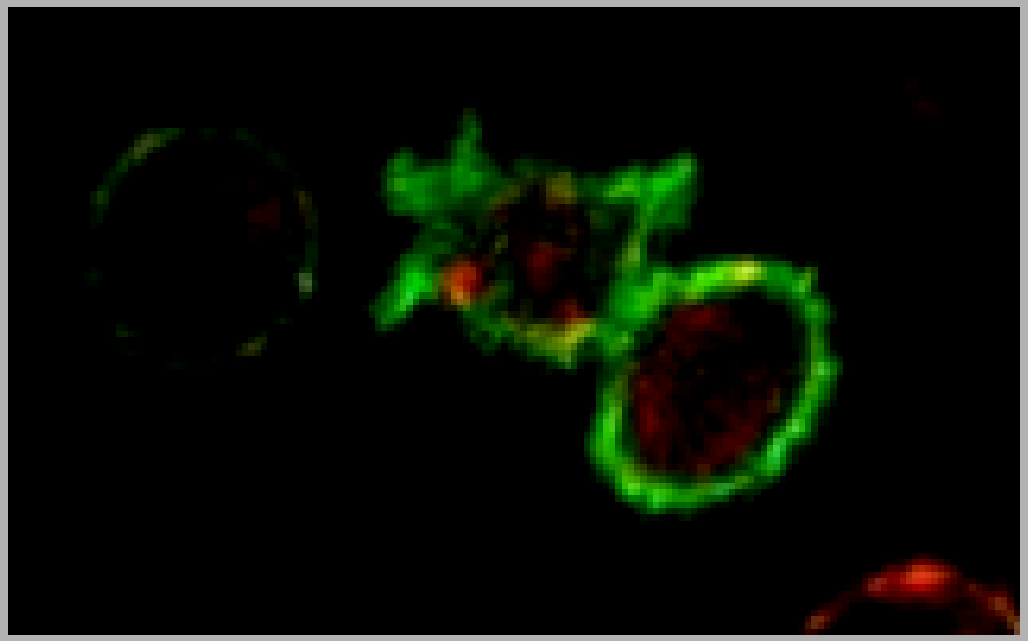
Confocal microscopy of DC phagocytosed fluorescence labelled liposomal vaccine and quantification of the process by flow cytometry

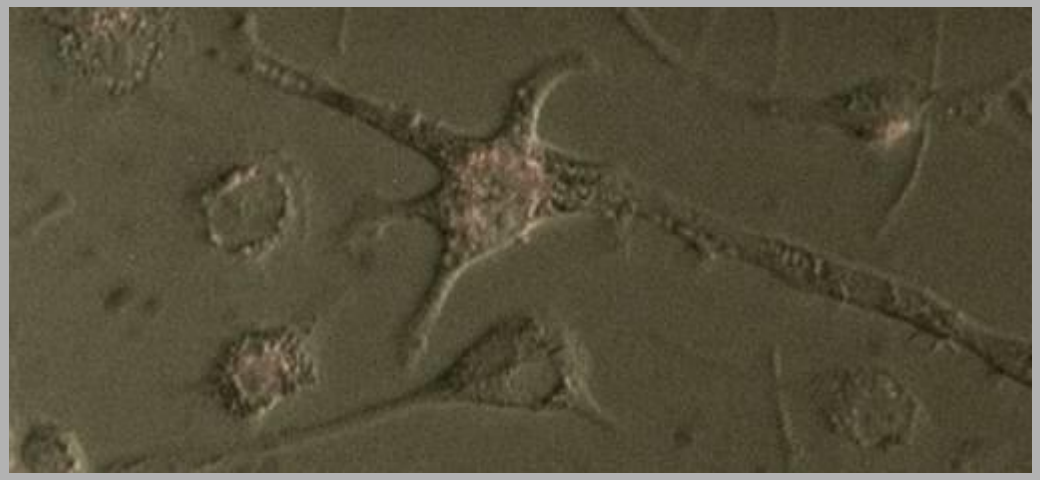


Bulky lipophilic residue



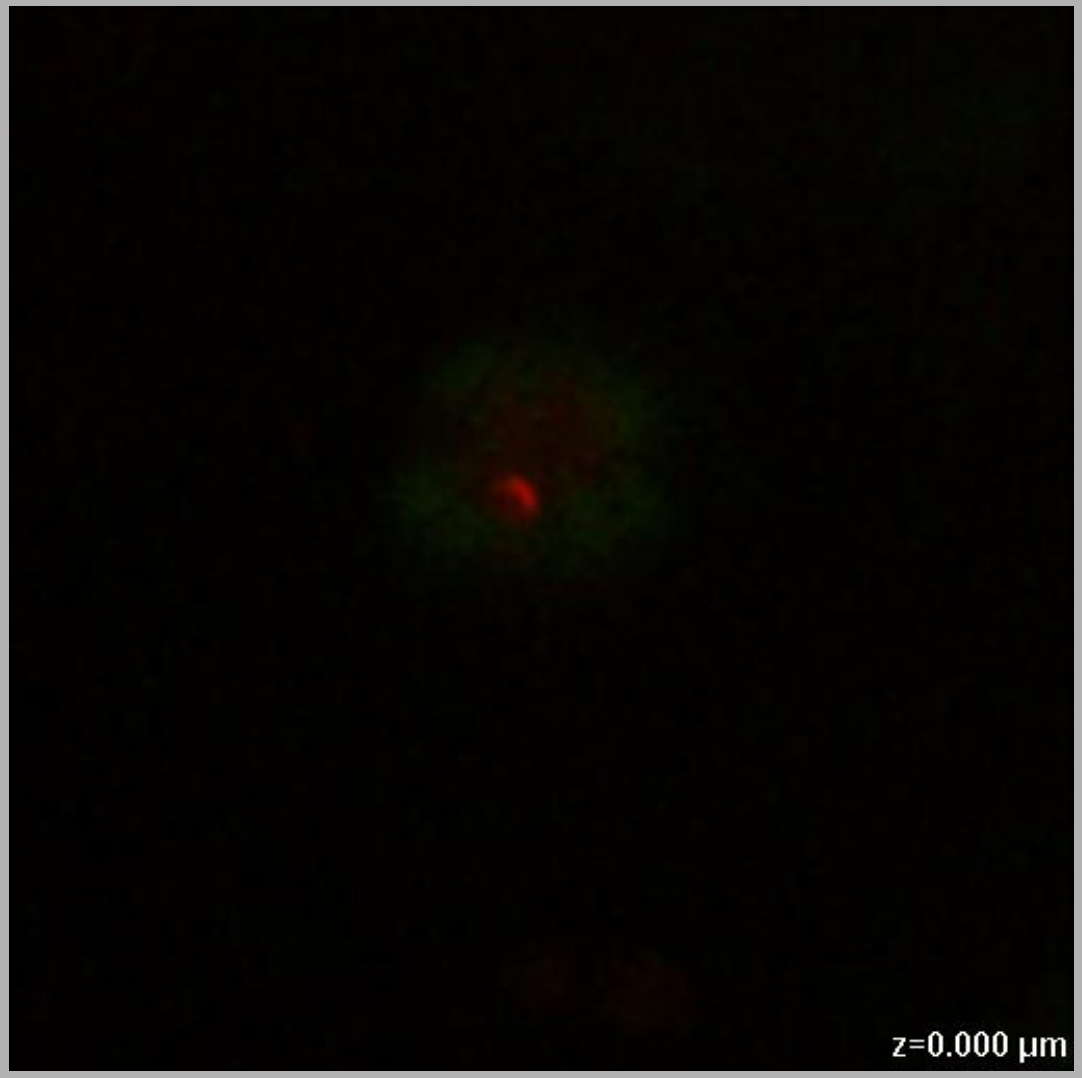
3D view of intracellular localisation of fluorescent liposomes in DC



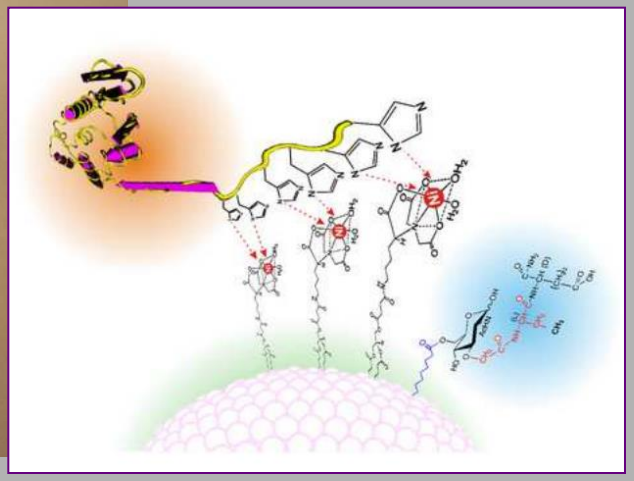


Intracellular localisation of liposomes

Confocal microscope Leica SP2



Lyophilised liposomal formulation of immunomodulator NorAbuMDP LiposIMM/MT05



Infection diseases transmitted by tick

Human ehrlichiosis – anaplazmosis – bakteria *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum*

Encephalitis - *Flaviridae* viruses

West Nile – *Flaviridae* viruses

Bartonellosis – bacterium *Bartonella henselae*

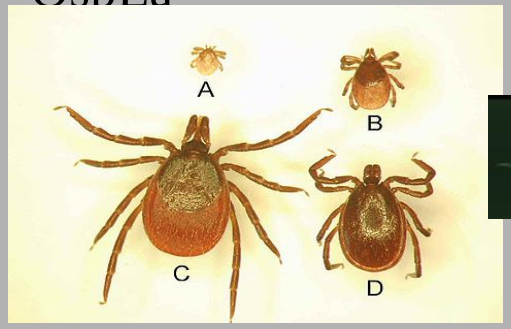
Babesiosis – protosoan *Babesia*

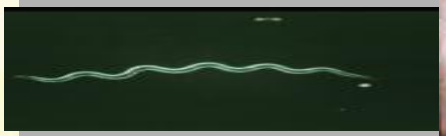
Rickettsiosis - bakteria Riskettsiaceae

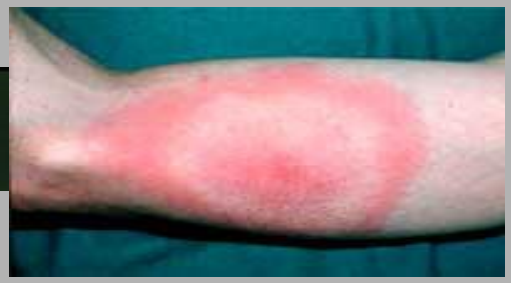
Tularemia - bakterium coccobacil *Francisella tularensis*

Q fiver - *Coxiella burnetti*

Borreliosis – *Borrelia b. surface antiges* OspA, OspB, **OspC**, OspD, OspEa

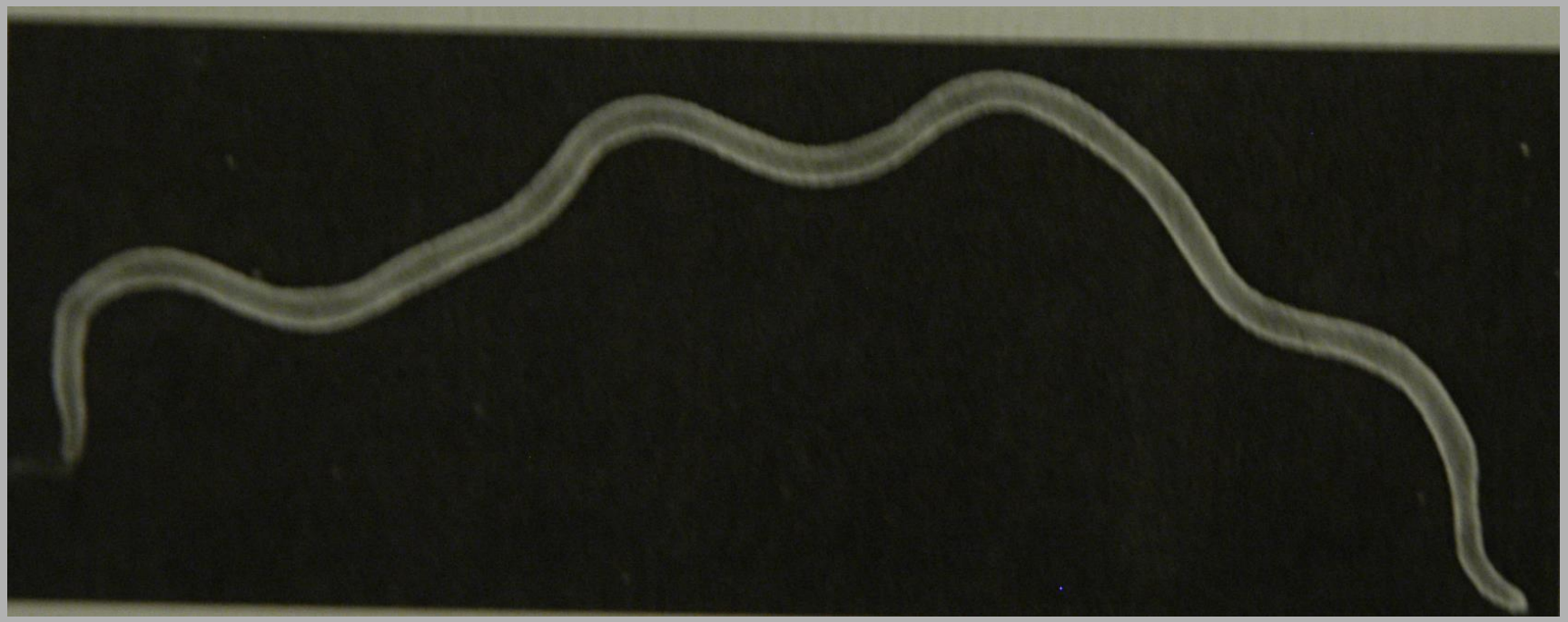




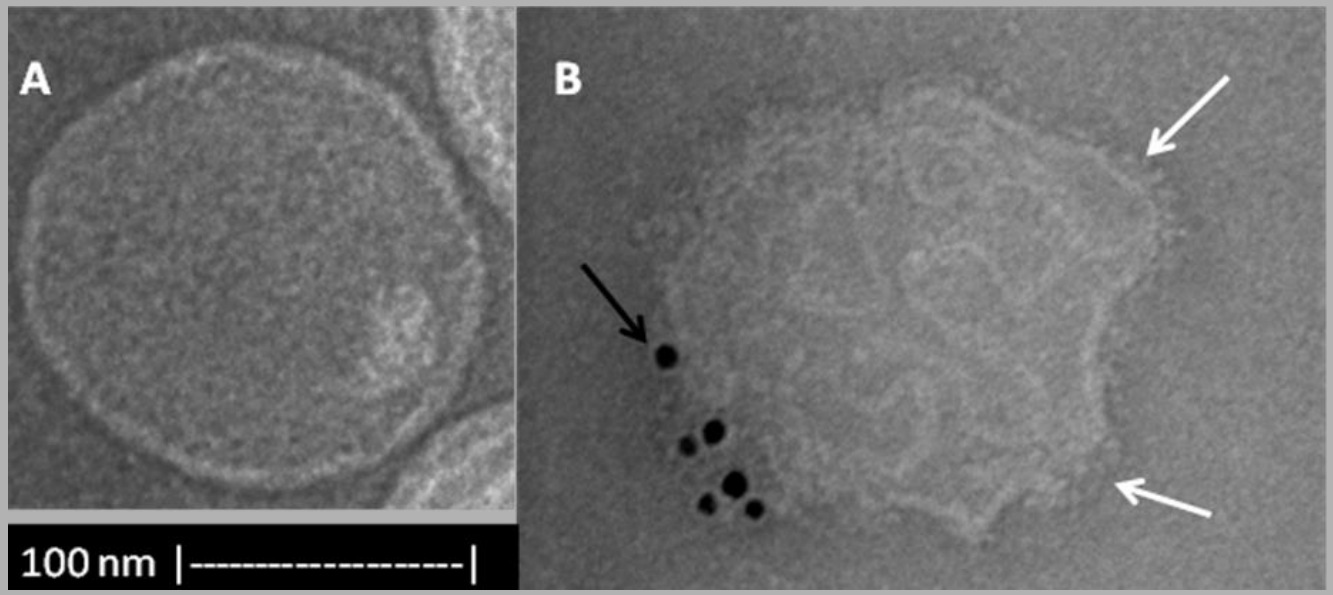


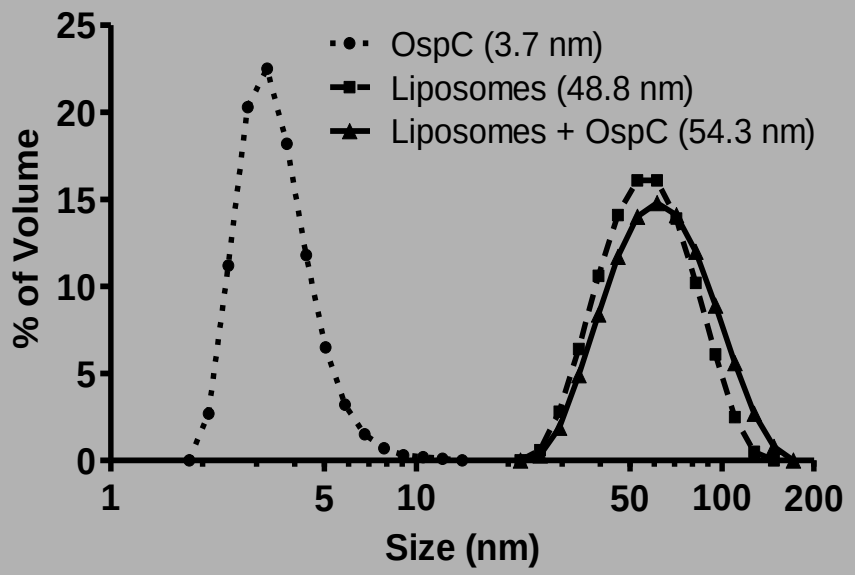
Lyme boreliosis

Pathogen: *Borelia burgdorferi, B afzelii, B. garinii*

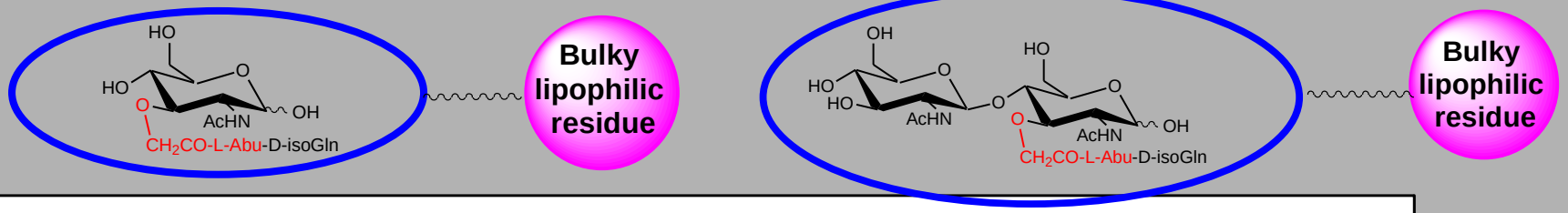


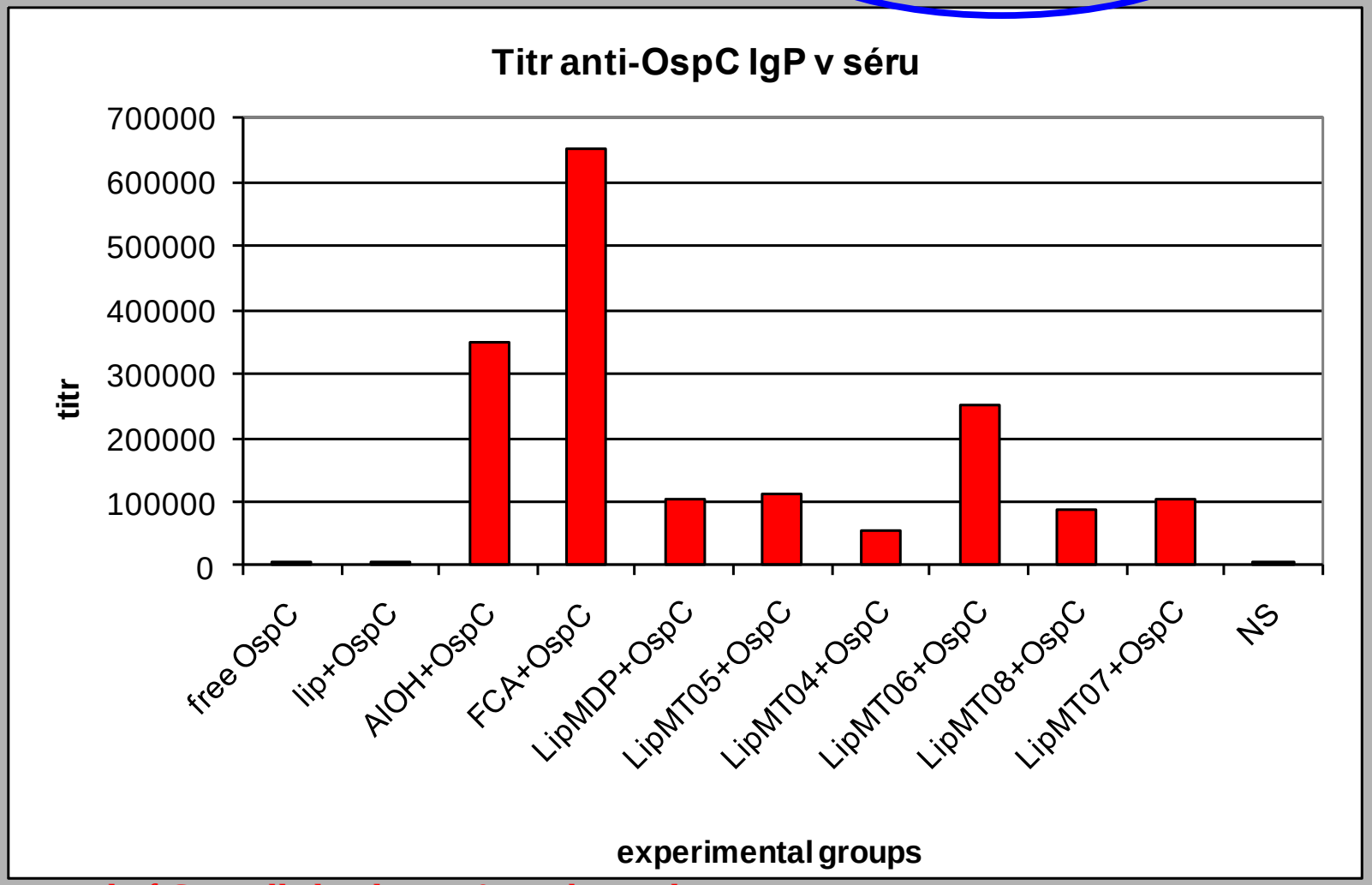
TEM of liposomal bound rOsp C





ELISA titers for specific anti rOspC antibodies

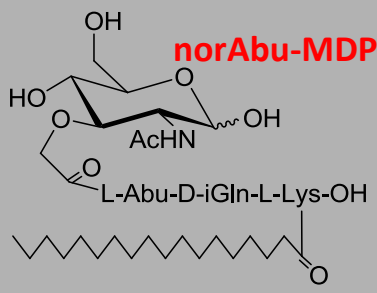




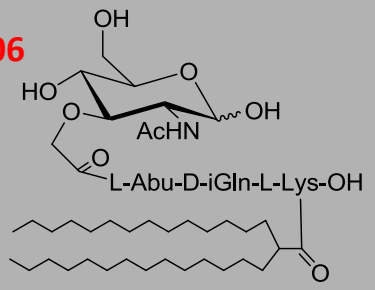
Journal of Controlled Release 2011 submitted

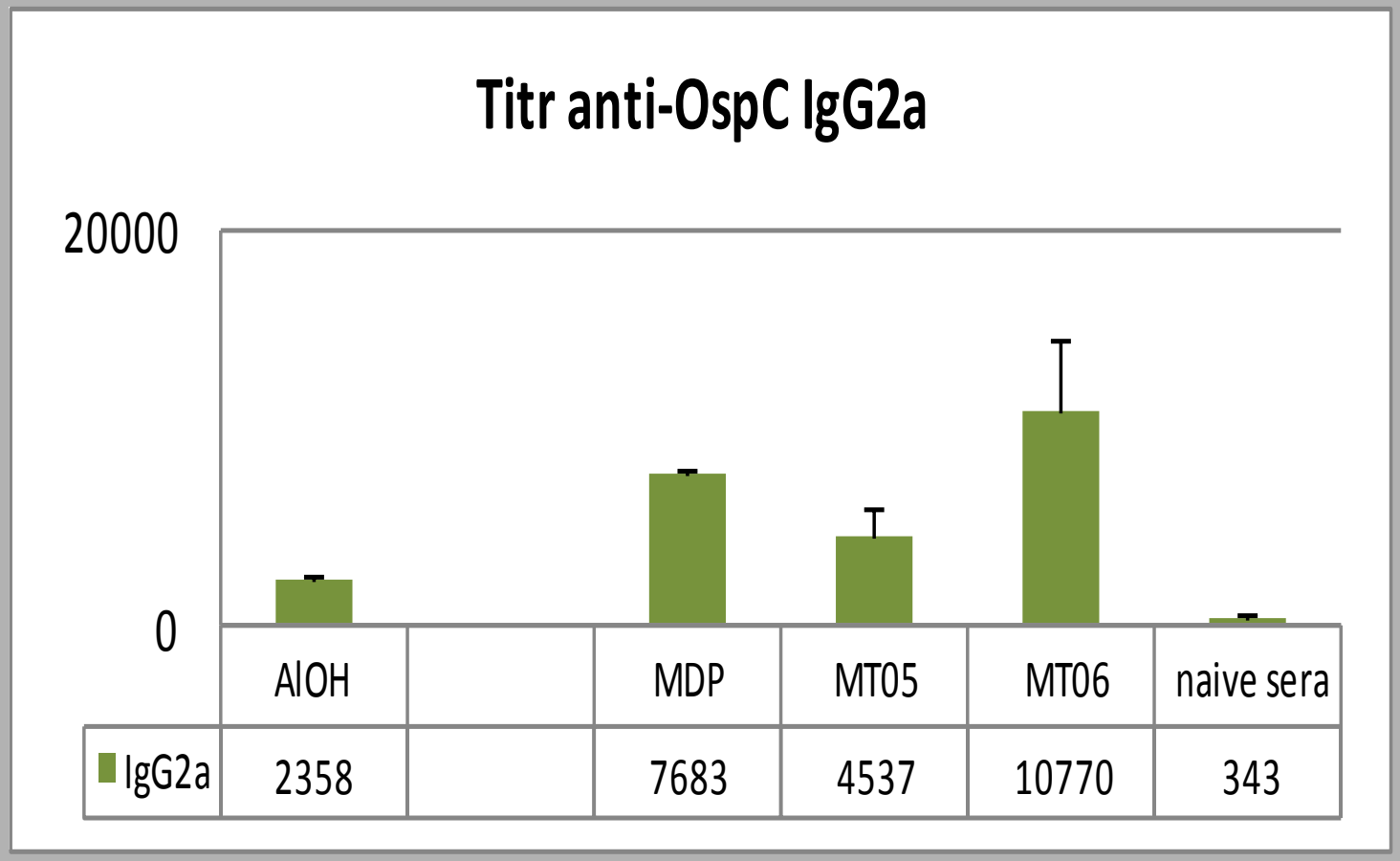
ELISA titers for specific anti OspC IgG subclasses

norAbu-MDP-Lys (L18) MT05



norAbu-MDP-Lys (B30) MT06





Safety of synthetic recombinant liposomal vaccine against cirkovirus (pigs, i.d. application)

DNA vaccines

Dna Vaccine Time Line

1983 In vivo expression of DNA (production of insulin in rats , DNA-liposome) (Nicolau et al.)

1992 Demonstration of immunogenicity (Tang et al.)
Genetic immunisation

1993 Early protection studies

1994 Naming of technolgy, WHO organised the conference in Geneva

1995 First phase 1 human trials

1996 FDA „Points to Consider“, (first US patent, VICAL, San Diego,CA)

1998 HIV-1, malaria, influenza, herpes, and hepatitis B in human trials

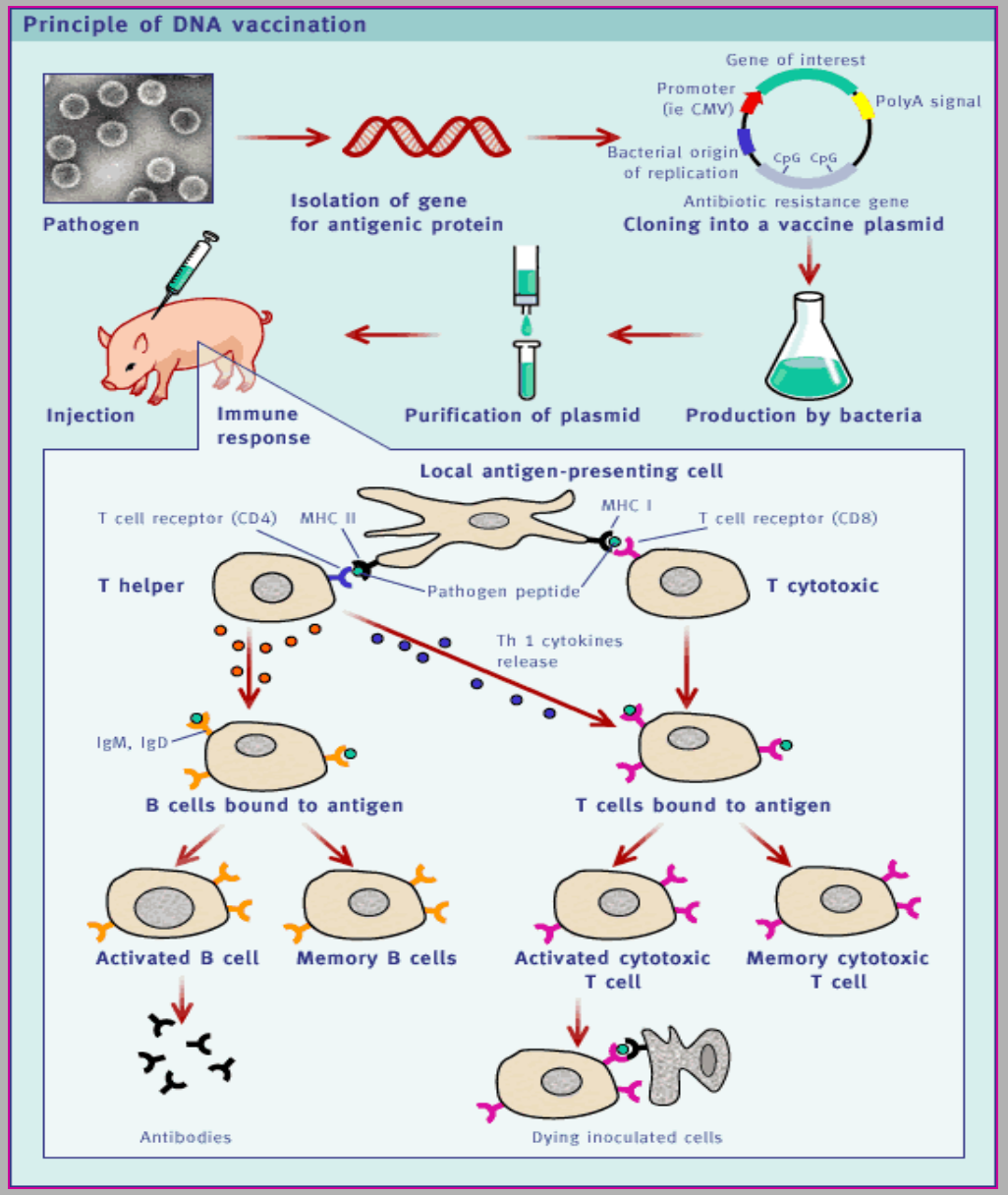
Principle of DNA Vaccination

Selection of antigen and construction of plasmid

Production of plasmid in industrial scale

Formulation of plasmid, development of carrier sytem, industrial scale production

Clinical trials



FROM GENE TO PATIENT

FERMENTATION

CELL RECOVERY

microfiltration, centrifugation

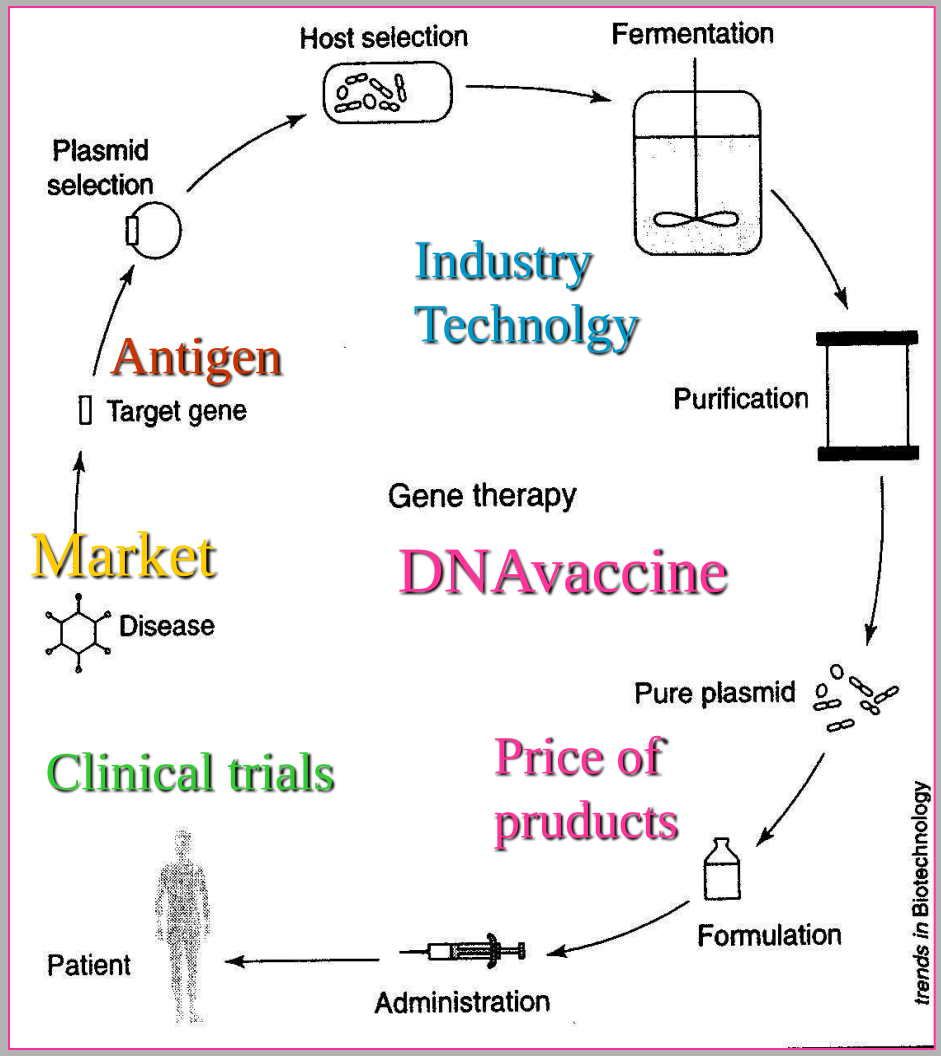
- CELL LYSIS - lysozyme, NaOH and SDS treatment
- **CLARIFICATION AND CONCENTRATION**

Ammonium-acetate ↓ PEG ↓
Potassium-acetate ↓
Isopropanol ↓ Selective ↓
(spermidine, polycations)
membrane processes
miF,UF,diaF

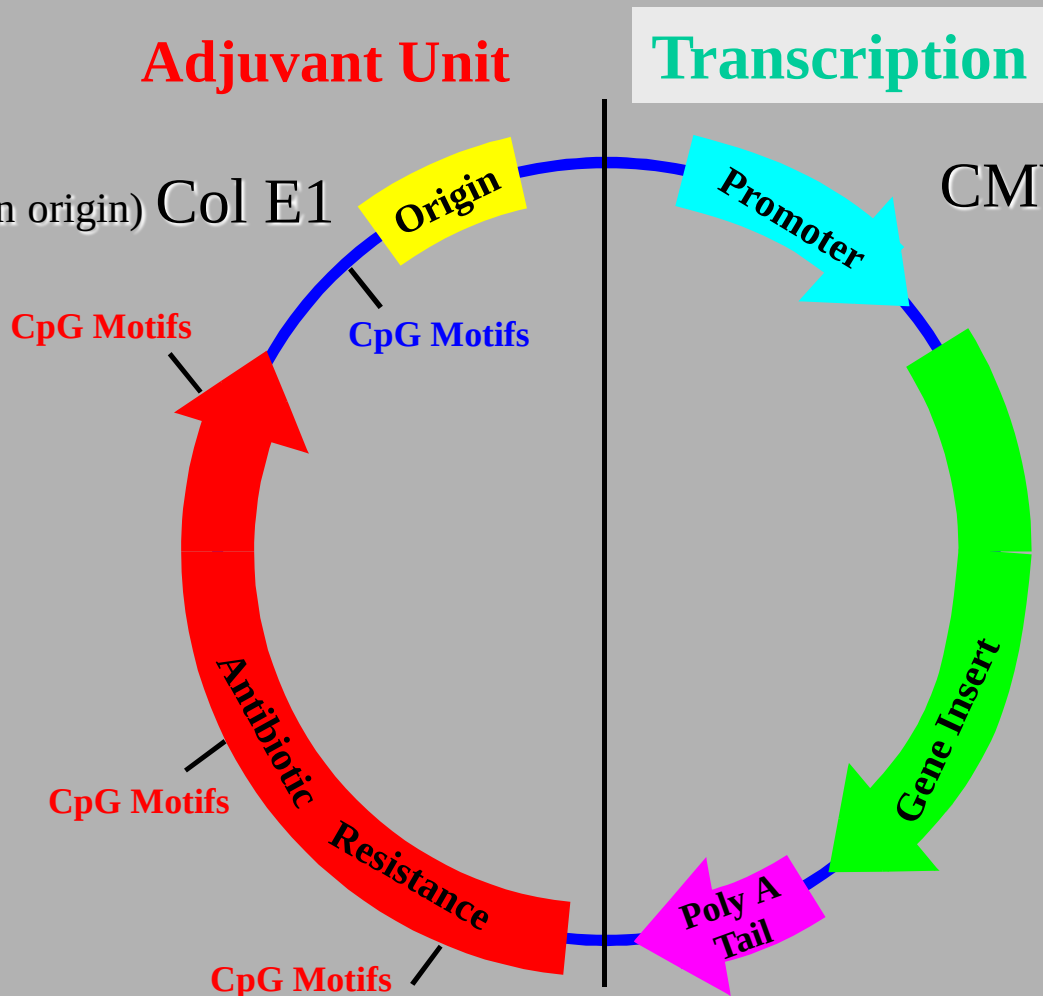
CHROMATOGRAPHY

Size exclusion, Ion exchange
HIC (Hydrophobic interaction chromatography)
RPC (Reversephase chromatography)

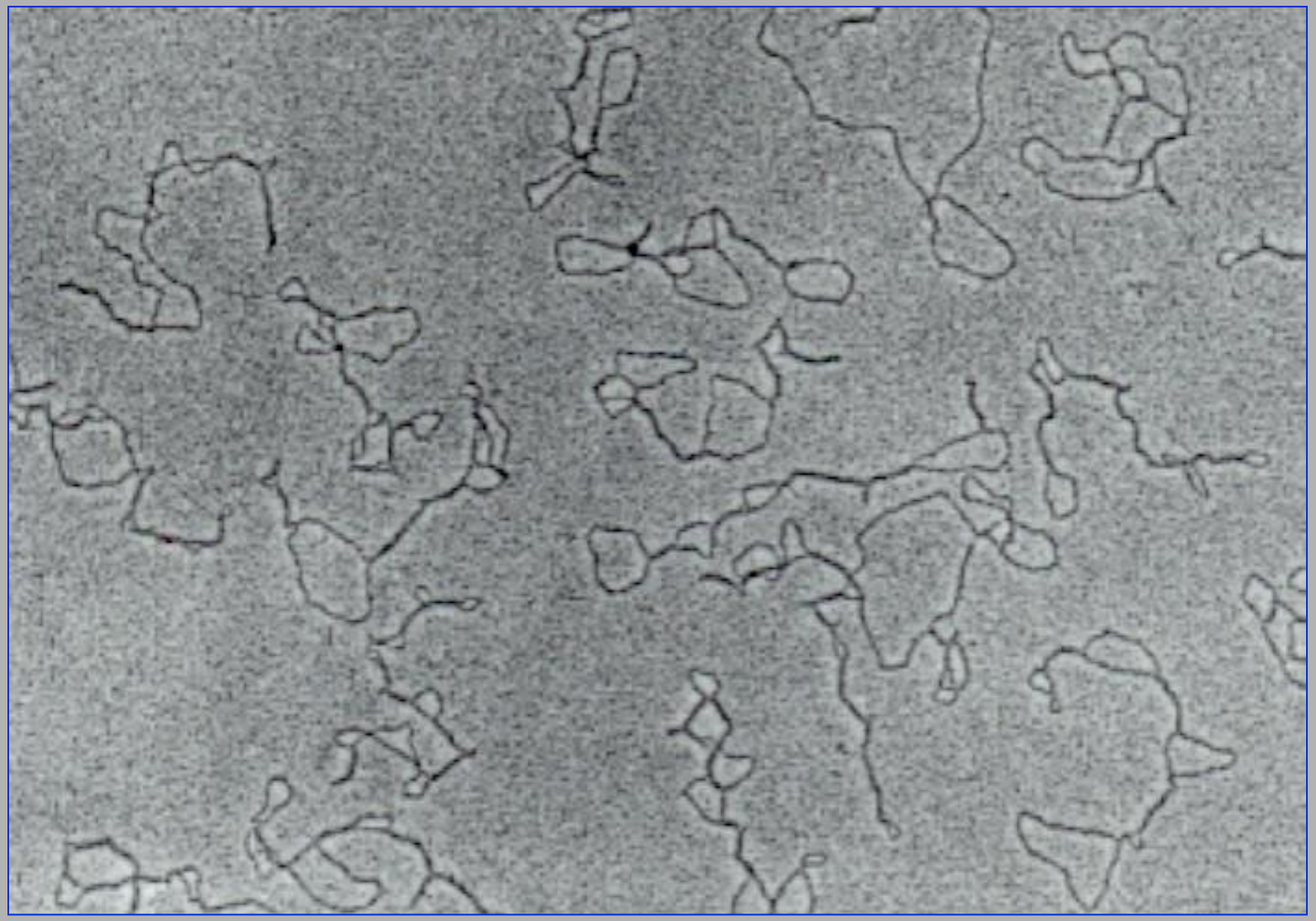
PURE PLASMID



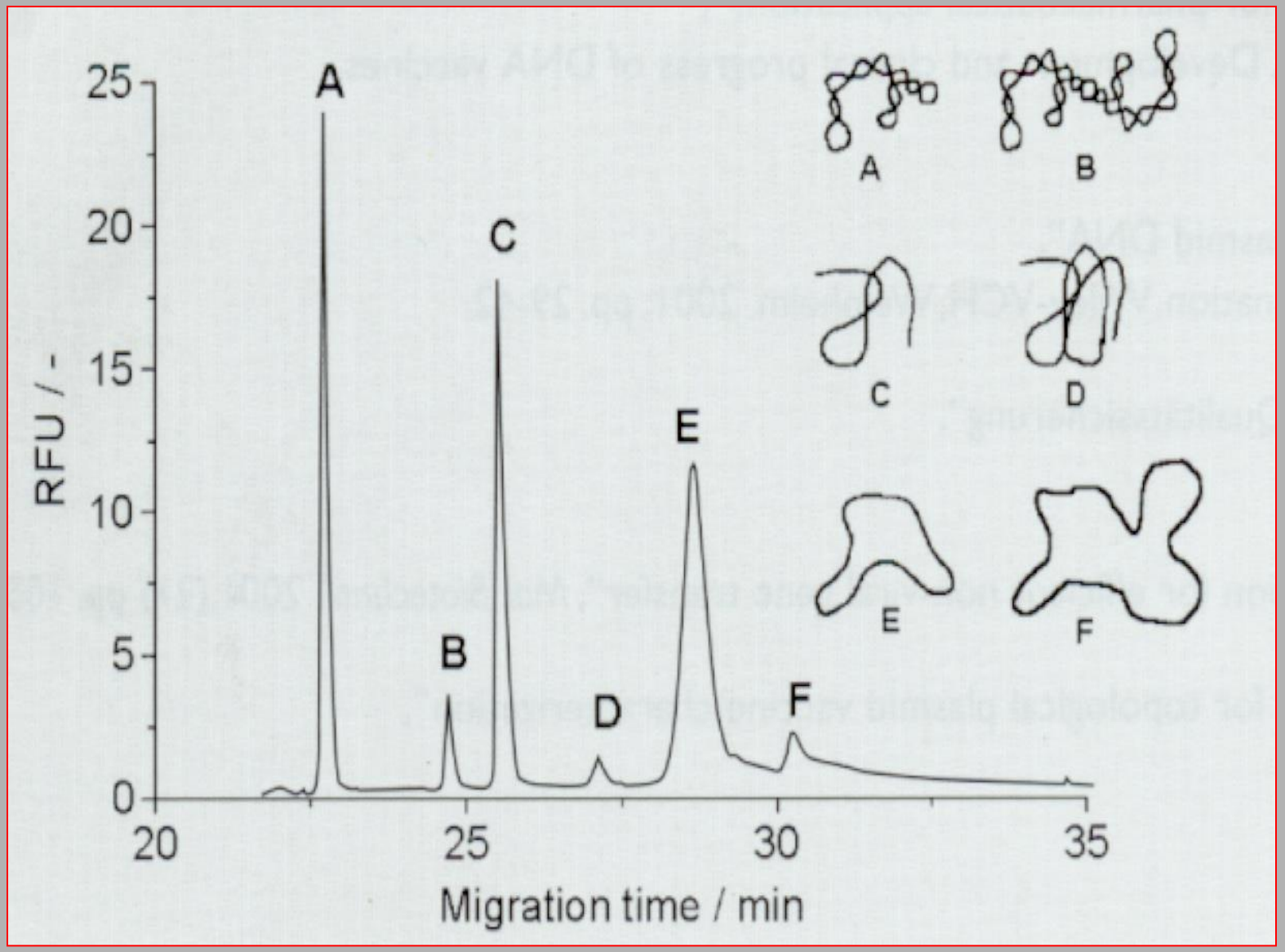
Plasmid Vector



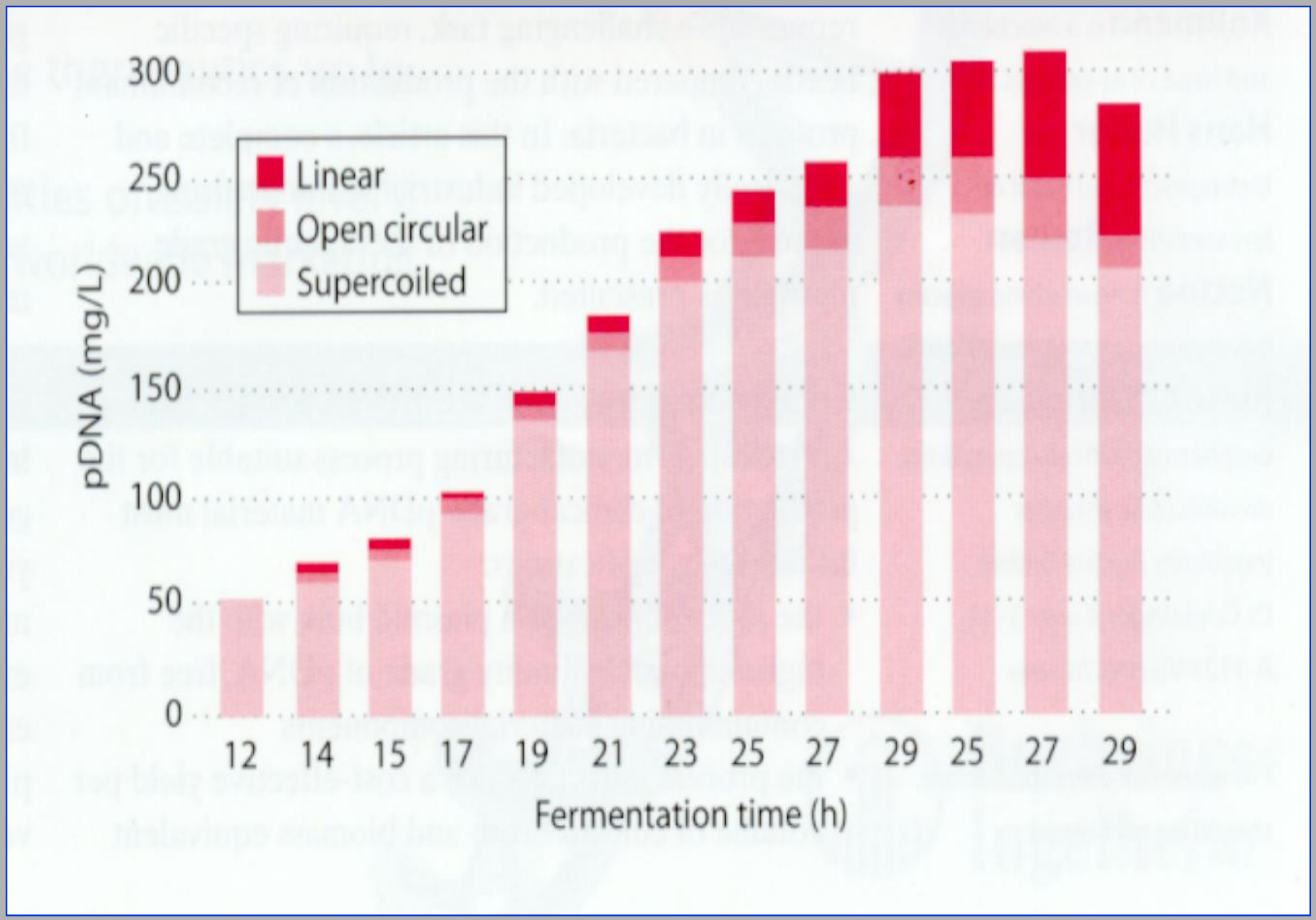
Electron micrograph of supercoiled plasmid



HP-CGE analysis of plasmide topologies



Optimalistion of a pDNA fermentation process



Plasmid

The market for gene therapy products could exceed 45 billion USD by 2010,

A typical dose size (patients with melanoma) is 0,3 μg but full treatments could require milligram quantities *(a role for suitable carriers)*

Large scale processes for plasmid preparation

Criteria of plasmid purity required for human application

Bacterial gDNA < 10ng per dose (< 10 ng gDNA/ μgcDNA)

Bacterial proteins < 10ng per dose (undetectable by BCA assay)

RNA non seen on agarose gel or HPLC

Endotoxin unit < 0.1EU per μg plasmid

Residual antibiotics !?.

Manufacturers

Cobra BioManufacturing UK

Fit Biotech Finland

Althea

Qiagen (Germany, USA)

Boehringer Ingelheim Austria

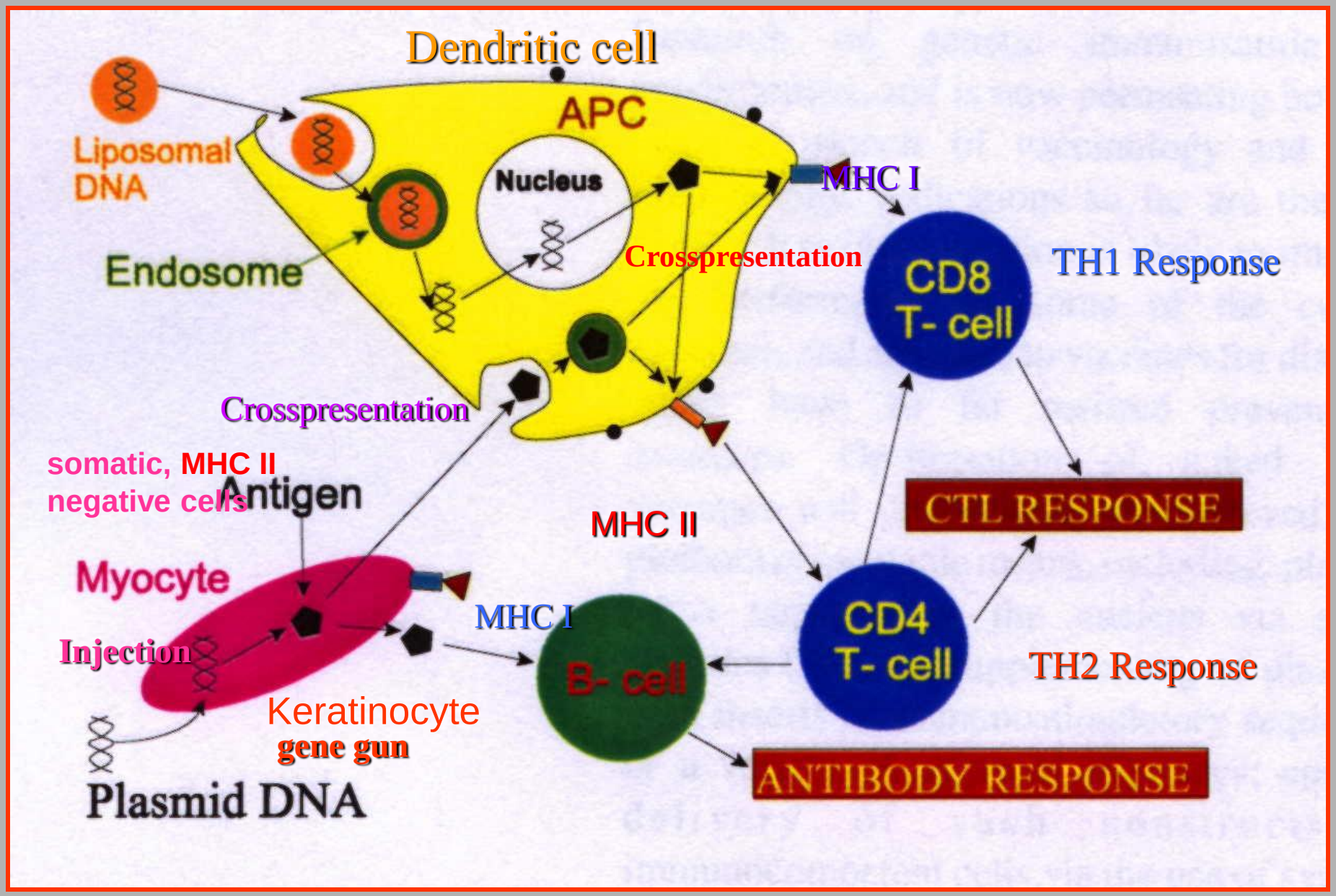
Fermentas Lithuania

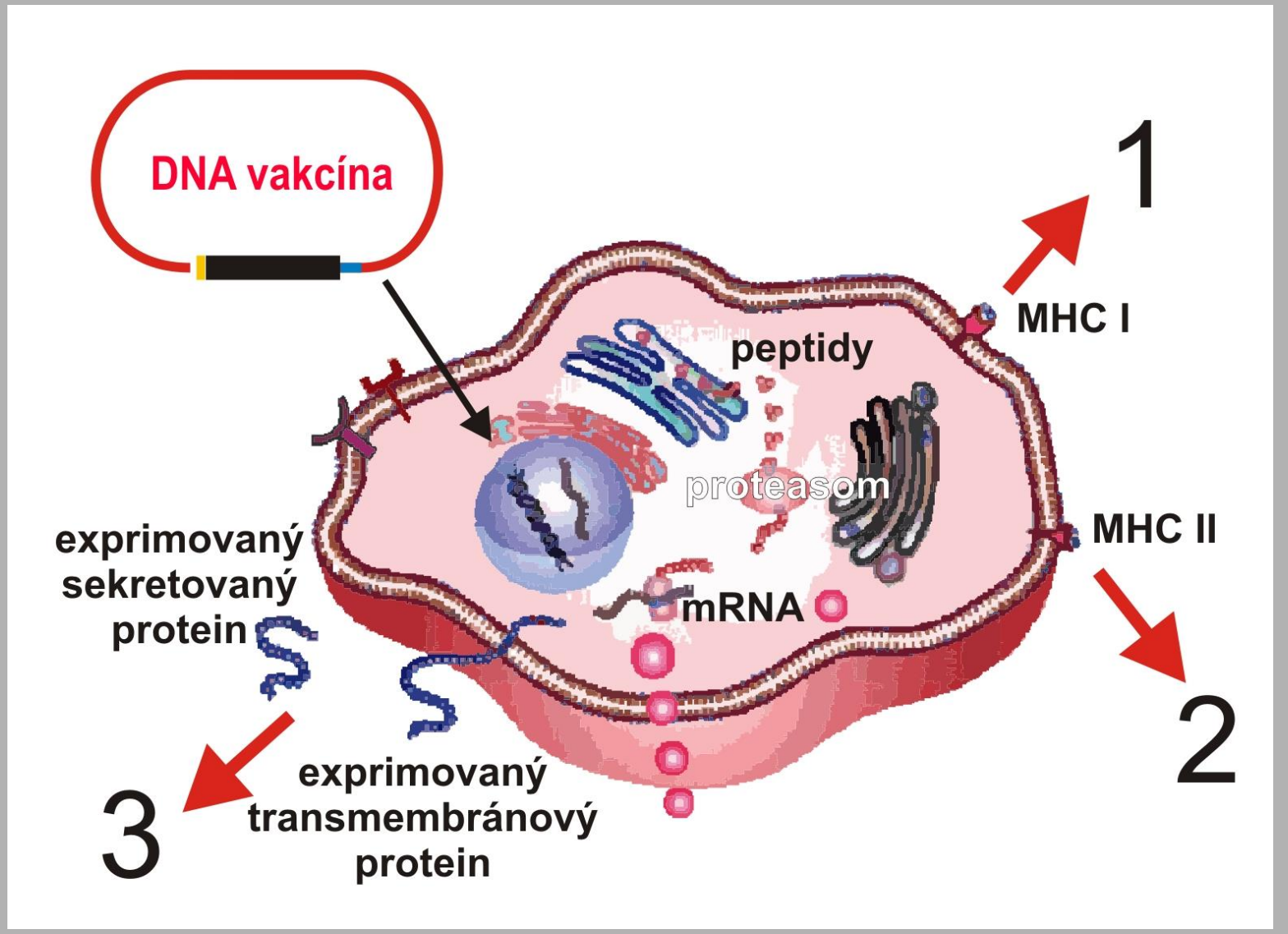
PlasmidFactory Germany

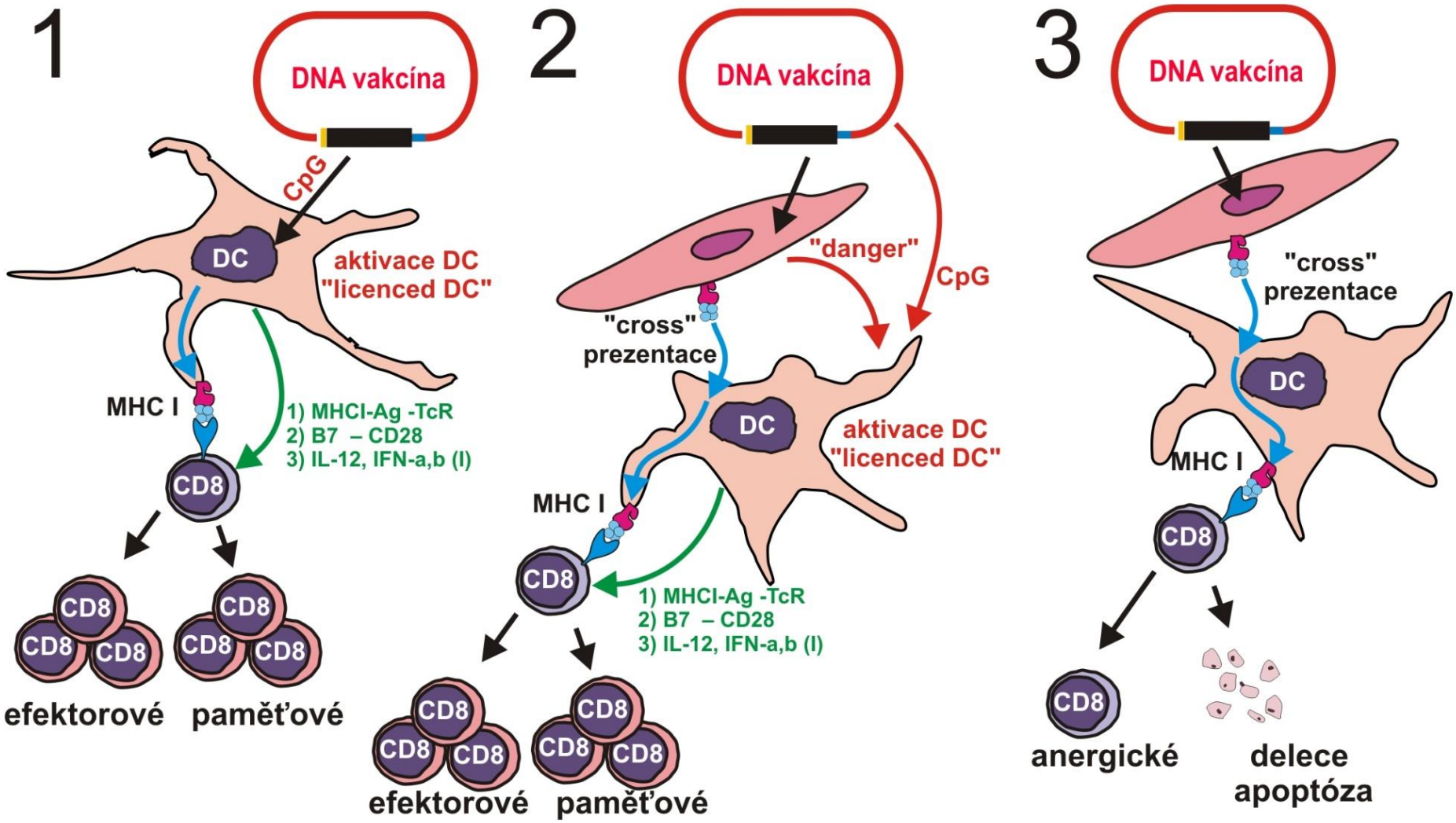
MoloGen Germany

1. **Snadná a rychlá příprava plasmidu**
2. **Snadná izolace, kontrola a skladování**
3. **Možnost rychlé modifikace**
4. **Neobsahuje složky s neznámým efekty**
5. **DNA není imunogenní – možnost opakované aplikace**
6. **V základní formě indukce Th1 odpovědi**

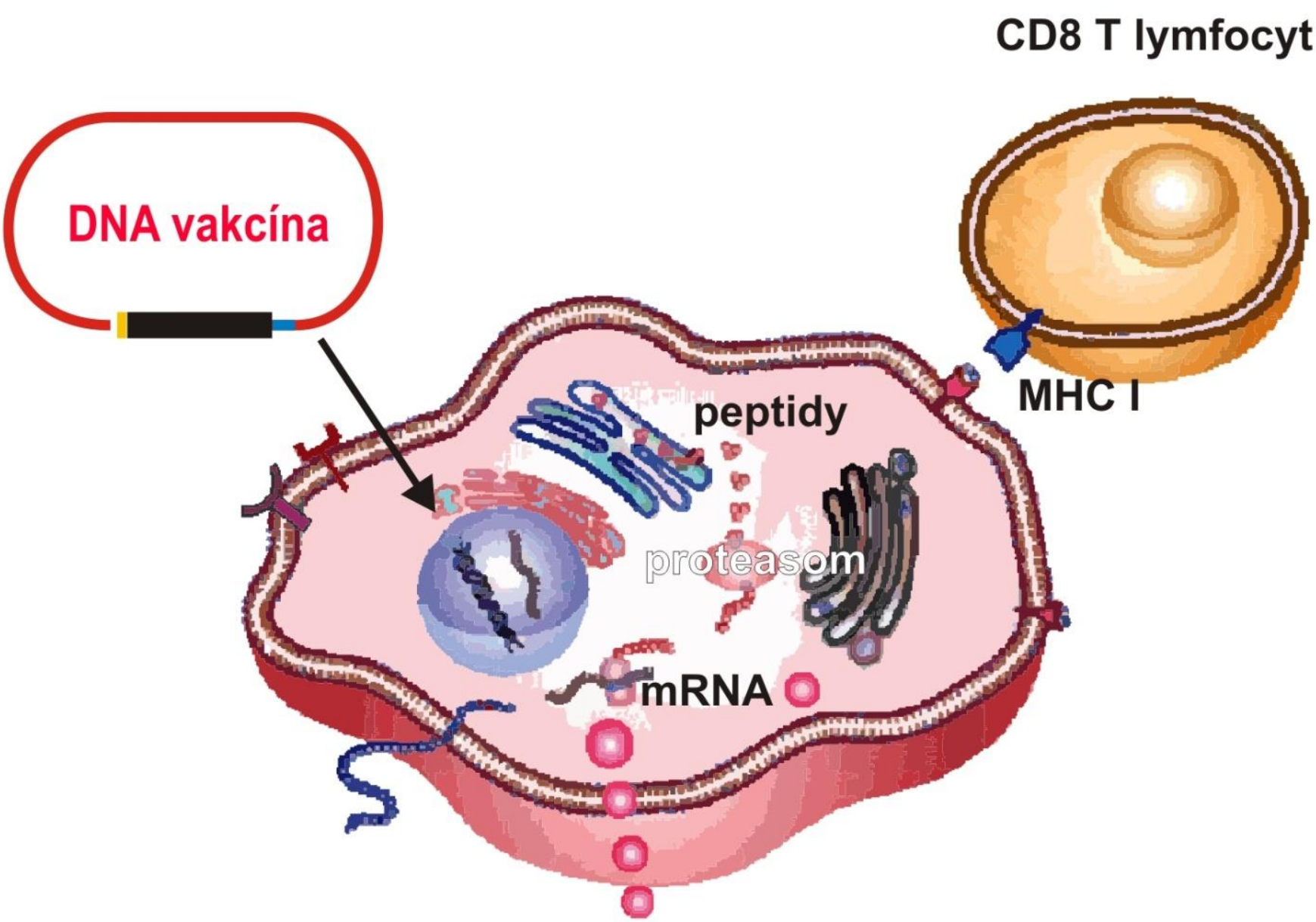
Mechanism of DNA immunisation

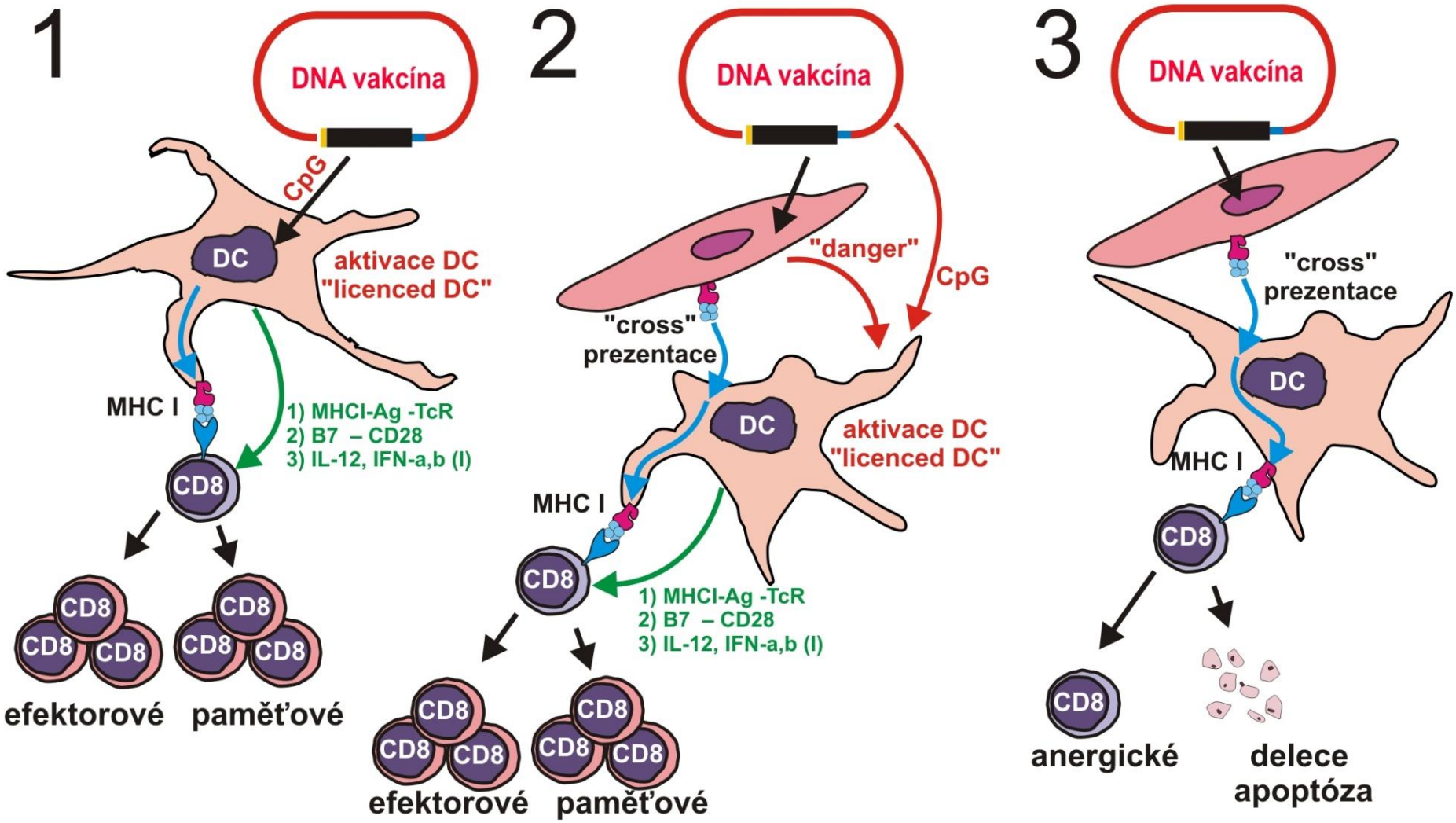



DNA vakcína
1
MHC I
peptidy
proteasom
MHC II
exprimovaný
sekretovaný
protein
mRNA
2
3
exprimovaný
transmembránový
protein

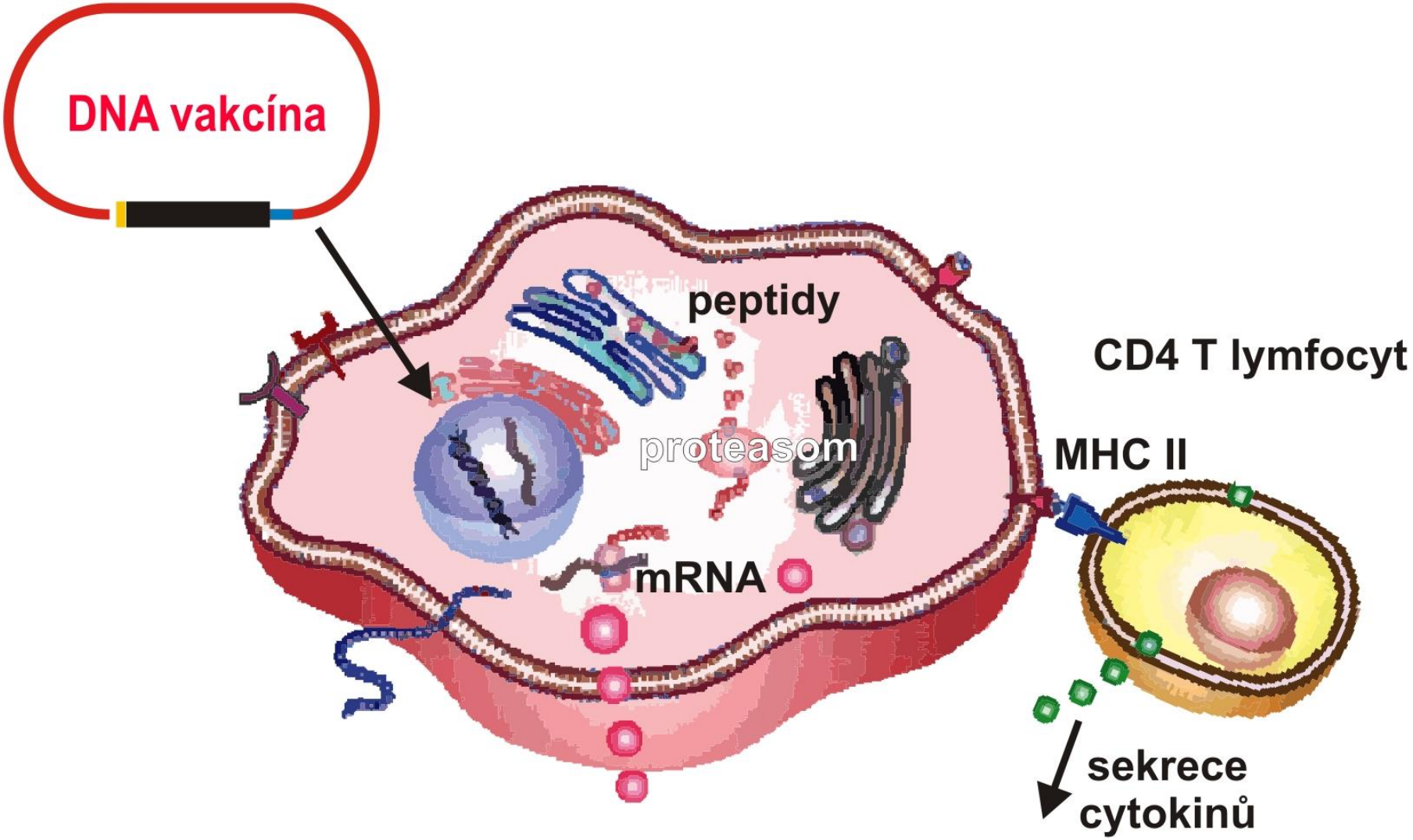

1
DNA vakcína
CpG
DC
aktivace DC
"licenced DC"
MHC I
1) MHCI-Ag -TcR
2) B7 – CD28
3) IL-12, IFN-a,b (I)
CD8
efektorové
paměťové
2
DNA vakcína
"danger"
CpG
"cross"
prezentace
DC
aktivace DC
"licenced DC"
MHC I
1) MHCI-Ag -TcR
2) B7 – CD28
3) IL-12, IFN-a,b (I)
efektorové
paměťové
3
DNA vakcína
"cross"
prezentace
DC
MHC I
anergické
delece
apoptóza

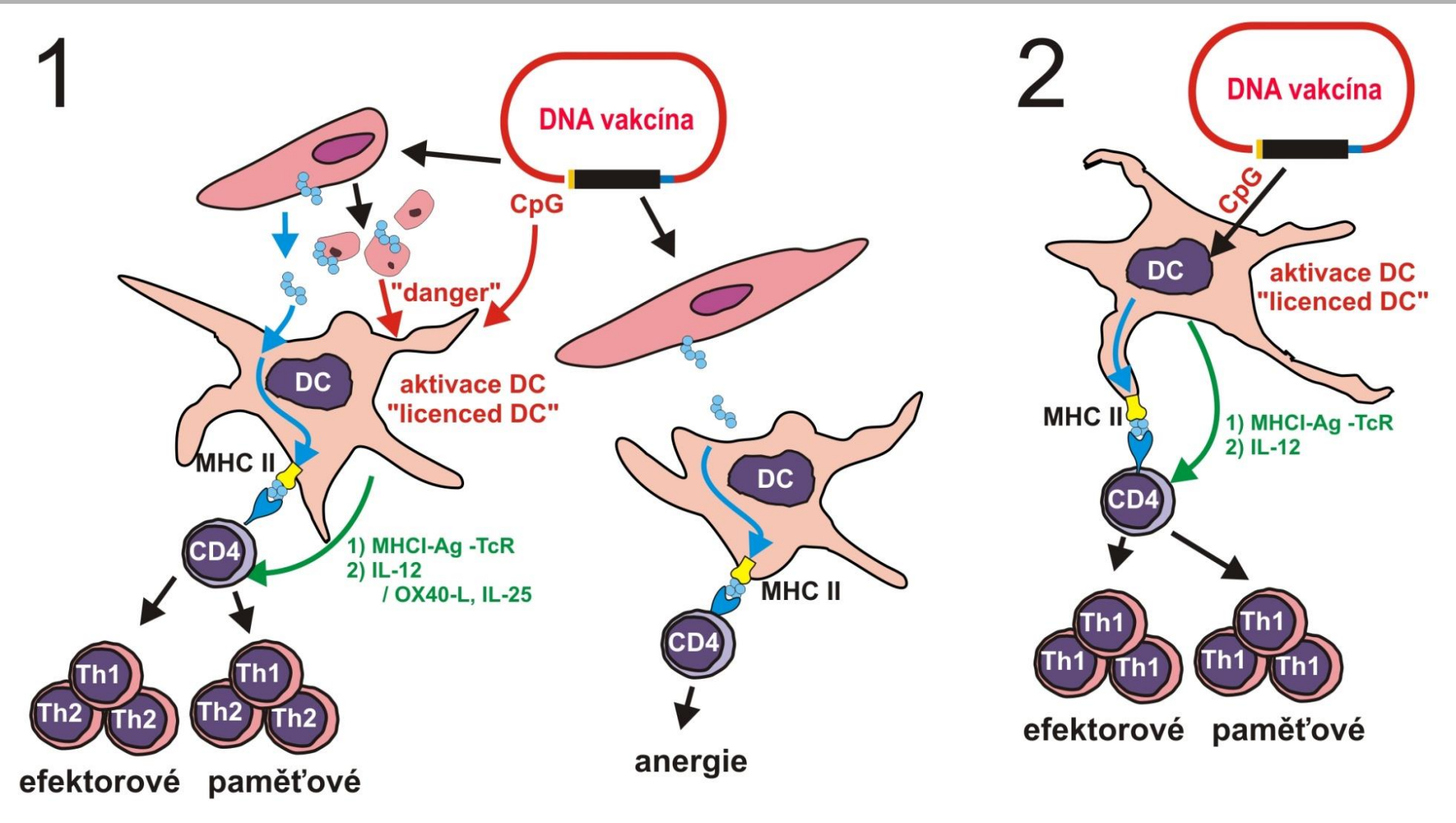
Mechanismus aktivace CD8 T lymfocytů

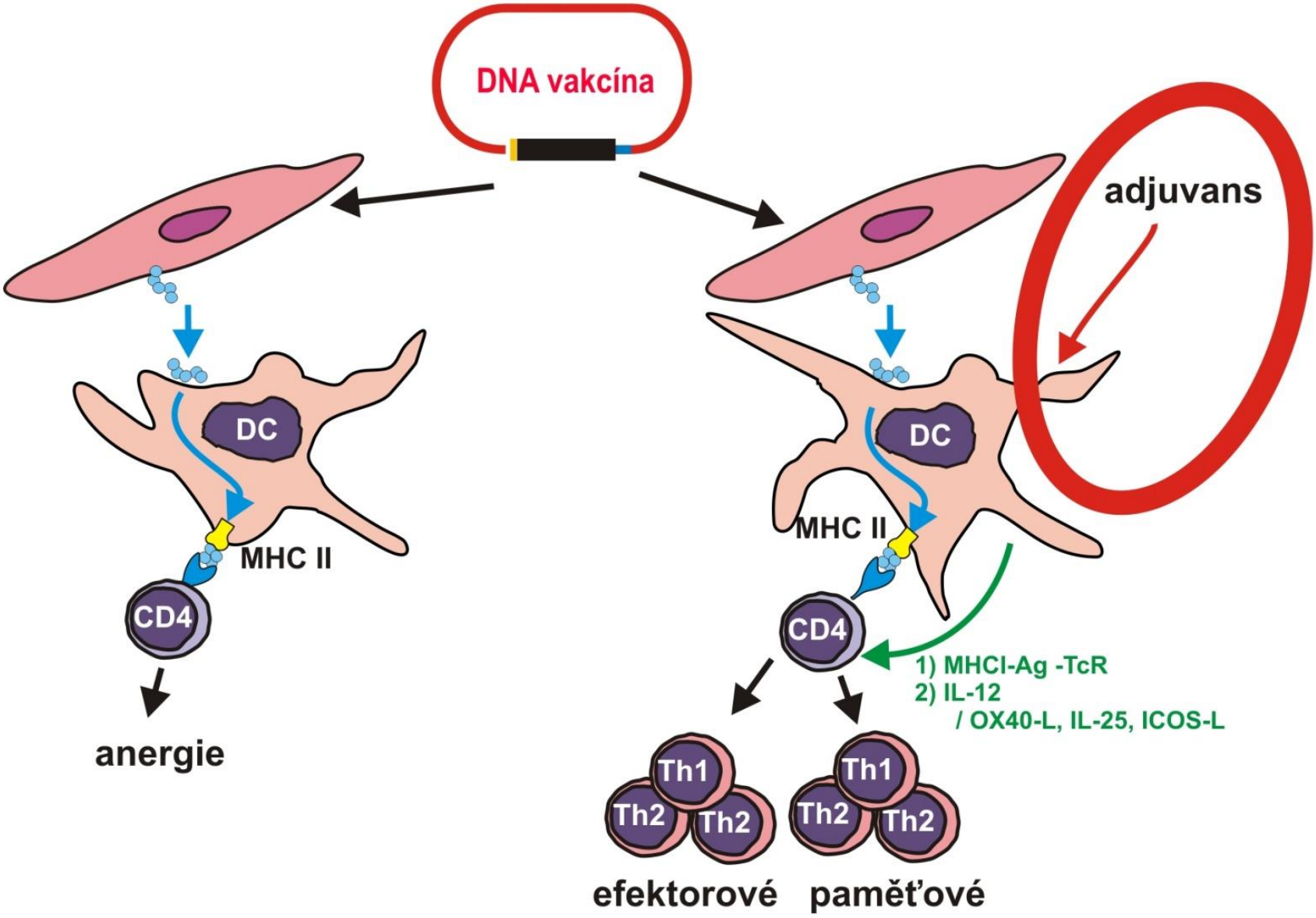

CD8 T lymfocyt
DNA vakcína
MHC I
peptidy
proteasom
mRNA


1
DNA vakcína
CpG
DC
aktivace DC
"licenced DC"
MHC I
1) MHCI-Ag -TcR
2) B7 – CD28
3) IL-12, IFN-a,b (I)
CD8
efektorové
paměťové
2
DNA vakcína
"danger"
CpG
"cross"
prezentace
DC
aktivace DC
"licenced DC"
MHC I
1) MHCI-Ag -TcR
2) B7 – CD28
3) IL-12, IFN-a,b (I)
efektorové
paměťové
3
DNA vakcína
"cross"
prezentace
DC
MHC I
anergické
delece
apoptóza

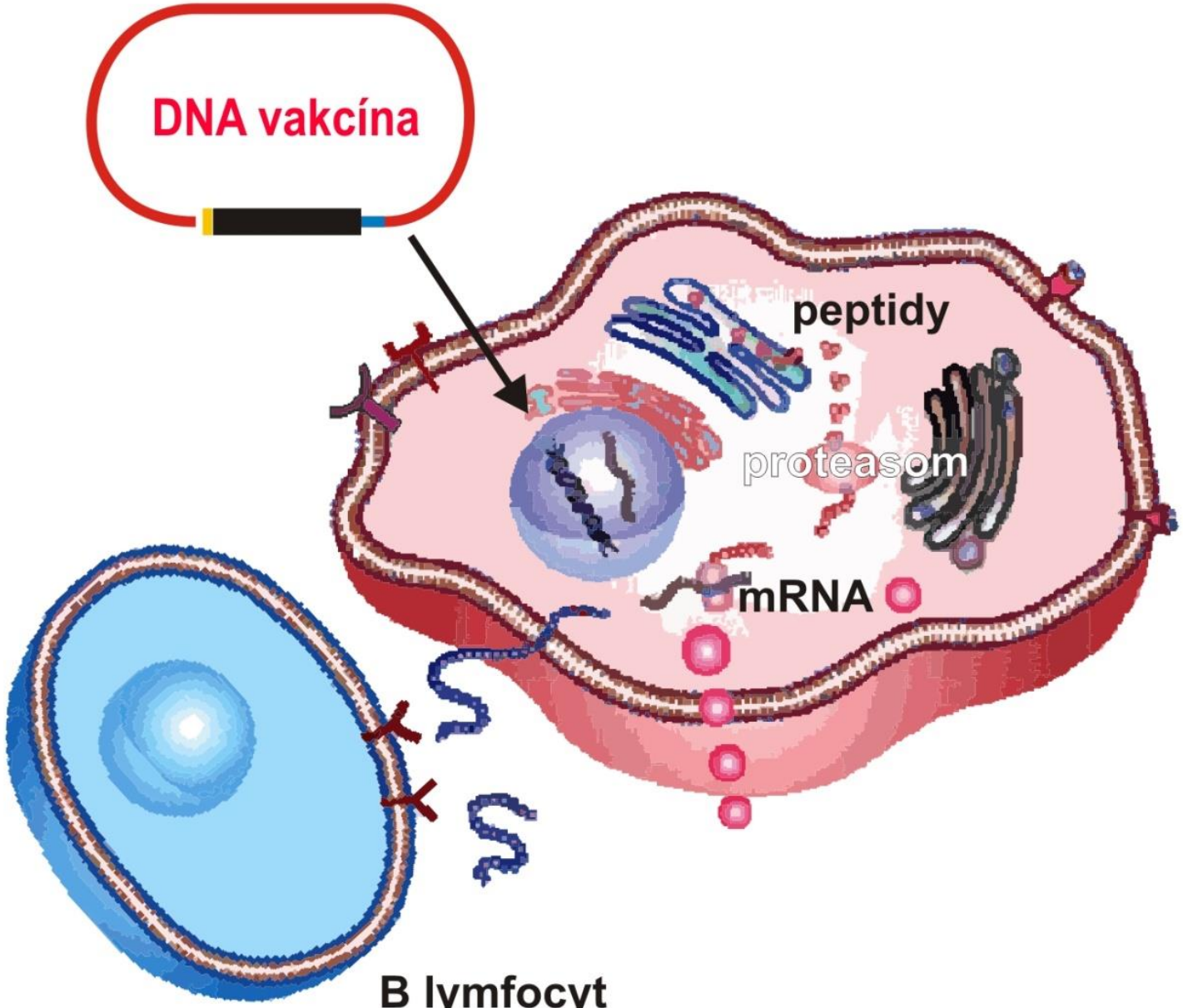
Mechanismus aktivace CD4 T lymfocytů


DNA vakcína
peptidy
proteasom
mRNA
CD4 T lymfocyt
MHC II
sekrece
cytokinů

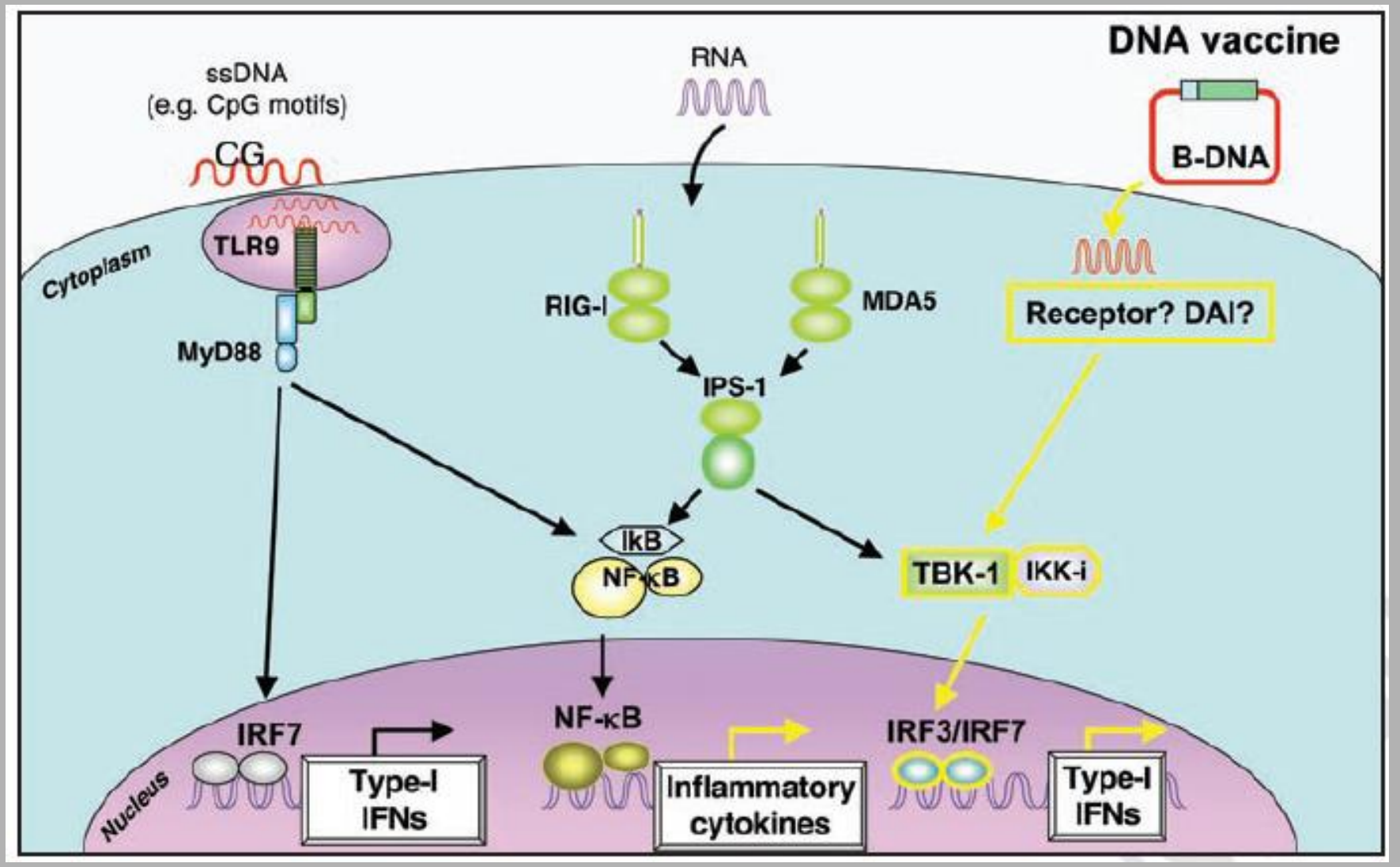

1
DNA vakcína
CpG
"danger"
DC
aktivace DC
"licenced DC"
MHC II
CD4
1) MHCI-Ag -TcR
2) IL-12
/ OX40-L, IL-25
Th1
Th2
efektorové
paměťové
DC
MHC II
CD4
anergie
2
DNA vakcína
CpG
DC
aktivace DC
"licenced DC"
MHC II
1) MHCI-Ag -TcR
2) IL-12
CD4
Th1
efektorové
paměťové


DNA vakcína
adjuvans
DC
MHC II
CD4
anergie
DC
MHC II
CD4
1) MHCI-Ag -TcR
2) IL-12
/ OX40-L, IL-25, ICOS-L
Th1
Th2
Th2
Th1
Th2
Th2
efektorové
paměťové

DNA vakcinace aktivuje B lymfocyty k tvorbě specifických protilátek


DNA vakcína
peptidy
proteasom
mRNA
B lymfocyt

Adjuvantní působení DNA



Coban et al, 2008

Mechanismus indukce imunitní CD8 T lymfocytární odpovědi na „exogenní“ antigen

Přenesená „cross“ prezentace

Přenesená prezentace „cross“

1. TAP závislá

a) korpuskulární antigen - fagosom (NOX2) – proteasom

b) solubilní antigen – receptor – časný endosom

- proteasom -ER

- proteasom – TAP na endosomu – MHC I

vazba antigenu na scavengerové receptory – MHC II

2. TAP nezávislá (vakuolární)

endosom – štěpení katepsin S - záměna peptidů MHC

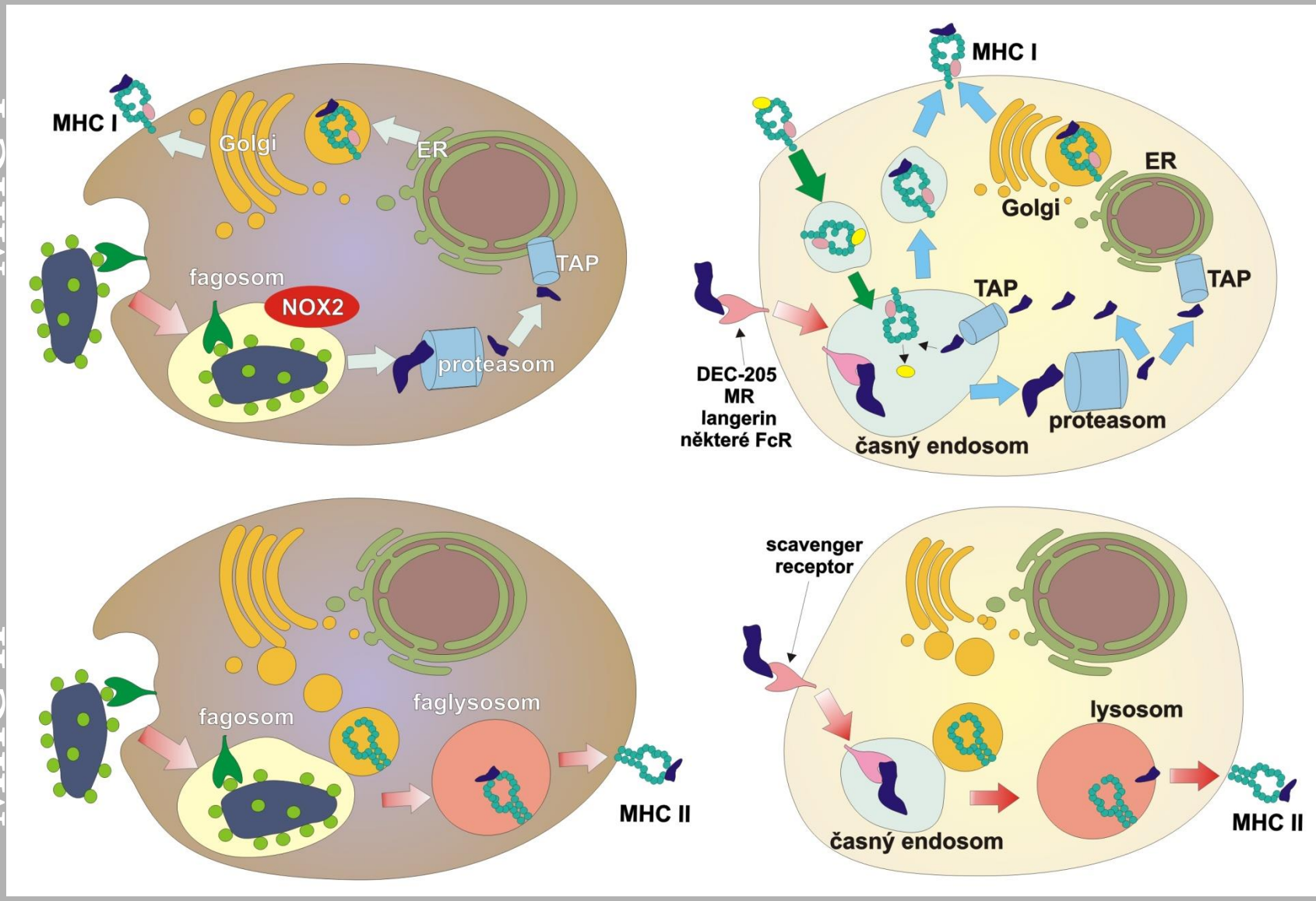
TAP – závislá „cross“ prezentace

korpuskulární antigen

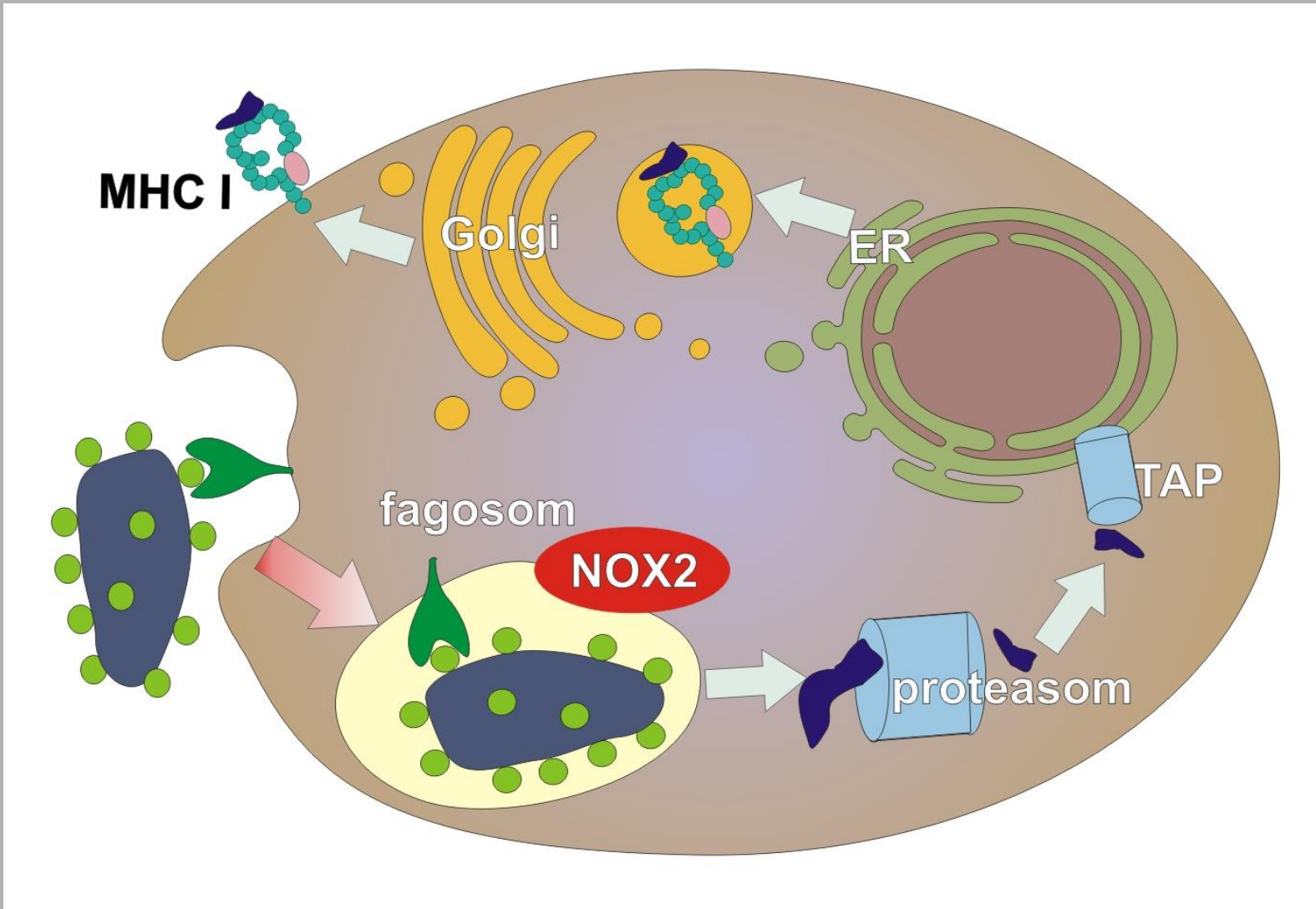
solubilní antigen

MHC I

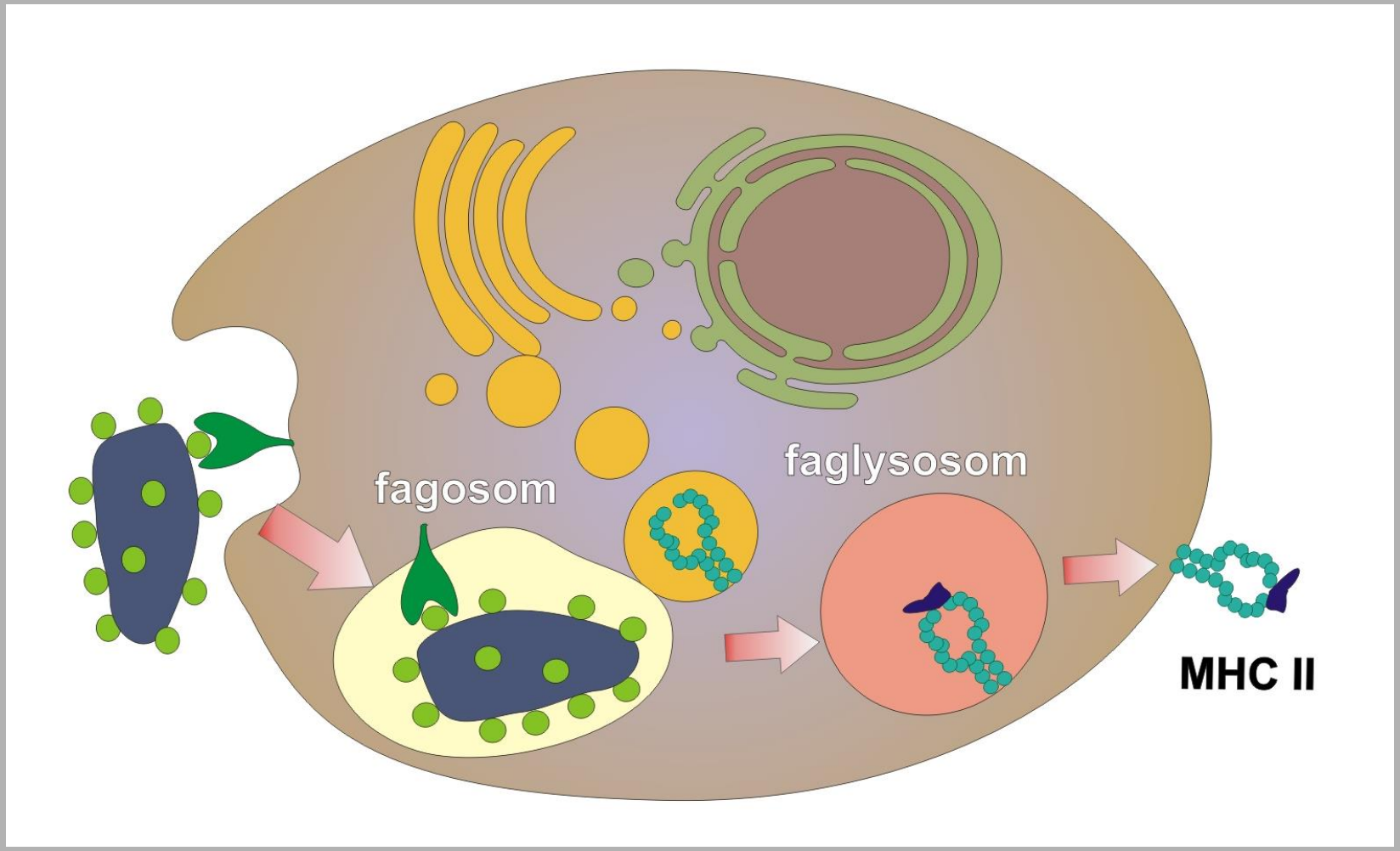
MHC II



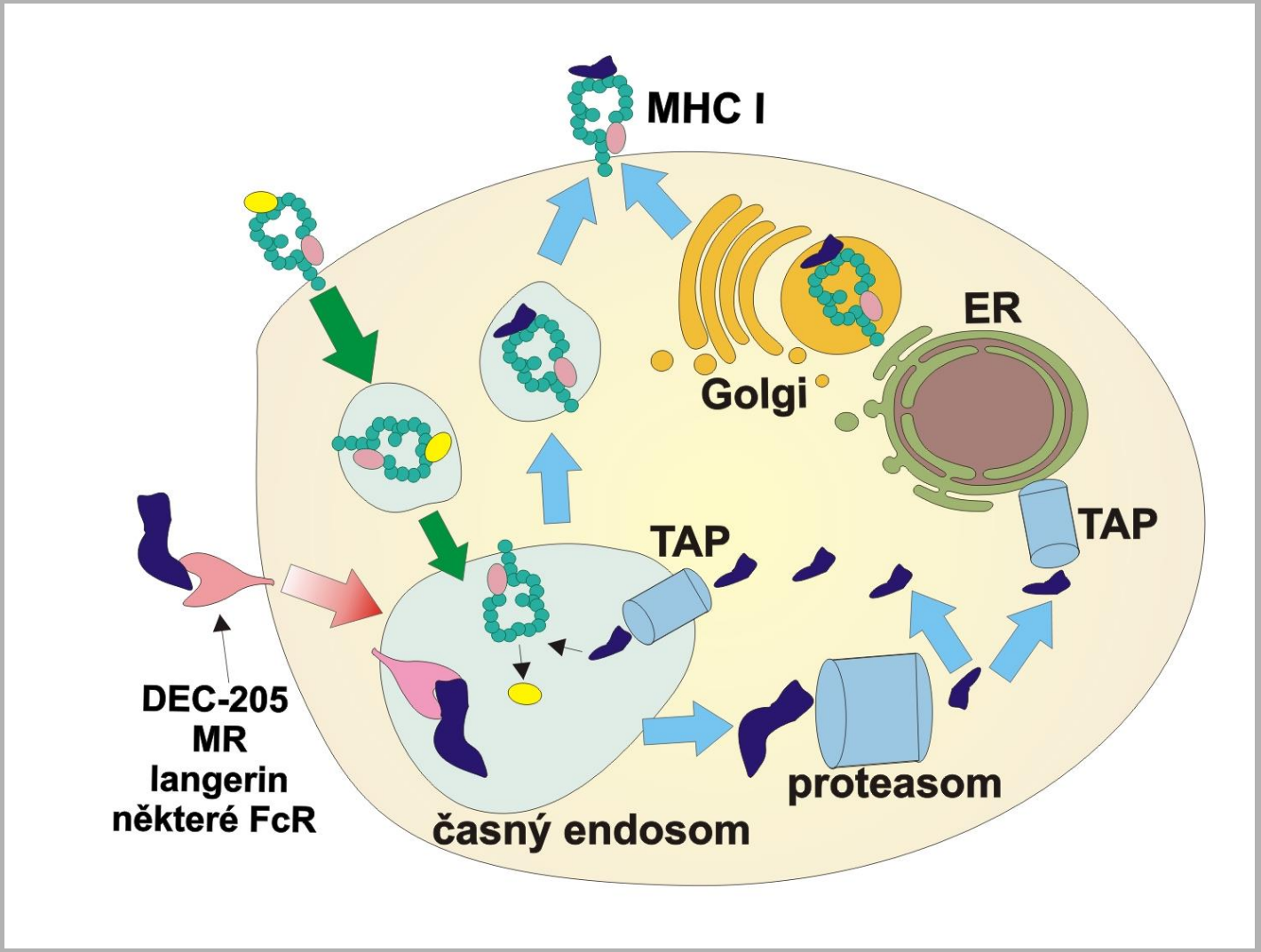
TAP závislá „cross“ prezentace – korpuskulární antigen



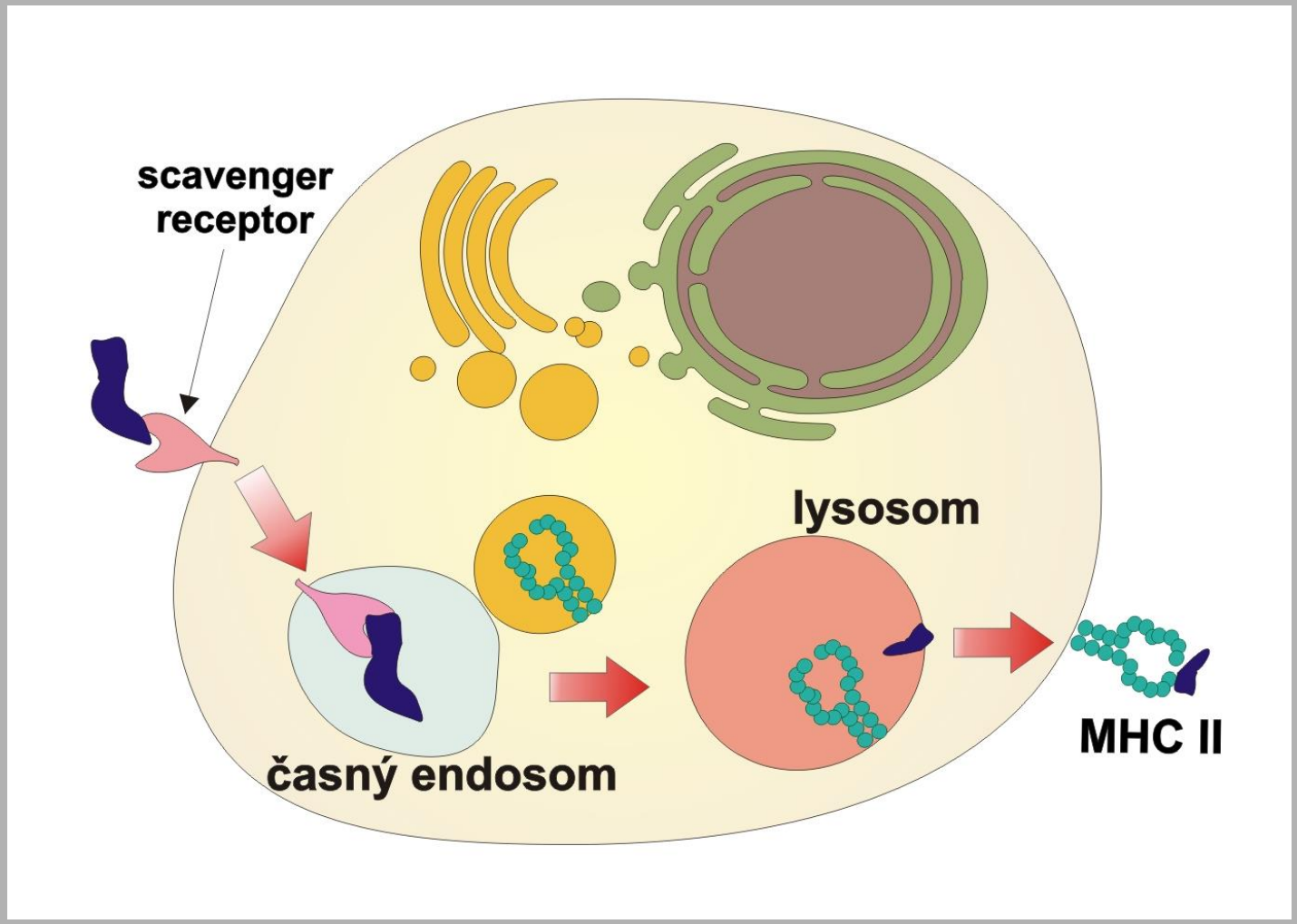
Pro srovnání prezentace korpuskulárního antigenu na MHC II



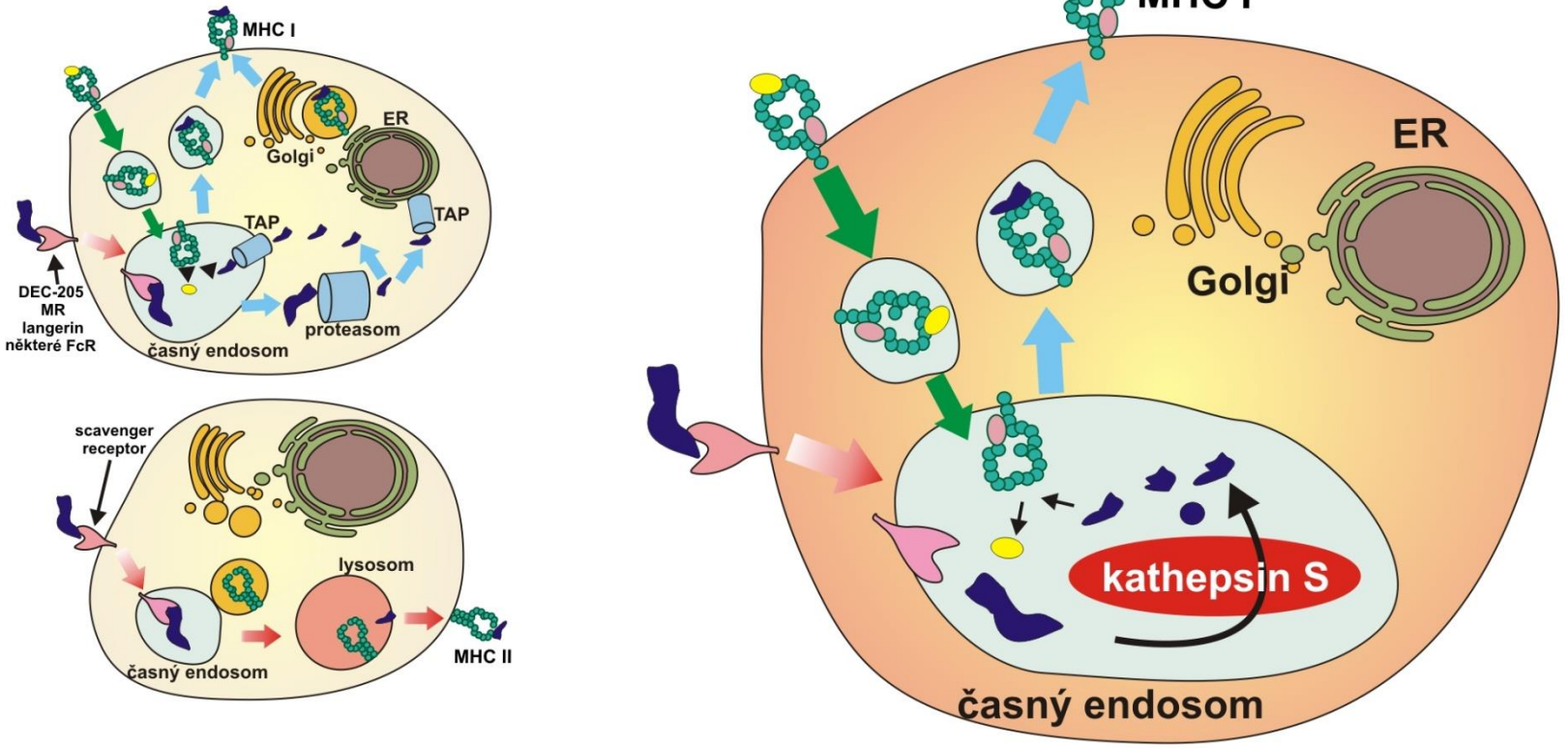
TAP závislá „cross“ prezentace – solubilní antigen



Pro srovnání prezentace solubilního antigenu na MHC II



TAP - nezávislá „cross“ prezentace



U člověka „cross“ prezentují solubilní i korpuskulární antigen konvenční DC (mDC-2) typizované $CD141^+$ (BDCA-3), $CD11c^+$

u myší konvenční DC $CD8^+$, $CD11c^+$

DNA Vaccine Carriers

Naked DNA (i.m., unprotected against degradation)

Pneumatic Jet Injection (particle free, mm-cm below the skin surface, strong shearing forces)

Needle (modified tatoo instrument), Electroporation

Gene Gun Delivery of Genes (epidermal, gold particles) TH2 response

Micro-Organisms: (Attenuated and genetically modified bacteria - Shigella, Salmonella, or Listeria) (mucosal immunisation, incorporation of pDNA into genome)

DNA-Containing Cochleates (oral)

Liposomes (all routes)

Polymers (biodegradable particles, mucosal, oral)

Dendritické buňky

Obtížná transfekce

I vitro lipofekce velmi málo účinná

Účinnost in vitro elektroporace kolem 10-15% pro Langerhansovy buňky nebo DC odvozené z progenitorů; DC odvozené z periferních monocytů jsou resistentní k transfekci

Rychlá degradace pDNA v dendritických buňkách, vrozená resistence k intracelulárním patogenům (viry, bakterie), výjimka HIV-1

Některé virové promotory mohou být i signálem pro ohrožení, snaha používat somatické promotory (promotor cytoskeletálního proteinu fascinu)

Svalové buňky

Poměrně dobře transfekovatelné – častý cíl pro genovou terapii (dlouhodobá transfekce, dlouhá doba života)

Nevirové vektory nejsou příliš účinné (na rozdíl od dobré účinnosti např. na transfekci jater nebo plic)

U myší transfekce svalových vláken volným plasmidem v rozmezí 2-5 týdnů (inhibiční efekt extracelulární matrix na penetraci pDNA, použití hyaluronidázy, rychlá degradace pDNA, 90% během prvních minut po aplikaci)

Způsoby přenosu DNA do buněk

Volná DNA

Virové vektory

- retrovirové vektory
- adenovirové vektory
- adeno-asociované viry

Nevirové vektory

- kationické liposomy
- polymery

Fyzikální metody

- gene-gun, jet-device
- elektroporace
- sonoporace

vektory odvozené od herpes simplex virů

hybridní virové vektory

Faktory ovlivňující úspěšnost DNA vakcinace

immunogennost antigenu patogena

frekvence a způsob podání, forma DNA vakcíny

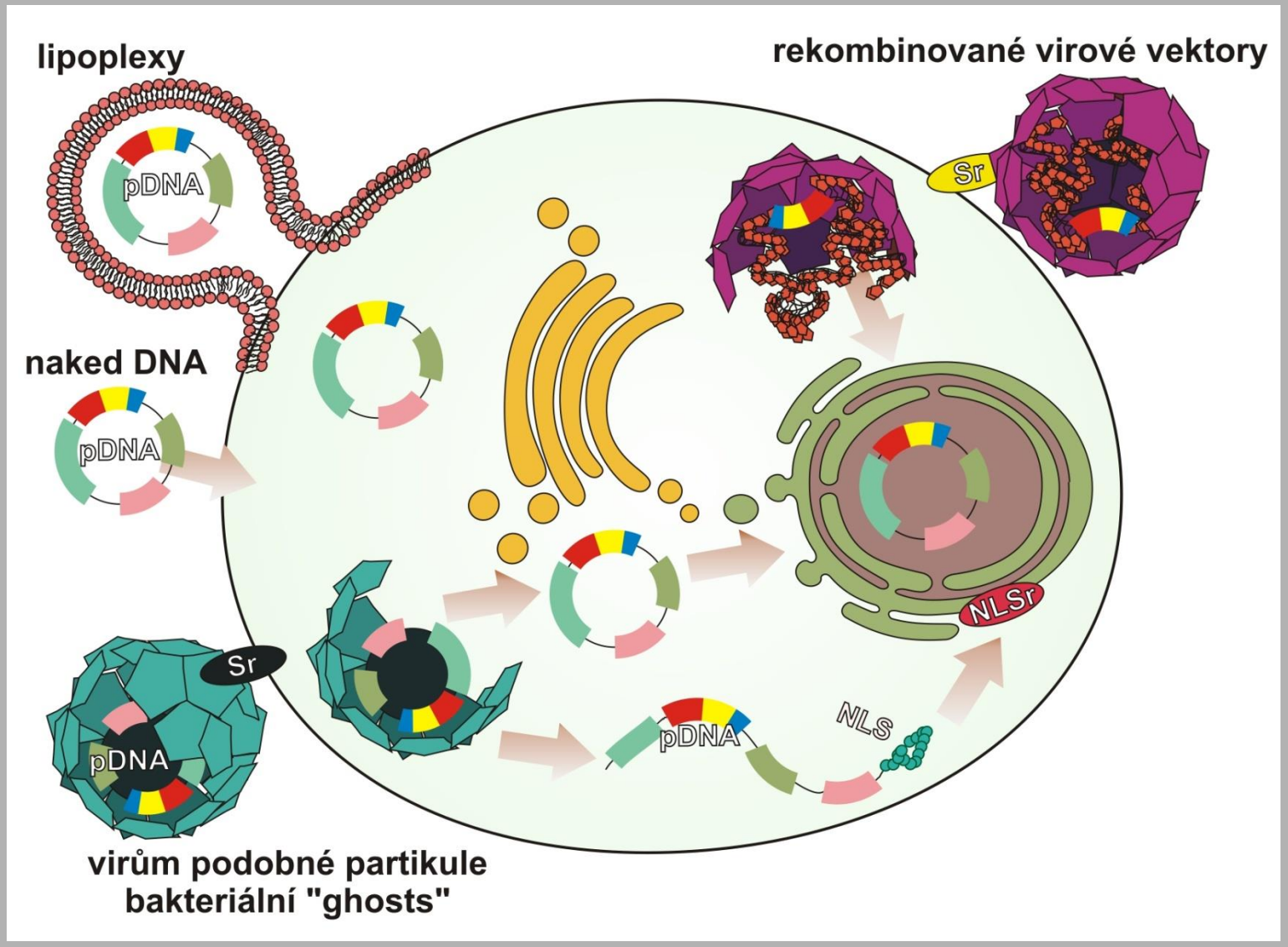
dávka pDNA

lokalizace antigenu (sekretovaný, membránový nebo cytoplasmatický)

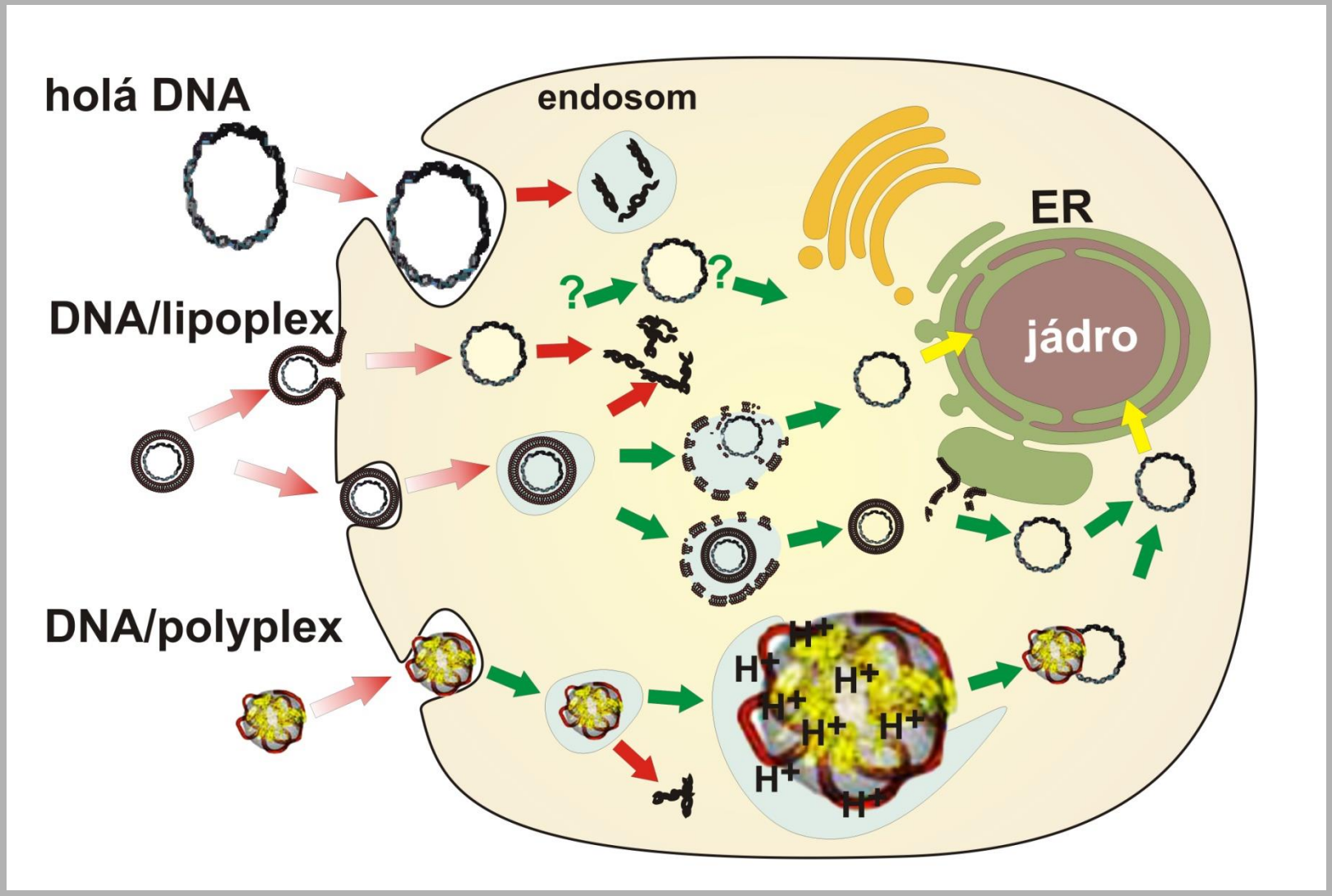
věk, zdravotní stav

živočišný druh

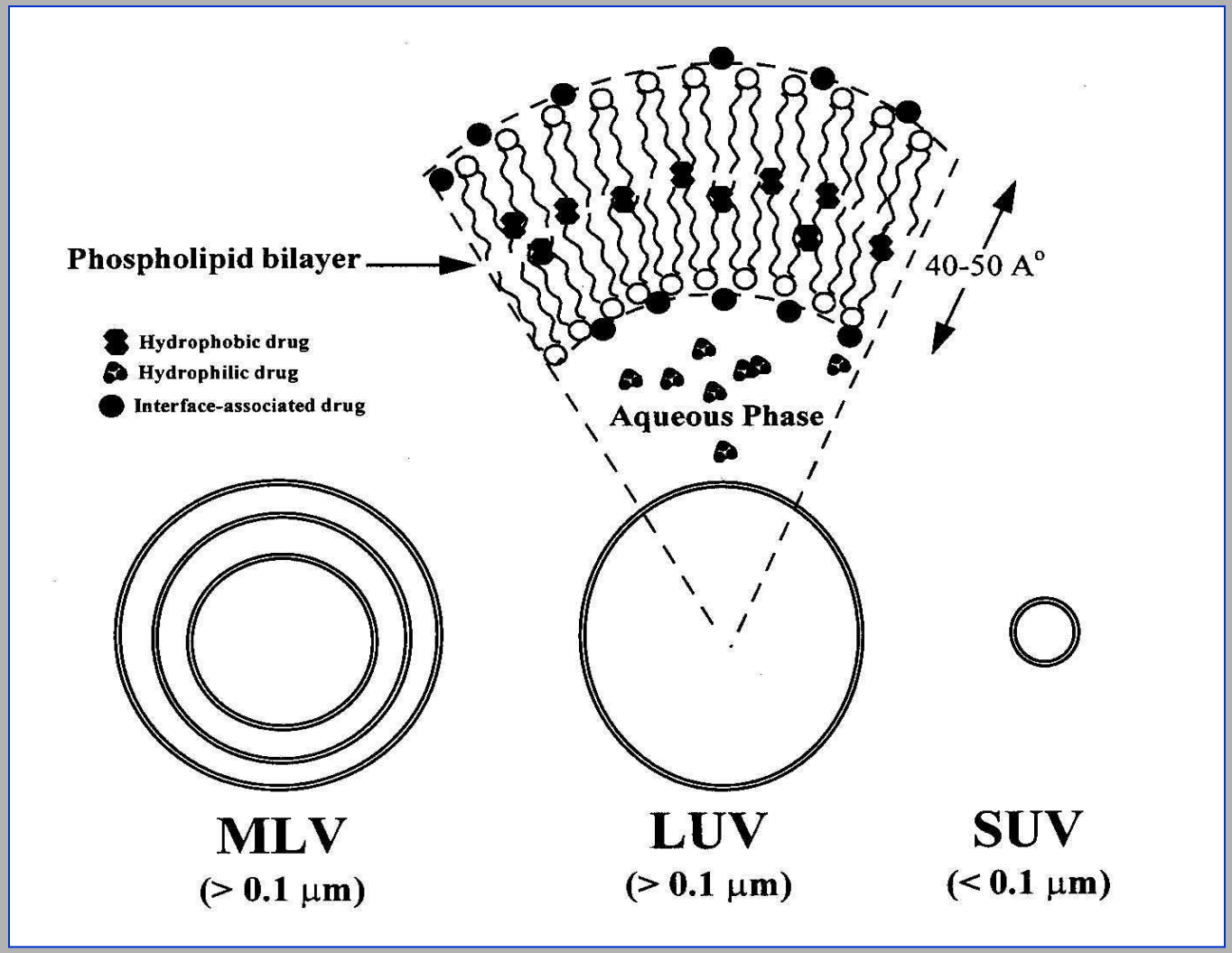
Formy aplikace DNA vakcín



Osud DNA v závislosti na použitém chemickém systému

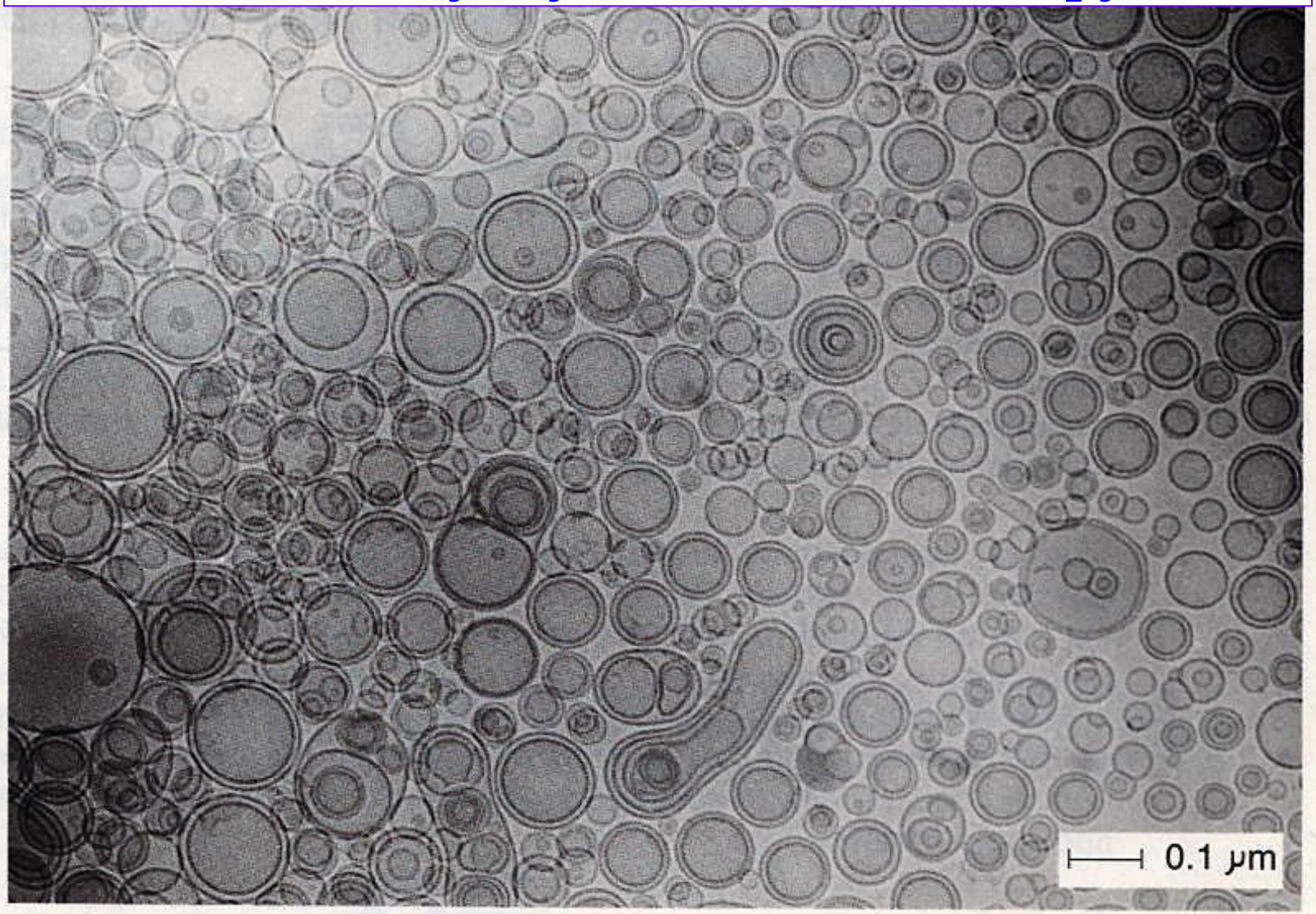


Classification of liposomes



Morphology of liposomes

Viewed by cryoelectron microscopy



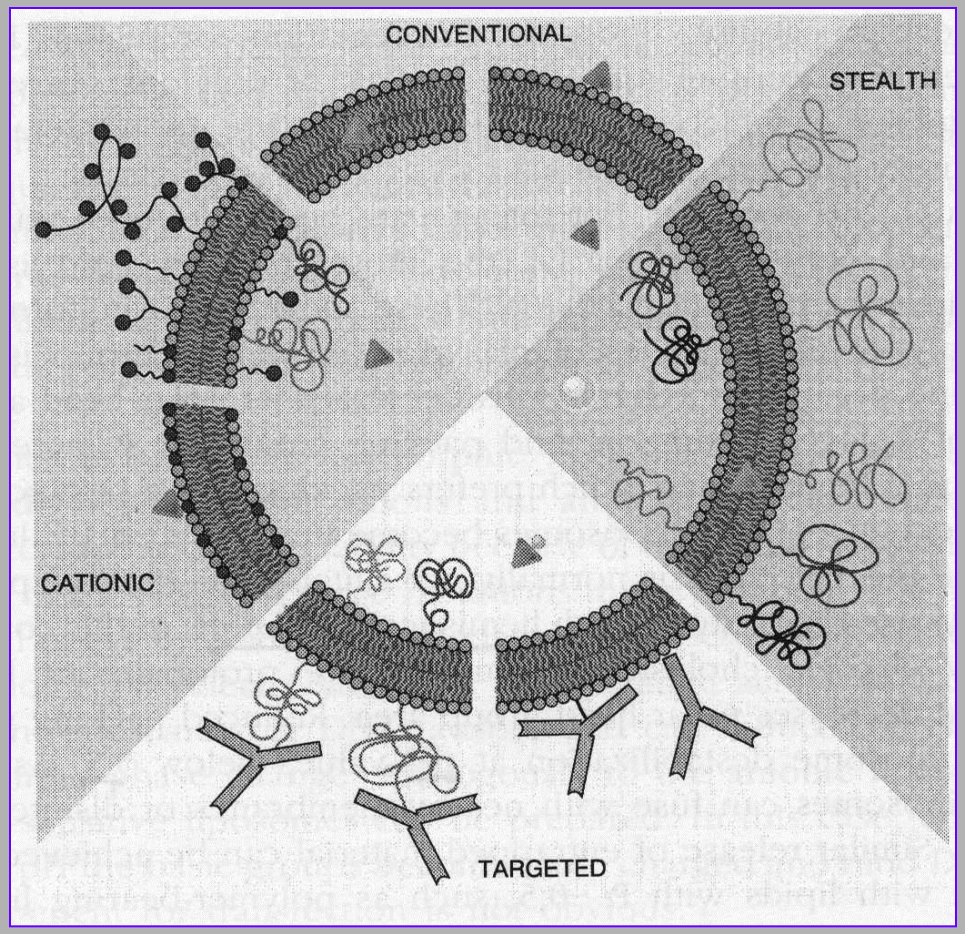
Classes of liposomes based on their functionality

Conventional liposomes *nonspecific interaction with melieu*

Stealth liposomes *sterically shielded , low non-specific interactions, long circulating*

Targeted liposomes *specific interaction via coupled ligand*

Polymorphic-*Cationic* *change their phase upon inter-action with specific agents, pH or temperature sensitive*



Artificial Virus

- Fully synthetic particles
- Deletion of undesirable structures

Immunosuppressors

Crossreacting antigens

- Selective targeting
- Production in cell free system
- Resembles a live atenuated vaccine

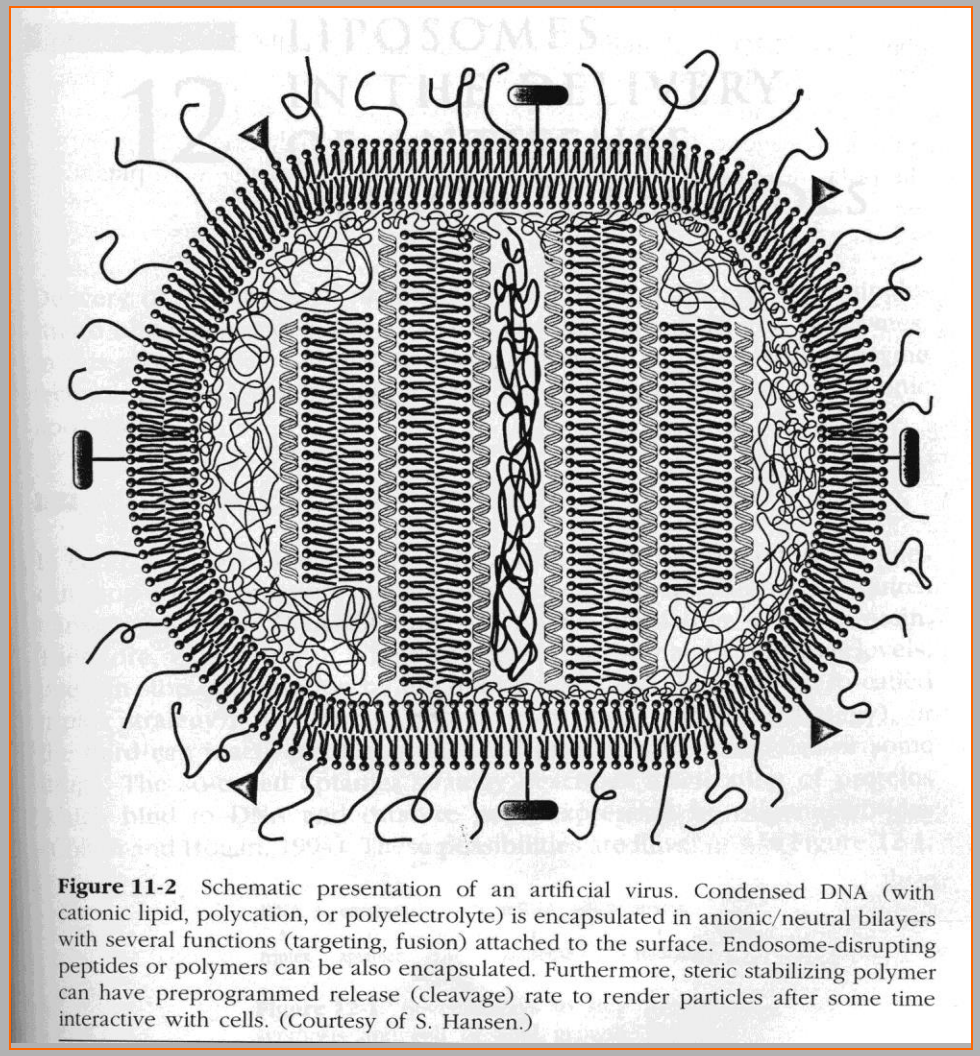
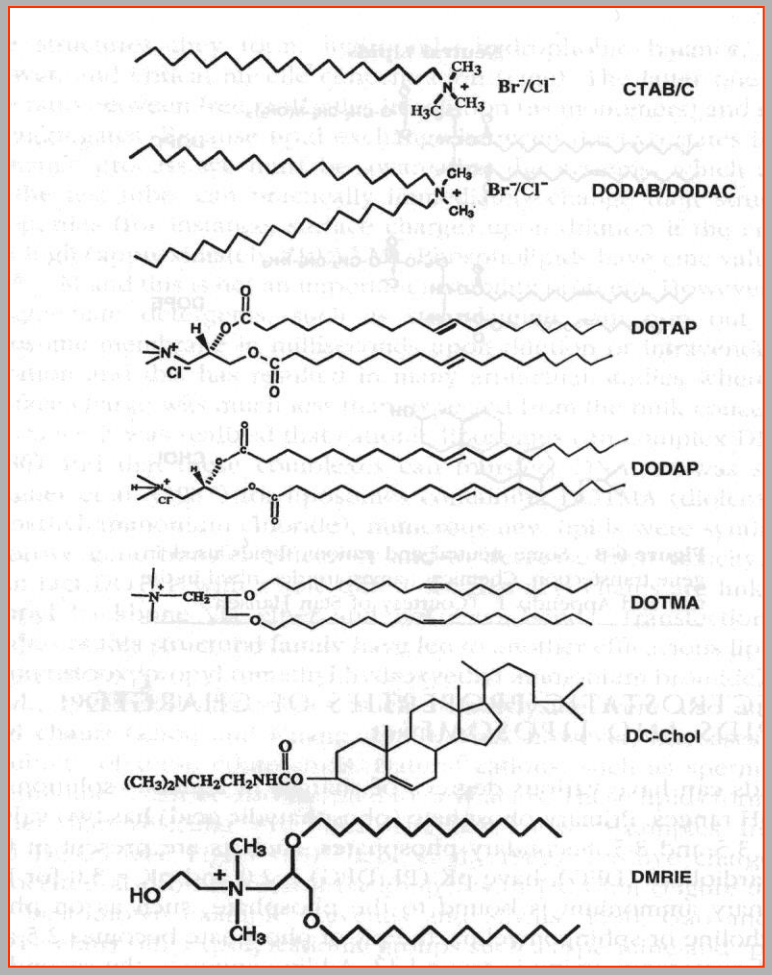
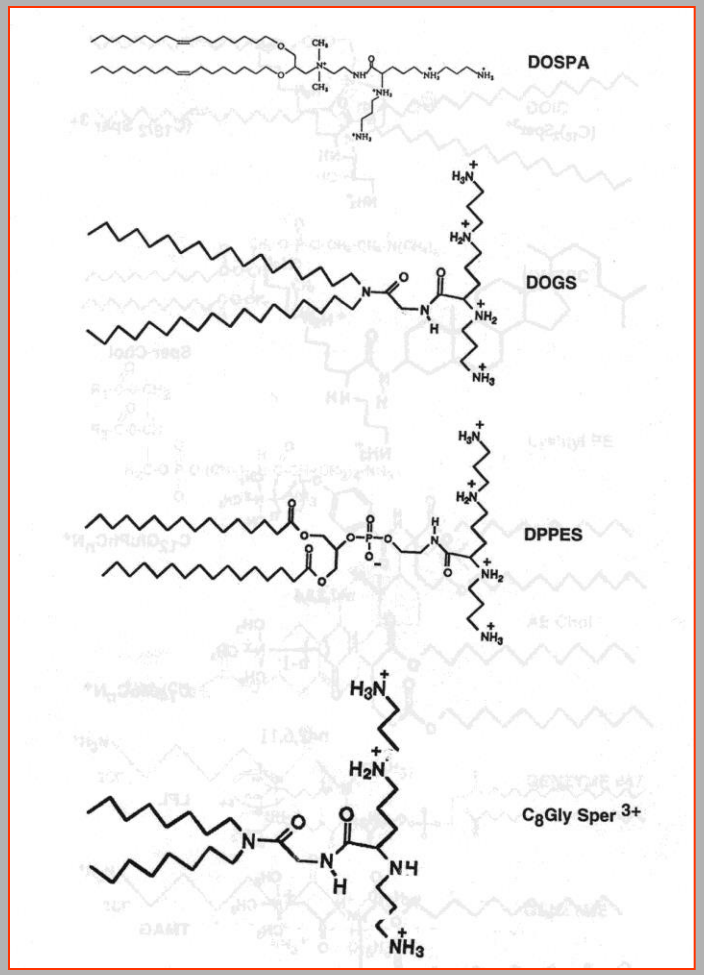


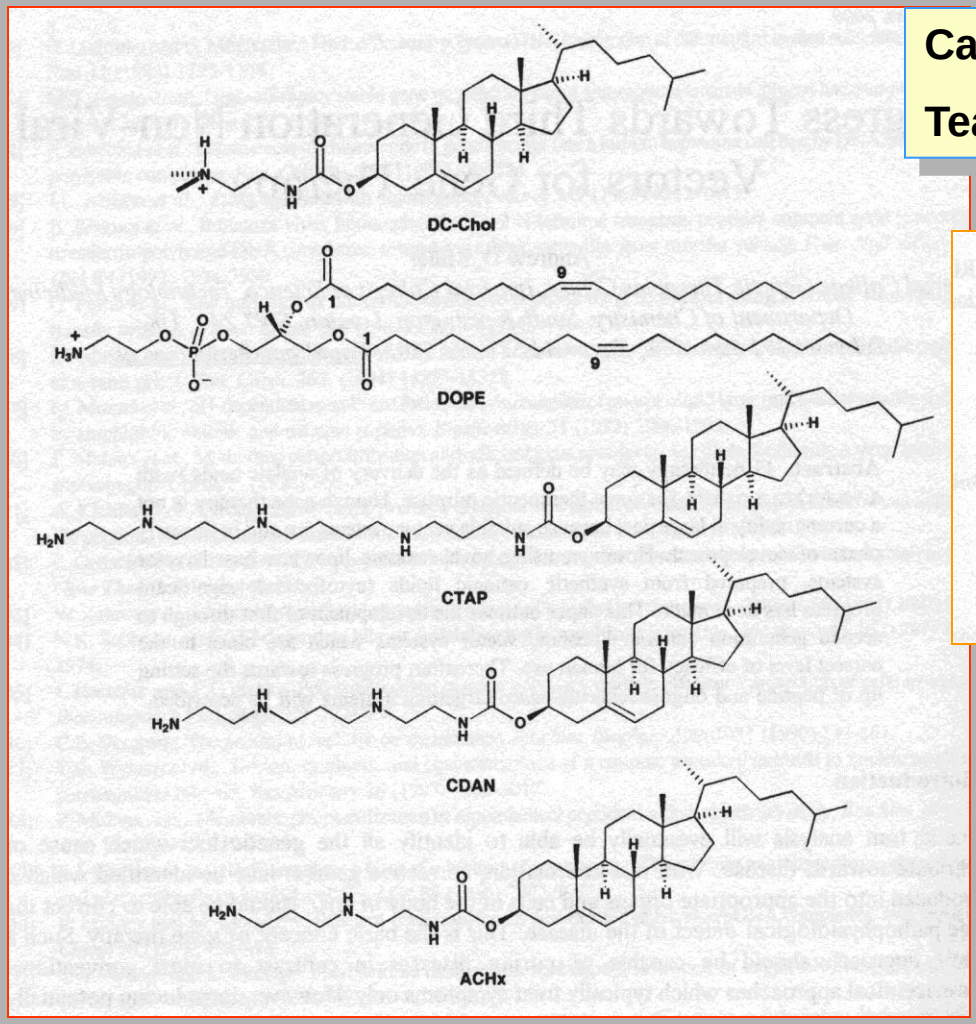
Figure 11-2 Schematic presentation of an artificial virus. Condensed DNA (with cationic lipid, polycation, or polyelectrolyte) is encapsulated in anionic/neutral bilayers with several functions (targeting, fusion) attached to the surface. Endosome-disrupting peptides or polymers can be also encapsulated. Furthermore, steric stabilizing polymer can have preprogrammed release (cleavage) rate to render particles after some time interactive with cells. (Courtesy of S. Hansen.)

Cataionic lipids



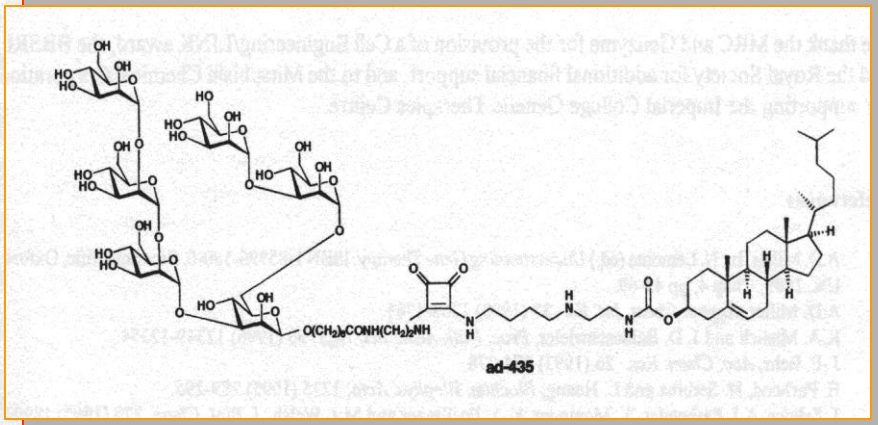


Cataionic lipids - Imperial College

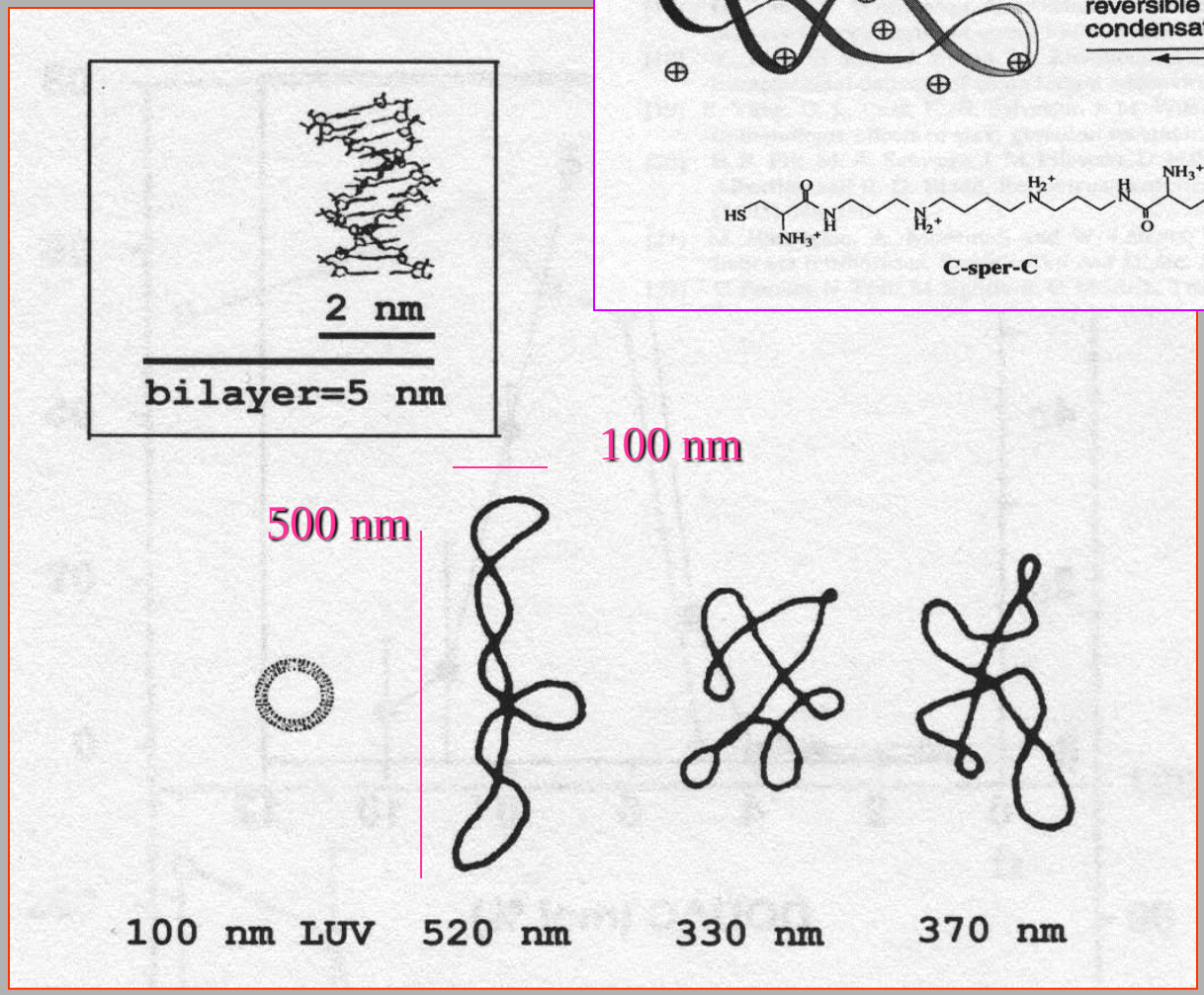


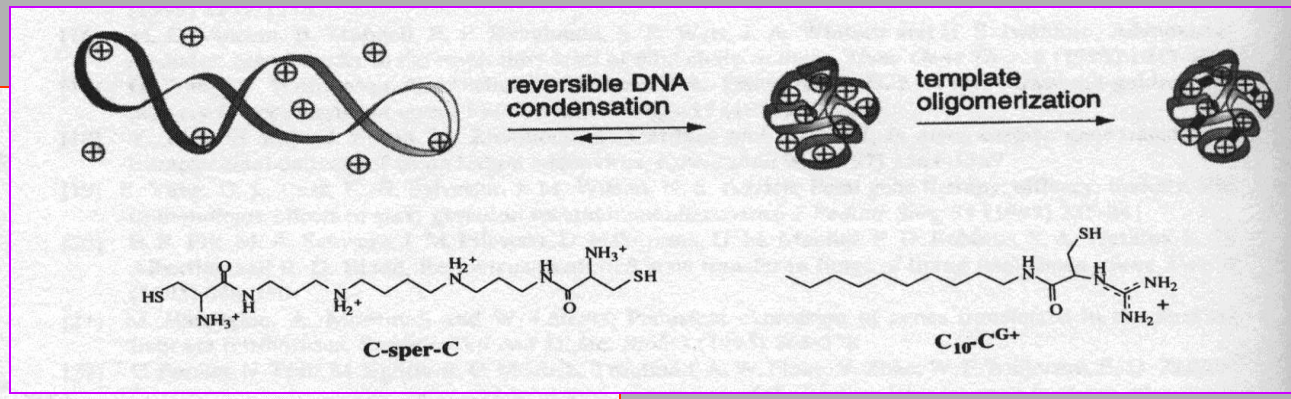
Cataionic lipids of the 3rd generation

Teargeting groups: peptides or saccharides

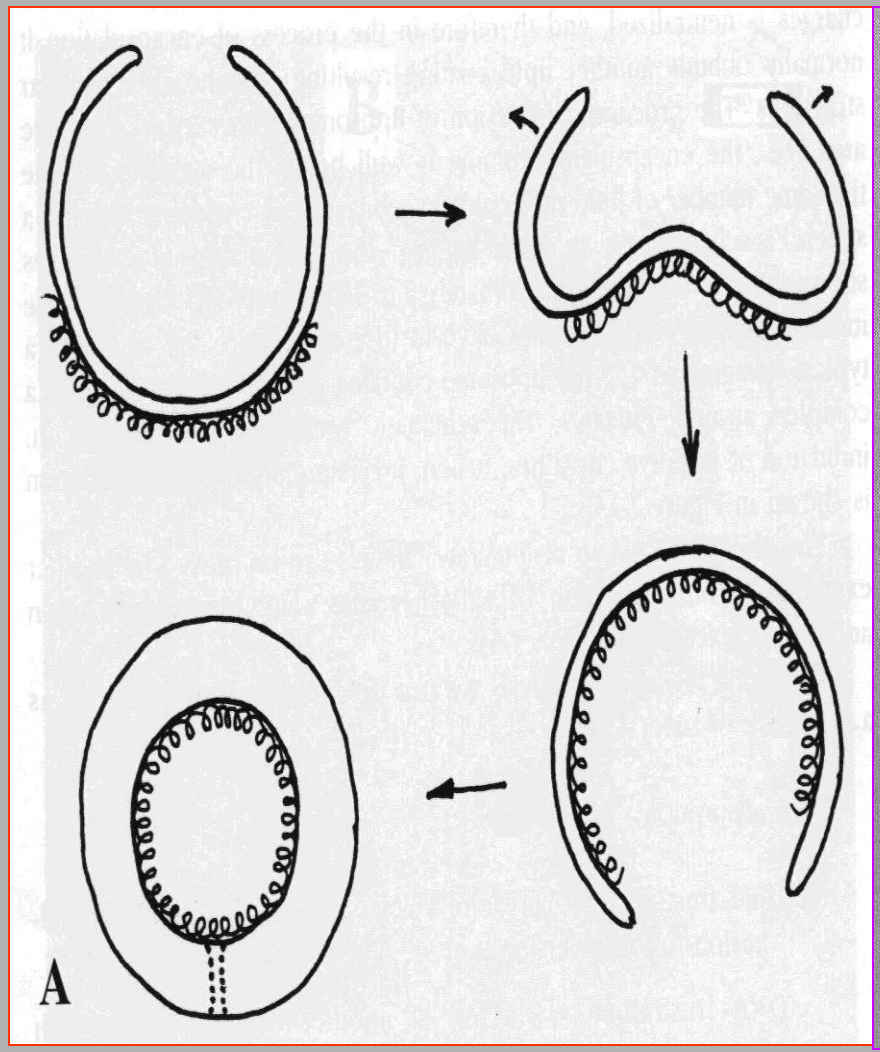


Encapsulation of plasmids into liposomes





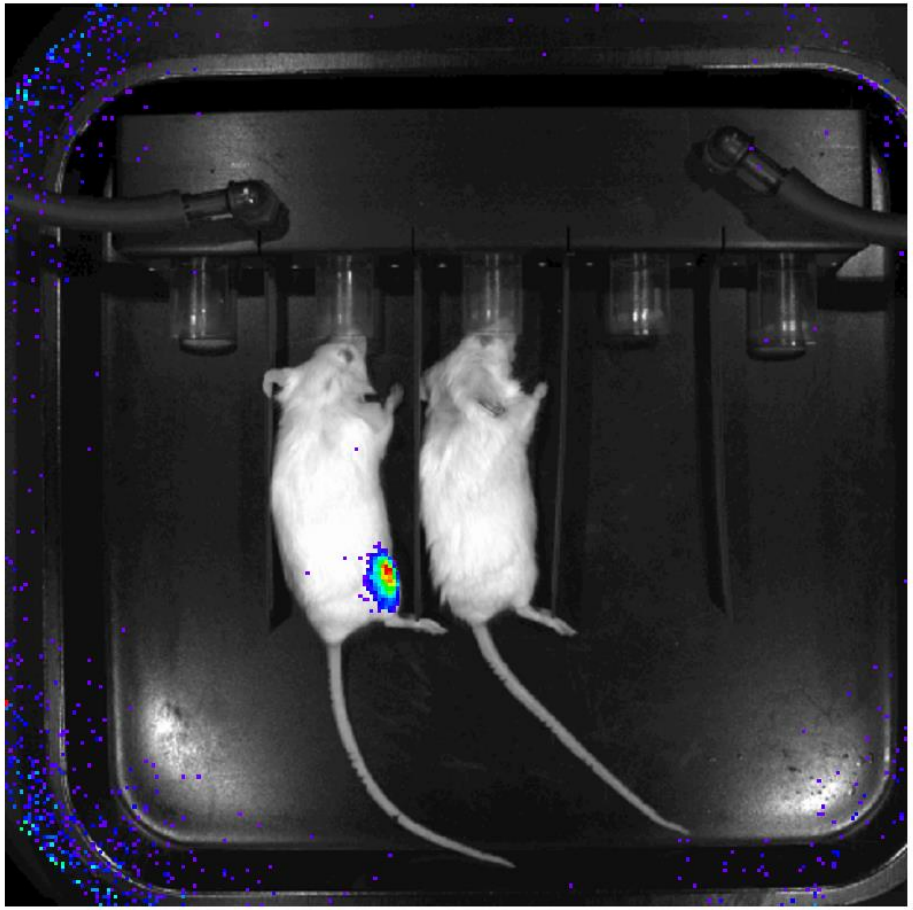
Interaction of cationic liposomes with DNA



Routes of Application

- Intramuscular
- Intraepidermal
- Mucosal: Oral, Nasal,Vaginal, Rectal, Lung, Sinovial
- Intravenous
- Intraperitoneal
- Subcutaneous

Aplikace DNA vakcíny do svalu



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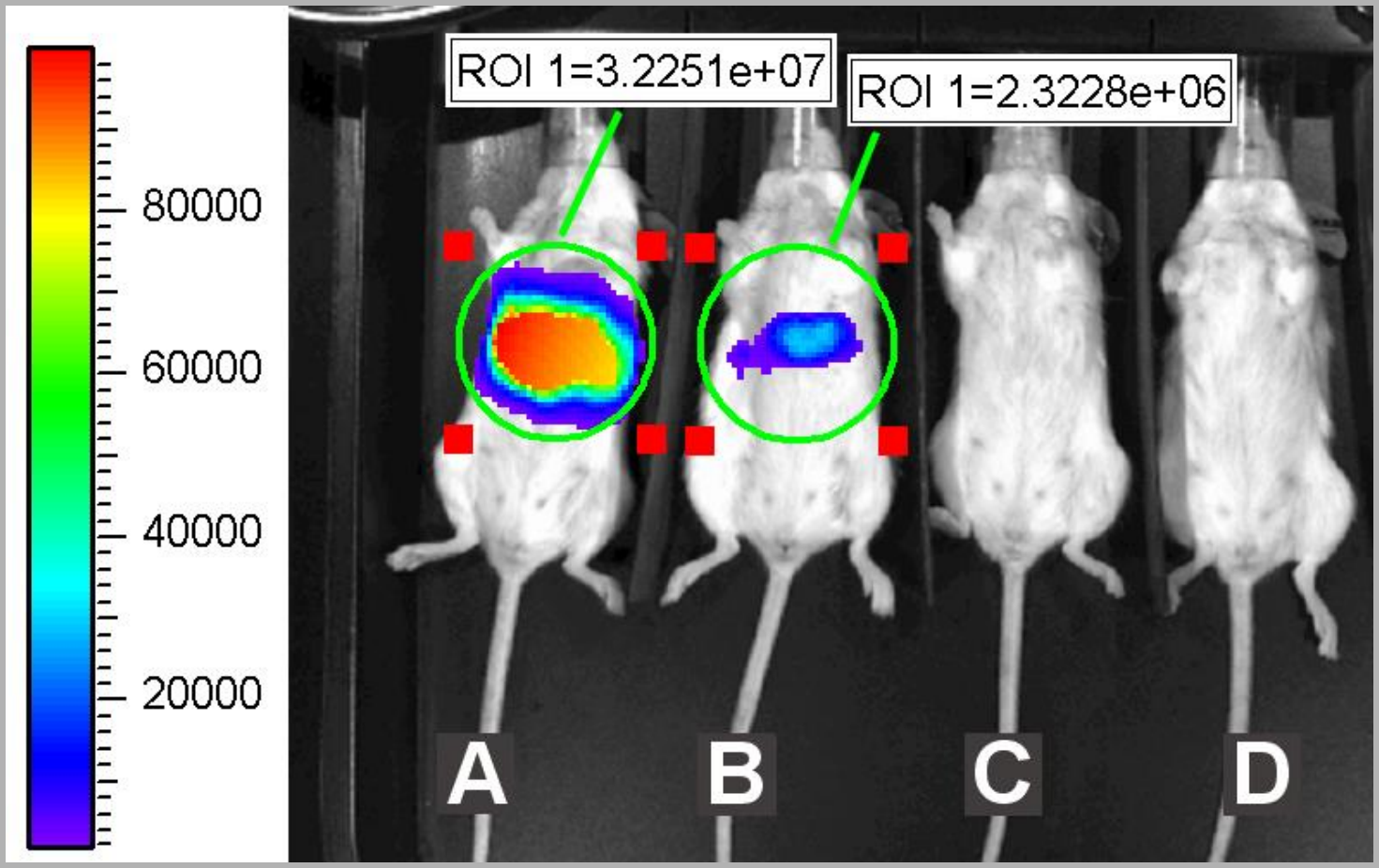
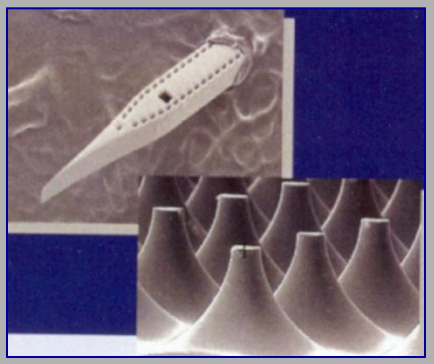
Tue, Jun 28, 2005 16:07:43
Bin:M (8), FOV25, f1, 120s
Filter: Open
Camera: IVIS 13083, SI620EEV

Group ID: Mouse11,12
Experiment: Mestecky imin
Label: 120s lateral 062805
Comment: bin 8
Investigator: Zinn/Mestecky

Aplikace DNA vakcíny do jater



Microenhancer Arrays



- Solid frusto-conical microprojections
- Designed to disrupt skin barrier function
- Enables delivery of DNA vaccines to epidermis

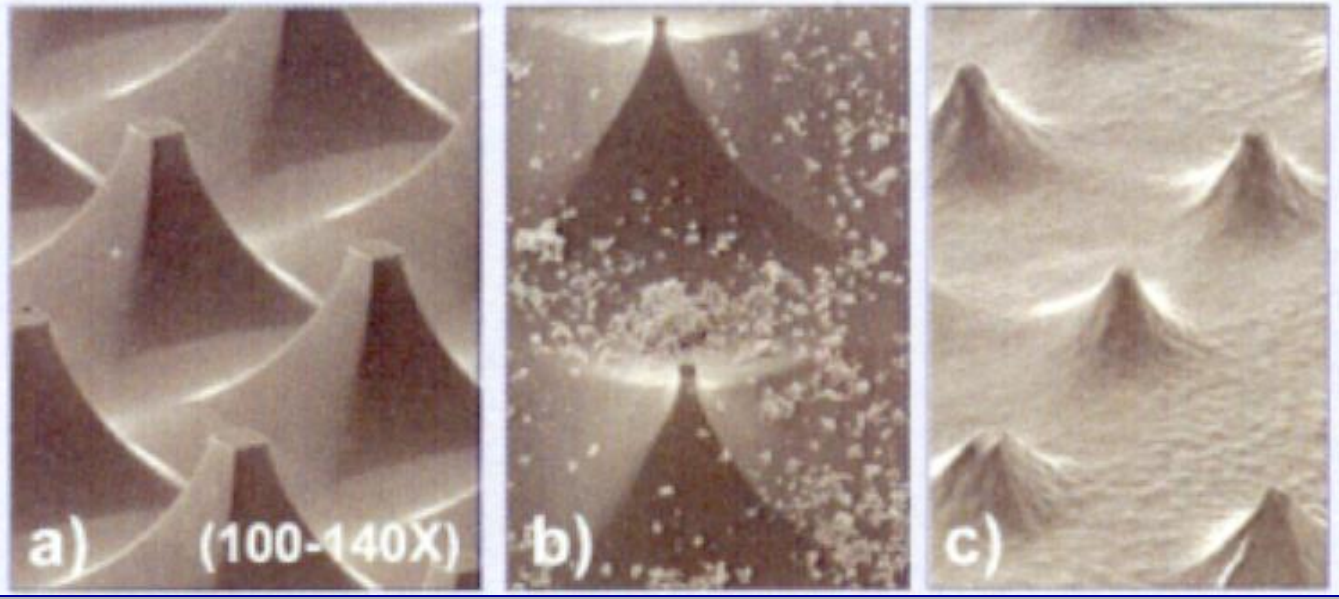
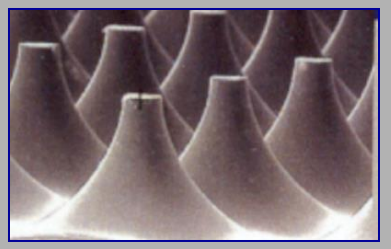


Figure 3. Electron micrographs of uncoated (a), powder-coated (b), or evaporative film-coated (c) MEAs.

Epidermal application



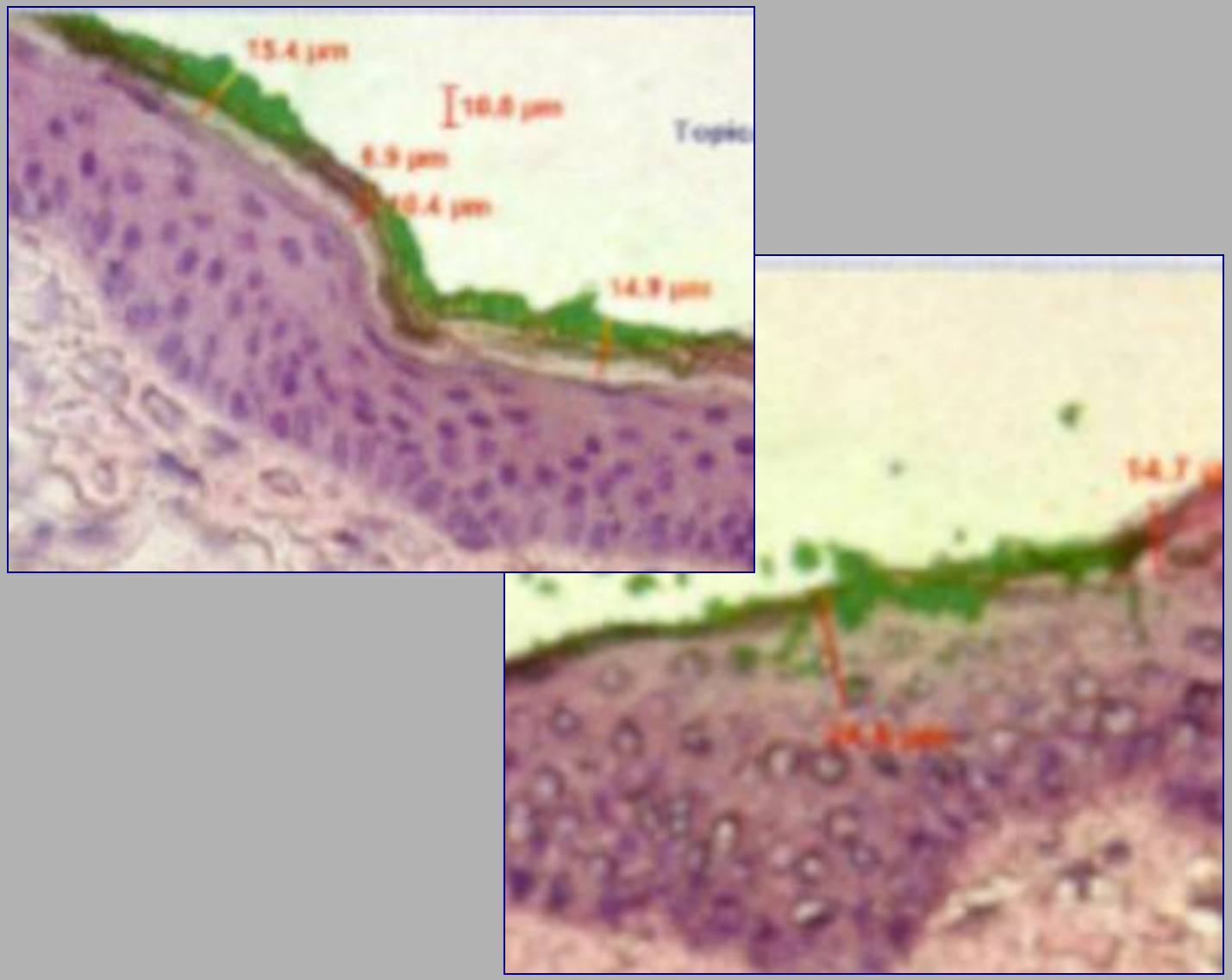


Figure 4. Tissue distribution of fluorescent microspheres applied to Yorkshire swine (Fig. 4a, b) or excised human cadaver skin (Fig. 4c,d) via 4 to 6 lateral MEA passes or unassisted topical application.

- MEAs disrupt stratum corneum allowing microspheres to access epidermis.
- Stratum corneum prevents access of topically applied microspheres to epidermis.

Intradermal application

Devices and Histology

MICRONEEDLES –

- Hollow microcannula
- Minute and minimally-invasive
- Needle length designed to restrict DNA vaccine delivery to the dermis

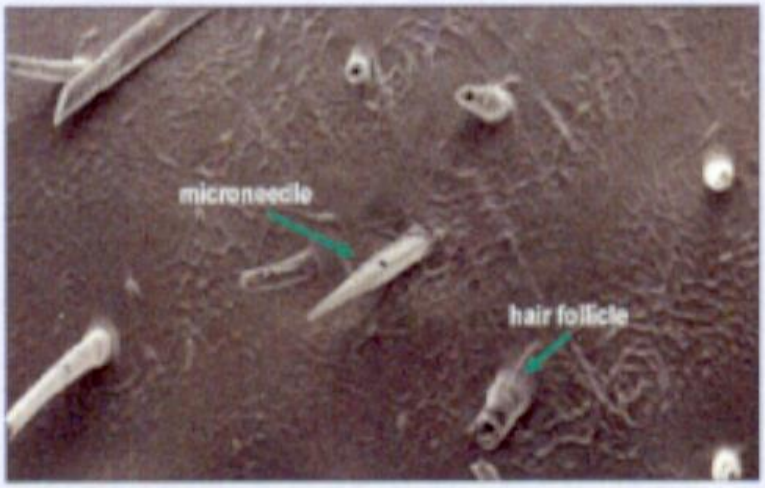


Figure 1. Microneedle penetrating swine dermis. *(Electron microscopy by H. Sugg, BD Technologies)*

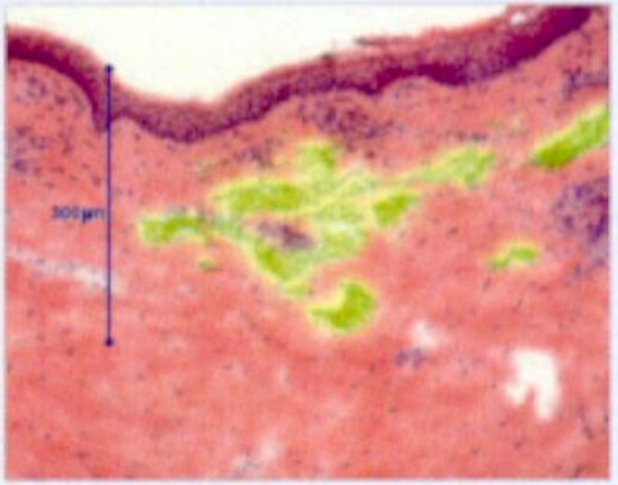
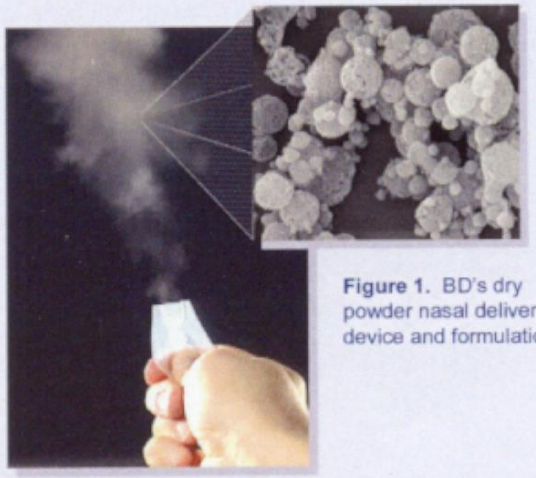


Figure 2. Delivery of fluorescent microspheres to the dermis by microneedle.

Intranasal delivery

BD Dry Powder Nasal Delivery Platform



- Human device
- Dry powder vaccine or drug is packaged in proprietary pressure capsule
- Dry powder vaccine or drug is delivered by 'popping' blister

Figure 1. BD's dry powder nasal delivery device and formulation.

Nasal Delivery Device for Rat Experiments

- Same driving force as human device
- Exit diffuser narrow
- Bulb at end of exit port to help locate device within the nasal cavity reproducibly

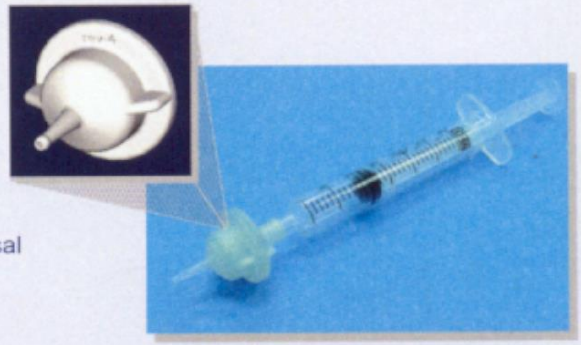
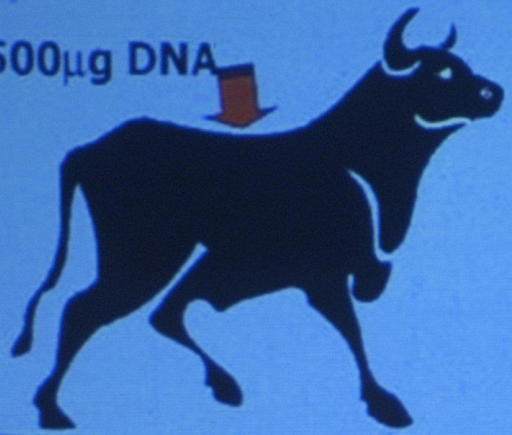


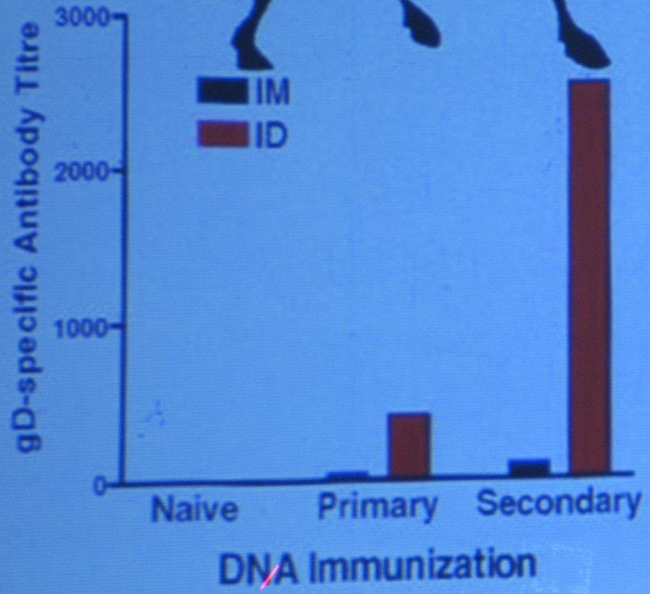
Figure 2. Nasal delivery device modified for

TRANSCUTANEOUS VACCINE DELIVERY SYSTEMS

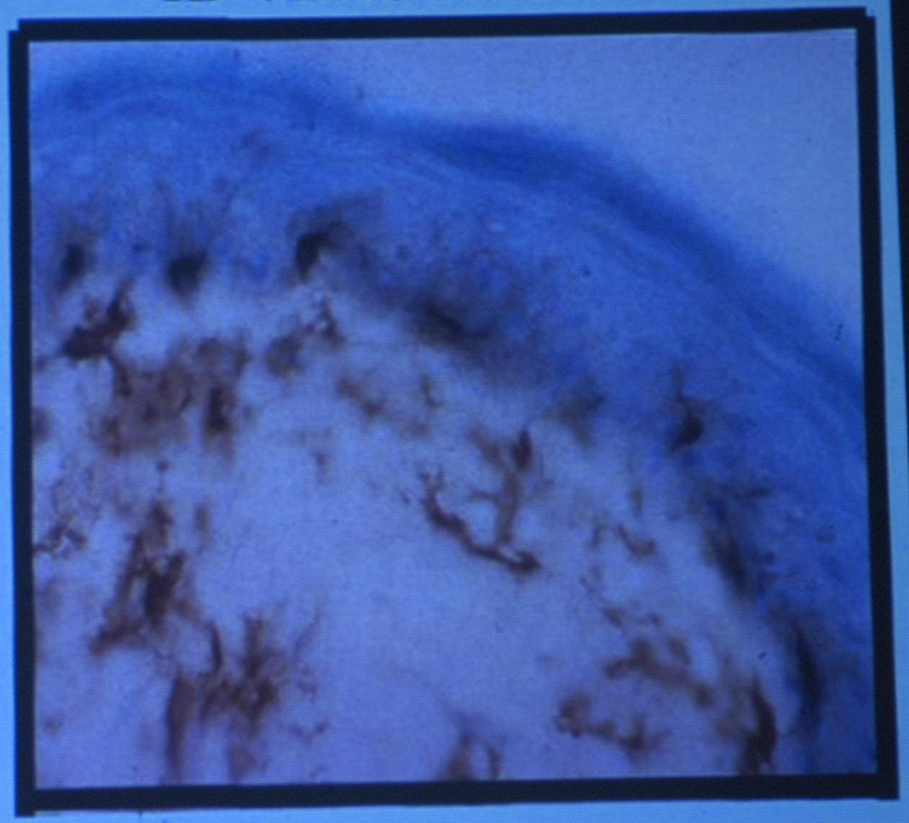
VIDO

Solutions through research





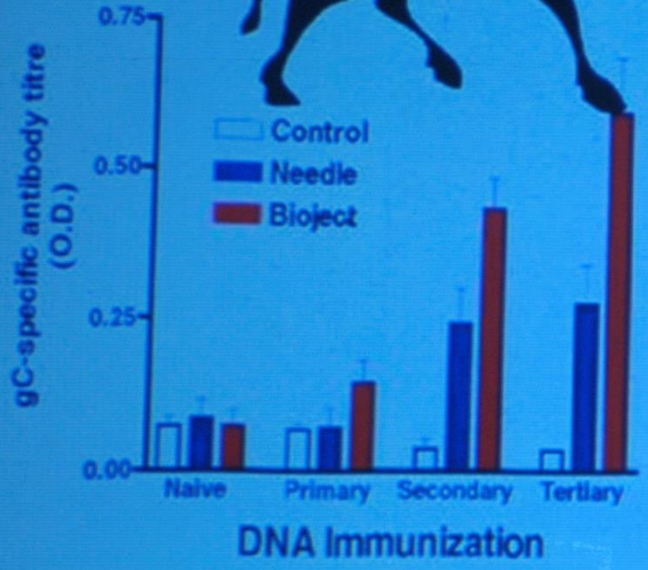
ID VERSUS IM



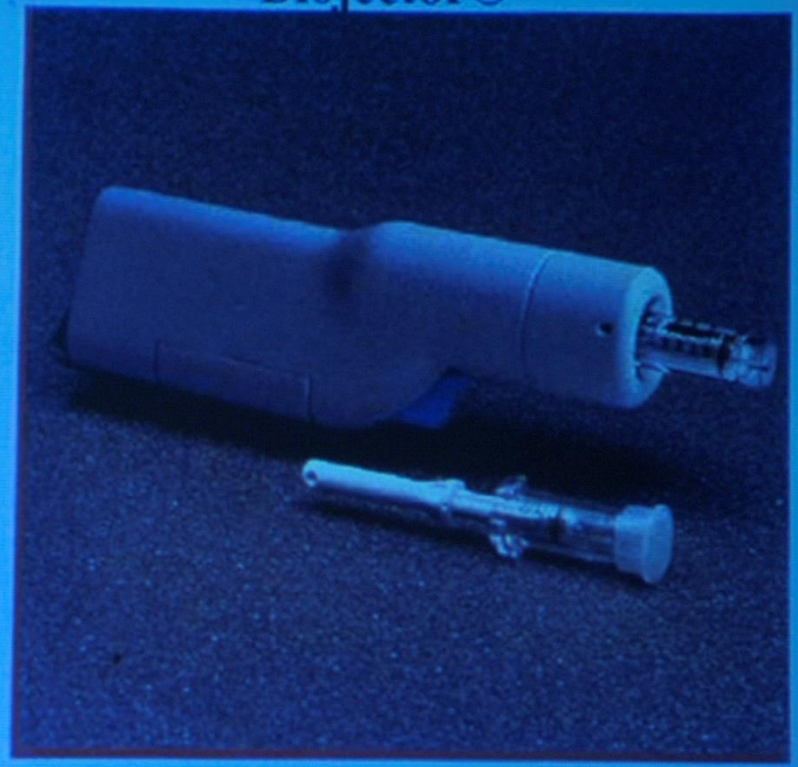
TRANSCUTANEOUS VACCINE DELIVERY SYSTEMS

VIDO

Solutions through research



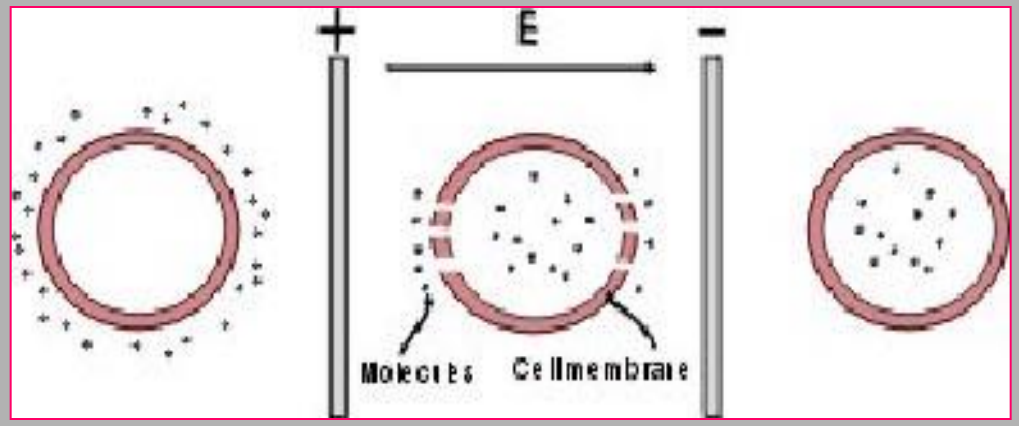
Biojector®

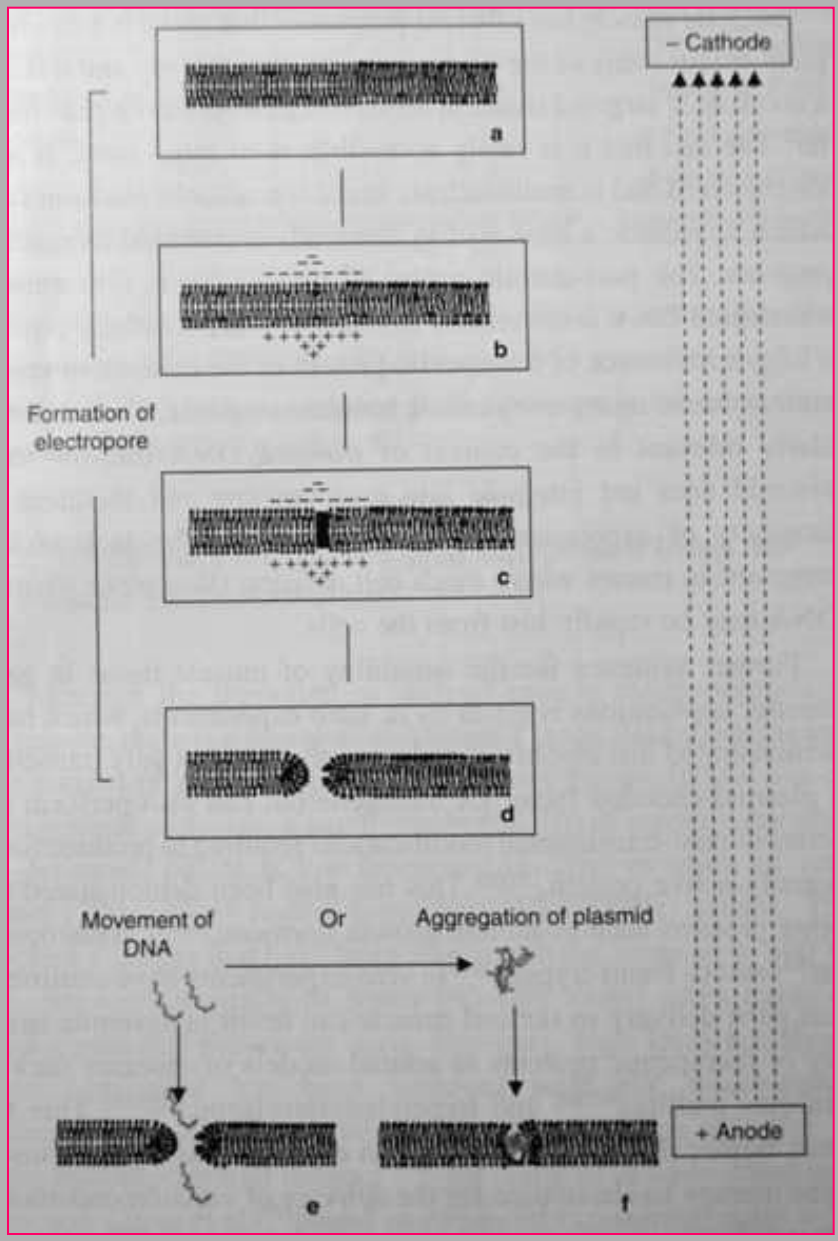


Elektroporace

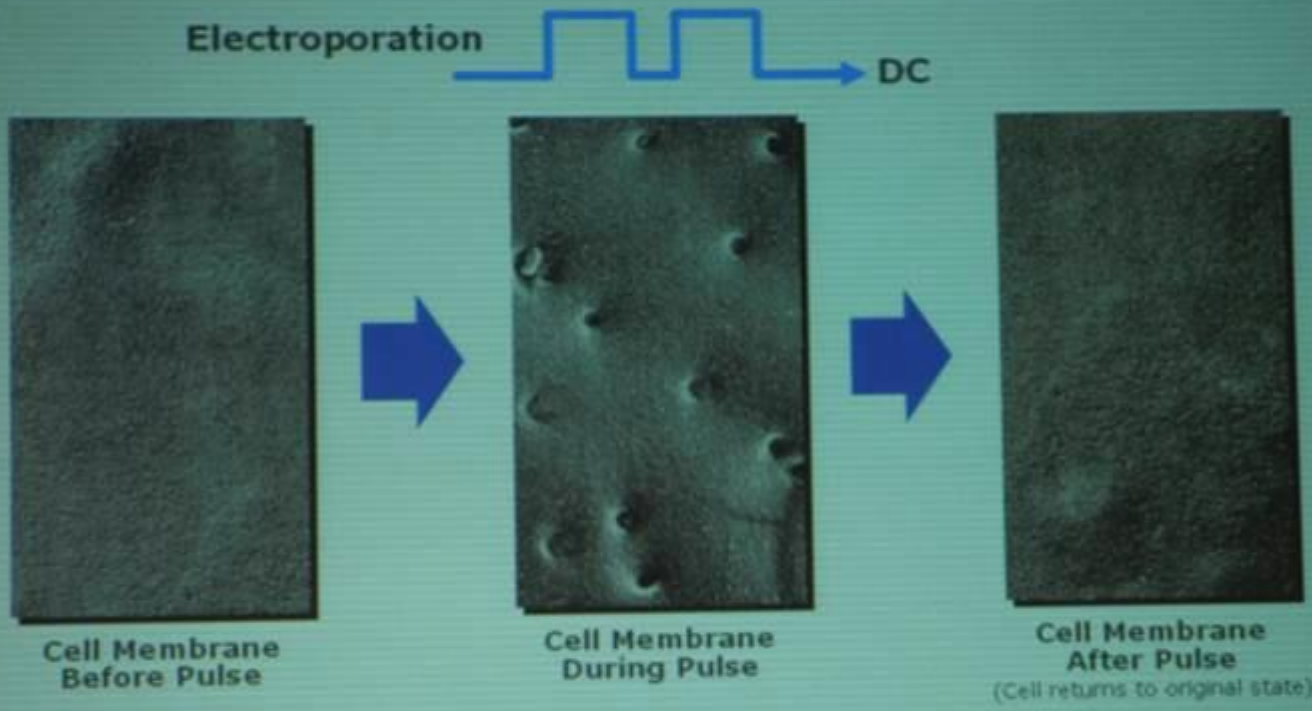
Princip elektroporace

- při aplikaci elektrického napětí dochází ke změnám transmembránového potenciálu, po dosažení kritické hodnoty se membrána stává permeabilní, dochází k tvorbě hydrofilních pórů
- průchod extracelulárních molekul do buňky





Therapeutic Platform Based on Electroporation



Electroporation applies brief electrical pulses, inducing pores to open in the cell membrane and dramatically increasing uptake of useful drugs, genes & DNA vaccines

A simple and effective system of delivering drugs or genes into cells

GENETRONICS BIOMEDICAL CORPORATION

Využití elektroporace

Vnášení farmakologicky významných molekul (hydrofilní povahy) do buněk

Genová terapie a DNA vakcíny - In vivo a in vitro transfekce buněk (antigeny, růstové faktory, cytokiny, enzymy) vnášení DNA do buněk pro účely genové terapie

Protinádorová elektrochemoterapie - zvýšení selektivity a účinnosti chemoterapeutik (bleomycin)

Elektroporace *in vivo*

Vliv na účinnost elektroporace

- velikost, tvar a morfologie buněk
- viskozita a vodivost extracelulární tekutiny (struktura extracelulární matrix)

Elektrické parametry

- nízký počet (2-10) středně dlouhých (1-50 ms) pulsů při nízké frekvenci (1-2 Hz)
- série velmi krátkých (200-500 μs) pulsů při vysoké frekvenci (10-1000 Hz)

Typy elektrod

- ploché elektrody, přiložené zevně ke svalu nebo kožnímu lemu
- jehlovité elektrody pro intramuskulární aplikaci
- Needle array – multilektrodové aplikátory s rotujícím elektrickým polem

Srovnání účinnosti elektroporační transfekce pomocí šestielektrodového a dvouelektrodového aplikátoru

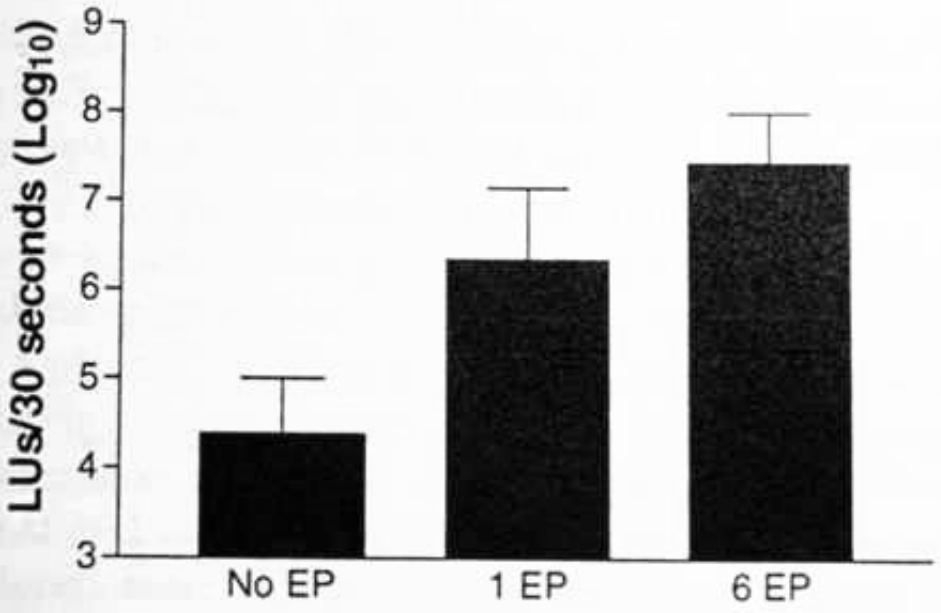
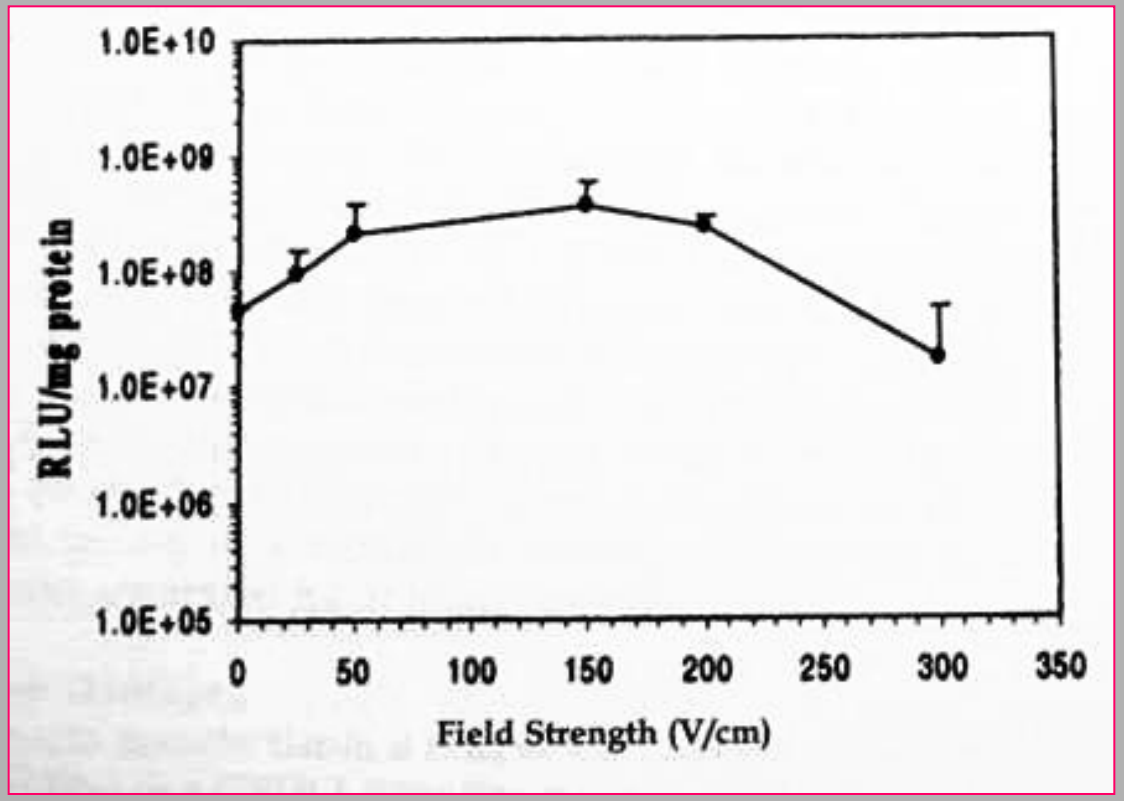


Fig. 1. Increase in gene expression in the muscle by electroporation. Porcine muscle was injected with 100 μg of pMAS-luc in 500 μl PBS at five different sites in the quadriceps muscle, followed by no electroporation, electroporation with a single-needle electrode (1 EP), or a six-needle array electrode (6 EP). Luciferase activity in the excised tissue was measured 24 h following administration of plasmid. No EP vs. 6 EP, $P < 0.05$ by one-way ANOVA followed by Tukey's multiple comparison test. Error bars represent S.E.M.

Babiuk at al. Vaccine 2002, 20,

Vliv intenzity elektrického pole na účinnost transfekce



doi:10.1006/mthe.2002.0540, available online at http://www.idealibrary.com on IDEAL

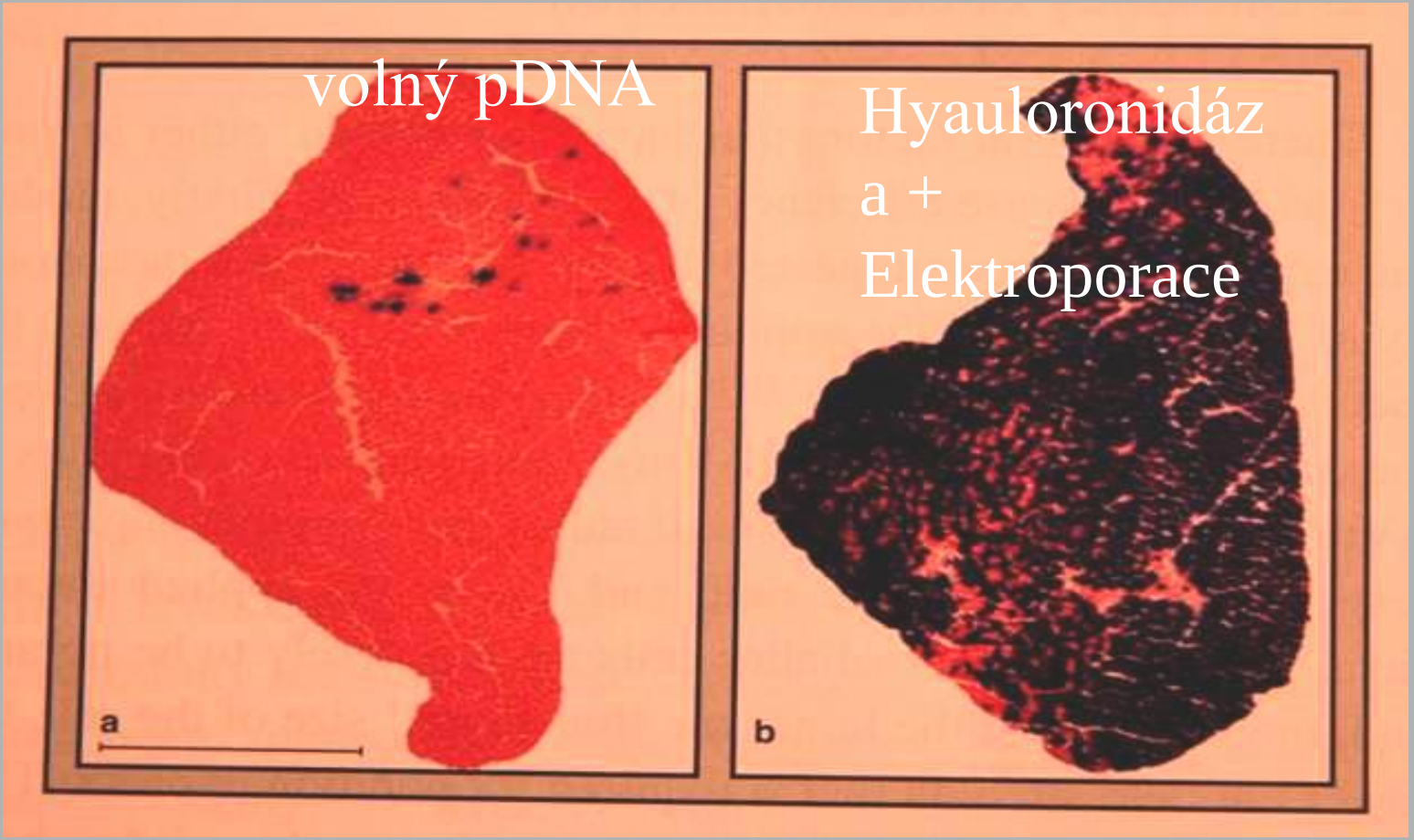
METHOD

A Syringe Electrode Device for Simultaneous Injection of DNA and Electrotransfer

Feng Liu and Leaf Huang*

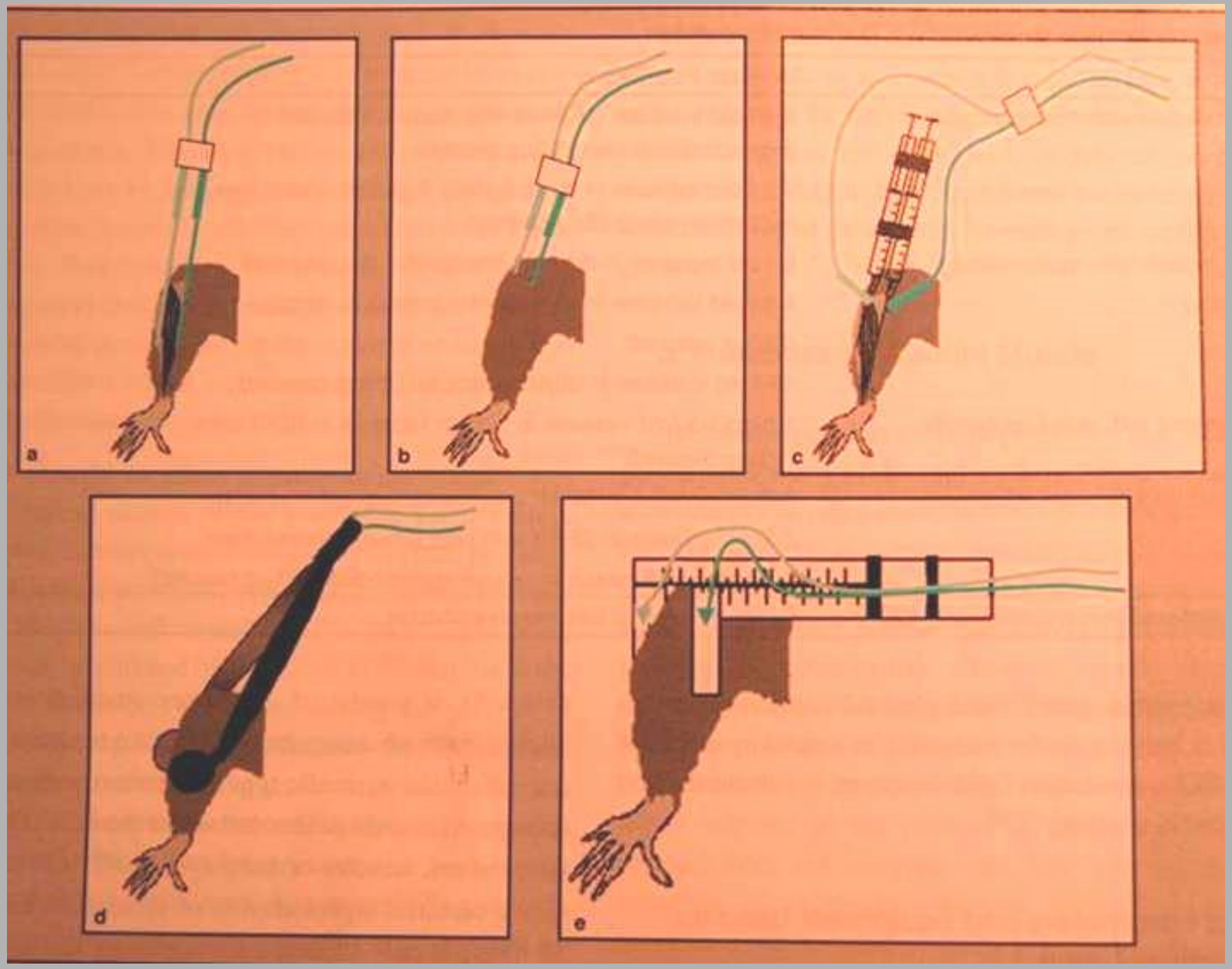
Center for Pharmacogenetics, School of Pharmacy, 633 Salk Hall, University of Pittsburgh, Pittsburgh, Pennsylvania 15213, USA

Vliv extracelulární matrix na elektrotransfekci myšího svalu



McMahon MJ and Wells DJBiodrugs 2004, 18,155-165

Typy elektrod



Typy elektrod

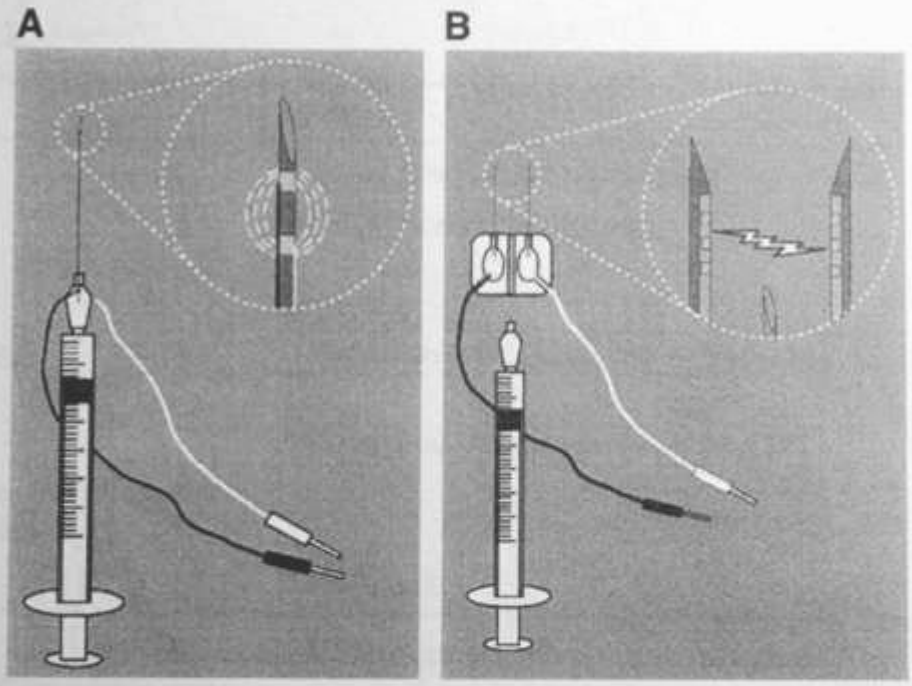
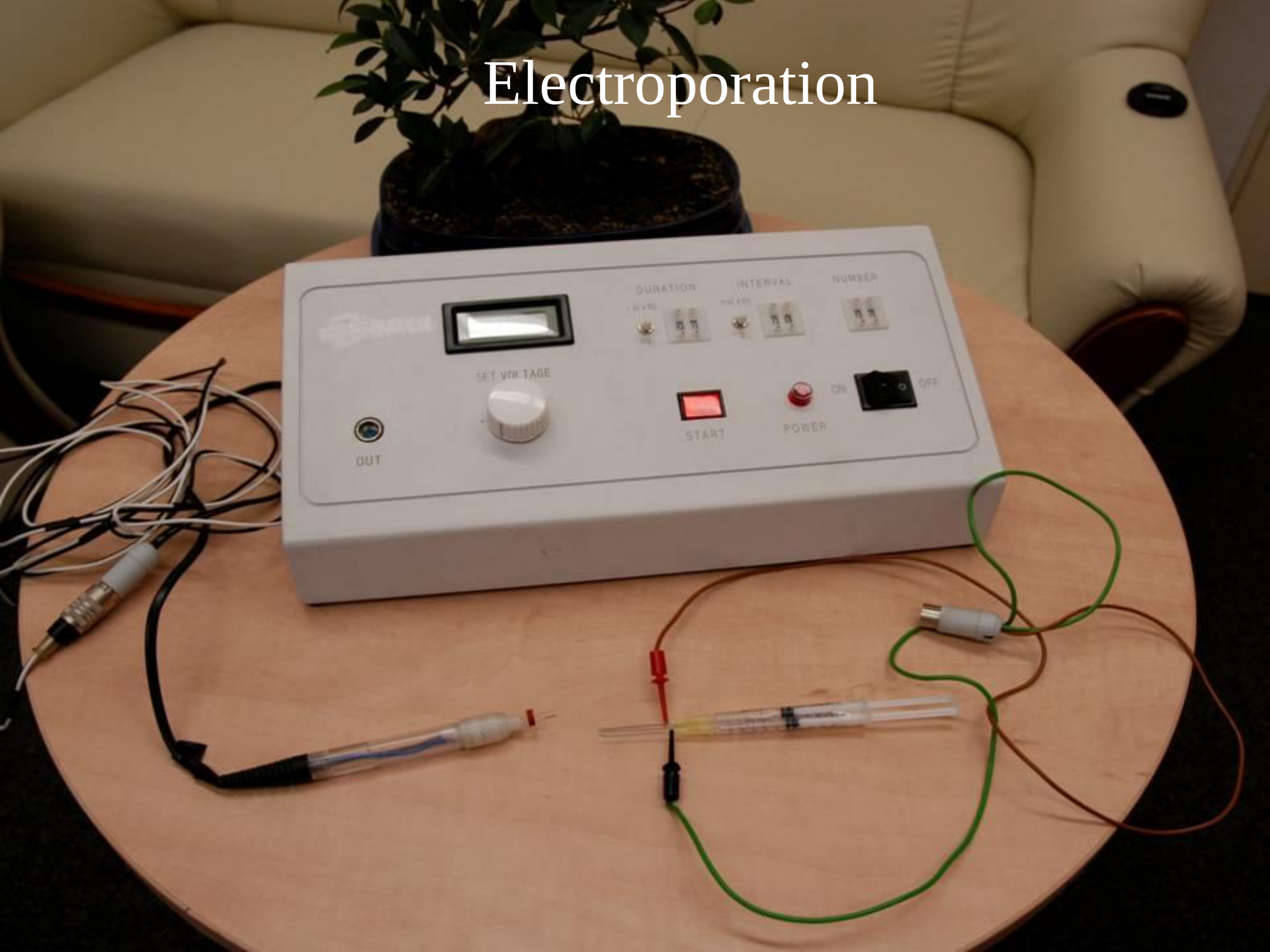
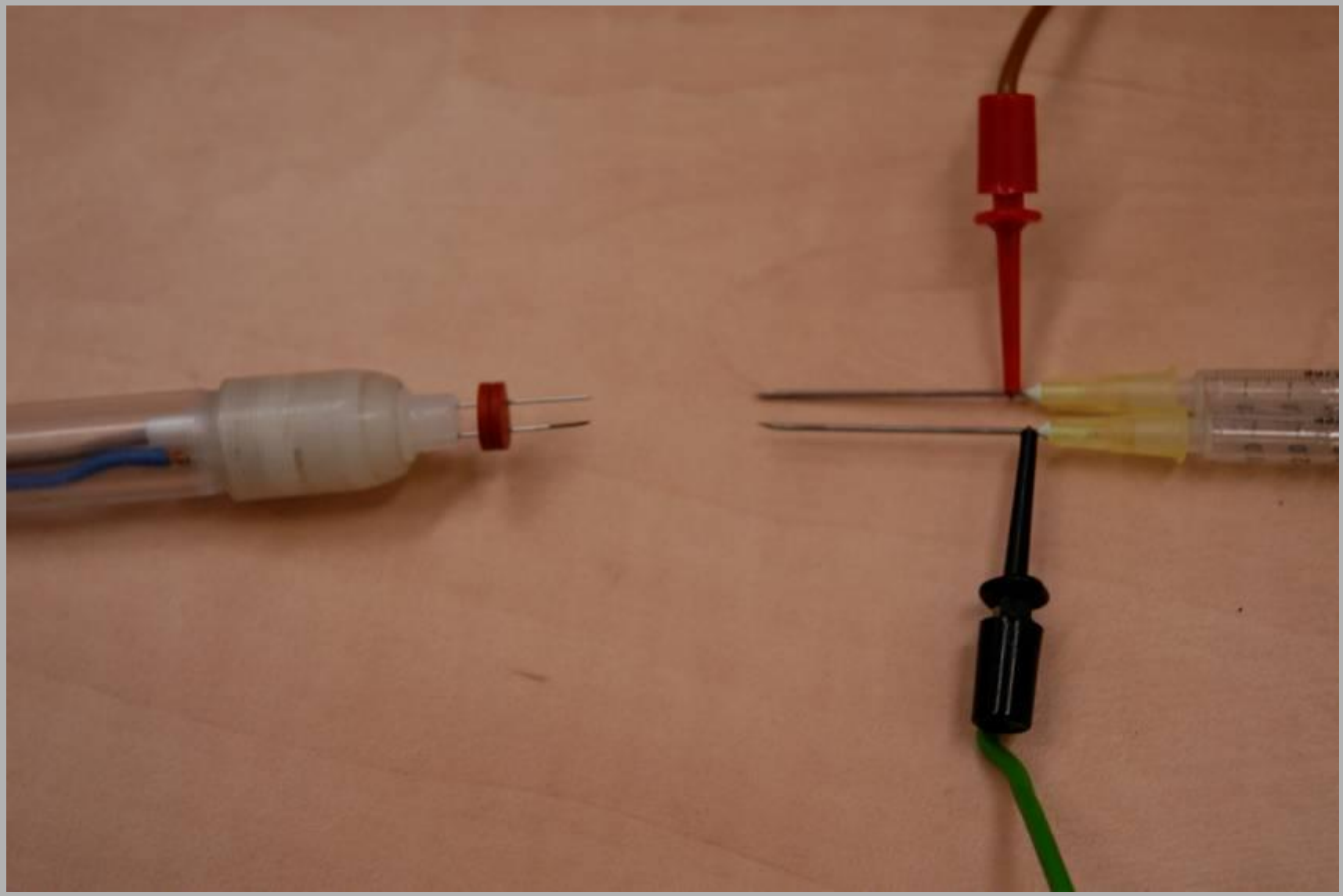


Figure 1 The electrodes used for DNA injection and electroporation. Panel A shows the electrode used in goats, a modified syringe for delivery of DNA, with two silver electrodes for generation of an electrical field. Panel B shows the electrode used in cattle, made of two syringes with gold electrodes for generation of an 'array-like' electrical field. The electrodes had fixed positions in polycarbonate, with a hole for injection of DNA through a standard syringe delivering the DNA at the right depth between the electrodes. The electrodes were connected to a Hear 6 bp stimulator (goats) or a generator made by INOVIO, Norway (cattle) for generation of the electrical field.

Electroporation



Aplicators




DURATION
INTERVAL
NUMBER
μs(x10)
ms
20
ms(x10)
S
01
06
START
POWER
ON
OFF

DURATIO

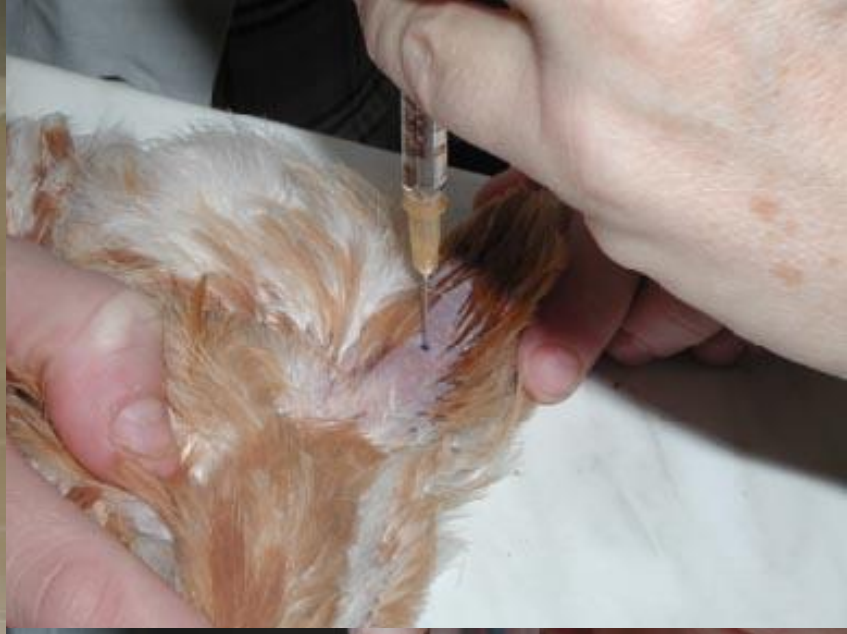
μs(x10)

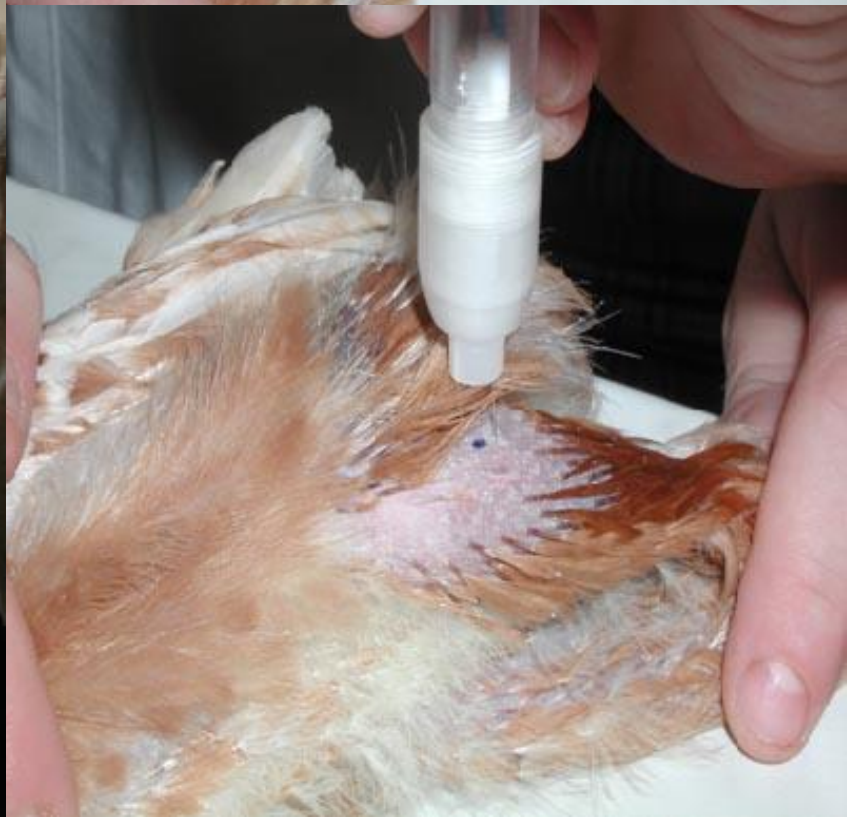
ms

SET VOLTAGE

OUT

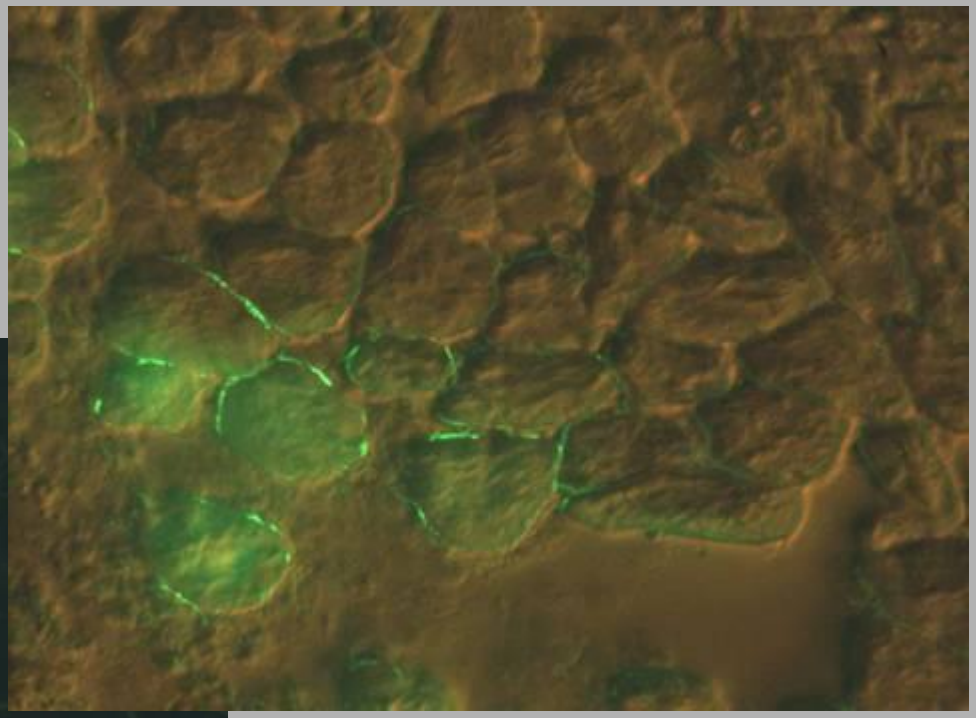
ST

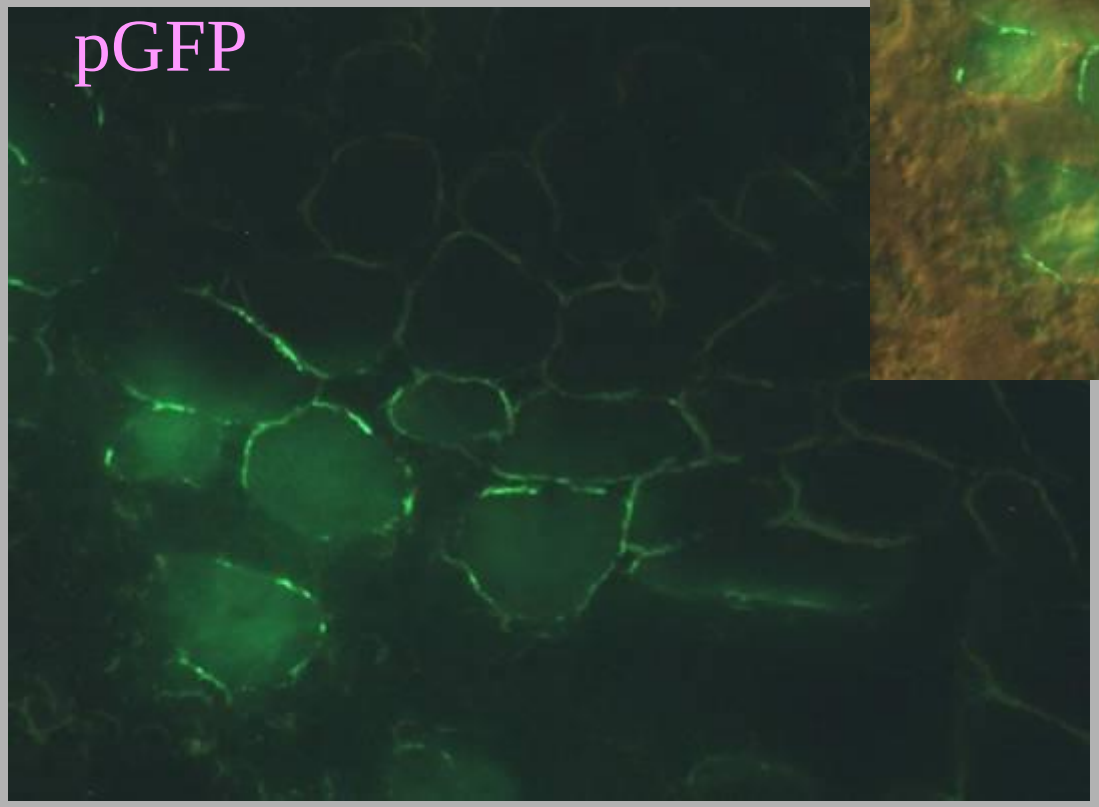




Transfekce *in vivo* u myší

transfekce lýtkového svalu myší pomocí elektroporace





Intenzita: 80 V/cm

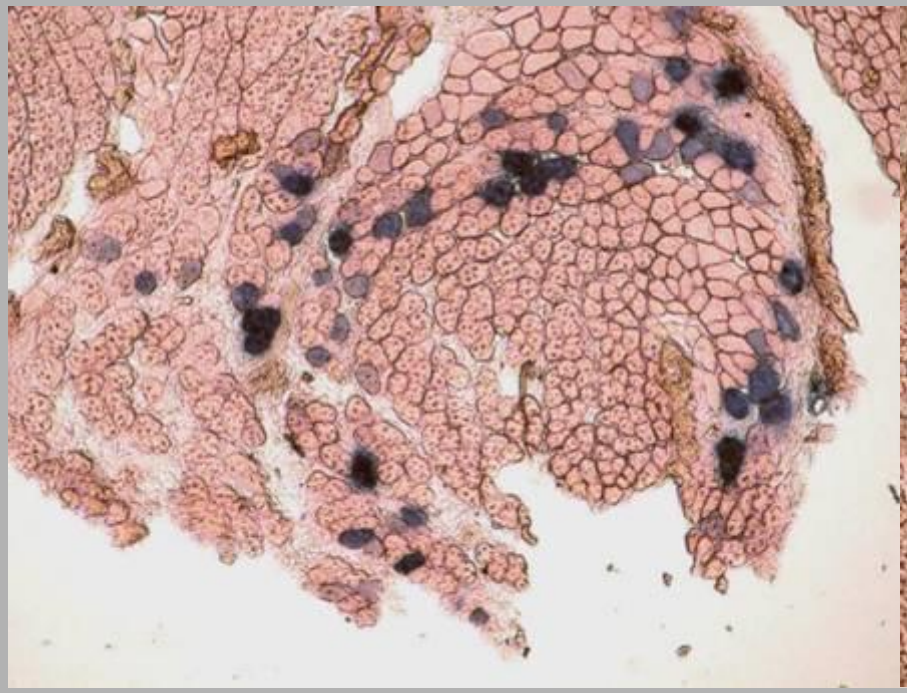
Doba pulzu: 20ms

Počet pulzů: 6

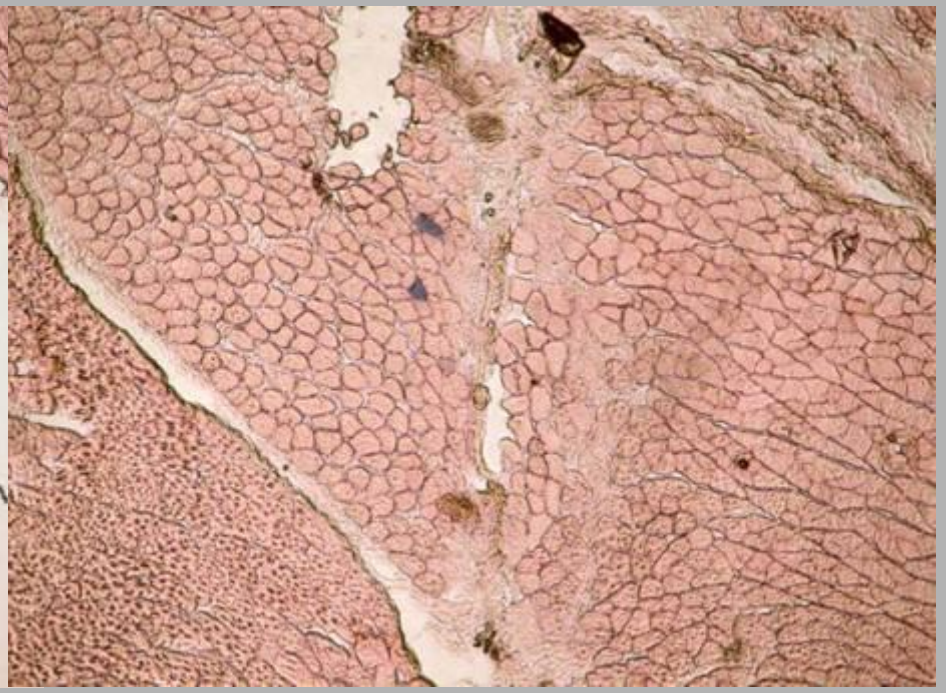
Frekvence: 1s

Transfekce *in vivo* u myší

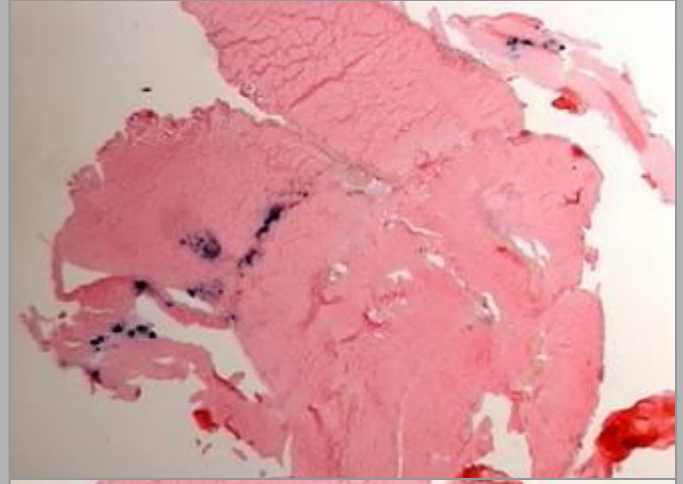
Balb/c, samice (stáří 8-9 týdnů), lýtkový sval 50μg pDNA (β-Gal), 48 h

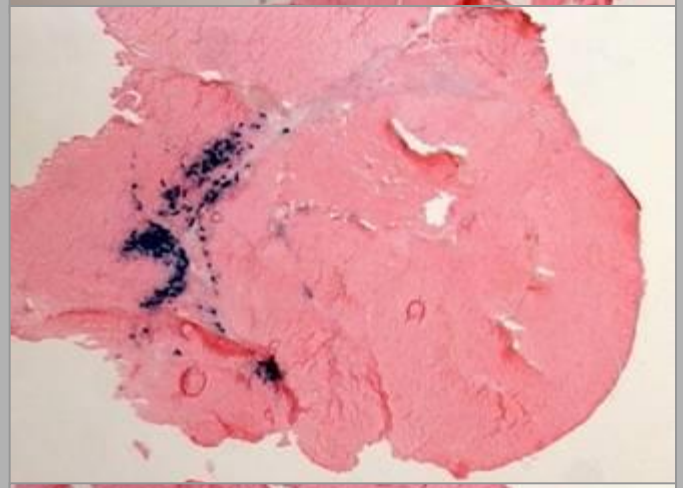


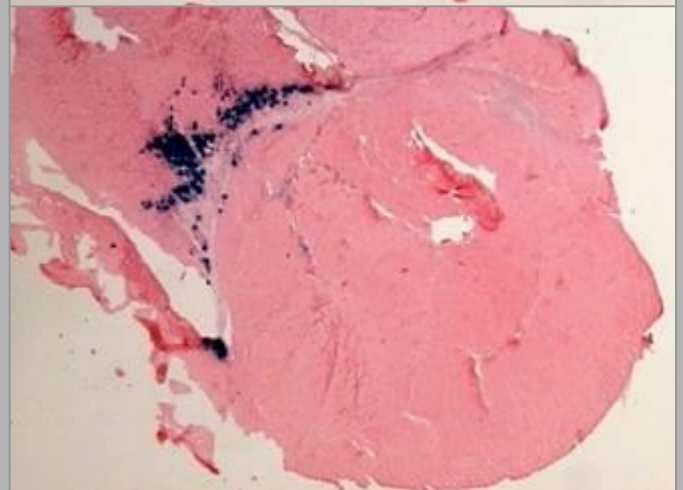
Elektroporace



Bez elektroporace







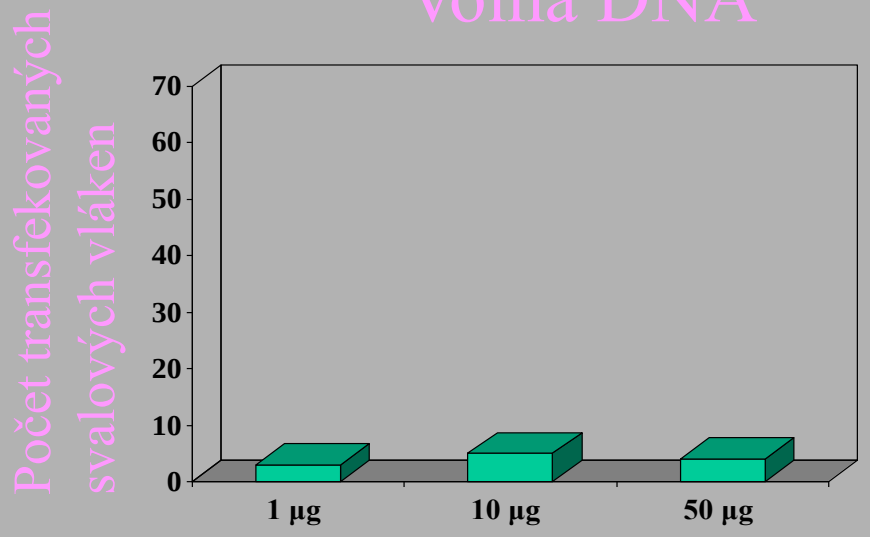
Transfekce *in vivo* s použitím elektroporace

Parametry elektroporace:
150 V/cm
6 pulzů
délka pulzu 20 ms
frekvence pulzů 1s

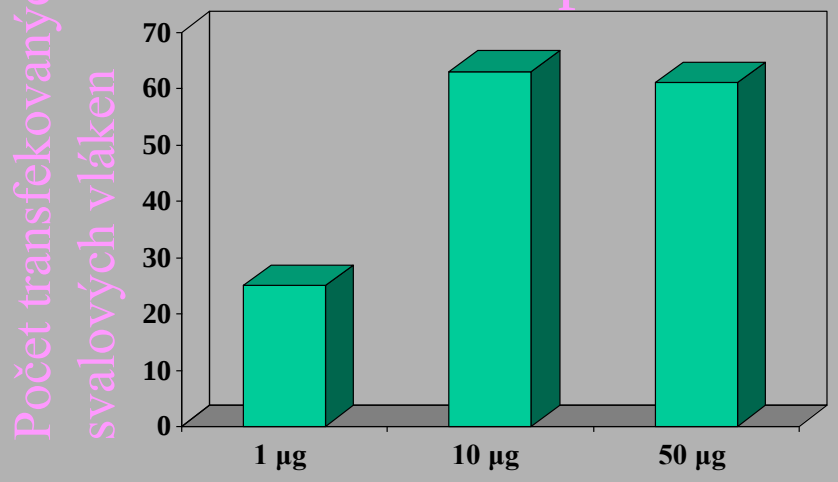
Zpracování svalu:
po 72 h. vypreparování lýtkového svalu
zmražení n-heptanem a nařezání 7 μm řezů
fixace řezů
detekce β-galaktozidázy pomocí X-gal

Transfekce *in vivo*

Volná DNA



Elektroporace

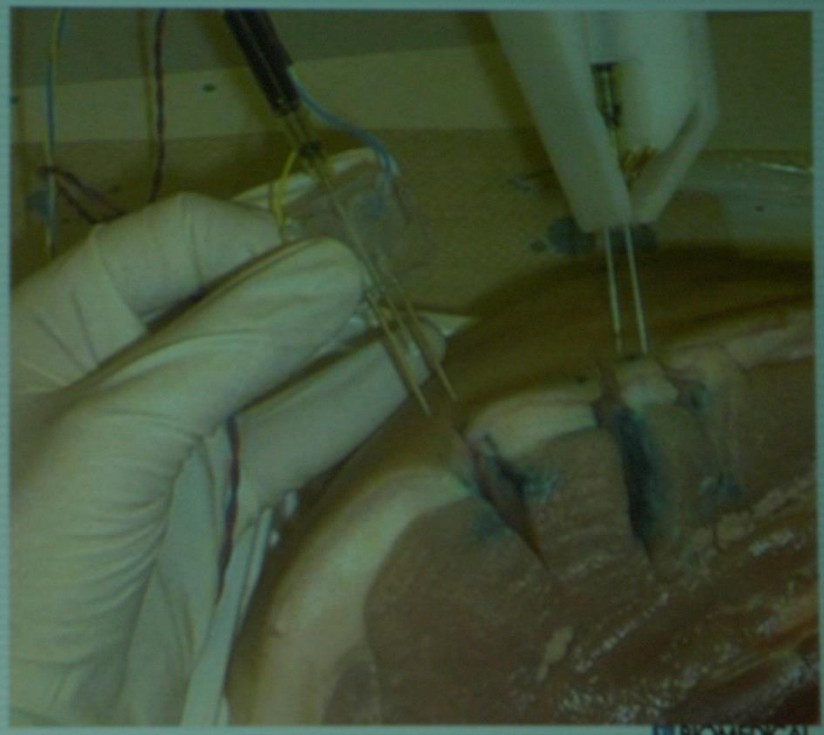


	+ elektroporace			- elektroporace		
1 µg	1L	2L	3L	1L	2L	3L
a	-	-	-	-	-	-
b	-	25	-	-	-	-
c	-	23	-	-	-	-
d	1	25	8	-	-	-
e	3	-	13	-	-	-
f	3	1	12	-	-	-
g		-		-	-	-
h		-		-	-	-
i				-		-
j				10		-
k				4		
l				4		
10 µg	1L	2L	3L	1L	2L	3L
a	2	7	-	-	-	-
b	11	14	-	-	-	-
c	21	15	-	-	-	1
d	95	18	75	-	-	1
e	-	12	-	-	-	4
f				-	-	14
g				-		
h				-		
50 µg	1L	2L	3L	1L	2L	3L
a	-	1	65	-	-	-
b	19	16	75	-	-	1
c	41	15	81	-	1	-
d	16	56	60	-	5	2
e	46		25	1	2	2
f	30		60	1	1	-
g	16		72	-	-	5
h	-		24	-	-	2
i			-	-		-
j				-		

Prototype testing

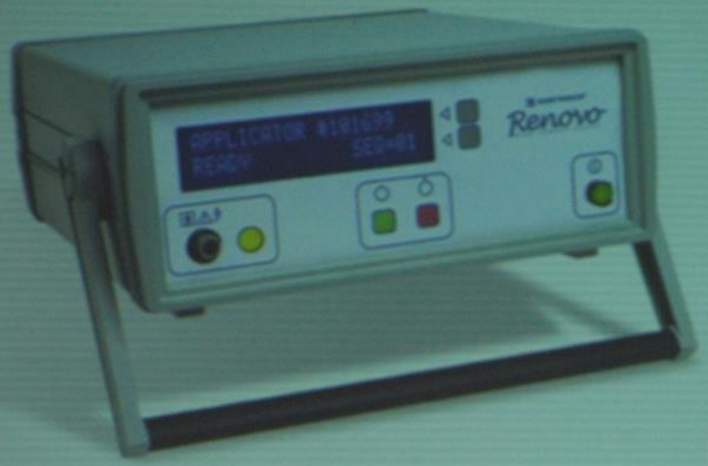
Distribution of ink in muscle tissue following injection **between** two needles **after** insertion or injection **through** two needles **during** insertion

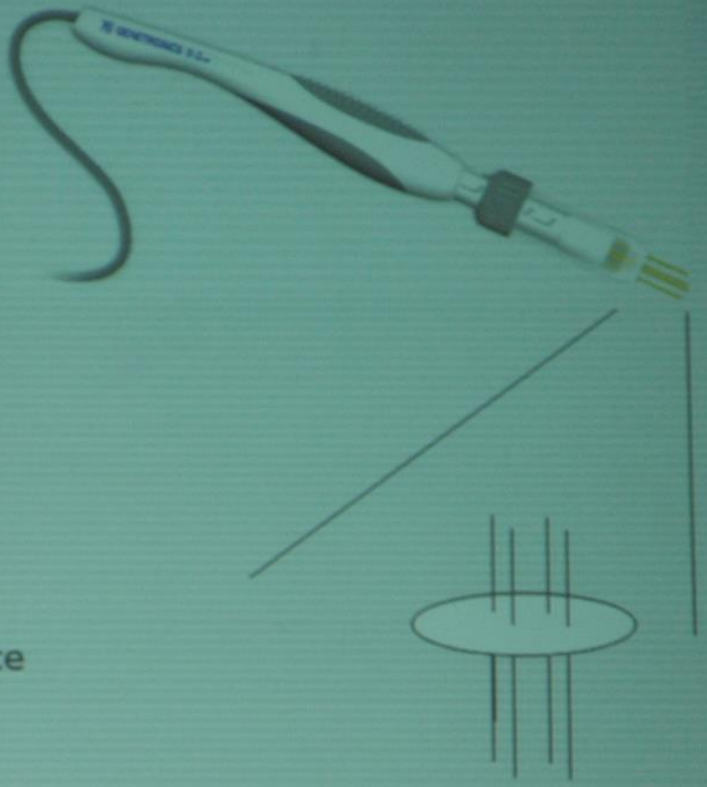
- Single needle injection results in distribution of injection volume around injection point
- Most of the injection volume is not located where the optimal electrical field is
- Controlled injection through two needles during penetration guarantees perfect distribution and ideal location between electric field and fluid



Genetronics' EPT Devices

EPT Gene Delivery



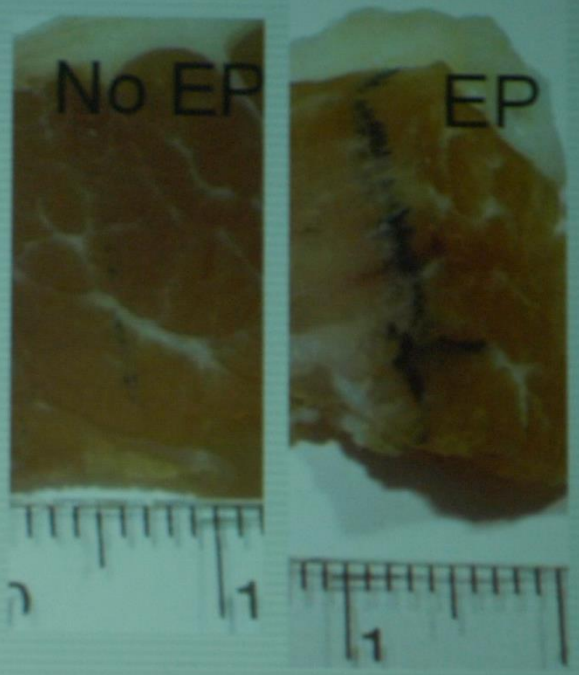


Renovo™

- Features same safety and performance characteristics as the Medpulser®
- Generates pulses optimized for delivery of plasmid DNA molecules

GENETRONICS BIOMEDICAL CORPORATION

Preliminary results from sheep

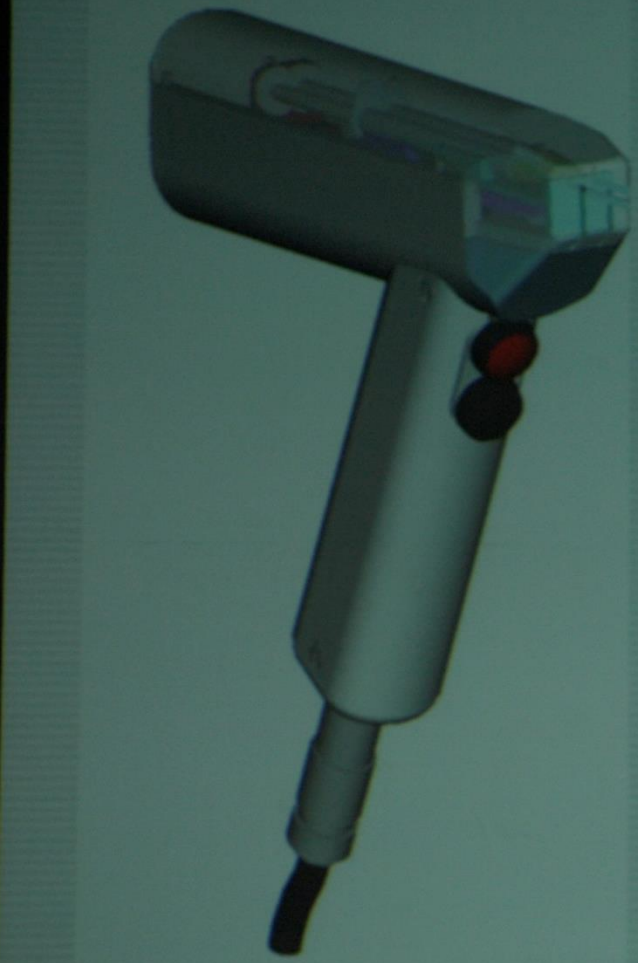


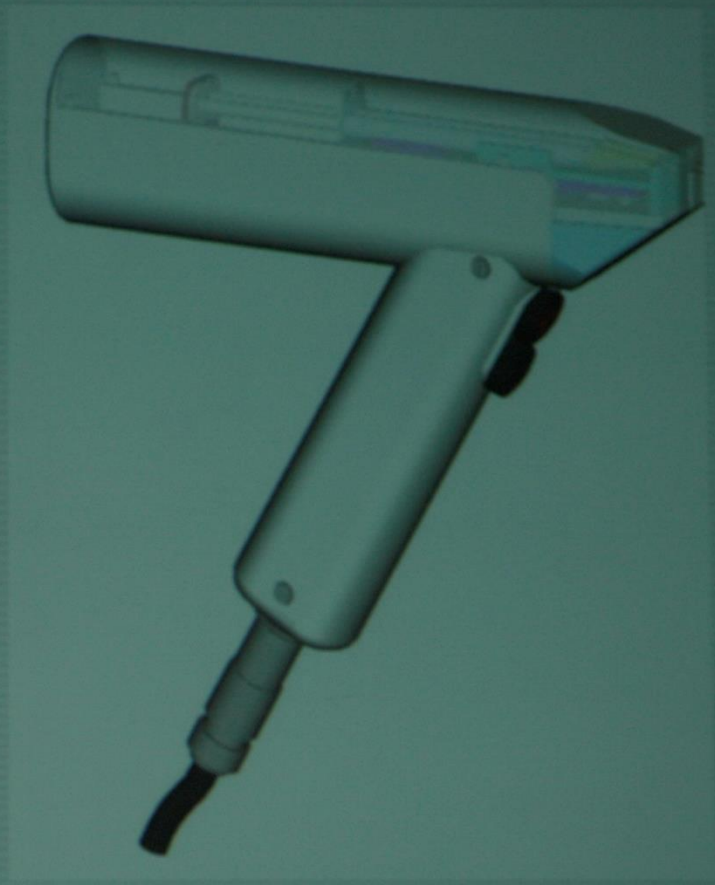
Each “needle path” has received 25 micrograms of DNA encoding β-gal encoding plasmid

Perfect match between electric field and injection of DNA

GENETRONICS BIOMEDICAL CORPORATION

Motorized injection – and insertion, close to final





GENETRONICS
BIOMEDICAL
CORPORATION

Aktuální situace u DNA vakcín

Tisíce DNA vakcín testováno u experimentálních zvířat

Tři vakcíny jsou chváleny pro veterinární použití

V současnosti (2011) není žádná DNA vakcína schválena pro klinické využití u lidí

Aktuální situace u DNA vakcín

Tisíce DNA vakcín testováno u experimentálních zvířat

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V současnosti (2011) není žádná DNA vakcína schválena pro klinické využití u lidí

Klinické studie s DNA vakcínami

- **prevence infekcí**
  - HIV, SARS, HPV, hepatitida B, chřipka, hemoragická horečka Ebola, západonilská horečka ...
  - malárie

- **terapie nádorových onemocnění**
  - karcinom ledvin, pankreatu, prsu, prostaty, plic, močového měchýře, hepatocelulární karcinom,
  - melanom

Počet klinických studií s DNA vakcínou přesahuje 500 (www.clinicaltrials.gov)

Závěr

Přenosný elektroporátor pro práci v terénu není dostupný (jsou ve vývoji)

Proces je do určité míry bolestivý (lokální nebo úplná anestesie)

Transfekce pomocí elektroporace vykazují 10-1000 násobně vyšší účinnost ve srovnání s volným plasmidem

Stále není optimalizovaný proces, velká variabilita výsledků

Testovány aplikace i.m., i.n. a i.d.

Adjuvans for DNA vaccines

Aluminium and Calcium Salts (*i.m.)*

Liposomal lentinan (glucans) (*p.o.*)

Monophosphoryl lipid A (*i.m.*)

Muramyldipeptide analogues

Cytokines (induced, external or encoded by plasmid) Th1/Th2

CpG motifs (integral part of plasmide DNA)

Cholera toxin, HSP

8Br-cAMP - enhencer of CMV promoter

The role of CpG motive in DNA vaccines

CpG - frequency 1/16 in bacteria and 1/60 in vertebrates (methylated in vertebrate DNA

Th1- like patern of cytokine (IL-12, INFγ)

Induction of strong CTL response

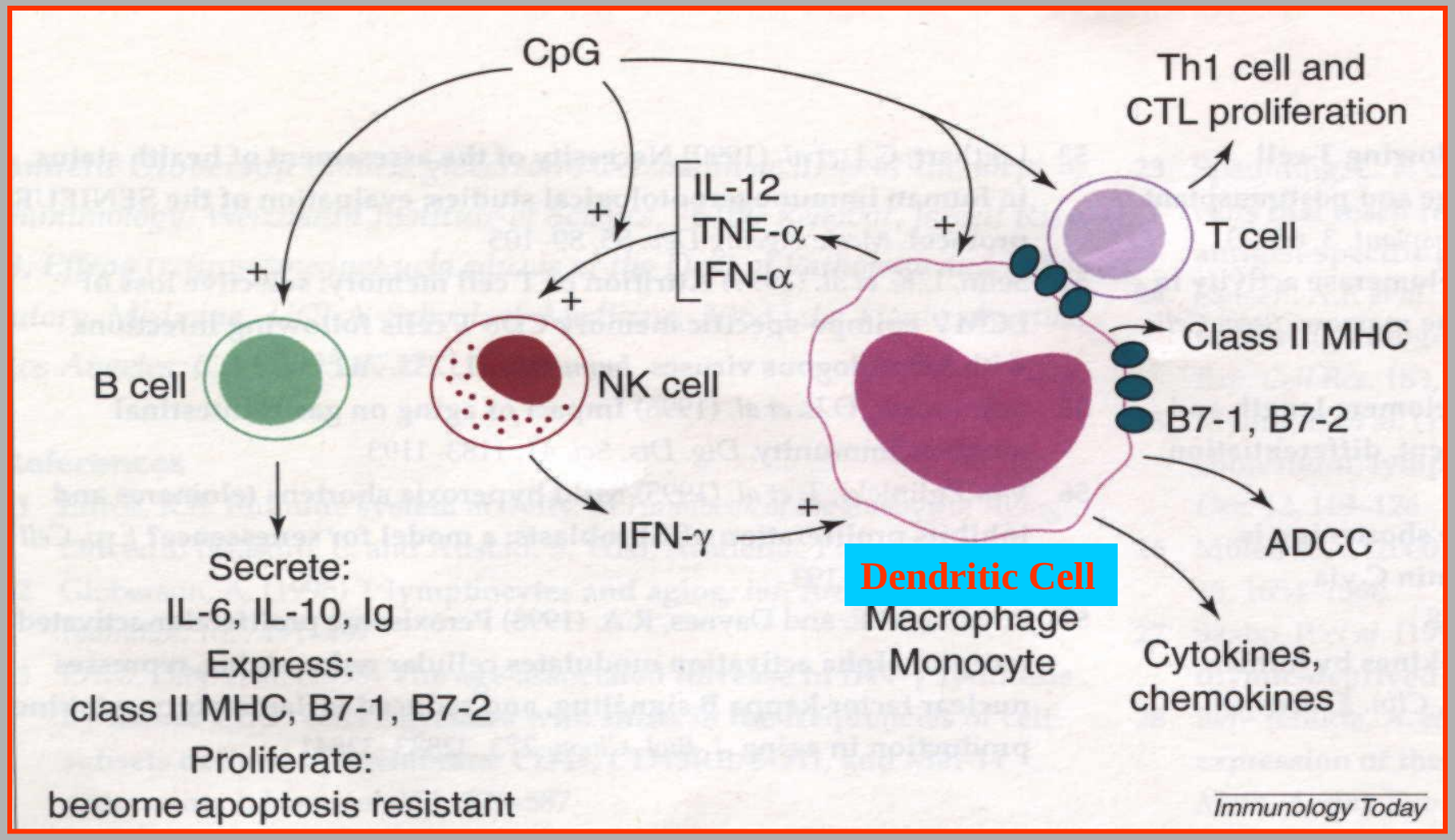
Immune response towards CpG - an evolutonary adaptation to augment innate immunity in response to bacterial infection

CpG DNA - T-cells independent and antigen nonspecific activation of **B-cells**, stimulation of B-cells to proliferate, secrete Ig, IL-6, IL-12, protection of B-cells from apoptosis,experession of MHCII and B-7

-direct activation of **monocytes** and **macrophages**

-activation of **dendritic cells** to secrete various cytokines and chemokines

Mechanism of immune stimulation by CpG DNA



Conclusion for DNA Vaccines

- CpG-DNA or CpG-ODN can be used as a vaccine adjuvants
- Two active parts of pDNA vaccine
- 1. insert encoding the protein antigen (accesory protein - cytokines)
- 2. CpG-S motifs directly stimulating B-cells and Th1 cytokine expression

Advantages of DNA Vaccines

- **Subunit immunisation with *no risk for infection***
- **Presentation by both MHC I and II**
- **Ability to raise TH1 and TH1/2 response**
- **Focused immune response**
- **Immunisation of neonates *(colostral immunity)***
- **Easy of development (cloning of genes) and production**
- **Stability of vaccine**

Disadvantages of DNA Vaccines

- Limited to protein immunogens
- Potential for atypical processing of bacterial and parasite proteins
- Lack of long term experience with use of DNA vaccines
- Patents: VICAL (San Diego,CA) , Wistar Institute (PA) University of Massachusetts (MA), GENEMEDICINE Inc. (TX)

Safety Issues

Production of pDNA and its formulation according to Good Manufacturing Practice

WHO:Guidelines Assuring the Quality of DNA Vaccines, 1997, Tech Rep Ser No-17

Integration into host genome undetectable for naked plasmid (construcion of plasmid, avoidance of the sequences possibly mediating chromosomal integration, retroviral, lentiviral) (effect of carrier - liposomes, bacteria)

Tolerance (neonates) only one case in mice immunised by MA

Autoimmunity risk no grater than that posed by live viral vaccines

Anti-DNA Antibodies (no evidence from the experiment with lupus-prone mice)

Effect of antibiotic resistance gene

Futre Promise

Preparation of monoclonal antibodies

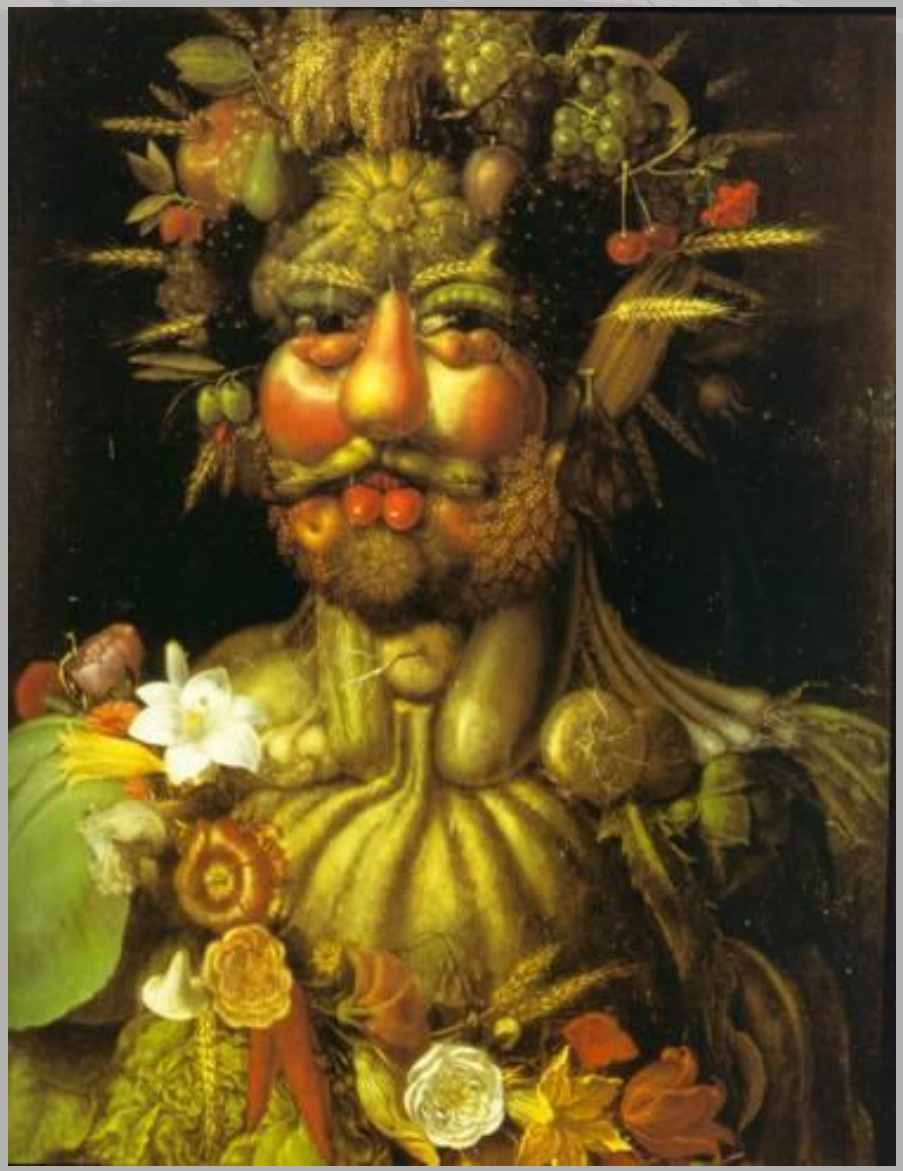
New approach and precise tool for the study of immune responses

Screening of protective effect of multiple different microbial antigenes and their combination (*library immunisation*)

Development of new efficient vaccines (HIV, malaria, cancer, tuberculosis, influenza, ebola, hepatitis B)

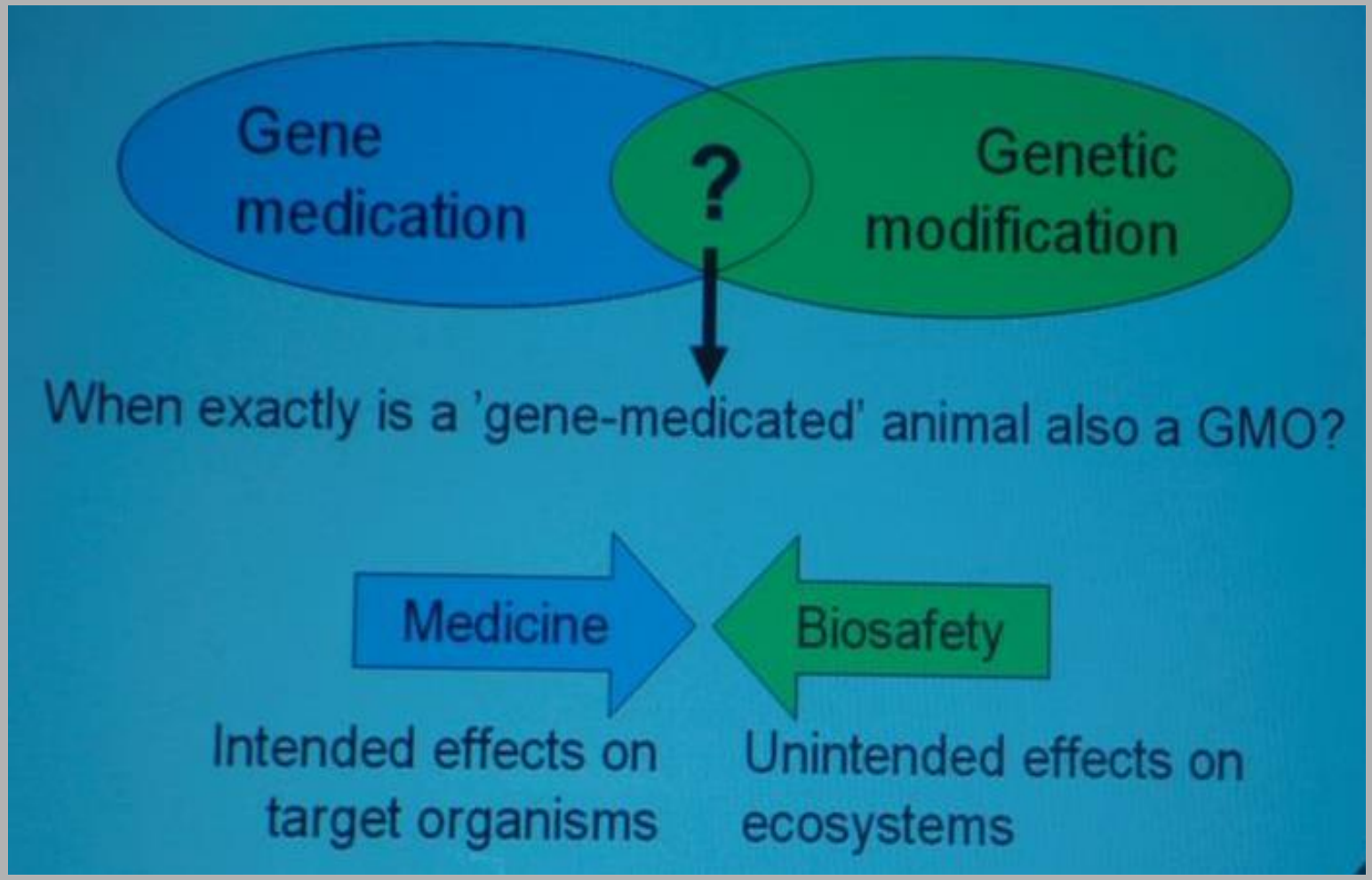
Development of vaccines for veterinary application

DNA Vaccines And Gentically Modified Organisms



DNA vaccines are not yet licensed in many countries, therefore national authorities are not experienced with this kind of product and do not differentiate between gene medication and gene modification. Within the EU two opposite points of view are maintained as regards DNA vaccinated animals.

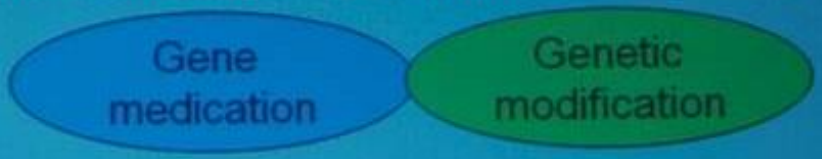
Gene medication or genetic modification?



Different regulations and interpretations

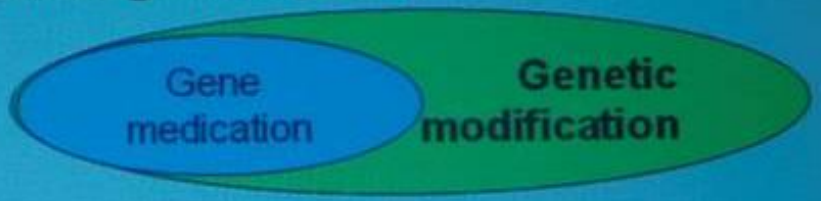
The British Agriculture and Environment Biotechnology Commission (2003)

"The foreign DNA is not expected to integrate into the host's genome and so the vaccinated animal is not genetically modified"



The Norwegian Directorate for Nature Management (2001)

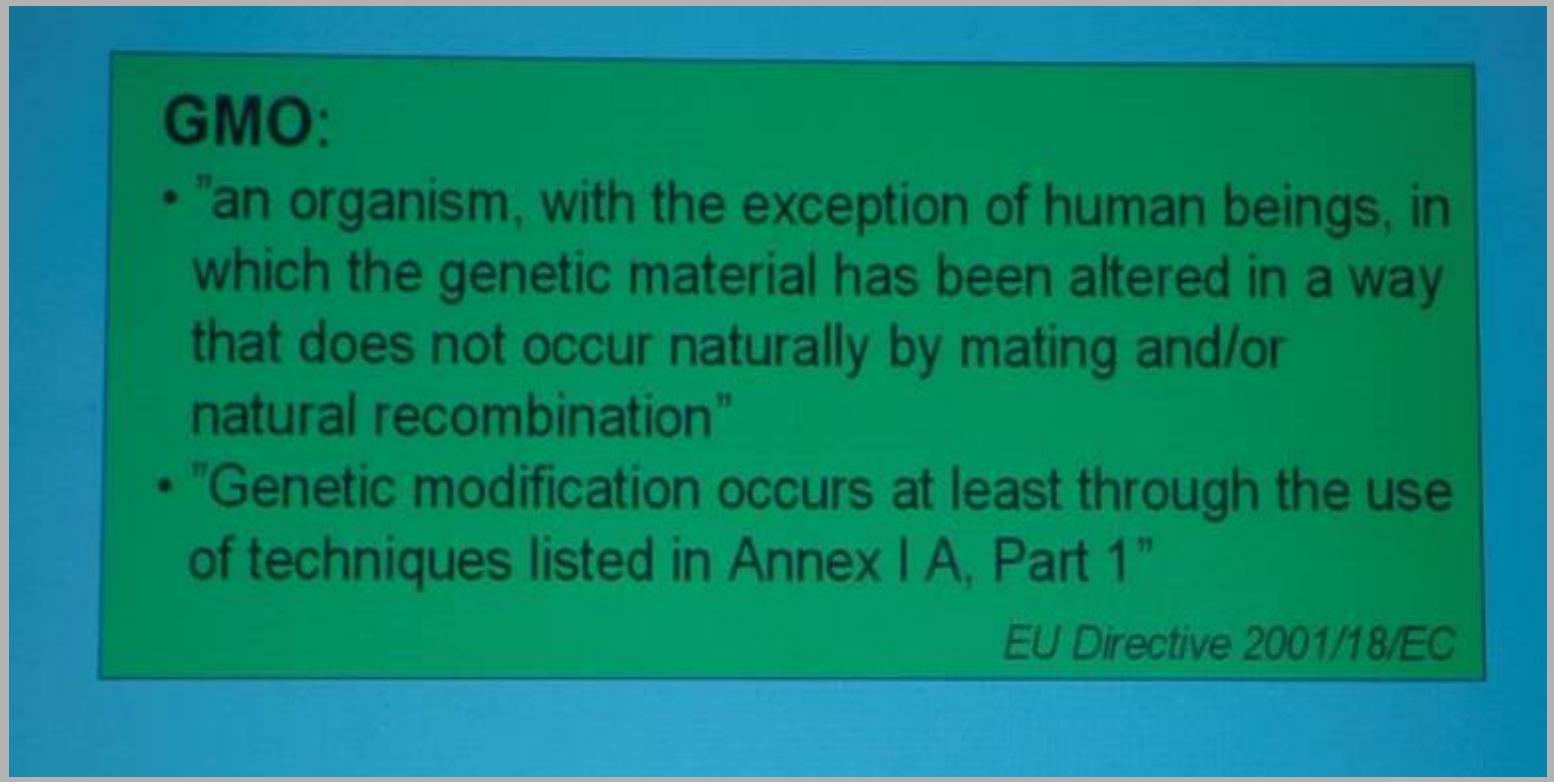
A DNA-vaccinated fish is to be considered genetically modified for as long as the added DNA is present



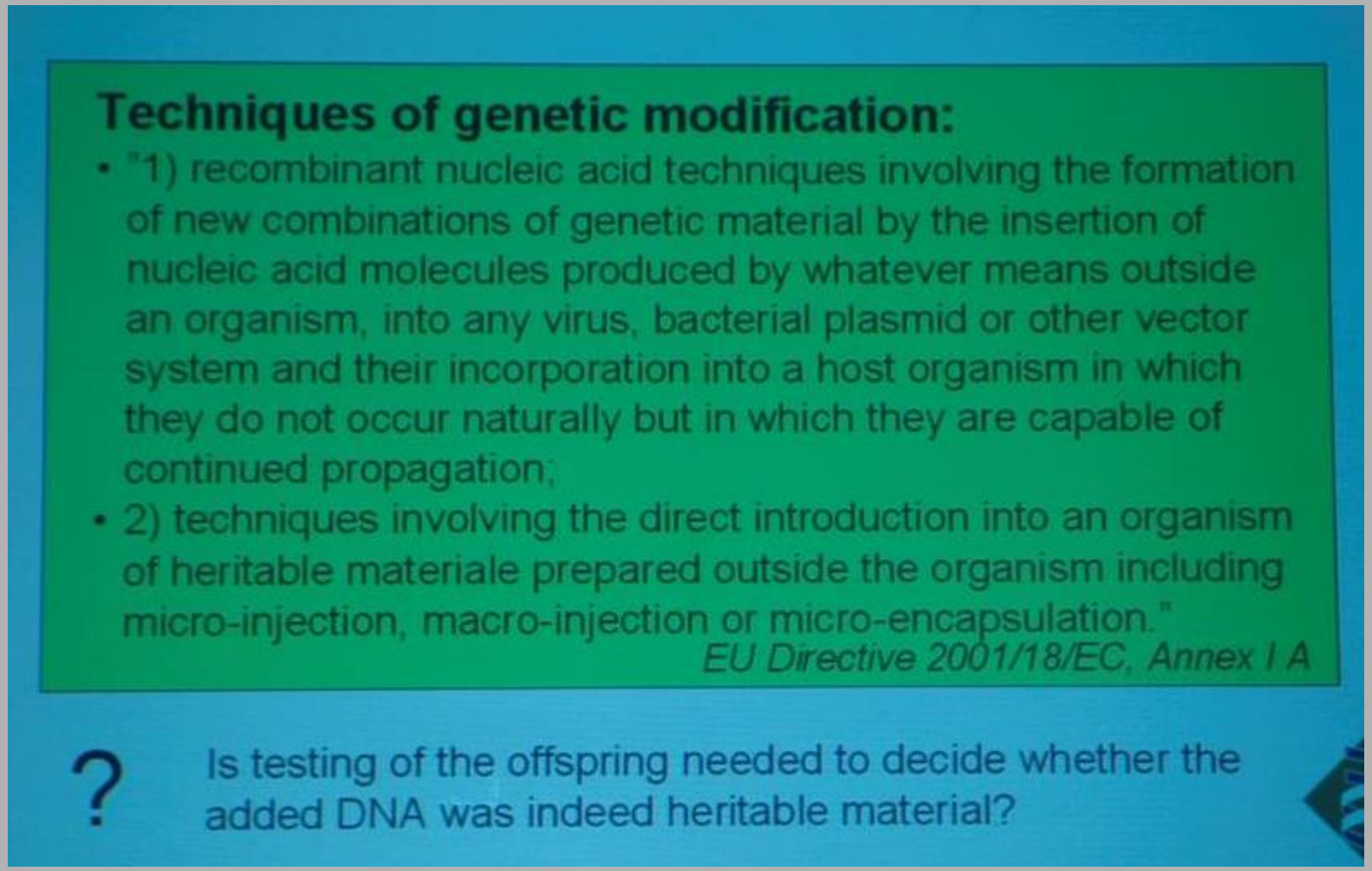
The US Food and Drug Administration (2003)

Genetic constructs used to create transgenic fish (and other animals) fall under the legal definition of drug

The EU definition of GMO



The EU difinition of genetic modification



DNA vaccines -Problems

Safety of DNA vaccines – proved

Efficacy – still real problem in large animals

Field experiments in large animals – condemnation of cadavers – economical as well as ethical problem

GMO – artificial problem (pig, broiler, mule)

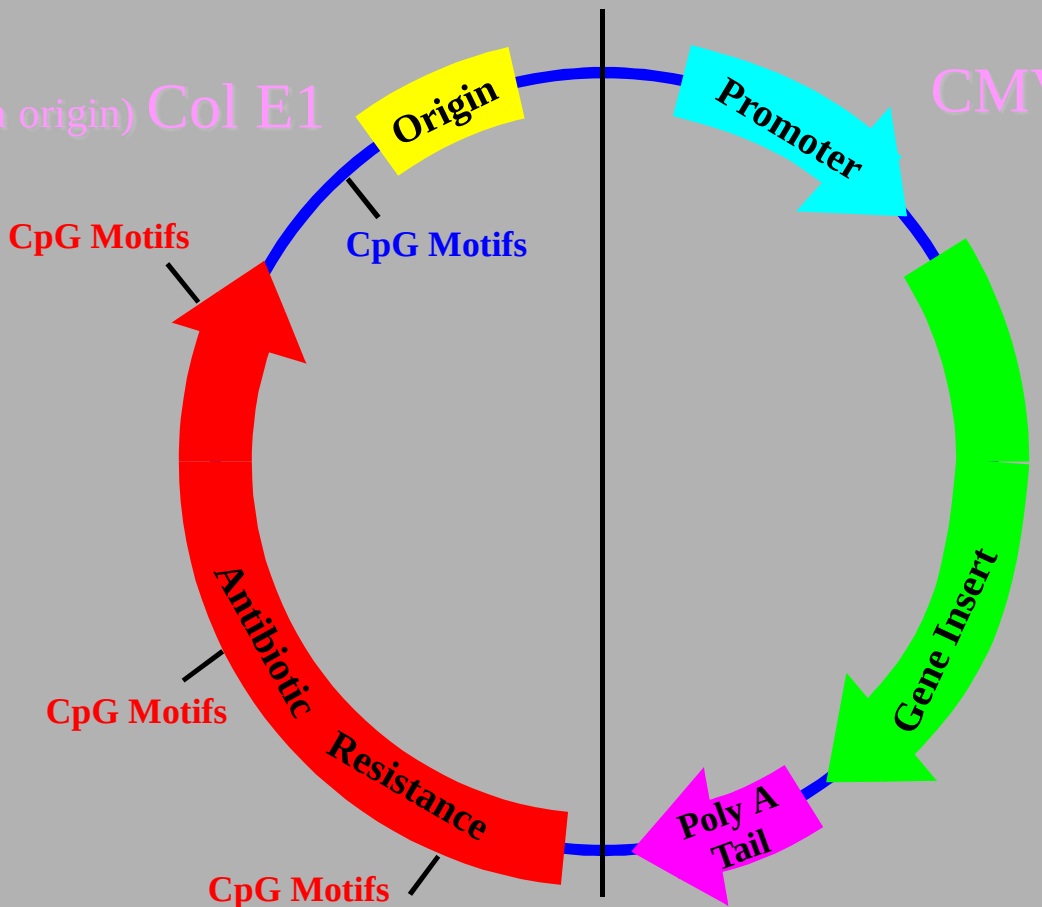
Food safety - ? *Consumption of residual plasmid? Spreading of the antibiotics resistance genes*

Plasmid Vector

Accesory and Adjuvant Unit

Transcription Unit - *minicircle*

(No mamalian origin) Col E1



CMV, RSV

Antigen Targeted AG,

Cytokine

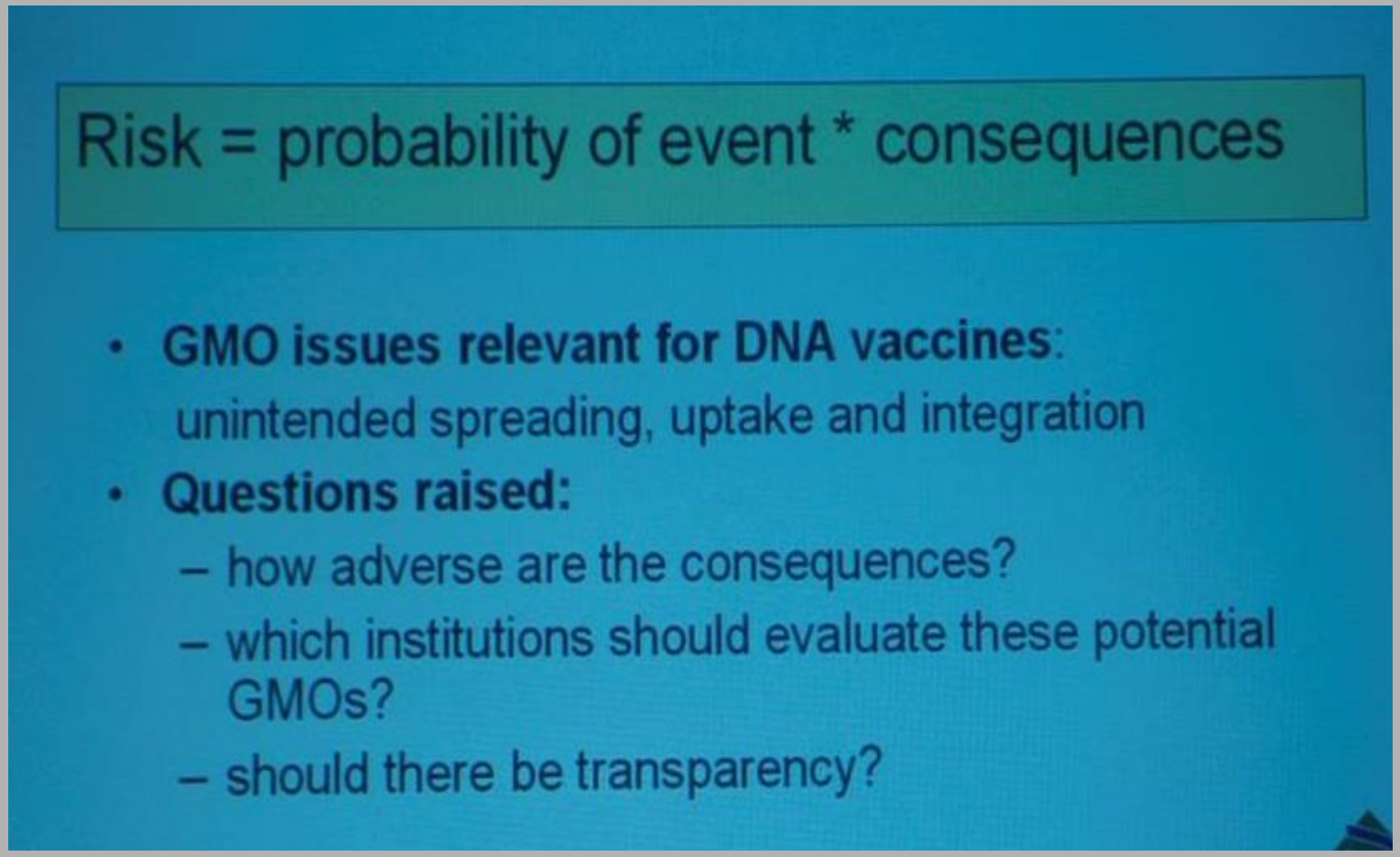
ILs (2,4,12,15,18), GM-CSF,IFN-γ, MIP-1α

CD86 (B7-1)

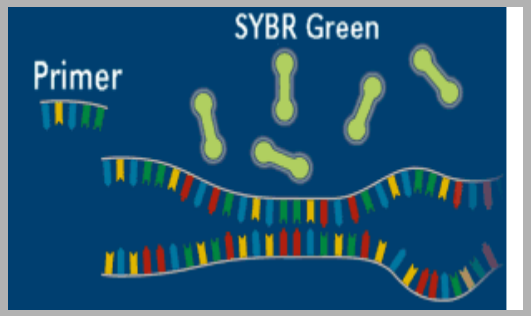
Heat Shock Proteins

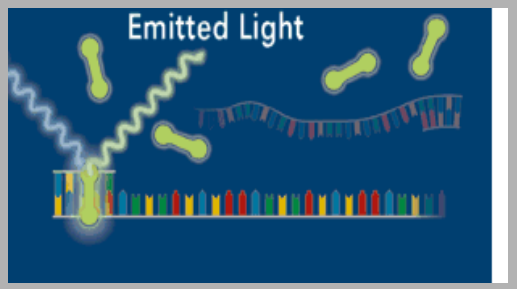
BGH, SV40

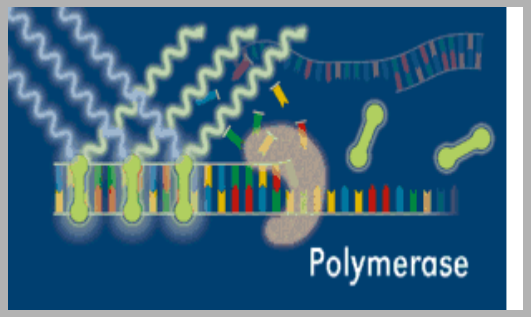
Risk and uncertainity

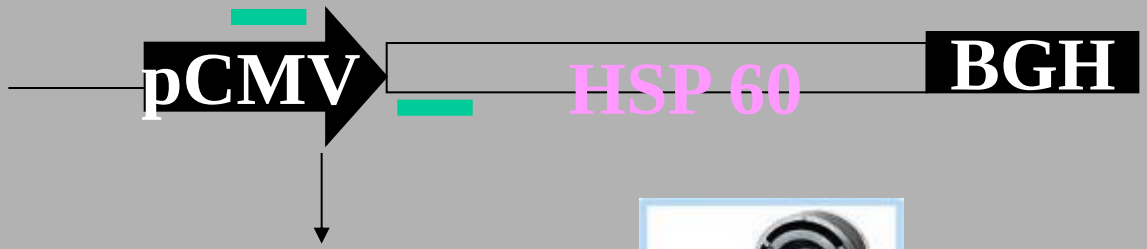


Real time PCR format – SYBER GREEN I









155 bp

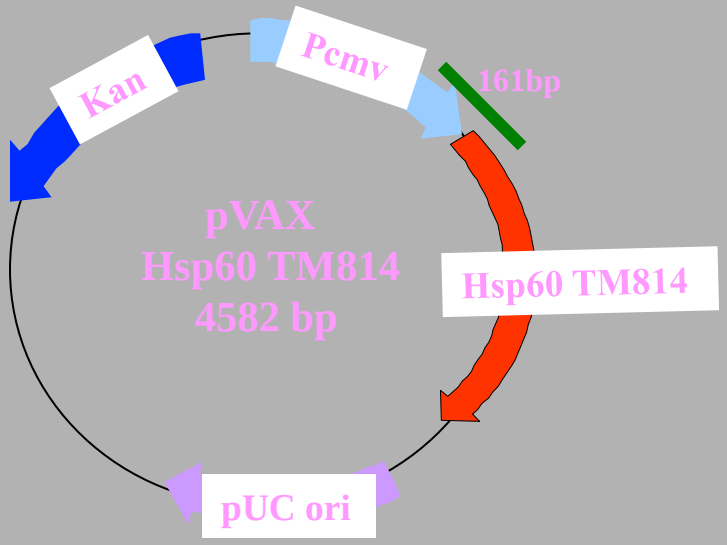
Lightcycler (Roche)

Quantitech Syber Green kit (Qiage

Cycling condition			
Initial actiovation	**15 min.**	**95°C**	**20**
Denaturation	**15 s**	**94°C**	**20**
Annealing	**25 s**	**55°C**	**20**
Extension	**10 s**	**72°C**	**20**

45 cycles

Plasmid pVAX Hsp60 TM814 (pDNA) persistence: Real Time PCR (RTPCR)



pDNA

↓

Muscle injection

↓

Necropsy, tissue sampling

↓

gDNA isolation (GuSCN:silica)

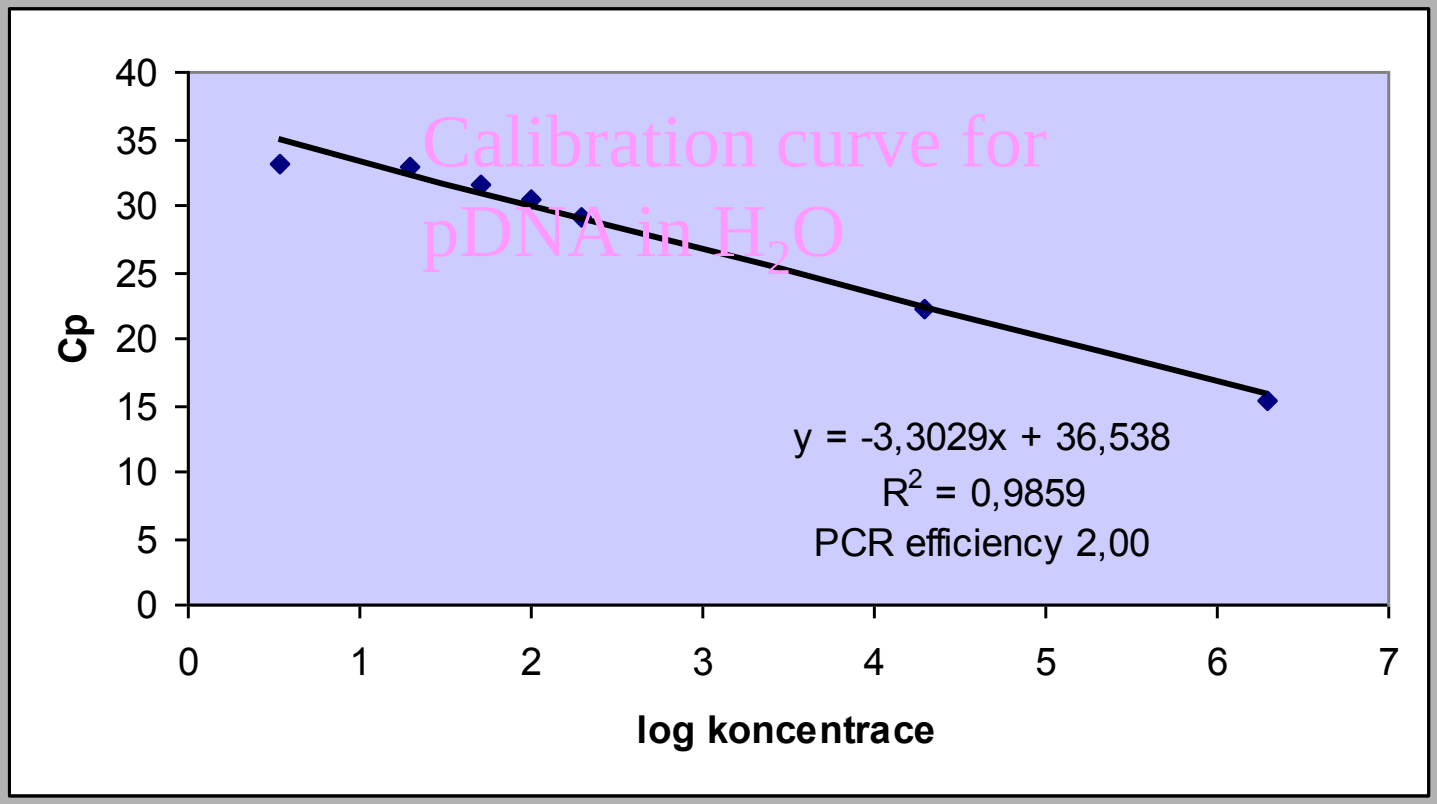
↓

RT-PCR: Syber Green format, Lightcycler
161bp PCR product

Validation RTPCR

- specificity – matrix inhibition, validation isolation method
- linearity (linear range) - 10^9 – 10^1 copies
- detection limit – 3 copies (≈7.5 ag) / 500ng gDNA

Quantification of the pDNA in bovine musles by the means of Real Time -PCR

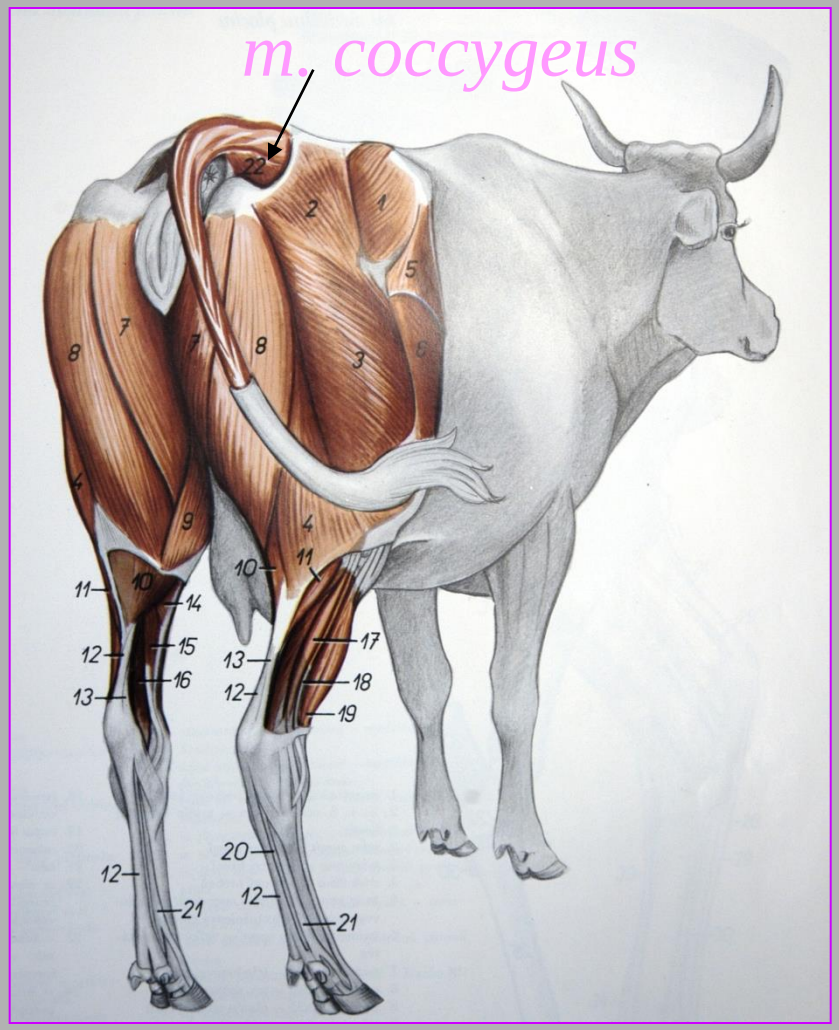


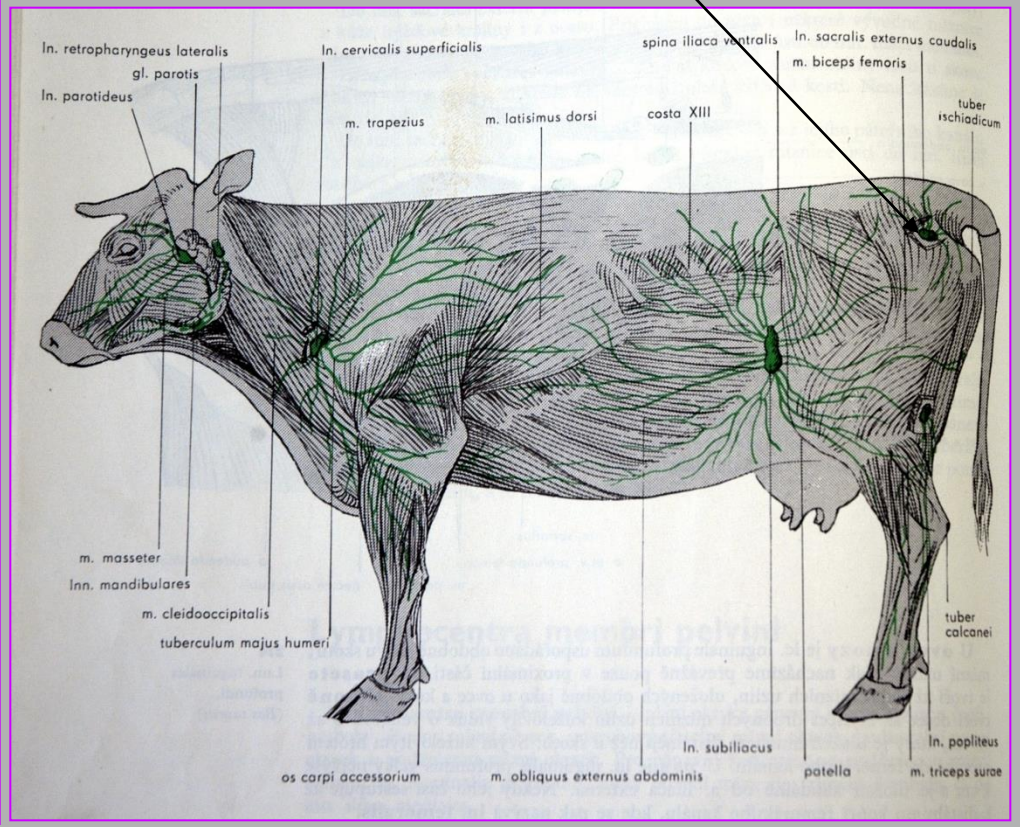
Detection limit in water: 2 copies/µl (per reaction)

LQL: value below linear limit of quantification (10 copies/reaction)

Detection limit in muscle tissue: (3 copies/ reaction) ≈3 000

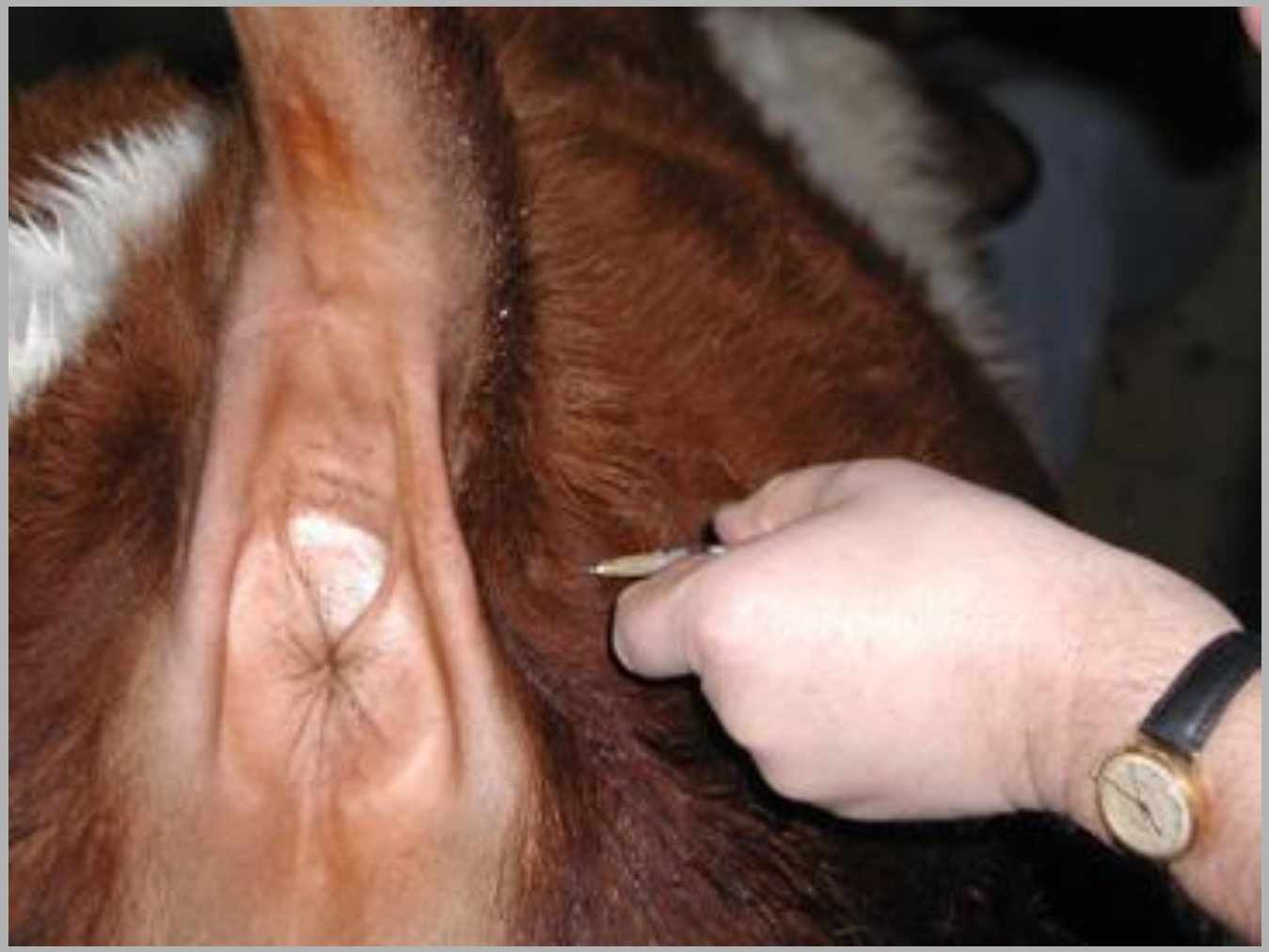
Site of pDNA vaccine application in cow





Vakccination of calves with the DNA vaccine against ringworm (trychophytosis)

m. coccygeus



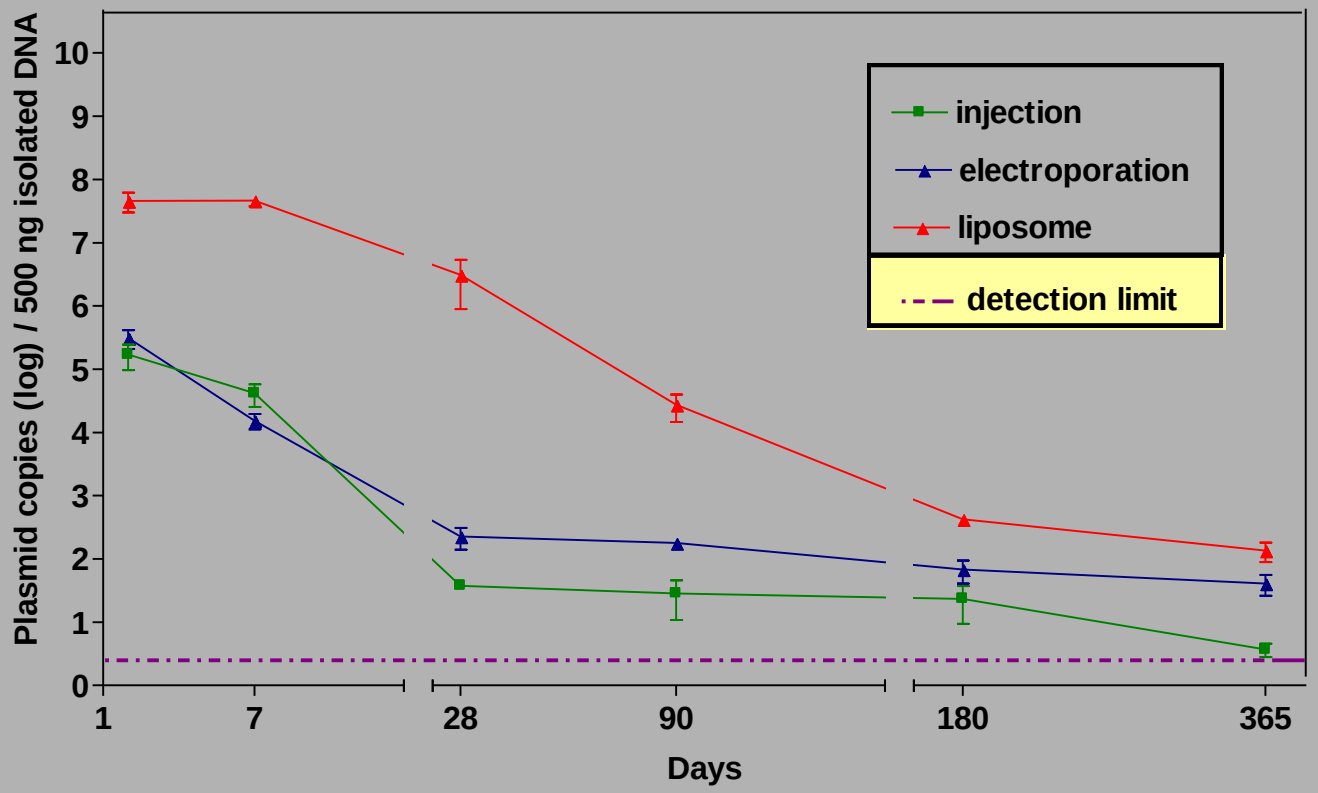
pDNA: 10 μg - i.m (calf muscle)

RTPCR: 500 ng gDNA - injection site

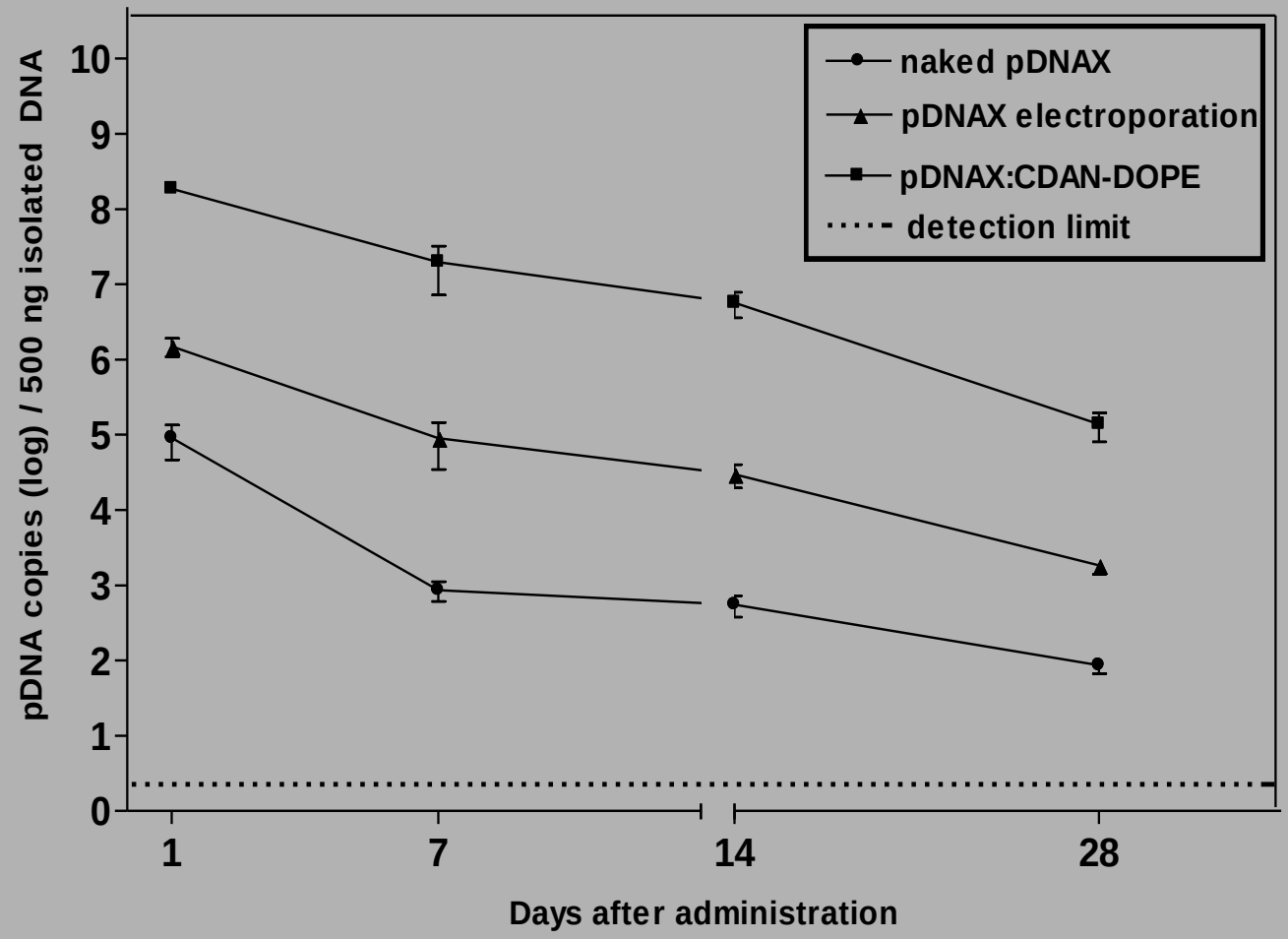
non-treated muscle - negative

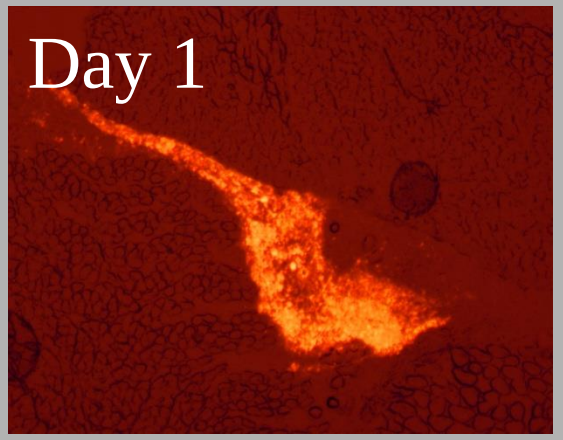
non-treated animals - negative

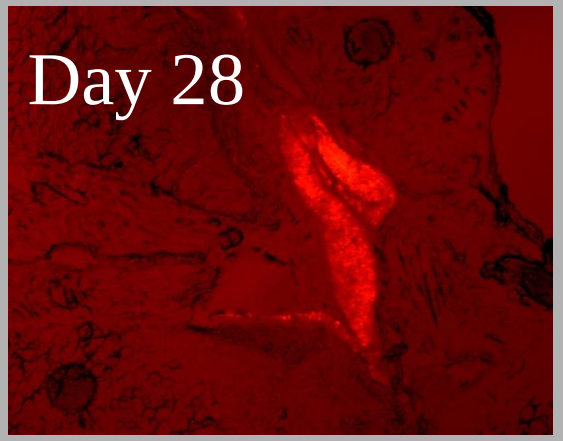
Persistence of pDNA at the injection site: 1, 7, 28, 90, 180, 365 days after injection



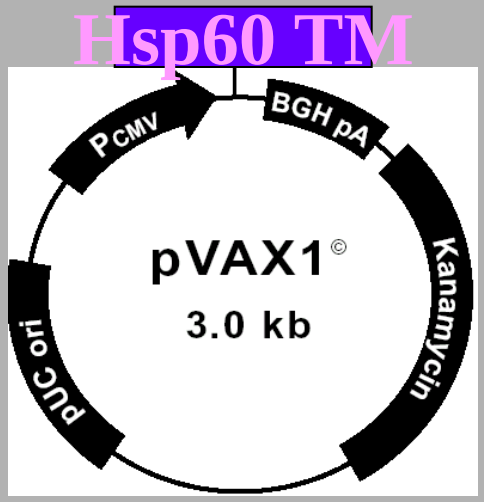
Plasmid levels in calf muscle (injection site) after administration of 10 μg pDNAX in 5 weeks old BalB/C mice.







Persistence of DNA vaccines in large animals



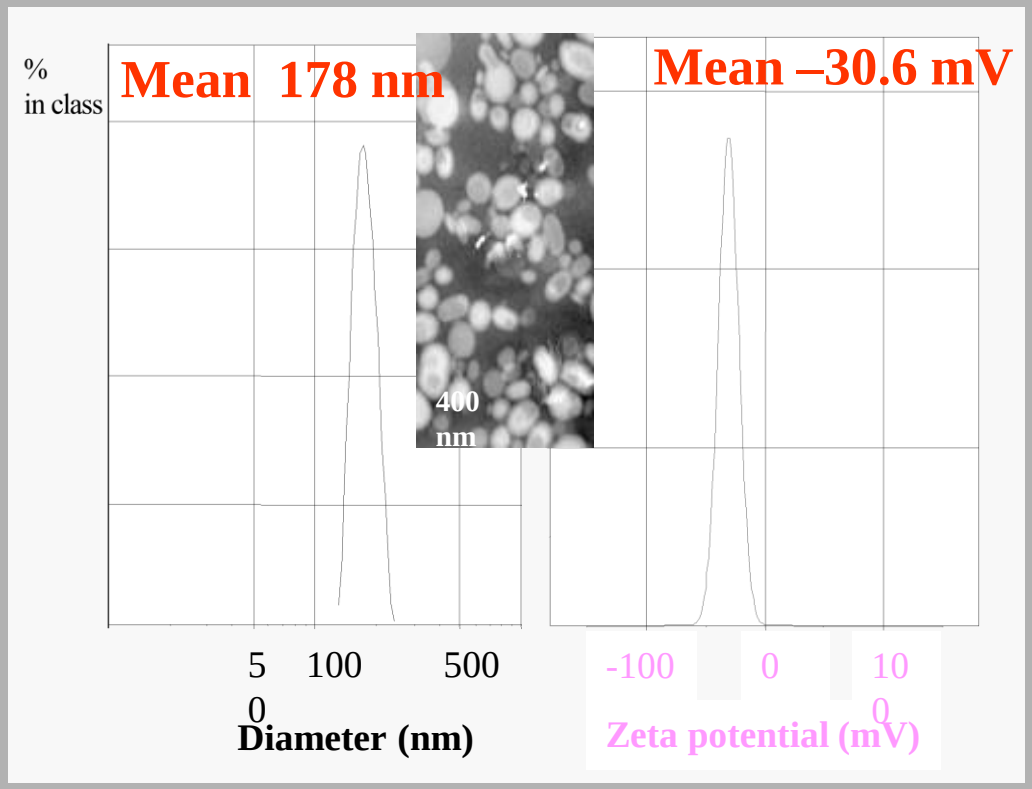
HSP 60 TM 814 pVAX

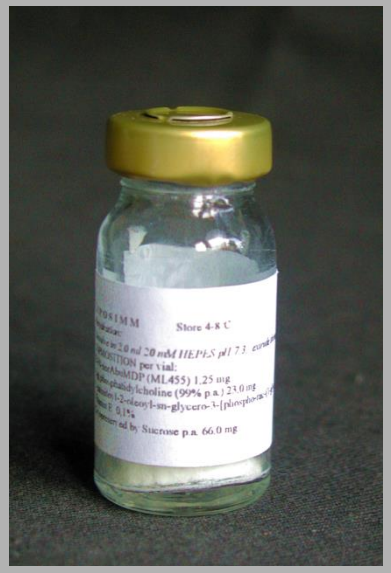
- heat shock protein 60 (Hsp60)
- Trichophyton mentangrophytes

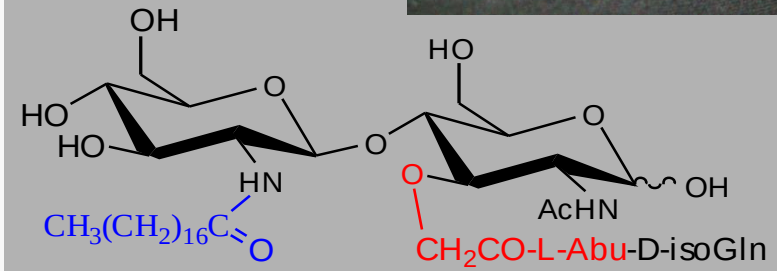
Code	Group (3-4 bulls/each group)	1. immunisation	2. immunisation
1	500 μg DNA	400 μl	500 μl
2	500 μg DNA + 500 μg ML-455	400 μl	500 μl
3	500 μg DNA + 2.5 mg CDAN-DOPE	600 μl	800 μl

500 μg DNA – 1.21 x 10*14 molecules7 months

LiposIMM-N-L18NAGMDP







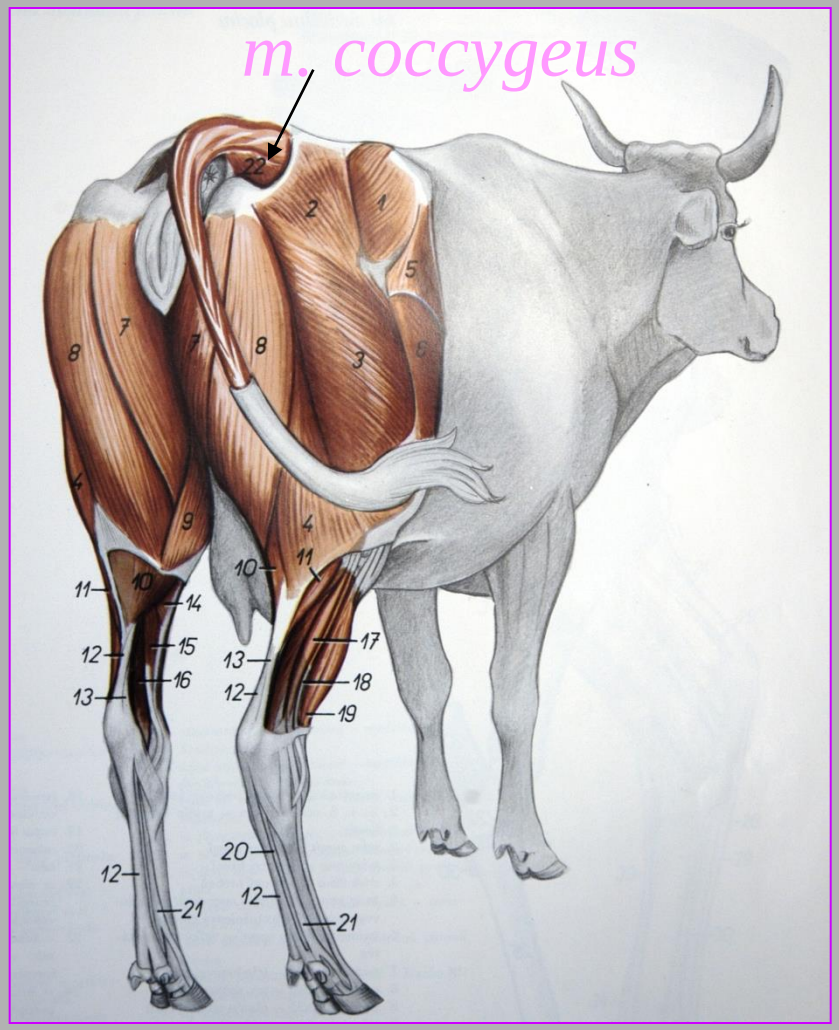
N-L18-norAbuGMDP

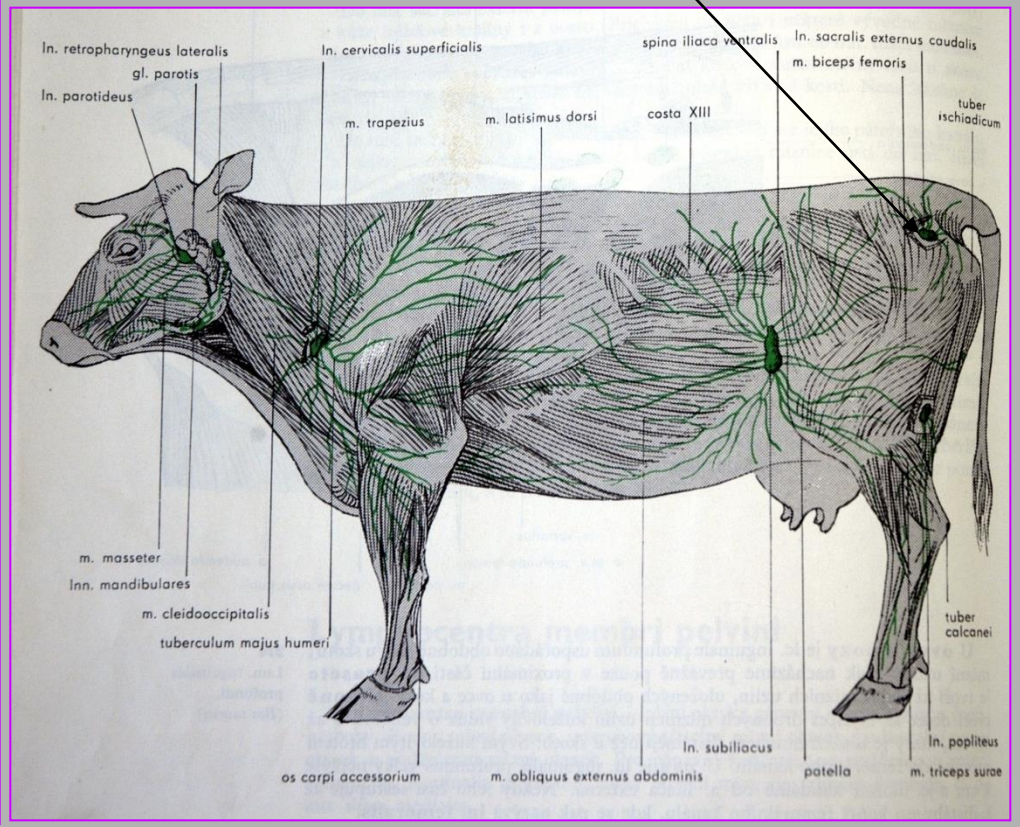
Applications:

Stimulation of innate immunity

(viral, bacterial, protozoal

Site of pDNA vaccine application in cow





Persistence of pDNAX in beef after i.m. administration

Beef cattle groups	Interval between 2nd immuni-sation and slaughter (days)	pDNA copies at the injection site copies/ 500 ng DNA (n=5)	pDNA copies opposite to- injection site muscle (n=4)	pDNA copies distant muscle (n=3)	pDNA copies DLN[a] total (n=6)
pDNA	242	<LQL (2); <DL (2); Neg. (1)	0/4	0/3	0/6
	277	74; 36; <LQL (2); <DL (1)	0/4	0/3	0/6
	284	<LQL (5)	0/4	0/3	0/6
DNA + B30-Nor-AbuMDP	242	20; 16; 15; <LQL (2)	0/4	0/3	0/6
	270	93; 29; 28; 92; 24; 24	0/4	0/3	0/6
	291	13; 13 ;11; <LQL (1); Neg. (1)	<DL(1/4)	0/3	0/6
DNA:cationic liposome complex	270	288; 220; 200; 30; <LQL (1)	0/4	0/3	0/6
	277	229; 170, 135; 39; 39	0/4	0/3	0/6
	291	149; 64; 46.; 19; 18	0/4	0/3	0/6
	298	<LQL (5)	0/4	0/3	0/6

The content of pDNA in bovine muscle tissue after 7 month from vaccination

Site of application(*M. coccygeus)*

: 0-3x10^6 copies/g muscle tissue

Symetric non-vaccinated muscle: 0 copies

M. coccygeus : 0 copies (Musce in the vicinity of the *M. coccygeus)*

Lymph node draining the site of vaccination: 0 copies

Characterisation of the site of application: no pathological changes in the muscle, no signs of inflamation, (increased local temperature, oadema, pain, tail mobility)