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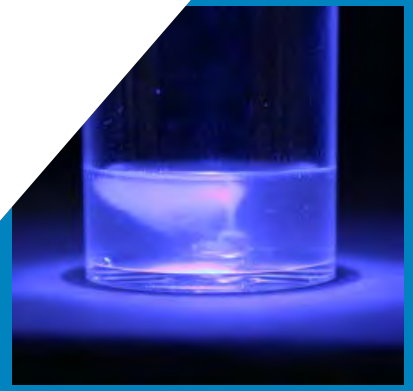
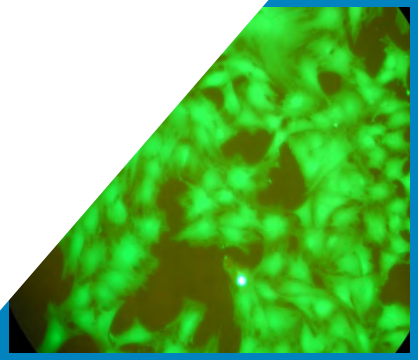
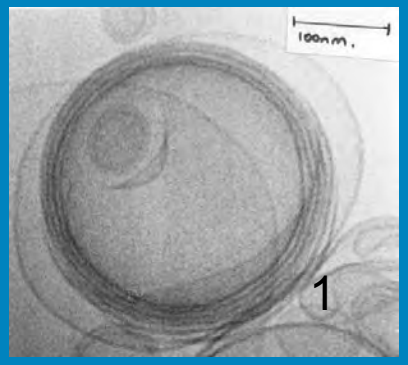
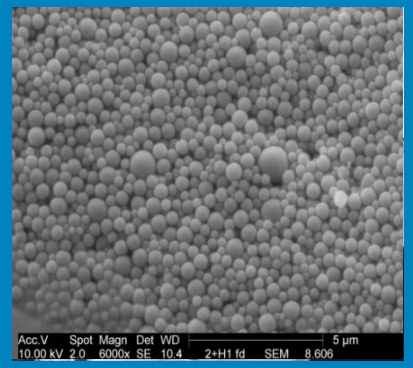
Liposomal DNA vaccines









Prof Yvonne Perrie et al.
y.perrie@aston.ac.uk

Vaccines & Public health

'The two public health interventions that have had the greatest impact on the World's health are clean water and vaccines'

– The World Health Organisation

Types of vaccines

efficacy

1. Live attenuated
   - BCG, polio
2. Inactivated (dead)
   - polio
3. Sub-unit
   - Hep B
4. DNA vaccines?

Safety

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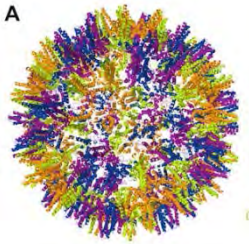
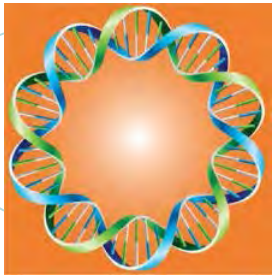
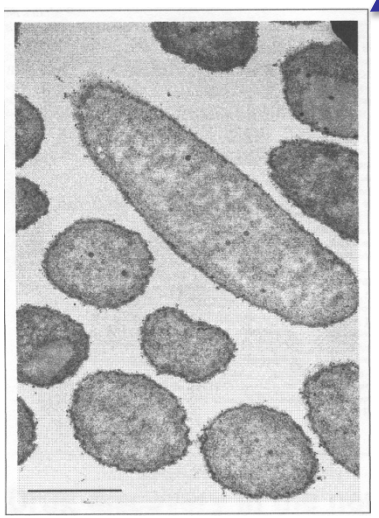
DNA vaccines



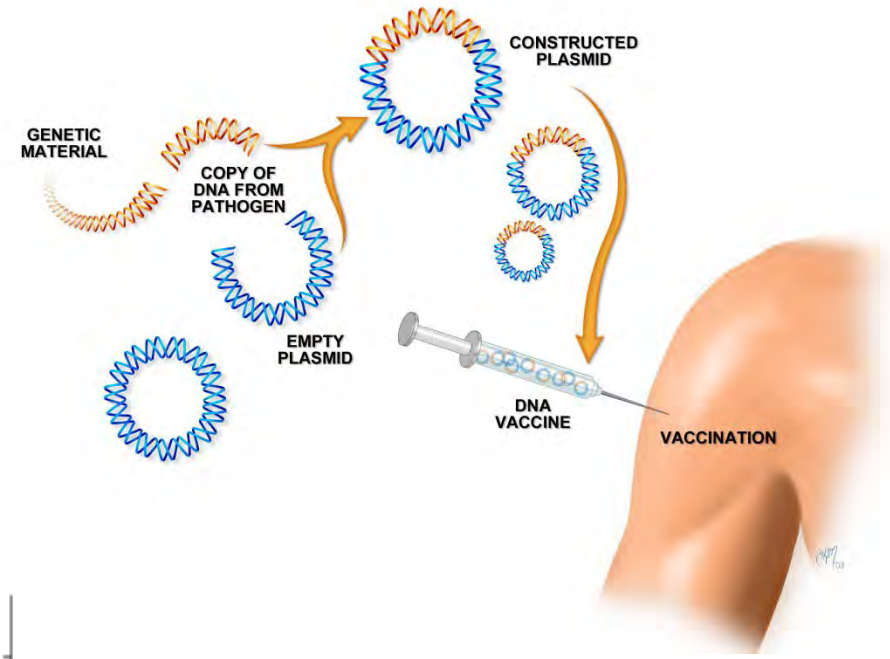
Hep B

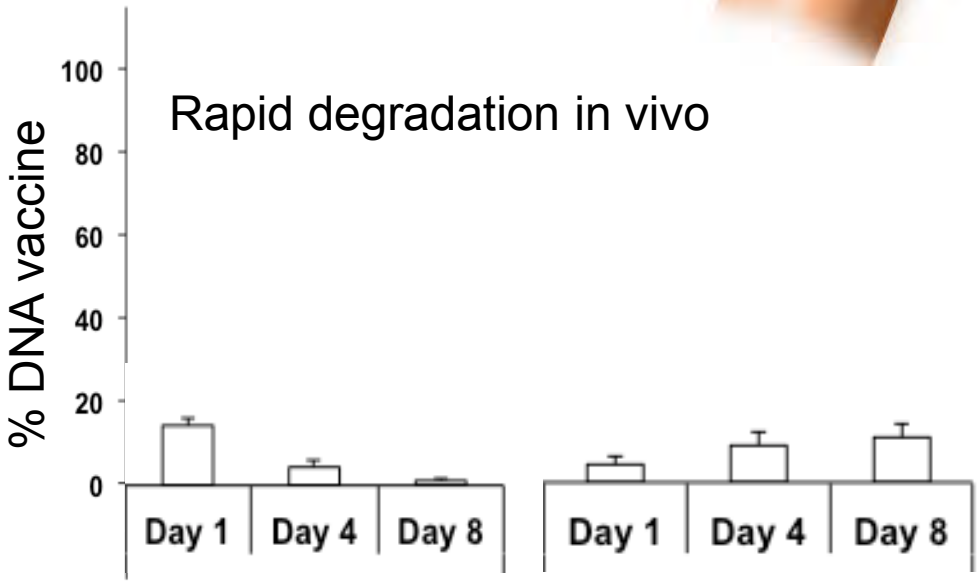


Plasmid encoding HBsAg



Sub-unit antigen





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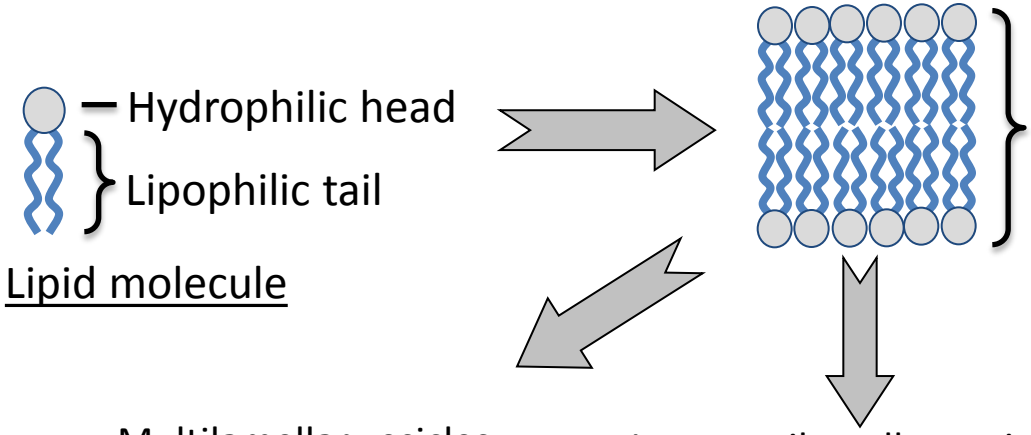
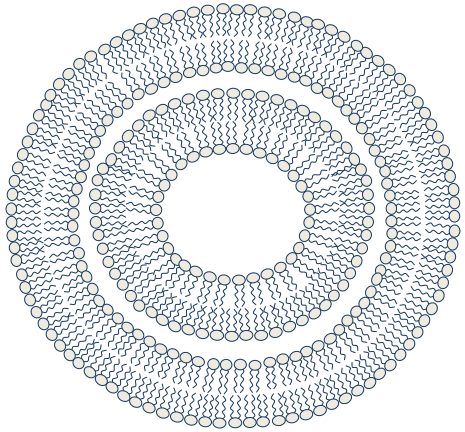
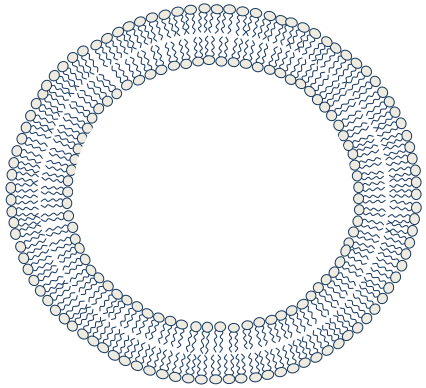
Liposomes as delivery systems:



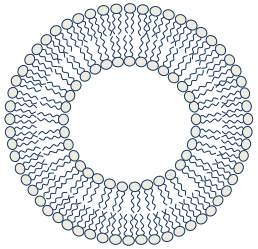
Multilamellar vesicles (MLV)

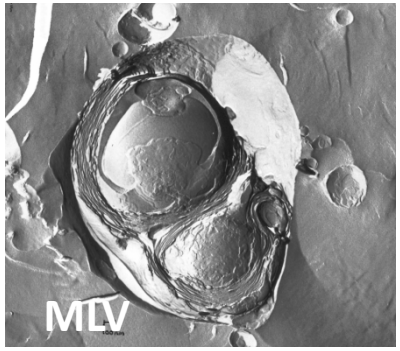


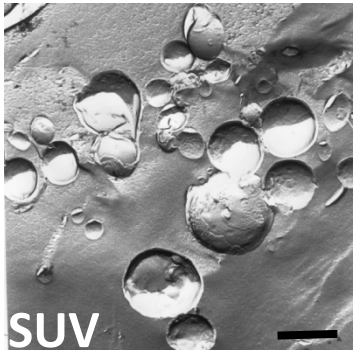
Large unilamellar vesicles (LUV)



Small unilamellar vesicles (SUV)

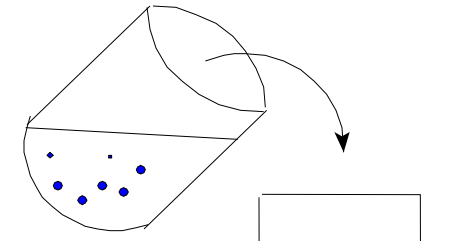






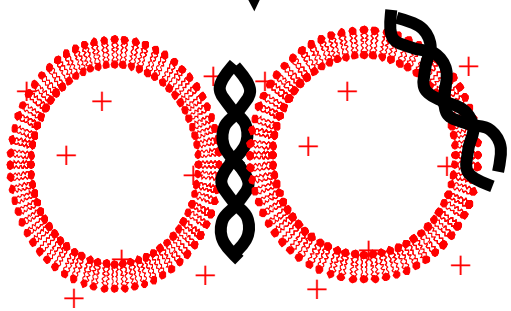
Entrapment of DNA into liposomes

SUV-DNA complexes



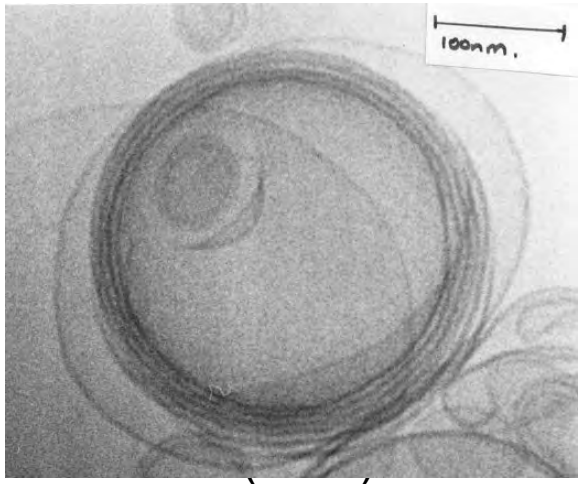
DNA

Liposomes



Dehydrate / rehydrate

DRV with entrapped DNA



Cyro-electron microscopy of DRV(DNA)

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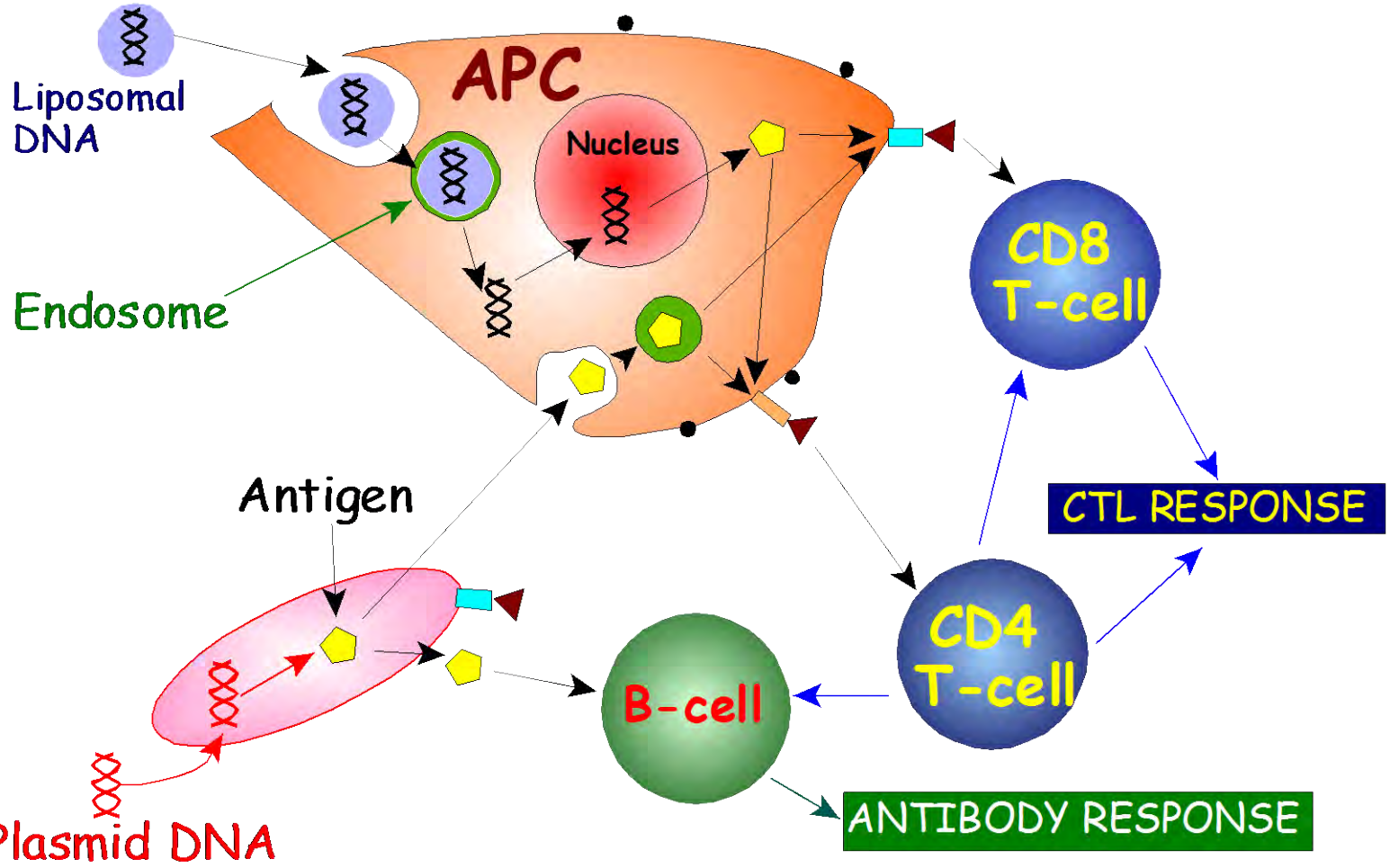
Perrie & Gregoriadis, 2000

DNA vaccines – mimic a viral response



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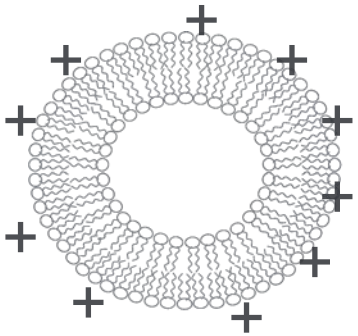
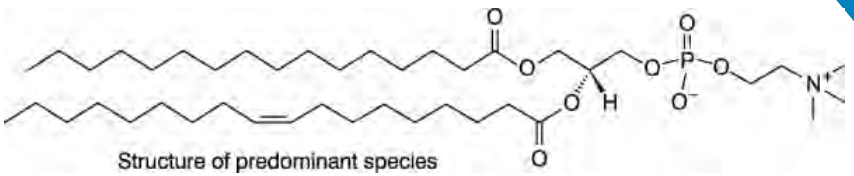
Perrie et al., 2001

Liposome formulations

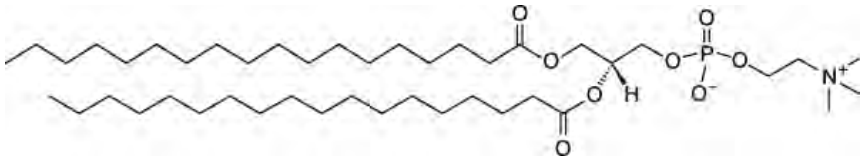


Phosphatidylcholine (PC)

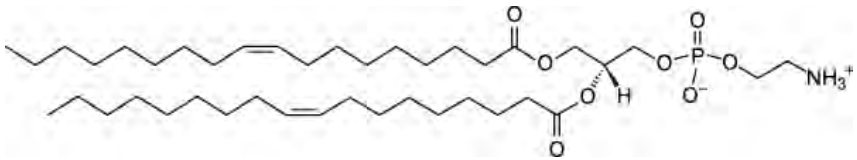


or

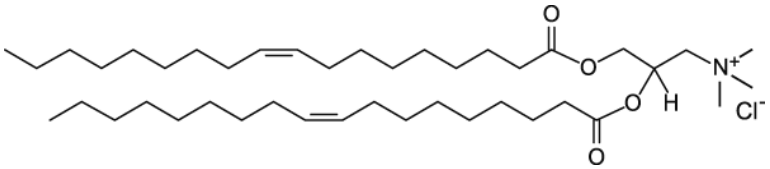
Disteroyl phosphaditylcholine (DSPC)



Dioleoyl phosphatidylethanolamine (DOPE)



Dioleoyl trimethylammonium propane (DOTAP)



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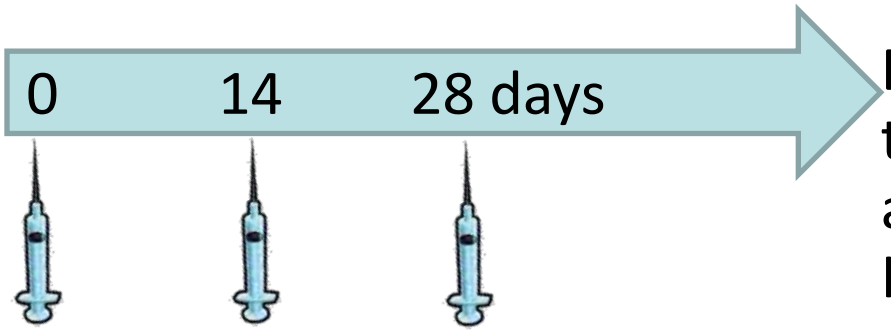
The effect of phospholipid T_c: Physicochemical characteristics

Liposomes	DNA incorporation (% of used)	Zeta Potential (mV)	Size (nm)
PC liposomes	94 ± 3	32.1 ± 0.3	679 ± 96
DSPC liposomes	91 ± 4	32.9 ± 0.4	1025 ± 153



IM injection 10 ug DNA

Blood serum samples taken at time intervals and tested for anti-HBsAg (S region) IgG subclasses by ELISA.

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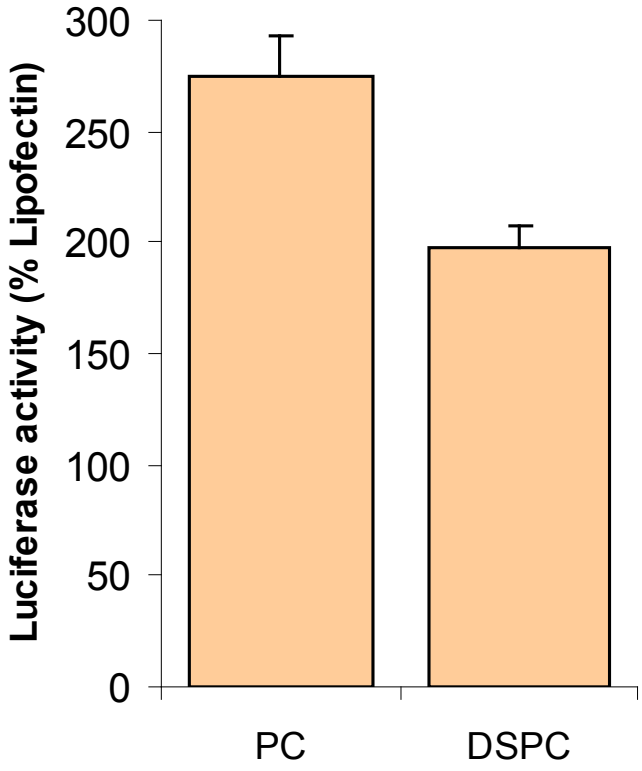
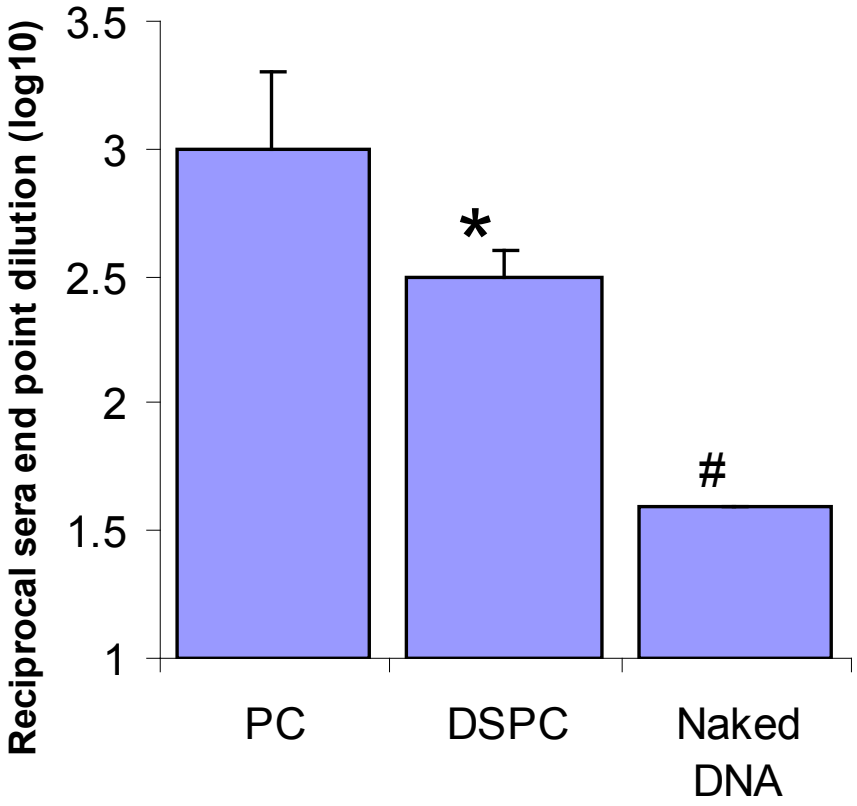
The effect of phospholipid T_c:

Transfection efficiency (in vitro, cos7):



Immune response:



* p<0.05 (compared with PC:DOPE:DOTAP)

# p<0.001 (compared with DRV(DNA))

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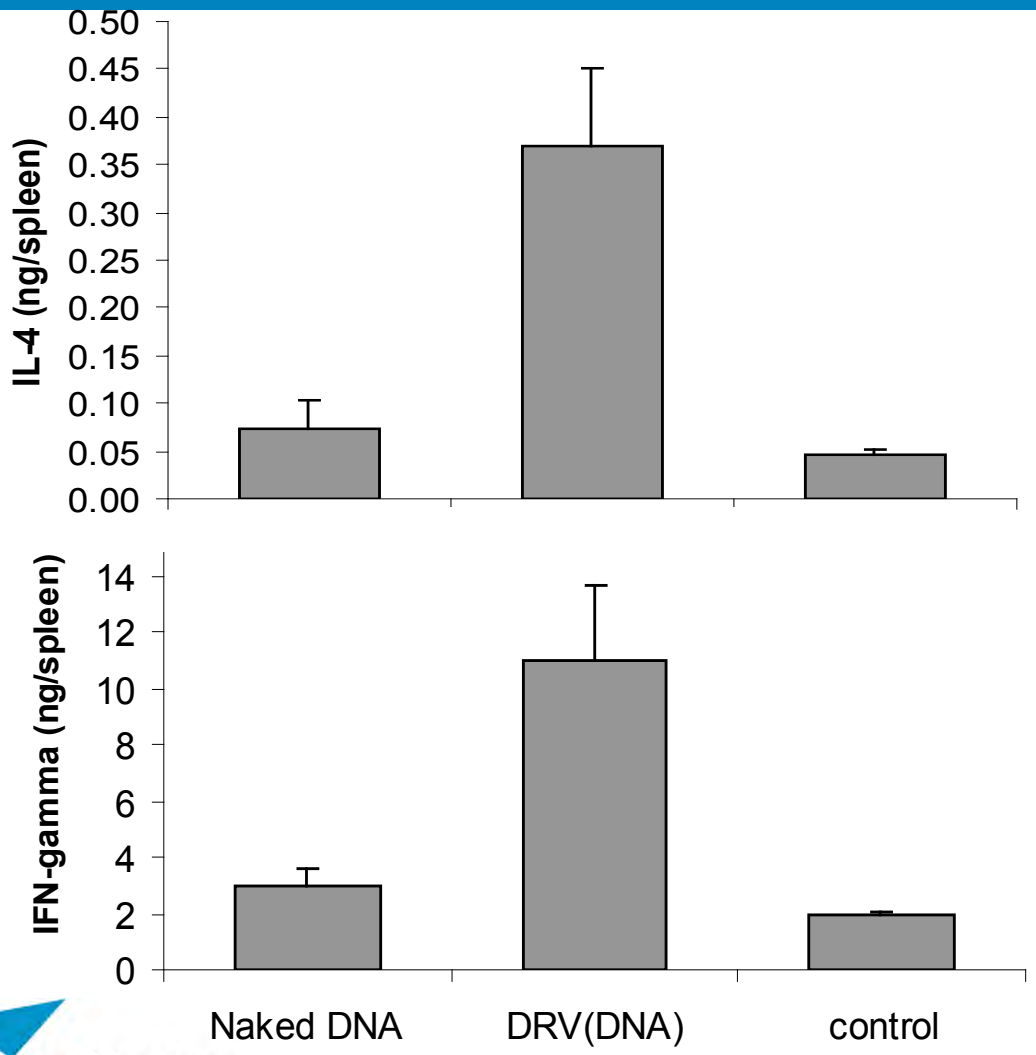
Cell-mediated responses



IL-4 and IFN-gamma levels in the spleens of mice immunised with naked of liposome-entrapped pRc/CMV HBS

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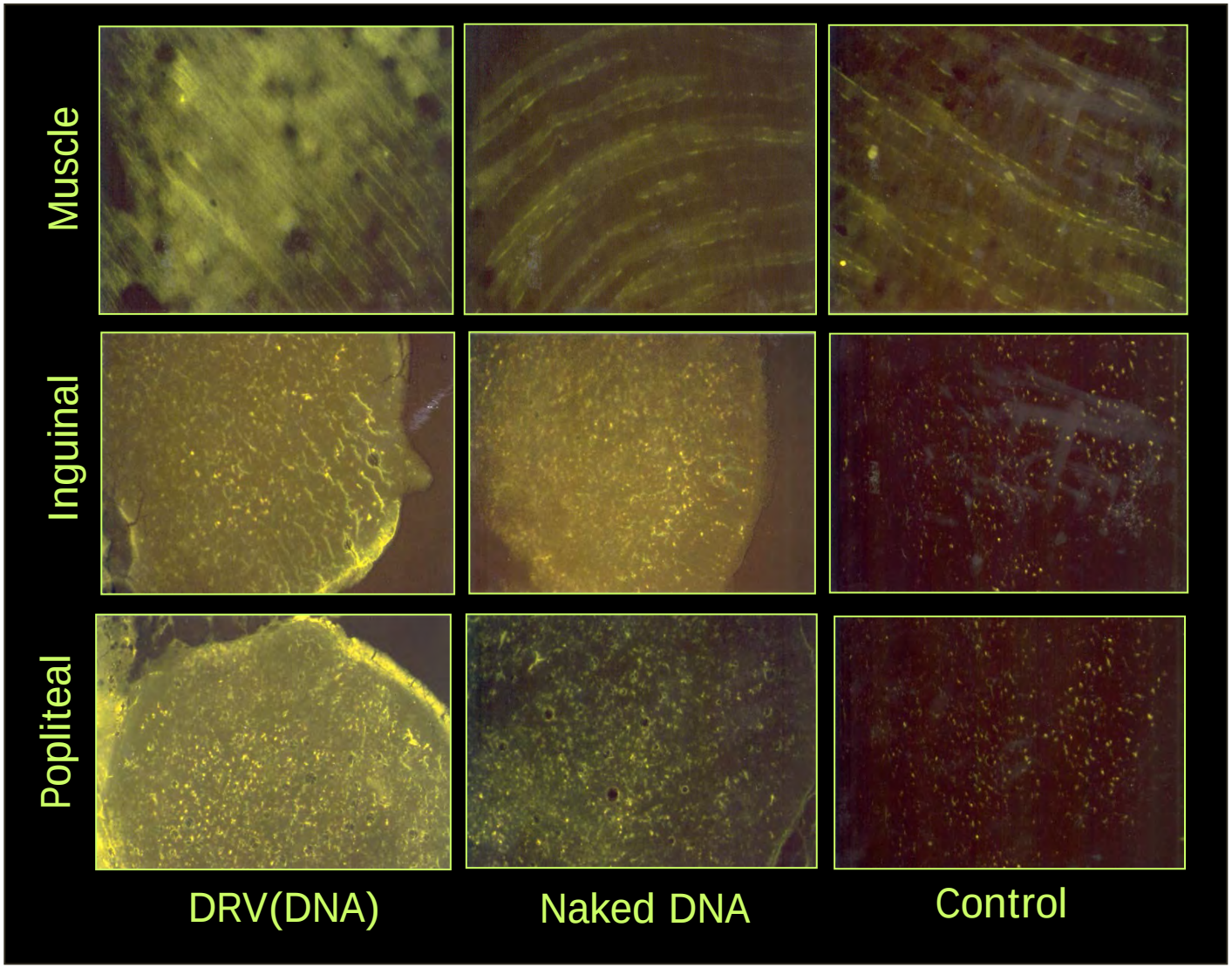
Gene Expression: Liposomal (pCMV.efgp)

Liposomal DRV(DNA) – PC:DOPE:DOTAP



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Types of vaccines

efficacy

1. Live attenuated
   - BCG, polio
2. Inactivated (dead)
   - polio
3. Sub-unit
   - Hep B

Safety

Adjuvants

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Adjuvants

Most subunit vaccines require adjuvants in order to induce protective immune responses to the targeted pathogens.

Adjuvant	Produced by	Disease
Aluminium salts	Various	Various
MF59® (squalene)	Novartis	Influenza
AS03 (squalene+ tocopherol)	GSK biologicals	Influenza
AS04 (MPL+aluminium hydroxide)	GSK biologicals	HPV, HBV
Virosome	Crucell	Influenza, HAV

Adjuvants

‘Immunologist’s dirty little secret’

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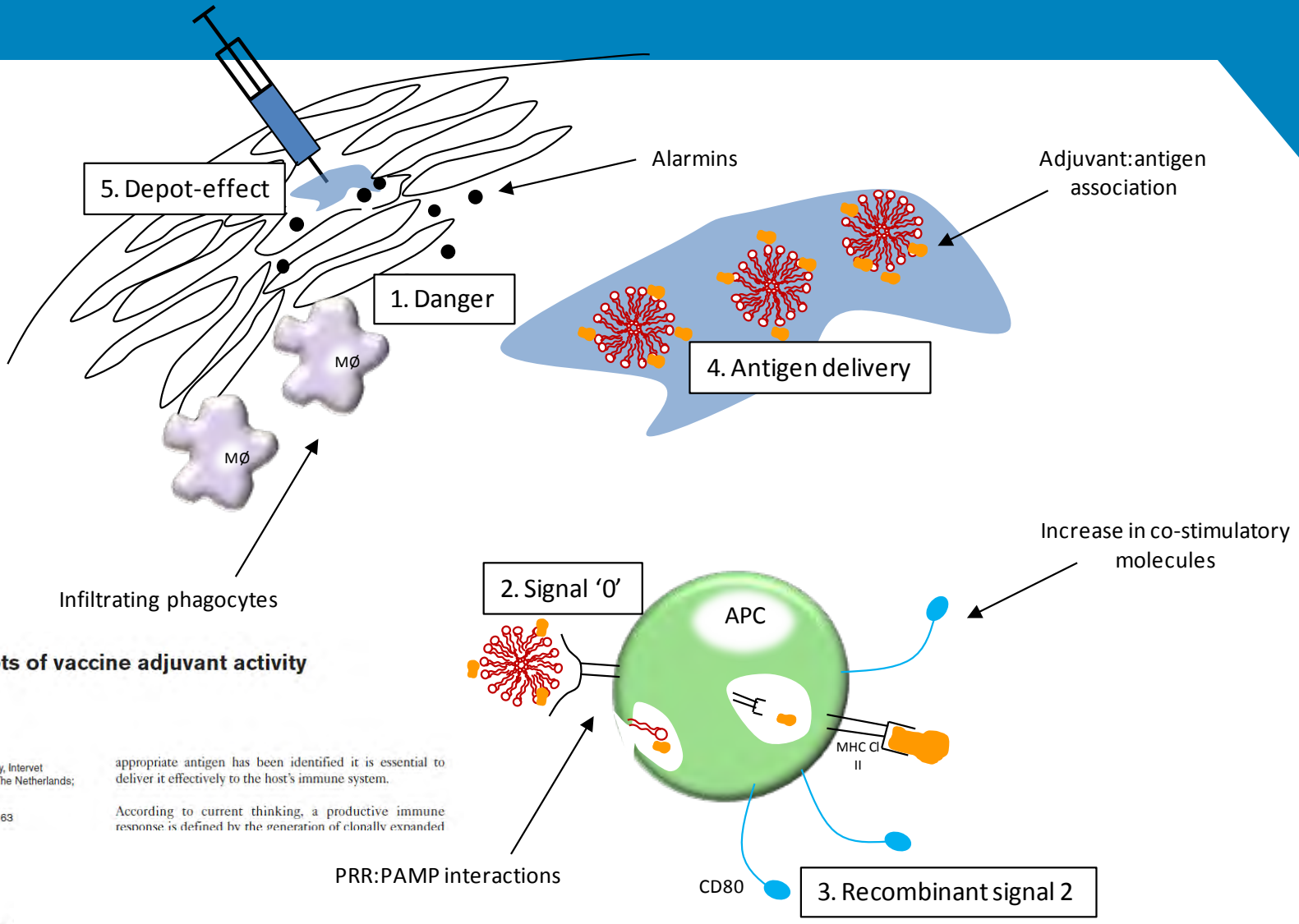
Adjuvants



Immunological concepts of vaccine adjuvant activity

Commentary

Virgil EJC Schijns

Addresses

Department of Vaccine Technology and Immunology, Intervet International BV, P.O. Box 31, 5830 AA Boxmeer, The Netherlands; e-mail: virgil.schijns@intervet.akzonobel.nl

Current Opinion in Immunology 2000, **12**:456–463

appropriate antigen has been identified it is essential to deliver it effectively to the host's immune system.

According to current thinking, a productive immune response is defined by the generation of clonally expanded

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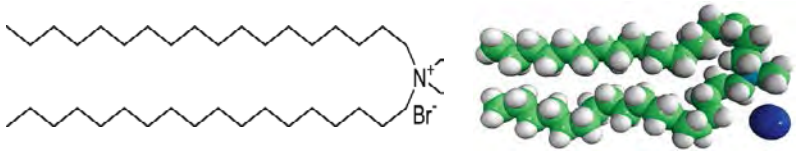
Cationic liposomal adjuvants

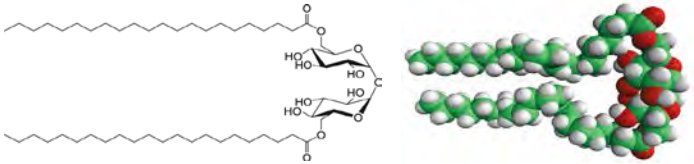
Cationic lipid

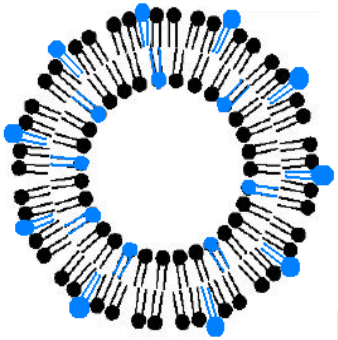
- Dimethyldioctadecylammonium (DDA)



Immunostimulator

- α,α'-trehalose 6,6´-dibehenate (TDB)





- moderate/strong T_H2
- Strong T_H1
- Carry antigen to APC

- Not effective without a delivery system
- Engages a TLR-independent Syk/Card9-dependent pathway

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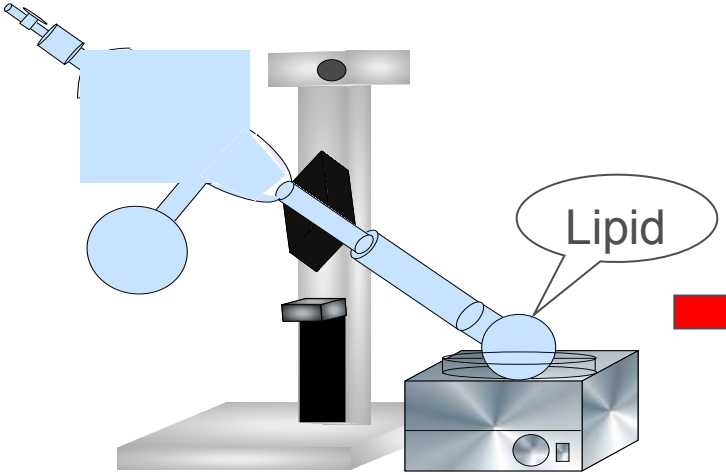
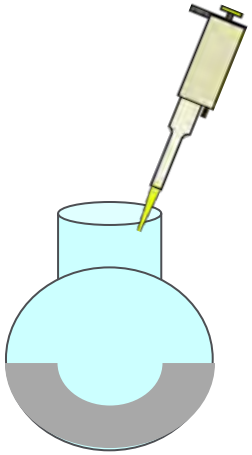
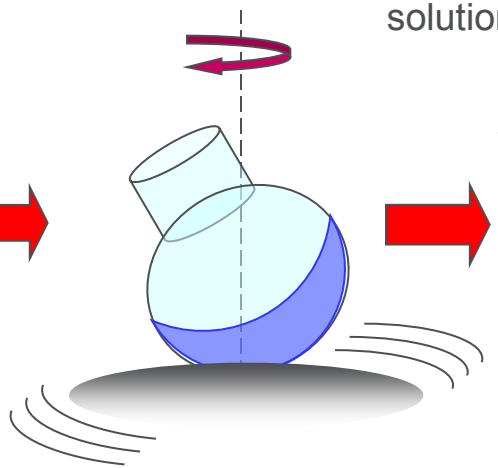
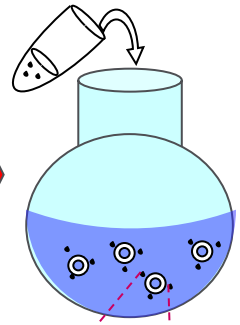
Preparation of liposomes







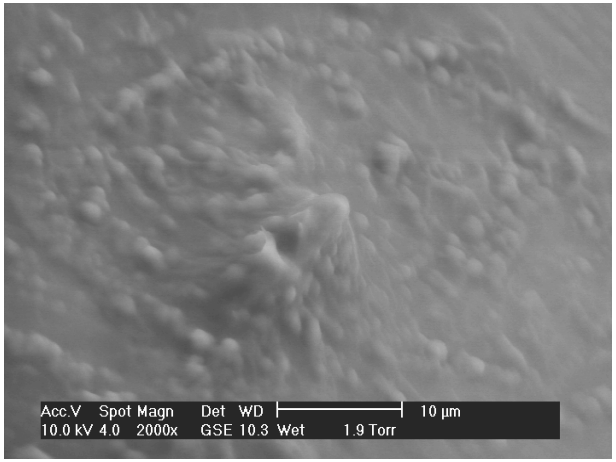


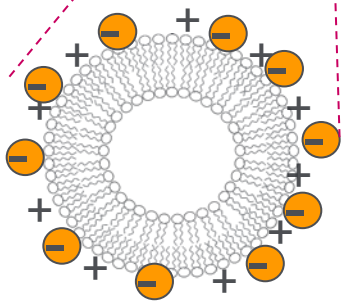
4. Add Antigen solution

1.evaporation

2. Addition of Buffer

3. Warm + Vortexing





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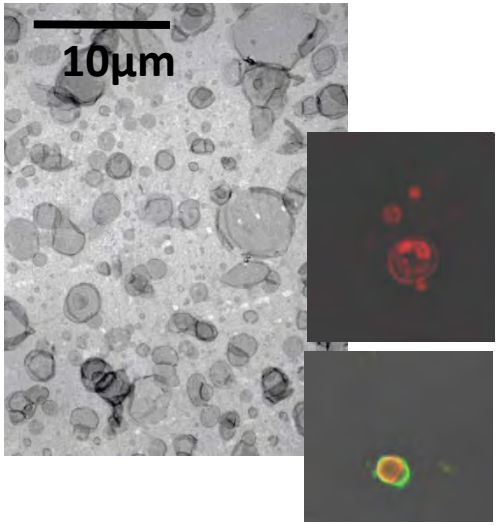
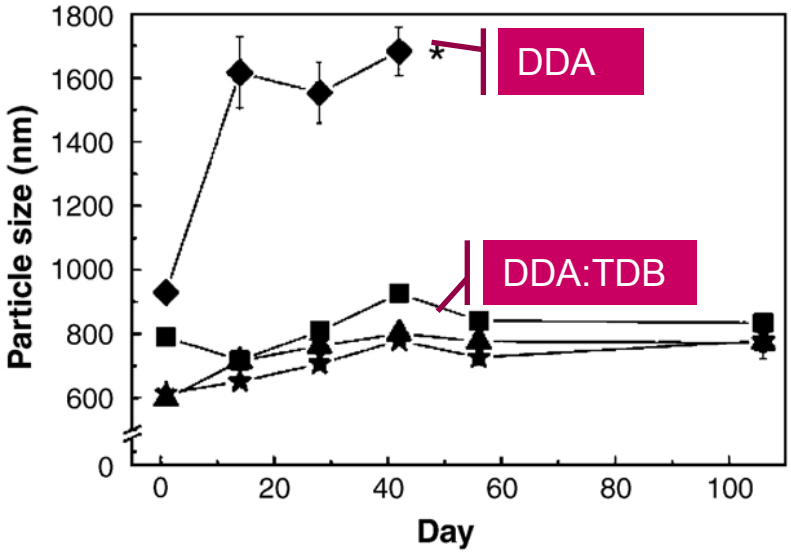
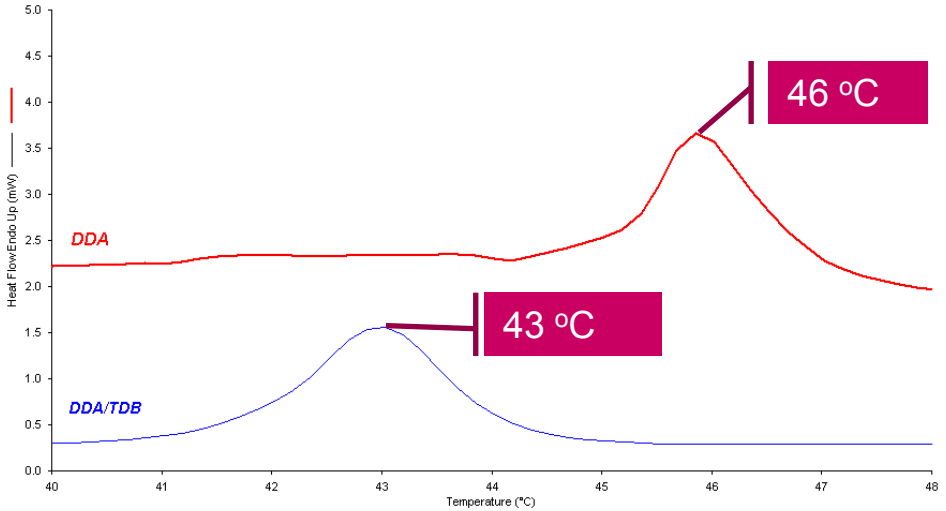
DDA:TDB liposomes



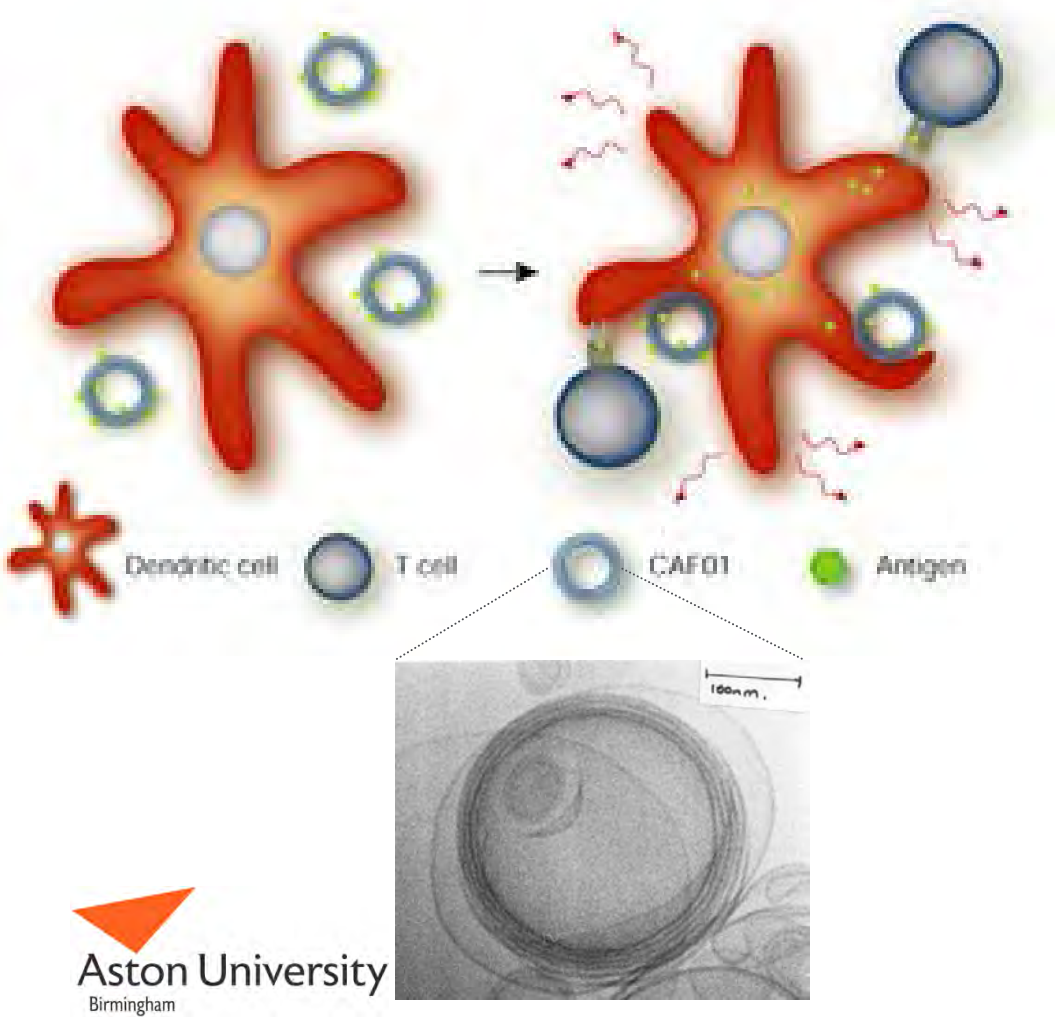
	Size (nm)	ZP (mV)	Loading (%)
DDA	488 ± 124	+46 ± 1.6	89 ± 10%
DDA:TDB	416 ± 40	+48 ± 5.1	87 ± 8%





Davidsen, Perrie et al., BBA, 1718 (2005) 22-31.

Cationic liposomes for vaccine delivery

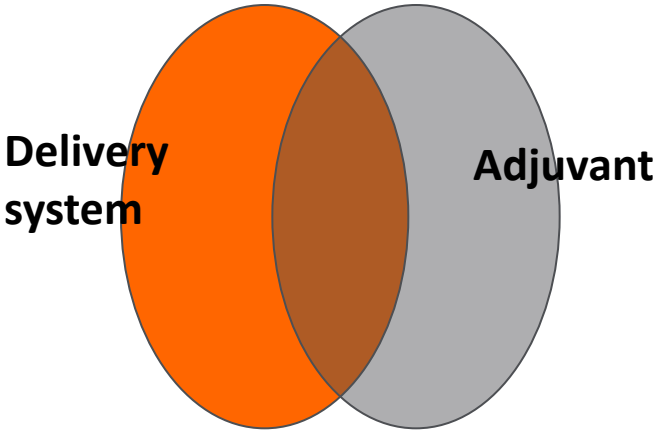


DDA

- Delivers antigen to DCs

TDB

- Activates DCs through Syk-Card9 signalling pathway and induces Th1 and Th17.



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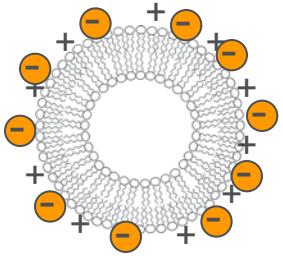
Liposomes for vaccine delivery:
Formulation & Function



Physical/chemical Characteristics

Stability

Pharmacokinetics and release

Biological function

Size,
Surface properties
Antigen loading/ release
Thermal analysis
Chemical analysis

Identify controlling parameters

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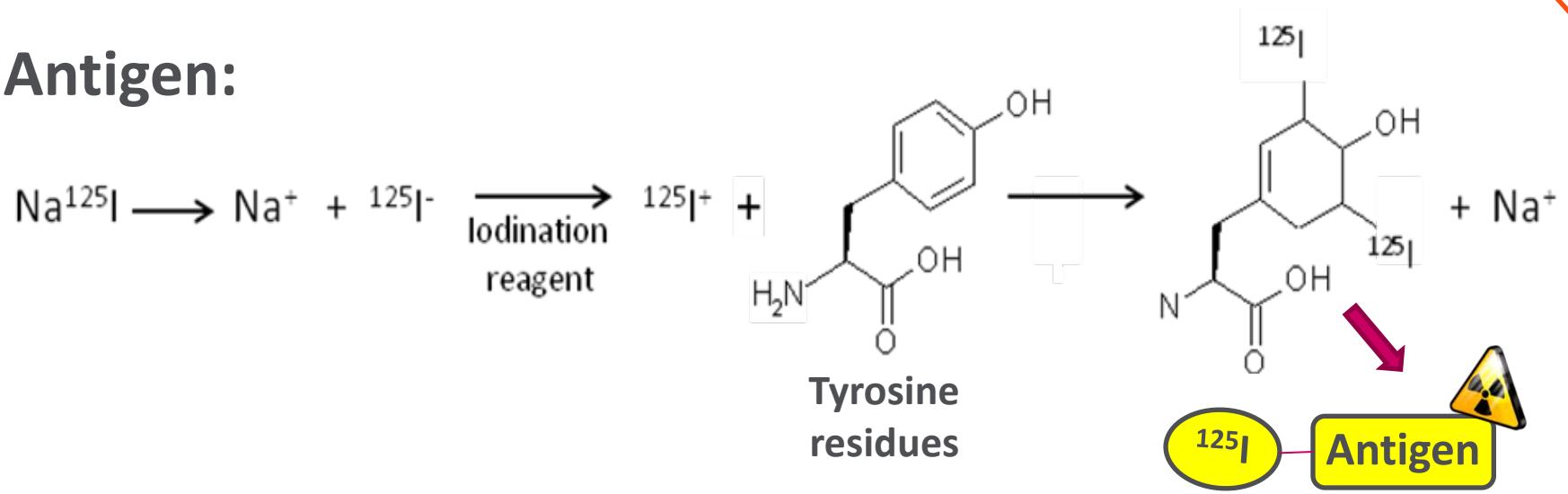
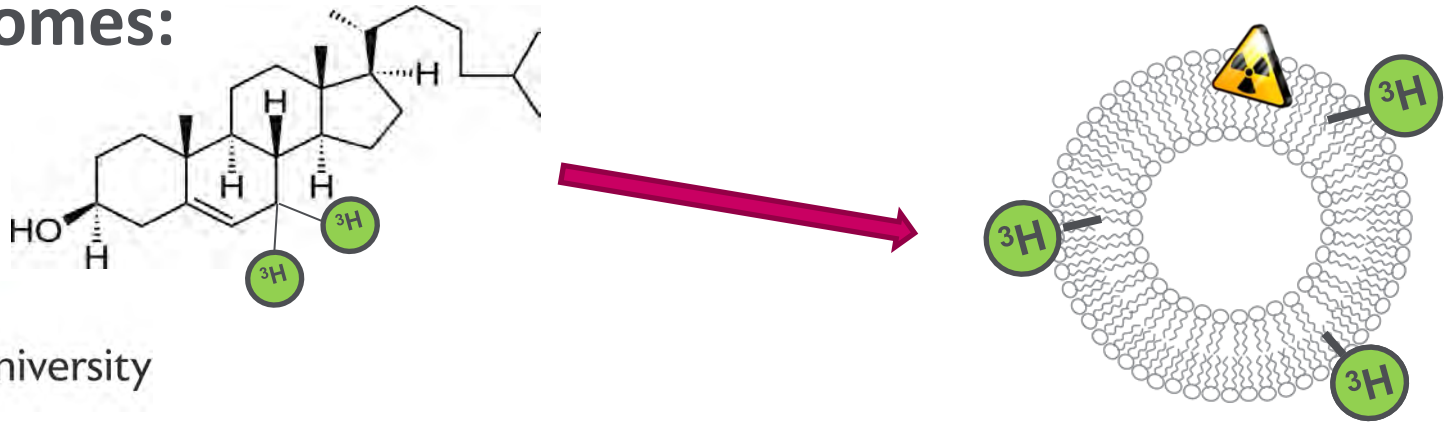
Considering delivery of liposomal adjuvants

Antigen:



Liposomes:



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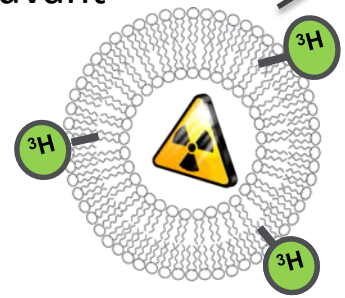
Investigating the bio-distribution and cell recruitment

Monocytes pre-stained using pontamine blue (2 – 3 days prior)

Dual labelled antigen and adjuvant



Gamma counting → Antigen levels

Tritium counting → CAF levels

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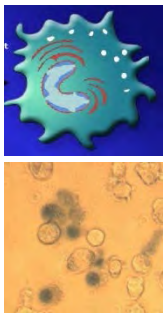
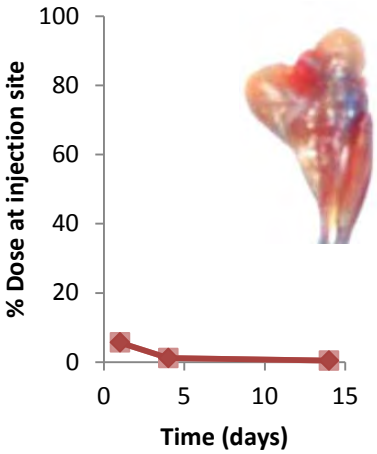
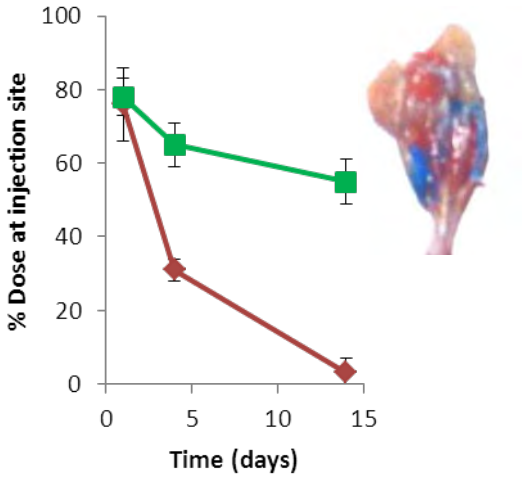
Liposomes promote depot effect and TDB promotes monocyte recruitment



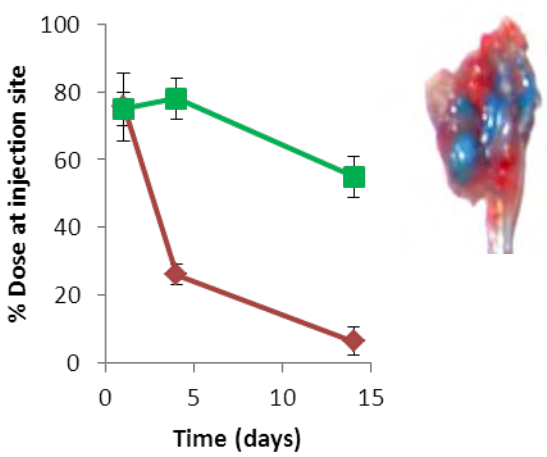
Antigen only

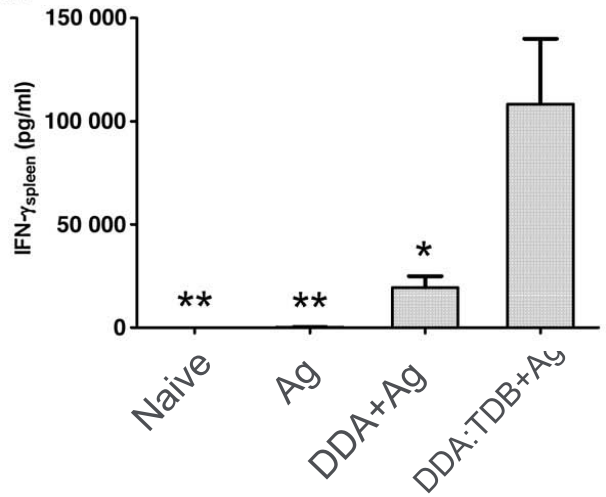


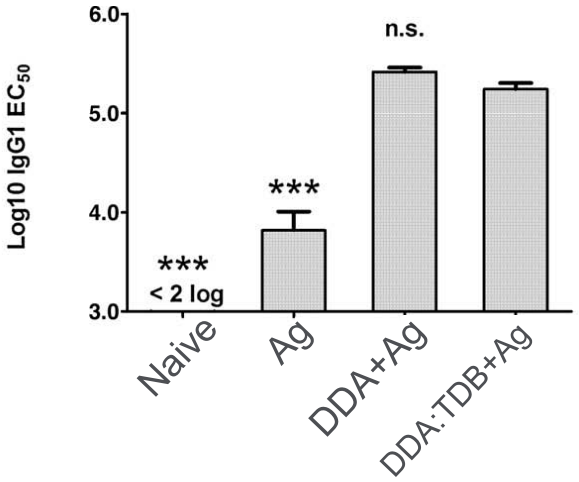
DDA + Antigen



DDA:TDB + Antigen



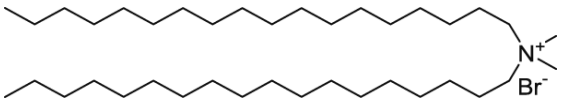


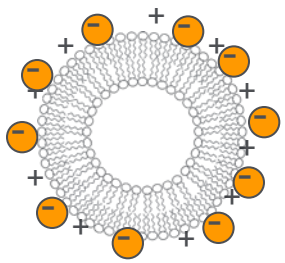


Henriksen., 2010a, Journal of Controlled Release.

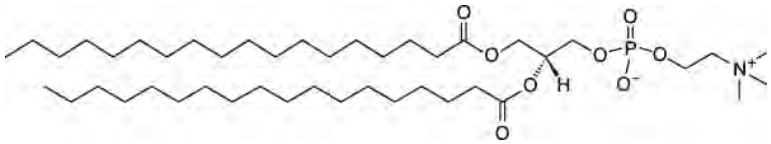
Controlled release of the liposomes from the depot site?

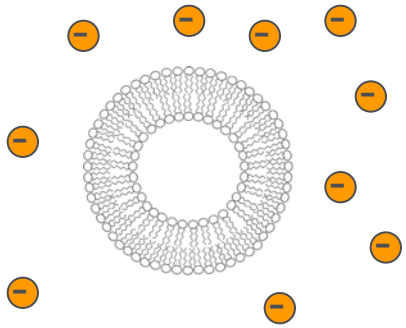
DDA - cationic

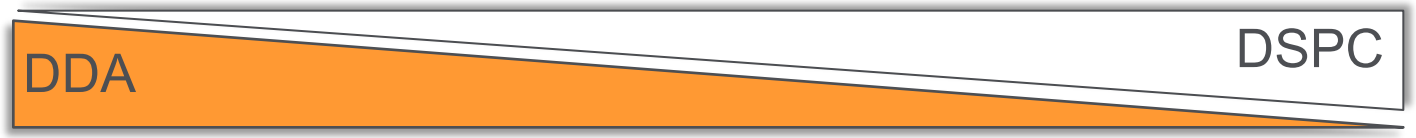




DSPC - neutral







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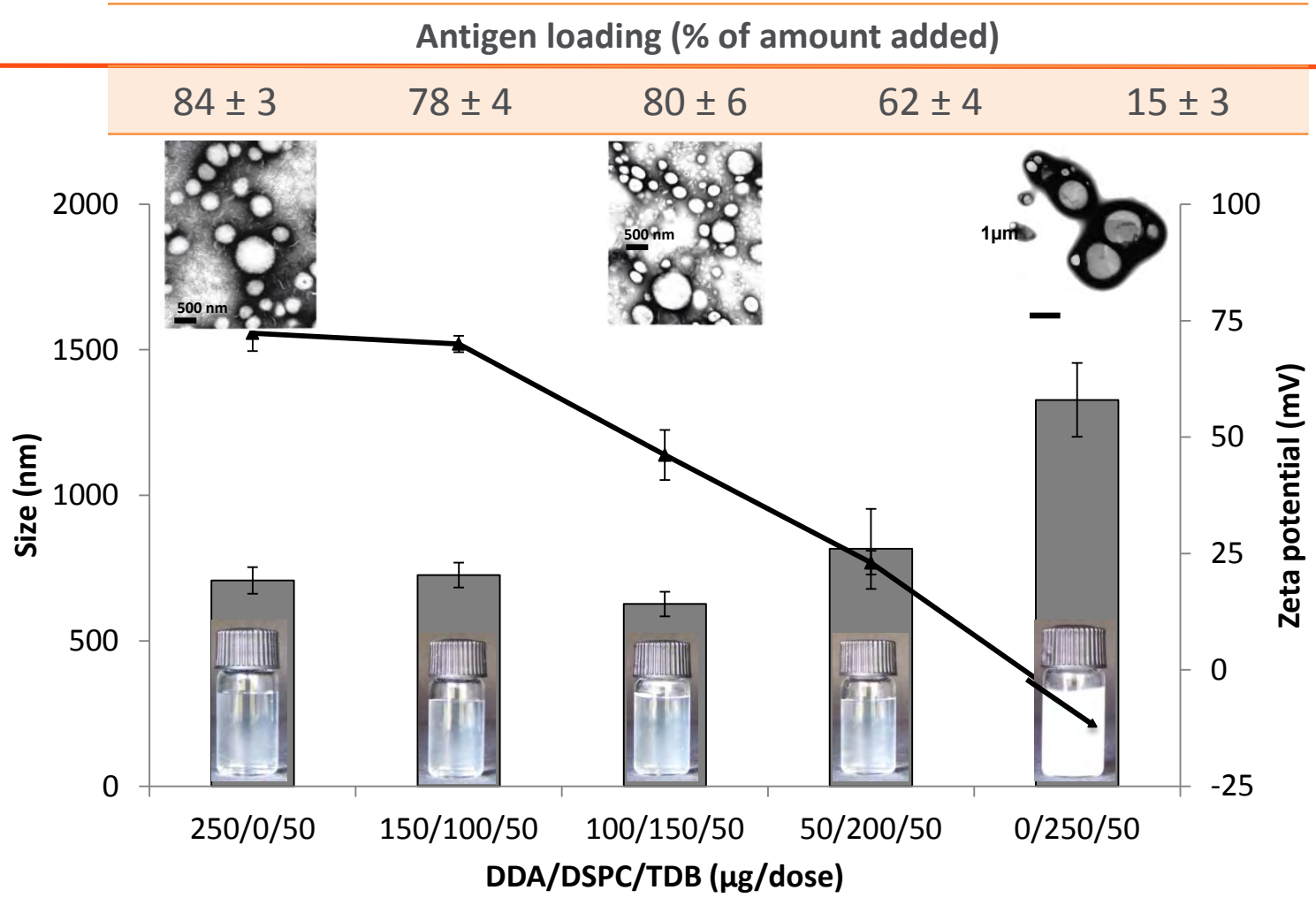
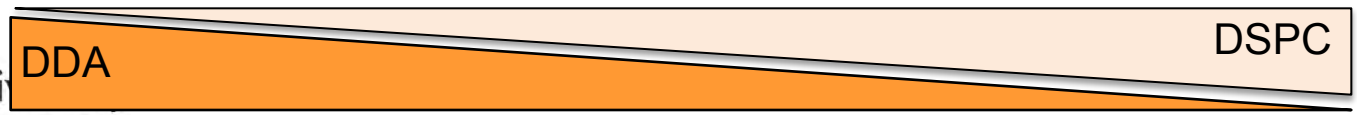
Characteristics: Role of DDA





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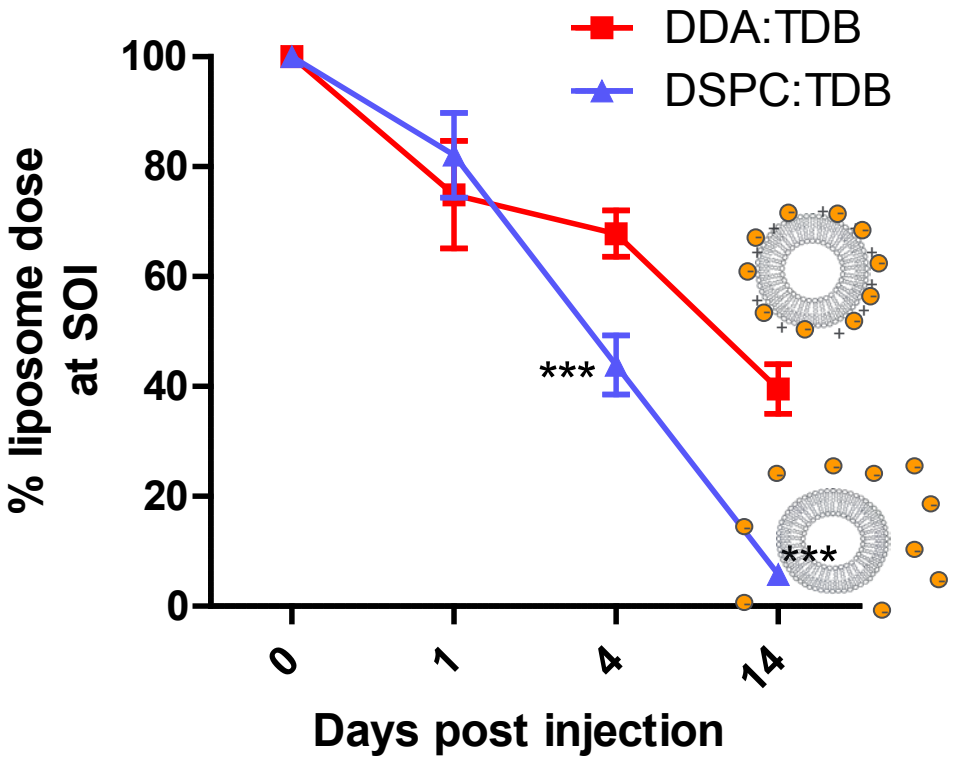
Hussain et al., 2014. Journal of Pharmacy and Pharmacology

Liposome & Antigen retention – site of injection



Monocyte influx

DDA/TDB

DSPC/TDB

Neutral liposomes cleared quicker

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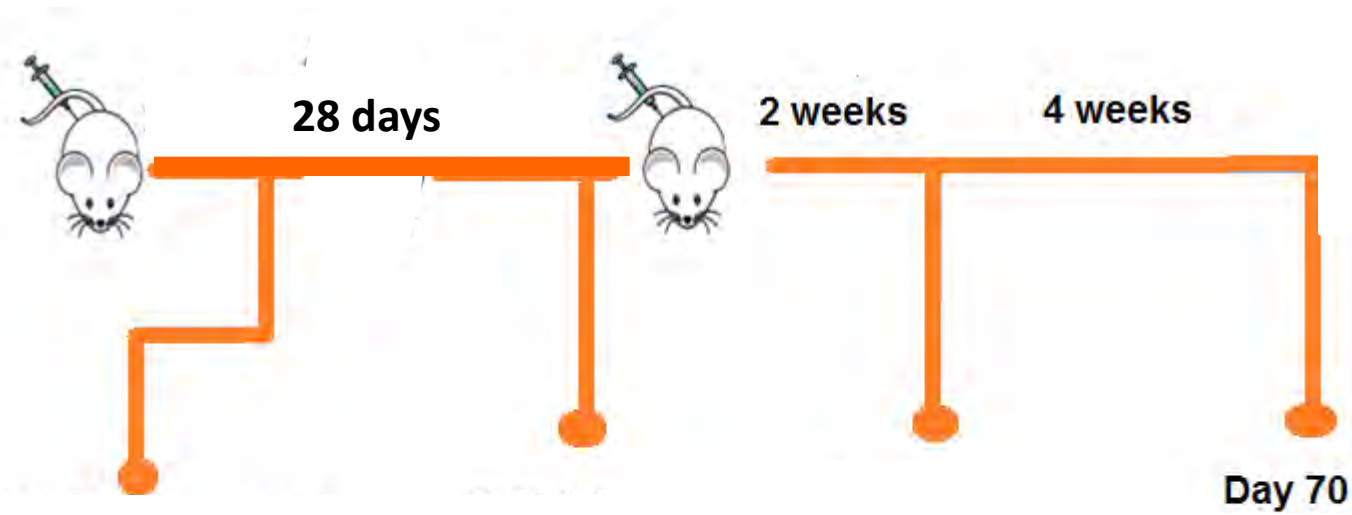
Henriksen., 2010b, Journal of Controlled Release.

Immunisation study time scale

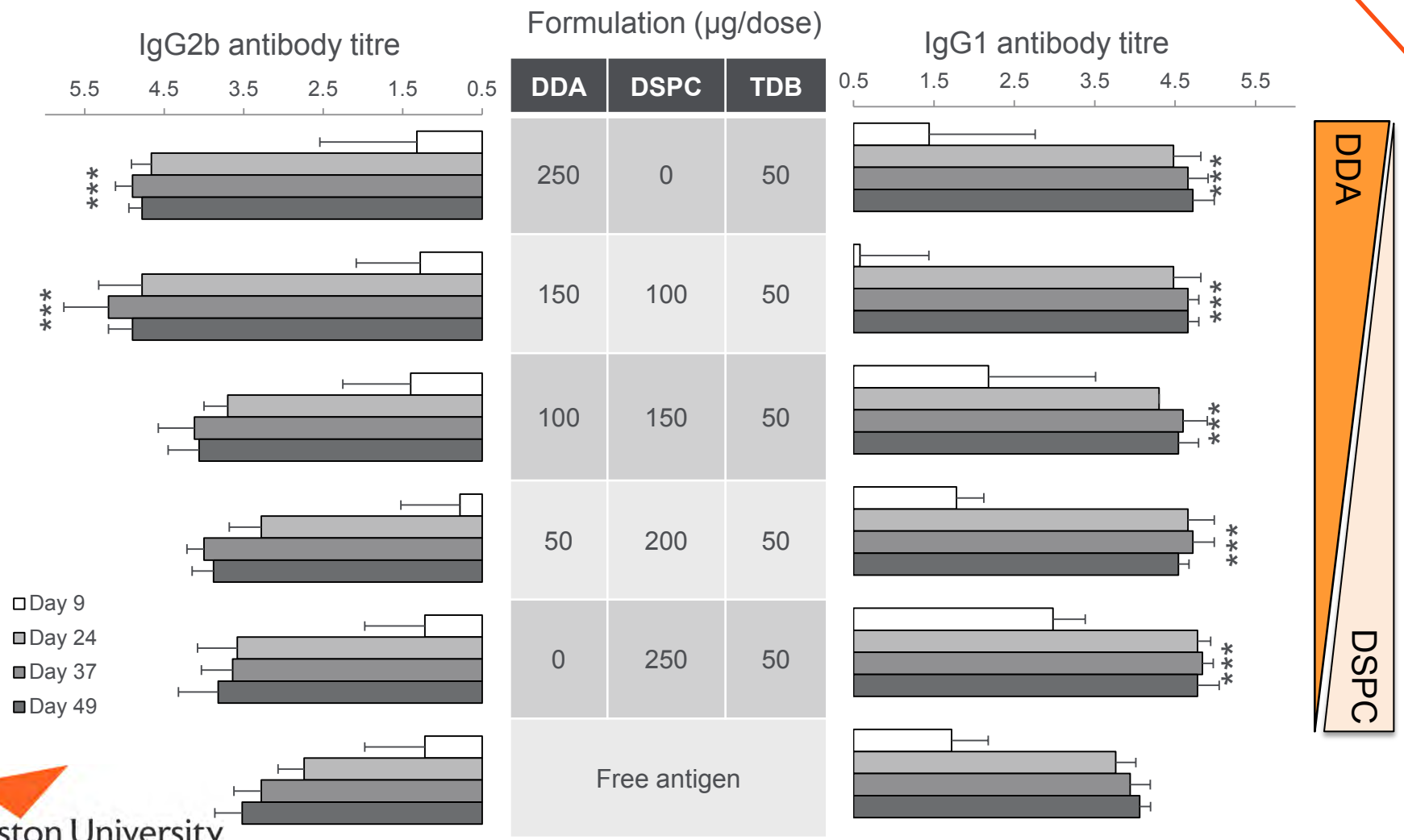


Day 9, 24, 37, 49 measure Ab responses

Day 70:

Measure Cytokines and cell proliferation

Reciprocal end point serum dilution (log_{10})



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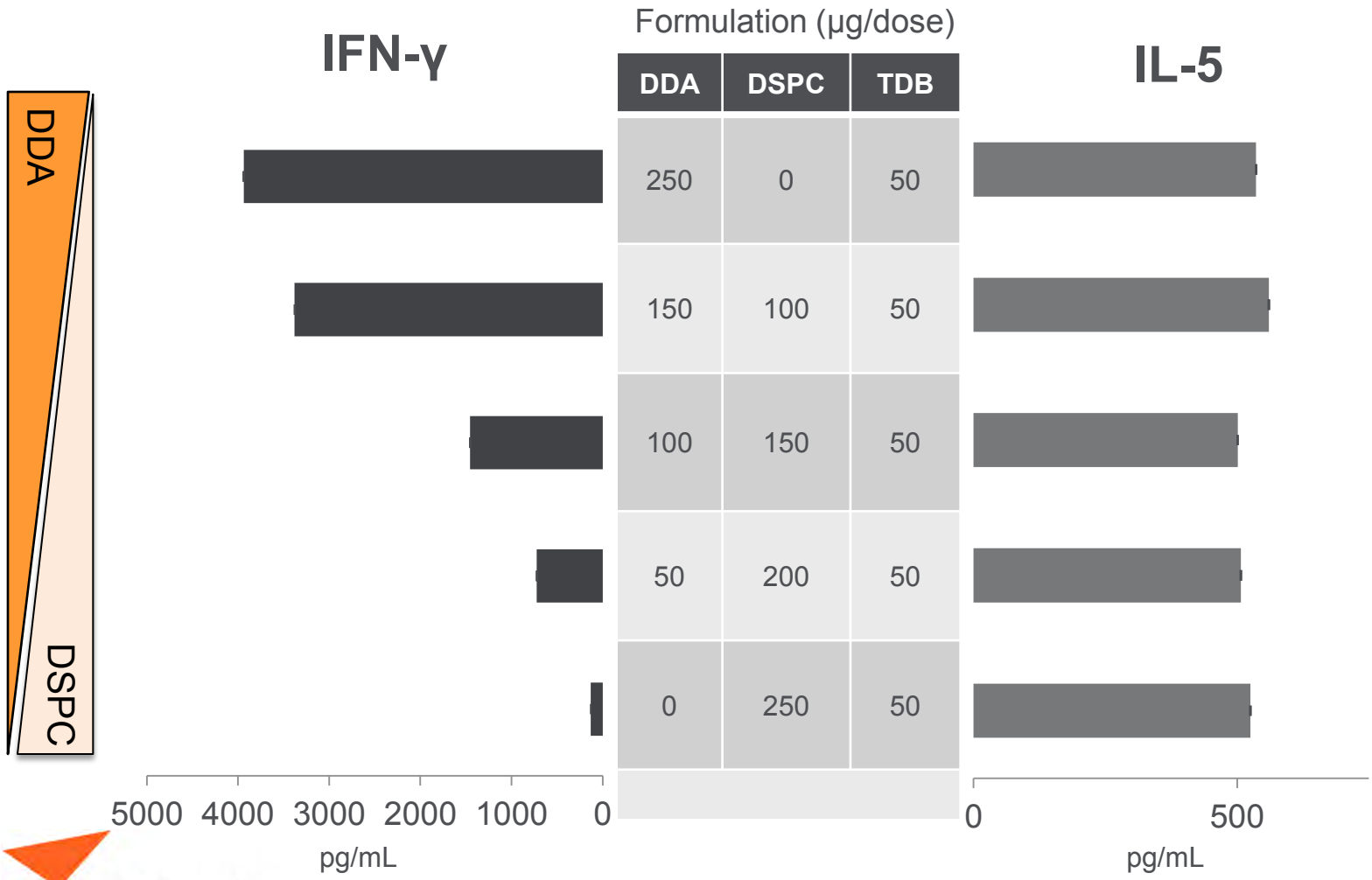
Hussain et al., 2014. Journal of Pharmacy and Pharmacology

DDA/DSPC ratio: Th1 responses are dependent, Th2 responses are independent.



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Hussain et al., 2014. Journal of Pharmacy and Pharmacology

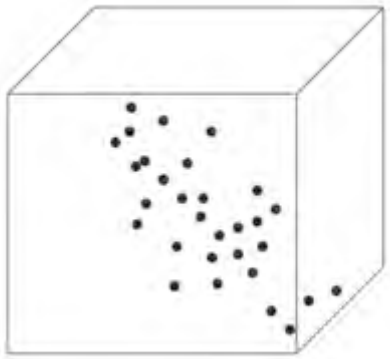
Multivariate Data Analysis (MVDA)

Data collection

Large amounts of data collected

Multiple variables, observations and responses

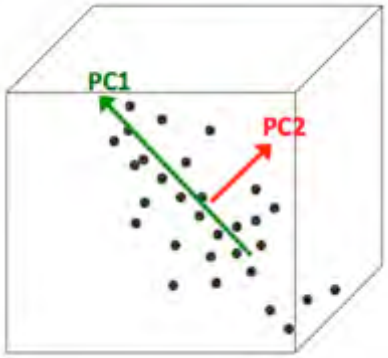
More than one variable



PCA

Principal Component Analysis

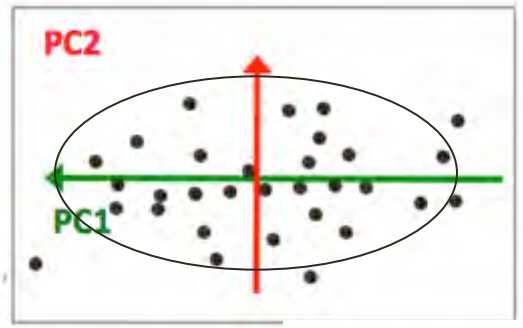
→ Data Mining
→ Clusters / Trends
→ Outliers



PLS

Partial least square Analysis

→ Multivariate regression analysis
→ Selection of important variables for a selected response
→ **Importance of variables**
→ **Correlation of variables to responses**



SIMCA
BY UMETRICS

MVA analysis of vaccine efficacy

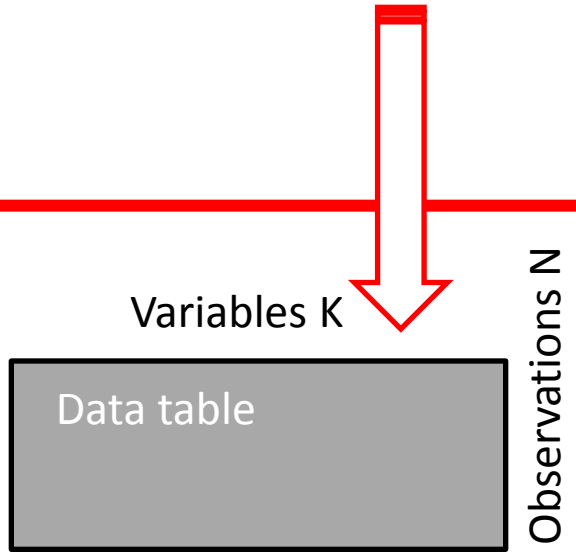
→ Link between Variables and responses
→ Cluster in immune responses
→ Most influential variable

Variables: Immune responses
Lipid composition
Size of Liposomes
(Zeta potential of liposomes)

Responses: Immunity
IL-2
IL-5
IL-6
IL-10
INF-g
Spleen Proliferation
IgG
IgG1
IgG2b

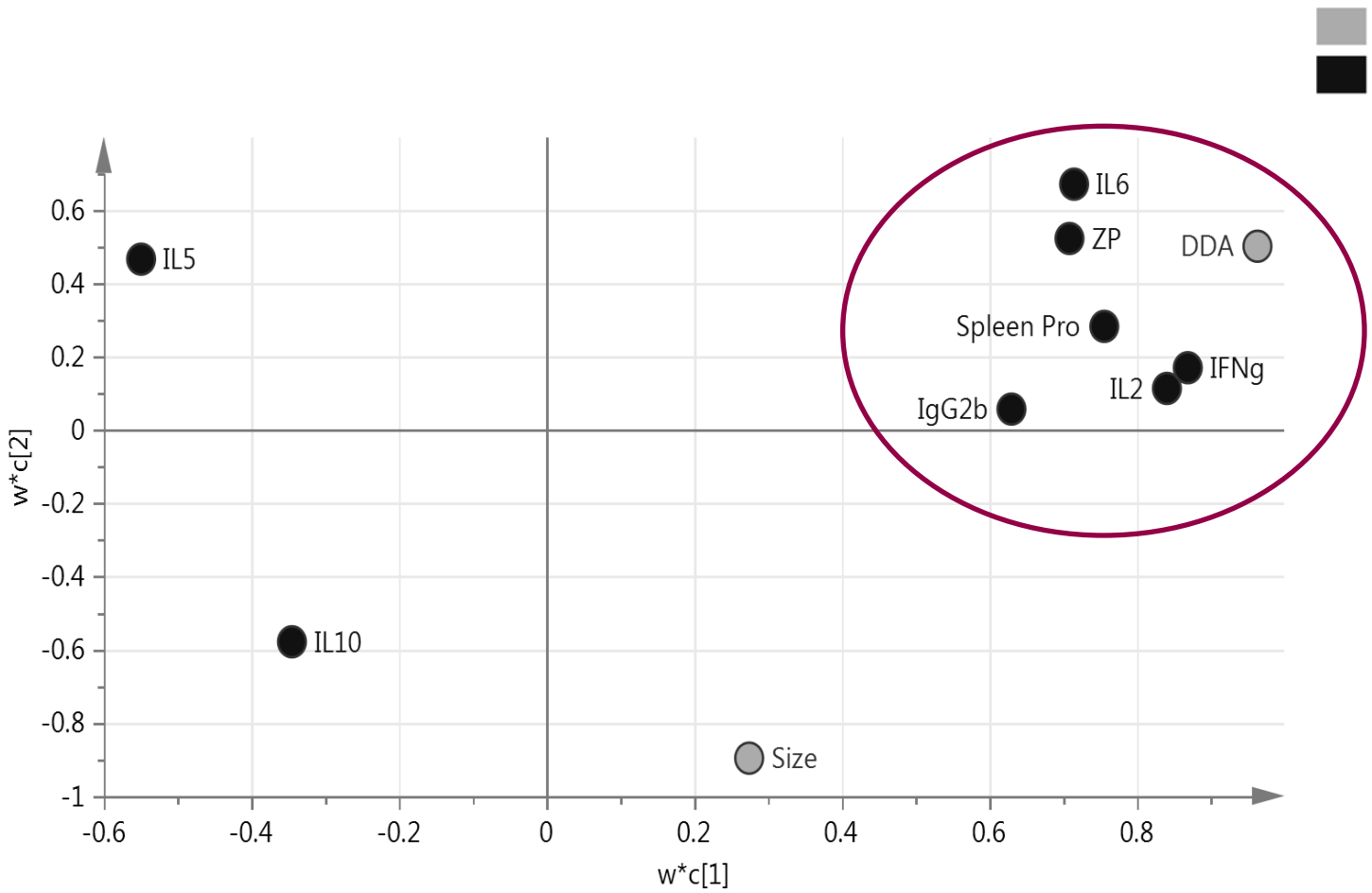
Chemometrics

Projection Methods
PCA, PLS



Underlying Assumptions
- X-variables are **not** independent
- X-variables may have errors
- Outlier detection
- Residuals may be structured

Clustering relationships – Th1 responses driven by DDA content



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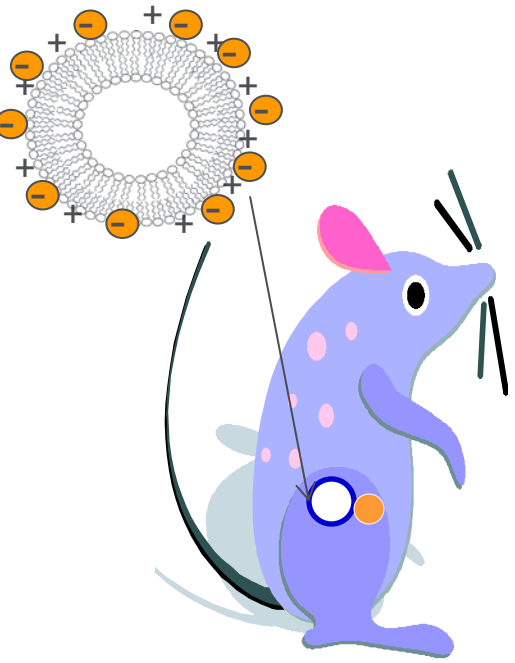
Kastner et al., 2014. Accepted.

Antigen needs to be adsorbed for co-delivery



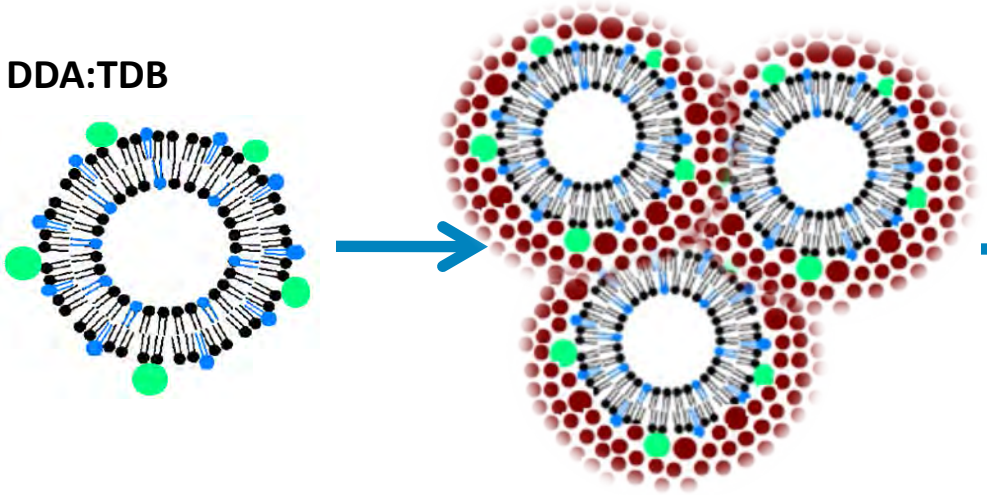
Both retained at SOI

Monocytes recruitment

Good protection

Henriksen et al., 2011, Molecular Pharmaceutics.

Keep the DDA content and enhance the flow?



Aggregation = Depot formation = Strong Th_1 response

Modify this depot effect?

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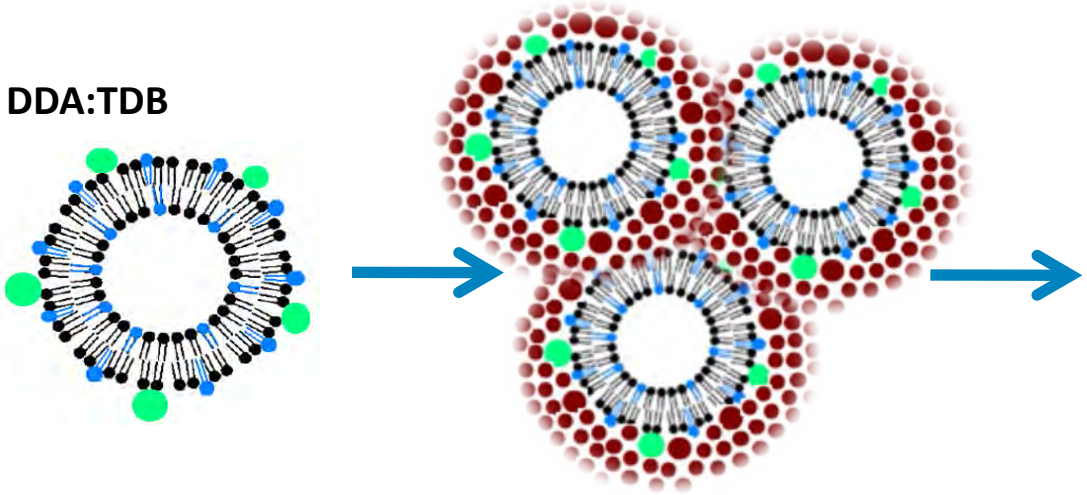
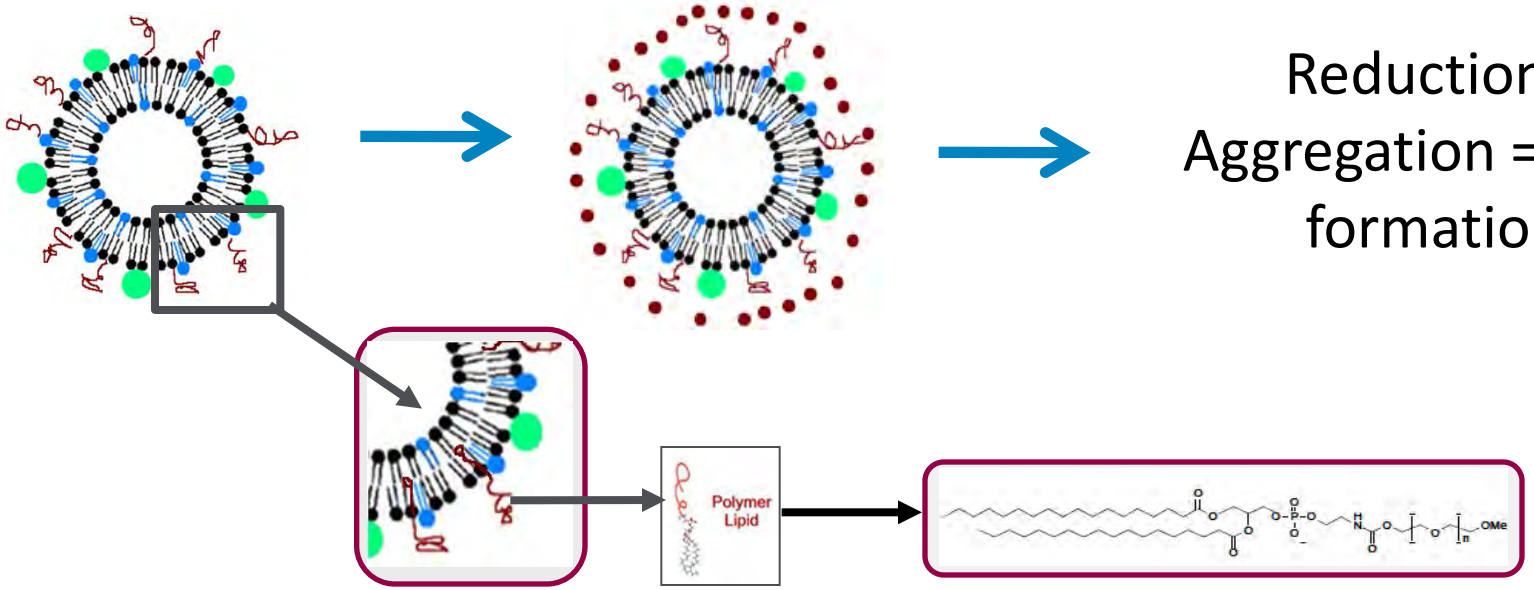
Retain DDA but mask the charge?

DDA:TDB



Aggregation = Depot formation = Strong Th_1 response

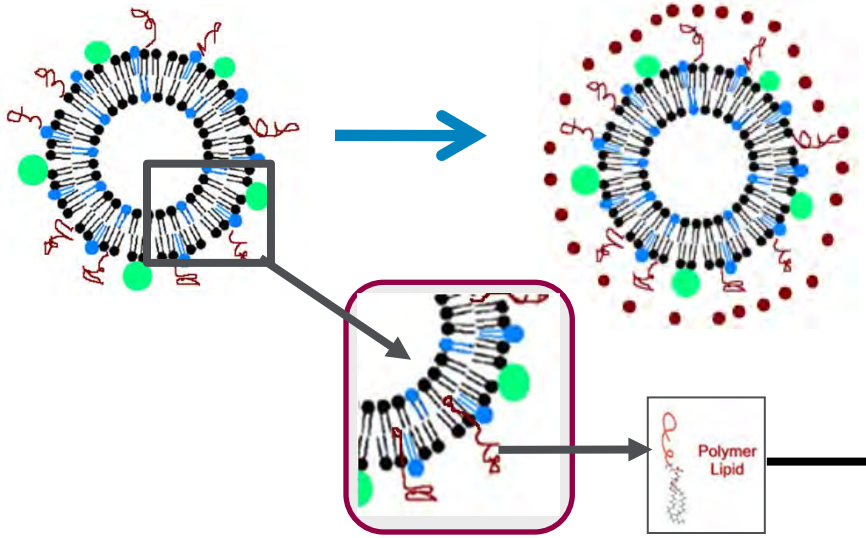
PEGylated DDA:TDB

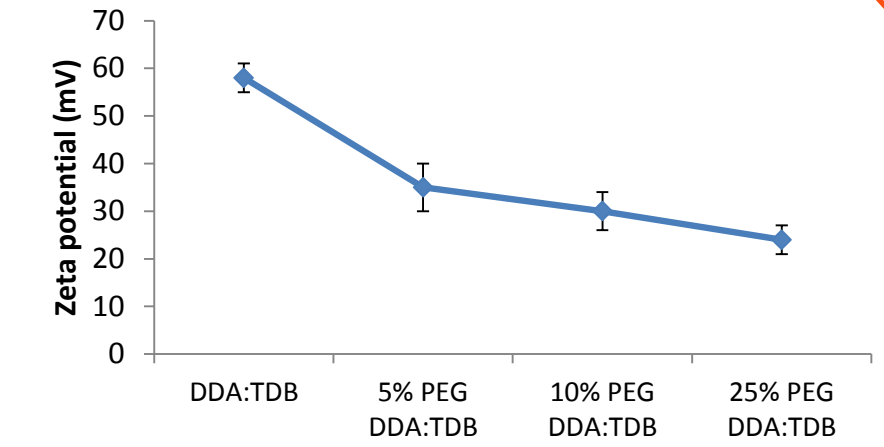


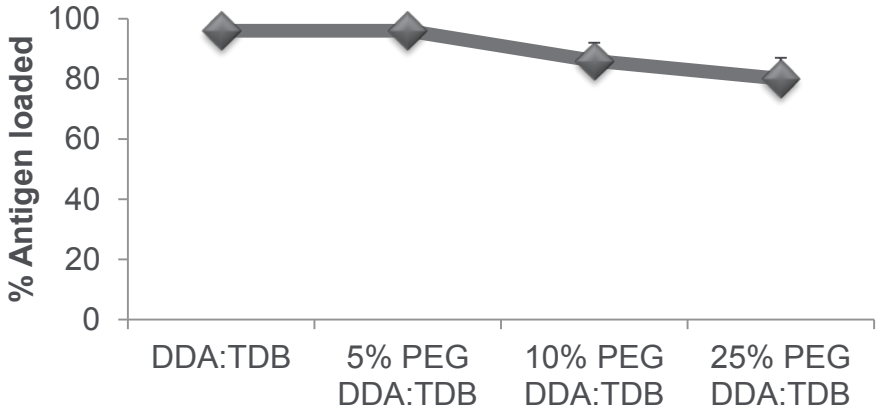
Reduction of Aggregation = Depot formation?

Pegylation of the liposomes – masking the charge

PEGylated DDA:TDB







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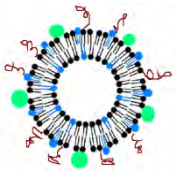
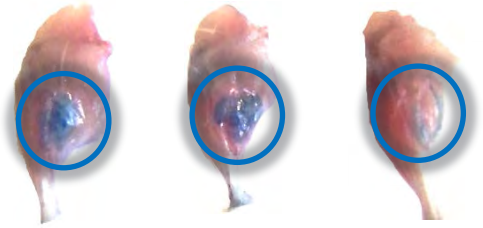
Small pegylated liposomes:

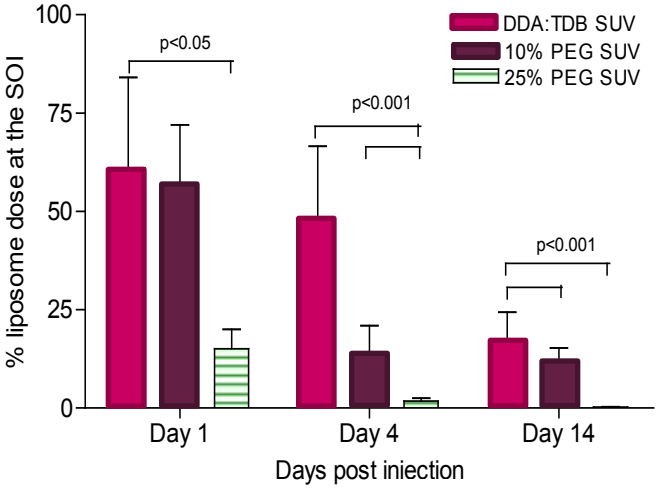


~120 nm

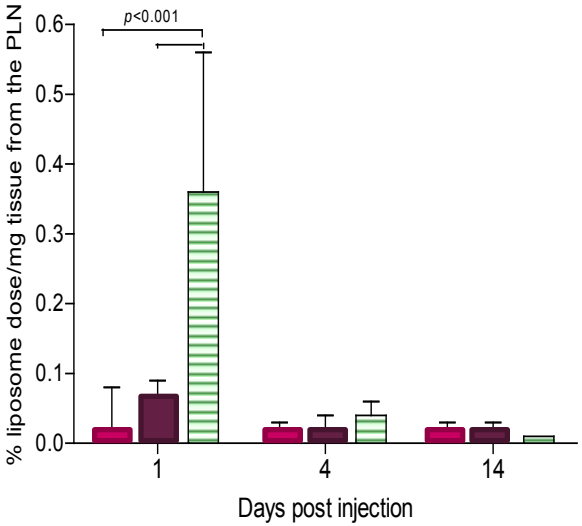
Injection site

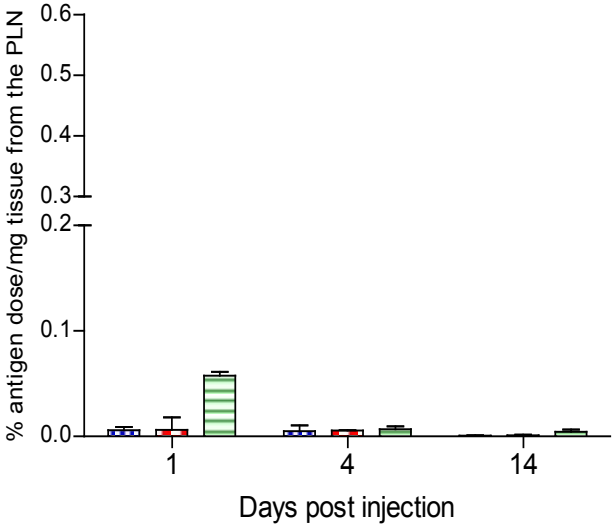


DDA:TDB | 10% PEG DDA:TDB | 25% PEG DDA:TDB



PLN





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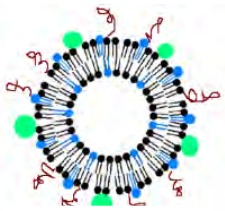
Kaur et al., 2012a and b, Journal of Controlled Release.

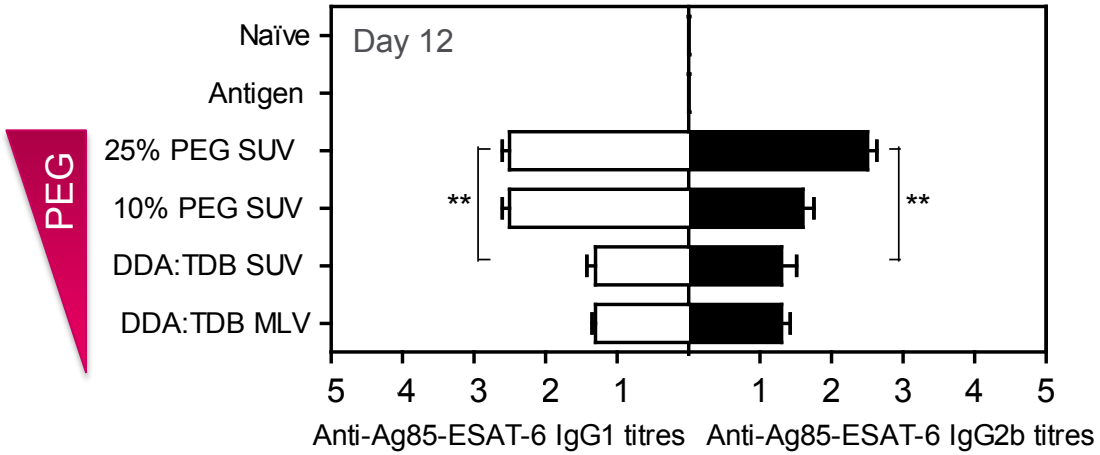
Pegylation promotes early immune responses



~120 nm

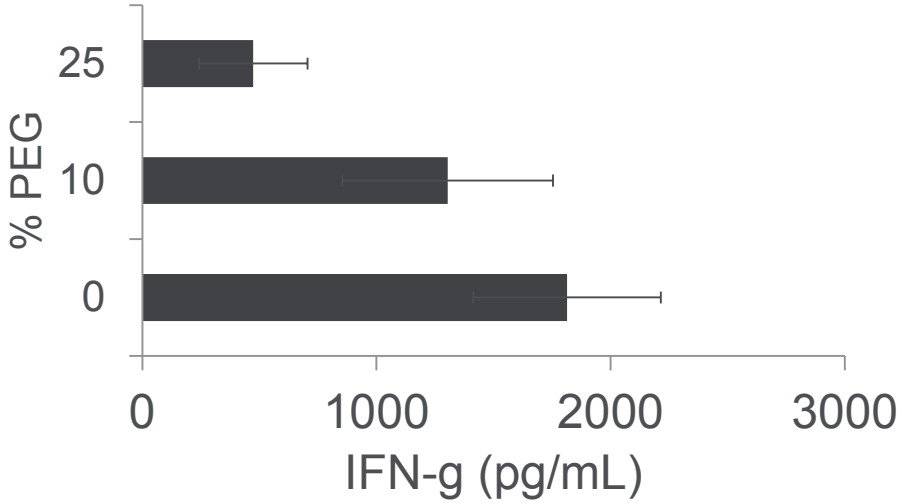
Early antibody response

PEG



IFN-g response

PEG



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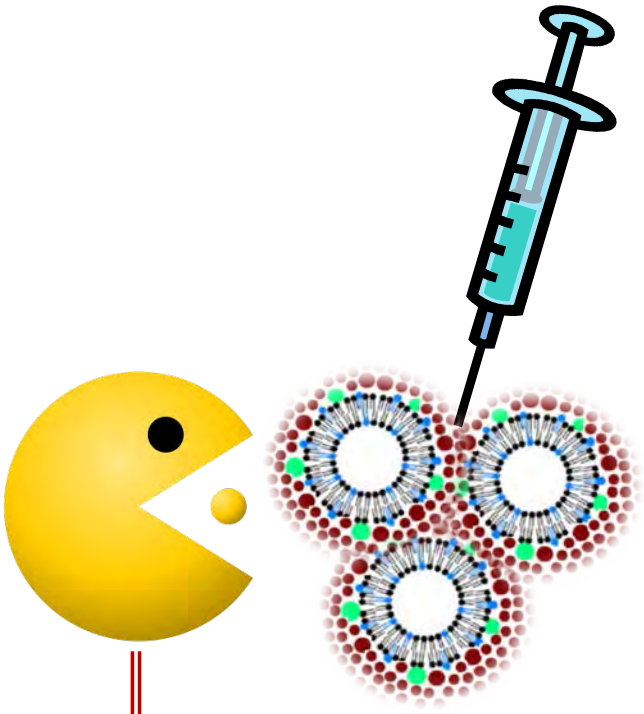
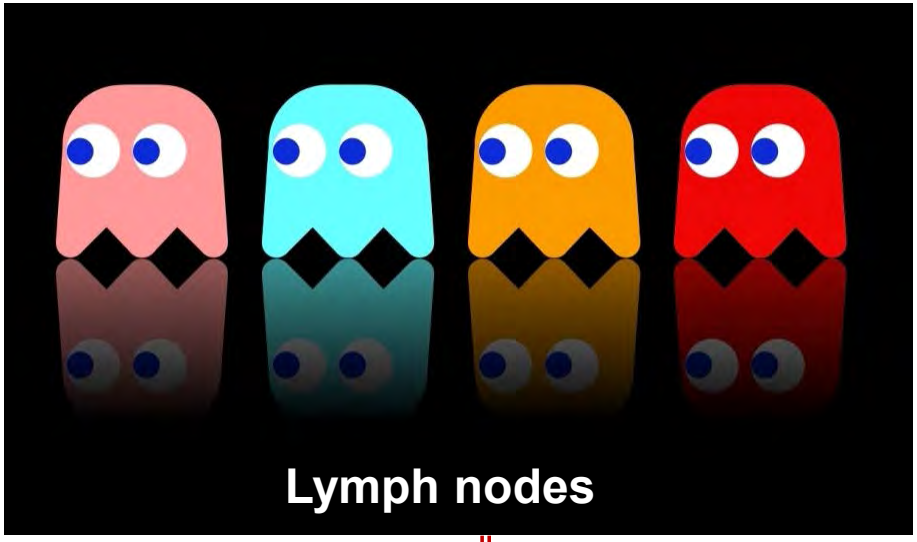
Kaur et al., 2012a and b, Journal of Controlled Release.

Summary – need co-delivery and possible controlled movement to lymphatics





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