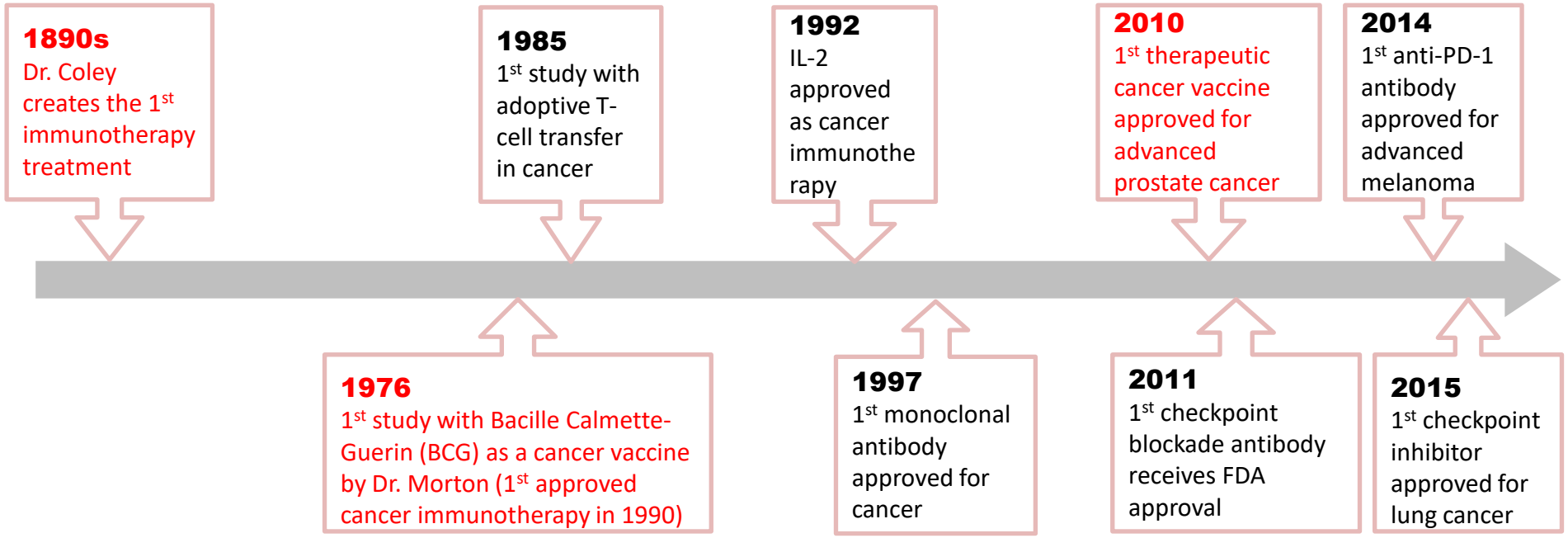
BIOENG-399 Immunoengineering

Prof. Li Tang

Lecture 10 and 11 Design of Immunogenic Vaccines (I and II)

Spring 2025

The History of Immunotherapy



Cancer vaccine: limited clinical success

New York Times July 29, 1908

ERYSIPELAS GERMS AS CURE FOR CANCER

Dr. Coley's Remedy of Mixed Toxins Makes One Disease Cast Out the Other.

MANY CASES CURED HERE

Physician Has
Years and T
Probably

Following news
two men have be
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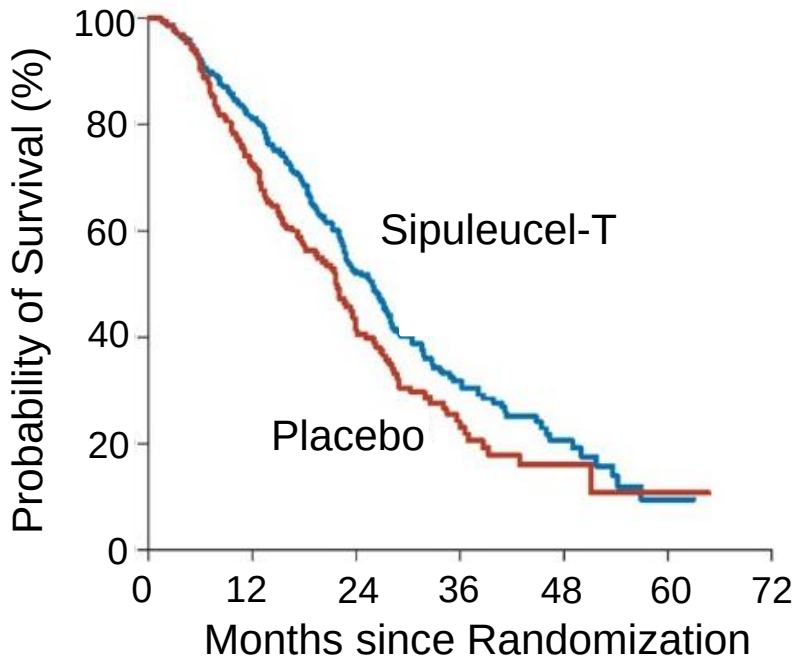
Sipuleucel-T to treat prostate cancer



Kantoff et al. , N Engl J Med. 2010 363(5):411-22.

- **>100 years**
- **1 therapeutic cancer vaccine**
- **4.1-month improvement in median survival**
- **>$ 100,000**

Major challenges in cancer vaccine development

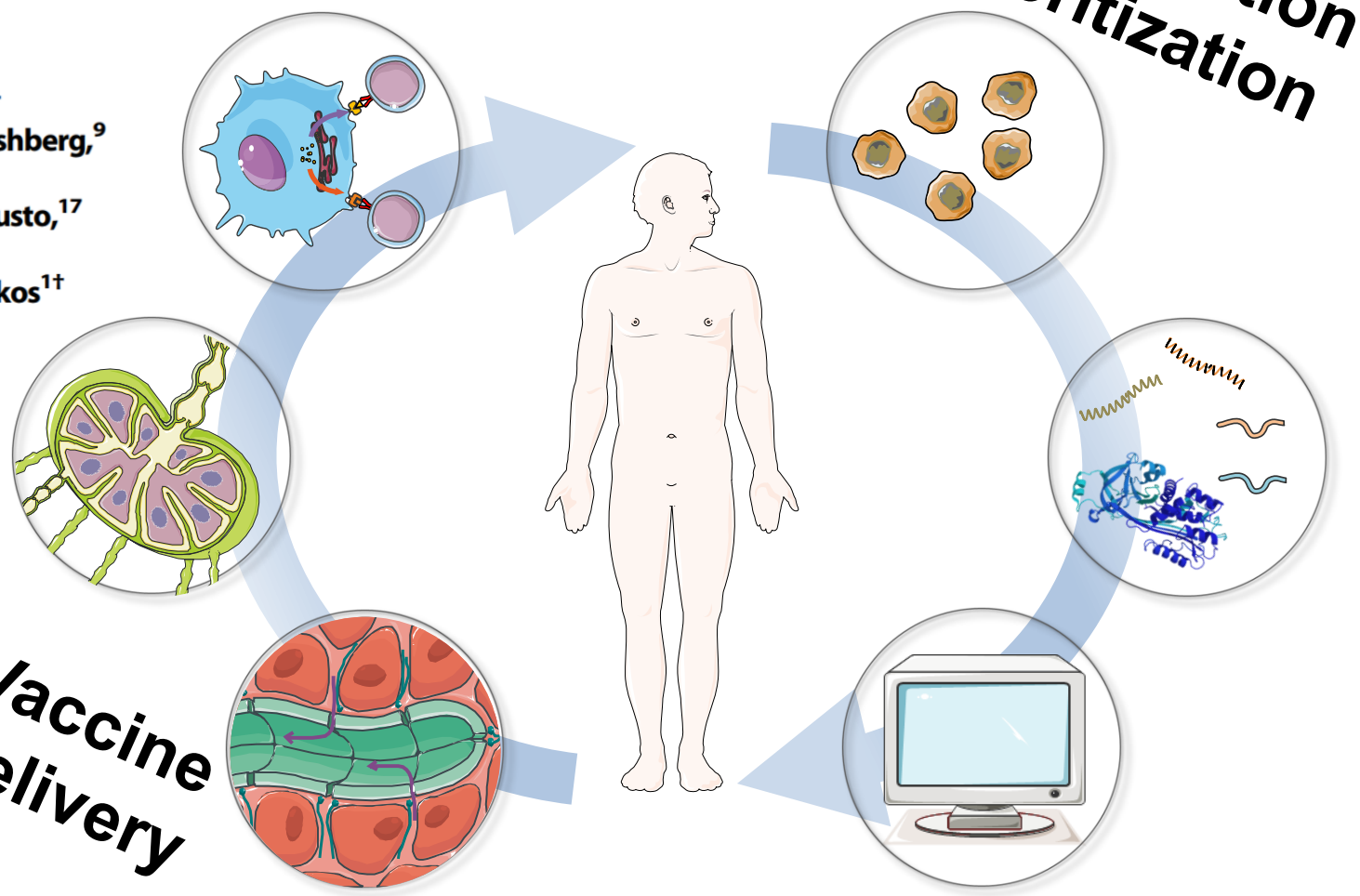
PERSPECTIVE

CANCER

The Human Vaccines Project: A roadmap for cancer vaccine development

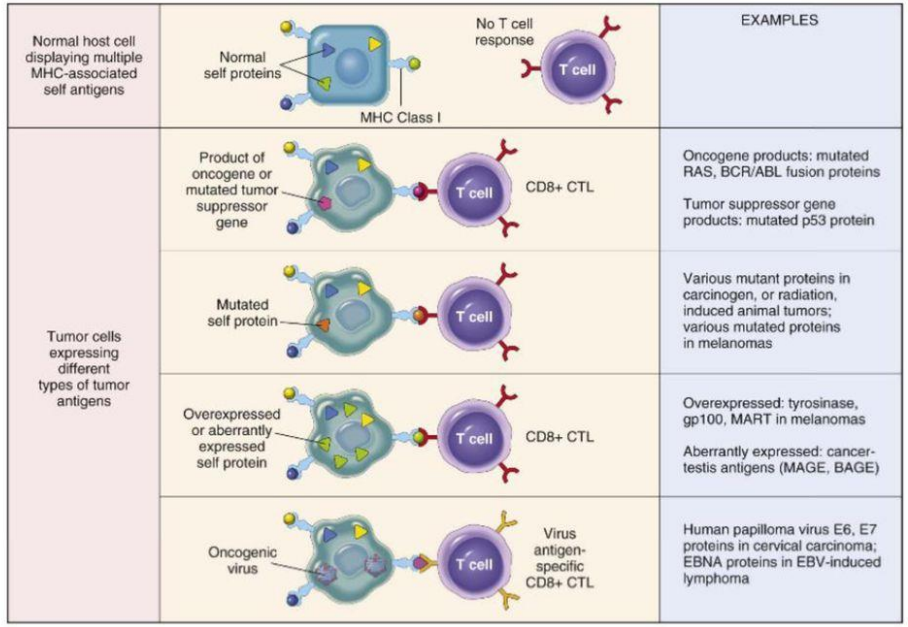
Pedro Romero,[1] Jacques Banchereau,[2] Nina Bhardwaj,[3] Mark Cockett,[4]* Mary L. Disis,[5] Glenn Dranoff,[6] Eli Gilboa,[7] Scott A. Hammond,[8] Robert Hershberg,[9] Alan J. Korman,[10] Pia Kvistborg,[11] Cornelis Melief,[12] Ira Mellman,[13] A. Karolina Palucka,[2,14] Irina Redchenko,[15] Harlan Robins,[16] Federica Sallusto,[17] Theodore Schenkelberg,[18] Stephen Schoenberger,[19] Jeffrey Sosman,[20] Özlem Türeci,[21] Benoît Van den Eynde,[22,23,24] Wayne Koff,[25] George Coukos[1†]

Remero et al ***Sci. Transl. Med.*** 8, 334ps339 (2016).

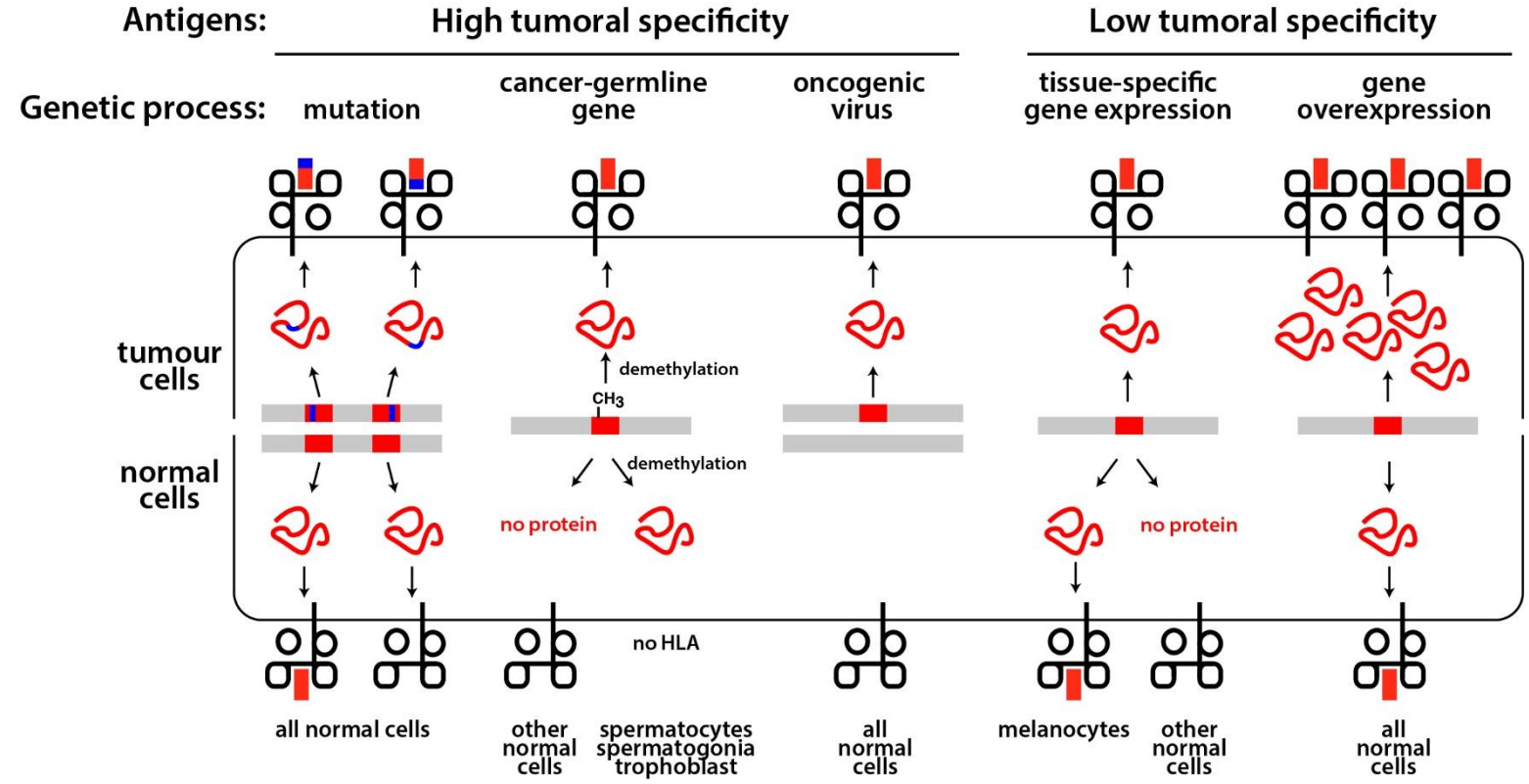


Guo et al ***Front Immunol*** (2018).

What antigens are T-cells responding to?



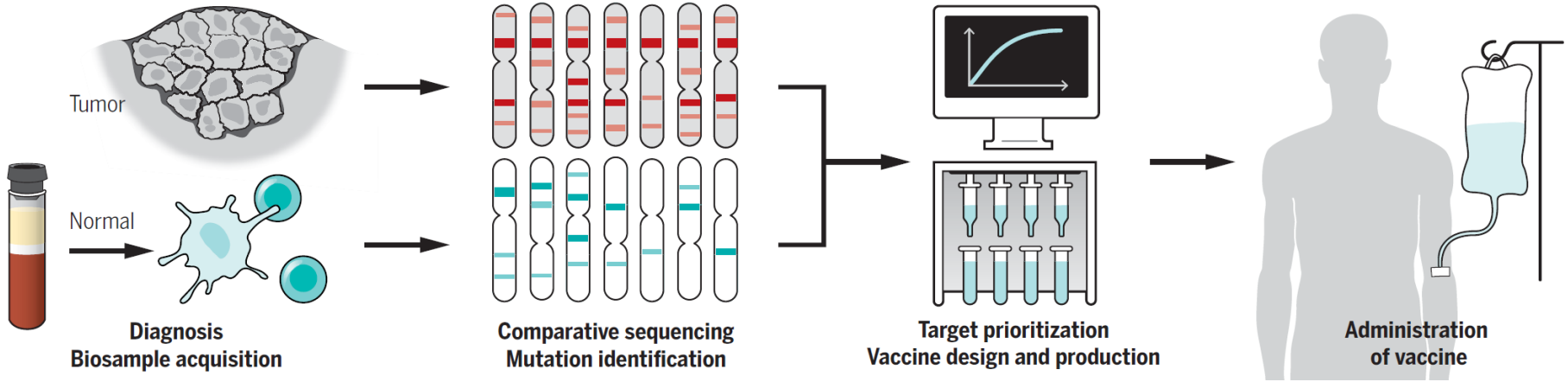
Tumor antigens recognized by CD8+ T cells.



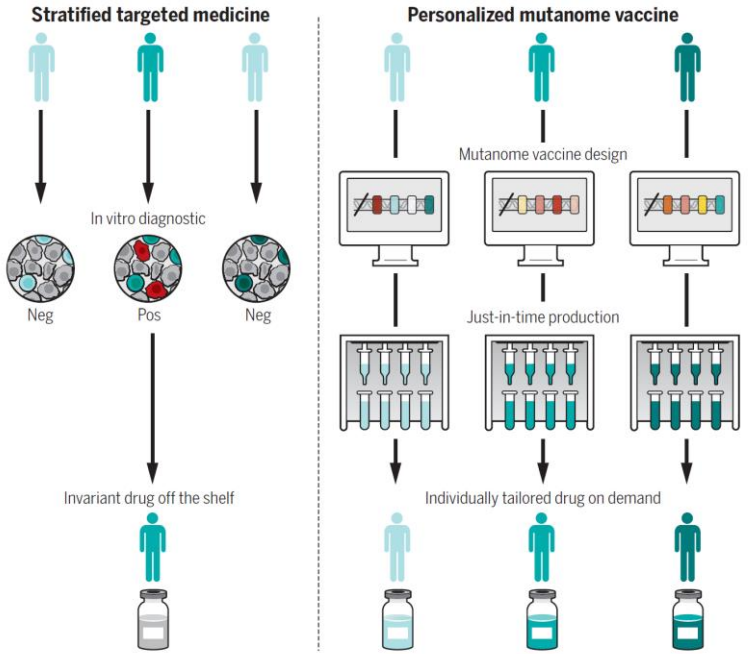
- Neo-antigens
- Cancer-testis antigens (CT Ags, or oncofetal Ags, such as MAGE, NY-ESO-1; expressed only in germ cells but not somatic (tissue) cells
- Viral antigens

http://slideplayer.com/slide/11983008/ https://www.slideshare.net/csbrprasad/neoplasia-robbins-path-13540987

Neoantigens – ideal cancer vaccine targets?



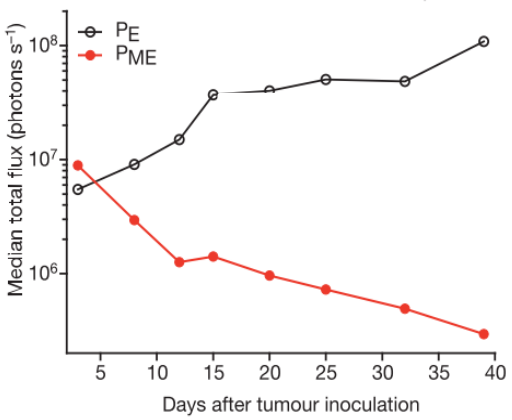
Technological advances in genomics, data science, and cancer immunotherapy now enable the rapid **mapping of the mutations** within a genome, rational **selection of vaccine targets**, and on-demand **production** of a therapy customized to a patient's individual tumor

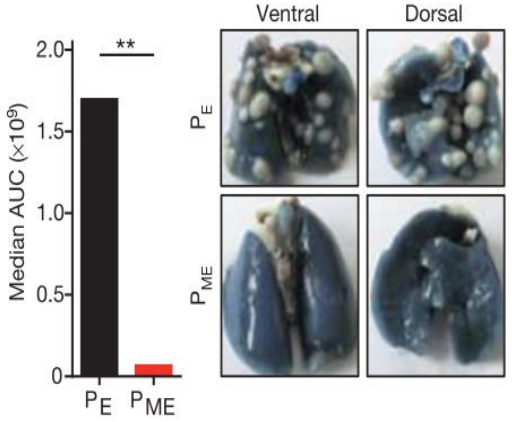


Sahin, U.; Tureci, O., Personalized vaccines for cancer immunotherapy. *Science* **2018,** *359*, 1355-1360.

Neoantigen cancer vaccines show the feasibility, safety, and immunotherapeutic activity

Mouse colon cancer





Sebastian Kreiter et al. ***Nature. 2015***

Human clinical trials

Personalized neoantigen peptide vaccine clinical trials (with ClinicalTrail.gov trial identifications).

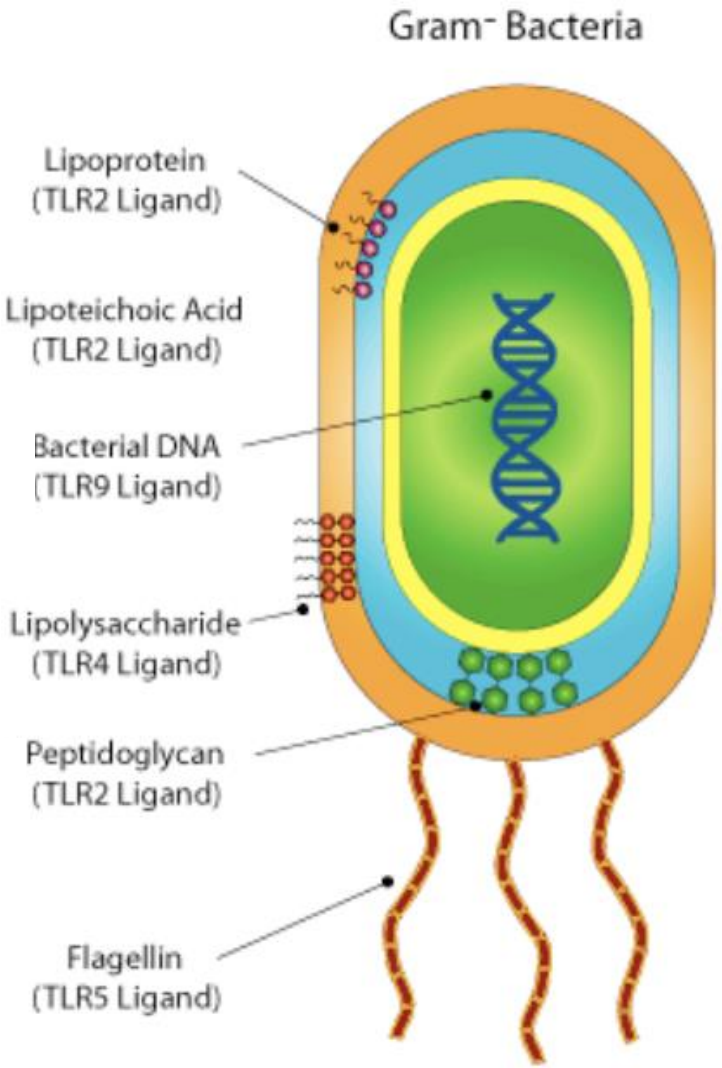
Institution	Trial ID	Details
DFCI	NCT01970358	**NeoVax:** Phase I trial for melanoma using SLP* neoantigens with poly IC:LC adjuvant[15,22,29,33,35,60]
DFCI	NCT02287428	**NeoVax:** Phase I trial for MGMT-unmethylated glioblastoma and glioblastoma multiforme using SLP neoantigens with poly IC:LC adjuvant[15,22]
Neon Therapeutics	NCT02897765	**NEOPV-01:** Phase I clinical trial for melanoma, non-small cell lung, and bladder cancers using personalized peptide vaccine with nivolumab[10,33]
Agenus	NCT02992977	**AutoSynVax:** Phase I trial for advanced cancer using synthetic peptides complexed to heat shock proteins with QS-21 Stimulon adjuvant[33,63]
WUSM**	NCT02427581	Phase I trial for triple negative breast cancer using SLP neoantigens with poly IC:LC adjuvant[22]
WUSM	NCT02510950	Phase 0 trial for glioblastoma multiforme astrocytoma using SLP neoantigens with poly IC:LC adjuvant[22]
MD Anderson Cancer Center	NCT02600949	Phase I trial for pancreatic and colorectal cancers using peptide vaccine plus IFA[22]

* SLP: synthetic long peptides.
** WUSM: Washington University School of Medicine.

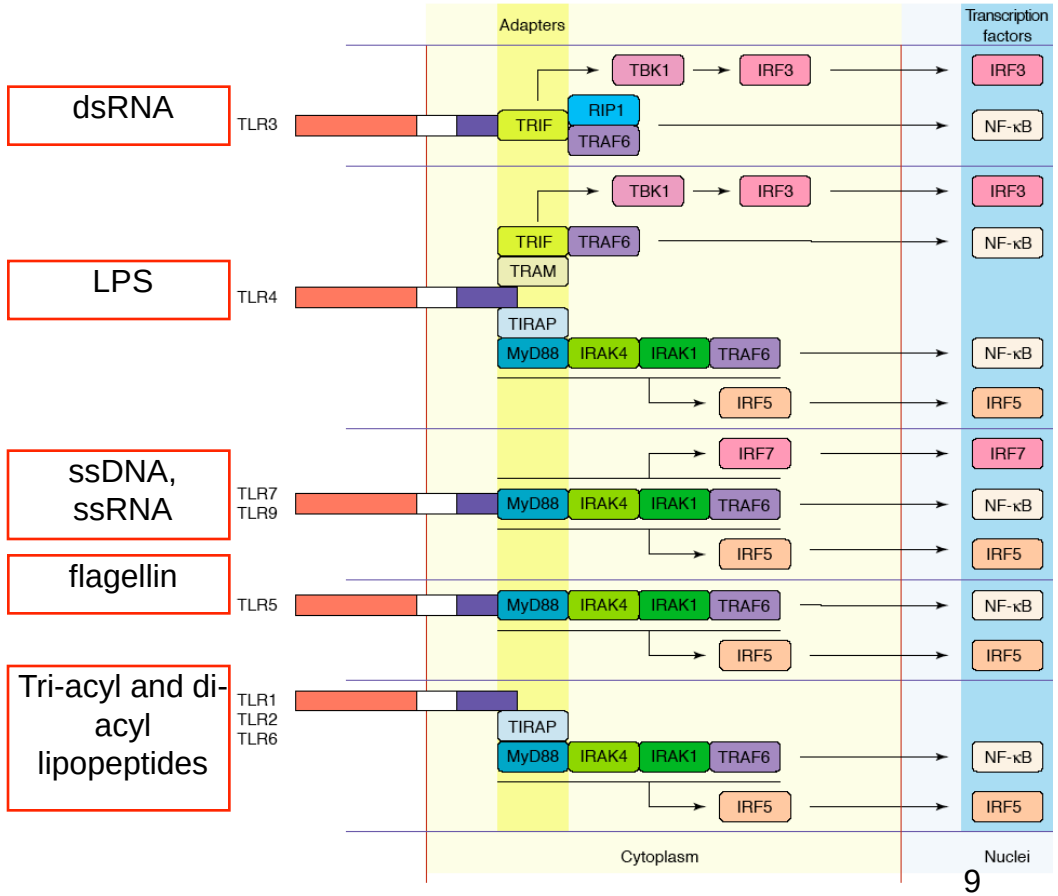
Amanda R. Aldous et al. Bioorganic & Medicinal Chemistry. ***2017***

What makes a vaccine?

Microbes co-deliver a package of antigen and multiple danger signals to dendritic cells

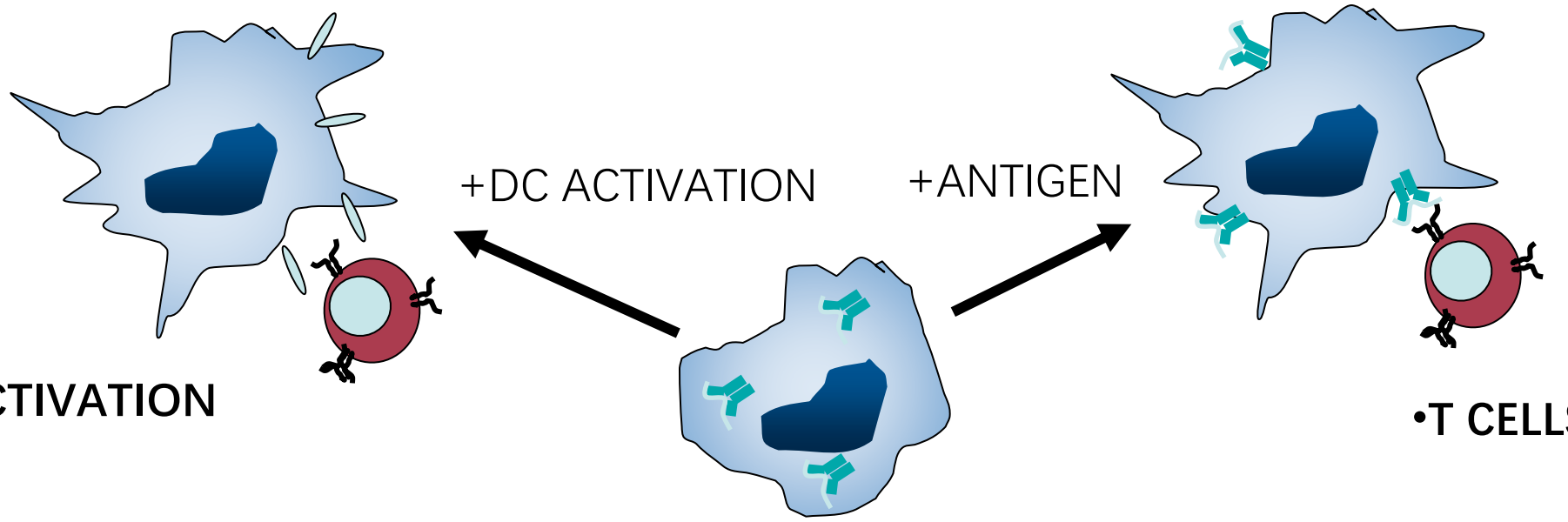


www.invivogen.com



Kawai and Akira, *Curr. Opin. Immunol*. **17** 338-344 (2005)

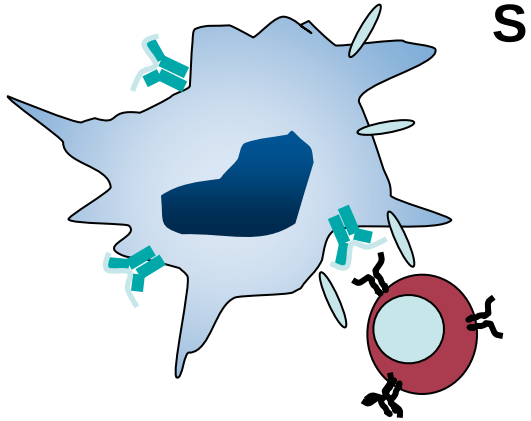
Antigen is one of (at least) two signals that must be delivered by a vaccine



•NO T CELL ACTIVATION

•T CELLS TOLERIZED

+ANTIGEN
+DC ACTIVATION



Signal 1 - antigen
Signal 2 – costimulation
Signal 3 - cytokines

•MAXIMAL T CELL PROLIFERATION
•GENERATION OF FULL EFFECTOR FUNCTIONS
•GENERATION OF MEMORY T CELLS

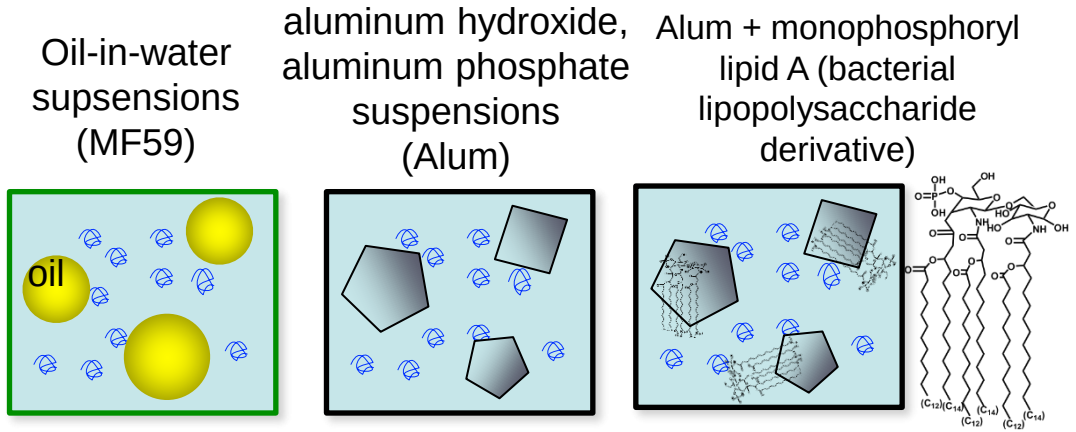
Rudimentary components of vaccines

Antigen:
any biological molecule from a microbe that is recognized by a T cell receptor (TCR) or B cell receptor (BCR)

Adjuvant:
molecules act specifically on DCs, and activate DCs

Traditional adjuvants lack characteristics suitable for potent cancer vaccines

Only 3 main adjuvants have been licensed for human use so far:



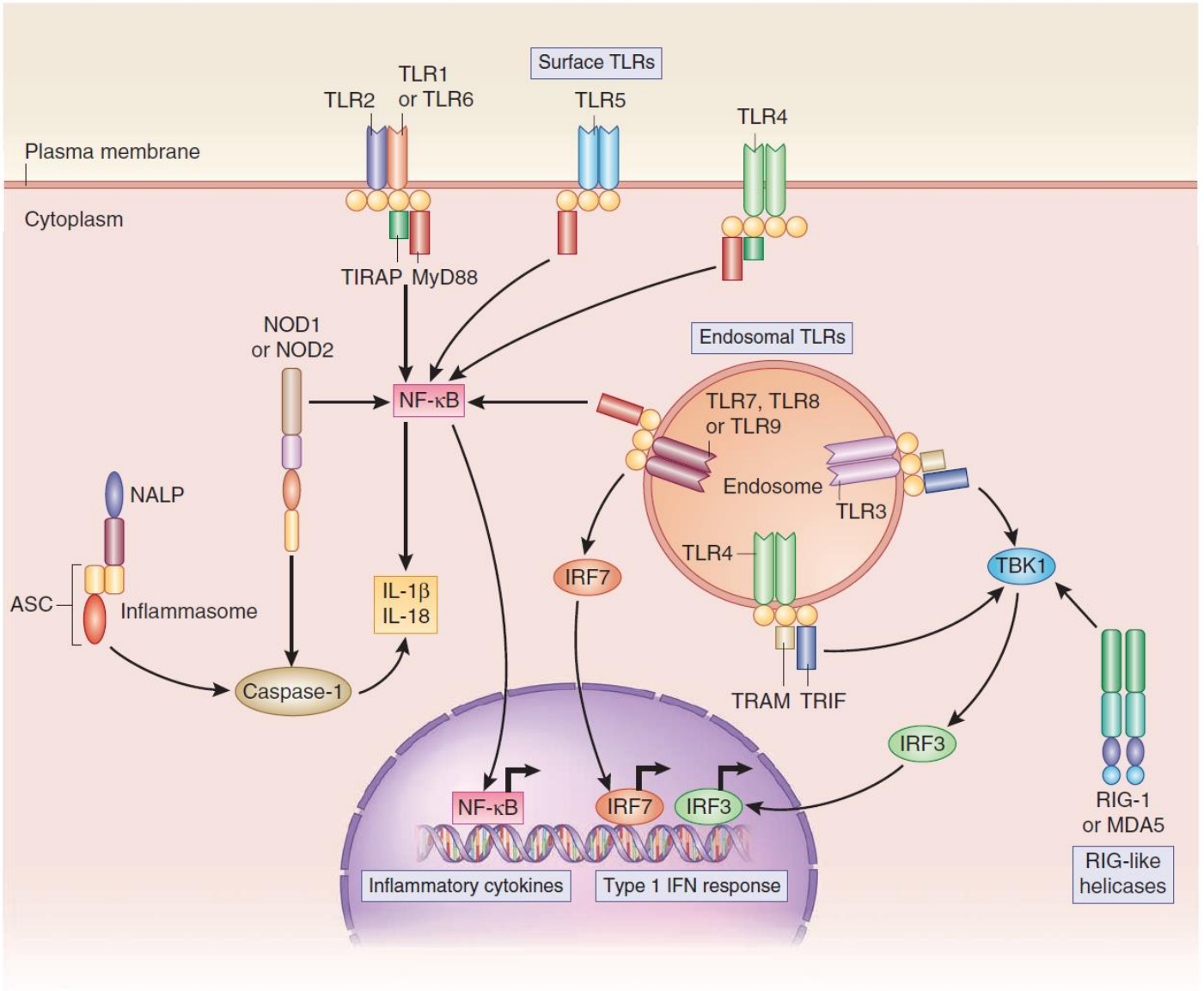
- **Poorly immunogenic on their own**
- **very safe**
- **readily manufactured**

Existing adjuvants:

- increase humoral response, but weak cellular responses and little or no CD8+ T-cell response
- Th2 bias to immune response - not the most effective for intracellular pathogens or cancer
- poor durability of immune response

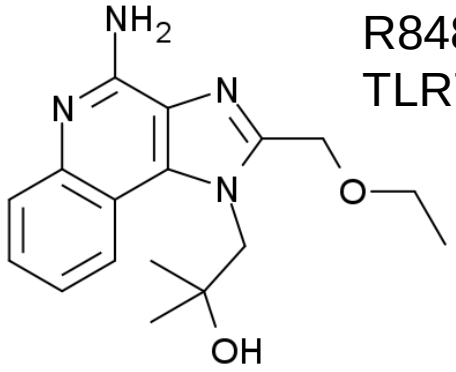
Key roles of adjuvants in modern vaccines

Year	Vaccine	Adjuvant and mechanism	Scientific findings
1885	Rabies	ssRNA TLRs 7 and 8	
1886			Briegen describes endotoxin
1889			Coley shows tumor necrosis with bacterial extracts
1911	Typhoid	LPS, DNA TLRs 1, 2, 4, 5, 6 and 9	
1916		Lipovaccine	More durable immune response to typhoid vaccine
1921	BCG for TB	DNA, lipoprotein TLRs 1, 2, 6 and 9	
1926		Aluminum salts	Enhanced antibody responses to diphtheria vaccine
1937		Incomplete Freund's adjuvant (IFA) (water-in-oil emulsion)	Enhanced cellular and antibody responses to TB
1942	Diphtheria, pertussis and tetanus	LPS, DNA TLRs 1, 2, 4, 5, 6 and 9	
1949	Whole-cell influenza	ssRNA TLRs 7 and 8	
1955	Inactivated polio vaccine	ssRNA TLRs 7 and 8	
1966			LPS structure determined
1979			Ribi makes detoxified endotoxin MPL
1991	Hepatitis A		MPL tested in clinic
1996			TLRs discovered
1997	Fluad	MF59 (oil-in-water emulsion)	
1997	Epaxal (for hepatitis A) Inflexal (for influenza)	Virosome	
1998			LPS shown to be TLR ligand
2004	Invivac (for influenza; Europe)	Virosome	
2005	Fendrix (for hepatitis B; Europe)	MPL Defined TLR4	
2007–2009	Pandemic influenza vaccines (Europe)	MF59, AS03 (oil-in-water emulsion)	
2009	Cervarix (for HPV16 and HPV18; USA)	MPL Defined TLR4	

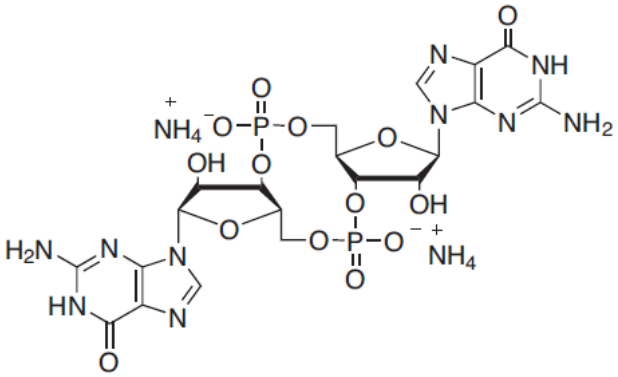


Nat. Med. **2013,** *19*, 1597-608.

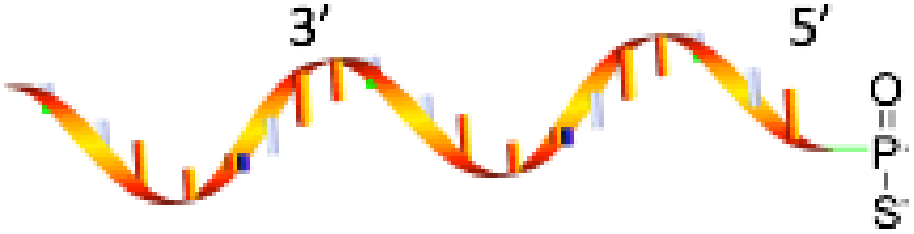
Some examples of TLR ligands—what are the problems then?



R848 (resiquimod)
TLR7 agonist



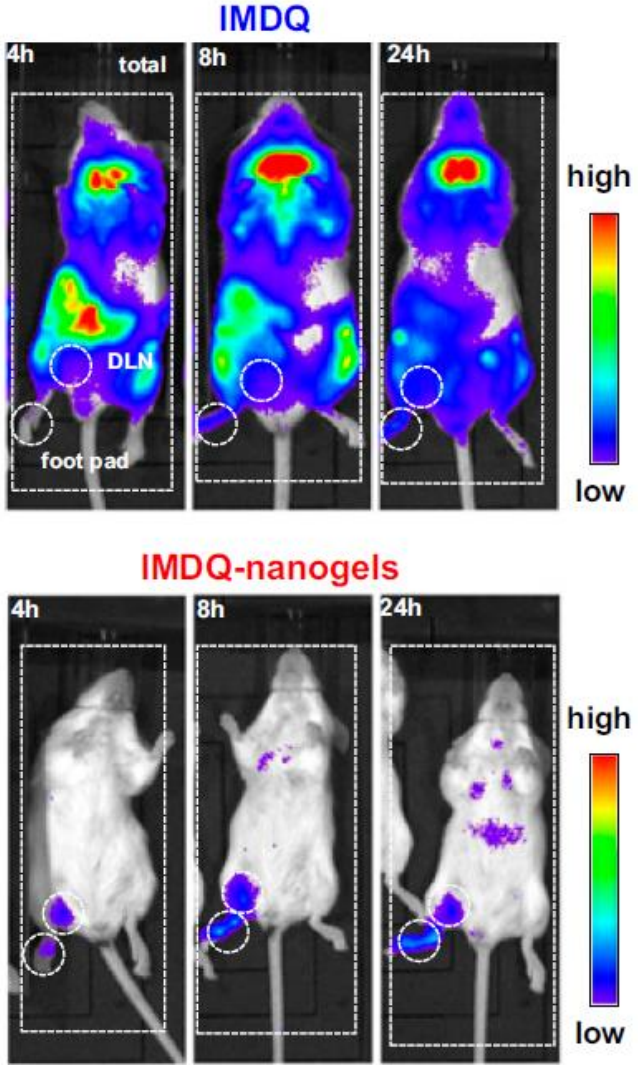
Cyclic di-GMP – ligand for STING



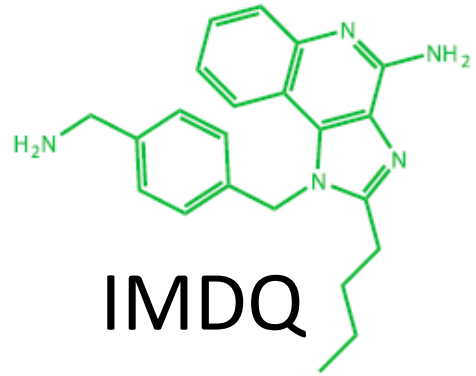
CpG DNA (20 base ssDNA - TLR9 agonist)

Systemic inflammatory toxicity of adjuvants

Footpad injection



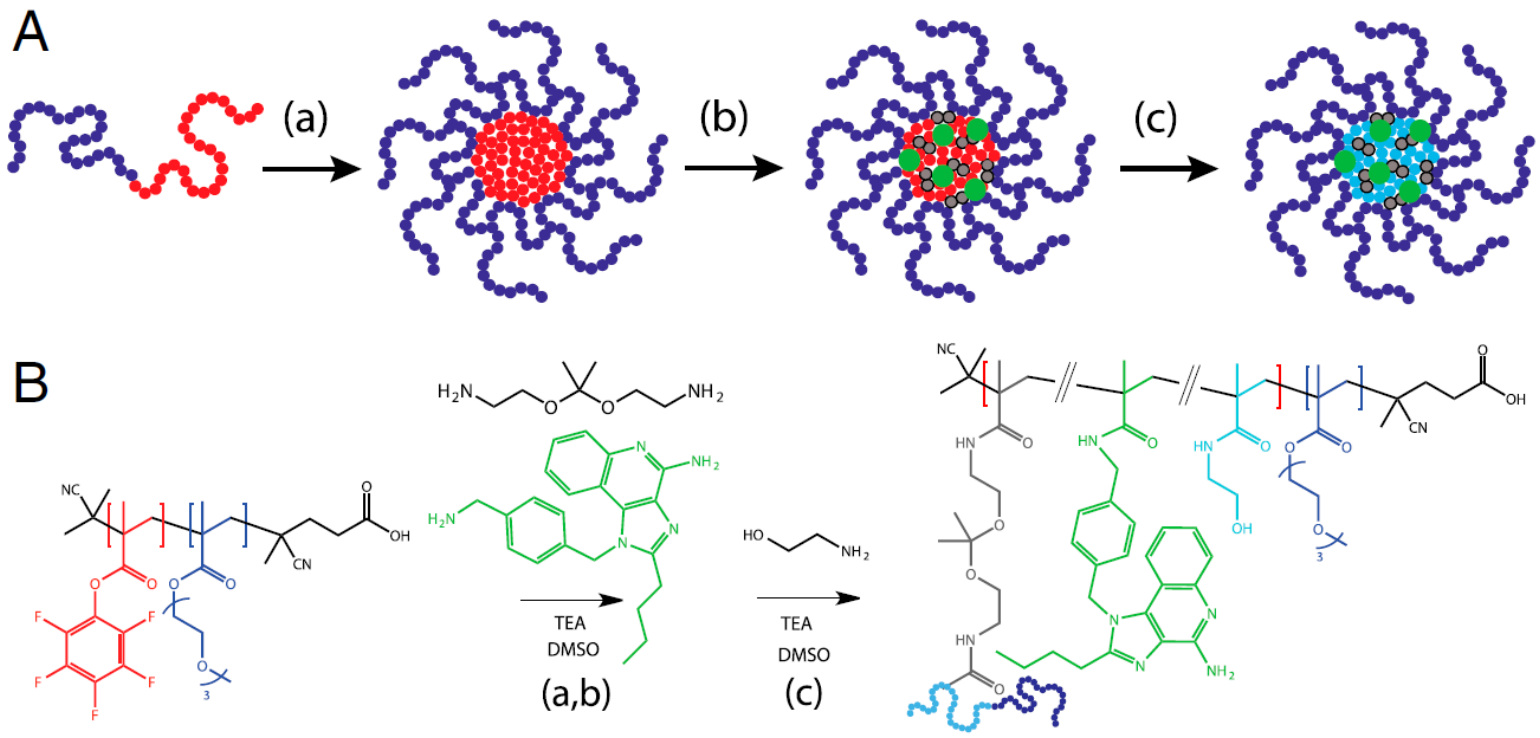
TLR7/8 agonist
1-(4-(aminomethyl)benzyl)-2-butyl-1H-imidazo[4,5-c]quinolin-4-amine (IMDQ)



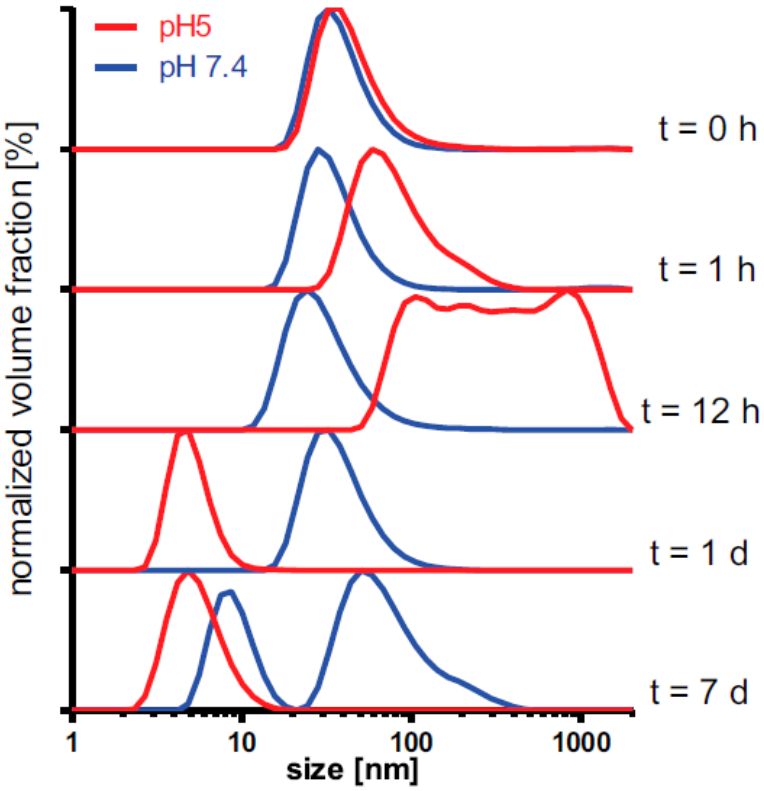
IFN-β reporter mice in which a firefly luciferase encoding sequence had been placed under the control of the IFN-β promoter

TLR7/8 agonists are indeed very potent inducers of type I IFN (including IFN-β), which are required for the adjuvant properties of TLR7/8 agonists but are **also a cause of severe inflammatory toxicity when induced systemically**

Proc. Natl. Acad. Sci. **2016,** *113*, 8098-103.



nanogels
with similar size around 50 nm
nanogels readily swell in response to acidic medium and fully degrade



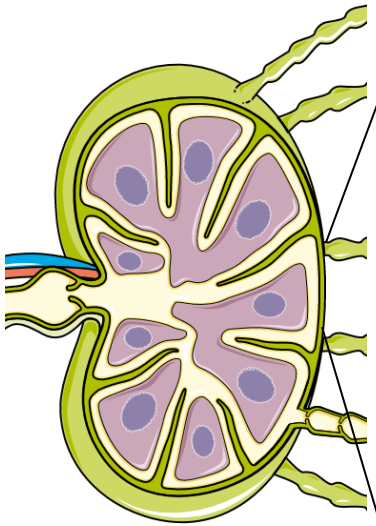
(a) Blockcopolymers self-assemble in DMSO into nanoparticles
(b) Covalent ligation of TLR7/8 agonist (green) and cross-linking
(c) Conversion of residual pentafluorophenyl ester with 2-ethanolamine yielding fully hydrated nanogels after transfer to the aqueous phase.

Proc. Natl. Acad. Sci. **2016,** *113*, 8098-103.

To achieve an effective vaccine

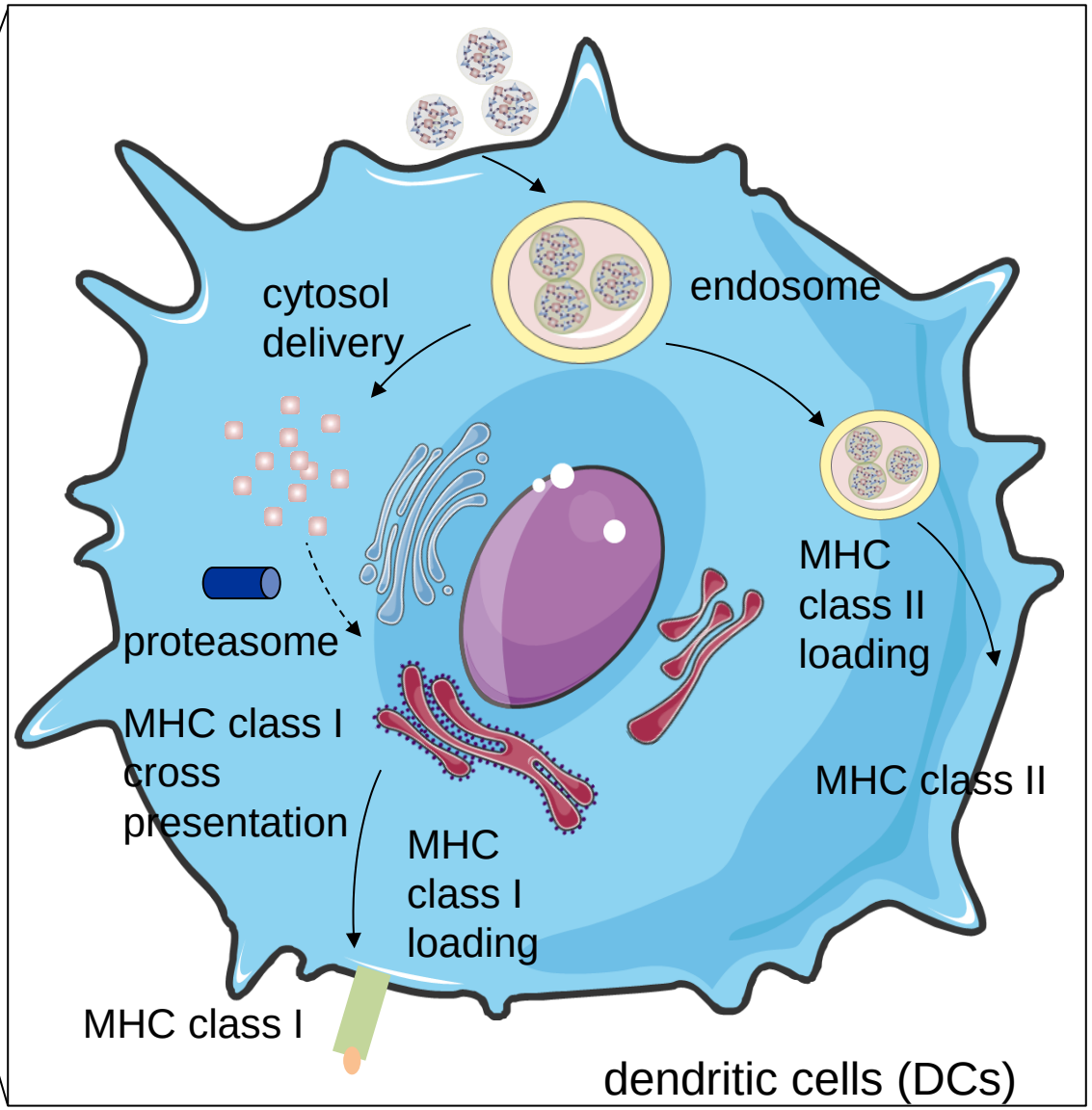
vaccines

1. LN/APC targeting



lymph node (LN)

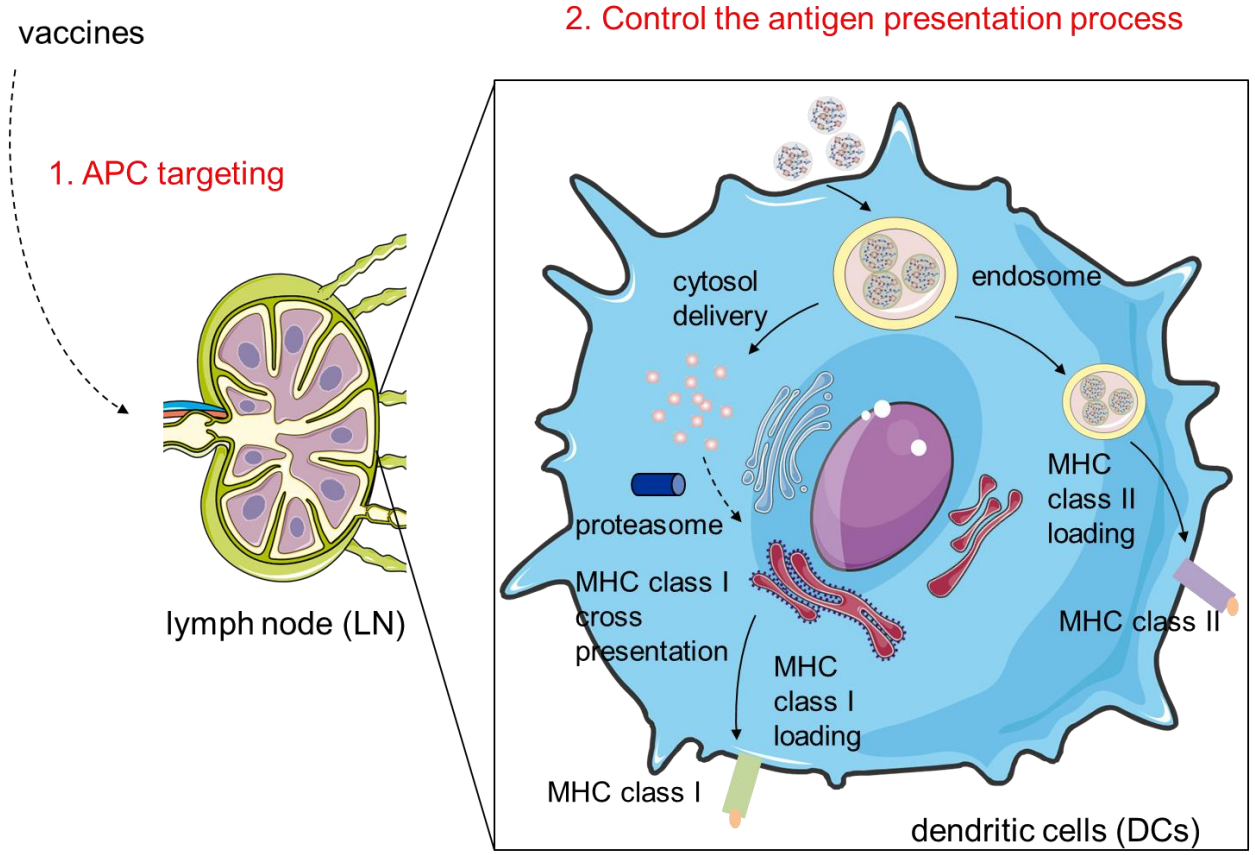
2. Control the antigen presentation process



To achieve an effective vaccine with engineered biomaterials

Using Biomaterials to control the trafficking of vaccines for APC targeting

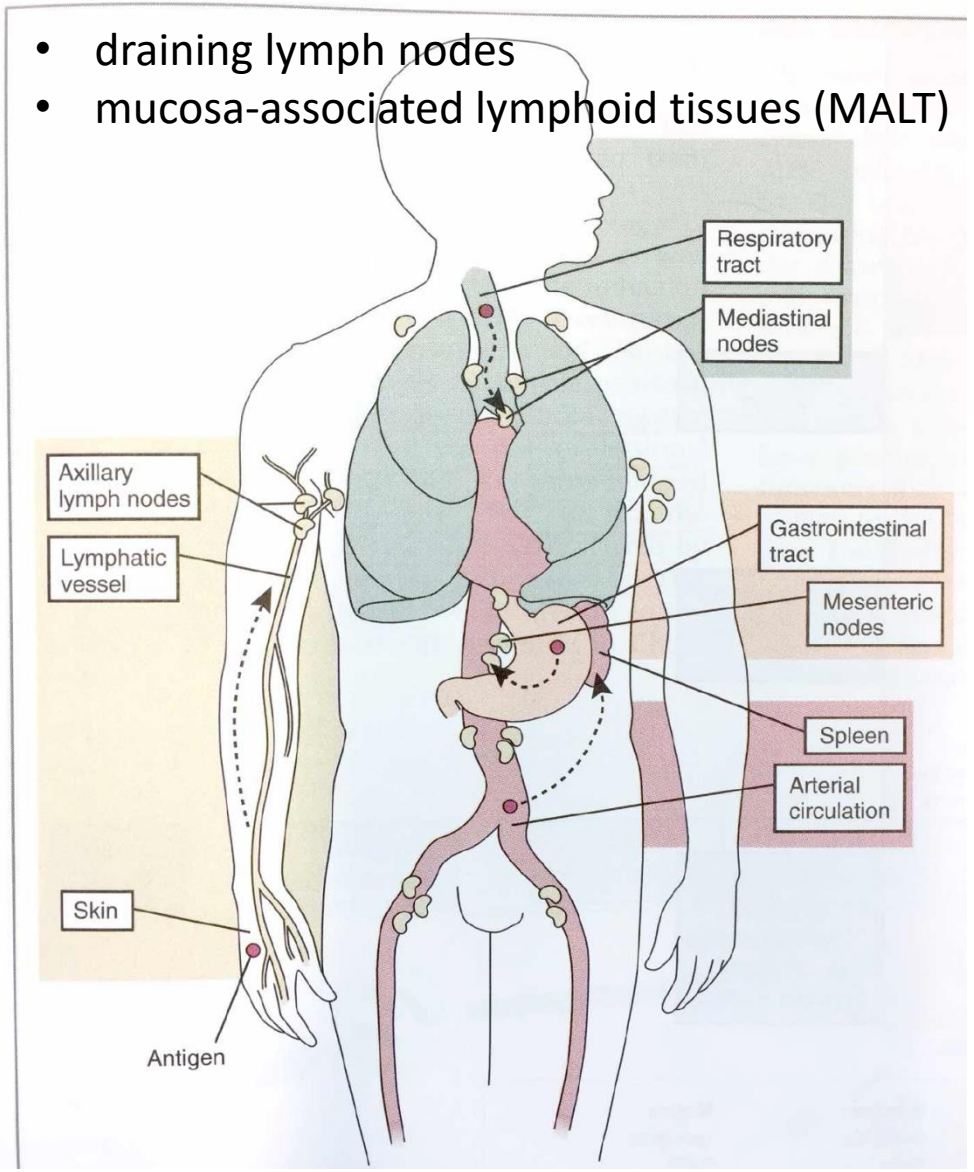
Using Biomaterials to control intracellular delivery and antigen presentation



Overcoming tissue barriers for vaccines with biomaterial engineering

1. Cutaneous immunization: Target Vaccines to Lymph Nodes

2. Mucosal immunization



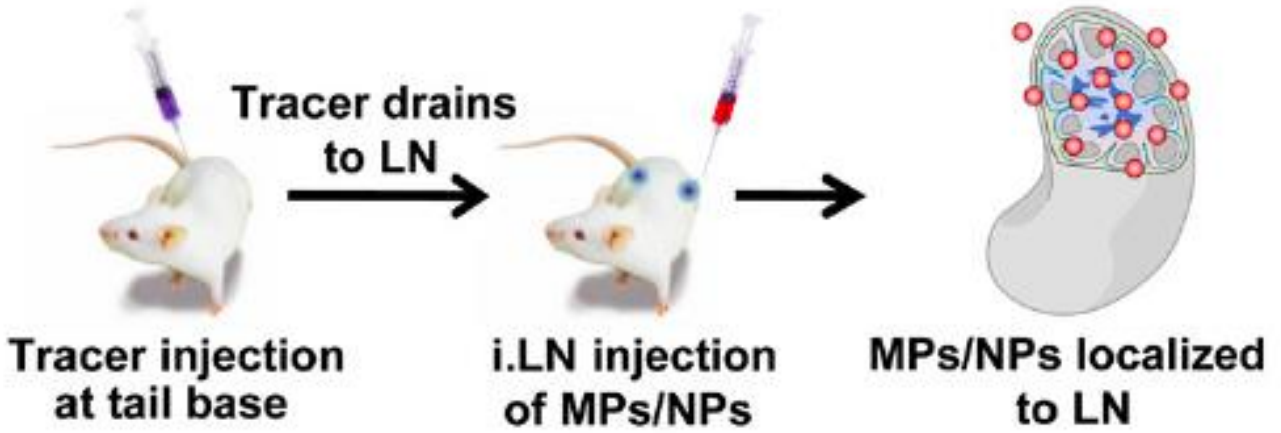
Chem. Rev. **115**, 11109-11146 (2015)

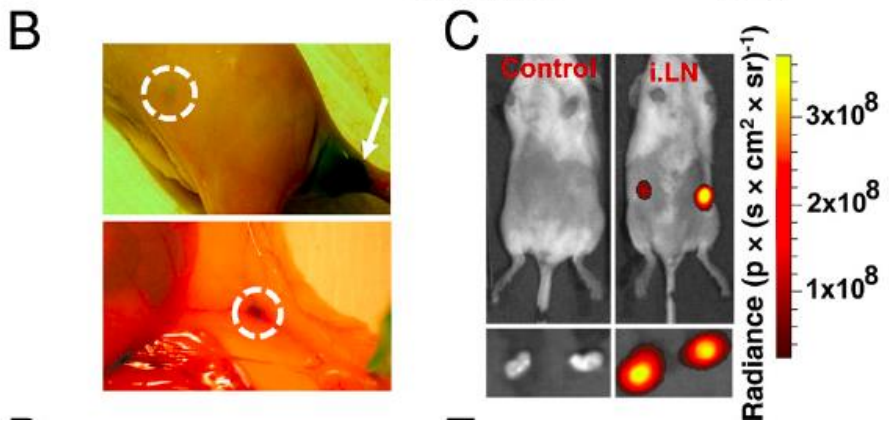
Abbas, A. K.,et al. *Cellular and Molecular Immunology*

Intranodal administration of particles

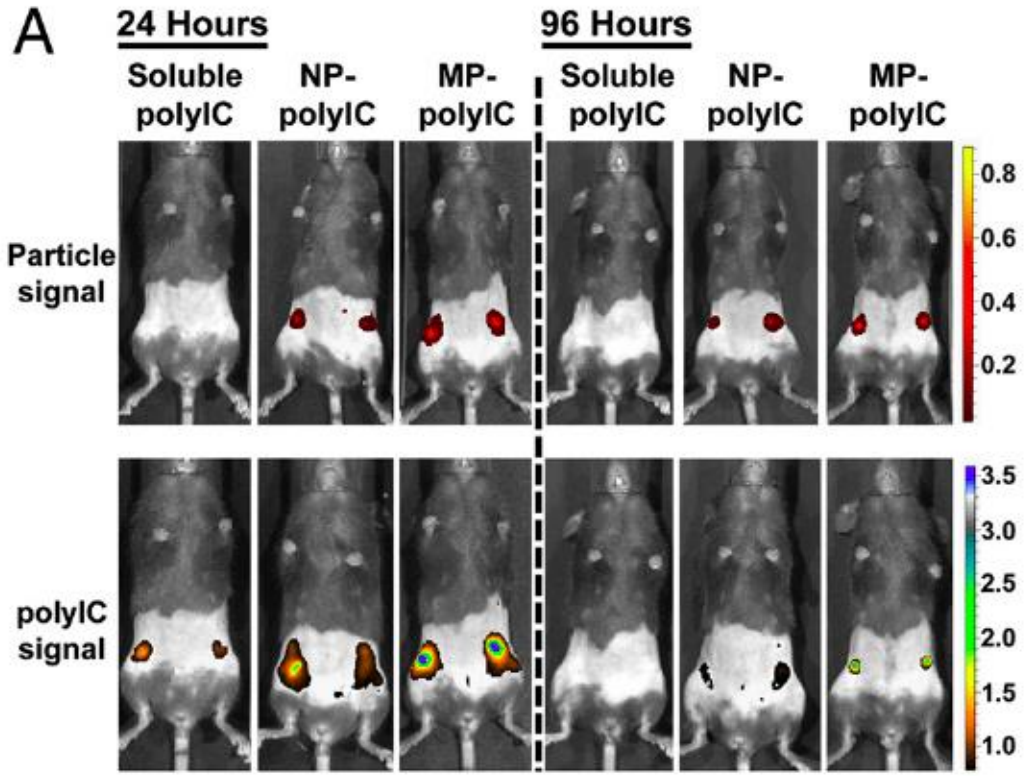
Ultrasound-guided intranodal administration of immunotherapies has been used in multiple phase 1 clinical trials
e.g., Melan-A/MART-1 DNA Plasmid Vaccine in Patients with Stage IV Melanoma

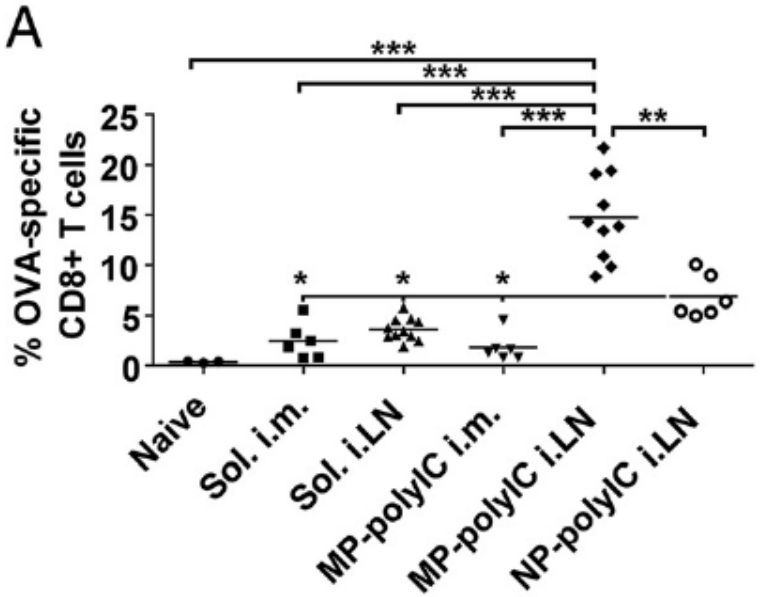
J. Immunother. 31, 215–223

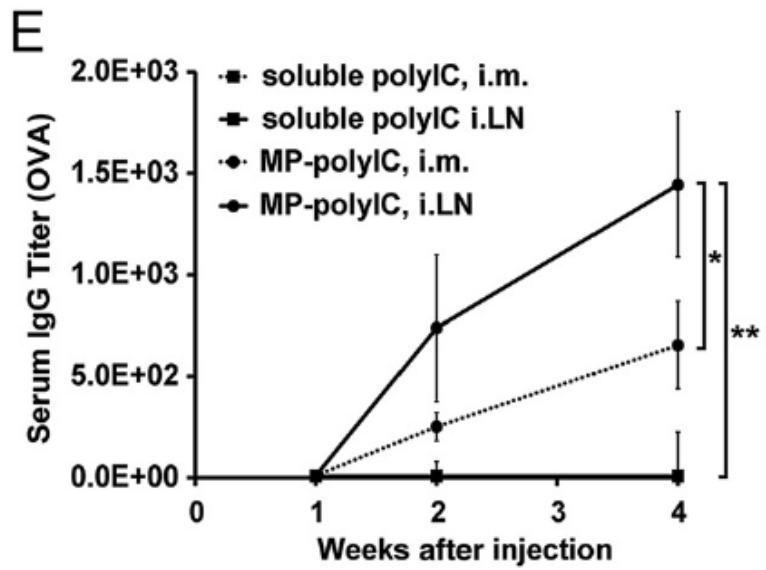




Proc. Natl. Acad. Sci. **108**, 15745-15750 (2011)







1. intranodal injection of the Toll-like receptor-3 (**TLR-3) agonists** poly(inosinic:cytidylic acid) (polyIC) in **micro-particulate (MP)** form substantially prolonged persistence of polyIC in lymph nodes when compared to soluble polyIC
2. intranodal injection of vaccines in MP induced much stronger humoral and cellular immune responses than intranodal injection of soluble vaccines

Proc. Natl. Acad. Sci. **108**, 15745-15750 (2011)

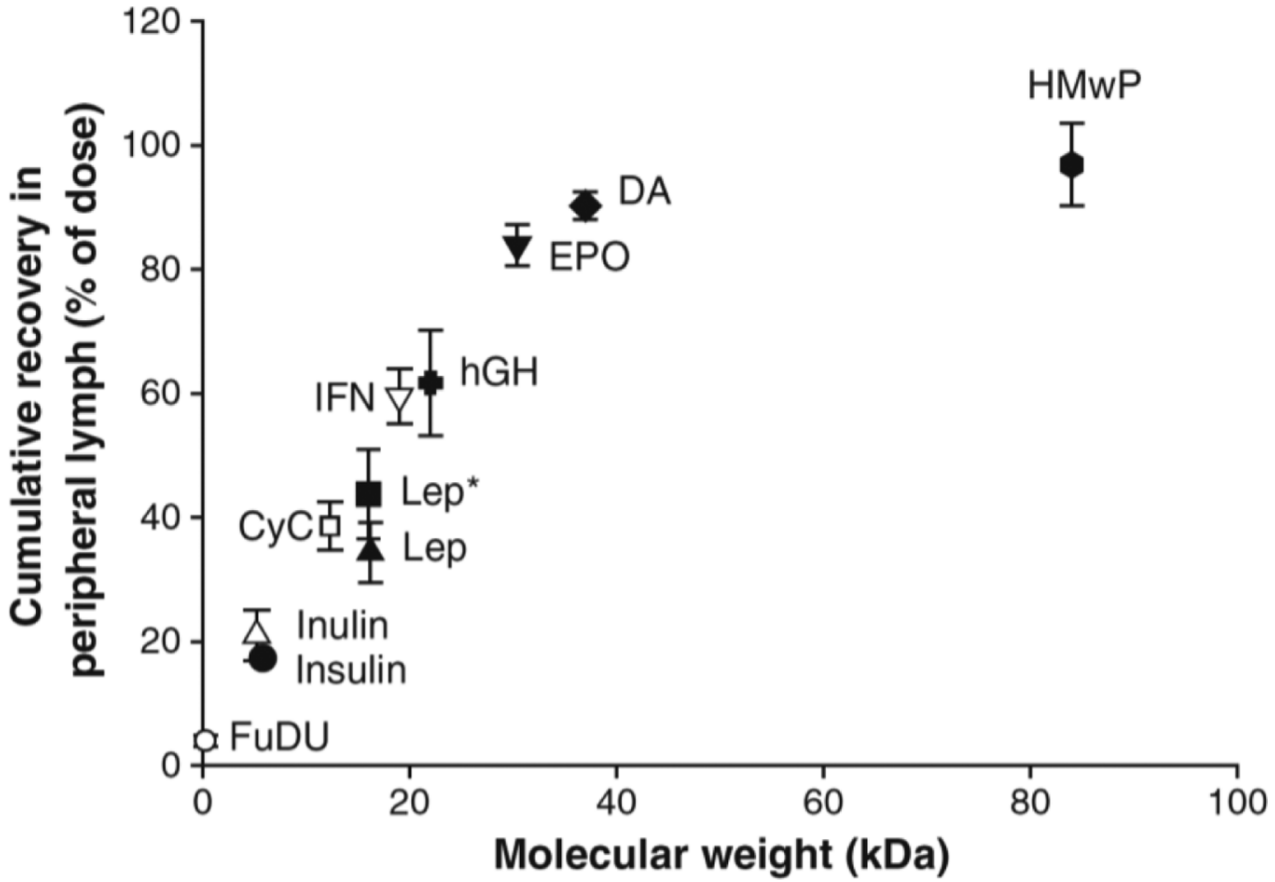
The antibody titer is a test that detects the presence and measures the amount of antibodies within a person's blood.

An **antibody titer** is a measurement of how much antibody an organism has produced that recognizes a particular epitope, expressed as the inverse of the greatest dilution (in a serial dilution) that still gives a positive result. ELISA is a common means of determining antibody titers.

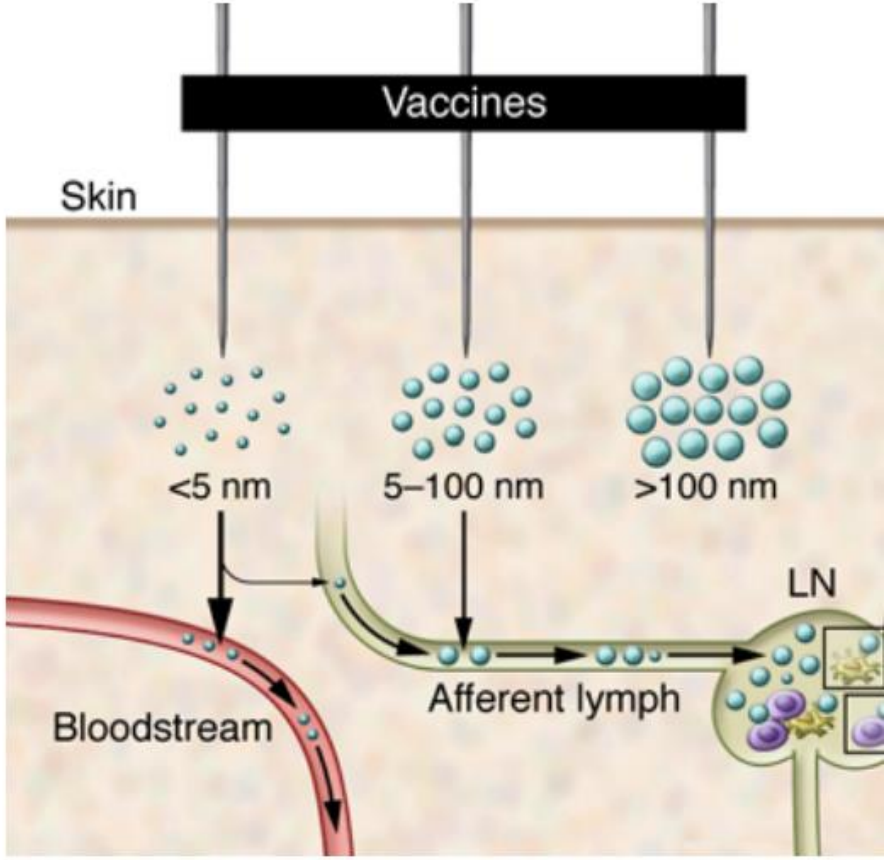
https://en.wikipedia.org/wiki/Antibody_titer

Size-Based LN Targeting

In general, materials larger than approximately 9 nm in diameter preferentially drain to lymphatics, whereas molecules/particles smaller than ~6 nm drain to the blood.

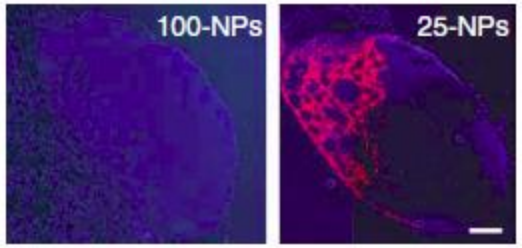


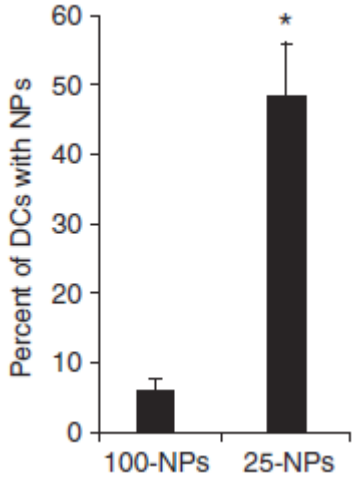
Adv. Drug Delivery Rev. 2011, 63, 890–900

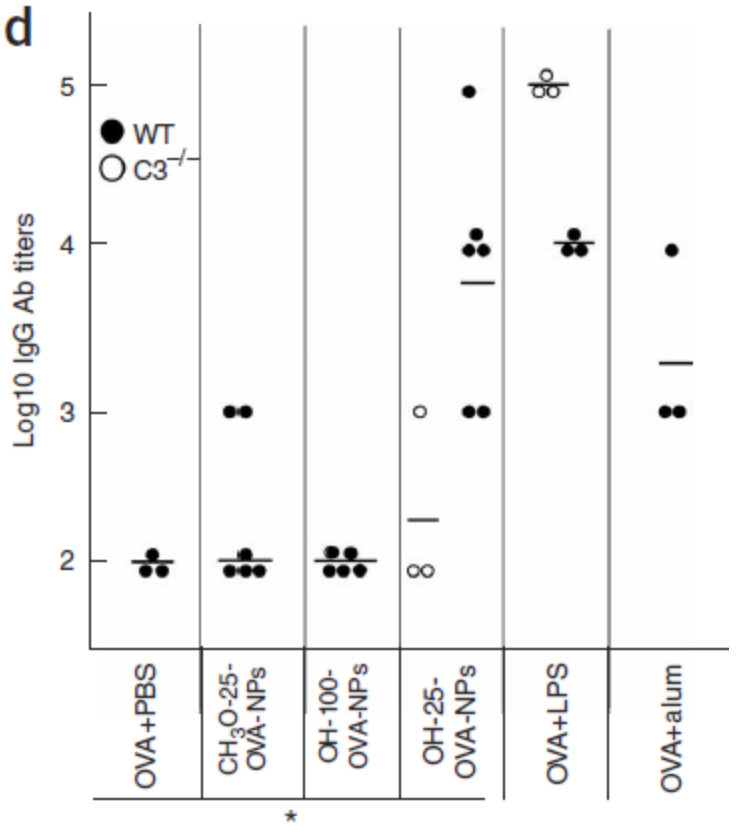


Moyer et al. *J. Clin. Invest.* **2016**
Melief et al. *J. Clin. Invest.* **2015**

Size-Based LN Targeting







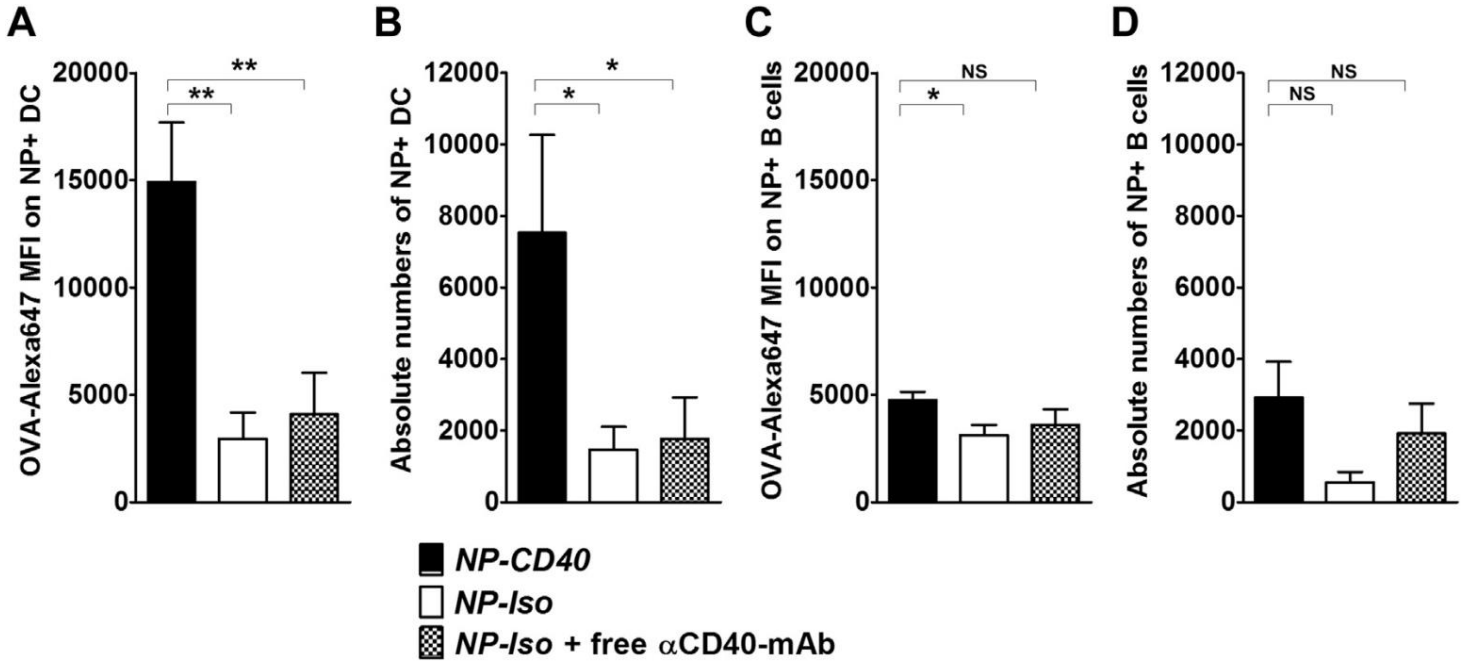
Larger particles with mean diameters of 100 nm were only 10% as efficient as the 25-nm particles at draining to lymph nodes, and 25-nm diameter particles subsequently induced the strongest immune responses

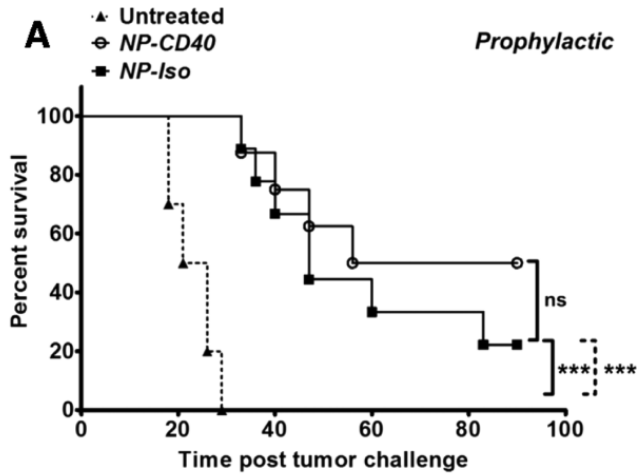
Nat Biotech **25**, 1159-1164 (2007)

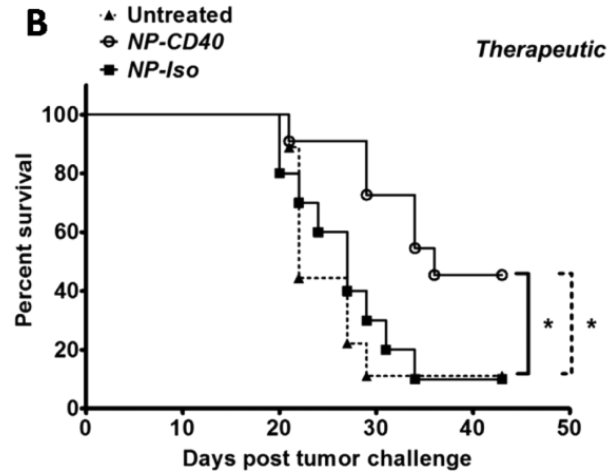
Promoting Vaccine Capture in Lymph Nodes

Incorporate targeting ligands for DCs, e.g.:

- Mannose
- Anti-CD40
- Anti-DEC-205
- Anti-CD11c

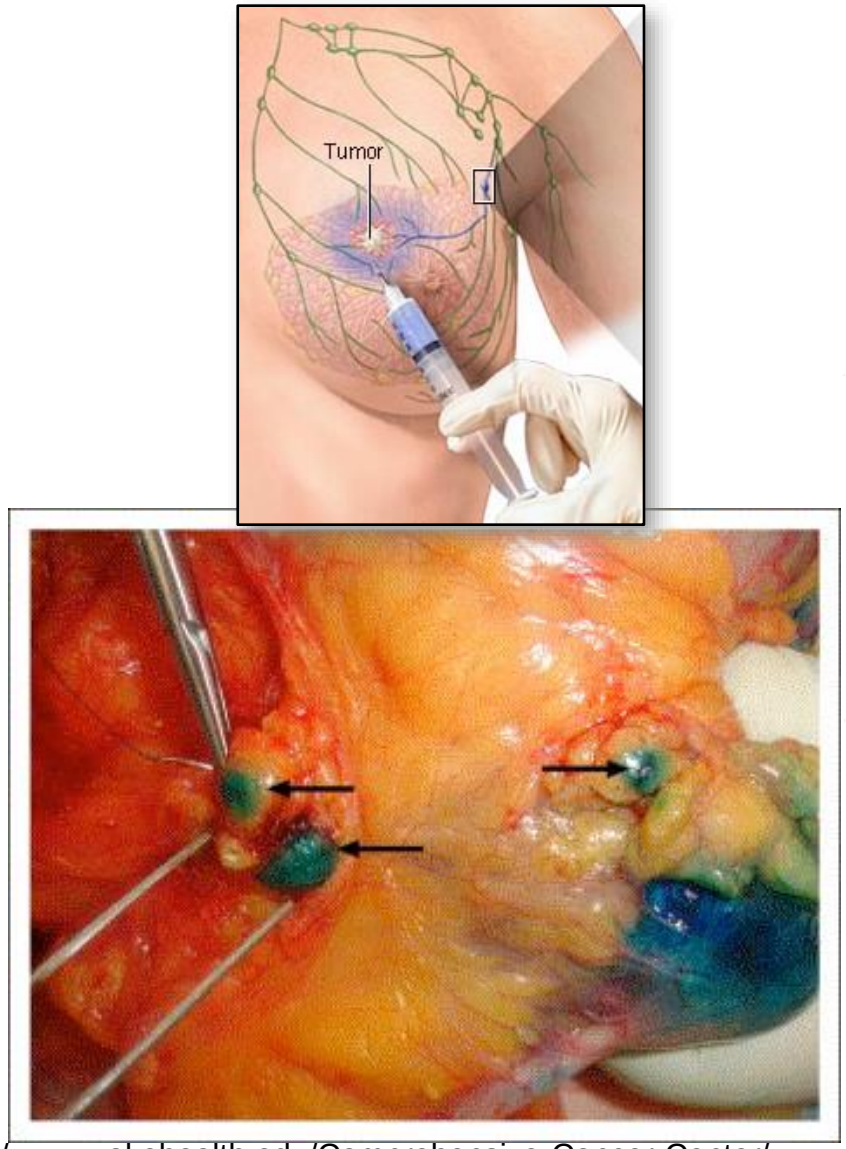




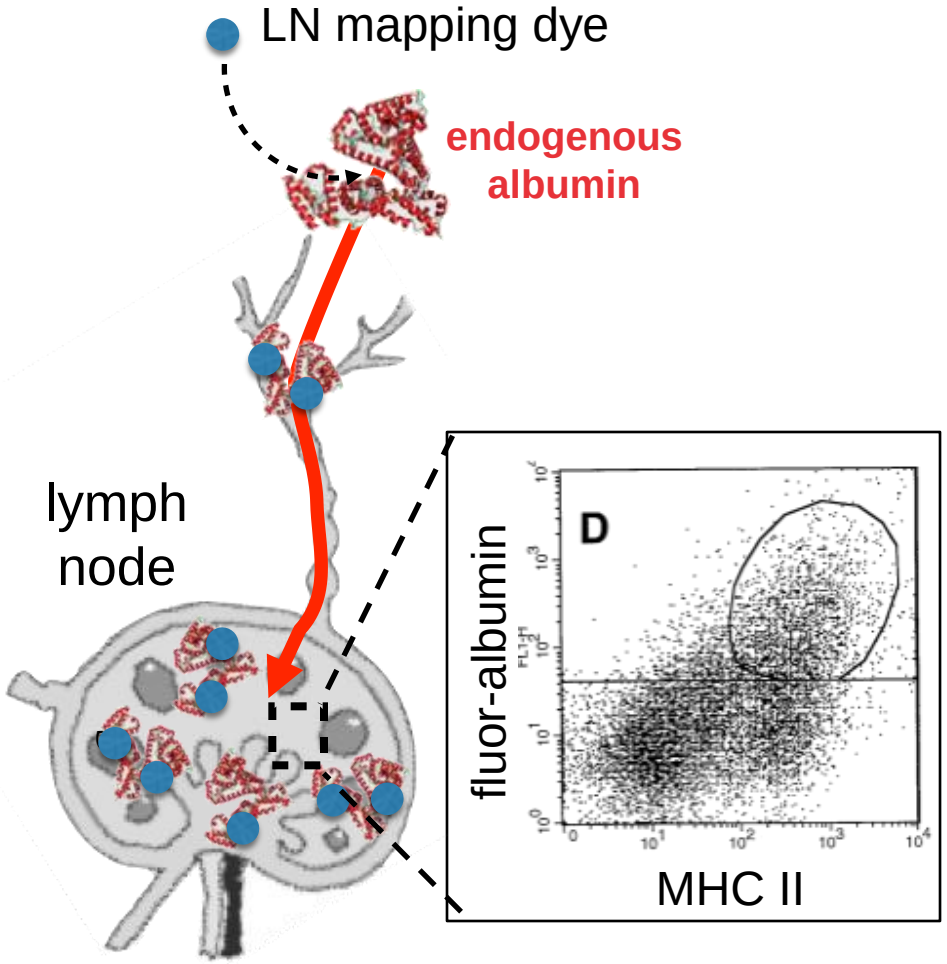


Biomaterials **40**, 88-97 (2015)

A clinically-validated strategy for LN targeting: sentinel lymph node mapping



http://www.wakehealth.edu/Comprehensive-Cancer-Center/

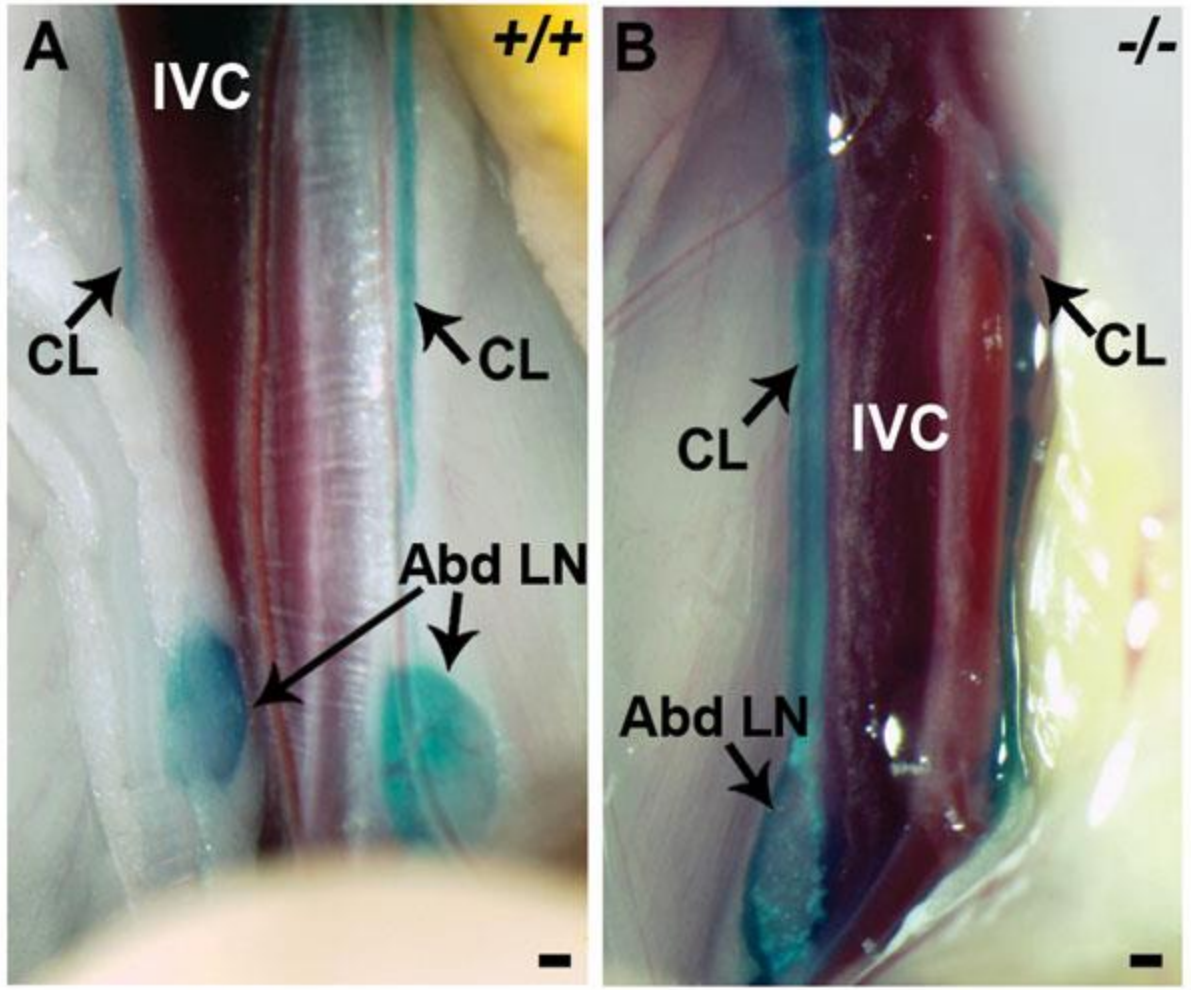


Tsopelas, C. & Sutton, R. *J. Nucl. Med.* **43**, 1377–1382 (2002); Faries, M. B. *et al. Ann. Surg. Oncol.* **7**, 98–105 (2000).

Saha, S. *et al. Am. J. Surg.* **191**, 305–310 (2006)

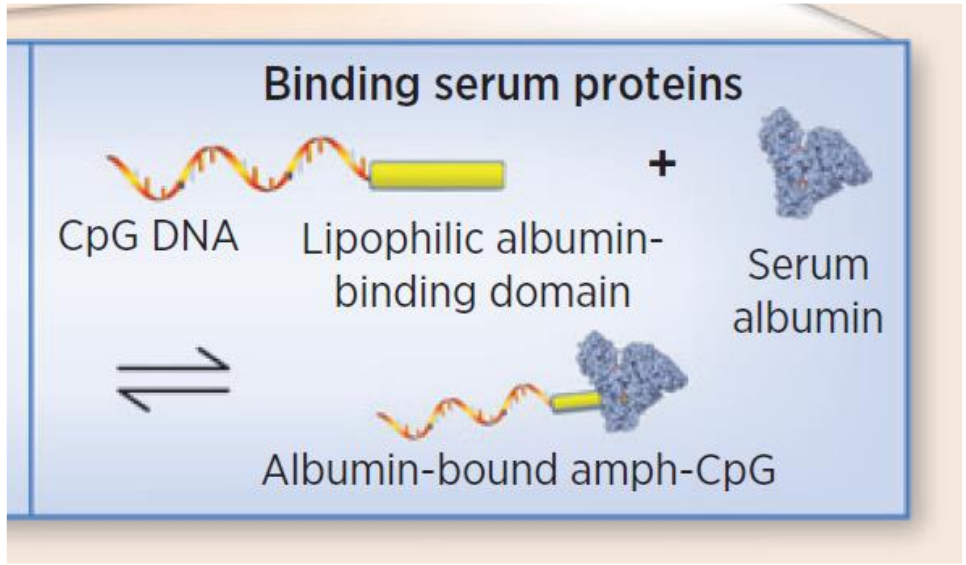
Lymph node mapping with albumin hitchhiking dyes

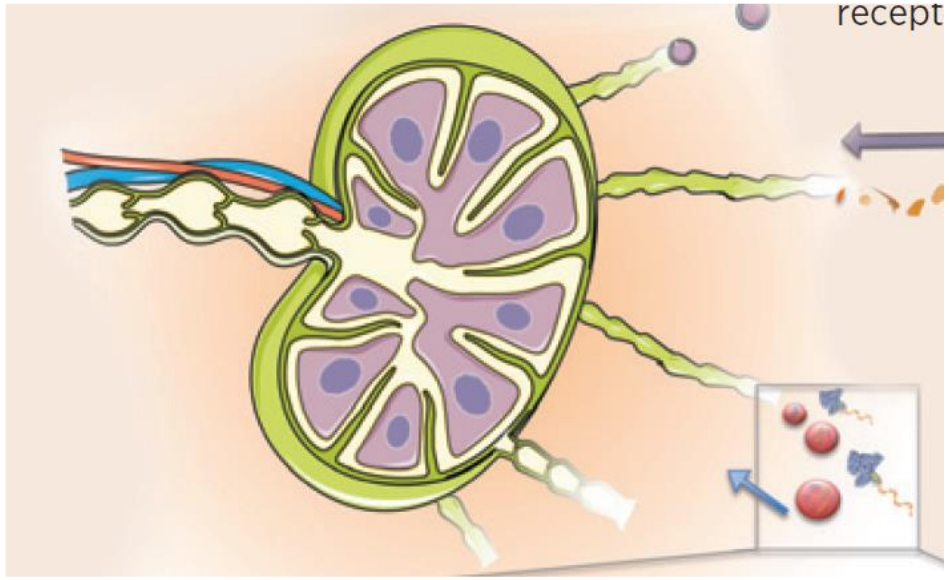
Evan's blue dye binds albumin and clearly map the lymph nodes

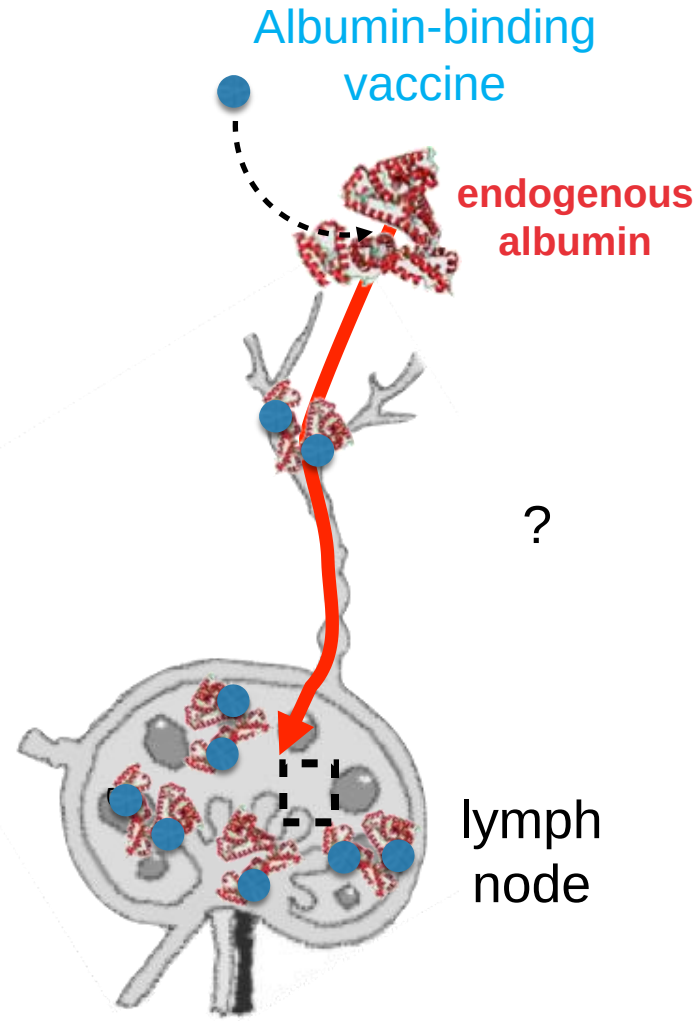


PNAS 2007 vol. 104 no. 2 606-611

“albumin hitchhiking” vaccine target lymph nodes?







Tsopelas, C. & Sutton, R. *J. Nucl. Med.* **43**, 1377–1382 (2002);
Faries, M. B. *et al. Ann. Surg. Oncol.* **7**, 98–105 (2000).

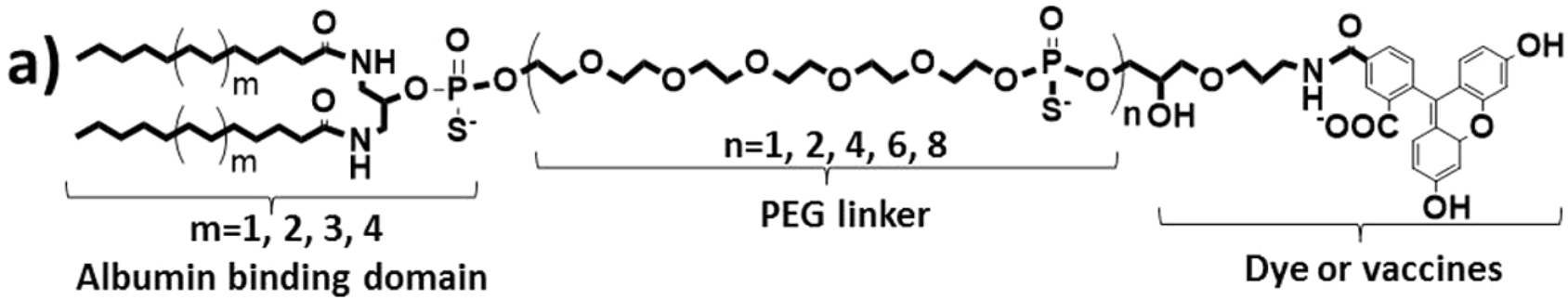
Saha, S. *et al. Am. J. Surg.* **191**, 305–310 (2006)

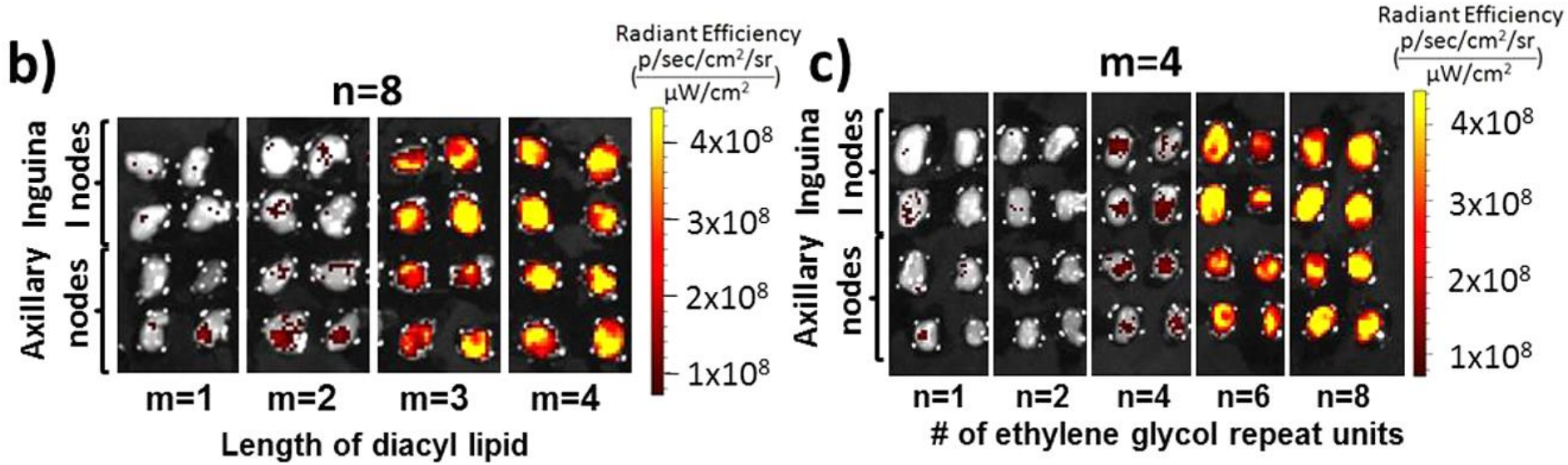
Cancer Immunology Research **2015,** *3*, 836-43.

“albumin hitchhiking” vaccines

This slide is not required.

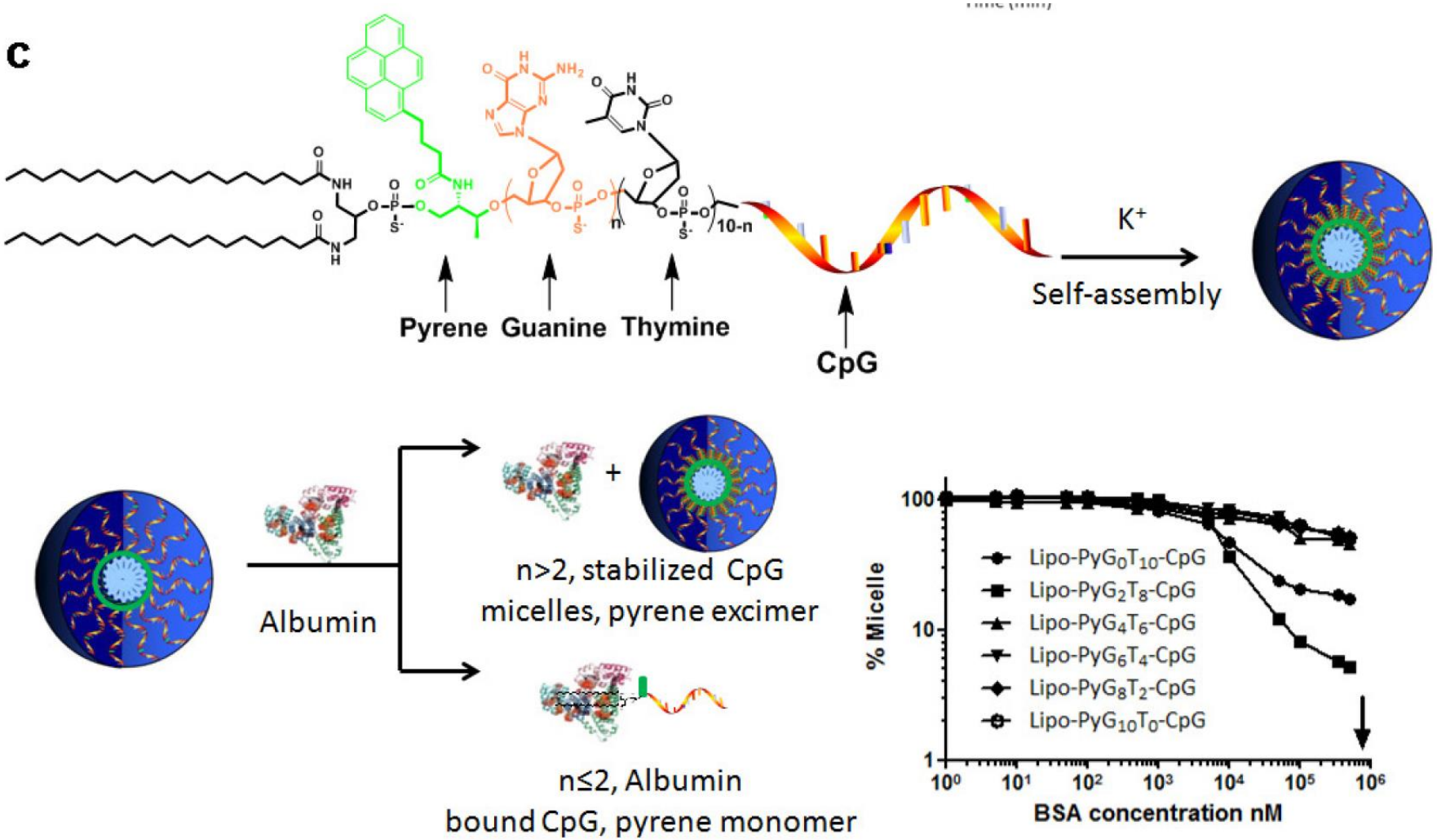
antigens or molecular adjuvants are covalently linked to a lipophilic albumin binding domain





Nature **507**, 519-522 (2014)

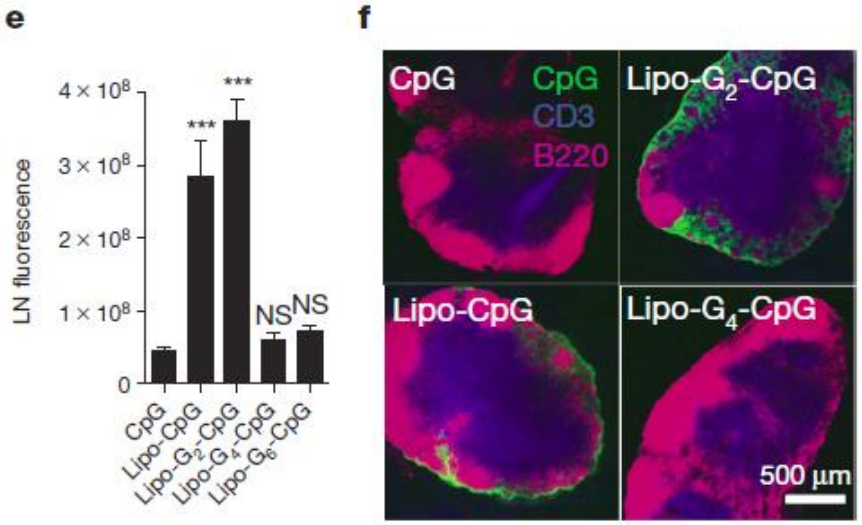
This slide is not required.

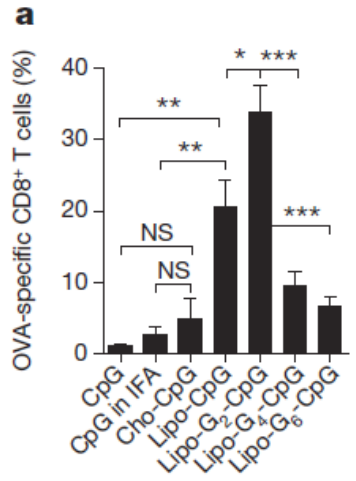


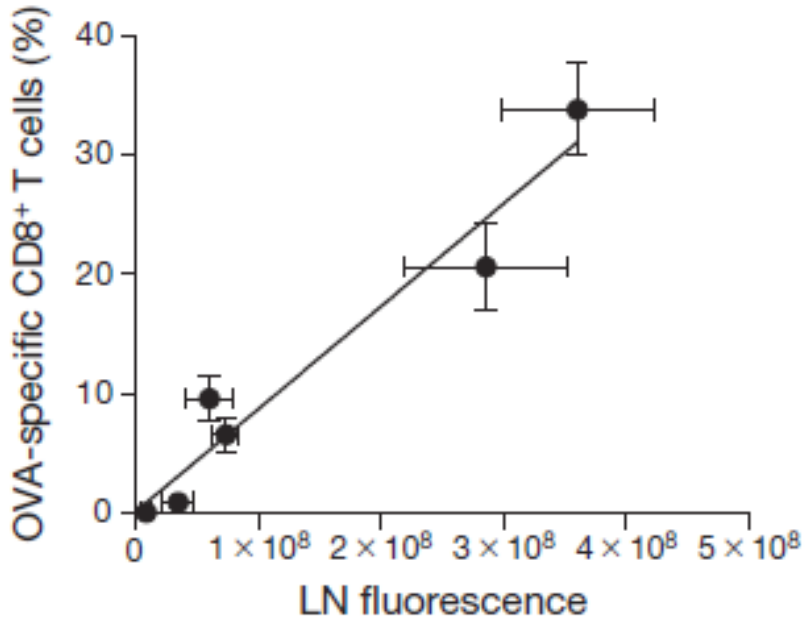
Nature **507**, 519-522 (2014)

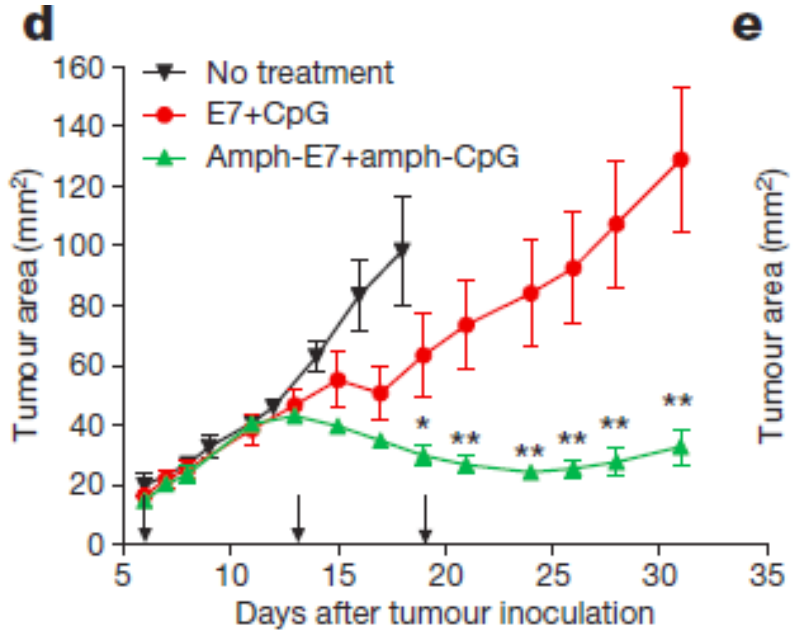
“albumin hitchhiking” vaccines

This slide is not required.



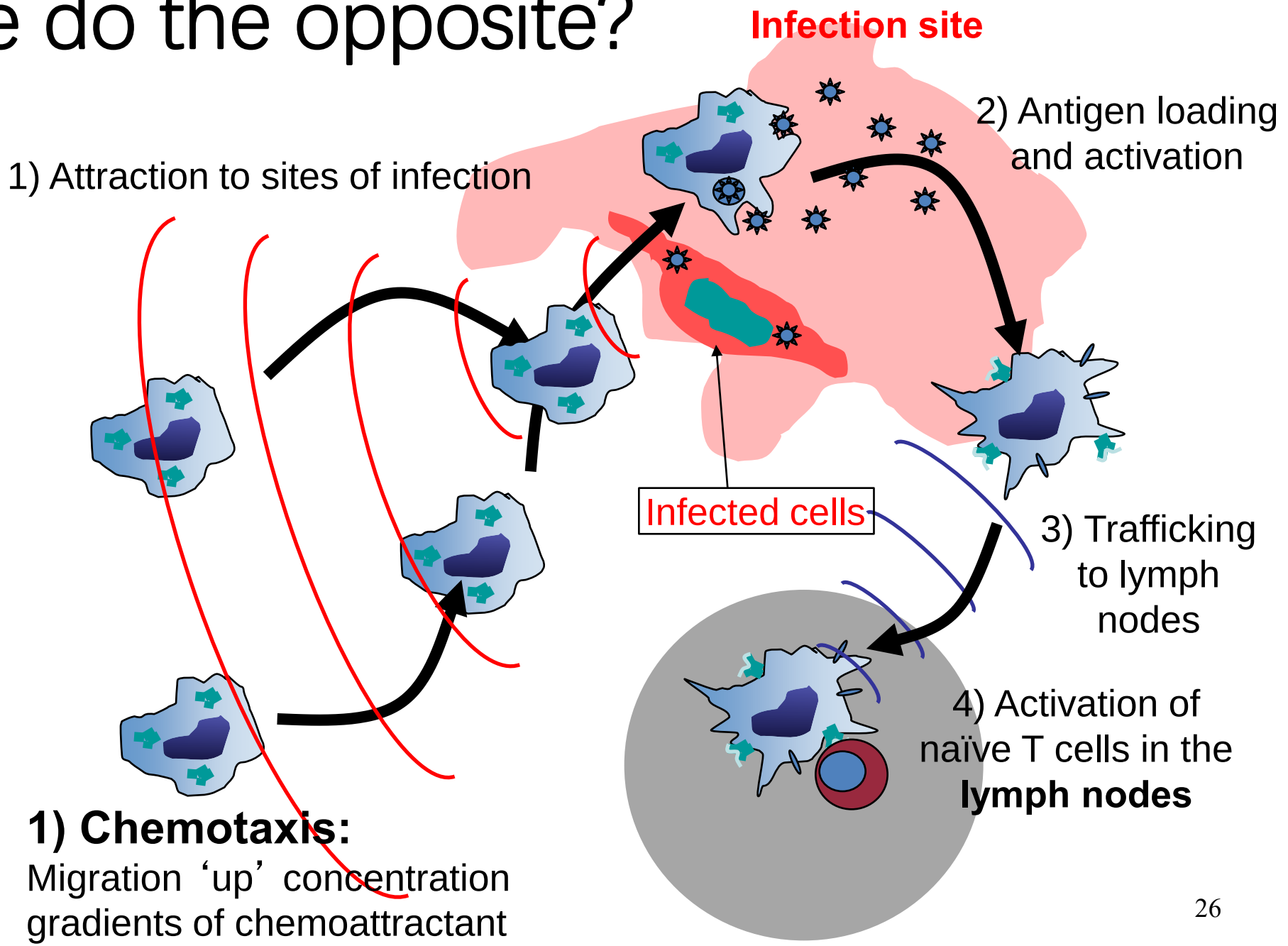






Nature **507**, 519-522 (2014)

Can we do the opposite?



1) Chemotaxis:
Migration 'up' concentration gradients of chemoattractant

Recruiting DCs to vaccine-loaded biomaterials

Attraction of target cells to device via chemotaxis

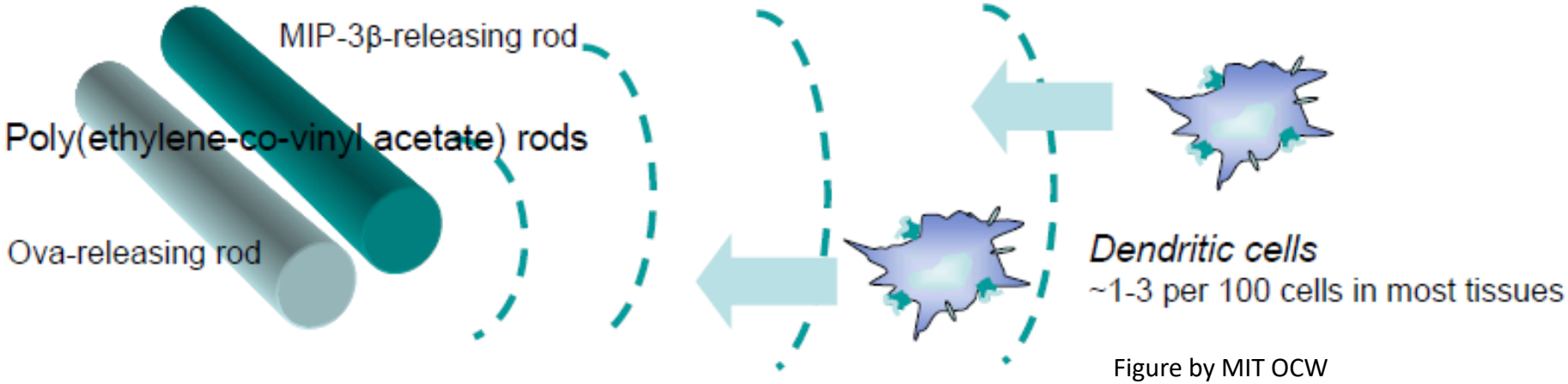
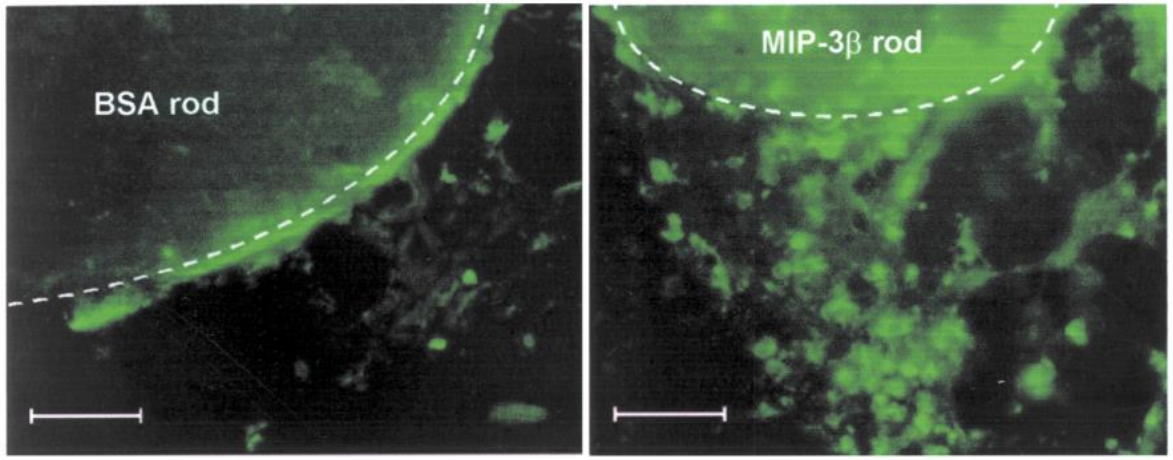


Figure by MIT OCW

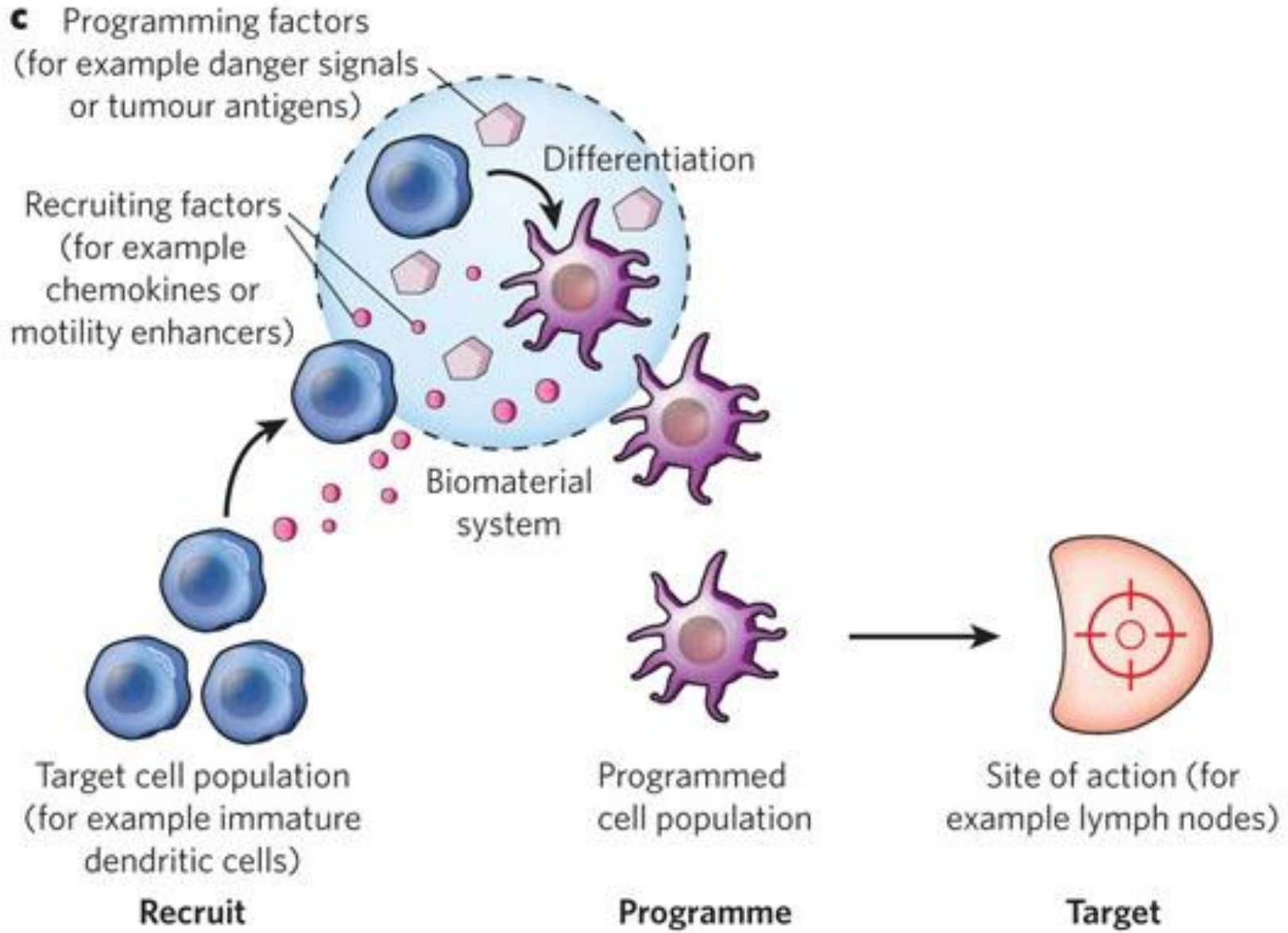
Attraction of target cells to device via chemotaxis:

1. Free chemokines are quickly cleared
2. Engineered concentration gradient of chemokines



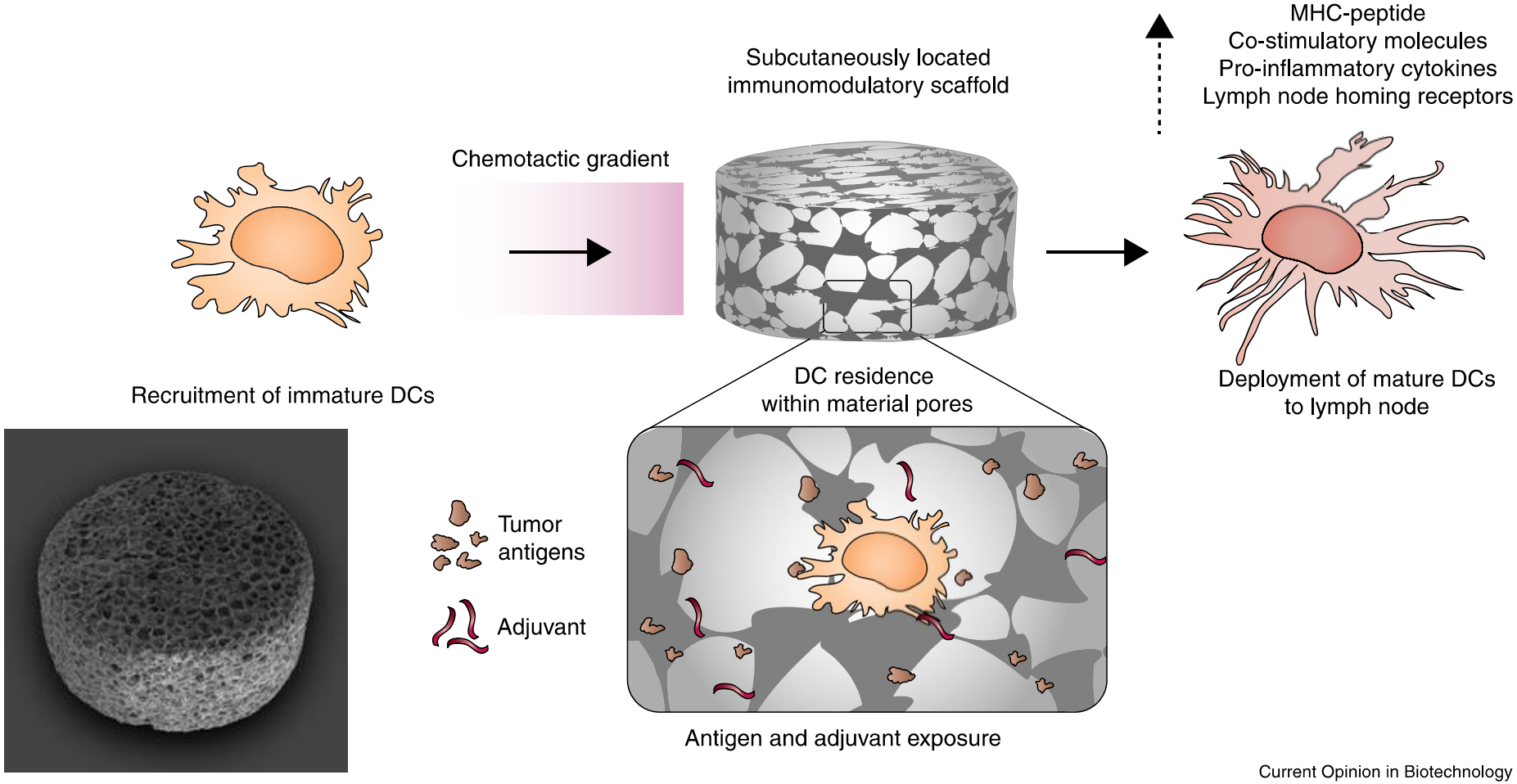
*Nat Biotechnol*20 (2002): 64-9.

Recruit DCs to vaccines



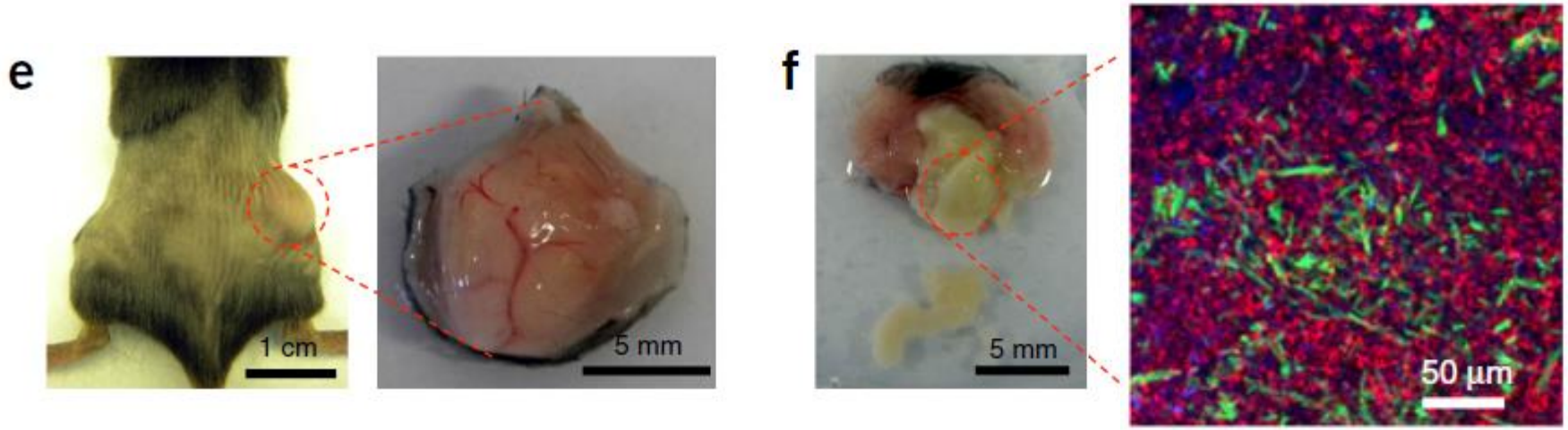
https://mooneylab.seas.harvard.edu

Improving DC vaccines: engineering DC generation, antigen loading, and activation in vivo

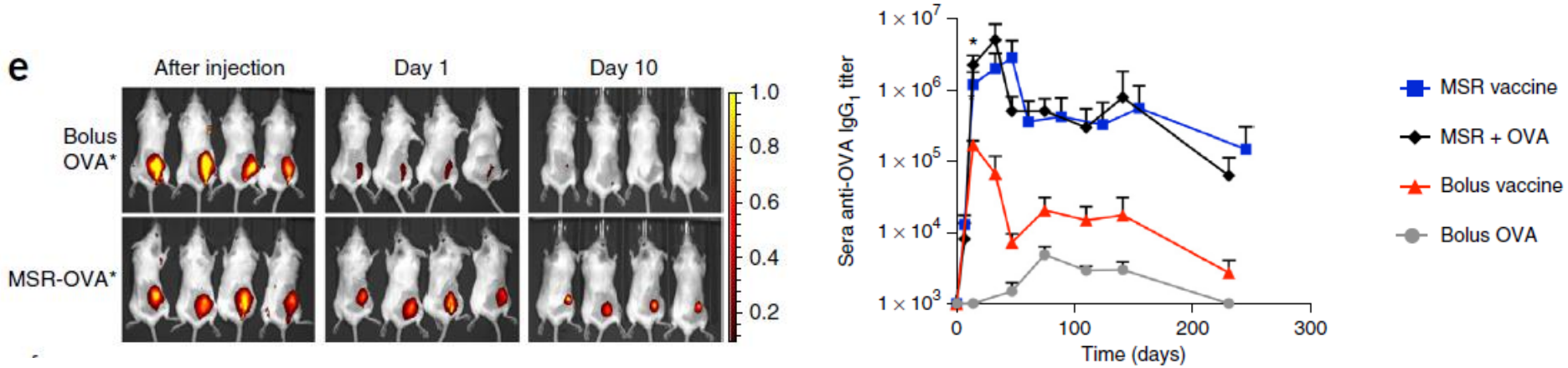


Koshy, S. T., & Mooney, D. J. (2016). *Current Opinion in Biotechnology*, *40*, 1–8. http://doi.org/10.1016/j.copbio.2016.02.001

Recruit DCs to vaccines

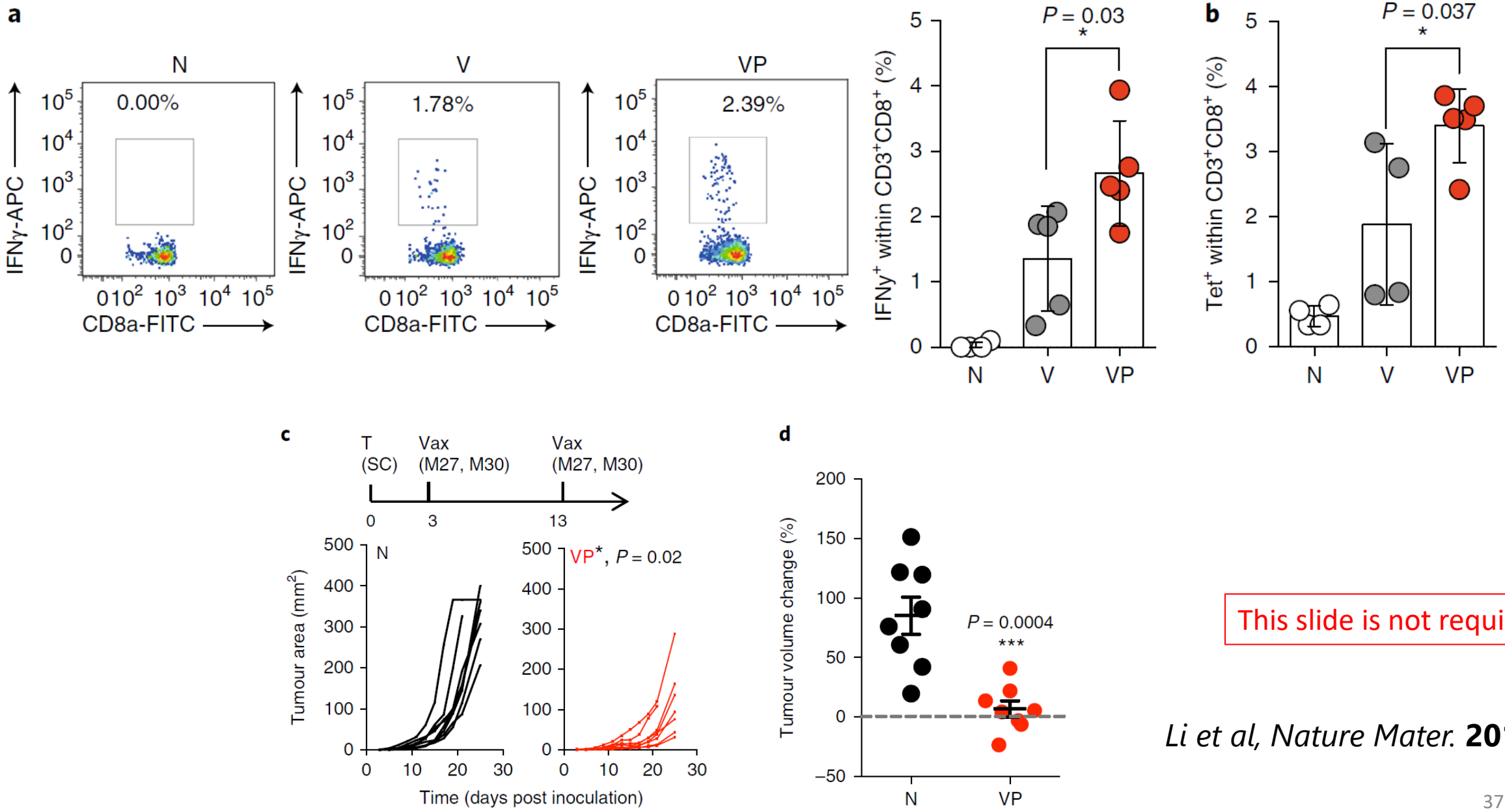


GM-CSF, CpG and OVA



Nat Biotech **33**, 64-72 (2015)

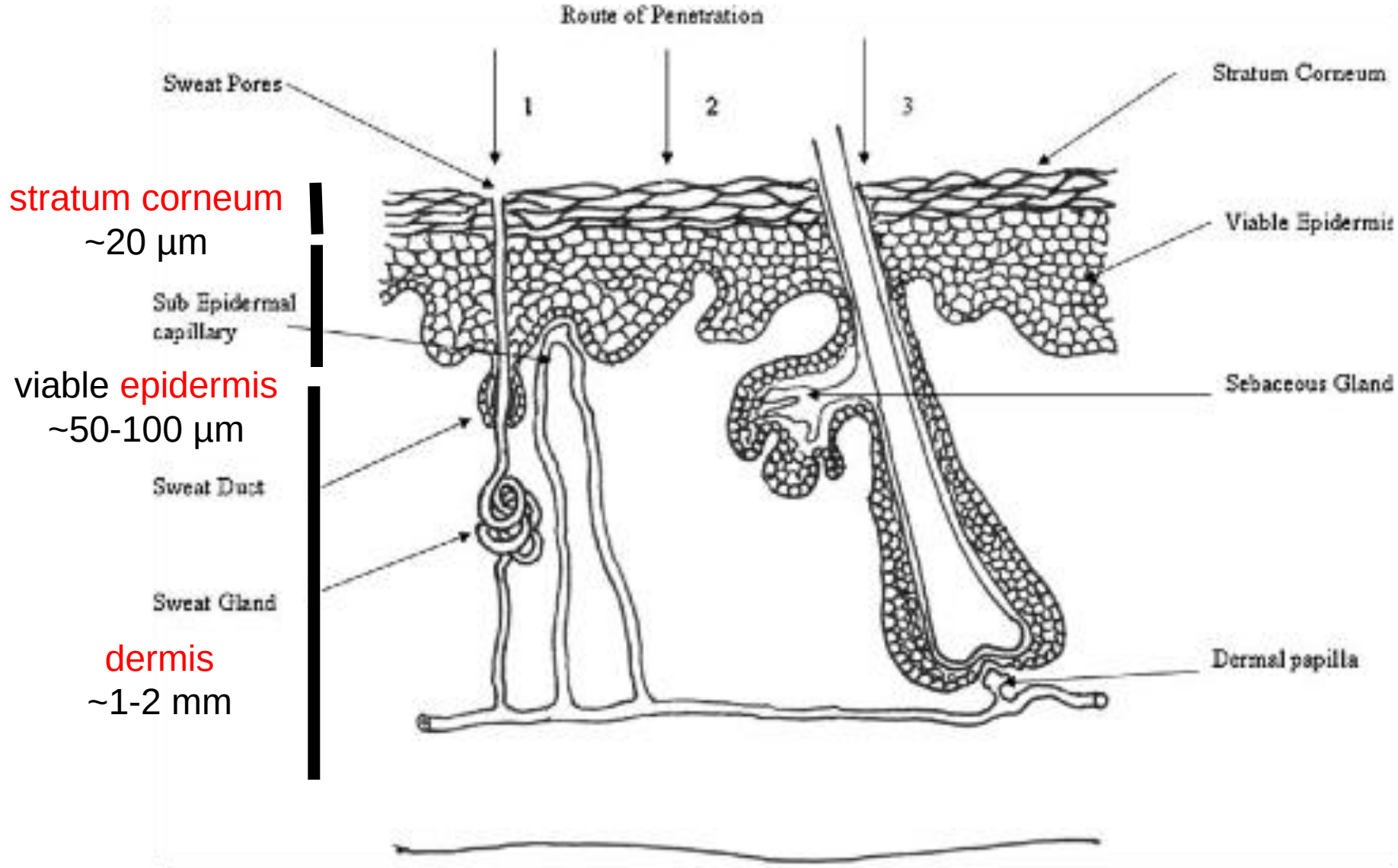
Recruit DCs to vaccines



This slide is not required.

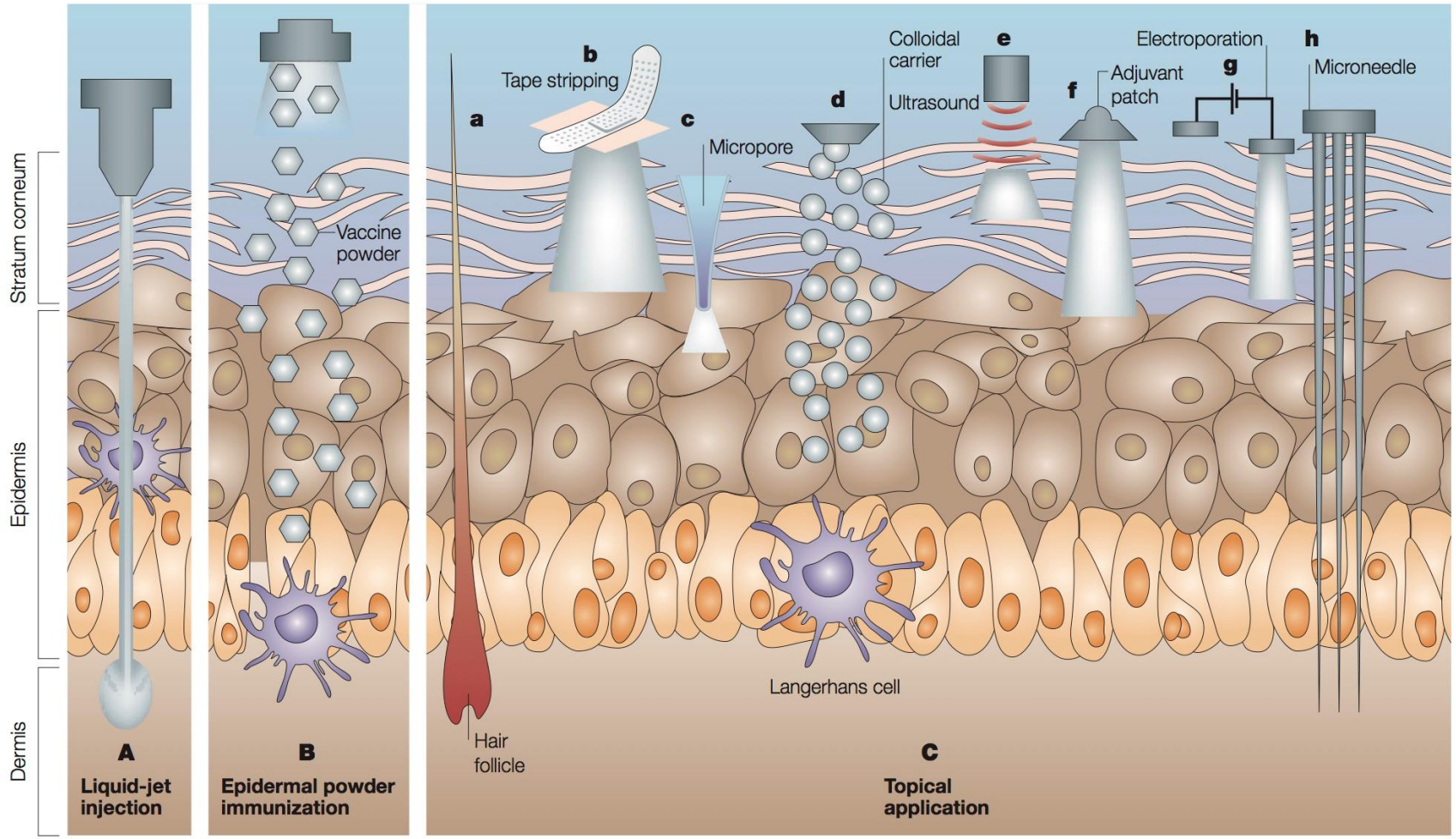
Li et al, Nature Mater. **2018**.

Delivering vaccines across skin:



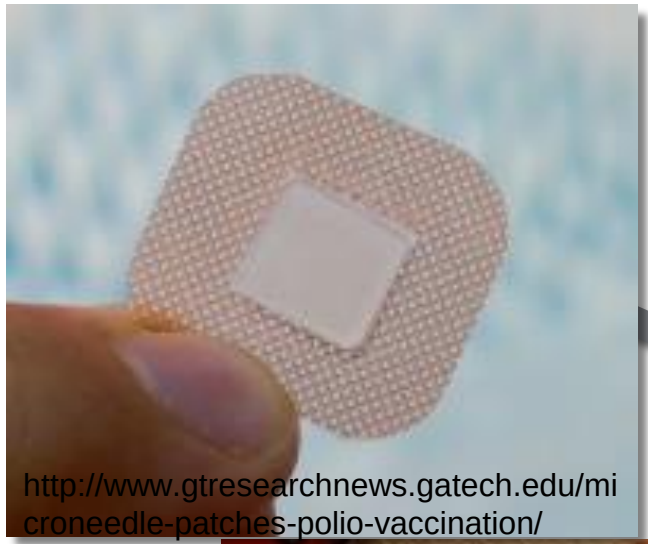
J Pharm Sci 97:3591–3610, 2008

Vaccine strategies for crossing the stratum corneum

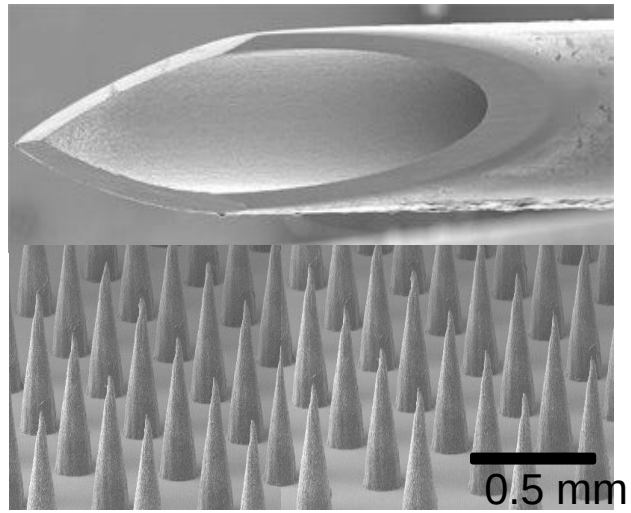


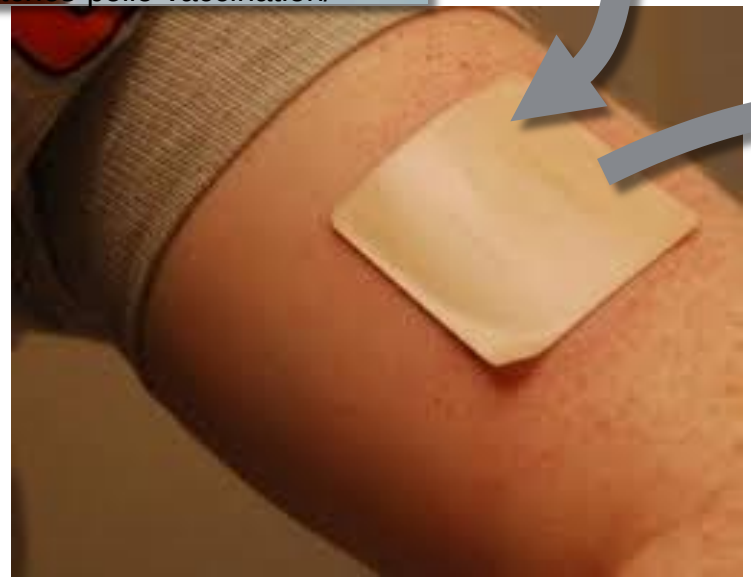
Mitragotri, S. (2005). Immunization without needles. Nature Reviews Immunology, 5(12), 905-916. doi:10.1038/nri1728

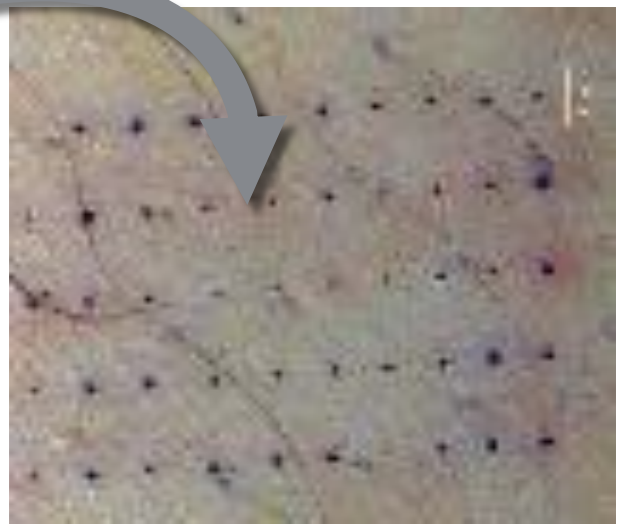
Accessing the skin or mucosal surfaces using “microneedle” patches



http://www.gtresearchnews.gatech.edu/microneedle-patches-polio-vaccination/

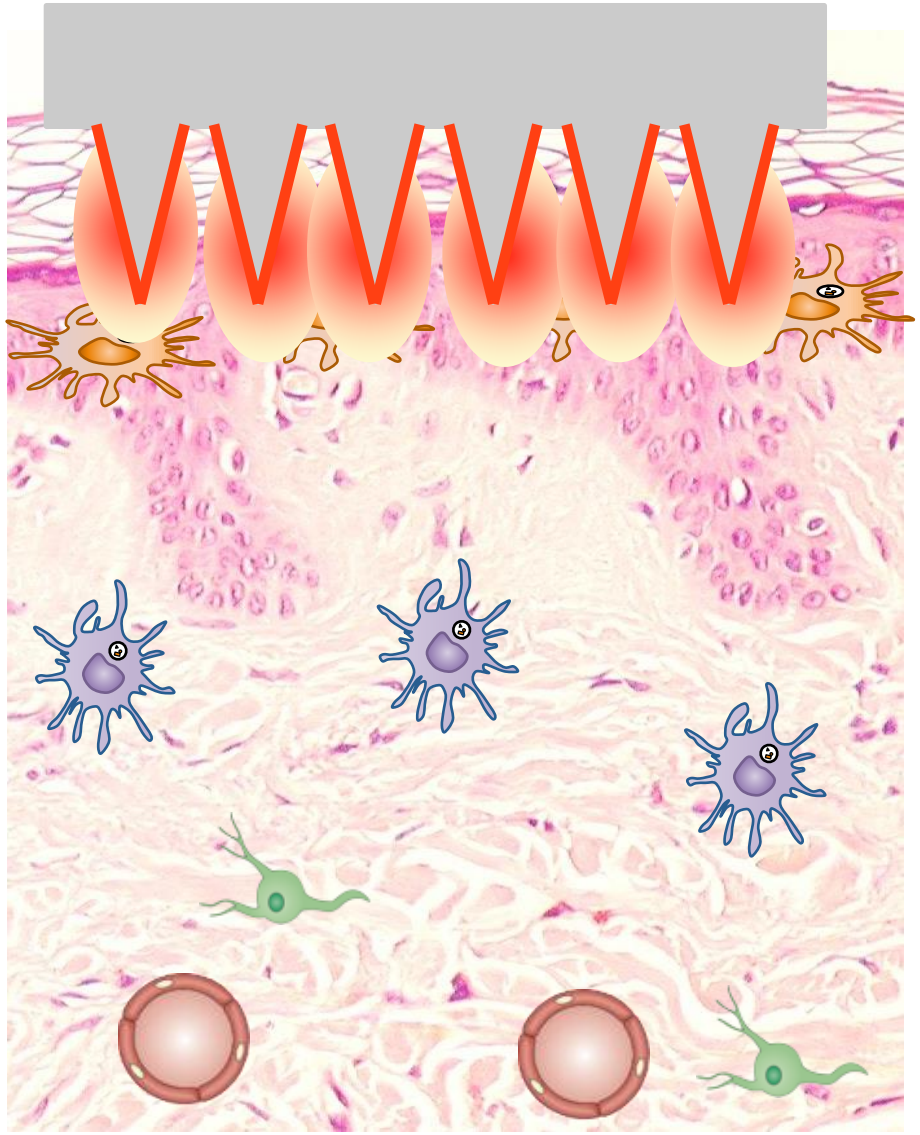




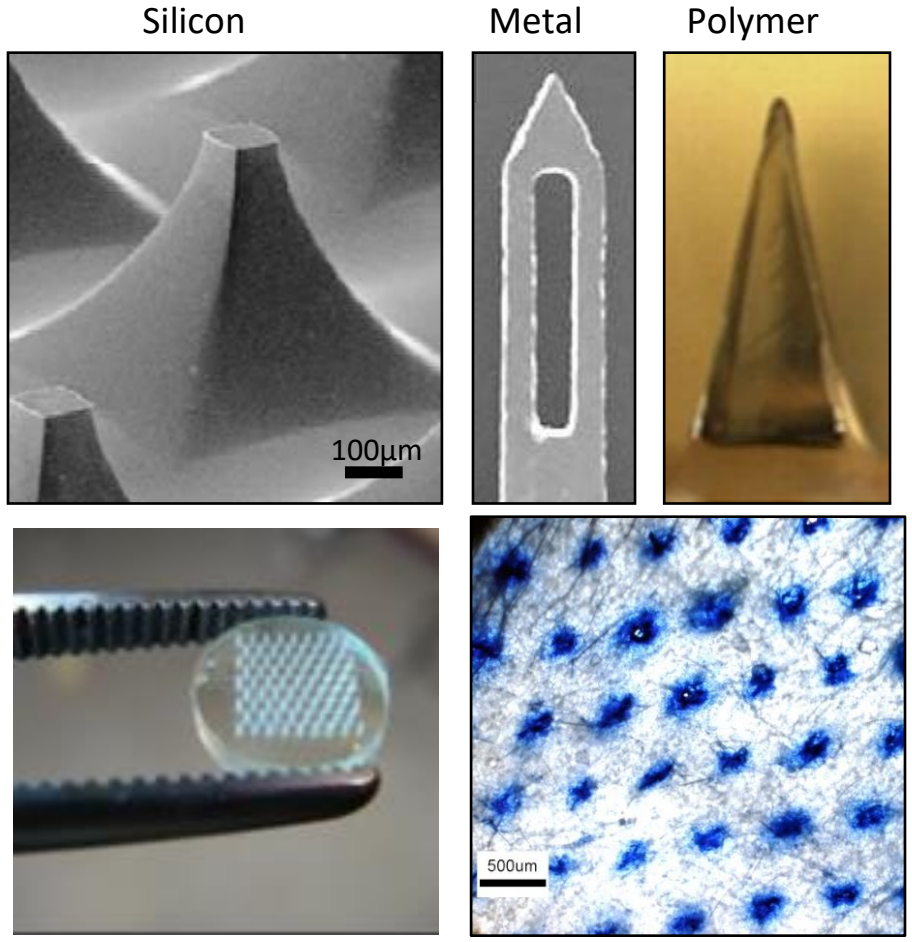


Wermeling, Prausnitz et al. (2008). *PNAS 105*(6), 2058–2063

Microneedles for noninvasive, pain-free delivery to the skin



Arrays of dry-coated microprojections:



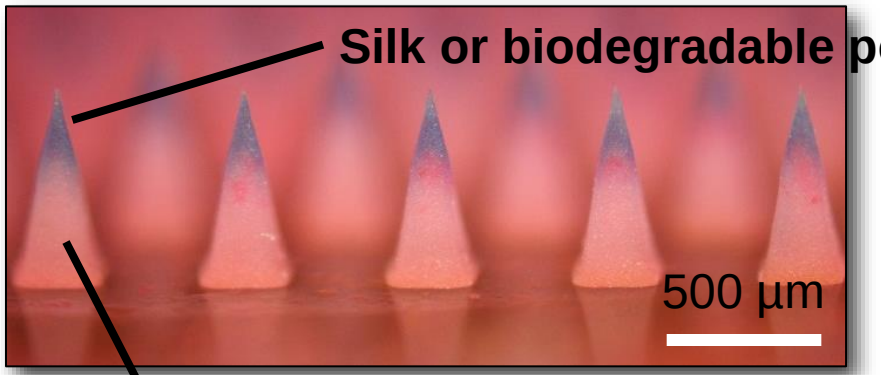
Sullivan, S.P. et al. (2010) Nature Medicine, 16: 915-920

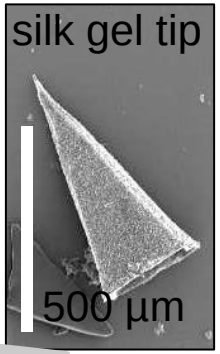
Lee J.W. et al. (2008) Biomaterials, 29: 2113-2124

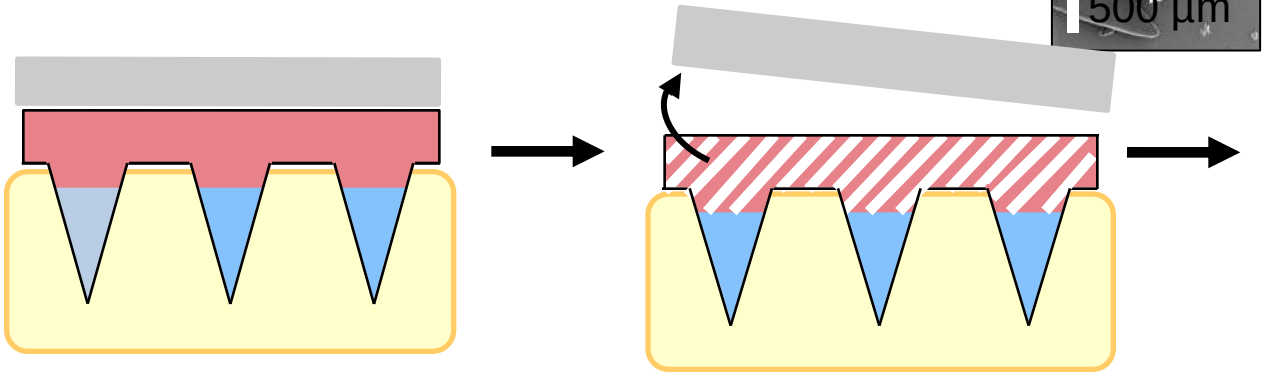
Gill, H.S. et al. (2007) Pharm. Res., 24: 1369-1380

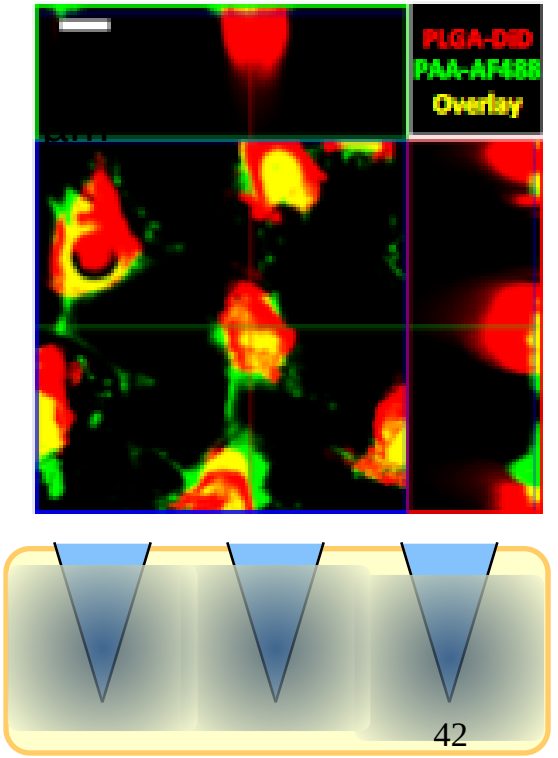
http://en.academic.ru

Implantable microneedles for sustained vaccine kinetics from a rapid-application skin patch





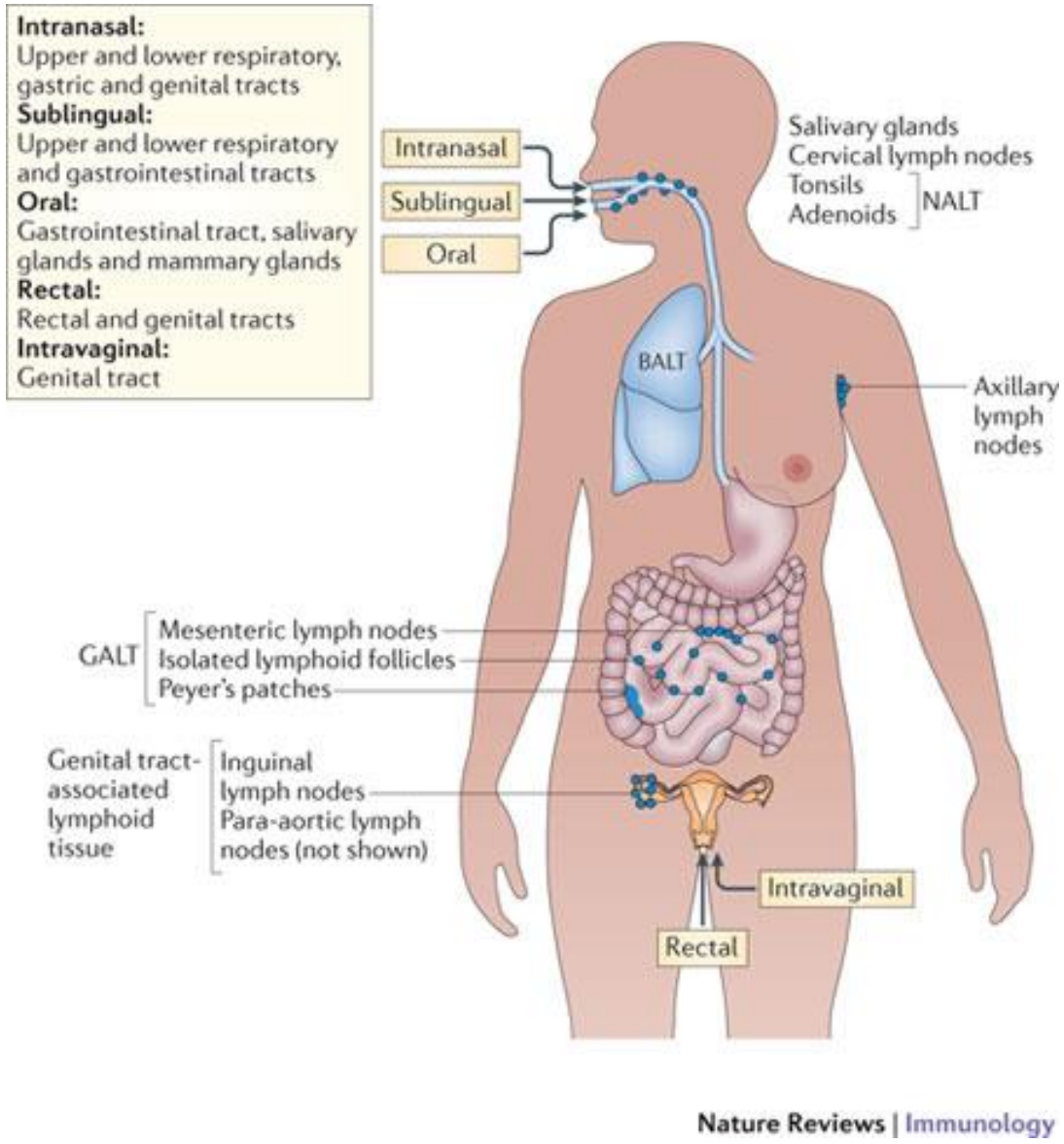




(DeMuth et al., *Adv. Funct. Mater.* 2013; *Adv. Healthcare Mater*. 2014)

Mucosal immunization

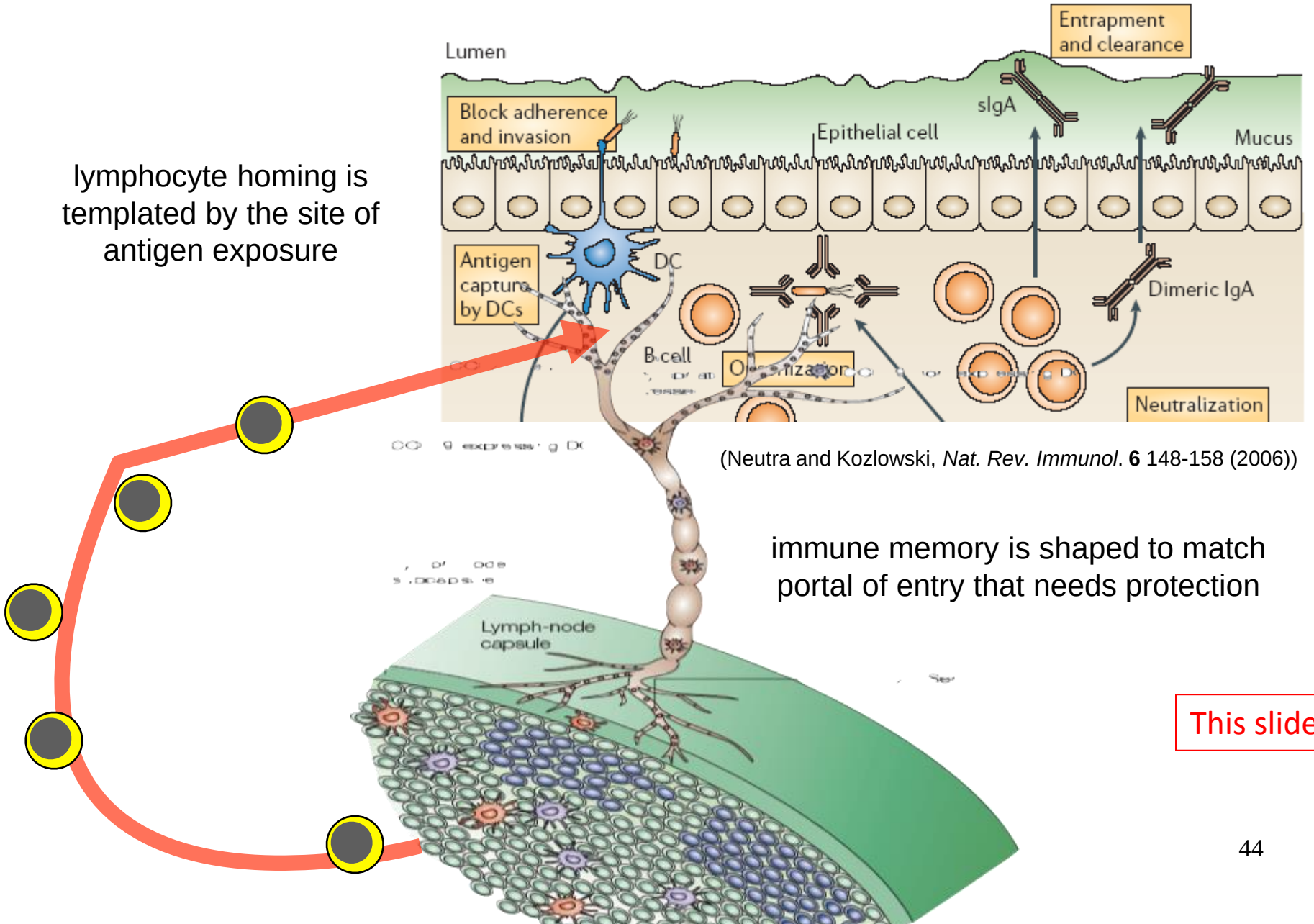
- Most pathogens infect through mucosal tissues
- the establishment of mucosa-homing T cells and B cells through mucosal immunization can be a key component of vaccine efficacy
- Nanoparticles are attractive mucosal vaccine/immunotherapy delivery vehicles
  - enhanced uptake by APCs of particulate antigen
  - the preferential draining of nanoparticles to lymphatics rather than to the bloodstream
  - the ability of nanoparticles to diffuse through mucus and cross mucosal barriers



Nat. Rev. Immunol. **12**, 592-605 (2012).

Why is mucosal immunization of interest?

lymphocyte homing is templated by the site of antigen exposure

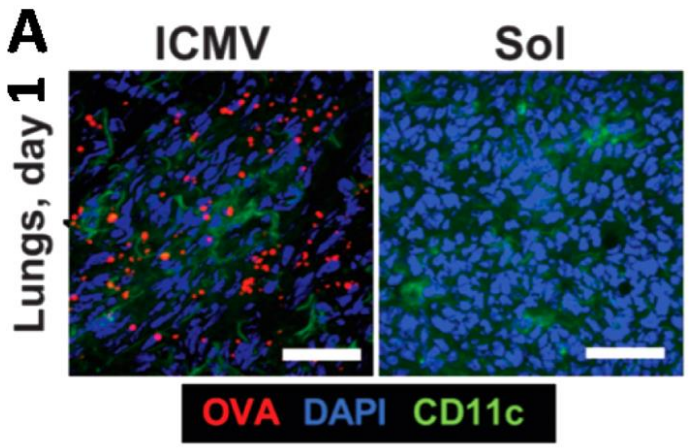


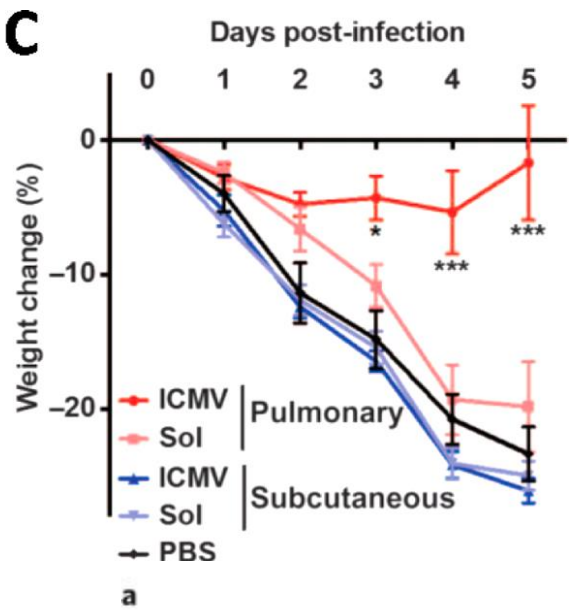
(Neutra and Kozlowski, *Nat. Rev. Immunol*. **6** 148-158 (2006))

immune memory is shaped to match portal of entry that needs protection

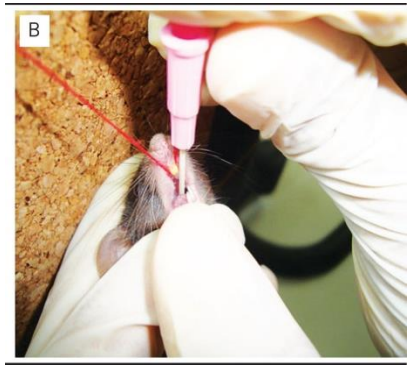
This slide is not required.

Airways and Nasal Mucosa

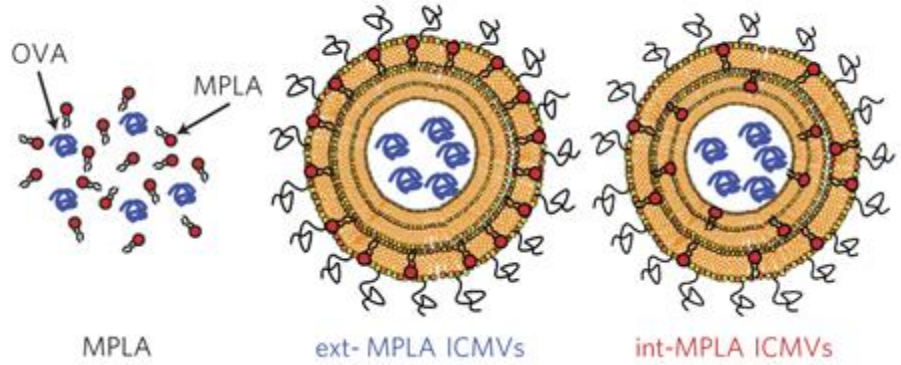




intratracheal immunization



http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1676-24442015000300183

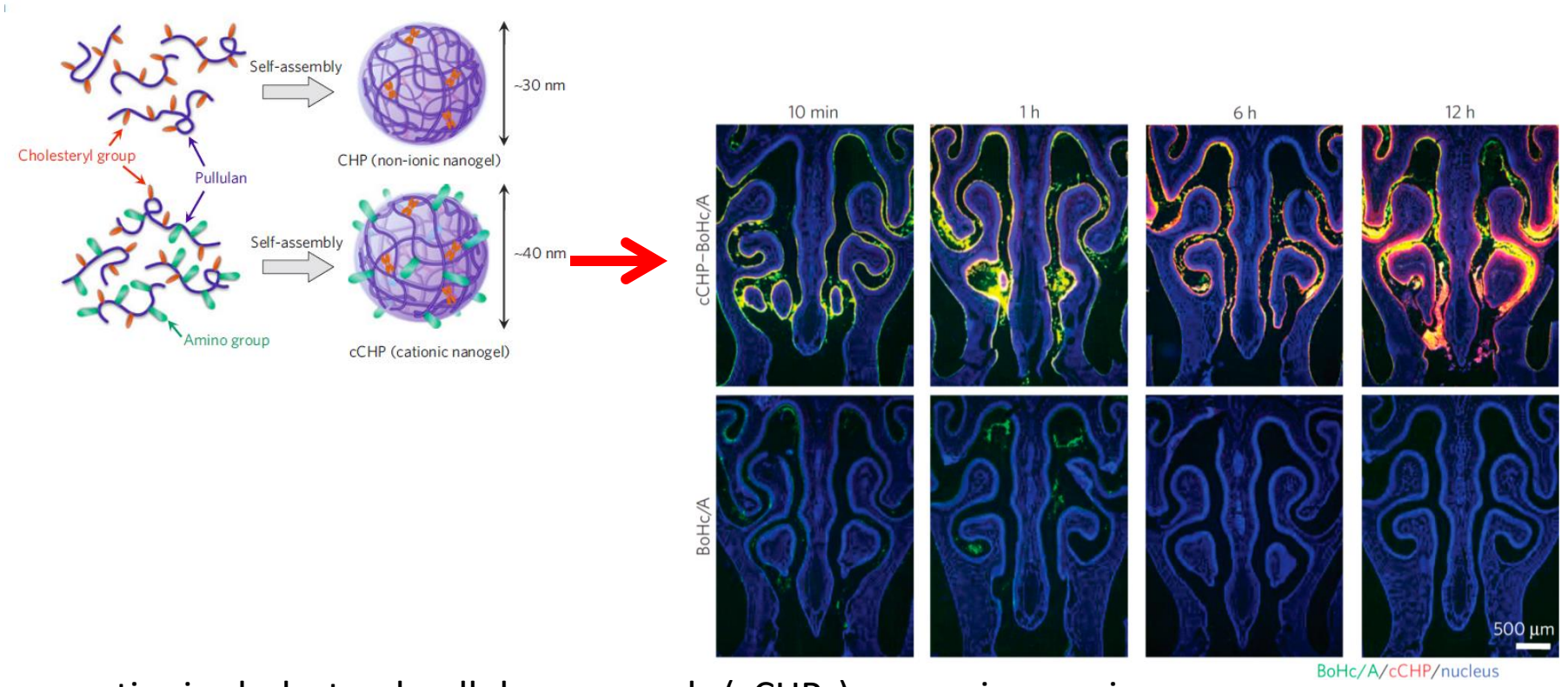


Using cross-link-stabilized lipid nanocapsules loaded with antigen and TLR agonist adjuvants, for greatly increased and prolonged antigen presentation in lung-draining lymph nodes and transformed an antigen/adjuvant combination that was completely nonprotective against a viral challenge as a soluble formulation to a 100% protective vaccine

Sci. Transl. Med. **5**, 204ra130 (2013).

Airways and Nasal Mucosa

employ nanoparticles that are muco-adhesive to increase the particles' residence time at the epithelial surface.



- cationic cholesteryl pullulan nanogels (cCHPs) as vaccine carriers
- increased retention in the nasal mucosa and uptake of antigen by mucosal dendritic cells
- enhanced antigen delivery translated to significantly increased mucosal antibody responses and protection from Clostridium botulinum challenge

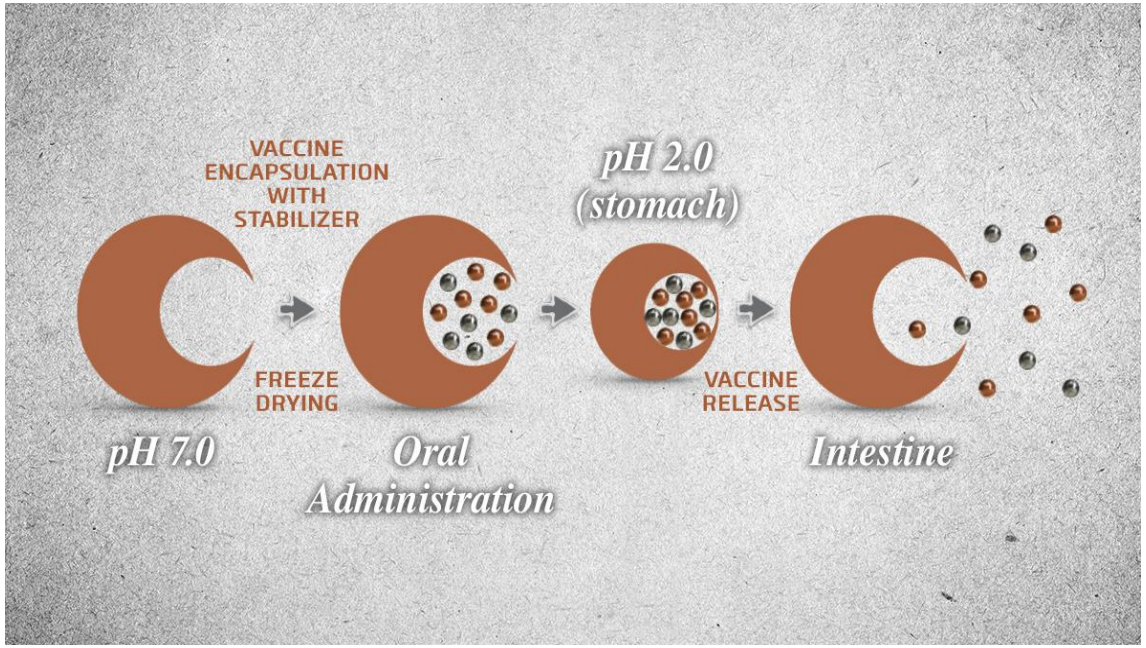
Nat. Mater. **9**, 572-578 (2010)

Gastrointestinal (GI) Tract

The oral delivery route offers a multitude of advantages for vaccines and immunotherapy treatment:

- dispense with risks from needle use (or reuse)
- amenable to self-administration with high patient compliance
- lead to the induction of both systemic and mucosal immunity

oral immunomodulators must first survive exposure to the stomach pH, proteolytic enzymes, and bile salts in the gastrointestinal (GI) tract and then transit through mucus and the gut epithelium to reach the gut-associated lymphoid tissue (GALT).



Biomaterials?

Protect vaccines/immunotherapeutics from degradation and effectively transport particles across the intestinal lumen

Delivering vaccines to gut mucosa

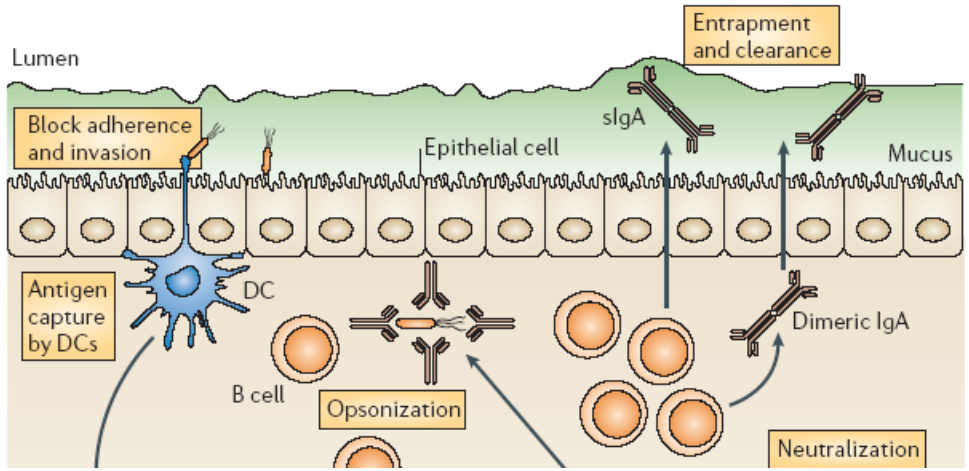
challenges:

rapid transit time...

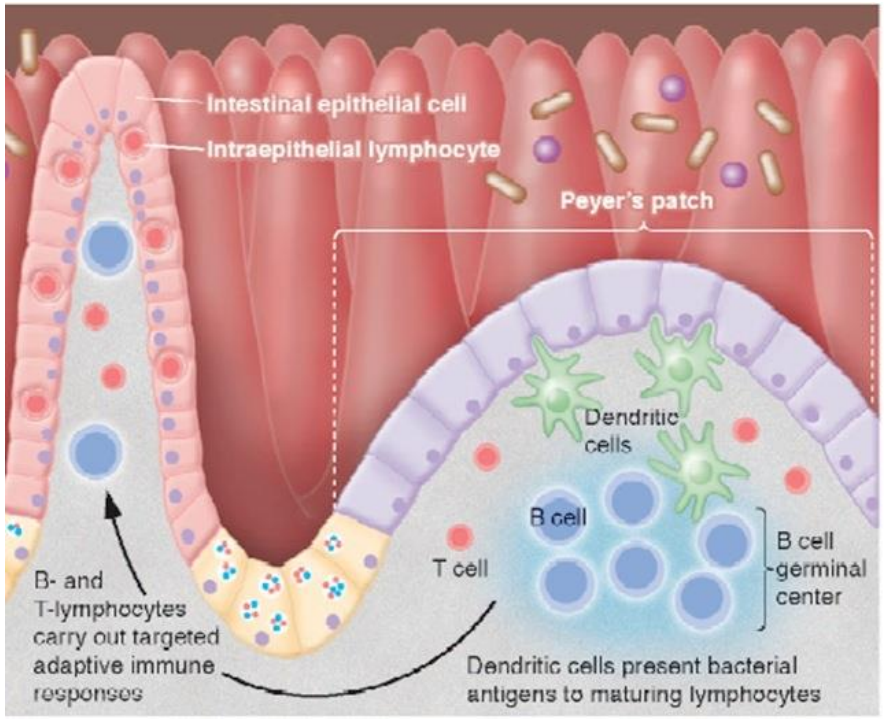
gut pH

lipases, degradative enzymes in gut lumen

Poor uptake at inductive sites



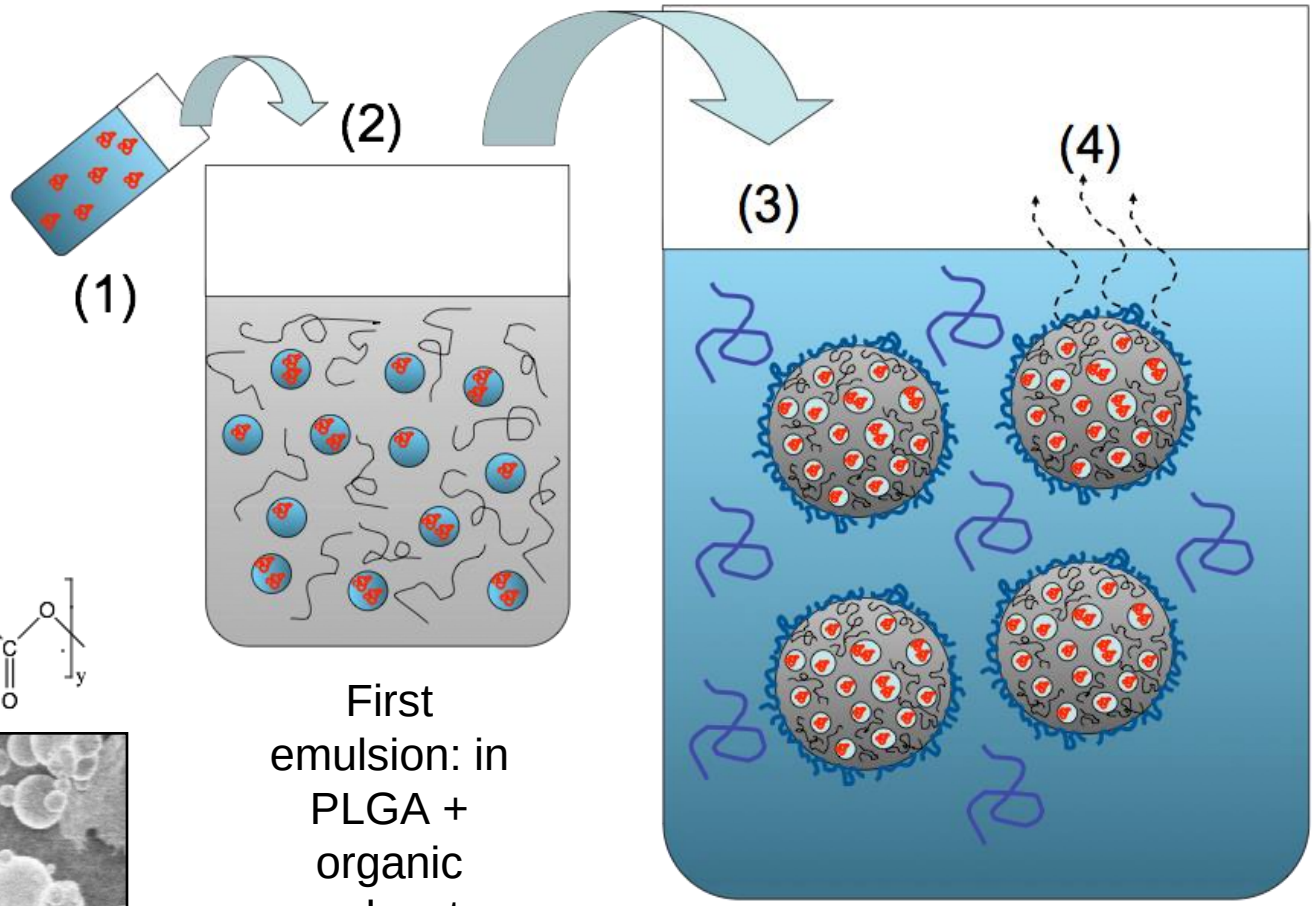
(Neutra and Kozlowski, *Nat. Rev. Immunol*. **6** 148-158 (2006))



How to protect antigens for transit to Gut-Associated Lymphoid Tissue?

antigen encapsulation in biodegradable polymer microspheres:
protection of antigen;
sustained release...

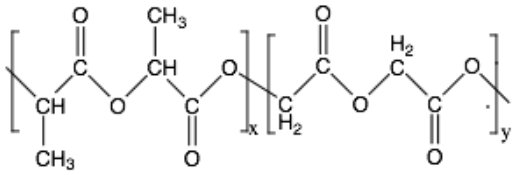
Antigen in aqueous solution

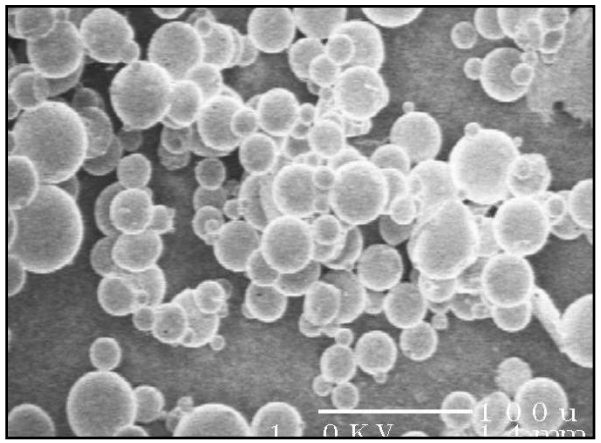


First emulsion: in PLGA + organic solvent

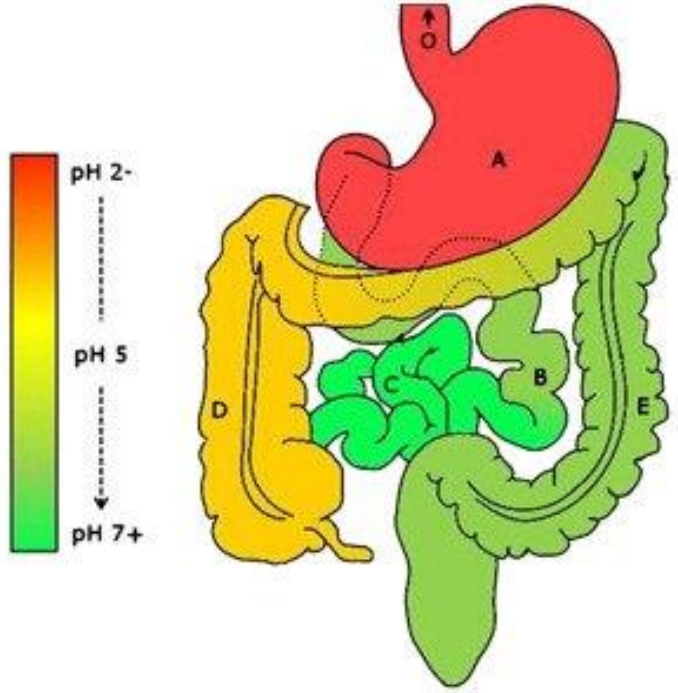
Second emulsion: stabilizer poly(vinyl alcohol) in water

poly(lactide-co-glycolide) [PLGA]



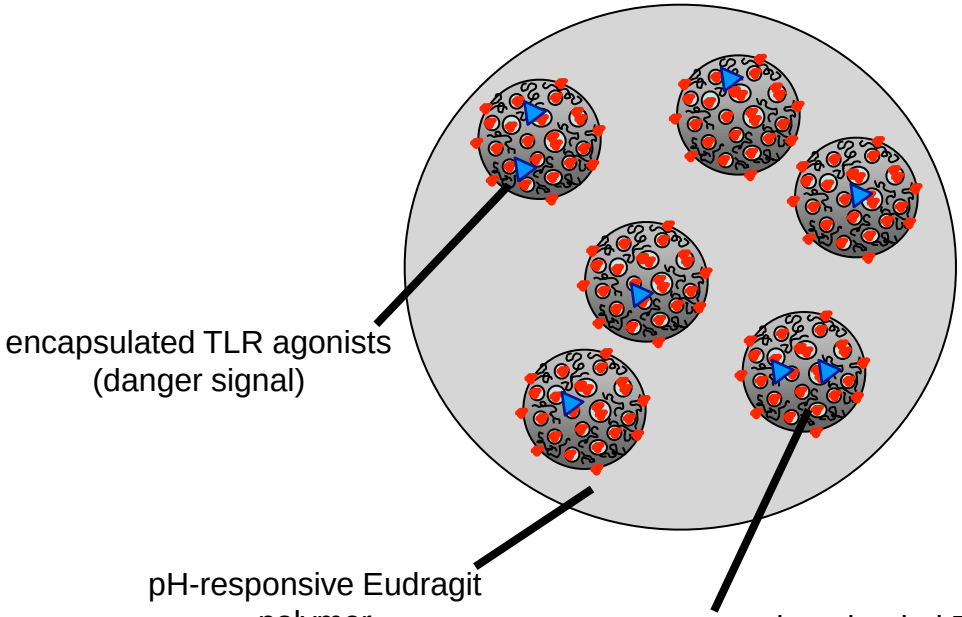


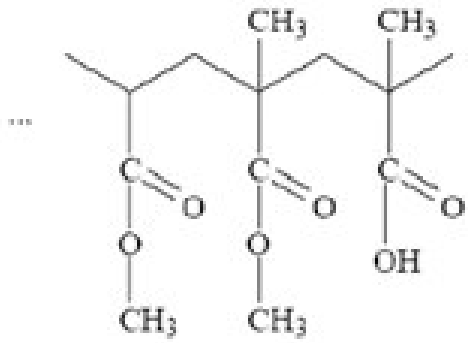
How to protect antigens for transit to Gut-Associated Lymphoid Tissue?



Letter	Region	pH	Transit Time
O	Oesophagus	~7.0	10-14 seconds
A	Stomach	1-2.5 (up to 5 fed)	Half emptying: ~80.5 mins
B	Proximal Small Intestine	6.15-7.35	3.2 ±1.6 hrs (combined)
C	Distal Small Intestine	6.80-7.88	
D	Ascending Colon	5.26-6.72	Highly variable, dependent on bowel evacuations
E	Descending Colon	5.20-7.02	

two-stage delivery capsules:





Zhu, Q. *et al. Nat Med* **18,** 1291–1296 (2012).

Targeting of vaccines to the large intestine

Eudragit polymer that dissolves at pH > 5.5

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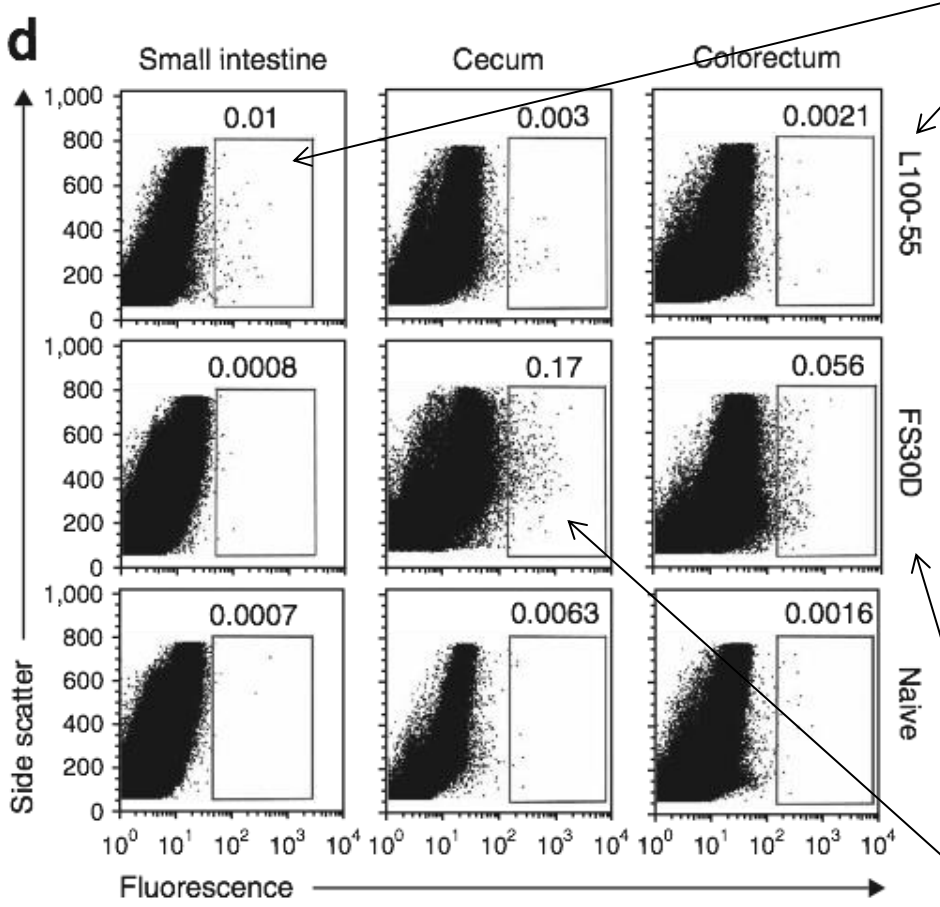
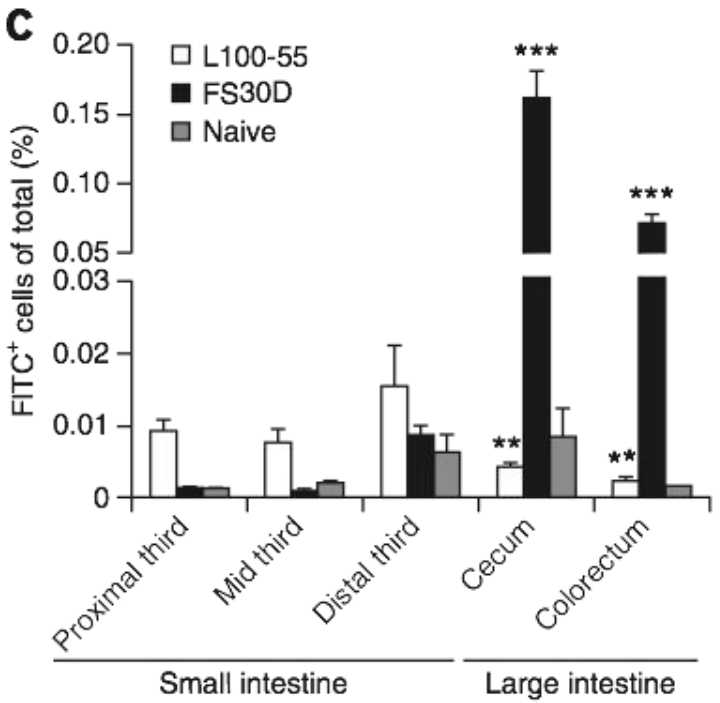


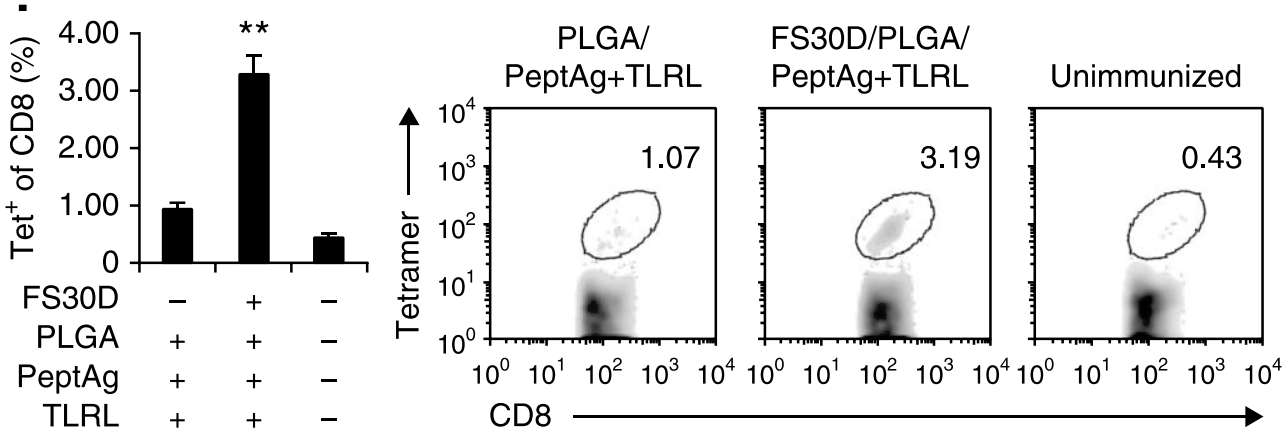


Eudragit polymer that dissolves at pH > 7.0
(only reached in terminal ileum)

Zhu, Q. *et al. Nat Med* **18,** 1291–1296 (2012).

Targeting of vaccines to the large intestine

Orally delivered FS30D-coated PLGA nanoparticle peptide vaccine



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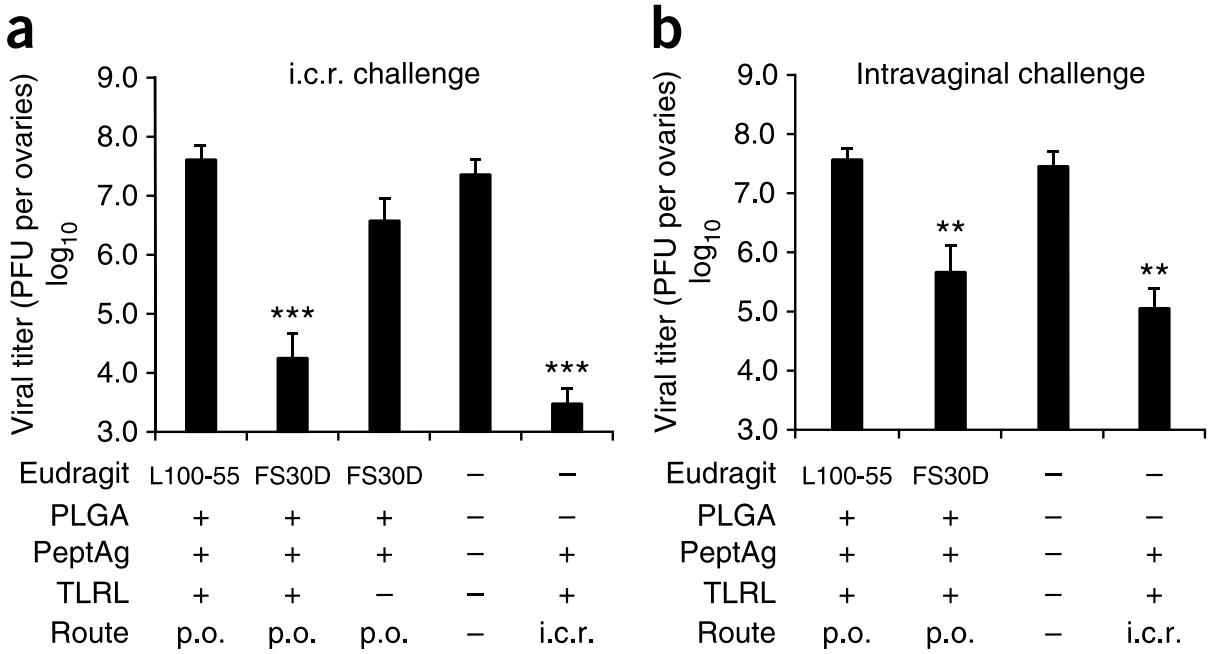
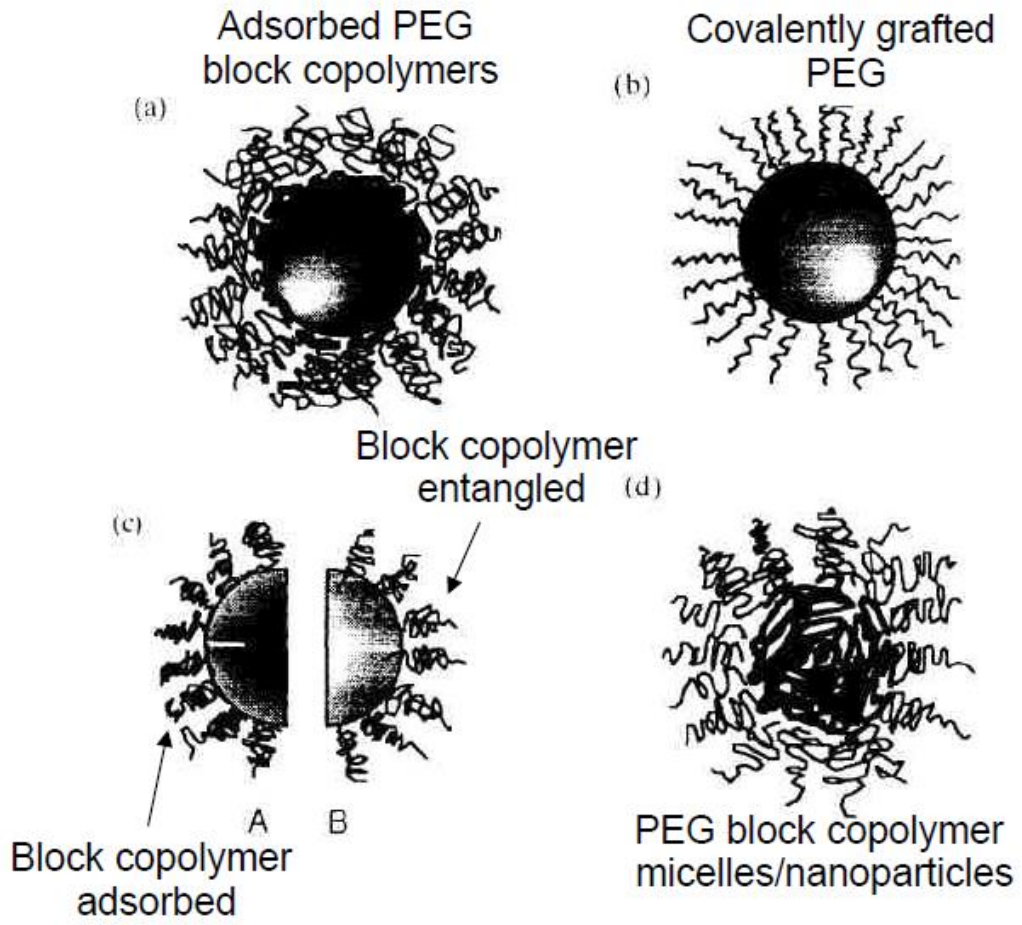


- direct intracolorectal (i.c.r.) administration
- p.o., per os (oral)

Zhu, Q. *et al. Nat Med* **18,** 1291–1296 (2012).

PEGylation of nanoparticles



Stolnik et al. 1995

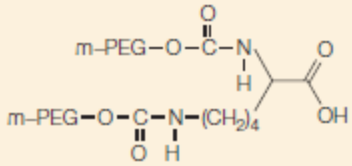
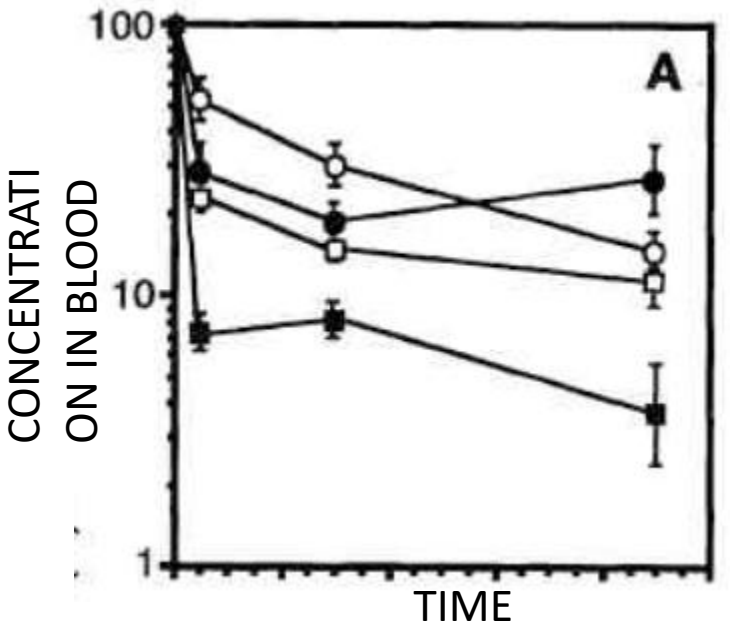
Linear PEG-OH	$H-(OCH_2CH_2)_n-OH$
Linear m–PEG-OH	$CH_3-(OCH_2CH_2)_n-OH$
Branched m–PEG_2	

Figure 1 | **Structural formulae of polyethylene glycol (PEG) molecules.** m–PEG, monomethoxy–PEG.

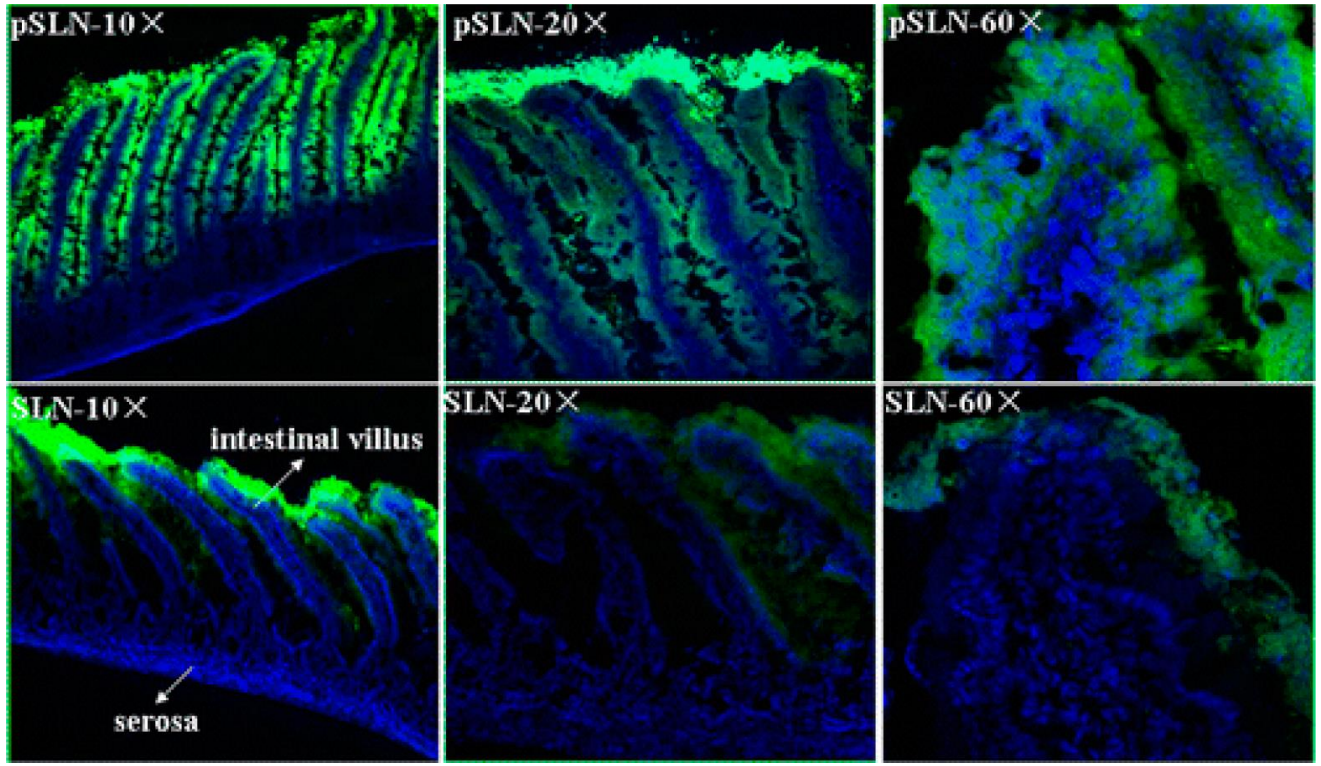
Nat. Rev. Drug Discov. **7**, 771-782 (2008).



Greg, Langer et al. Science 263 1600-1603 (1994)

Gastrointestinal Tract

PEGylated nanoparticles



PEGylation of polystyrene nanoparticles enabled widespread nanoparticle penetration of the intestinal tract in both healthy and ulcerative colitis mouse models

PEGylation has also been demonstrated to enhance the intestinal tract permeation of solid lipid nanoparticles (SLNs).

Mol. Pharm. **10**, 1865-1873 (2013).

Oral vaccines

Table 1 | **Licensed mucosal vaccines**

Vaccine	Trade name (developer)	Composition	Dosage and formulation	Mechanism of protection	Efficacy	Refs
Influenza type A and B viruses	FluMist (MedImmune)	Live viral reassortant with trivalent mix of H1, H3 and B strains of haemagglutinin and neuraminidase genes in an attenuated donor strain	Intranasal in young children, 2 doses	Haemagglutinin- and neuraminidase-specific mucosal IgA and systemic IgG responses; possibly a role for cell-mediated immunity; heterotypic protection effective in children	>85% in children, variable in adults	9,34, 37,81
H1N1 influenza virus (swine flu)	NASOVAC (Serum Institute of India)	Monovalent live attenuated vaccine	Intranasal spray, 1–2 doses	Haemagglutinin- and neuraminidase-specific mucosal IgA and serum IgG antibodies; possibly cell-mediated immunity	Unavailable	148
Rotavirus	RotaTeq (Merck); Rotarix (GlaxoSmithKline)	Monovalent, live attenuated human rotavirus and multivalent animal–human reassortant rotavirus	Oral, 3 doses	Mucosal IgA and systemic neutralizing IgG antibodies specific for homotypic or heterotypic VP4 and VP7 antigens	>70–90% against severe disease	27,38, 39,42
Poliovirus	Many	Trivalent, bivalent and monovalent vaccines	Oral, 3 doses	Mucosal IgA and systemic IgG neutralizing antibodies	>90% in most of the world	25,149, 150
Salmonella Typhi	Vivotif (Crucell); Ty21A	Live attenuated S. Typhi (Ty21A)	Oral, 3–4 doses of Ty21A	Mucosal IgA and systemic IgG antibody responses and CTL responses	Variable, but >50%	3,4,25,29, 35,48,151
Vibrio cholerae	Orochol (Crucell)	Live recombinant vaccine lacking CTA (CVD 103HgR)	Oral, single dose	Vibriocidal antibodies (possibly not the main effector mechanism but correlate well with protection)	Poor effect in a field trial	4,5,22,36, 43–47,114
Cholera	Dukoral (Crucell); Shanchol (Shantha Biotechnics)	Whole killed *Vibrio cholerae* O1 classical and El Tor biotypes with (Dukoral) or without (Shanchol) CTB	Oral, 2–3 doses	Gut antitoxin- and CTB-specific IgA and antibacterial and LPS-specific antibodies	Strong herd protection; >85% short term; >60% 3–5 years	4,5,22,36, 43–47,114

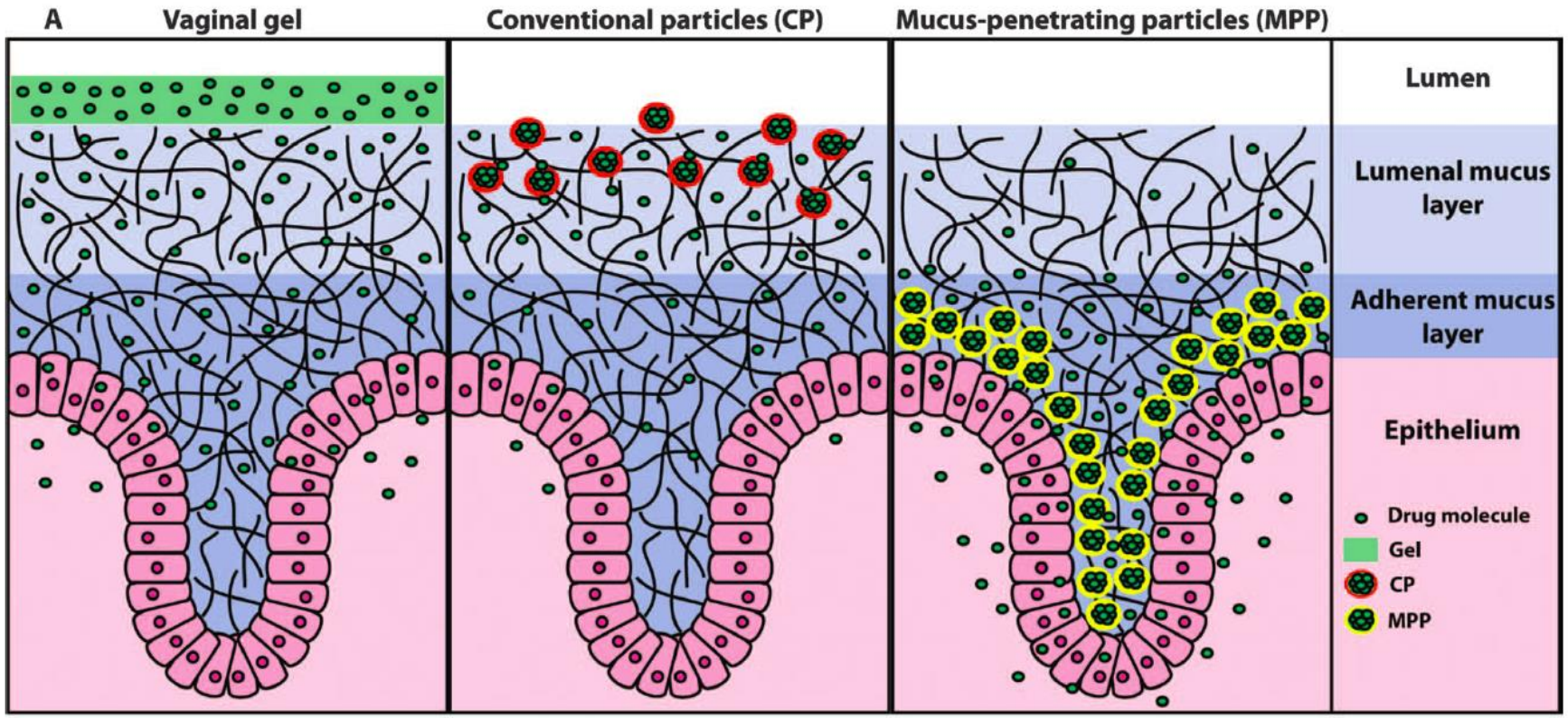
CTA, cholera toxin subunit A; CTB, cholera toxin subunit B; CTL, cytotoxic T lymphocyte; LPS, lipopolysaccharide.

Nat. Rev. Immunol. **12**, 592-605 (2012).

Reproductive Tract

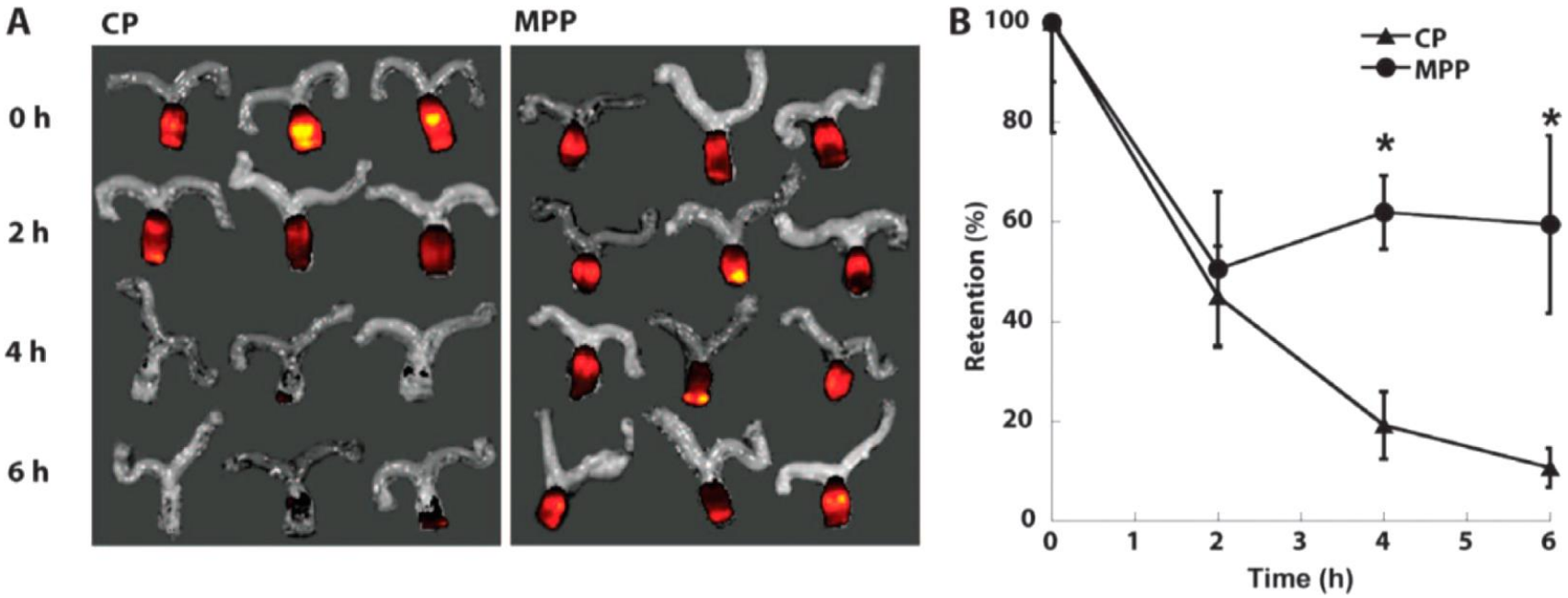
There are three significant barriers to effective vaginal delivery of immunotherapies:

- penetrating mucus
- crossing the epithelial layer
- subsequently trafficking to the draining lymph node



Sci. Transl. Med. 2012, 4, 138ra79.

Reproductive Tract



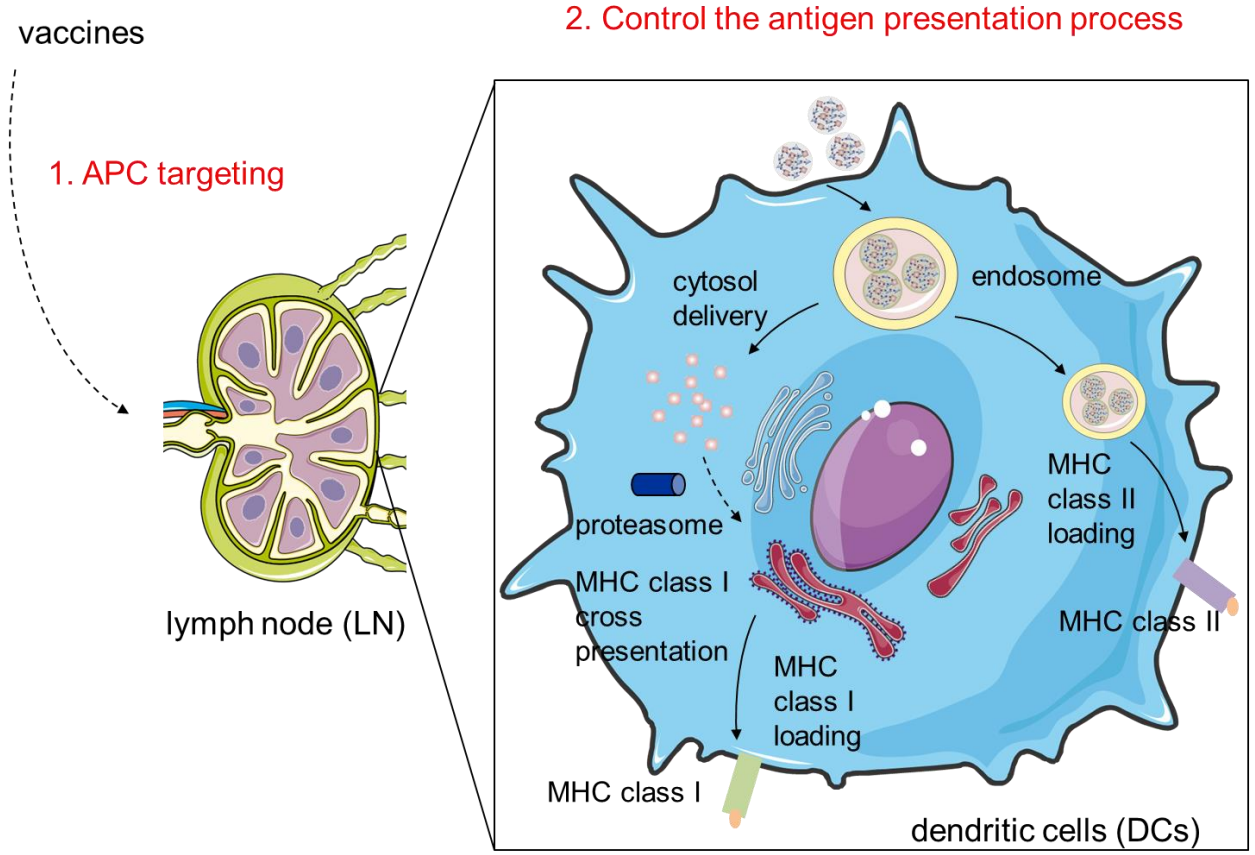
- low molecular weight PEG at high surface-coating densities penetrates cervical–vaginal mucus (CVM), but high molecular weight PEG adheres to CVM in vitro
- conventional **polystyrene nanoparticles (CPs)** Vs. CPs with a **high-density coating of low molecular weight PEG** to be mucus-penentrating particles (MPPS)
- MPPs were uniformly distributed across the murine vaginal epithelium, while CPs were aggregated in vaginal mucus.
- MPPs were retained in the cervicovaginal tract, whereas CPs were almost entirely flushed out in 6 h

Sci. Transl. Med. 2012, 4, 138ra79.

To achieve an effective vaccine with engineered biomaterials

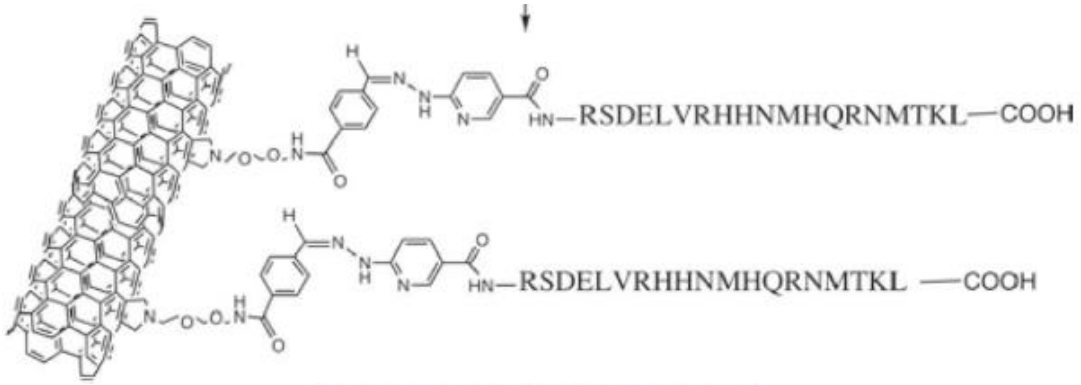
Using Biomaterials to control the trafficking of vaccines for APC targeting

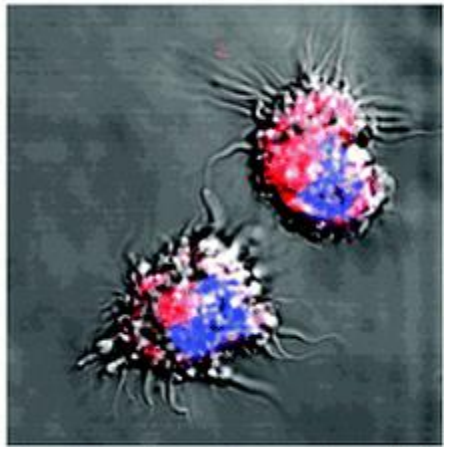
Using Biomaterials to control intracellular delivery and antigen presentation



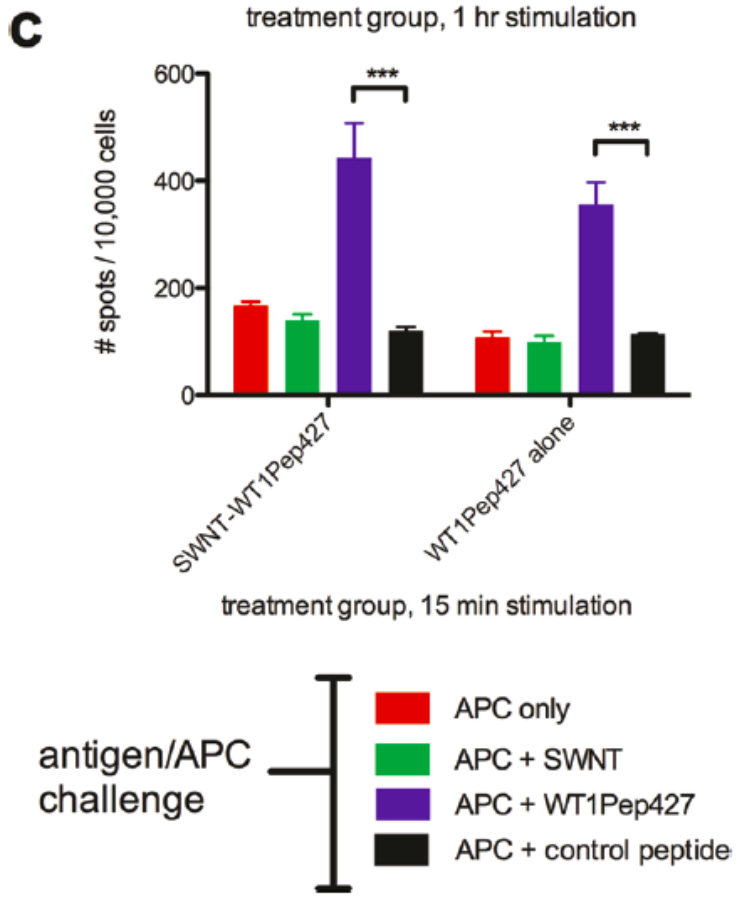
Enhancing Antigen Presentation to CD4+ T Cells

nanoparticle carriers can promote antigen delivery to APCs for CD4+ T cell priming





single-walled carbon nanotubes (SWNT): Mice immunized with 50−400 nm peptide conjugated carbon nanotubes along with a water-in-oil emulsion adjuvant induced peptide-specific IgG responses that were not present following immunization with the peptide and adjuvant alone.

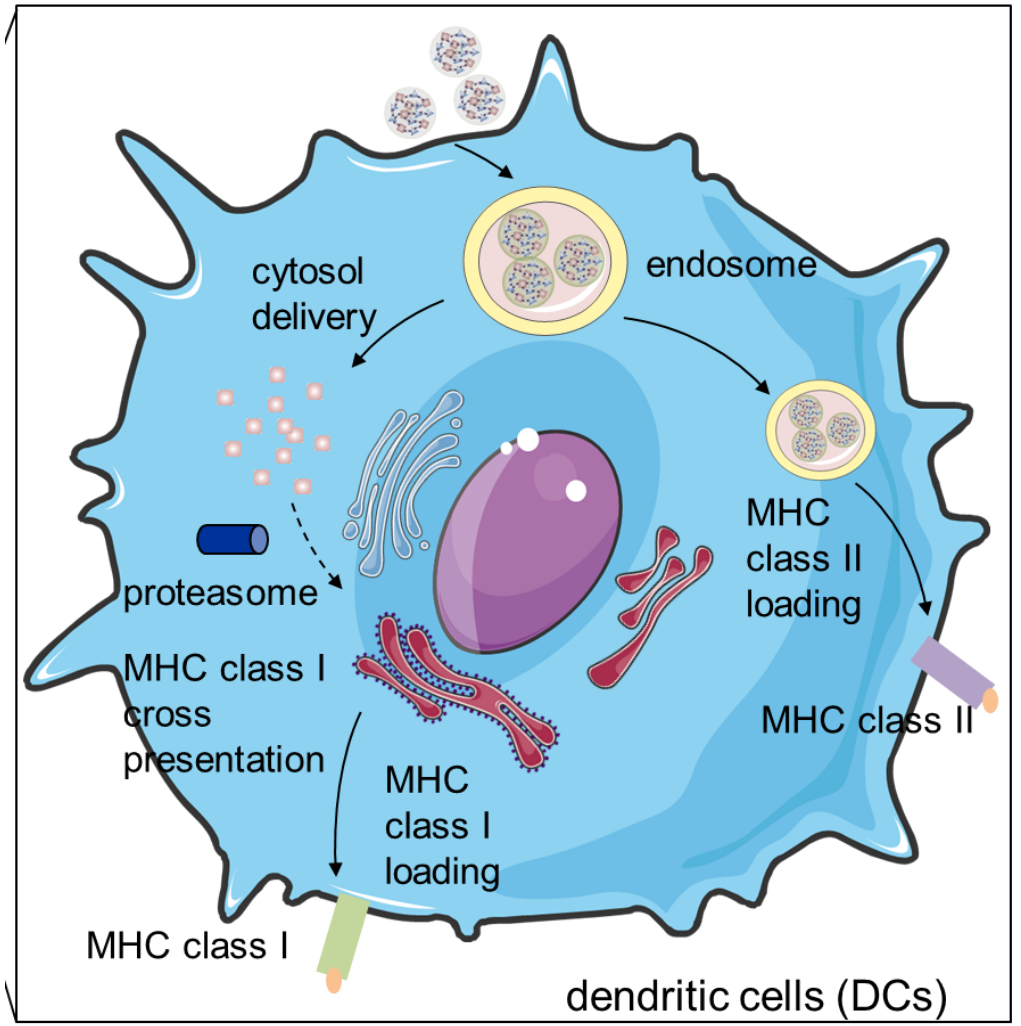


CD4 T cells from a healthy donor were stimulated twice with APCs that were pulsed with SWNTs, WT1Pep427, or WT1Pep427/SWNT

ACS Nano **5**, 5300-5311 (2011).

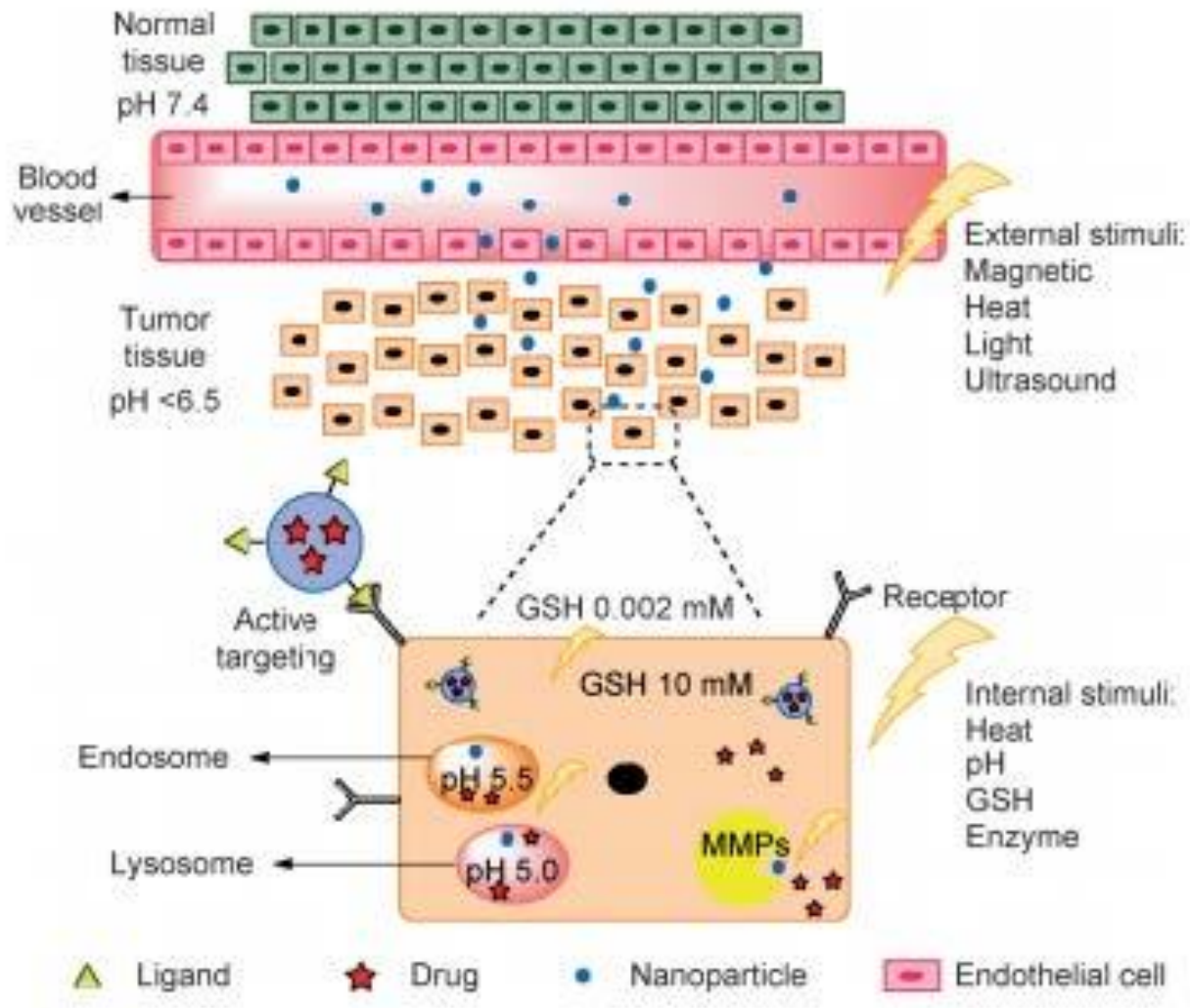
Enhancing cross presentation: Cytosolic delivery of large macromolecules

- CD8 T cell response is required to eradicate some infected cells (e.g., HIV) or tumors
- typically only live infections elicit strong CD8+ T cell priming
- class I MHC molecules that present peptides to CD8+ killer T cells are usually only loaded with antigens located in the cytosol of DCs
- **cross presentation**: APCs shuttle a fraction of the antigen to the cytosol or to deliver it to special vacuoles where class I MHC loading can occur



Chem. Rev. **115**, 11109-11146 (2015)

Intracellular vs. extracellular environment



AIMS Materials Science, 2016, 3(1): 289-323

Endosomal escape facilitated by biomaterials

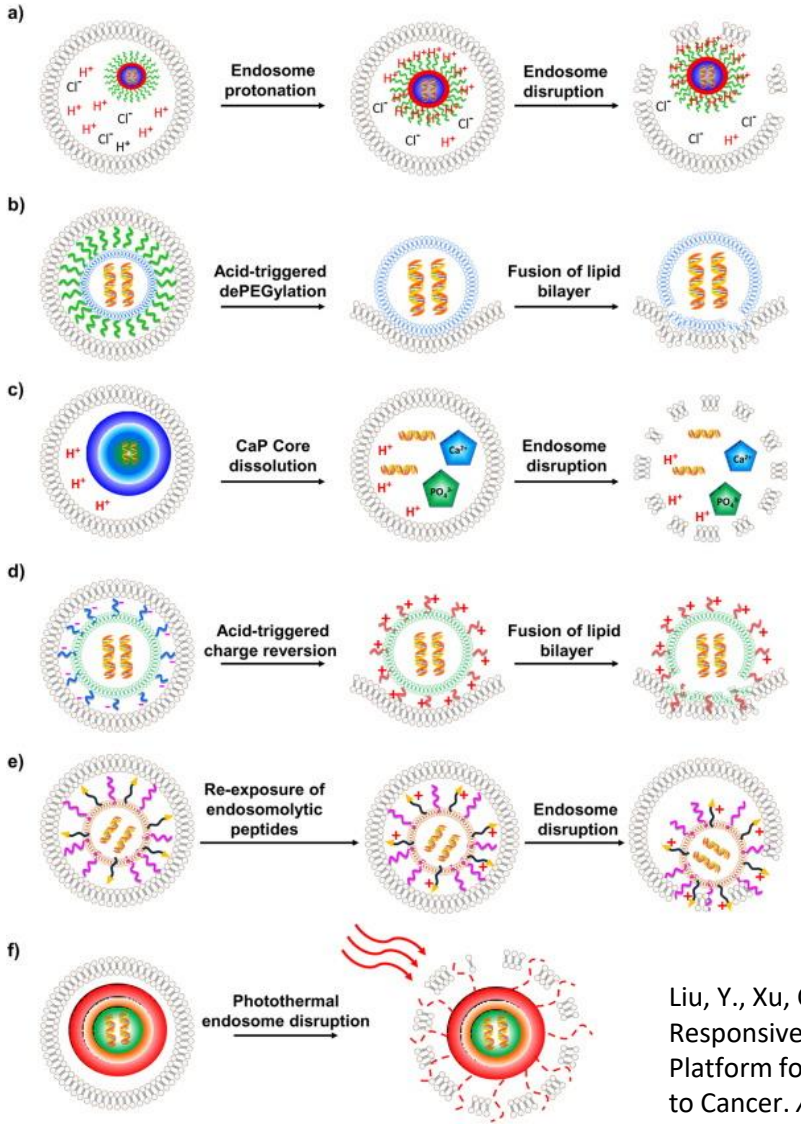
- pH-buffering effect (the proton sponge effect)
- direct membrane interactions: fusion or disruption

This slide is not required.

Table 1
Endosomal escape agents.

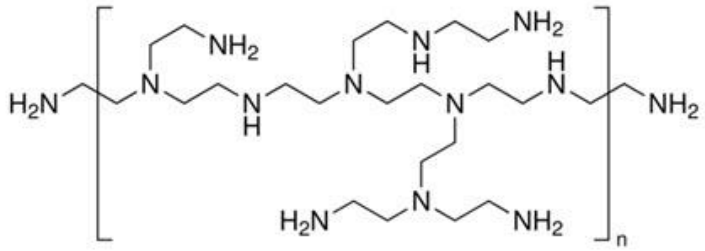
Category	Agent	Mechanism of endosomal escape
Proteins and peptides	Virus derived agents	
	haemagglutinin (HA2) [50–52]	Fusion
	(HA2)/poly (L-lysine) (PLL) [53]	Fusion
	diINF-7 [54–58]	Fusion
	penton base [59]	Pore
	gp41 [60,61]	Pore/fusion
	gp41/polyethylenimine (PEI) [62]	Pore/fusion/proton sponge
	TAT [63–68]	Unclear
	L2 from Papillomavirus [69]	Fusion
	envelope protein (E) of West Nile virus [70]	Fusion
	Bacteria derived agents	
	listeriolysin O (LLO) [72–78]	Pore
	Pneumococcal pneumolysin (PLO) [79]	Pore
	Streptococcal streptolysin O (SLO) [79]	Pore
	Diphtheria toxin (DT) [80–83]	Fusion
	Pseudomonas aeruginosa exotoxin A (ETA) [84–88]	Pore
	Shiga toxin [89]	Pore
	cholera toxin [90]	Pore
	Plant derived agents	
	Ricin [90,91]	Unclear
	Saporin [92–94]	Unclear
	Gelonin [92–94]	Unclear
	Human / animal derived agents	
	human calcitonin derived peptide, hCT [95–98]	Unclear
	fibroblast growth factors receptor (FGFR3) [99,100]	Unclear
	Melittin [101–106]	Pore
	Synthetic peptides	
	(R-Ahx-R)(4) AhxB [107–110]	Unclear
	glycoprotein H (gpH) from herpes simplex [111]	Fusion
	KALA [112–115]	Fusion
	GALA [116–120]	Fusion
	Synthetic surfactants [121,122]	Fusion
	Penetratin (pAntp) [123,124]	Unclear
	R6-Penetratin with arginine-residues [125]	Unclear
	EB1 [126]	Unclear
	bovine prion protein (bPrPp) [127–129]	Pore
	Poly (L-histidine) [130–134]	Proton sponge
	Sweet Arrow Peptide (SAP), proline-rich [135,136]	Fusion
Chemicals		
	polyethylenimine (PEI) [137]	Proton sponge
	Poly(amidoamine)s (PAAs) [141–143]	Proton sponge
	poly(propylacrylic acid) (PPAA) [144]	Proton sponge
	ammonium chloride [145]	Proton sponge
	chloroquine [145]	Proton sponge
	methylamine [145]	Proton sponge

J. Controlled Release **151**, 220-228 (2011).

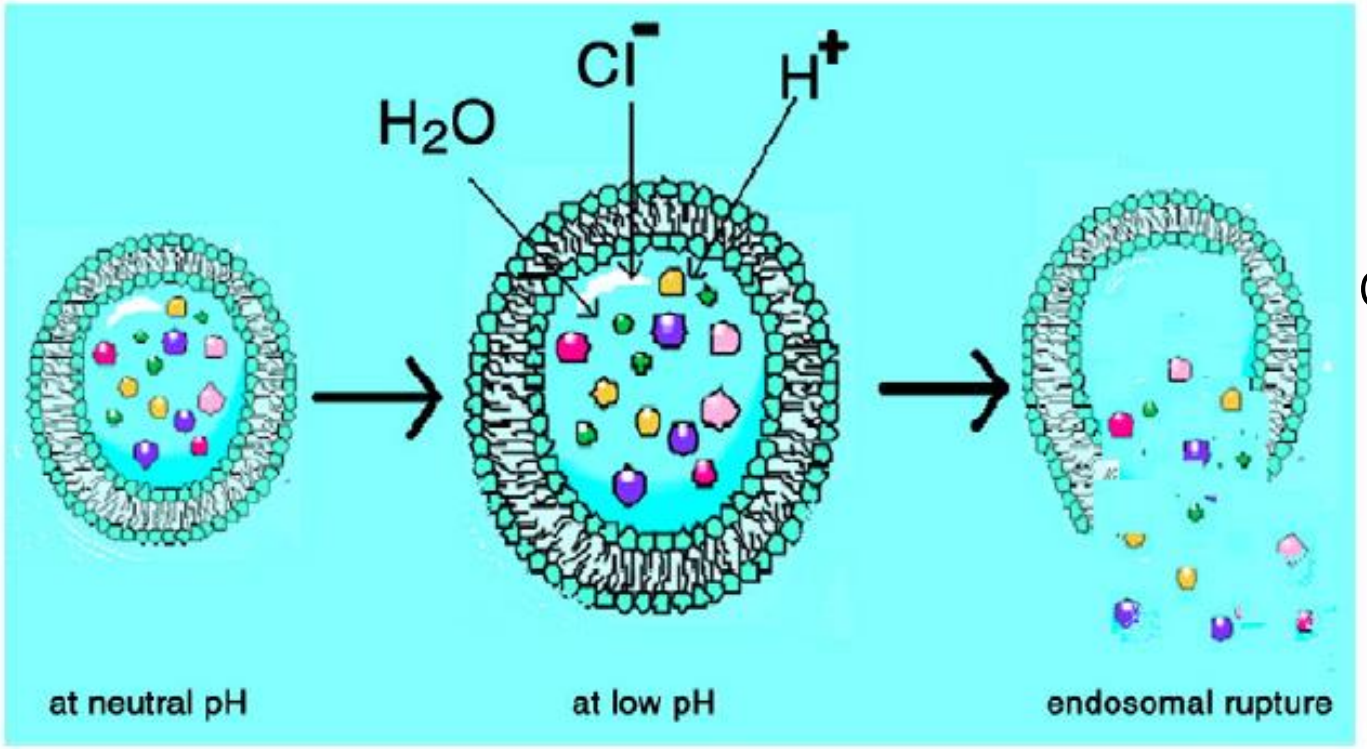


Liu, Y., Xu, C.-F., Iqbal, S., Yang, X.-Z. & Wang, J. Responsive Nanocarriers as an Emerging Platform for Cascaded Delivery of Nucleic Acids to Cancer. *Adv. Drug Deliv. Rev. 2017*

Endosomal escape facilitated by biomaterials



“proton sponge” effect



Osmotic pressure change

$-NH_2 \rightarrow -NH_3^+$

J. Controlled Release **151**, 220-228 (2011).

Intracellular release of cargos

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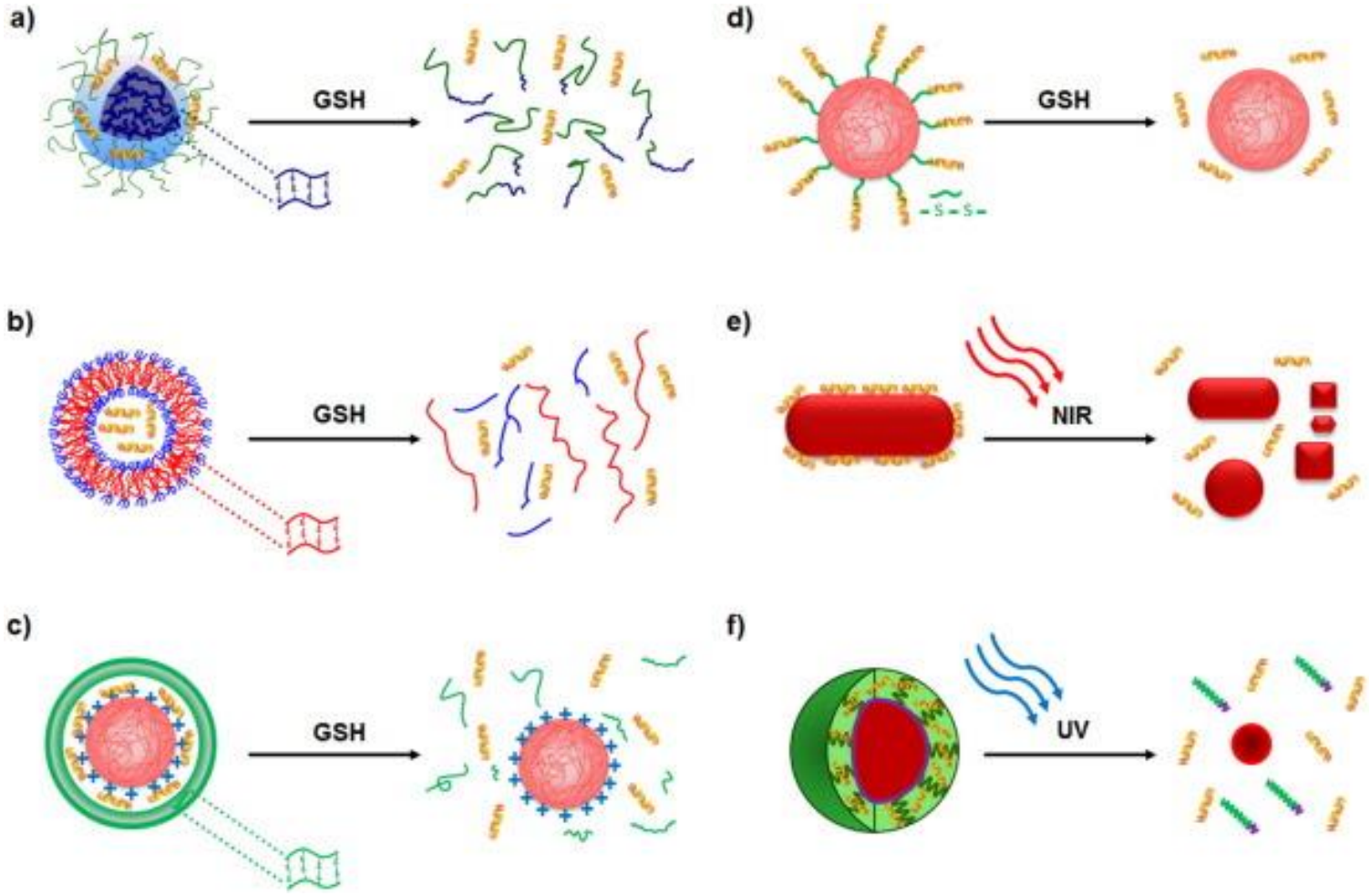
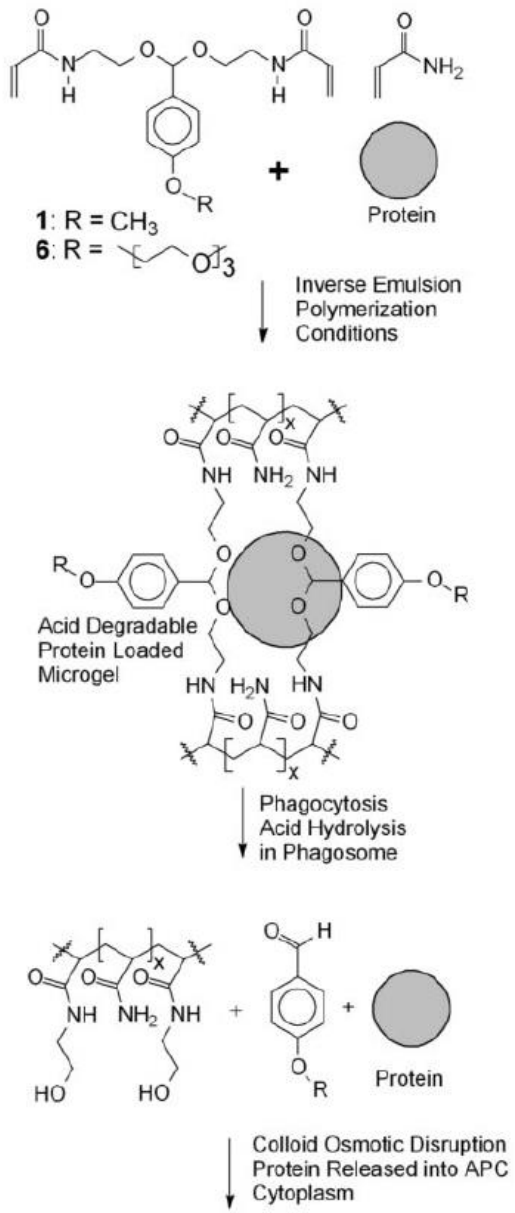
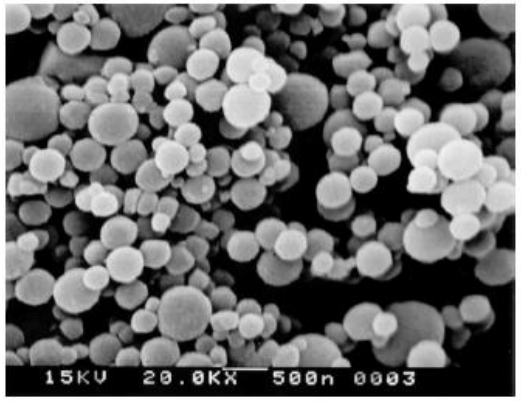
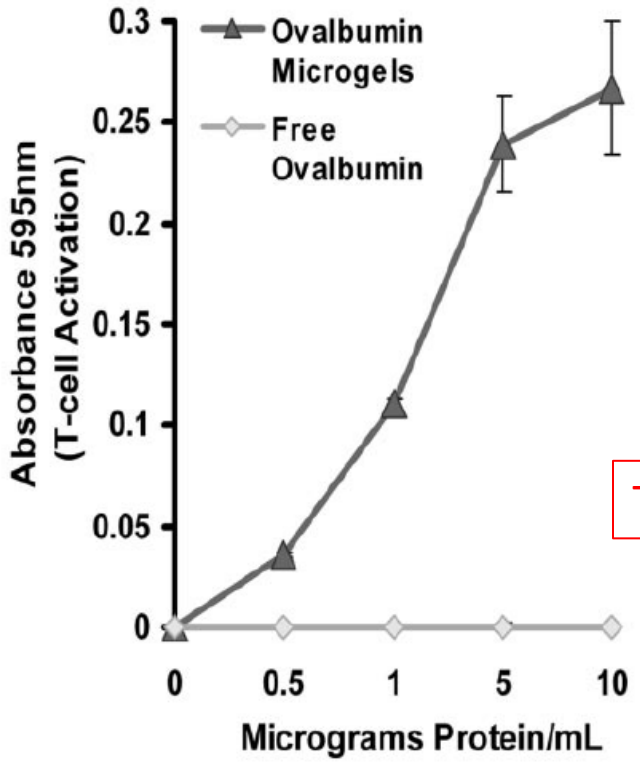


Liu, Y., Xu, C.-F., Iqbal, S., Yang, X.-Z. & Wang, J. Responsive Nanocarriers as an Emerging Platform for Cascaded Delivery of Nucleic Acids to Cancer. *Adv. Drug Deliv. Rev. 2017*

Endosomal escape facilitated by biomaterials







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Proc. Natl. Acad. Sci. **100**, 4995-5000 (2003)

pH responsive NP for cytosol delivery of antigen

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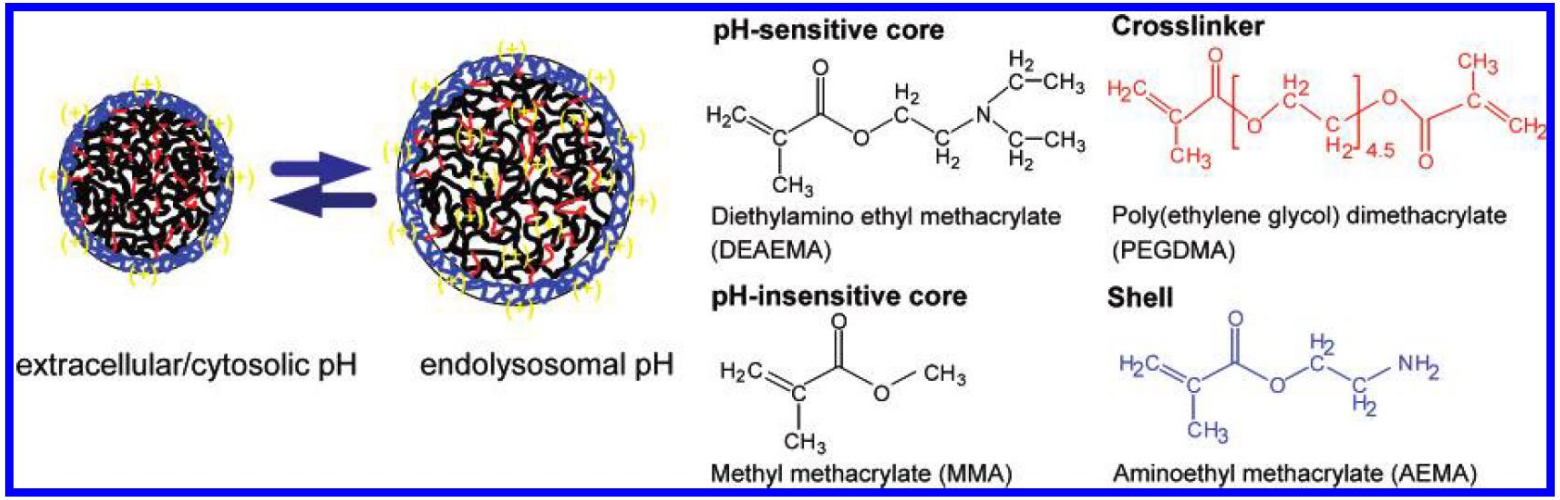
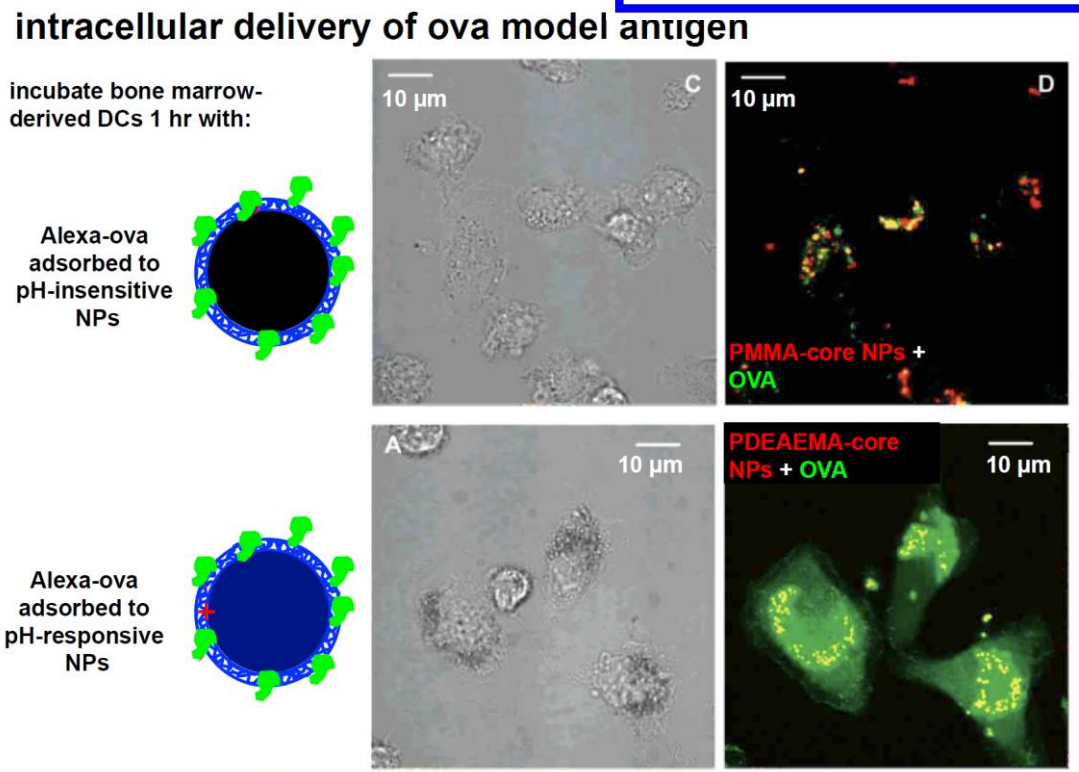
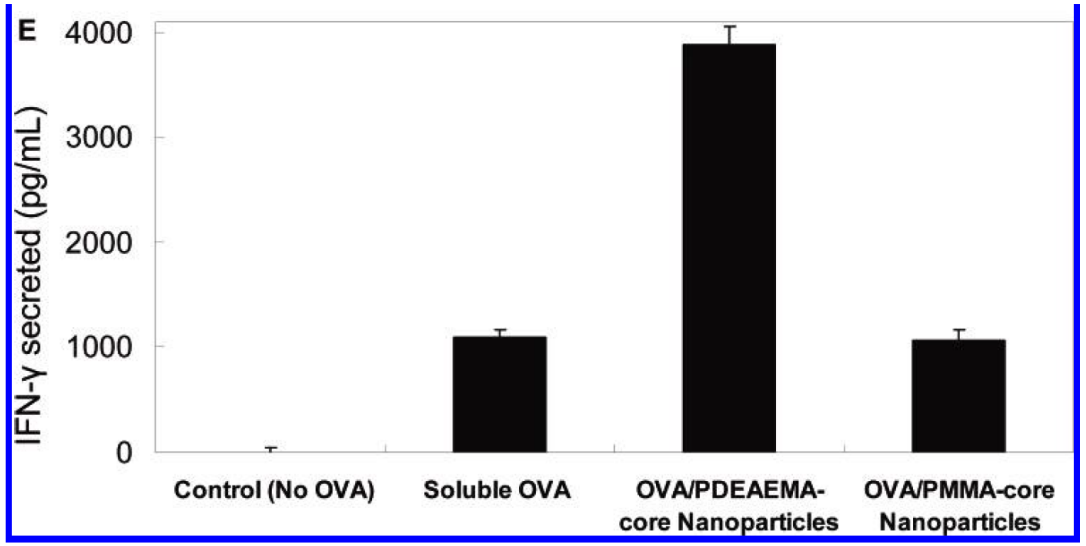


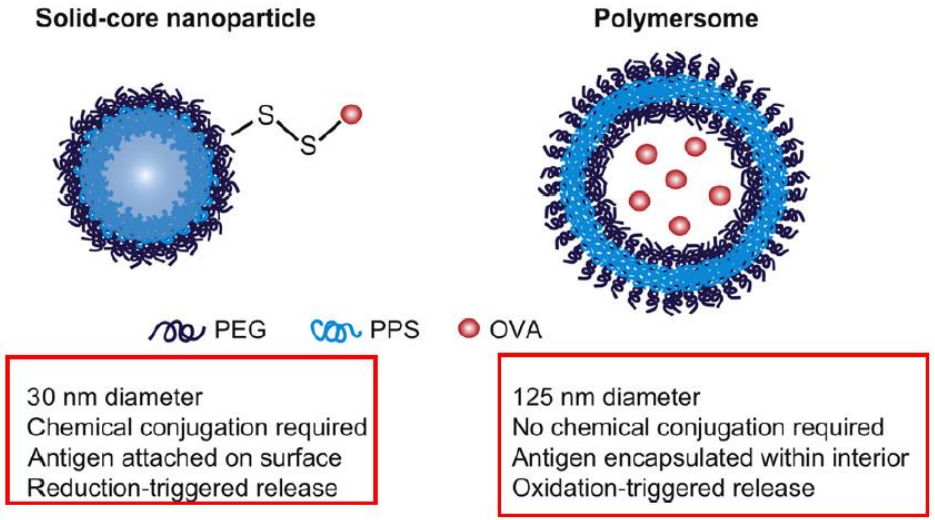


(Hu et al. *Nano Lett.* **7**(10) 3056-3064 (2007))



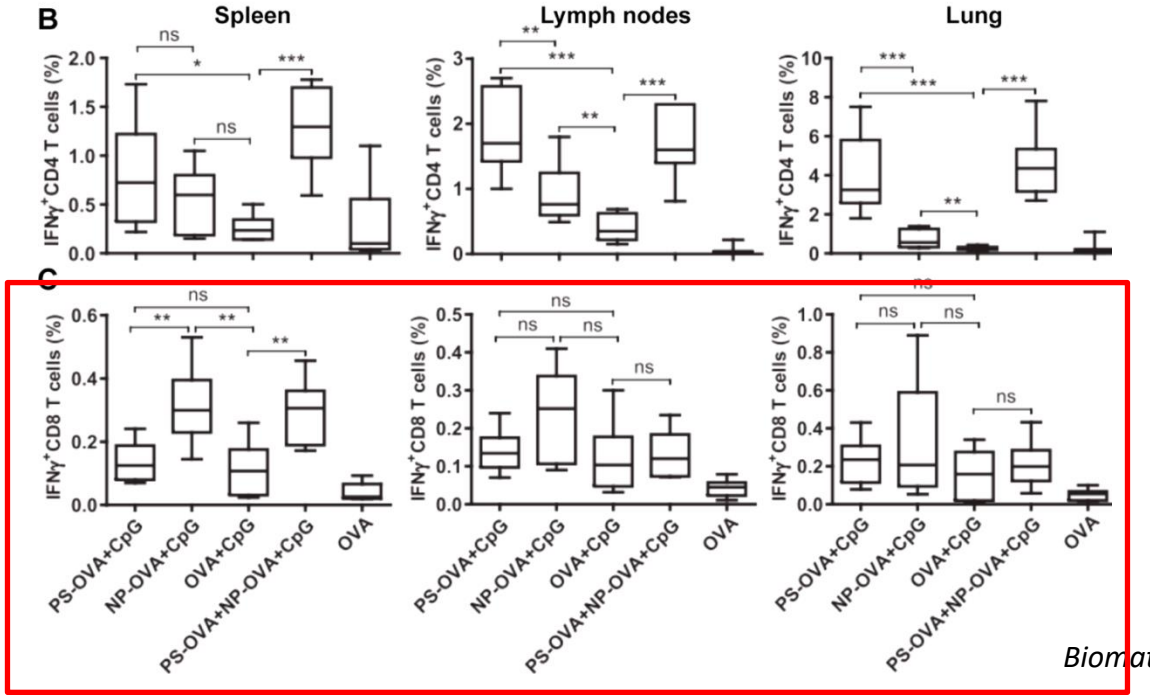
Nano Lett. **7**, 3056-3064 (2007).

Nanoparticles promote cross presentation for vaccines



OVA alone
OVA and CpG (OVA + CpG)
OVA loaded in PSs (PS-OVA + CpG)
OVA conjugated onto NPs (NP-OVA + CpG)
a mixture of both (PS-OVA + NPeOVA + CpG)

cross presentation



Biomaterials **34**, 4339-4346 (2013)