DRUG TARGETING

Focusing drug actions at target tissue sites

20.380 S10 workshop

What is drug targeting?

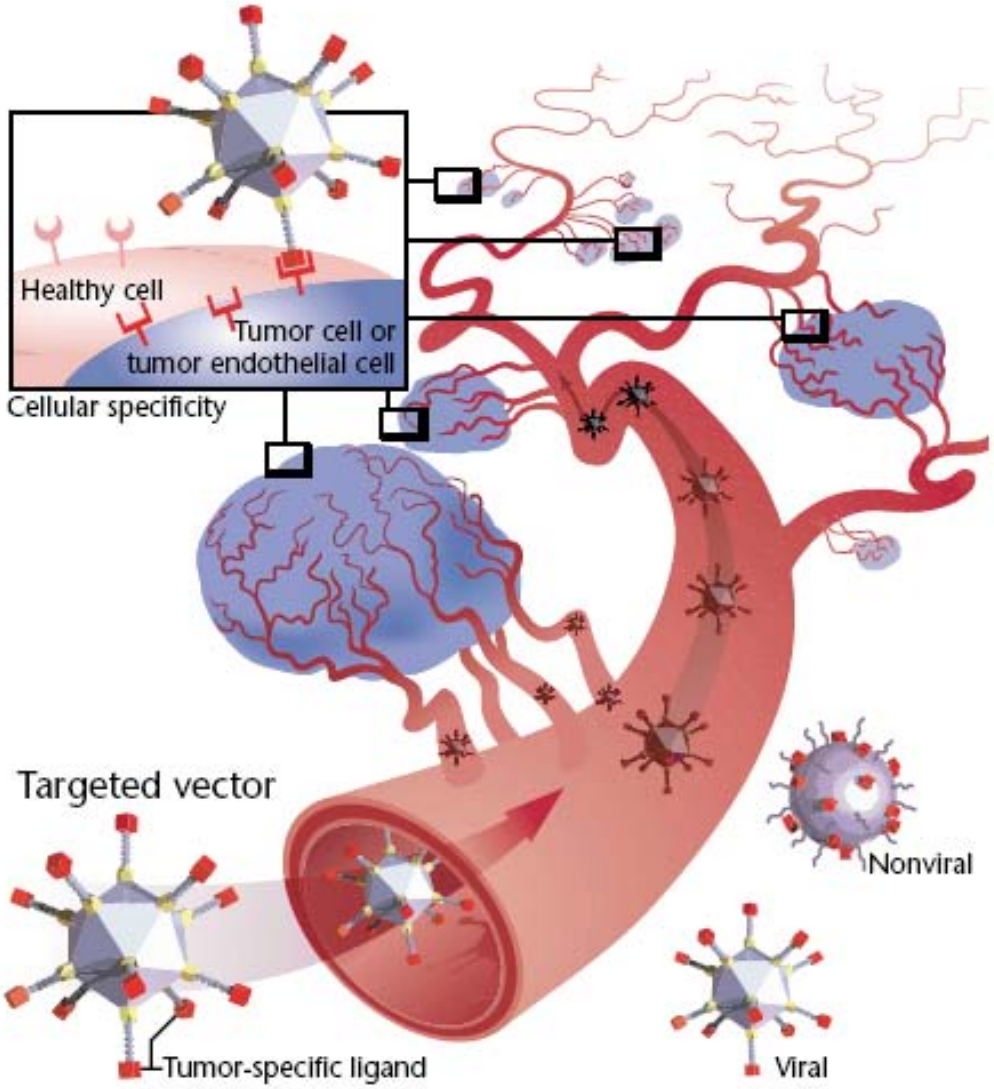


Fig. 1 One of the ultimate goals of targeted gene transfer is to engineer the vectors so that they can be administered through the circulation. When thus administered, the vehicle can potentially reach disease that is either disseminated (making injection difficult) or too small to be detected. These vehicles are now being modified to avoid interactions with their native receptors (for viral-based vehicles) and with blood components (antibodies or serum opsonizing proteins) that can bind to the particle and result in clearance in the lung or liver. Also incorporated into the vector are tumor-specific ligands that permit specific uptake into the tumor. The ligands recognize tumor cell-specific receptors, tumor endothelial-specific receptors, or even tumor matrix. Specificity may be further enhanced through the use of tumor cell–specific, tumor endothelium–specific, or radiation- or chemotherapy-induced promoters that drive gene expression.

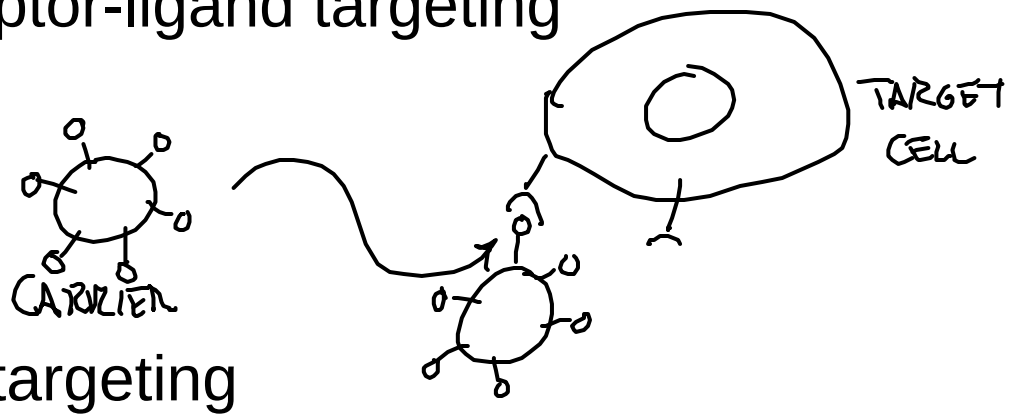
(Wickham, 2003)

Motivation for drug targeting: General

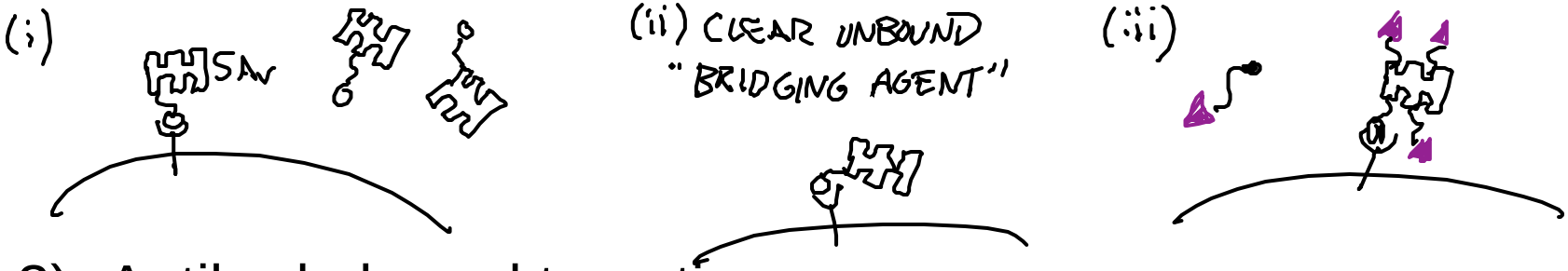
- Many drugs are toxic if delivered systemically:
  - Nonspecific radio/chemotherapeutic drugs
    - Top 6 chemotherapeutics nonspecifically kill proliferating cells
      - …thus lower doses used
      - …in cancer, tumor has time to mutate, leading to development of drug resistant tumors
  - Protein drugs may act specifically on many tissues distal to target tissue

Major approaches for targeted delivery

1) receptor-ligand targeting



2) Pre-targeting



3) Antibody-based targeting

4) “Reverse” targeting

Issues to consider:

- Where is the target molecule expressed?
  - Is it expressed by normal tissues?
  - Is it stably expressed?
    - Can select out evasive tumor cells/viruses

↳ SOME HEALTHY TISSUES CAN BE ABLATED IN AN ACCEPTABLE MANNER

- What is the affinity of binding?
- DOES BINDING TRIGGER ENDOCYTOSIS? COULD BE GOOD OR BAD
- immune response to targeting agent

↳ PARTICULARLY ACUTE FOR mAb TARGETING

	$t_{1/2}$ IN HUMANS
MOUSE Ab	<20 MIN
HUMAN Ab	12 d

(1) Receptor-ligand mediated targeting

Application	Cellular target	Molecular target	Targeting ligand	Ligand type
Anti-cancer therapy	Various tumor cells	Folate receptor EGF receptor	Folate EGF	Protein ligand for target receptor preferentially expressed on target cells
	Neovascular tissue	B-FN (fibronectin isoform)	anti-B-FN antibody	antibody against fibronectin isoform only expressed during embryonic development and in aggressive tumors
Anti-cancer therapy, pulmonary, cardiovascular, and inflammatory diseases	Endothelial cells	E-selectin P-selectin	sialyl Lewis^X receptor	receptor expressed at sites of inflammation
Anti-cancer therapy (leukemias and B cell lymphomas)	Transformed B lymphocytes	CD20	Anti-CD20 antibody	Antibody against target cell-surface protein unique to target class of cells (e.g. B cells)
Anti-cancer therapy (T cell lymphomas)	Transformed T lymphocytes	IL-2Rα (interleukin-2 receptor a chain	Anti-IL-2Rα antibody	Antibody against target cell-surface protein not expressed on normal resting cells

Cytotoxic drugs
- AraC
- Doxorubicin

Anti-tumor cytokines
- Interleukin-2
- Interleukin-12

OVEREXPRESSED ON 95% OVARIAN CARCINOMAS

LOSS OF HEALTHY B CELLS OK: BONE MARROW STEM CELL TRANSPLANT (AUTOLOGOUS)

Table 1 | **Some ligands that have been used in LTTs**

Targeting ligands and antibodies	Alternative names (trade name)	Target	Example of tumour target	References
Non-antibody				
RGD		Cellular adhesion molecules, such as αvβ3-integrin	Vasculature endothelial cells in solid tumours	19
NGR		Aminopeptidase N (CD13)	Vasculature endothelial cells in solid tumours	100
Folate		Folate receptor	Cancer cells that overexpress the folate receptor	101,102
Transferrin		Transferrin receptor	Cancer cells that overexpress the transferrin receptor	103,104
GM-CSF		GM-CSF receptor	Leukaemic blasts	62
Galactosamine		Galactosamine receptors on hepatocytes	Hepatoma	92
Antibody				
Anti-VEGFR	2C3	Vasculature endothelial growth-factor receptor (FLK1)	Vasculature endothelial cells in solid tumours	105
Anti-ERBB2	Trastuzumab (Herceptin)	ERBB2 receptor	Cells that overexpress the ERBB2 receptor, such as in breast and ovarian cancers	7
Anti-CD20	Rituximab (Rituxan), ibritumomab tiuxetan (Zevalin)	CD20, a B-cell surface antigen	Non-Hodgkin's lymphoma and other B-cell lymphoproliferative diseases	8
Anti-CD22	Epratuzumab, LL2, RFB4	CD22, a B-cell surface antigen	Non-Hodgkin's lymphoma and other B-cell lymphoproliferative diseases	33,52
Anti-CD19	B4, HD37	CD19, a pan-B-cell surface epitope	Non-Hodgkin's lymphoma and other B-cell lymphoproliferative diseases	49,52
Anti-CD33	Gemtuzumab, ozogamicin (Mylotarg)	CD33, a sialo-adhesion molecule, leukocyte differentiation antigen	Acute myeloid leukaemia	37,67
Anti-CD33	M195	CD33, a T-cell epitope	Acute myeloid leukaemia	37
Anti-CD25	Anti-Tac, LMB2	CD25, α-subunit of the interleukin-2 receptor on activated T cells	Hairy-cell leukaemia, Hodgkin's and other CD25+ lymphoma haematological maliganancies	106
Anti-CD25	Denileukin diftitox (Ontak)	Interleukin-2 receptor	Cutaneous T-cell lymphoma	46,47
Anti-HLA-DR10β	Lym1	HLA-DR10β subunit	Non-Hodgkin's lymphoma and other B-cell lymphoproliferative diseases	32
Anti-tenascin	81C6	Extracellular-matrix protein overexpressed in many tumours	Glial tumours, breast cancer	107
Anti-CEA	MN-14, F6, A5B7	CEA	Colorectal, small-cell lung and ovarian cancers	28,108
Anti-MUC1	HMFG1, BrE3	MUC1, an aberrantly glycosylated epithelial mucin	Breast and bladder cancer	28,109
Anti-TAG72	CC49, B72.3	TAG72, oncofetal antigen tumour-associated glycoprotein-72	Colorectal, ovarian and breast cancer	28,110

CEA, carcinoembyonic antigen; GM-CSF, granulocyte–macrophage colony-stimulating factor; LTTs, ligand-targeted therapeutics; NGR, Asn–Gly–Arg tripeptide; RGD, Arg–Gly–Asp tripeptide; TAG72; oncofetal antigen tumour-associated glycoprotein-72; VEGFR, vascular endothelial growth-factor receptor.

Targeting agents may bring cargo to specific cell type or just localize the cargo at the tissue area

Example approach: receptor-ligand-mediated targeting to vasculature at sites of inflammation

Mimicking lymphocyte responses to inflammation:

Figure removed due to copyright restrictions.
See Figure 1 from Hogg, Nancy et al. "T-Cell Integrins:
More Than Just Sticking Points." *Journal of Cell Science* 116 (2003).

Example approaches: receptor-ligand-mediated targeting to vasculature

Diagram of mimicking lymphocyte responses to inflammation removed due to copyright restrictions.

(2) Pre-targeting drug delivery with bispecific antibodies

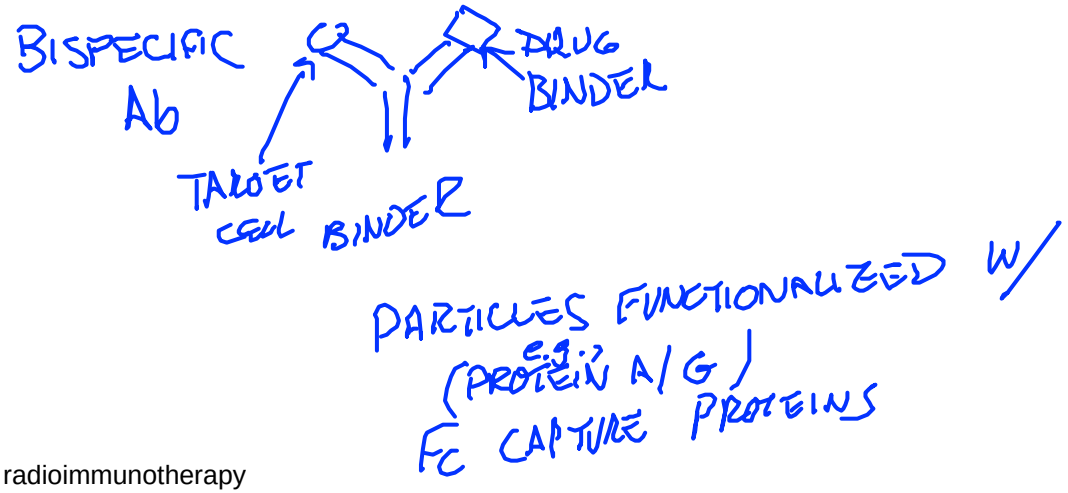


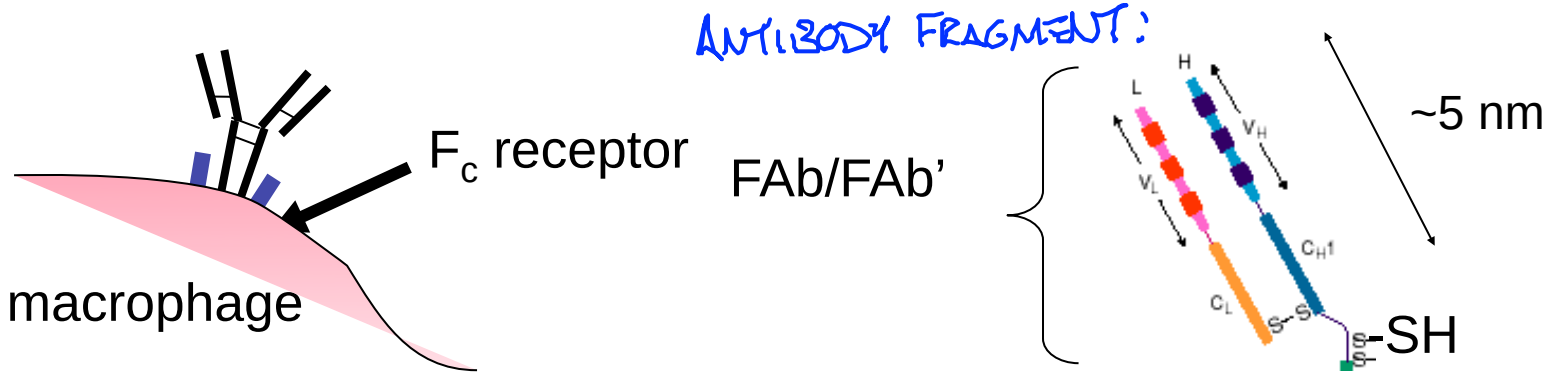
Figure showing schematic of three-step pretargeting radioimmunotherapy from Drugs of the Future journal removed due to copyright restrictions. See Figure 2 in Lam, L. X. Liu, and Y. Cao. "Pretargeted Radioimmunotherapy, A Potential Cancer Treatment." *Drugs of the Future* 28, no. 2 (2003).

(Cao and Lam, 2003)

(3) Antibody-based targeting

General structure of IgA, IgE, IgD, IgG:

Figure removed due to copyright restrictions.
See Figure 2 from Kalsi, Jatinderpal et al.
"Structure–Function Analysis and the Molecular Origins of Anti-DNA Antibodies in Systemic Lupus Erythematosus." *Expert Reviews in Molecular Medicine* 1, no. 7 (1999).



Generation of monoclonal antibodies against selected molecular targets

Figure showing the standard procedure for development of monoclonal antibodies removed due to copyright restrictions. See Figure 4-12 from "Immunology: Understanding the ImmuneSystem" by Klaus D. Elgert (1996).

MONOCLONAL: ALL Abs ARE IDENTICAL

PROS OF Ab TARGETING:

1. SPECIFICITY
2. HIGH AFFINITY POSSIBLE

TYPICALLY

K_D 0.1–100 nM

$t_{1/2}$ (37°C) ~ 30–60 min

(Elgert 1996)

Synthesizing antibodies which avoid recognition by the immune system

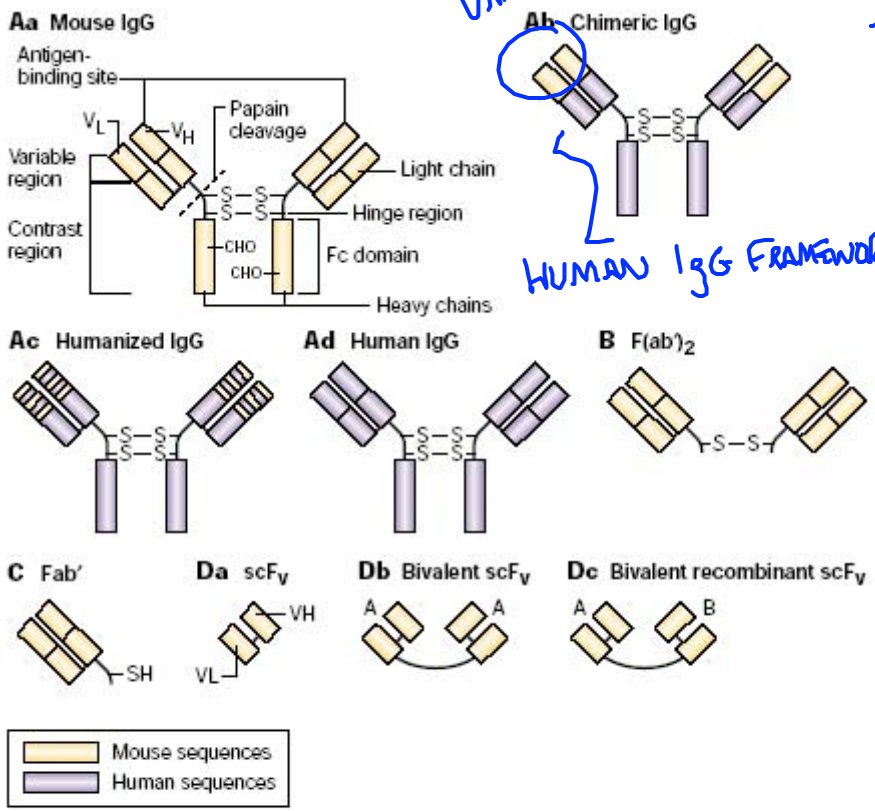


Figure 2 | **Antibodies and antibody fragments.** Targeting antibodies are normally monoclonal immunoglobulin G (IgG) (**Aa**) or IgG fragments (**B–D**). $F(ab')_2$ (**B**) or Fab' (**C**) fragments can be made by enzymatic cleavage of the whole monoclonal antibody (mAb) (**Aa**) or by molecular biological techniques — for example, Fab' (**C**), scF_v (**Da**), bivalent (**Db**) or recombinant fragments (**Dc**). mAbs that are made from the traditional hybridoma technique are murine in origin. Recent developments have led to improved techniques for the production of chimeric, humanized or fully human antibodies or fragments (**Ab–d**). V_H, variable heavy chain; V_L, variable light chain.

MOUSE VARIABLE REGIONS

HUMAN IgG FRAMEWORK

* CAN'T GENERATE FULLY HUMAN Abs IN MICE (YET)

↓

HOW TO MAKE 'HUMANIZED' ANTIBODIES

↓

RETAIN ONLY CRITICAL Ag-BINDING SEQUENCES

$t_{1/2}$ IN HUMANS:

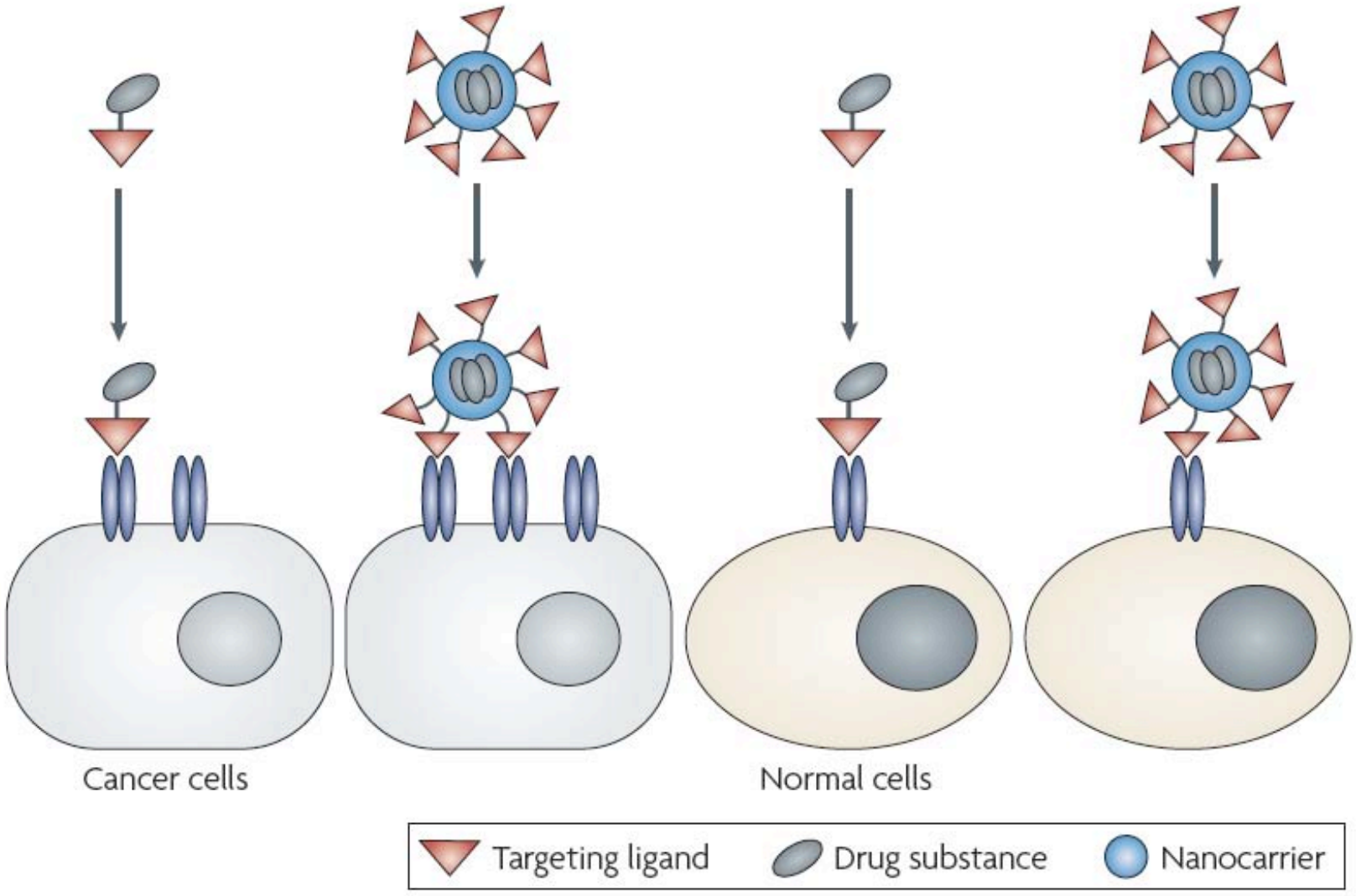
MOUSE Ab	< 20 MIN.
HUMAN IgG	12d

(Allen 2002)

Strategies for conjugation of antibodies to biomaterials

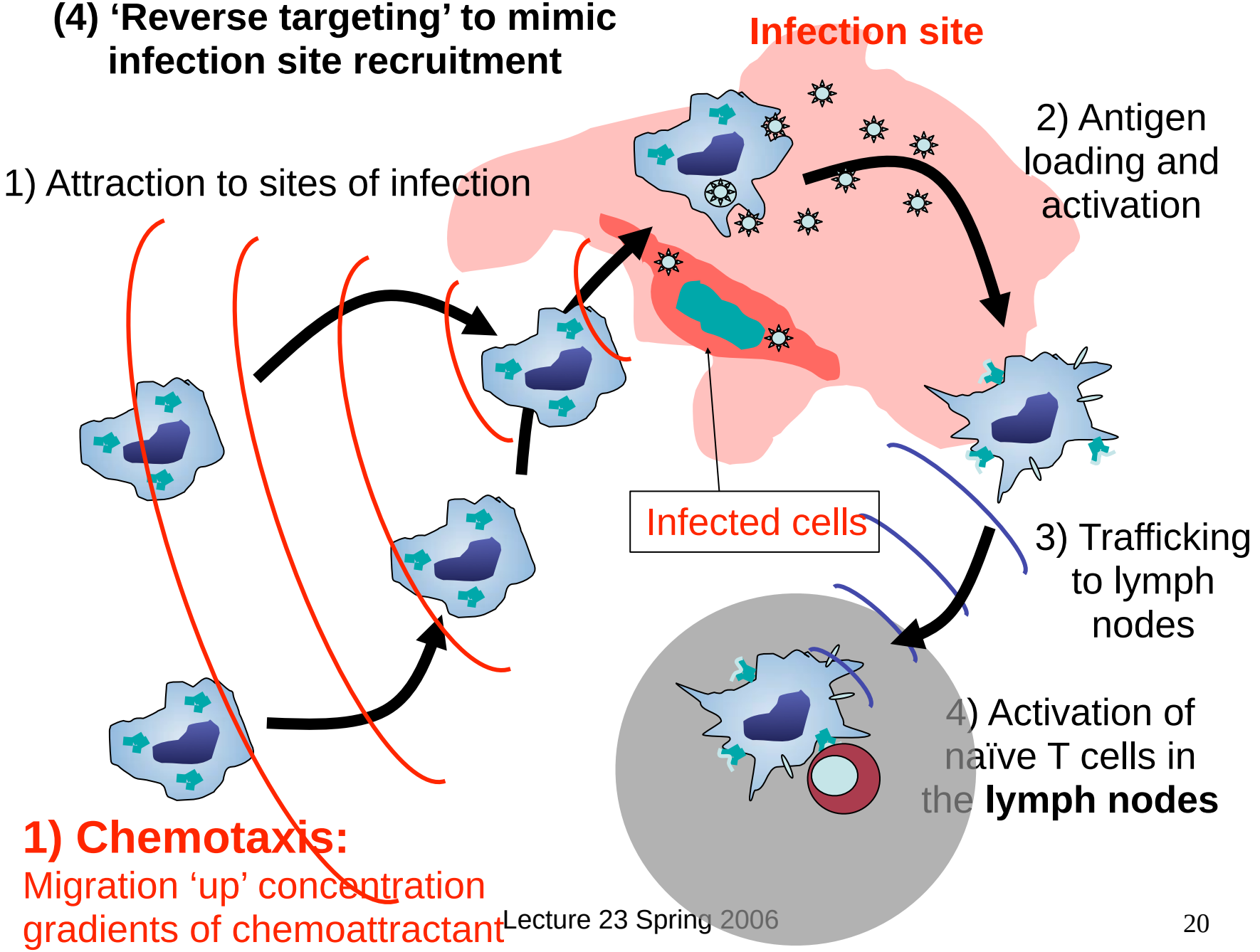
Figures showing pepsin and papain enzyme digestion of antibodies removed due to copyright restrictions.

Role of nanoparticle carriers in promoting multivalent interactions with targets

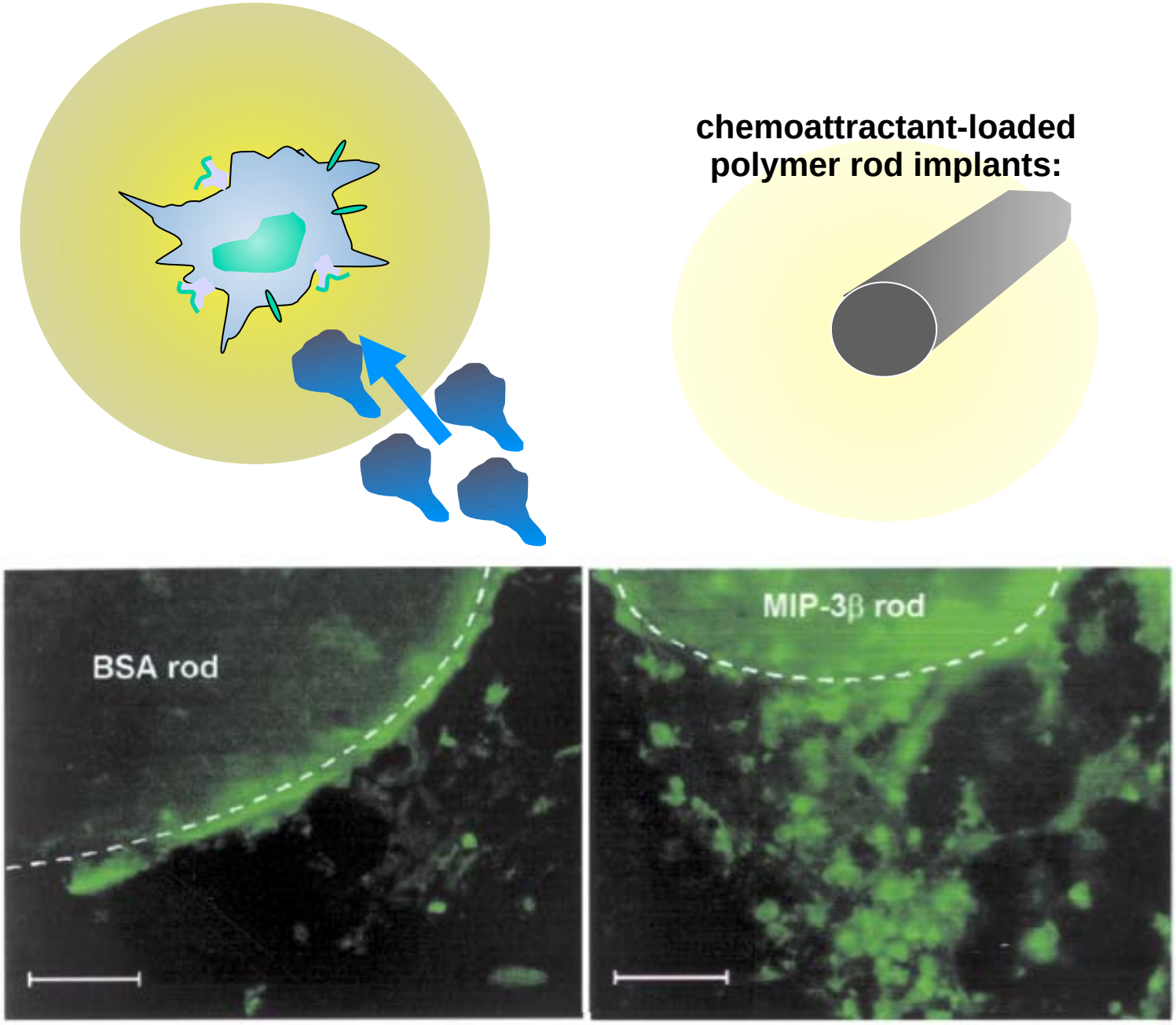


(Davis et al. Nat. Rev. Drug Disc. 7 771-782 2008)

(4) 'Reverse targeting' to mimic infection site recruitment



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

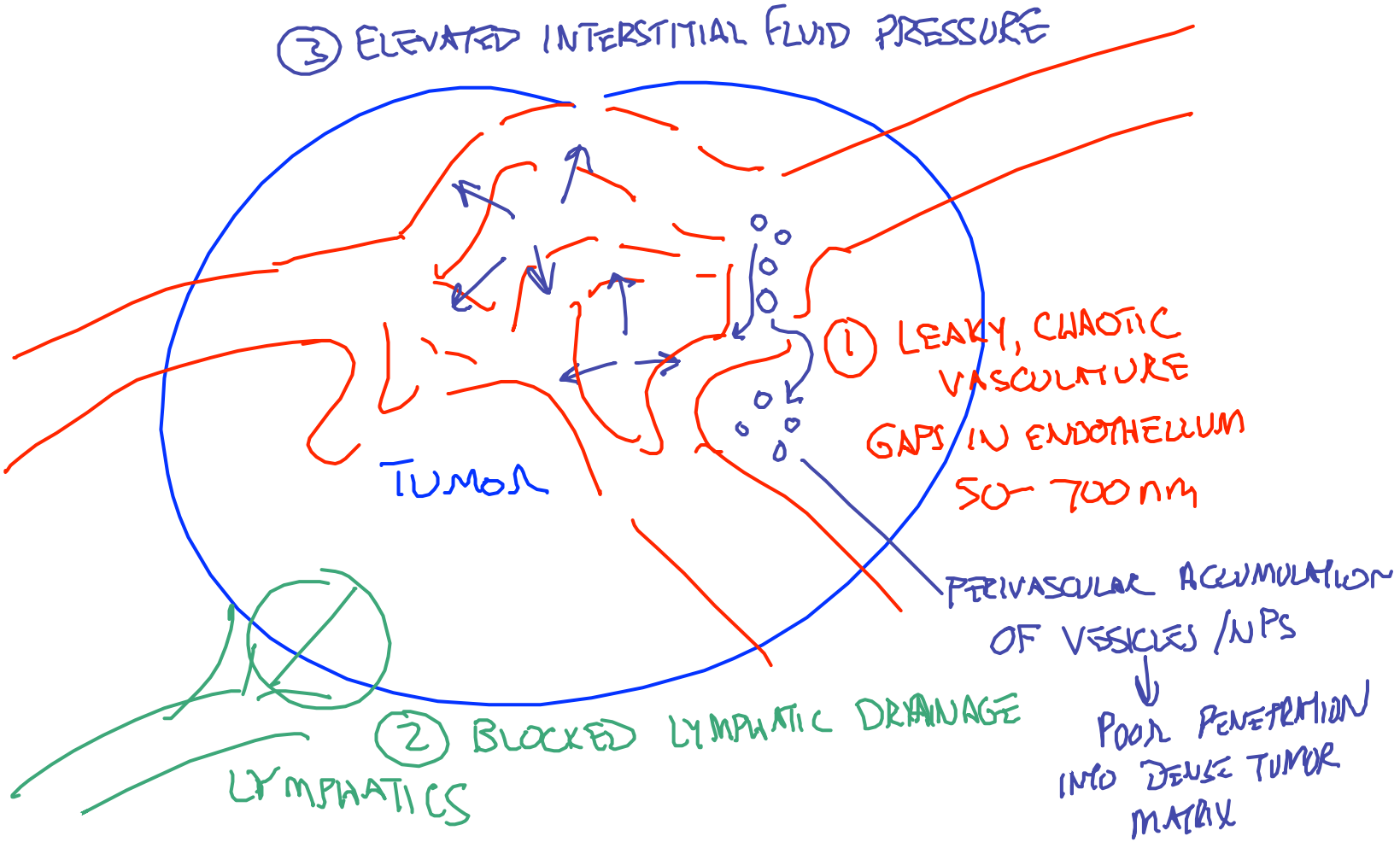


Kumamoto et al, *Nat. Biotech.* **20**, 64-69 (2002)

Electron micrograph images of dendritic cells and T-cells attracted to a tissue site removed due to copyright restrictions.

IMPACT OF TARGETING IN VIVO

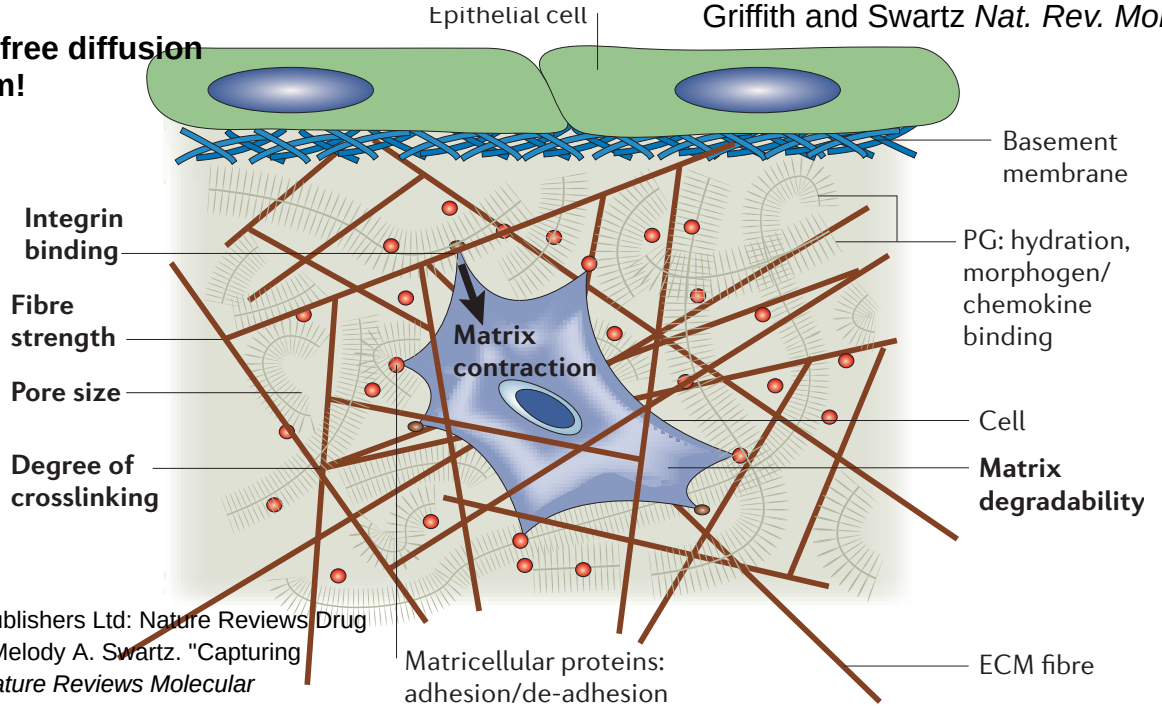
PASSIVE TARGETING OF TUMORS:
Enhanced permeation and retention (EPR) effect in tumors:

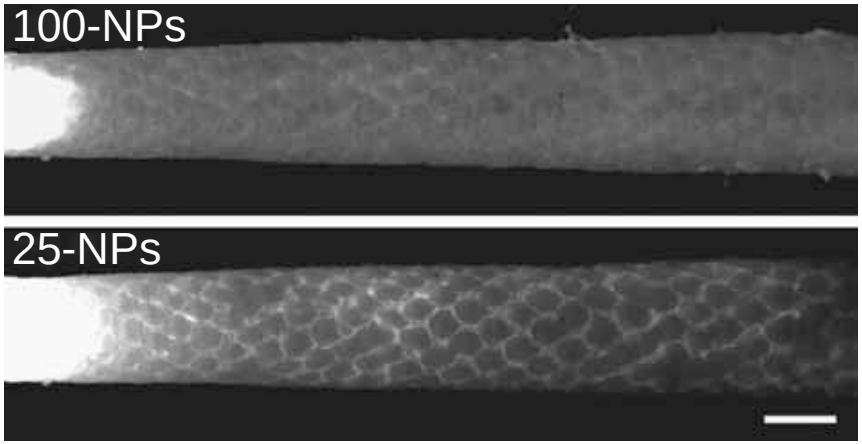


Key to remember: vasculature is not the only barrier to diffusion in vivo

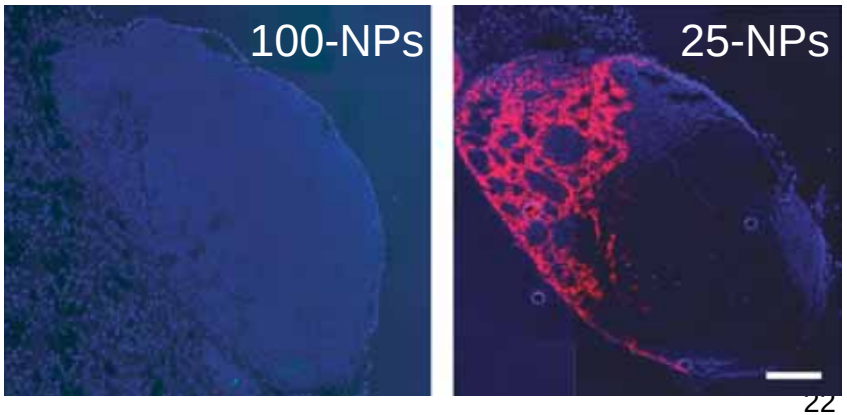
cut-off particle size for free diffusion through ECM ~40-50 nm!

Griffith and Swartz *Nat. Rev. Mol. Cell Biol.* **7** 211 (2006)

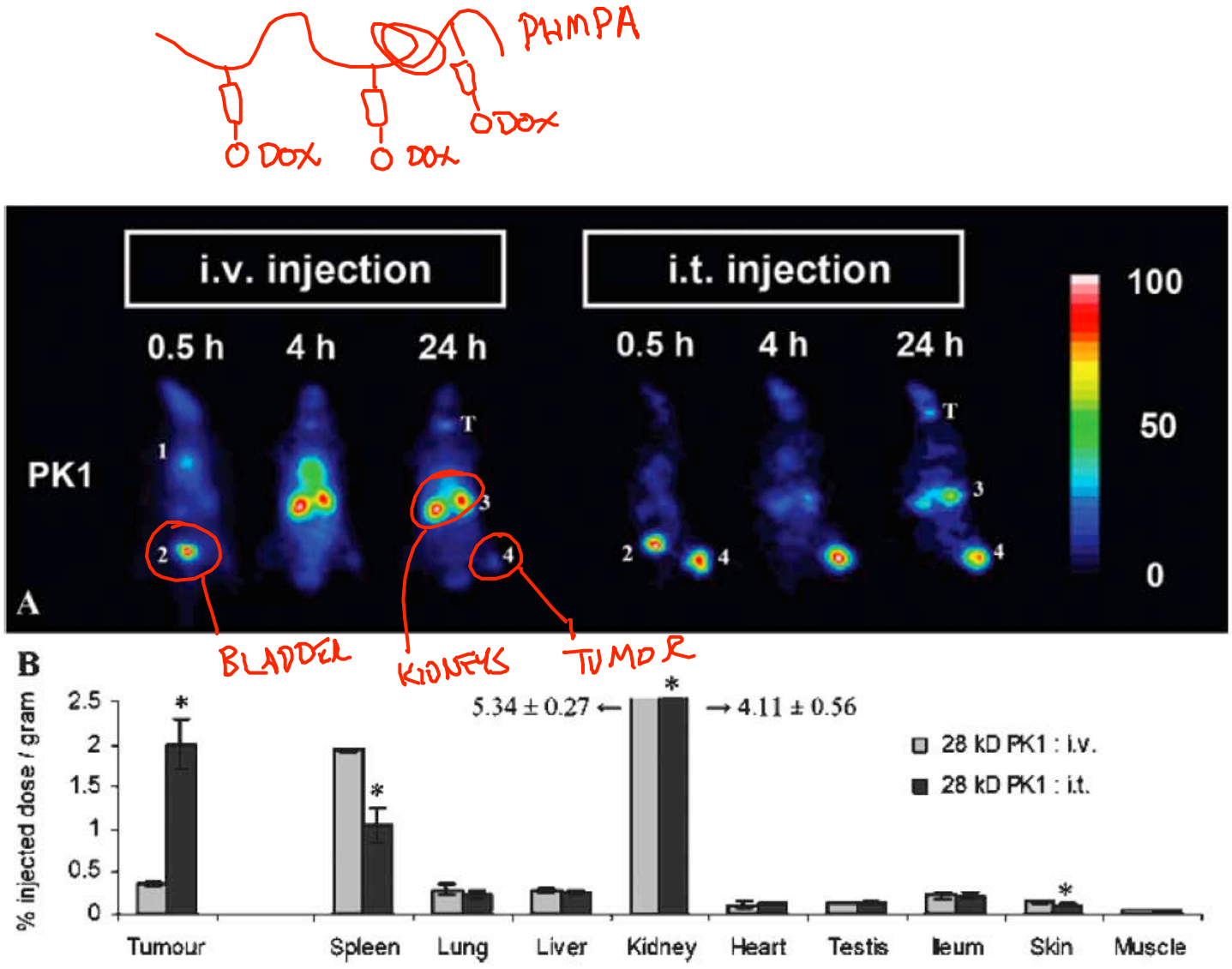




(Reddy, Hubbell, Swartz et al. *Nat. Biotech.* 2007)



Enhanced permeation and retention (EPR) effect in tumors:



(Lammers et al. *Neoplasia* **8** 788-795 (2006))

Results from mAb-targeting: targeting tumors

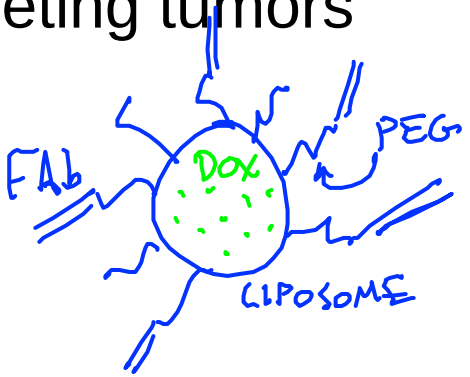


Figure removed due to copyright restrictions.
See Figure 2 from Park, John W. et al. "Anti-HER2 Immunoliposomes: Enhanced Efficacy Attributable to Targeted Delivery." *Clinical Cancer Research* 8, no. 4 (2002).

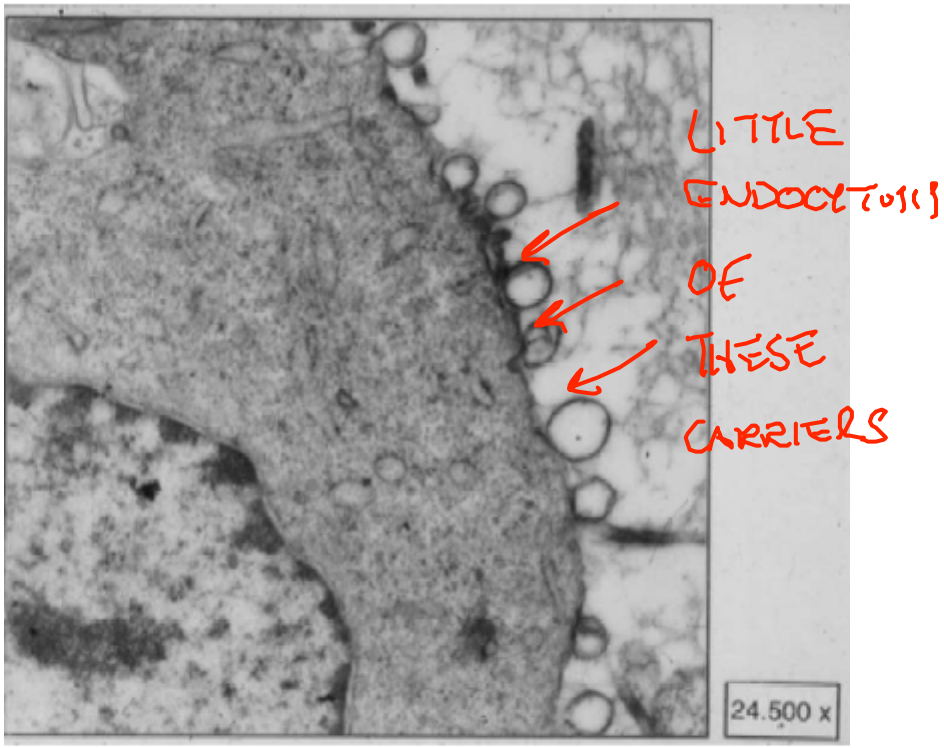


Fig. 4. Immunoliposomes binding to the surface of an ovarian carcinoma cell. This electron micrograph depicts a human OVCAR-3 cell taken from the peritoneal cavity of nu/nu mice after injecting the animals intraperitoneally with OV-TL3-Fab'-immunoliposomes. A more detailed analysis of the cell-immunoliposome interaction showed very little endocytic uptake. A search was started to identify endocytosis inducing antibodies. mAB with human ovarian cancer cell specificity were identified (e.g., mAB 425). These 425 immunoliposomes loaded with DTA and a pH-dependent fusogen (diINF-7) were tested in vitro [15,17].

Results from mAb-targeting: Targeting tumors

Figures removed due to copyright restrictions. See Figures 1 and 3 from Kirpotin, Dmitri B. et al. "Antibody Targeting of Long-Circulating Lipidic Nanoparticles Does Not Increase Tumor Localization but Does Increase Internalization in Animal Models." *Cancer Research* 66 (2006).

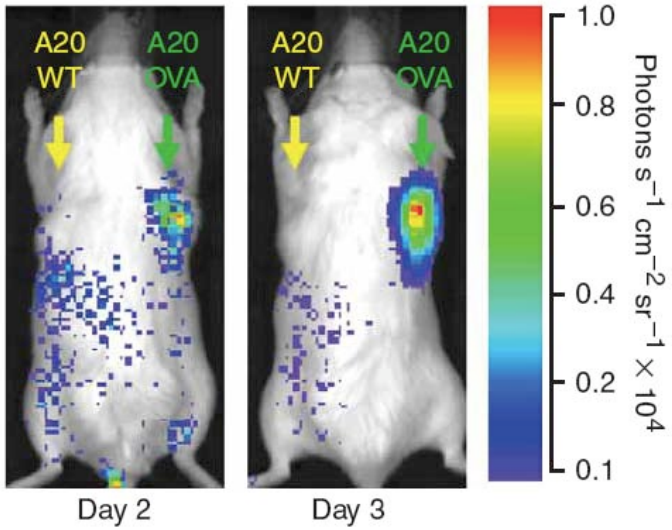
ONE MORE IDEA, time permitting: CELL-MEDIATED TARGETING

lymphocyte homing to target tissue sites: the basis of adoptive T-cell therapy for cancer

adoptive T-cell therapy

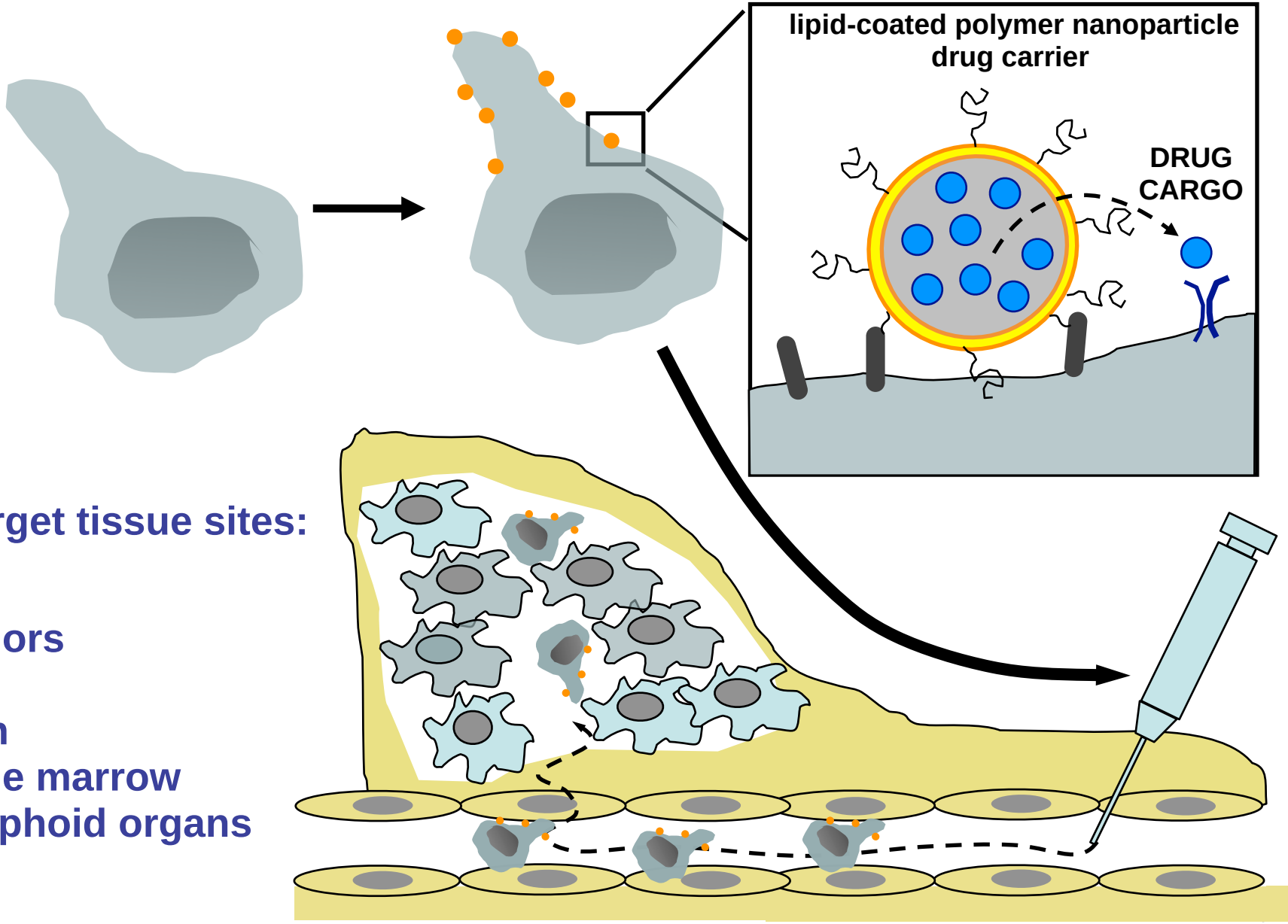
Figure removed due to copyright restrictions.
See Figure 1 from Rosenberg, Stephen A. et al.
"Adoptive Cell Transfer: A Clinical Path to Effective
Cancer Immunotherapy." *Nature Reviews Cancer* 8 (2008).

Imaging the trafficking of tumor-specific T-cells following i.v. injection:



(Santos, Brentjens et al. *Nat. Med.* **15** 338-344 (2009))

Concept: Combine adoptive cell therapy with nanoparticle delivery

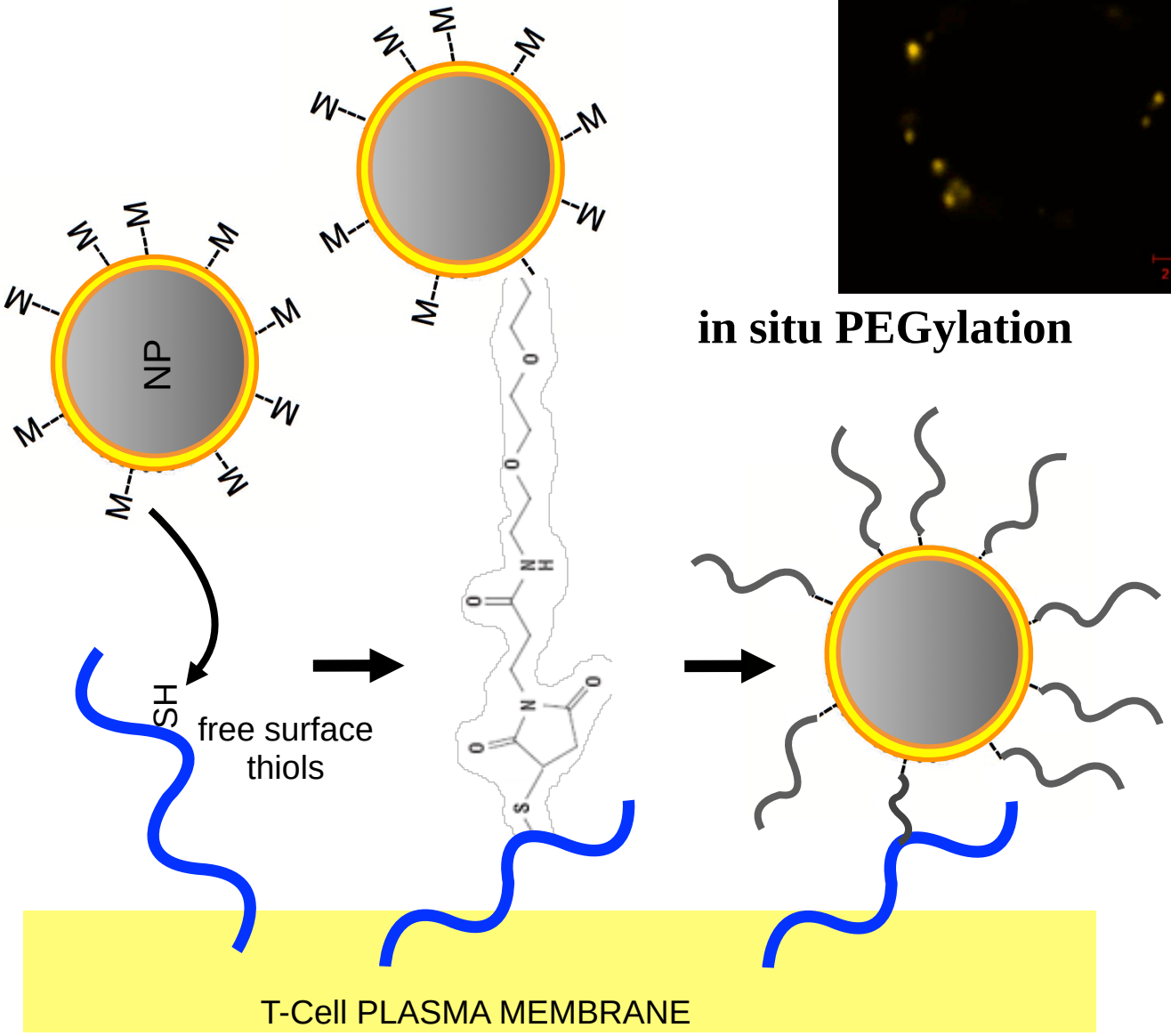


Target tissue sites:

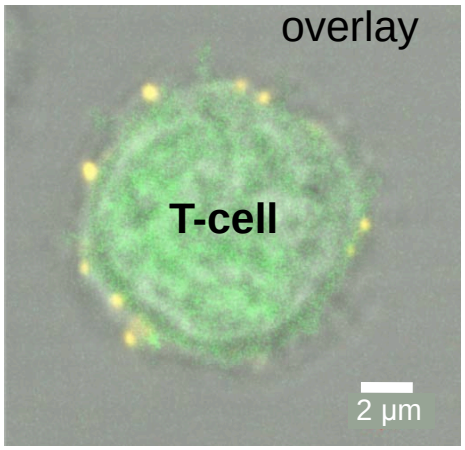
?
tumors
gut
skin
bone marrow
lymphoid organs

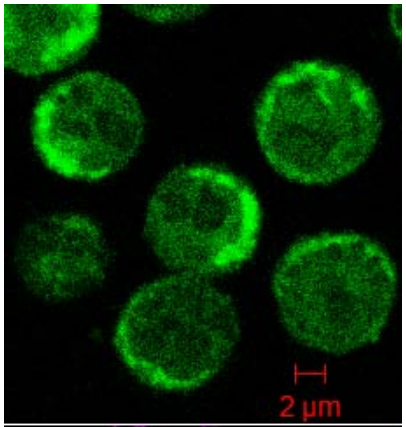
How to stably link nanoparticles to the surfaces of living cells?

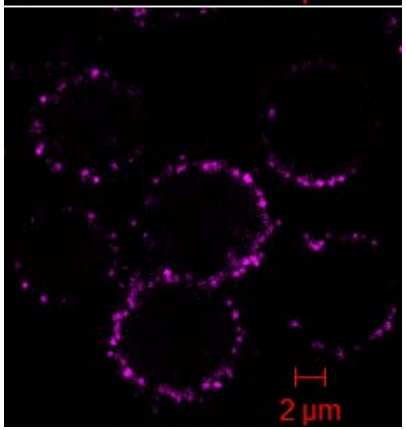
coupling to free surface thiols



in situ PEGylation

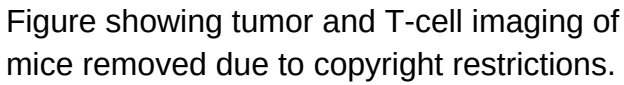






Images showing nanoparticle accumulation in tumors is more effective when carried there by T-cells have been removed due to copyright restrictions.

T-cells “armed” with particles releasing cytokine IL-15 exhibit greatly enhanced antitumor activity



20.380J / 5.22J Biological Engineering Design

Spring 2010