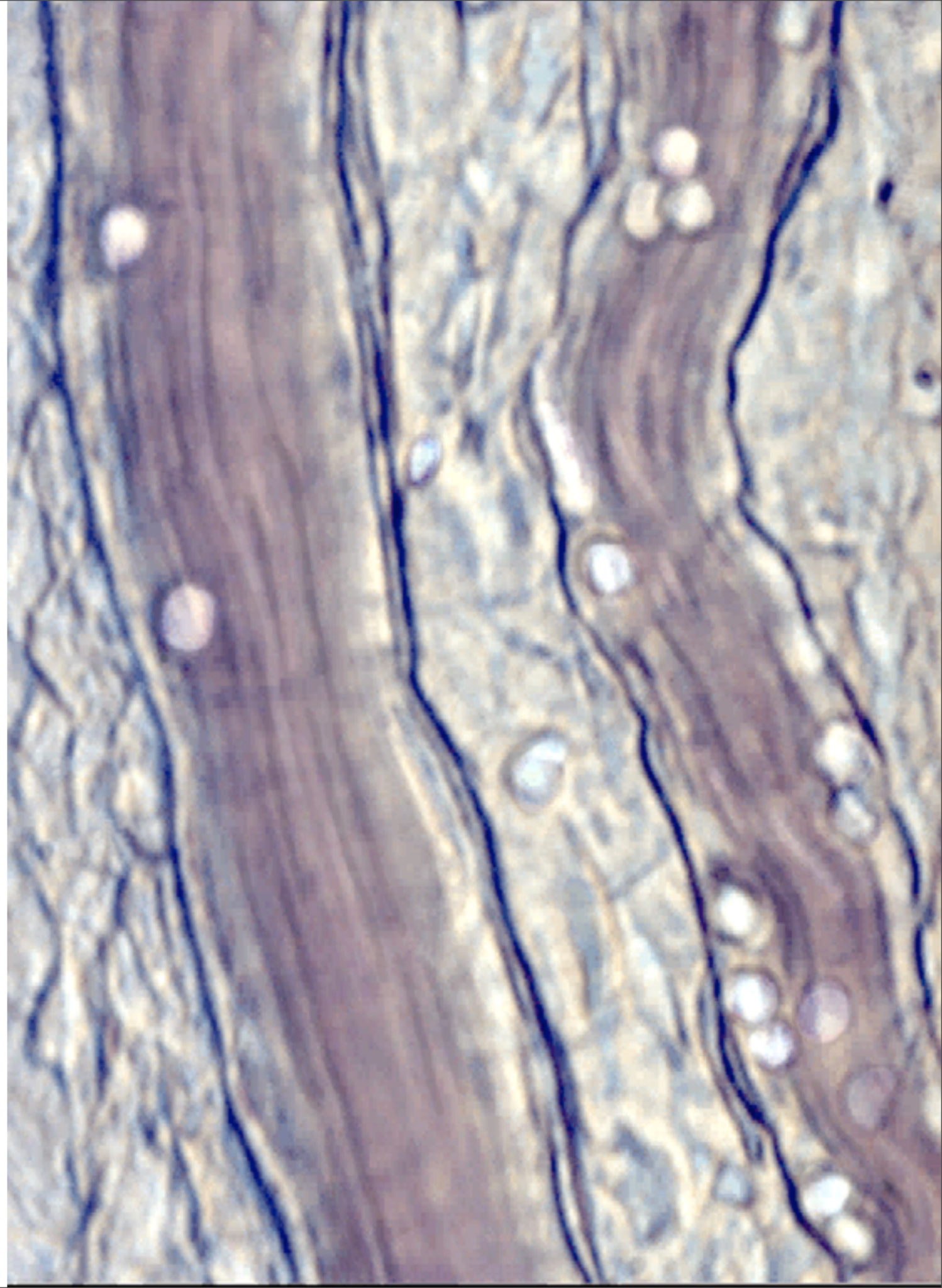


# Leukocyte adhesion: mechanisms and applications

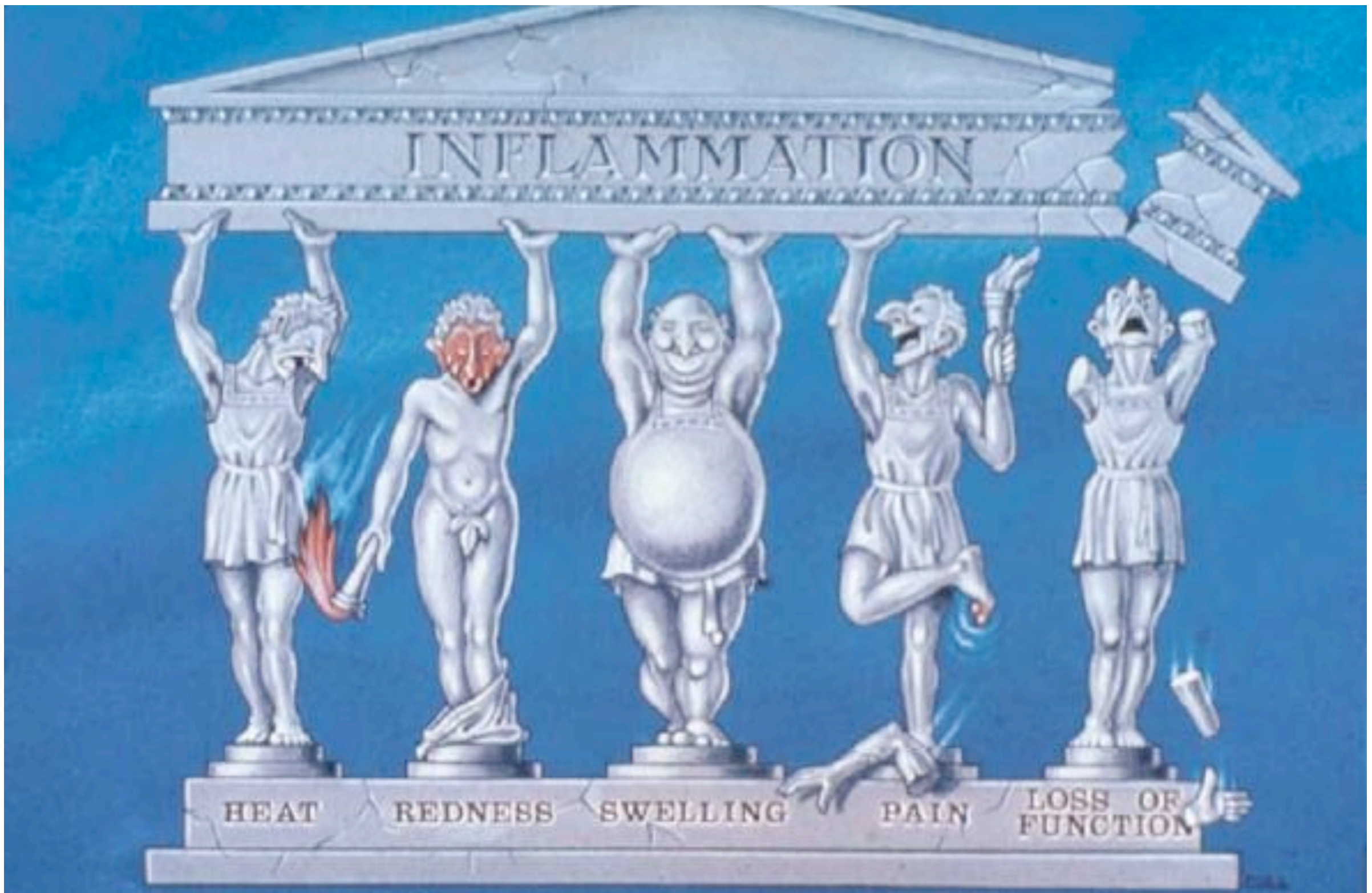
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Ellie Hughes  
Microcirculation group

Contact: [f.gavins@imperial.ac.uk](mailto:f.gavins@imperial.ac.uk)

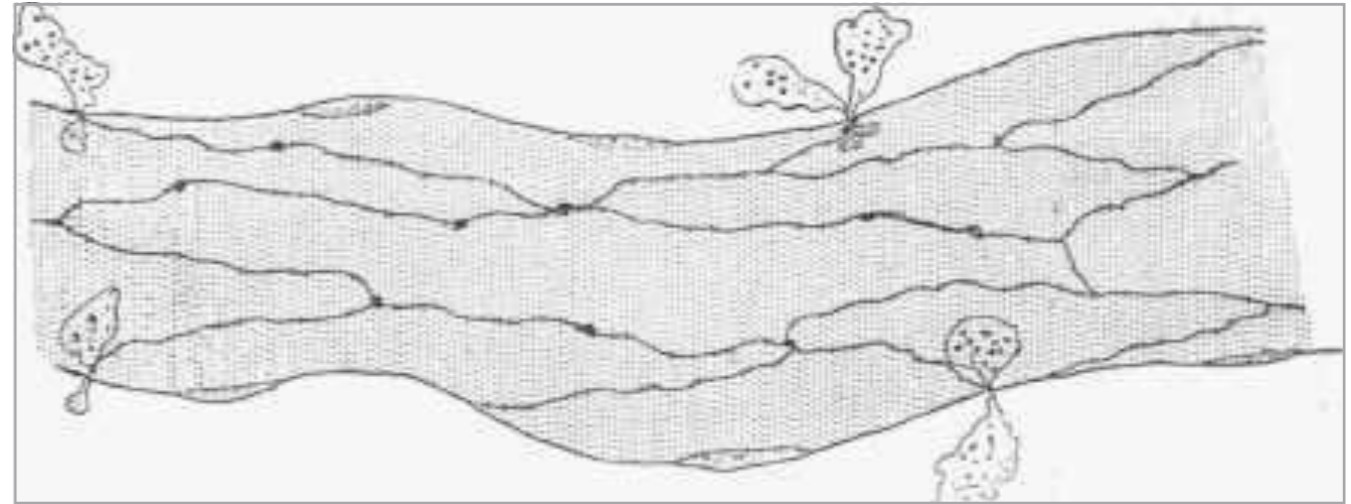


- Principles of leukocyte-endothelium interactions
  - the leukocyte recruitment cascade
  - adhesion molecules: classes, structures, involvement in recruitment
- Diseases caused by defects in adhesion molecules
- Adhesion molecules as drug targets
- Methods of imaging leukocyte adhesion (as a marker of inflammation)

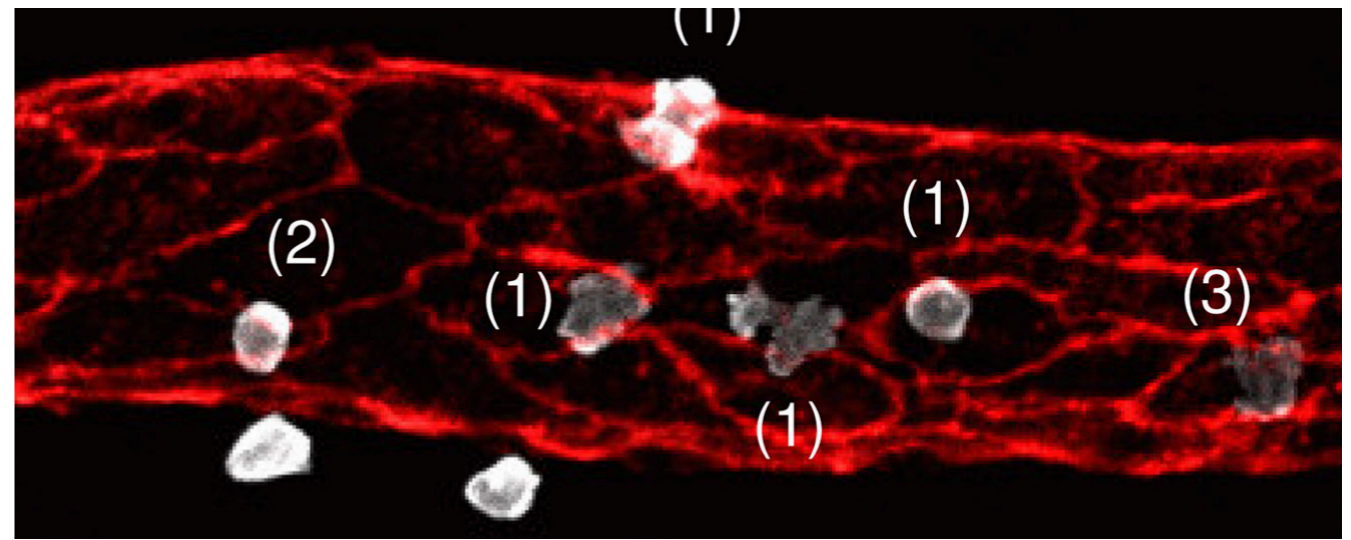


Early work

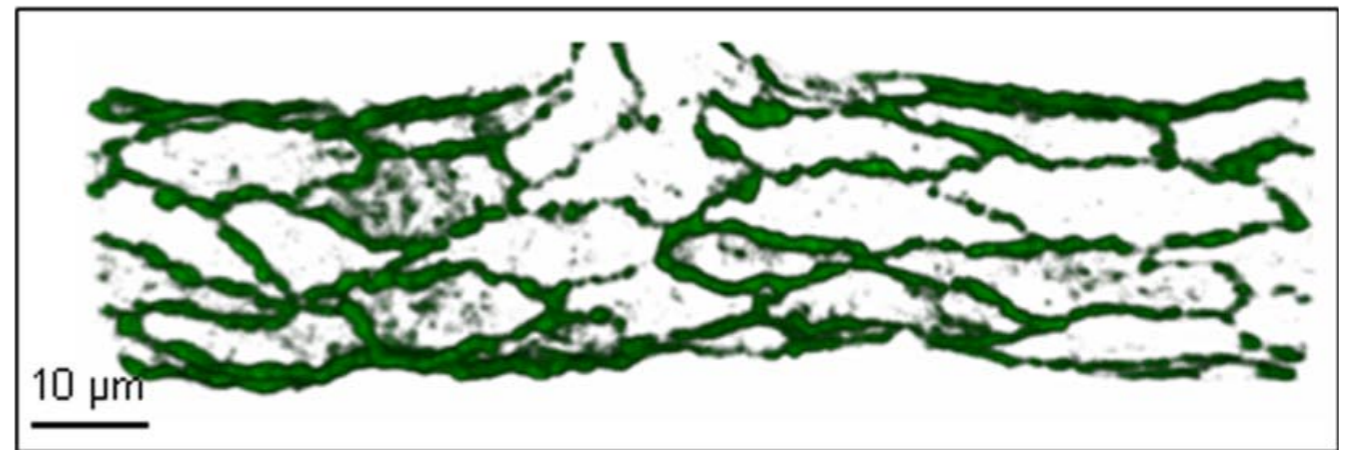
Cardinal signs observed by Celsus (200 AD)

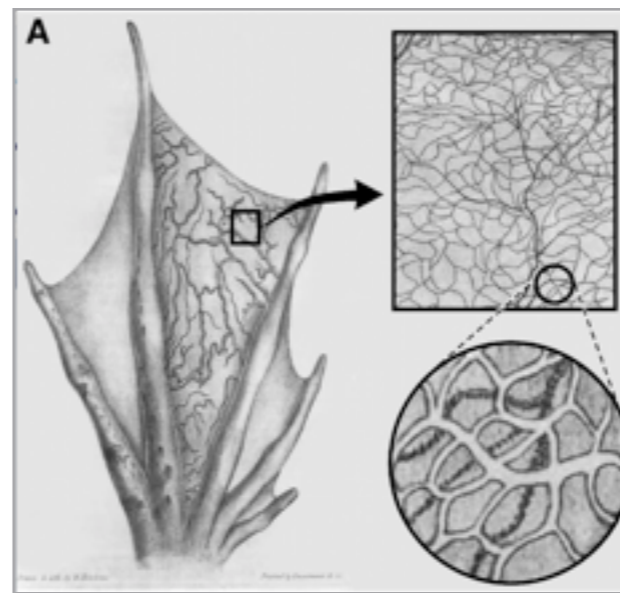


“It is possible that chemical agents seep into interendothelial junctions and thereby attract paved leukocytes, but there is, as yet, no evidence for this concept.”

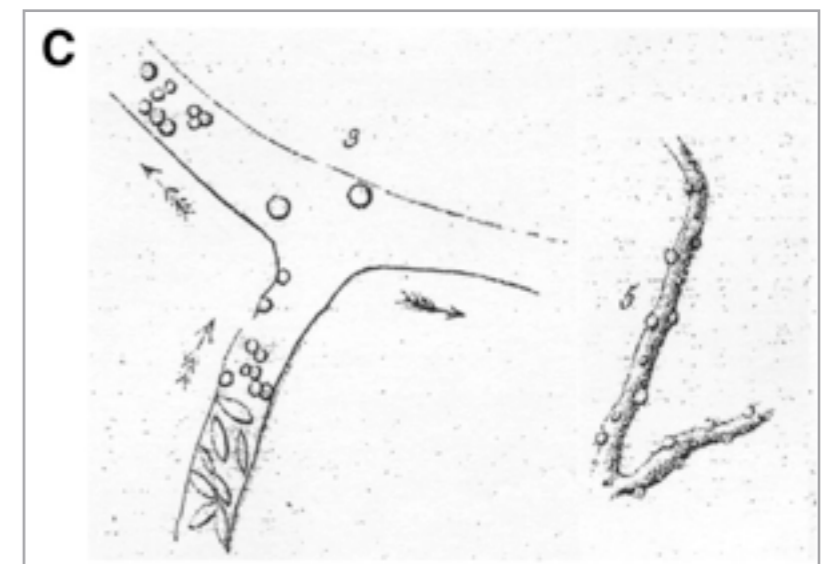
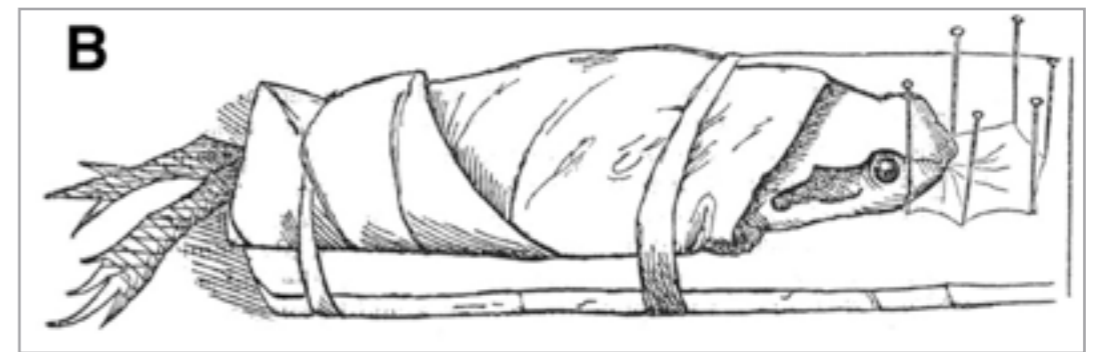


Arnold, 1870's

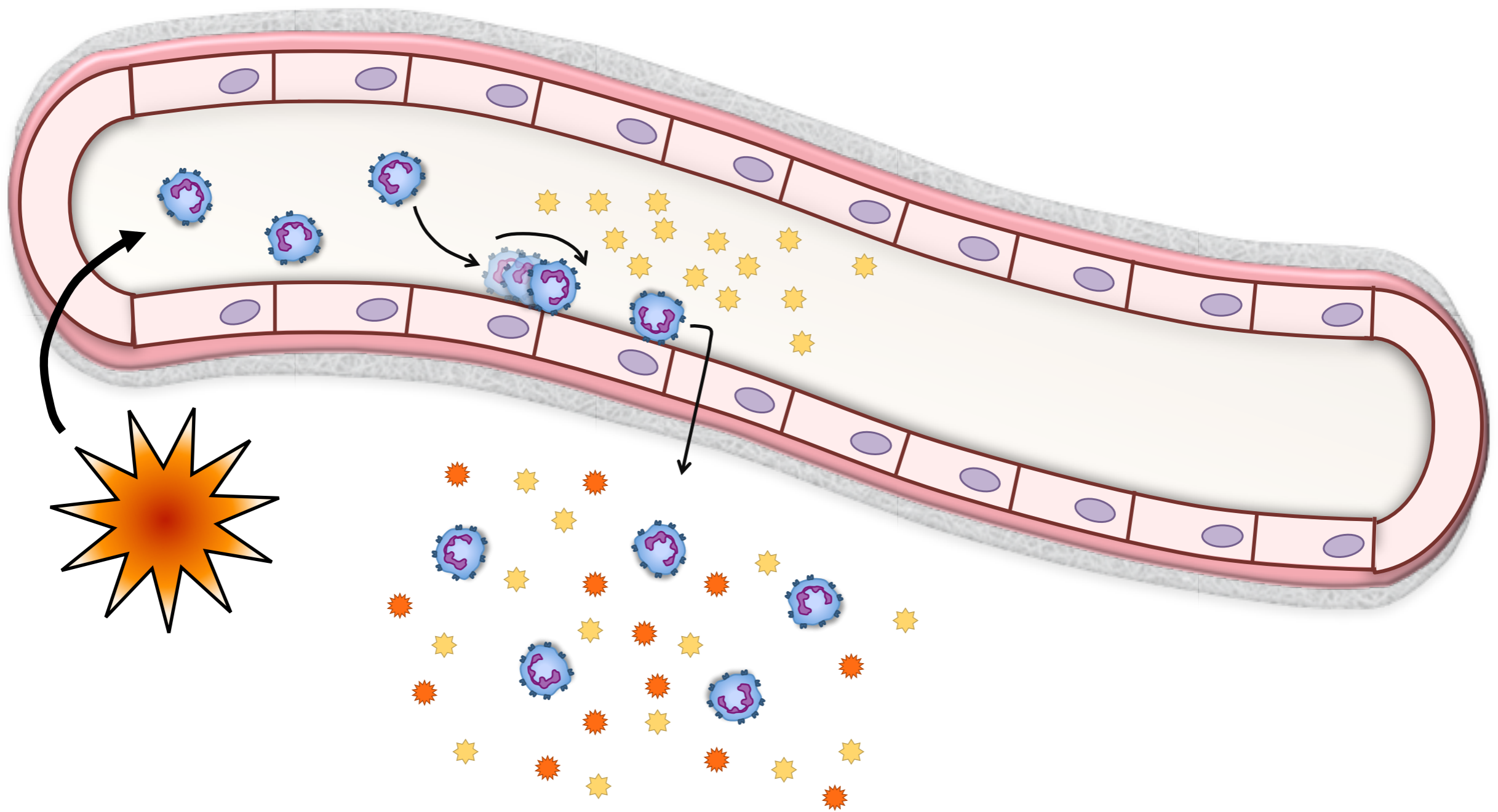




“Inflammation is the expression and consequence of a molecular alteration in the vessel walls. By it, adhesion between the vessel wall and the blood is increased.”

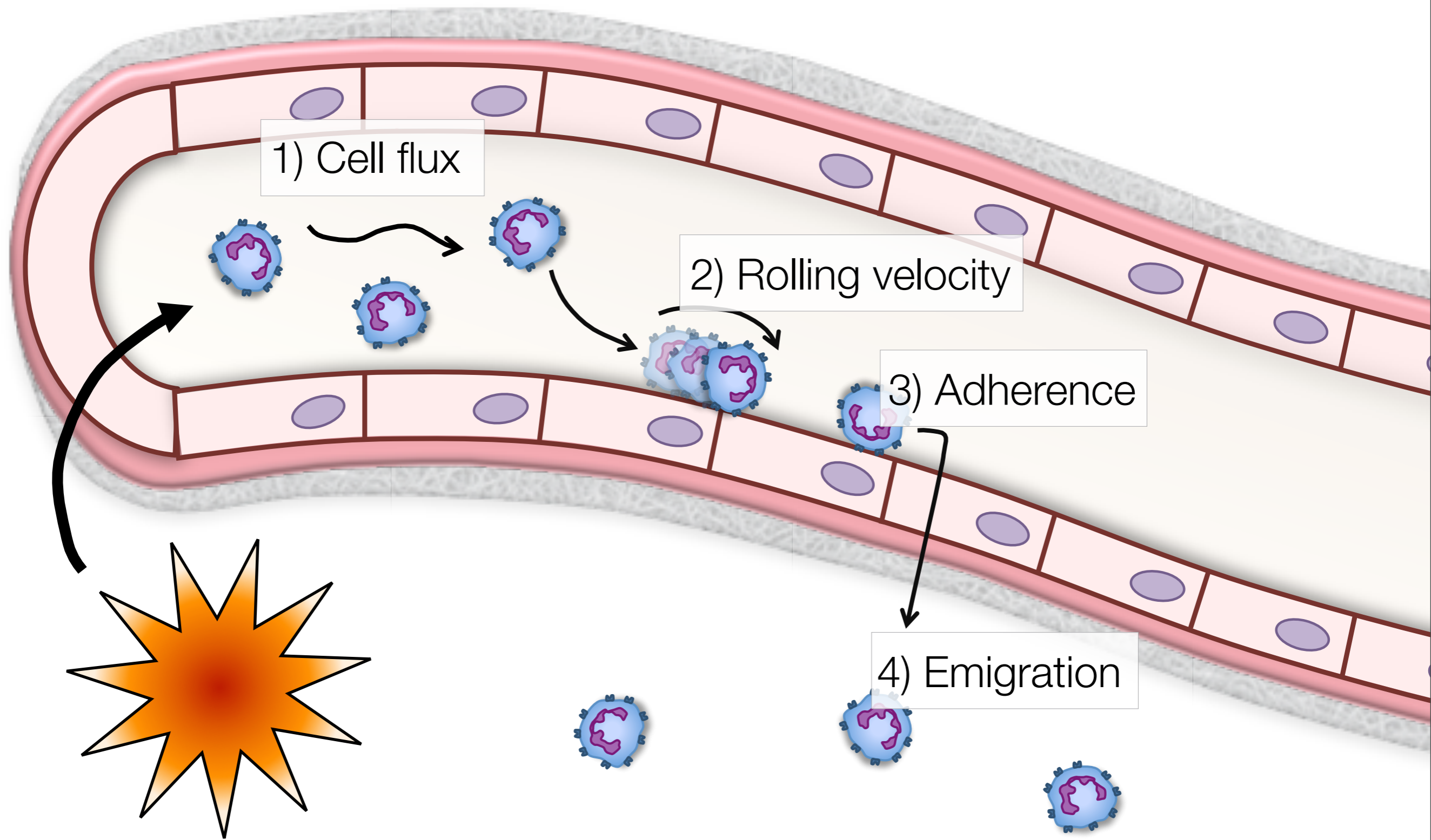


Julius Cohnheim, 1882

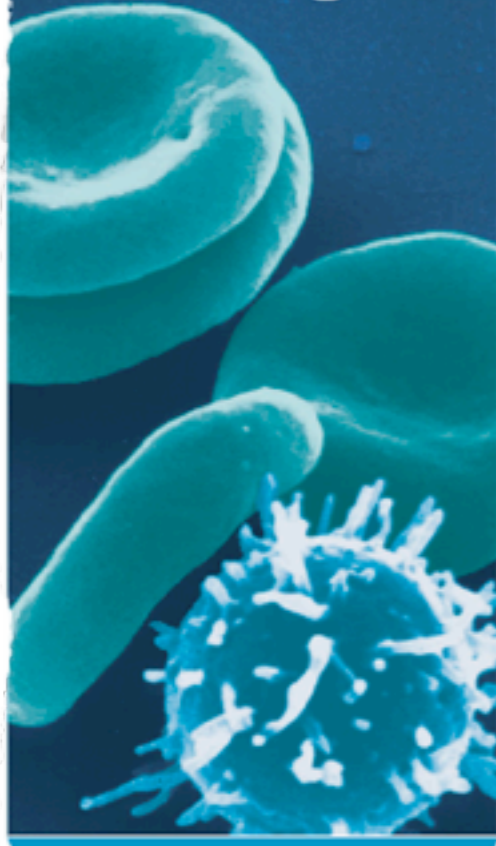


Leukocyte recruitment

Measuring inflammation by intravital microscopy



# CD Antigens



**Text & Colour Key:**

**Text abbreviations**

|      |  |
|------|--|
| HLA  | Major Histocompatibility Complex         |
| HSP  | Heat shock protein                       |
| IG   | Immunoglobulin                           |
| ITAM | Immunoreceptor tyrosine activation motif |
| ITIM | Inhibitory tyrosine immunoreceptor motif |
| ITSM | Inhibitory tyrosine signaling motif      |
| ITSM | Inhibitory tyrosine signaling motif      |
| ITSM | Inhibitory tyrosine signaling motif      |
| ITSM | Inhibitory tyrosine signaling motif      |
| ITSM | Inhibitory tyrosine signaling motif      |
| ITSM | Inhibitory tyrosine signaling motif      |
| ITSM | Inhibitory tyrosine signaling motif      |
| ITSM | Inhibitory tyrosine signaling motif      |

**Key to Conjugations**

|        |             |
|--------|-------------|
| Blue   | Alumination |
| Green  | Coupling    |
| Yellow | Coupling    |
| Red    | Coupling    |
| Purple | Coupling    |

**Key to Research Topics**

|        |            |
|--------|------------|
| Blue   | Stem Cells |
| Green  | Stem Cells |
| Yellow | Stem Cells |
| Red    | Stem Cells |
| Purple | Stem Cells |

[www.abcam.com/CDmarkers](http://www.abcam.com/CDmarkers)

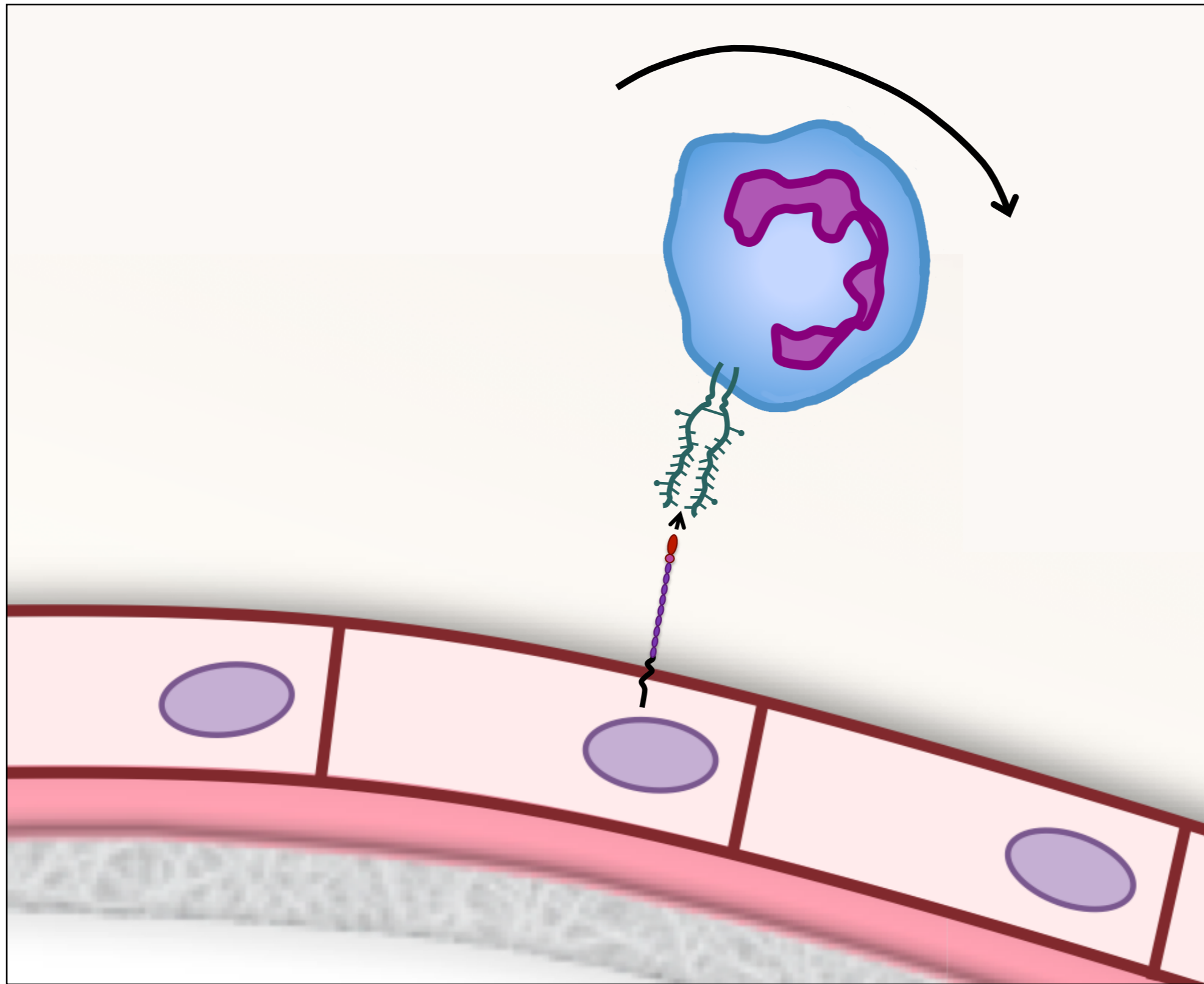
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1 Kendall Square, Ste 301  
Cambridge, MA 02138-1817  
USA  
Tel: 1 877-234-2032  
Tel: 617 486 2700  
Fax: 1 888 731-9288

**Abcam in Europe**  
880 Cambridge Science Park  
Wilton Road  
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Tel: +44 (0) 1223 326000  
Fax: +44 (0) 1223 326030

**Abcam in Japan**  
Abcam KK  
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Katsushika-ku  
Chūryū  
Tokyo 125-8514 Japan  
Tel: +81-3-6271-0940  
Fax: +81-3-6271-0941

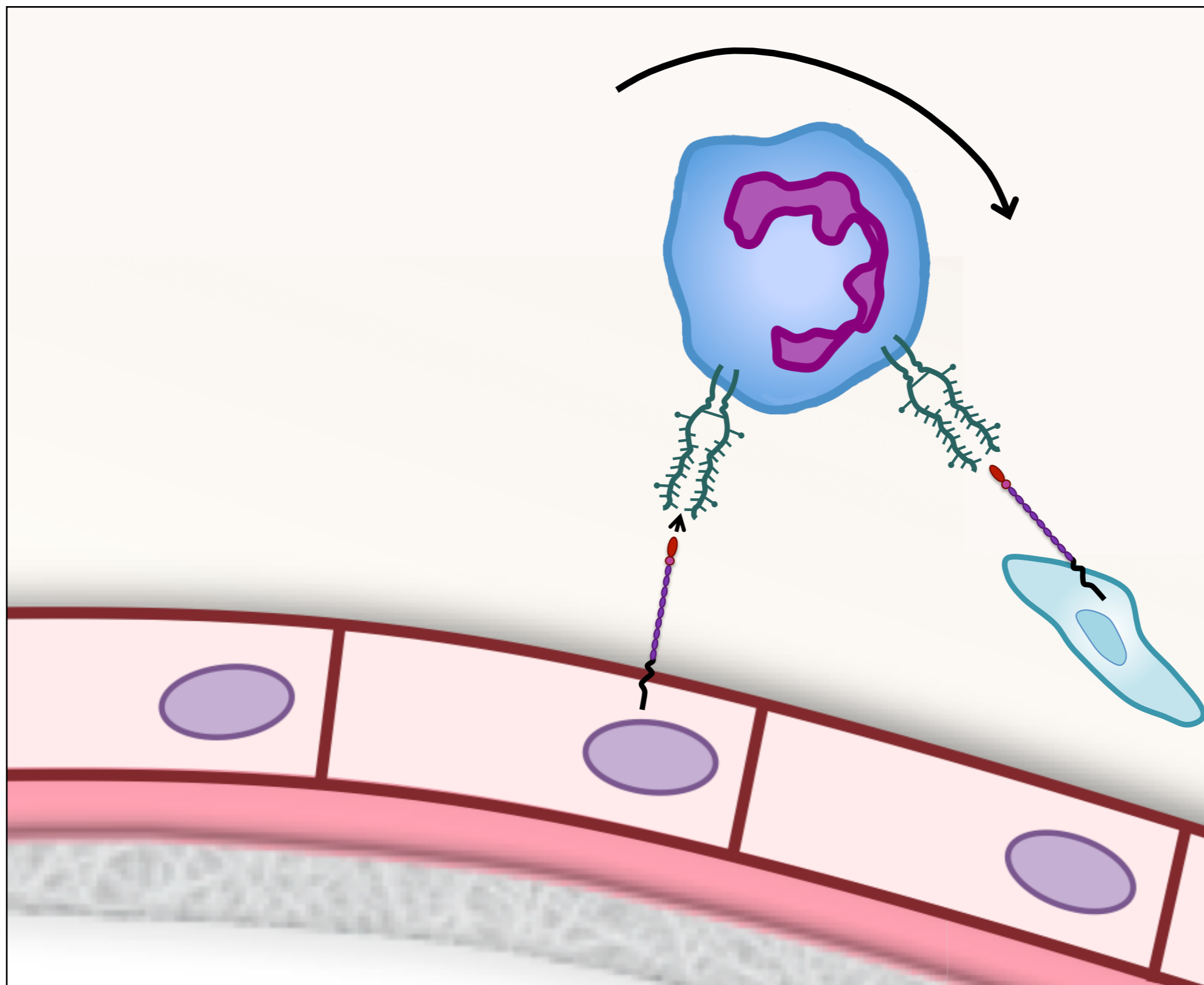
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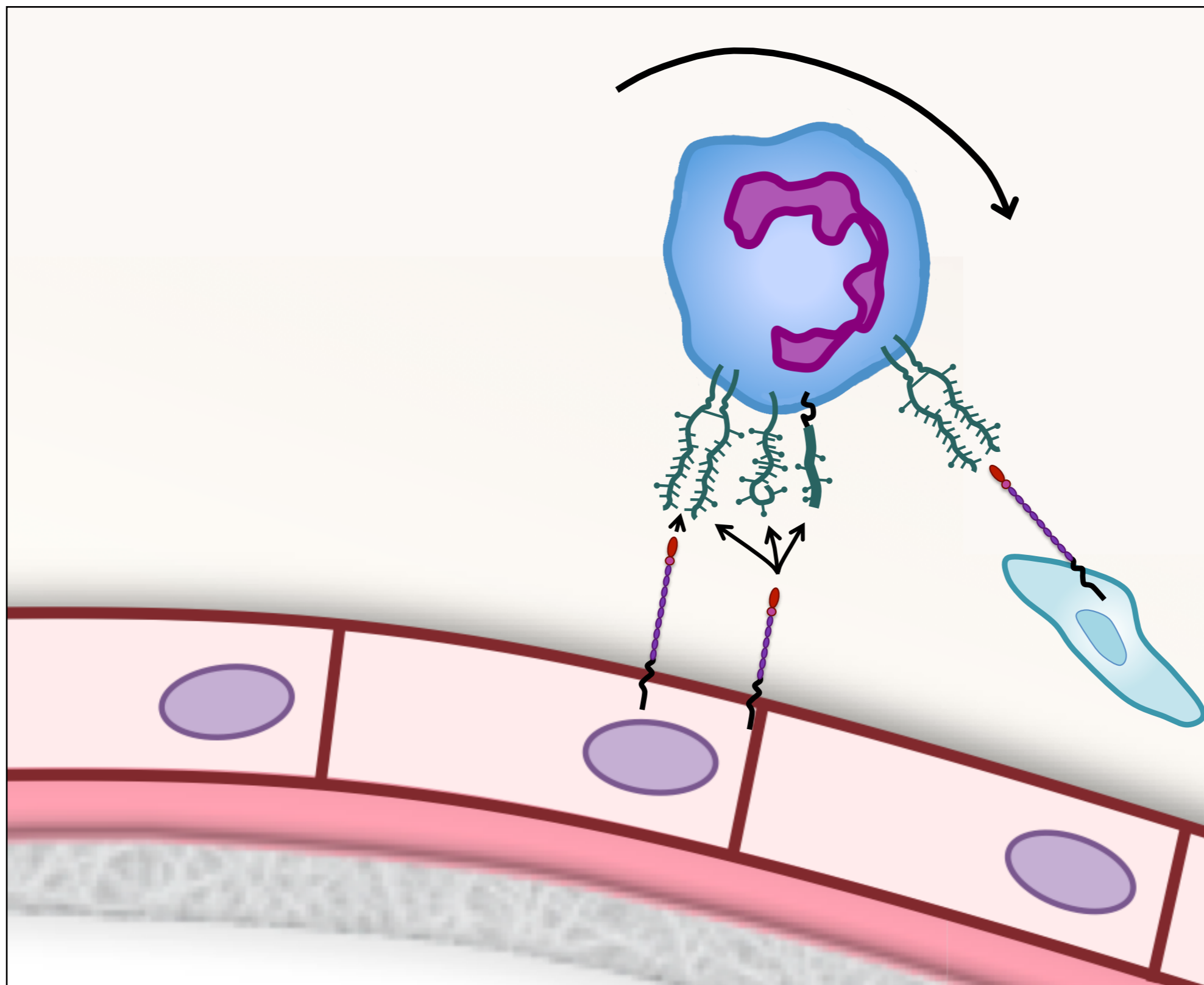
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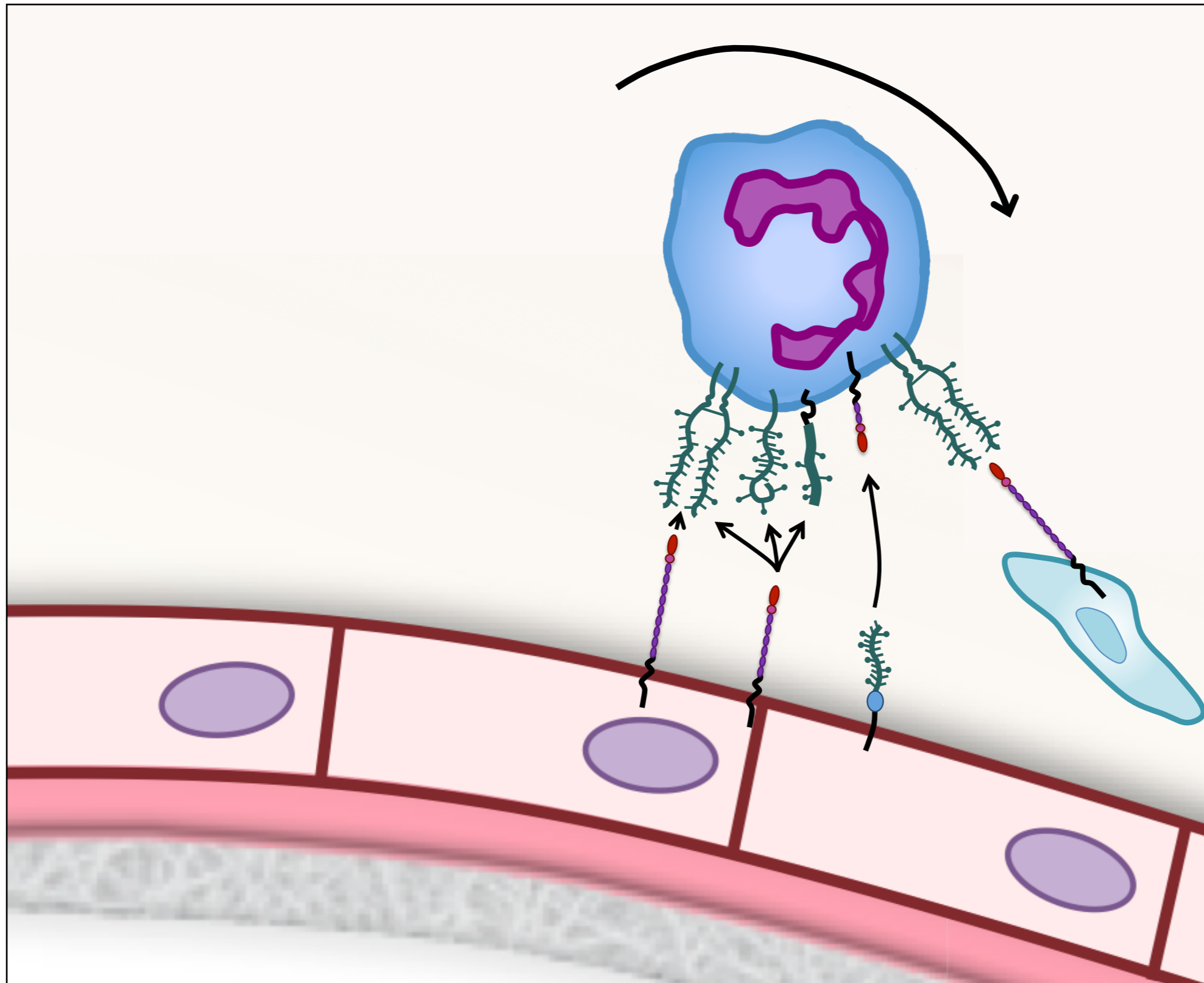


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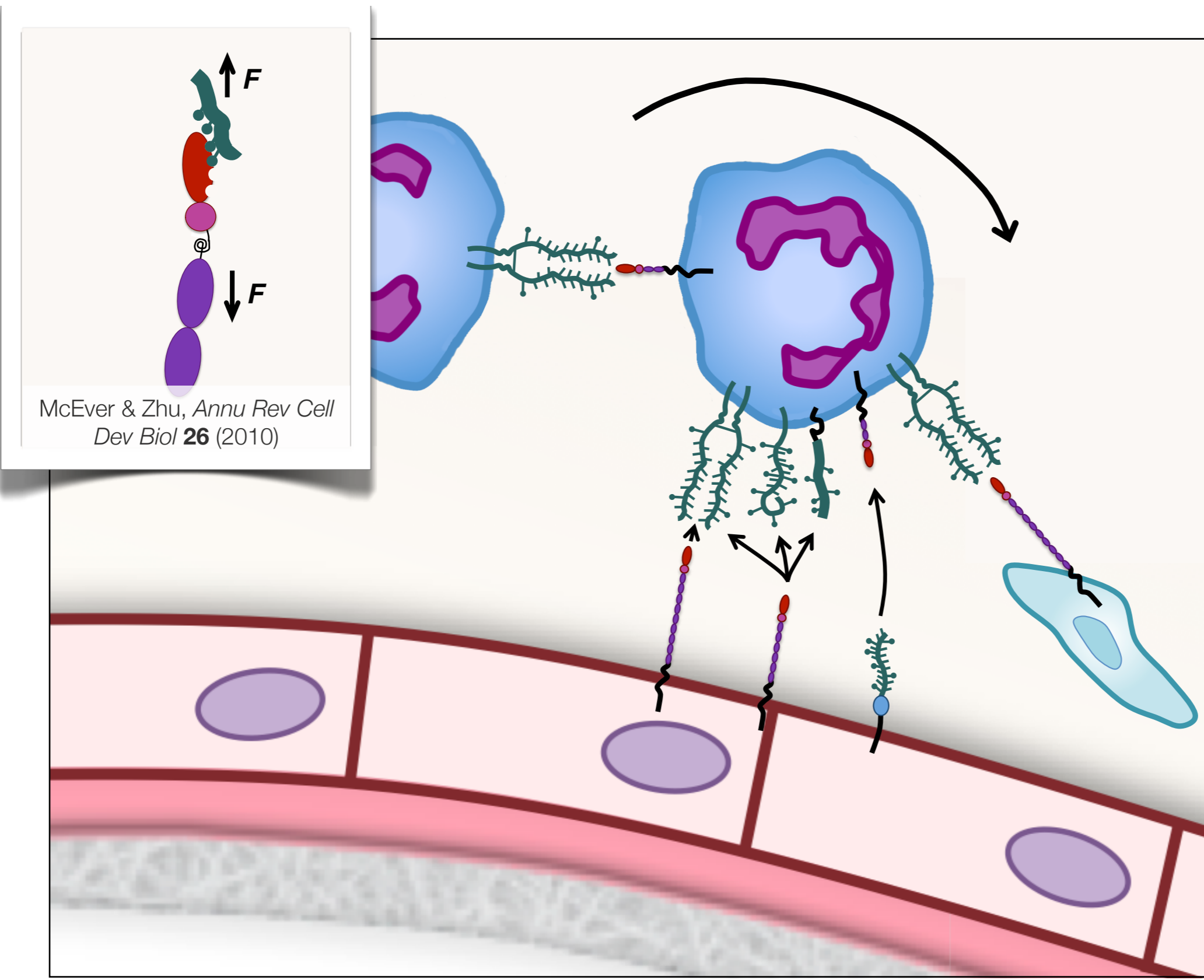
PSGL-1



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- CD44**
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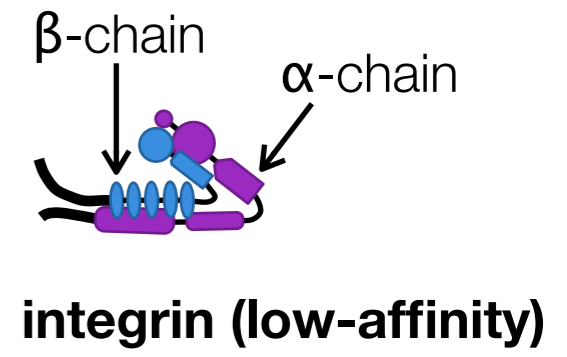
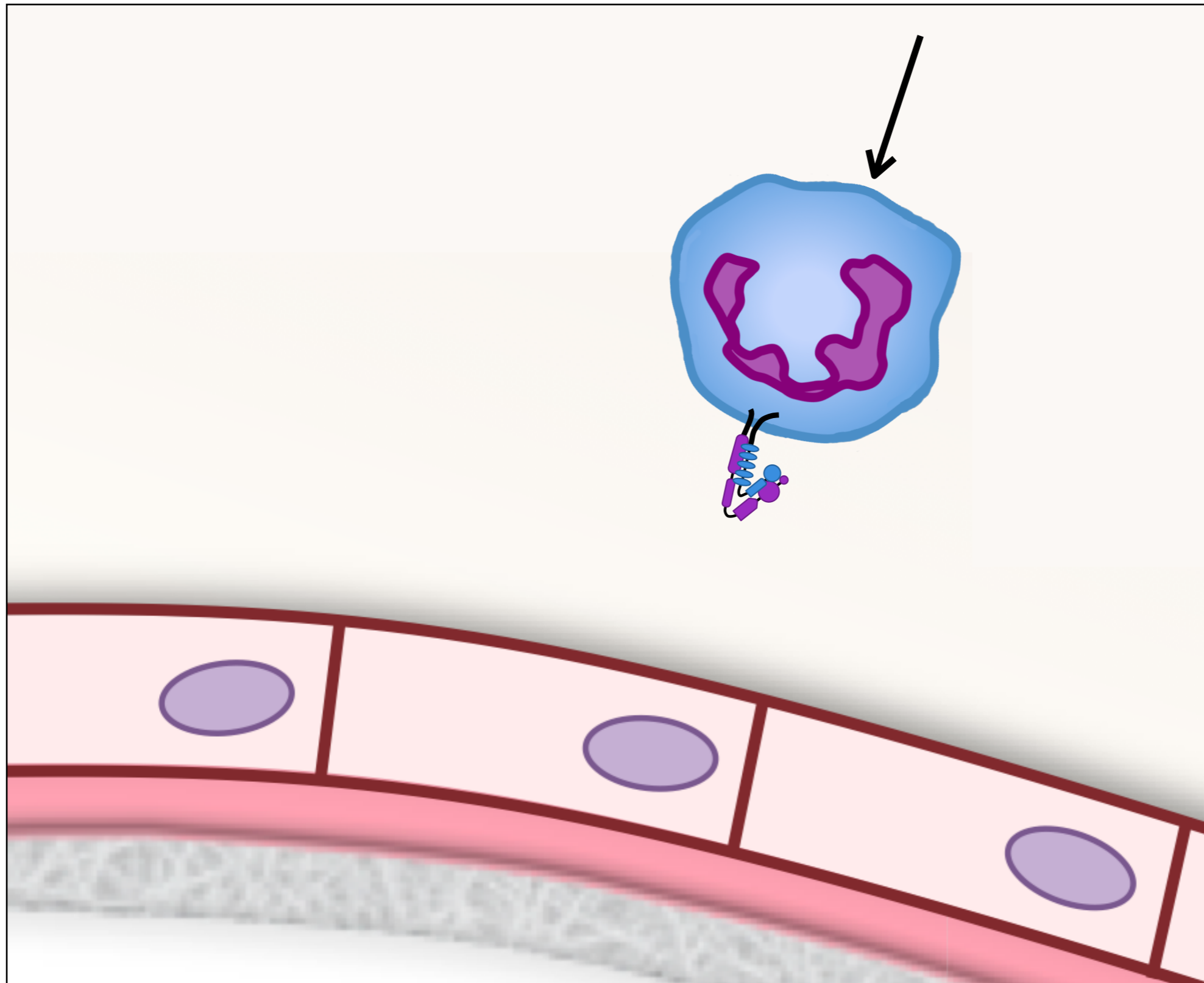
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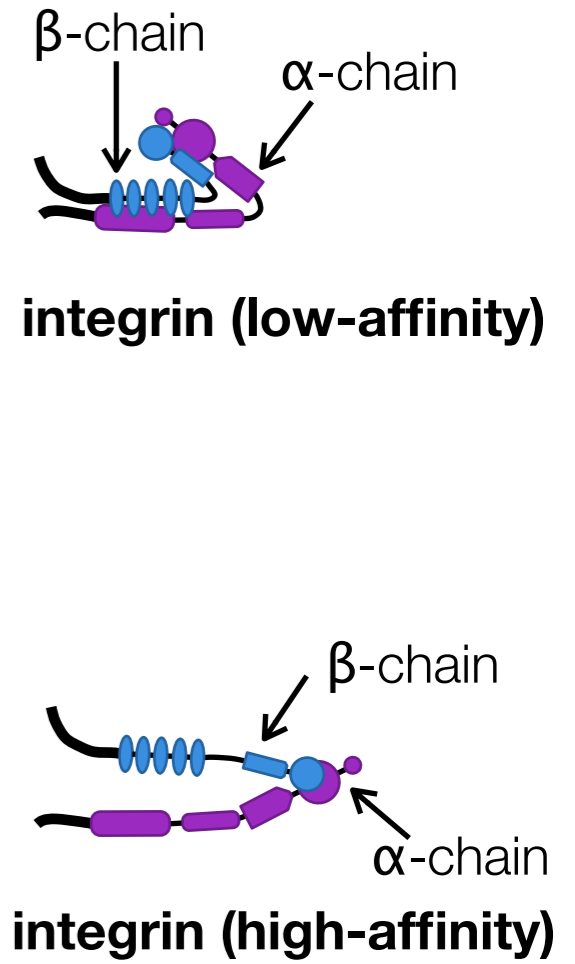
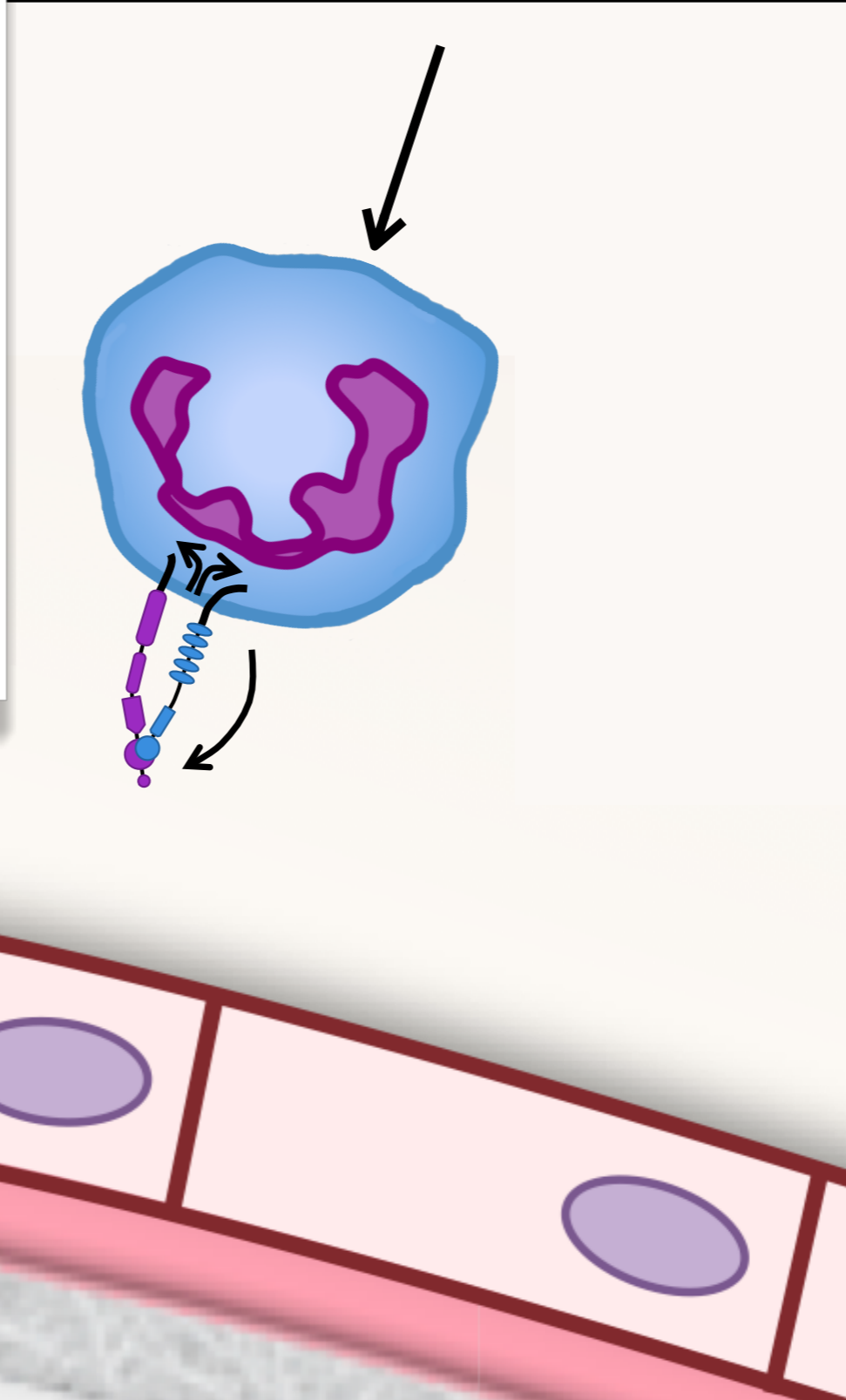
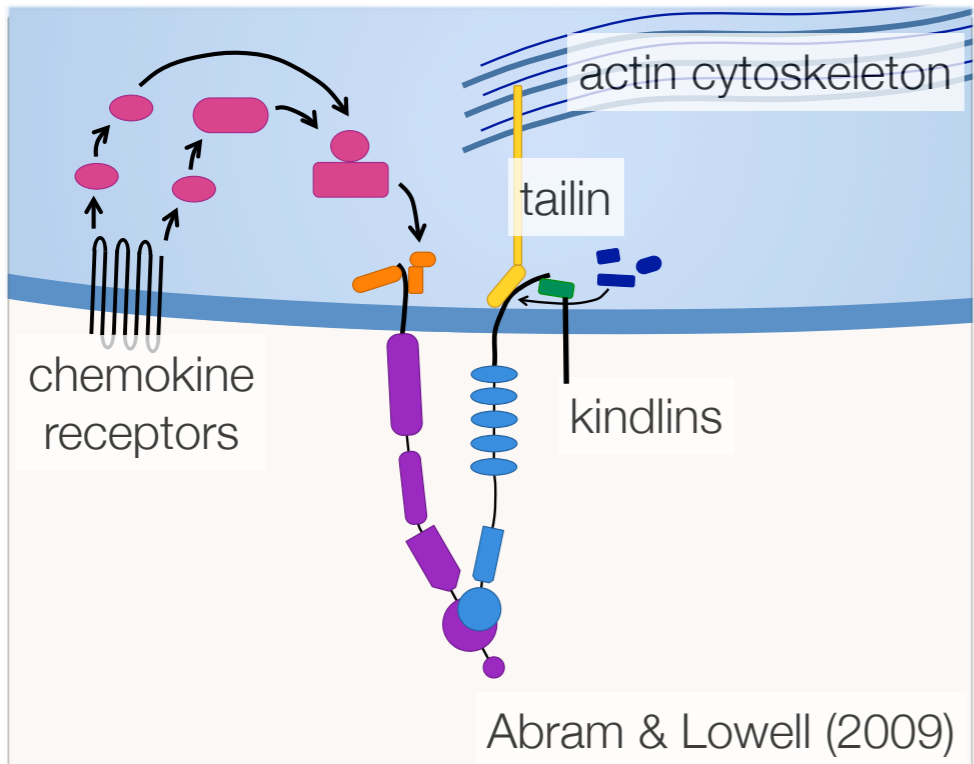


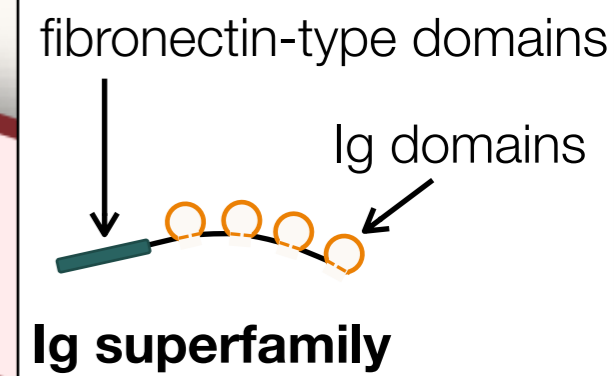
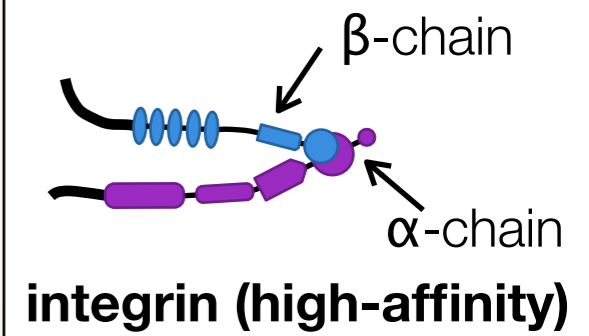
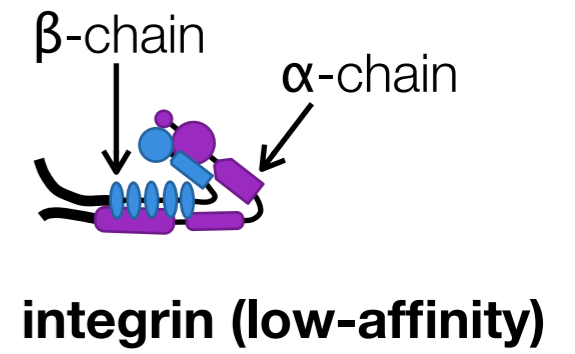
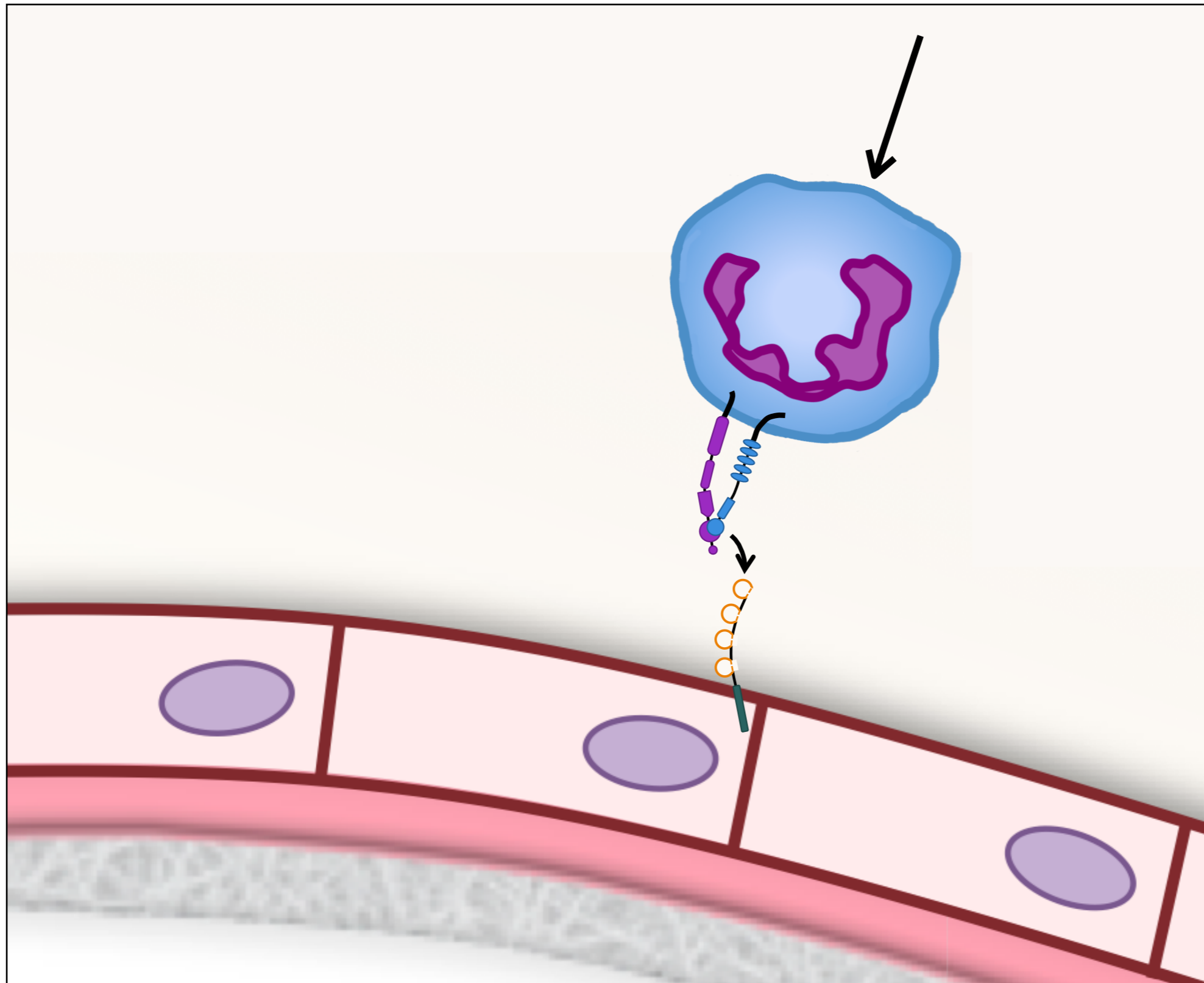
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- P-selectin
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  - PSGL-1
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  - ESL-1

Adhesion molecules

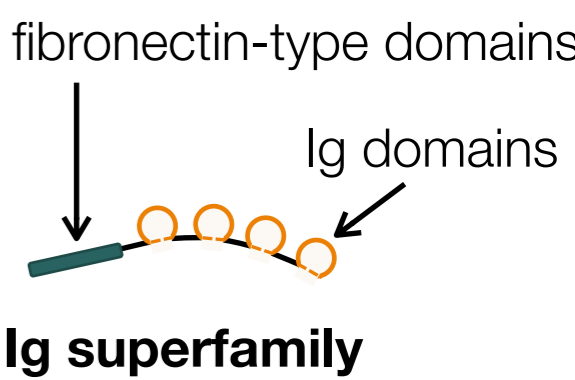
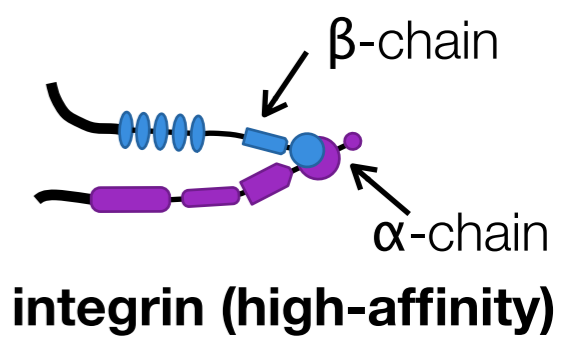
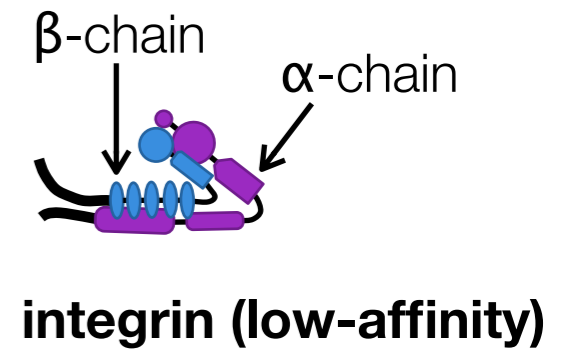
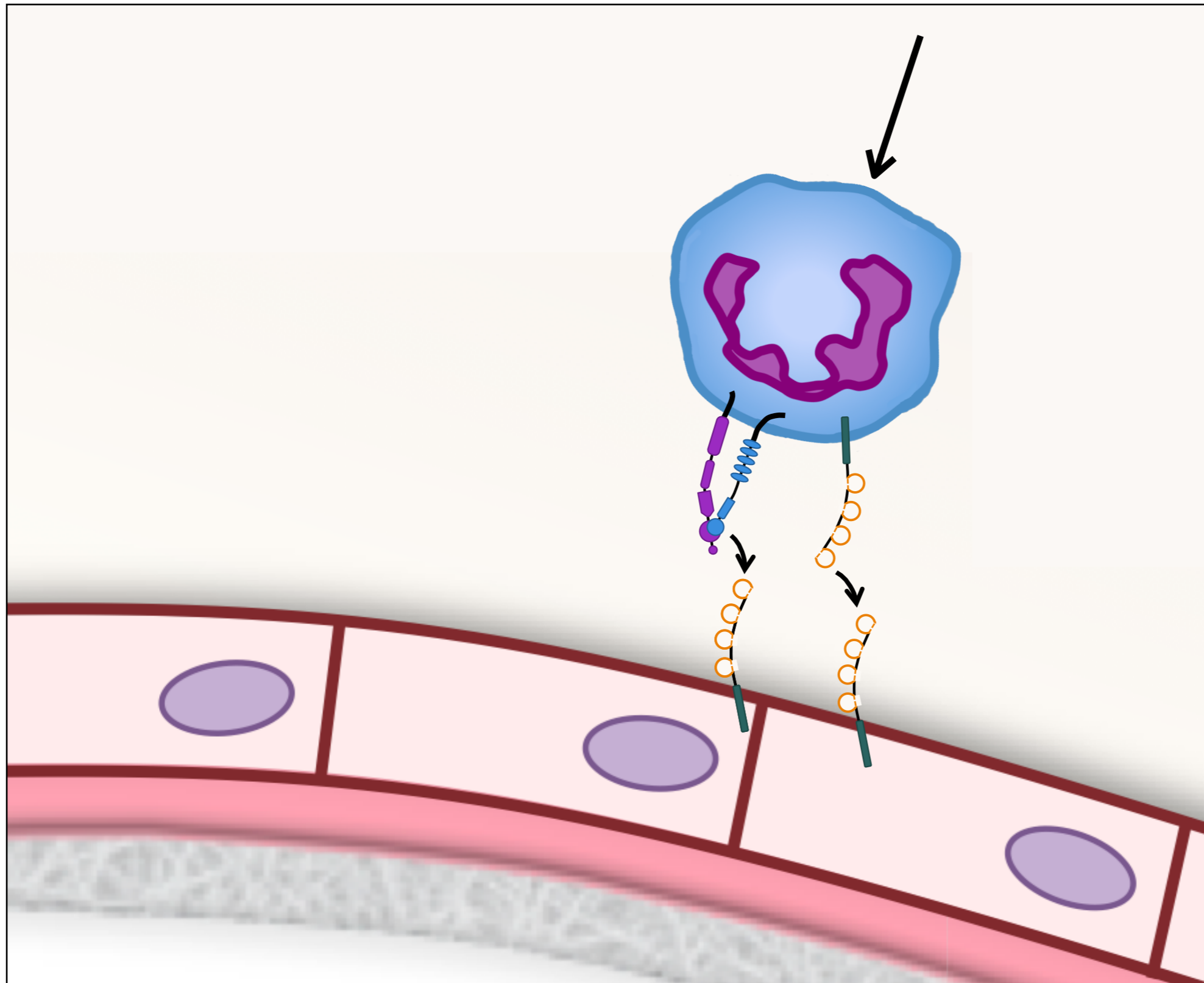
Selectin-mediated rolling



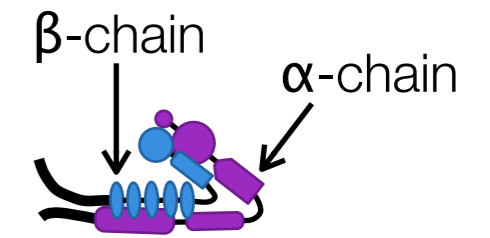
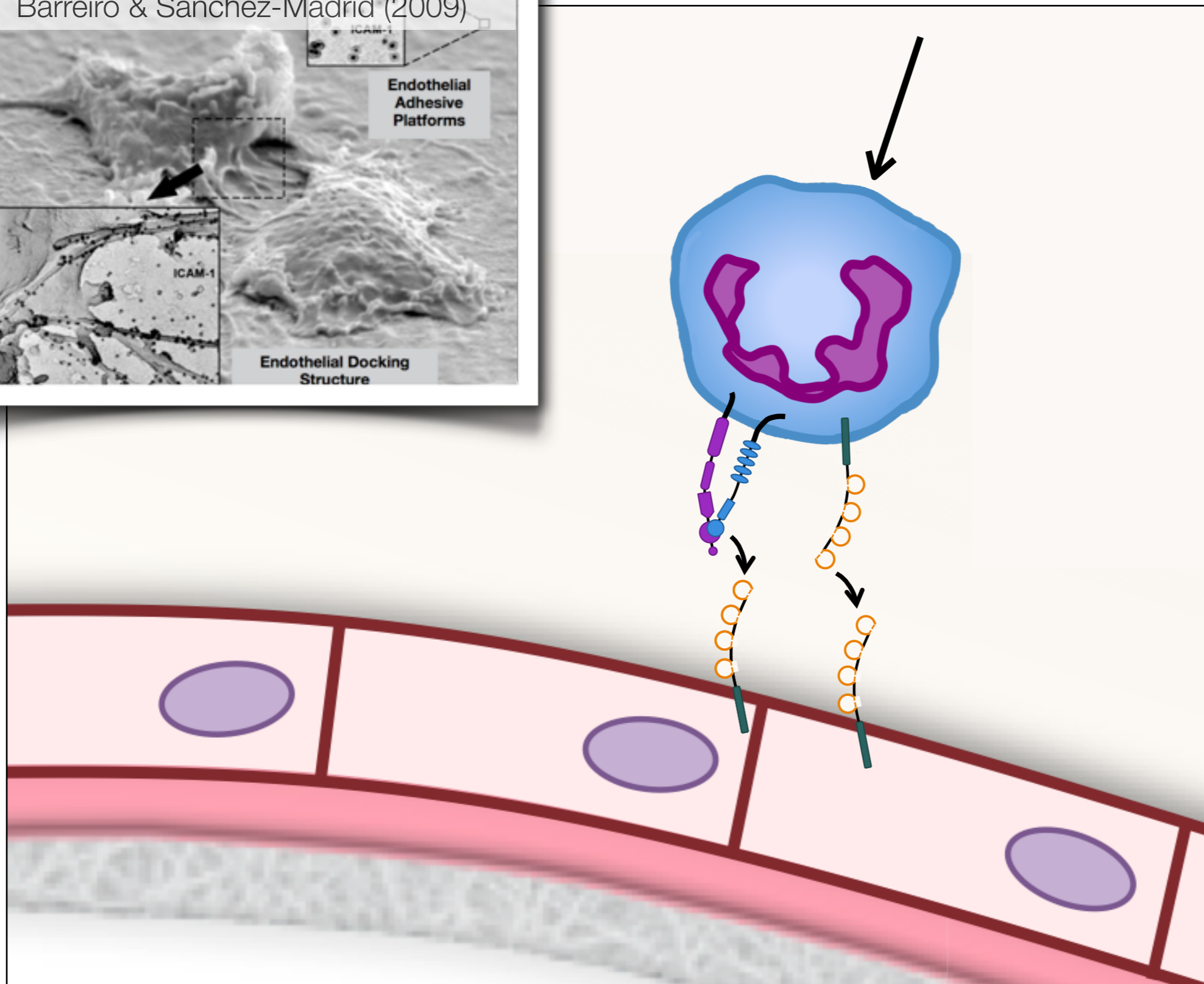
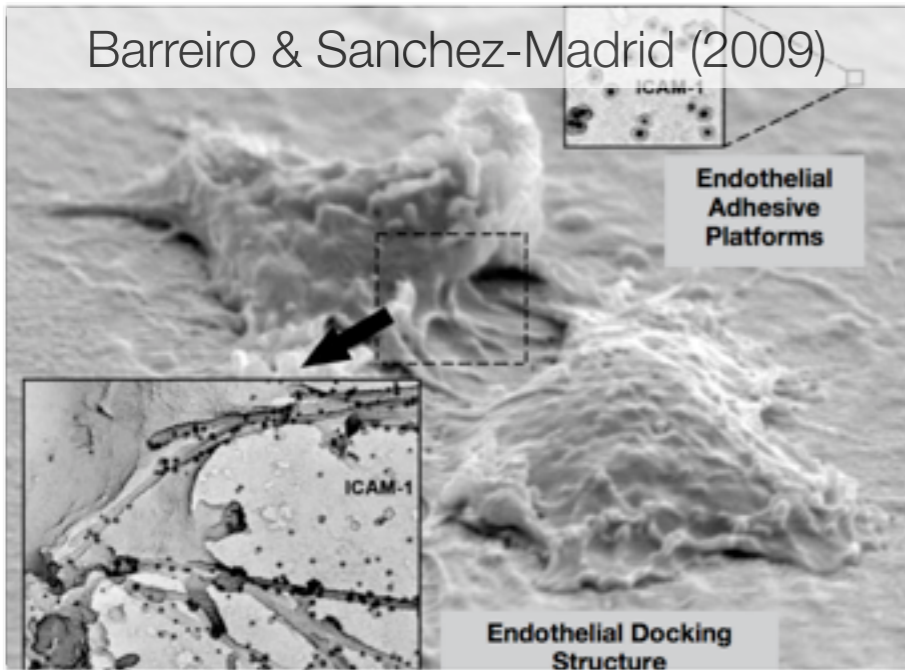




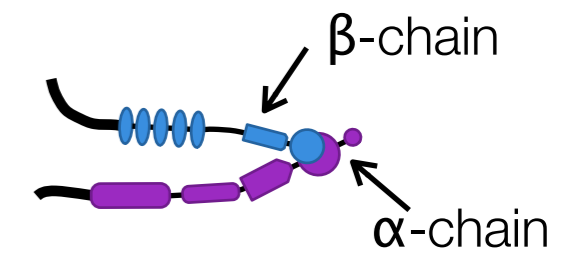




Barreiro & Sanchez-Madrid (2009)

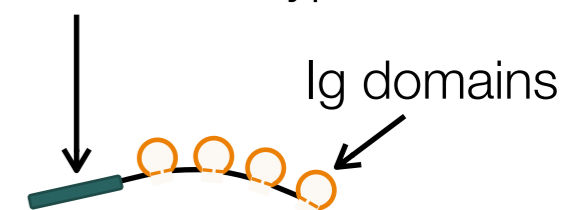


**integrin (low-affinity)**



**integrin (high-affinity)**

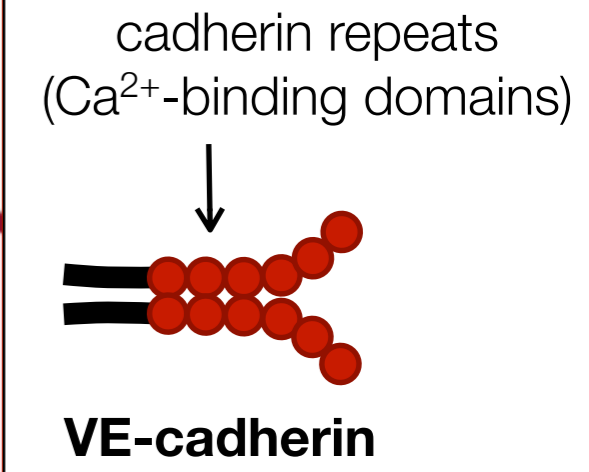
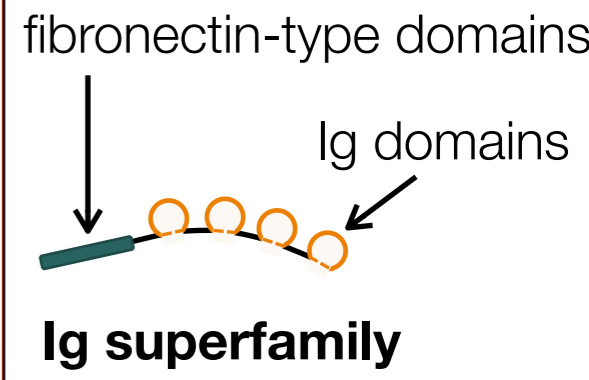
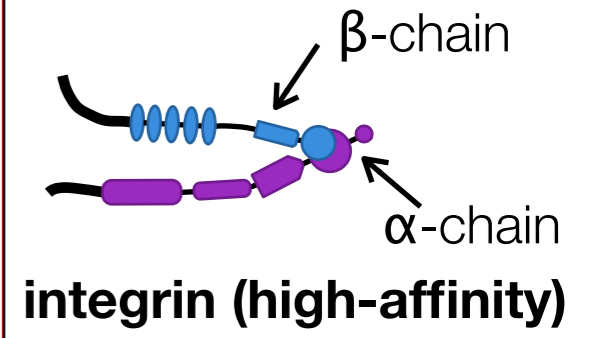
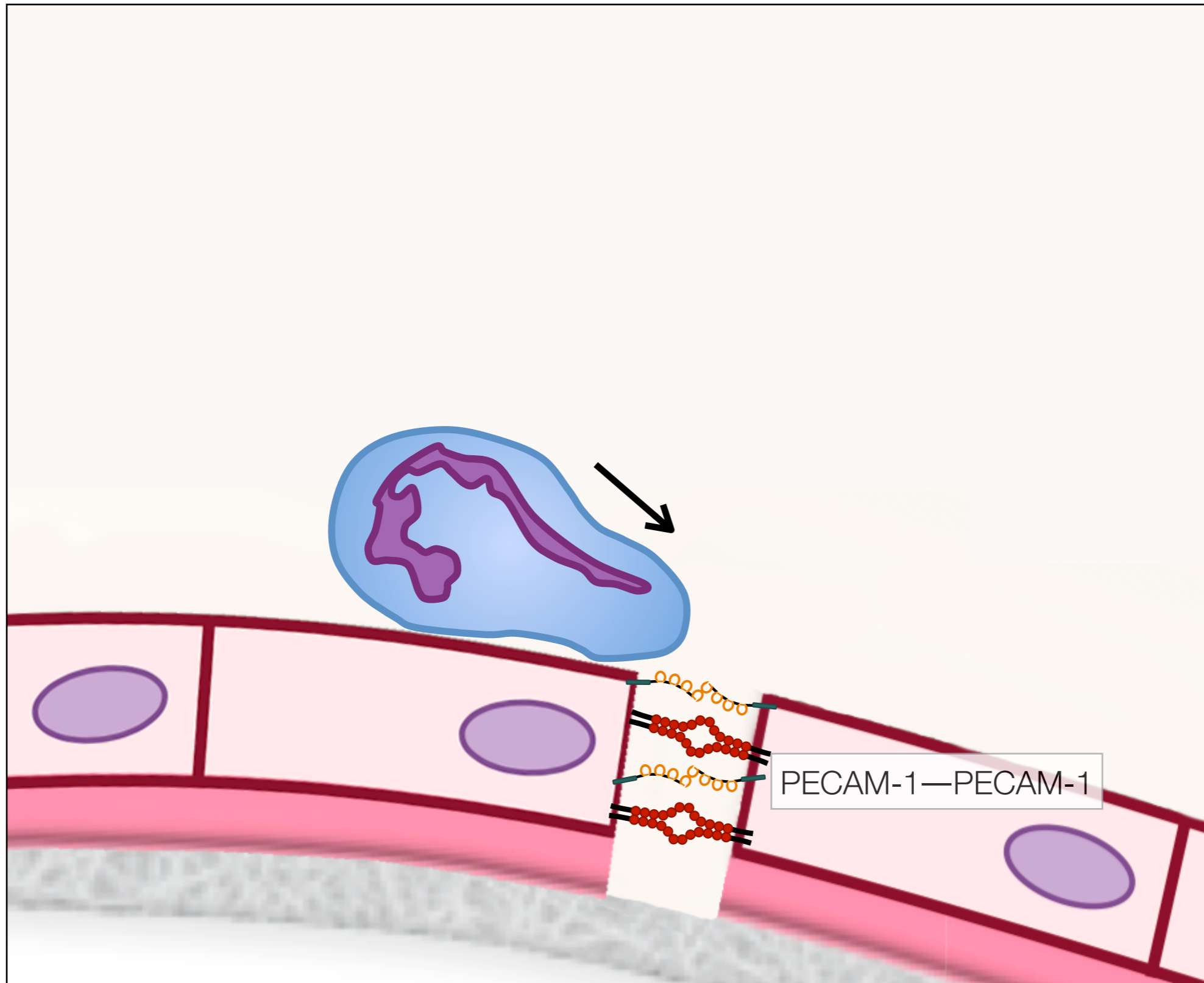
fibronectin-type domains



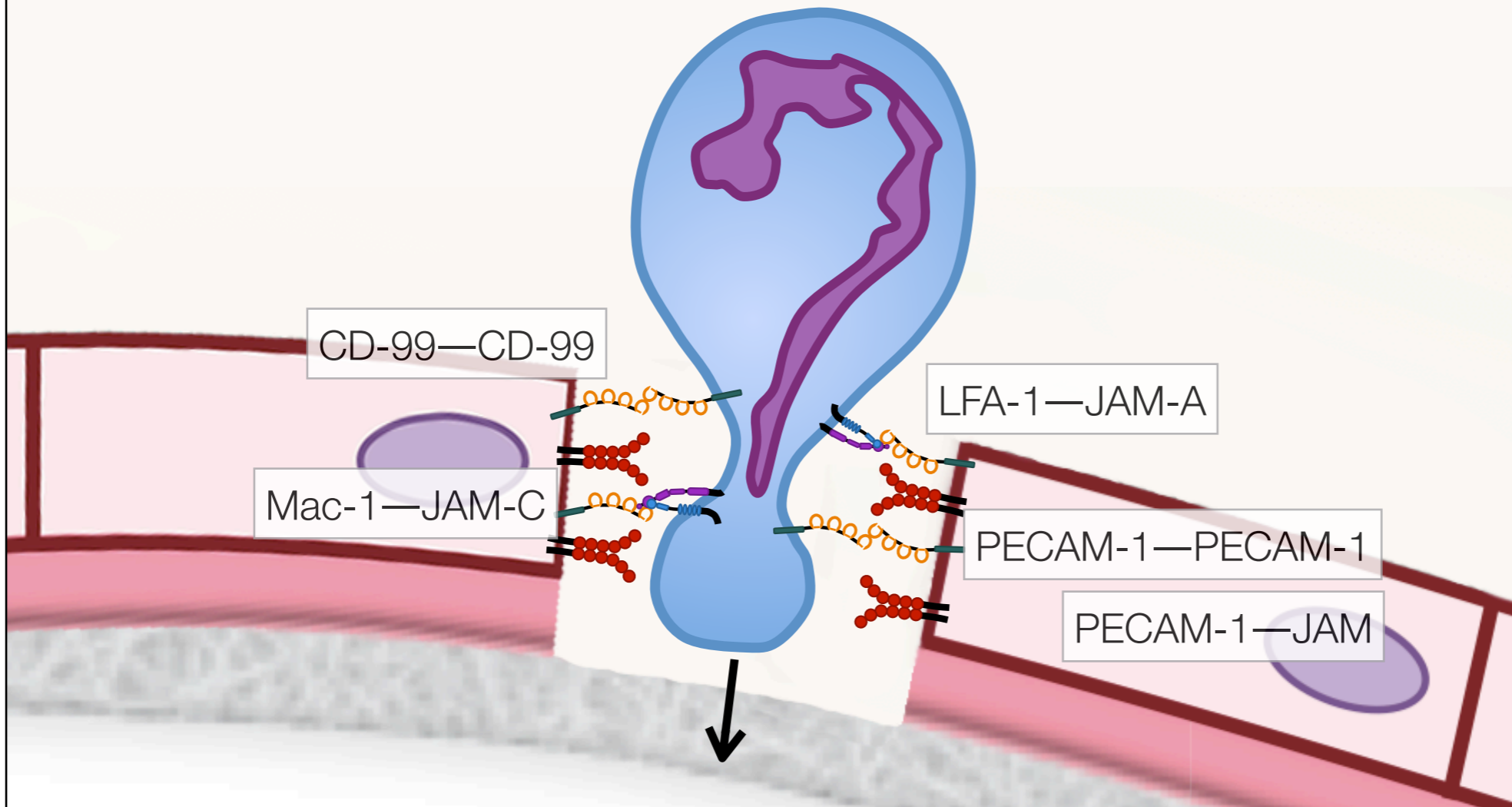
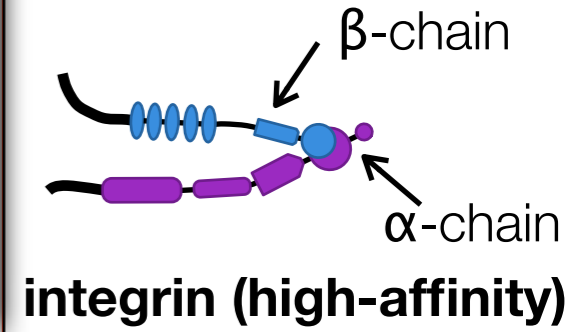
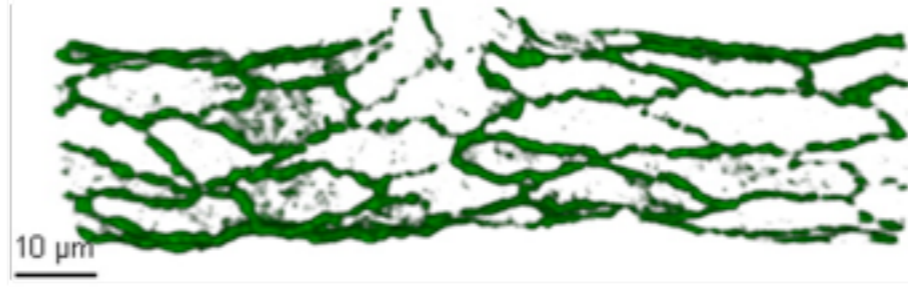
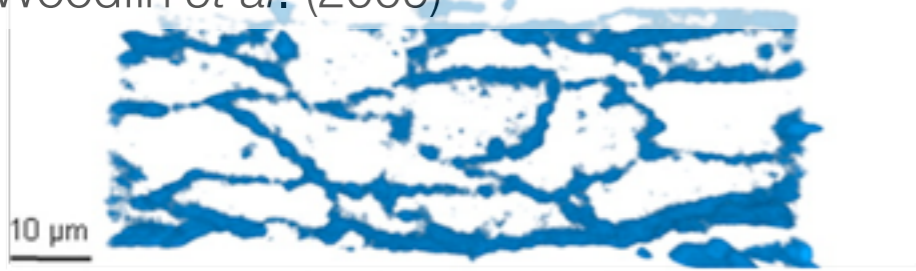
**Ig superfamily**

Adhesion molecules

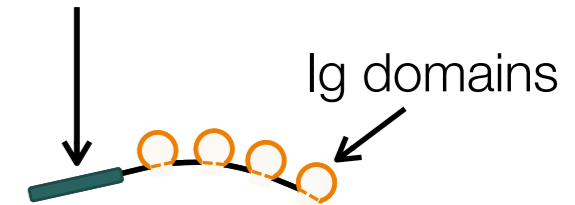
Adhesion and crawling: integrins and Ig superfamily



Woodfin *et al.* (2009)

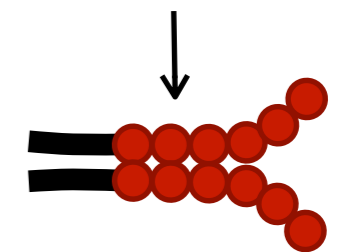


fibronectin-type domains



**Ig superfamily**

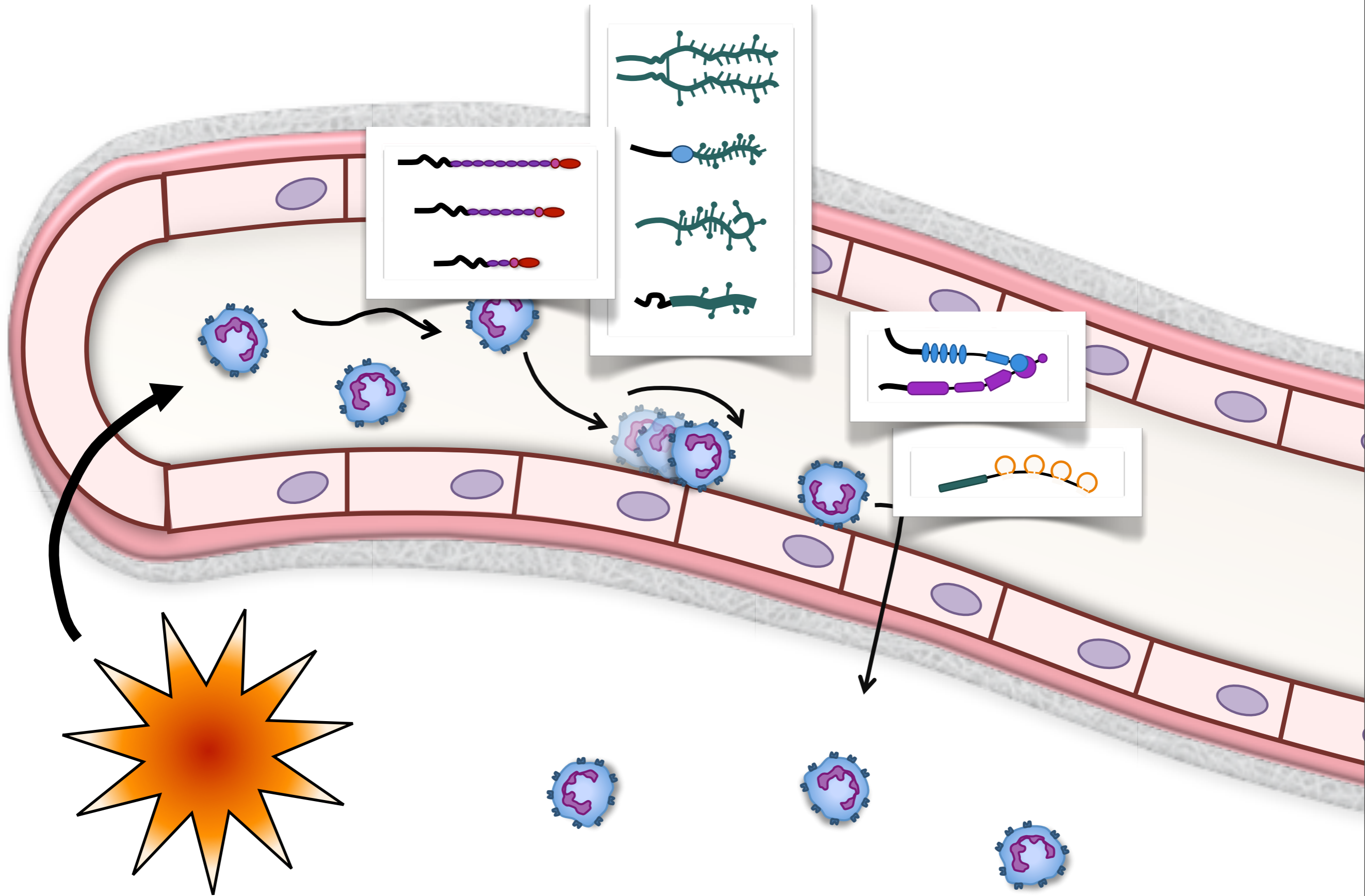
cadherin repeats  
(Ca<sup>2+</sup>-binding domains)



**VE-cadherin**

Adhesion molecules

Emigration: interactions within endothelial junctions



| Selectins             | Mucin-like | Integrins                 | IgSF              |
|-----------------------|------------|---------------------------|-------------------|
| L-selectin<br>(CD62L) | GlyCAM-1   | $\alpha 4\beta 1$ (VLA-4) | ICAM-1,-2,-3      |
| P-selectin<br>(CD62P) | CD34       | $\alpha 6\beta 1$ (VLA-6) | VCAM-1            |
| E-selectin<br>(CD62E) | PSGL-1     | $\alpha L\beta 2$ (LFA-1) | LFA-2, -3         |
|                       | MAdCAM-1   | $\alpha M\beta 2$ (Mac-1) | MAdCAM-1          |
|                       |            |                           | JAMs              |
|                       |            |                           | PECAM-1<br>(CD31) |

| Syndrome                   | Phenotype   | Defect  |
|----------------------------|---|---|
| LADI                       | severe, recurrent infections; delayed wound healing; no pus formation   | $\beta_2$ integrin causing diminished expression of CD18 on leukocytes (firm adhesion)            |
| LADII                      | recurrent infections in early life; decreased chemotaxis; motor retardation; short stature; facial stigmata; Bombay blood group phenotype | defective fucose metabolism causing absence of SLe <sup>x</sup> (leukocyte rolling)               |
| LADIII                     | LADI + severe bleeding tendency   | mutation in kindlin 3 causing general activation defects in all $\beta$ integrins                 |
| Glanzmann's thrombasthenia | bleeding tendency of varying degrees  | mutated $\alpha IIb\beta 3$ integrin on platelets, which are unable to aggregate                  |
| Wiskott-Aldrich syndrome   | raised pathogen susceptibility; eczema; small platelets; thrombocytopaenia  | mutations in WASp mean monocytes cannot form podosomes and have limited phagocytotic capabilities |



## Selectin antagonists

### *Small molecule inhibitors*

- Bimosiamose (pan-selectin) - allo-/xenograft rejection, asthma, COPD, psoriasis

PHASE 2

### *Carbohydrate-based*

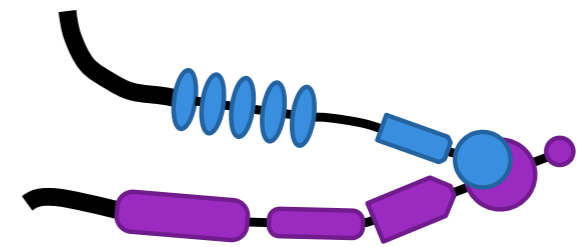
- GMI-1070 (pan-selectin) - SCD crisis

PHASE 2

### *Peptides/recombinant proteins*

- YSPSL (rPSGL-1) - renal I/R

PHASE 2



## Integrin antagonists

### *Antibodies*

APPROVED

- Efalizumab ( $\alpha I\beta 2$ ) - psoriasis
- Natalizumab ( $\alpha 4$  chain) - MS, Crohn's disease

APPROVED

### *Non-peptide inhibitors*

- Tirofiban ( $\alpha II\beta 3$ ) - platelet aggregation
- Valategrast ( $\alpha 4\beta 1$ ) - asthma

WITHDRAWN

APPROVED

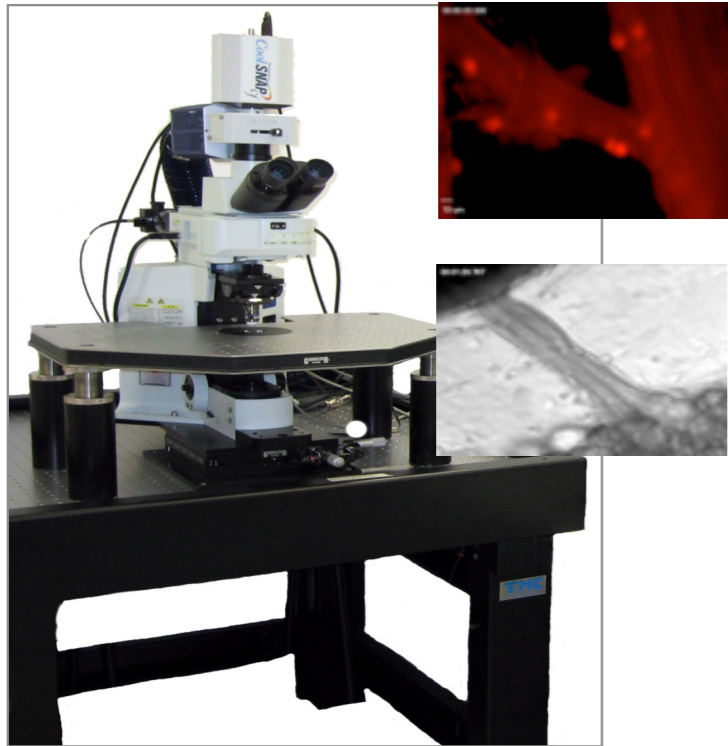
### *Peptide mimetics*

- Cilengitide ( $\alpha v\beta 3$ ) - glioblastoma (via angiogenesis)
- Eptifibatide ( $\alpha II\beta 3$ ) - anti-platelets

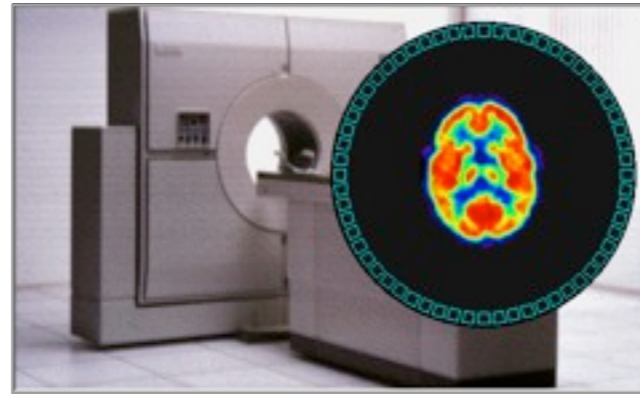
PHASE 3

APPROVED





Optical



PET



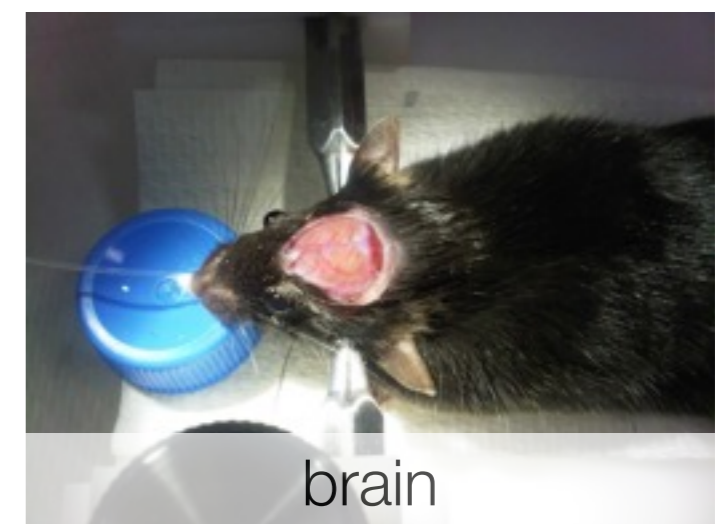
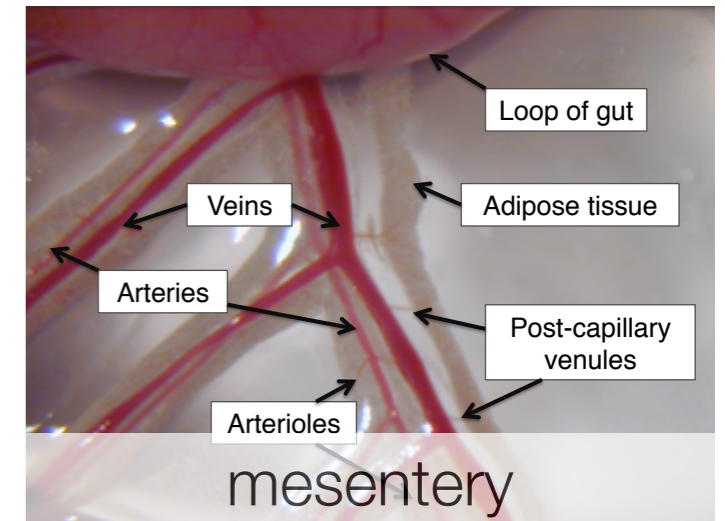
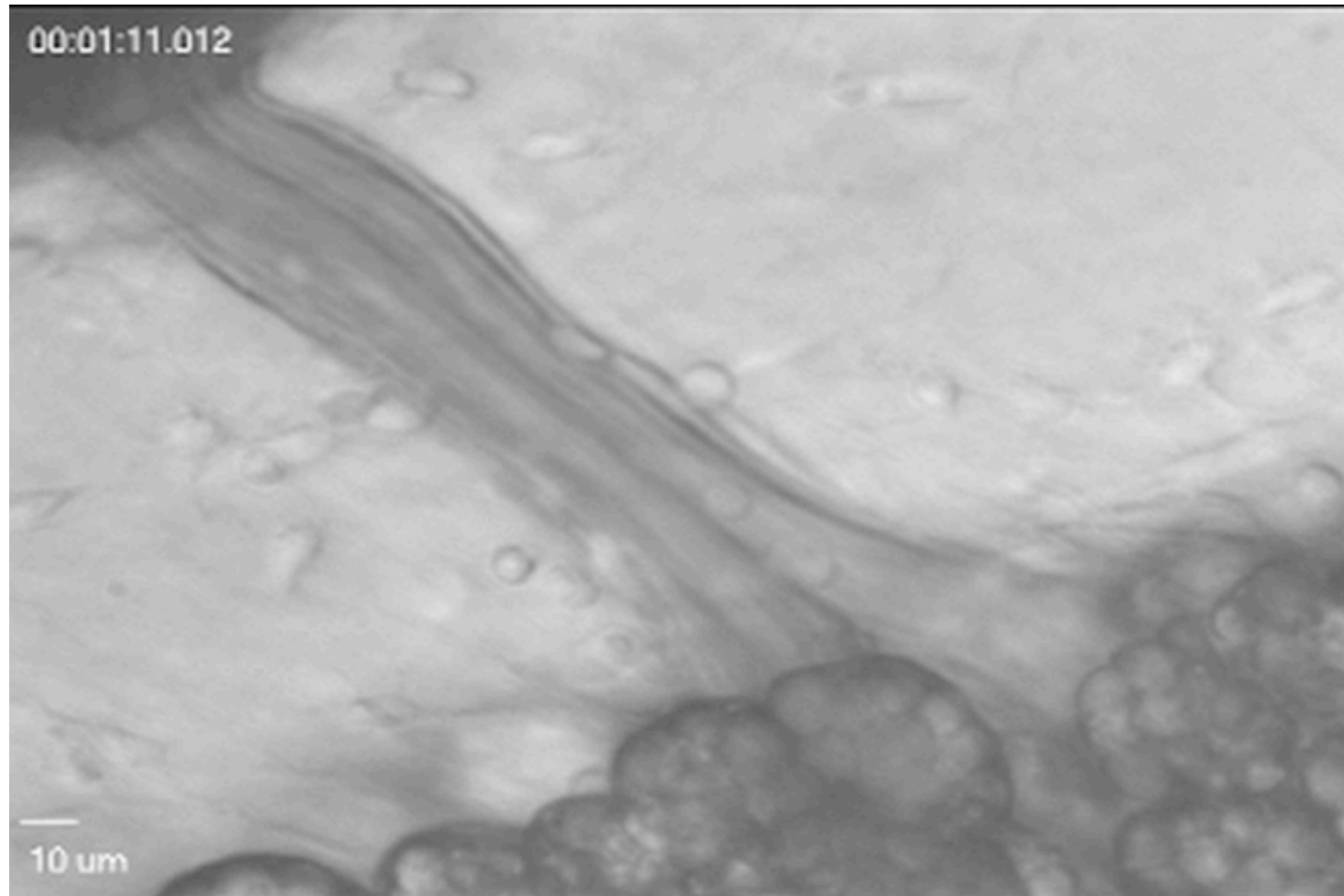
Scintigraphy

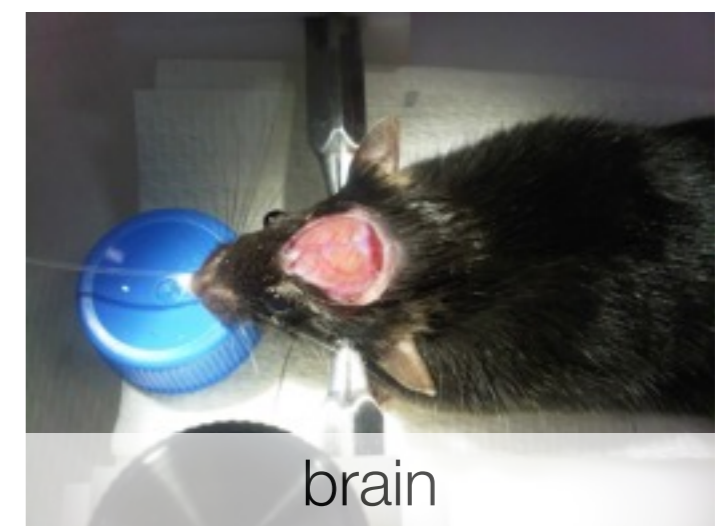
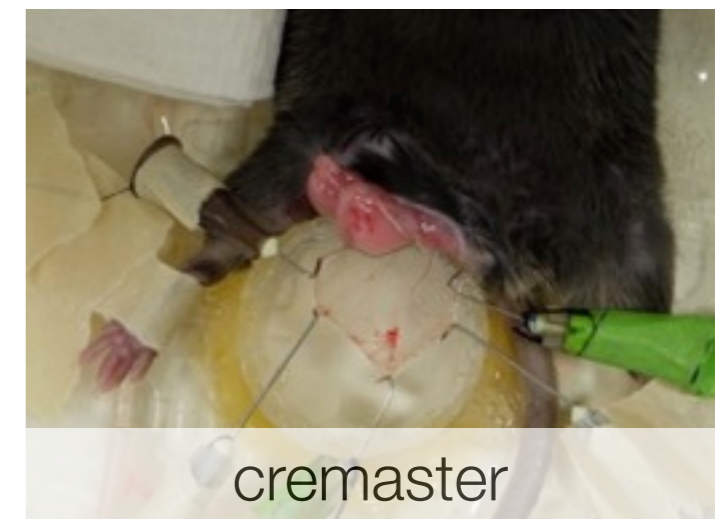
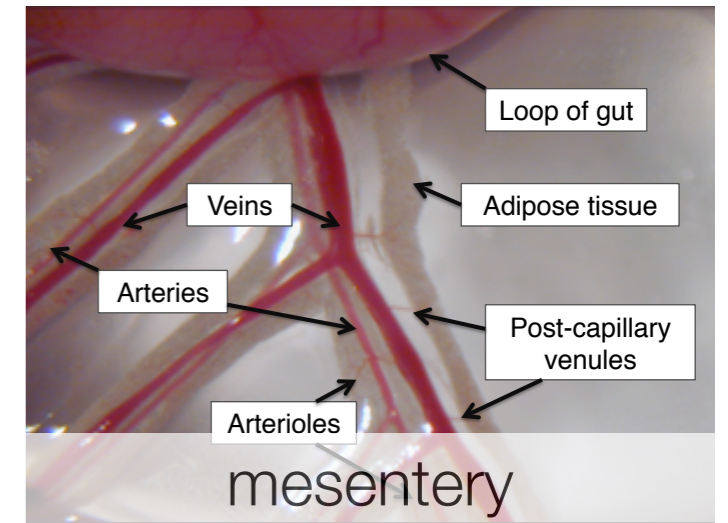
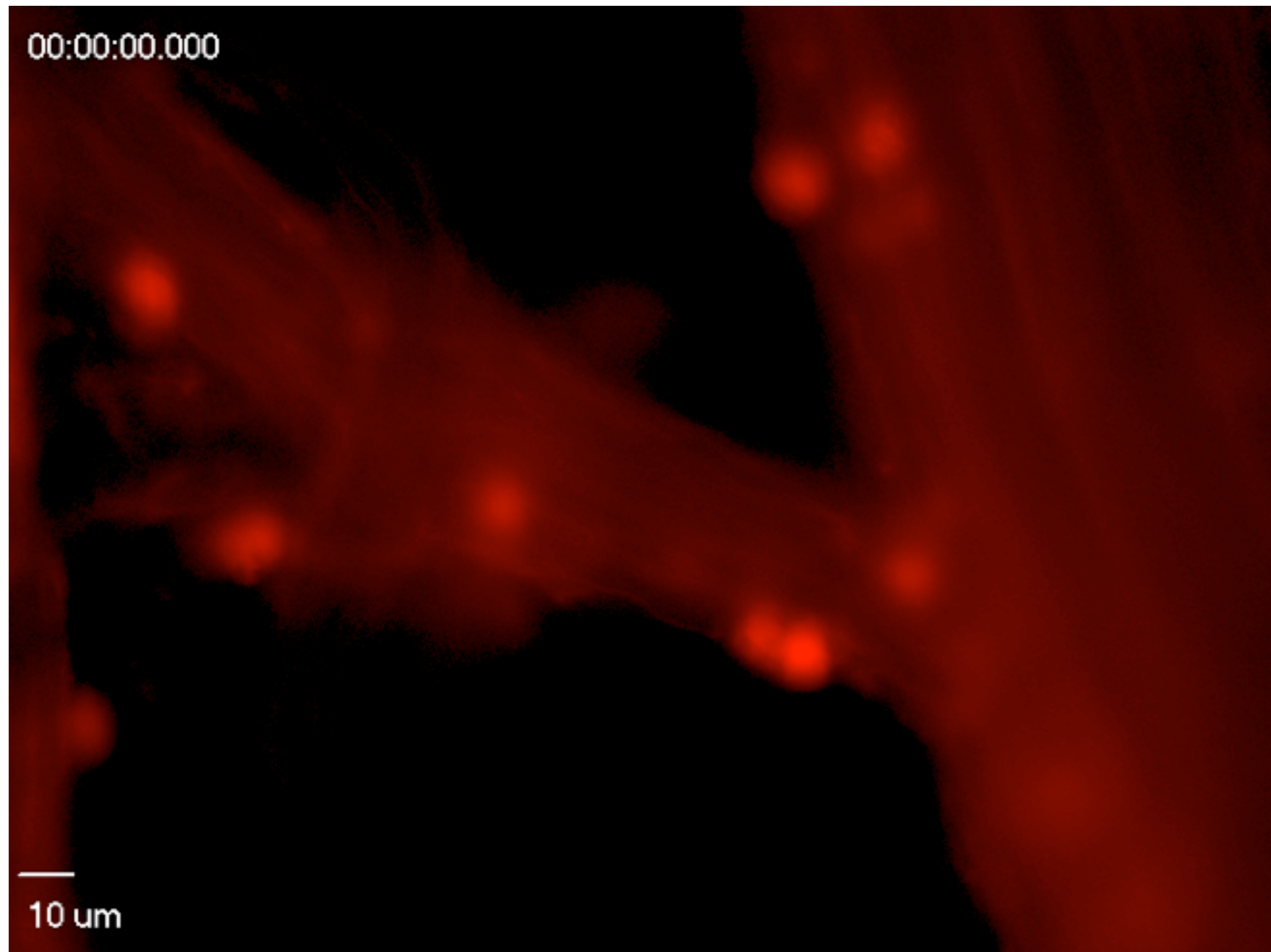


Ultrasound

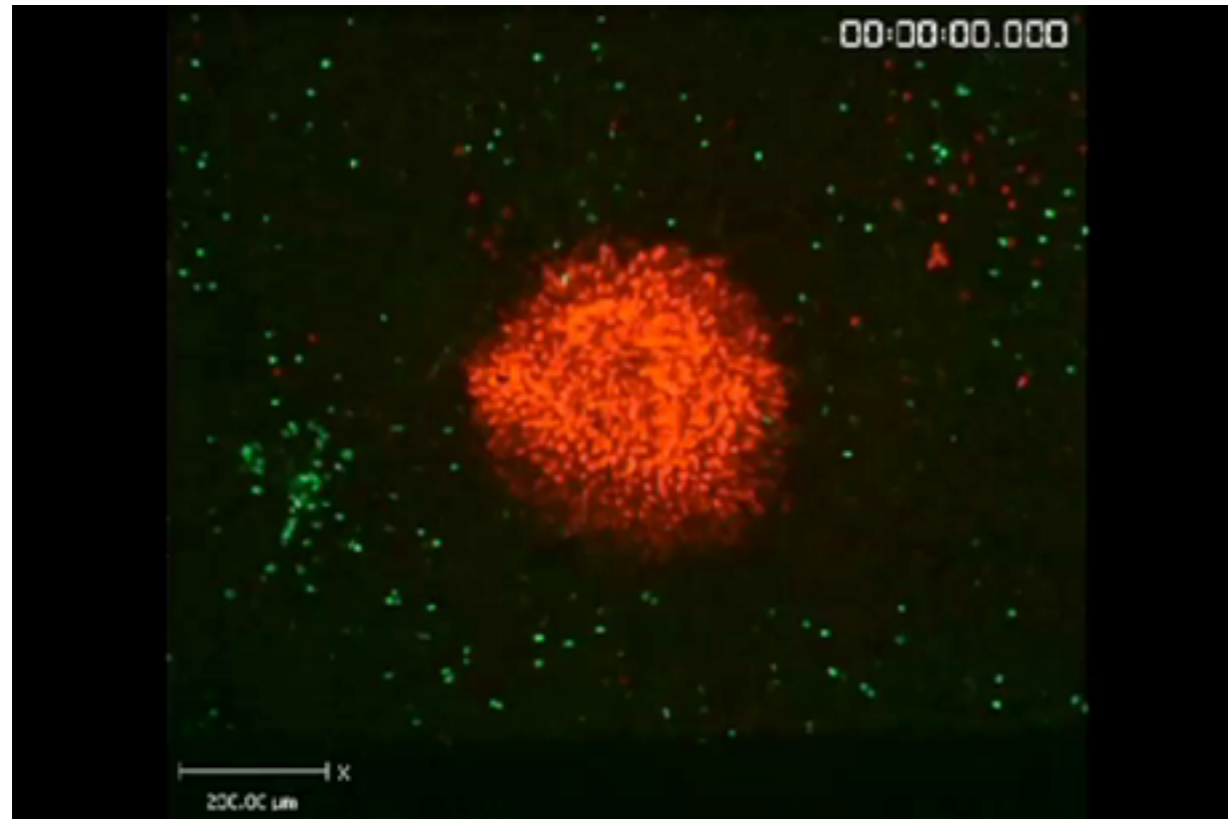


MRI

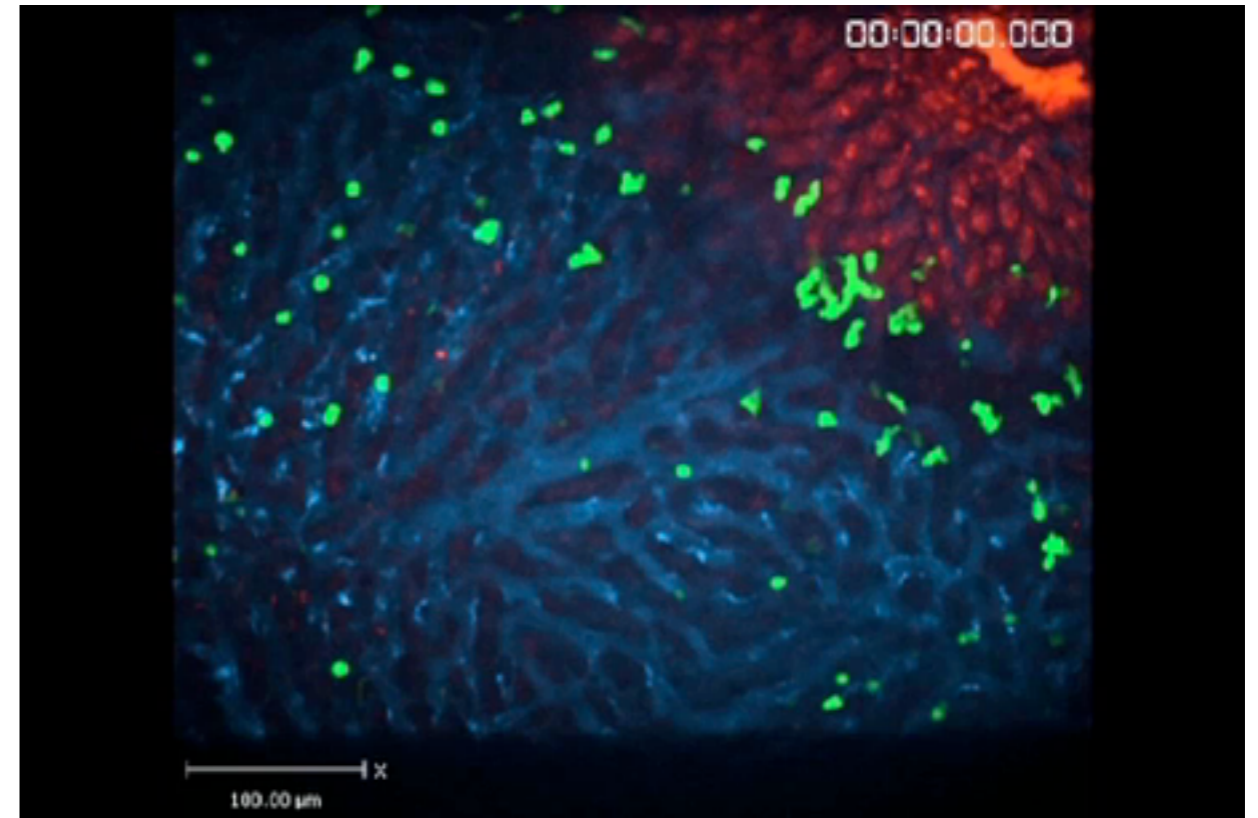




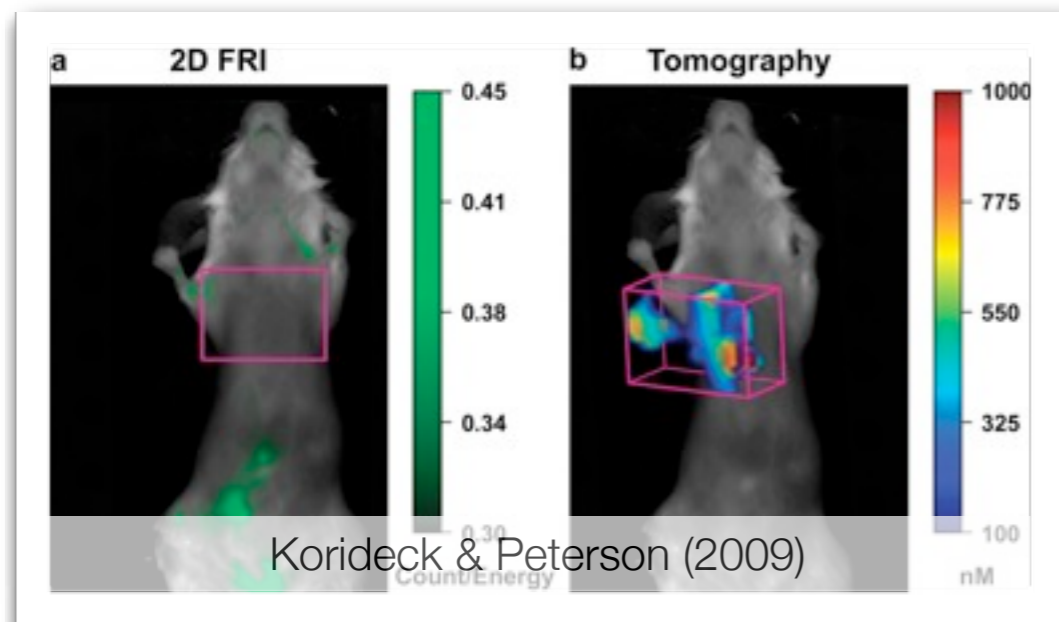
## Neutrophil migration towards a focal necrotic lesion in the liver



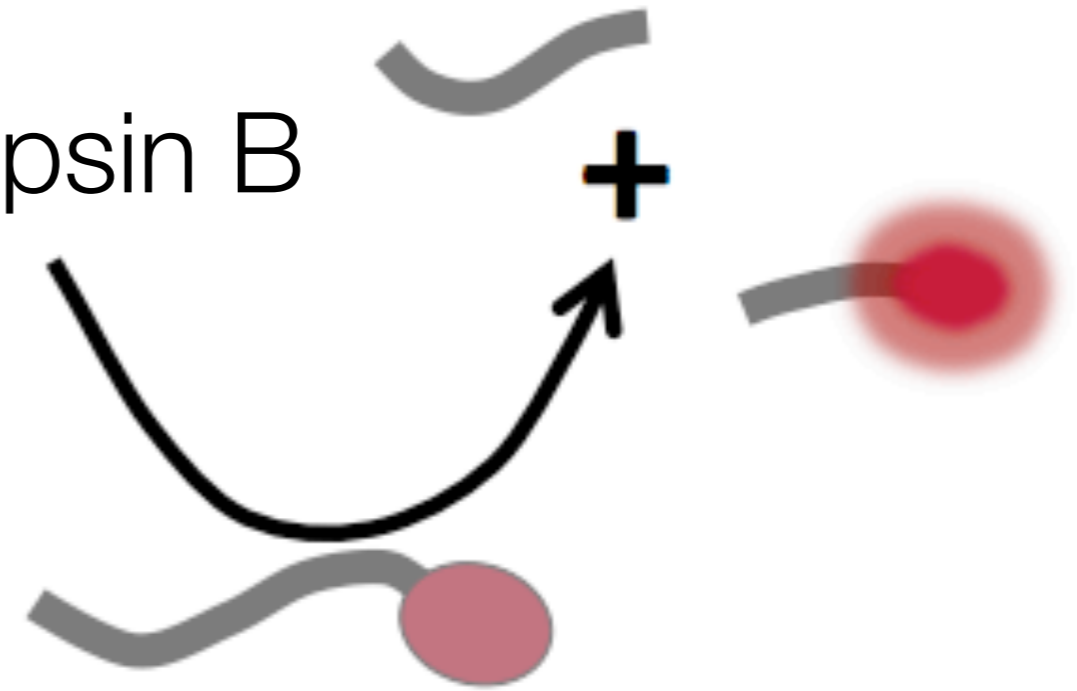
## Intravascular routes of neutrophil chemotaxis



McDonald et al. (2010)



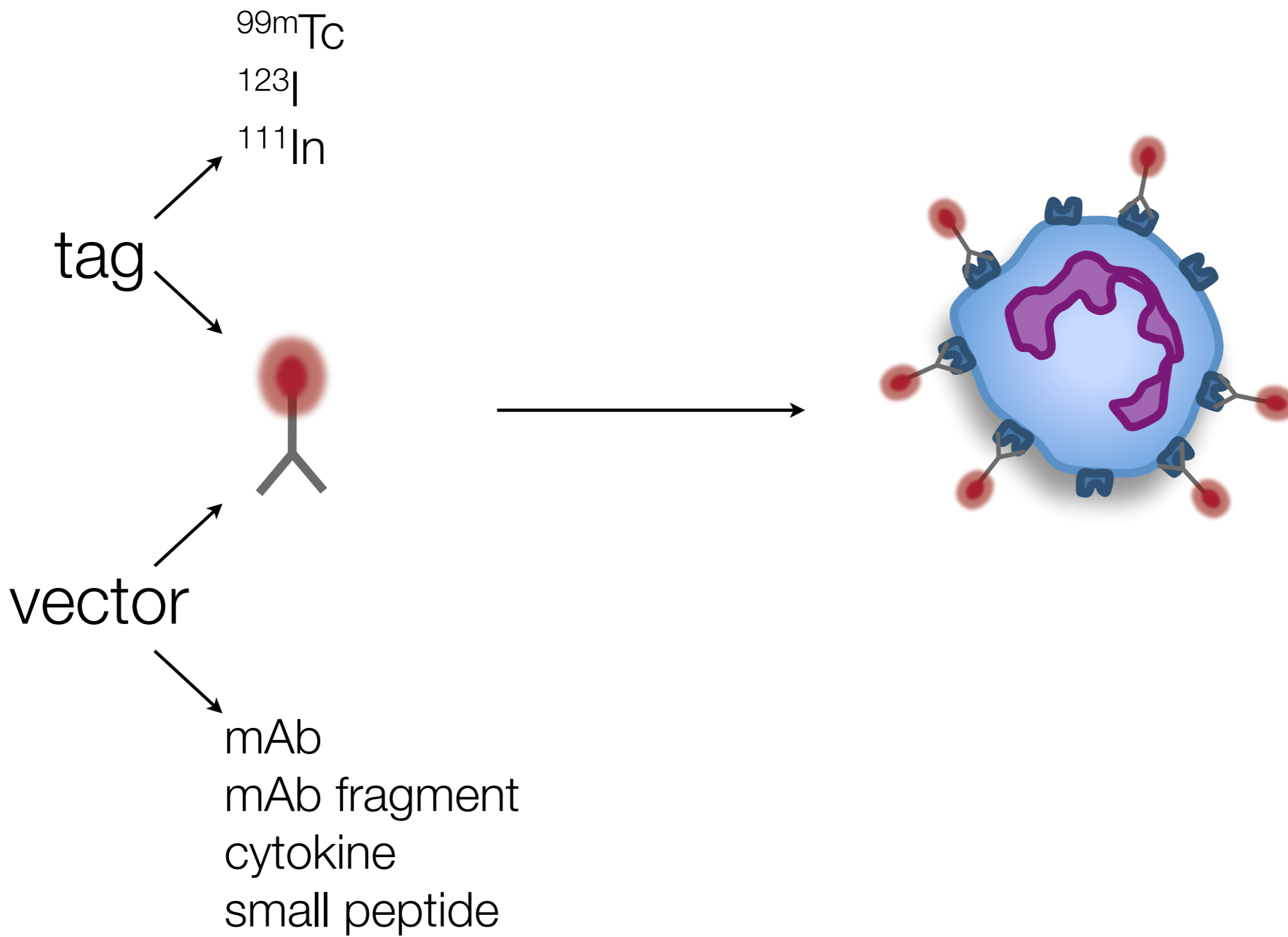
cathepsin B

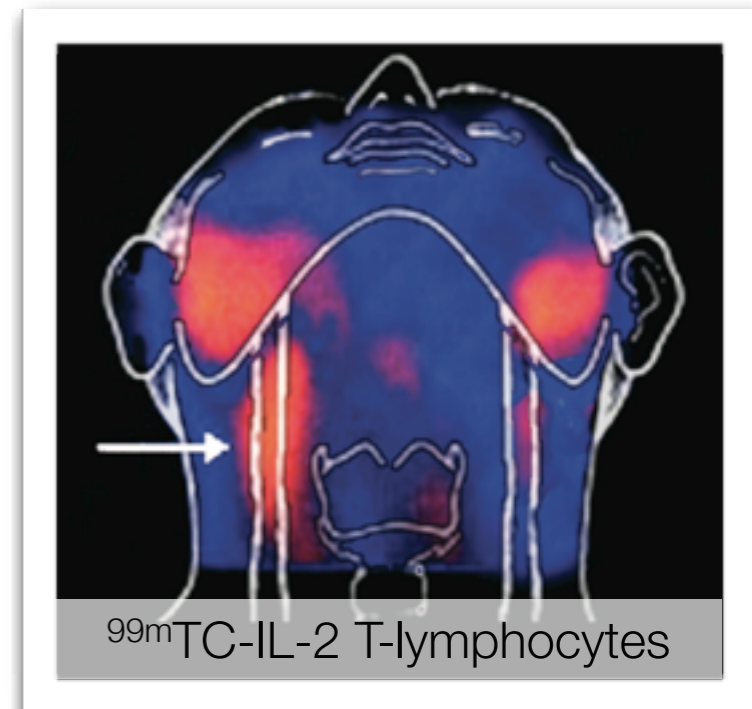
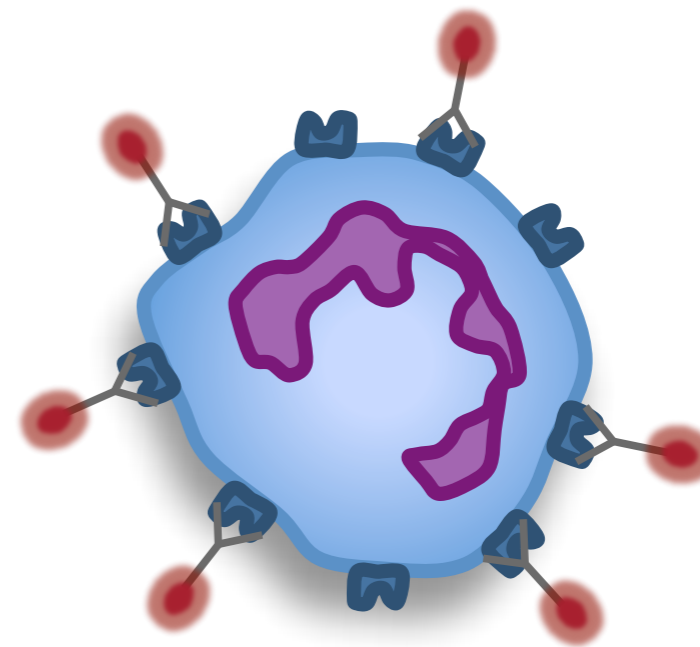
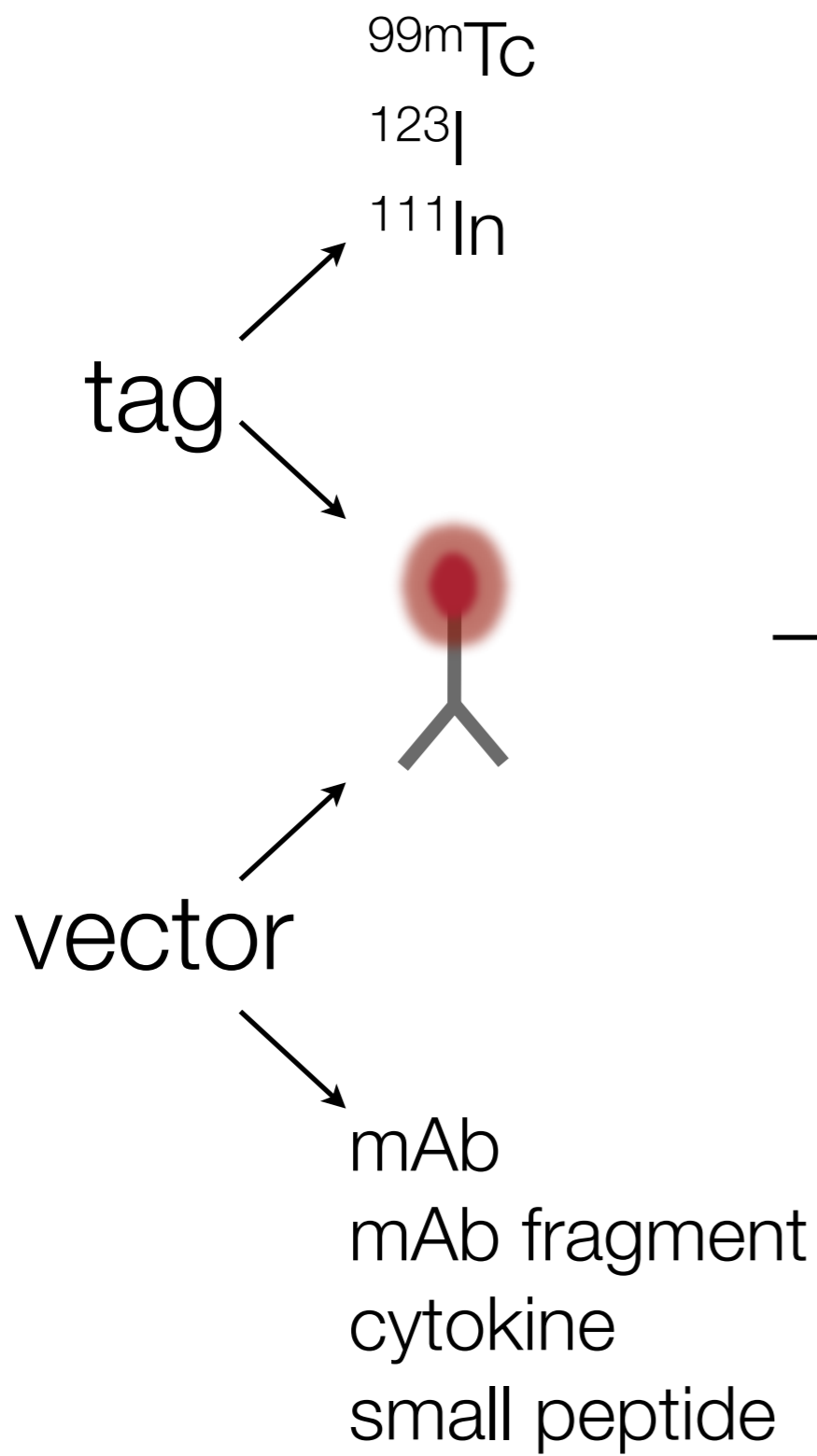


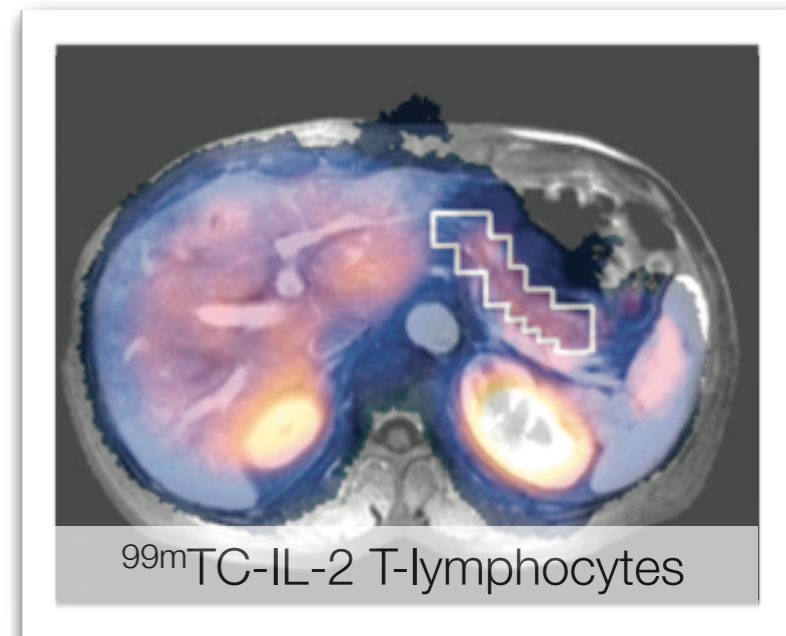
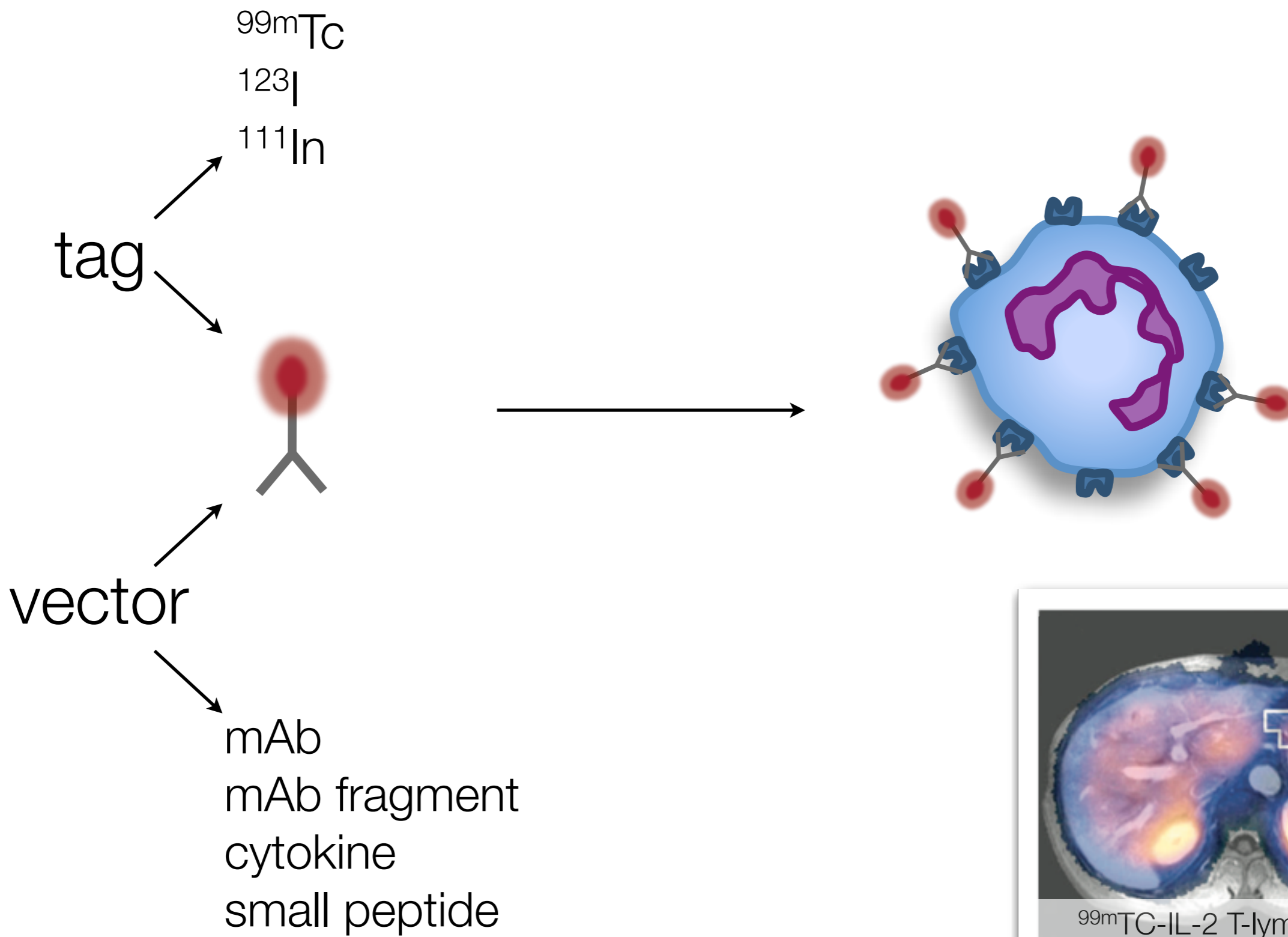
## ProSense 680

- An inducible fluorescent imaging probe
- Used in arthritis and cancer models
- Shows local density of pro-inflammatory cell infiltrates

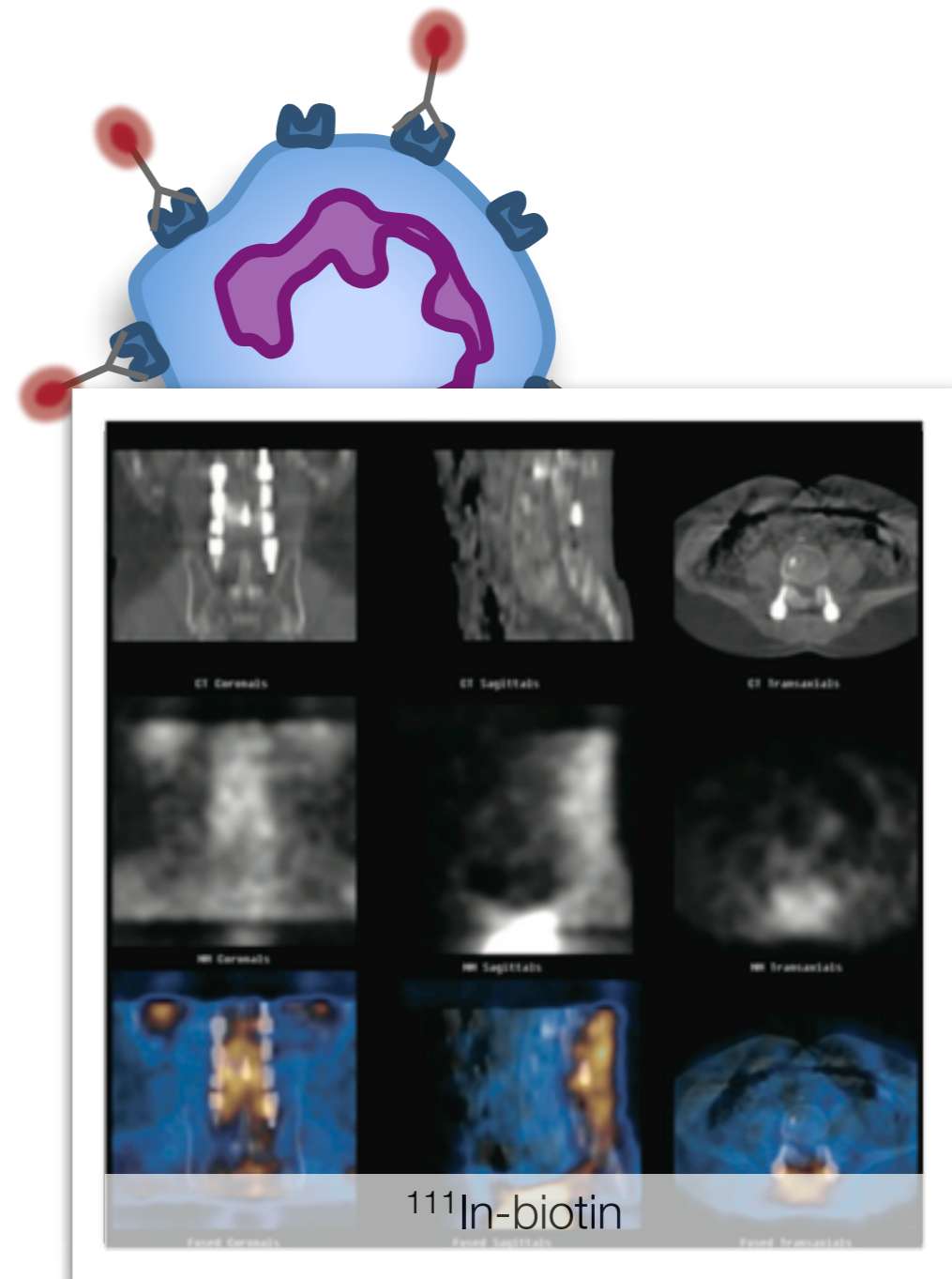
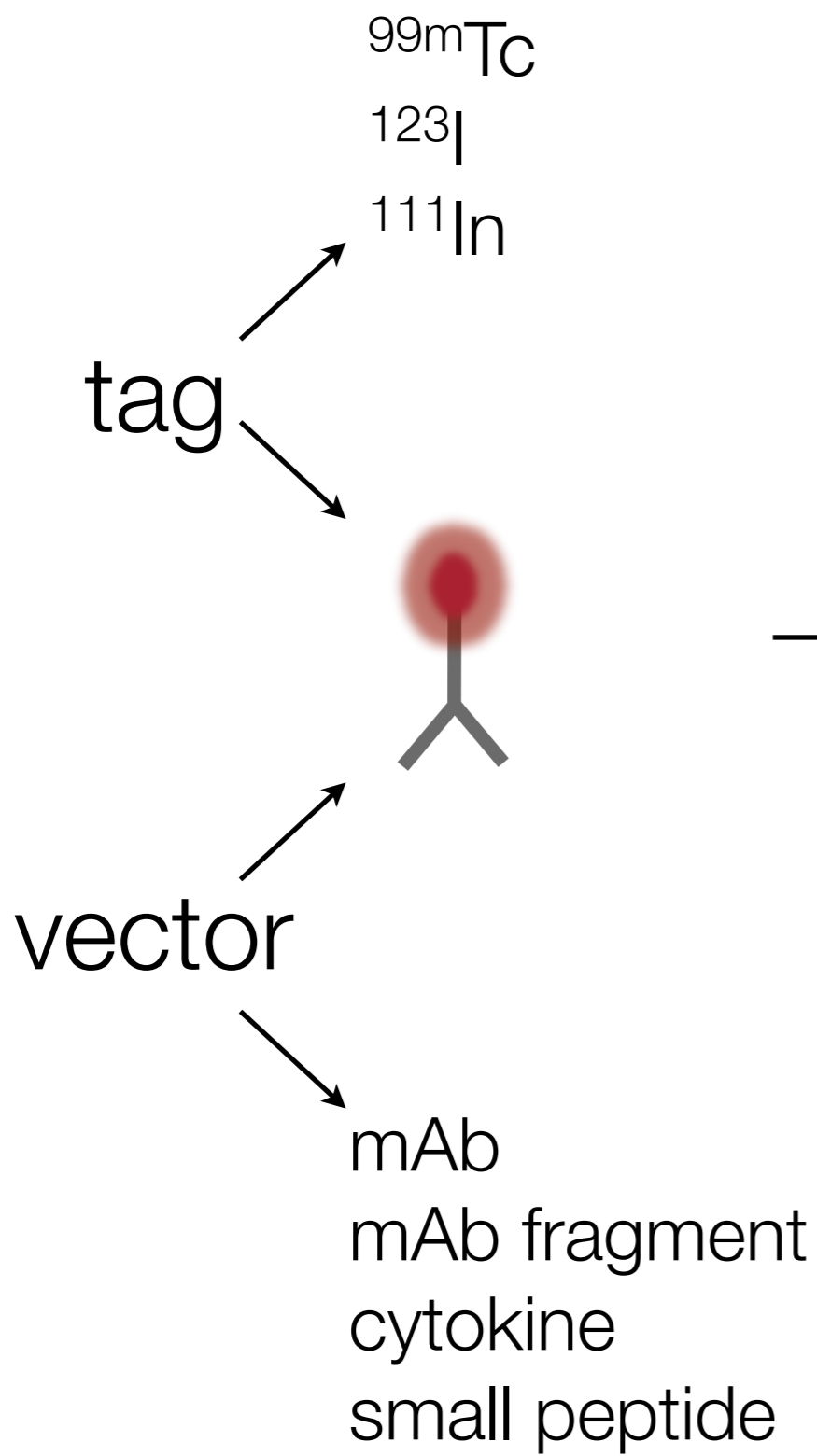


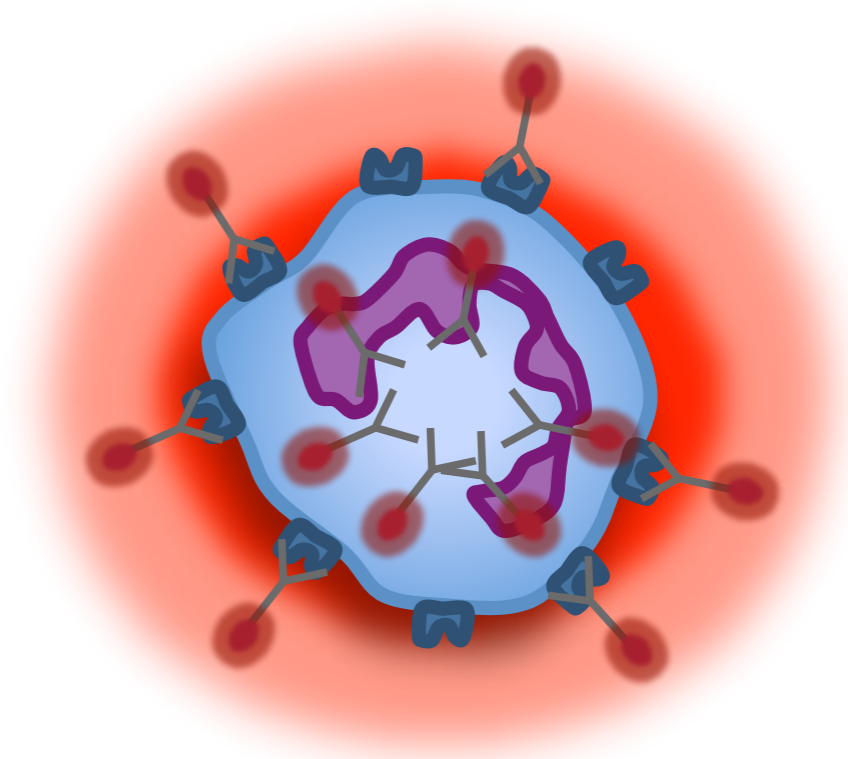
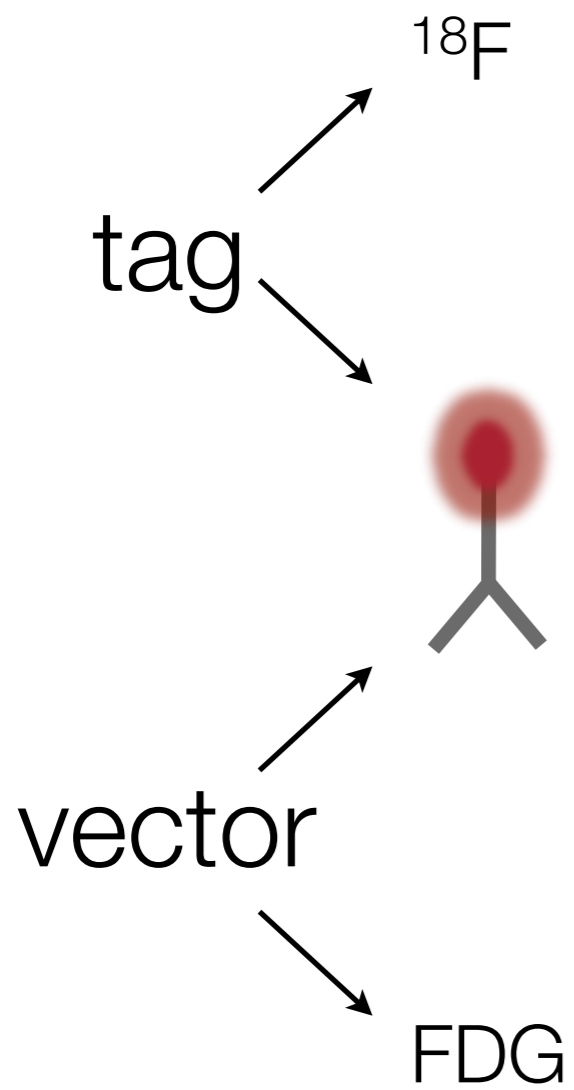


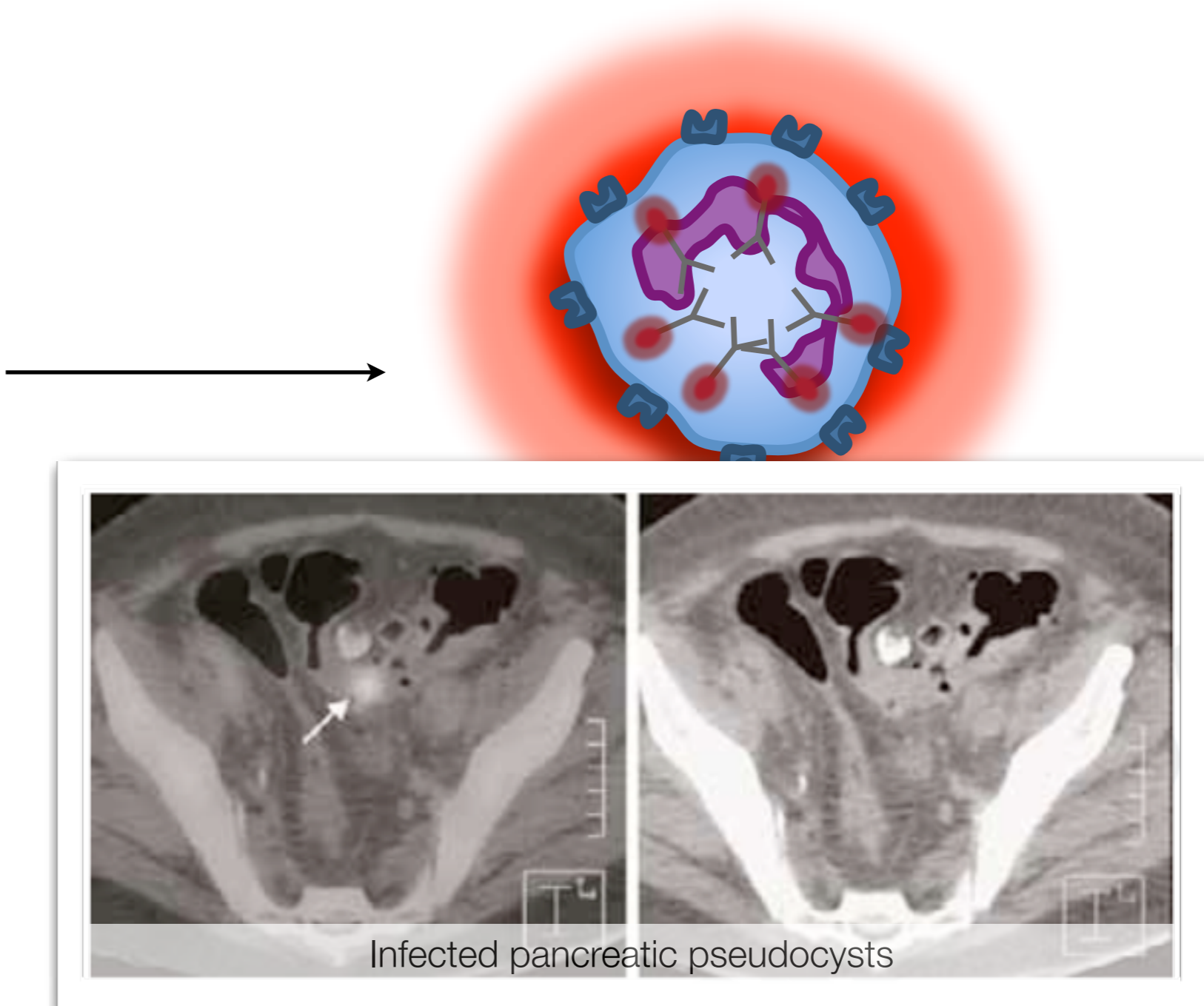
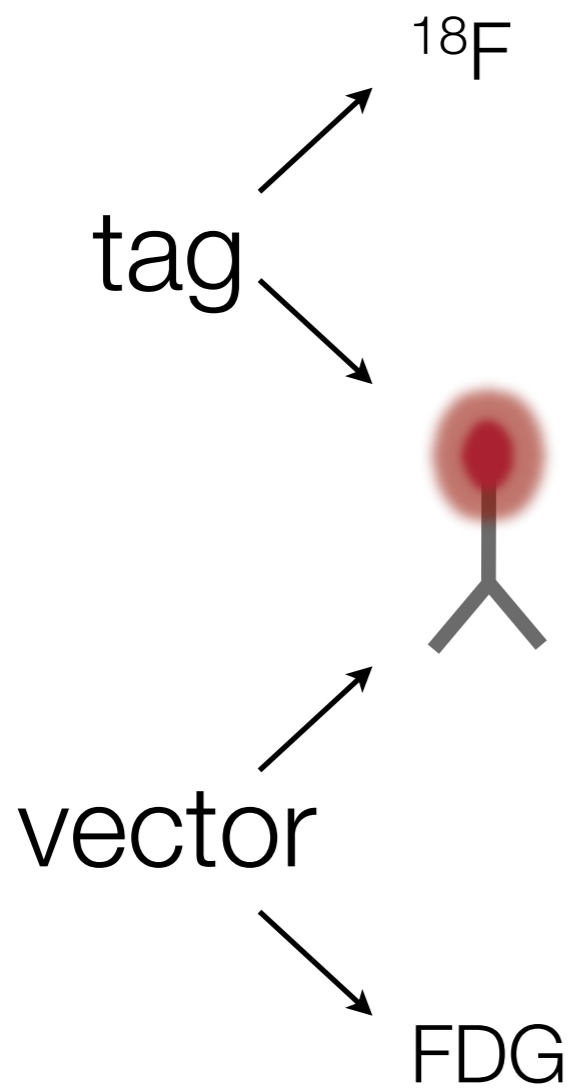


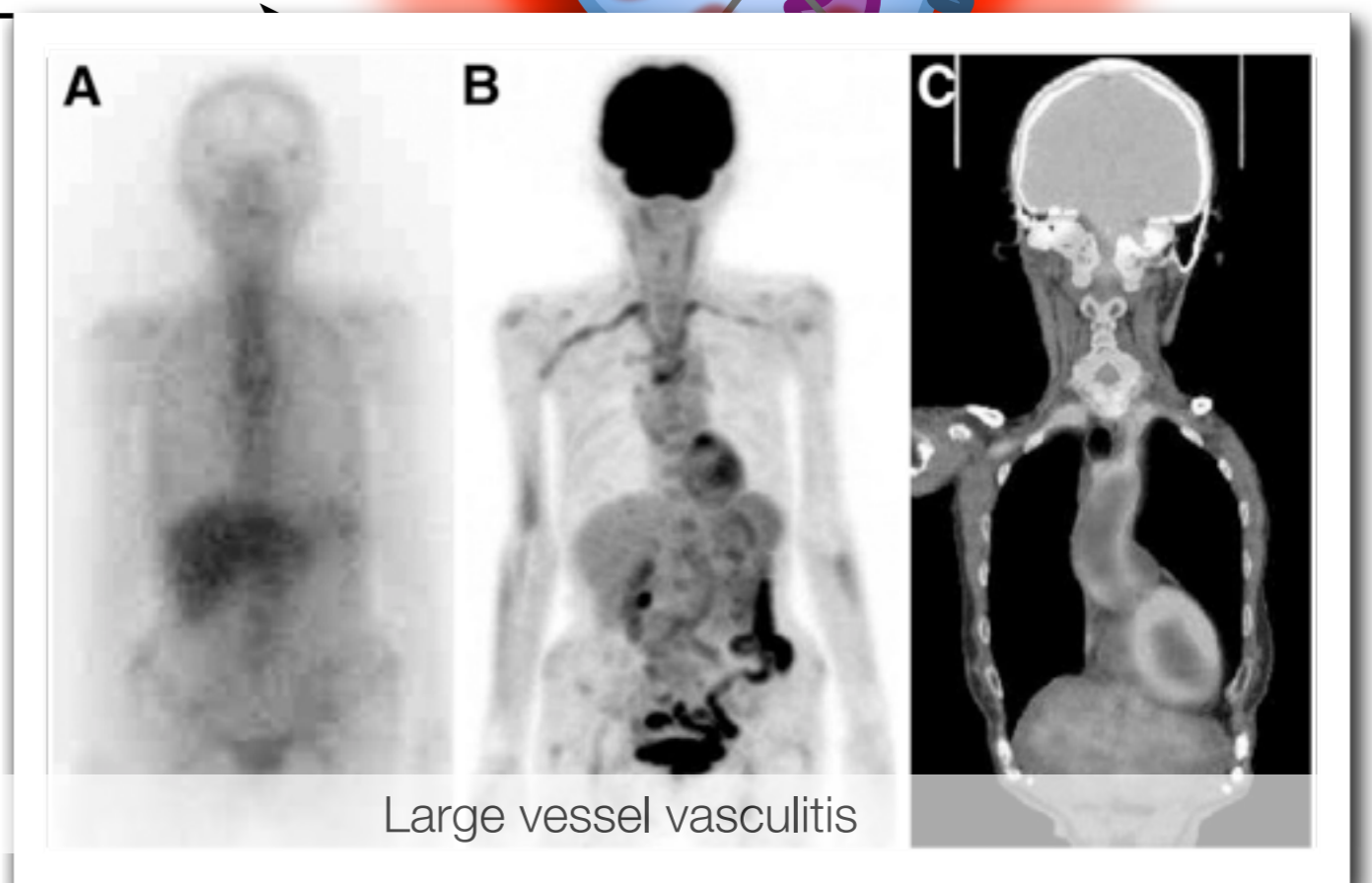
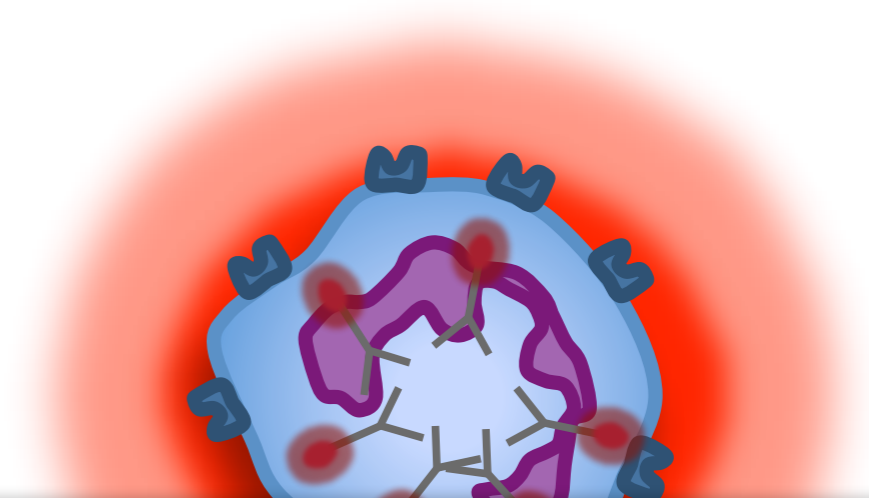
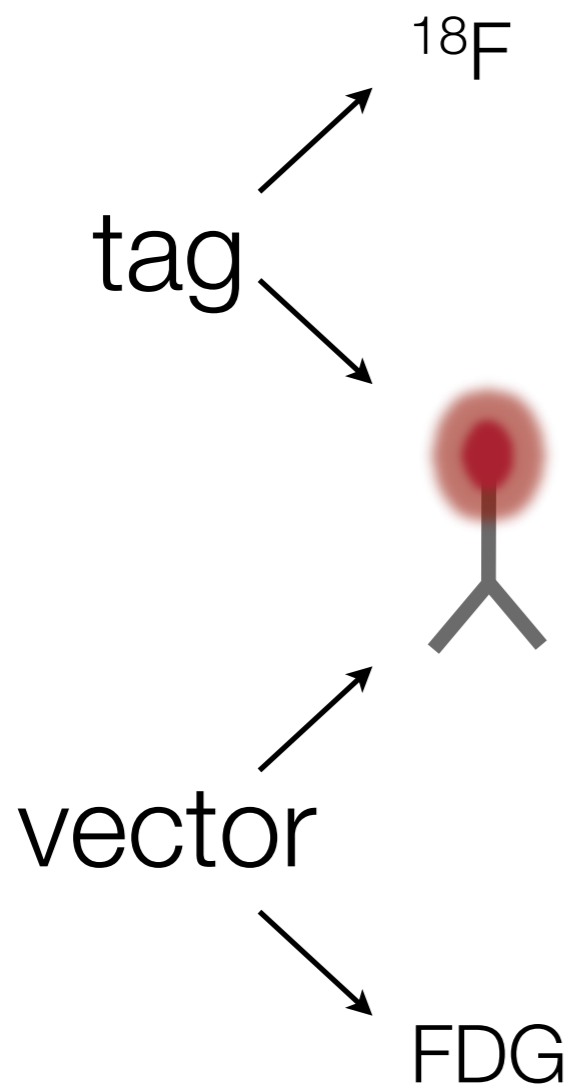


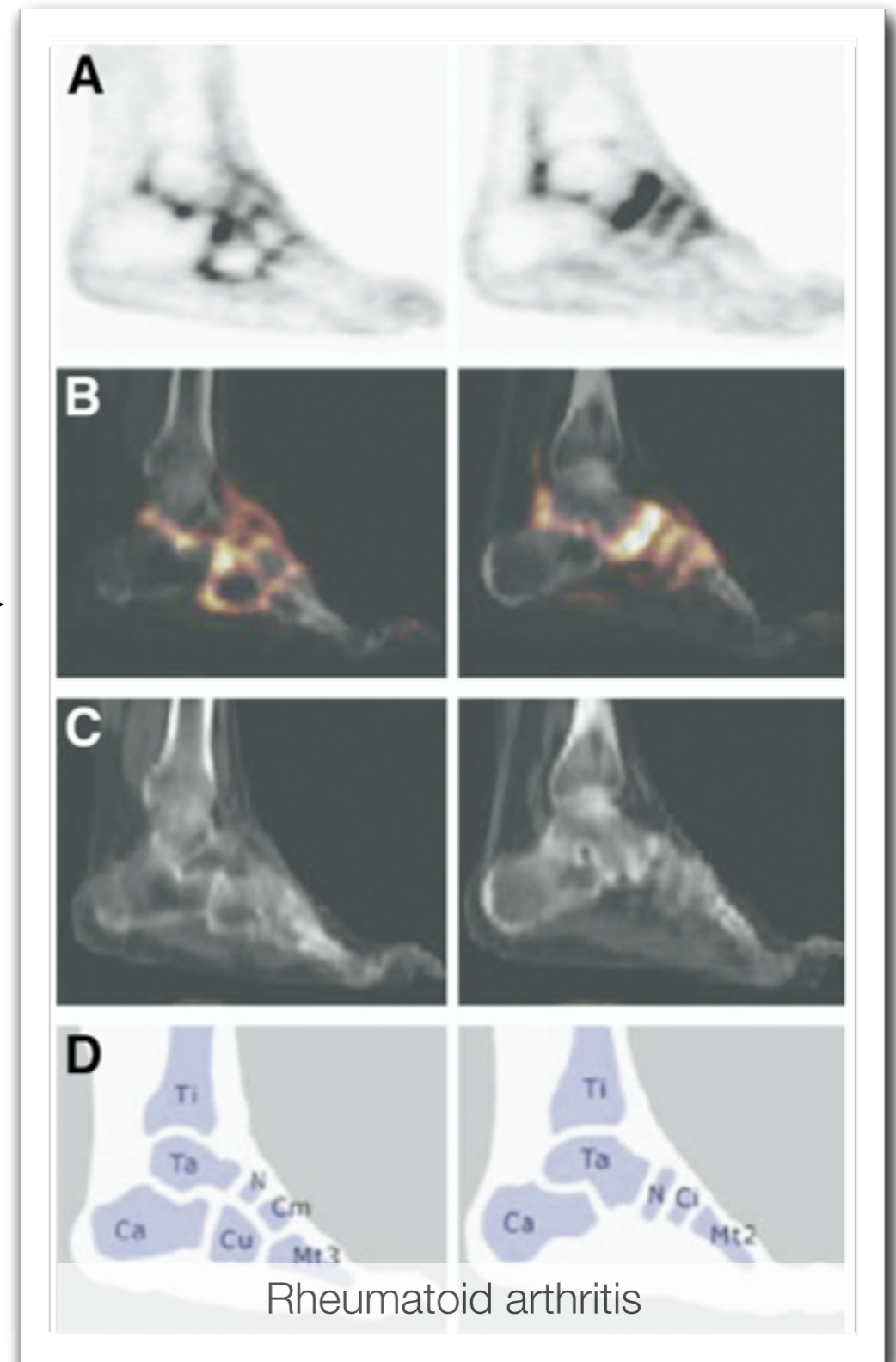
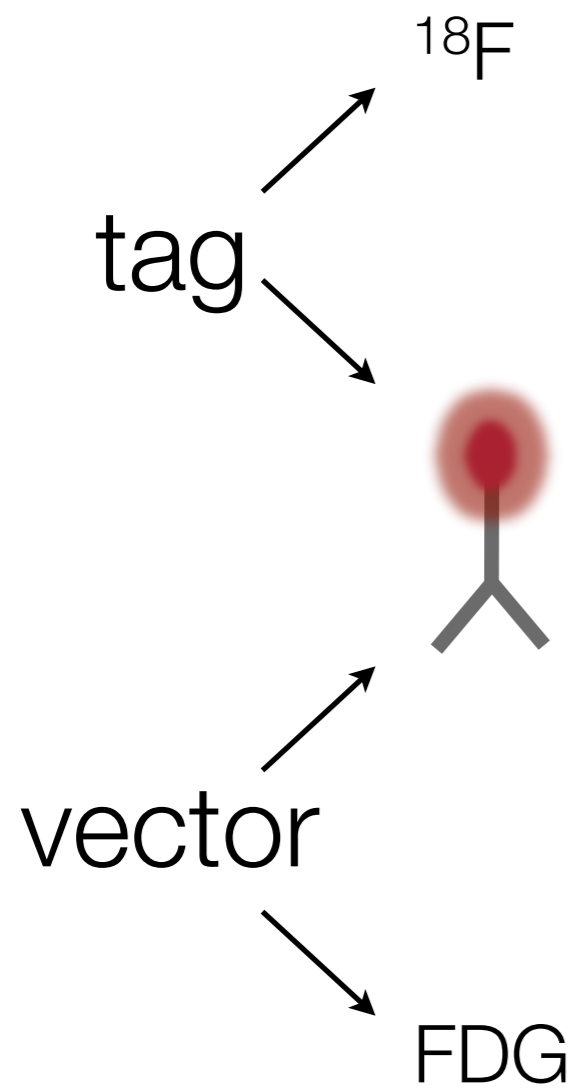








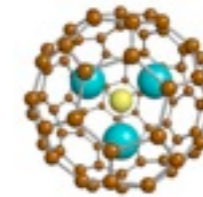




# MRI contrast agents

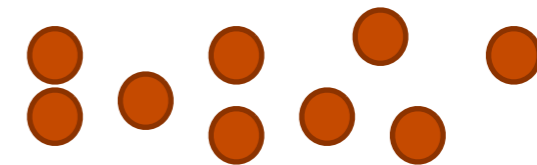


**gadolinium complexes**



**iron oxide nanoparticles**

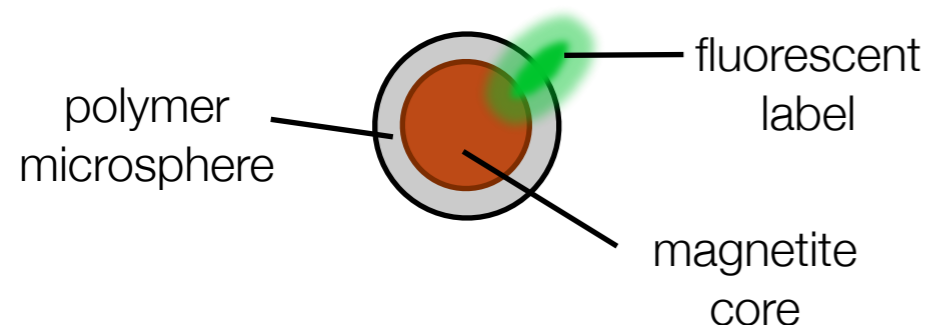
(5-10nm)



- ultrasmall superparamagnetic iron oxide (USPIO)
- cross-linked iron oxide (CLIO)
- superparamagnetic iron oxide (SPIO) coated with eg. dextran, starch, albumin, silicon

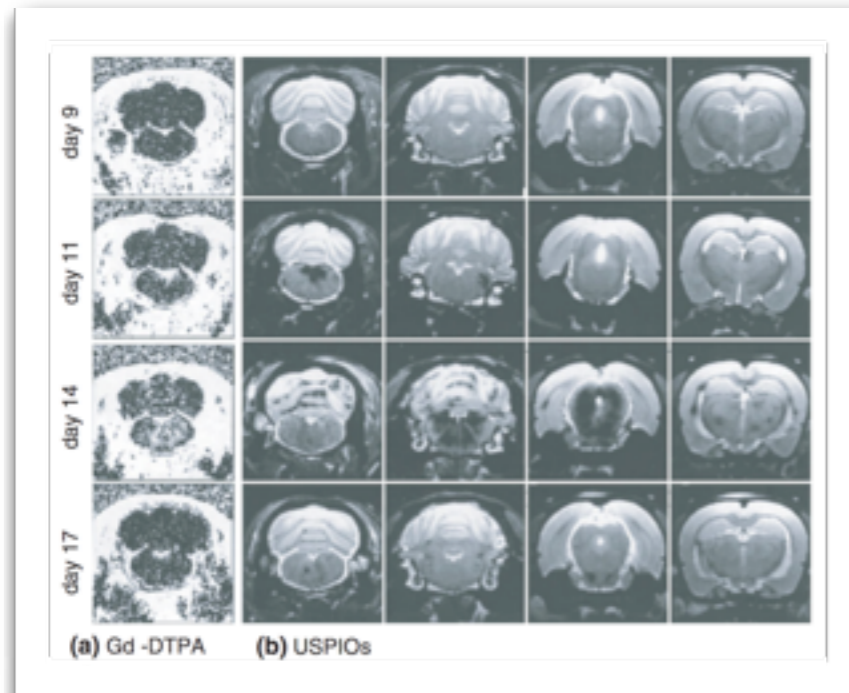
**magnetofluorescent nanoparticles (MFNPs)**

(0.4-1.6 $\mu$ m)

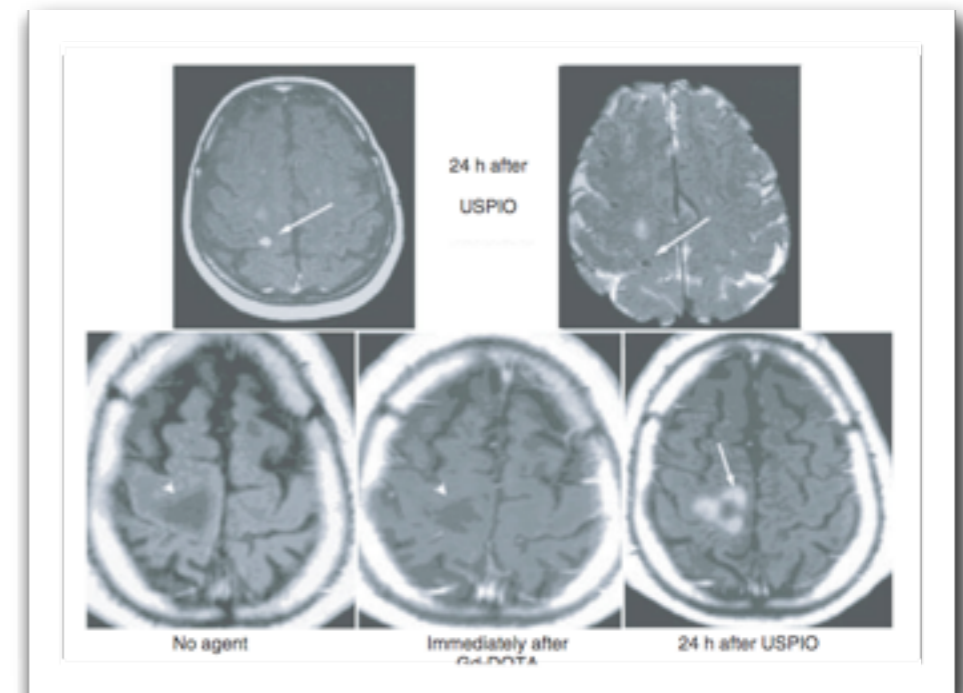


# Reproducibility between animal models and human patients

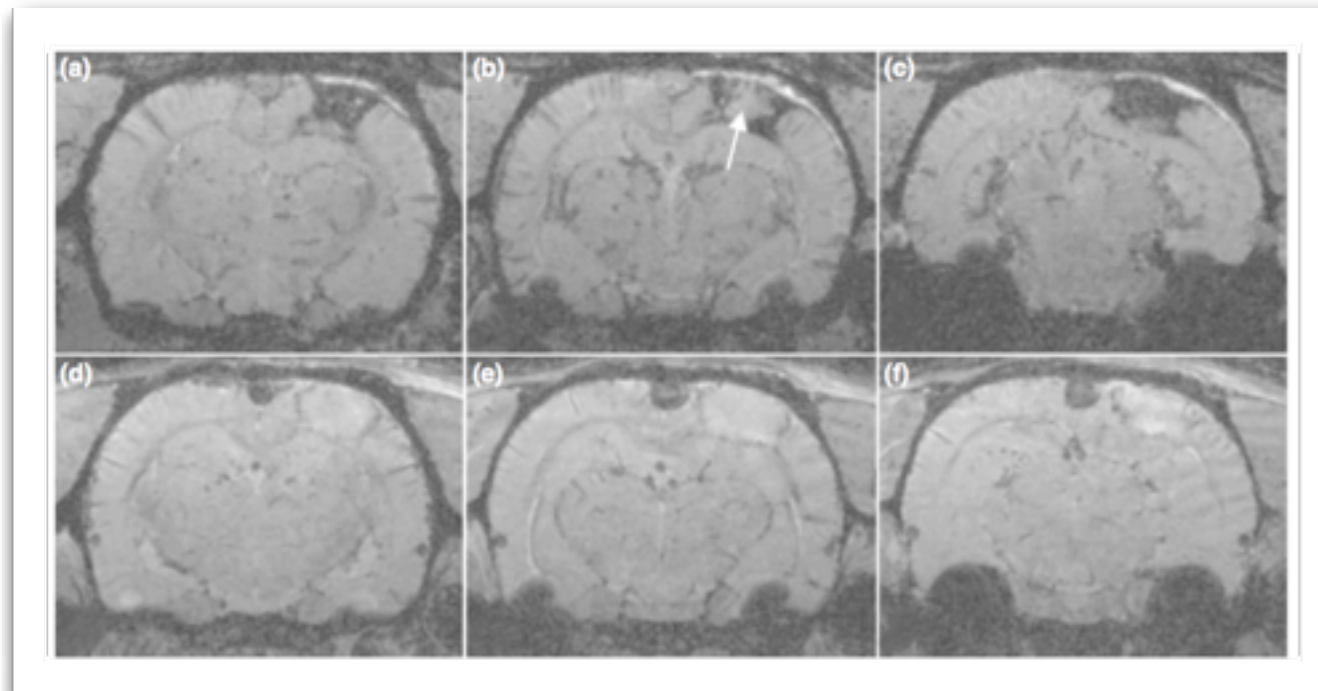
EAE (mouse)



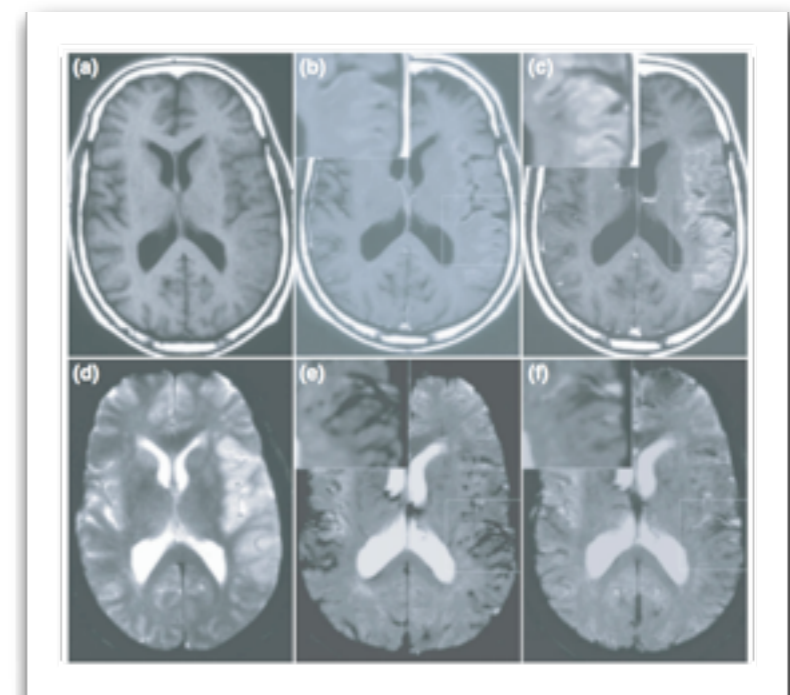
MS



Photochemically-induced thrombosis (rat)



Ischaemic stroke



- L/EC interactions are mediated by a variety of adhesion molecules
  - rolling - selectins
  - adhesion - integrins and IgSF
  - emigration - integrins and IgSF (with some cadherin involvement)
- Genetic mutations in adhesion molecules are the cause of several diseases
  - LAD I, II, III
  - Glanzmann's thrombasthenia
  - Wiskott-Aldrich syndrome
- Emerging novel therapies
  - Targeting adhesion molecules
  - Adhesion molecules as drug delivery agents
- Imaging leukocyte adhesion: advantages and disadvantages of different modalities
  - Optical imaging - intravital microscopy, activated optical probes
  - Radiopharmaceuticals - scintigraphy, PET
  - MRI - contrast agents



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