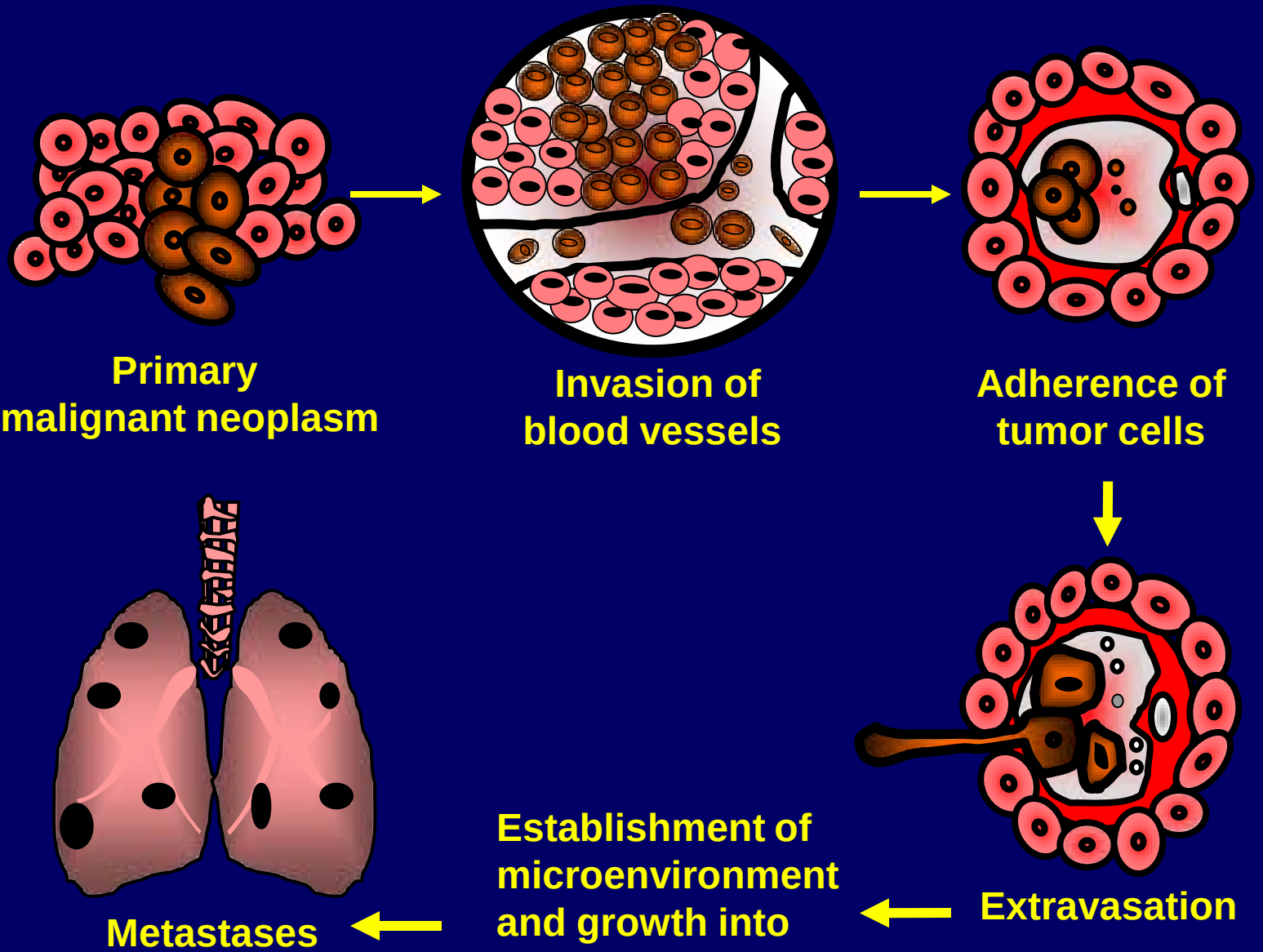
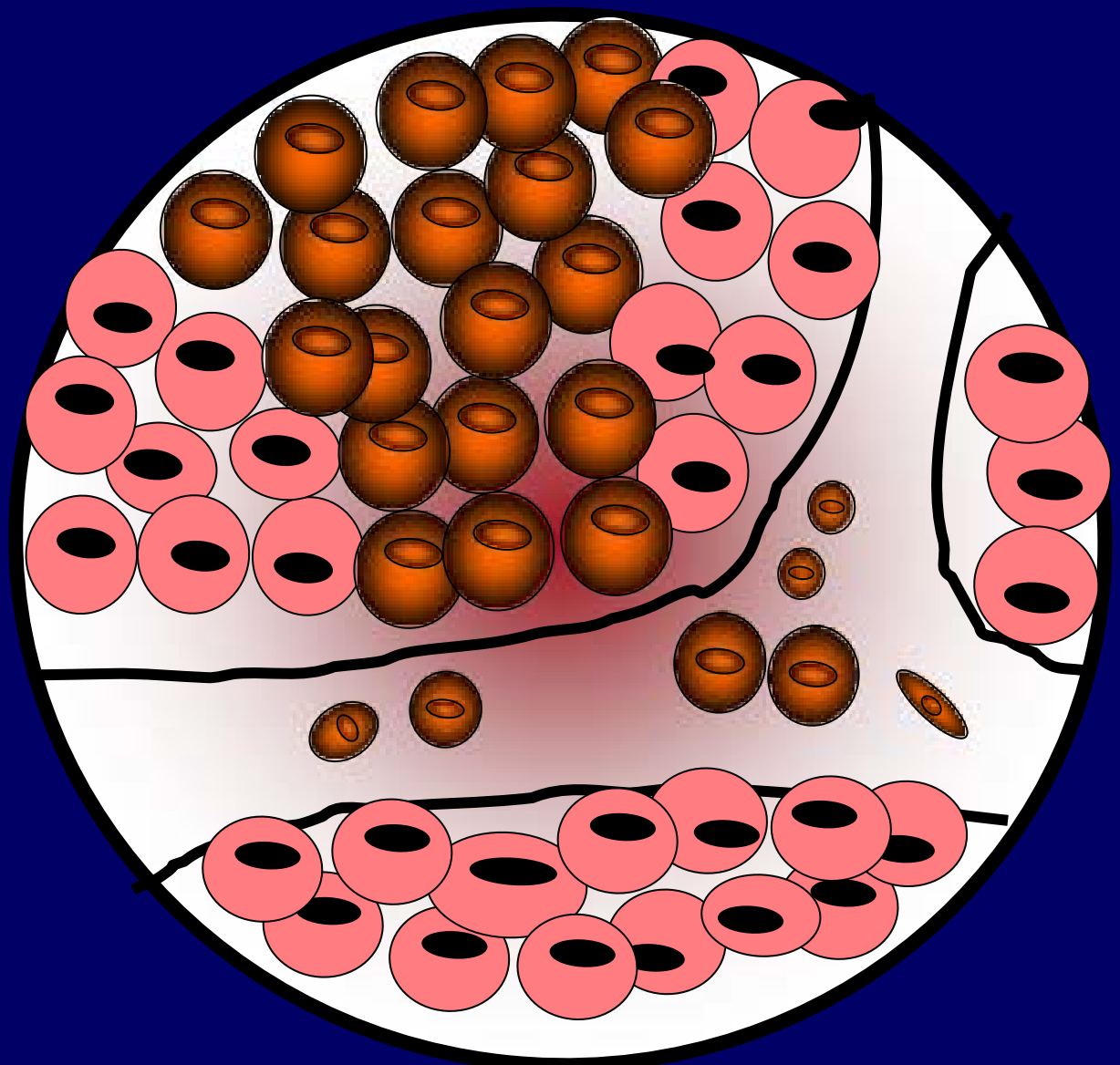
The Pathogenesis of a Metastasis



Invasion of surrounding tissues and blood vessels



ECM
Extracellular Matrix

Composition of ECM

Lamina densa

Entactin
Nidogen

TYPE IV
COLLAGEN

Proteoglycans
Glycosaminoglycans
Proteoglycans

Fibronectin

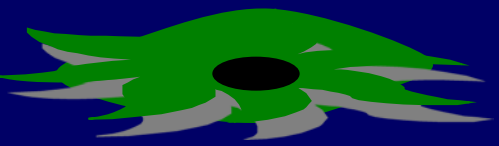
Lamina reticularis

Collagen types
I, II, III, V, others

Proteoglycans

Glycosaminoglycans

Intestinal stroma



Fibroblasts

Amorphous substance

Glycosaminoglycans
Proteoglycans
Glycoproteins

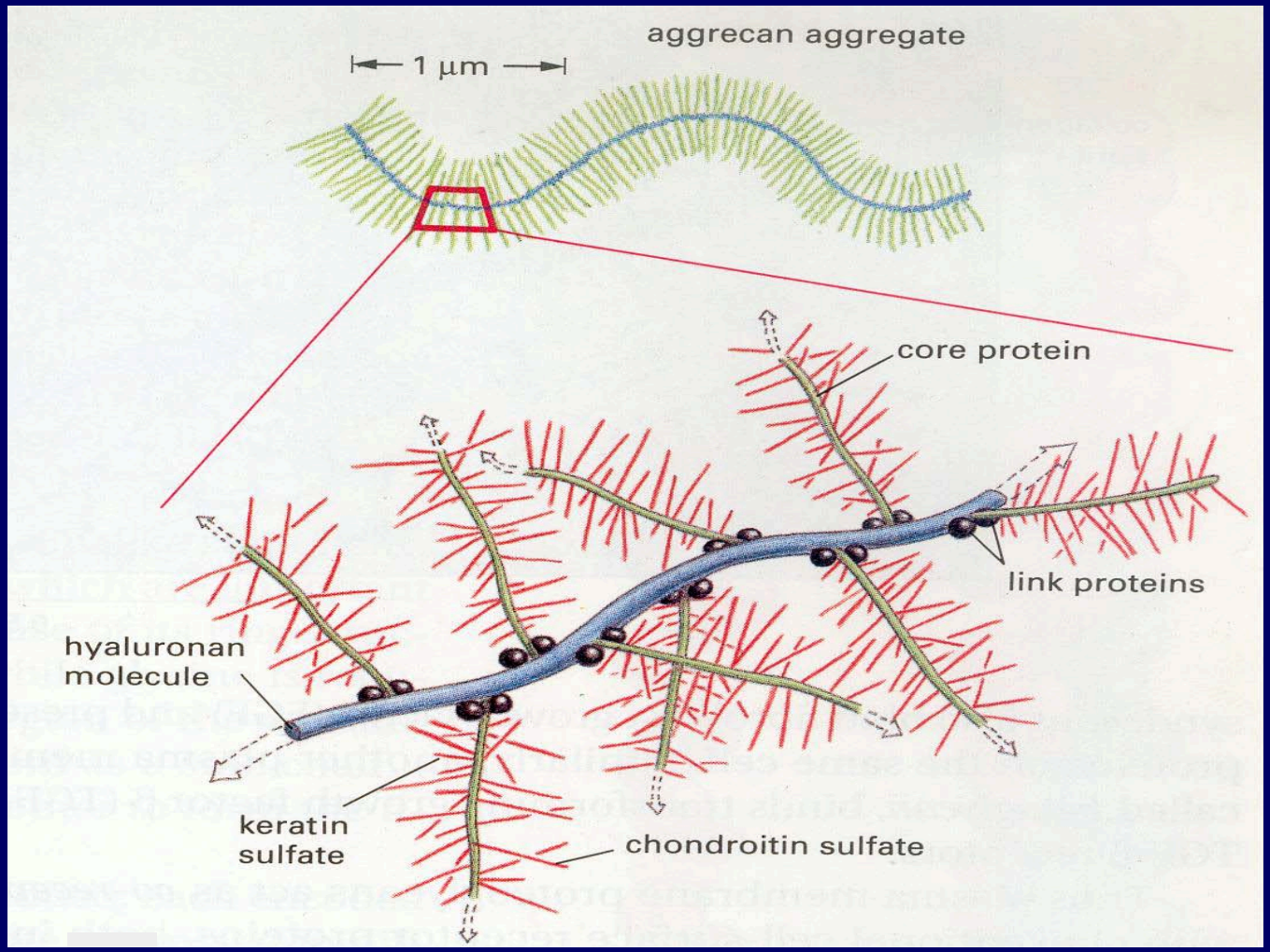
Collagen fibers

Collagen types
I, II, III, IV, others
Elastin

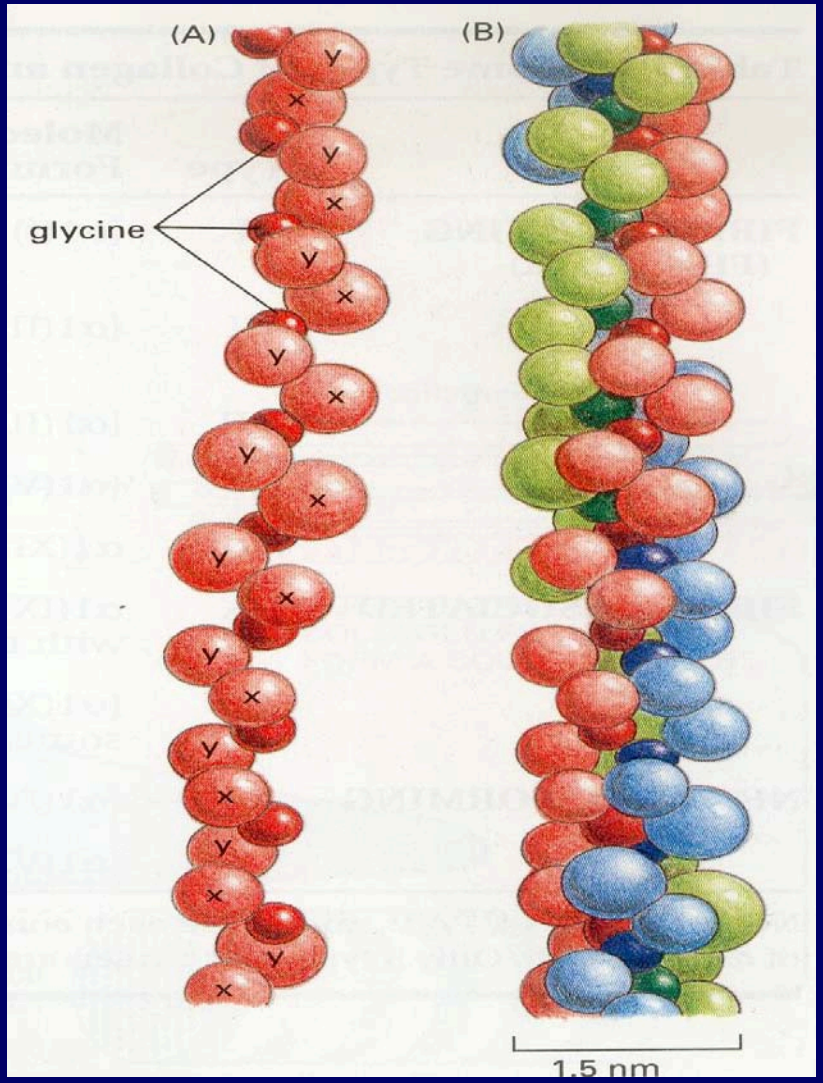
The Glycosaminoglycans

Glycosamino-glycan	MW [kDa]	Repeating Disaccharide(A-B)$_n$ Monosaccharide A	Monosaccharide B	Sulfates per (A-B) unit	Linked to protein
Hyaluronic acid	4-8000	D-glucuronic acid	N-acetyl-D-glucosamine	0	-
Chondroitin sulfate	5-50	D-glucuronic acid	N-acetyl-D-galactosamine	0.2-2.3	+
Dermatan sulfate	15-40	D-glucuronic acid or L-iduronic acid	N-acetyl-D-galactosamine	1.0-2.0	+
Heparan sulfate	5-12	D-glucuronic acid or L-iduronic acid	N-acetyl-D-glucosamine	0.2-2.0	+
Heparin	6-25	D-glucuronic acid or L-iduronic acid	N-acetyl-D-glucosamine	2.0-3.0	+
Keratan sulfate	4-19	D-galactose	N-acetyl-D-glucosamine	0.9-1.8	+

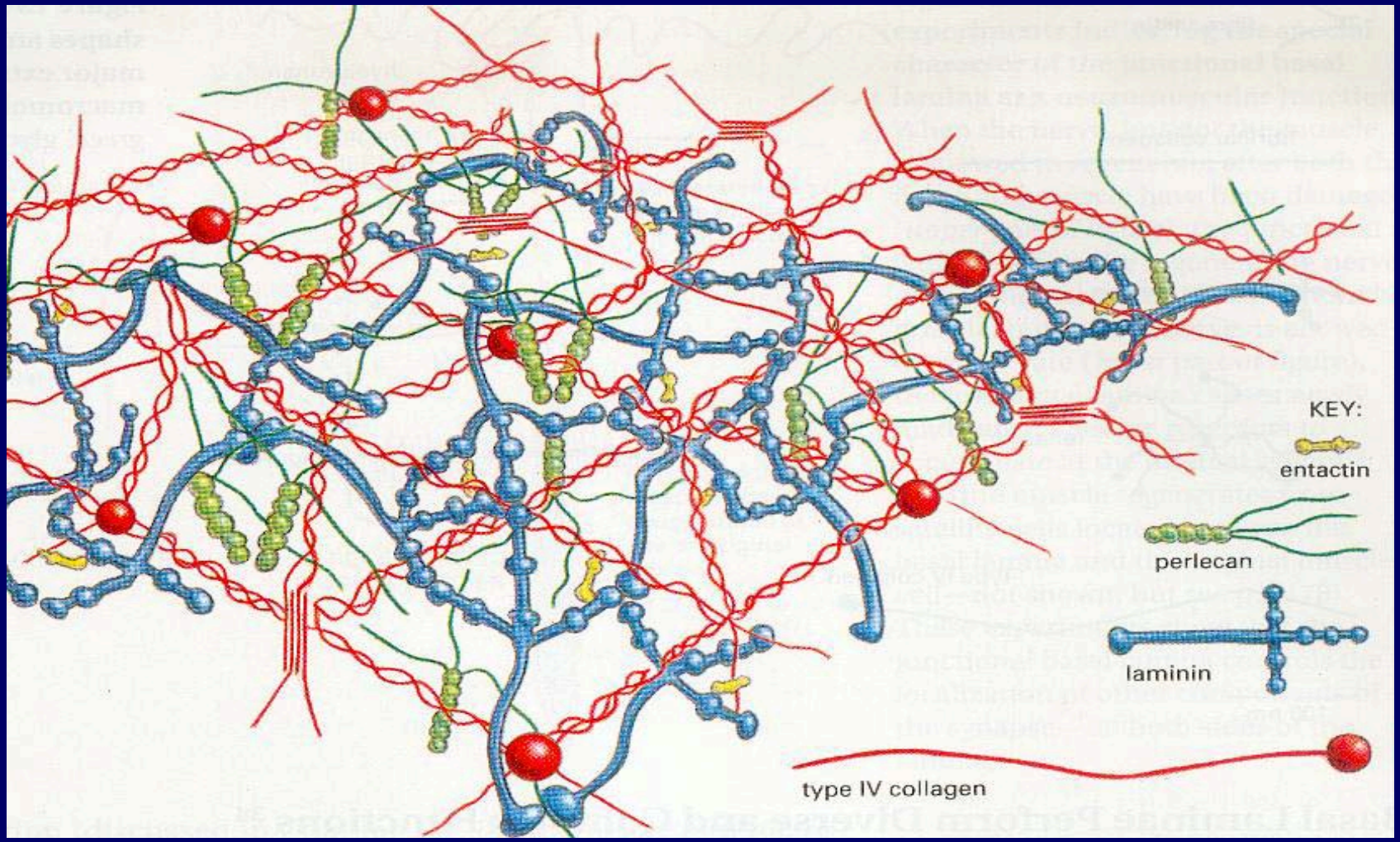
GAG chains may be highly organized in the ECM



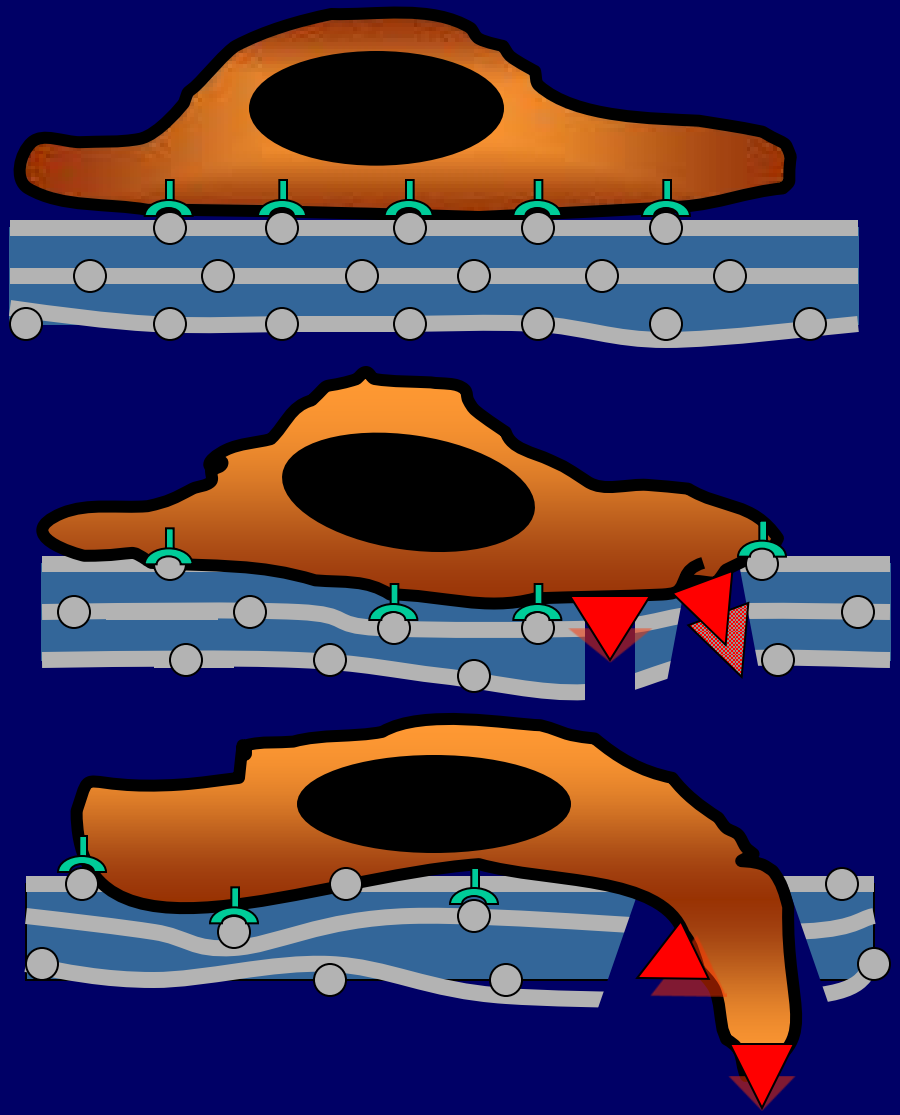
The structure of a typical collagen molecule



Model of the molecular structure of a basal lamina



Crossing a basal lamina



Binding to laminin

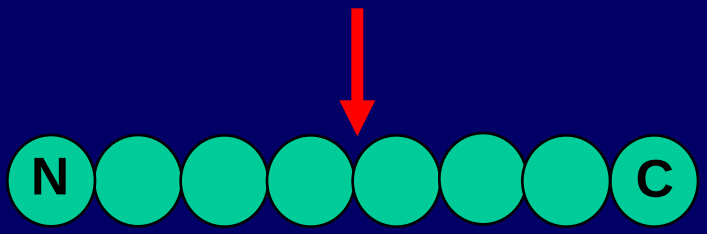
Digestion of basal lamina

Motility

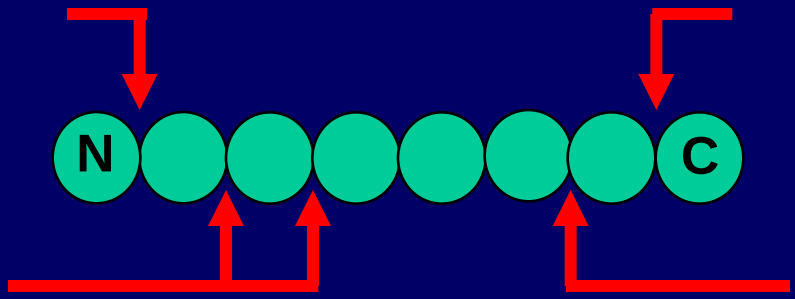
Proteinases

Proteases
(Protein hydrolases)

Endopeptidases
(proteinases)



Exopeptidases
(peptidases)



Endopeptidases

- Serine proteinases
- Cysteine proteinases
- Aspartyl proteinases
- Metalloproteinases

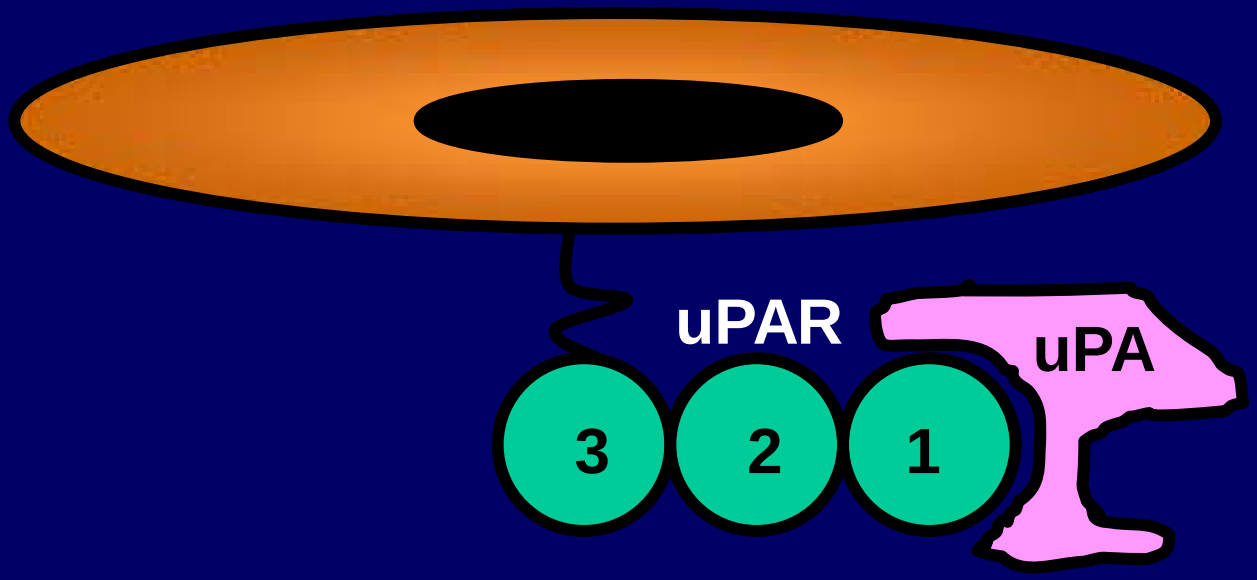
Enzymes degrading ECM

Enzymes	Collagen Types							Gelatins	Fibronectin	Laminin	GAG
	I	III	IV	V	VII	IX	X				
Serine proteinases											
Plasmin								XX	XX	XX	
Metallo-proteinases											
Intestitial collagenases	X	X			X		X				
72-kDa gelatinase			XX	XX	XX		XX	XX	XX		
92-kDa gelatinase			XX	XX	XX		XX	XX	XX		
Stromelysin	X		X	X		X		XX	XX	XX	
Cysteine proteinases											
Cathepsins (B, D)	X	X						XX	XX		
Glycosidases											XX

Serine proteinases

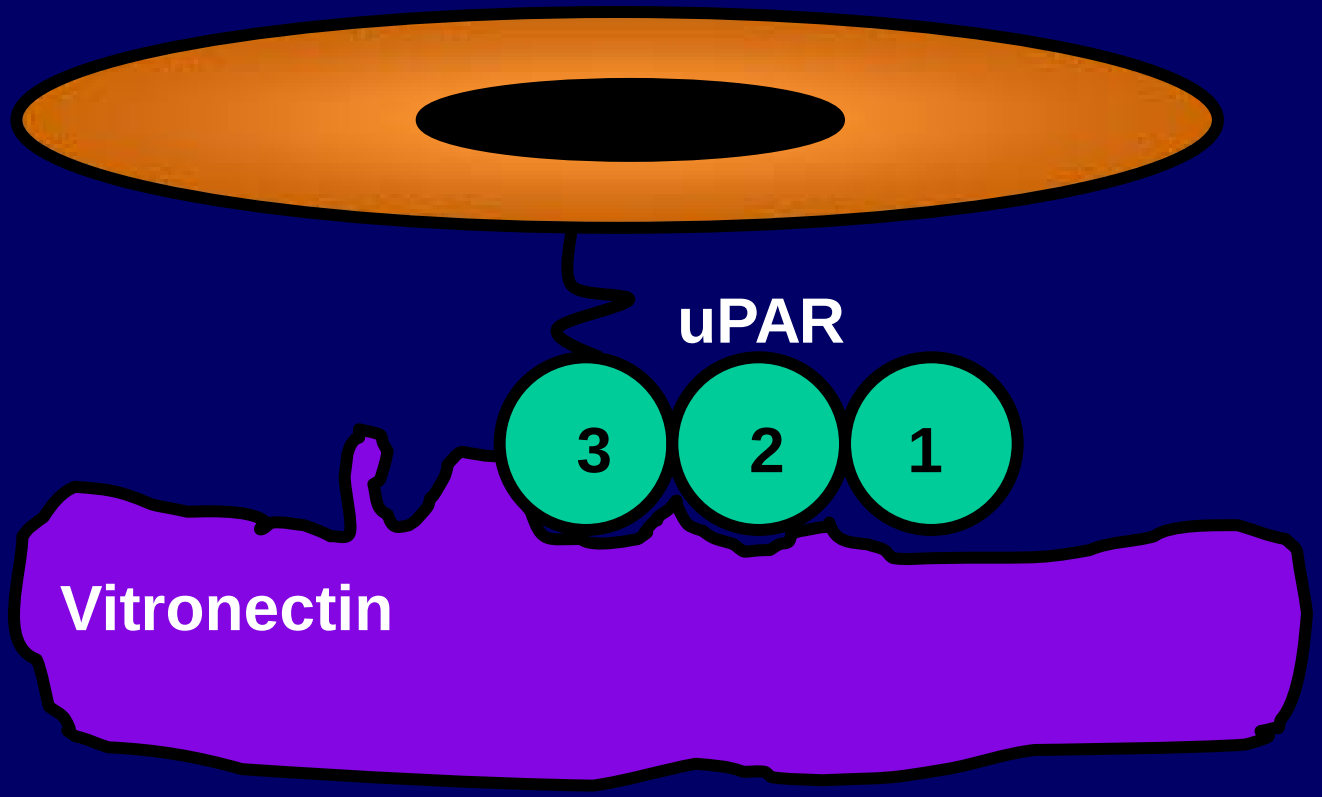
uPA
Urokinase-type plasminogen activator

- Serine proteinase with high specificity for plasminogen
- Cleaves plasminogen at a single site to form plasmin
- It is secreted as a single-chain molecule with poor activity
- It is activated by plasmin or by binding to its surface receptor uPAR
- It is inhibited by PAI-1 or 2 (plasminogen activator inhibitor)


uPAR
uPA
3
2
1

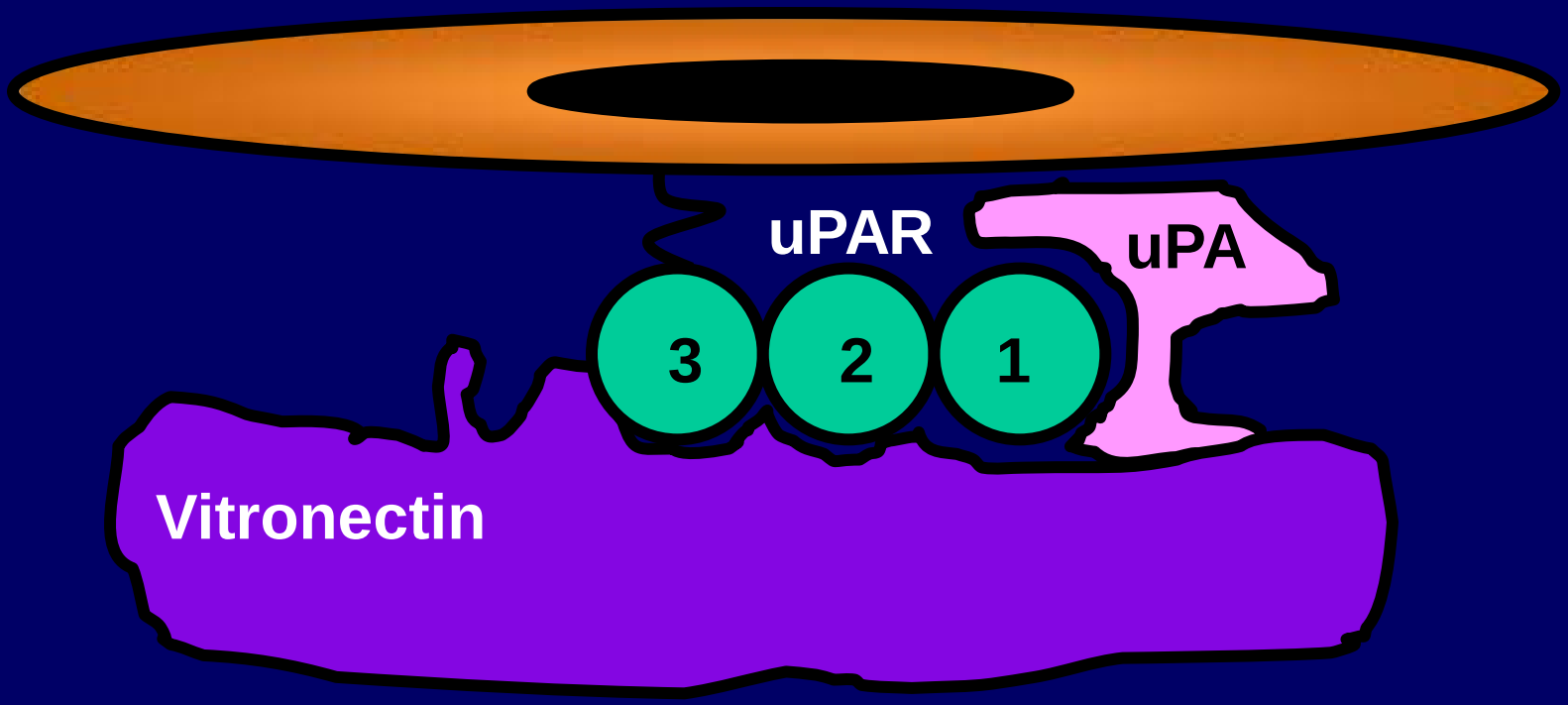
uPAR-vitronectin binding:

Cell adhesion

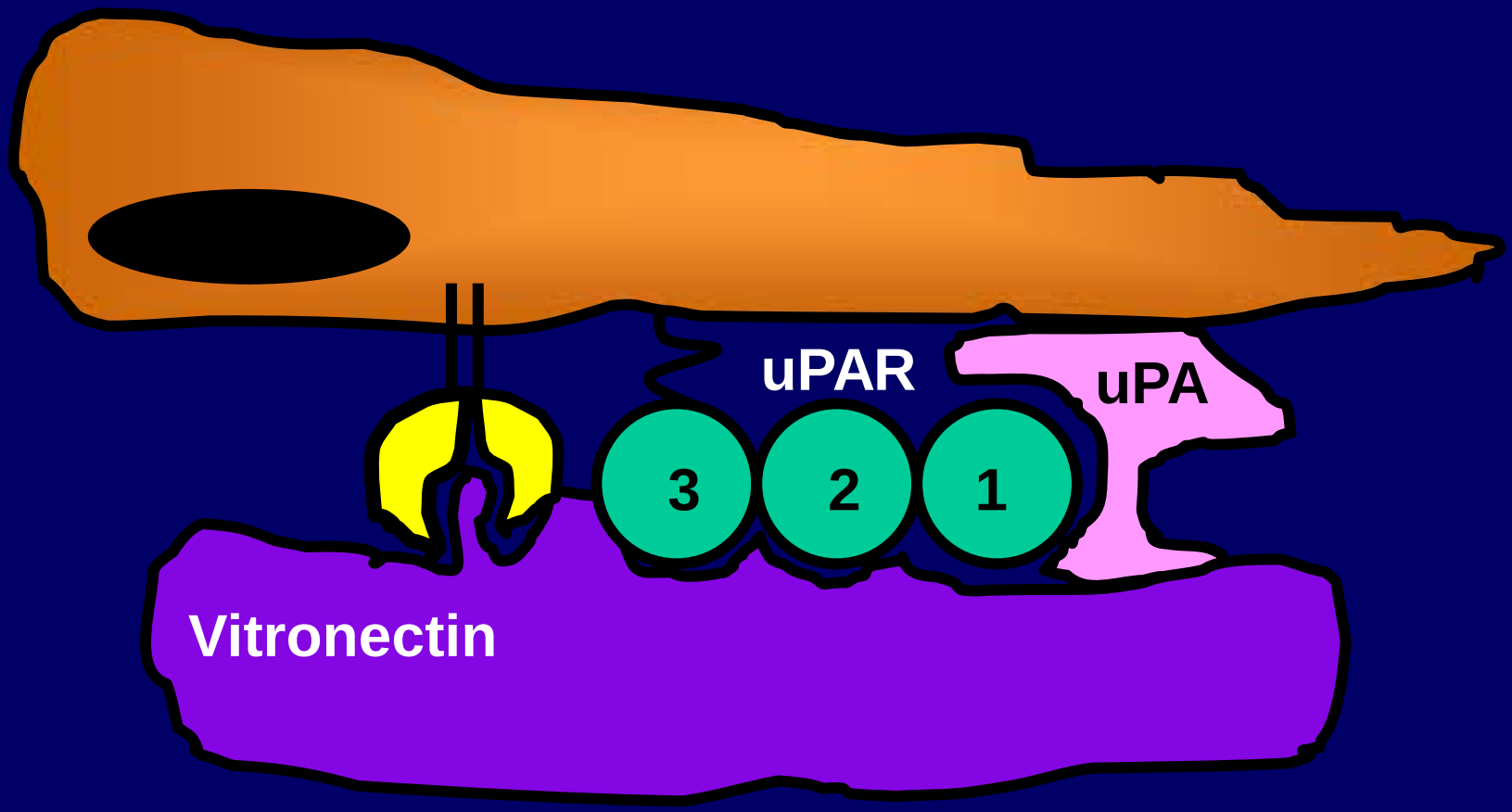


uPAR uPA-vitronectin binding:

Increased cell adhesion

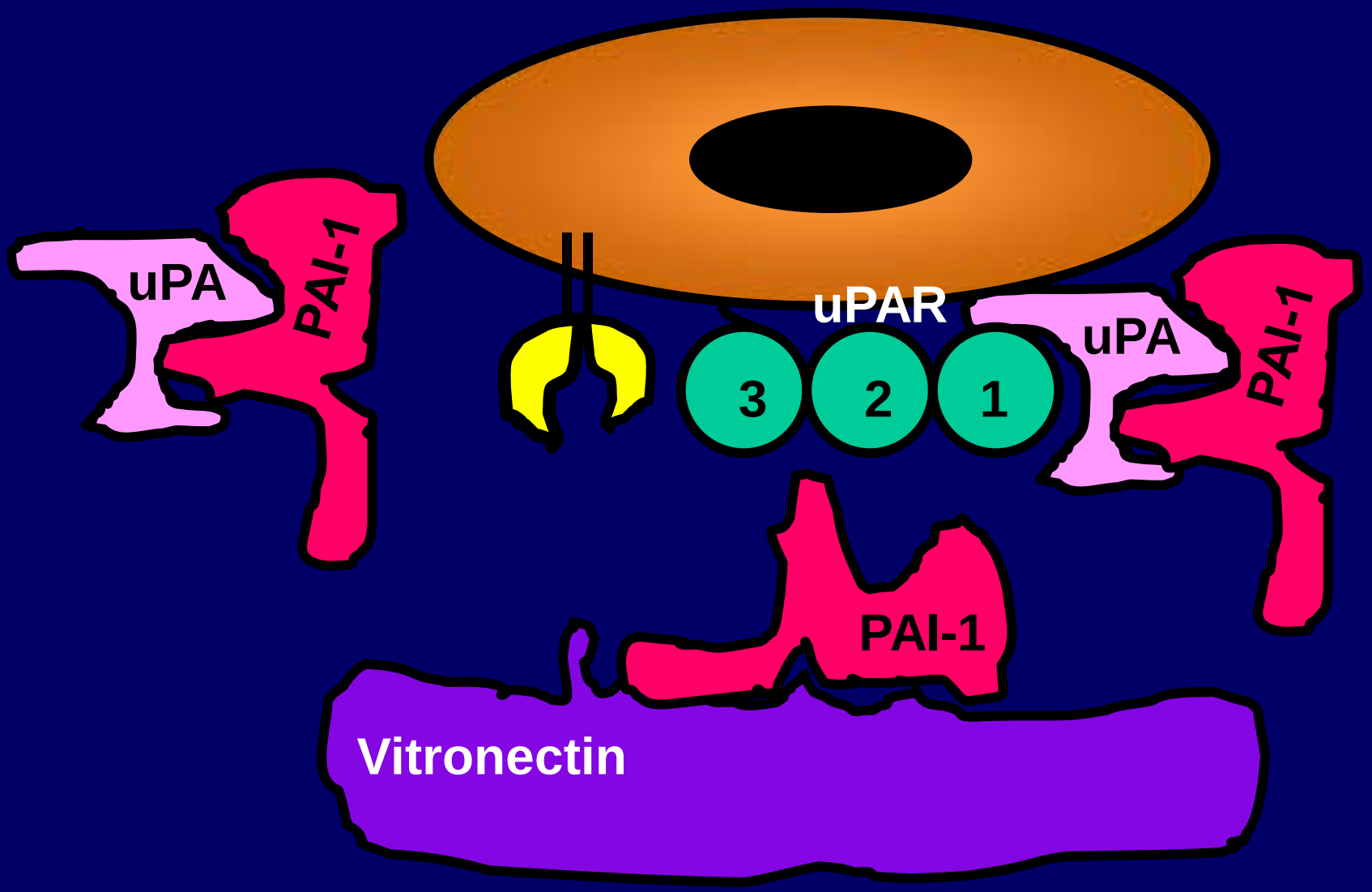


uPAR*uPA-integrin binding:
Enhanced spreding and migration



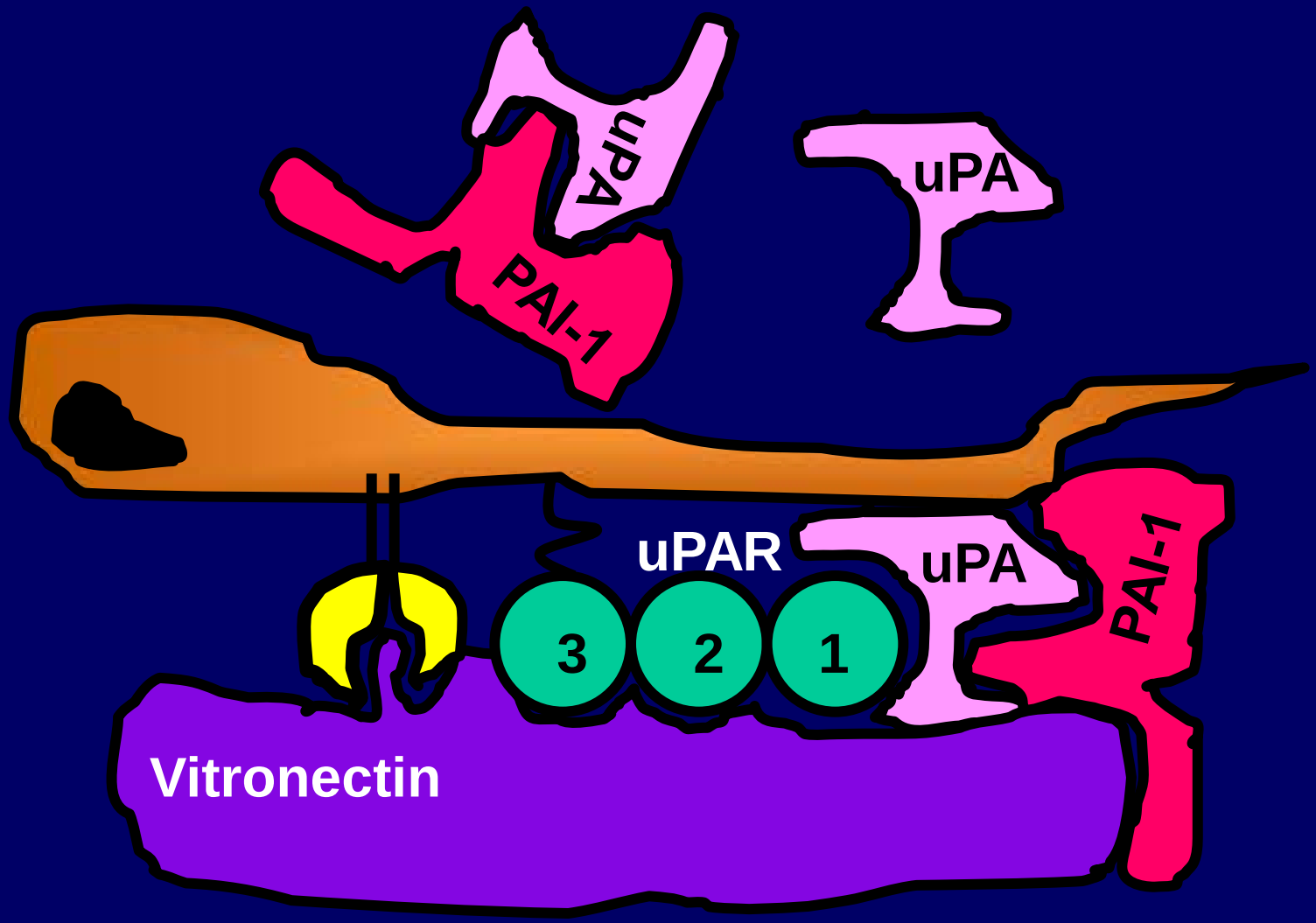
Excess PAI-1:

Detachment and inhibition of migration

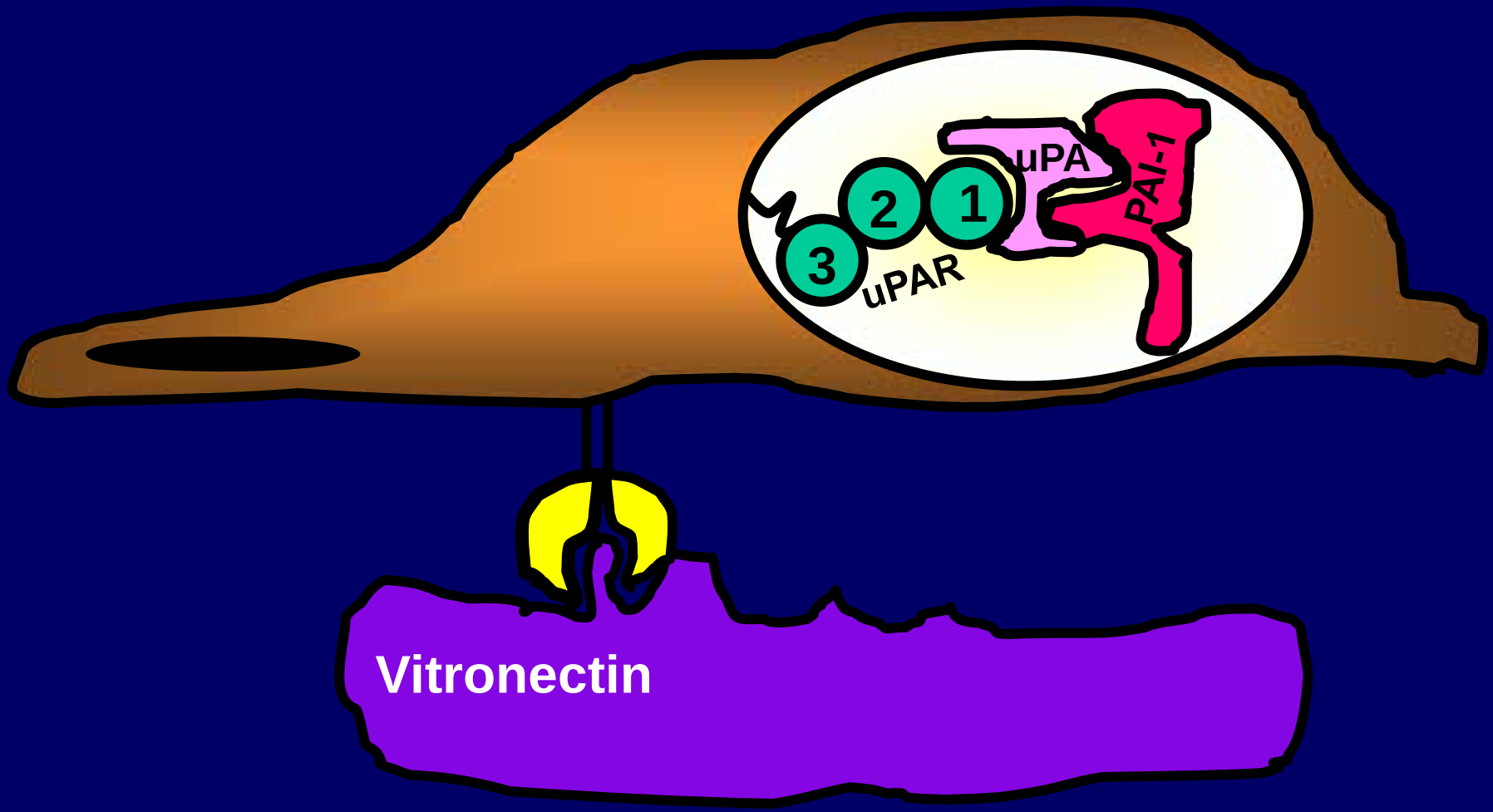


Excess uPA:

Increased attachment and migration

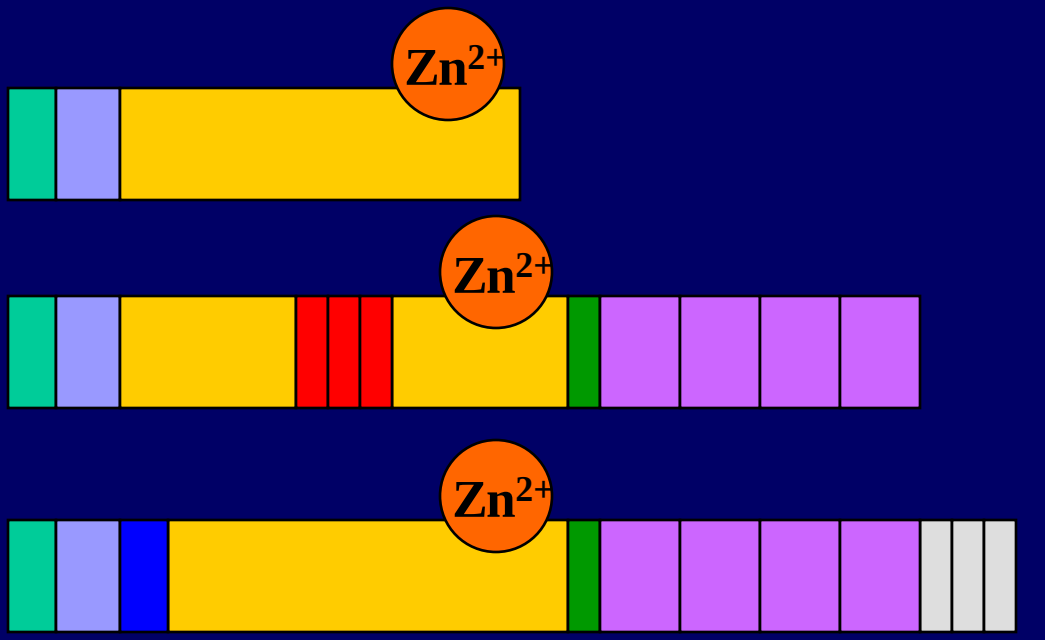


Internalization of uPAR*uPA*PAI-1:
Migration



Metalloproteinases

MMPs



Matrilysins

Gelatinases

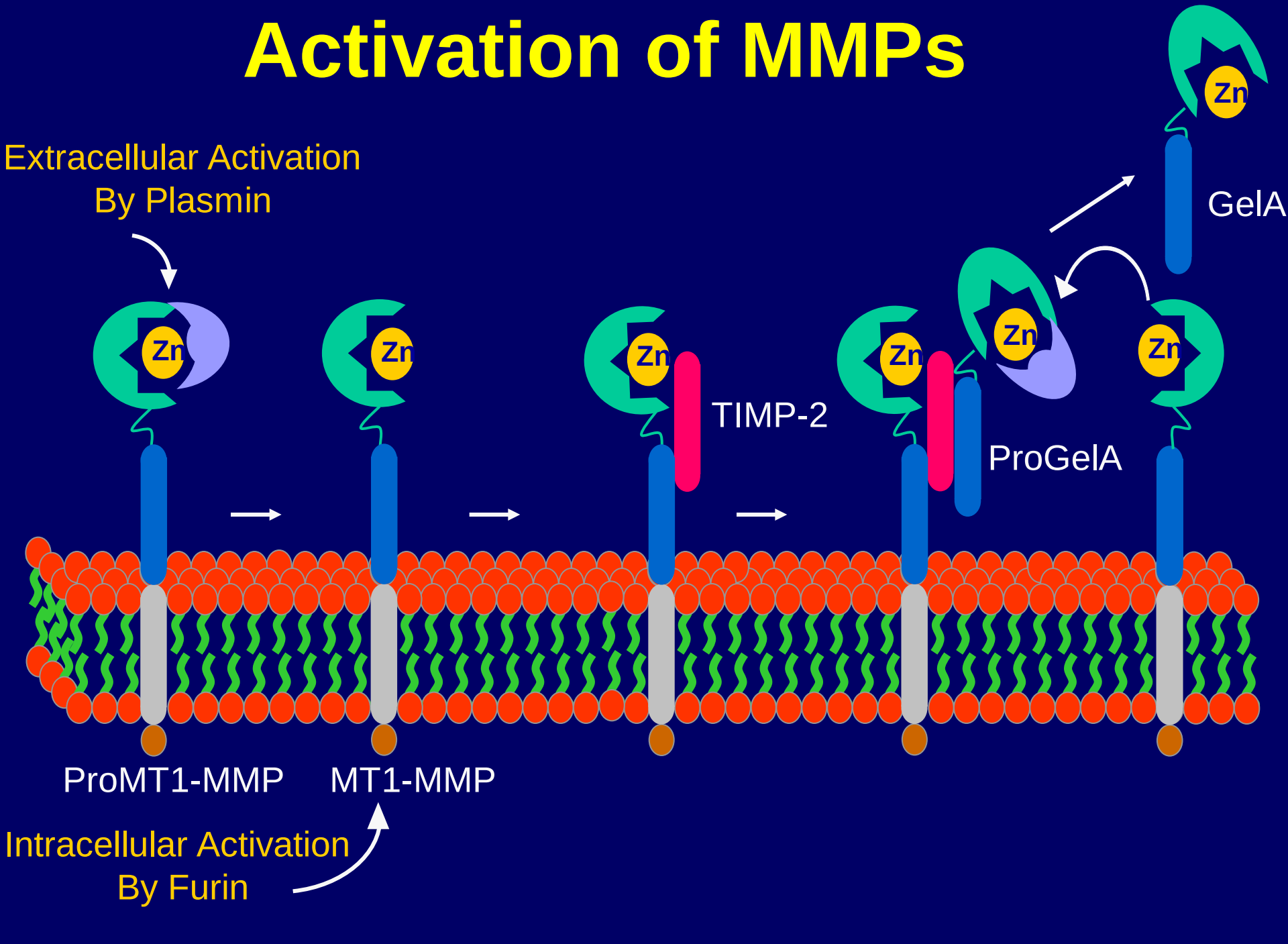
Membrane-associated

- Signal peptide
- Propeptide
- Catalytic domain
- Fibronectin type II modules
- Hinge/linker
- Hemopexin domain
- Convertase cleavage site
- Linker, transmembrane and cytoplasmic domains

Regulation of MMP proteolytic activity

- Transcription
- Proenzyme activation
- Inhibition

Activation of MMPs

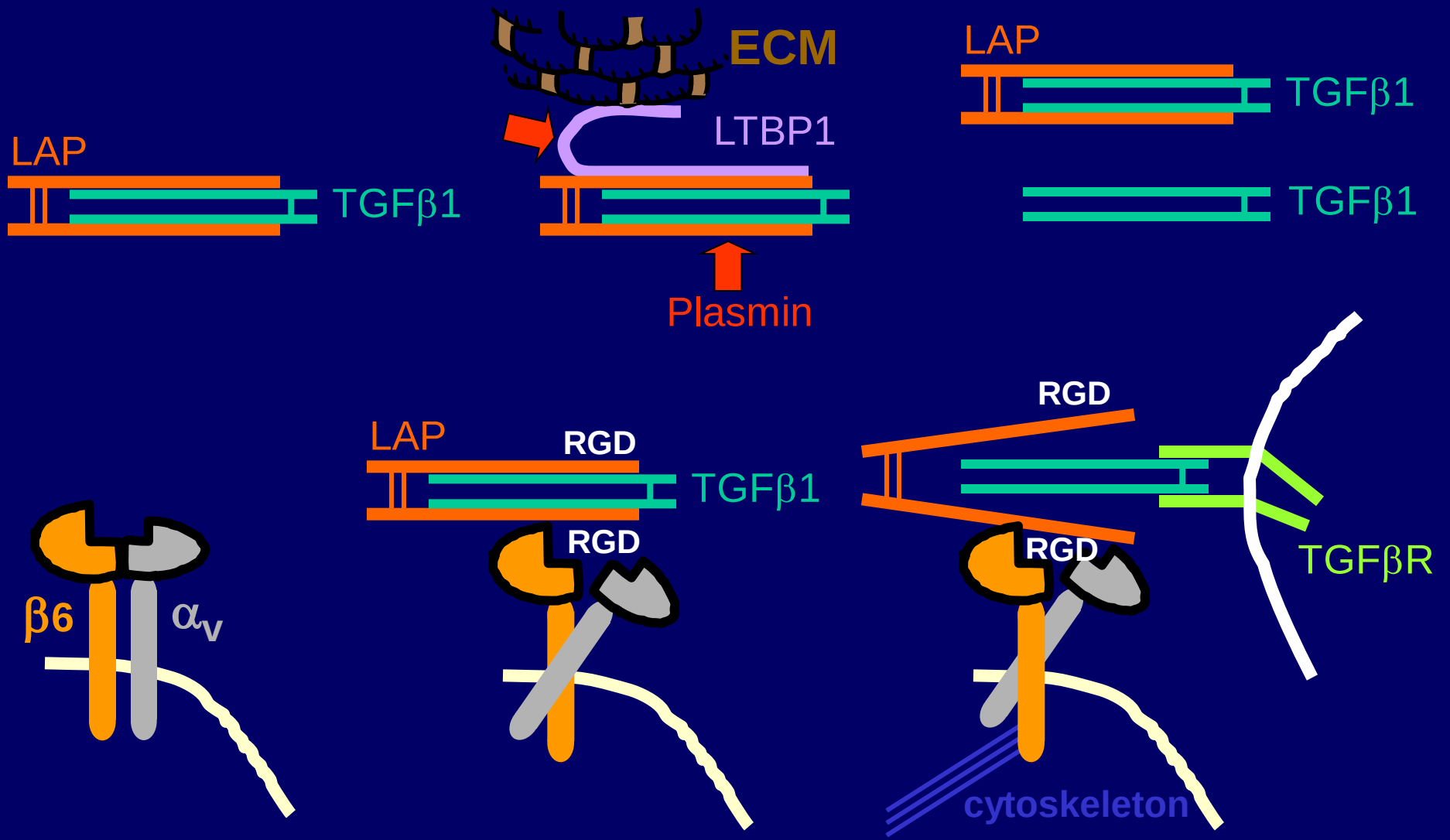


Proteinases function in cancer

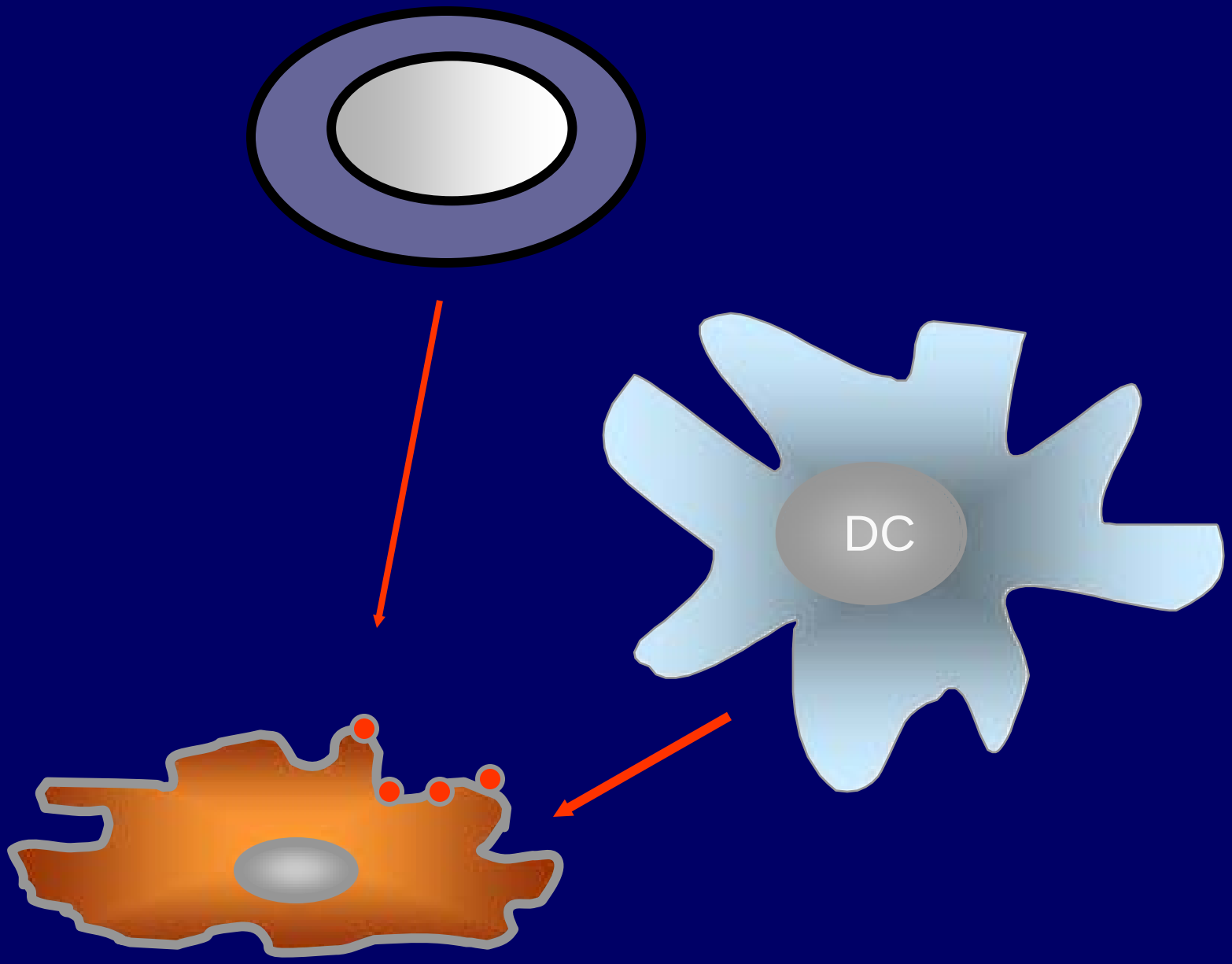
- ECM and basement-membrane degradation
- Release and activation of growth regulators
- Processing of cell adhesion molecules
- Destruction of chemokine gradients
- Regulation of angiogenesis

Release and activation of growth regulators

Activation of TGFβ



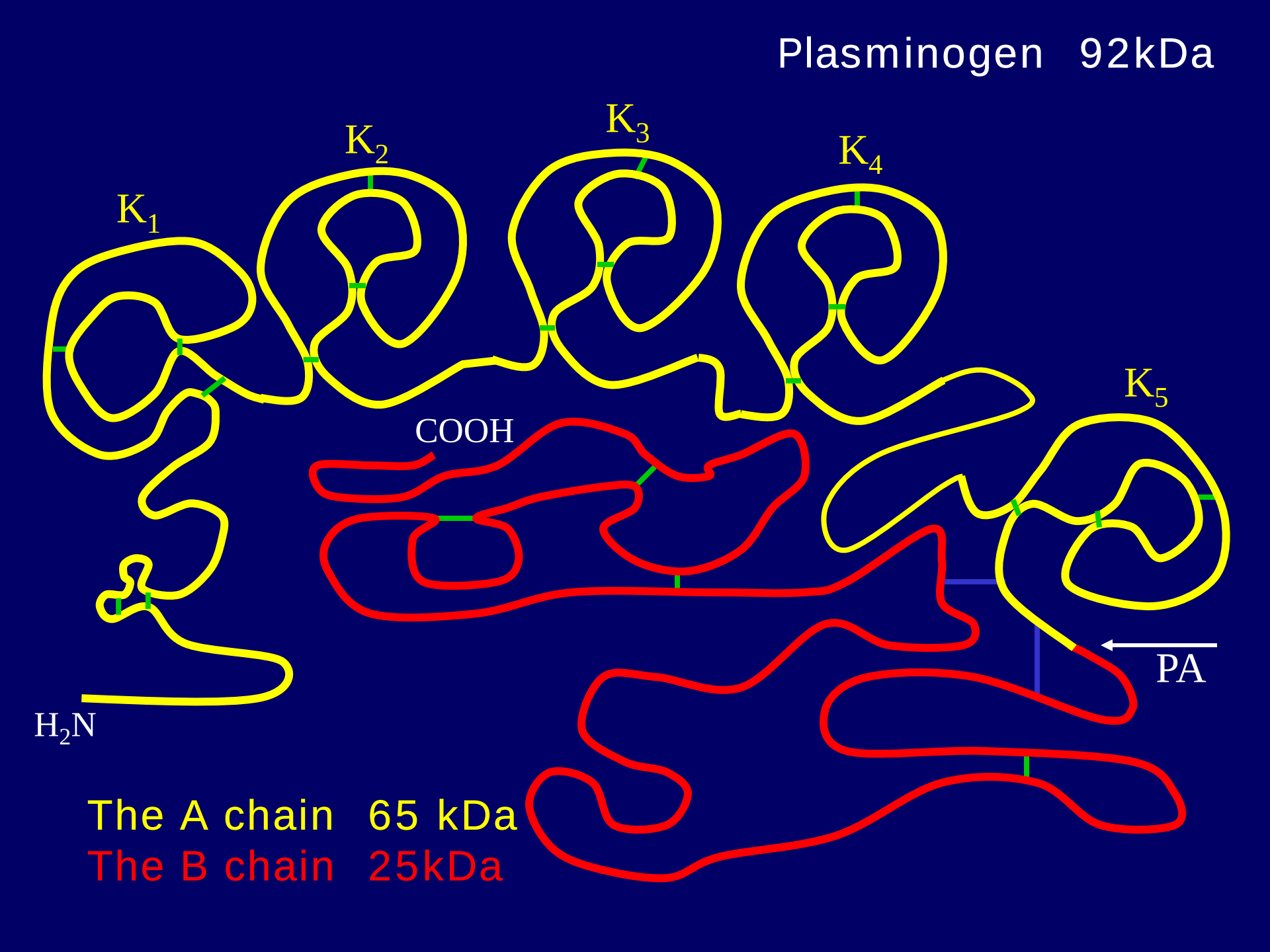
Destruction of chemokine gradients


DC
Tumor cell

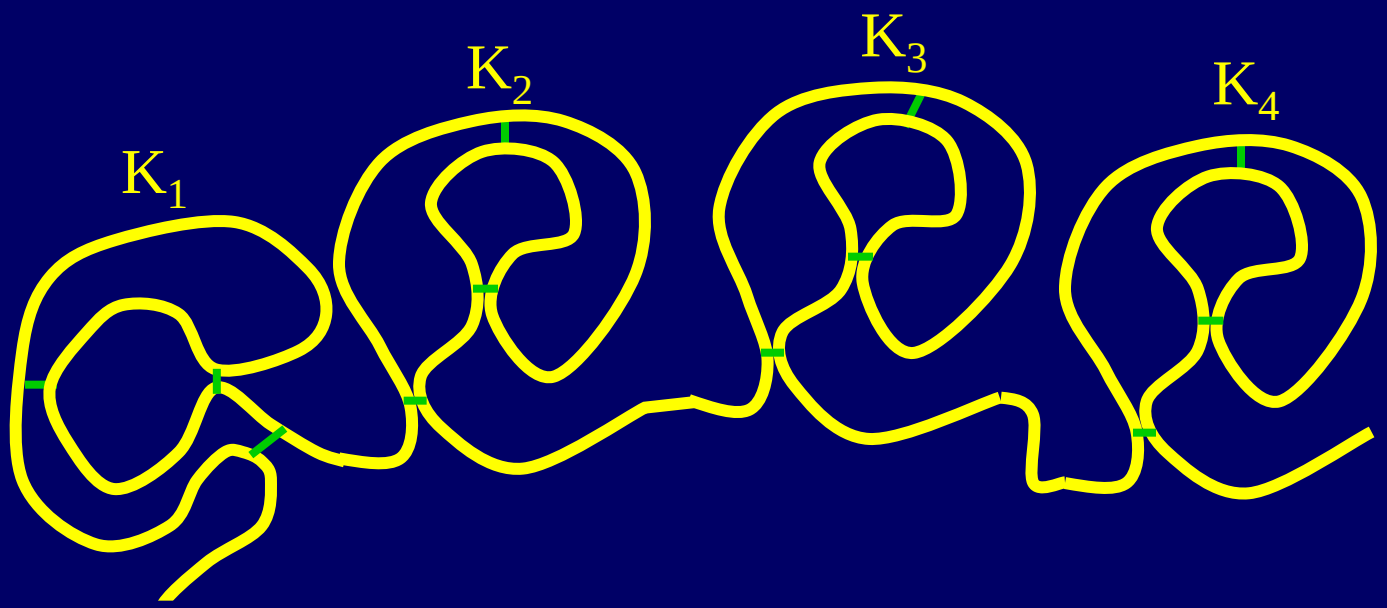
MMPs downregulate the activity of chemokines

- MCP-3:MMP2, MMP13, MT1-MMP
- MCP-1, 2, 3, 4:MMP1, MMP3
- SDF-1α, SDF-1β: MMP1, MMP2, MMP3, MMP9, MMP13, MT1-MMP

Regulation of angiogenesis

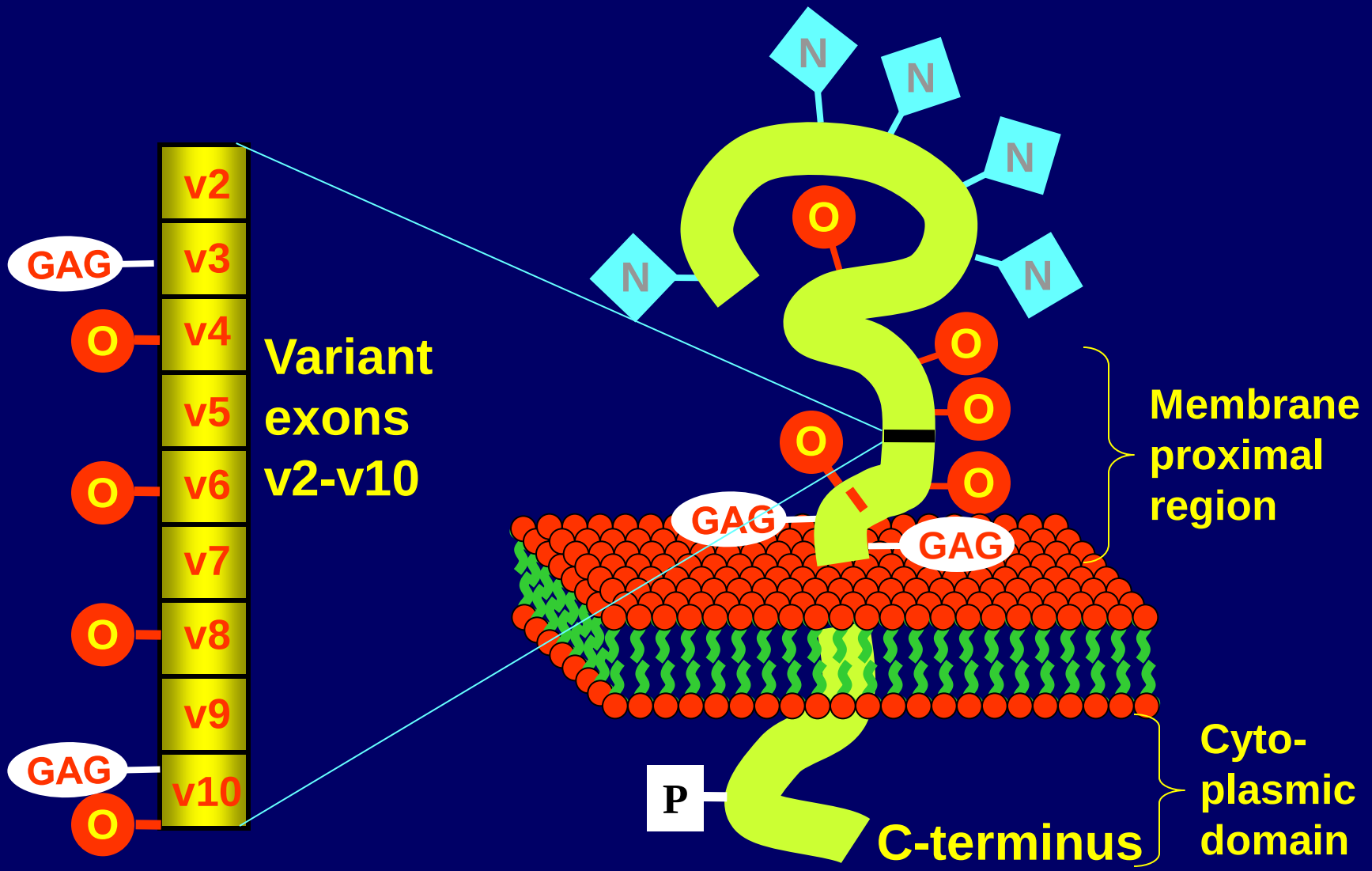

Plasminogen 92kDa
K1
K2
K3
K4
K5
COOH
H2N
PA
The A chain 65 kDa
The B chain 25kDa

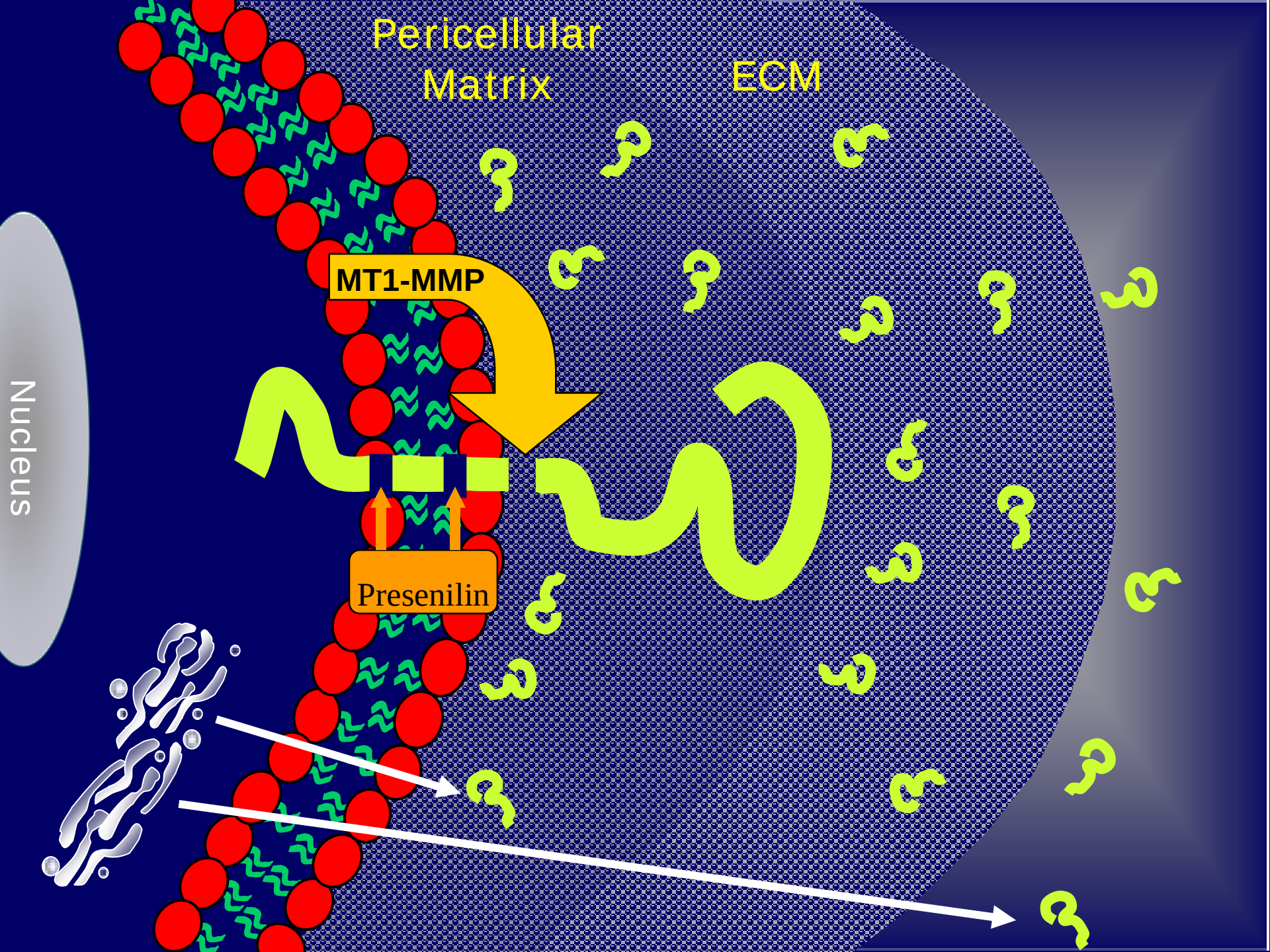
Angiostatin



Processing of cell adhesion molecules

CD44


v2
v3
v4
v5
v6
v7
v8
v9
v10
GAG
O
GAG
O
O
O
Variant exons v2-v10
N
N
N
N
N
O
O
O
O
GAG
GAG
Membrane proximal region
P
C-terminus
Cyto-plasmic domain

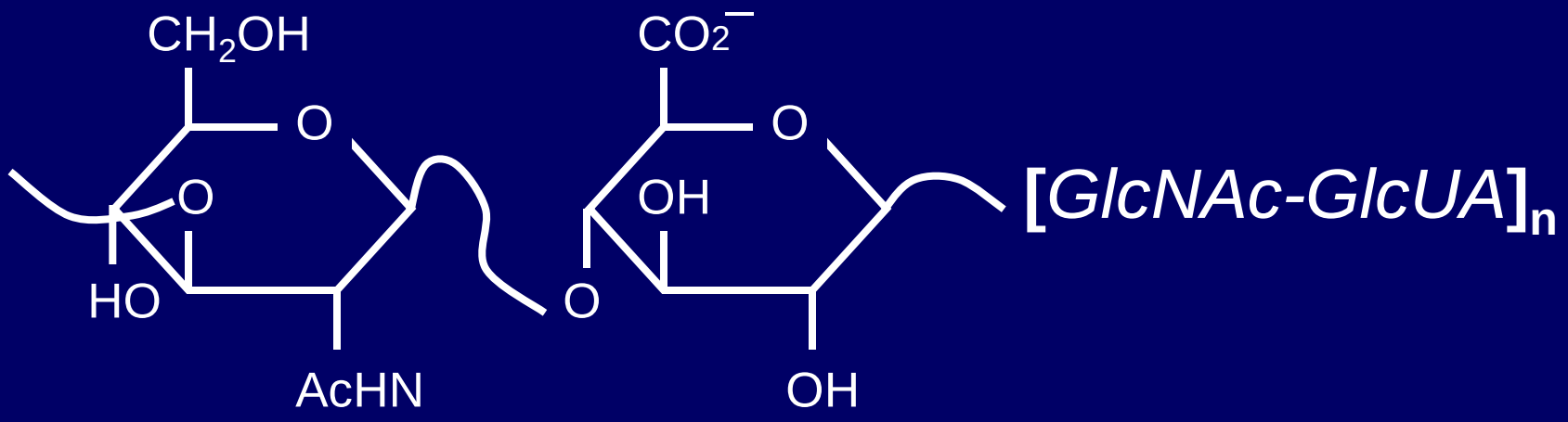

Pericellular
Matrix
ECM
MT1-MMP
Presenilin
Nucleus

Role of CD44 in cell trafficking

CD44 mediates cell-cell and cell-matrix interactions

- Leukocyte extravasation at inflammatory sites
- Tumor metastasis

Hyaluronan



N-acetylglucosamine Glucuronic acid

GlcNAc *GlcUA*

Hyaluronan (HA)

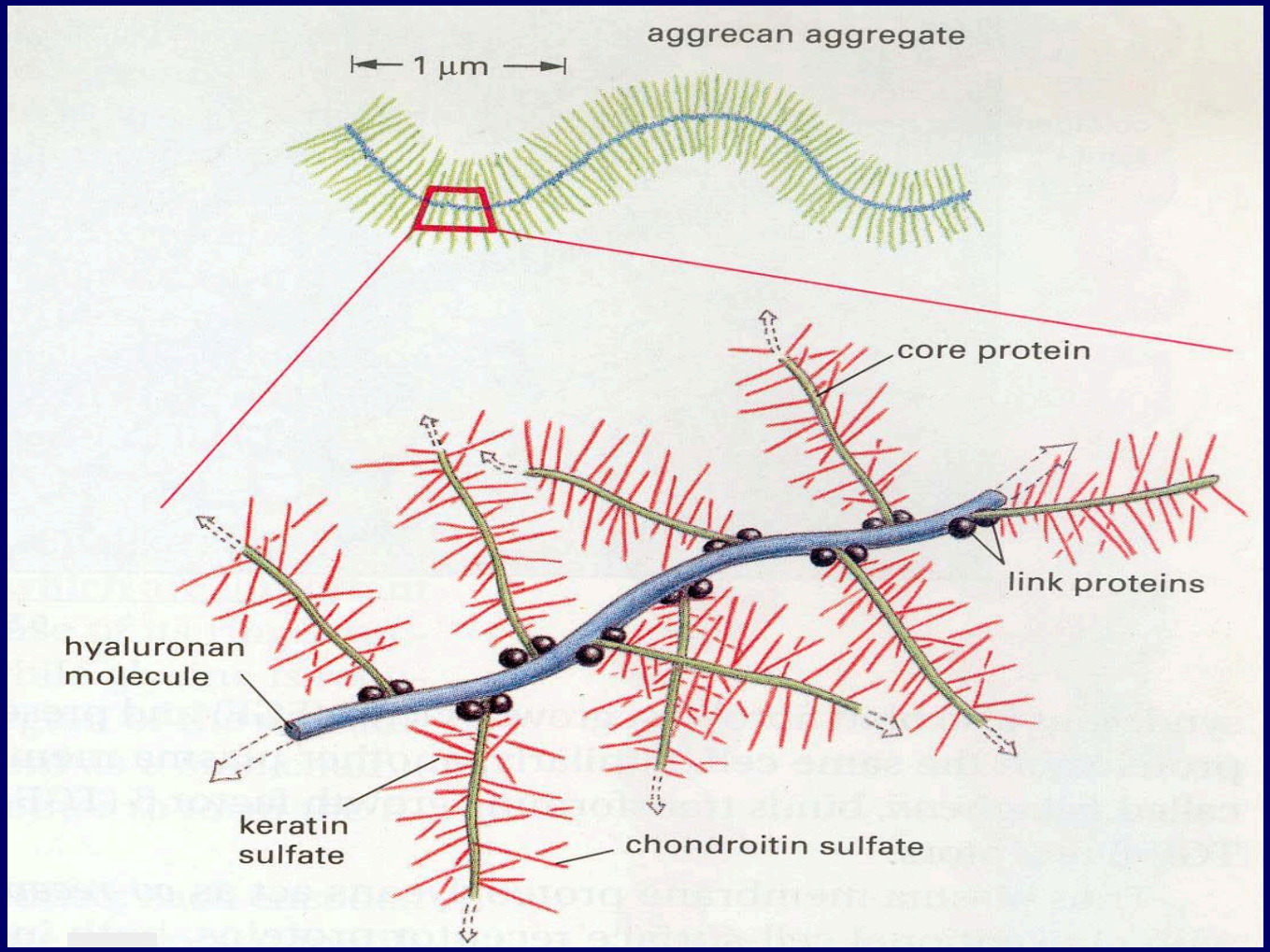
Basal
Predominantly HMW (>10^6 Da)

- Extracellular Matrix
- Connective Tissue
- Proteoglycan Aggregates
- Synovial Fluid
- Blood vessel walls

Upregulated
Accumulation of LMW (<$5x10^5$ Da)

- Healing Wounds
- Sites of Inflammation
- DTH Sites
- Embryonic Development
- Invasive Tumors

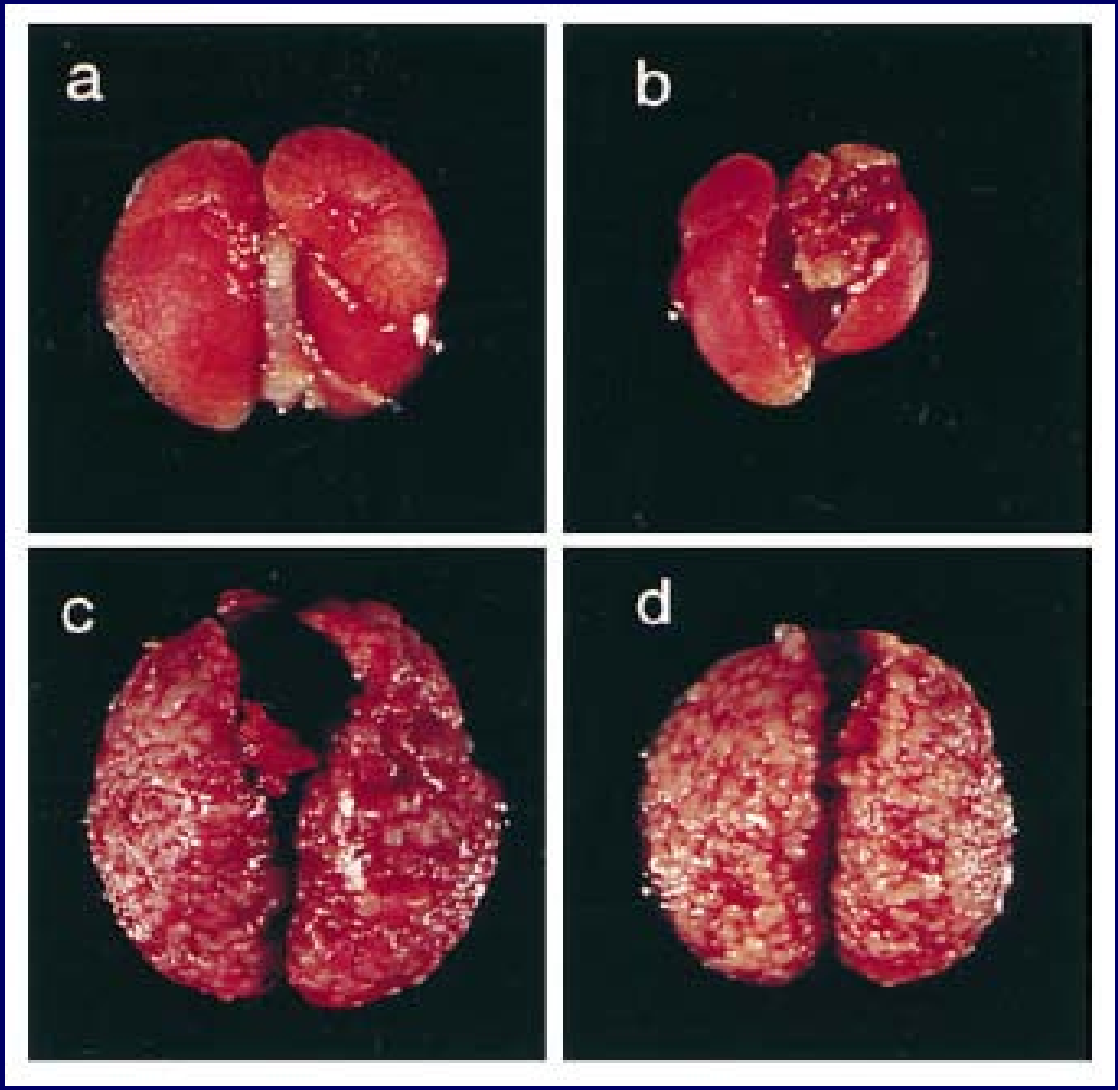
HA in the ECM


aggrecan aggregate
1 μm
core protein
link proteins
hyaluronan molecule
keratin sulfate
chondroitin sulfate

CD44 is implicated in tumor growth and metastasis

- Enhanced tumorigenesis of some tumors depends on the ability of CD44 to bind HA
- CD44 mutants which can not bind HA fail to promote growth and metastasis of some tumors
- Transfection of cells originating from a non-metastatic tumor with a CD44 cDNA isolated from a metastatic cell line confered metastatic behavior
- Expression of HA is often increased around colonies of metastatic cells

Expression of sCD44 prevents lung metastasis of mammary cancer



1h after iv injection

Arrest in pulmonary Blood vessels

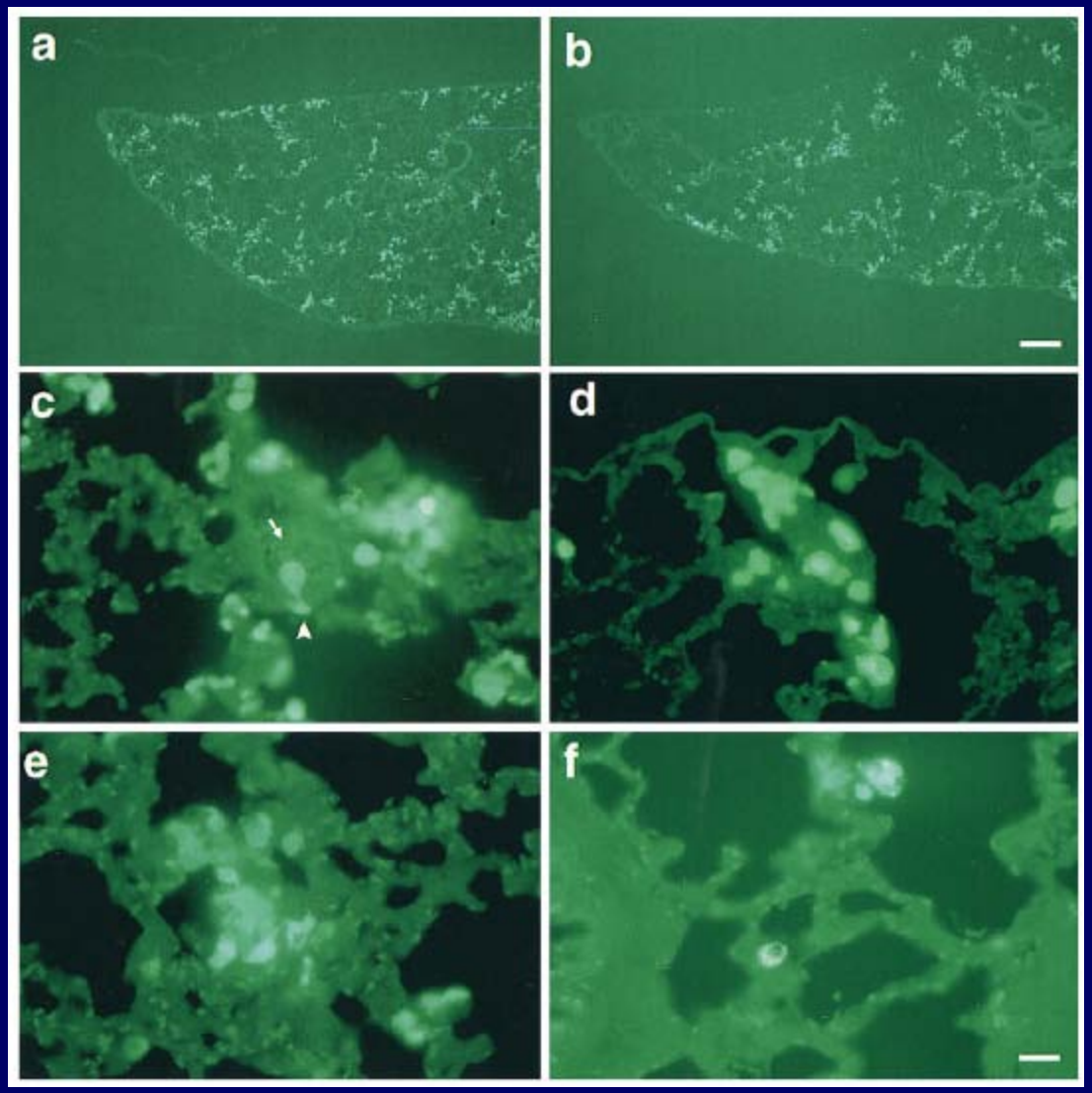
1h after iv injection

Penetration the pulmonary interstitium

48h after iv injection

Cluster formation

Tumor cells Tumor cells +sCD44

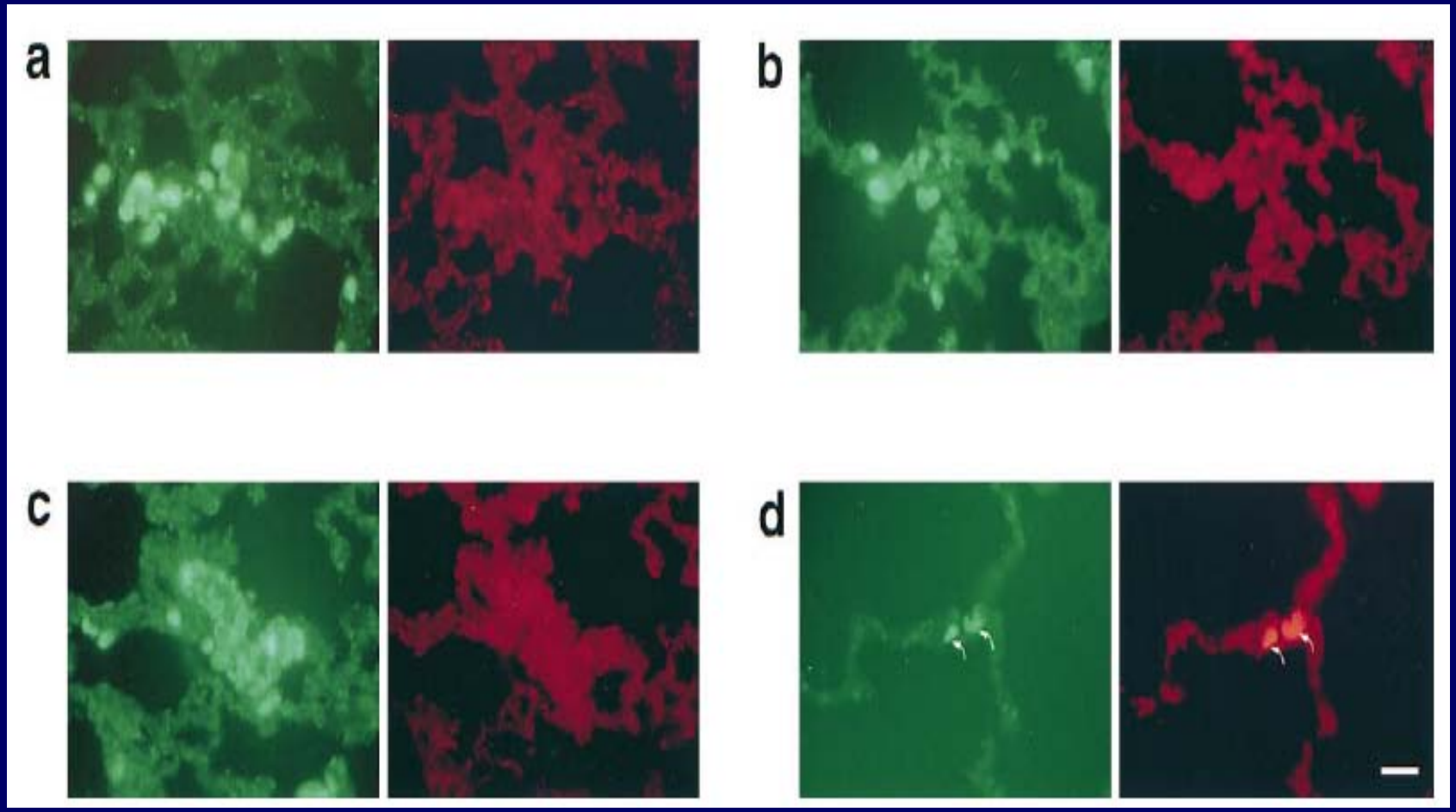


Tumor cells

Tumor cells +sCD44

1h

48h



Role of the microenvironment in the secondary growth