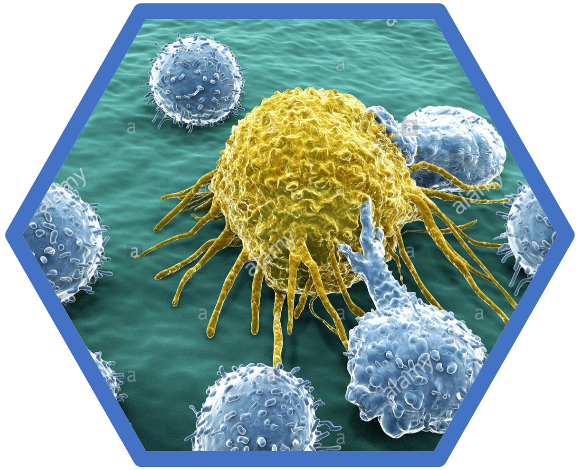
Biomaterials for MX

MSE – 471

Maartje Bastings



Course 6: Materials for Cell Adhesion

Course Content & Time Table

BLOCK 1: Introduction and materials overview

11-9	Lecture 1.	Intro to biomaterials and biology
18-9	Lecture 2.	Naturally derived biomaterials
25-9	Lecture 3.	Polymers and nanoparticles
2-10	Lecture 4.	Surfaces

BLOCK 2: Interactions and specific applications

9-10	Lecture 5.	Materials for drug delivery and targeting
16-10	**Lecture 6.**	**Materials for cell adhesion**
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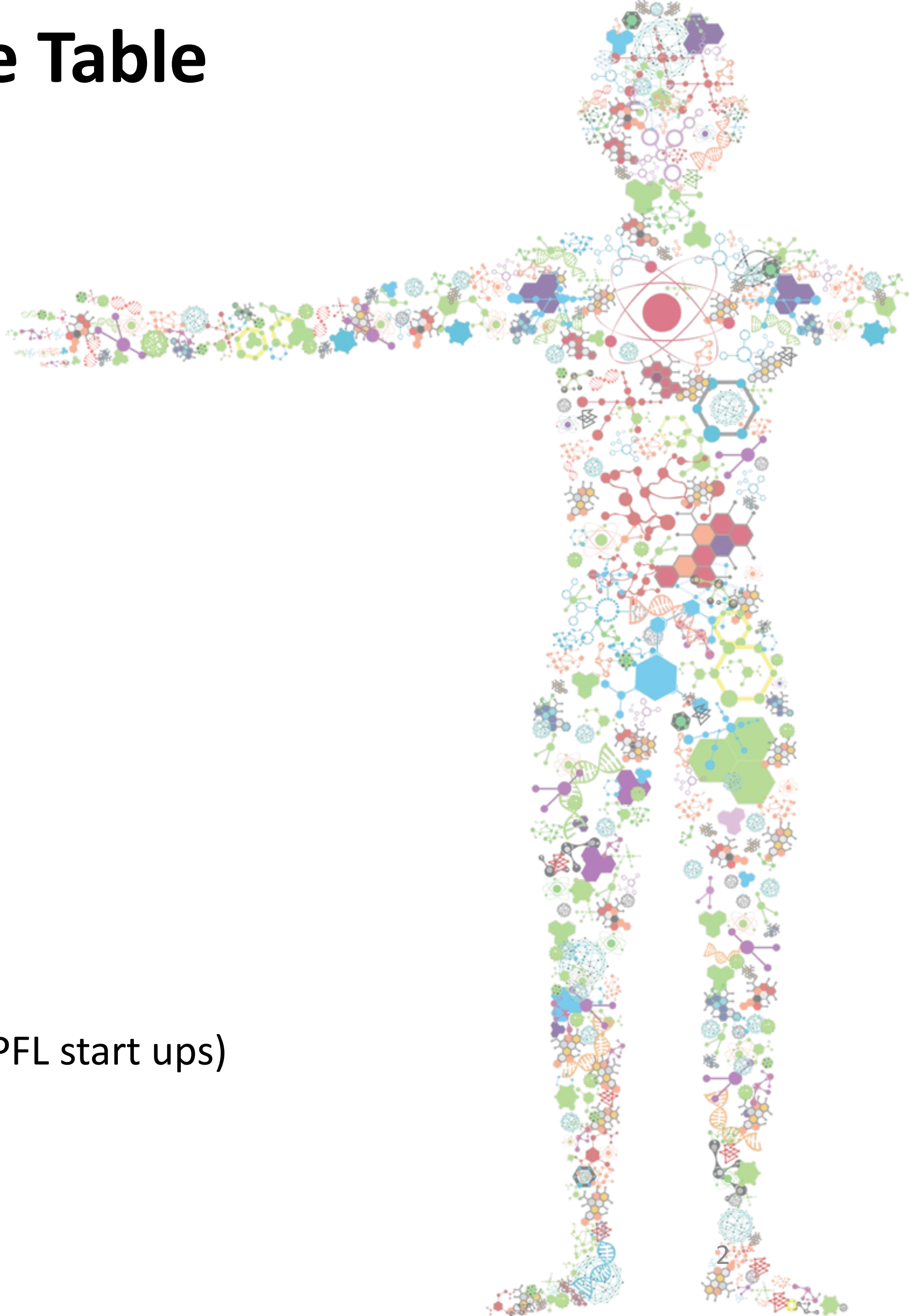
30-10	Lecture 7.	Materials for immune engineering
6-11	Lecture 8.	Materials for tissue engineering

BLOCK 3: Measurements, regulation and translation

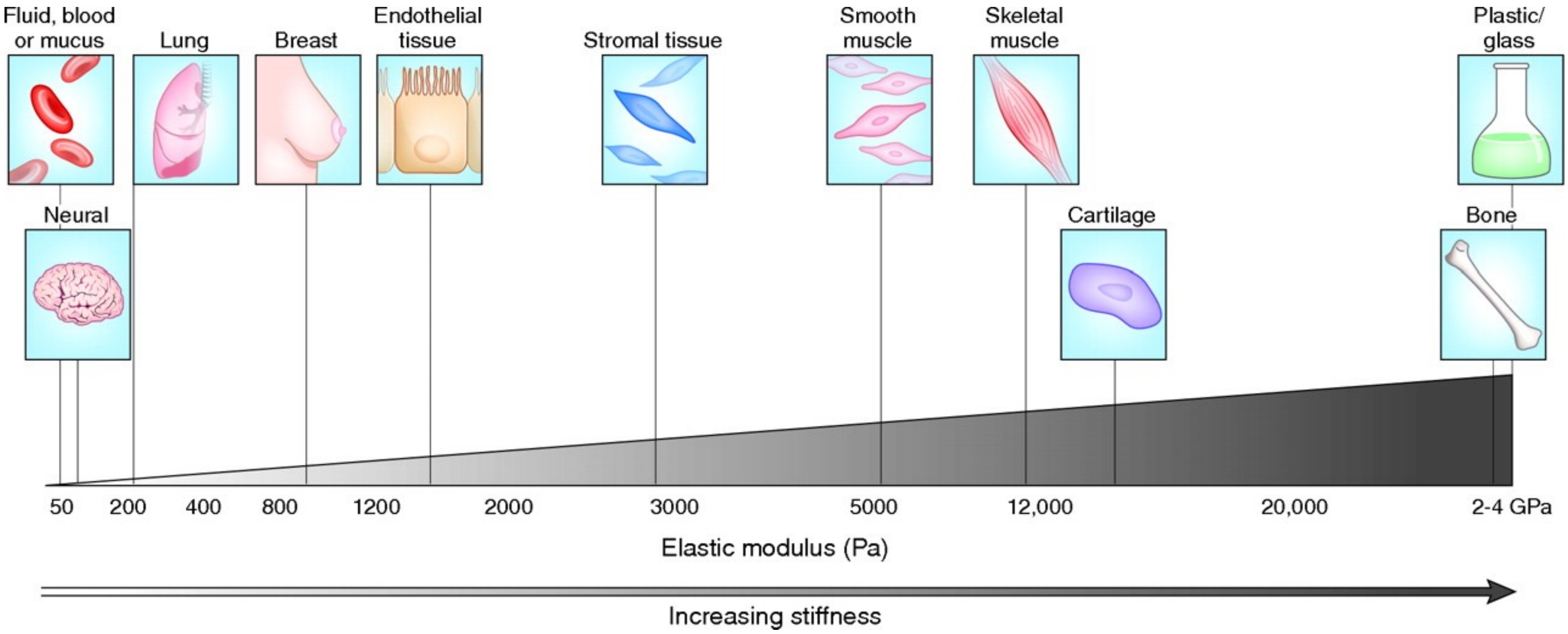
13-11	Lecture 9.	Characterization and performance
20-11	Lecture 10.	Sensors and diagnostic devices
27-11	Lecture 11.	Translation to industry, patents, spin-offs (EPFL start ups)
4-12	Lecture 12.	Regulatory aspects and trials (EPFL TTO)

11-12	Lecture 13.	Revision and conclusion
18-12	Open discussion and hand in of lab papers	



Why do cells need to adhere?

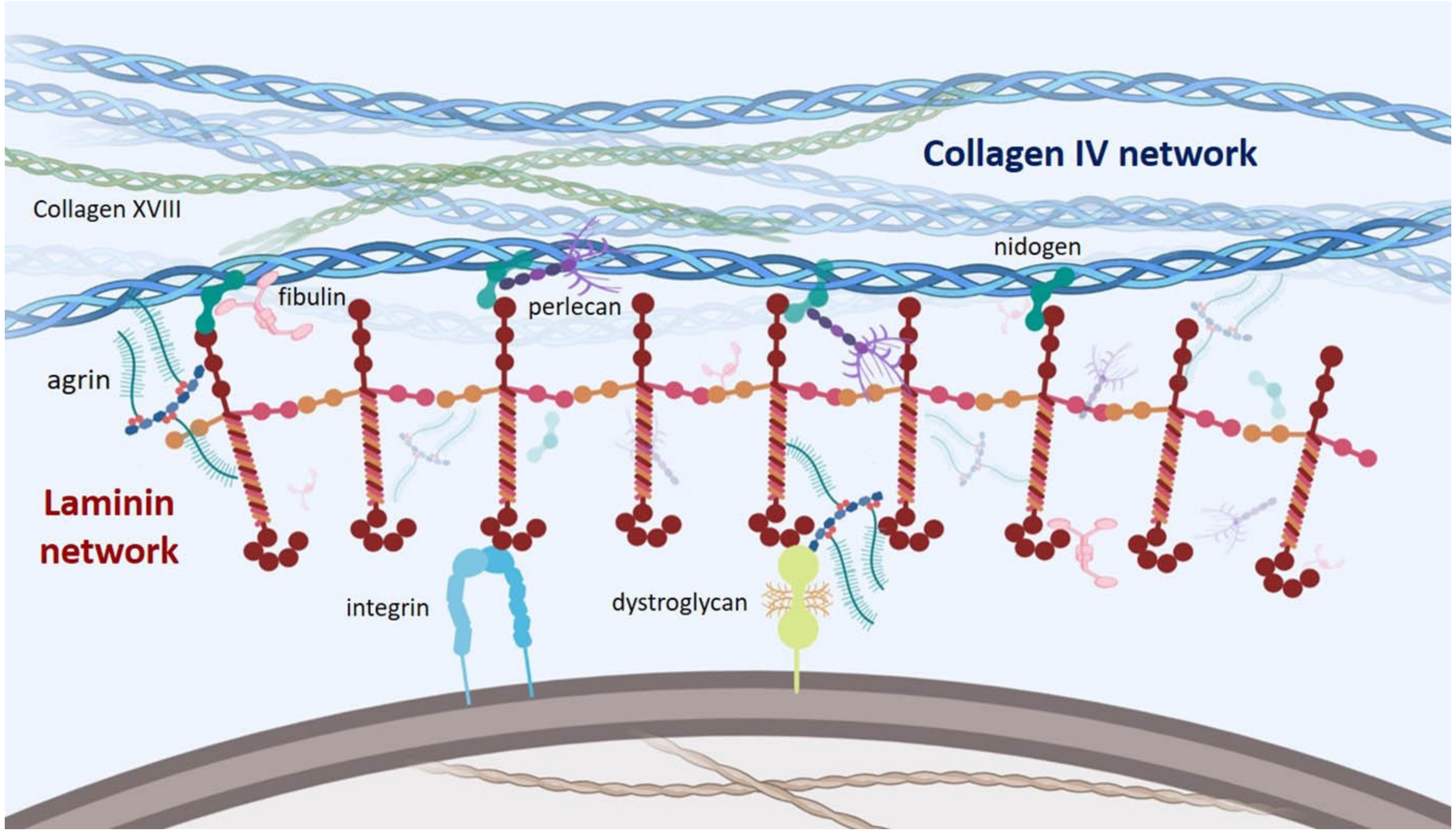
Mechanical properties



Disease Models & Mechanisms 2011 4: 165-178; doi: 10.1242/dmm.004077

Basement Membrane

Network architecture of all epithelia



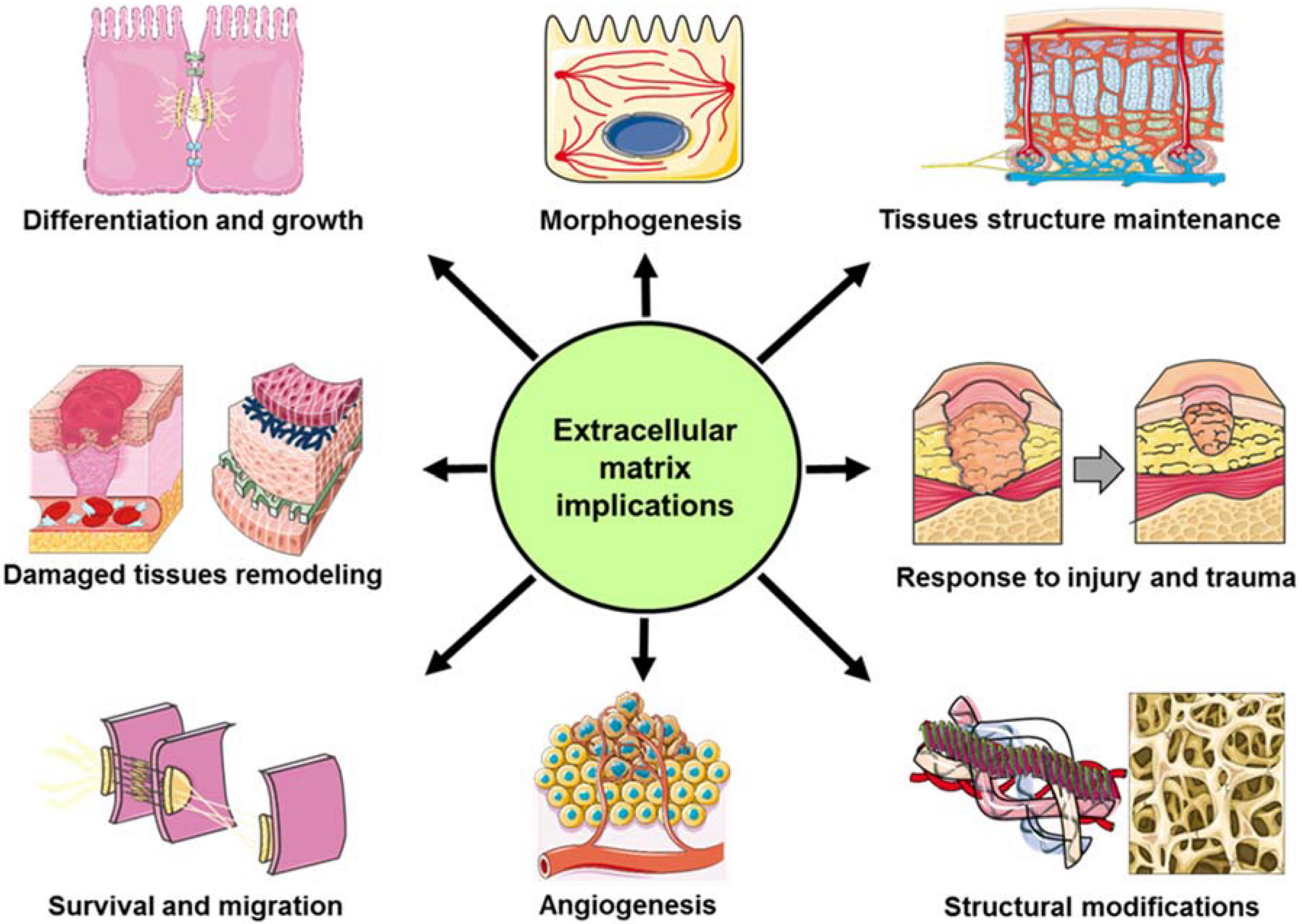
Type IV Collagen

Proteoglycans

Laminin

Illustration by Maryline Fresquet (University of Manchester, UK).

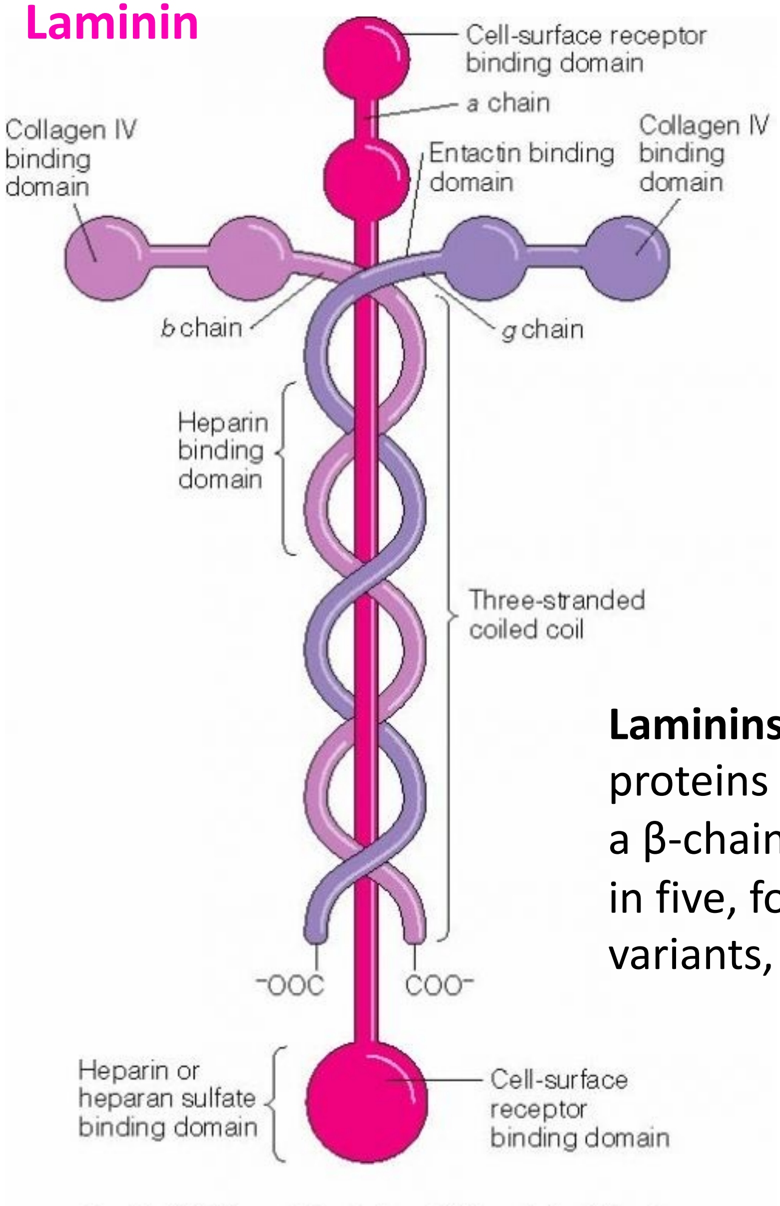
The extracellular matrix functions



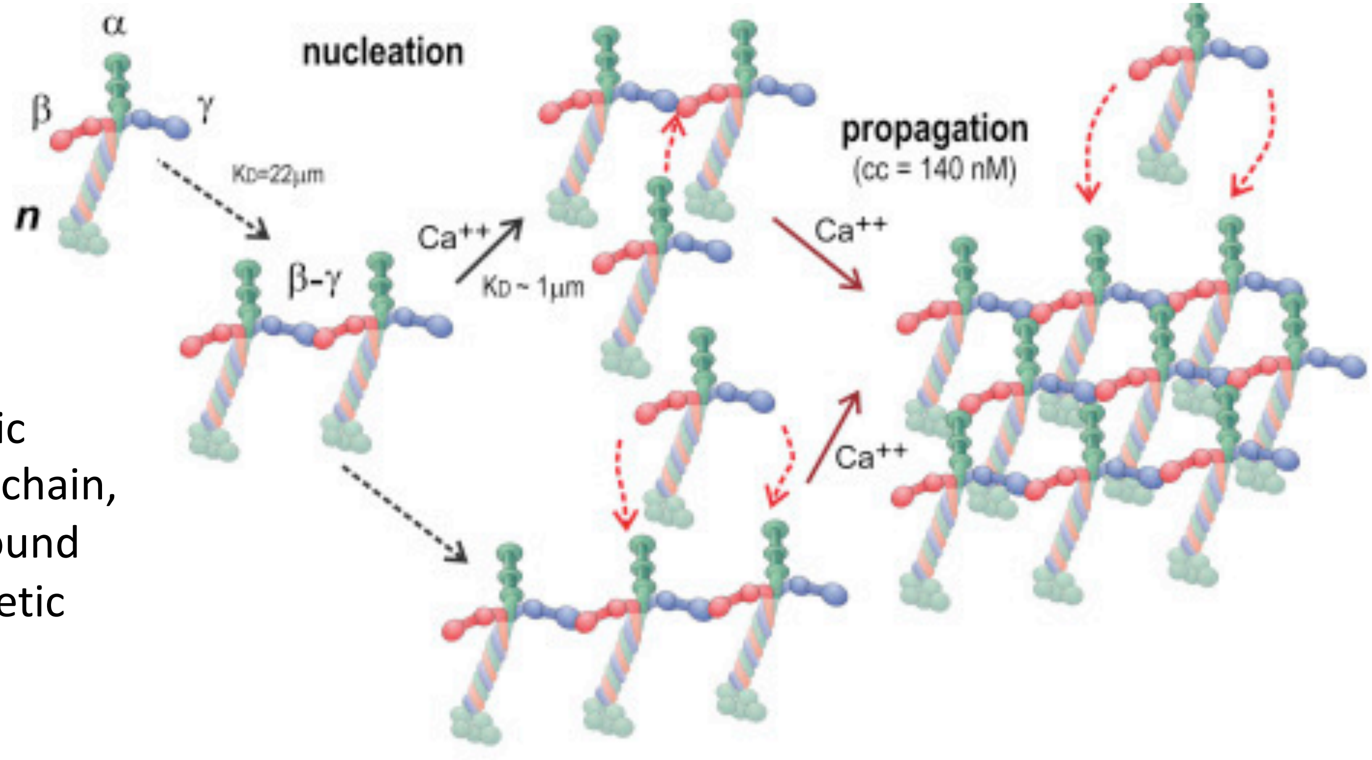
Appl. Sci. 2020, 10(7), 2388;
https://doi.org/10.3390/app10072388

Laminin: Critical Organizer

Laminin



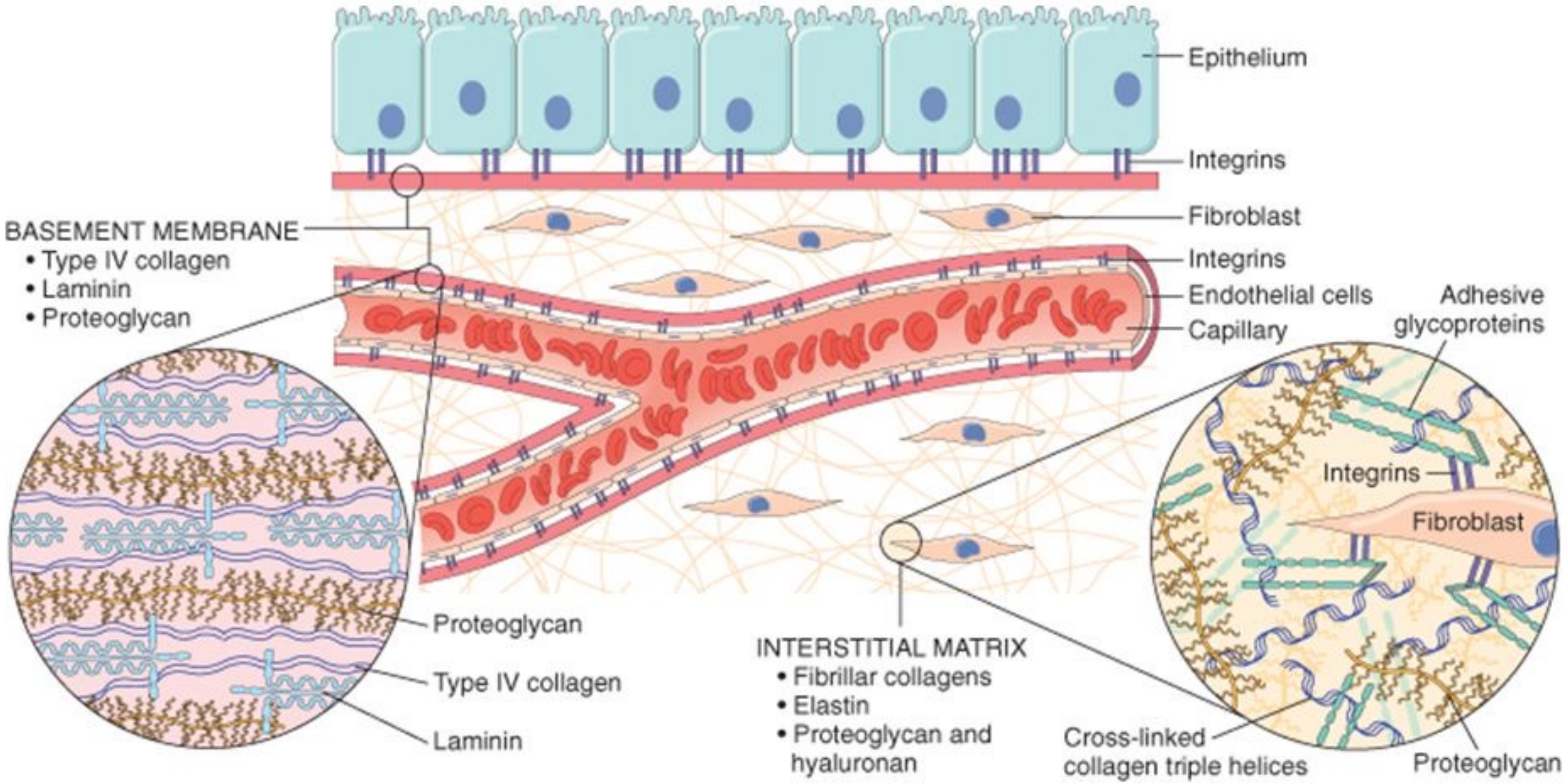
Laminins are the major component of the basal lamina.
They influence cell differentiation, migration, and adhesion.



Laminins are heterotrimeric proteins that contain an α-chain, a β-chain, and a γ-chain, found in five, four, and three genetic variants, respectively.

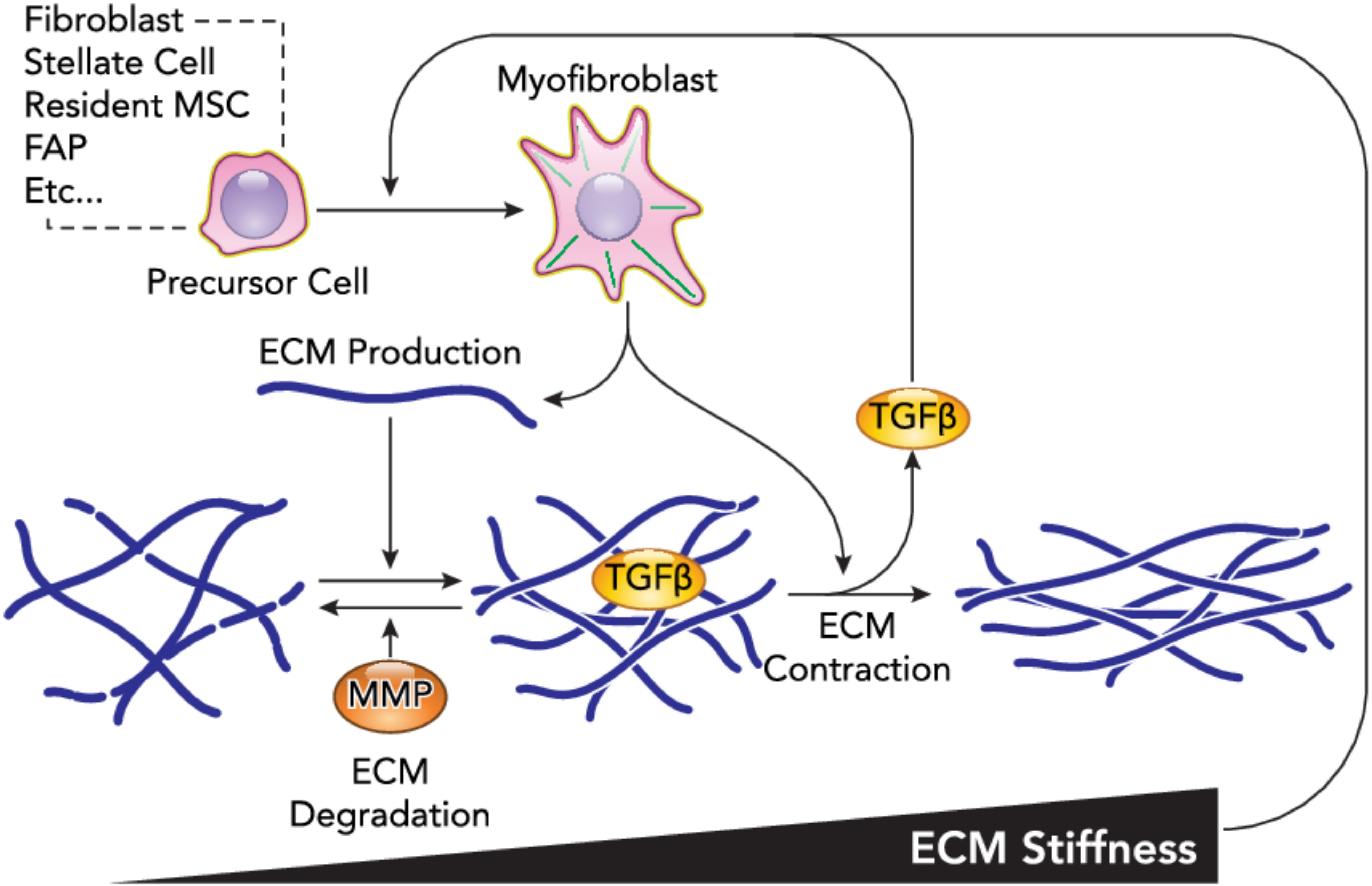
https://doi.org/10.1016/bs.ctm.2015.05.001

The Space Between Cells

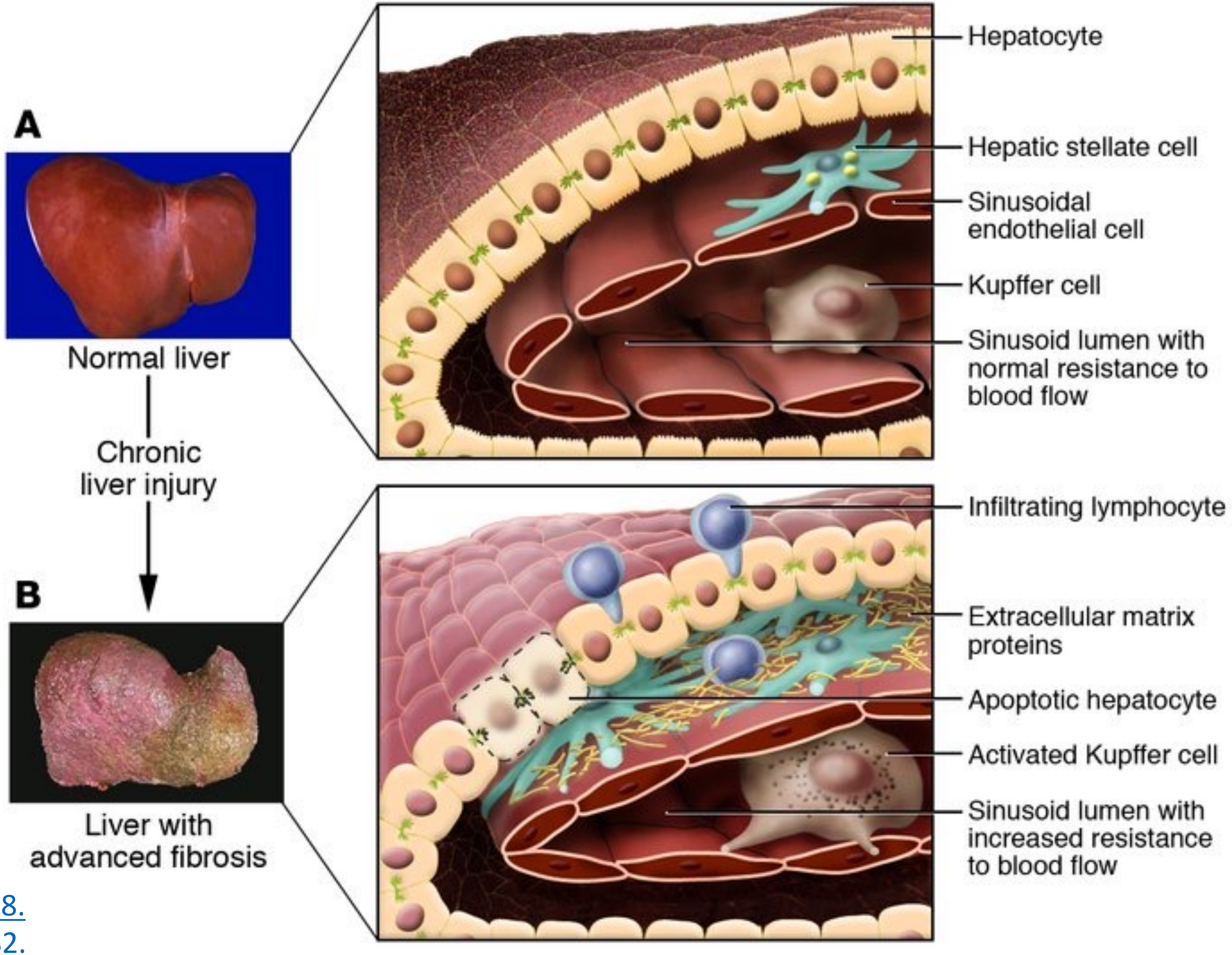


Can there be too much ECM?

When there is too much ECM: Fibrosis



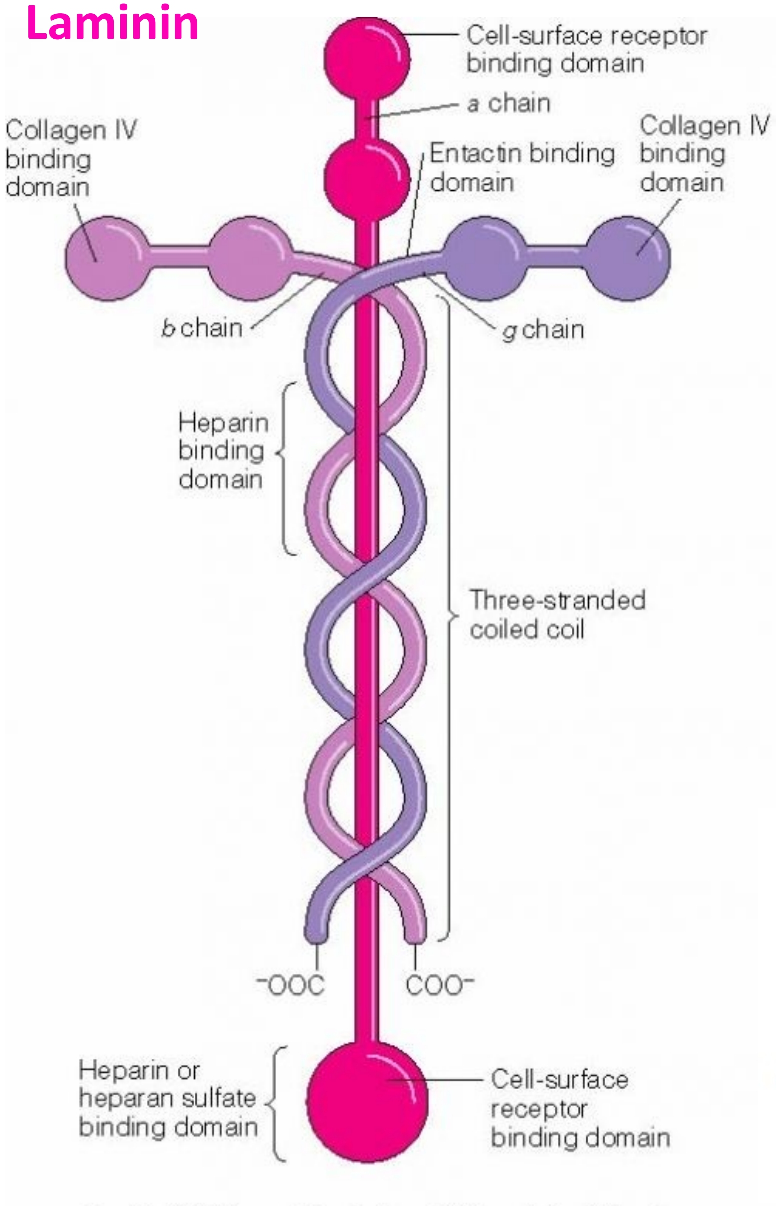
Liver Fibrosis

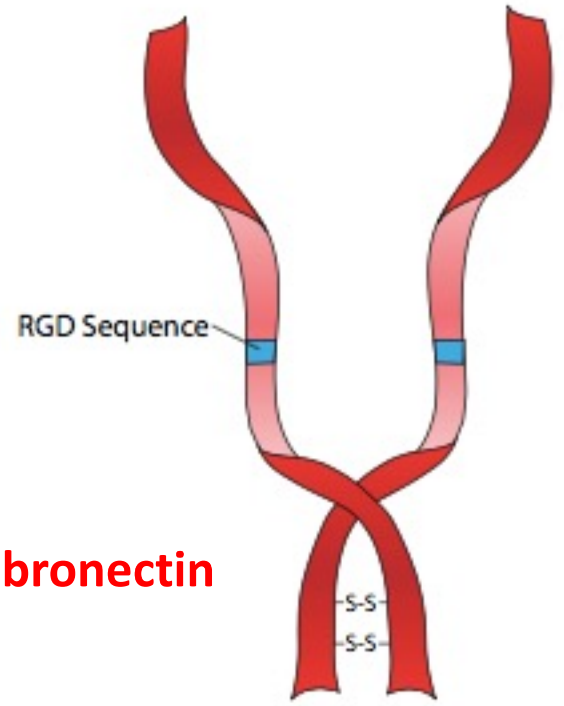


J Clin Invest. 2005;115(2):209-218.
https://doi.org/10.1172/JCI24282.

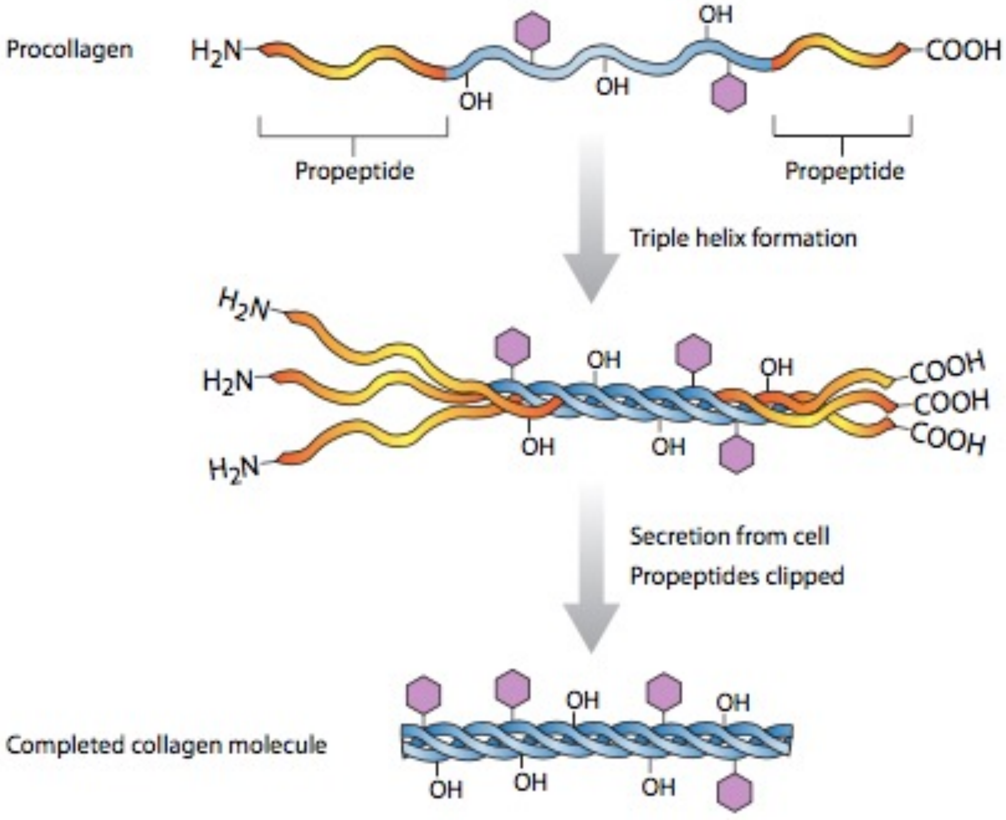
The extracellular matrix components

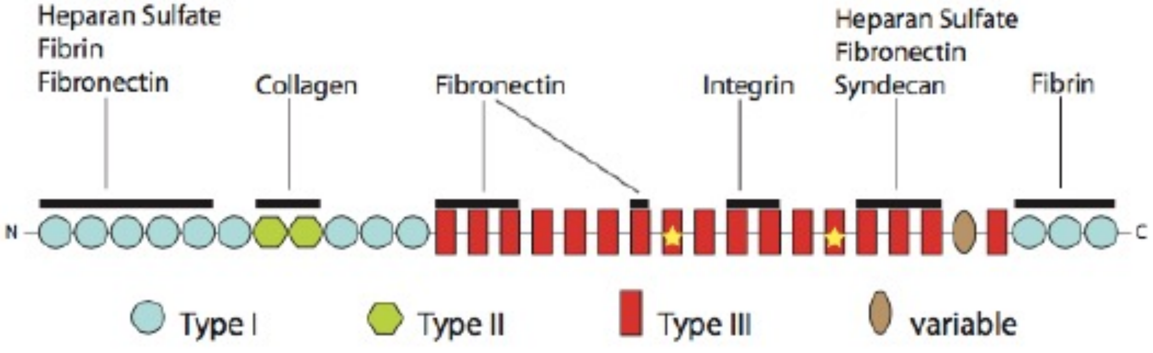
Laminin





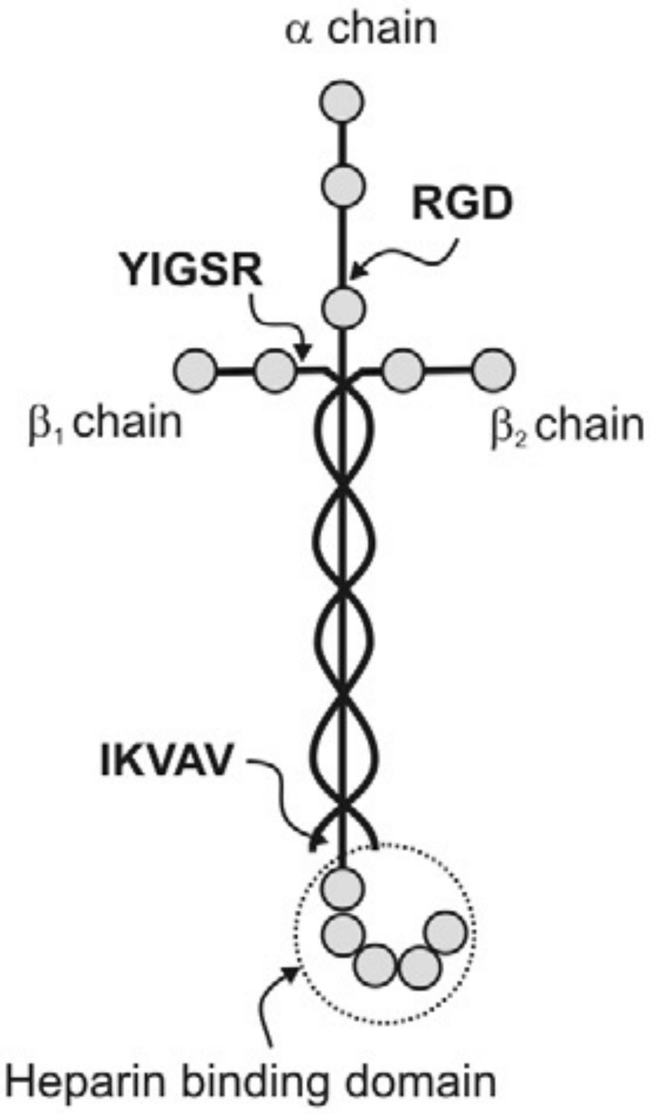
Fibronectin

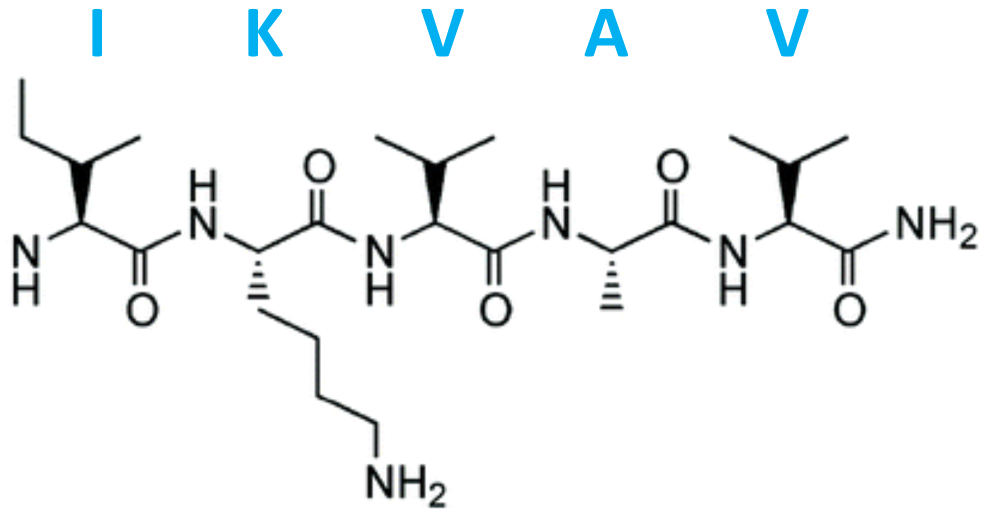




Collagen

Mimic Laminin: IKVAV



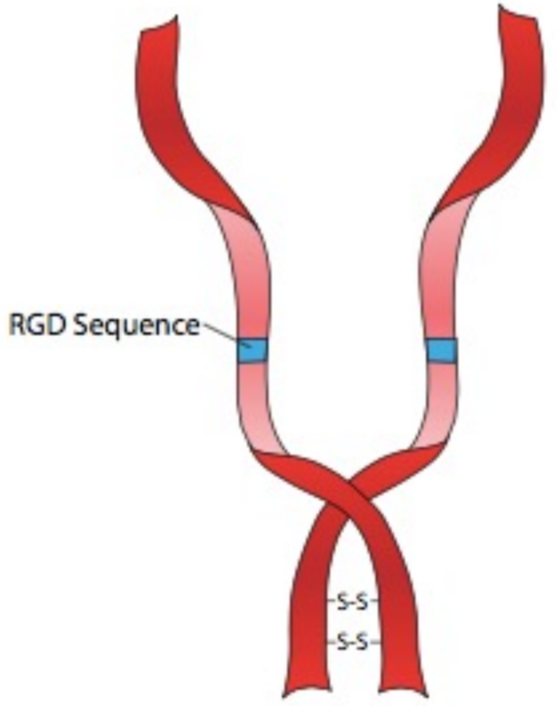


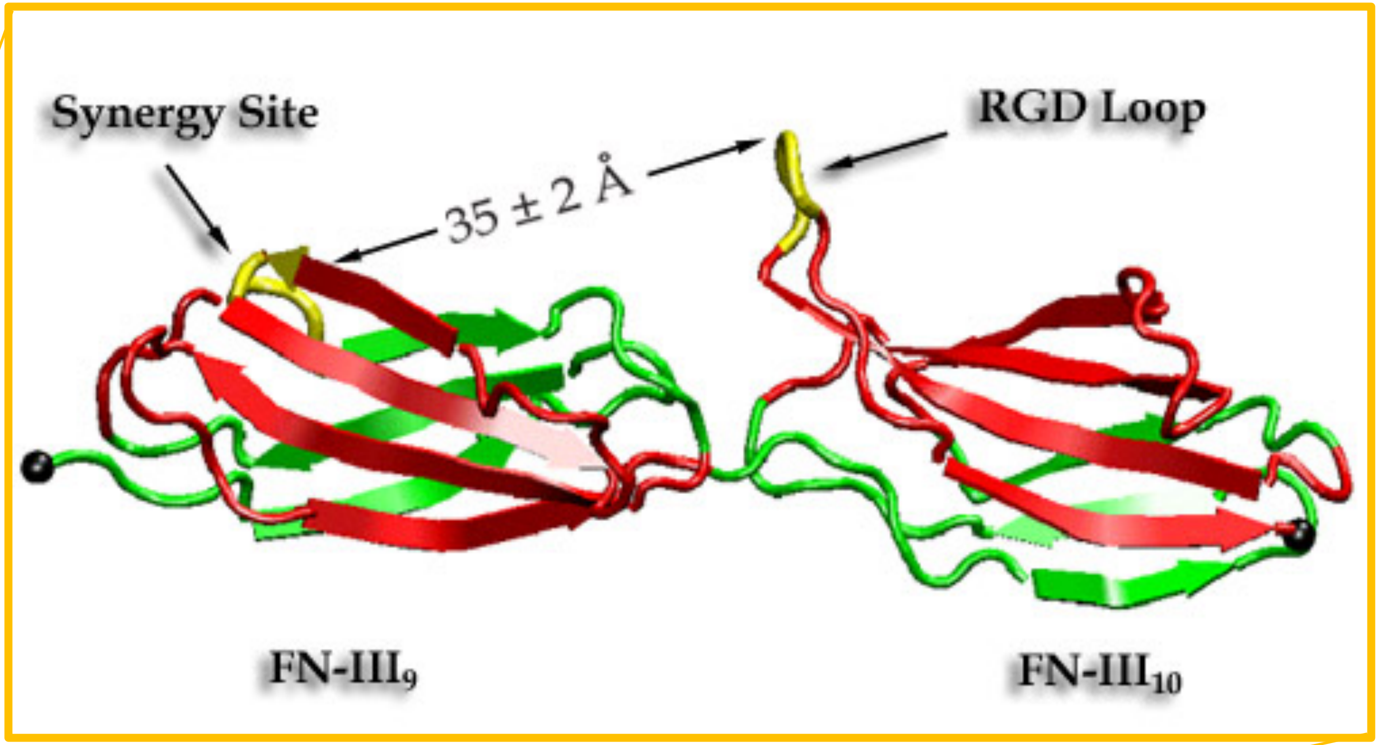
Stimulates neural growth

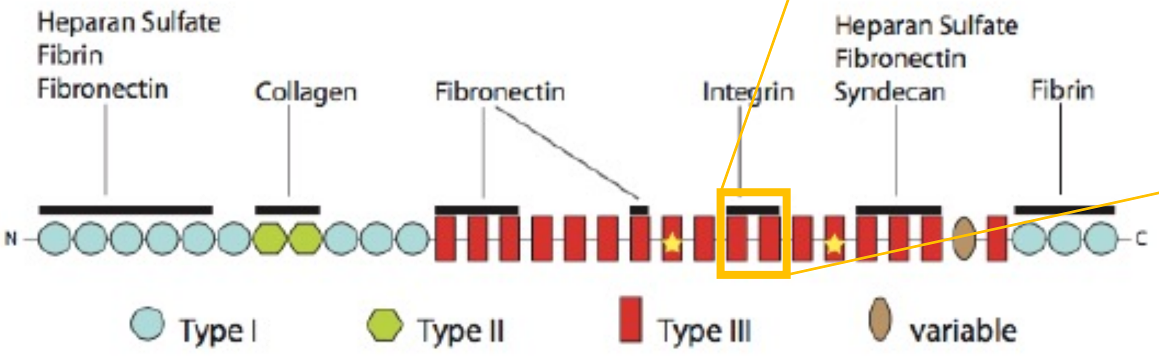
IKVAV is known to be bound through combinations of α3β1, α4β1, and α6β1

J Biol Chem
. 1989 Sep 25;264(27):16174-82.

Fibronectin



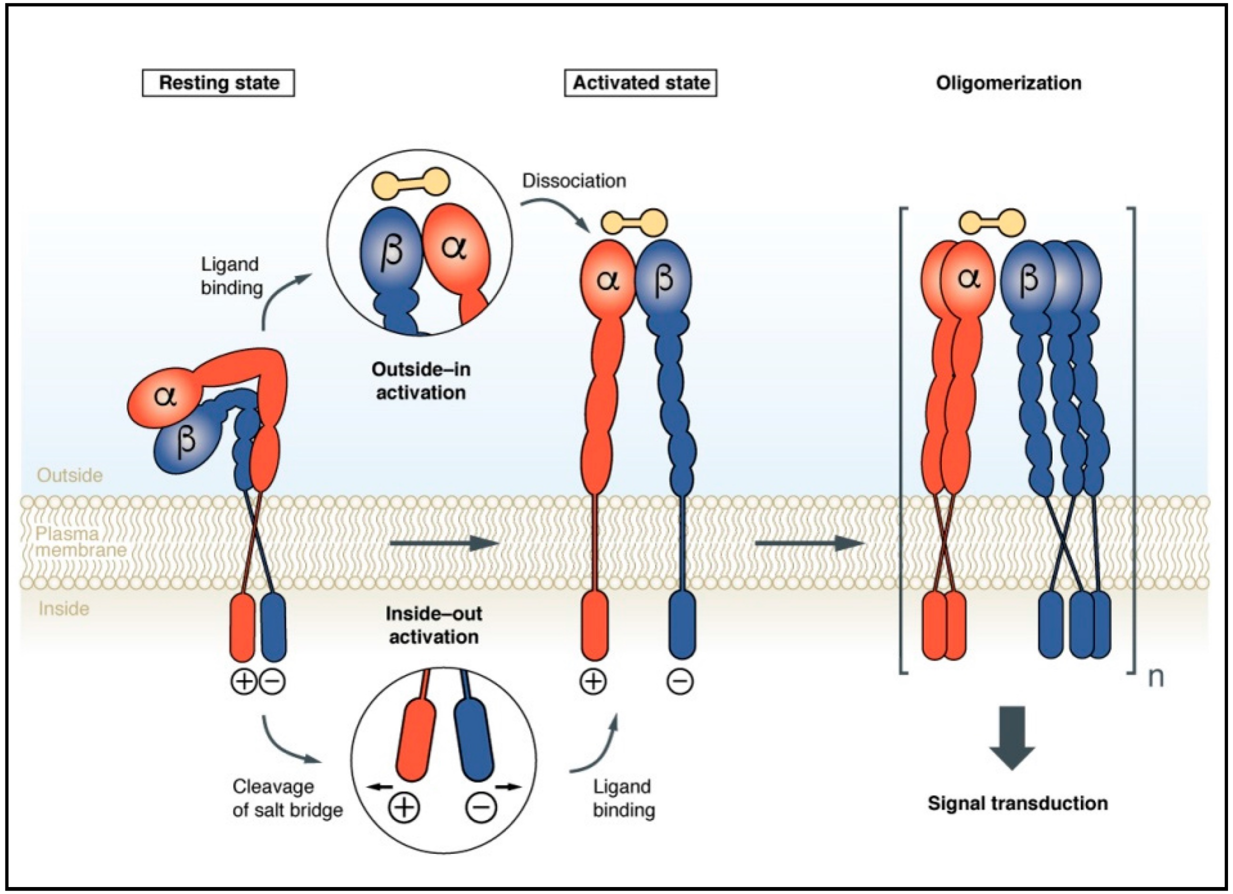


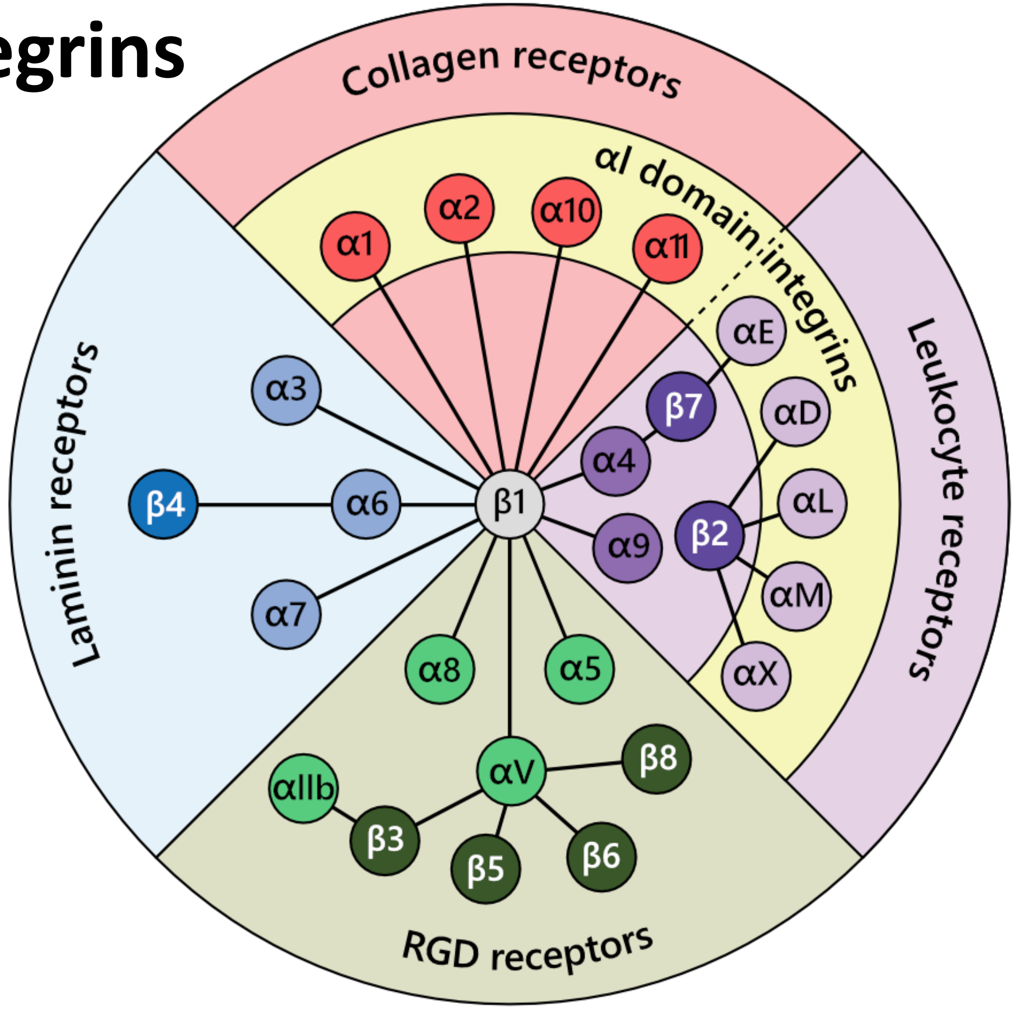


Fibronectin is a high-molecular weight (~500 kDa) glycoprotein of the extracellular matrix that binds to membrane-spanning receptor proteins called integrins. **Fibronectin** also binds to other extracellular matrix proteins such as collagen, fibrin, and heparan sulfate proteoglycans (e.g. syndecans).

Cell adhesion on the molecular level: Integrins

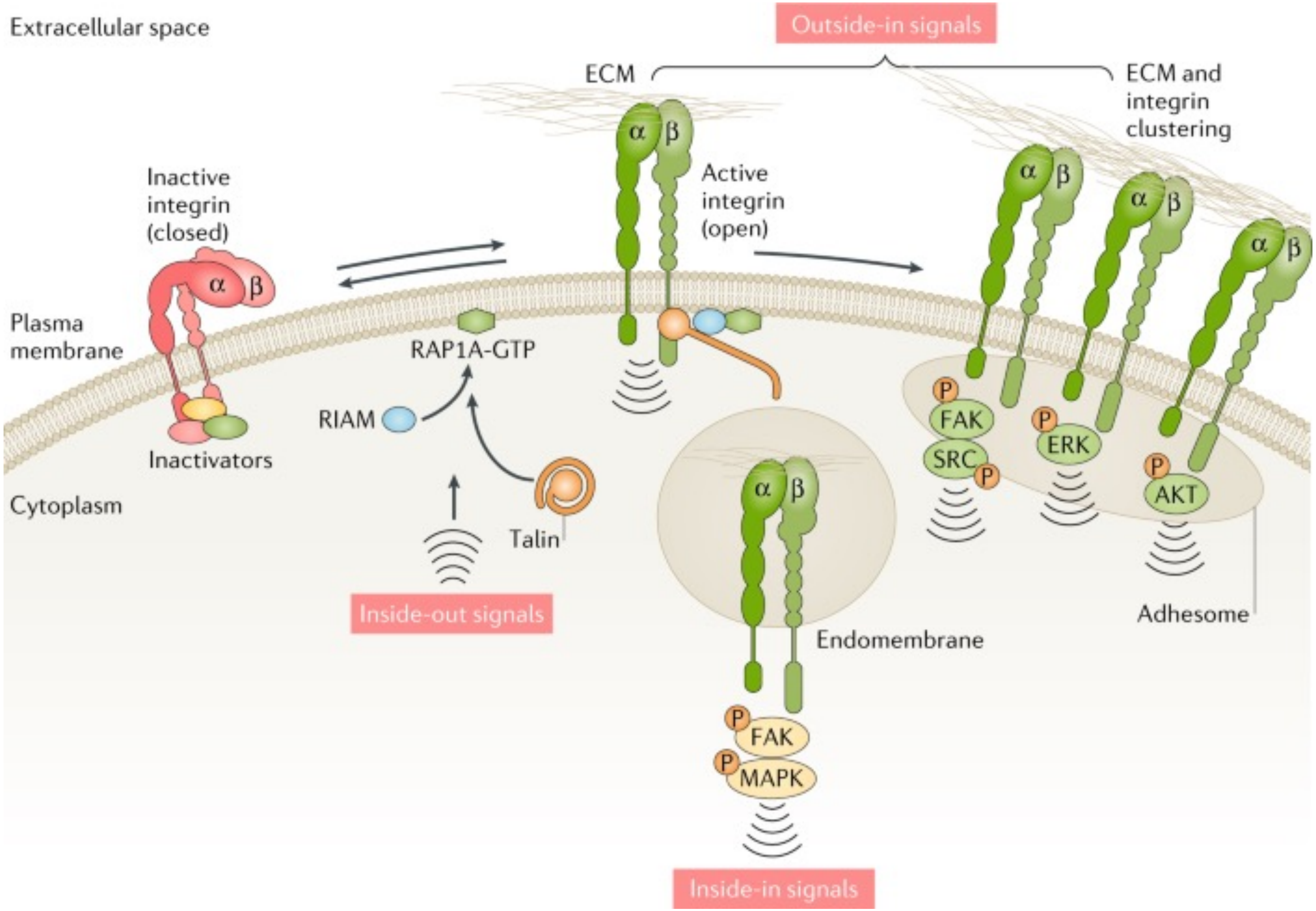
Integrins are the principal receptors used by animal cells to bind to the extracellular matrix. They are heterodimers and function as transmembrane linkers between the extracellular matrix and the actin cytoskeleton.





Both α and β subunits are transmembrane proteins that pass through the membrane just once. All integrins but α6β4, connect to the actin cytoskeleton through the b subunit cytoplasmic domain. The α6β4 integrin links to the intermediate filaments, in part because the β4 cytoplasmic domain is very large and extends further into the cytoplasm.

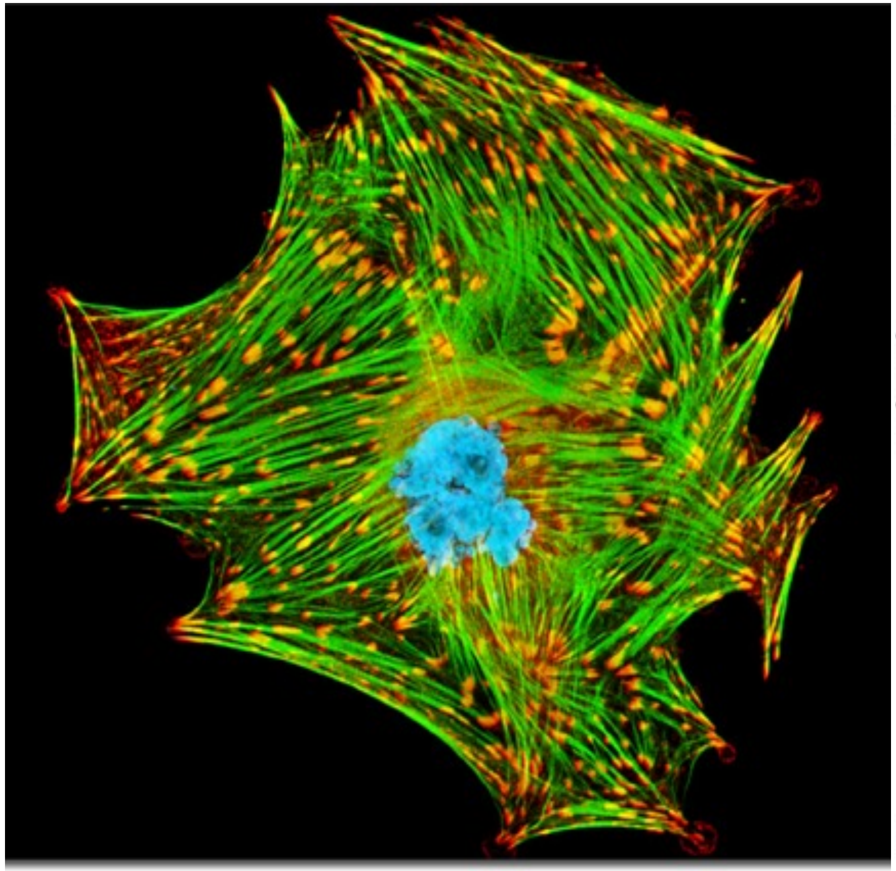
Focal Adhesions

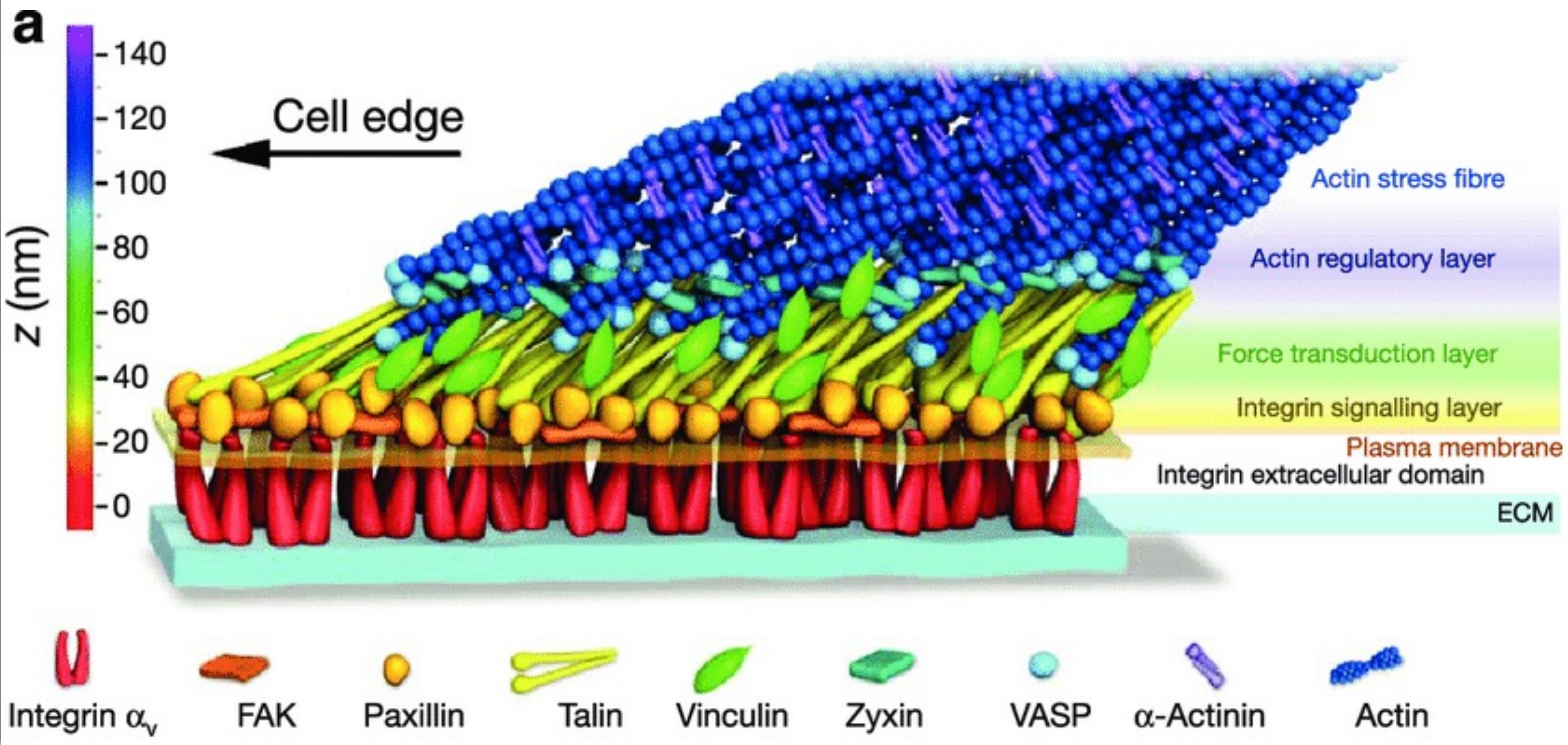


Binding to Integrins

Actin structures are linked to **focal adhesions** that provide the pathway of **force transmission** from inside the cell to the elastic matrix and associated with the focal-adhesion complexes are a number of well-known signaling molecules that are well-placed to act as **mechano-transducers**

At the molecular scale, cells to pull against the matrix and, secondly, a cellular mechano-transducer(s) **generates signals based on the force** that the cell must generate to deform the matrix.

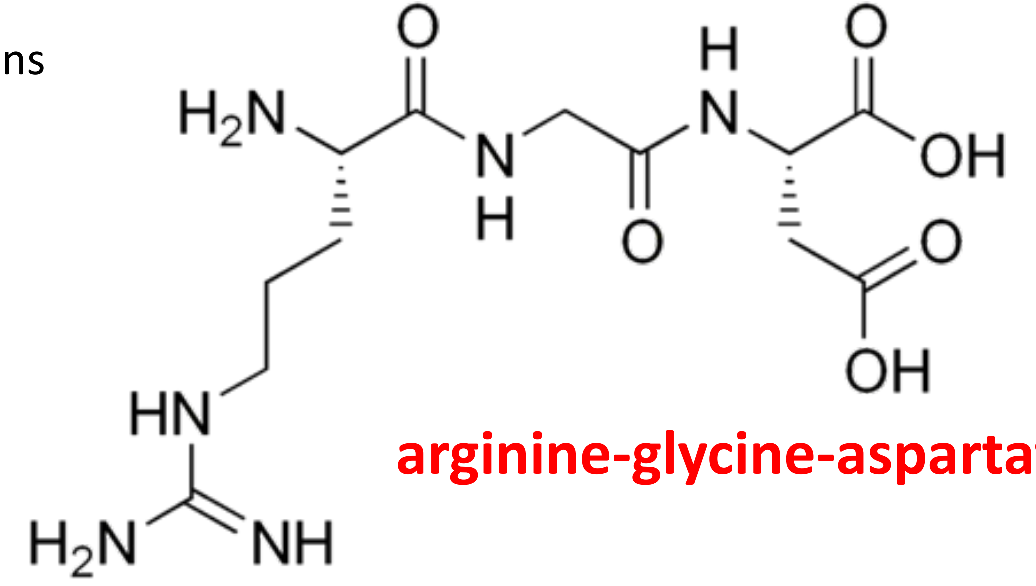




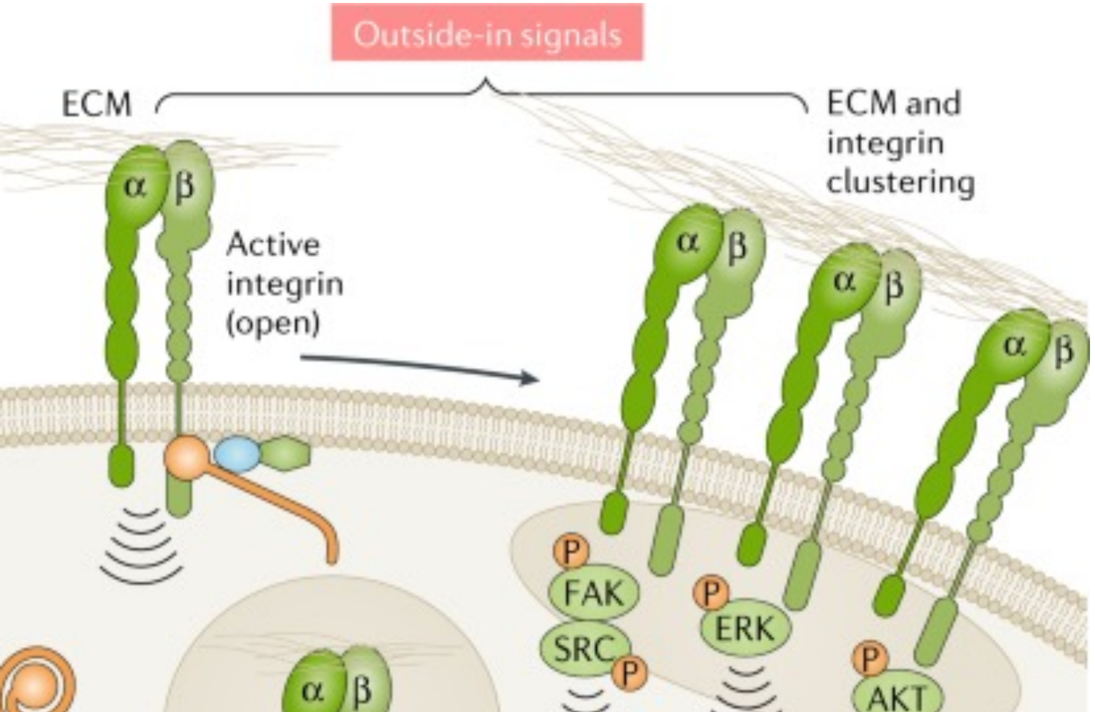
Synthetic Adhesion: RGD

The most widely studied adhesive peptide in the biomaterials field is the tri-amino acid sequence, arginine-glycine-aspartate, or “**RGD**”.

Principal integrin-binding domain present within ECM proteins

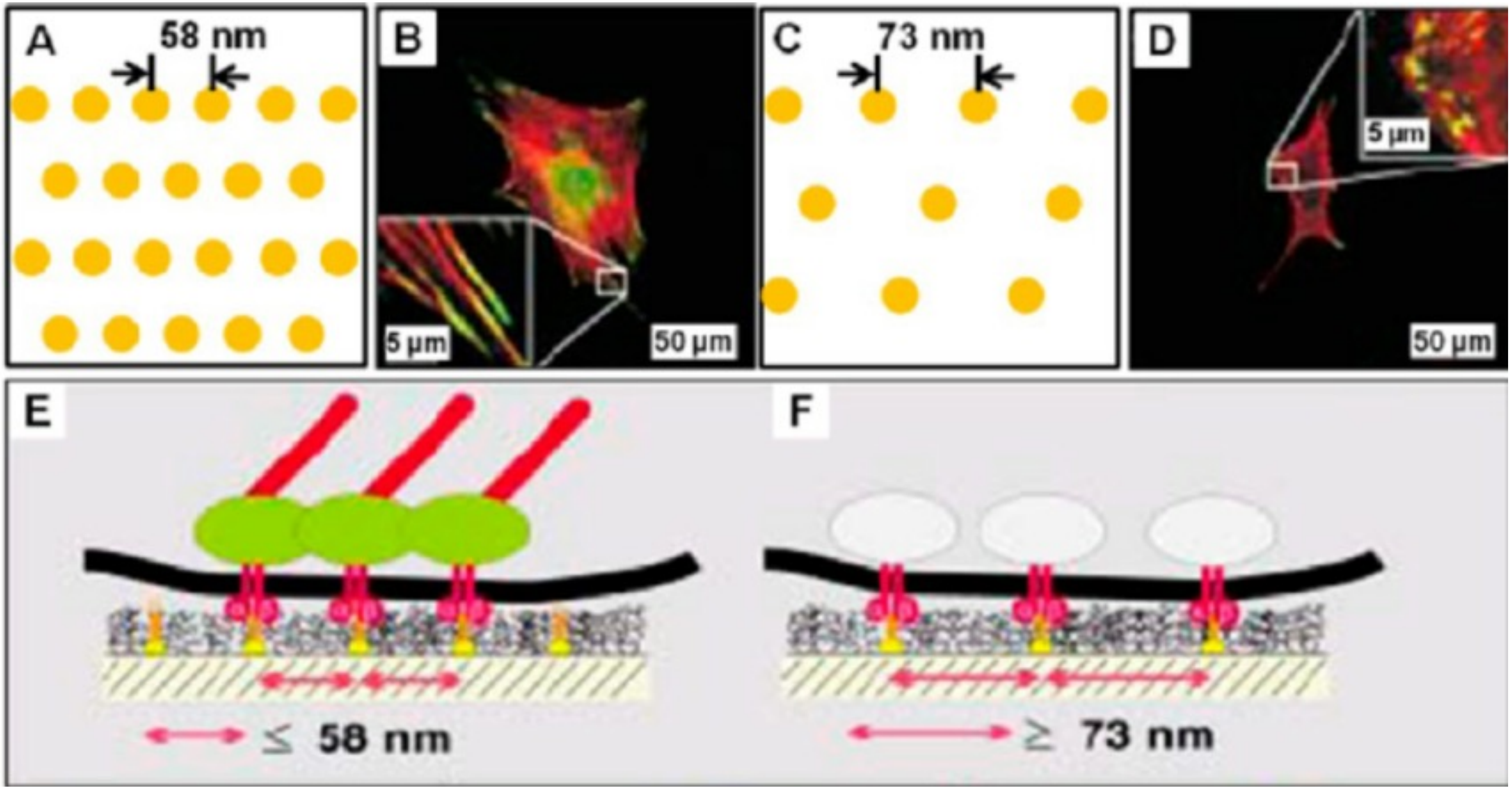


arginine-glycine-aspartate



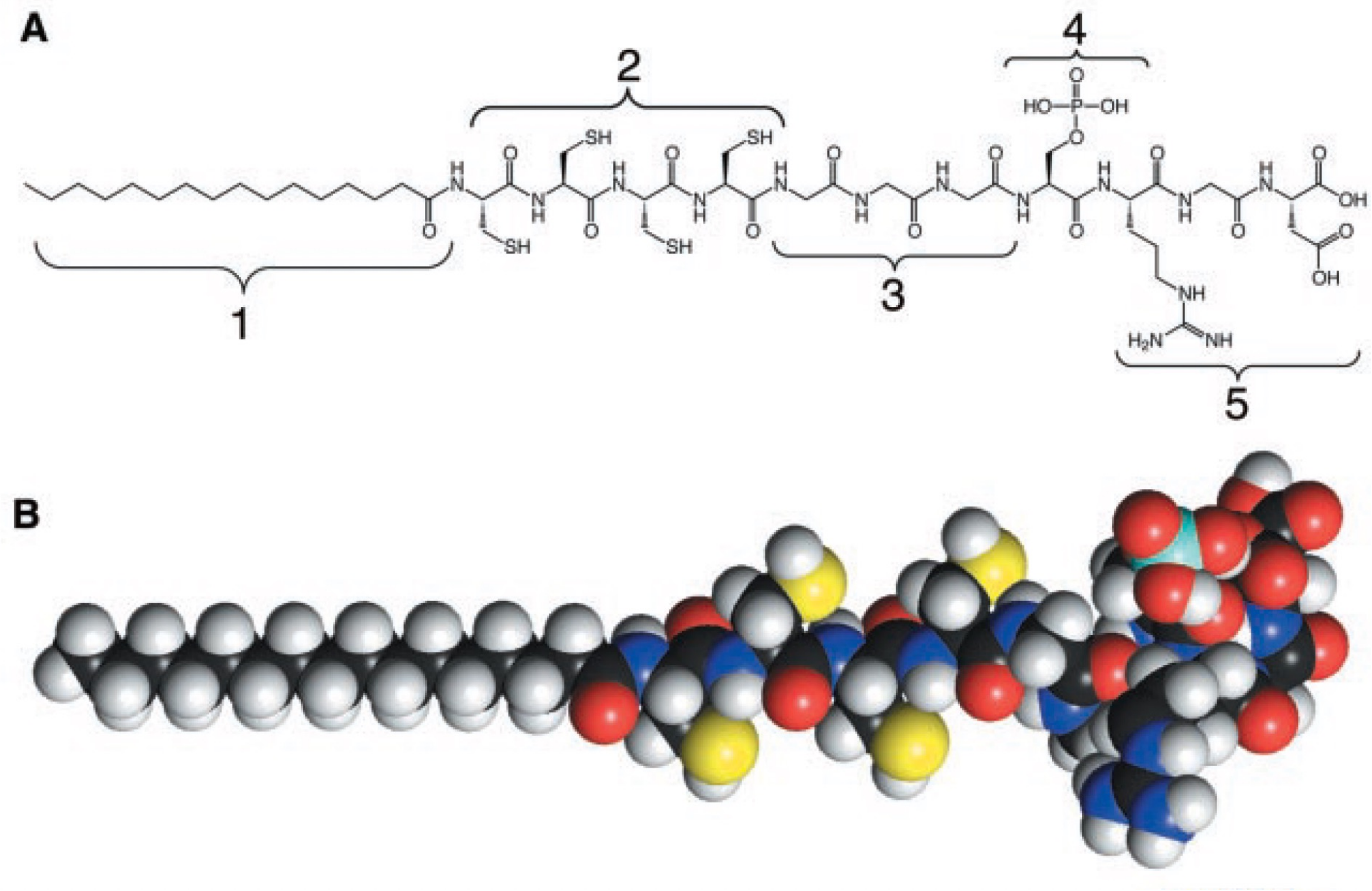
Synthesis is simple and inexpensive
Easy sterilization
Minimizes the risk of immune reactivity
Controlled densities and orientations.

Synthetic Adhesion: effect of nanospacing



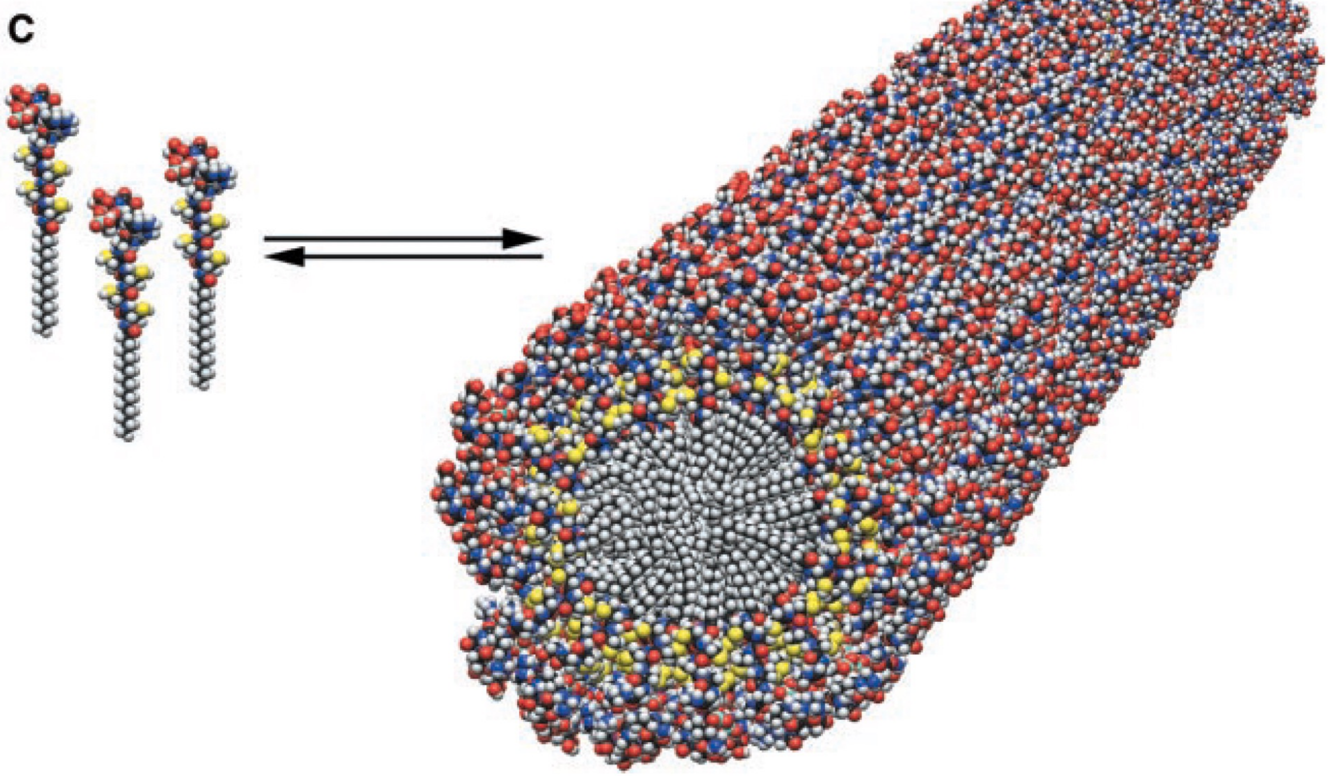
Li, Jie-Ren et al (2012). Biochemistry. 51. 5876-93.

Peptide Amphiphiles (PAs)

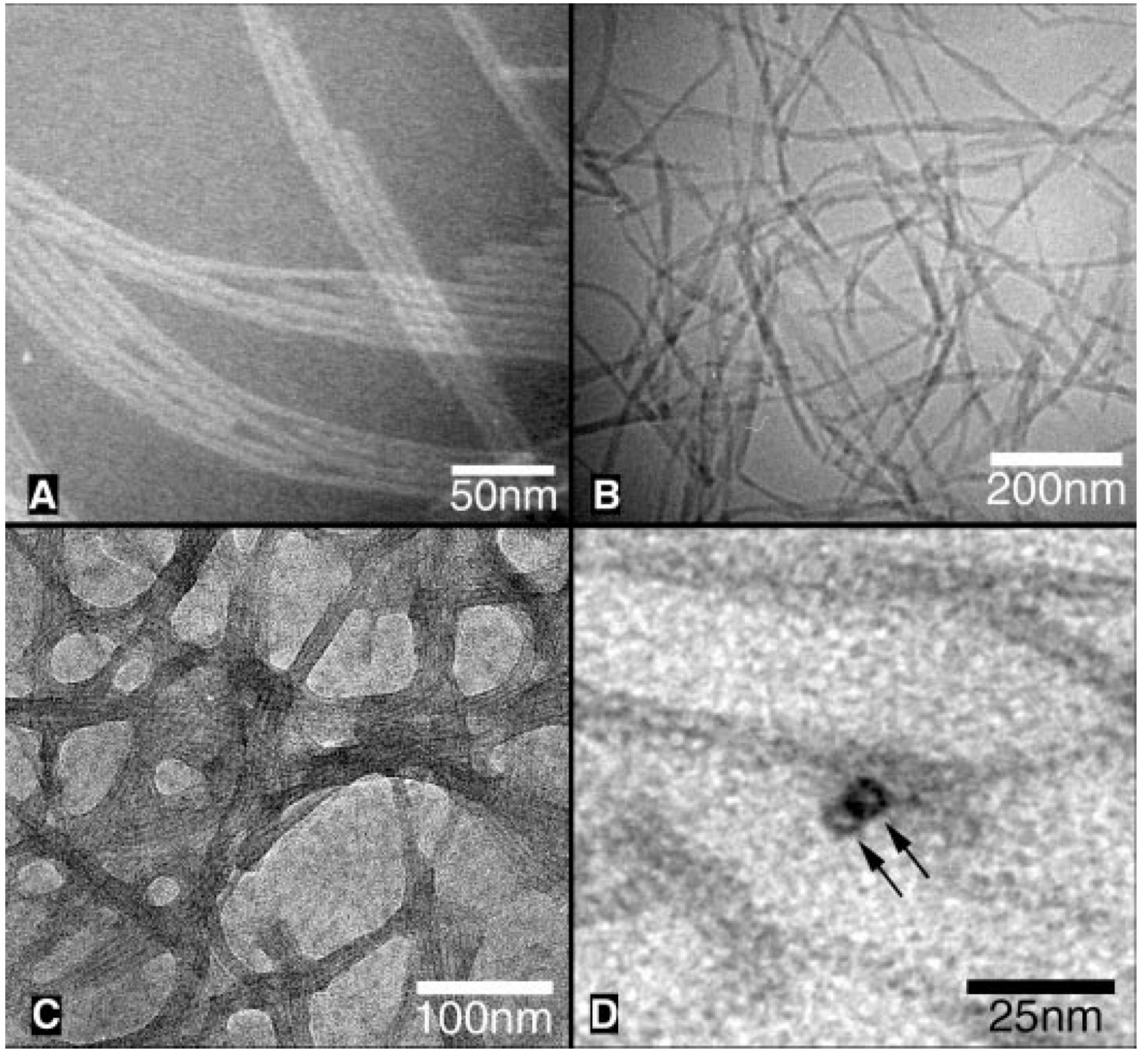


2001 VOL 294 SCIENCE

Peptide Amphiphiles (PAs)



High density of RGD!

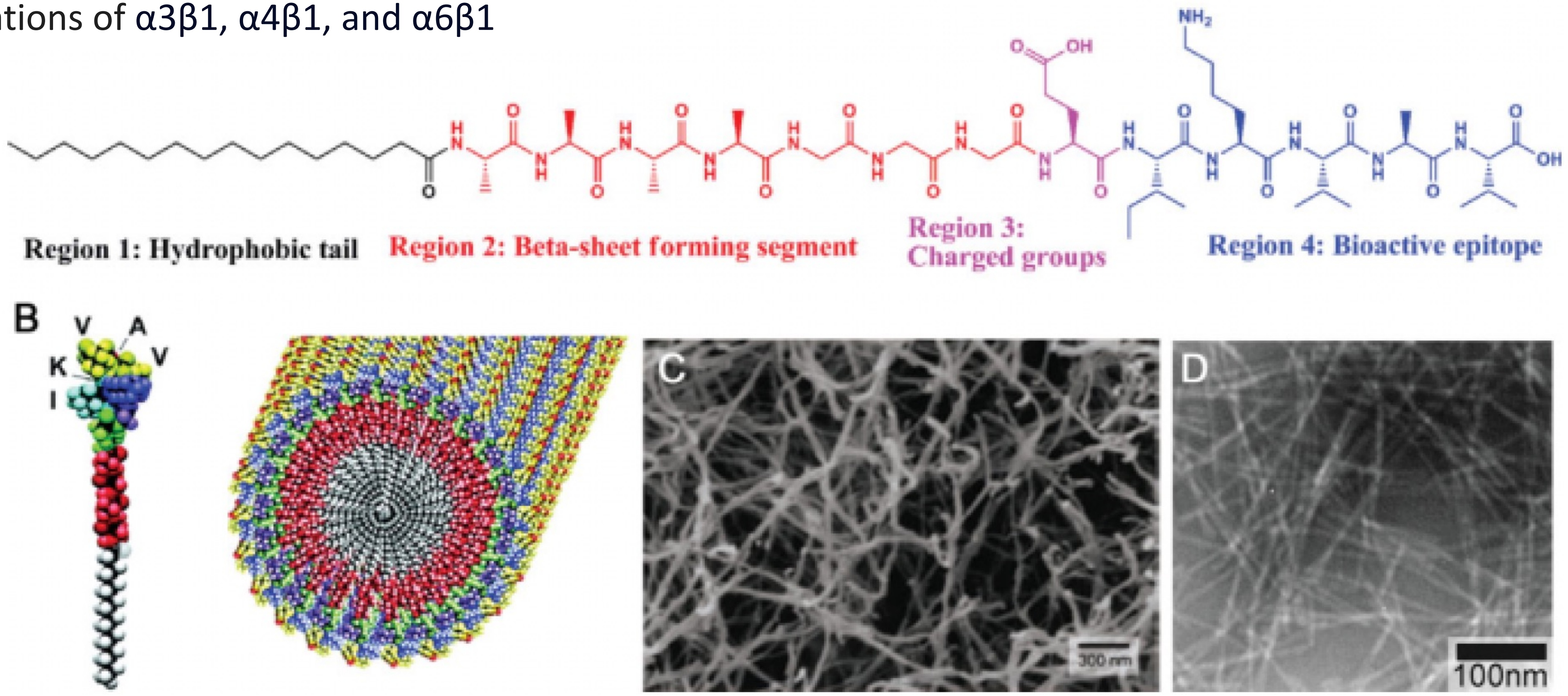


Peptide Amphiphiles (PAs)

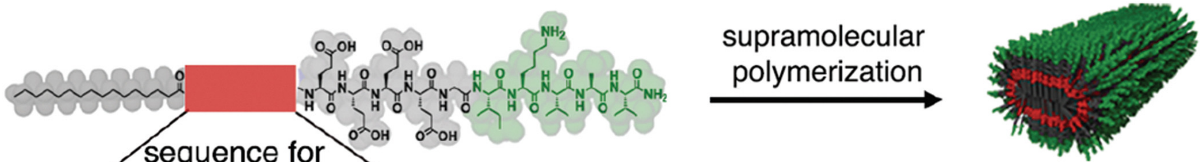
MODULAR!

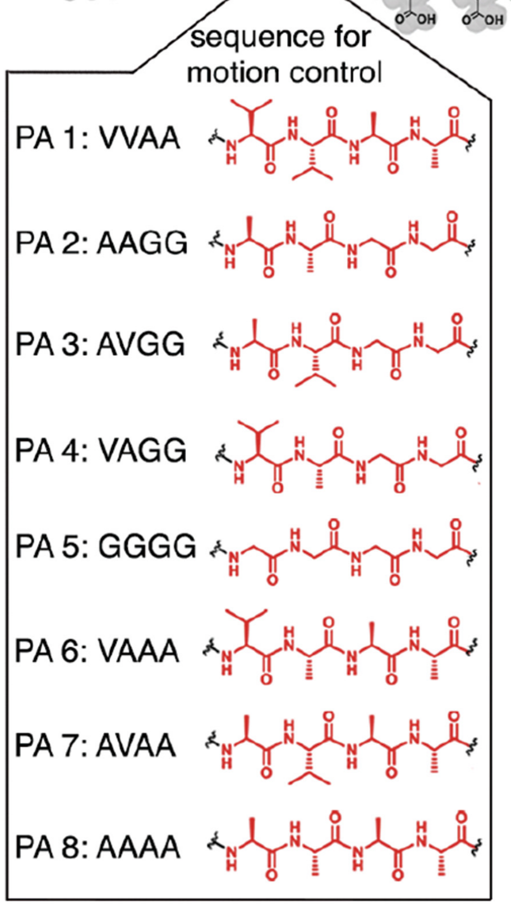
Stimulates neural growth

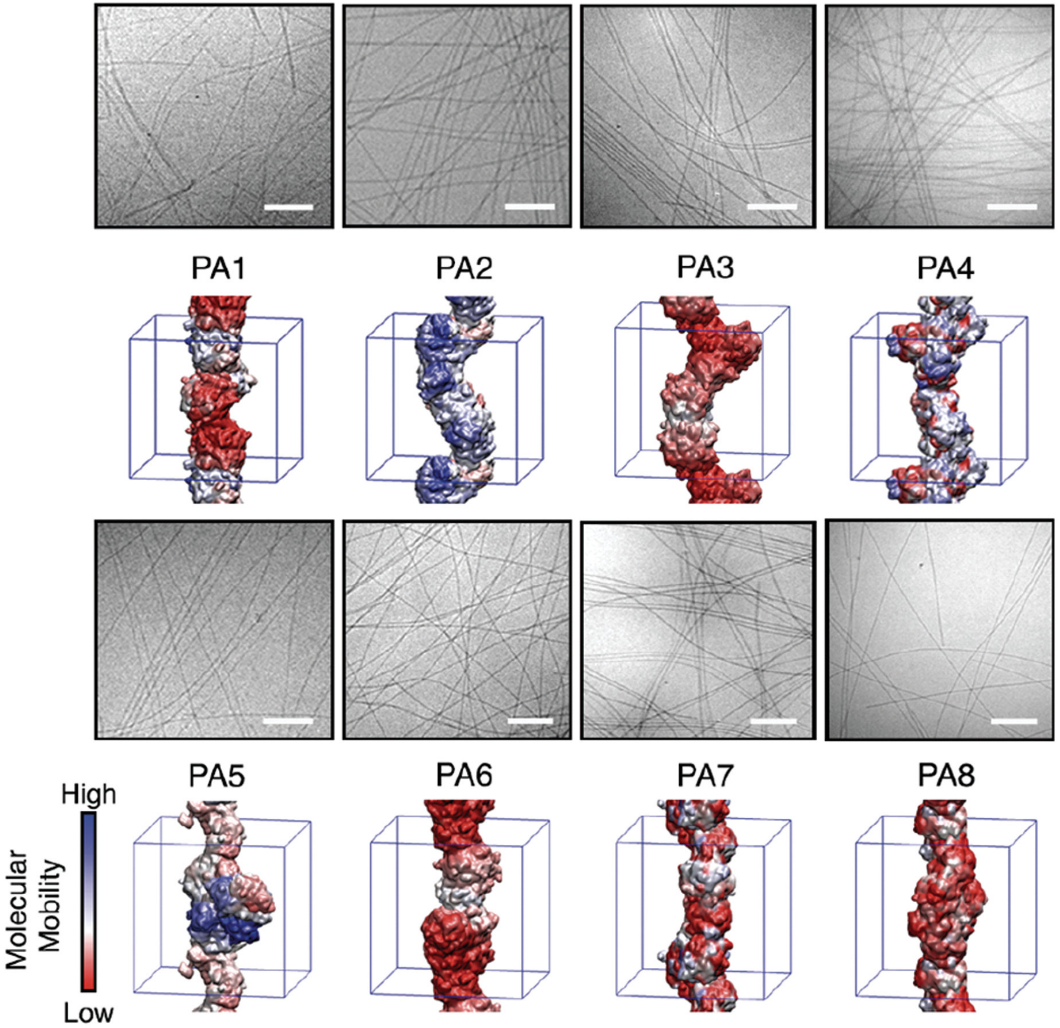
IKVAV is known to be bound through combinations of α3β1, α4β1, and α6β1

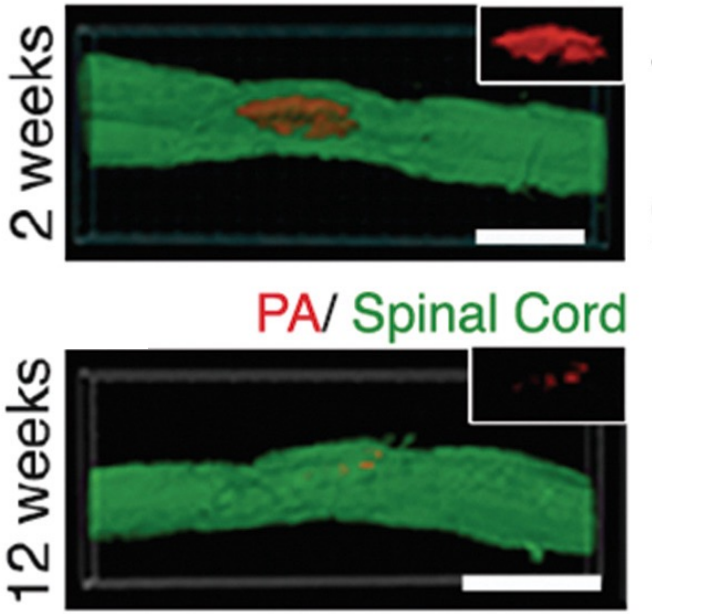


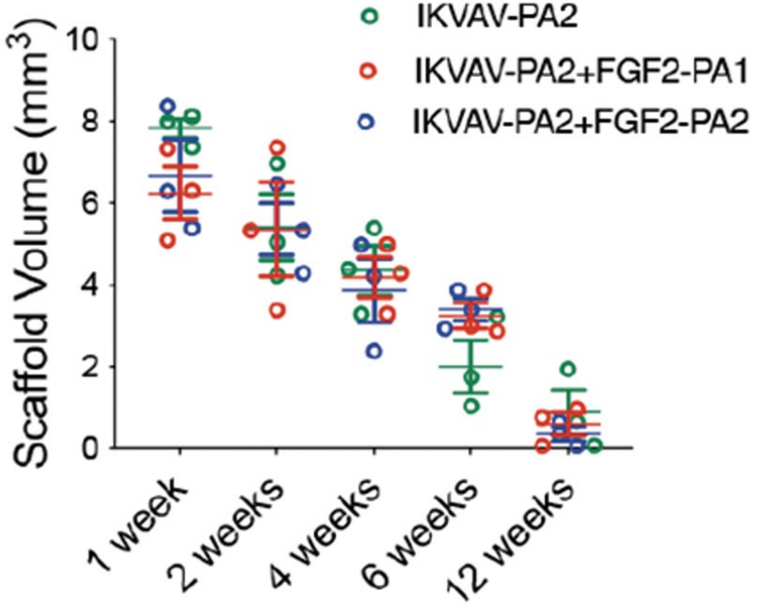
Spinal Cord Injury Repair









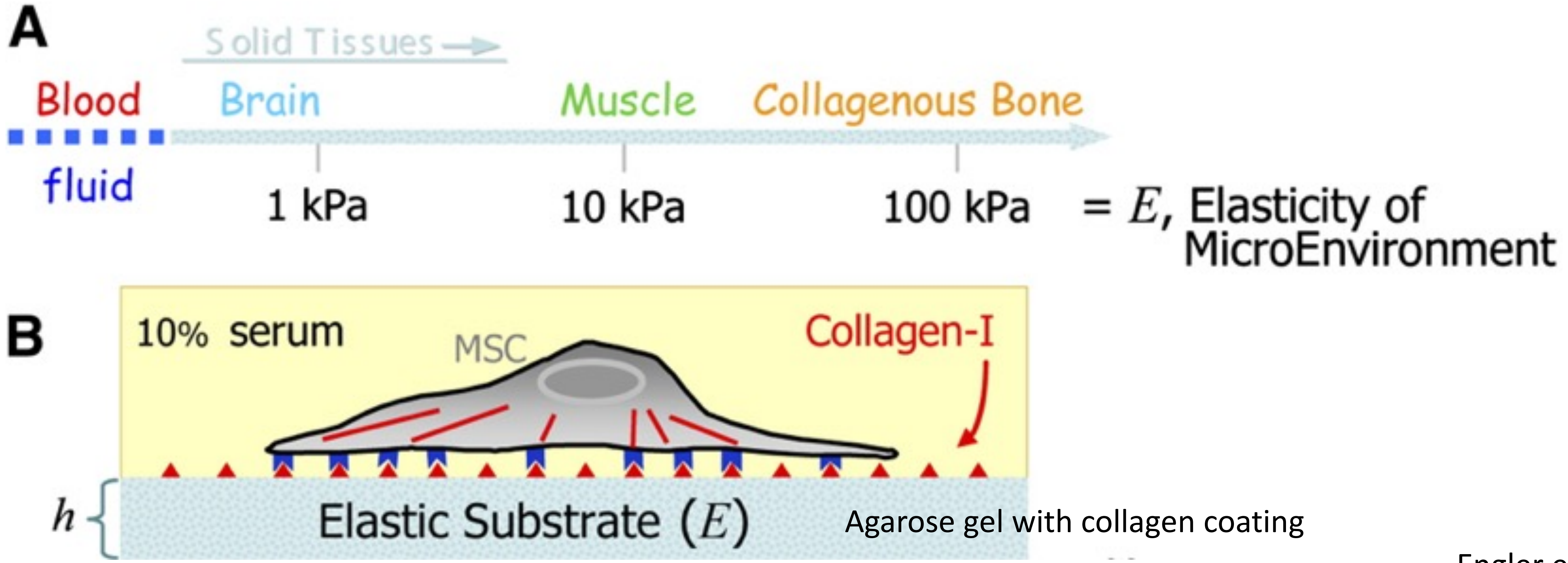


DYNAMICS

Effect of stiffness

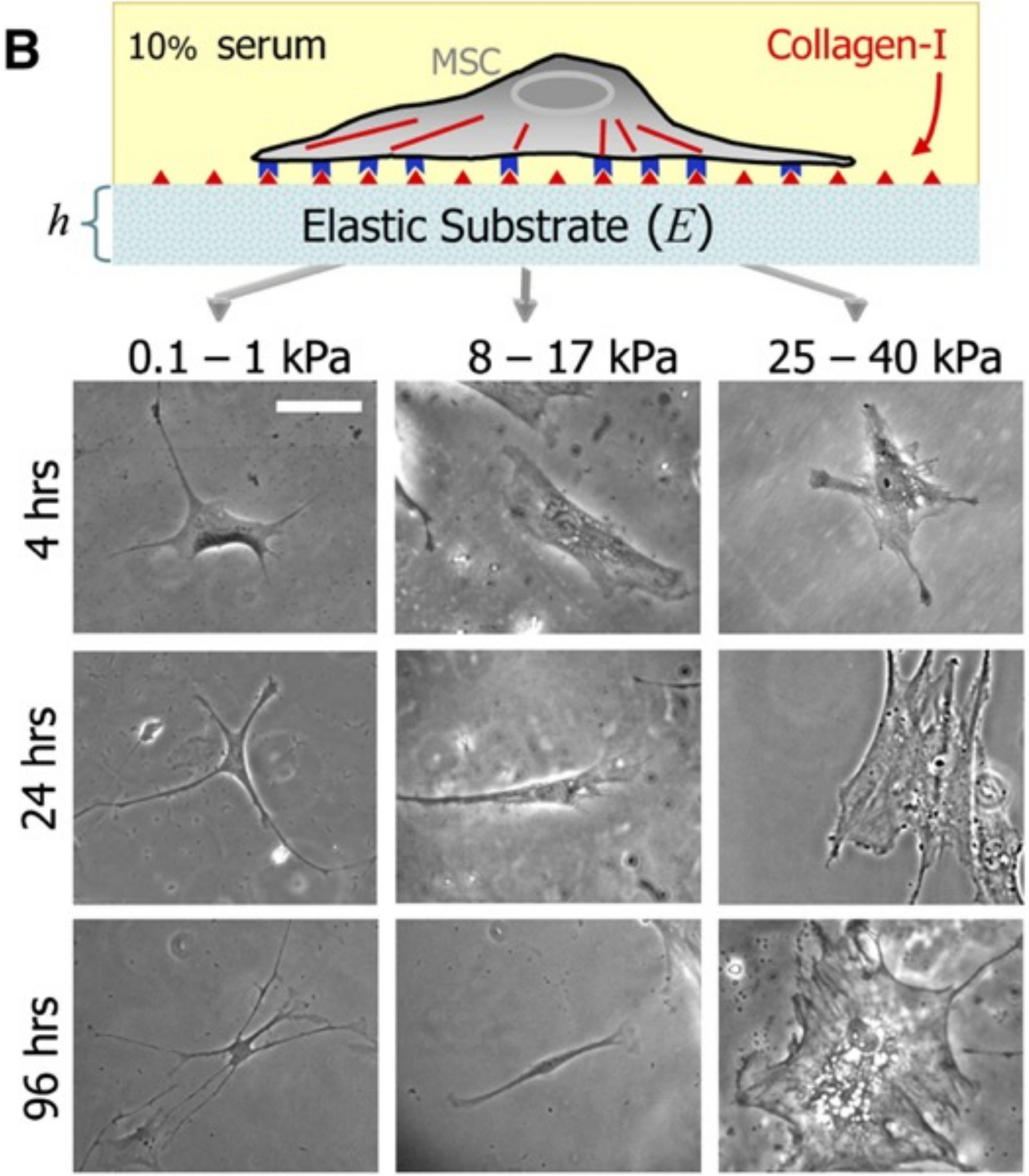
Naive mesenchymal stem cells (MSCs) have been shown to specify lineage and commit to phenotypes with extreme **sensitivity to tissue-level elasticity**.

Soft matrices that mimic brain are neurogenic, stiffer matrices that mimic muscle are myogenic, and comparatively rigid matrices that mimic collagenous bone prove osteogenic.



Agarose gel with collagen coating

Engler et al. Cell 2006



Effect of stiffness

The in vitro gel system allows for control of E through crosslinking, control of cell adhesion by covalent attachment of collagen-I, and control of thickness, h.

Naive MSCs of a standard expression phenotype are initially small and round but develop increasingly branched, spindle, or polygonal shapes when grown on matrices respectively in the range typical of
~Ebrain (0.1–1 kPa),
~Emuscle (8–17 kPa),
or stiff crosslinked-collagen matrices (25–40 kPa).

Scale bar is 20 μm.

Engler et al. Cell 2006

Matrigel

Matrigel is the trade name for a gelatinous protein mixture secreted by Engelbreth-Holm-Swarm mouse sarcoma cells produced by Corning Life Sciences. Matrigel resembles the complex extracellular environment found in many tissues and is used by cell biologists as a substrate for culturing cells

Matrigel is **limited** in its applicability to cellular biology, therapeutic-cell manufacturing and drug discovery, owing to its complex, ill-defined and variable composition.

Variations in the mechanical and biochemical properties within a single batch of Matrigel —between batches — have led to uncertainty in experiments and a lack of reproducibility.

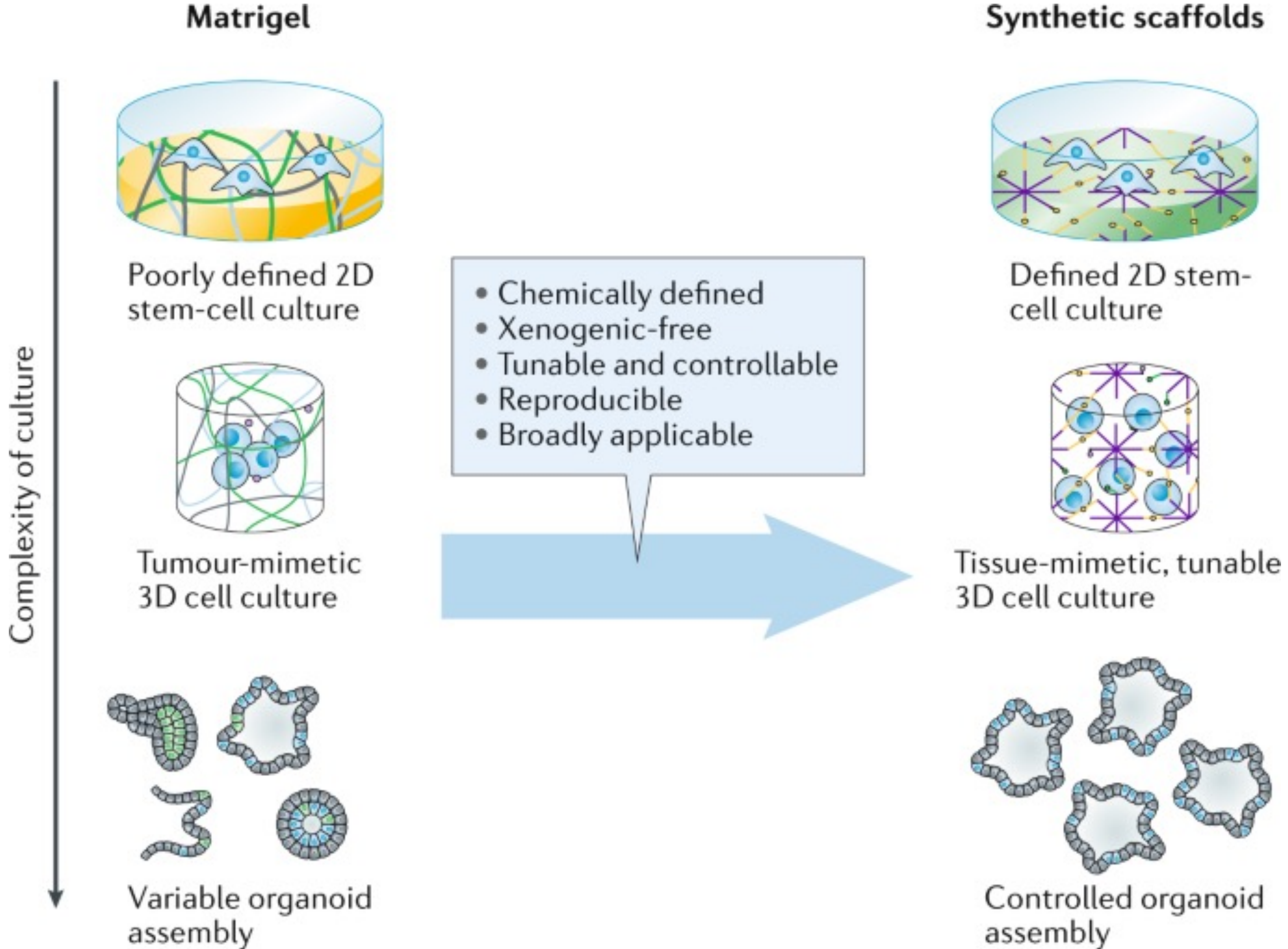
Moreover, Matrigel is **not conducive to physical or biochemical manipulation**, making it difficult to fine-tune the matrix to promote intended cell behaviours and achieve specific biological outcomes.

Composition:
laminin (~60%),
collagen IV (~30%),
entactin (~8%)
perlecan (~2–3%)

Aisenbrey, E.A., Murphy, W.L. Synthetic alternatives to Matrigel. *Nat Rev Mater* **5,** 539–551 (2020).

Aisenbrey, E.A., Murphy, W.L. Synthetic alternatives to Matrigel. *Nat Rev Mater* **5,** 539–551 (2020).

Artificial ECM advantages



Artificial Intestinal Organoid Matrix

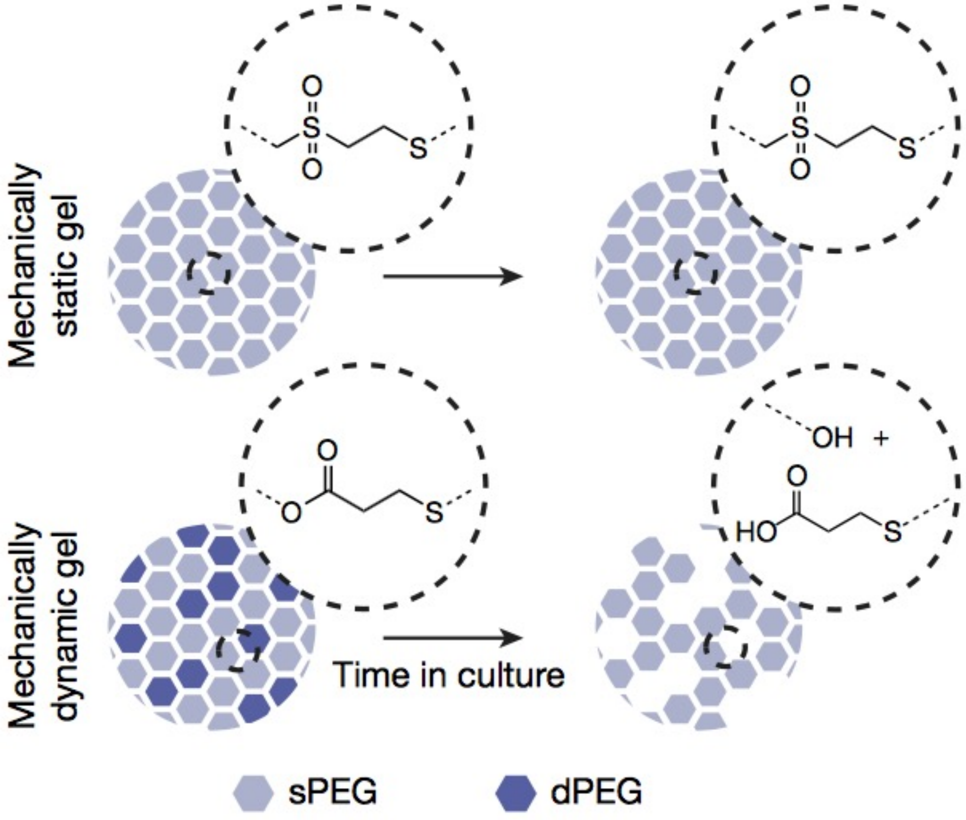
Modular synthetic hydrogel networks to define the key extracellular matrix (ECM) parameters that govern intestinal stem cell (ISC) expansion and organoid formation

Separate stages of the process **require different mechanical environments** and **ECM components**.

Fibronectin-based adhesion (RGD) was sufficient for ISC survival and proliferation.

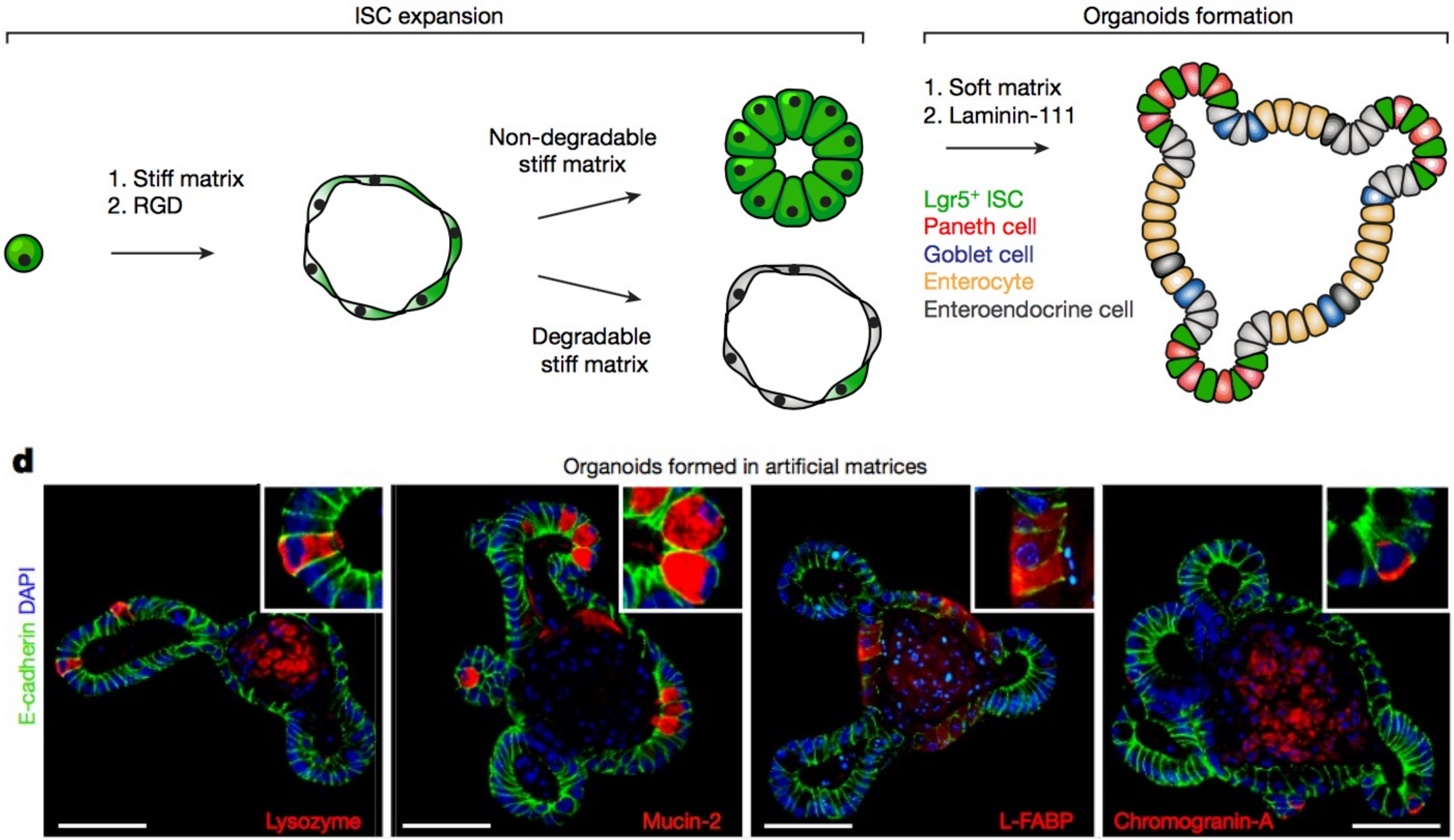
High matrix stiffness significantly enhanced ISC expansion through a yes-associated protein 1 (YAP)-dependent mechanism.

ISC differentiation and organoid formation required a **soft matrix** and **laminin-based adhesion**.



Nature 539, 560–564(2016)

Artificial Intestinal Organoid Matrix



Nature 539, 560–564(2016)

Conclusion

The ECM is a crucial part of every tissue, and is constantly in dynamic reciprocal remodeling

Main components are Laminin, Collagen, Fibronection and proteoglycans

Cells adhere to the ECM via cell surface receptors called Integrins, that can bind and sense the stiffness of the surrounding. A clustered integrin / actin / ECM complex is called a Focal Adhesion Komplex.

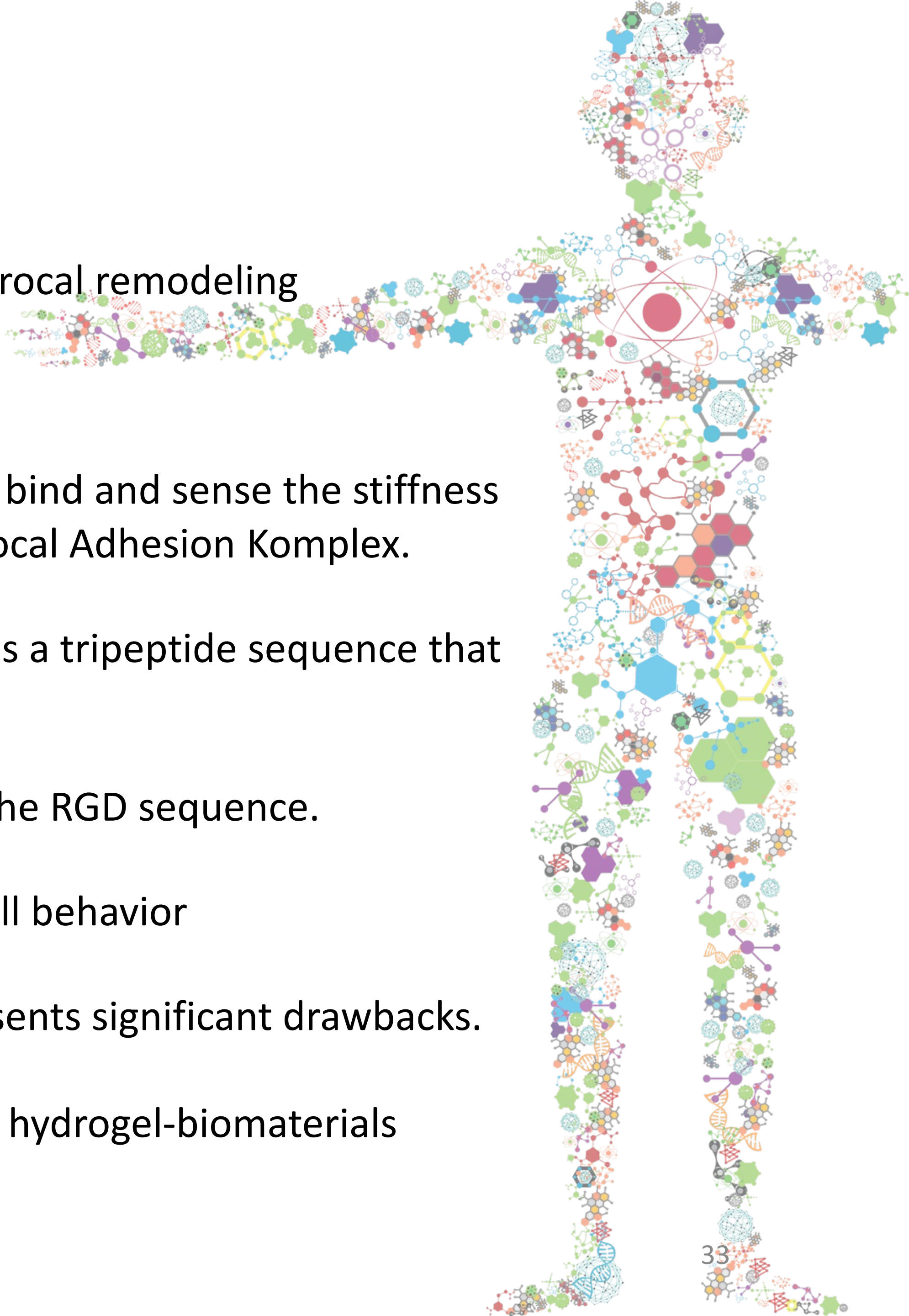
The binding site for many integrins in RGD, found on a.o. Fibronectin. This is a tripeptide sequence that is extremely suited for synthetic production.

Surfaces and scaffolds can be made cell-adhesive through the addition of the RGD sequence.

Spacing and stiffness of synthetic substrates are incredibly important on cell behavior

Matrigel is a commonly used hydrogel for cell / tissue engineering, yet presents significant drawbacks.

Exploring the best synthetic ECM is currently one of the main challenges in hydrogel-biomaterials engineering research.



Test Questions

What is the difference between the ECM and the basement membrane?

Why is matrigel not suited for in vivo therapy?

How is the ECM made?

How do cells sense the mechanical properties of their environment?

Design an artificial material where cells can grow on in a circular pattern. Motivate your choice of scaffold material, functional adhesion sites, and indicate size / dimensions.