Mechanobiology

(old name Ing Tess e bioreattori)

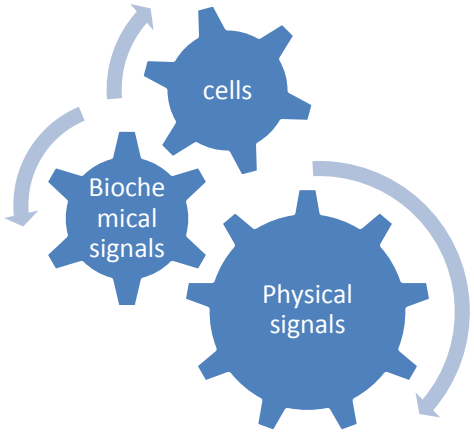
The course book: **Fondamenti di ingegneria dei tessuti per la medicina rigenerativa.** Author/sMantero S, Remuzzi A, M.T. Raimondi, Ahluwalia A ISBN Code978-88-55-3039-2 Publisher :Patron: Number of pages212

What is the course about?

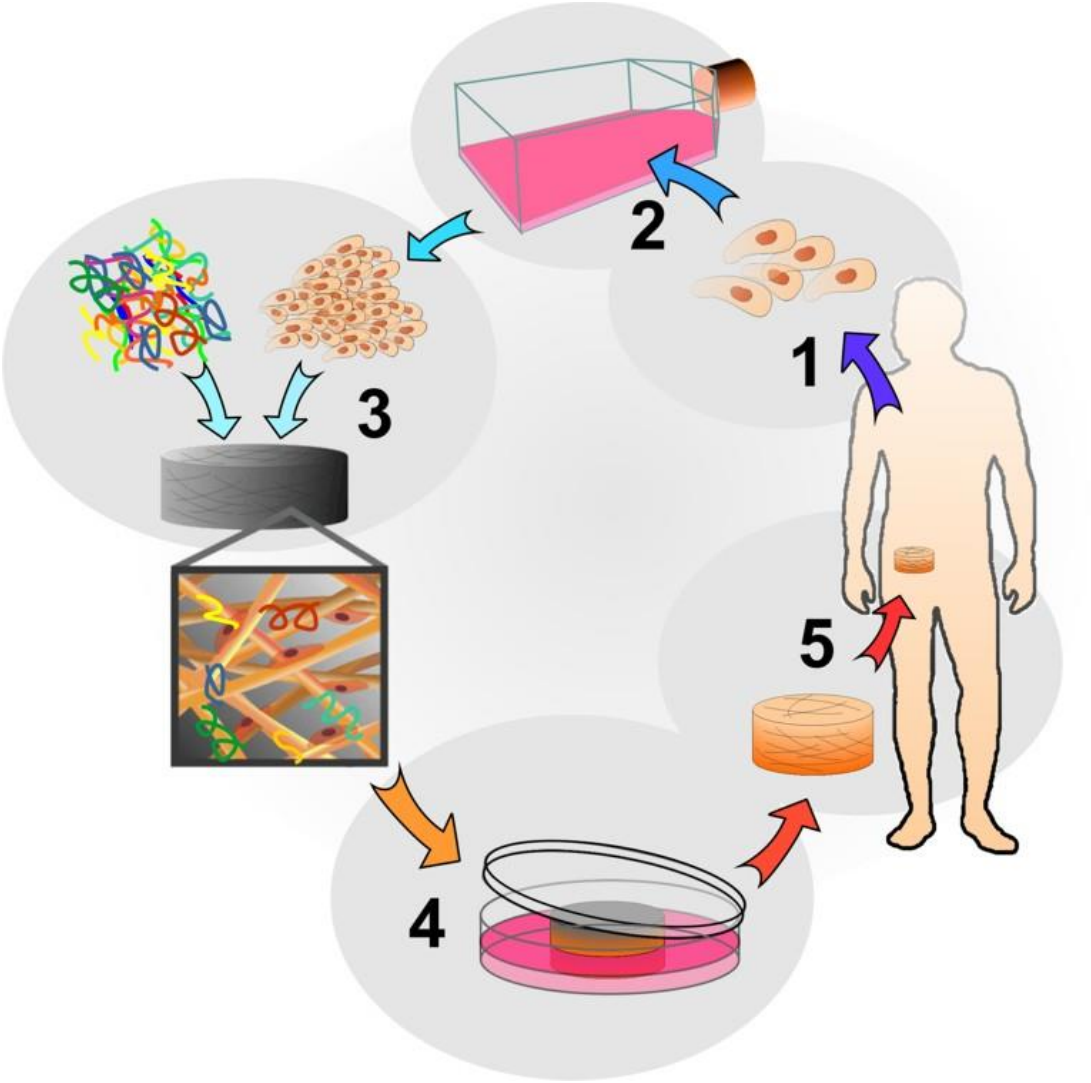
- Tissue engineering
- Regenerative Medicine
- Physiological models
- Biomimicking tissues, organs and systems

Why?

- ATMP is the bioengineering of the future.
- Biological engineering
- Design downscaled biomimetic in-vitro systems, understand how the big picture comes together.



What is Tissue engineering?



The old cells on a scaffold approach

21 century tissue engineering (regenerative medicine)

Allopathy:a system of medical practice that aims to combat disease by use of remedies (as drugs or surgery) producing effects different from or incompatible with those produced by the disease being treated

New Regenerative medicine uses ATMP (advanced therapy medicinal products)

An ATMP is a medicinal product which is either:

- a gene therapy medicinal product
- a somatic cell therapy medicinal product (allogenic, autologus, or xenogenic)
- a tissue engineered product

They all involve a degree of manipulation in-vitro

Why do we need it?

(Lack of donor organs used to be the reason)

Allopathy cannot “cure” 21° century diseases

like :

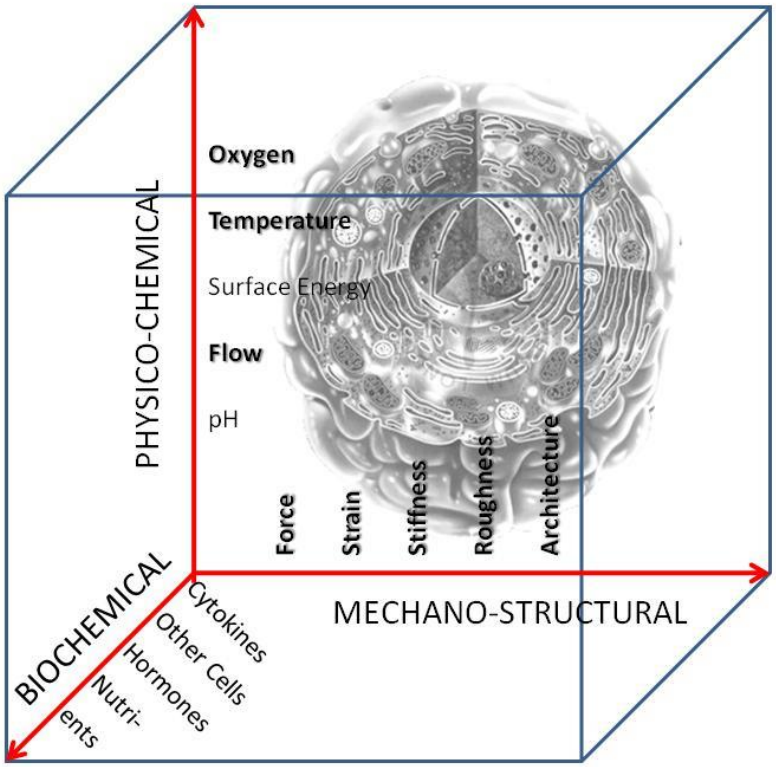
- Ageing & degeneration
- Auto immune diseases
- Cancer
- Obesity
- Or genetic disorders

(what do they have in common?, what diseases can be cured with allopathy?)

The main ingredient we manipluate in-vitro is the cell

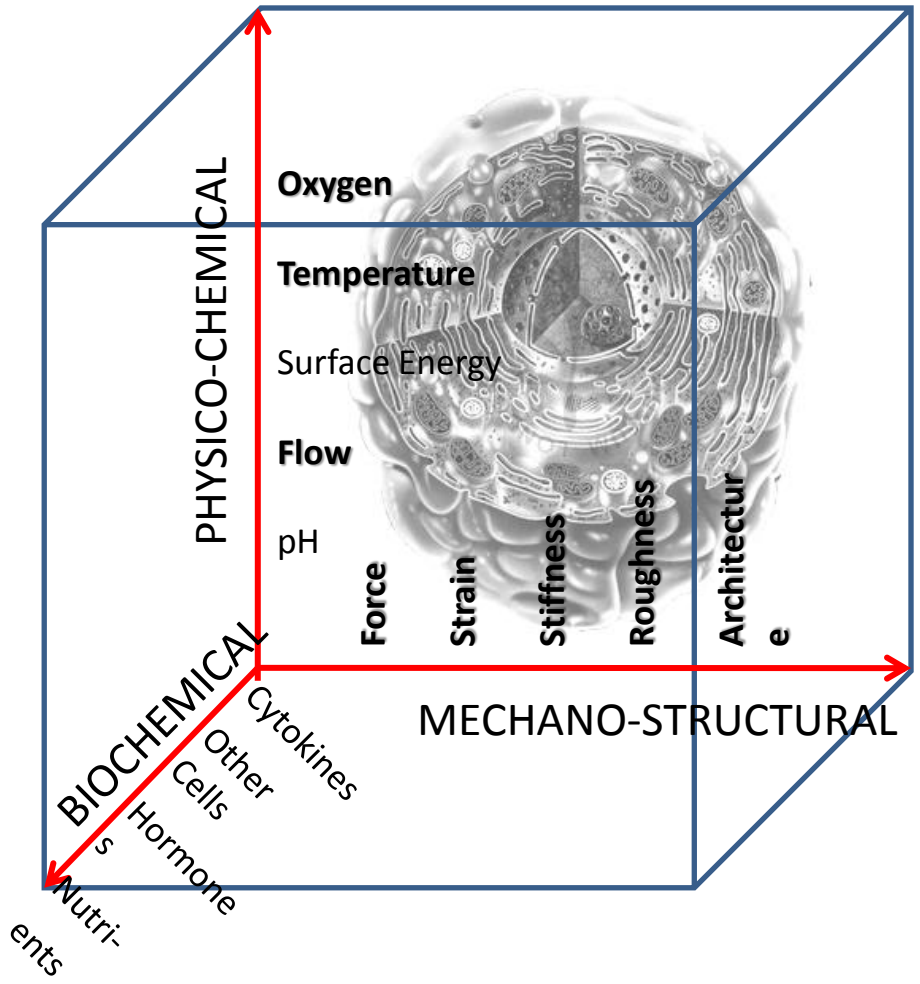
Stimuli

- Biochemical
- Physico-chemical
- Mechano-structural

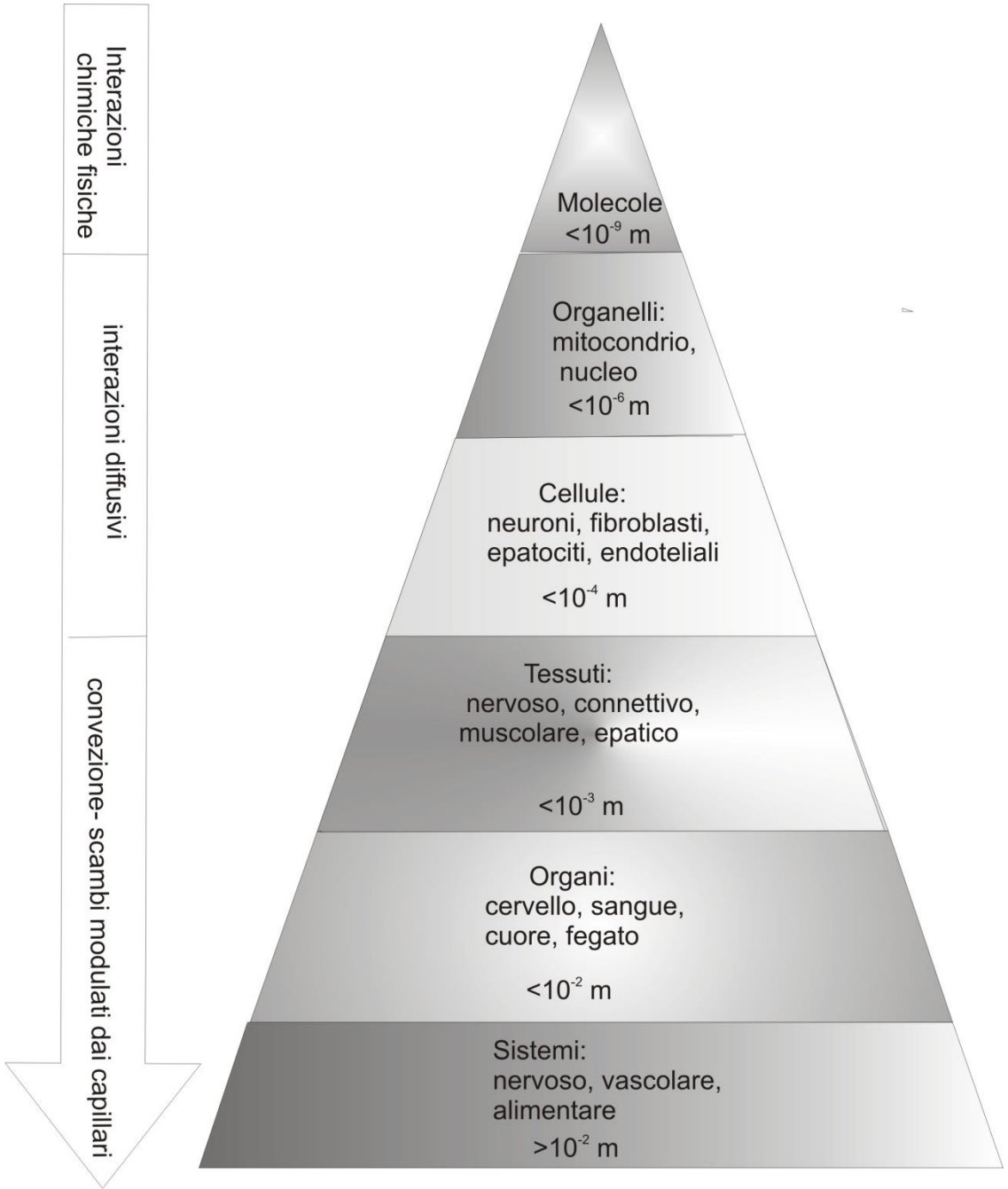


Note even time has a role- thus a *dynamic* environment, is fundamental in all biological processes.

monitoring/sensing/control is essential



Hierarchical organisation



Il corso- adesso Meccanobiologia, prima Ing Tessutale e Bioreattori

Faremmo un approccio bottom up

Sviluppo e morfogenesi : modelli Steinberg, Wolpert

Controllo biochimico, adesione e forza di adesione

Crescita e differenziazione.(cellule staminali, iPSC)

Recettori e communicazione: binding e secrezione

Controllo geometrico e tensegrity

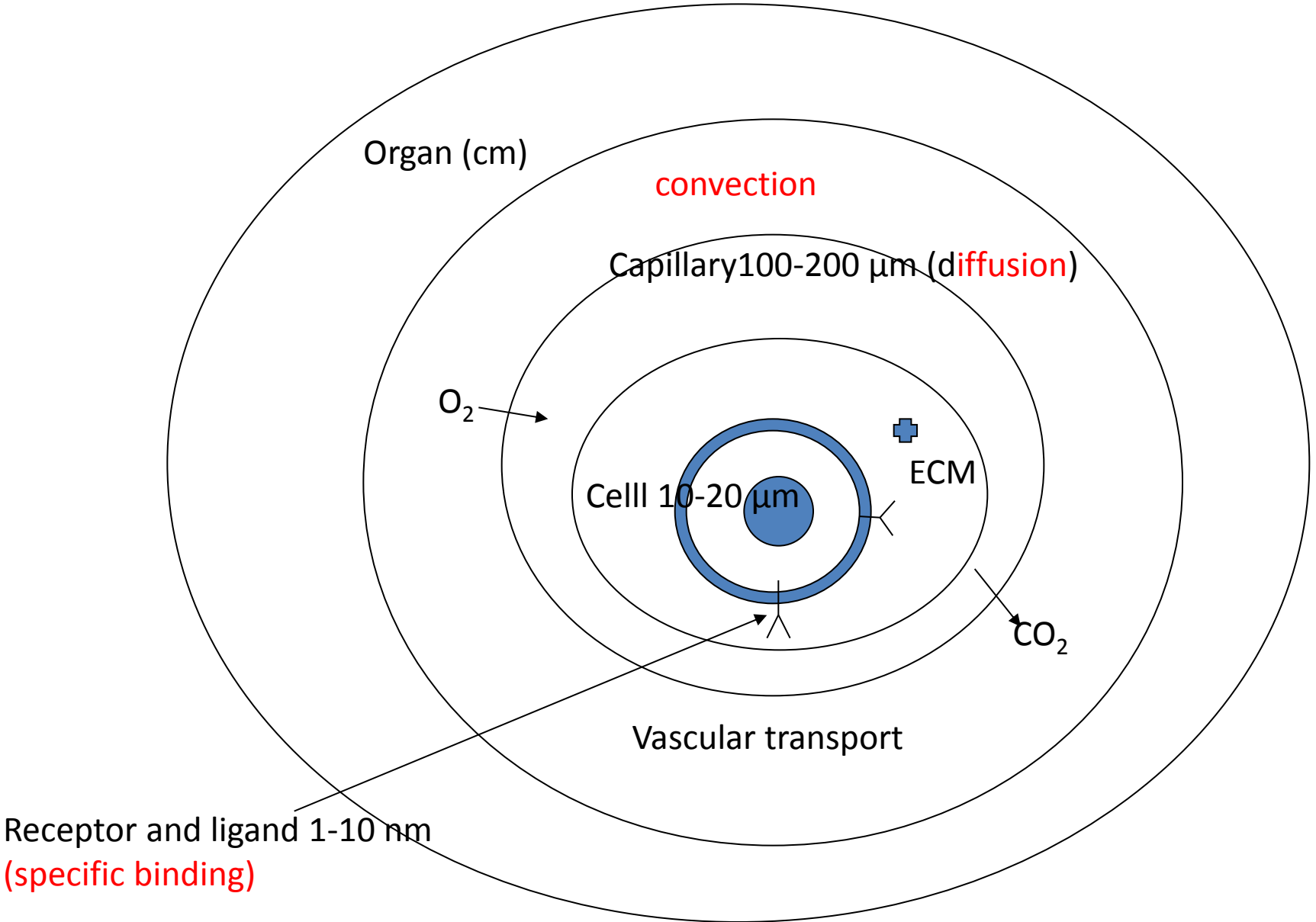
Progettazione usando allometria e apporto nutrienti

I biomateriali e gli scaffold

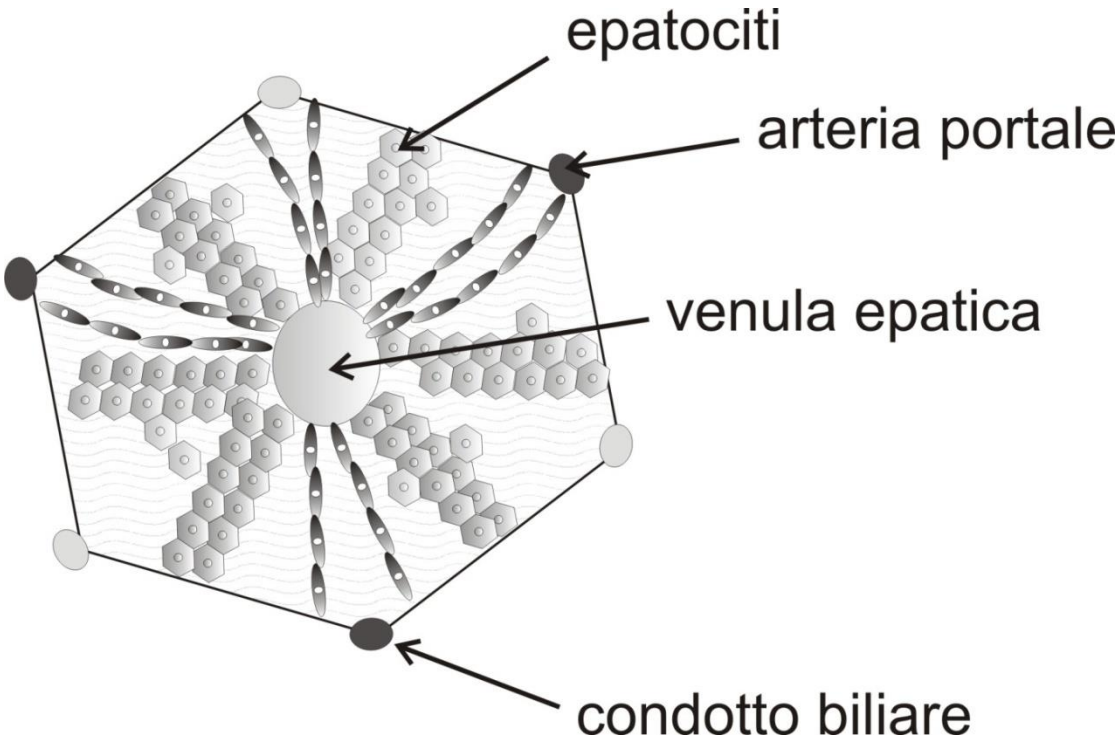
Alcuni approci, pancreas, fegato, pelle ecc

Bioreactors and environmental control

Characteristic distance 100-200 µm

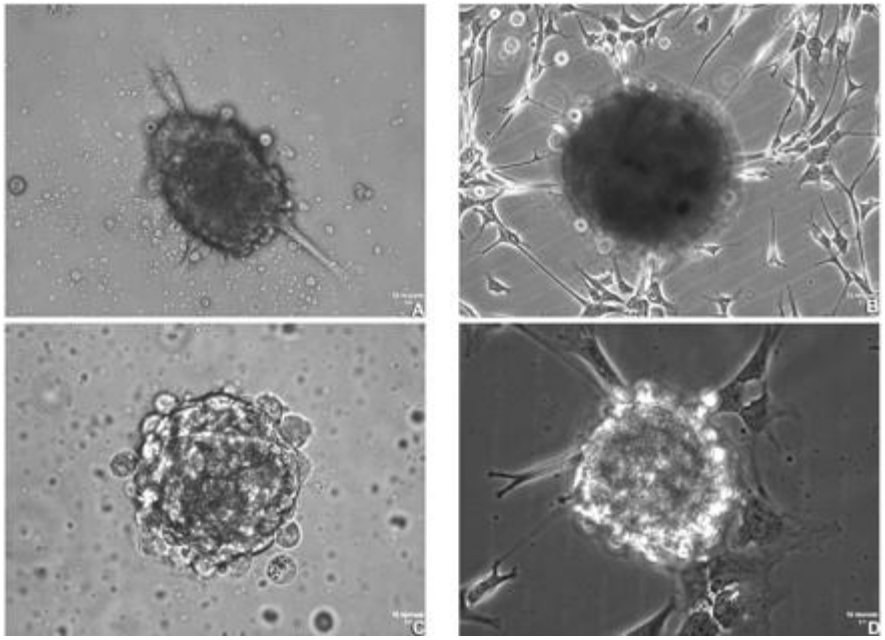


Functional unit: collection of functional (parenchymal) and support (stromal or non-parenchymal) cells which do not require a capillary network. Is equivalent to a cube of 100 micron sides. In vitro these units are usually referred to as ORGANOIDS



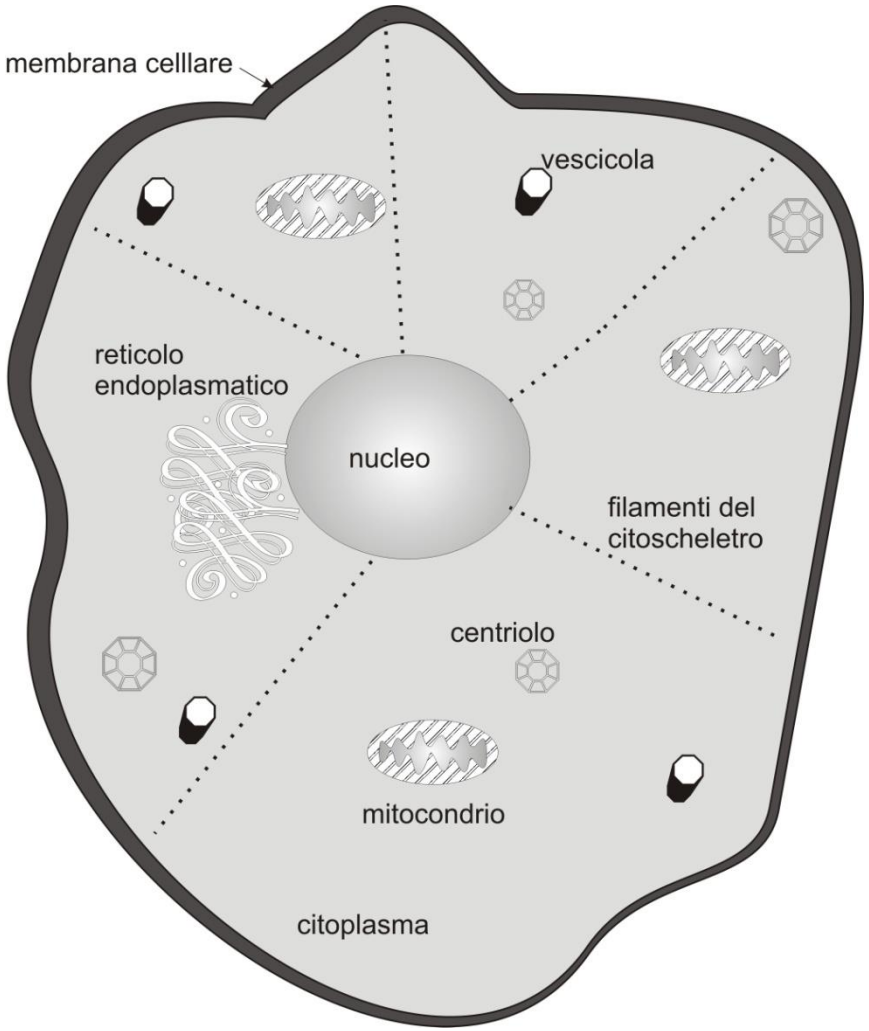
Functional unit

- Each organ is a network of the parallel functional units, composed of groups of functional cells or parenchymal supported by stromal cells, each unit has dimensions of a few hundreds of microns, and responds with characteristic times in the order of minutes. The micro-functional domains are repeated both in morphology and function.



Cardiospheres are a good example

La cellula



Dovete sapere le funzioni dei componenti citoplasmici

Quali sono i processi cellulari fondamentali?

Divsione
Moto
Differenzazione .....

Morte
Adesione

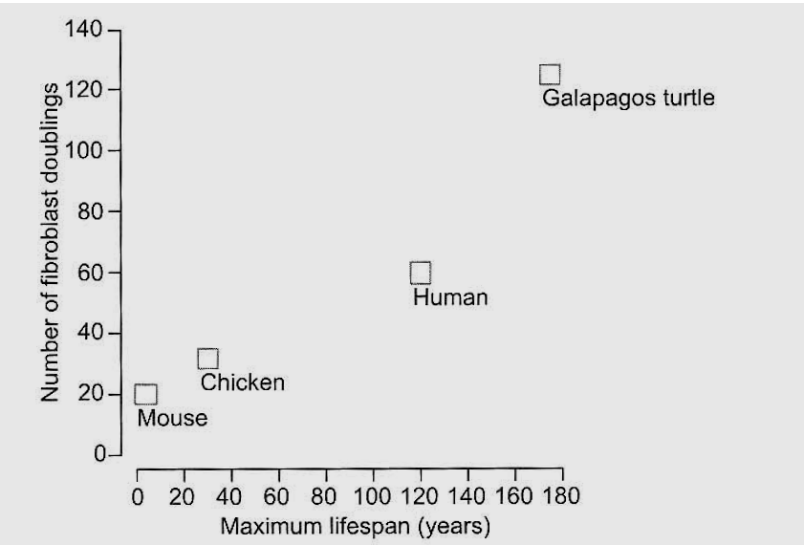
Quali invece sono specifici a cellule specifiche?
Fenotipo
Genotipo
Epigenotipo

Le funzioni cellulari sono diverse da cellula a cellula e da tessuto a tessuto, e definiscono il **fentotipo** cellulare. Però alcuni processi sono comuni a tutte le cellule. I processi cellulari più noti sono:

- Proliferazione o crescita
- Migrazione
- Differenzazione
- Morte (apoptosi, necrosi)
- Metabolismo, respirazione
- Adesione
- Espressione proteica

Define: phenotype, genotype, epigenotype

Cell growth: Hayflick limit and population doublings



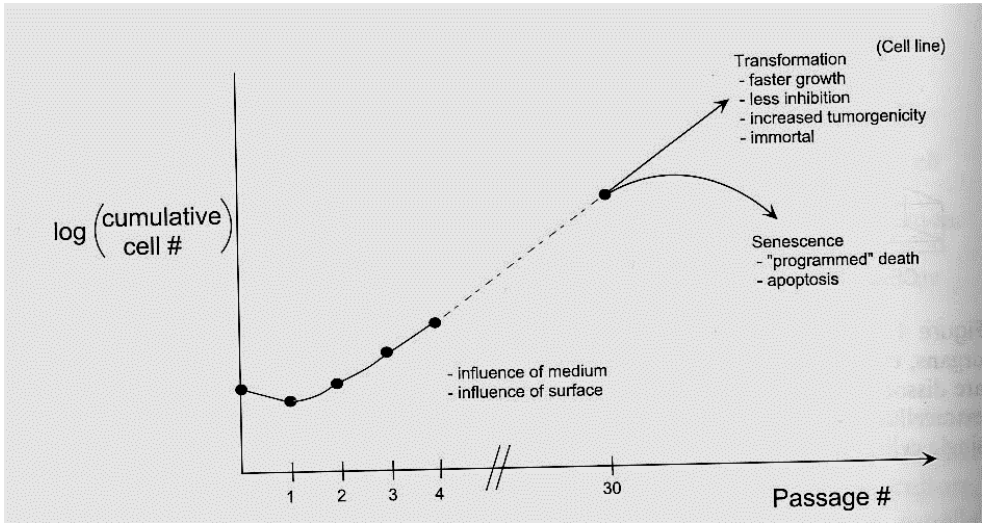
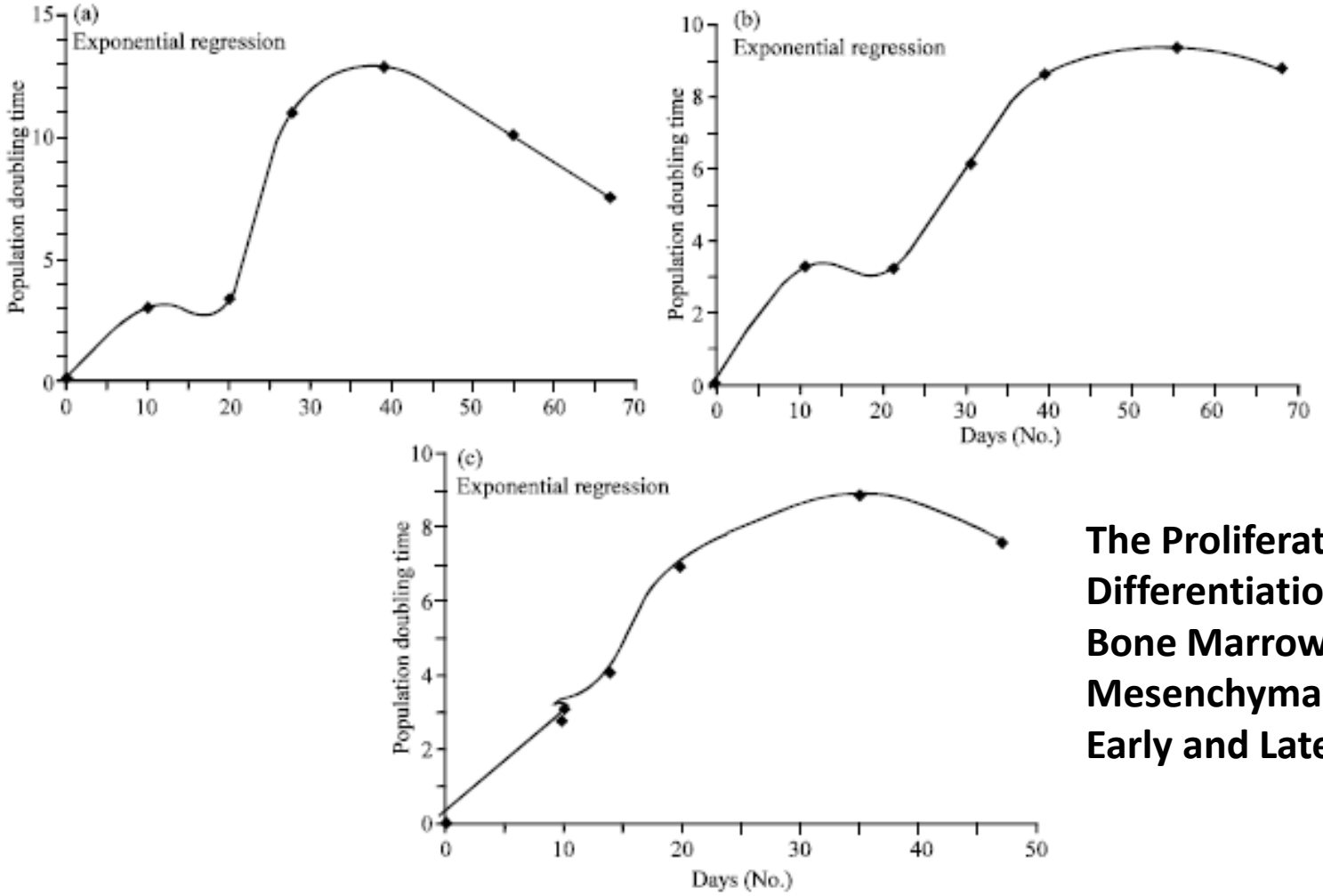


Fig. 1(a-c):*In vitro* population doubling time (PDT) of human bone marrow derived MSCs cultures in three sets. (a) Set 1 (b) Set 2 and (c) Set 3



The Proliferation and Differentiation Capacity of Bone Marrow Derived- Human Mesenchymal Stem Cells in Early and Late Doubling

Rate of cell proliferation is proportional to cell number

$$\frac{dN}{dt} \propto N$$

$$\frac{dN}{N} = kdt$$

$$N = N_o e^{kt}$$

$$2N = N_o e^{kt_d}$$

$$t_d = \frac{k}{\ln 2}$$

N= cell population

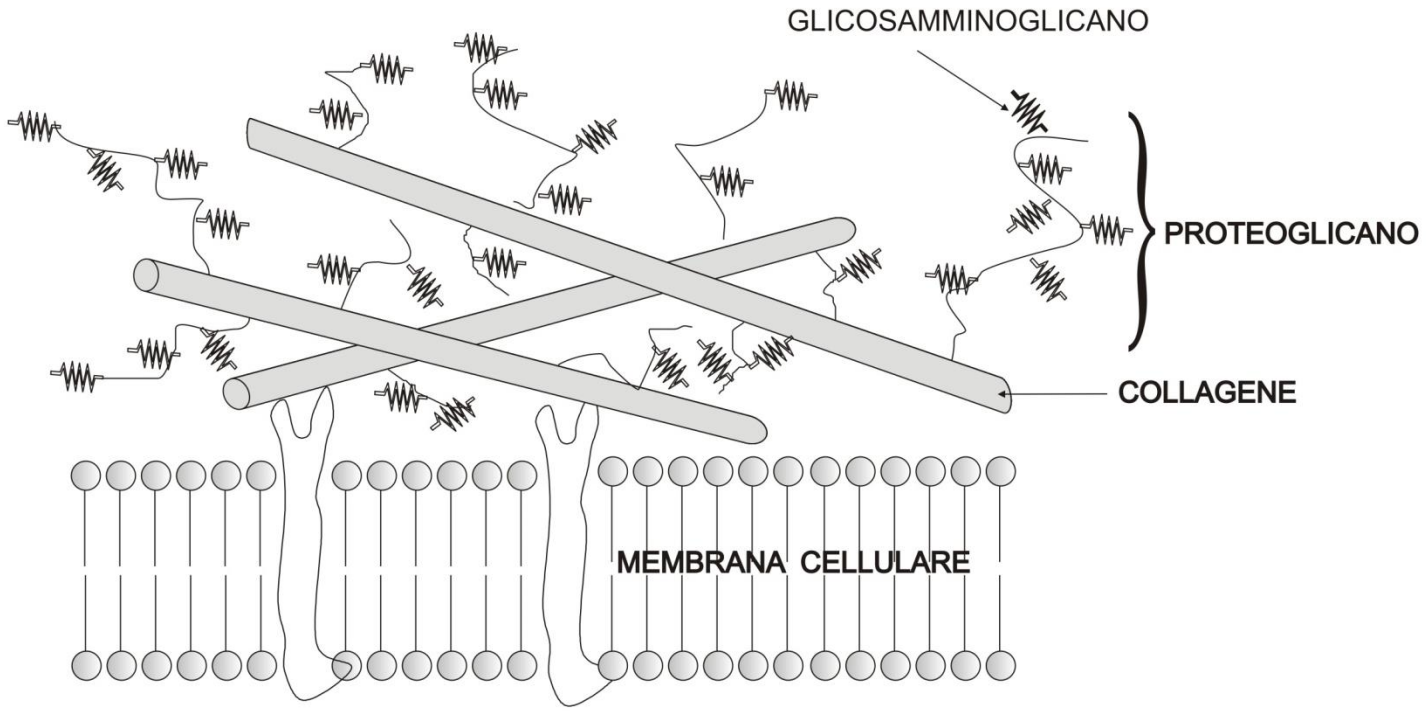
N_o= initial population @t=0

t_d =population doubling time

La Matrice Extra Cellulare

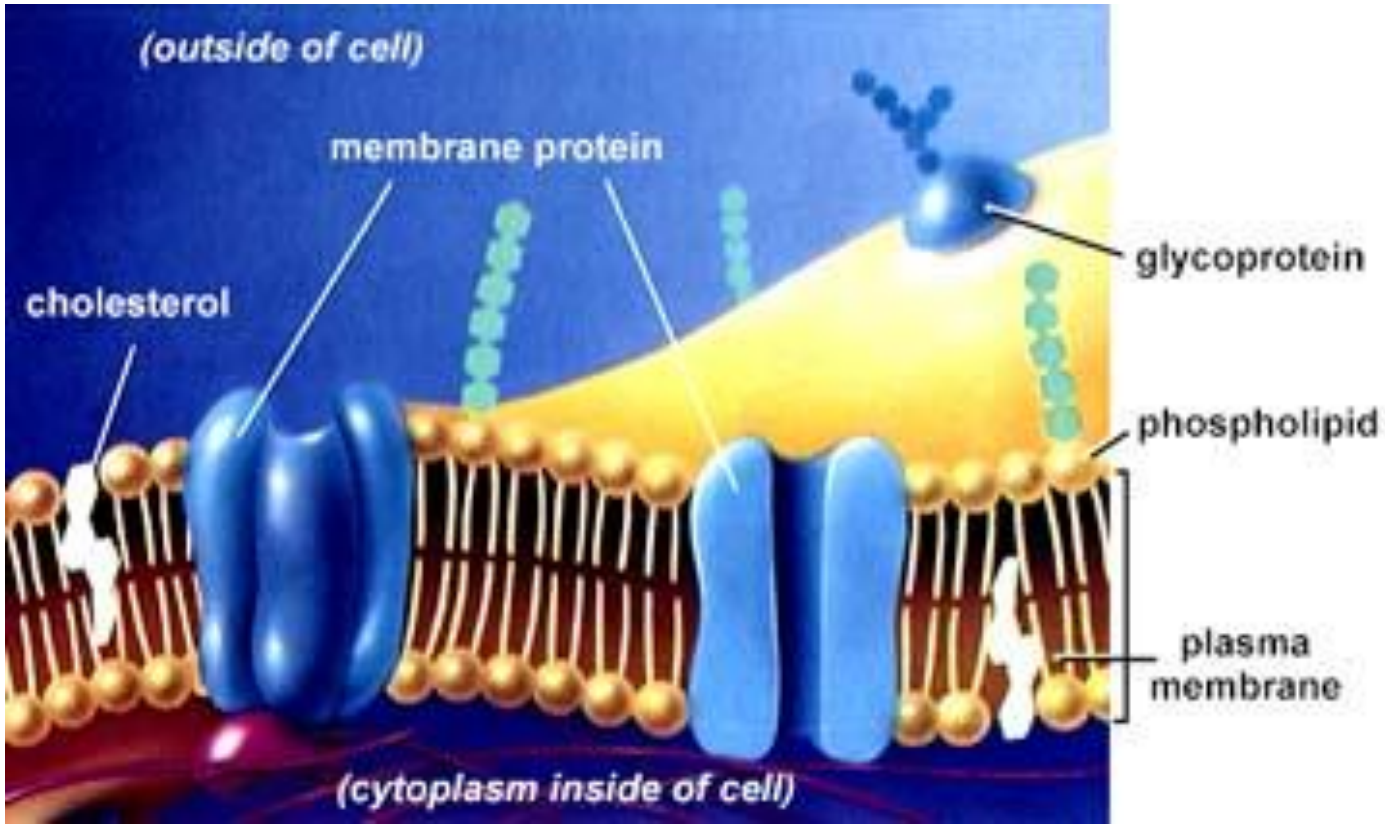
(vedere anche la roba di biomeccanica sul ECM)

Matrice Extra Cellulare	
Componente	Funzione
Acqua	E' il mezzo di trasporto, ed è la componente più importante degli organismi viventi. Rende inoltre incomprimibile L'ECM,.
Sali Minerali	Mantengono un sistema tamponato
Elastina	Proteina strutturale
Fibronettina, laminina ..	Proteine adesive specializzate, spesso glicosilate
Glicosamminoglicani	Disaccaridi (ad esempio:acido ialuronico, eparina, eparan solfato) che formano un complesso con le proteine per formare i proteoglicani
Proteoglicani	Complessi zuccheri-proteine che formano un reticolo macromolecolare o gel idratato, figura 4
Collagene	Proteina strutturale e ligando adesivo

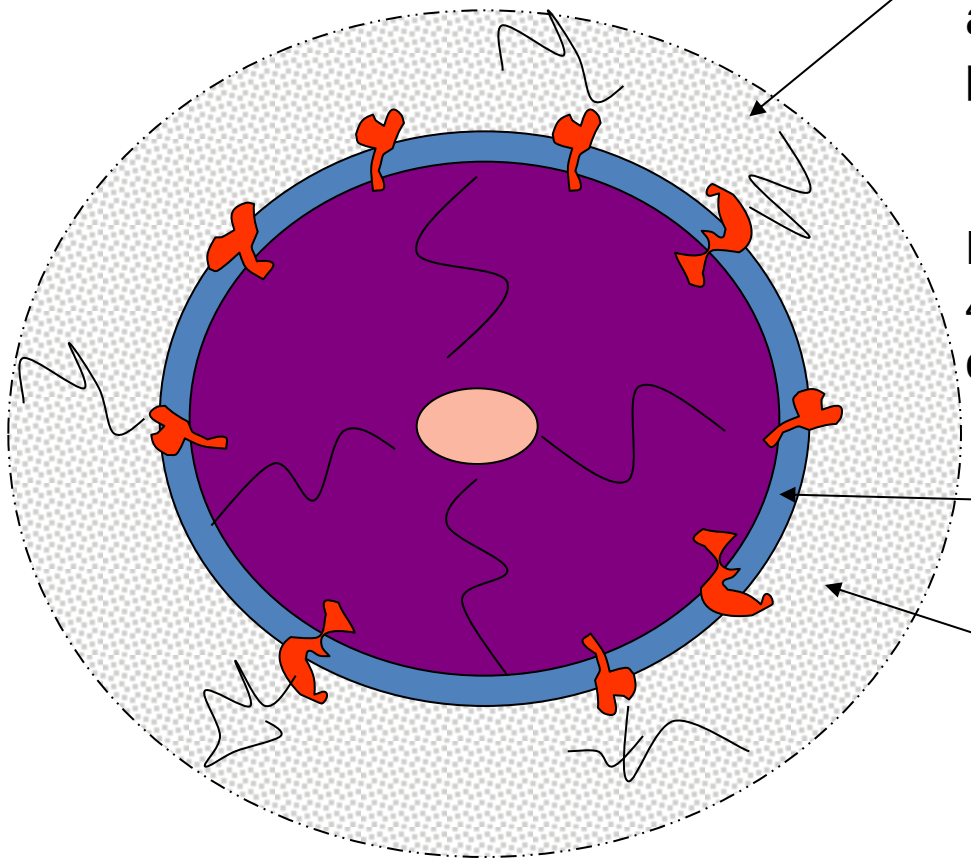

GLICOSAMMINOGLICANO
PROTEOGLICANO
COLLAGENE
MEMBRANA CELLULARE

LIGAND BINDING/RECEPTORS

Libro di Lauffenburger e Linderman


(outside of cell)
membrane protein
glycoprotein
cholesterol
phospholipid
plasma membrane
(cytoplasm inside of cell)

Binding



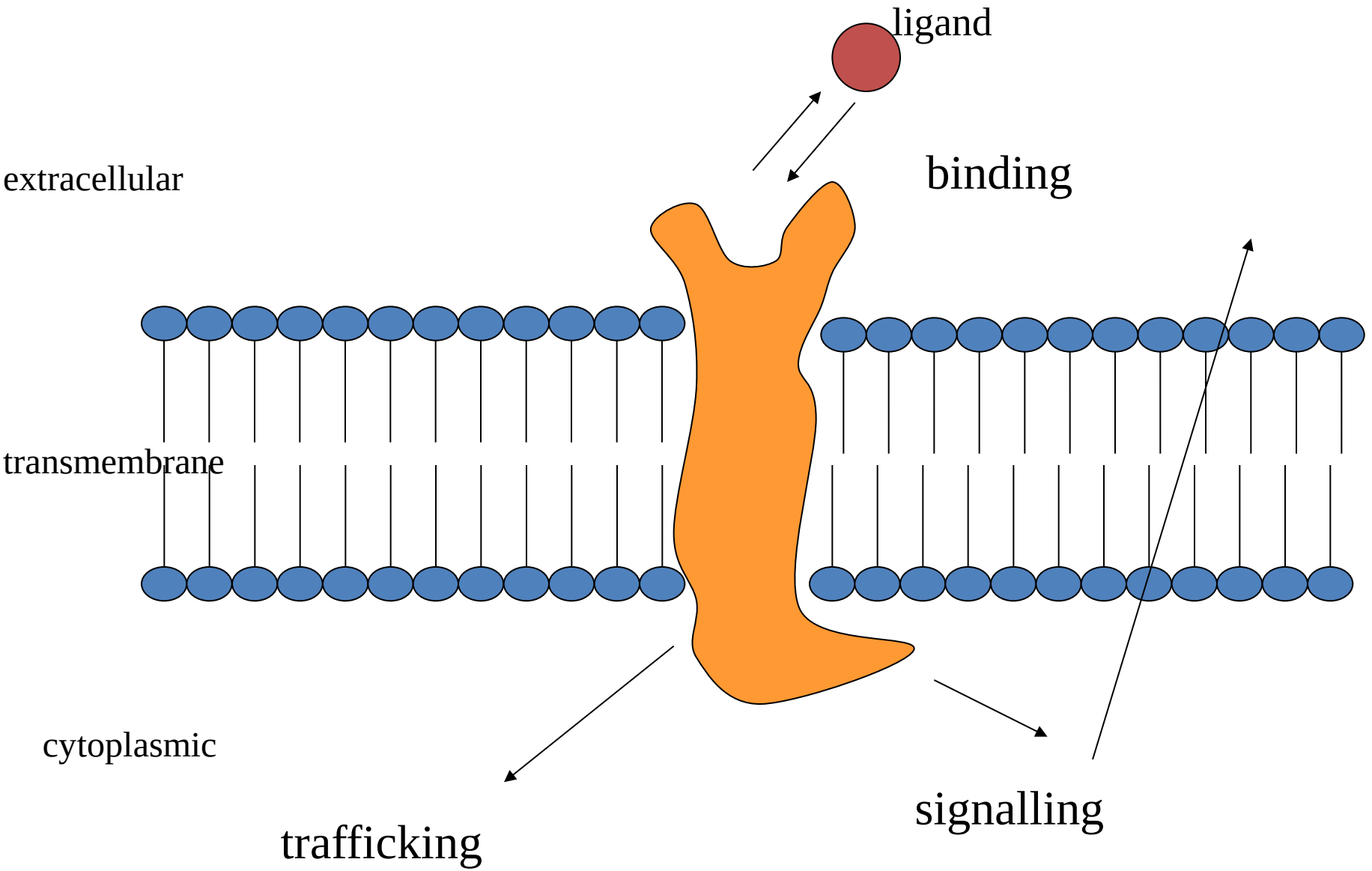
Glycocalyx: carbohydrates adsorbed on transmembrane proteins. It is negative, why?

Membrane is 40% protein, 45% lipid and 5% carbohydrate

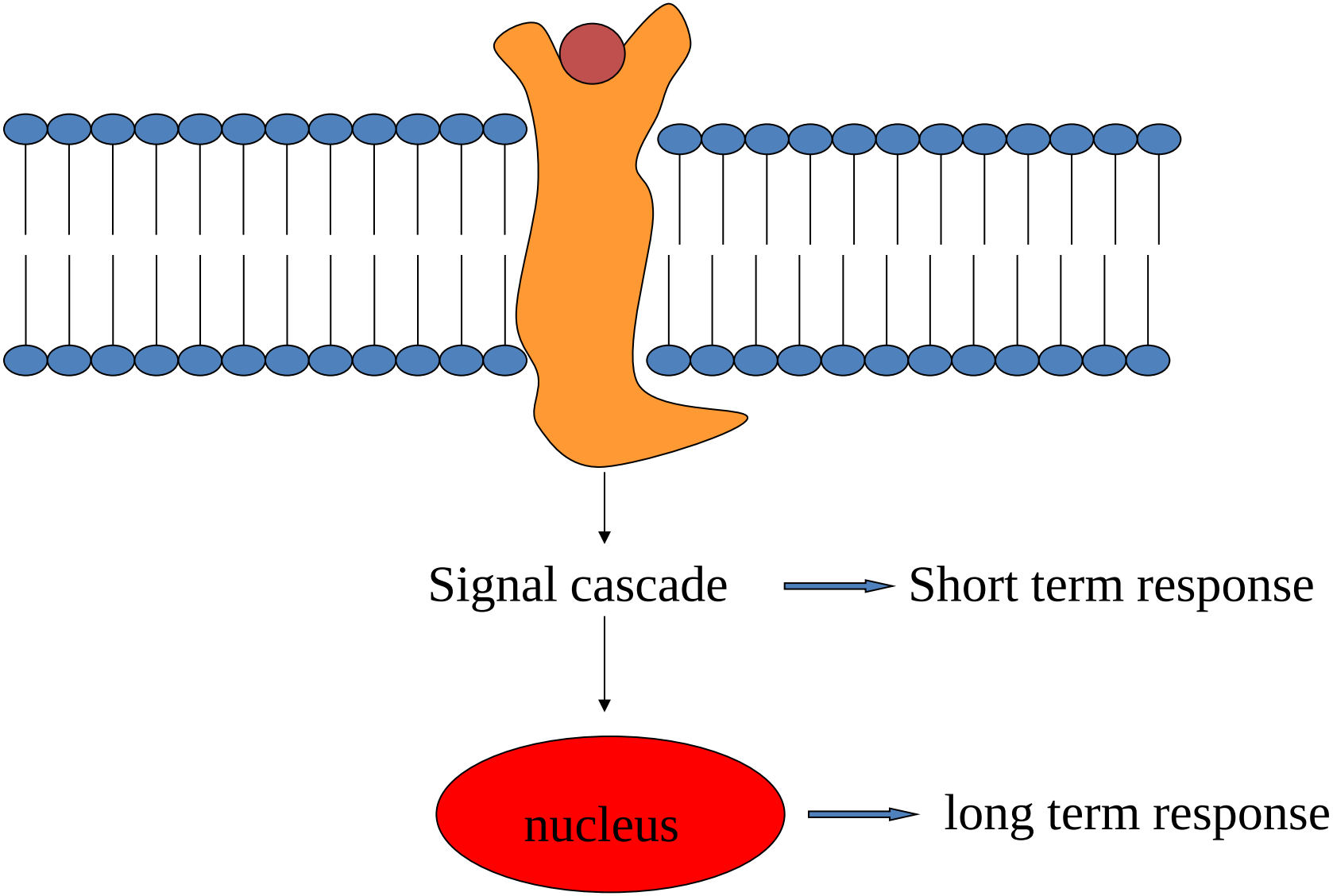
40 A

100-200 A

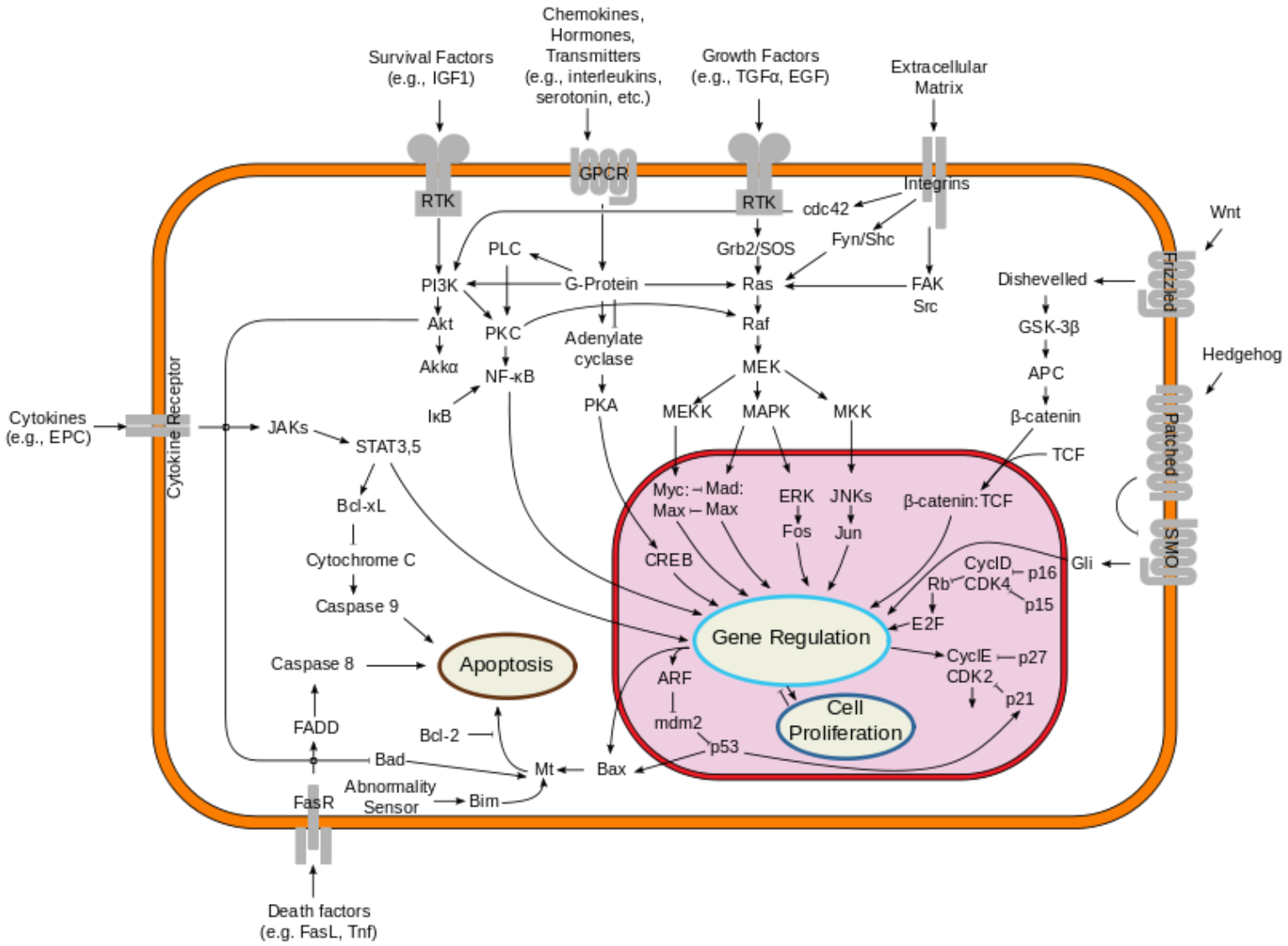
Eukaryotc Cell responses are regulated and controlled by receptor interaction with the environment. So parameters such as growth, death, differentiation, are studied by analysing receptor-ligand binding and the associated trafficking and signalling events.



INSIDE OUT- OUTSIDE IN


Signal cascade
Short term response
nucleus
long term response

An example of signal transduction pathways



Signal transduction occurs when an extracellular signaling[1] molecule activates a specific receptor located on the cell surface or inside the cell. In turn, this receptor triggers a biochemical chain of events inside the cell, creating a response.[2] Depending on the cell, the response alters the cell's metabolism, shape, gene expression, or ability to divide.[3] The signal can be amplified at any step. Thus, one signaling molecule can cause many responses.[4]

Receptors: Cell surface receptors (CSR). They interact with the extra cellular environment giving rise to four types of signals:

- Nerve transmission
- Hormone release
- Muscle contraction
- Growth stimulation

There are four types of messenger molecules.

- steroids
- small organic or inorganic molecules
- peptides
- Proteins

The messengers may be

- Endocrine: usually hormones
- autocrine
- paracrine : usually cytokines
- juxtacrine

There are 4 classes of ligand bound receptor signal transduction models

- ion channel receptor (fast ms, low affinity)
- G protein linked receptor (second messenger involved)(medium, mins, med affinity) (GPCR)
- Receptors which are also enzymes (slow, high affinity)
- Tyrosine kinase linked receptors (enzyme which adds a phosphate group to proteins at tyrosine residues...ie phosphorylation)

A variety of messengers can bind to various tissues.

Various cellular responses may occur, depending on the tissue.

Either positive or negative responses may occur, even in the same tissue, depending on the type of receptor.

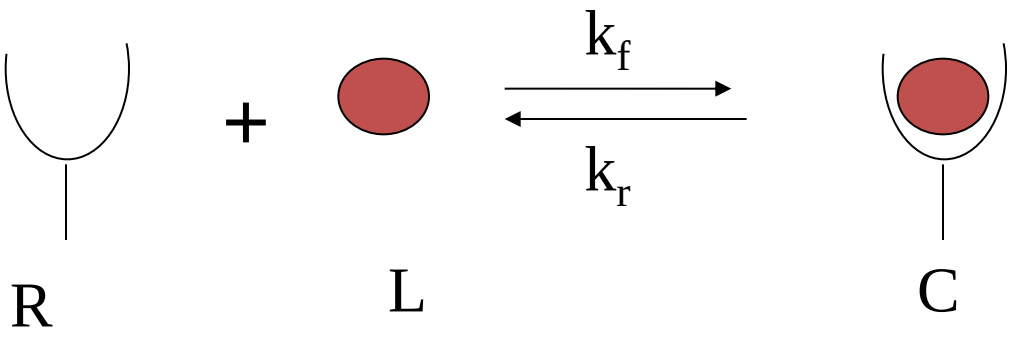
The response of a cell to a messenger depends on the number of receptors occupied.

A typical cell may have about 1000-3000 receptors.

Only a small fraction (10%) of the receptors need to be occupied to get a large (50%) response.

Receptors may have a dissociation constant of about 10^{-11}; this is the concentration of messenger at which they are 50% saturated. Thus very low concentrations of messengers may give a large response.

Receptor	Ligand	Cell	R_T(#/cell)	Kf (M^{-1} min^{-1})	Kr (min^{-1})	Kd (M)	$T_{95\%}$ (min)
Fc	Fab	macrophage	7.1e5	3e6	0.023	7.7e-10	650
EGF	EGF	Rat lung	2.5e4	1.8e8	0.12	6.7e-10	12.5
Fibronectin	Fibronectin	fibroblasts	5e5	7e5	0.6	8.6e-7	2.5
Transferrin	Transferrin	hepatocytes	5e4	3e6	0.1	3.3e-8	15



We consider a model of receptor-ligand binding in which binding is monovalent and interfering effects are absent. kf and kr are the kinetic association and dissociation constants.

R=number of receptors per cell

C=number of complexes per cell

L=conc of ligand in the ECM (moles/liter)

$kr=t^{-1}$

$kf=M^{-1}t^{-1}$

N=number of cells per unit volume

ok