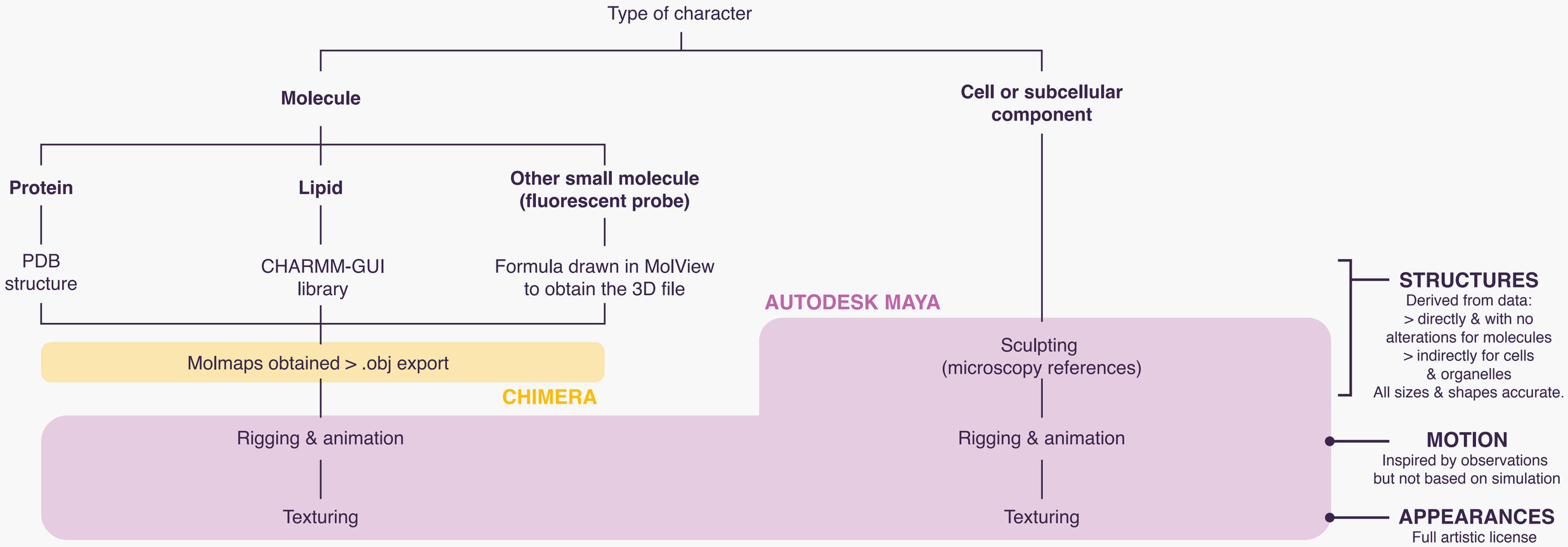
Behind the scenes: Principles & workflows

(see next pages for more details)



- The cell landscape is purely fictionnal and undefined.
- Molecular crowding is depicted but the exact composition of the cytosol & extracellular environment haven't been taken into account.
- All molecular movements have been slowed down for clarity.

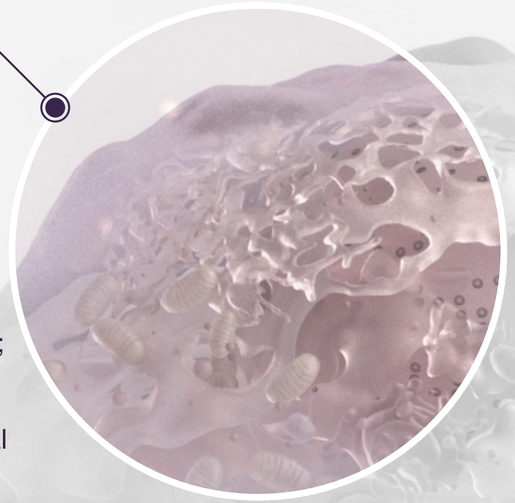
The cell & its environment

The environment

> Purely fictionnal & indefinite.

The cell (human fibroblast)

A fibroblast is a type of cell that produces the structural framework for our tissues to stay cohesive. It is about 15-20μm and can for example be found in the dermis, below the skin.

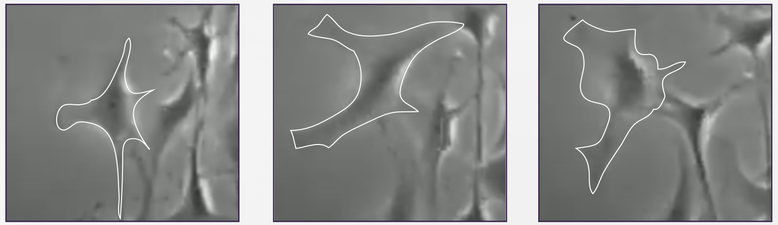


> The **structures** (shapes, sizes...) were sculpted based on electron microscopy pictures; in theory, it is possible to use softwares to directly reconstruct the model from the dataset, but it was not the case here *(see the box for more details about the acquisition of the images)*.

Some numbers: one sheet of endoplasmic reticulum is 40-50nm thick; the nucleus is on average 5-10μm in diameter and has around 2000 pores; a fibroblast typically has a couple hundreds mitochondria, but cells that need a lot of energy (such as muscle cells) can have several thousands!

> The **appearances** (textures, colors...) fall fully under artistic license.

> The **movements** of the cell are based on observations by light microscopy *(see representative screenshots below; the outline of one given cell is highlighted & followed in time)*.

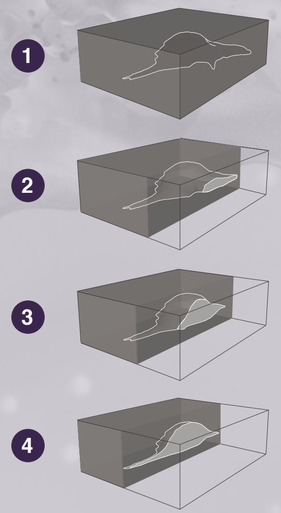


Time

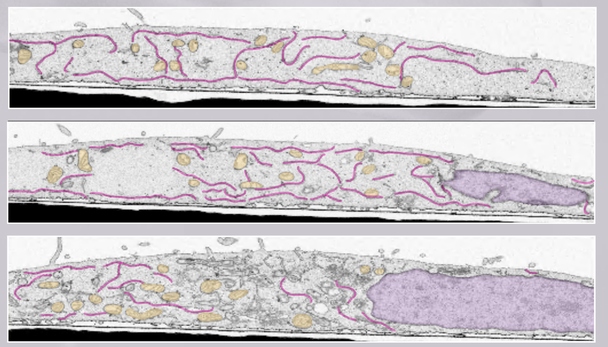
Watch the full microscopy movie:
https://www.youtube.com/watch?v=609SS3NM0nI

Getting 3D data by Electron Microscopy

The pictures used for reference were obtained thanks to a new technique called «Focused Ion Beam milling combined with Scanning Electron Microscopy», by Dr. Vincent Mercier (University of Geneva).



1: The specimen to image is included into a block of resin.
2: The ion beam removes a thin slice of the block, and the newly-exposed face is imaged.
3-4: The process is repeated, so that the series of images can be used to reconstruct a 3D volume.



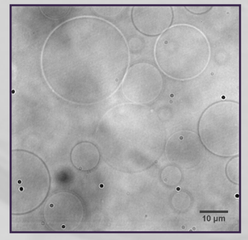
▲ Representative images from the reference stack. (The cell has been 'cut' on the right & left sides; Purple: nucleus; Orange: mitochondria; Pink: endoplasmic reticulum)

The artificial membranes

Giant Unilamellar Vesicle (GUV)

> Giant Unilamellar Vesicles (GUVs) are liquid-filled spherical membranes often used as a basic model for the study of biological membranes.

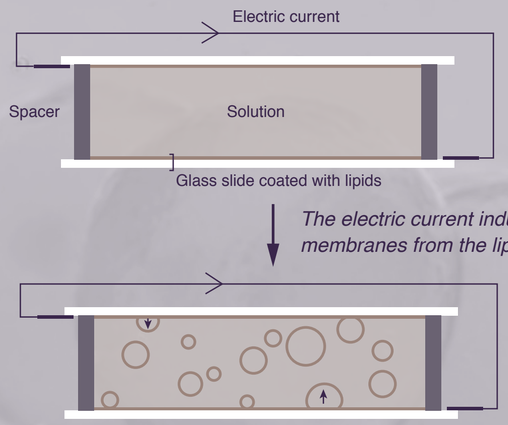
> Their structure (shape & size) is depicted here accurately, but their appearance (texture & color) falls fully under artistic license.



Representative picture of GUVs observed under a light microscope.

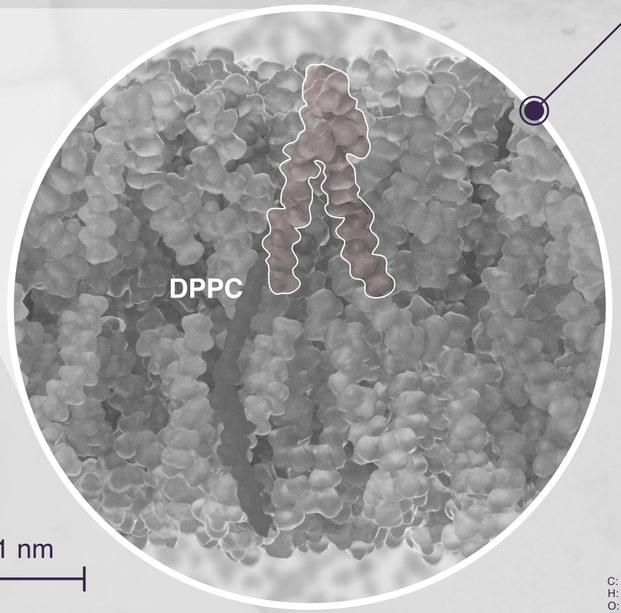
Kriegsmann & al, *ChemBioChem* (2009)

How are GUVs formed?



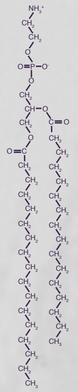
The electric current induces the formation of spherical membranes from the lipids put on the slides.

x 1000



GUV membrane

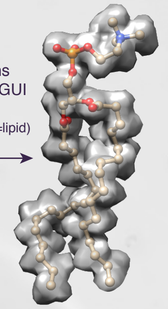
> The composition of the GUV membrane is decided by the scientists.
In this case, it was made from only one kind of lipid, called « dipalmitoylphosphatidylcholine» (DPPC).



DPPC chemical formula

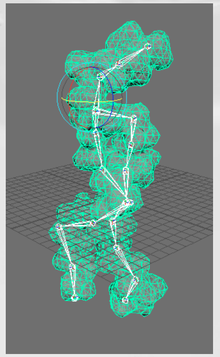
C: carbon atom
H: hydrogen atom
O: oxygen atom
N: azote atom
P: phosphate atom

Several structure conformations downloaded from the CHARMM-GUI lipid library (www.charmm-gui.org/?doc=archive&lib=lipid)



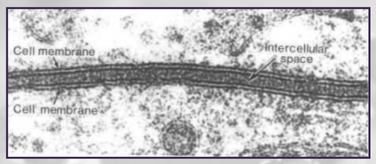
Representative conformation of DPPC 3D molecular structure visualized in Chimera (molecular visualization software)

> The surface was imported, rigged & animated in Autodesk Maya. The molecular movements are not based on specific simulation data.

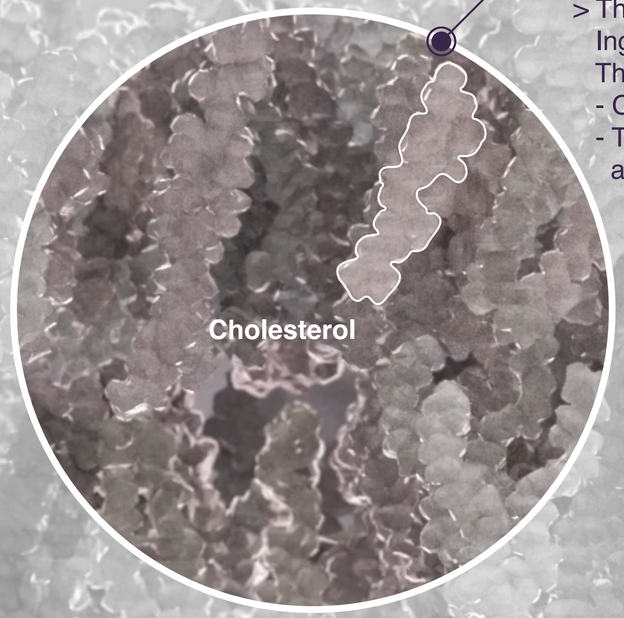


Rigged DPPC as seen in the Autodesk Maya interface
The textures & colors used for rendering have no physical reality.

The plasma membrane



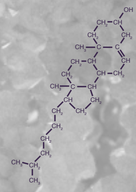
Electron Microscopy image of the plasma membrane.
Bloom & Fawcett, *A Textbook of Histology*, Chapman and Hall, N.Y., 12th ed (1994)



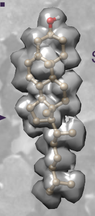
Lipids

> The plasma membrane of a human cell contains between 50 and 100 different kinds of lipids, which also varies depending on the cell type.
> The composition depicted here is based on data from the following paper: Ingolfsson & al, *J. Am. Chem. Soc.* (2014)
The data have been slightly simplified:
- Only lipids making up for more than 1% of total lipids are showed.
- The asymmetry between inner and outer leaflets composition has been accounted for, but not the lateral heterogeneity.

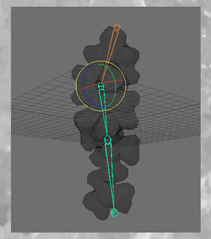
WORKFLOW EXAMPLE:



Structure conformations downloaded from the CHARMM-GUI lipid library
(www.charmm-gui.org/?doc=archive&lib=lipid)



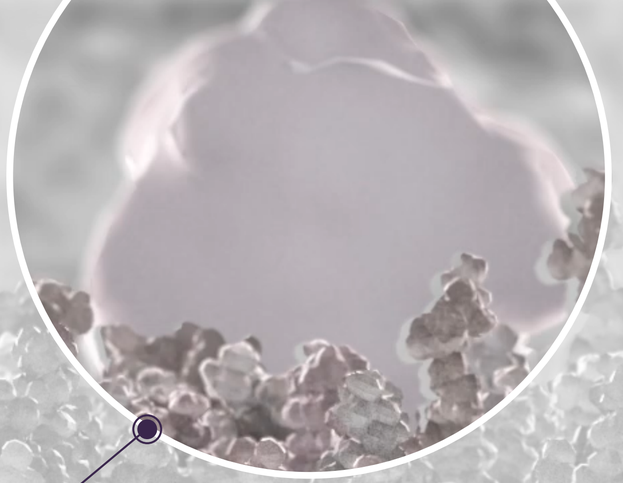
Surface were imported, rigged & animated in Autodesk Maya.



Cholesterol chemical formula
C: carbon atom O: oxygen atom
H: hydrogen atom

Representative conformation of cholesterol 3D molecular structure visualized in Chimera

Rigged Cholesterol as seen in the Maya interface
The textures & colors used for rendering have no physical reality.



Membrane proteins

> The membrane actually contains 50% of proteins in weight. Here is featured a protein called DDR2, which binds to the collagen filaments on the extra--cellular side of the membrane.
> This **structure** comes from Protein Data Bank reference 2WUH, obtained by X-ray cristallography.

> **STRUCTURES** (shapes, sizes): all used without alteration from experimental data; surfaces were obtained in Chimera (molecular visualization software), before being imported and animated in Maya.
> **MOTION & CROWDING:** Molecules are constantly moving, but the random motion showed in this animation is not supported by specific simulation data. The compostion of the intra- & extra-cellular environment are also not accurate; random molecules are depicted to tshow crowding.
> **APPEARANCES** (textures, colors): full artistic license

Representative cryo-EM image of an actin filament.

Murakami & al, *Cell* (2010)

Actin cytoskeleton & cytoplasm

> The actin cytoskeleton is a dynamic network of protein filaments (made of actin & other associated proteins). It is connected to the plasma membrane & gives the cell its shape & resistance, like a skeleton.
> This **structure** comes from Protein Data Bank reference 3G37, obtained by cryo-electron microscopy.

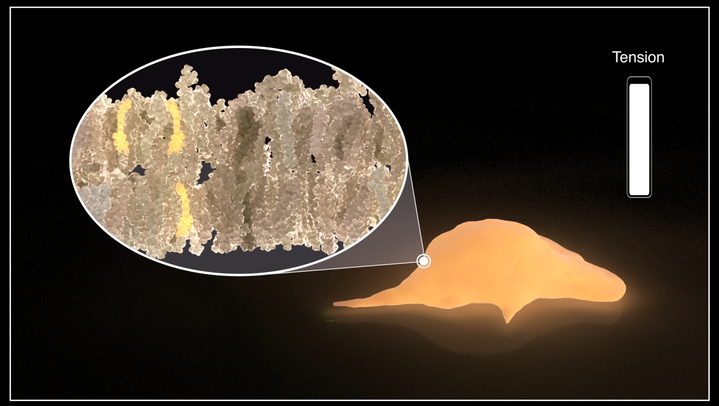
Tension measurement by Fluroscence Lifetime Imaging Microscopy (FLIM)

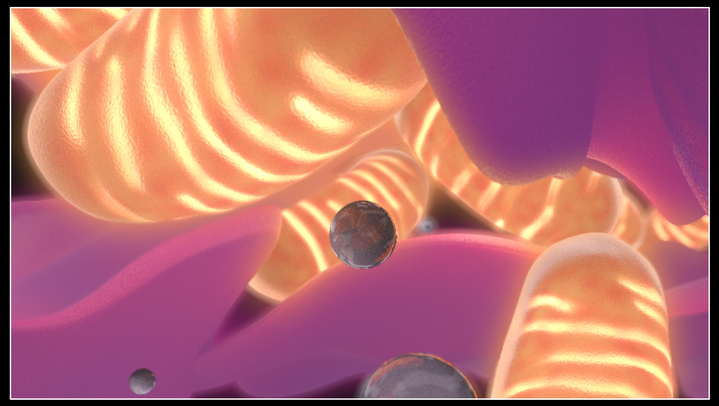
And why the colors used in all the fluorescence scenes are ‘fake’ colors!

> All **structures** of tension probes are accurate.

For reference & more details about their synthesis:
Fin & al, *Angewandte Chemie* (2012)
Dal Molin & al, *J. Am. Chem. Soc.* (2015)
Soleimanpour & al, *Chem. Commun.* (2016)

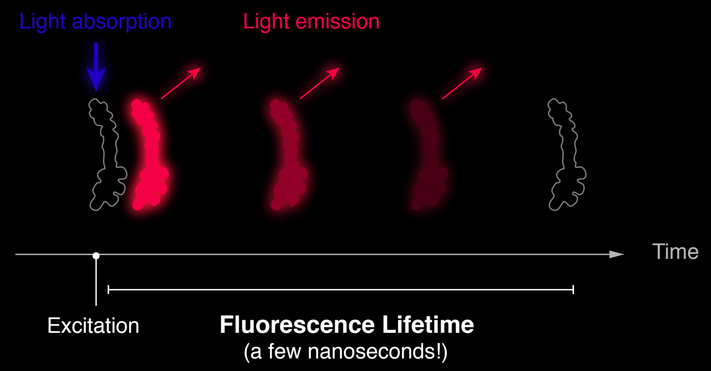
> All **appearances** are fictive.





> Fluorescent molecules are able to transitorily emit light of specific color when ‘excited’ by a light of another precise color.
The amount of time during which the molecule is able to emit light before it goes ‘off’ again, is called «fluorescence lifetime».

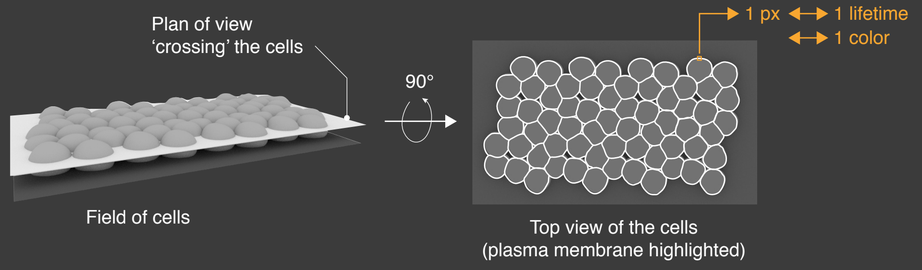
> Scientists have tools to measure both of these colors, as well as their intensities, and the fluorescence lifetime.
In this study, scientists have focused on the lifetime, because they had observed that it was the parameter that was best suited to study membrane tension (colors & intensities change slightly with tension, but not a lot).
Also, the probes depicted in this animation actually **ALL** emit only **RED** light!!

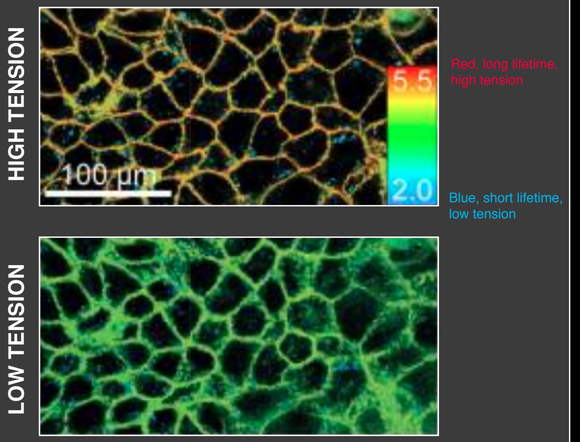


How FLIM works

The microscope ‘scans’ a cross-section of the field of view; for each pixel of the membrane region (containing the probe), a lifetime is measured:
1) the fluorescent molecule is ‘excited’ so that it emits light
2) the time it takes to stop emitting is measured

Lifetimes are just numbers, so scientists visualize them through arbitrary color-coding. As lifetime & tension are related, these colors are in turn representative of membrane tension.





Representative images of cells’ plasma membrane color-coded according to the lifetime of the probe they contain (Colom & al, *Nat Chem*, 2018)