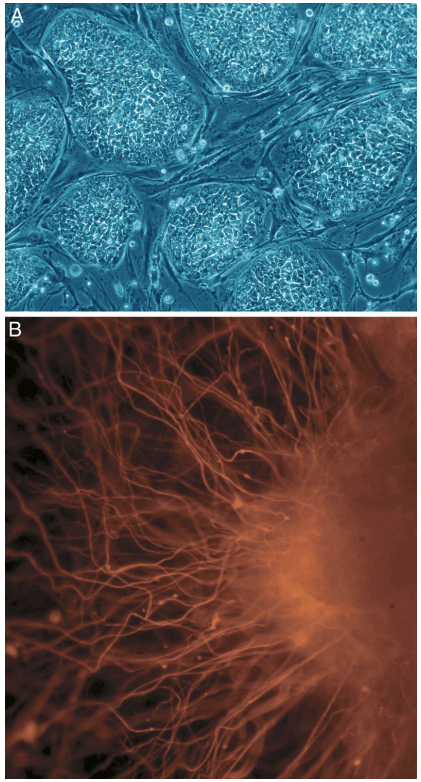
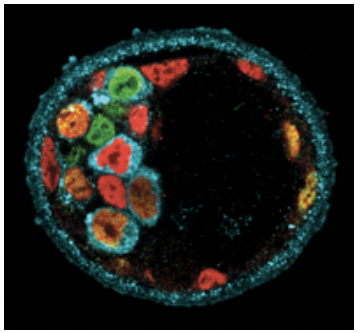
STEM CELLS AND REGENERATIVE MEDICINE CONTINUED

Stem cell potency, induced pluripotency, cancer stem cells, stem cell niches, and challenges of regenerative medicine

Review: Stem cell potency

- Potency – ability of cell to take on different fate
- Totipotent – has potential to take on all fates
  - Zygote, very very early embryo
- Multipotent/Pluripotent – fates are narrowed down, restricted to a few fates
  - Commitment – process of committing to some lineage; choices of fates are reduced
  - Embryonic stem cells, adult stem cells
- Bipotent – can be one of two fates
- Unipotent – can only have one fate
  - Differentiated/fully committed cells



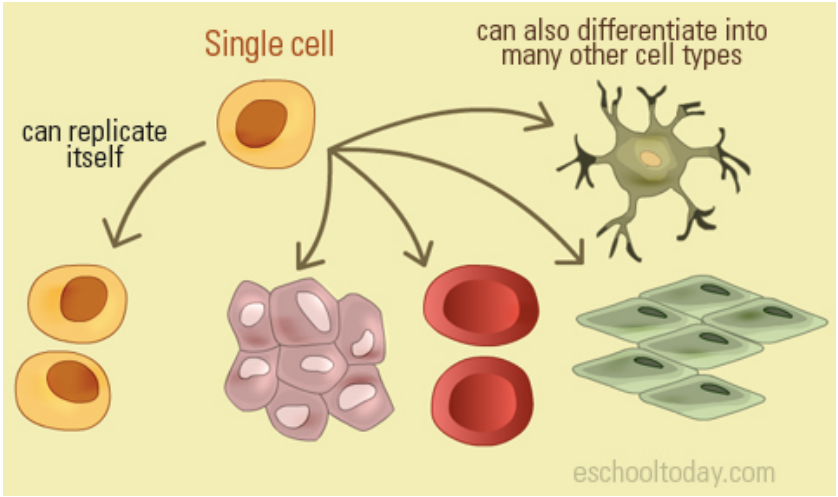


Molecular heterogeneity during mouse blastocyst patterning. Cells expressing Nanog (green), Gata6 (red) or Serpinh1 (blue).

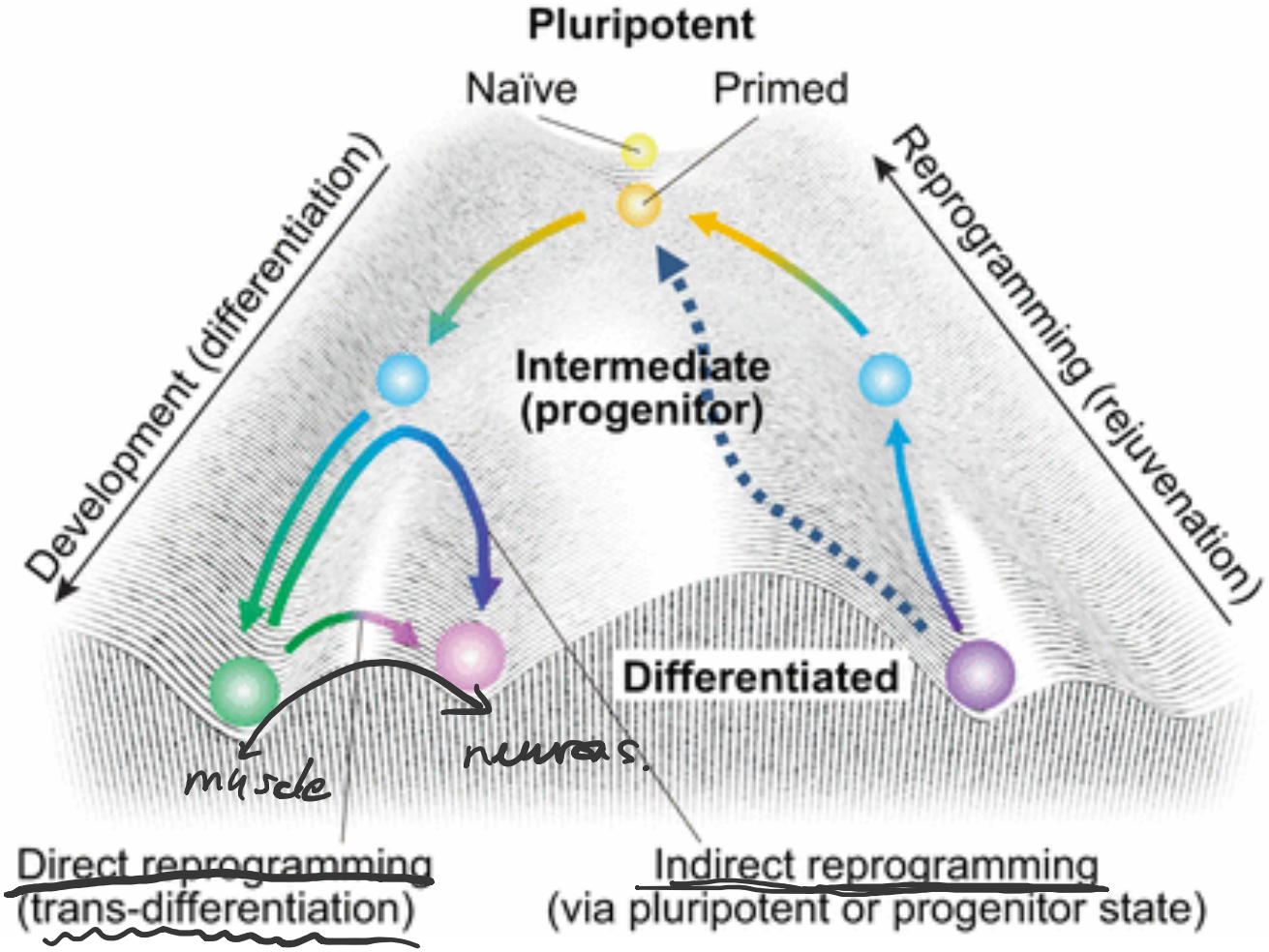
http://www.embl.de/research/units/dev_biology/hiiragi/

How to define a stem cell

- Self-renewal
- Differentiate (potency)
- E.g. in HSC, a single HSC must be able to re-populate the whole blood system
- http://ed.ted.com/lessons/what-are-stem-cells-craig-a-kohn
- Fun facts about blood (~120 days); intestine (~1 week); hair (~4 years); skin (~2-4 weeks)

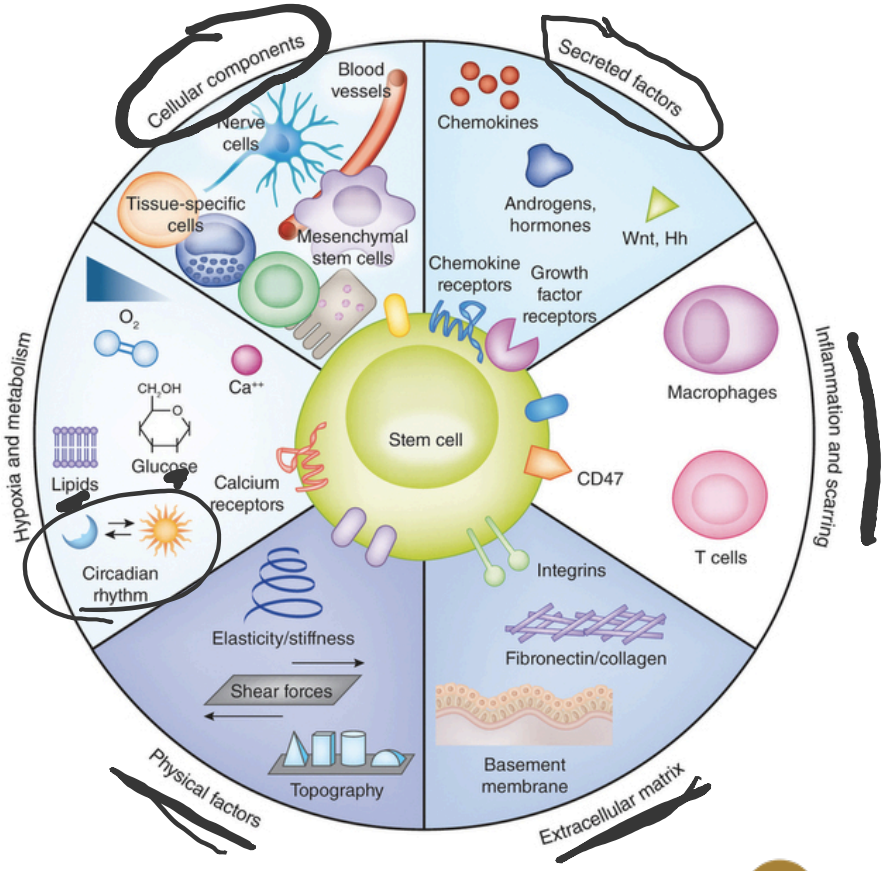


Commitment and differentiation



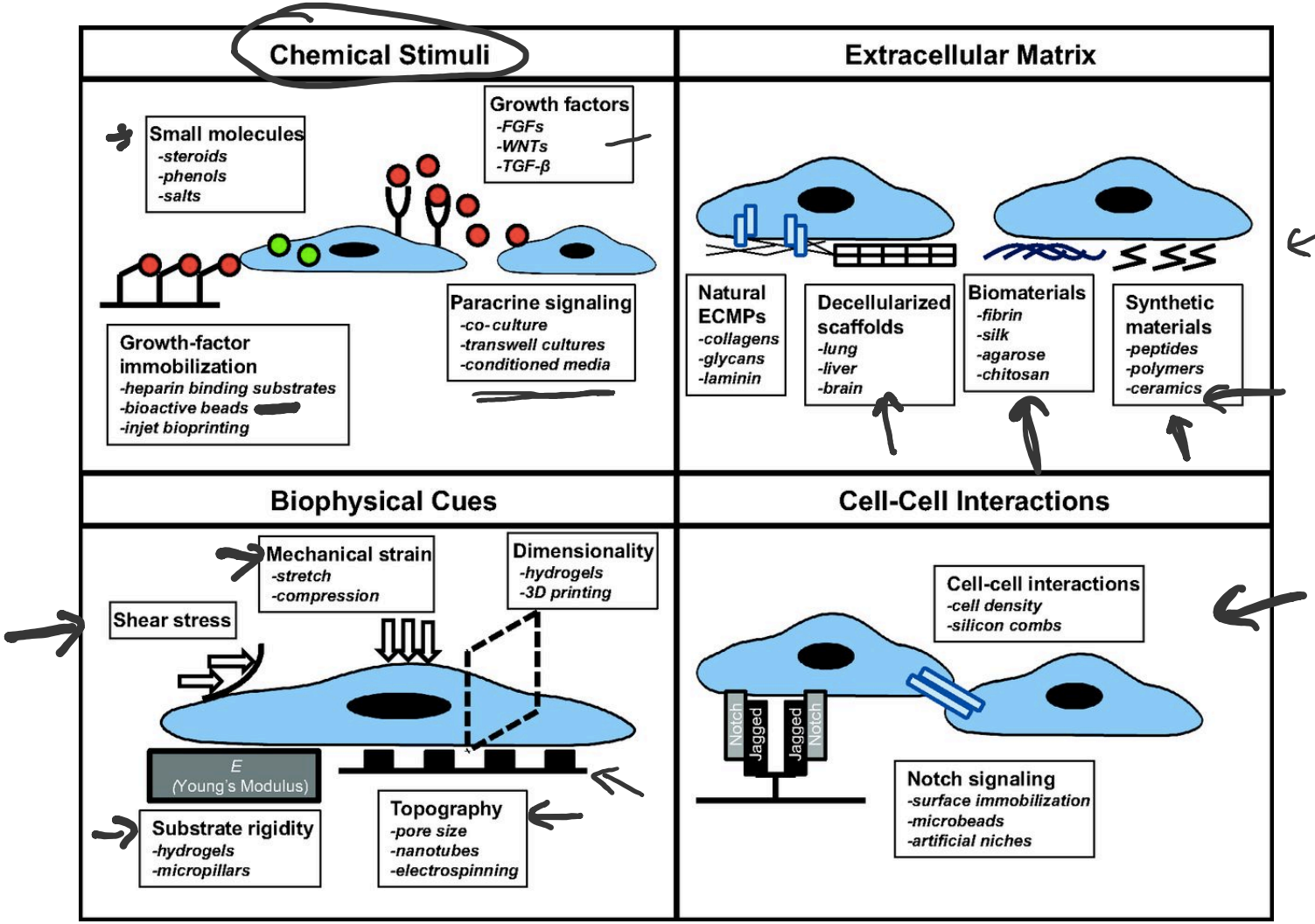
Stem cell niche

- Regulation and homeostasis is important for stem cells
- Normally SCs are kept quiescent (in a dormant/resting state) and not dividing or differentiating
  - They are kept quiescent by surrounding cells (the niche)
- Stimulus from the environment may activate/trigger the SC
  - E.g. in muscle, it could be an injury to the tissue; in blood, it could be an immune response to infection

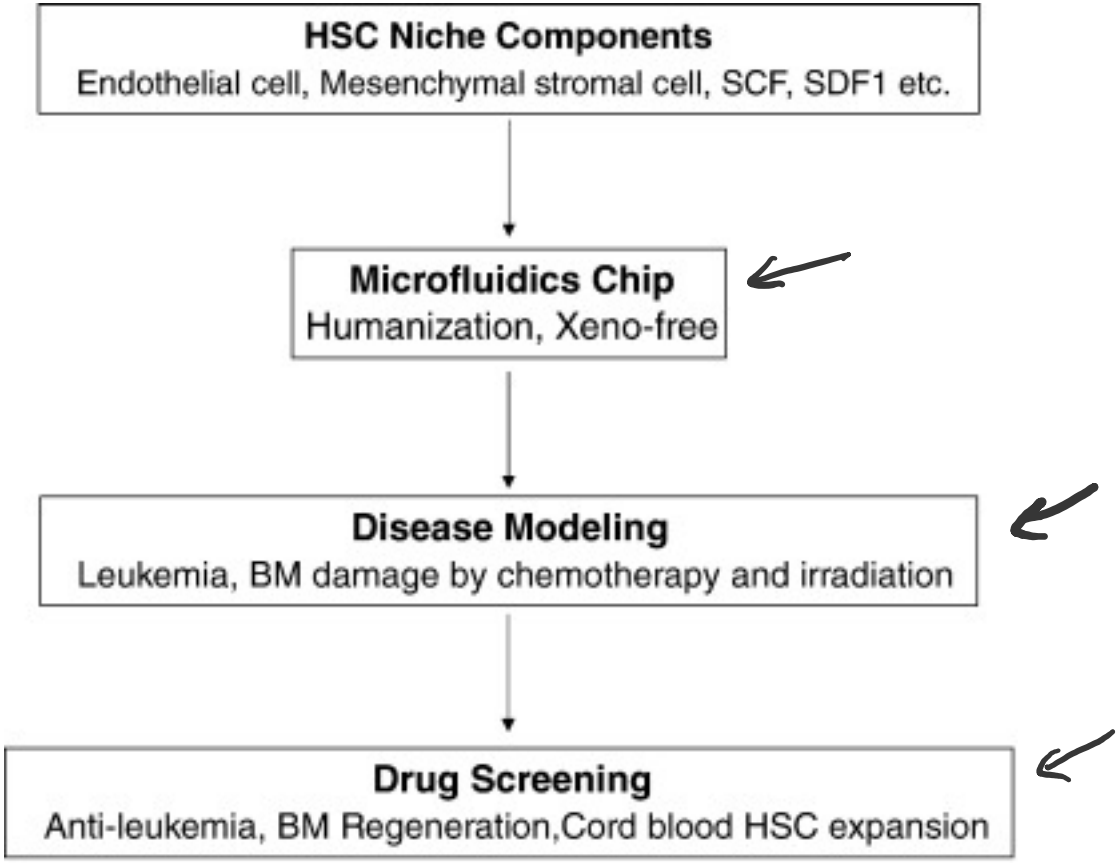


Lane, Williams, & Watt, "Modulating the stem cell niche for tissue regeneration", Nature Biotech, 2014

Bioengineering of the niche



Bioengineering of the niche

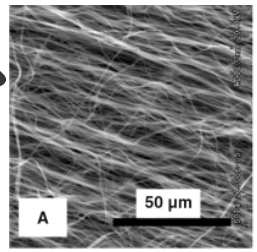


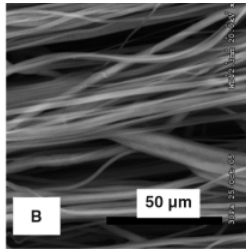
Synthetic extracellular matrix

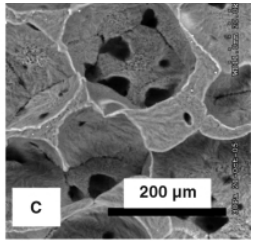
- Using electrospun biocompatible polycaprolactone (PCL) scaffolds to improve alignment of chondrocytes when differentiating from mesenchymal SC into cartilage tissue

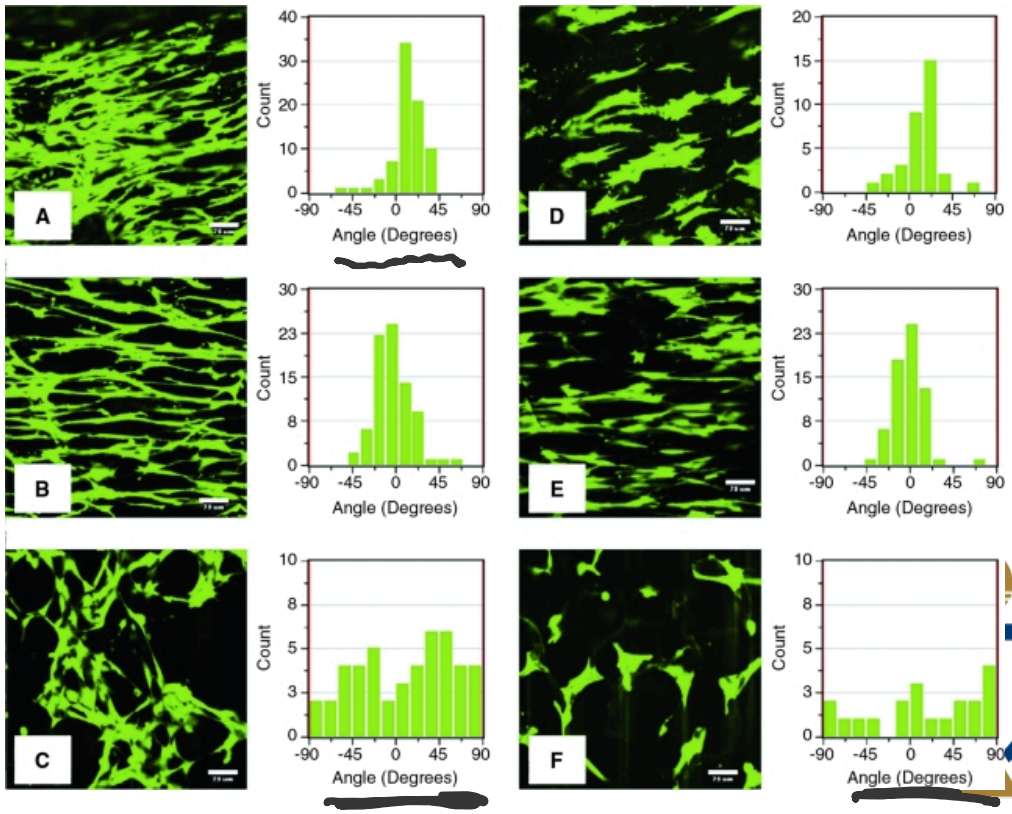
Scaffold

MSCs → Chondrocytes





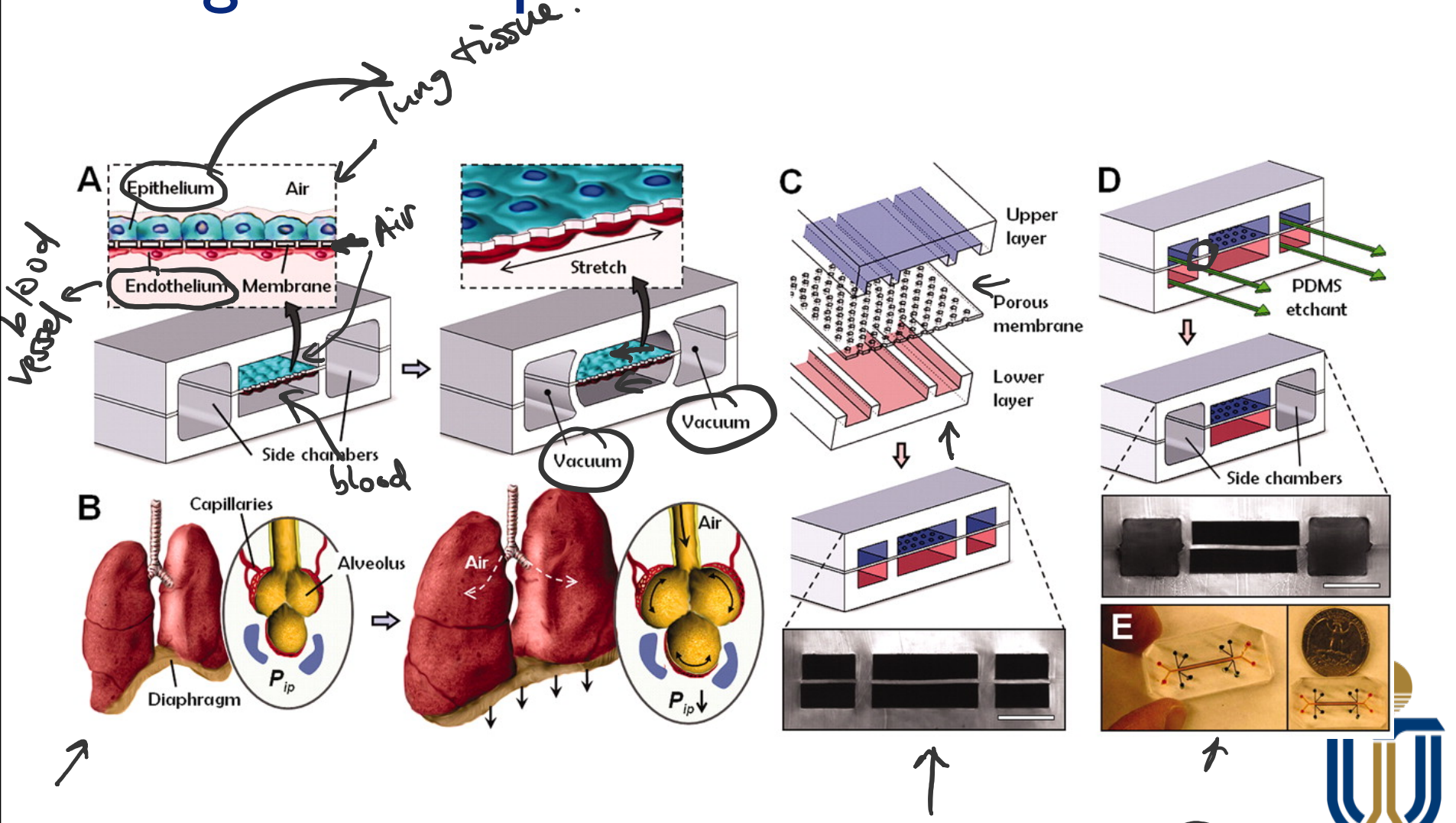




*SEM images of oriented electrospun PCL fibrous scaffolds. The average fiber diameter was estimated using an image processor: (**A**) 500 nm; (**B**) 3000 nm. (**C**) shows nonelectrospun porous PCL film.*

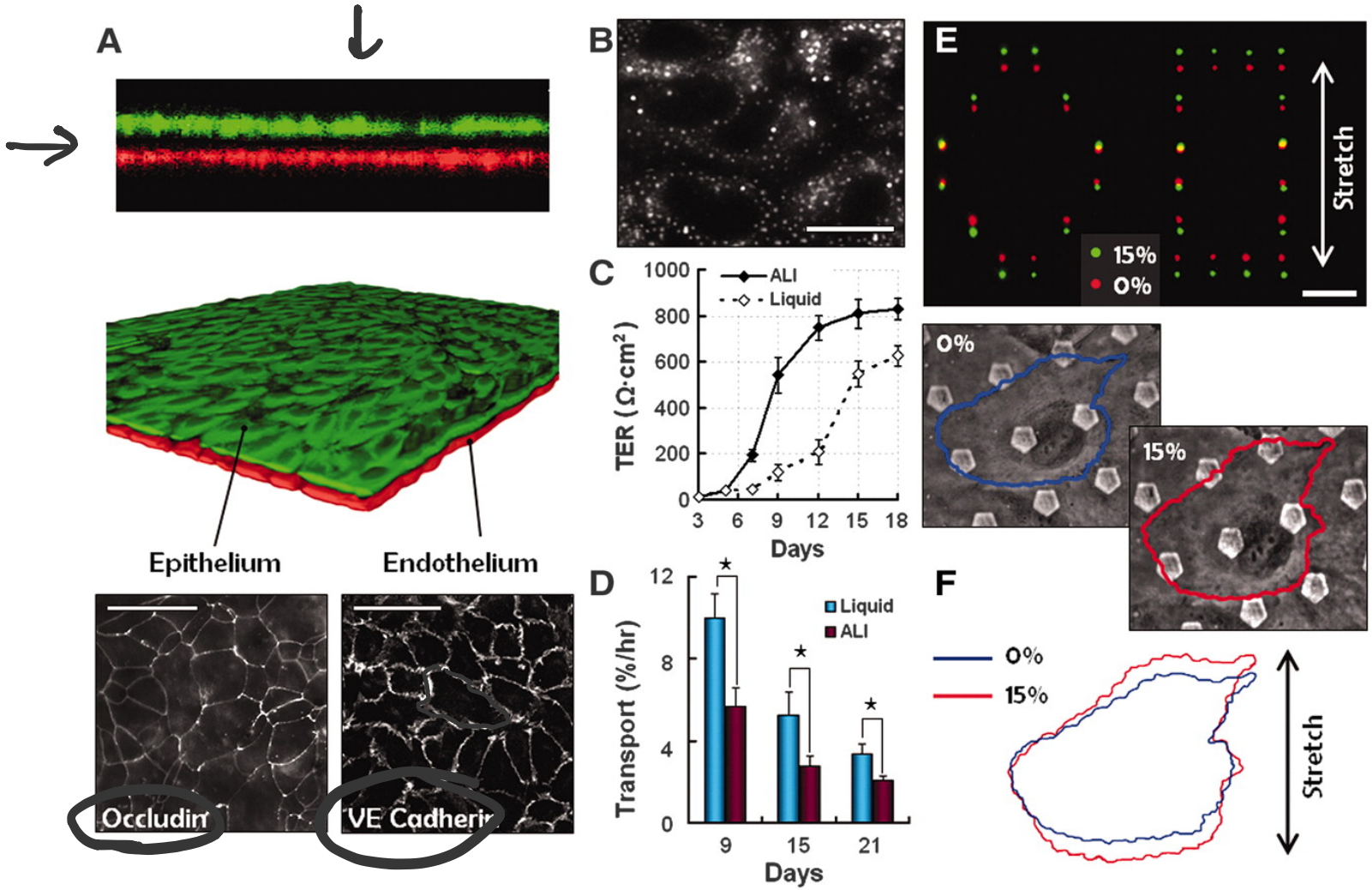
Wise et al., Tissue Eng Part A. 2009

Lung-on-a-chip



Huh, Matthews, Mammoto, Montoya-Zavala, Hsin, and Ingber, Reconstituting Organ-Level Lung Functions on a Chip, Science, 2010

Lung-on-a-chip



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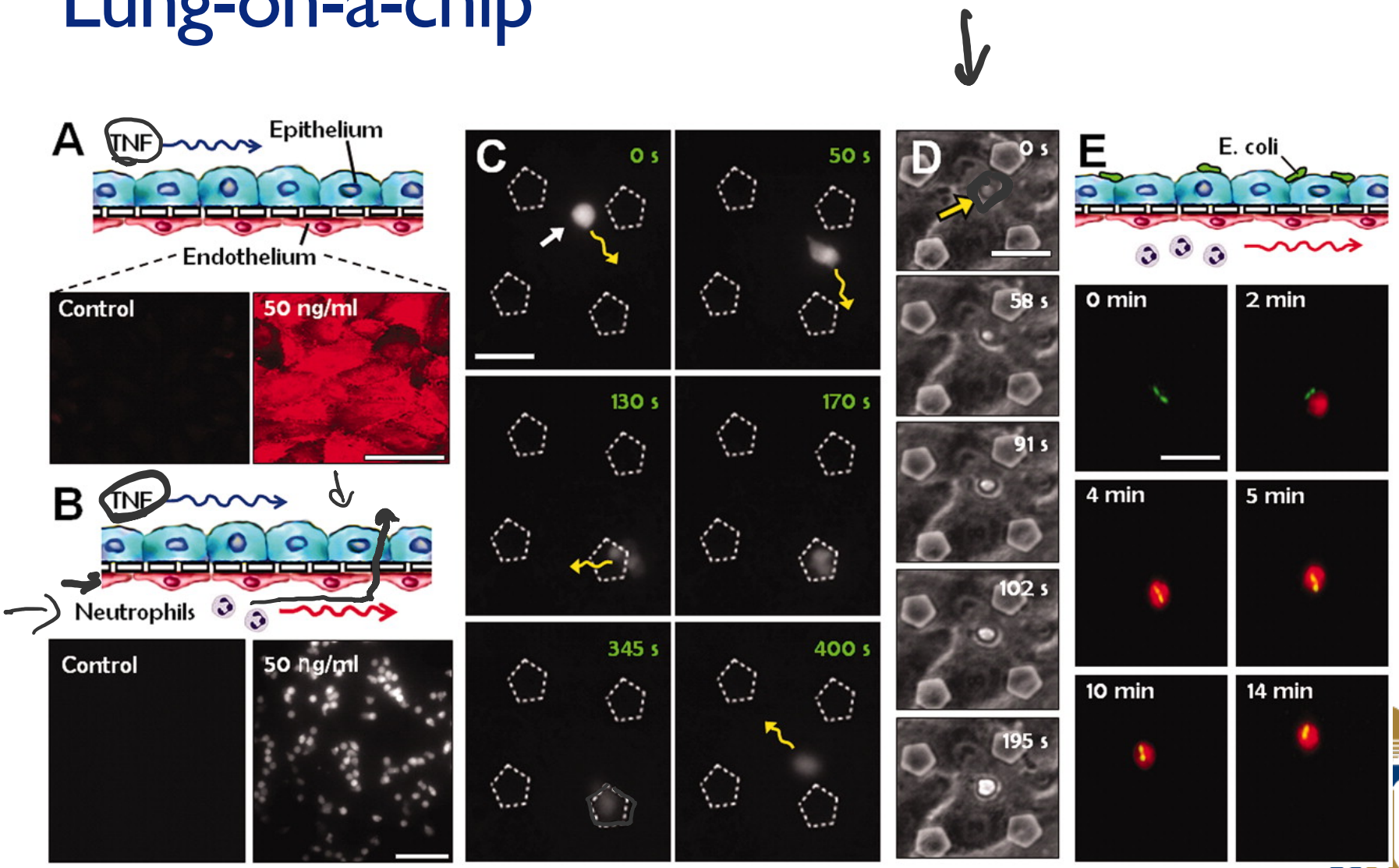

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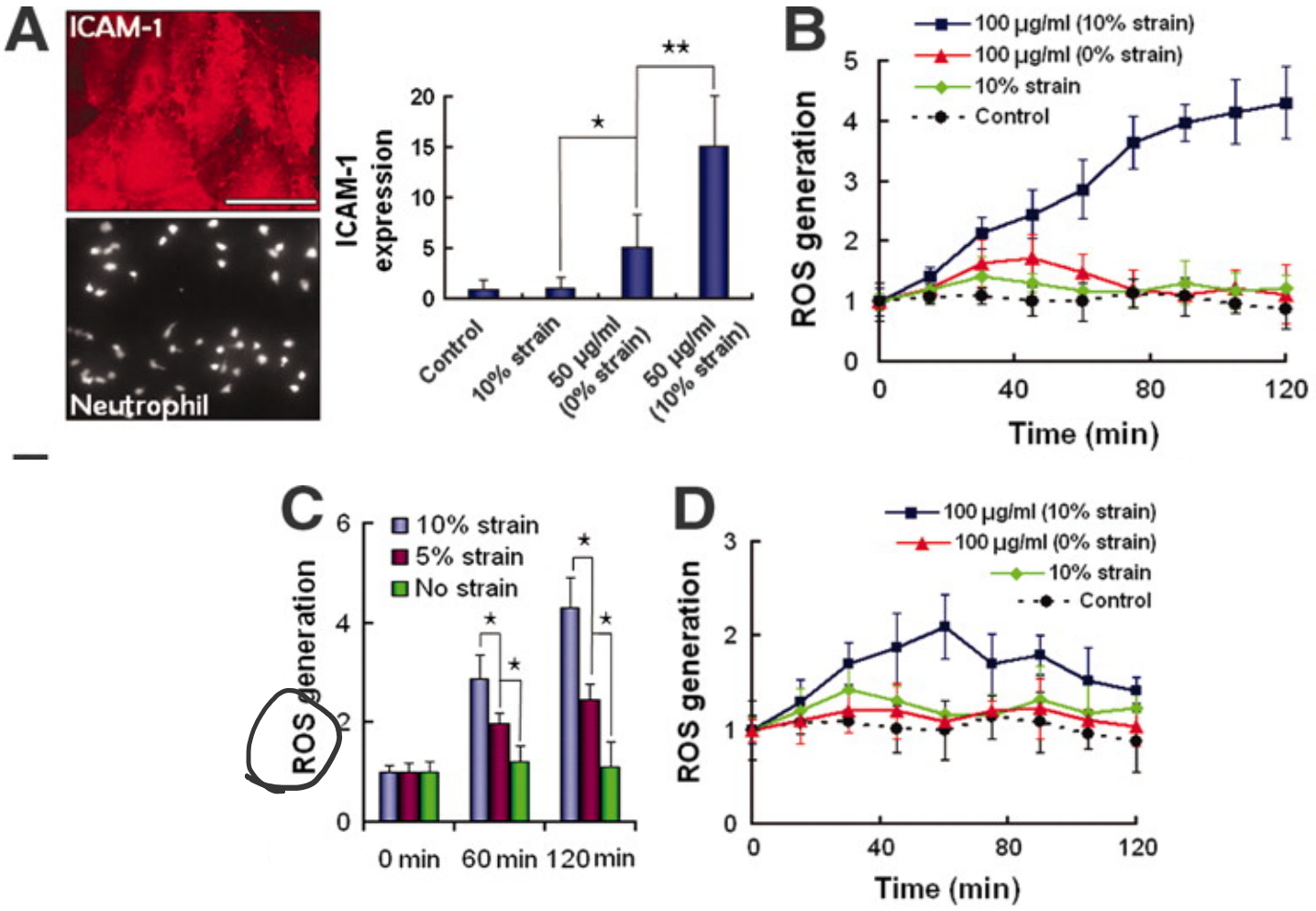

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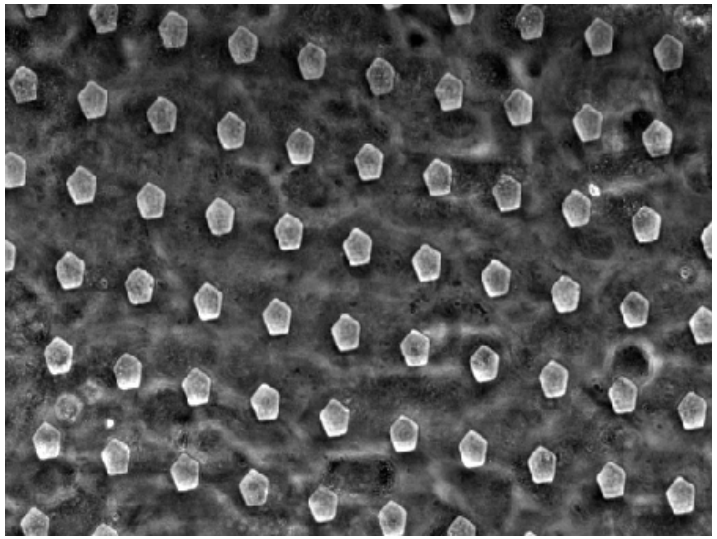
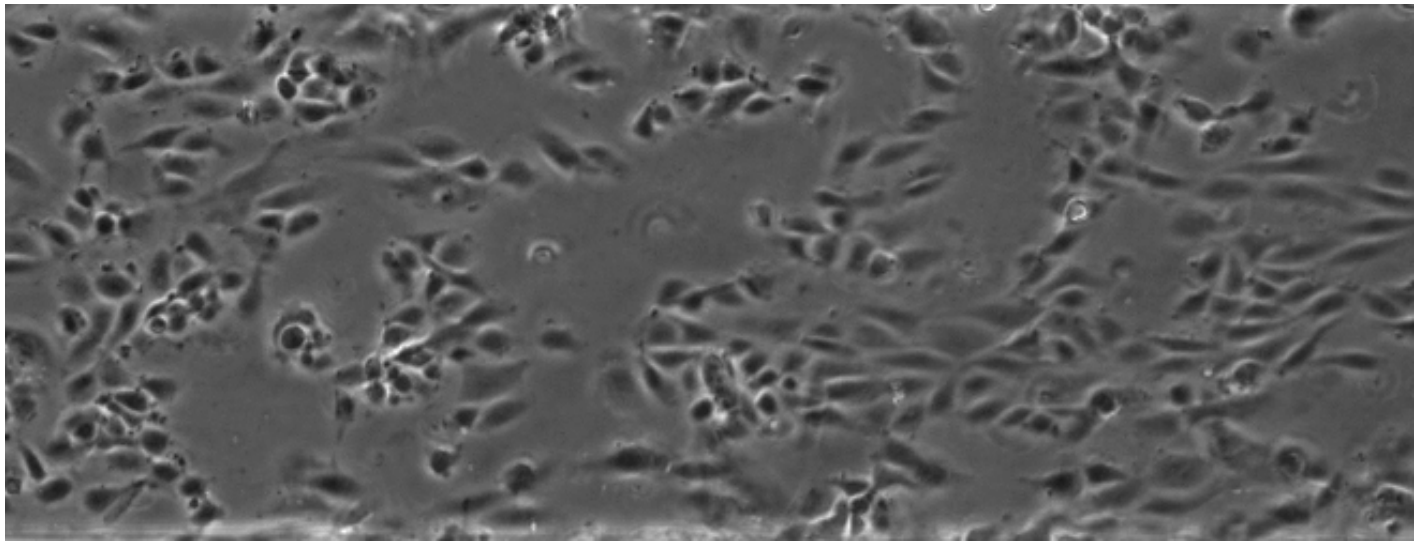
Lung-on-a-chip

Stretching of the cells on PDMS membrane



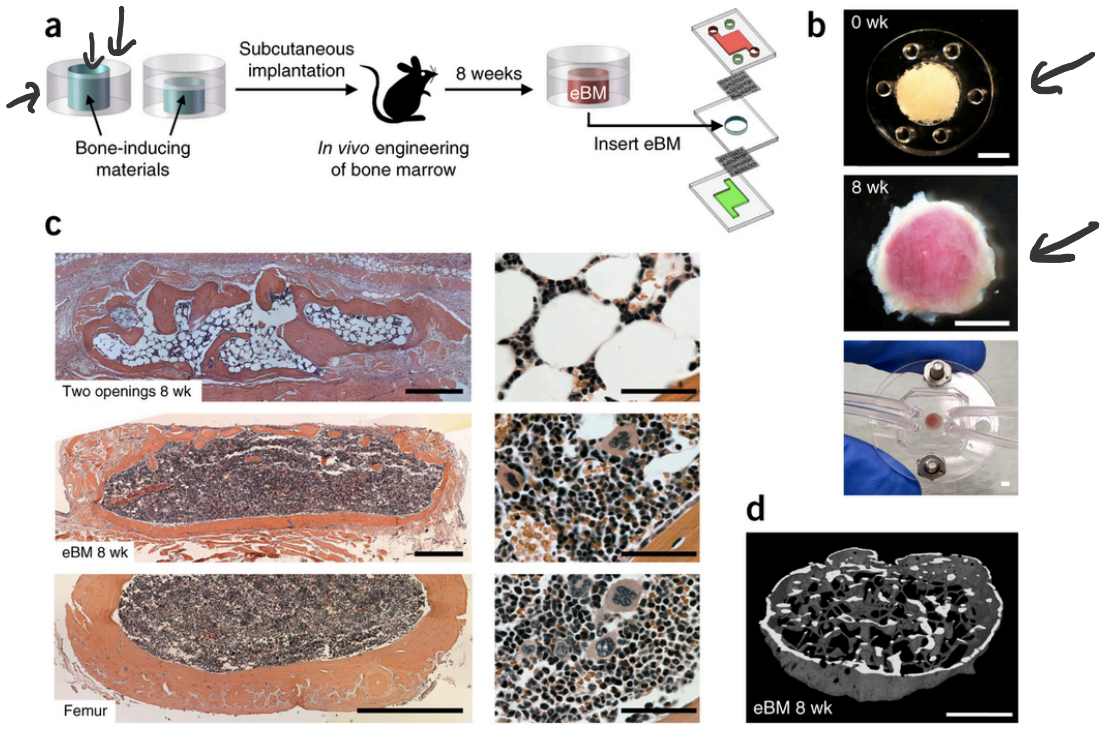
Reorientation and alignment of cells in response to cyclic stretch of 10% strain at 1 Hz over the period of 10 hours



Huh, Matthews, Mammoto, Montoya-Zavala, Hsin, and Ingber, Reconstituting Organ-Level Lung Functions on a Chip, Science, 2010

Bone marrow on a chip

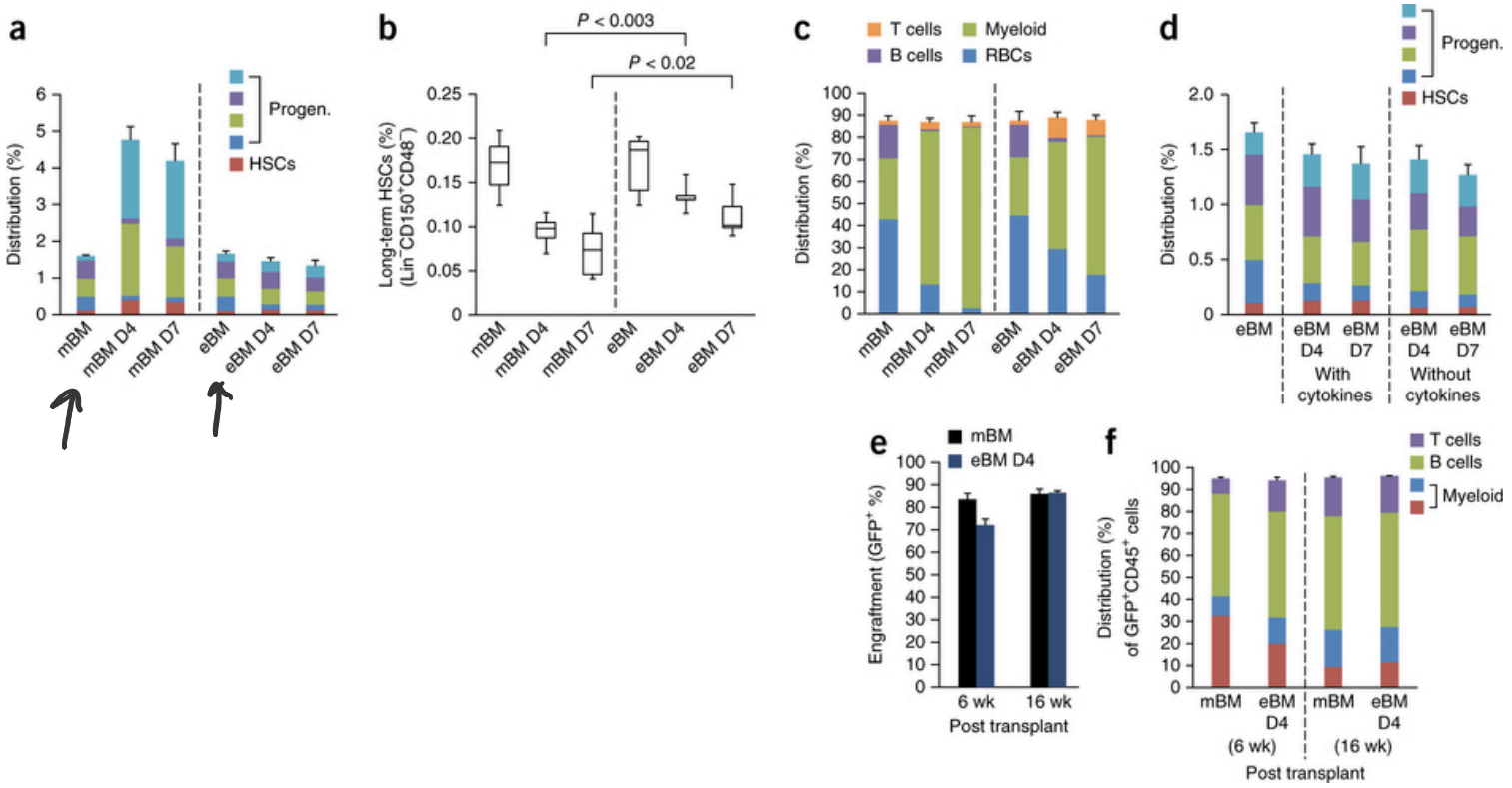
- Microfabricated PDMS device with a central tube-like cavity
- Hollow compartment filled with type I collagen gel (contains bone-inducing demineralized bone powder, bone morphogenetic proteins BMP2, BMP4)
- Implanted the device subcutaneously in the back of a mouse



Torisawa et al., Bone marrow–on–a–chip replicates hematopoietic niche physiology in vitro, Nature Methods, 2014

Bone marrow on a chip

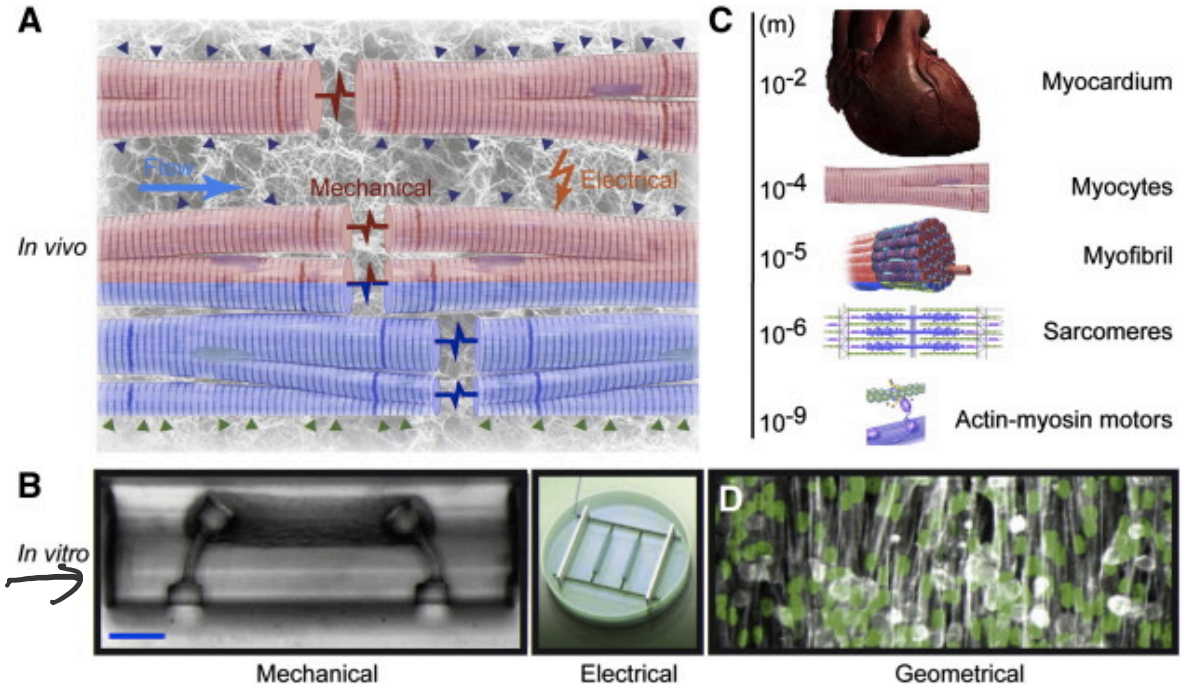
- Results of culturing HSCs in the bone-marrow chip



Torisawa et al., Bone marrow–on–a–chip replicates hematopoietic niche physiology in vitro, Nature Methods, 2014

Other methods of tissue engineering

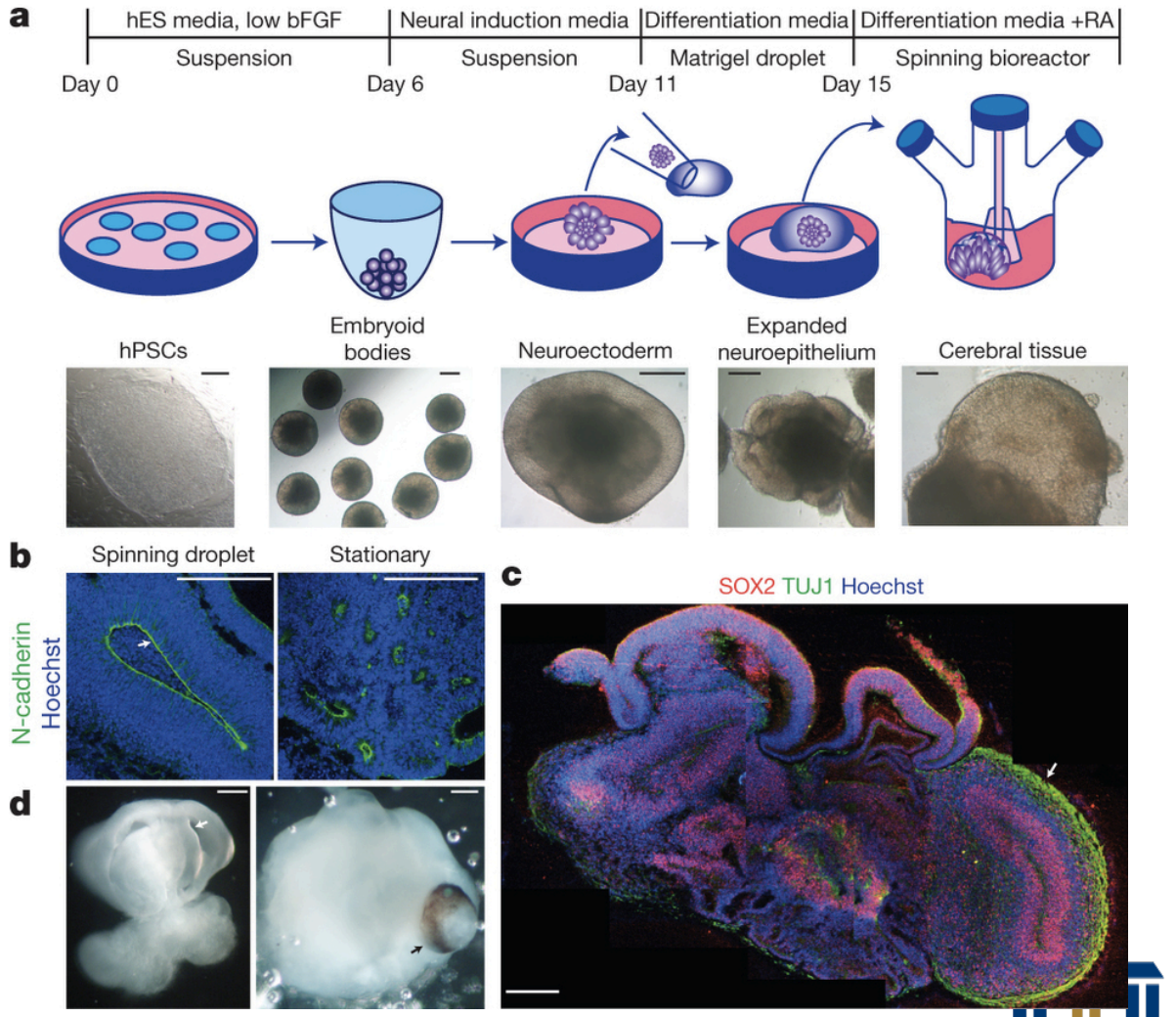
- Biophysical stimuli – mechanical, electrical, biochemical – to drive cardiomyocyte maturation
  - Mechanical forces regulate cardiac development, and mutations affecting contractile proteins can cause heart malformations
  - Electrical forces required for preserving cardiac chamber morphology, where they act as a key epigenetic factor in cardiac remodeling



Parsaa, Ronaldsona, Vunjak-Novakovica, Bioengineering methods for myocardial regeneration, Advanced Drug Delivery Reviews, 2016

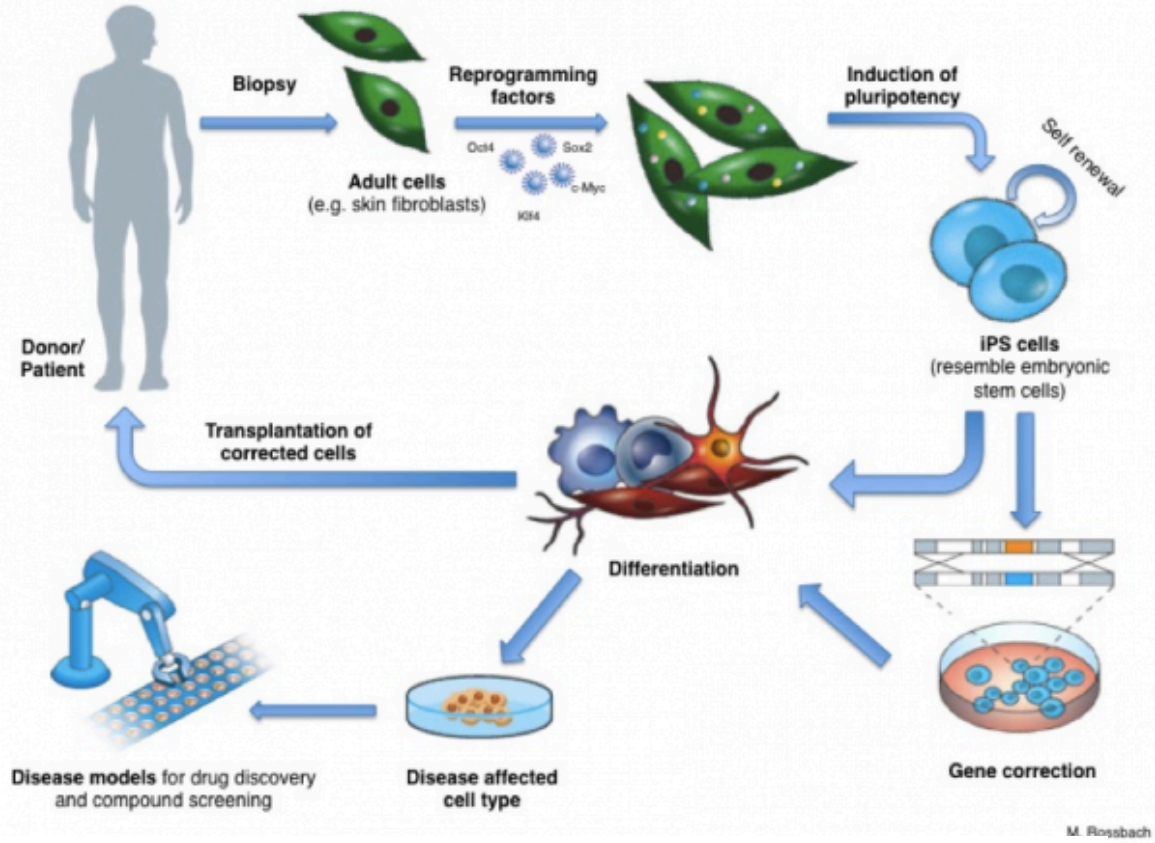
Organoids

- An **organoid** is a 3D organ-bud grown *in vitro* that shows realistic micro-anatomy
- Gene expression appears to be closely recapitulating the organ (see further reading)
- Lack of vascularization is one major concern
- http://hub4organoids.eu/organoid-technology/



Lancaster et al., Cerebral organoids model human brain development and microcephaly, Nature, 2013

Induced pluripotency



- **iPSCs: induced pluripotent stem cells**
- Discovered in 2006 by **Shinya Yamanaka**; awarded Nobel Prize in Physiology in 2012
- Introduction of specific transcription factors can convert adult stem cells back into pluripotent stem cells – reversing the processing of differentiation
  - Yamanaka factors: **Oct4, Sox2, c-Myc, Klf4** transcription factors
- Advantages – Uses patient's own cells so no rejection (autologous); avoids ethical issues of obtaining ESCs
- http://www.eurostemcell.org/toolkititem/stem-cells-future-introduction-ips-cells

Induced pluripotency

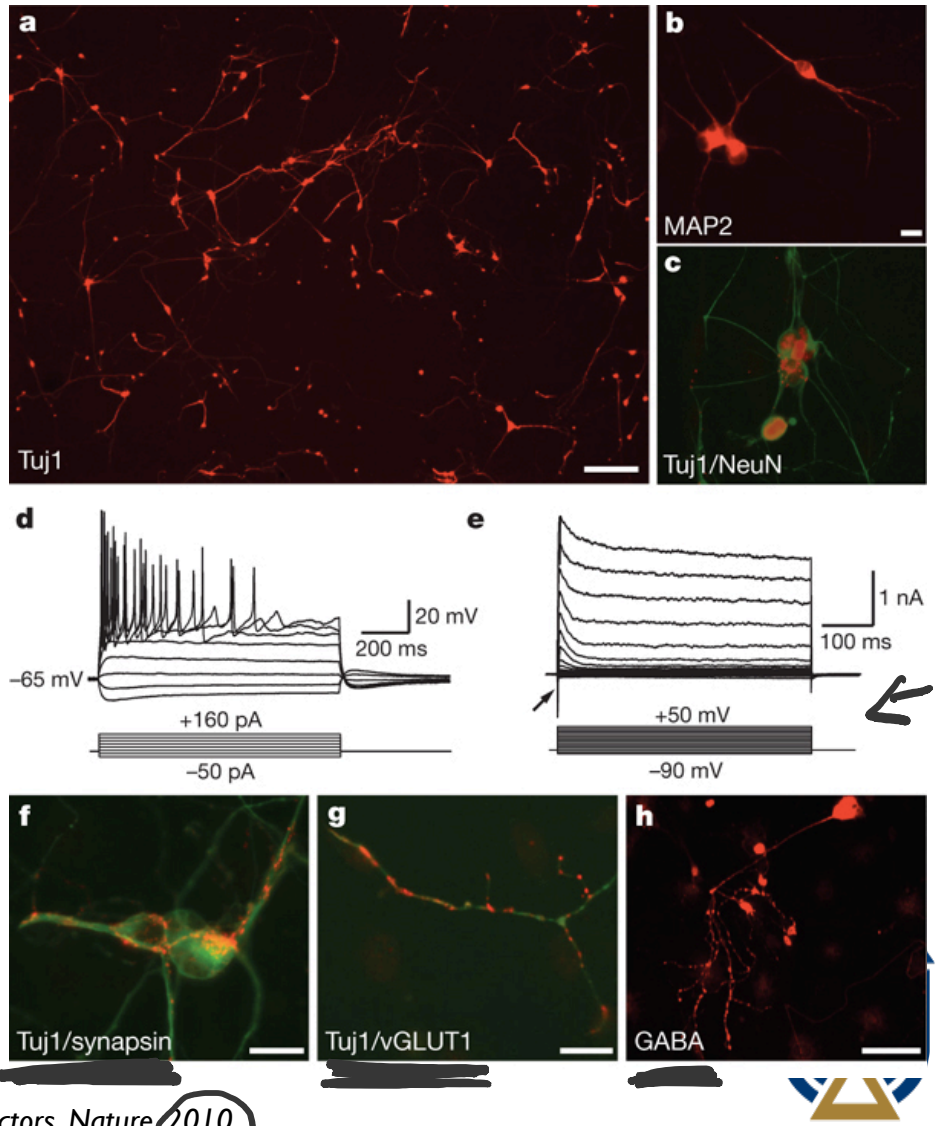
Year	Group	Strategy	Contribution
2006	Yamanaka et al.	First to demonstrate	iPS cells were first generated using retroviruses and the four key pluripotency genes; failed to produce viable chimera.
2007	Yamanaka et al.	Different Selection Method	iPS cells were generated again using retroviruses, but this time produced viable chimera (they used different selection methods).
2007	Thomson et al.	Vector	iPS cells were generated again using lentiviruses, and again produced viable chimera.
2008	Melton et al.	Small Compound Mimicking	Using HDAC inhibitor valproic acid compensates for C-Myc.
2008	Ding et al.	Small Compound Mimicking	Inhibit HMT with BIX-01294 mimics the effects of Sox2, significantly increases reprogramming efficiency.
2008	Hochedlinger et al.	Vector	The group used an adenovirus to avoid the danger of creating tumors; however, this led to lower efficiency.
2008	Yamakana et al.	Vector	The group demonstrated reprogramming with no virus (they instead used a plasmid)
2009	Ding et al.	Proteins	Used recombinant proteins ; proteins added to cells via arginine anchors was sufficient to induce pluripotency.
2009	Freed et al.	Vector	Adenoviral gene delivery reprogrammed human fibroblasts to iPS cells.
2009	Blelloch et al.	RNA	Embryonic stem-cell specific microRNAs promted iPS reprogramming.
2011	Morrisey at al.	RNA	Demonstrated another method using microRNA that improved the efficiency of reprogramming to a rate similar to that demonstrated by Ding.

- Remaining issues –
  - Reprogramming efficiency: Initial methods had 0.01-0.1% efficiency; recent method that down-regulates nucleosome re-modeling and deacetylation complex, NuRD, can achieve almost 100% efficiency, but downstream effects of NuRD not fully explored
  - Genomic insertion of 4 Yamanaka factors – mutations possible; viral vector-based methods avoid insertion but are lower efficiency/throughput and could trigger oncogenesis from the viral vector itself
  - Tumorigenesis – use of c-myc as induction factor sometimes results in cancer; method not using myc is less efficient; methods of screening for the proto-oncogenic cells?

Marius Wernig Group.

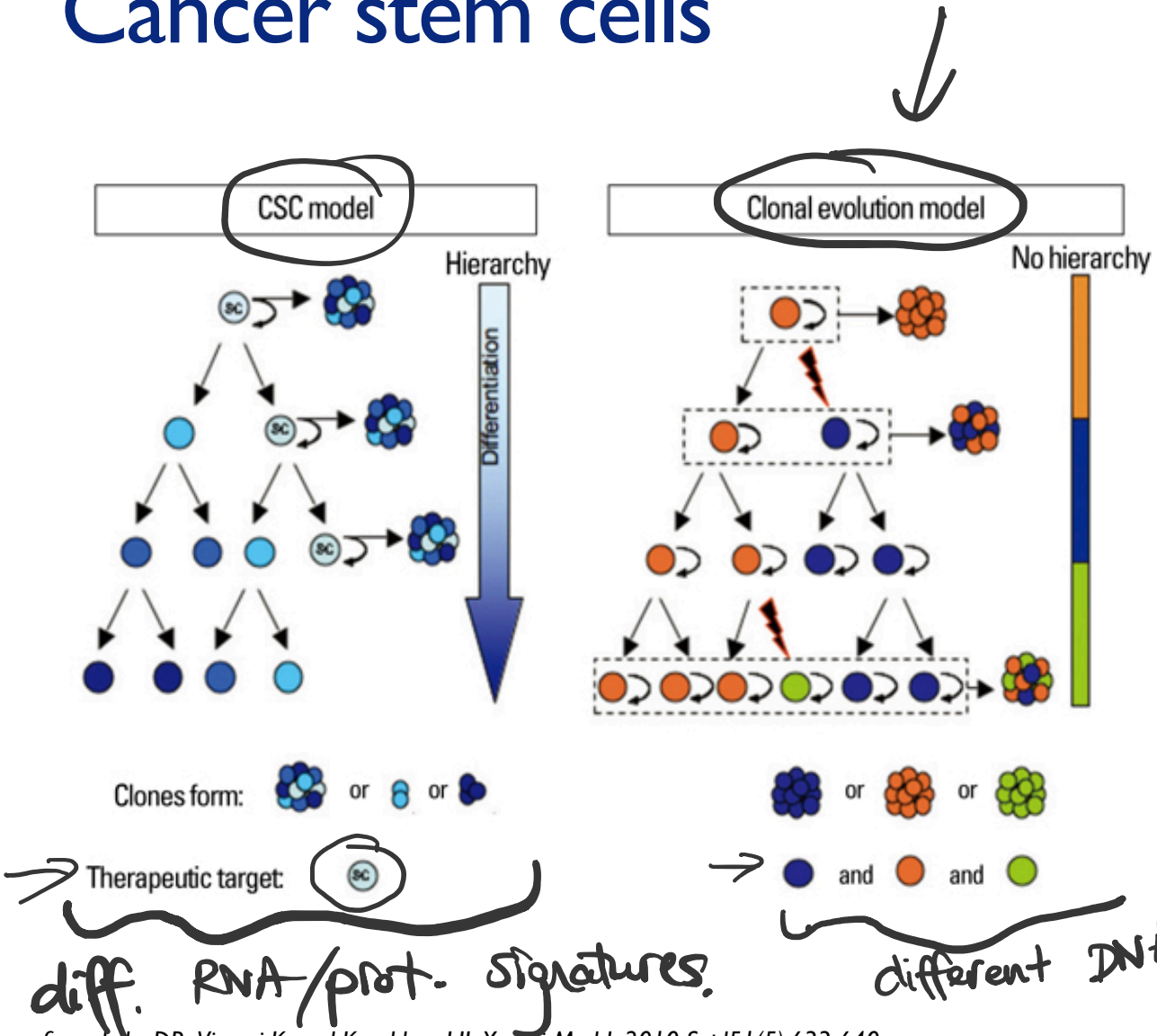
Trans-differentiation

- Direct reprogramming of fibroblasts into neurons
  - Combinatorial expression of **neural-lineage-specific TFs** can directly convert fibroblasts into neurons
  - Three factors: **Ascl1, Brn2 (aka Pou3f2) and Myt1l** can rapidly and efficiently convert mouse embryonic and postnatal fibroblasts into functional neurons *in vitro*
  - Induced neuronal (iN) cells express multiple neuron-specific proteins, **generate action potentials and form functional synapses**



Vierbuchen, Direct conversion of fibroblasts to functional neurons by defined factors, Nature, 2010

Cancer stem cells



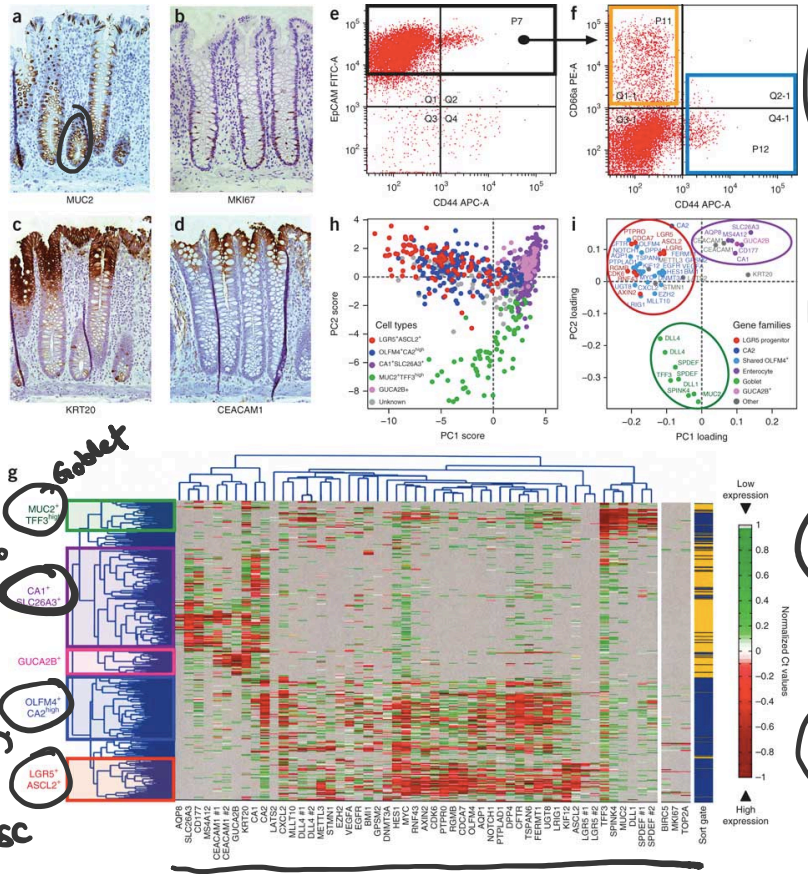
- Currently two models of tumor evolution:
  - Clonal evolution described by genotype heterogeneity (i.e. DNA mutations)
  - Cancer stem cell model described by phenotype heterogeneity (i.e. different cell types)

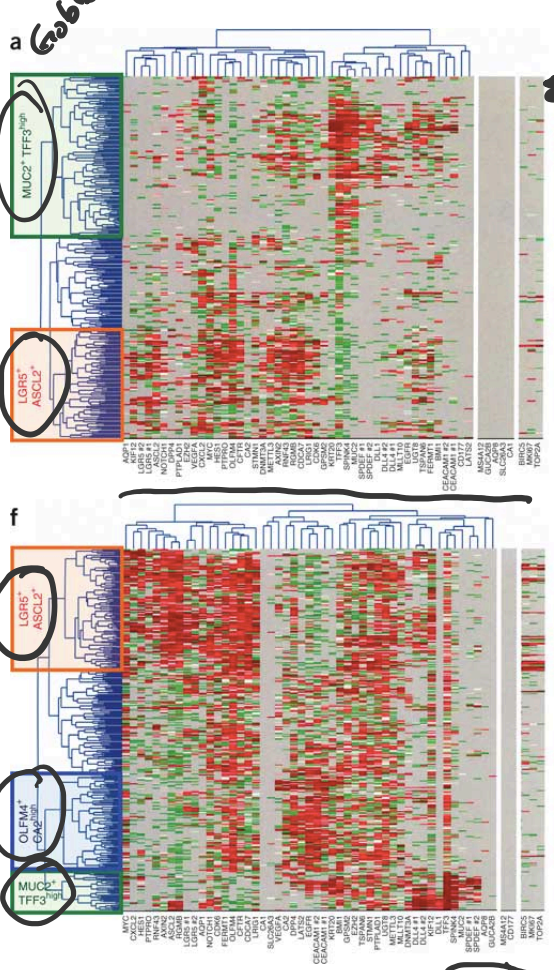
Figure from: Laks DR, Visnyei K, and Kornblum HI, Yonsei Med J. 2010 Sep151(5):633-640

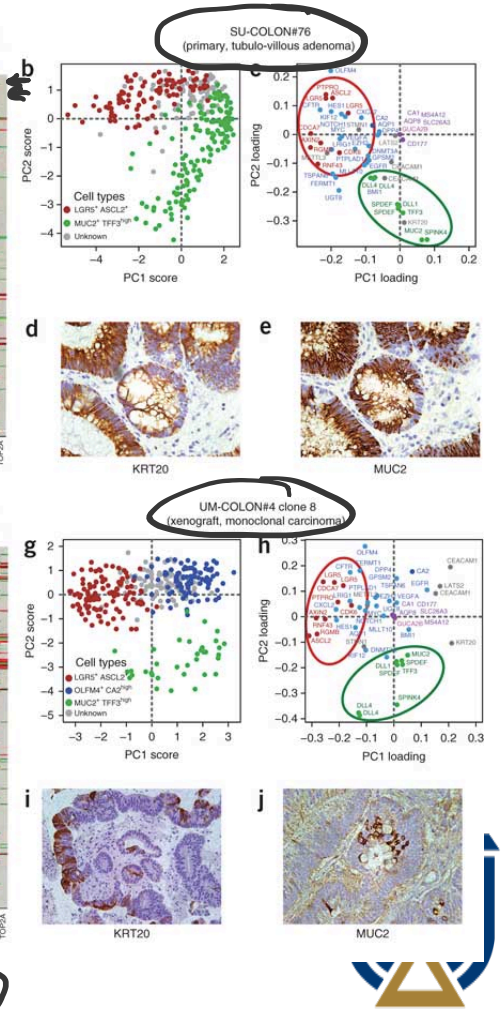
Cancer stem cells

Normal human colon

Tumor Xenografts from single cells
(removal of genetic factor)







Dalerba et al., Single-cell dissection of transcriptional heterogeneity in human colon tumors, Nature Biotech, 2011

Additional reading

Stem cell characterization, identification:

- Loh et al., Mapping the Pairwise Choices Leading from Pluripotency to Human Bone, Heart, and Other Mesoderm Cell Types, Cell, 2016 (http://dx.doi.org/10.1016/j.cell.2016.06.011)
- Tumbar et al., Defining the Epithelial Stem Cell Niche in Skin, Science, 2004 (http://science.sciencemag.org/content/303/5656/359)

Stem cell therapy related:

- Chhabra et al., Hematopoietic stem cell transplantation in immunocompetent hosts without radiation or chemotherapy, Science Translational Medicine, 2016 (http://stm.sciencemag.org/content/8/351/351ra105)

Tissue Engineering

- http://www.nature.com/news/tissue-engineering-how-to-build-a-heart-1.13327

Reprogramming and trans-differentiation:

- Lujan et al., Early reprogramming regulators identified by prospective isolation and mass cytometry, Nature, 2015 (http://www.nature.com/nature/journal/v521/n7552/full/nature14274.html)
- Wapinski et al., Hierarchical Mechanisms for Direct Reprogramming of Fibroblasts to Neurons, Cell, 2013 (http://www.sciencedirect.com/science/article/pii/S0092867413011653)
- Treutlein et al., Dissecting direct reprogramming from fibroblast to neuron using single-cell RNA-seq, Nature, 2016 (http://www.nature.com/nature/journal/v534/n7607/abs/nature18323.html)
- Rais et al., Deterministic direct reprogramming of somatic cells to pluripotency, Nature, 2013 (http://www.nature.com/nature/journal/v502/n7469/full/nature12587.html)

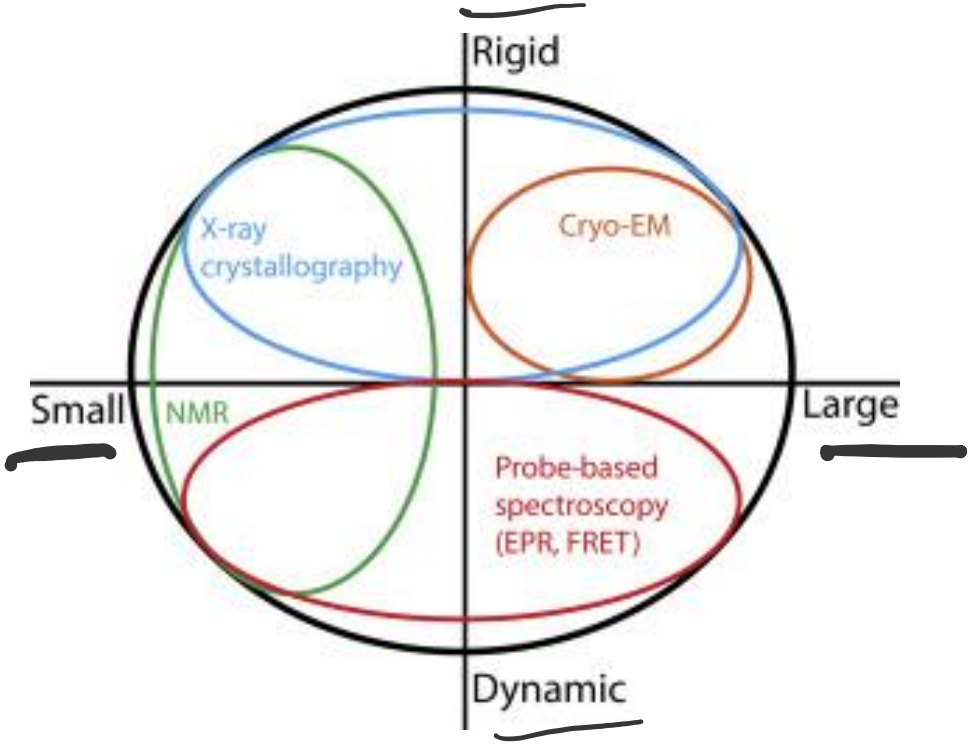
Organoids:

- Camp et al., Human cerebral organoids recapitulate gene expression programs of fetal neocortex development, PNAS, 2015 (http://www.pnas.org/content/112/51/15672.abstract)
- Fujii et al., Efficient genetic engineering of human intestinal organoids using electroporation, Nature Protocols, 2015 (http://www.nature.com/nprot/journal/v10/n10/full/nprot.2015.088.html)

BIOPHYSICAL CHARACTERIZATION OF MACROMOLECULES

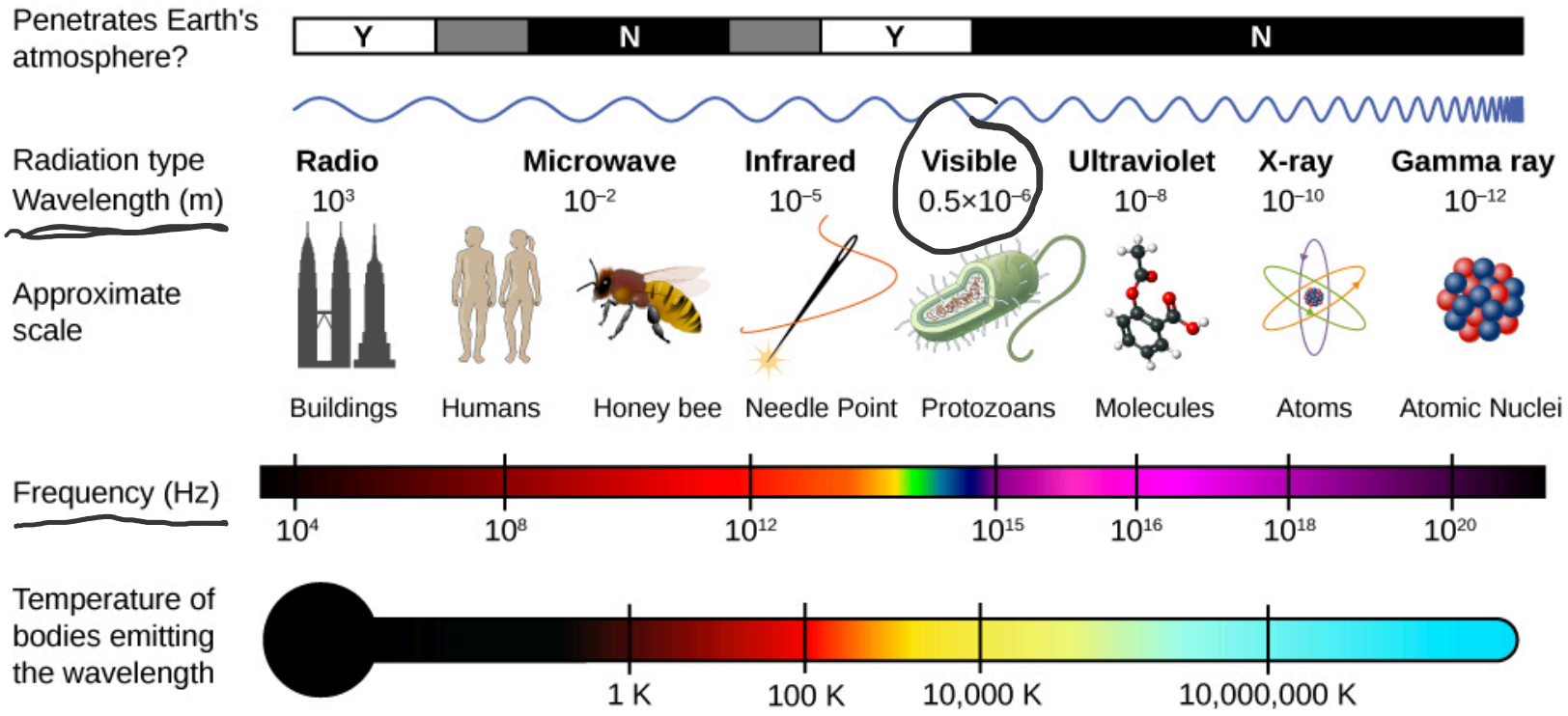
An overview of molecular and physical methods

Characterizing Protein Structure



Claxton, D. P. et al., Methods in Enzymology; Qin, P. Z. et al., *Electron Paramagnetic Resonance Investigations of Biological Systems by Using Spin Labels, Spin Probes, and Intrinsic Metal Ions, Part B,* Academic Press, 2015

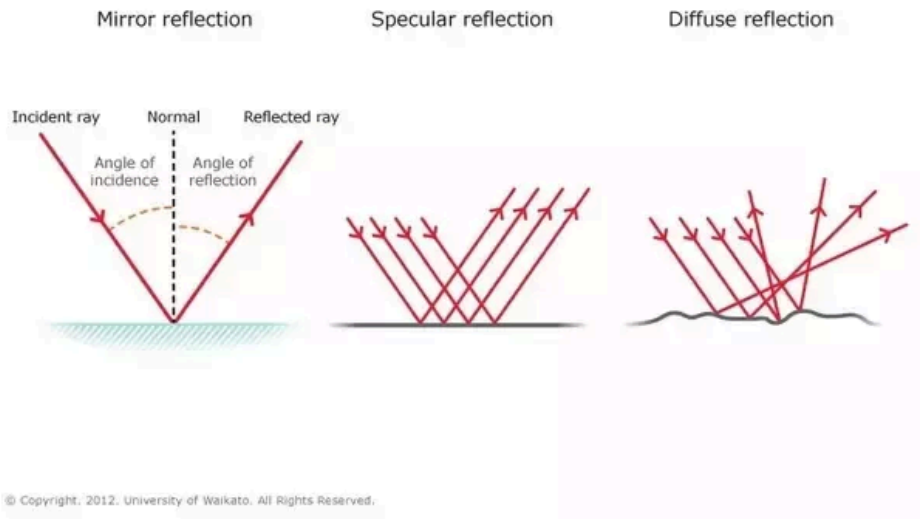
First, a quick physics recap!

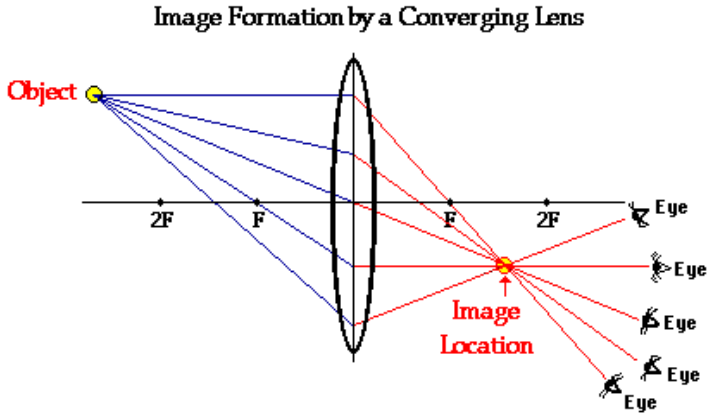


Electromagnetic Waves

$$f = \frac{1}{\lambda}$$

Some things we cannot observe by eye





We can only see things within the visible spectrum:

1) Lenses cannot focus very short wavelengths (why?)
2) The cells in our eyes cannot efficiently sense wavelengths outside the visible spectrum

General rationale:

- We cannot observe atomic structures by eye:
  - Wavelength of visible light 400-700 nm, but atomic spacing = 0.15 nm apart, so the resolution would not be high enough! Therefore, light microscopy would NEVER work
  - X-rays are 0.08-0.6 nm, therefore each ray passing between atom will be reflected separately, so we can measure distances between structures by measuring the x-rays passing through them.
- A reminder about waves and their behavior:

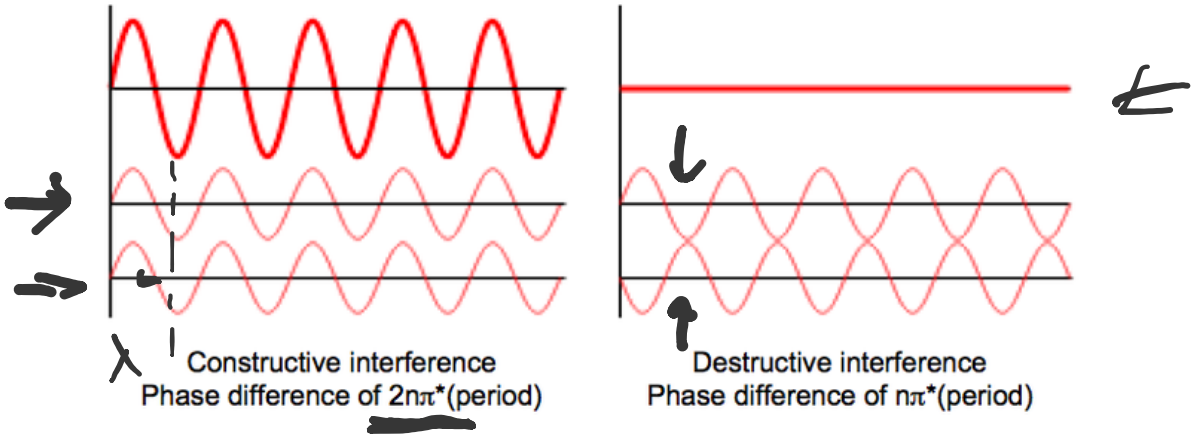
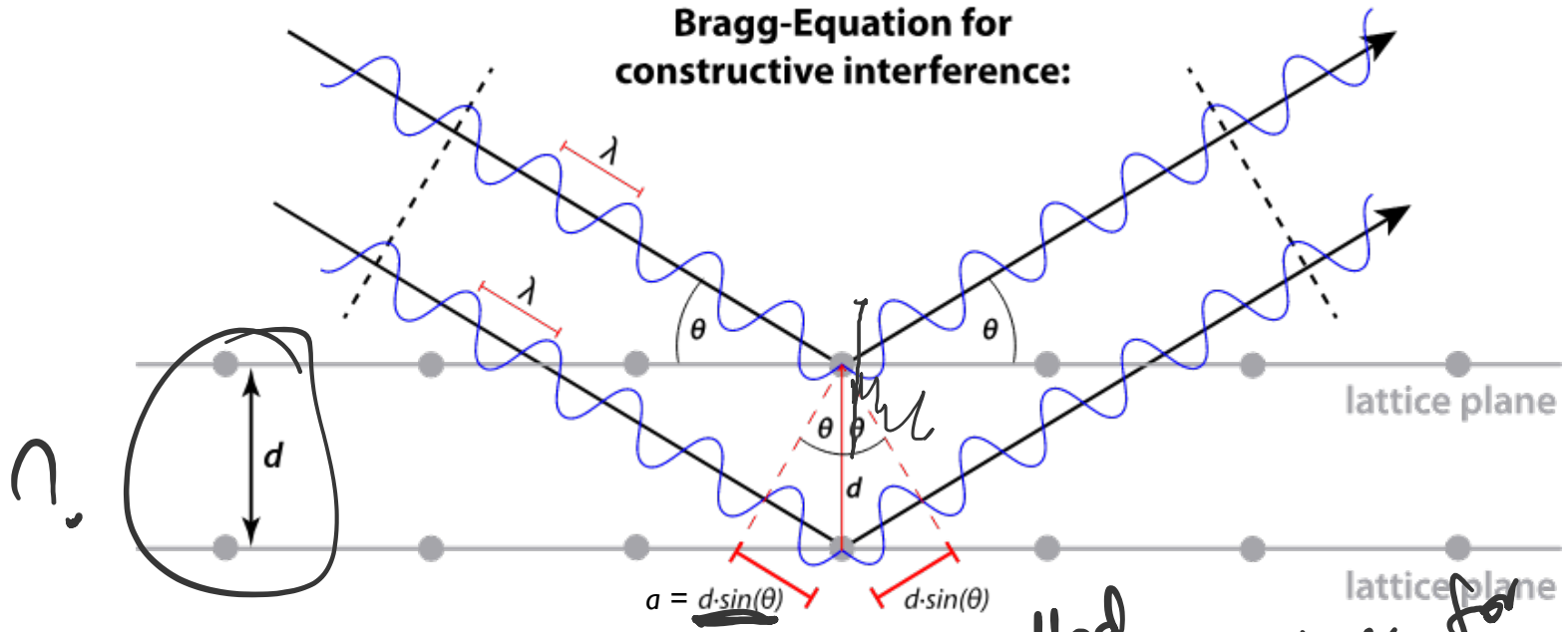


Figure from: https://courses.lumenlearning.com/introchem/chapter/interference-and-diffraction/

Bragg's Law: A simple lattice example



a = difference in path length

Constructive interference occurs when:

$$n\lambda = 2a$$

$$sin\theta = \frac{a}{d}$$

$$a = d \cdot sin\theta$$

$$n\lambda = 2 \cdot d \cdot sin\theta$$

Constructive interference only happens when this condition is met

$$n\lambda = 2 \cdot d \cdot sin\theta$$

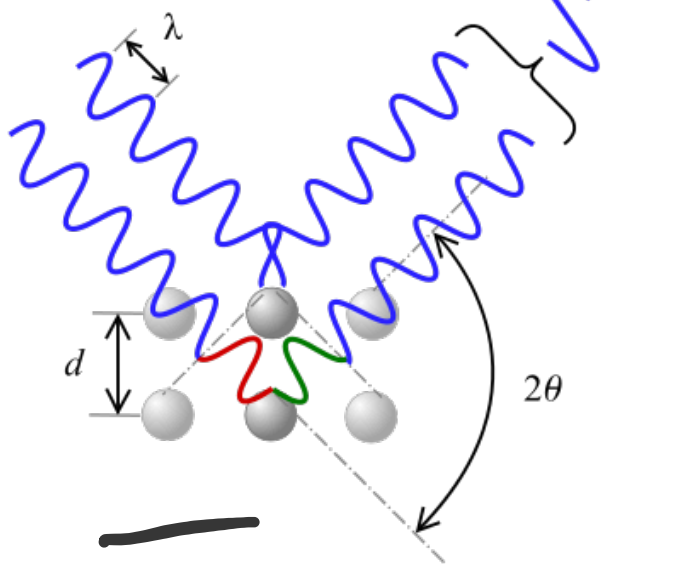
or

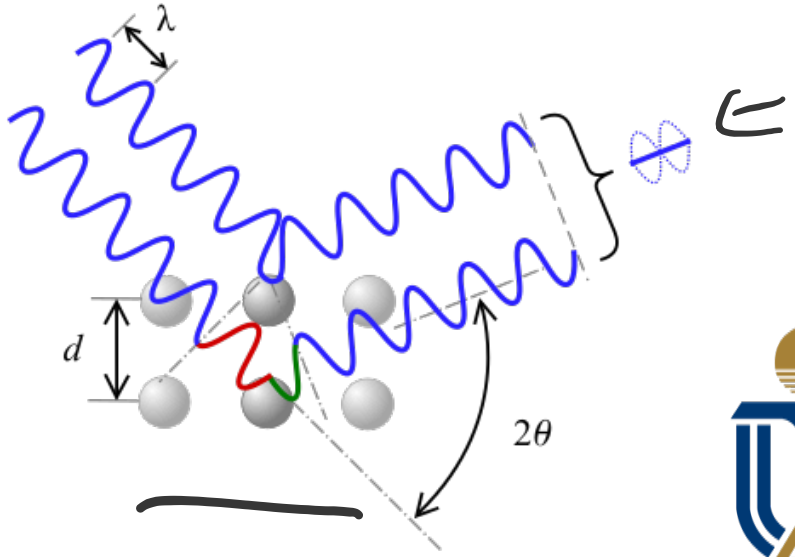
$$d = \frac{n\lambda}{2 \cdot sin\theta}$$

nλ is fixed, since we know the input wavelength (x-ray)

d is the unknown

Therefore, we can test all angle θ to find the value that gives the maximum diffracted intensity, and solve for d





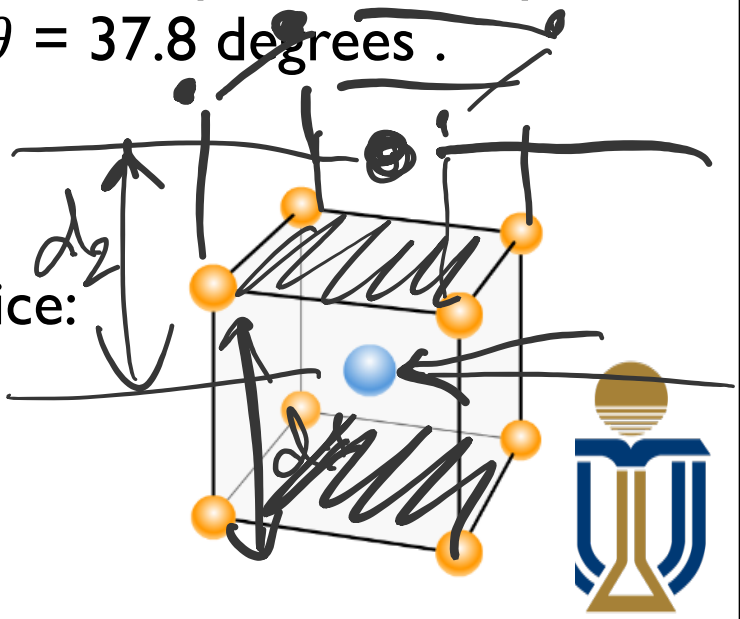
Exercise

- **Q1:** What is the distance between the adjacent planes of atoms if the first order reflection from X-rays of wavelength 239pm occurs at 27.5 degrees?

- **Q2:** The distance between two planes is 398.6 pm. An X-ray beam produces a strong interference 2θ = 37.8 degrees . What is the X- ray wavelength?

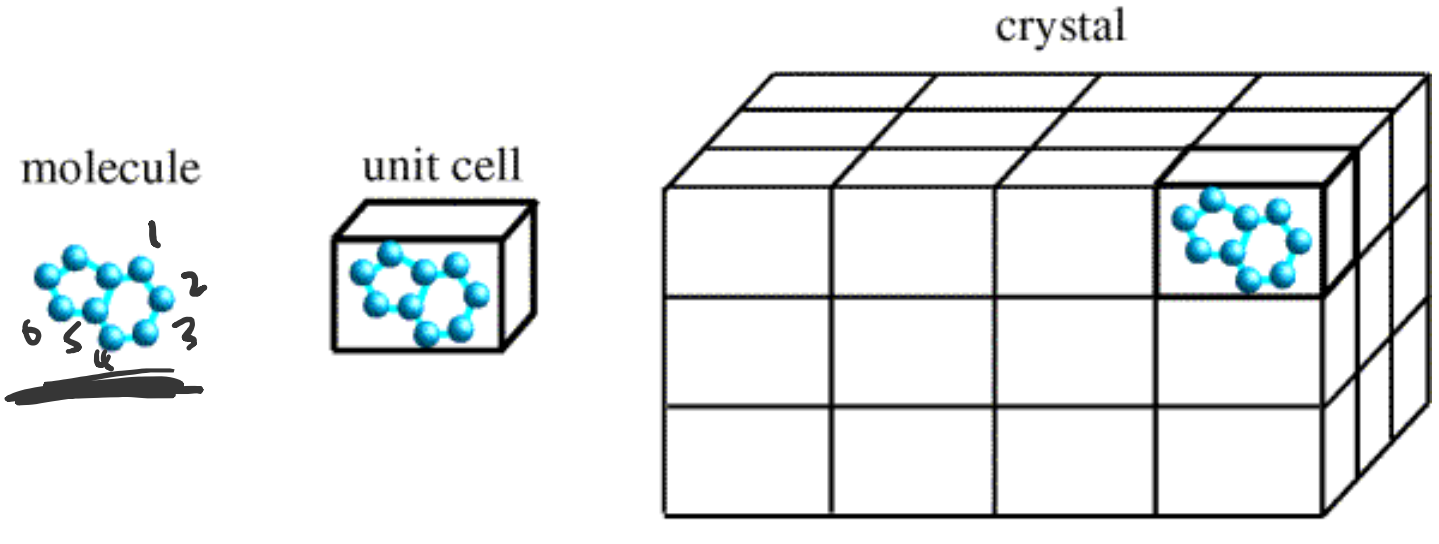
- **Q3**: Consider a body centred cubic lattice:

  What would you expect to see in the interference patterns for this sample as you change θ?

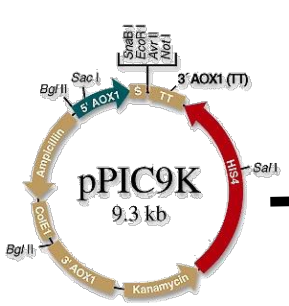


How to extrapolate the concept to proteins?

- Make proteins into neatly aligned crystals with lattice structure
- Do the same as before – measure interference intensities upon shooting with X-ray
- The measured intensities would now be the linear combinations of all the waves (whether after constructive or destructive or partial interference)

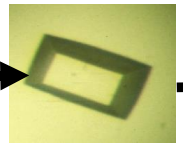


X-ray crystallography workflow

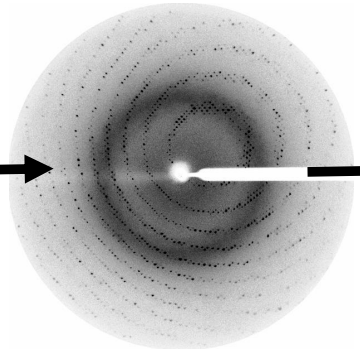


Construct for protein expression

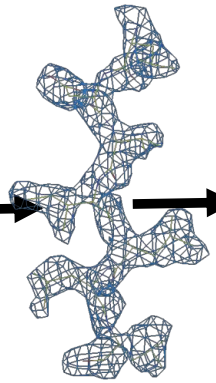
Protein purification



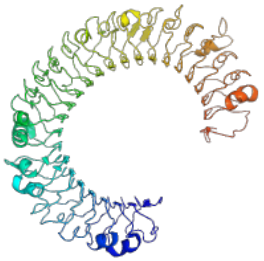
Crystallization



X-ray Diffraction



Electron density



3D structure

fail = no structure.

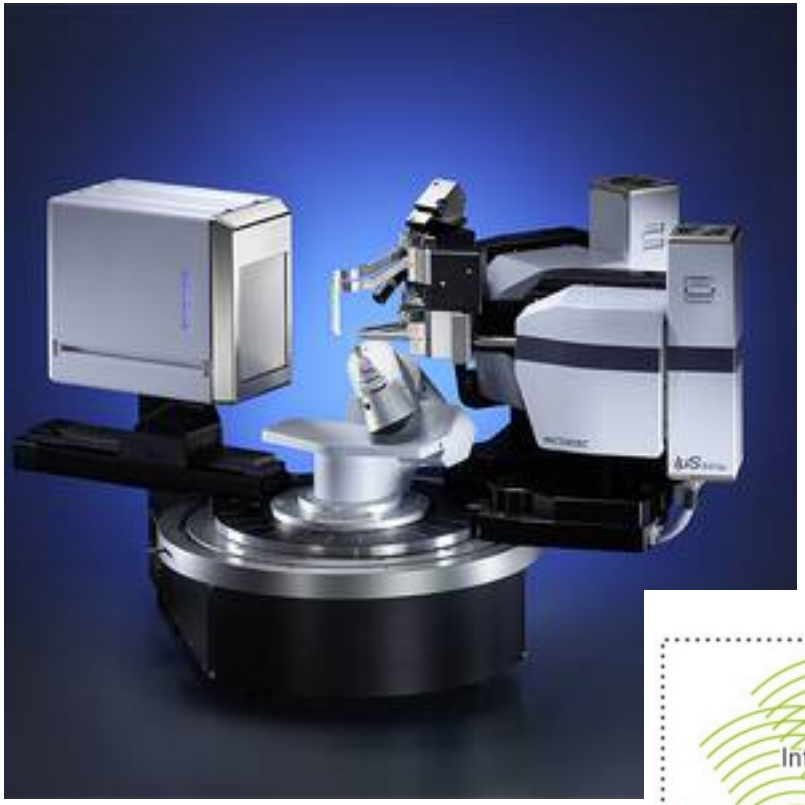
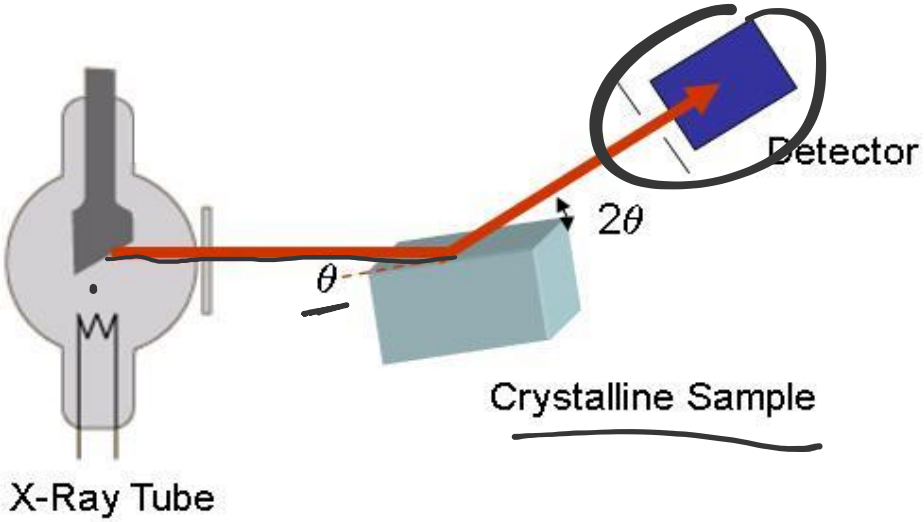
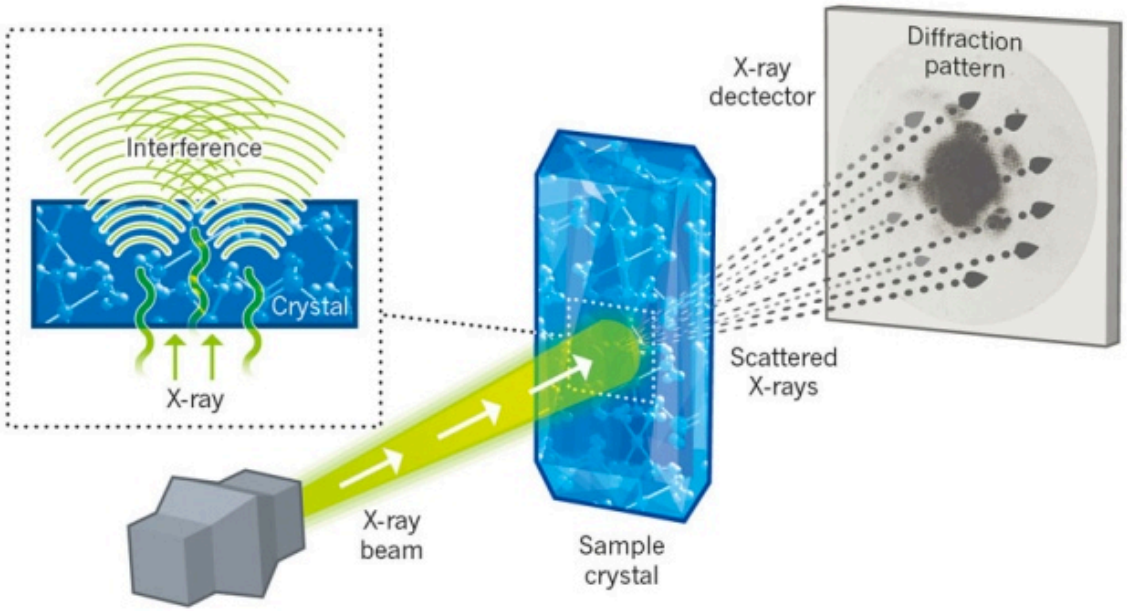


Photo from Bruker

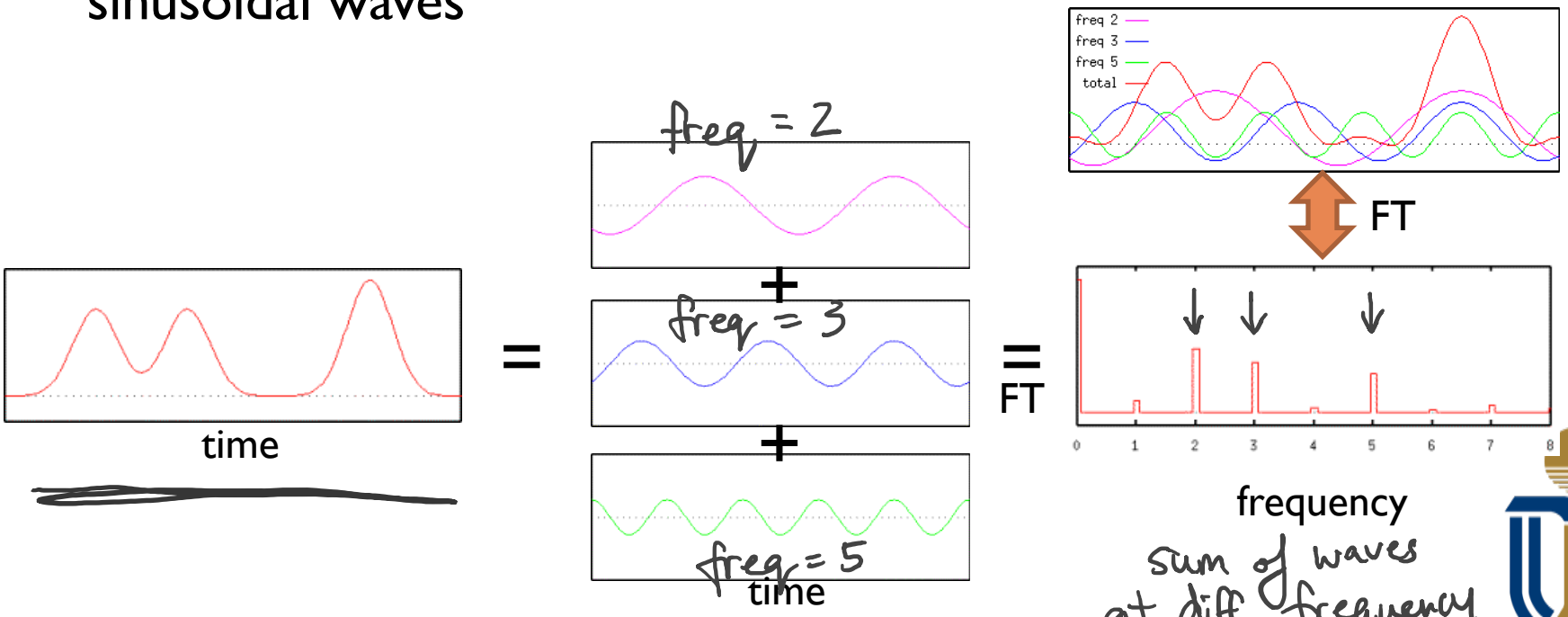




https://www.nature.com/news/crystallography-atomic-secrets-1.14608

Using Fourier Transforms to help solve the protein structure

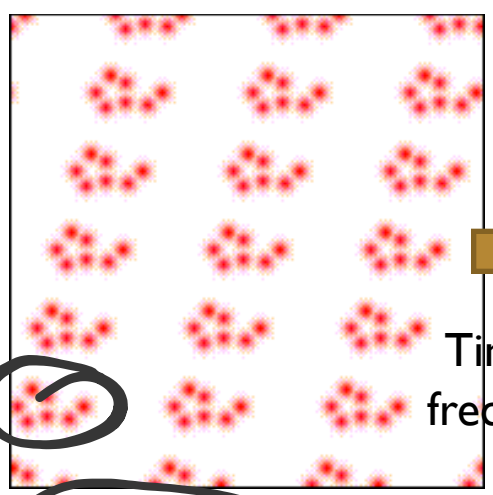
- Fourier transform is just using a different "unit" of accounting
- Instead of working in time, we can work in frequency
- As long as the signal measured is a linear combination of sinusoidal waves



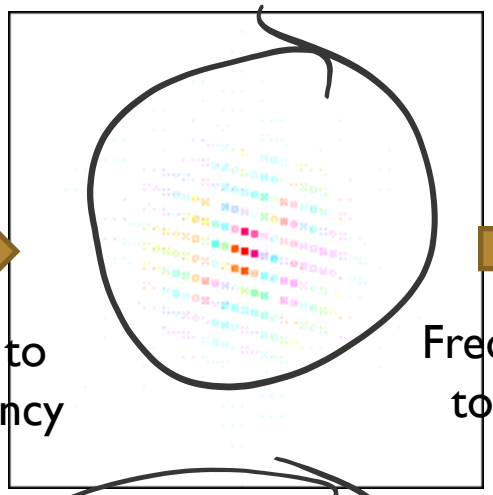
[Example taken from: http://pruffle.mit.edu/atomiccontrol/education/xray/fourier.php](http://pruffle.mit.edu/atomiccontrol/education/xray/fourier.php)

Using Fourier Transforms to help solve the protein structure

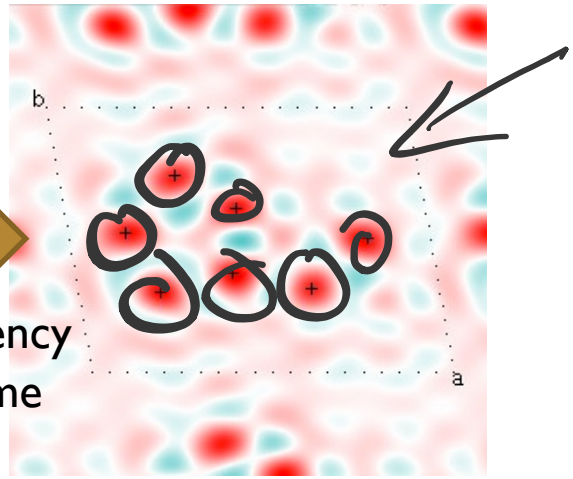
- So, this means we can use linear algebra to go back and forth



Time to frequency



Frequency to time



This is a grid arrangement of a molecule with seven atoms

This is what the diffraction pattern would look like. It is the FT of the of the molecule (but we only get amplitude)

This (zoomed in) electron density map is generated from the measured interference signal using FT. Note that it is noisy.

Example taken from: http://www.ams.org/publicoutreach/feature-column/fc-2011-10

More resources to understand Fourier Transforms

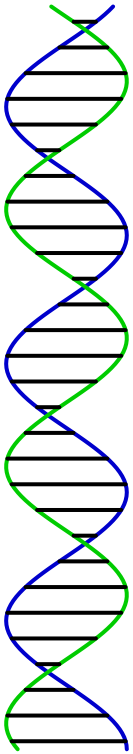
- Basics of Fourier transforms for laymen: https://betterexplained.com/articles/an-interactive-guide-to-the-fourier-transform/

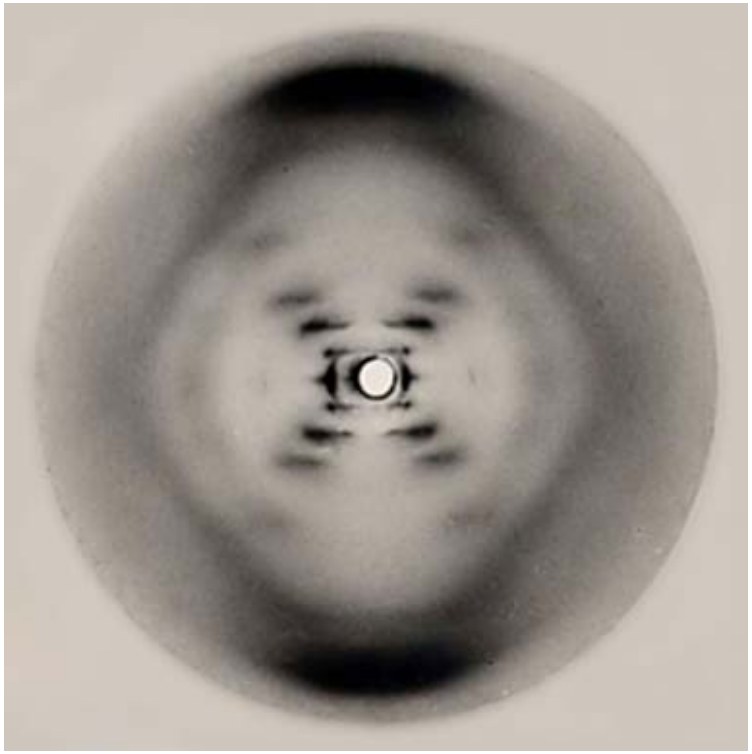
- Video: https://www.khanacademy.org/science/electrical-engineering/ee-signals/ee-fourier-series/v/ee-fourier-series-intro

- Fourier transforms and X-ray crystallography, very nice explanation and more images: http://www.ysbl.york.ac.uk/~cowtan/fourier/crys1.html
  - Click "More" at the bottom to progress through the entire series

An Historic Fourier Transform

This is an x-ray crystallographic image of DNA, and it shows the Fourier transform of the structure of DNA.





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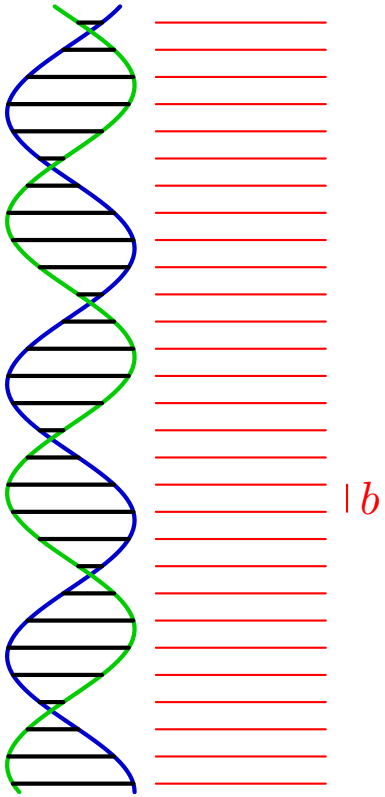
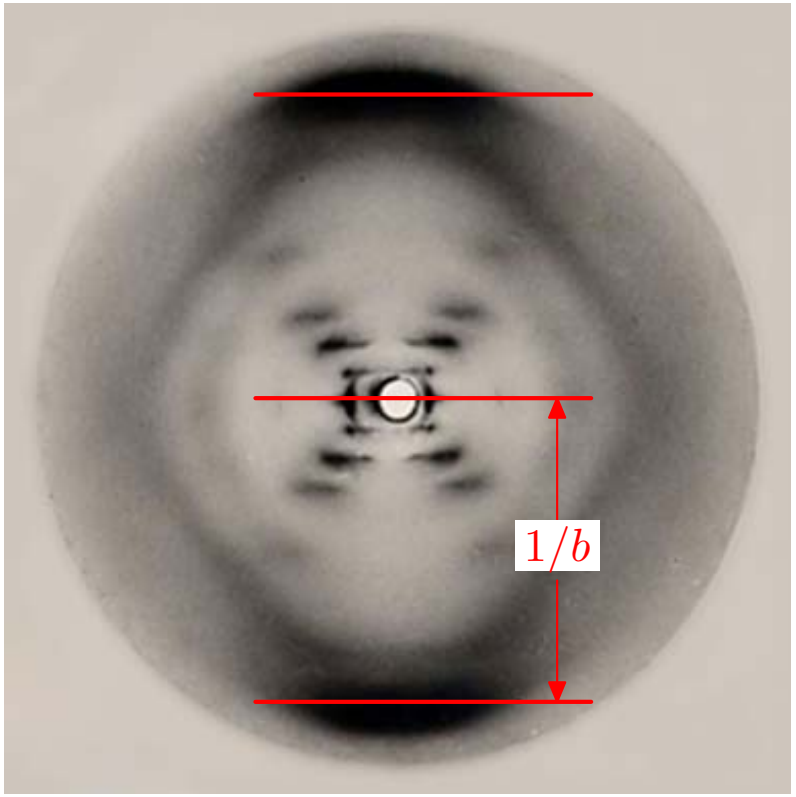
https://ocw.mit.edu/courses/electrical-engineering-and-computer-science/6-003-signals-and-systems-fall-2011/lecture-videos/MIT6_003F11_lec20.pdf

An Historic Fourier Transform

High-frequency bands indicate repeating structure of base pairs.





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https://ocw.mit.edu/courses/electrical-engineering-and-computer-science/6-003-signals-and-systems-fall-2011/lecture-videos/MIT6_003F11_lec20.pdf

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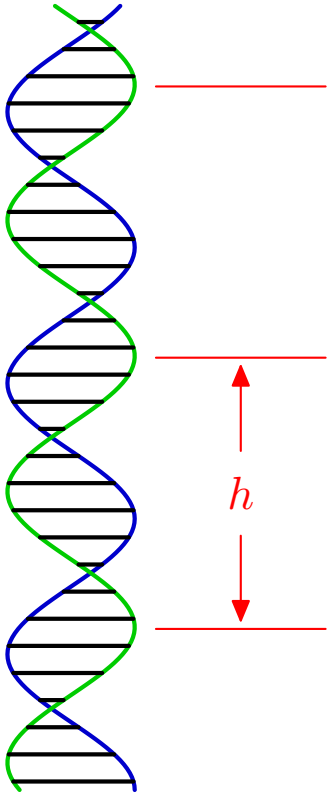
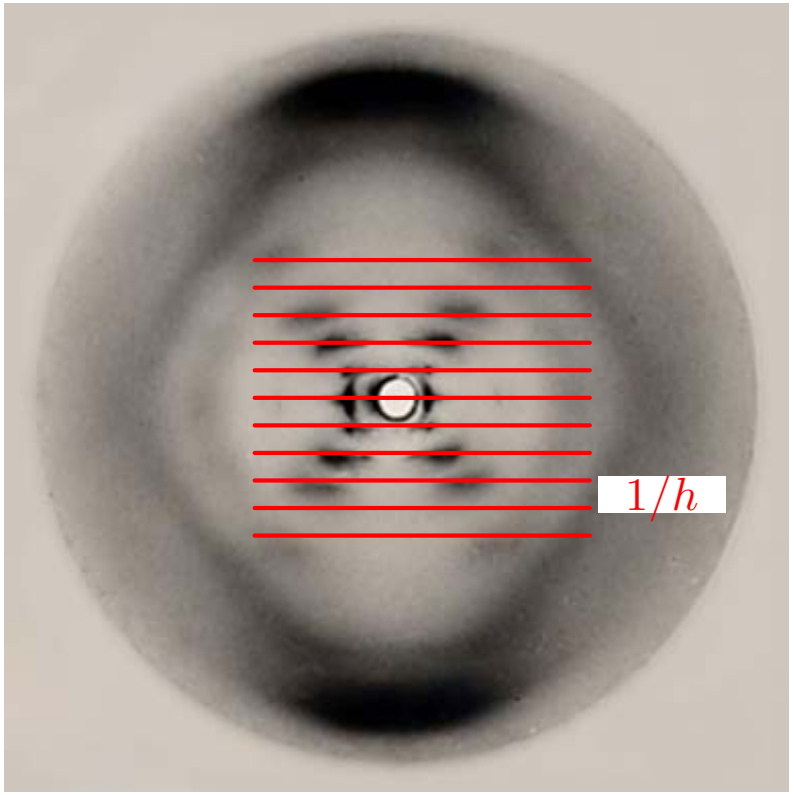
An Historic Fourier Transform

Low-frequency bands indicate a lower frequency repeating structure.





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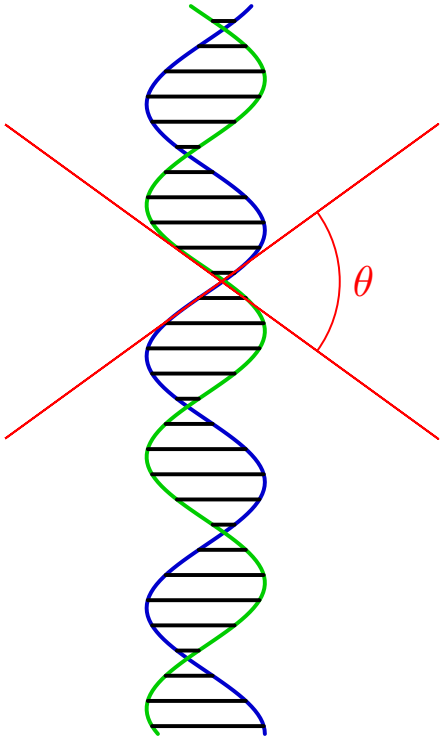
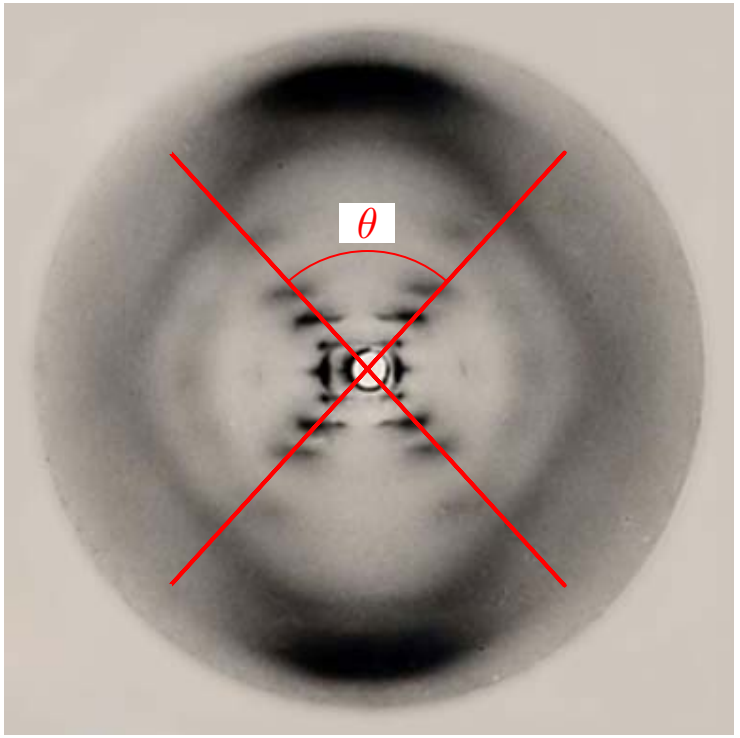
https://ocw.mit.edu/courses/electrical-engineering-and-computer-science/6-003-signals-and-systems-fall-2011/lecture-videos/MIT6_003F11_lec20.pdf

An Historic Fourier Transform

Tilt of low-frequency bands indicates tilt of low-frequency repeating structure: the double helix!





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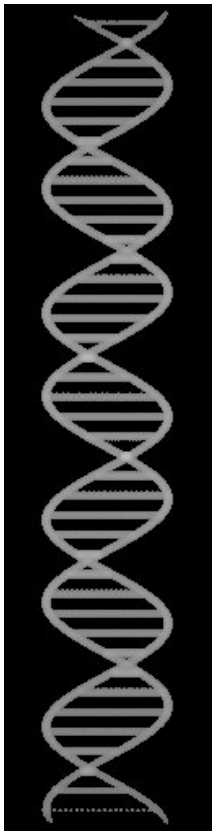
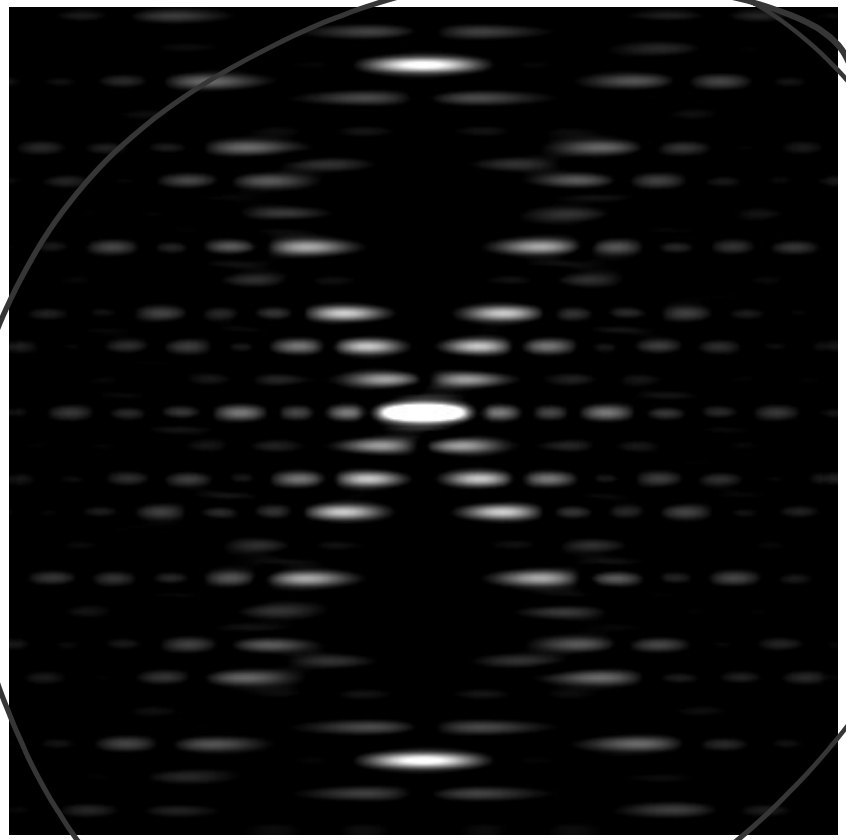
https://ocw.mit.edu/courses/electrical-engineering-and-computer-science/6-003-signals-and-systems-fall-2011/lecture-videos/MIT6_003F11_lec20.pdf

Simulation

Easy to calculate relation between structure and Fourier transform.

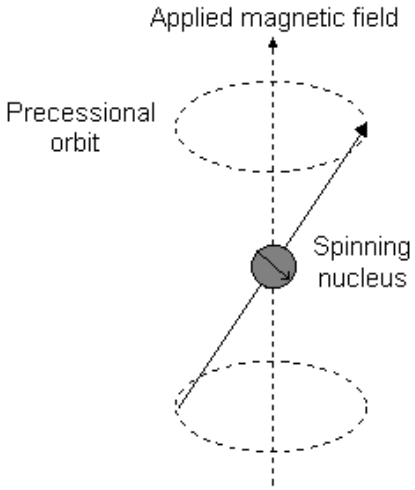




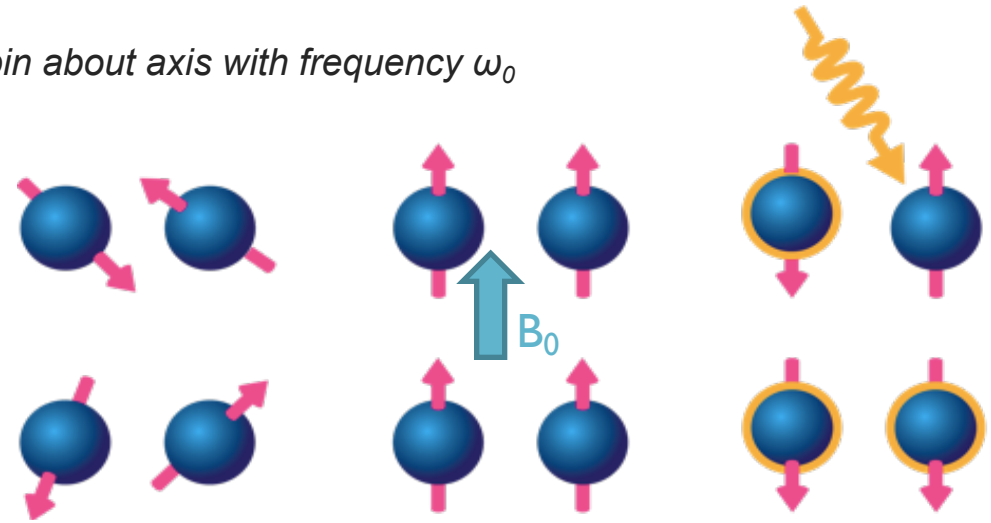
https://ocw.mit.edu/courses/electrical-engineering-and-computer-science/6-003-signals-and-systems-fall-2011/lecture-videos/MIT6_003F11_lec20.pdf 42

What is NMR?

- Nuclear Magnetic Resonance Imaging – detection of the magnetic spin of protons using very powerful magnets



Spin about axis with frequency ω_0



No external magnetic field: Spin in random directions

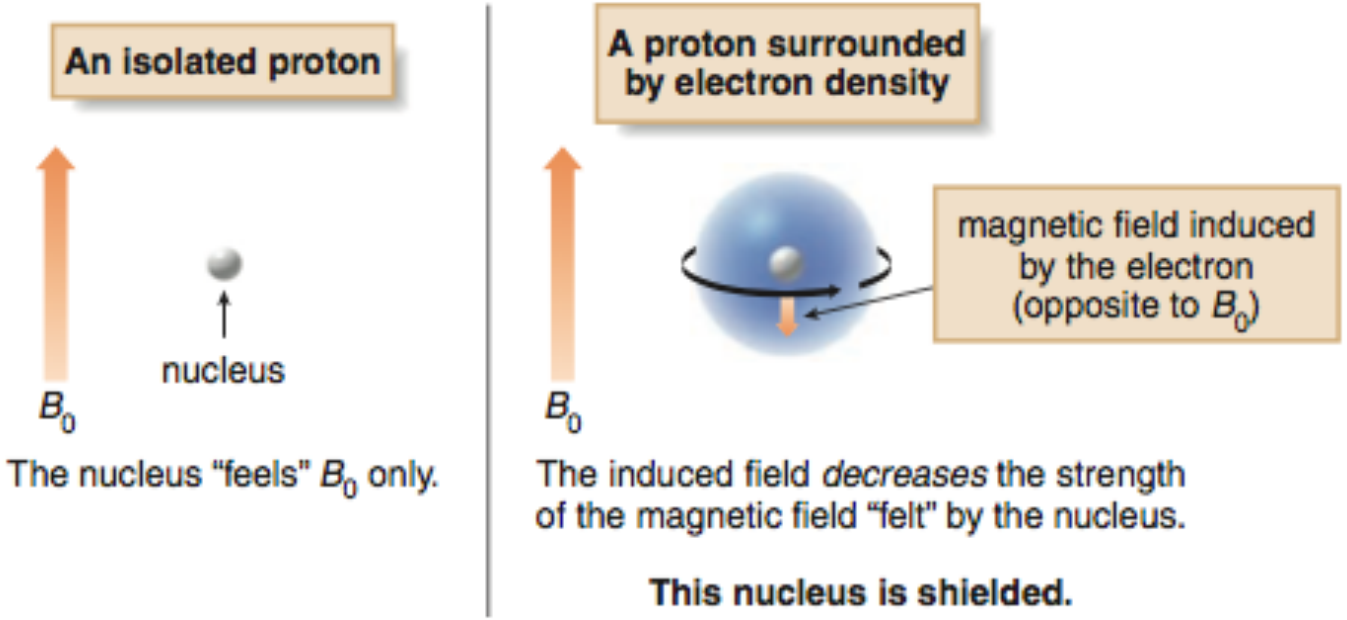
Apply external magnetic field B_0: Axis of spin aligns with external field

Add RF pulse: Proton spins get synced and "knocked down" due to excitation, gradually recovering over time

http://wikidoc.org/index.php/Basic_MRI_Physics
Khan Academy - Chemical and physical sciences

NMR for peptides

Which one is higher, E1 or E2?



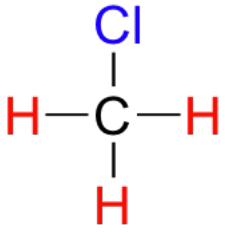
E1 – energy required to "flip" **deshielded** proton into anti-parallel alignment (aka into resonance)

E2 – energy required to "flip" **shielded** proton into anti-parallel alignment (aka into resonance)

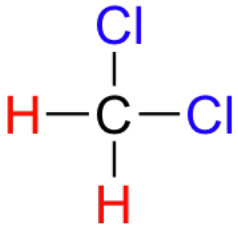
https://socratic.org/questions/what-is-shielding-and-deshielding-in-nmr-can-you-give-me-an-example

Which one is more shielded?

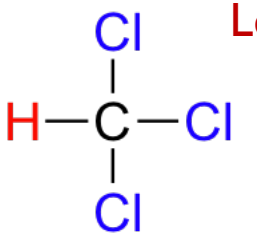
Most shielded



3.05 ppm

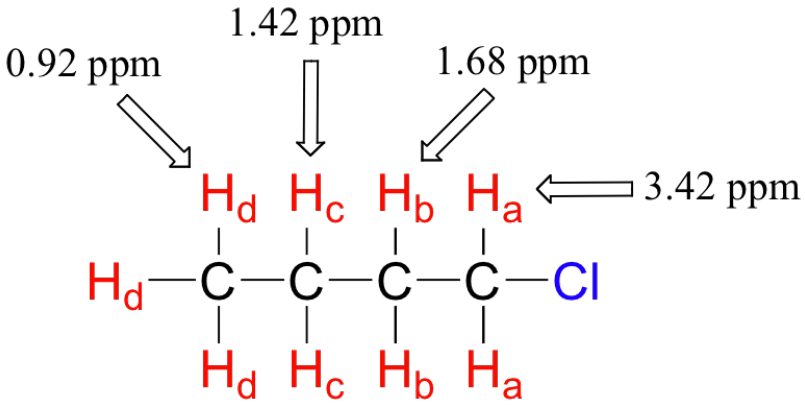


5.30 ppm



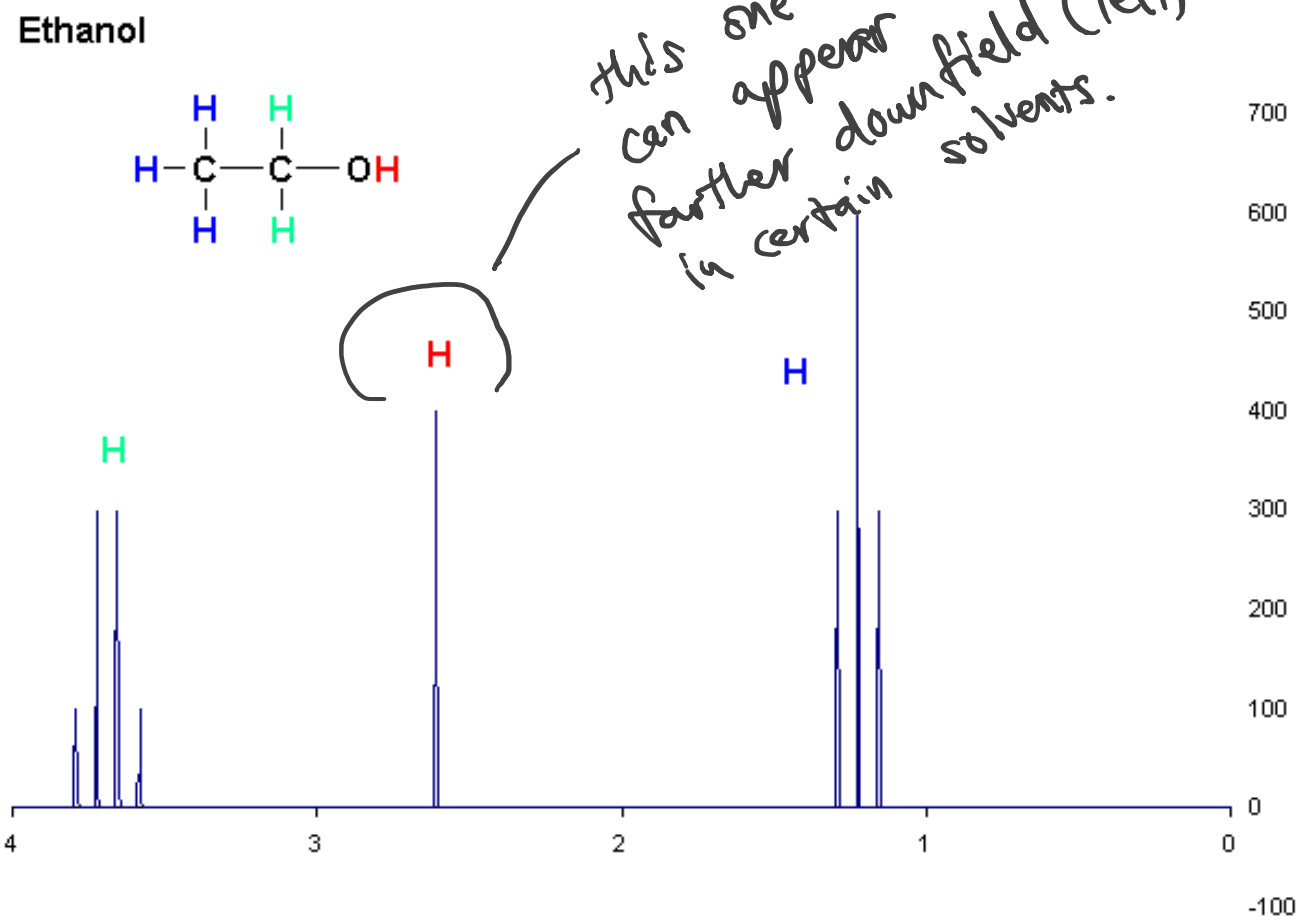
7.27 ppm

Least shielded



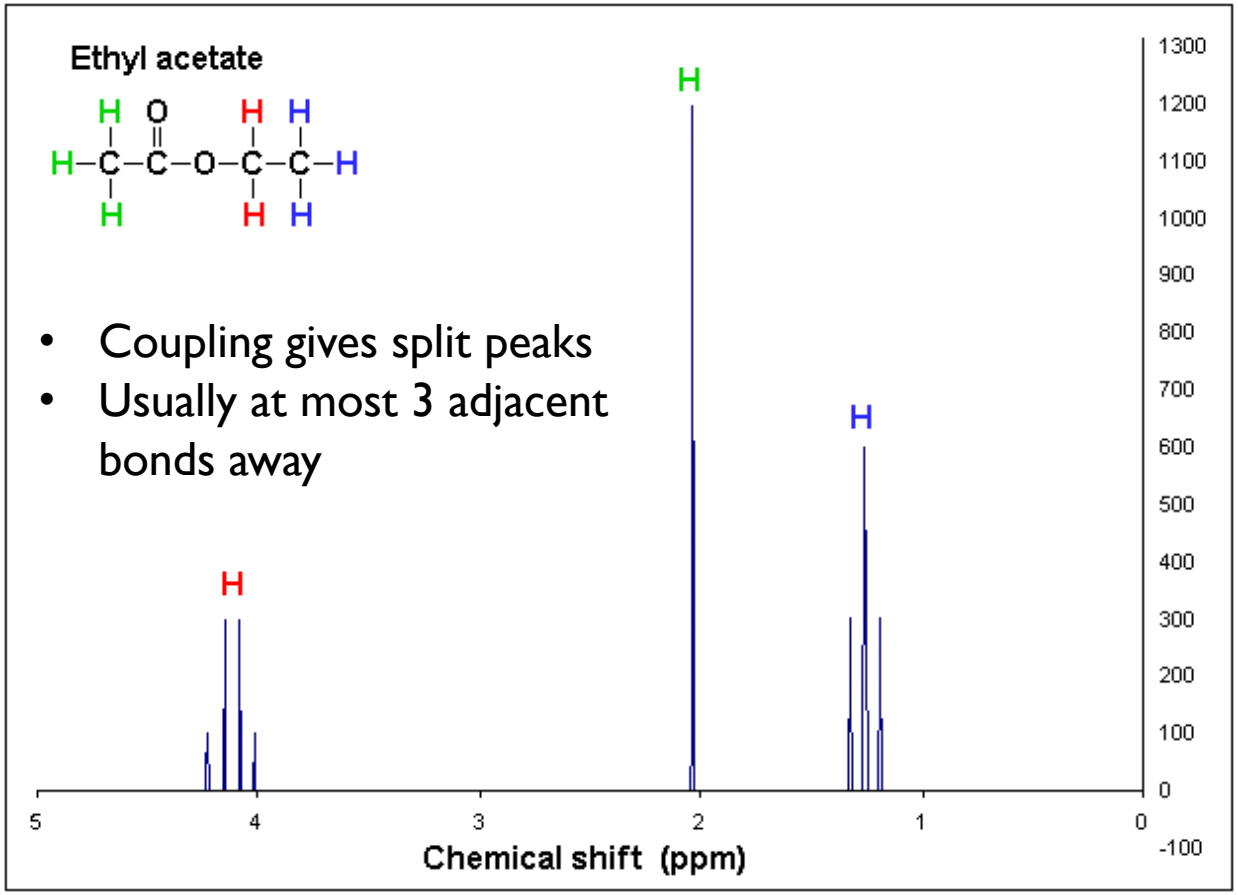
https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Book%3A_Organic_Chemistry_with_a_Biological_Emphasis_(Soderberg)/Chapter_05%3A_Structure_Determination_II/5.4%3A_The_basis_for_differences_in_chemical_shift

Coupling – when protons affect nearby protons with their own tiny magnetic field



By T.vanschaik - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=8452750

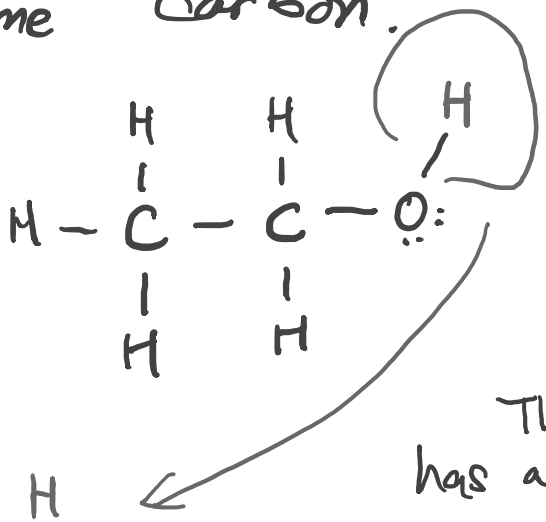
Coupling – when protons affect nearby protons with their own tiny magnetic field



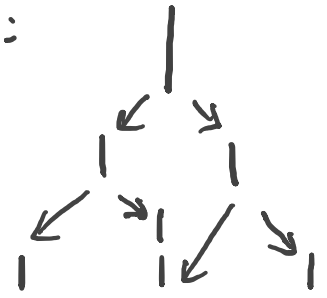
- Coupling gives split peaks
- Usually at most 3 adjacent bonds away

By 1H_NMR_Ethyl_Acetate_Coupling_shown.GIF:T.vanschaikderivative work: H Padleckas (talk) - This file was derived from: 1H NMR Ethyl Acetate Coupling shown - 2.png:, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=18159618

I made a mistake in class regarding coupling. Coupling is the effect of protons on ADJACENT carbons on the protons in question, not the effect from protons attached to the same carbon.

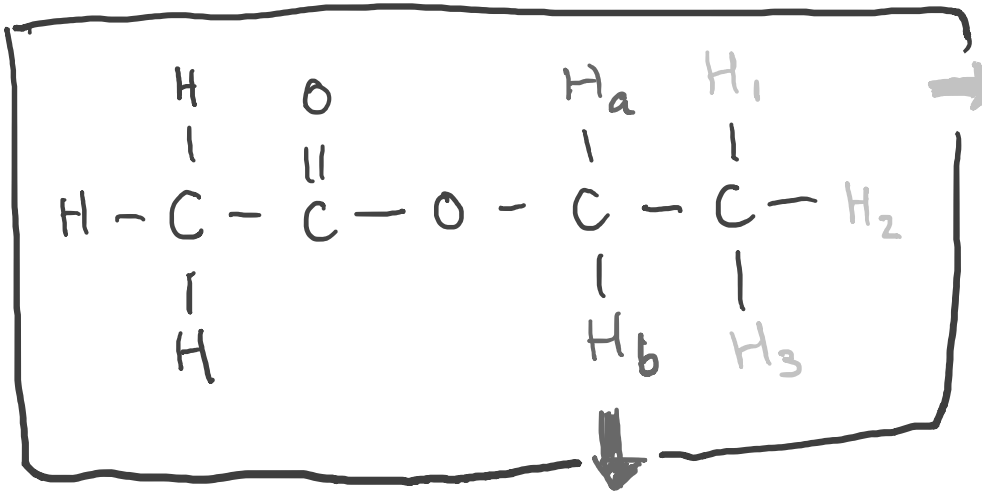


Ex: Red H are coupled by the two blue H, so the peak is split TWICE:



The final result has a higher middle peak because it is the sum of the two split peaks

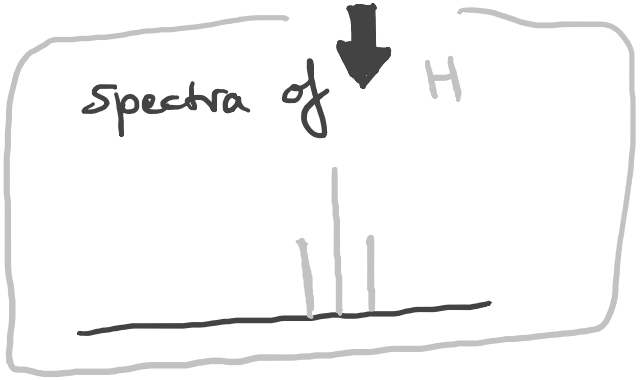
This H is special because of the attached O. In certain solvents, it does appear furthest downfield.



coupling from two green H

coupling Ha

coupling Hb



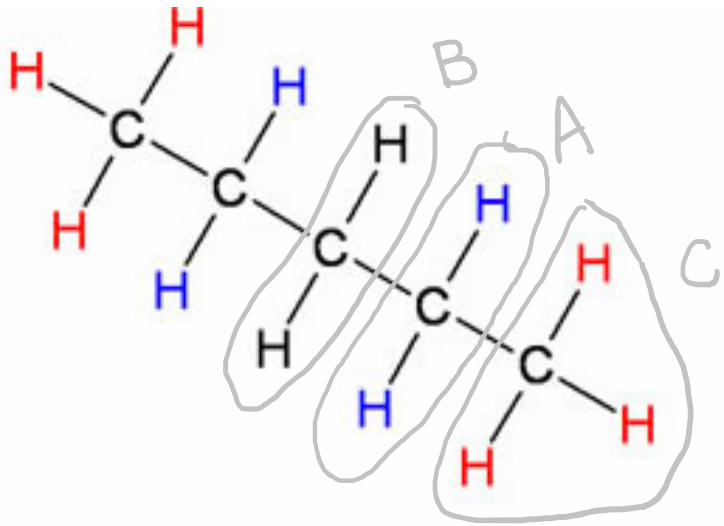
Coupling from three yellow H

Coupling with H1

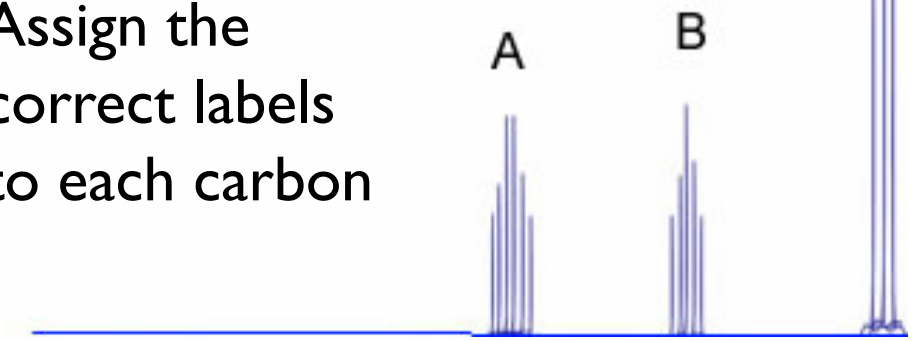
coupling with H2

coupling with H3

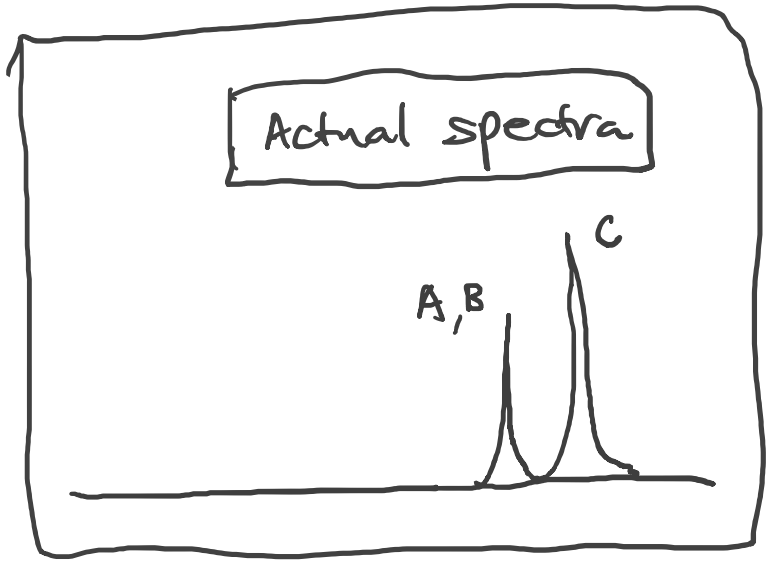
Exercise



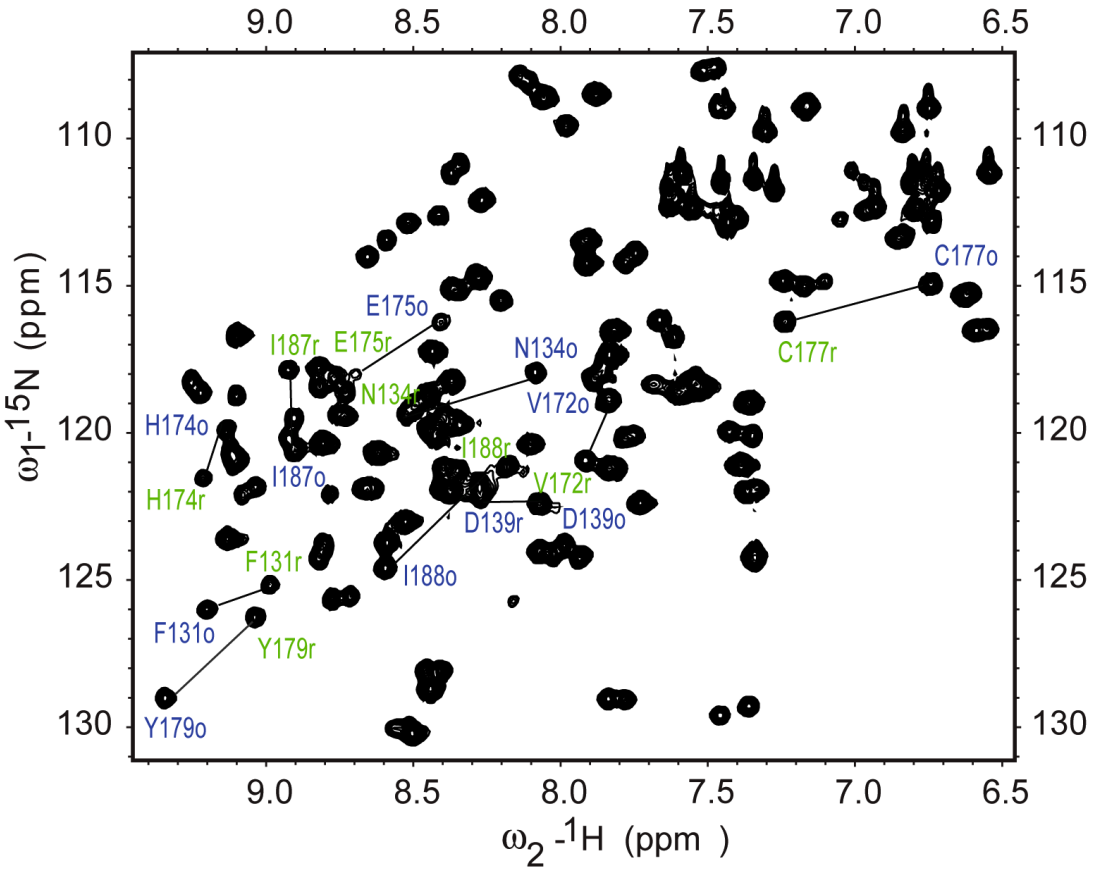
Assign the correct labels to each carbon



This question is contentious, because in reality the resolution of the spectrum is usually not high enough to distinguish A (blue) and B (black), since they are in similar environment. They will merge into one peak, and be downfield (to the left) of the peak for C. However, for the sake of the problem set, the idealized spectra is acceptable.



2D NMR spectra for proteins

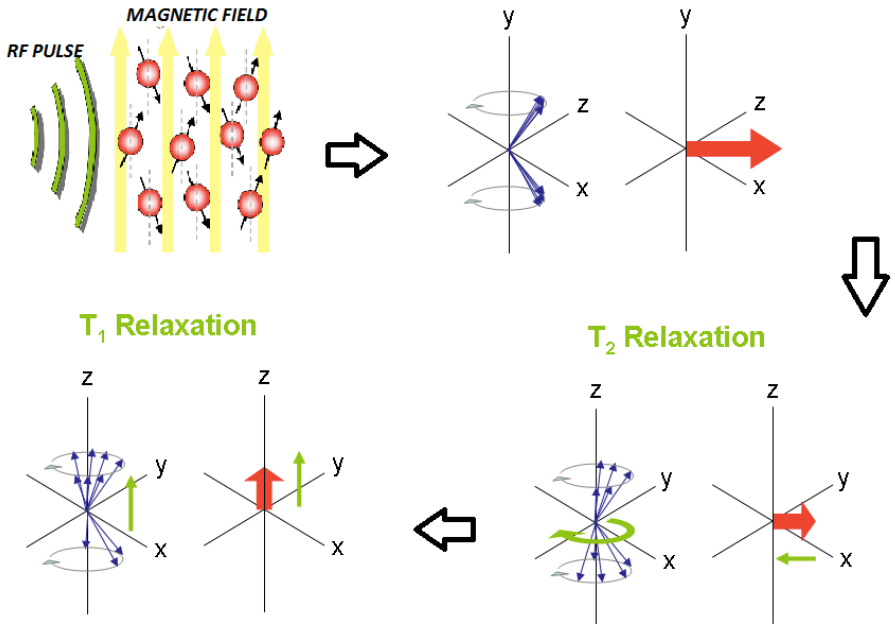


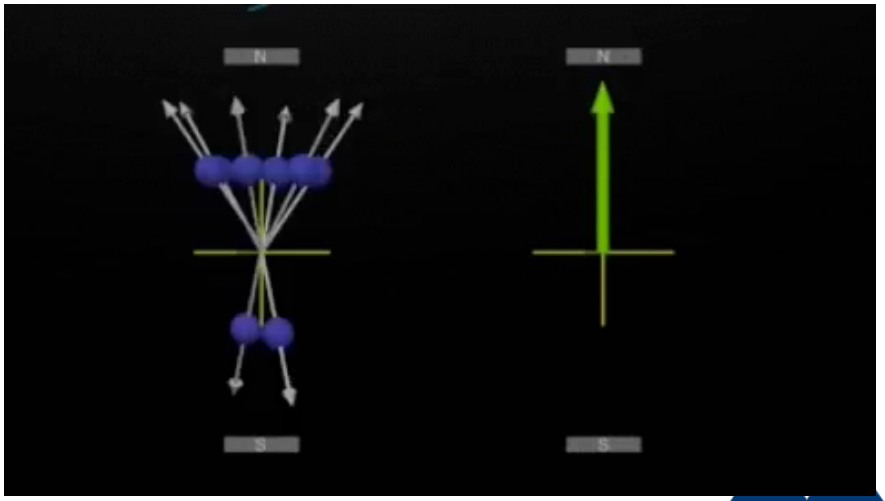
By See above citation - Wu, Bin; Skarina, Tatiana, Yee, Adelinda, Jobin, Marie-Claude, DiLeo, Rosa, Semesi, Anthony, Fares, Christophe, Lemak, Alexander, Coombes, Brian K., Arrowsmith, Cheryl H., Singer, Alexander U., Savchenko, Alexei, Stebbins, C. Erec (June 2010). "NleG Type 3 Effectors from Enterohaemorrhagic Escherichia coli Are U-Box E3 Ubiquitin Ligases". PLoS Pathogens 6 (6): e1000960. DOI:10.1371/journal.ppat.1000960. Retrieved on 29 June 2011., CC BY 2.5, https://commons.wikimedia.org/w/index.php?curid=15650313

Great video to help understand proton resonance, precession in MRI:
https://youtu.be/7aRKAXD4dAg?list=PL5F910D93AEB724CE

NMR in MRI – Magnetic Resonance Imaging

- Measurement of relaxation of protons after RF pulse as they de-phase and return to alignment with B_0 tells us about the composition of the material
- T2* = observed T2 in actual imaging environment, where magnetic field inhomogeneities contribute to faster decay than predicted





http://mriphysics.github.io/teaching-mri-intro.html
http://physiology-physics.blogspot.hk/2010/06/understanding-basic-principles-of.html