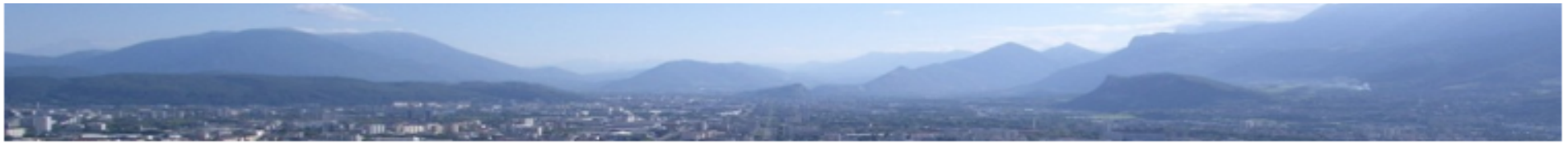

European School On Nanosciences & Nanotechnologies

Advanced biophysics for micro-system design

Learning from nature the future of nanobiotechnology

Ruud Hovius & Joachim Piguet

Laboratory for Physical Chemistry of polymers and Membranes

Ecole Polytechnique Fédérale - Lausanne

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ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

ESONN' european school on nanosciences & nanotechnologies

Structure of course

Part I – Biological systems
Labelling and Interactions

- Number and scale in biology
- Labelling proteins
- Complex chaos
- Interactions and networks
- Molecular construction
- Molecular machines

Part II - Molecular characterization
Methods and results

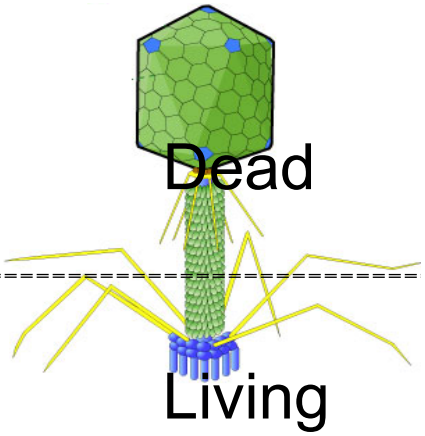
- Fluorescence and
  Single molecule detection
- Tracking of objects
- Super-resolution methods
- Fluctuation methods
- Single molecule experiments
- Single or few molecule devices

The “n” in biology

Nanoscience & -technology deal with the study, design & control of matter on an atomic and molecular scale of the size ≤ 100 nm.

Nano-objects in biology are either molecules or molecular assemblies

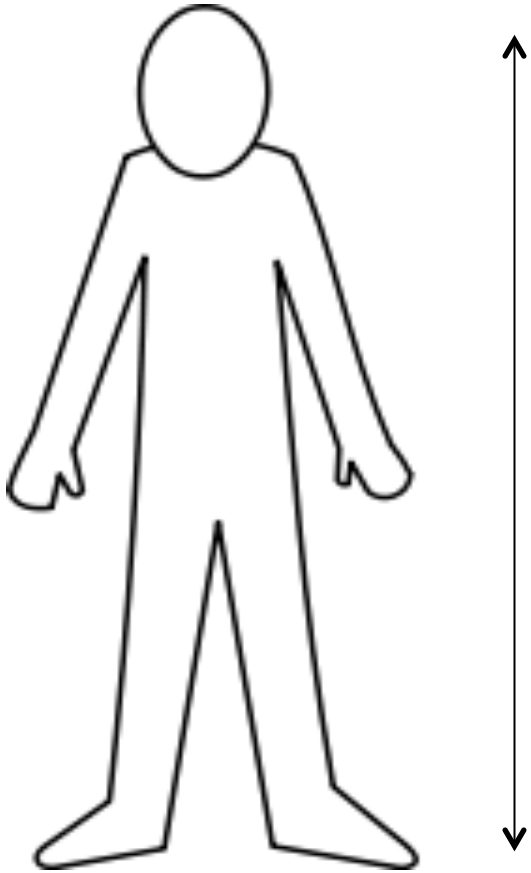
Solute molecules	1 Å - few nm
Proteins	1 - 100 nm
DNA	2.5 nm in diameter & 10^7 nm in length
Membranes	4 nm thick & 10^3 to 10^9 nm in length
Viri	10^1 to 10^2 nm
Bacteria	10^2 to 10^3 nm
Eukaryotic cells	µm to m’s



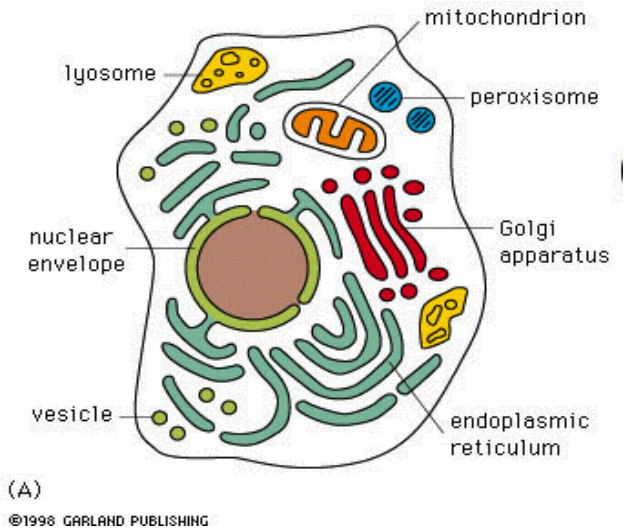
Cell Talk

Communication between the cells that constitute our body

One human is composed of ~10’000’000’000’000 cells.



2 meter



~0.01 millimeter

Communication

There are two complementary parts :

- Sending a message
- Perception of the message

Perception on the scale of man

The five senses

sight, hearing, smell, taste & touch

Perception on the scale of a cell

Protein or DNA-like molecules

=> induction of an electrical or chemical signal

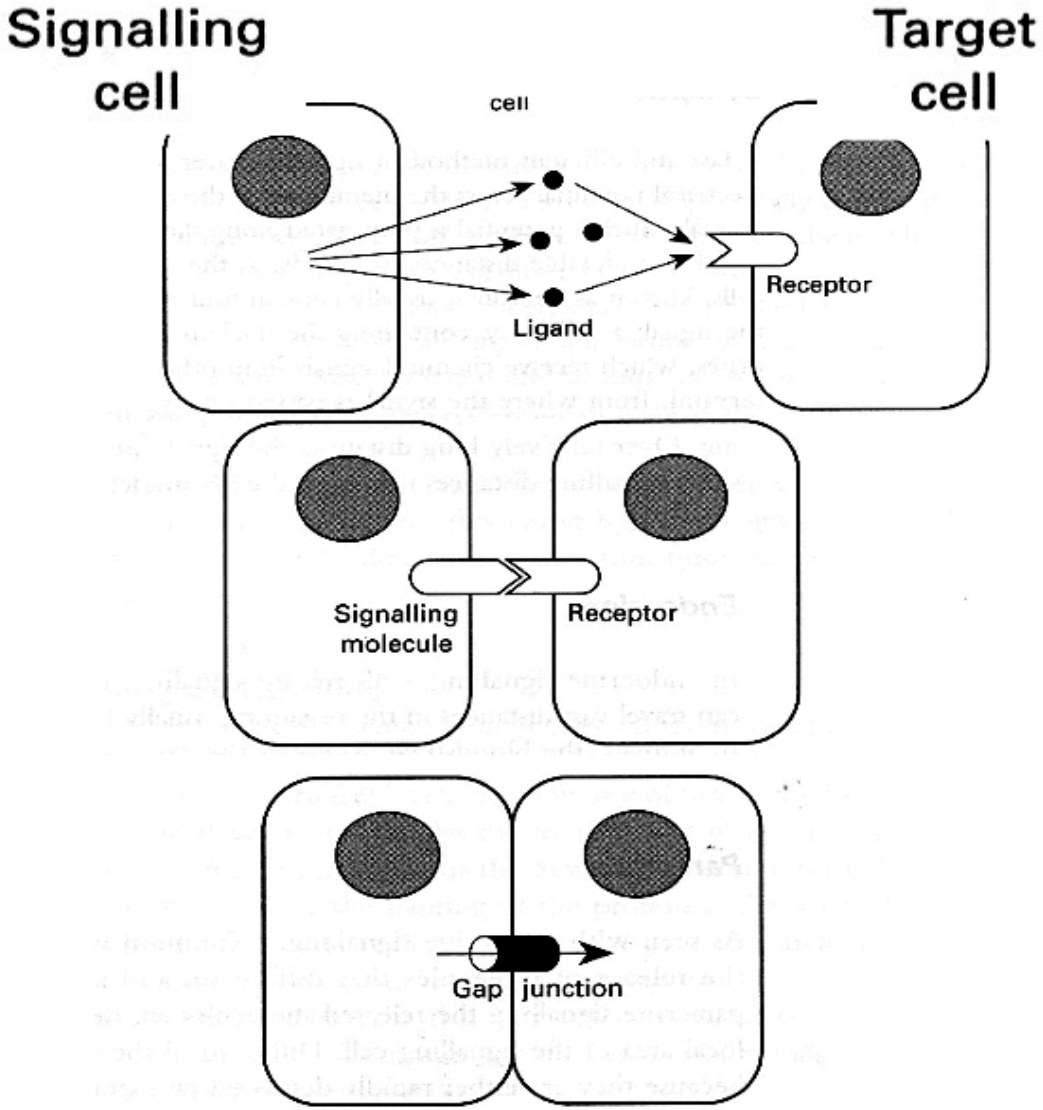
How do cells communcate ?

- Sending chemical compounds

- Contact detection

  “touch”

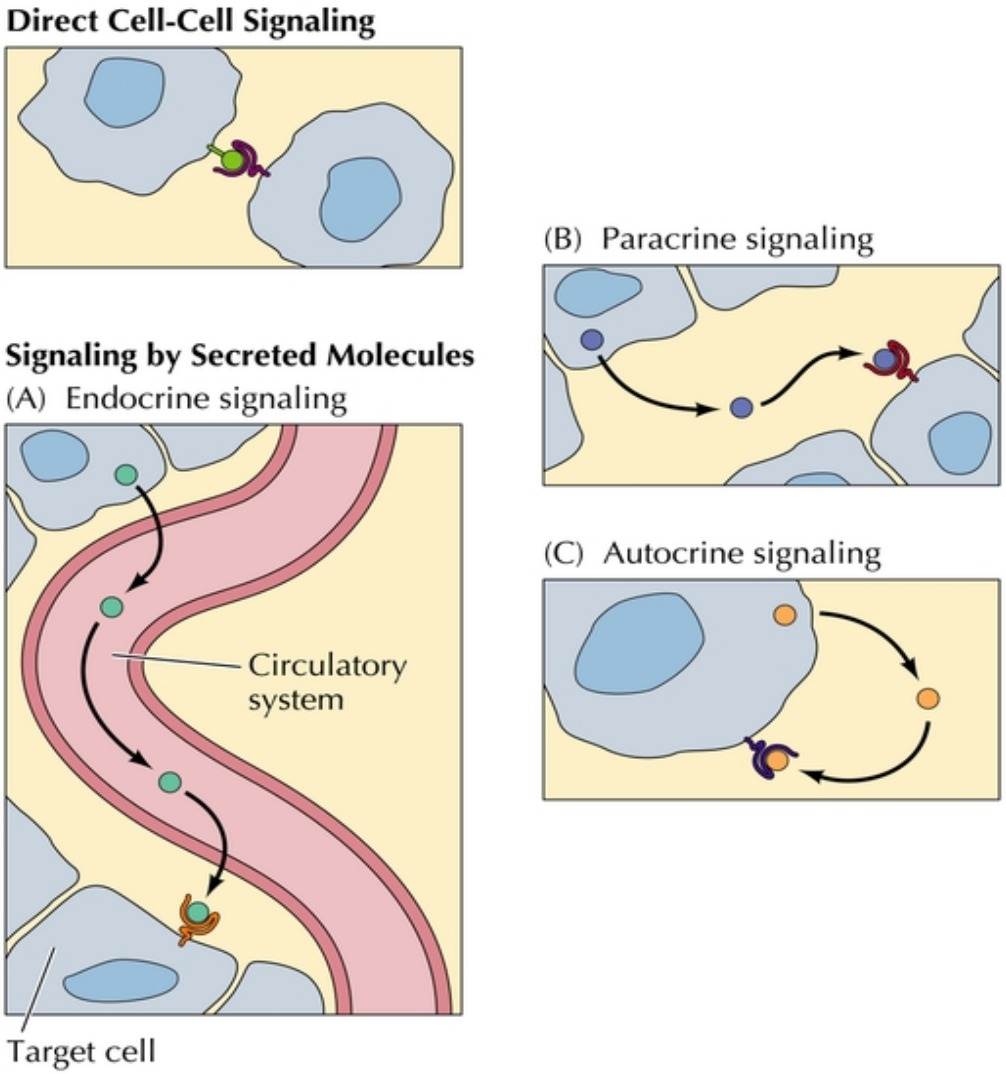
- Exchange of content



Sending chemical compounds

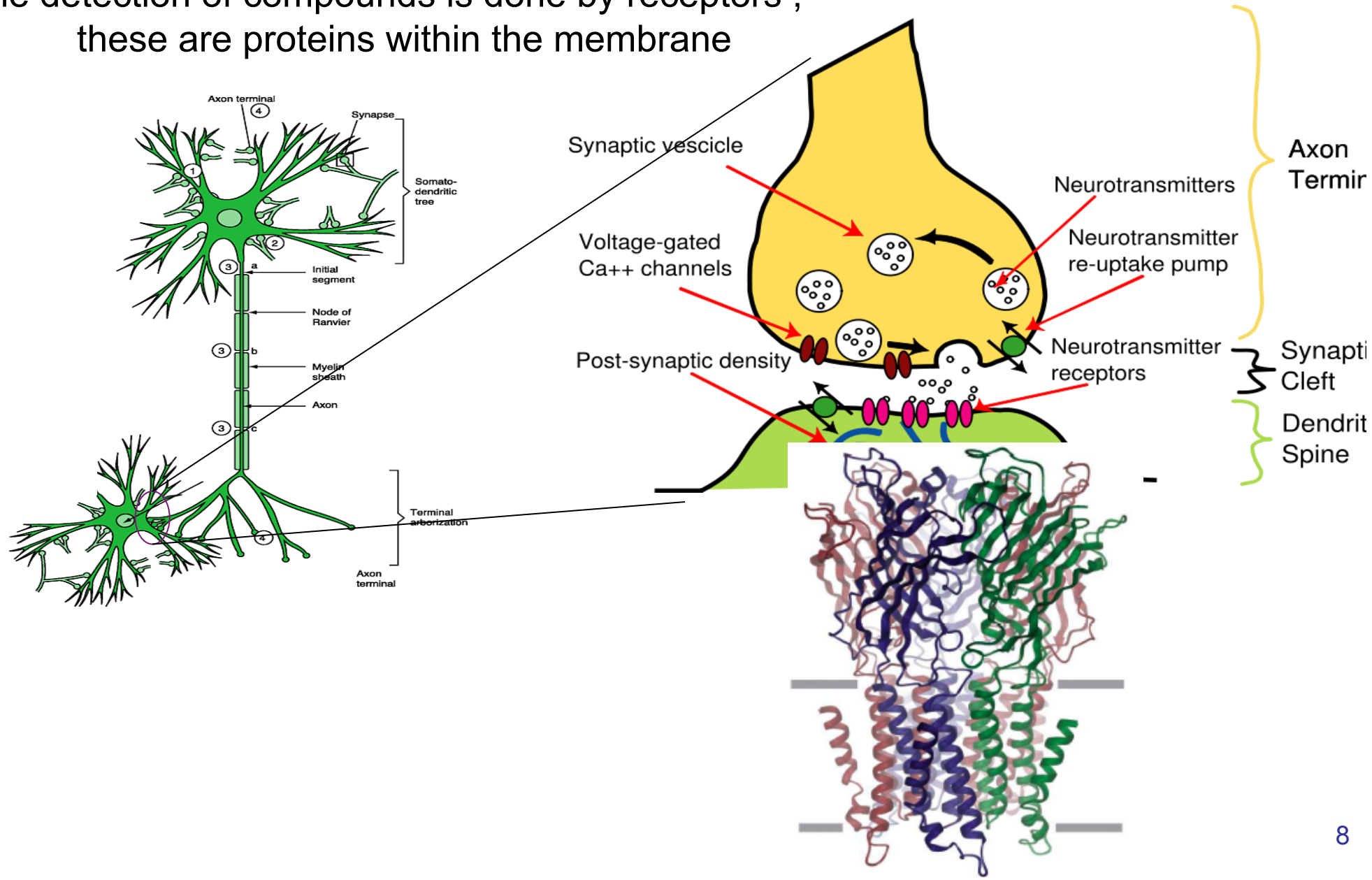
- Excretion => towards the exterior of the organisme

- Secretion => within the organisme
  - Endocrine :: long distance
  - Paracrine :: short distance
  - Juxtacrine :: neighbour by contact
  - Autocrine :: self
  - Synaptic :: neighbour



Detecting chemical signals by neurons

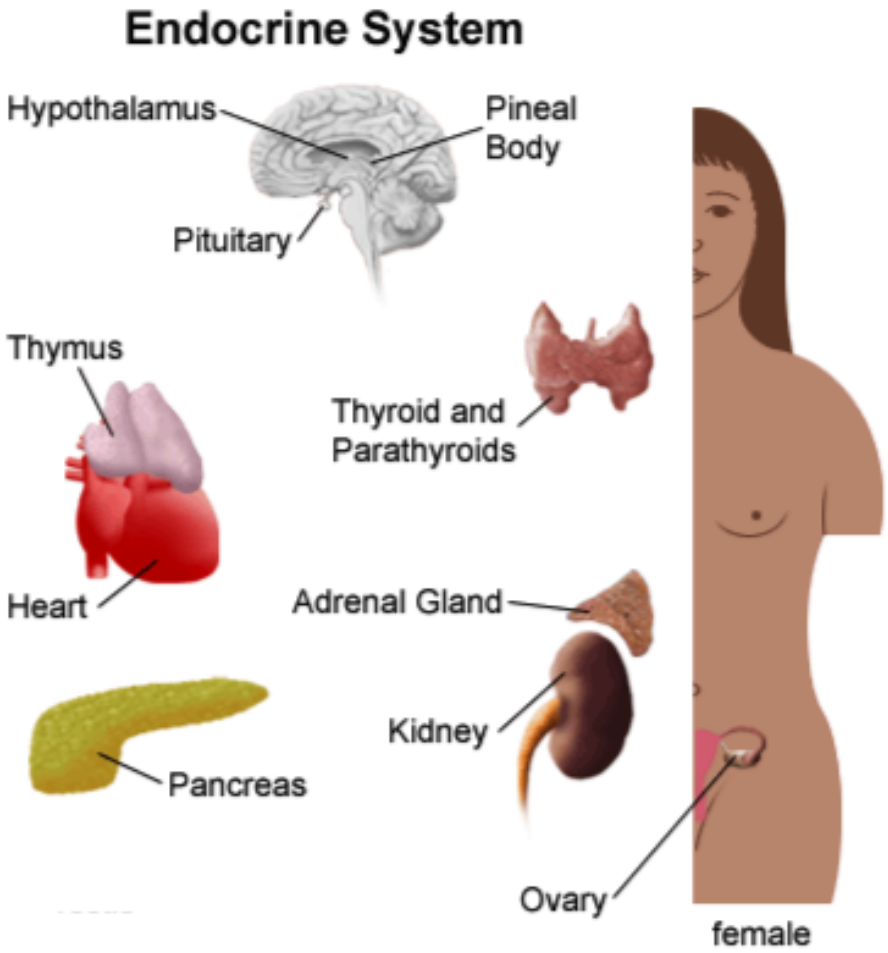
- The detection of compounds is done by receptors ; these are proteins within the membrane



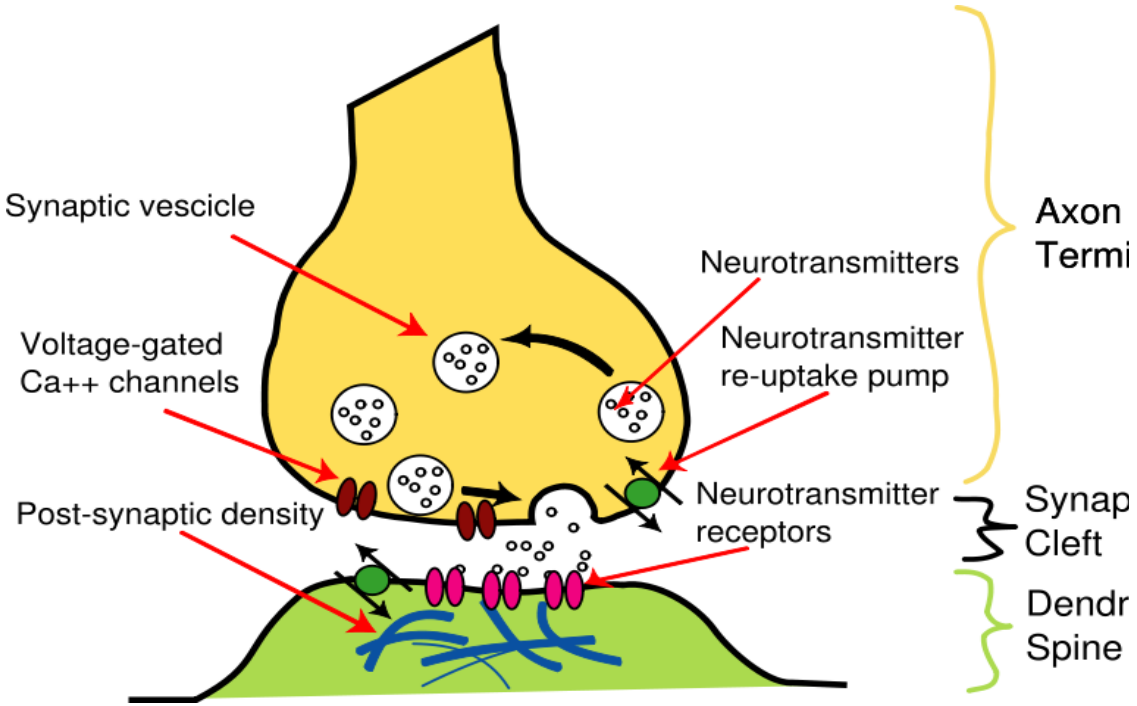
Scale of distance

The distance between sending and receiving cells can differ dramatically

÷ Endocrine



÷ Synaptic contact between neurons



50 nanometer

0.000’000’050 meter

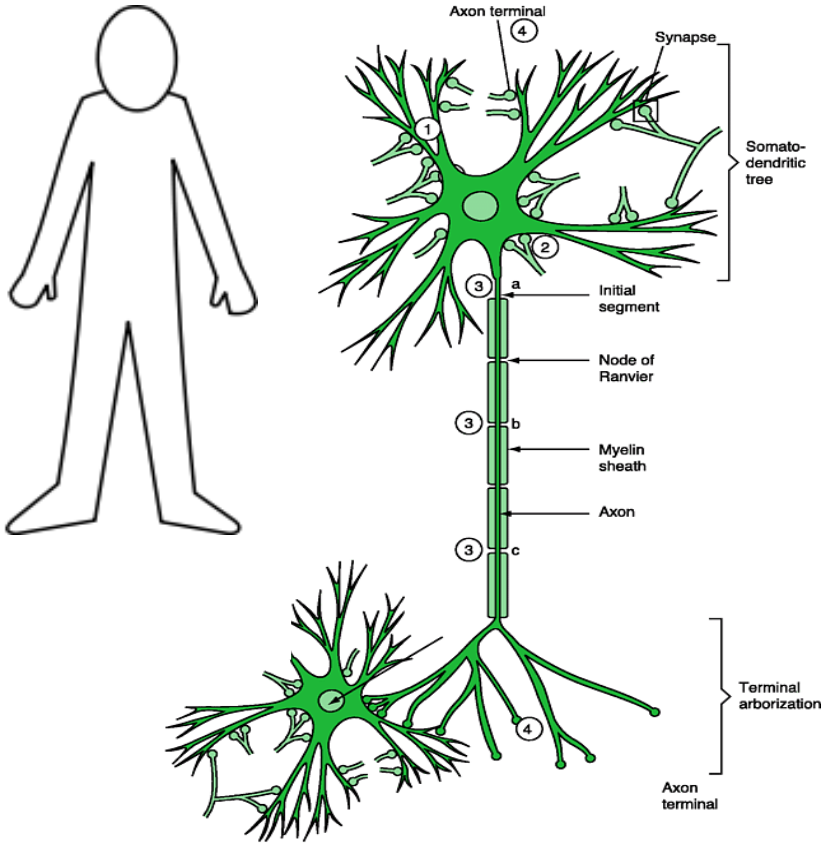
Long distance signalling between cells

Two complementary solutions :

÷ Long nerve cells

up to 1 meter

=> need ~ n^2 neurons

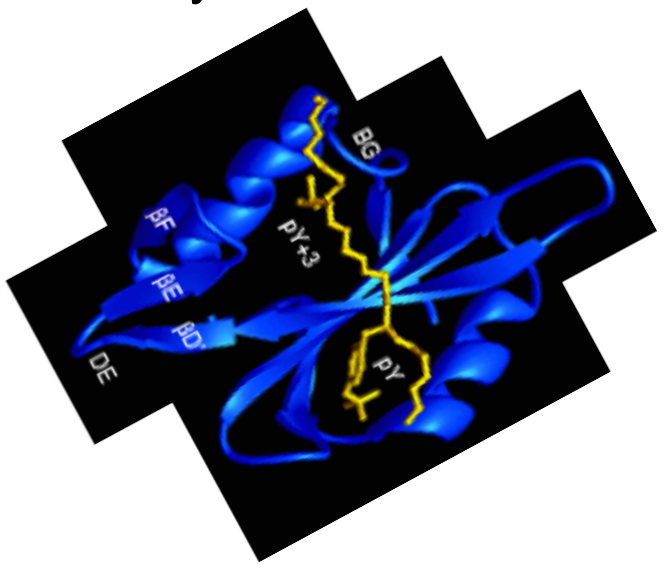


÷ Precise targetting of chemical signals

combined with

highly sensitve detection

=> Message & receptor with complex and complementary structures

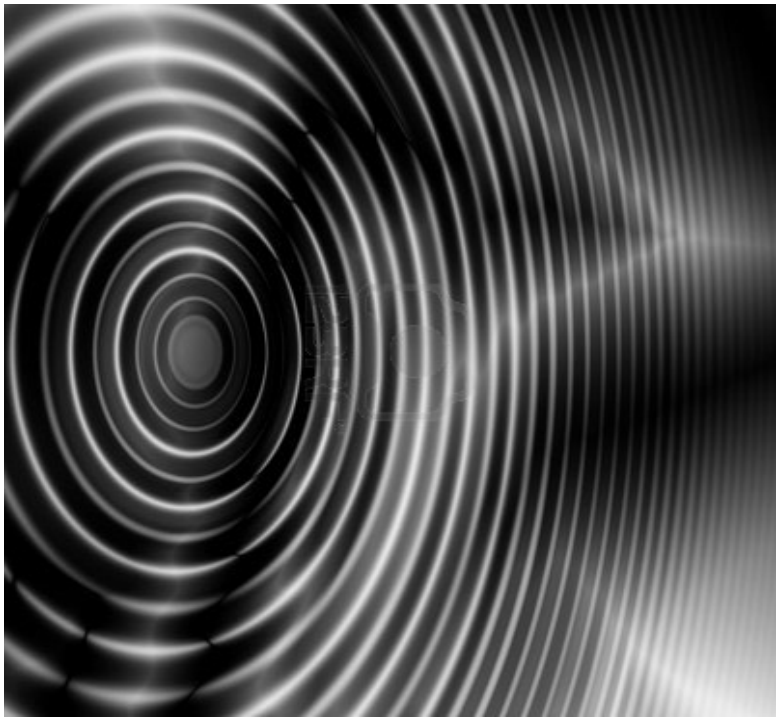


÷ Comparaison: telephone number

00 41 21 123 4567

The consequences of long distances

The signal diminishes with the distance **r**



In quantitative terms :
When one is at a distance **r**
from the source

÷ in two dimensions
the signal diminishes with $\mathbf{r^2}$

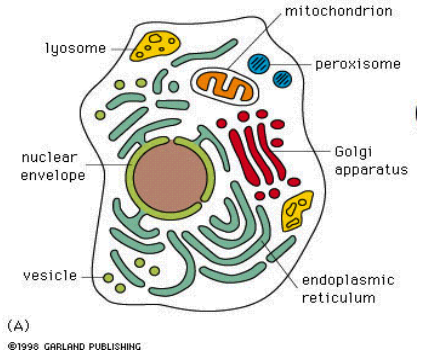
÷ in three dimensions
the signal diminishes with $\mathbf{r^3}$

Thus: at 50 nanometers
=> strong signal
=> High concentration

vs at 2 meter
=> extremely weak signal
=> highly diluted

Quantities in biology

	Macroscopic world		Cellular world
Length	1 decimeter		1 μm
Volume	1 liter		10^{-15} liter or 0.000'000'000'000'001 liter

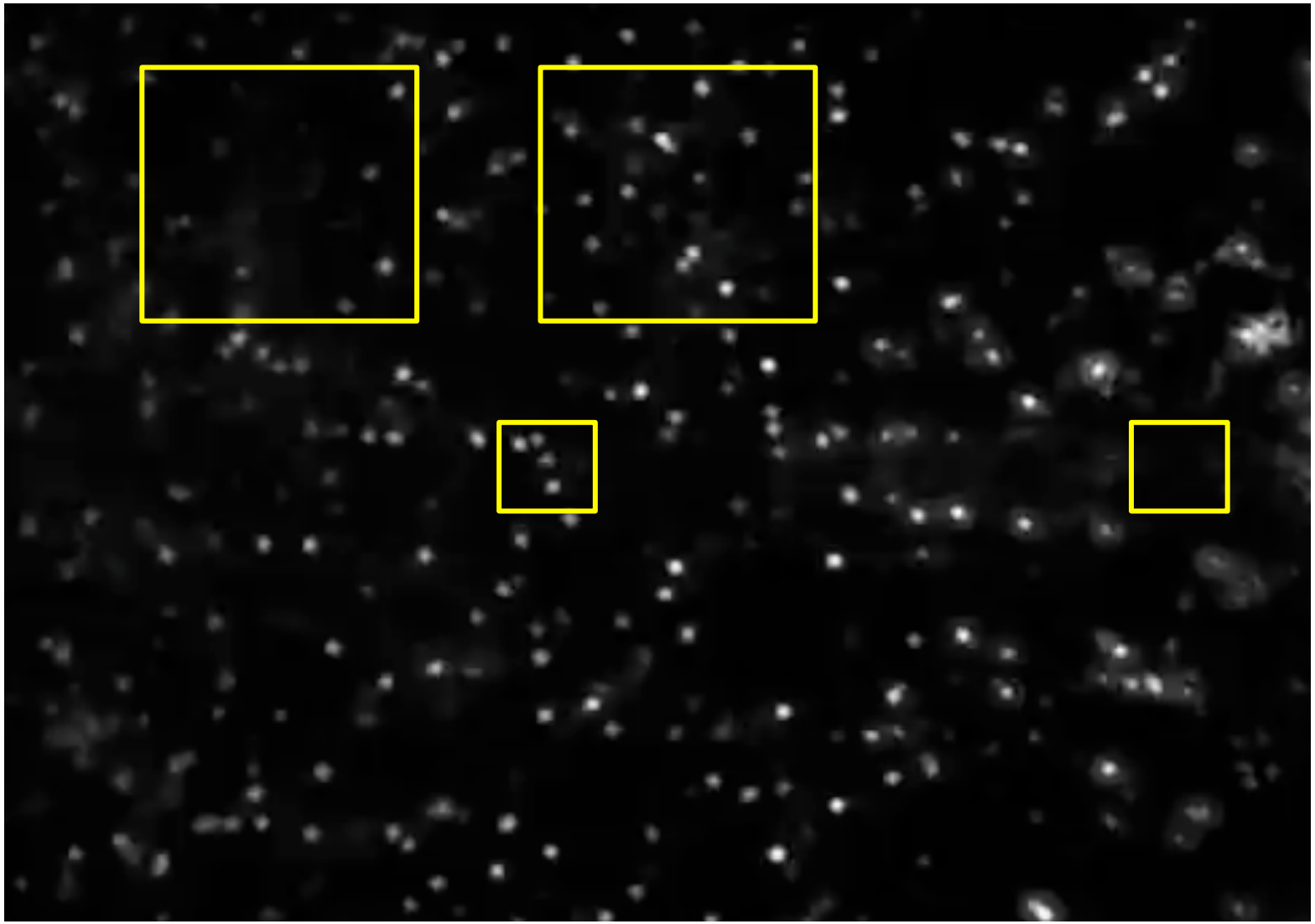


- Consequence of scale on the number of molecules present

	Macroscopic world	Cellular world
Salts	10^{23}	100'000'000
Synaptic transmitter	10^{21}	1'000'000
Hormone	10^{15}	1

Consequence of a small number

A solution filled with particles – How many particles are there in a given volume ?



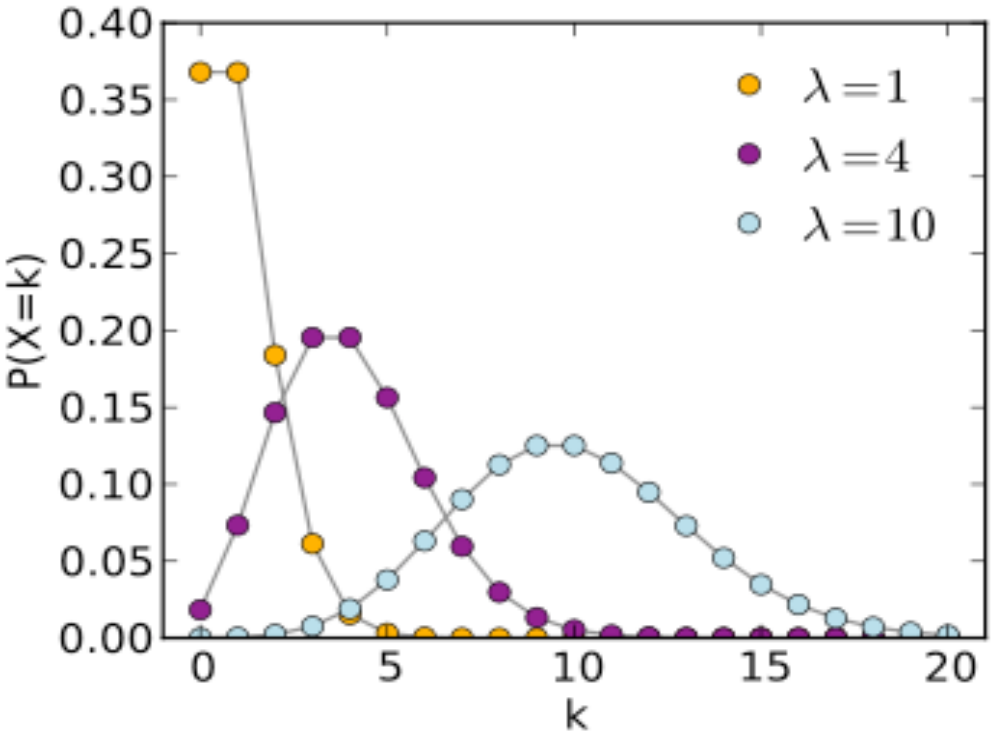
Law of Poisson

Siméon Denis Poisson
1781 - 1840

“when the average is small,
the change with find nothing is not null !”

In fact, there is a distribution of the average ***λ*** with different probabilities ***p*** to observe value ***k*** *:*

$$p(k) = P(X = k) = e^{-\lambda}\frac{\lambda^k}{k!}$$



The “n” in biology

Some effects of small numbers

- Detection of molecular properties of N molecules

  signal $\propto$ N *standard deviation* $\propto \sqrt{N}$

  $N = 10^6$ => $SD = 10^3$

  $N = 1$ $SD = 1$

- Imagine a molecule with an equal change to be red or white

  $N = 10^6$ => average properties => pink

  $N = 1$ => individual character => either red or white

=> individual molecules with individual & changing properties !!

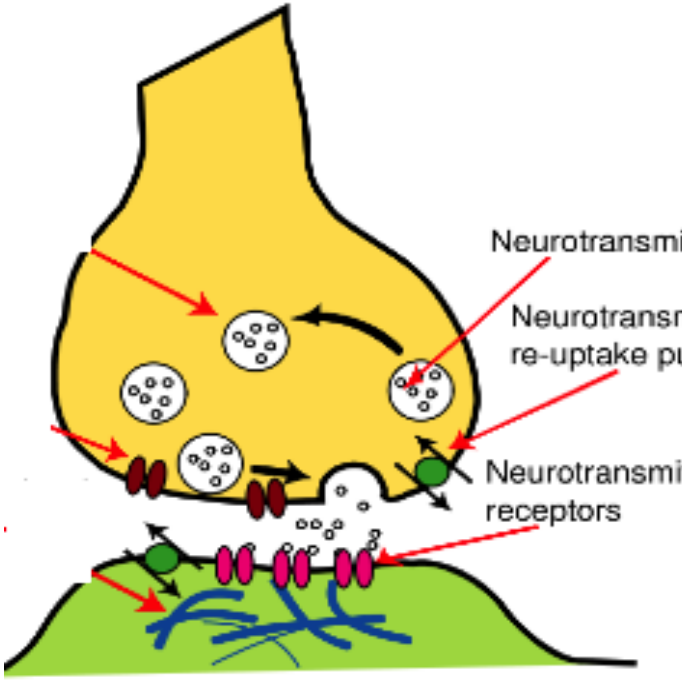
One of the consequences of Poisson

The metric average is not always valid in the cellular world.

An example,

In the synaptic contact between neurons
there are 10 receptors present
that are active for only 10 % of the time

=> on the average
there is **only 1** receptor active at the time



• Consequence of Poisson:

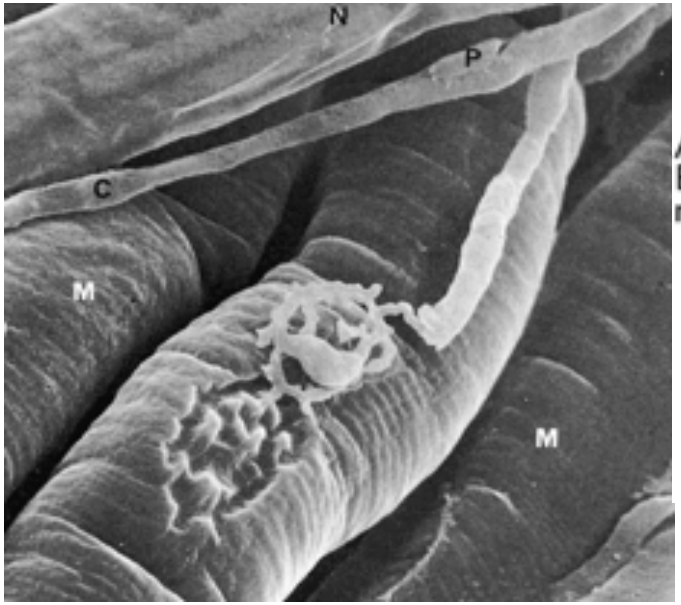
=> There is 37 % chance that none of these 10 receptors is active !!

One of the consequences of Poisson

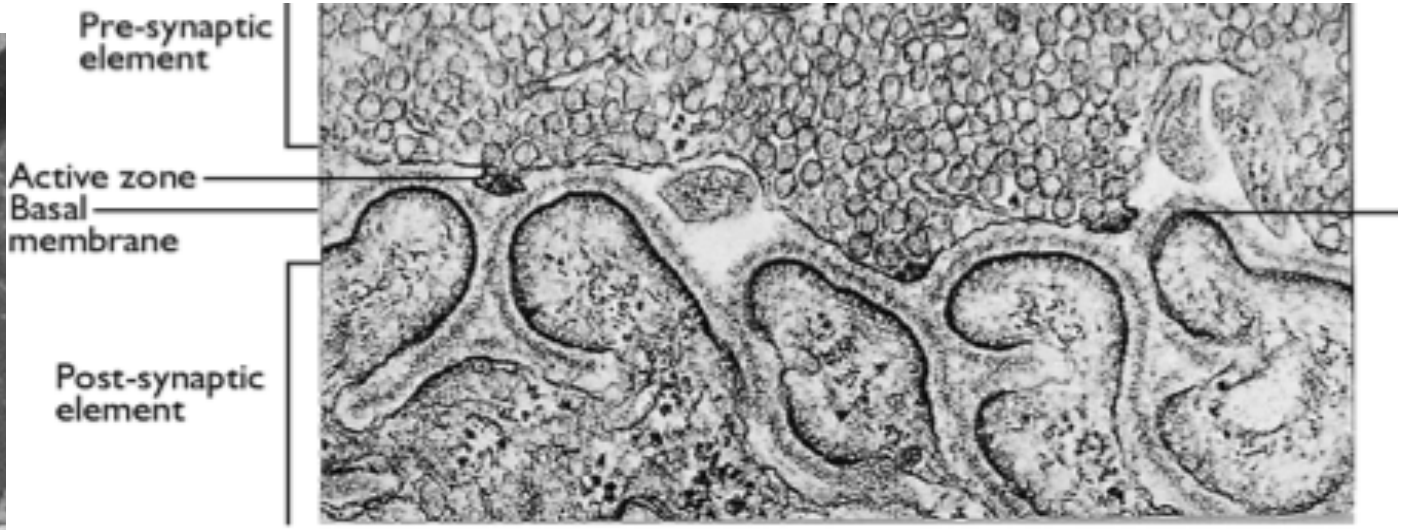
How to make a synapse that always works ?

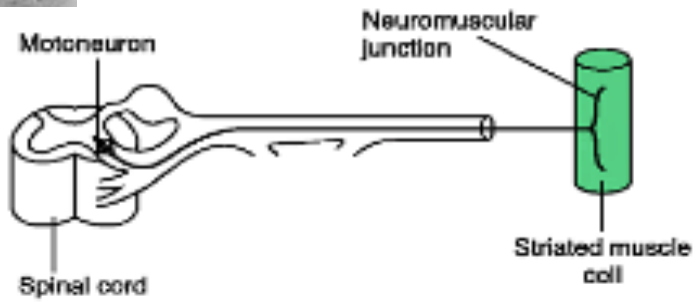
=> Many receptors & high concentration of transmitter

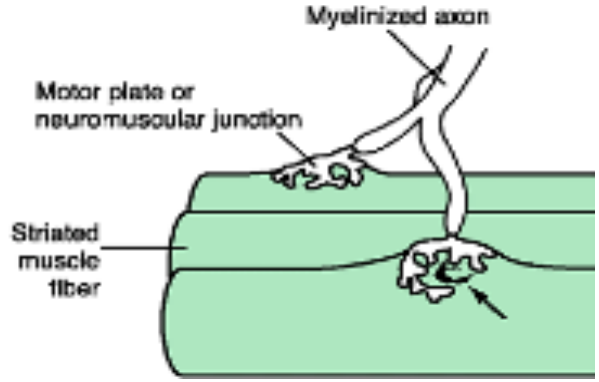
Text book example: the neuro-muscular junction.



5 µm





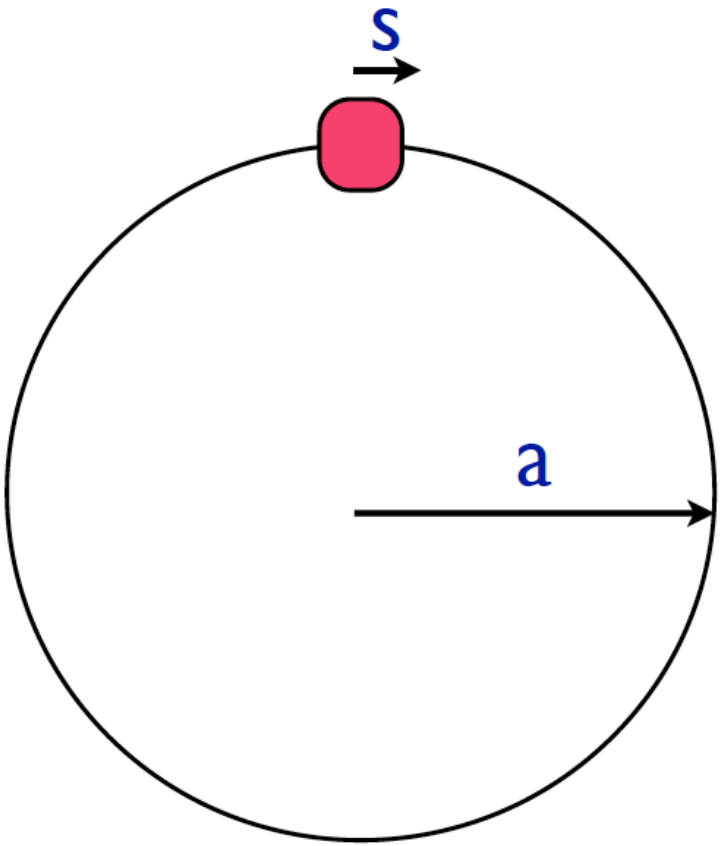


Receptors sample extracellular ligand concentration

Or rather fluctuation is the concentration **c** of a ligand.

How many receptors  does a cell need to do this accurately?

Berg & Purcell, 1977



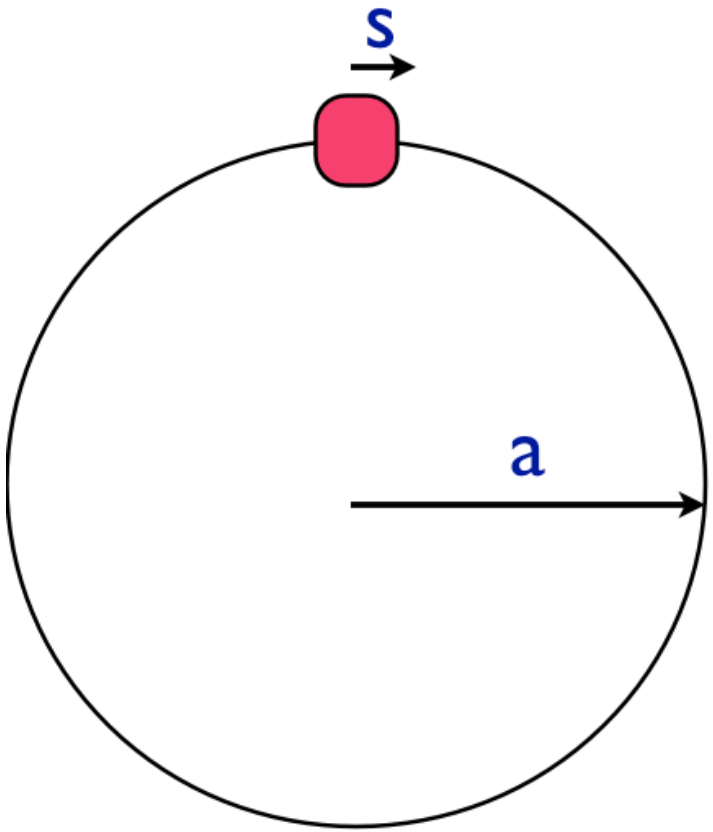
Assumptions:

- Receptor occupancy reflects cellular response
- Ligand binding events are independent

? what is the smallest error Δc possible ?

? How many receptors are needed per cell ?

Receptors sample extracellular ligand concentration



=> The error Δc in concentration c detected in a time interval T equals

$$\frac{\Delta c}{c} \cong \frac{1}{\sqrt{T \cdot c \cdot a \cdot D}}$$

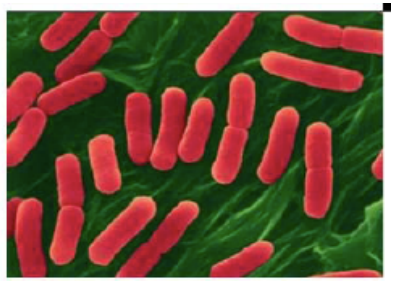
where: D is the ligand's diffusion coefficient
c is [Ligand] in molecule.cm^{-3}

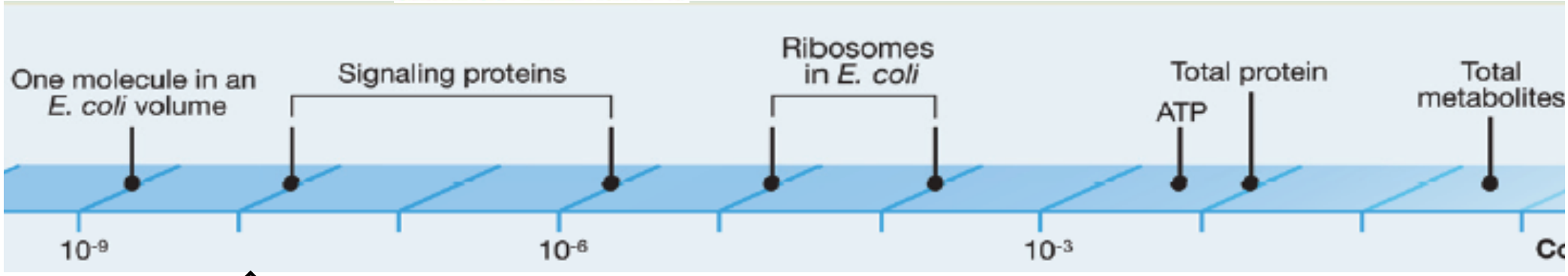
=> The number N of receptors needed is

$$N = \frac{a}{s}$$

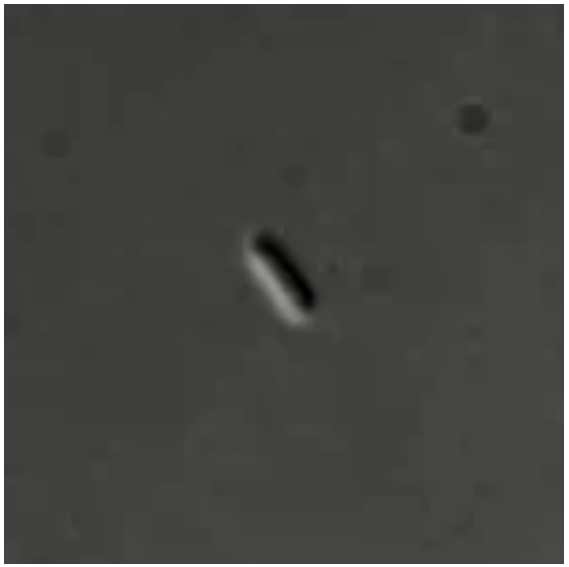
The “n” in biology

Escherichia coli:
Volume 10^{-15} L





Several important molecules are rare, almost alone
=> these molecules are decisive or deciding !!



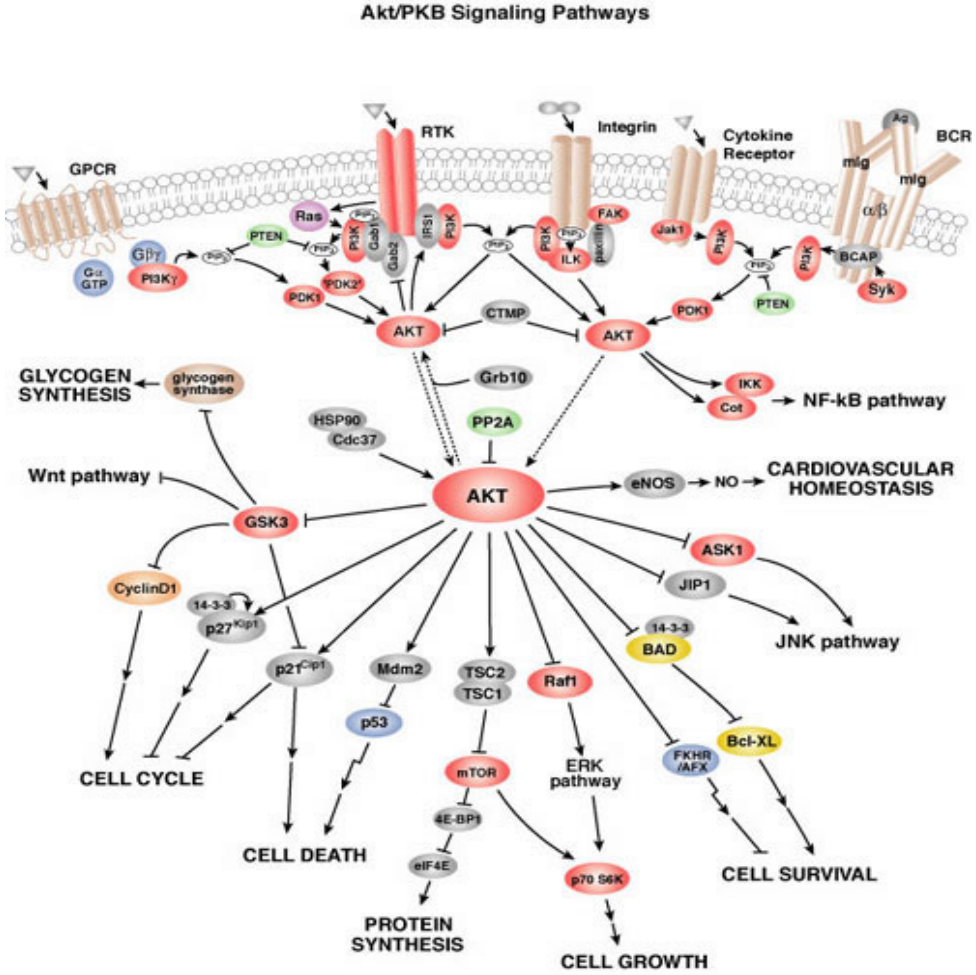
SNAP shot Key numbers in Biology (2010) Cell 141
S.Xie (2006) Science Vol 311

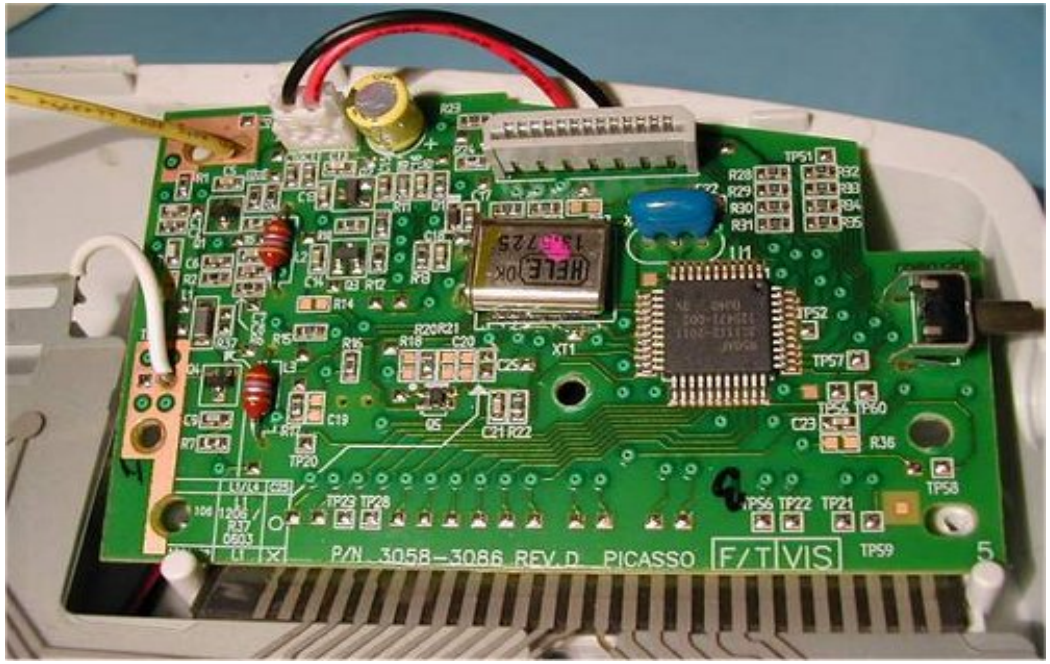
Molecular interactions - Biological networks

- The cell is extremely complex

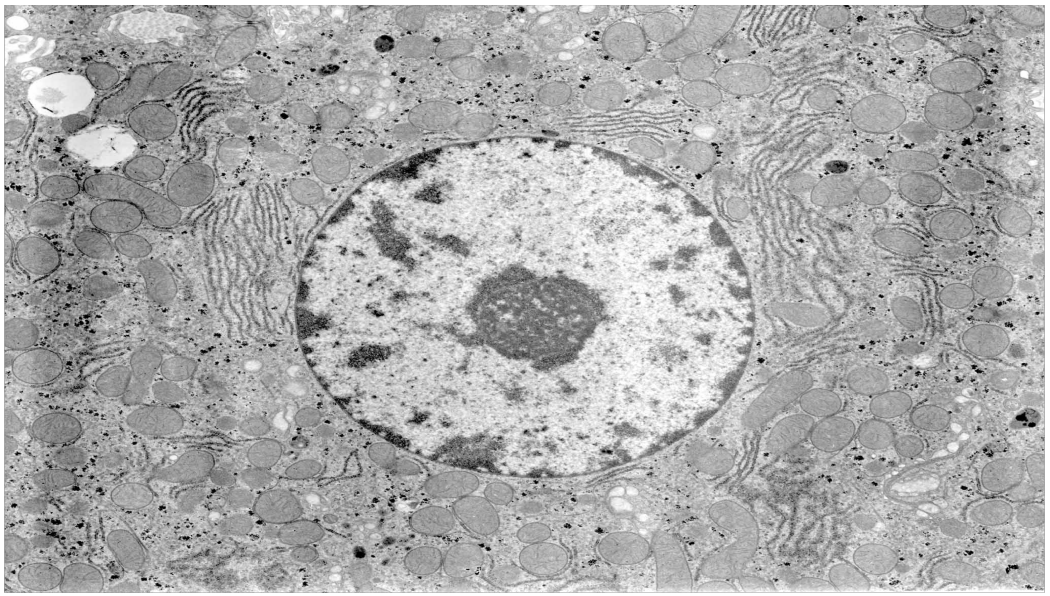
  many intertwined signaling and metabolic **multi-dimensional networks**

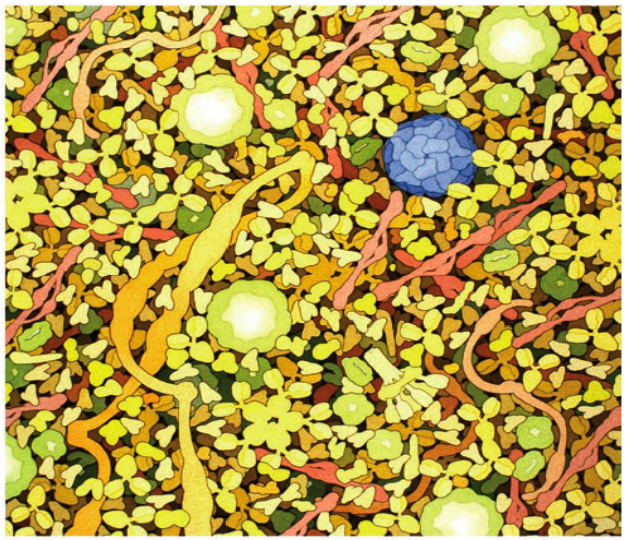
  a strong **spatial-temporal organization**





Cells - Very complicated conditions





Goodsell, Nat Chem Biol 3 (2007) p.681

- Highly heterogeneous - compartments with very different contents
- Diffusion, transport and separation
- Highly crowded
- Many different components - 5’000 different gene products

  => Very far from an ideal system in physicochemical terms

=> Systems biology :

Molecular description in space (x,y,z) and time (t) of a cell or an organism

Effects of Crowding: Diffusion

versus

- Macroscopically, the diffusion slows down
  - => the diffusion coefficient D decreases strongly
  - => larger molecules are more affected

The time ***t*** to travel a certain distance increase as $t \propto D^{-2}$

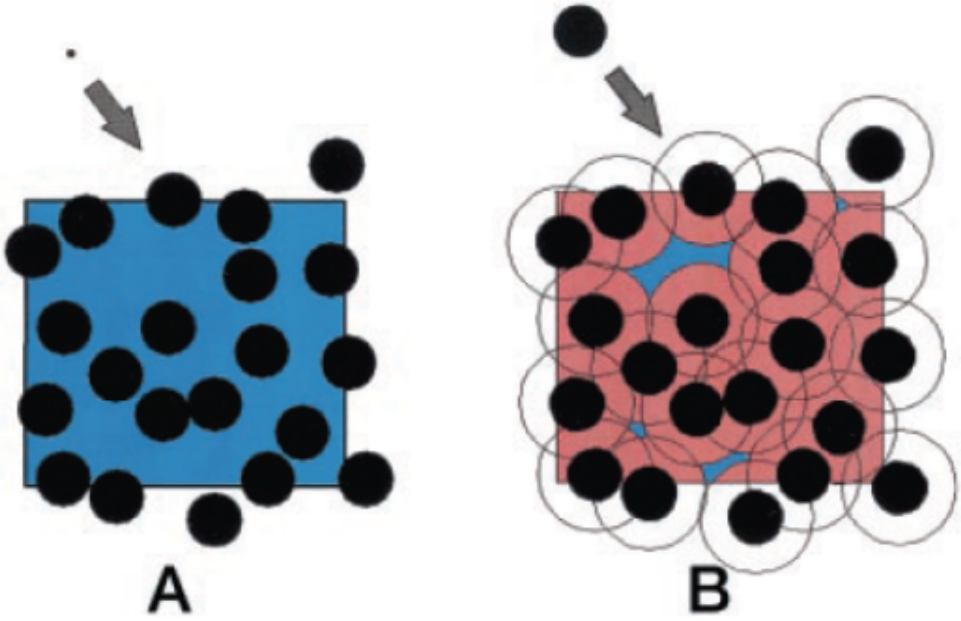
e.g. GFP diffuses 10 times slower in the cytosol than in an aqueous buffer
=> takes in cytosol 100 time more time to travel the same distance

Effects of Crowding : Excluded volume and activity

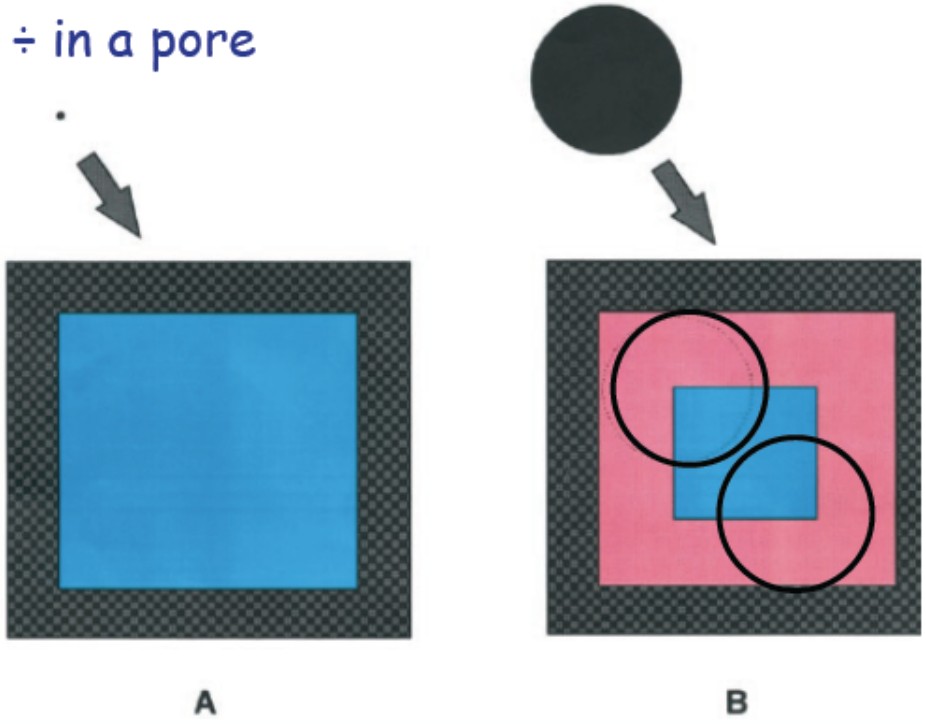
Size matters !!

Compare a small (A) and a large (B) molecule for accessible to centre of mass (blue) and excluded (pink) volume with in a solution containing large solutes

÷ in bulk solution



÷ in a pore



Minton, JBC, 2001

Effects of Crowding: Chemical equilibria

Ideal system: No interactions between molecules

This condition is met in systems like : - gases at low pressure
- very diluted solutions

Imagine a polymerisation reaction: $nA \rightleftharpoons A_n$

characterized by a dissociation constant $K_d = \frac{[A]^n}{[A_n]}$

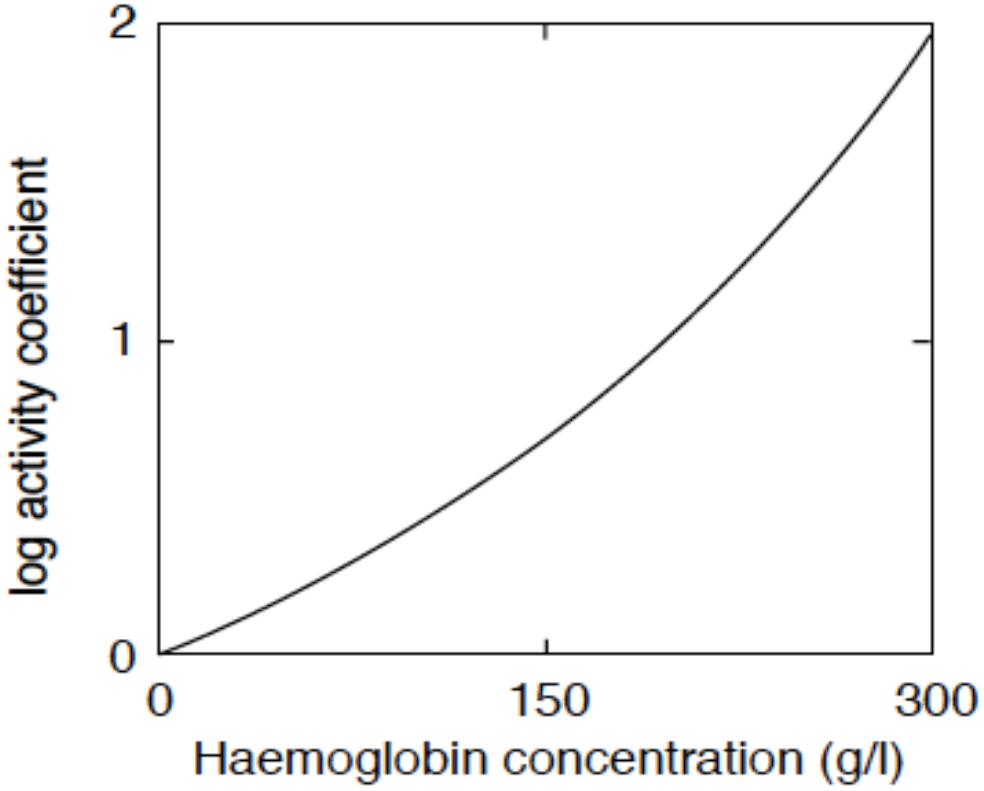
Under non-ideal conditions the concentrations of each species has to be multiplied by a correction factor - the activity constant ***a***

$$K_d = \frac{a_A^n \cdot [A]^n}{a_{An} \cdot [A_n]} \approx \frac{a_A^{n-1} \cdot [A]^n}{[A_n]}$$ if ***n*** is small

Effects of Crowding: Chemical equilibria

Activity coefficient of a species depends on molecular size & total solute density

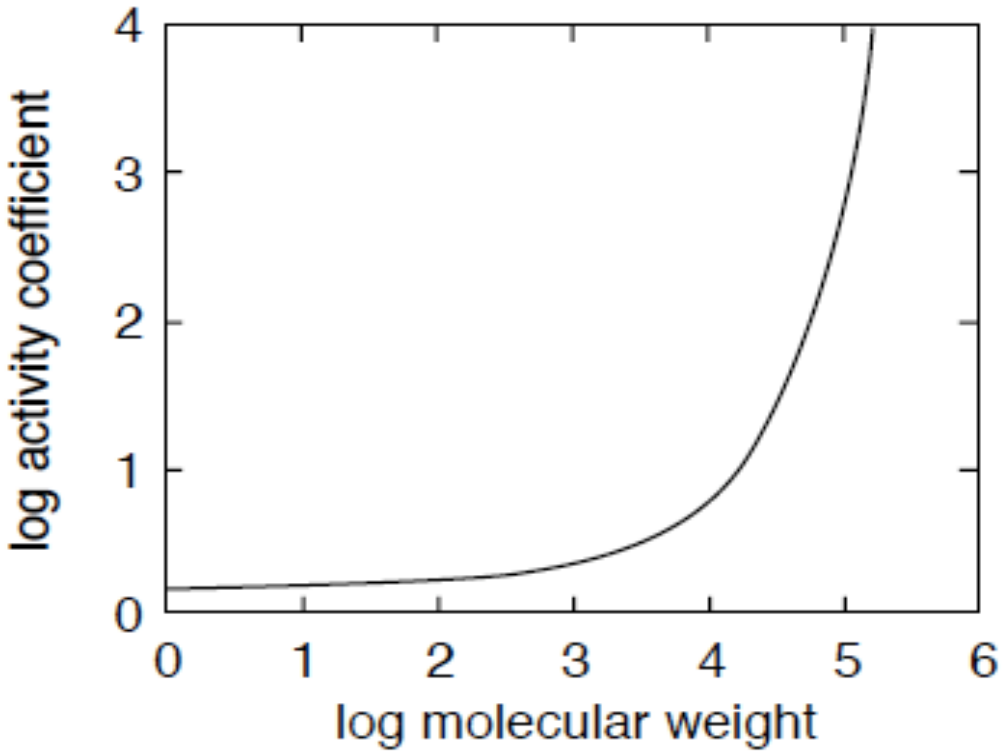
- Haemoglobin in water



at low conc. $a = 1$

in blood cell $a \approx 100$

- Molecules dissolve in a solution of haemoglobin of 300 g/l

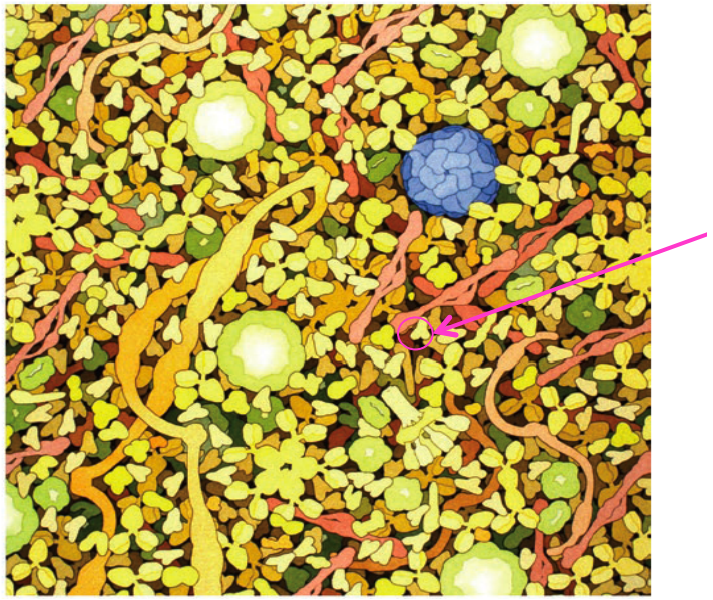


at low MW $a = 1$

at high MW a explodes

Methods: Fluorescence labelling & microscopy

Fluorescent labeling in needed to discriminate between molecules of interest and matrix in a living system



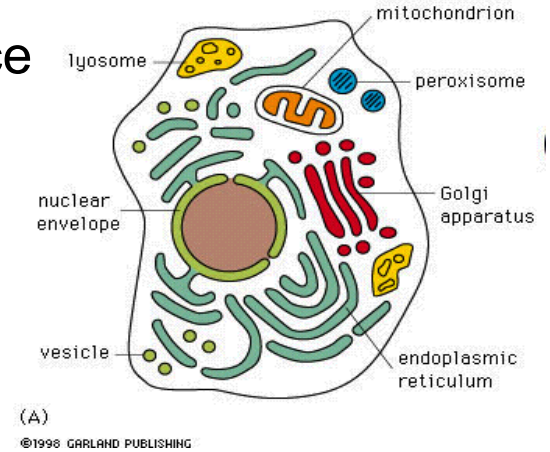
Advantages of fluorescence methods

- Non-invasive => *in vivo*
- Sensitive => down to a single molecule
- On line => direct info from ns to days
- High spatial & temporal resolution
- High information content
  - Location, movement & distance
  - Number of molecules
  - Microenvironment
  - Molecular interactions

Focus:
Bio-orthogonal labelling of proteins

Where & When ?

- On the cell surface
- Within the cell
- Anywhere

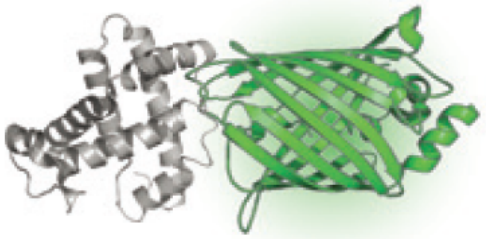


- Temporarily
- Always
- Reversible

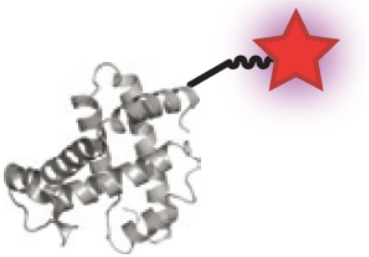
How to label your protein of interest “POI” ?

- During biosynthesis of protein

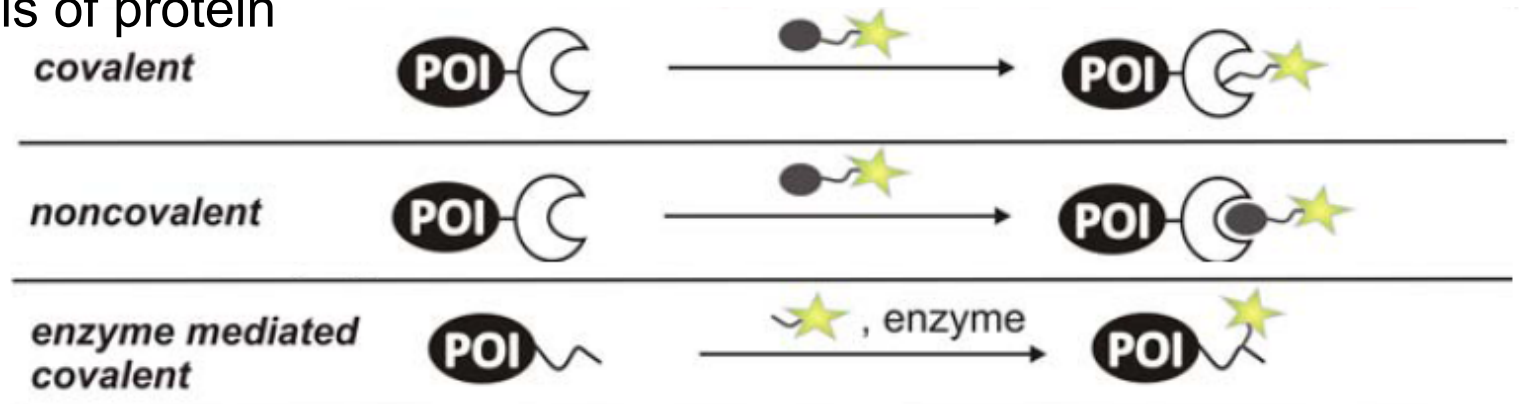
Autofluorescent protein



Non-natural amino acid



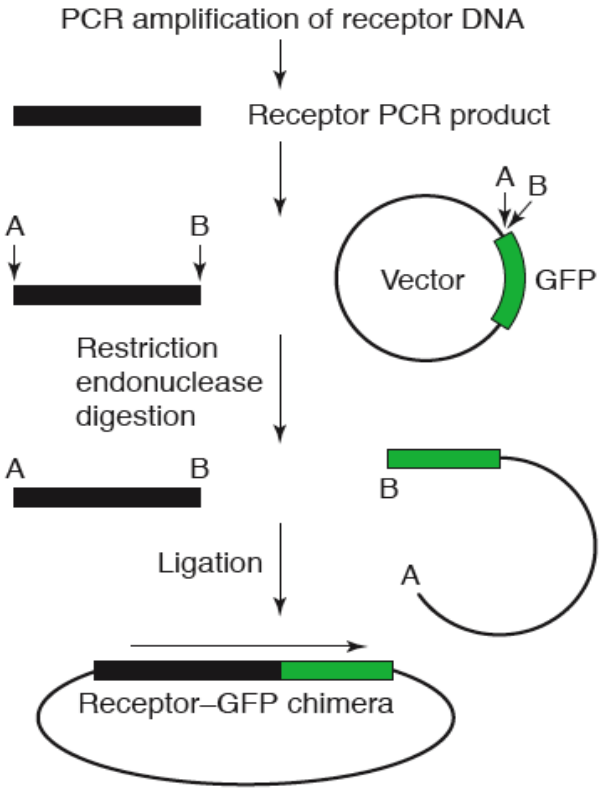
- After biosynthesis of protein



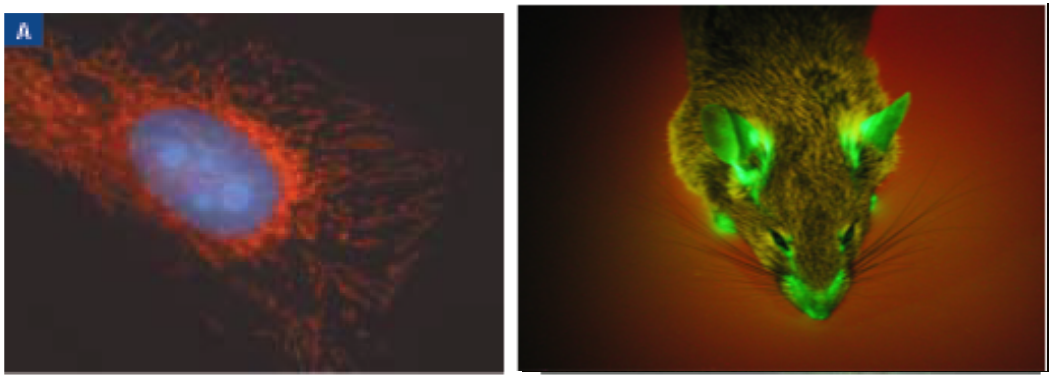
Biosynthetic labeling: Fluorescent proteins

- Fusion to protein of interest

  N- or C- terminal, internal

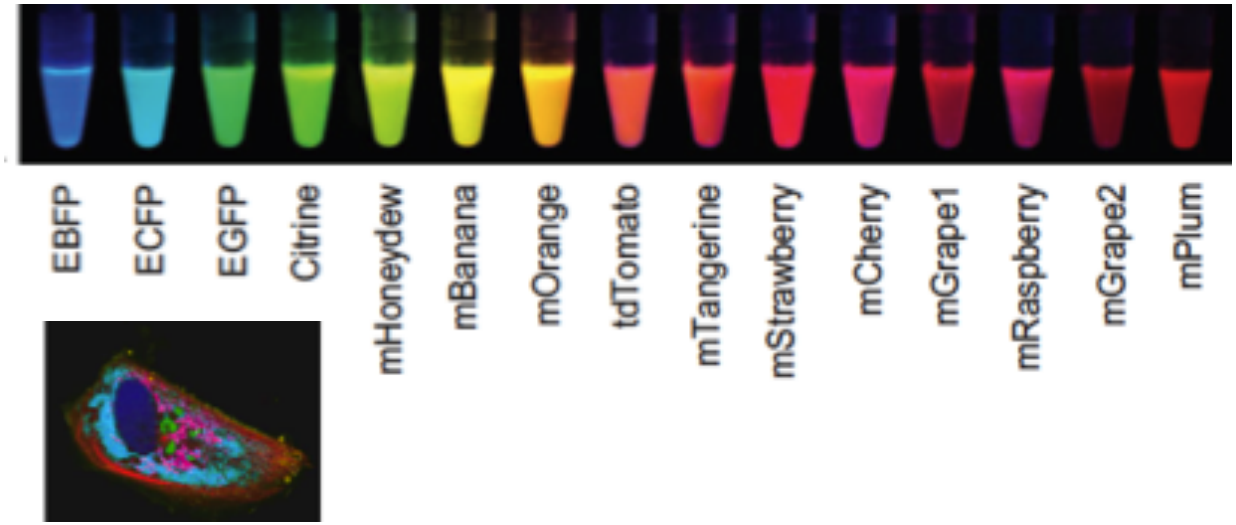


- Within single cells or living organisms



R. Y. Tsien

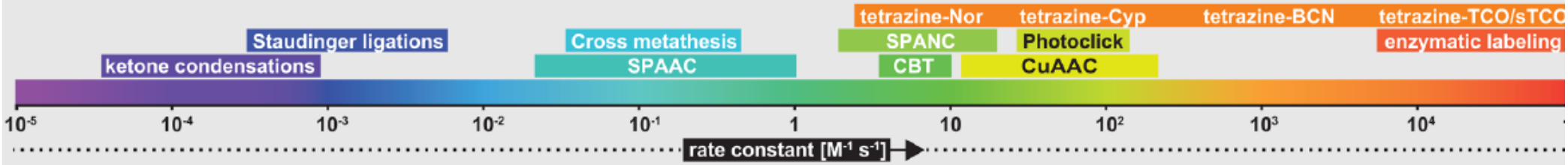
- Parallel labeling of several proteins with different fluorescent proteins



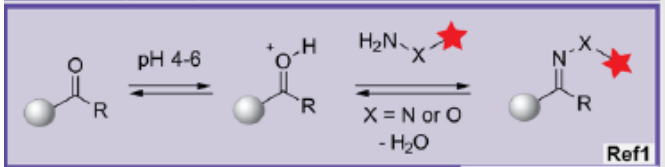
T. Kogure, Nat Biotech 24 (2006) 577

Wang (2008)

BIOORTHOGONAL REACTIONS FOR LABELING PROTEINS

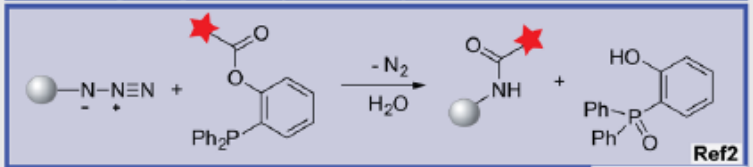


ketone/hydroxylamine condensations



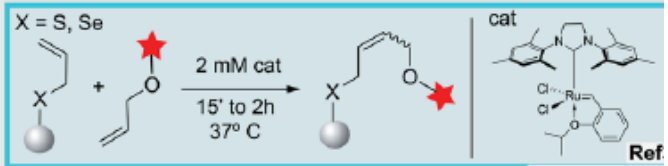
on cell surface, acidic pH — k ~ 10^{-4} - 10^{-3} $M^{-1}s^{-1}$

Staudinger ligations (traceless)



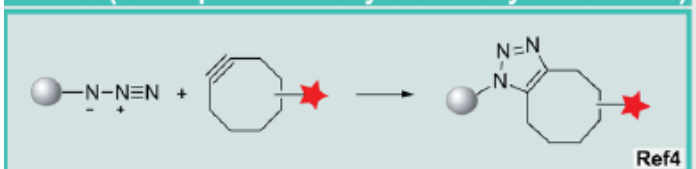
in vivo, phosphines are oxidation-sensitive — k ~ 10^{-3} $M^{-1}s^{-1}$

Cross-metathesis



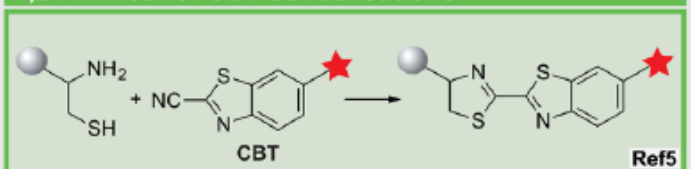
in vitro — k ~ 0.3 $M^{-1}s^{-1}$

SPAAC (strain-promoted alkyne-azide cycloadditions)



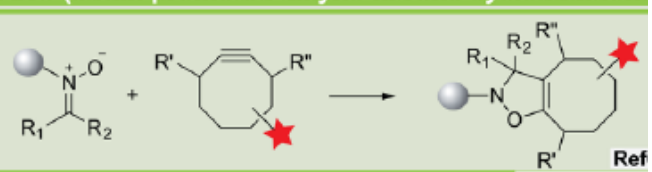
in vivo, synthesis of alkynes often low-yielding — k ~ 10^{-2} - 1 $M^{-1}s^{-1}$

1,2-Aminothiol-CBT condensations



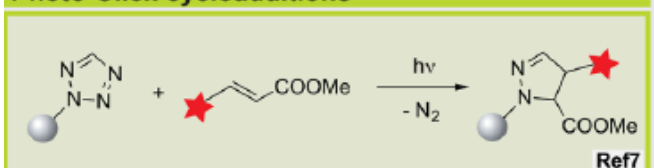
mainly *in vitro* — k ~ 10 $M^{-1}s^{-1}$

SPANC (strain promoted alkyne-nitrone cycloadditions



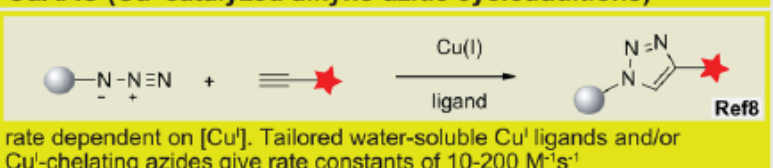
mainly *in vitro*, nitrones are prone to hydrolysis — k < 30 $M^{-1}s^{-1}$

Photo-Click cycloadditions



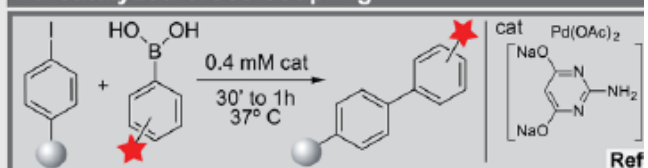
in vivo, fluorescent product, UV-inducible — k < 60 $M^{-1}s^{-1}$

CuAAC (Cu^{I}-catalyzed alkyne-azide cycloadditions)



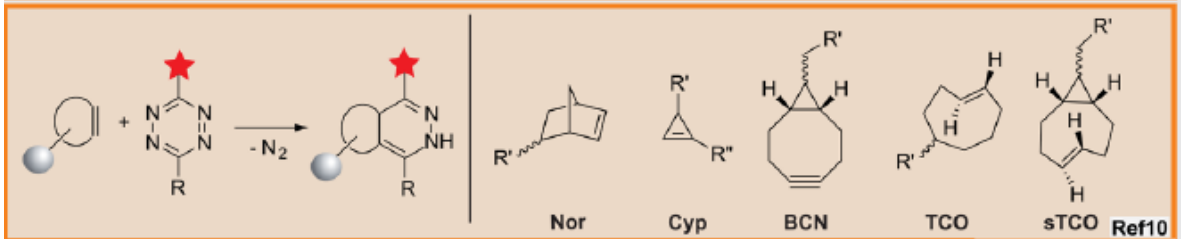
rate dependent on [Cu^{I}]. Tailored water-soluble Cu^{I} ligands and/or Cu^{I}-chelating azides give rate constants of 10-200 $M^{-1}s^{-1}$ in the presence of 10 - 500 µM Cu^{I}. — k ~ 10 - 200 $M^{-1}s^{-1}$

Pd-catalyzed cross coupling



on bacterial cell surface, rate constants not reported

Diels-Alder cycloadditions between tetrazines and strained alkenes/alkynes



cell surface and intracellular labeling, very fast, fluorogenic — k ~ 1 - 10^{4} $M^{-1}s^{-1}$

Kathrin Lang and Jason W. Chin

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(2) a) Saxon E. et al., *Science* **2000**, 287, 2007; b) Saxon E. et al., *Organic Letters* **2000**, 2, 2141; c) Prescher, J.A. et *Nature* **2004**, 430, 873; d) Tsao, M.L. et al., *Chembiochem* **2005**, 6, 2147; e) Kiick, K.L. et al., *PNAS* **2002**, 99, 19.

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(4) a) Agard N.J. et al., *JACS* **2004**, 126, 15046; b) Baskin J.M., *PNAS* **2007**, 104, 16793; c) Ning, X. et al., *Angew. C Int. Ed.* **2008**, 47, 2253; d) Plass, T. et al., *Angew. Chem. Int. Ed.* **2011**, 50, 3878

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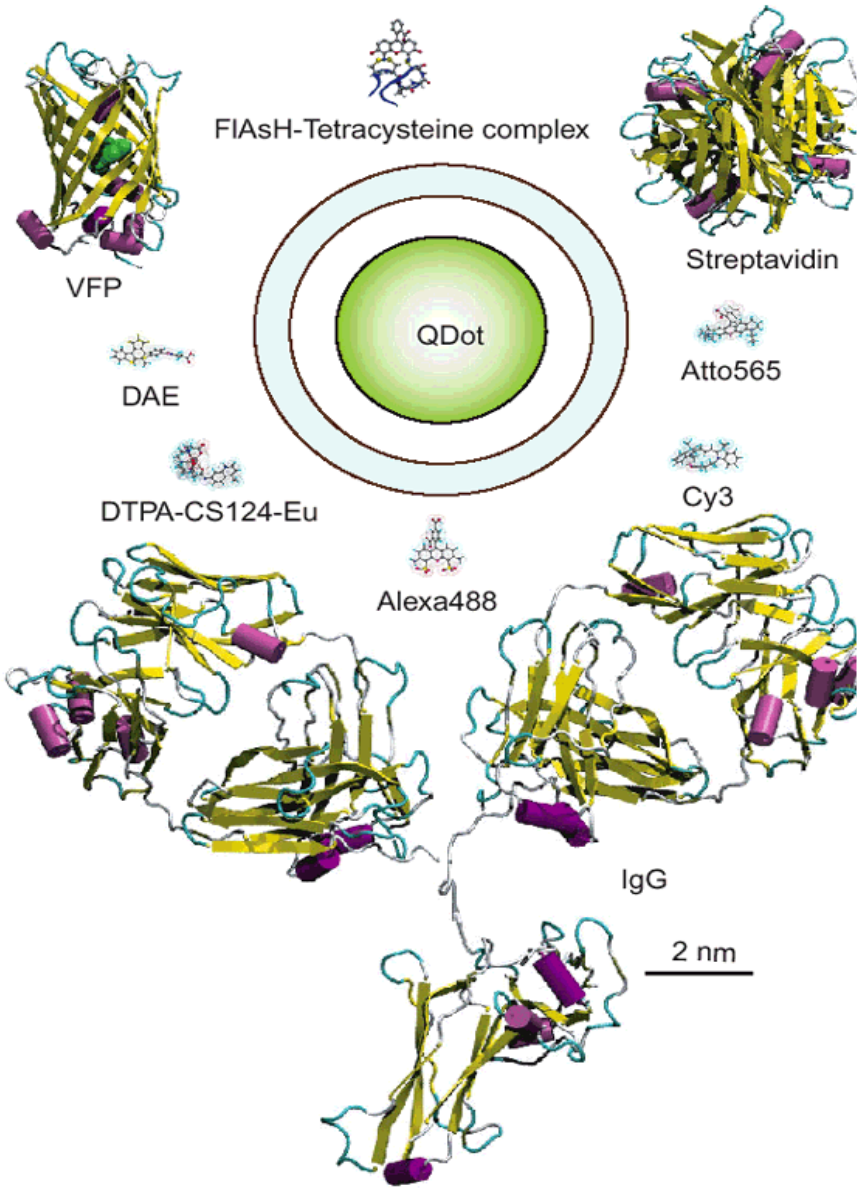
(10) a) Blackman, M.L. et al., *JACS* **2008**, 130, 13518; b) Devaraj , N.K. et al., *Bioconjugate Chem.* **2008**, 19, 2297; c) Lang, K. et al., *Nature Chem.* **2012**, 4, 298; d) Plass, T. et al., *Angew. Chem. Int. Ed.* **2012**, 51, 4166 ; e) Lang, K. al., *JACS* **2012**, 134, 10317; f) Yang, J. et al., *Angew. Chem. Int. Ed.* **2012**, 51, 7476; g) Seitchik, J.L. et al., JACS **2012**, 134, 2898; h) Elliott, T. et al., unpublished data.

Labeling, with what?



Ideally,

- the probe should be:
  - Small is beautiful
  - Bright
  - Stable
  - Non-perturbing
  - ..
- the labeling should be/allow:
  - Specific
  - Stoichiometric
  - Inside or on surface of cell
  - Permanent or reversible
- the duration of labeling should match the duration of the process of interest

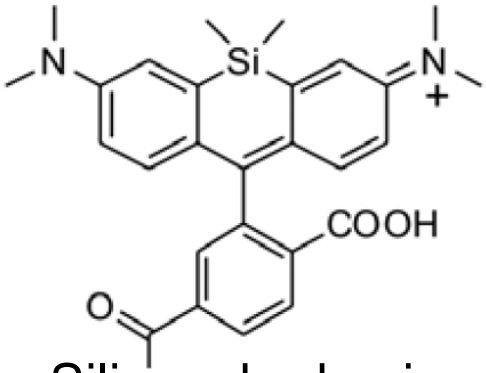
E. A. Jares, Nat BioTech 21 (2003) 1387
Resh-Genger, Nat Methods (2008)

Labeling with organic dyes

Very large choice of commercially available (reactive) dyes from e.g.

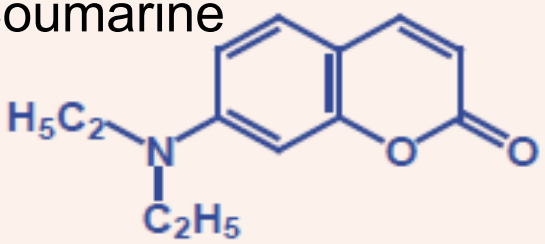
- Invitrogen ***Alexa*** & ***Bodipy***
- GE ***Cy*** dyes
- Atto-tec ***Atto*** dyes
- Toronto Research Chemicals, Biotium, Dyomics,

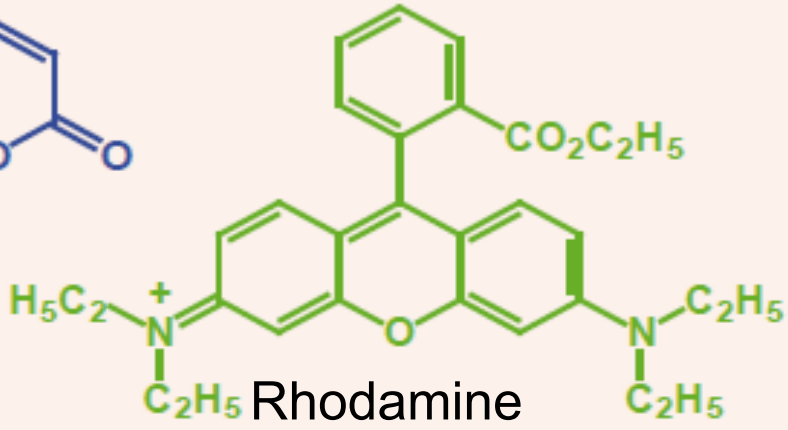
SPIROCHROME Probes for Bioimaging



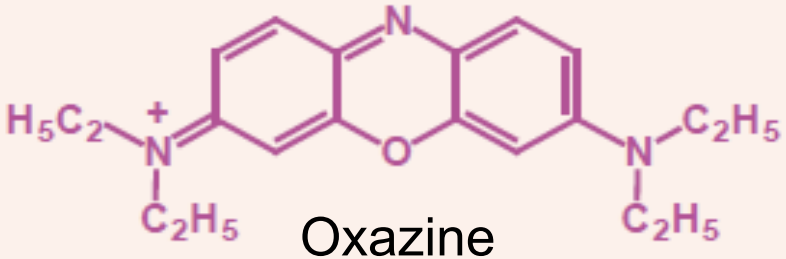
Silicon rhodamine

Coumarine

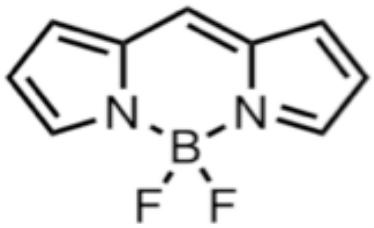




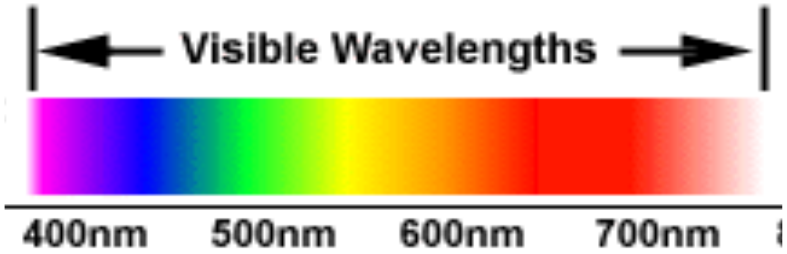
Rhodamine

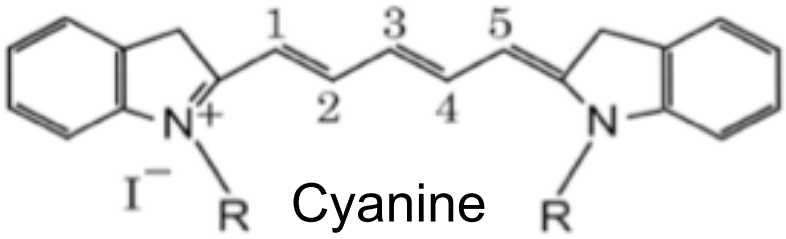


Oxazine



Bodipy

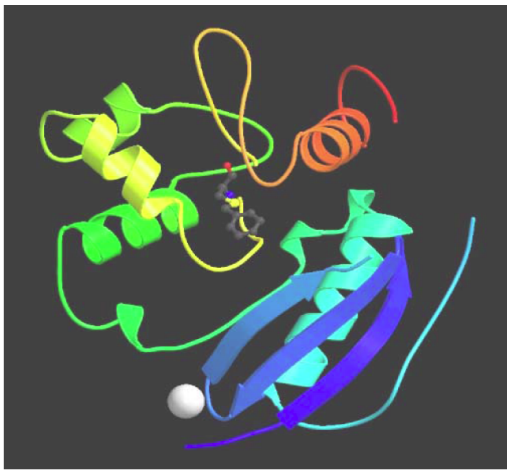




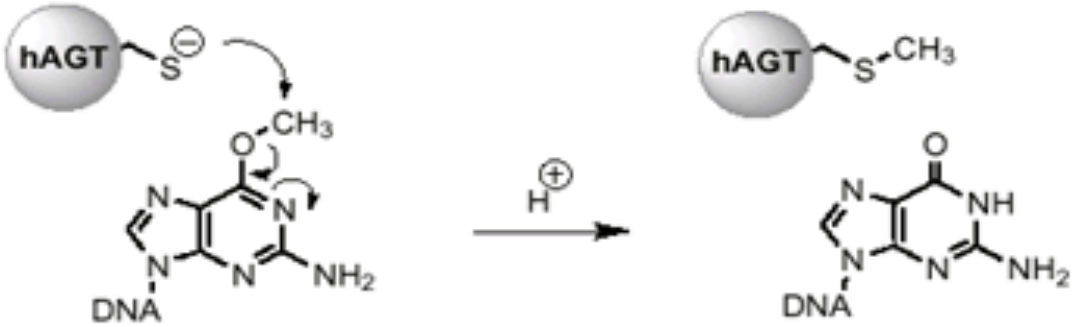
Cyanine

Enzyme-mediated covalent labelling : *SNAP*-tag

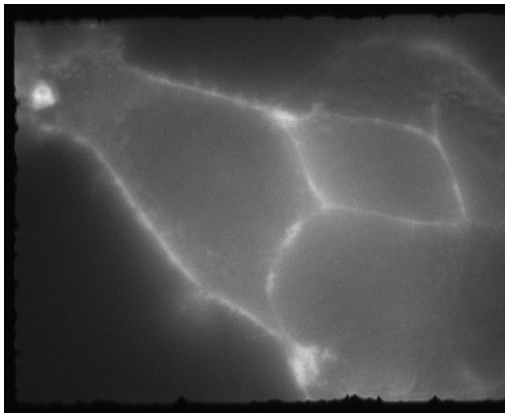
- O^6-Alkylguanine-DNA Alkyltransferase (hAGT) is a DNA-repair "enzyme".
- The nucleotide-modifying **LABEL** is covalently bound to AGT.



Natural role:



Labeling:



+ Some substrates are membrane impermeable => specific cell-surface labeling

Developed in the Johnsson lab

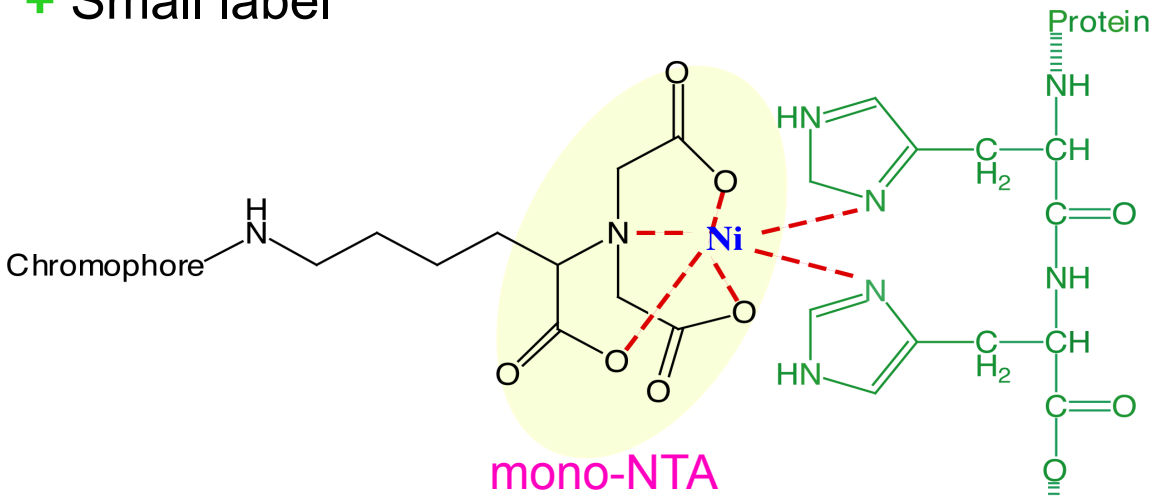
Keppler, Nat BioTech 21 (2003) 86

*Commercially available as **SNAP**-tag from* NEW ENGLAND BioLabs Inc.

Reversible labelling : Peptide tags & NTA-Probes

Selective & revesible binding of NTA-Ni^{2+} to oligohistidine sequences added to proteins of interest by genetic engineering

+ Wide choice of chromophores
+ Small label



Guignet (2004) Nature Biotech

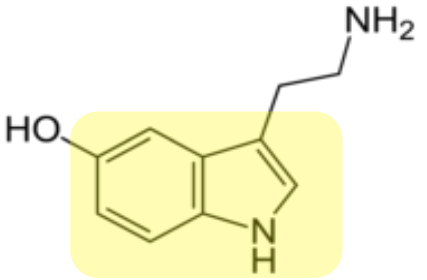
	K_d:	$t_{1/2}$:
mono-NTA	5'000'000 pM	5 sec

Reversible labelling : Ligand for serotonin receptor

How to couple a fluorophore to a ligand ?

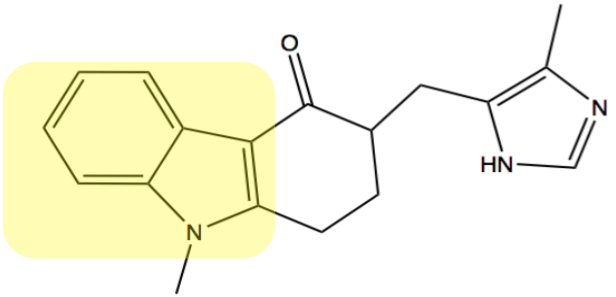
i) On the chemically reactive groups of the ligand

e.g. serotonin : -OH -NH_2 or NH



=> evaluate binding & effect of conjugate to receptor

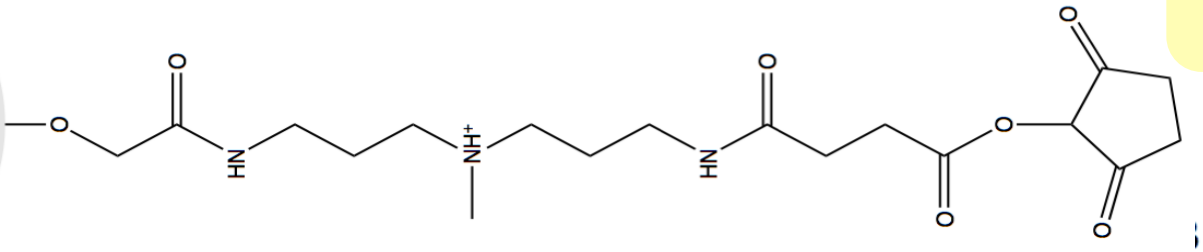
ii) Try ligand analogues, e.g. GR-67330

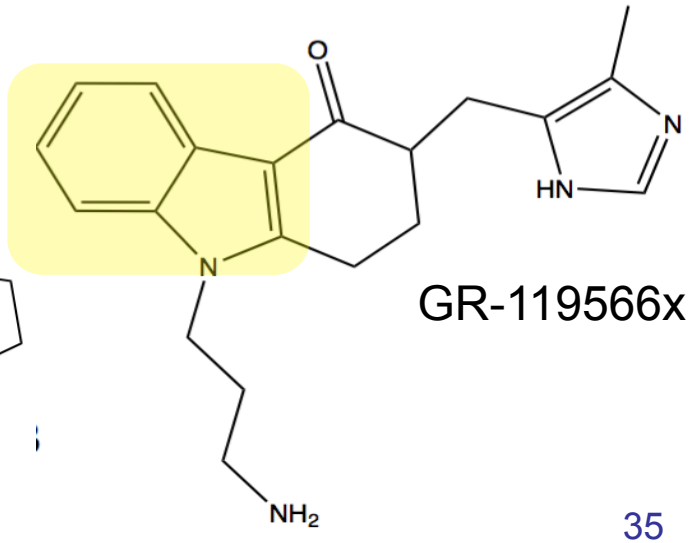


iii) Look in literature !

=> GR-119566x has been used for receptor purification by affinity chromatography

µm-sized bead

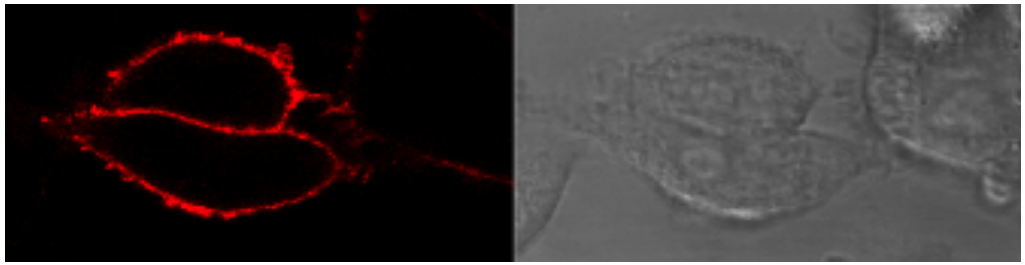


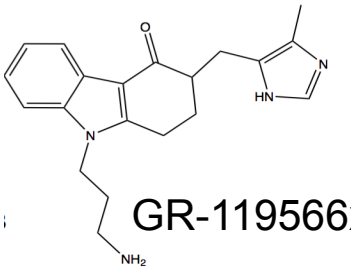


GR-119566x

Reversible labeling of receptors with fluorescent ligands

e.g. GR-119566x labeled with Alexa-647

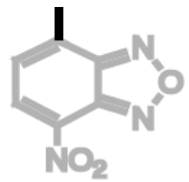




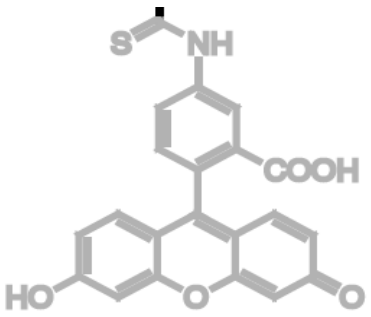
GR-119566x

Properties of conjugates :

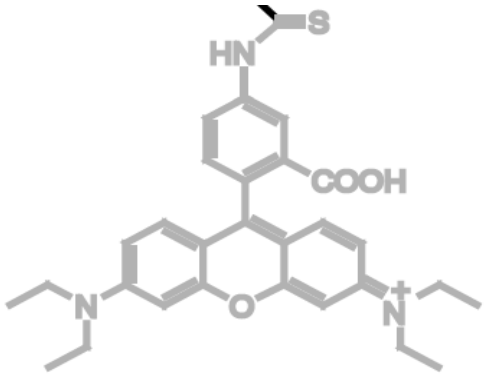
	Affinity (pM)	
GR-H	30	
GR-Fluorescein	300	fluorescence decreases upon binding
GR-NBD	500	hardly fluorescent
GR-Rhodamine	800	aggregates receptor
GR-Cy5	18’000	high non-specific membrane staining



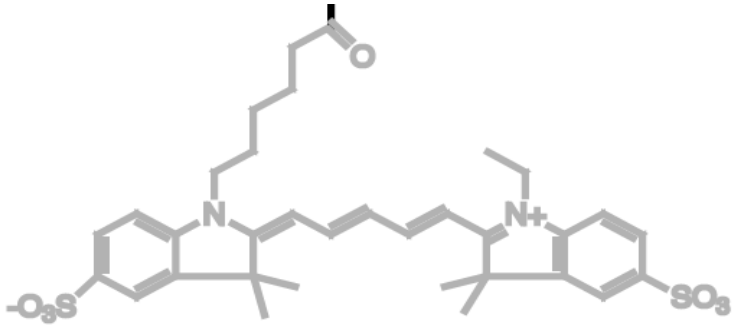
NBD



Fluorescein



TetraEthyl-Rhodamine



Cy5

Reversible vs covalent labeling

Covalent

+ once it's there it is there to stay
- upon photo bleaching your labeled molecule is invisible

Reversible

Depending on affinity and off rate complexes can have a

- short lifetime of seconds to minutes
    - Cannot wash or label in advance
    + Upon photobleaching can be replaced
    + Repetitive labeling under same or different conditions

- long lifetime of hours to days => almost as covalent labeling

Labeling

The ***ideal*** label and labeling method do not exist.

=> What do you would like to learn and where?

÷ Localisation and movement ÷ In live cells

÷ Molecular interactions ÷ Reconstituted systems

÷ Structural changes

=> Each protein & each process needs an individual approach.

=> Combine orthogonal methods for multiple specific labeling.

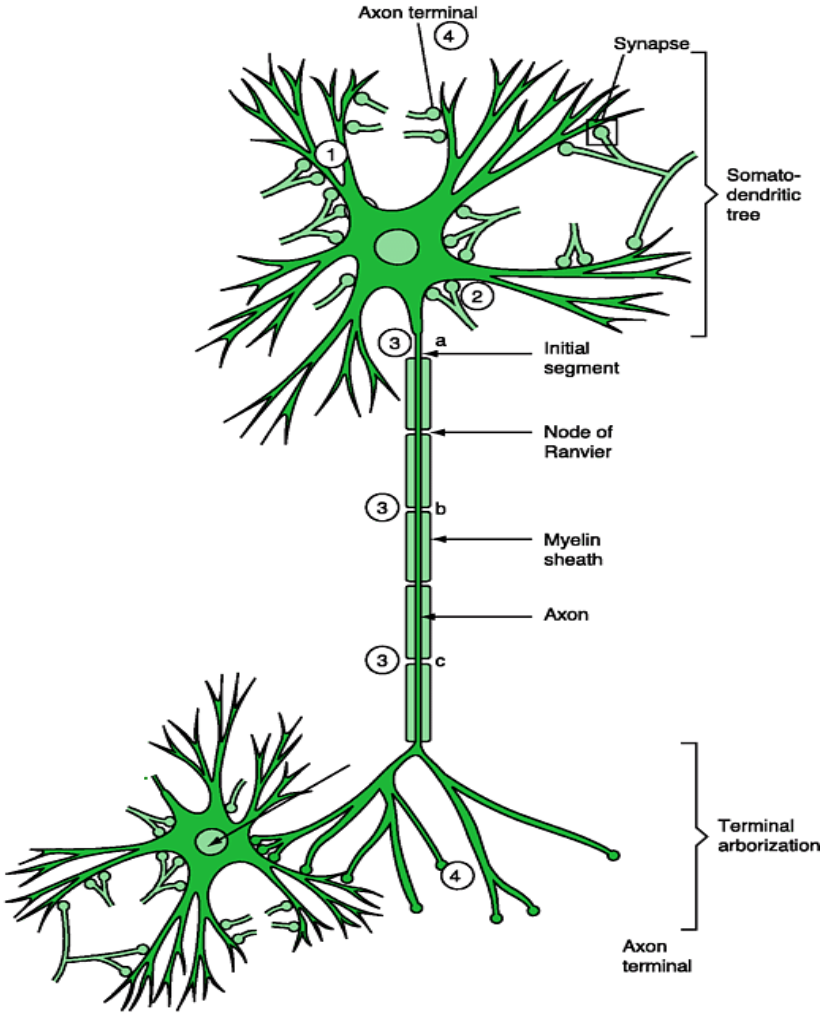
=> Beware of nonspecific labeling and artefacts.

=> Combine measuring techniques.

=> Do lots of careful experiments.

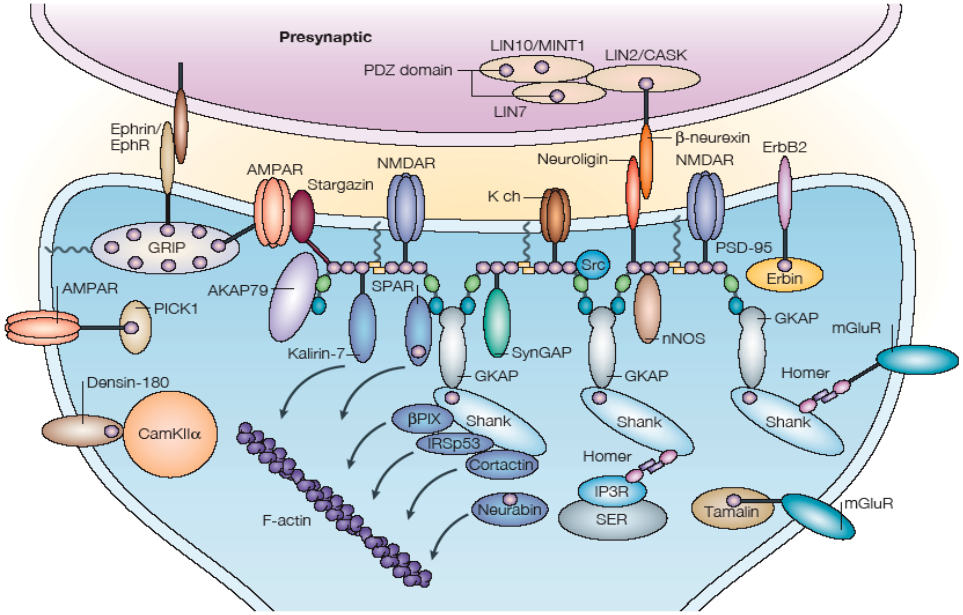
Biological networks - Molecular interactions

- Interacting neurons



Hammond "Cell & Mol Neurobiology"
Kim, Nat Rev Neurosci 5 (2004) 772

- Synaptic organization



Signaling events within a cell

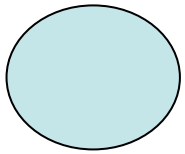
- Þ Right place
- Þ Right time
- Þ Proper arrangement
- Þ Specificity & right partners

through *reversible* and *adjustable*
molecular interactions.

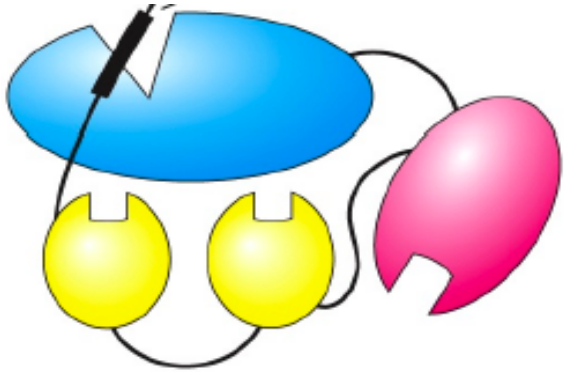
Proteins - Modular assembly of functional domains

- Proteins must be multi-functional
  - specific interaction
  - regulation of interaction
  - effector

- Proteins are in general not monolithic globules



but rather have a beads-on-a-string multi-domain structures,
where ***each domain has a specific function***



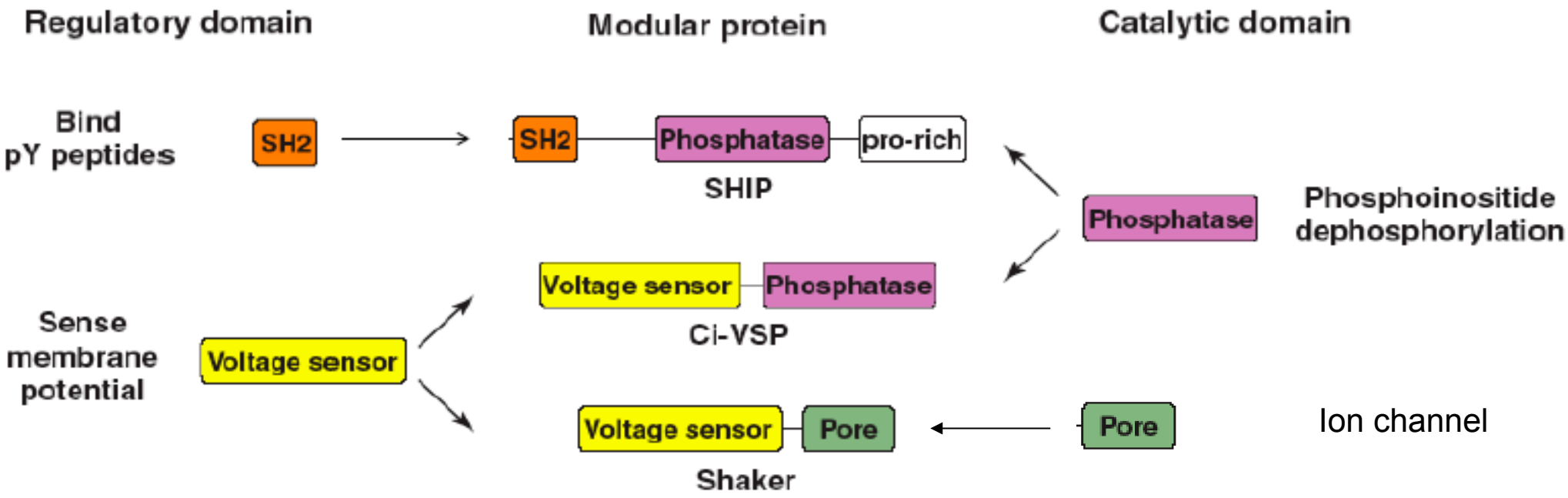
- Domains: small (70-140 amino acids) autonomously folding sequences
  specific function e.g. catalysis or binding
  > 1'000 type of domains present in human genome

- Linear combination of a few domains yields an infinite variation of proteins
  with each unique properties

"Modular Protein Domains" Cesareni e.a. (Eds) 2005 Wiley

Domain combinations - Variations on a theme

"Combinatorial" protein design to create proteins with new properties



Reversible & adjustable protein interactions

Examples of protein domains involved in molecular recognition:

Domain	Sequence recognised	Abundance human genome
SH2	-pY-x-x-hydrophobic-	352
PTB	-hydrophobic-x-N-P-x-pY-	141
SH3	-P-x-x-P-x	894
WW	-P-P-x-Y-	307
14-3-3	-R-S-x-pS-x-P-	19
PDZ	-E-S/T-D/V- *C*-terminus	918
PH	phospholipids	±250
C2	phospholopids	641

NB: human genome encodes approximately 3.10^4 proteins

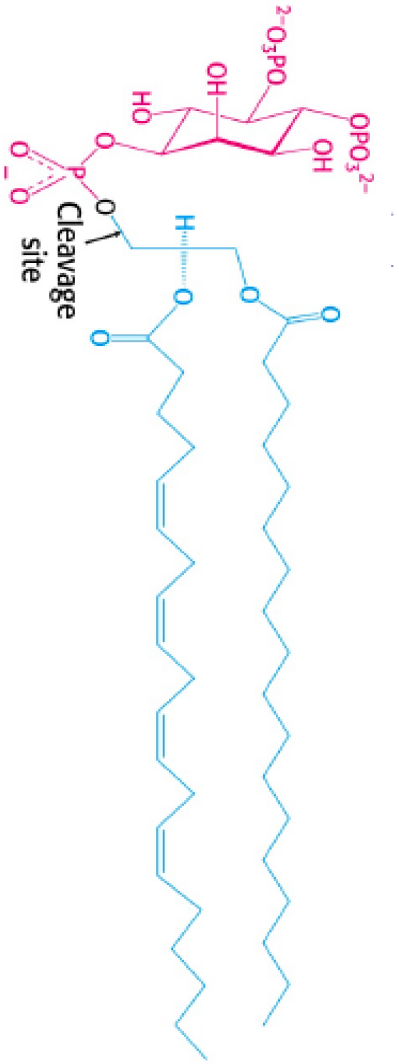
M-M Zhou, Bhattacharyya Ann Rev Bc 2006

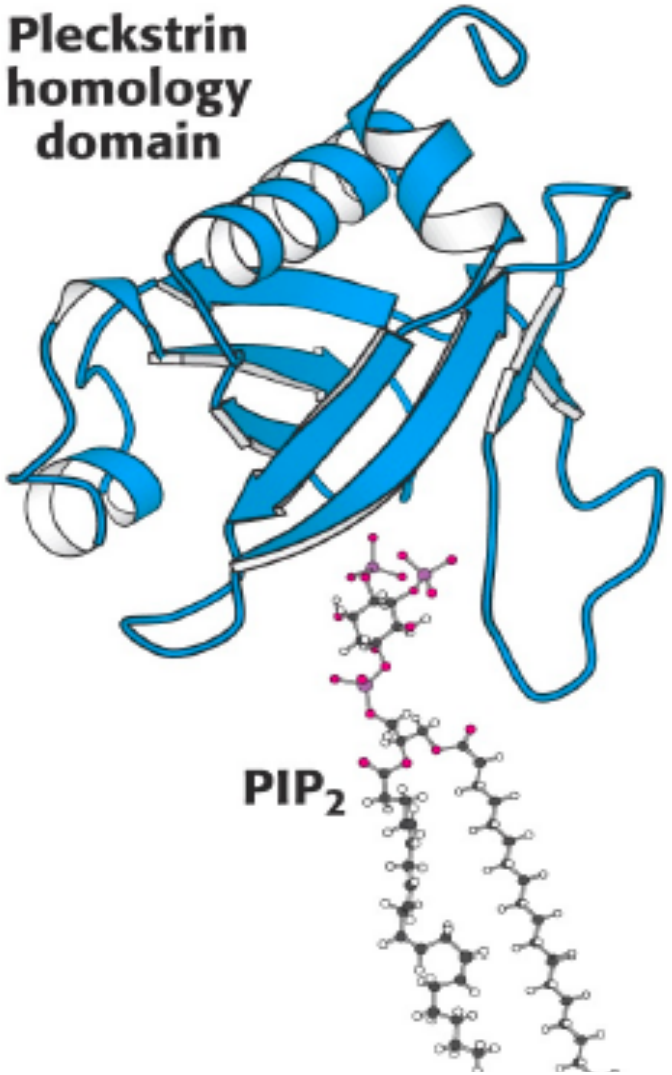
Reversible & adjustable membrane binding

Pleckstrin homology (PH) domains binds specifically to so-called “PIP-lipids”

- some bind to “PIP_2” an abundant lipid in **only** the plasma membrane

PIP_2





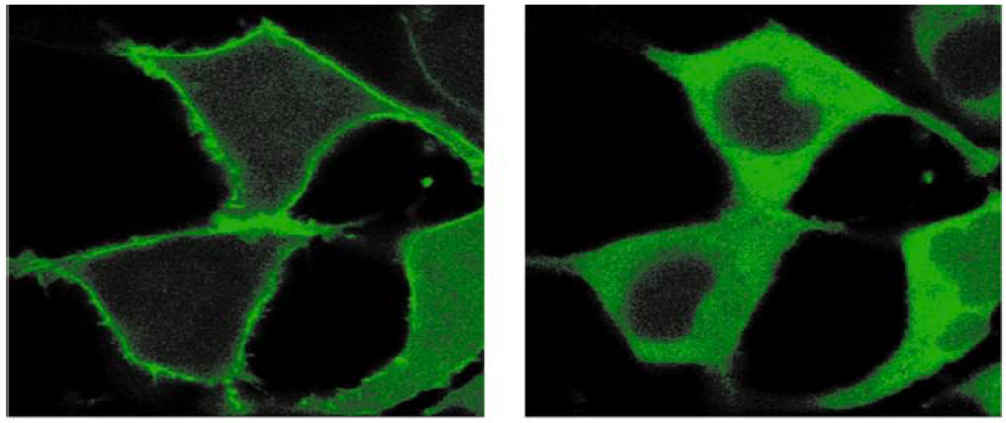
Imaging PIP_2 *in vivo* using PH-GFP chimeras

- PIP_2 is rapidly broken down upon activation of e.g. the angiotensin receptor

- A fusion protein PH_GFP, composed of a green fluorescent protein (GFP) and a PH domain binds to PIP_2 in the plasma membrane

- Angiotensin addition leads to PIP_2 breakdown

  => PH-GFP can not bind to membrane anymore



Control AngII 30 s

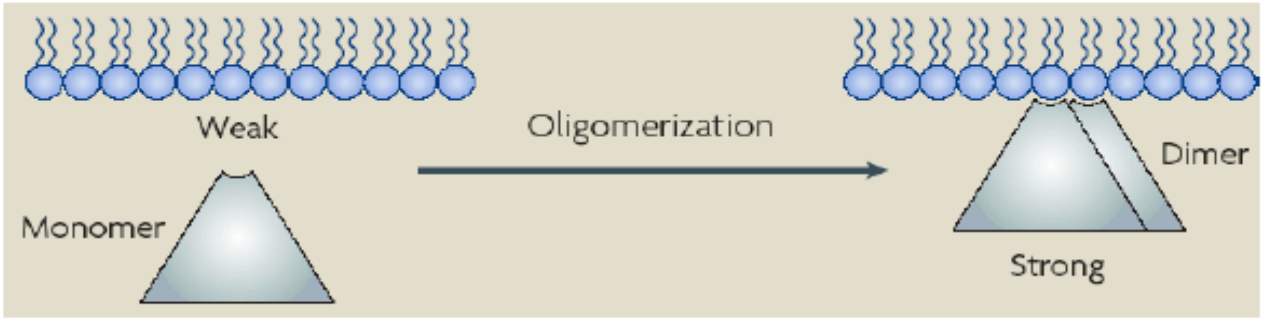
=> Regulated localisation

Balla, TiPS 2000

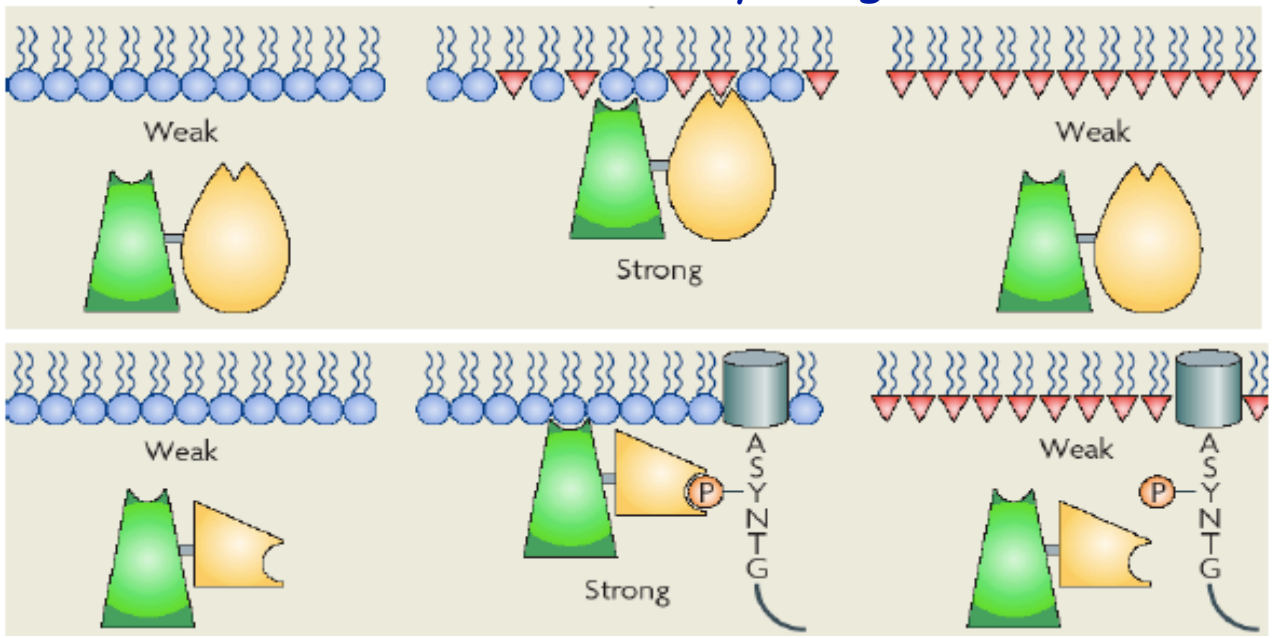
Combination of domains for co-incidence analysis

What now if one interaction domain is strong not enough for binding?

=> Stronger by more of the same



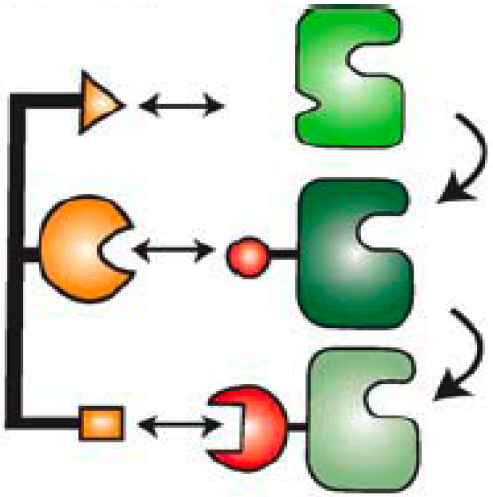
=> Stronger by **co-incidence** & more **selective** by **integration** of different inputs

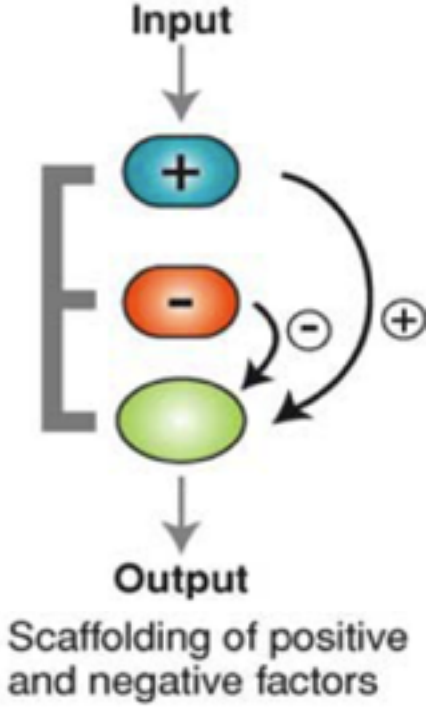


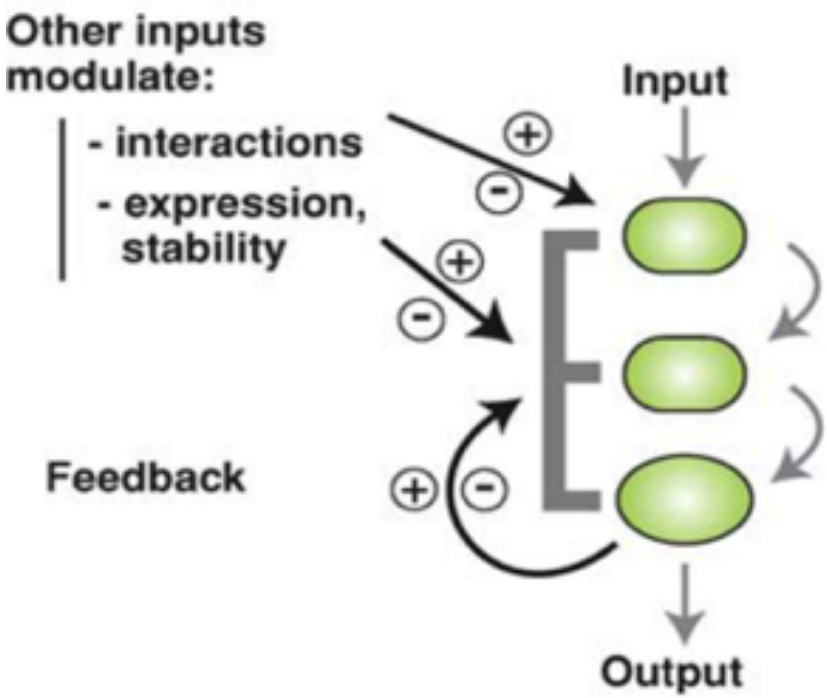
Lemmon, 2008

Organizing molecular interactions using domains

- Scaffold bring proteins together







"biological integrated circuit"

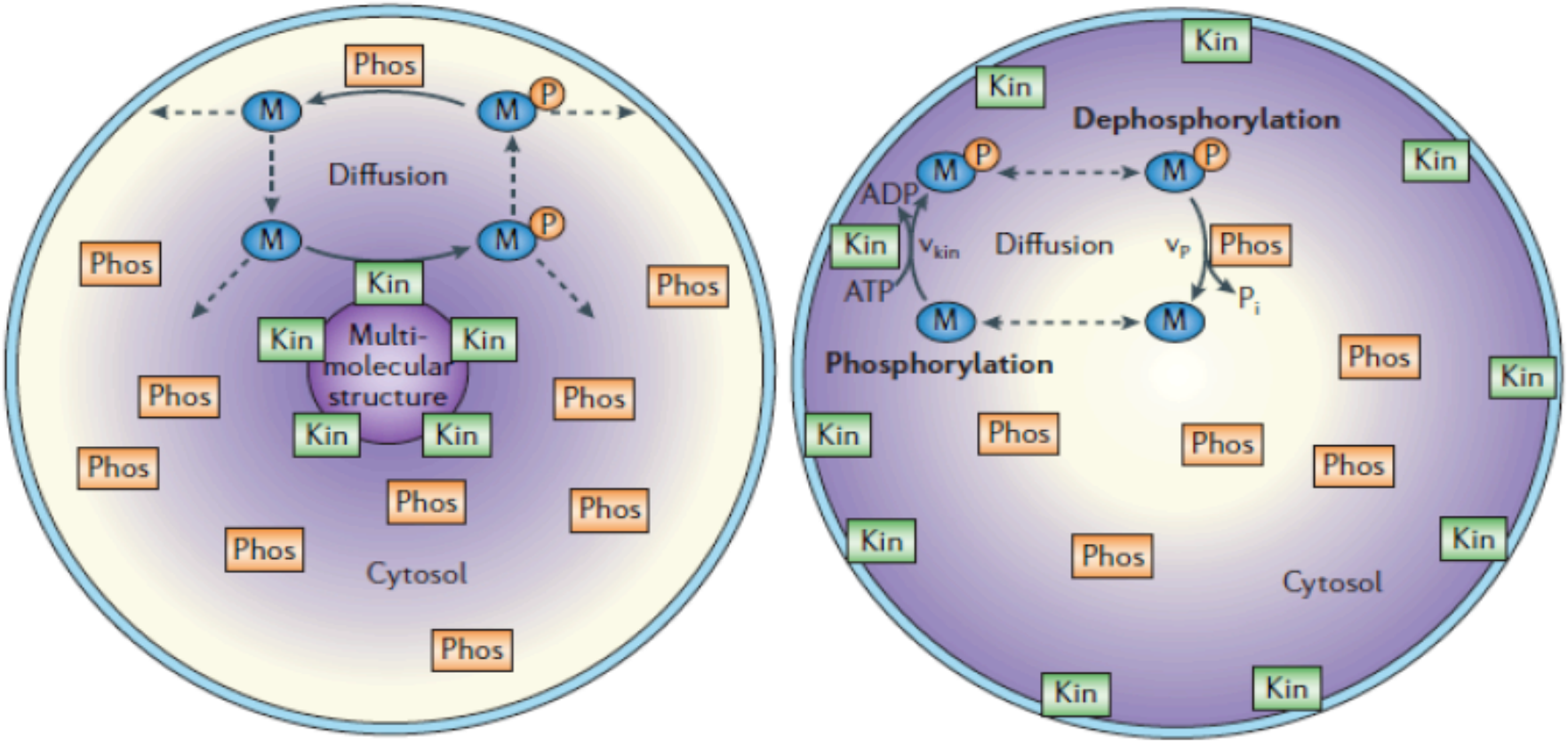
- Scaffolds allow **regulation** and **integration** of signals and **organization** of signaling molecules in **space** and **time**
- Modification of scaffold or proteins that bind affect output
- **Coincidence** of events is important for both control and out-put

Space & time in signalling: A thought experiment

E.g. for the phosphorylation state of protein M (purple hue indicate [M-Pi].

- the phosphatase **Phos** is homogeneously distributed in the cytosol, but
- the kinase is bound to

a central structure or the plasma membrane



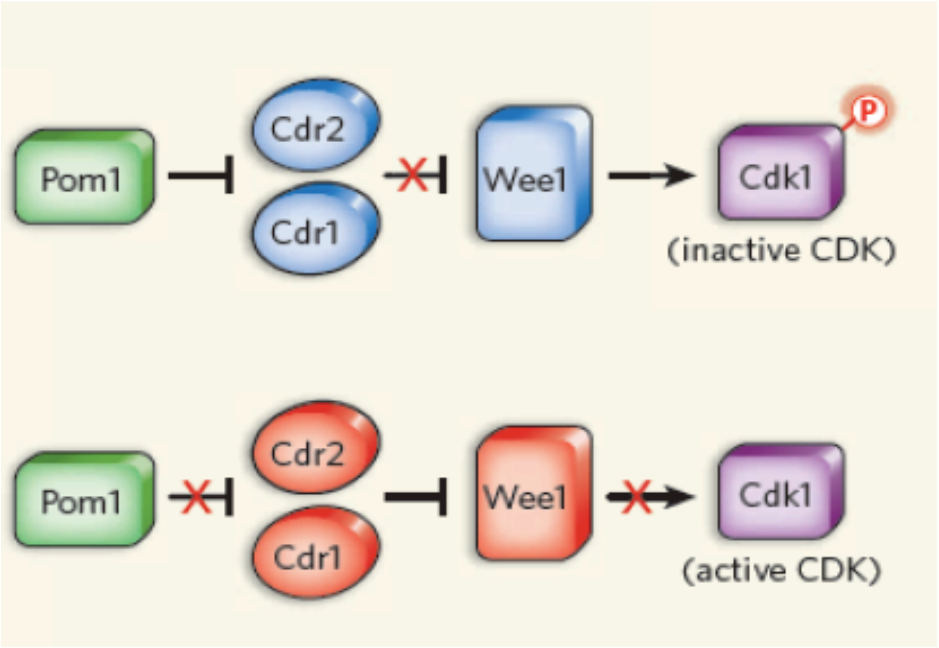
Kholodenko, Nat Rev Mol Cell Biol 2006

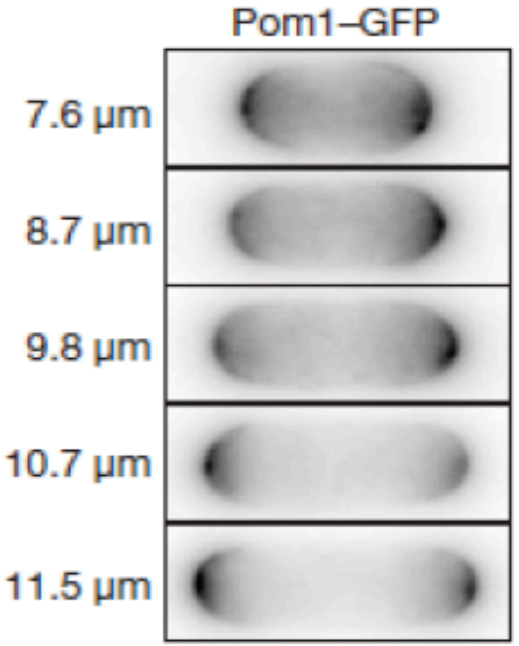
=> Localized activity creates concentration gradients

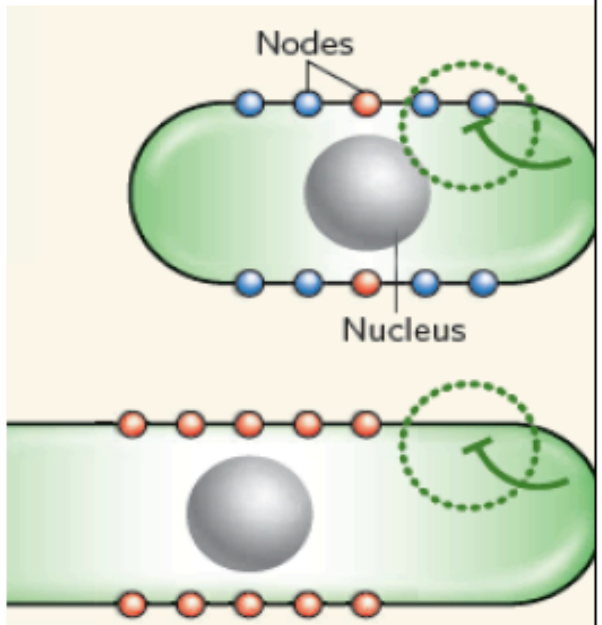
Space & time in signalling: A cellular mechanism

E.g. regulation of cell division of yeast.

Observations:
- only big cells divide
- all components needed seem to be present continuously
- When nucelar Cdk1 is active => cell division
- Pom1 inhibits Cdk1 via a cascade



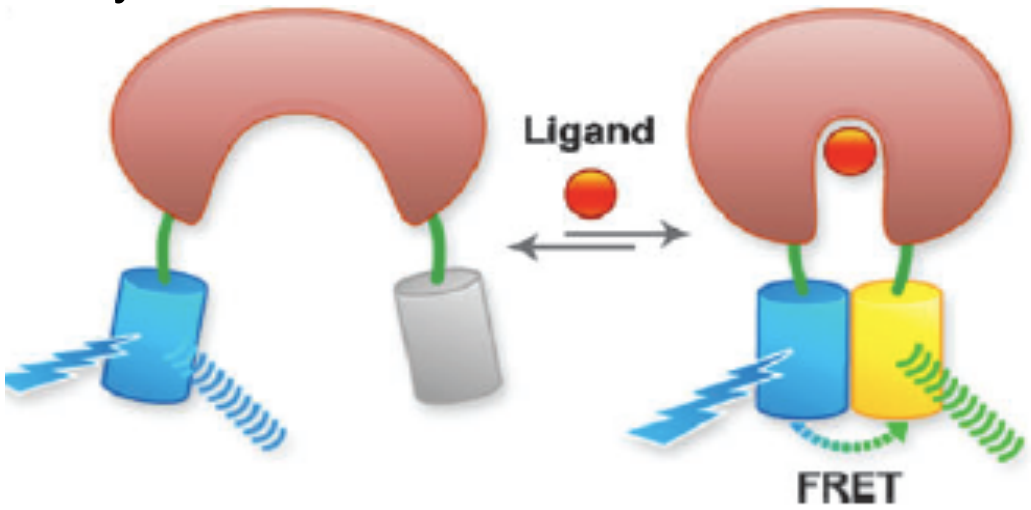




Moseley, Nature 2009

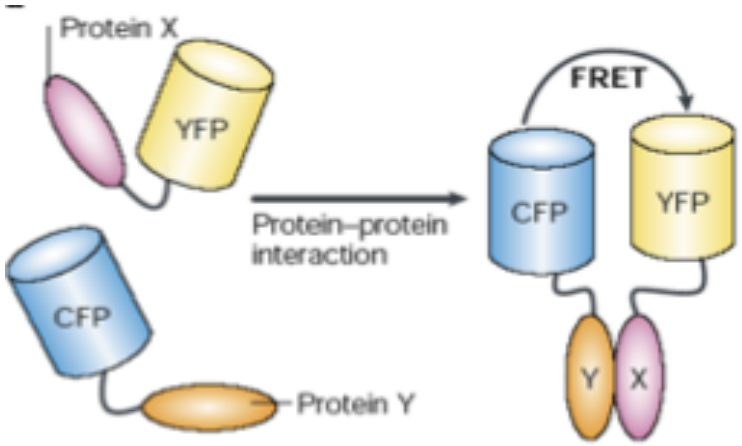
Sensors based on fluorescent proteins and molecular interaction

Analyte detection



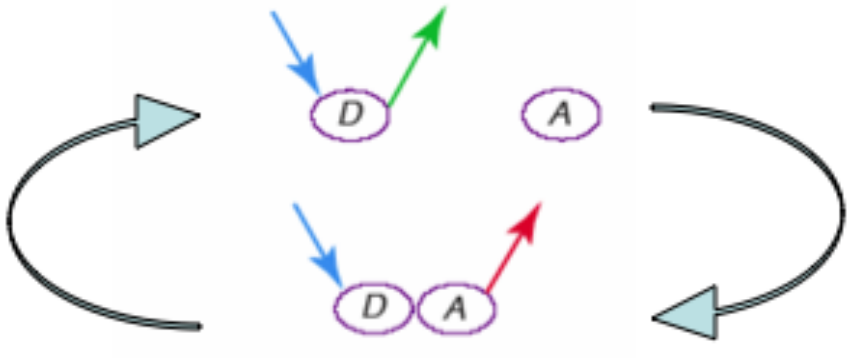
Frommer (2009)

Molecular interactions



J. Zhang, Nat Rev MolCelBiol 3 (2002) 906

Föster resonance energy transfer (FRET):



Transer of excitation energy from one fluorophore “Donor” to an “Acceptor”; possible only when Donor and Acceptor are within a few nm.

Up-to-date list:
biosensor.dpb.carnegiescience.edu/

Limitations of fluorescent protein-based sensors

- Fluorescent protein-based sensors
  - limited photo-stability
  - not so bright
  - limited visible range of spectrum
  - always “on”
  - conformational change needed

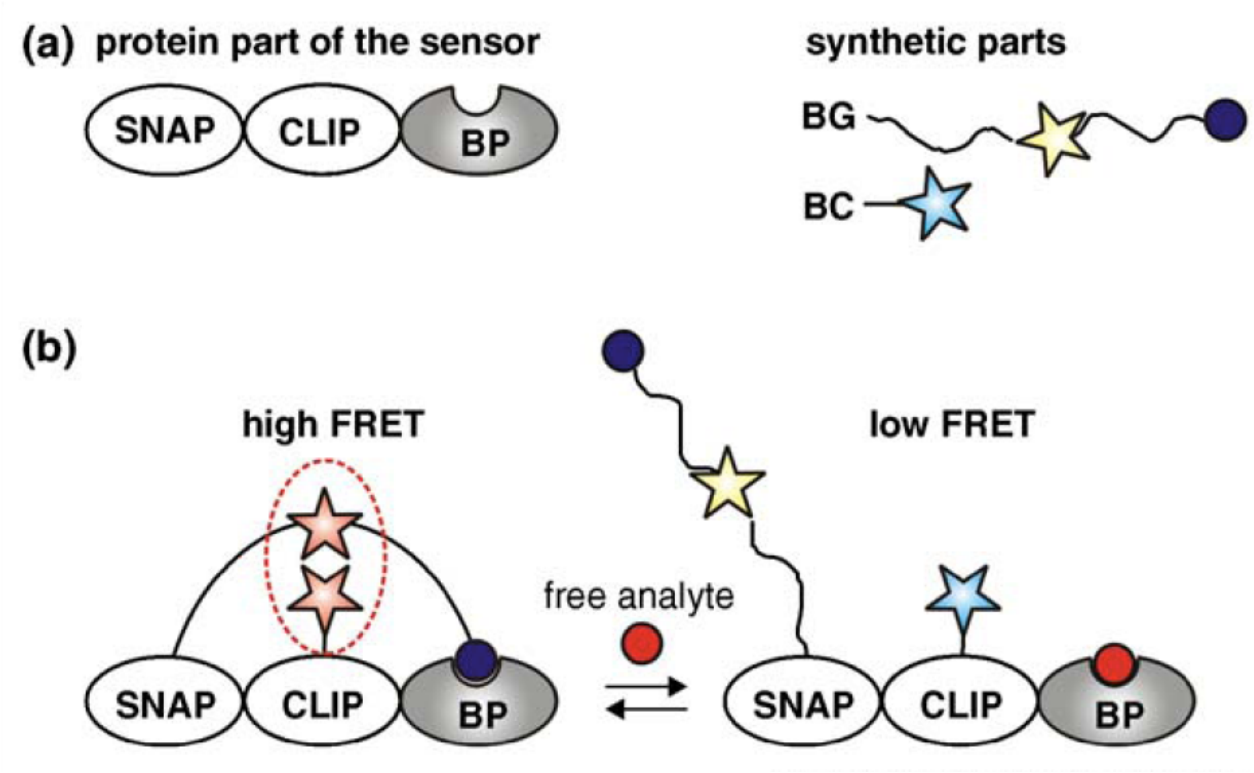
- Semi-synthetic sensors with self-labeling tags offer the possibility to :
  - introduce photo-stable and red-shifted dyes
  - measure longer, increase S/N, penetrate deeper in tissue
  - choose time of labeling, pulse labeling, multi-color labeling
  - employ rigid binding domains

Semi-synthetic sensors

Snif-it's (**SN**AP-tag based **I**ndicator proteins with a **F**luorescent **I**ntramolecular **T**ether) for visualizing the concentration of metabolites.

Additional innovation:

Binding domain does not have to undergo structural change !!



CLIP :
a mutant of SNAP
not reacting with BG,
but only with BC

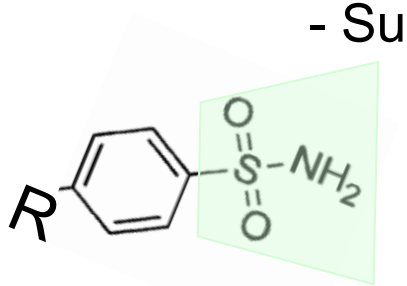
BP :
Binding protein for ●

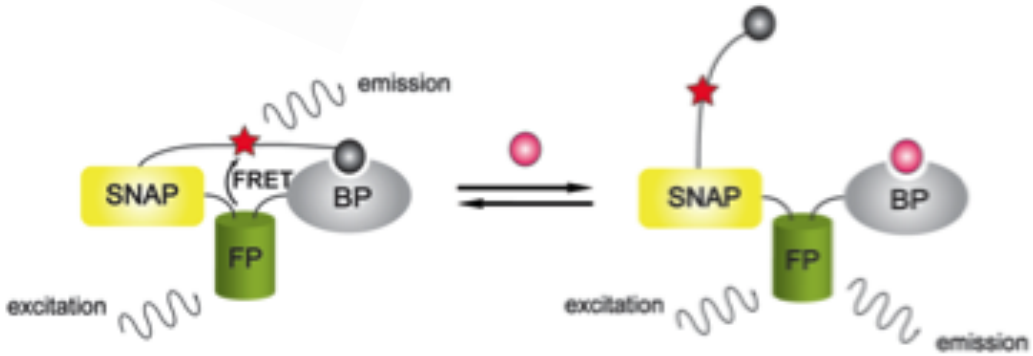
[Brun (2009) JACS 131, 5873]

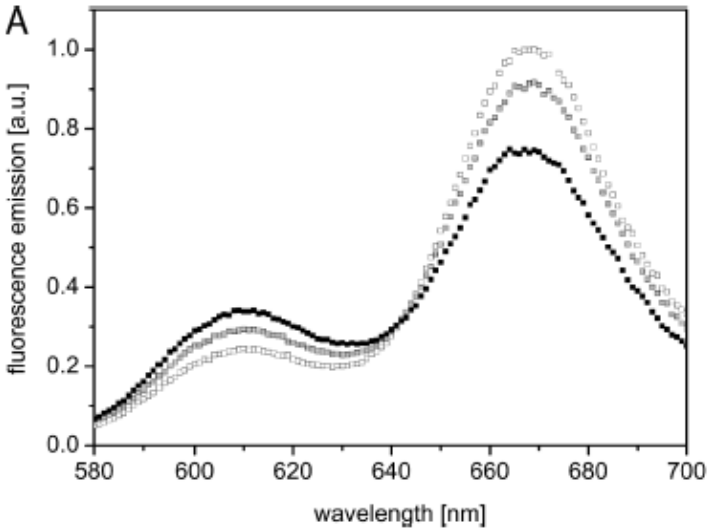
Semi-synthetic sensors – Snif-it's

An example: - Human Carbonic Anhydrase (**HCA**) as binding protein (BP)

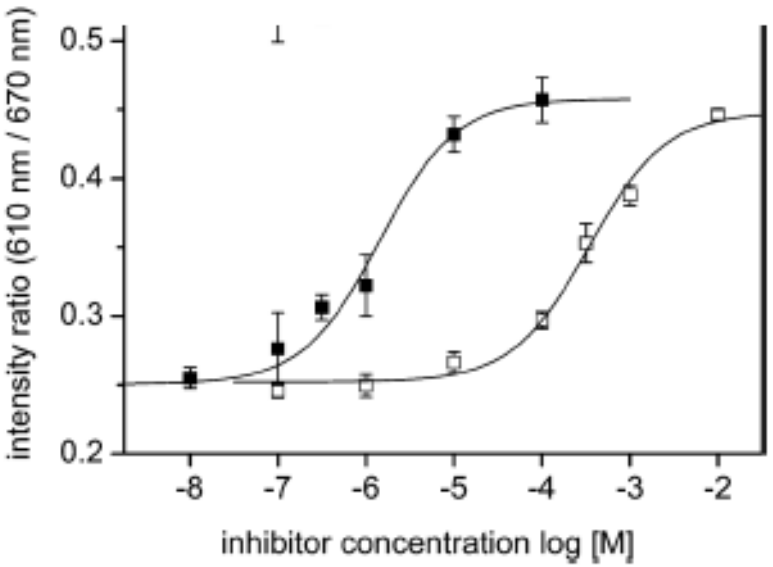
- Sulfonamides as analytes







- mCherry & BG-PEG$_{11}$-Cy5



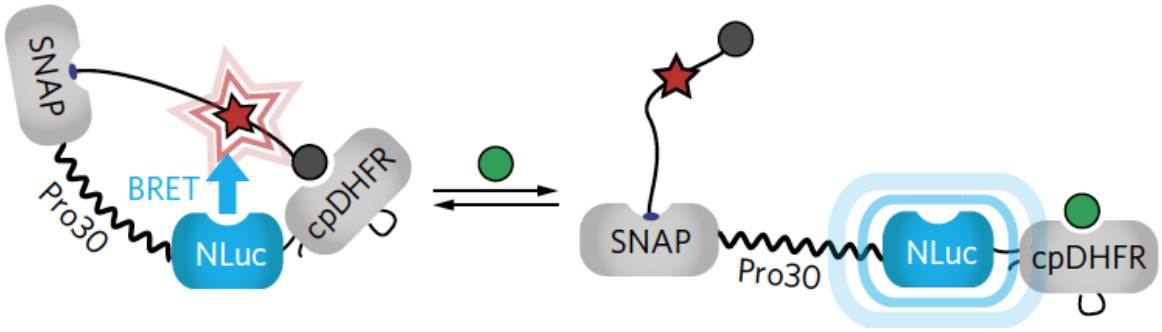
=> Principle works !

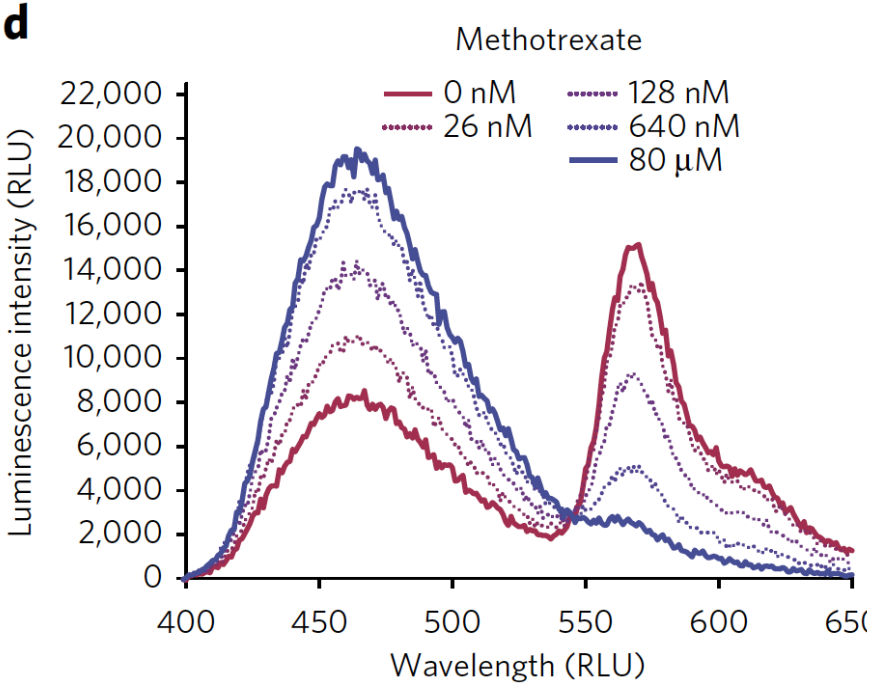
[Brun (2009) JACS 131, 5873]

Snif-it's for drug monitoring : LUCID's

- **LUCID's** : **Luc**iferase-based **i**ndicators of **d**rugs

  e.g. for the anti-cancer drug methotraxate





Griss (on-line June 2014) Nat Biotech *Schena (on-line July 2015) Nat Commun*

Interacting molecules - Nanoscopic building blocks

Constructing supra-molecular functional entities using nature's components.

=> in solution or on surfaces

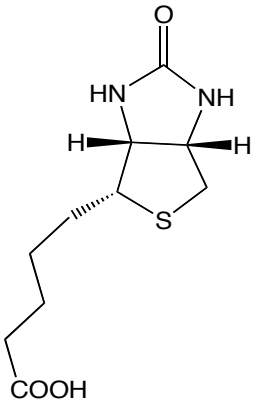
Commonly used constructing materials:

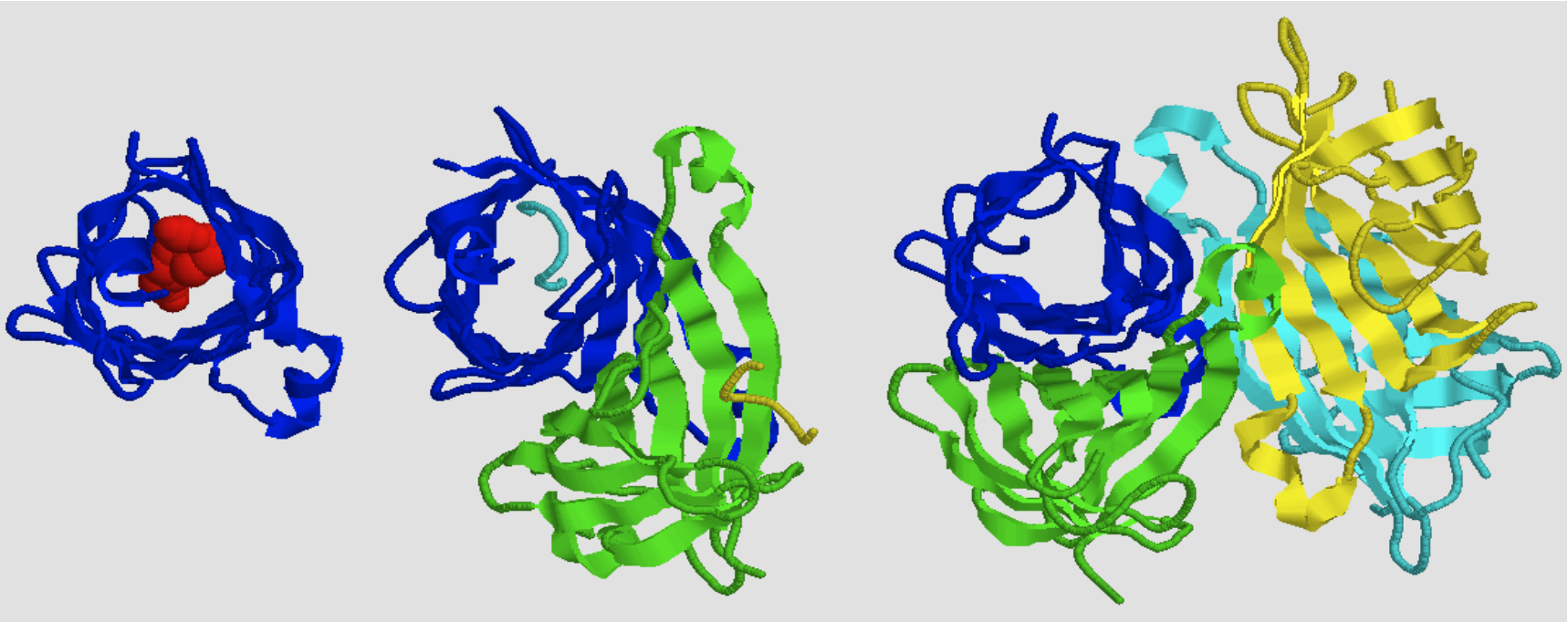
- Streptavidin	Biotin
- Immunoglobulins	Antigens
- Oligonucleotides	Oligonucleotides

- Natural or engineered binding proteins

Interacting molecules: Streptavidin

- Tetrameric protein from *Streptomyces avidinii*, about 65 kDa (approx 5x5x5 nm)
- each monomer binds very tightly to **biotin** : $K_d \sim 10^{-14}$ M or $\Delta G \sim 32$ kT.
  Biotin, also known as vitamin H or B7





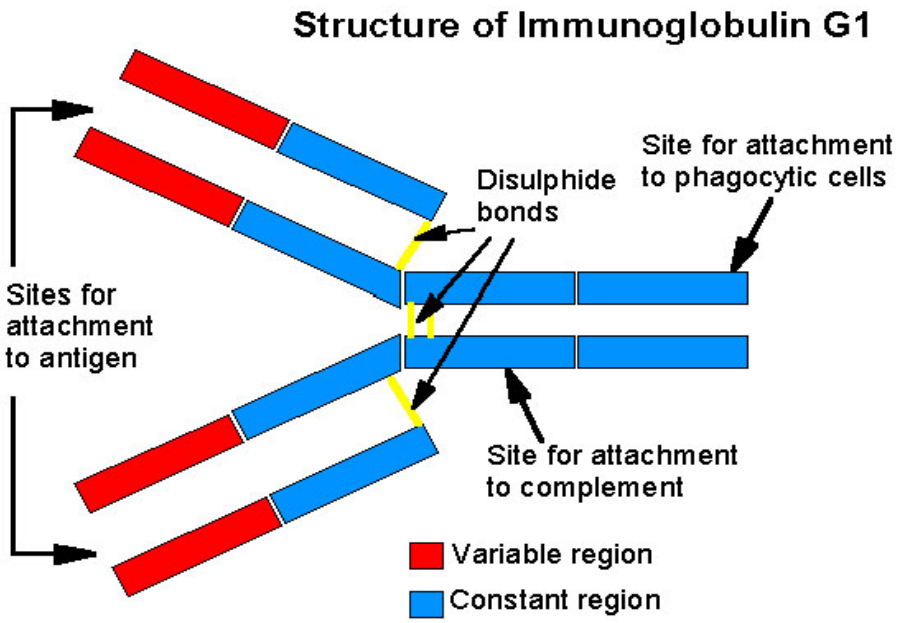
- Avidin is a glycoprotein from bird eggs with comparable structure and properties.
  It prevents biotin absorption in the gastrointestinal tract.

- Biotin can easily be linked chemically to many substances.

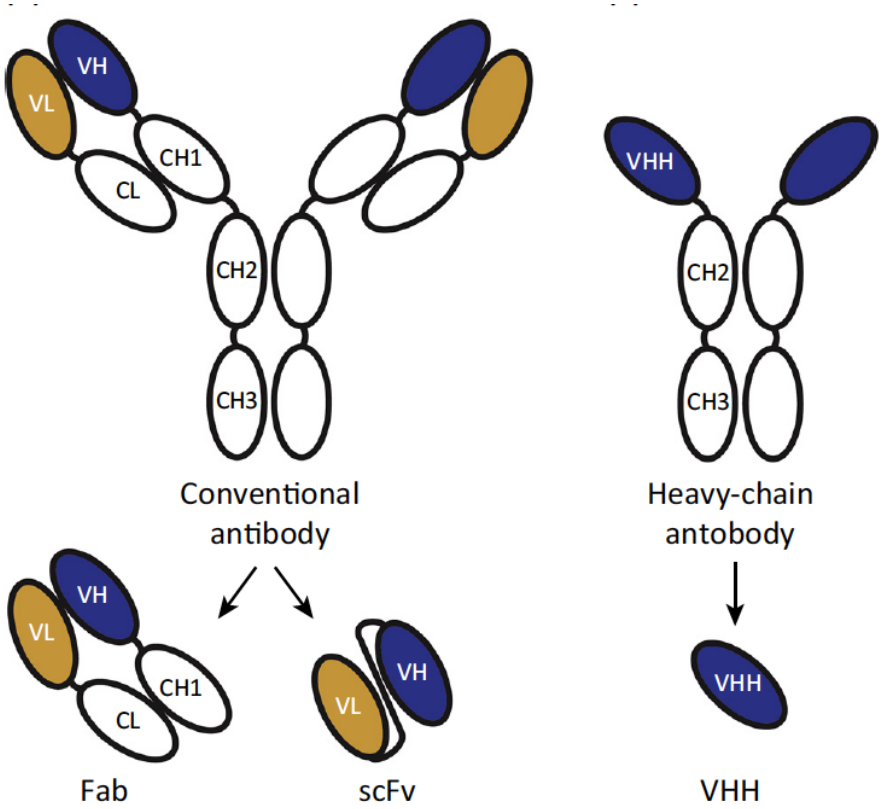
Interacting molecules: Immunoglobins

A binding partner to almost anything, the immune system uses immuno-globins, also called antibodies:

- the antigen is bound to the variable regions
- genetic recombination yield infinite variations



Antibody of IgG class



- Each domain is approx 50 kDa and 5 nm long.
- Dissociation constants are nM for good antibodies.

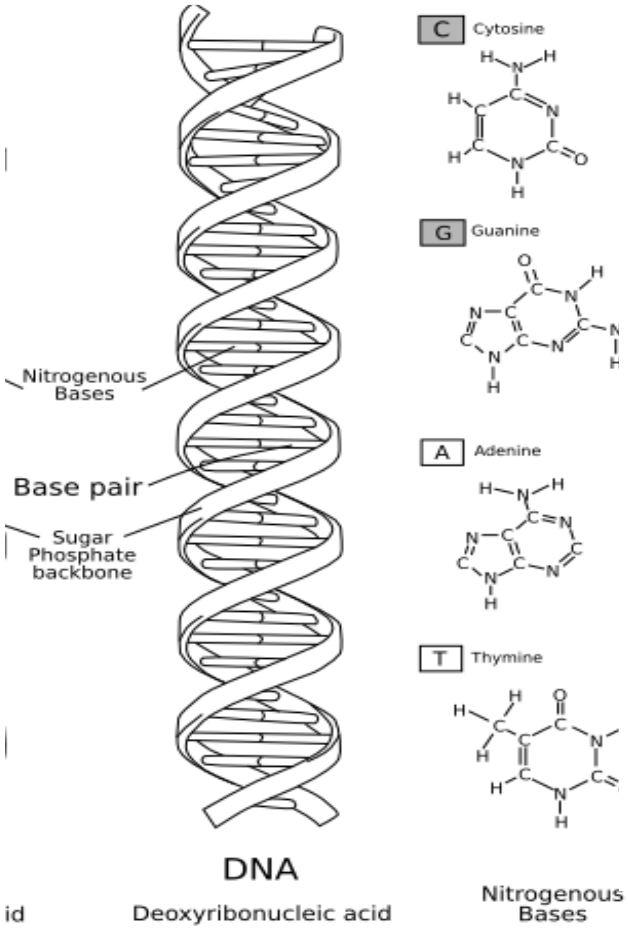
Smaller derived binding domains, e.g . VHH

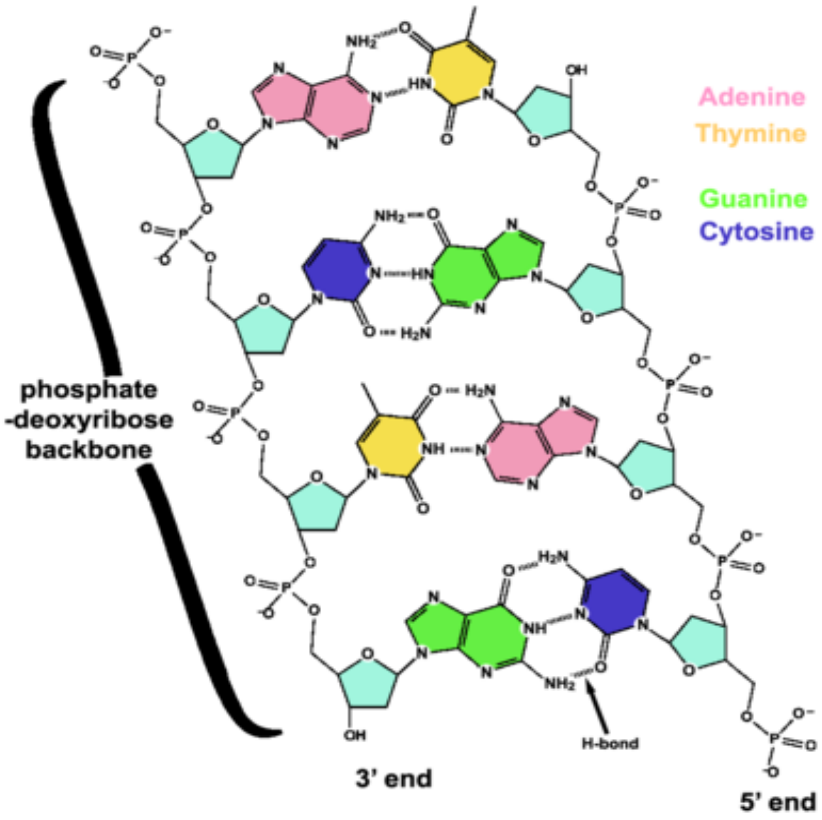
De Meyer, TiBioTech 32 (2014)

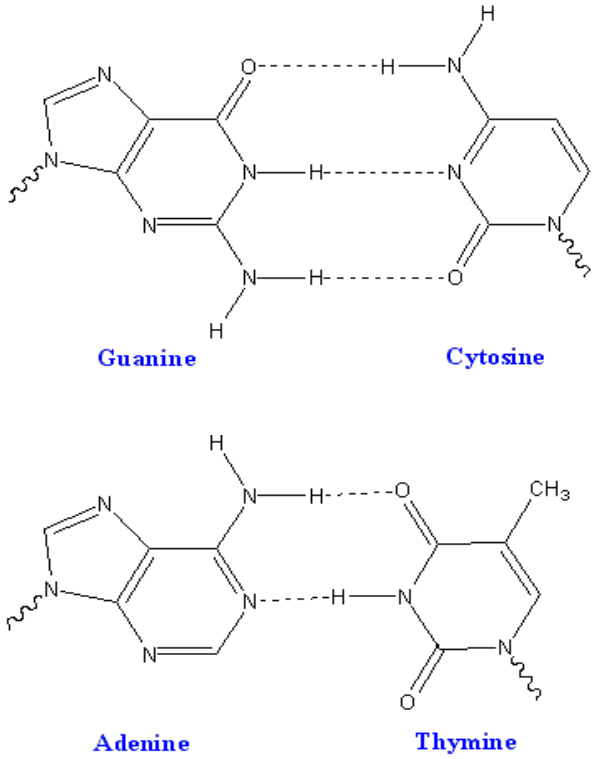
Interacting molecules: Oligonucleotides

Oligonucleotides are polymers made of the 4 (ribo)nucleobases : A C G T

Complementary antiparallel strands hybridize through specific hydrogen-bond directed base pairing and sequence recognition

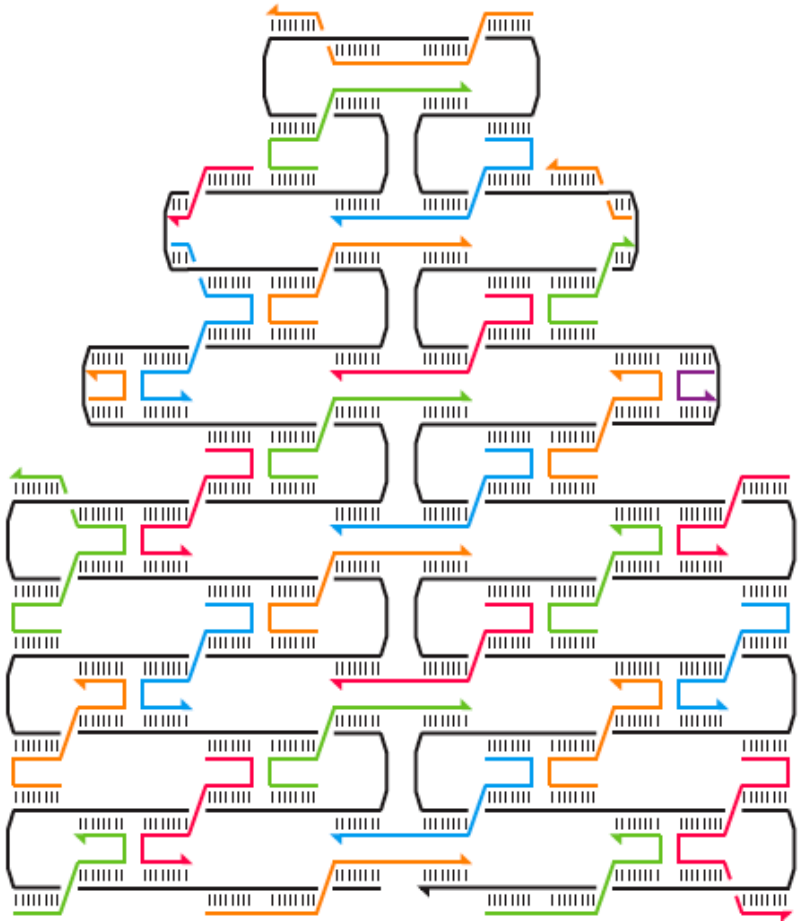


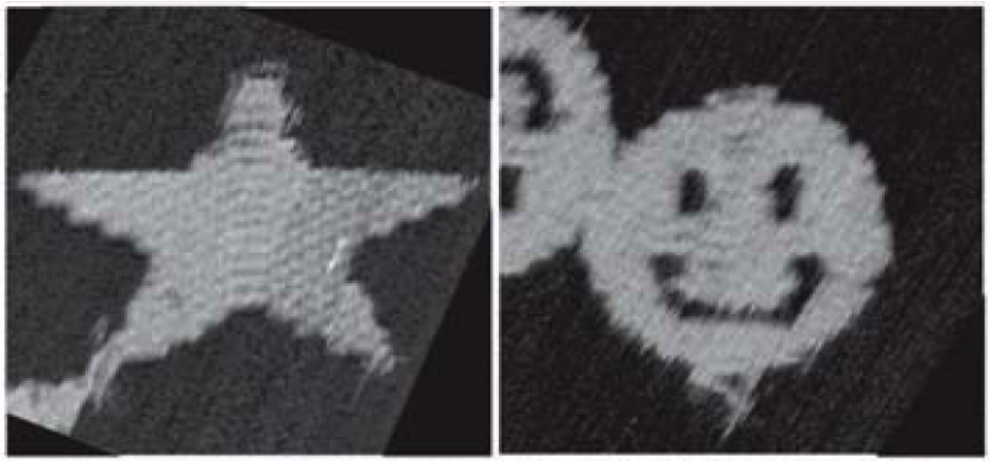




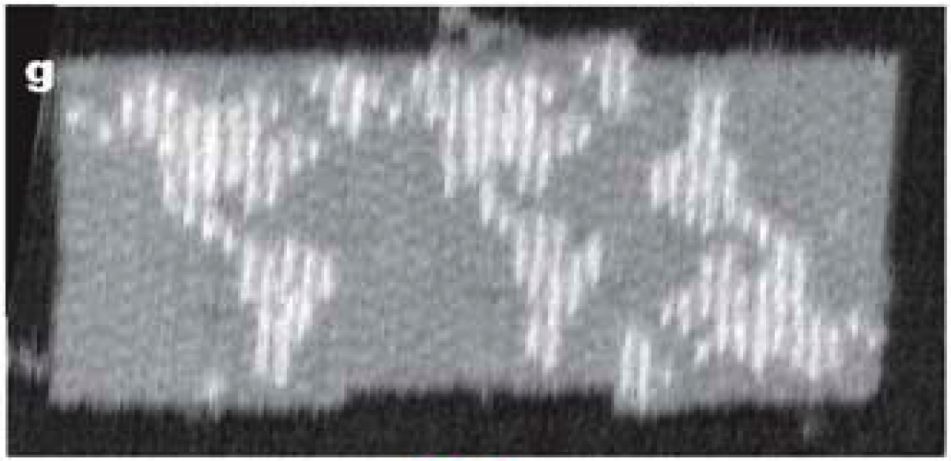
Molecular origami with oligonucleotides

Base pairing & sequence recognition can be used e.g. to construct complicated structures:





165x165 nm

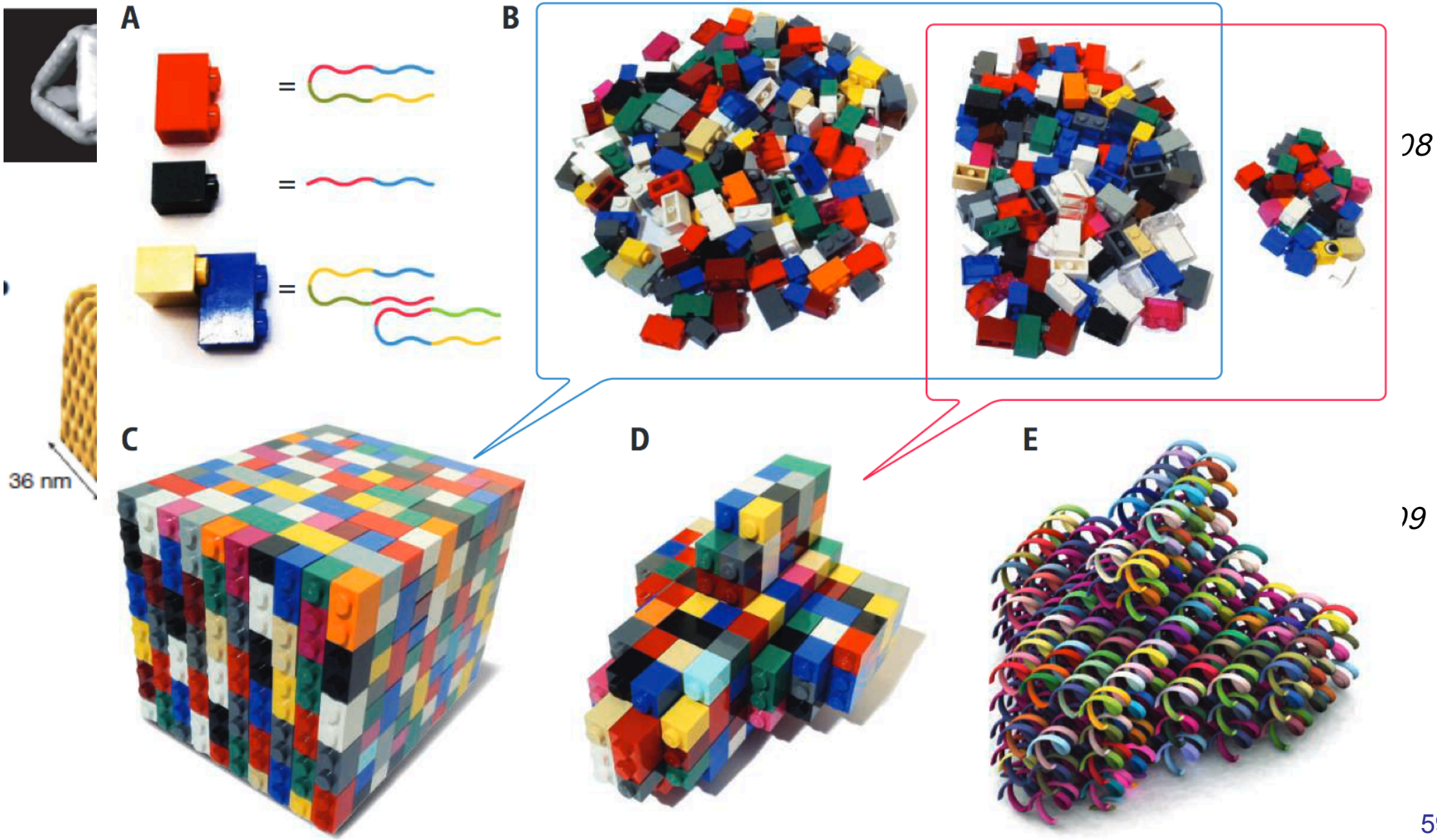


Scale: 1 to 2.10^{14}

Rothemund, Nature 440 (2006) 297

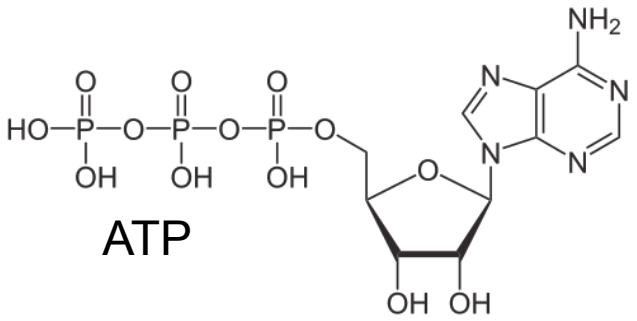
Molecular origami with oligonucleotides

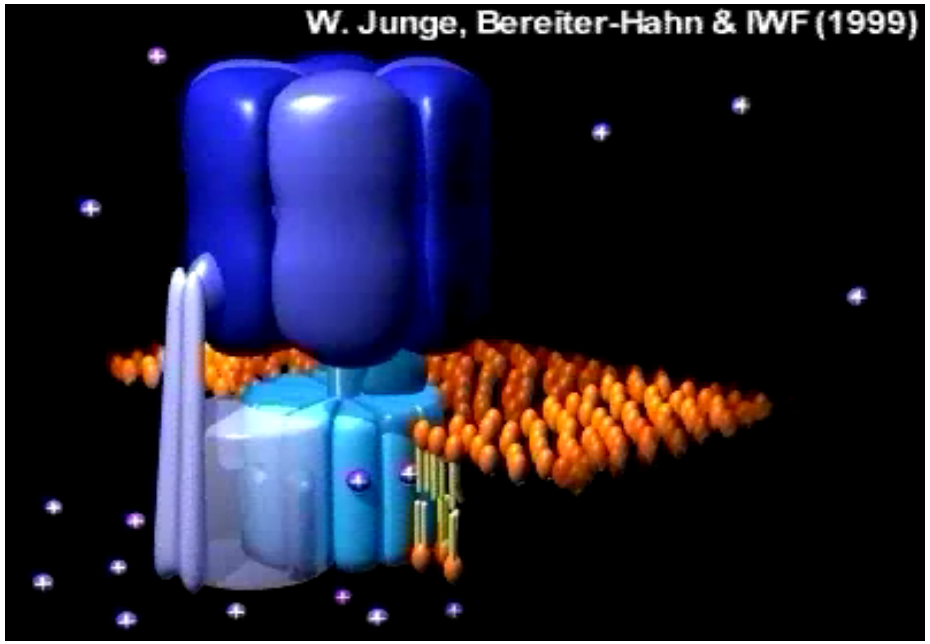
Also 3-D structures can be made:



Molecular machines

- Energy conversion
- Linear motors
- Rotational motors



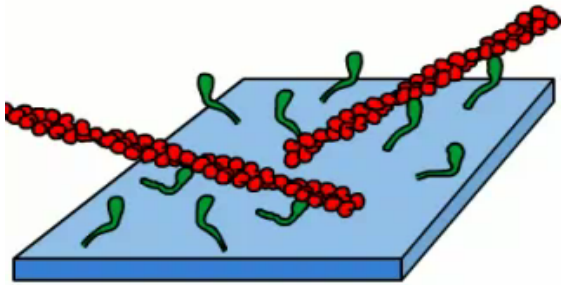


ATPase uses H^+- gradient to **produce ATP**: 3-5 H^+ needed per ATP

Energy conversion:	Electro-chemical >> mechanical >> chemical
Dimensions:	Diameter about 10 nm
Rotations:	> 3000 rpm => >10’000 ATP/min

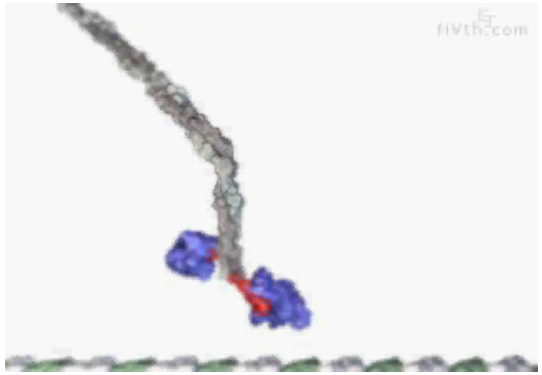
Molecular machines

- Energy conversion
- Linear motors
- Rotational motors

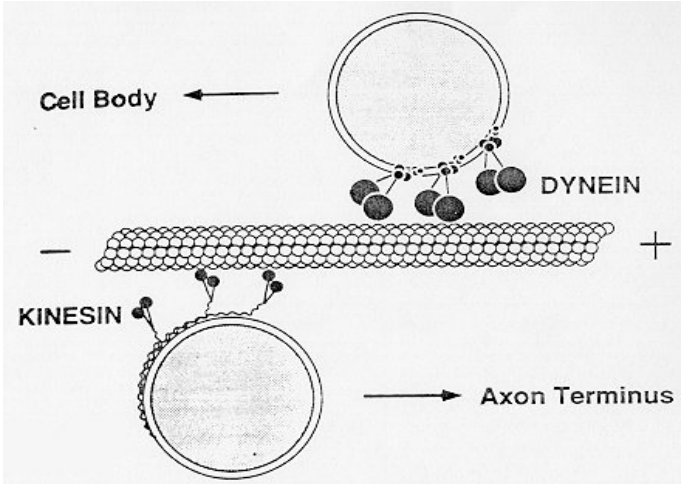


Actin filaments slide on myosin motors

Motors walk on filament



Kinesin on microtuble



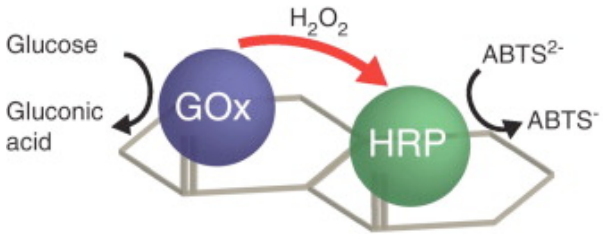
Typical speed: about 1 μm/s
Step size: about 10 nm
Energy conversion: ATP => motion

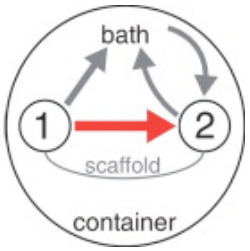
Bio-inspired nanotechnology => Nanomachines?

÷ Molecular interactions
÷ Nanoscopic structures

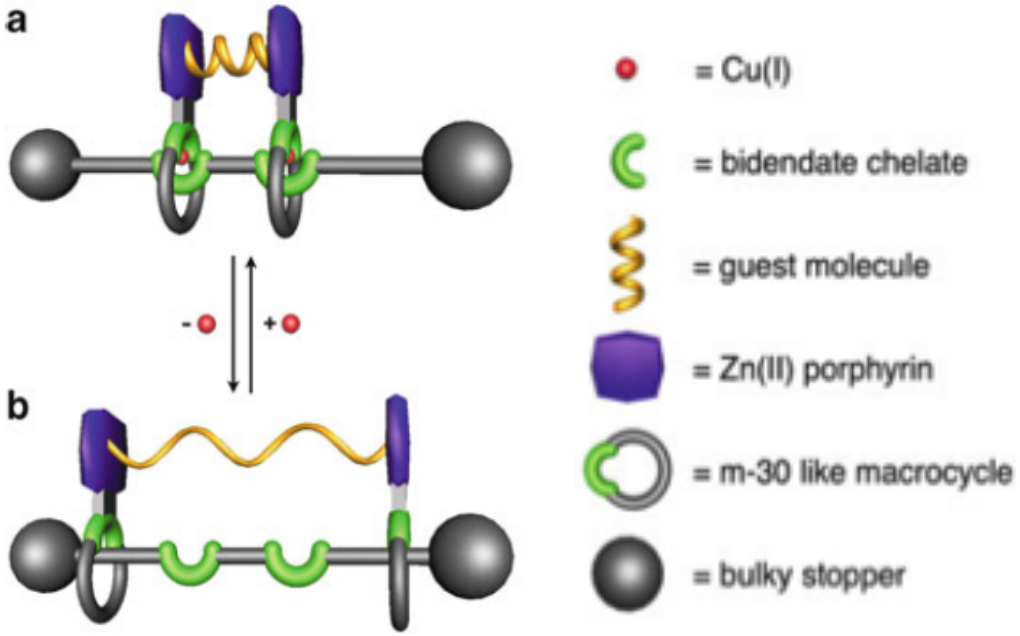
=> Nanobiomachines

Scaffolds for "assembly lines"

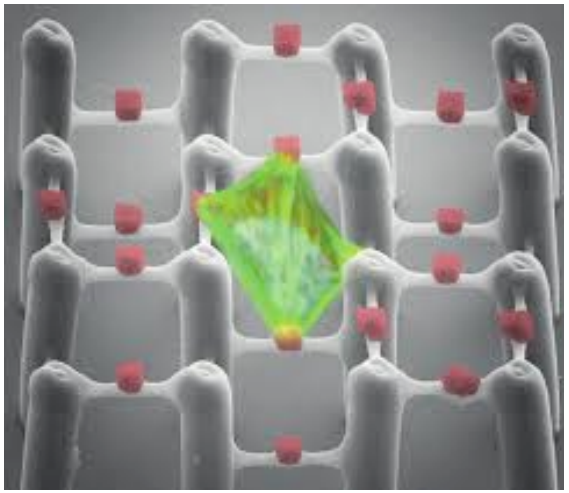




Responsive sensing

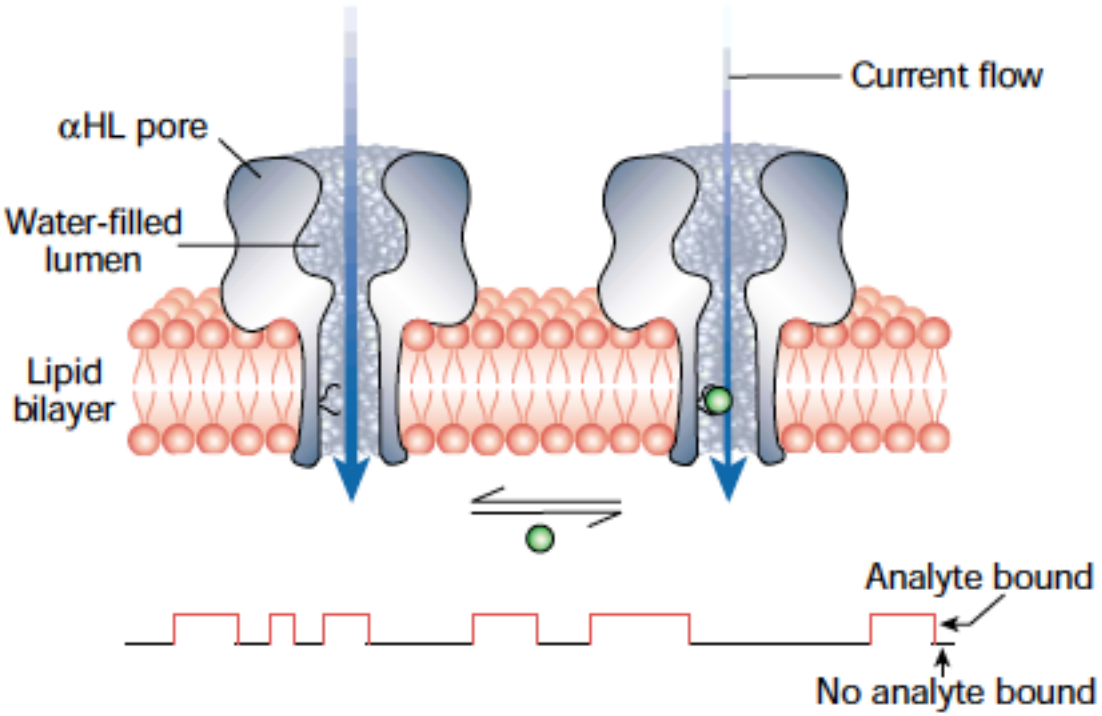


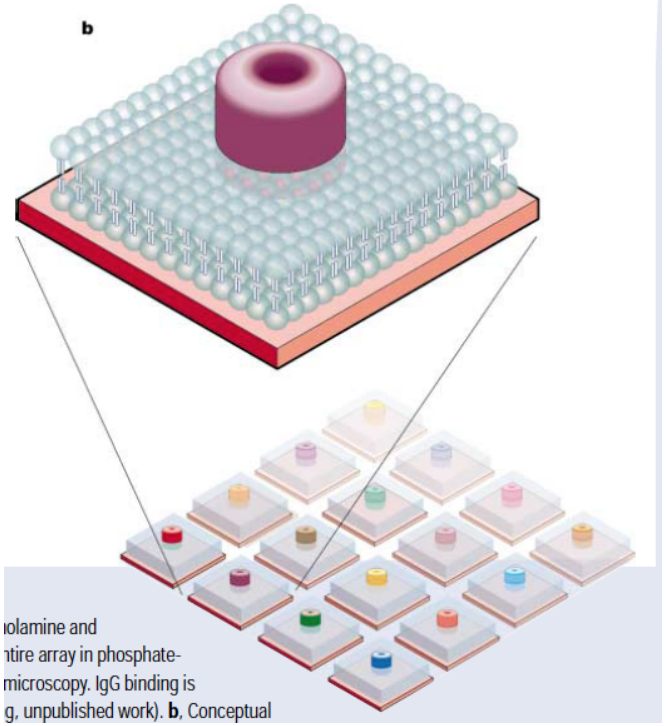
Cellular Mechanics



Nanopore sequencing : From dream to reality

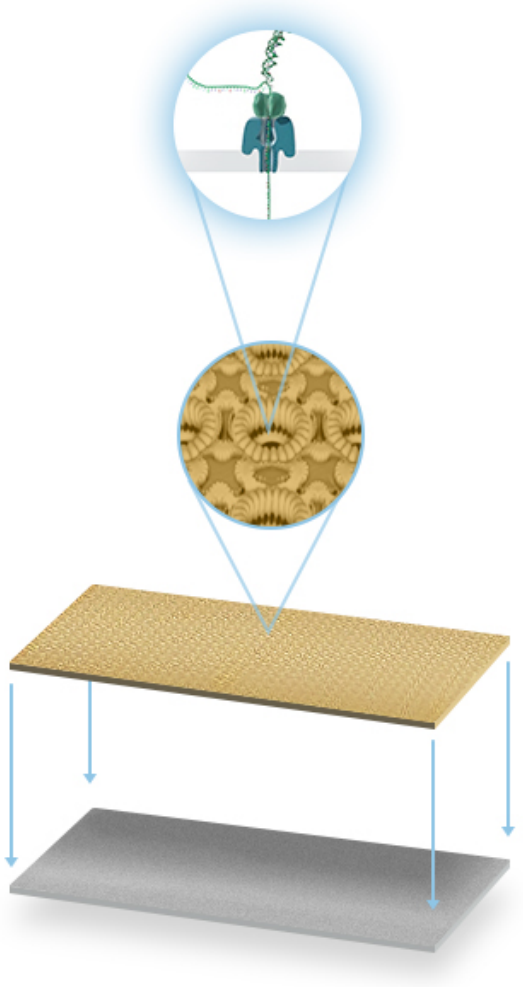
~2000 : The dream Hagan Bayley





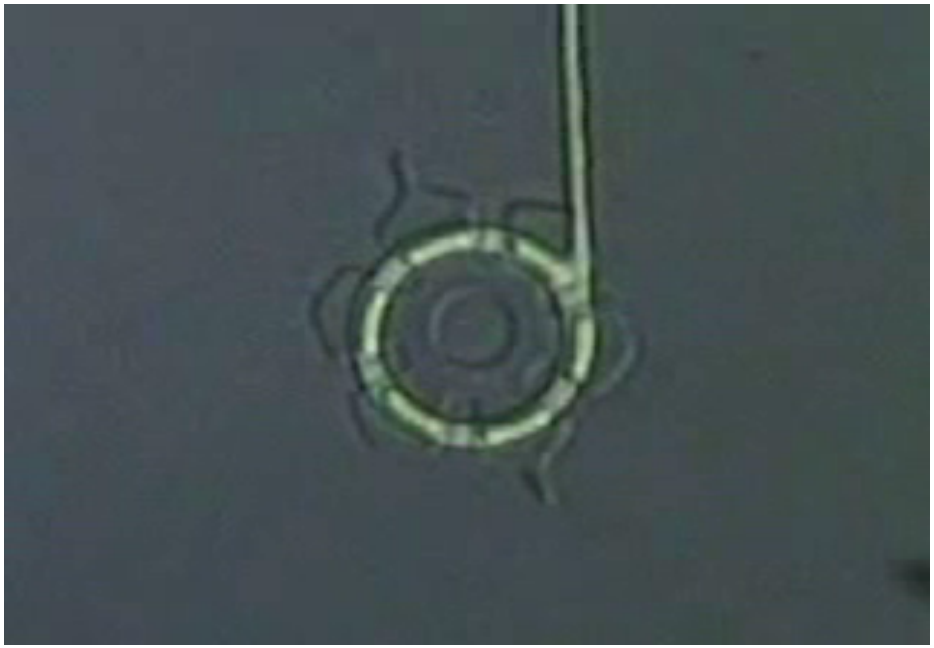
Nanopore sequencing : From dream to reality

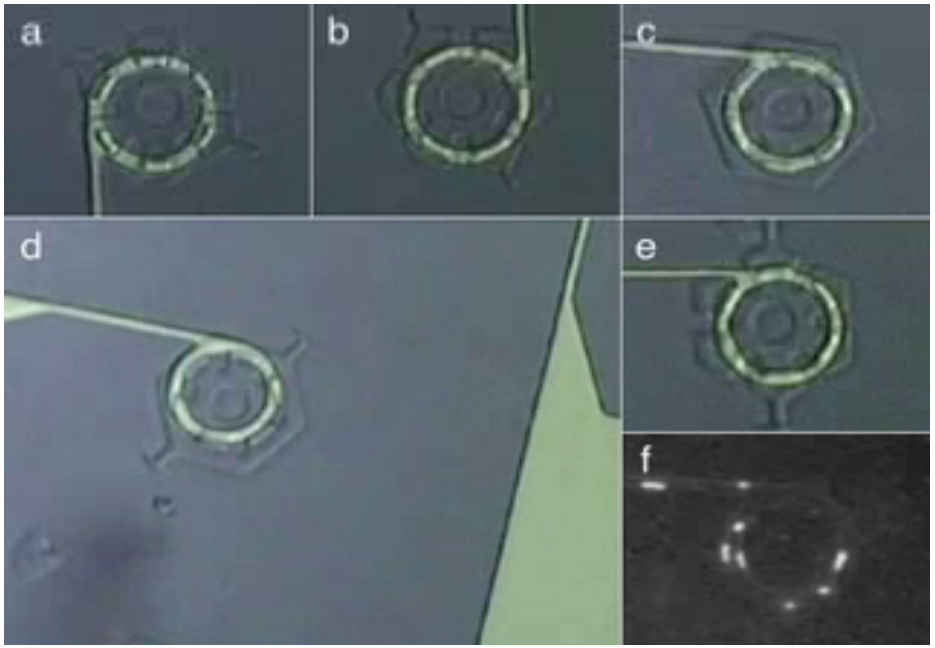
Today's reality Minion from Oxforn Nanopore

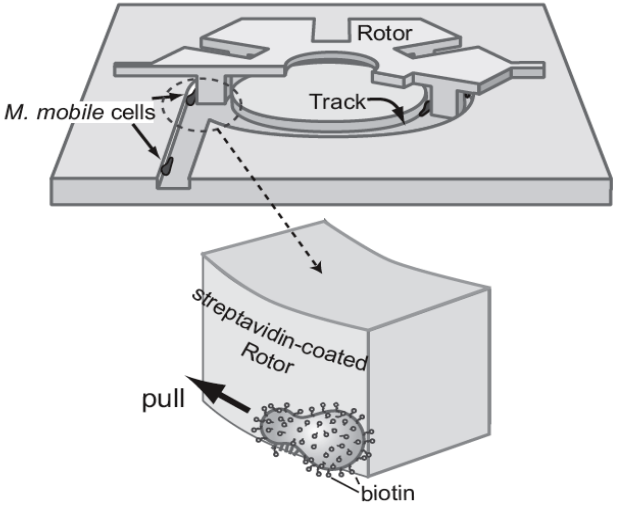


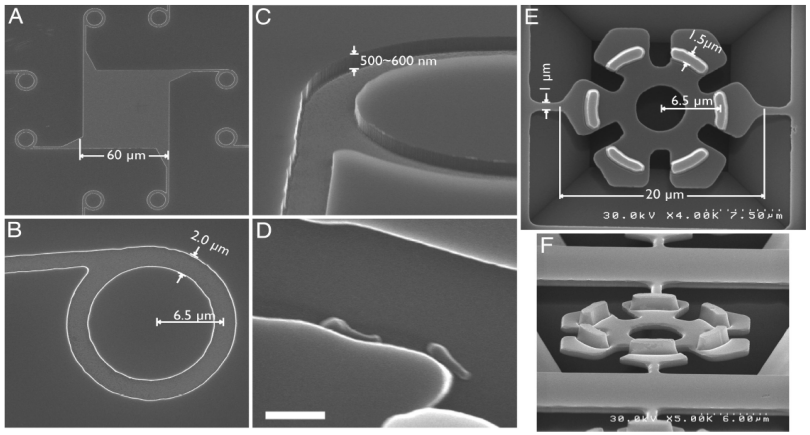
Biomolecular nanotechnology => Nanomachines?

Nanobiomachines : A recent example of a completely different approach









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