Single-molecule membrane biology

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Single-molecule membrane biology: Outline

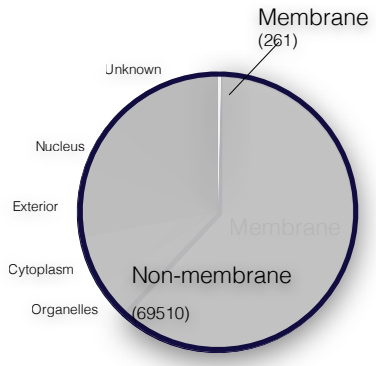
- Cell membranes.
- Why single-molecules?
- Current applications.
  - Ion channels.
  - Protein assembly and dimerisation.
  - The role of lipids in membrane protein function.
  - Do lipid rafts exist?

...(aka Presentation / Extended essay topics).

Resources

- Slides
  - wallace.chem.ox.ac.uk/teaching
- Books
  - Lakowicz, Principles of Fluorescence Spectroscopy.
  - Knight, Single Molecule Biology.
  - Hinterdorfer & Van Oijen, Handbook of Single-molecule biophysics.
  - Leake, Single-molecule cellular biophysics.

Membrane protein as drug targets



Heart Disease

Cancer

Cystic Fibrosis

Chronic Pain

Diabetes

High Blood Pressure

Schizophrenia

Alzheimer's Disease

....

The cell membrane, a reminder...

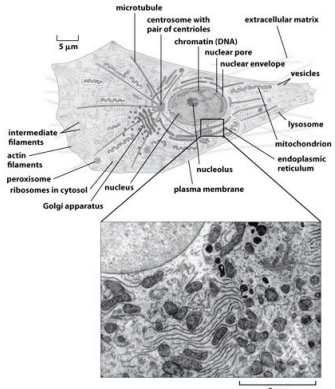


Figure 2.13 Physical Biology of the Cell (© Garland Science 2009)

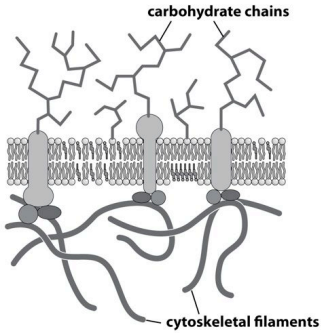
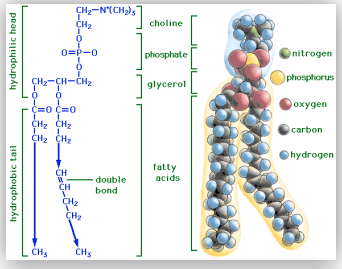
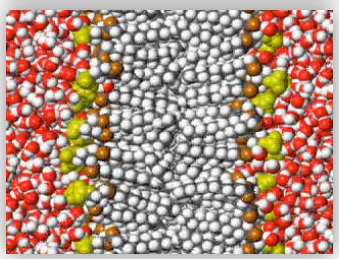
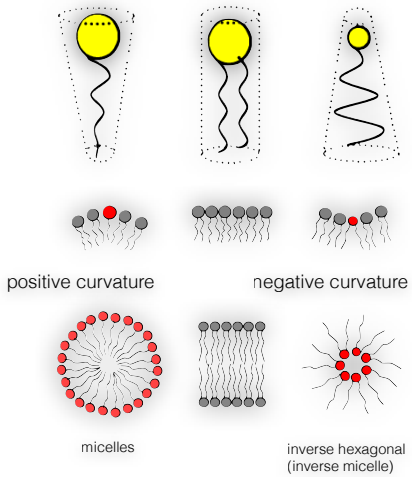


Figure 11.4c Physical Biology of the Cell (© Garland Science 2009)

Lipids self assemble







http://faculty.abe.ufl.edu/~chyn/age2062/lect/lect_06/

Constituents of the cell membrane

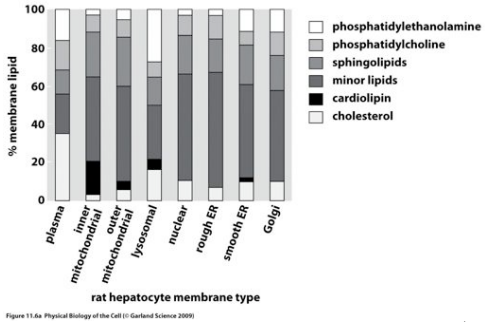
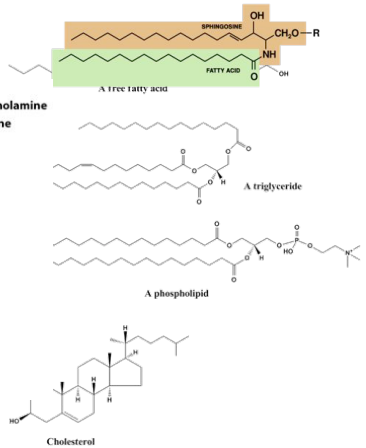


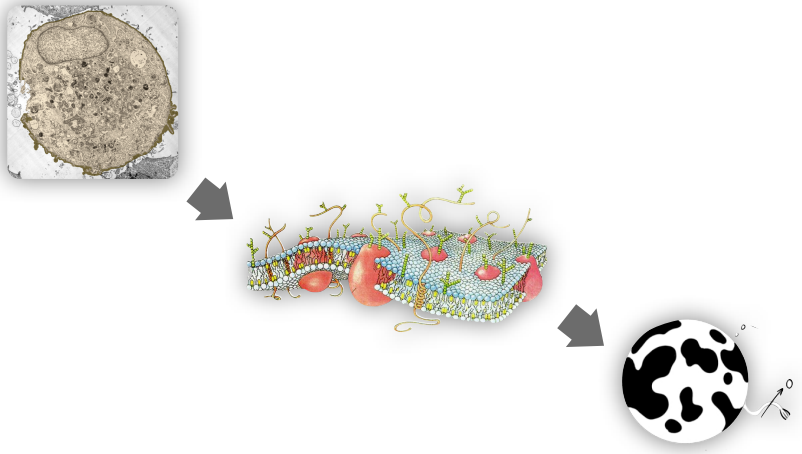
Figure 11.6a Physical Biology of the Cell (© Garland Science 2009)



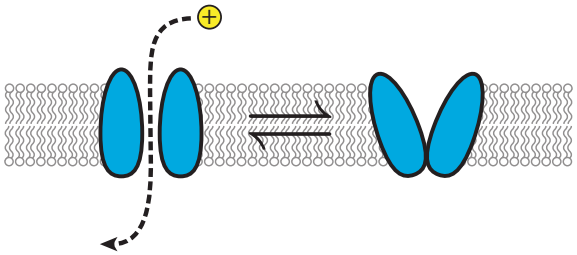
Why single molecules?

- No ensemble averaging
- Kinetics at equilibrium
- Detect rare events

Simplifying the cell membrane



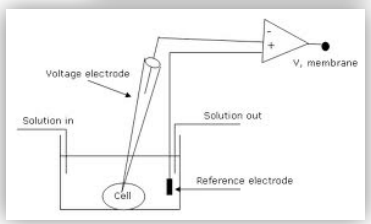
Ion channels for physicists

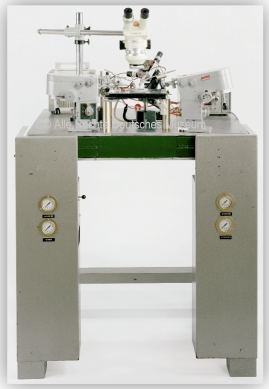


Presentation / Extended essay topics

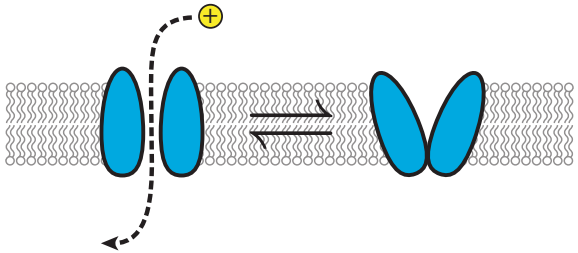
- Single-molecule methods for studying ion channels.
- Single-molecule methods for protein assembly and dimerisation
- The role of lipids in membrane protein function.
- Do lipid rafts exist?

The patch clamp

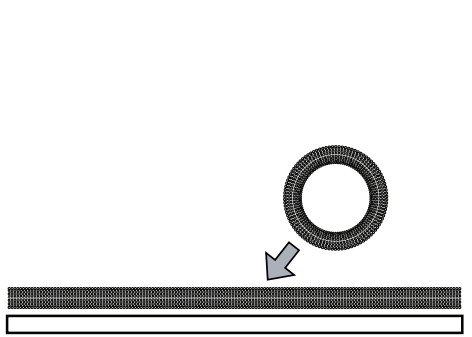


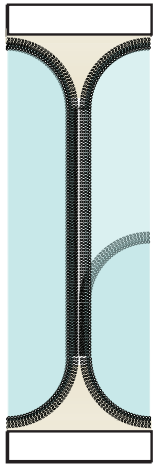


Ion channels for physicists

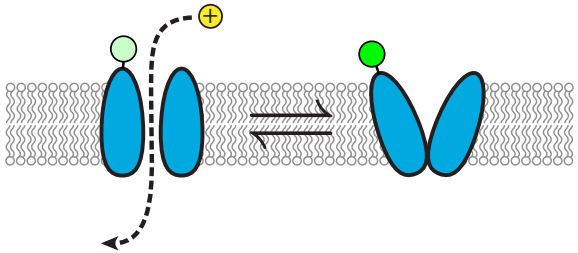


Methods for producing artificial bilayers

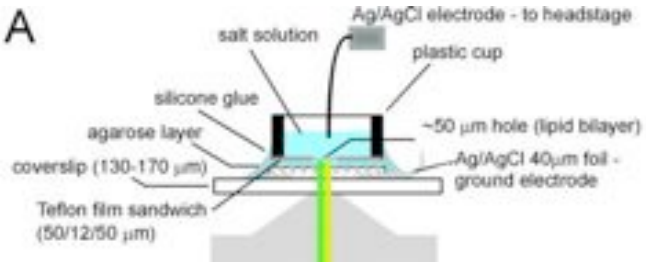




Dynamic Structure & Function?



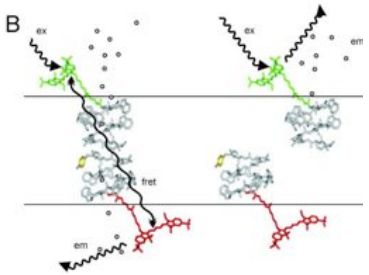
Simultaneous optical and electrical recording of single gramicidin channels



Borisenko V,et al. Biophys J. 2003 Jan;84(1):612-22.

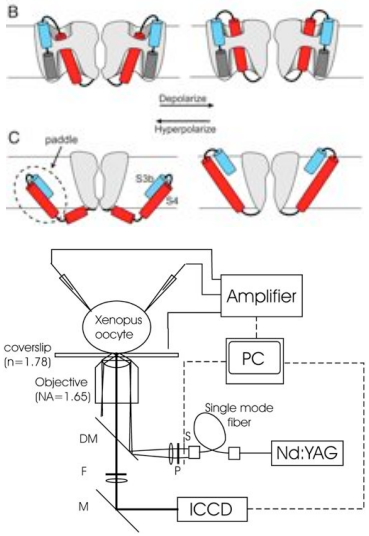
Simultaneous optical and electrical recording of single gramicidin channels

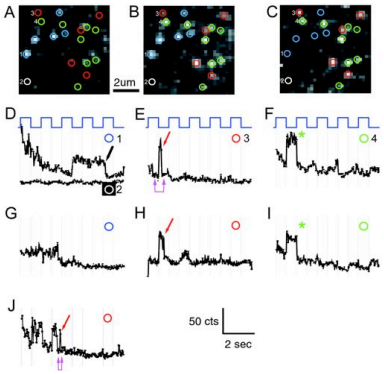
- FRET labelled Gramicidin
- Hydrophobic linear polypeptide, with alternating d-aa.
- Forms channels in phospholipid membranes that are specific for monovalent cations.
-



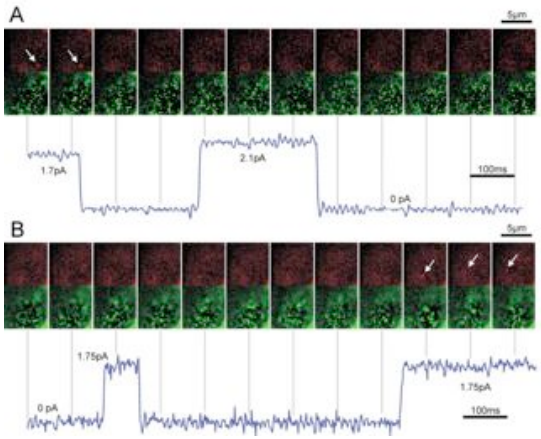
Borisenko V,et al. Biophys J. 2003 Jan;84(1):612-22.

Structural rearrangements in single ion channels detected optically in living cells





Sonnleitner A,et al. PNAS. 2002 October 1; 99(20): 12759–12764.



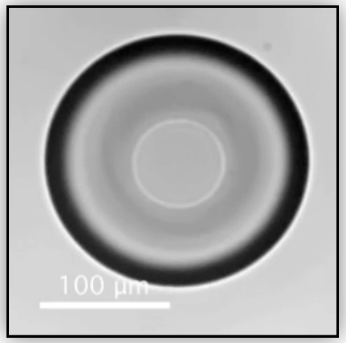
Borisenko V,et al. Biophys J. 2003 Jan;84(1):612-22.

A droplet bilayer

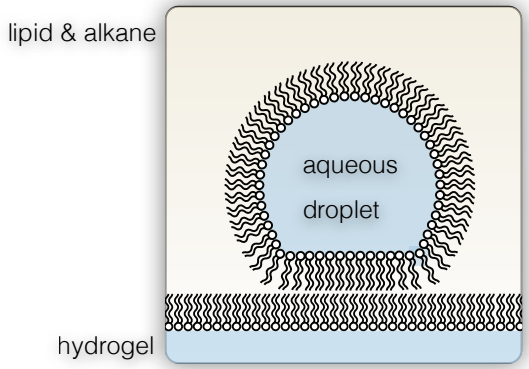
hydrogel

A droplet bilayer

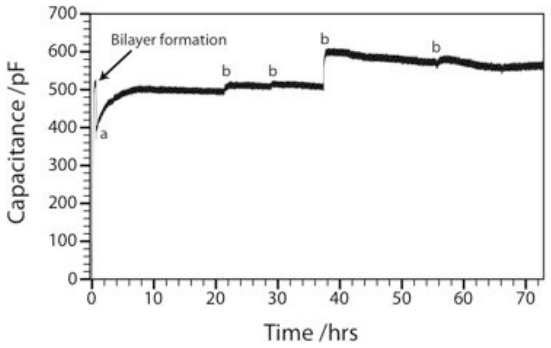
A droplet bilayer



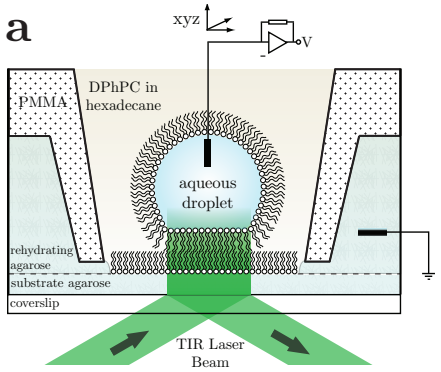
A droplet bilayer

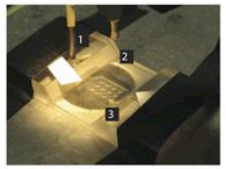


Bilayer Stability

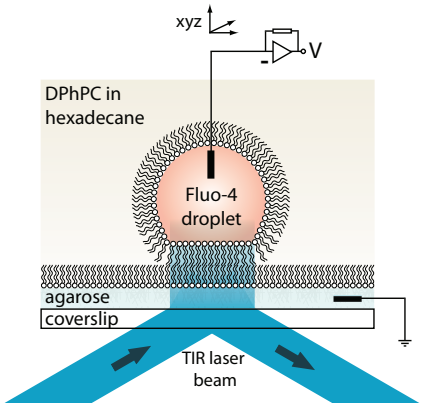


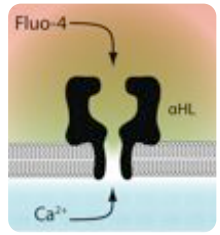
Droplet bilayers



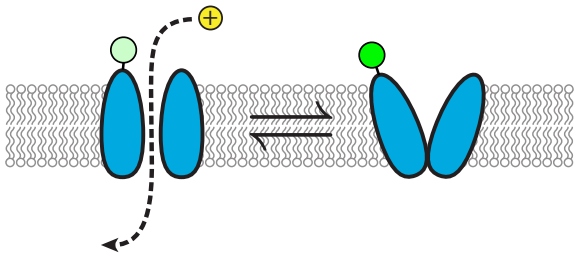


A model system

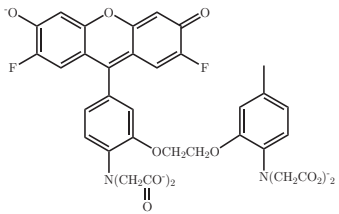


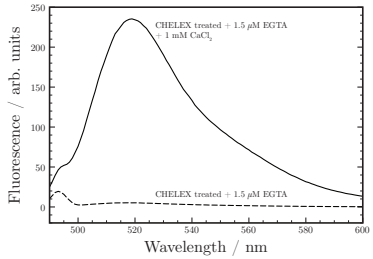


Ion channels

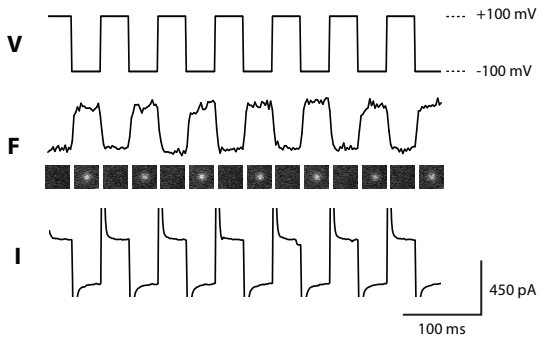


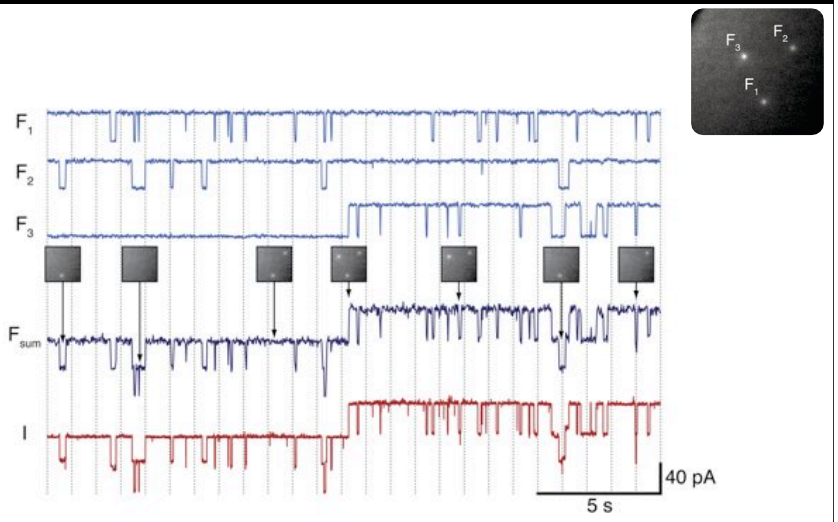
Ca^{2+} indicator, Fluo-4

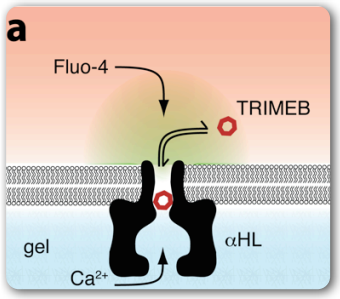


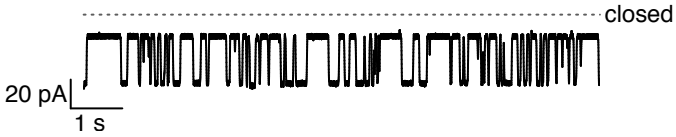


SMF & SCR







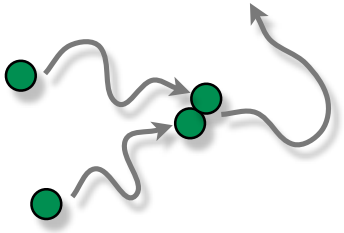


Stochastic blocking of αHL by TRIMEB

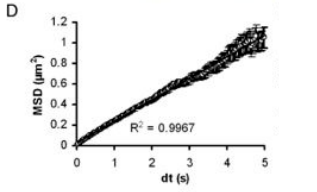
1 kHz electrical acquisition
100 Hz fluorescence acquisition

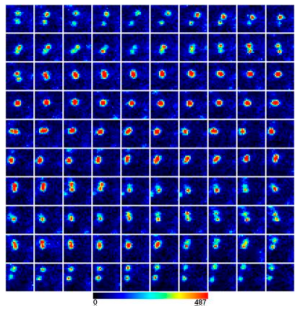
Protein-protein interactions in the membrane

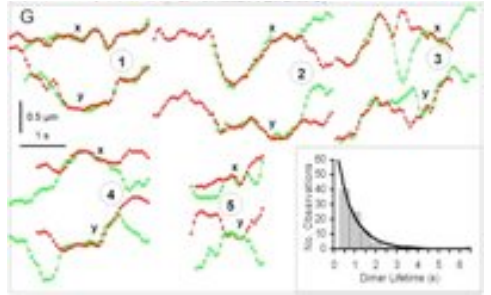
- Protein complex assembly
- Receptor dimerisaton
- Signalling
- Ligand binding



M1 muscarinic receptor dimers seen by TIRF imaging of single molecules



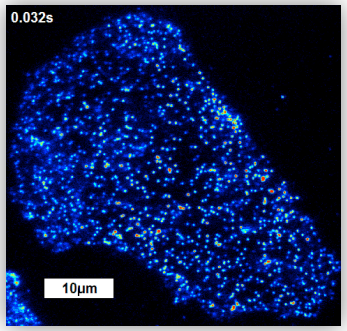




Hern,et al. PNAS. 2010 107(6): 2693–2698.

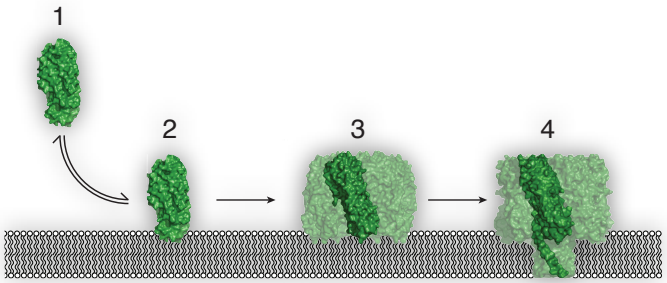
Formation and dissociation of M1 muscarinic receptor dimers

- Muscarinic acetylcholine receptor
- GPCR with important role in cognition and memory
- M1 receptors moving on a single CHO cell.
- Fluorescent telenzepine agonist binds receptors



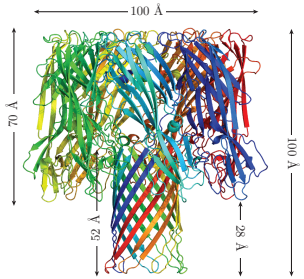
Hern JA,et al. Proc Natl Acad Sci U S A. 2010 107(6): 2693–2698.

α-hemolysin assembly

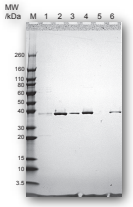


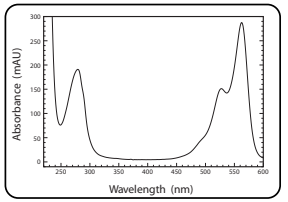
α-hemolysin

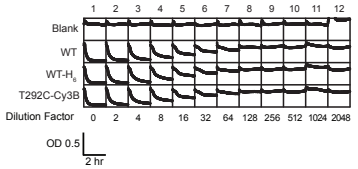
- Pore-forming toxin secreted by Staphylococcus aureus
- DNA nanopore
- A simple model for many membrane protein processes



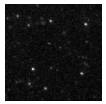
Fluorescent α-hemolysin

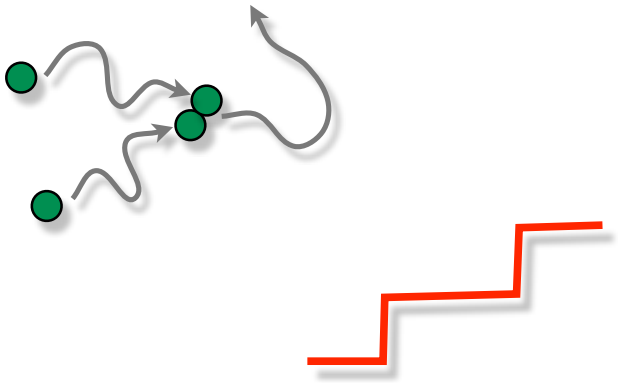




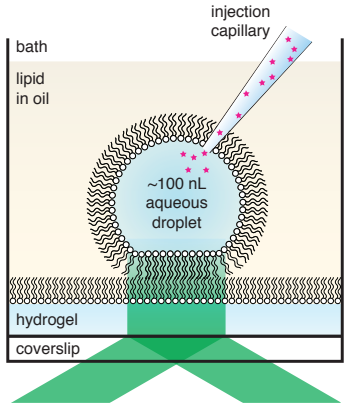


The idea

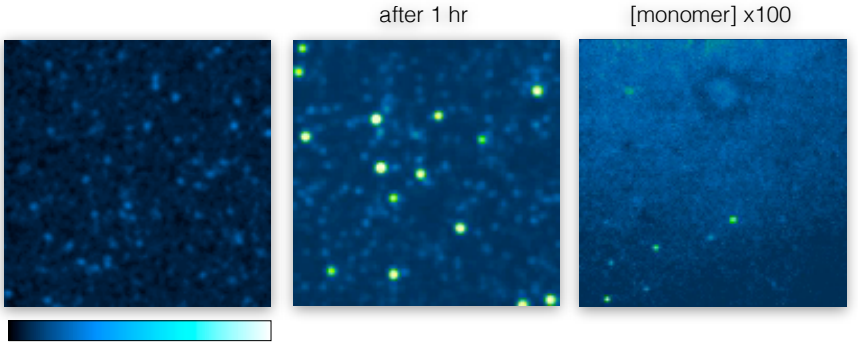




droplet nanoinjection



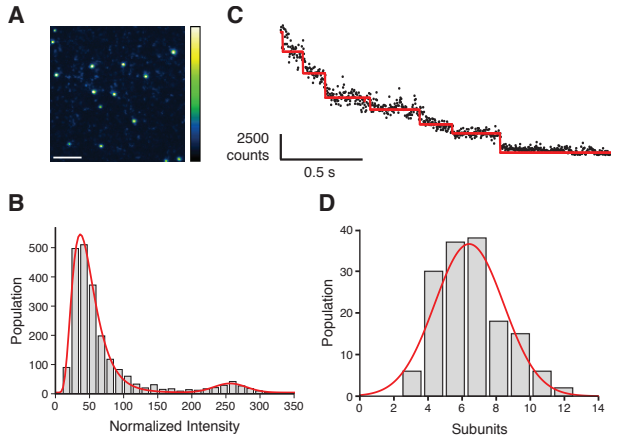
Diffusing aHL



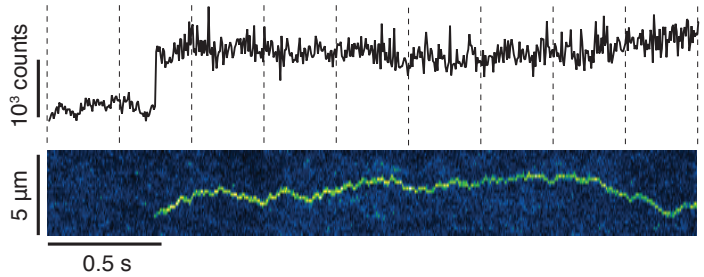
10 ms @ 25 fps playback, 90x magnification

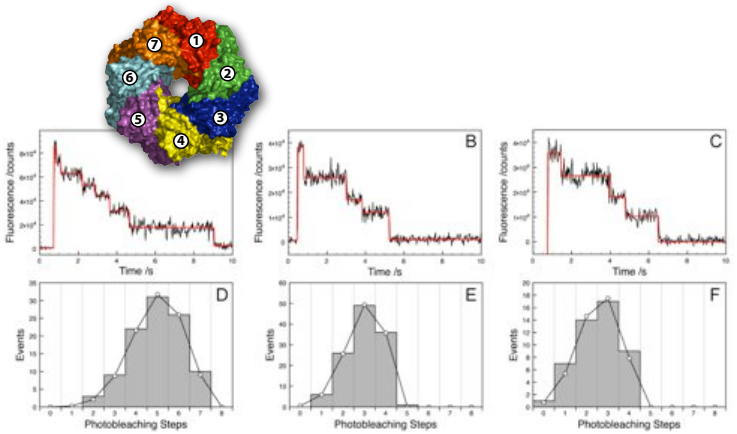
50 ms @ 100 fps playback, 60x

Stoichiometry

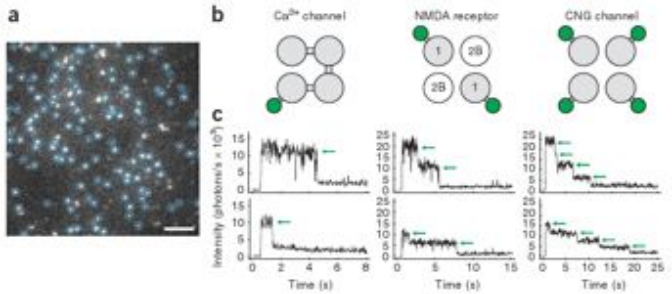


Assembly is rapid



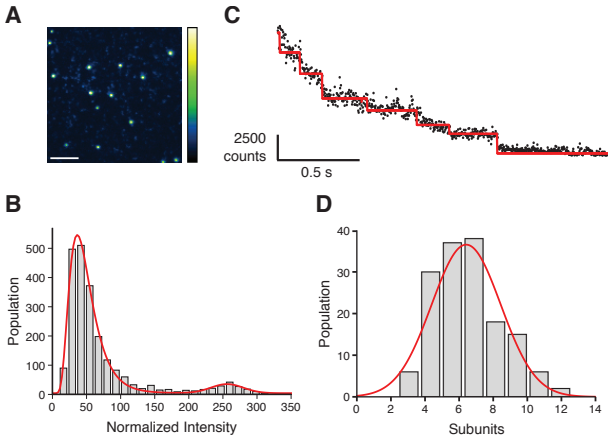


Subunit counting in membrane-bound proteins



Ulbrich, M. H. & Isacoff, E. Y.. Nat Methods 4, 319–321 (2007).

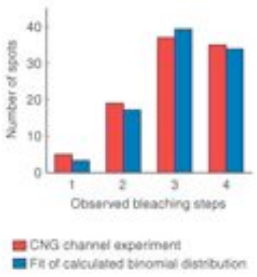
Stoichiometry



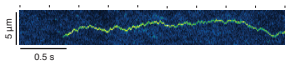
Binomial distributions

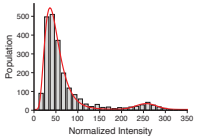
Event	Number of heads	Probability
TTTT	0	1/16
HTTT THTT TTHT TTTH	1	4/16
HHTT HTHT HTTH THHT THTH TTHH	2	6/16
THHH HTHH HHTH HHHT	3	4/16
HHHH	4	1/16

$$P(\mathrm{X}=x)=\frac{\mathrm{n}!}{x!(\mathrm{n}-x)!}\mathrm{p}^{x}(1-\mathrm{p})^{\mathrm{n}-x}$$



Observations





Heptamer assembly is rapid.

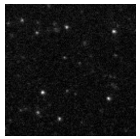
Heptamers appear in less than 5 ms.

No intermediates.

Intermediates survive no longer than 5 ms.

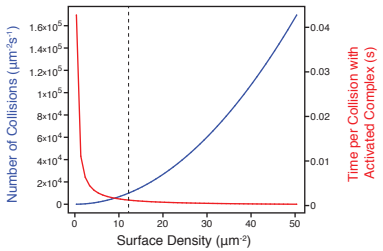
... so how rare is an assembly event?

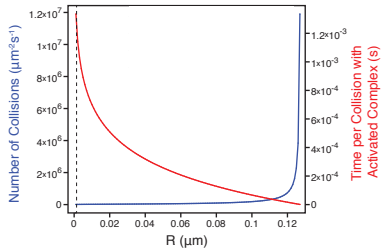
monomer-monomer collision frequency



3D: $\phi = 4\pi D' R\,[A]\,[B]$

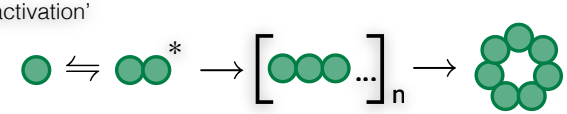
2D: $\phi = \dfrac{2\pi D'}{\ln(b/R) - \gamma + \ln\sqrt{2}}\rho_A\rho_B$



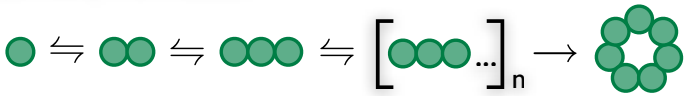


Models for αHL assembly

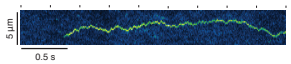
Rare dimer 'activation'



Reversible 'sticky' intermediates

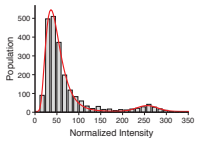


Observations



Heptamer assembly is rapid.

Heptamers appear in less than 5 ms.



No intermediates.

Intermediates survive longer than 5 ms.

$\phi = \dfrac{2\pi D'}{\ln(b/R) - \gamma + \ln\sqrt{2}}\rho_A\rho_B$

Heptamer formation is rare.

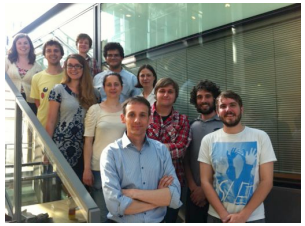
~10^5 monomer collisions for every new heptamer.

Mark Wallace
Lauriane Angue
Matthew Baker
Oliver Castell
Brid Cronin
John Danial
Charlotte Dodson
Linda Gross
David Marshall*
Jason Sengel
Stuart Thomas
James Thompson
Eve Weatherill

Hagan Bayley
Shuo Huang
Mercedes Romero-Ruiz

Stephen Tucker
Marc Szabo

Gregor Anderluh
Nejc Rojko



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Resources

- Slides
  - wallace.chem.ox.ac.uk/teaching
- Books
  - Lakowicz, Principles of Fluorescence Spectroscopy.
  - Knight, Single Molecule Biology.
  - Hinterdorfer & Van Oijen, Handbook of Single-molecule biophysics.
  - Leake, Single-molecule cellular biophysics.