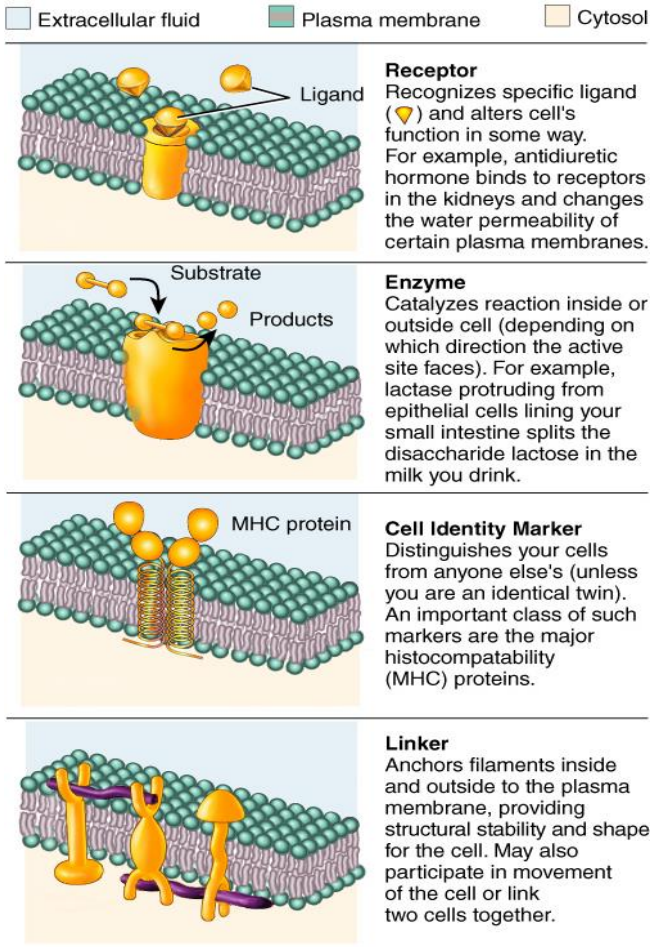
Membrane Proteins

- Membrane proteins account for 25-30 % of all open reading frames
  - A reading frame in a sequence of nucleotides in DNA that contains no termination codons and so can potentially translate as a polypeptide chain.
- Wide range of central functions

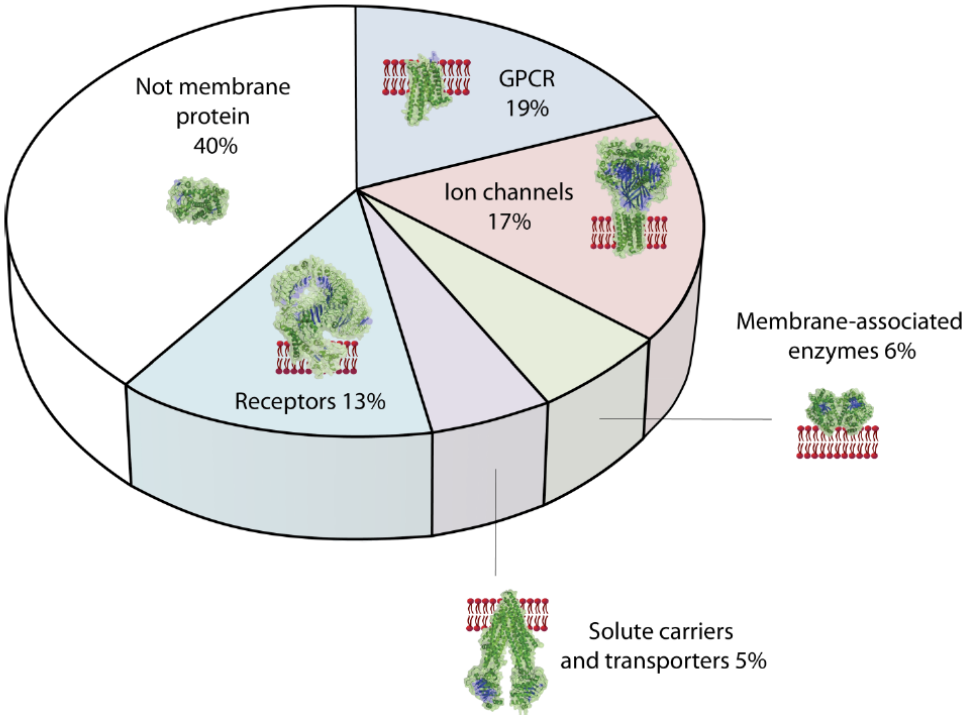


Membrane Proteins

- Human membrane proteins of different classes represent about 60% of all protein drug targets:

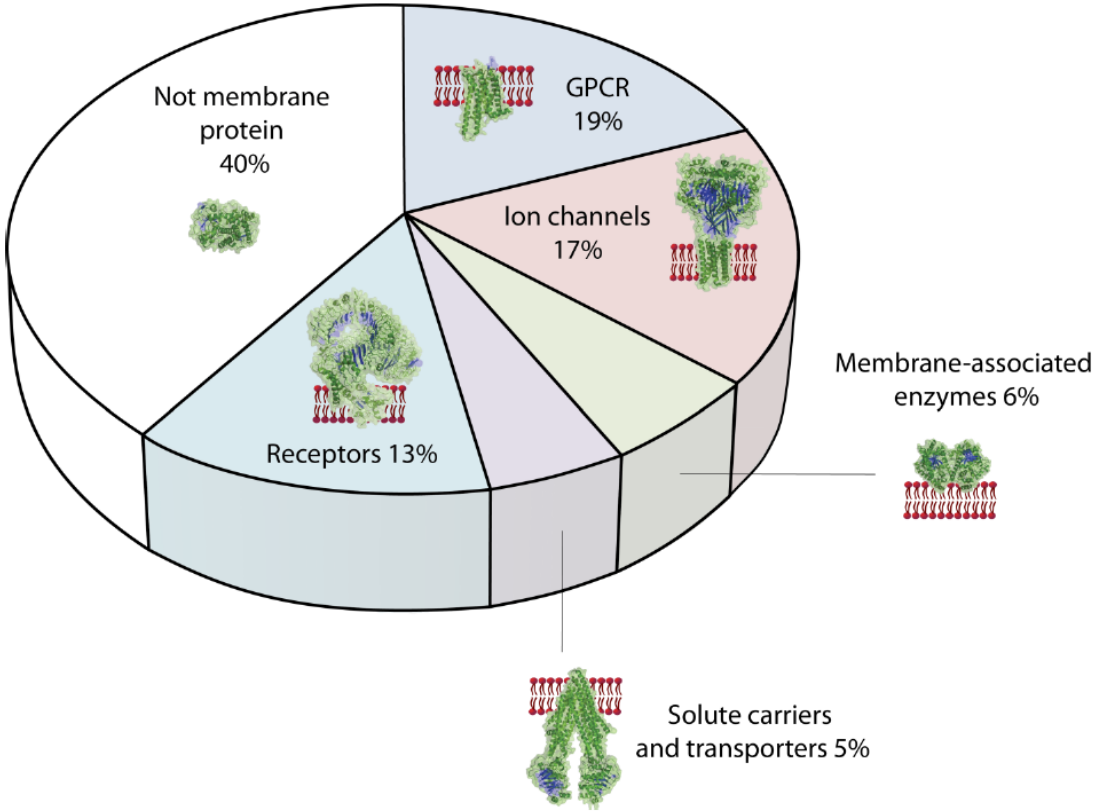
  - G Protein Coupled Receptors (GPCRs) (most prevalent)
  - ion channels and receptors
  - Membrane-associated enzymes,
  - solute carriers and transporters

Figure 5. Human membrane proteins as drug targets.



Materials 2012, 5, 2205-2242; doi:10.3390/ma5112205

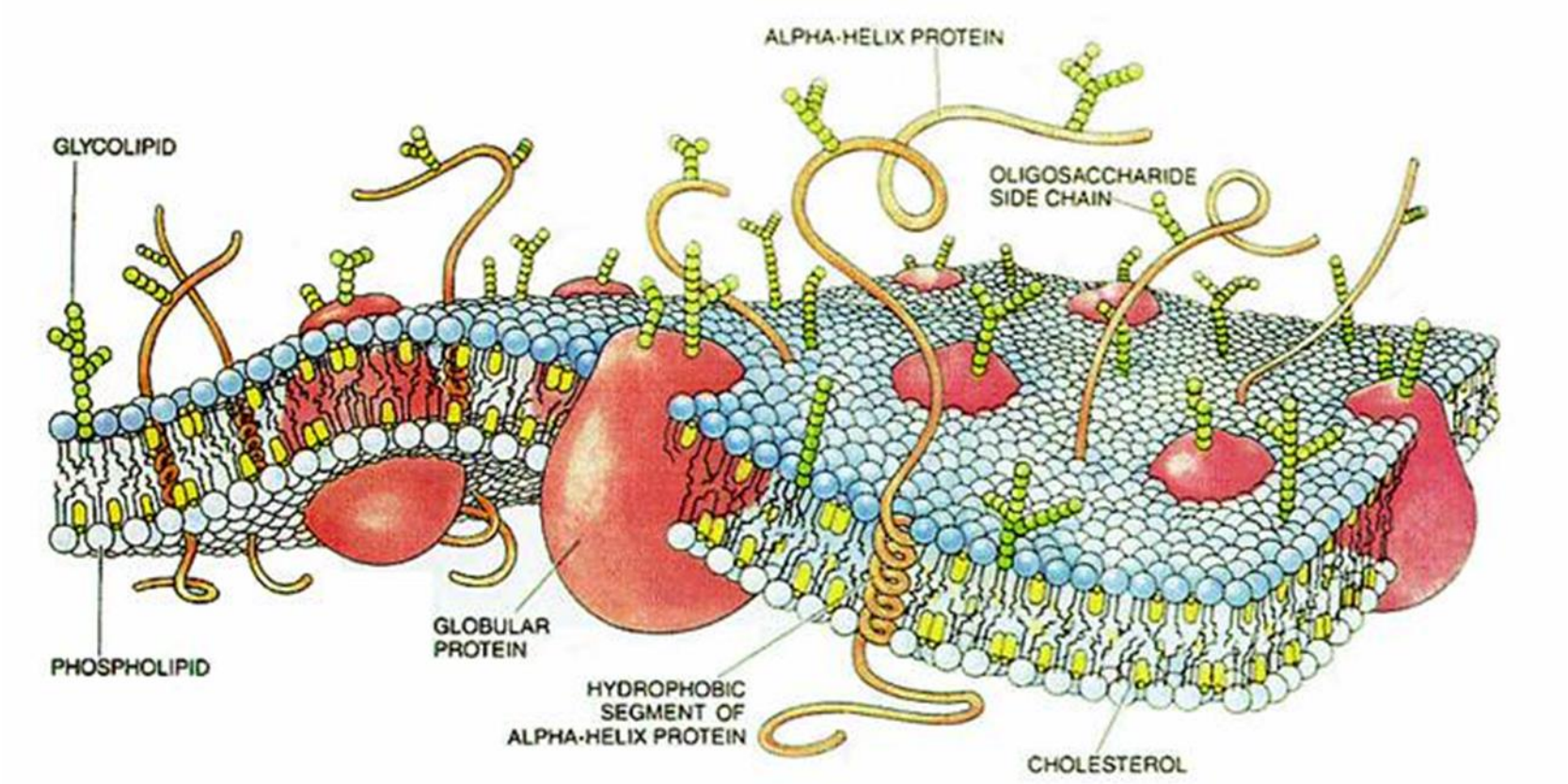
Figure 5. Human membrane proteins as drug targets.



- G Protein Coupled Receptors (GPCRs) perceive many extracellular signals and transduce them to G proteins, which further transduce these signals intracellular to appropriate downstream effectors and thereby play an important role in various signaling pathways.

- G proteins, also known as guanine nucleotide-binding proteins, are a family of proteins that act as molecular switches inside cells, and are involved in transmitting signals from a variety of stimuli outside a cell to its interior.

Cell Membrane

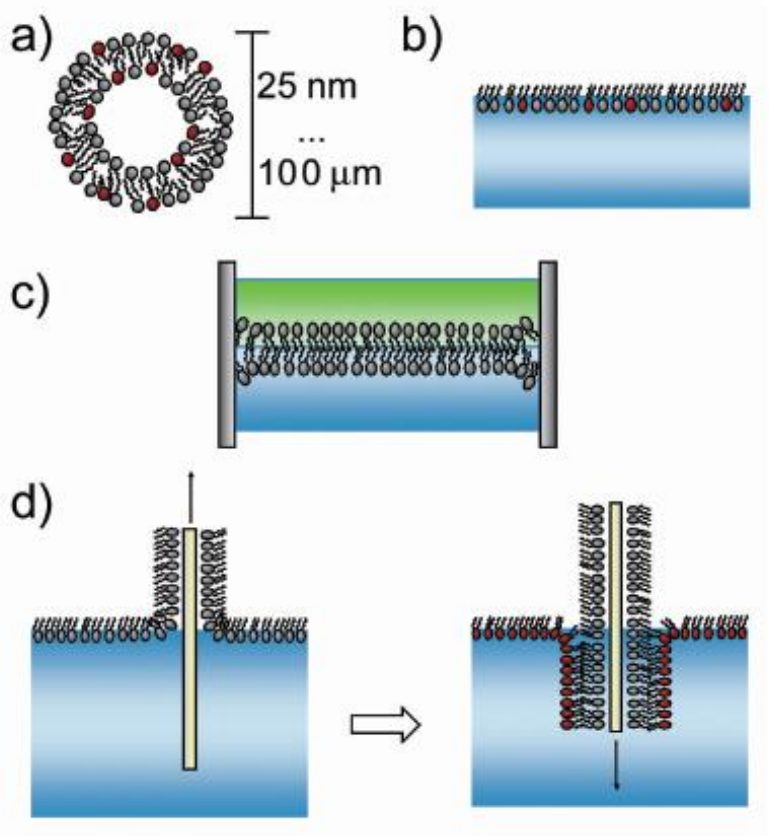


Membrane Protein Biosensors

- **Soluble protein**s (e.g. most enzymes): biosensor methodologies are well developed
- **Membrane proteins:** there is a functional requirement of an intact lipid bilayer and such structures are difficult to attach to biosensor surface
- Whole cells & membrane preperations are too large and heterogeneous

Model Membrane Systems

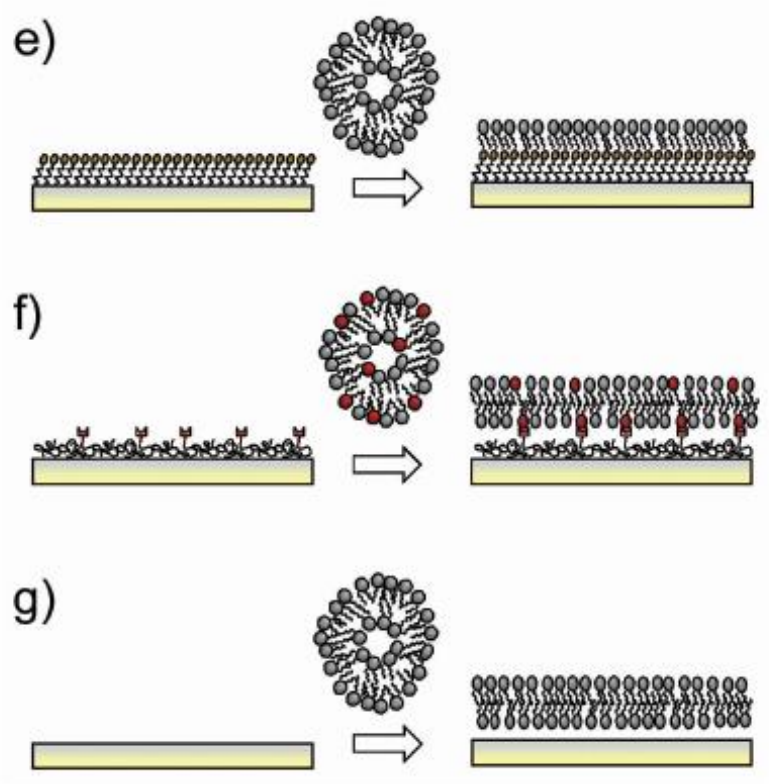
- to offer a natural environment for the membrane proteins



(a) liposomes: hollow spheres (25 nm to 100 µm in diameter) enveloped by a bilayer of lipid molecules; **(b) lipid monolayers at the air-water interface; (c) black lipid membranes** suspended over an aperture between two aqueous phases; **(d) Langmuir-Blodgett method**, which allows the transfer of lipid mono- and multi-layers from the air-water interface to a solid support;

Supported lipid membranes, Materials Today Volume 6, Issue 11, November 2003,Pages 32–37

Model Membrane Systems

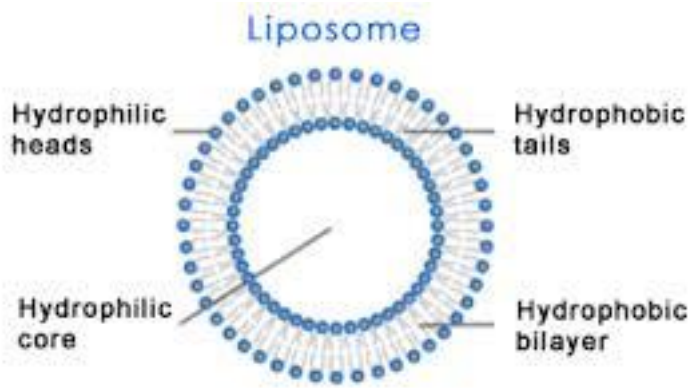


(e) self-assembled monolayers (SAMs, e.g. thiols on Au or silanes on glass or silica), a second lipid layer can be deposited by spontaneous disruption of liposomes; (f) **deposition of a polymer coating with tethers** followed by the spontaneous spreading of liposomes, so that the polymer creates a cushion between support and bilayer; (g) **spontaneous spreading of liposomes** or membranes on mica, glass, and silica.

Supported lipid membranes, Materials Today Volume 6, Issue 11, November 2003,Pages 32–37

Liposome & Liposome Spreading

- Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a Phospholipids bilayer molecule.



Liposome spreading →

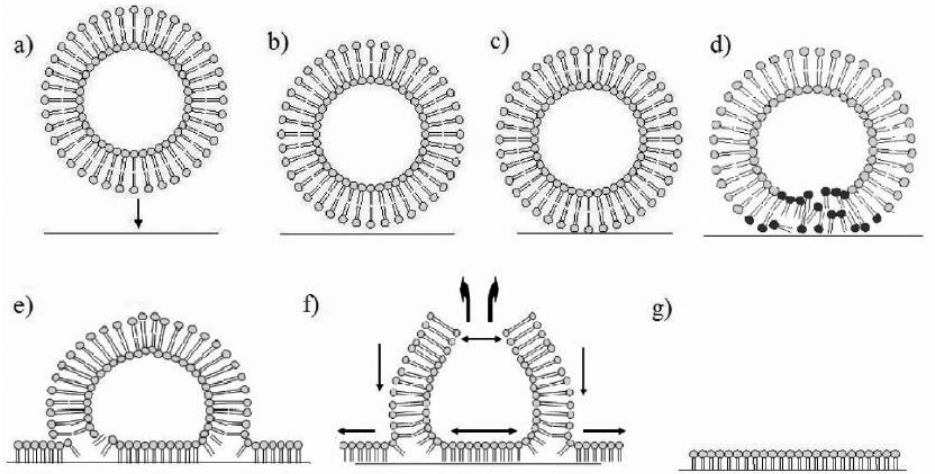
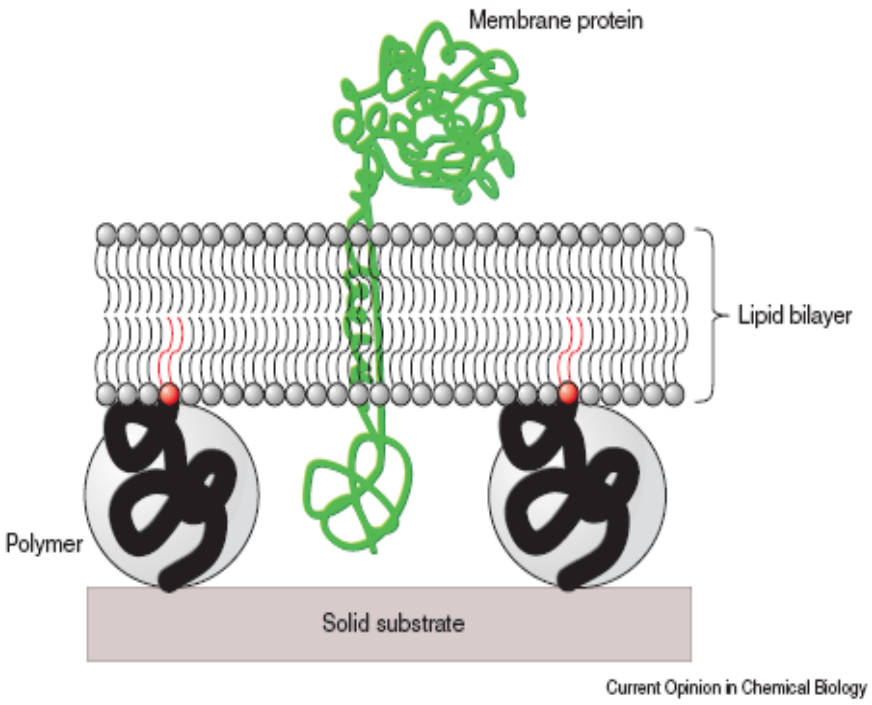


Fig. 8. Mechanism of adhesion-spreading of liposomes on a mercury electrode. (a) Mass transport from the bulk to the electrode surface. (b) Interaction of the electrical double layers of the liposome and the electrode. (c) Direct contact of the liposome with the electrode (docking). (d) Bilayer opening resulting in (e) a partially adsorbed liposome with increased lateral tension. (f) Rupture of the liposome and spreading, resulting in (g) an adsorbed lipid island.

The Electrochemistry of Liposomes, DOI: 10.1560/IJC.48.3-4.169

Tethered Bilayer Membranes (tBLMs)

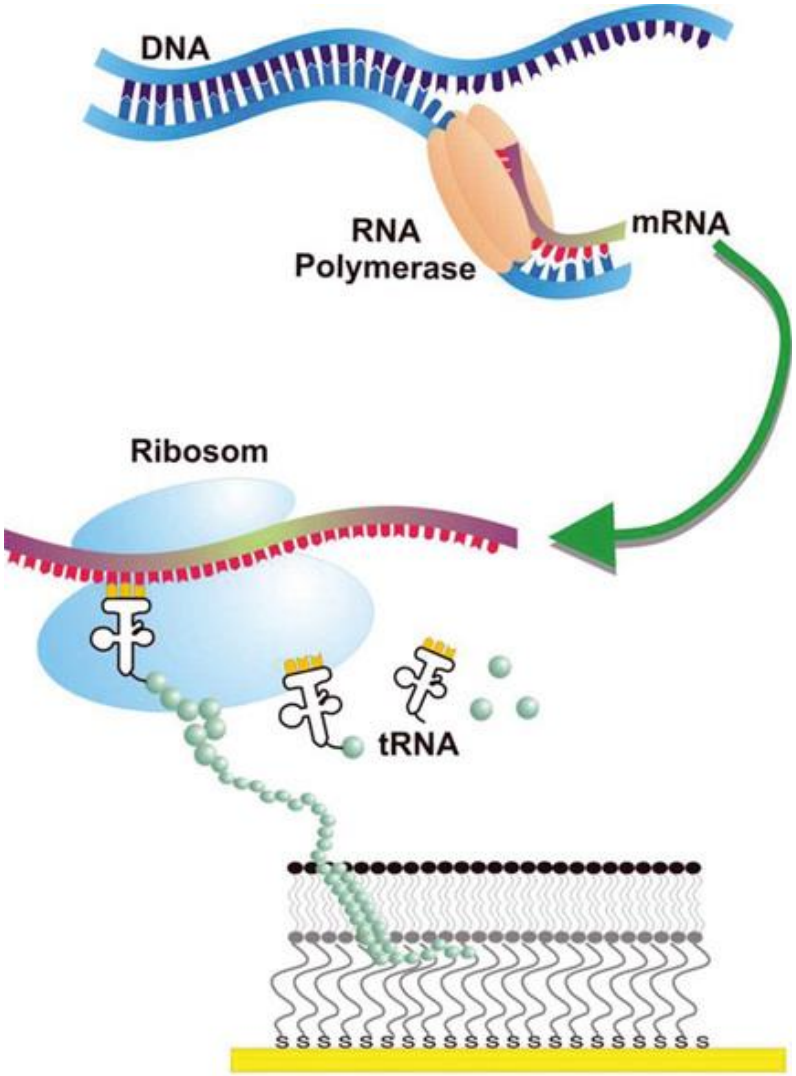


Sinner ve Knoll (2001) Current Opinion in Chemical Biology, 5: 705-711

tBLMs are advantageous because:

- ✓ they are stable over long periods of time
- ✓ the water layer between lipid bilayer and the solid surface results a fluid state rather than a rigid, crystalline one
- ✓ this layer also allows insertion of integral proteins in their active form

Molecular Nose
Odorant reseptors

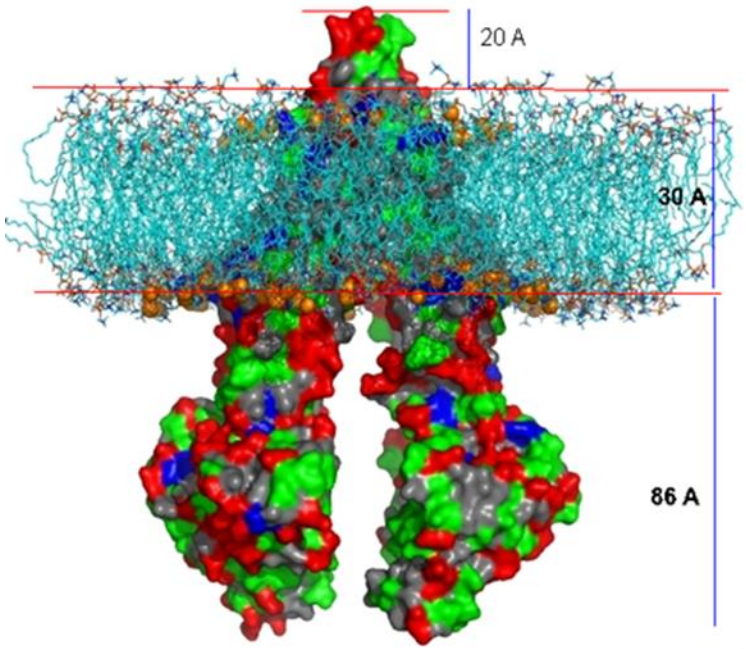


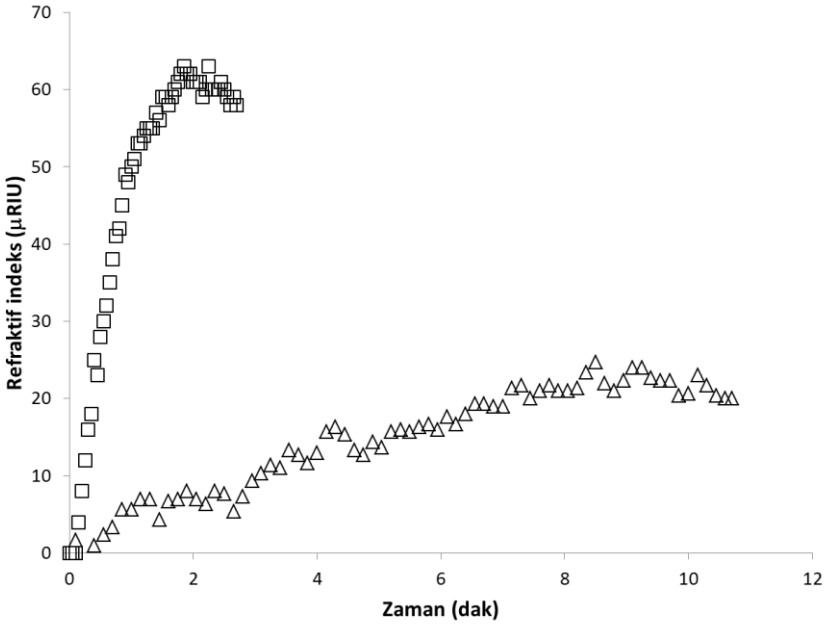
- **Odorant receptors in artificial lipid membranes**
  - for the first time natural functions of such membrane proteins in situ was examined.
  - This is of considerable importance to pharmaceuticals research, as it means that new active agent screenings can now be carried out using receptors that were inaccessible until now.

Diagram of the in vitro synthesis of a membrane protein and its subsequent insertion into an artificial membrane. The artificial membrane consists of two lipid layers and its structure resembles that of a cell membrane. In the (simplified) diagram, the ribosomes of the cell-free extract insert the assembled amino acid chains directly into the artificial membrane, just as they would insert them into a natural cell membrane in a real cell

Robelek, R.et al, Angewandte Chemie, 2007

Drug / p-glycoprotein Interactions





(☐) 0.05 mg/ml pravastatin
(Δ) 0.01 mg/ml pravastatin

Drug / p-glycoprotein Interactions

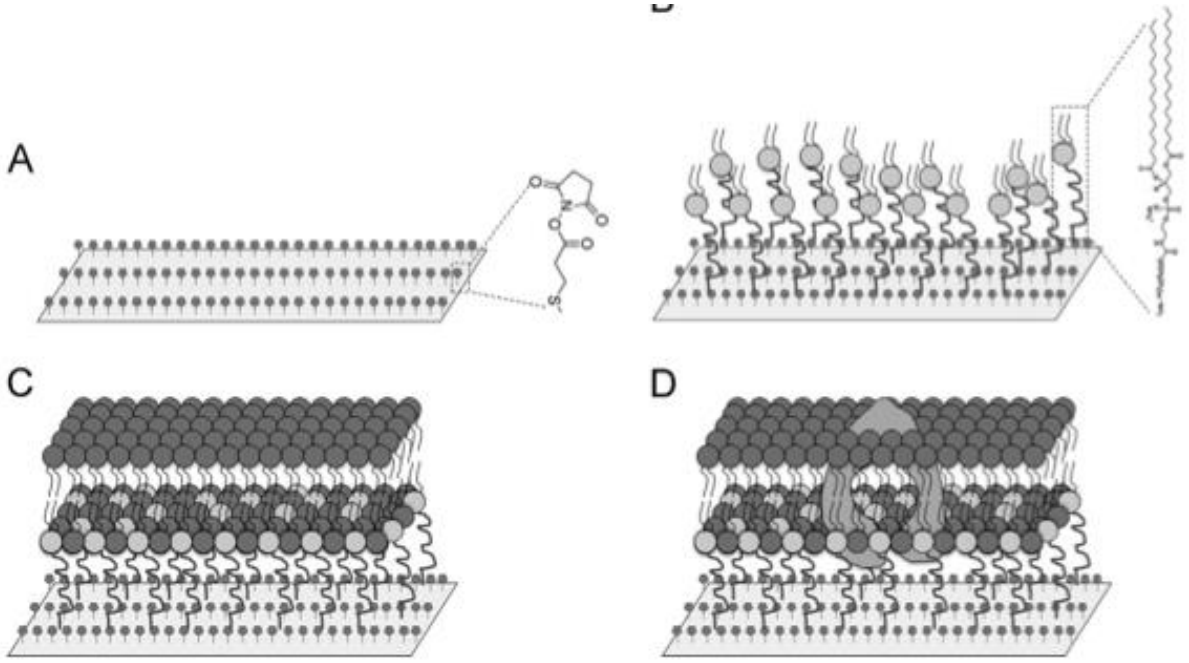
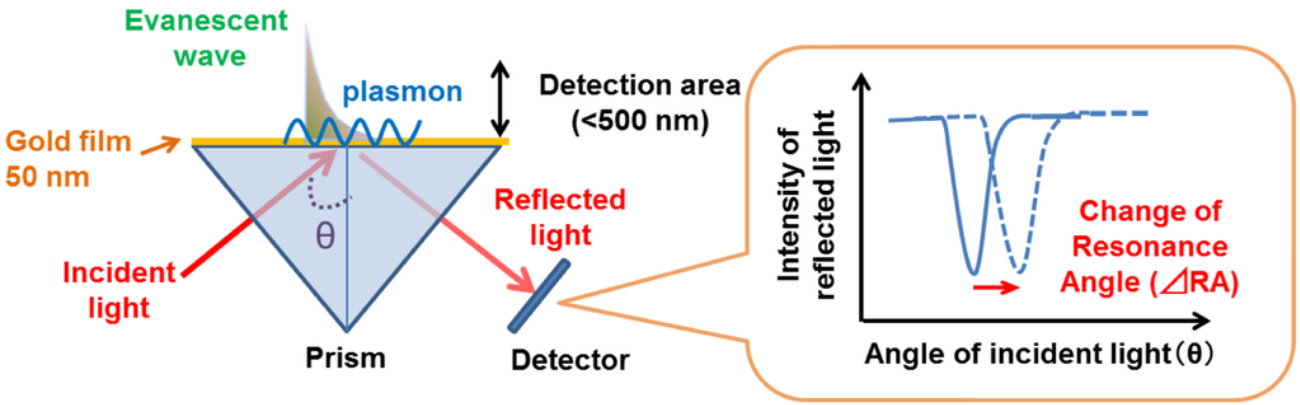


Fig. 1. Construction of tBLM on gold-coated surface. (A) Activation of the surface with DTSP. (B) DSPE-PEG modification. (C) Construction of protein-free tBLM. (D) Construction of protein-incorporated tBLM (molecules were not presented in their actual sizes).

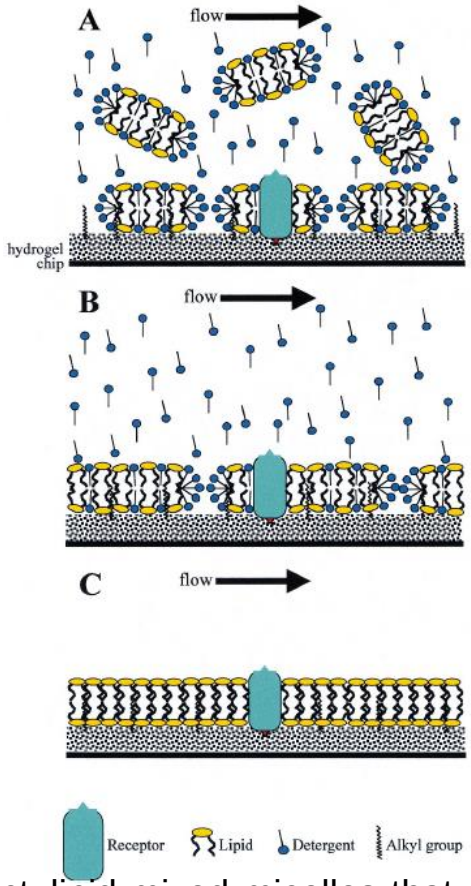


Surface Plasmone Resonance System (SPR)

Surface Plasmone Resonance System (SPR)

- Surface **plasmons** are electromagnetic waves that can be excited at certain metal-dielectric interfaces.
- When polarized light is incident upon this interface, photons of the correct frequency are absorbed by oscillating free electrons at the surface of the metal.
- This transfer in energy excites surface plasmons.
- The surface plasmon wave oscillates along the metal-dielectric interface and is therefore extremely sensitive to any changes in the vicinity of the border.

Ex: On-surface reconstitution Rhodopsin- light switch



(A) Detergent–lipid mixed micelles that are injected, as described in Fig. and attach to the alkyl chains on the L1 surface and other nonpolar groups like hydrophobic parts of immobilized membrane proteins. (B) When amphiphile-free buffer is running through the flowcell, a detergent monomer concentration is maintained in the mobile phase leading to a very quick extraction of the detergent from the surface. (C) The lipids remain attached to the surface and are able to reconstitute the function of membrane proteins. The relative proportions of the components in the diagram are not displayed in scale.

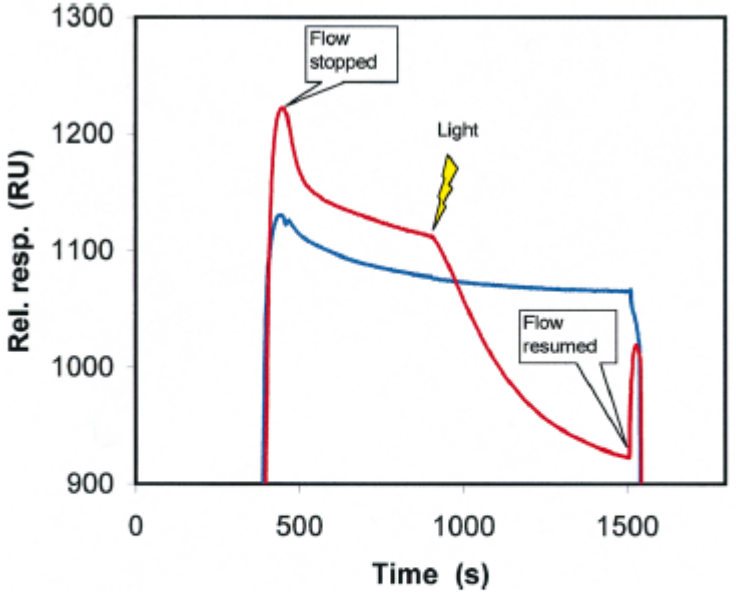


FIG. 4. Signaling of reconstituted receptor. Transducin (2.3 μM) and GTP (200 μM) were injected. When maximal binding was reached the flow was stopped (459 s). At stabilization of the signal in the POPC-reconstituted rhodopsin channel (red) relative to the signal in the POPC-only reference channel (blue), the flowcells were illuminated (902 s). The activation of the receptor was recorded as a surface mass decrease caused by dissociation of the activated transducin from the membrane.

Karlsson & Lofas, 2002

Rhodopsin, also called visual purple, pigment-containing sensory protein that converts light into an electrical signal.

- It is made up of opsin (a colourless protein) and 11-cis-retinal (11-cis-retinaldehyde), a pigmented molecule derived from vitamin A.

- When the eye is exposed to light, the 11-cis-retinal component of rhodopsin is converted to all-trans-retinal, resulting in a fundamental change in the configuration of the rhodopsin molecule.

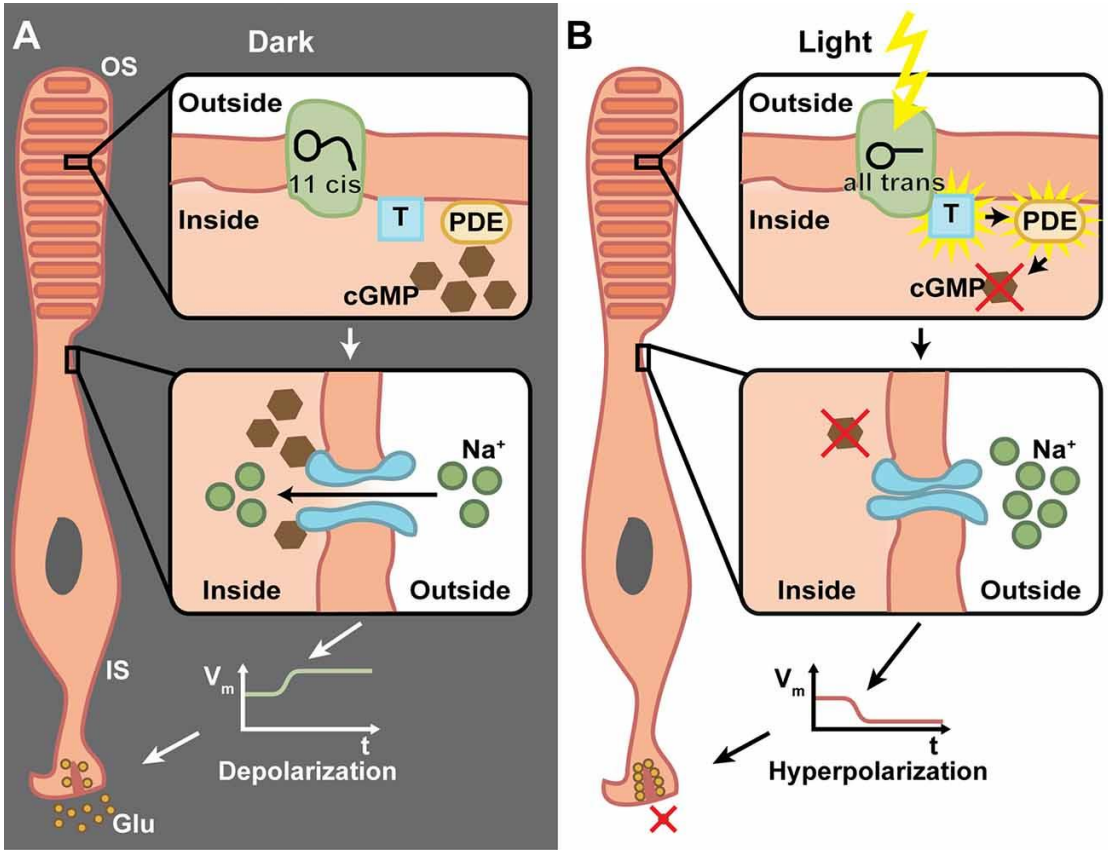
- The change in configuration initiates a phototransduction cascade within the rod, whereby light is converted into an electrical signal that is then transmitted along the optic nerve to the visual cortex in the brain.

- The change in configuration also causes opsin to dissociate from retinal, resulting in bleaching.

The phototransduction cascade in vertebrate photoreceptors.

(A) In the dark, 11-cis retinal is bound to rhodopsin, which is located inside the membrane of the outer segment's (OS) discs. The G-protein transducin (T) and phosphodiestherase (PDE) are inactive (upper panel). Cyclic guanosine monophosphate (cGMP) triggers the opening of cation channels in the photoreceptor membrane, which mediate a Na+ influx (middle panel). This in turn depolarizes the membrane potential of the inner segment (IS) and triggers the release of glutamate (Glu) from ribbon synapses (lower panel).

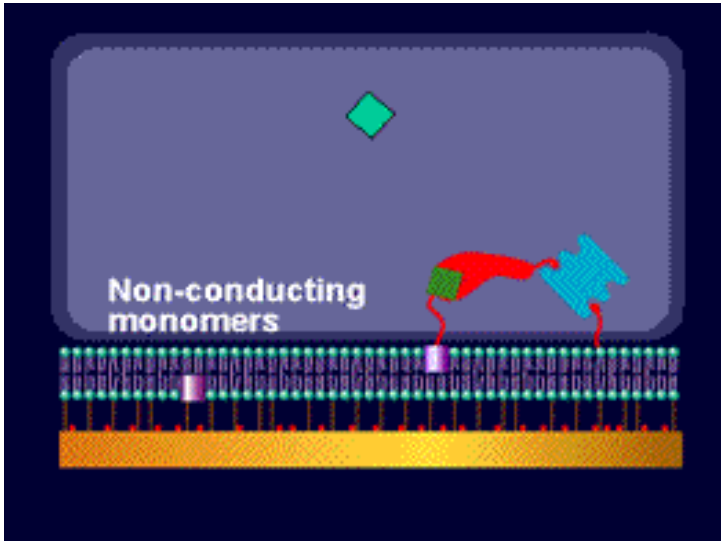
(B) Upon light absorption, 11-cis retinal is converted to all-trans retinal and dissociates from the rhodopsin. This activates the transducin, which in turn activates PDE, whose catalytic activity degrades cGMP (upper panel). This effectively lowers the cGMP concentration, which leads to closure of cation channels (middle panel). Thus, the IS is hyperpolarized and the Glu exocytosis is stopped (lower panel). Due to the G-protein involved in the transduction, a very high amplification of single photon responses is possible.

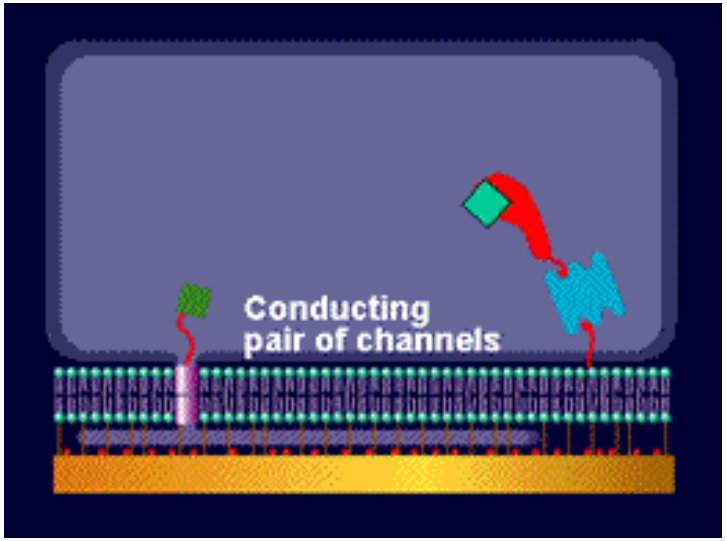


Front. Syst. Neurosci., 02 September 2016 | http://dx.doi.org/10.3389/fnsys.2016.00074

A Commercial Example
Ambri ICS™ Biosensor (Australia)

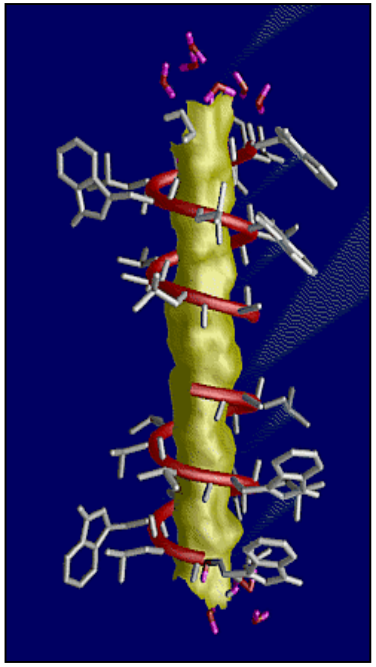
- A biological switch: a membrane which can detect the presence of specific molecules and signal their presence by triggering an electrical current
- The Ambri Ion Channel Switch (ICS™) Biosensor is a two molecular layer self assembled membrane based on the ion channel gramicidin
- The design can be made to work in competitive manner:

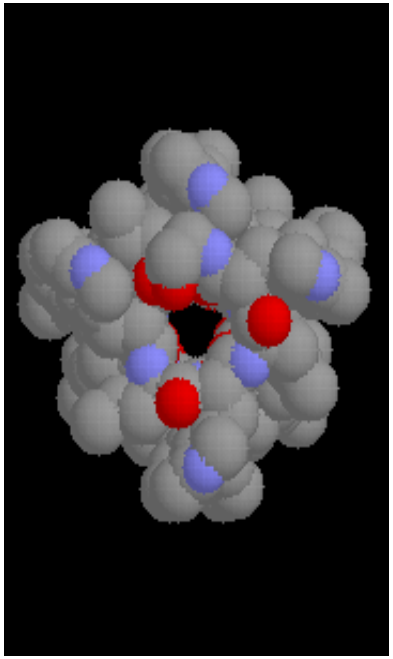


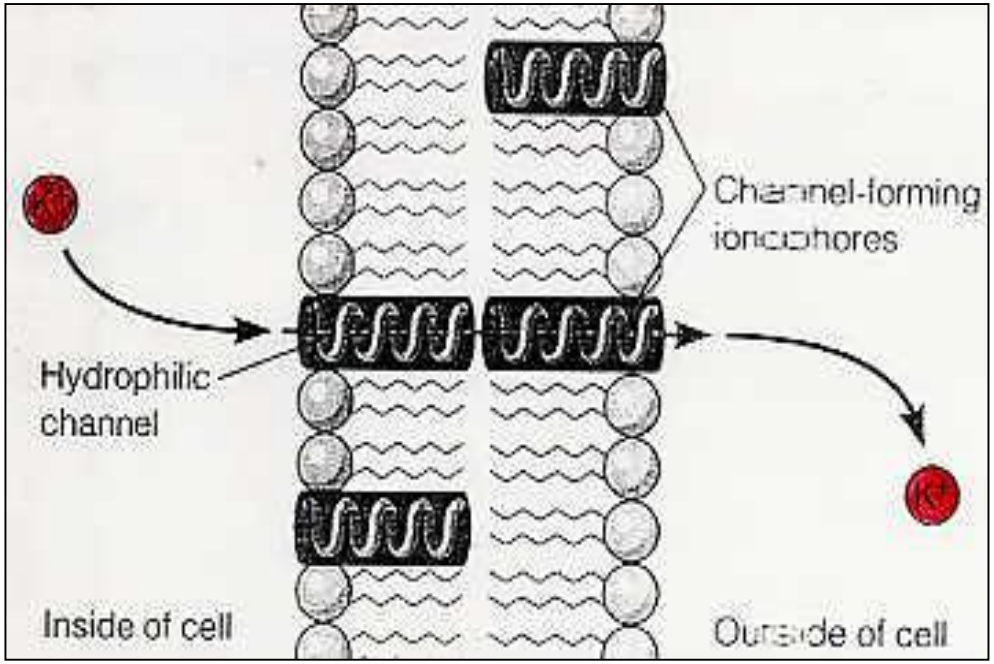


Ion channels: ionophores

- **Gramicidin is an antibiotic obtained from the bacterial species *Bacillus brevis***
- **Gramicidin is a peptide of 15 amino acids**
- **Its sequence contains alternatively D- and L-amino acids and the molecule builds a helix with an inner pore of 0.4 nm in diameter.**
- **Two molecules build a transmembrane, unspecific cation channel through which K^+ and Na^+ can permeate. The channel is open whenever the two molecules are in position with each other**

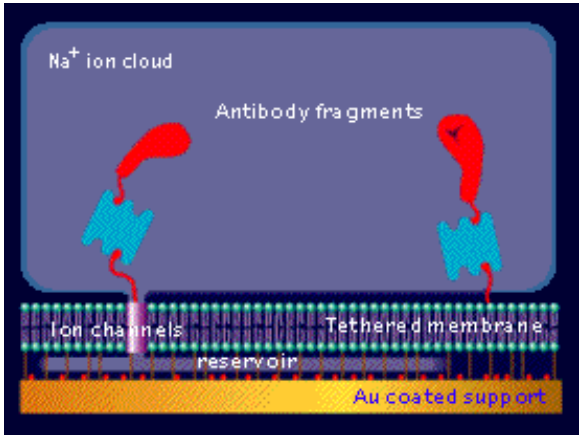


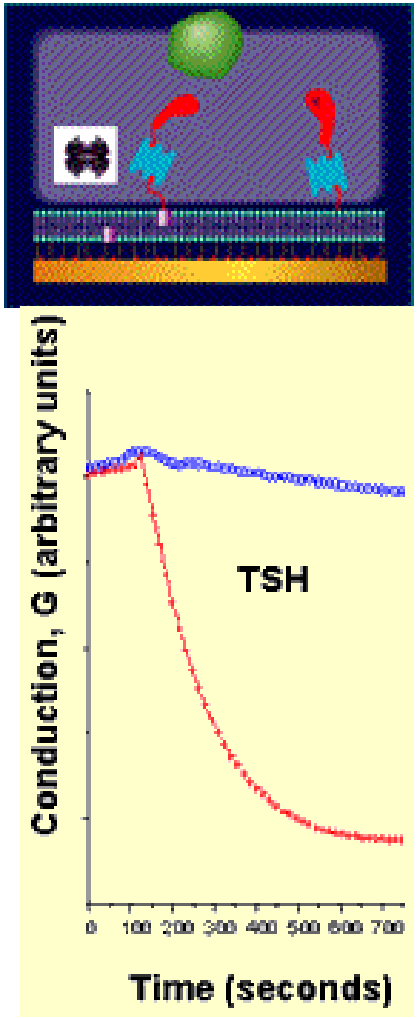




A Commercial Example
Ambri ICS™ Biosensor (Australia)

Or direct measurement is possible:





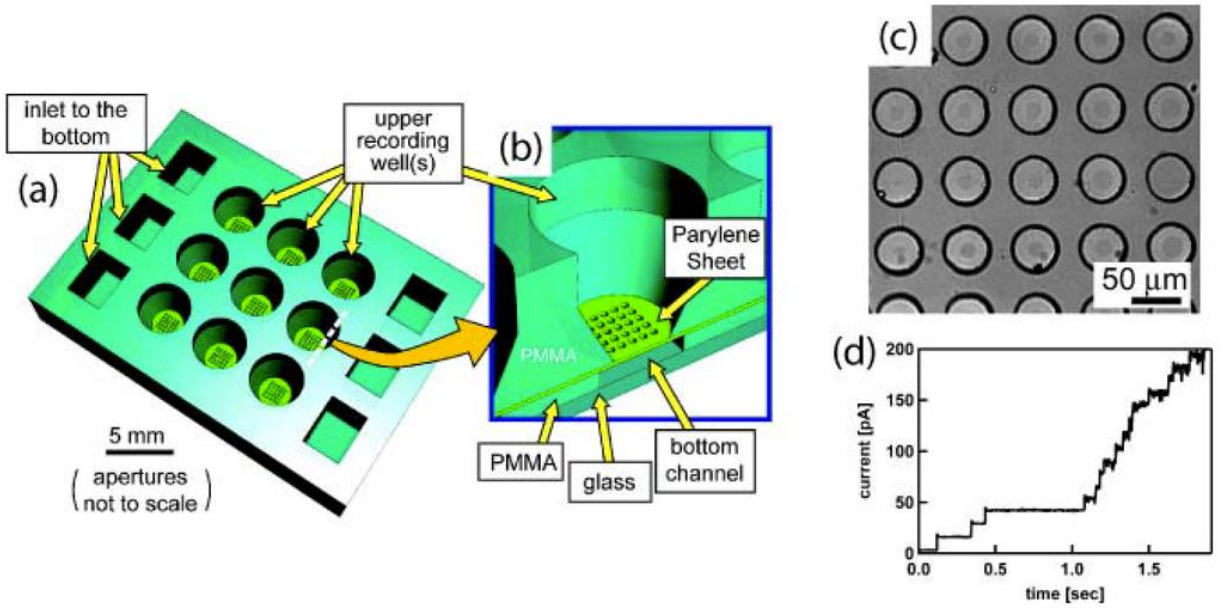
https://www.youtube.com/watch?v=6Ti83oO2ml4

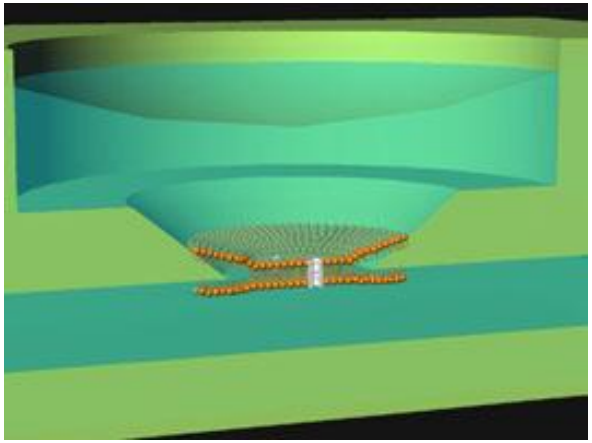
When a test chemical binds to the capture sites on the membrane and ion channels, the channel is split and the circuit broken. The electrical signal generated is a measure of the presence of the test chemical.

Membrane Protein Chips

Anal. Chem. **2008**, *80*, 328–332

Lipid Bilayer Microarray for Parallel Recording of Transmembrane Ion Currents



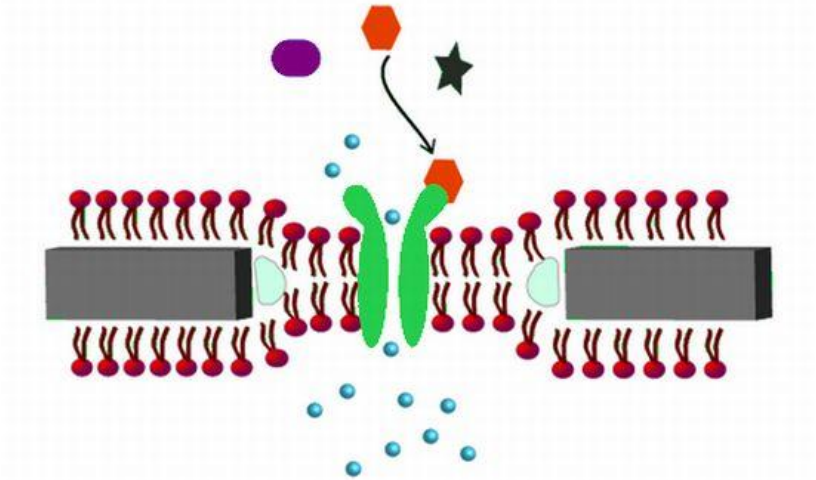


This paper describes a multiwell biochip for simultaneous parallel recording of ion current through transmembrane pores reconstituted in planar lipid bilayer arrays. Use of a thin poly(p-xylylene) (parylene) film having micrometer- sized apertures (O) 15-50 µm, t) 20 µm) led to formation of highly stable bilayer lipid membranes (BLMs) for incorporation of transmembrane pores; thus, a large number of BLMs could be arrayed without any skillful technique. We optically confirmed the simultaneous formation of BLMs in a 5 × 5 matrix, and in our durability test, the BLM lasted more than 15 h. Simultaneous parallel recording of alamethicin and gramicidin trans- membrane pores in multiple contiguous recording sites demonstrated the feasibility of high-throughput screening of transmembrane ion currents in artificial lipid bilayers.

Materials Used for Artificial Bilayers and Microfluidic Systems

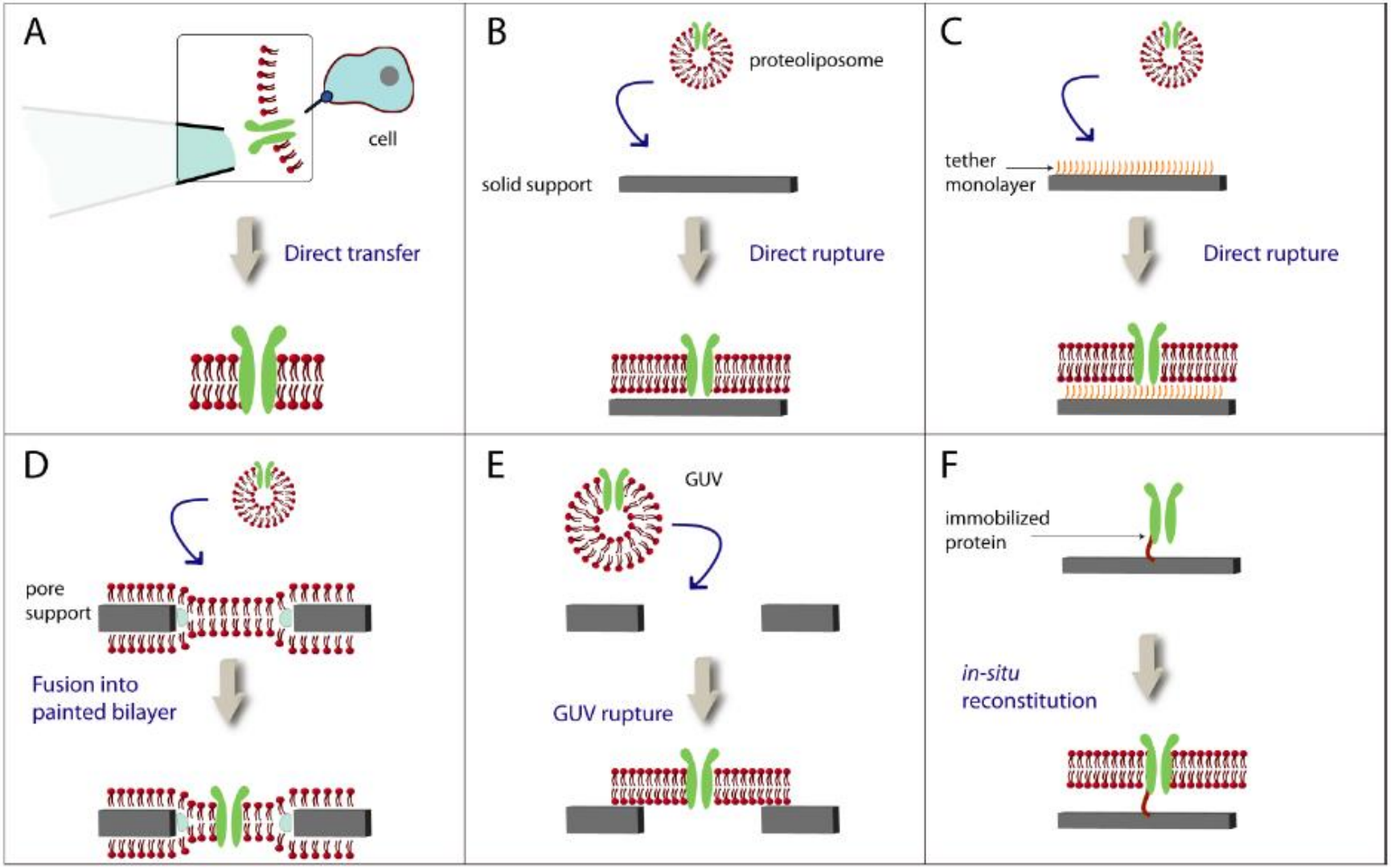
A material suitable to bear lipid bilayers and transporting fluids meets the following requirements:

(1) Resistant to organic solvents;
(2) Surface properties such as wettability and roughness should be well defined or can be adjusted through chemical modifications of activated material
(3) Technologies are available for making structures of micro- and nanometer dimensions within the material and the resulting structures remain stable;
(4) The properties of the material allow electrochemical detections;
(5) For optical detection the material is transparent



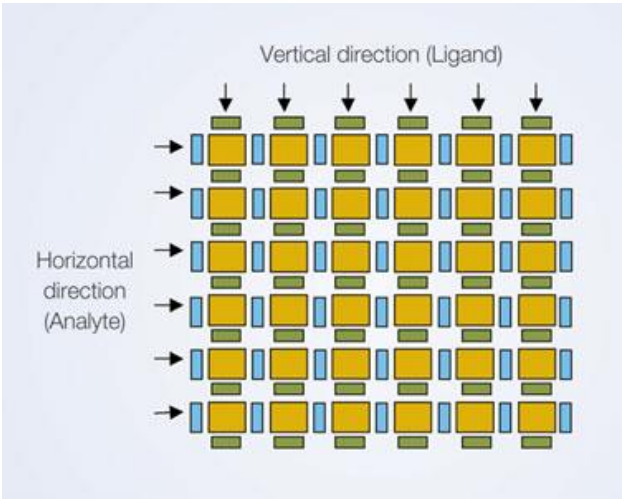
Tiefenauer and Demarche, 2012

Methods Used to construct Artificial Bilayers in Microfluidic Systems

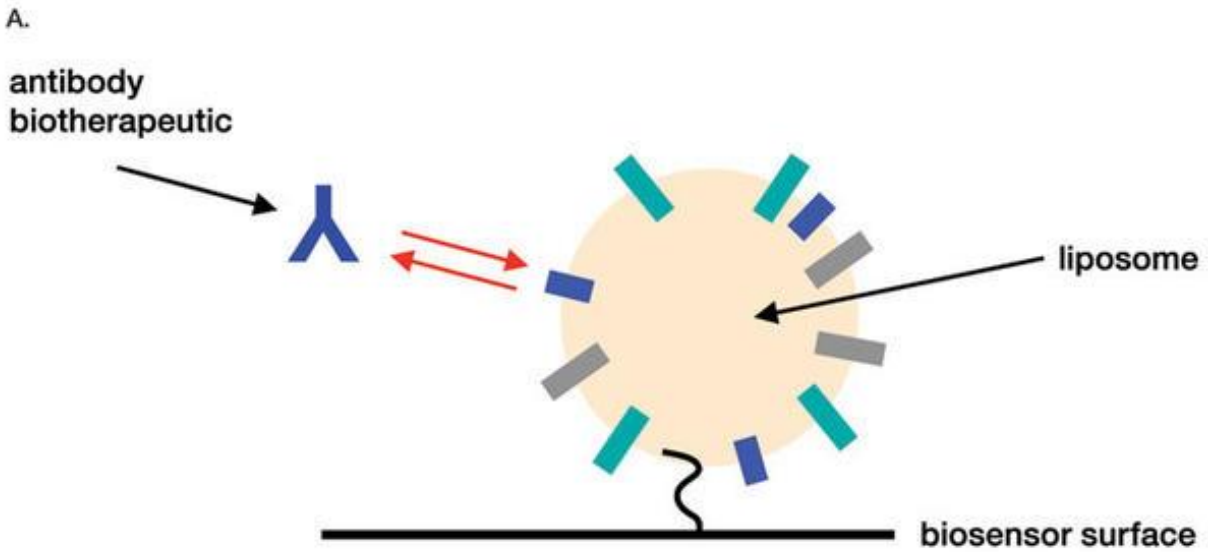


Commercial Example: BIO-RAD ProteOn

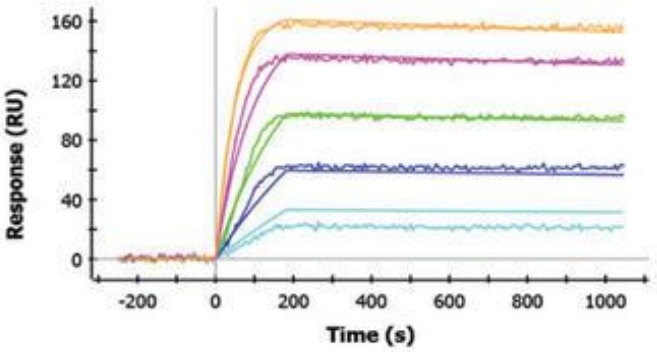
- Integral Molecular's lipoparticle technology + array-based ProteOn biosensor
- Lipoparticles (ca. 150 nm)
- Membrane protein surface concentrations 10-100 fold higher than those found in cells
- Easily attached to array surface (carboxylated alginate + antibodies)



Commercial Example: BIO-RAD ProteOn

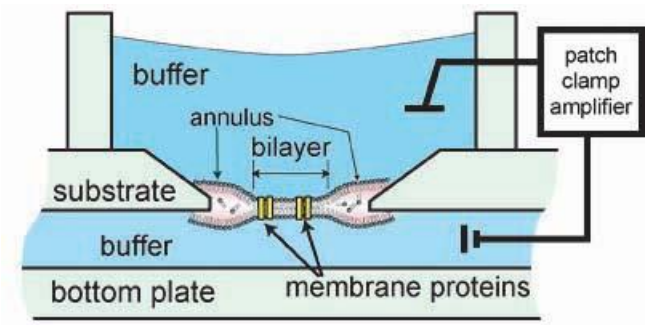


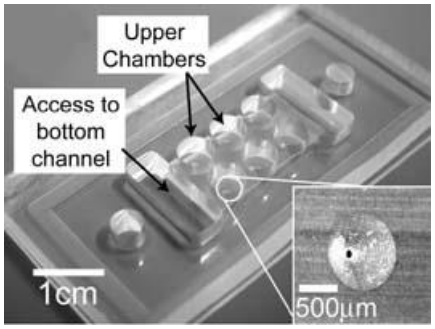
B.



K_a (1_{-Ms})	1.97×10^{6}
K_d (1^{-s})	6.05×10^{-5}
R_{max} (RU)	166
K_D (M)	3.06×10^{-11}

Membrane Protein Chips and More

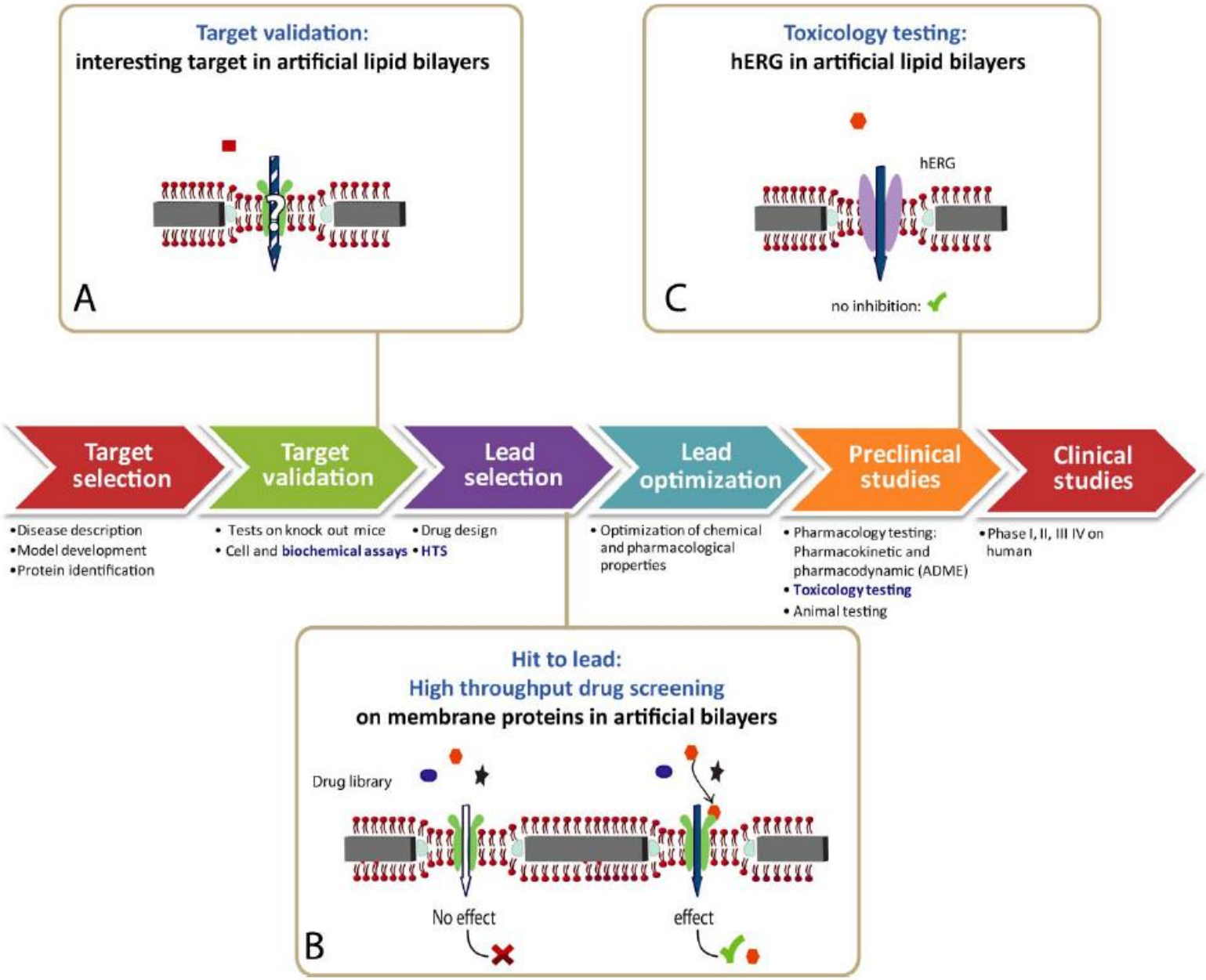




Suziki, H, 2007

- ☑ Sandison ME, et al, Micromachined glass apertures for artificial lipid bilayer formation, *J. Micromech. Microeng.* 17:189-196
- ☑ Janshoff A and Steinem C (2006) Transport across artificial membranes - an analytical perspective, *Anal Bioanal Chem* 385 (3): 433-451
- ☑ Tanaka M and Sackmann E (2006) Supported membranes as biofunctional interfaces and smart biosensor platforms, *Physica Status Solidi A - Applications and Materials Science* 203 (14): 3452-3462
- ☑ Abdiche YN and Myszka DG (2004) Probing the mechanism of drug/ lipid membrane interactions using Biacore, *Anal Biochem* 328 (2): 233-243

Figure 6. Drug discovery applications.



Clinical Trial Phases

Phase	Participants	Purpose	Special Features
Phase 1	Small number (20–80) of participants, usually healthy volunteers, in some cases patients with advanced disease (e.g., cancer)	To evaluate safety, identify side effects, determine a safe dose range, and learn how the agent is absorbed and handled by the body (pharmacokinetics/dynamics).	Often first time tested in humans
Phase 2	Larger number (hundreds) of patients with the condition under study	To further evaluate safety and to determine if the agent has the intended effect in humans.	Sometimes randomized controlled trials
Phase 3	Larger still (thousands) of people with the condition under study	To confirm or further evaluate an agent's effectiveness, monitor side effects, compare it to commonly used treatments, and collect other information that will be used to determine whether the agent should be approved and marketed.	Usually randomized, controlled trials
Phase 4 (postmarketing study)	Various populations	To collect additional information after an agent is approved and marketed regarding its risks, benefits, and use in various populations over a longer period of time.	