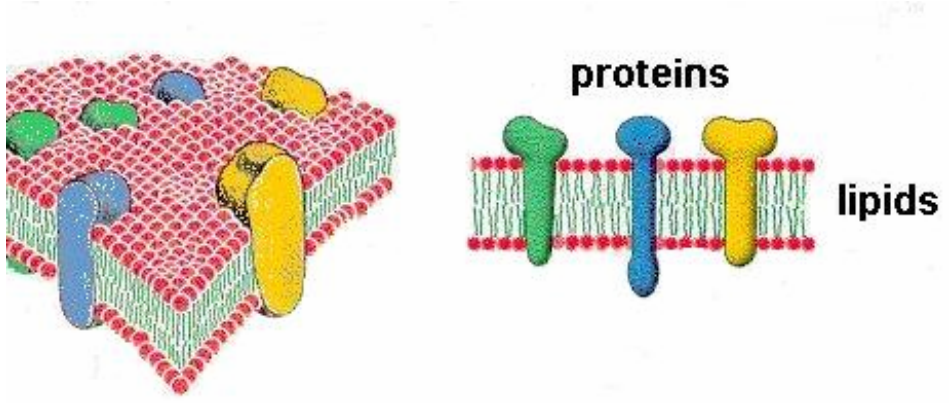
Examining the interaction of drugs and membranes to improve selectivity



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Enhancing Screening Strategies with Cell-Based Assays
Munich, Germany, February 5-7th, 2007
Informa-Life Science, Informa plc, London, UK

The pharmacologist's and toxicologist's key question …

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  - composition and lipid-distribution heterogeneity
  - structure and micro/macrostructure – influence from and on proteins
  - fusion and rupture
- Drug disposition: membranes and penetration/efflux
  - the case of two fluoroquinolones
- Drug toxicity toxicity
  - the case of aminoglycosides, azithromyccin, and lipoglycopeptides
- Drug activity activity
  - the case of new anti Gram (+) antibiotics (daptomcyin, telavancin, … and lantibiotics) and a few words on rafts as drug targets

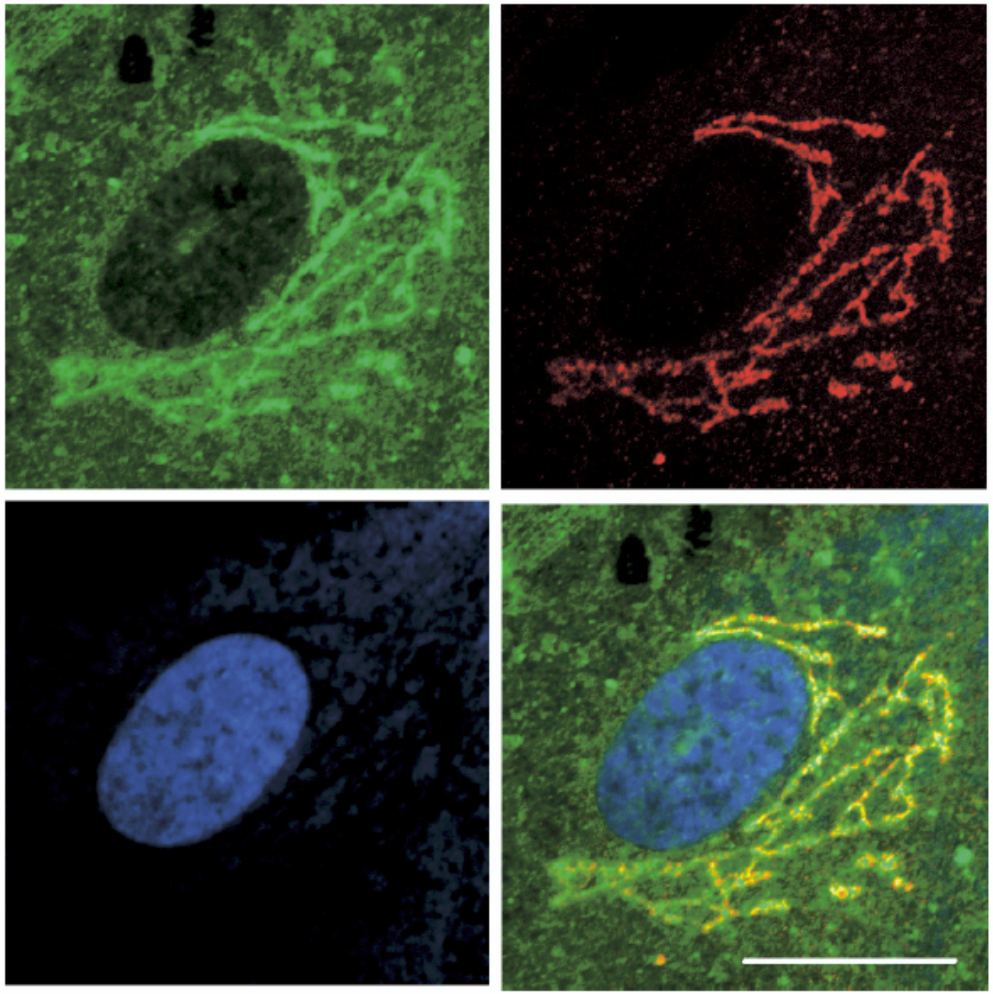


Fig. 1. PEG-Chol Labels the Golgi Apparatus in Human skin Fibroblasts

Cells were fixed, permeabilized and triply labeled with fPEG-Chol (green), anti-TGN46 (Golgi, red), and TOPRO-3(nucleus, blue). The lower right figure shows the merge. Bar, 20 μm. Golgi labeling by PEG-Chol indicates the accumulation of cholesterol in the organelle in human skin fibroblast. Photograph courtesy of Kumiko Ishii.

Heterogeneity of lipid distribution among organelles: the case of cholesterol macro-distribution

Kobayashi et al. Biol Pharm Bull. 2006 Aug;29(8):1526-3.

Membrane microheterogeneity: role of P-glycoprotein and impact on cholesterol trafficking

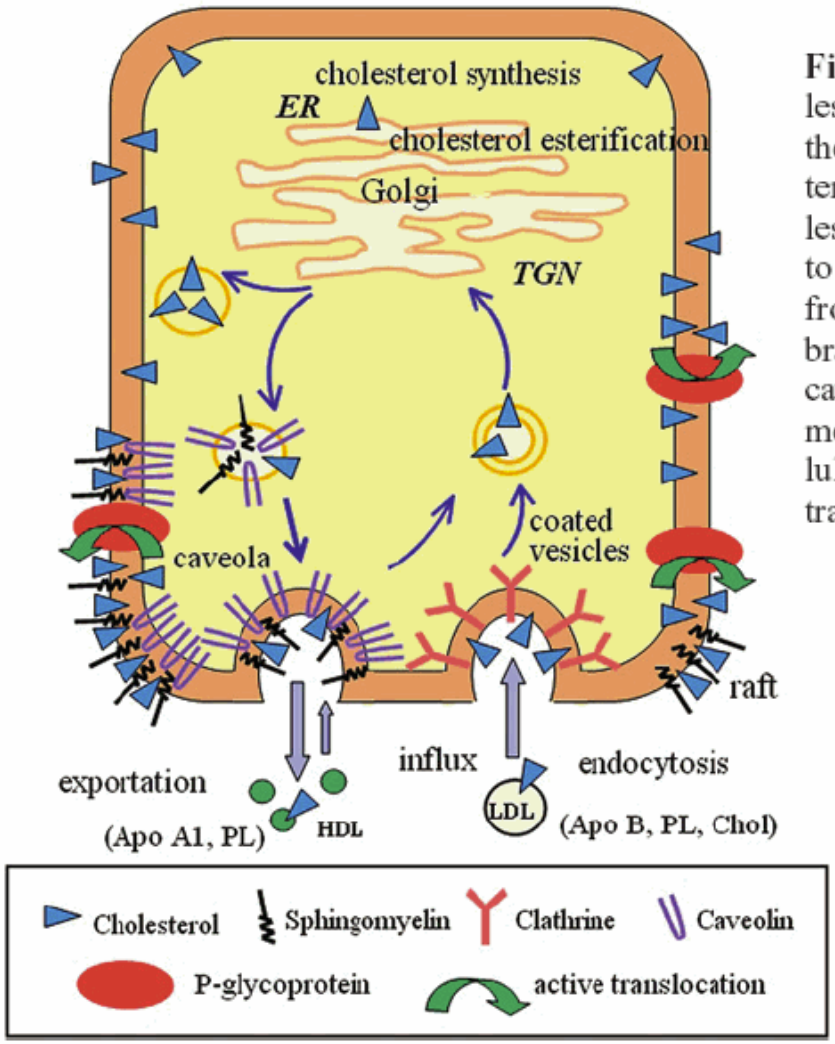
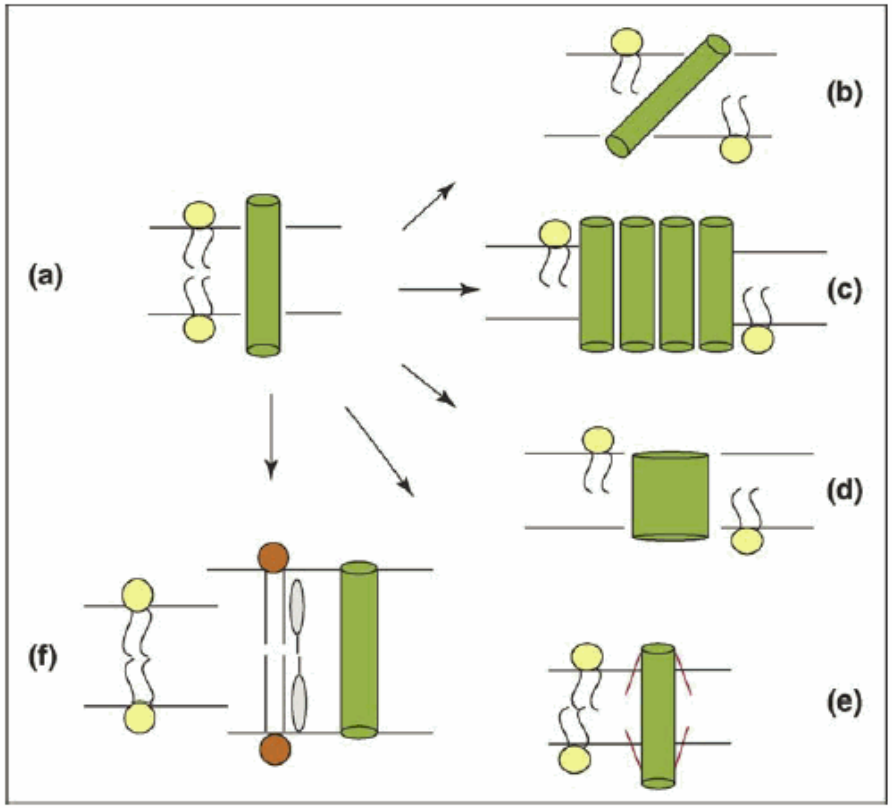


Figure 5. Functional scheme of P-gp involvement in cellular cholesterol trafficking. In cells, according to an oversimplified view, the cholesterol amount is regulated between endogenous cholesterol biosynthesis and esterification in the ER, an exogenous cholesterol import from LDL by endocytosis and a cholesterol export to HDL [133, 134]. The active cholesterol flux mediated by P-gp from the cytosolic to the exoplasmic leaflet of the plasma membrane supports a role for P-gp in cholesterol enrichment of rafts and caveolae, leading to increased integration of caveolin-1 in plasma membrane, and possibly to upregulation of other steps in intracellular cholesterol trafficking. (ER, endoplasmic reticulum; TGN, trans-Golgi network.)

Orlowski et al. Cell Mol Life Sci. 2006 May;63(9):1038-59

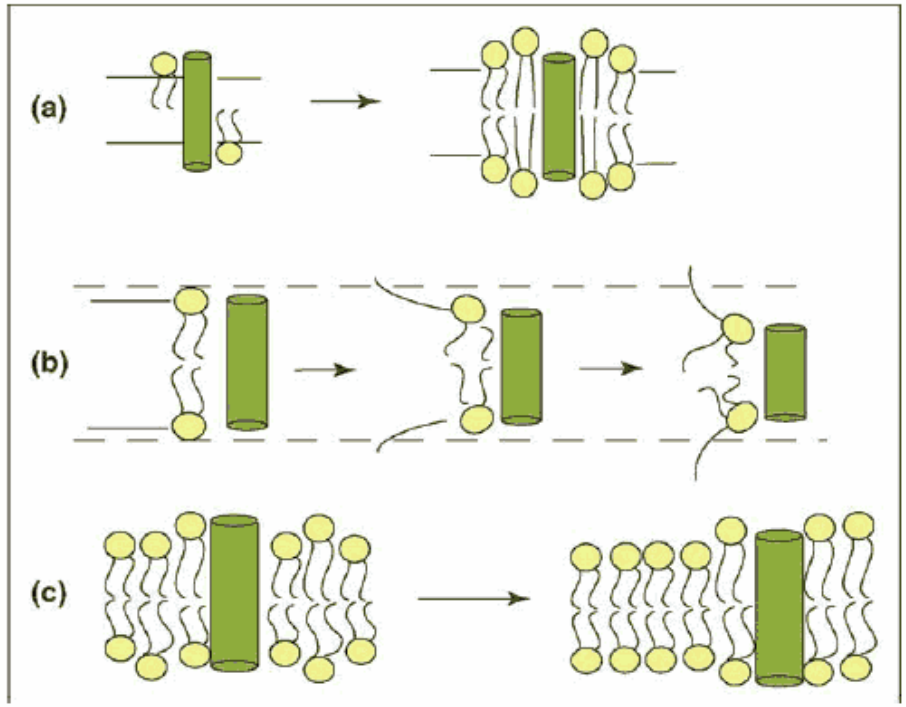
How lipids influence proteins structure and organization…



Possible consequences of hydrophobic mismatch for protein structure and organization. The green cylinder represents the hydrophobic part of a membrane protein. **(a)** Positive mismatch by itself would lead to exposure of hydrophobic groups to a hydrophilic environment at the interface. Possible adaptations are **(b)** tilting of transmembrane segments to reduce their effective length, **(c)** self-association, **(d)** changes in backbone conformation or **(e)** changes in the orientation of the sidechains. **(f)** In multicomponent systems, transmembrane segments that are too long might partition into thicker domains.

Current Opinion in Structural Biology 2006, **16**:473–479 www.sciencedirect.com

How protein influence membrane micro-organization



Possible mismatch-induced effects of proteins on lipids are **(a)** stretching of lipids under conditions of positive mismatch, **(b)** disordering of the lipid acyl chains and formation of non-lamellar structures under conditions of negative mismatch, and **(c)** sorting of lipids by recruitment of lipids with the best-matching length from mixtures of lipids.

www.sciencedirect.com **Current Opinion in Structural Biology** 2006, **16**:473–479

Rafts: a typical example of microheterogeneity ...

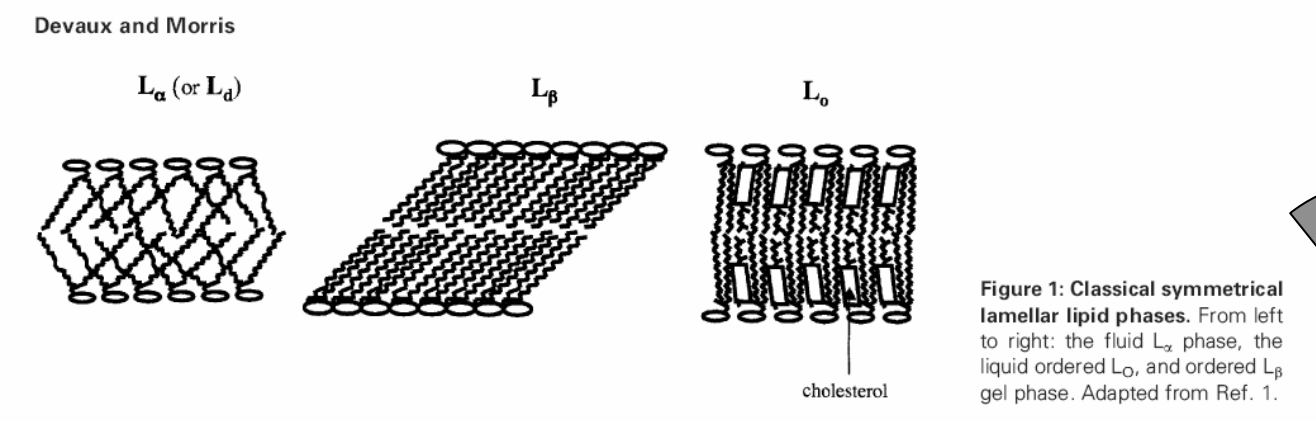
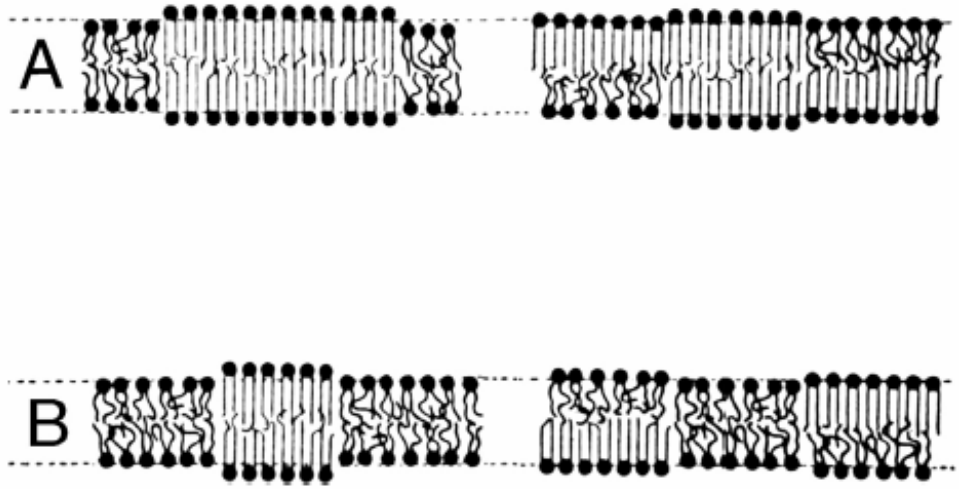


Figure 1: Classical symmetrical lamellar lipid phases. From left to right: the fluid L_α phase, the liquid ordered L_O, and ordered L_β gel phase. Adapted from Ref. 1.

Lipid rafts are defined as specialized, dynamic microdomains that can be found in plasma membrane, and they are enriched with cholesterol and sphingolipids.



Devaux & Morris: Traffic. 2004 Apr;5(4):241-6.

What are rafts and how are they formed ?

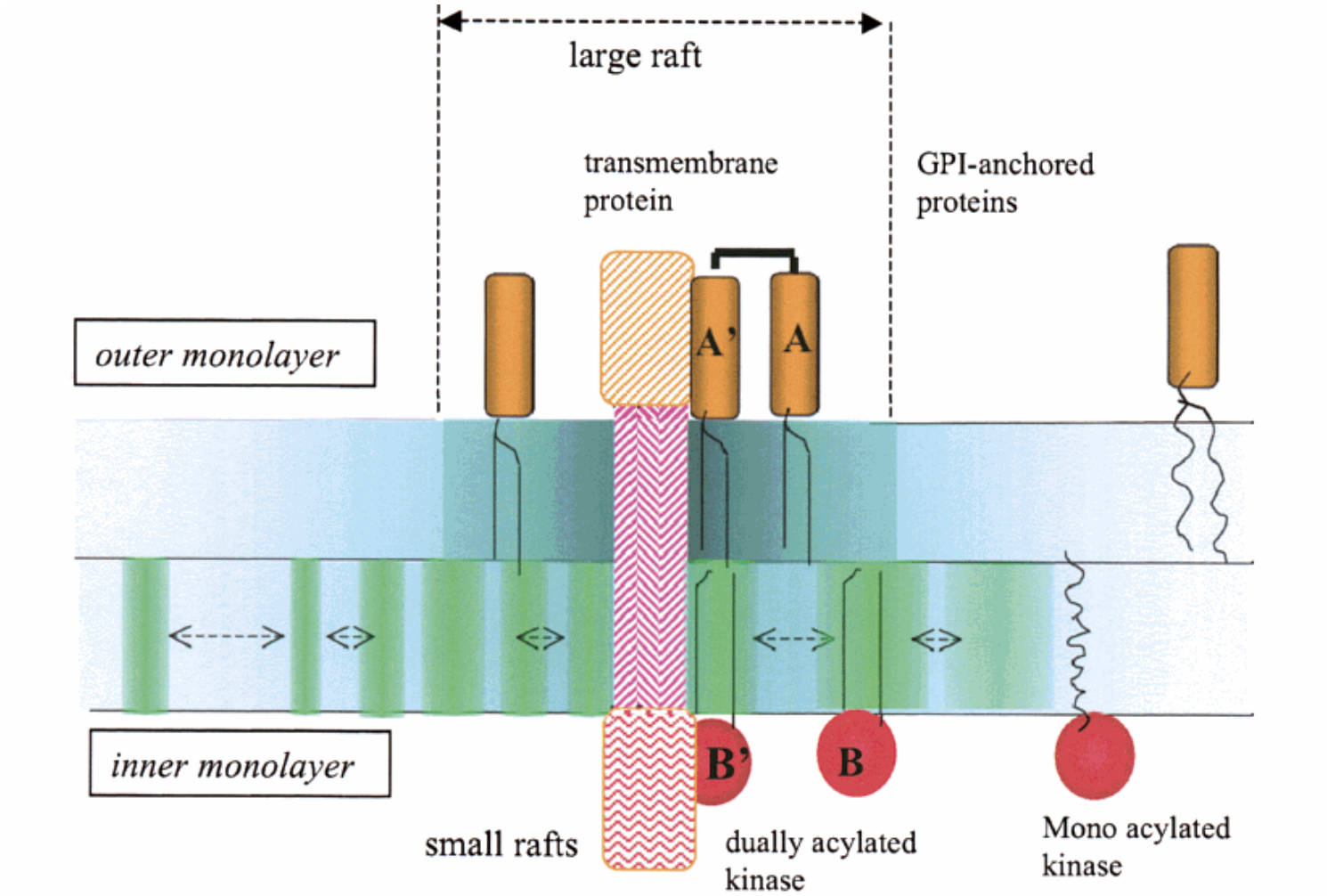


Figure 3: Schematic representation of biomembrane rafts emphasizing the asymmetry of the rafts in the two monolayers, which have different sizes (and probably different lifetimes). Both monolayers have liquid-ordered phases with cholesterol but the phospholipids interacting with cholesterol are different and hence induce slightly different L_O phases. This figure suggests that superposition of rafts in two monolayers requires a coupling via a transmembrane protein. The association can be also fortuitous and is likely to be temporary.

Devaux & Morris: Traffic. 2004 Apr;5(4):241-6.

How proteins may create lipid microdomains (rafts)

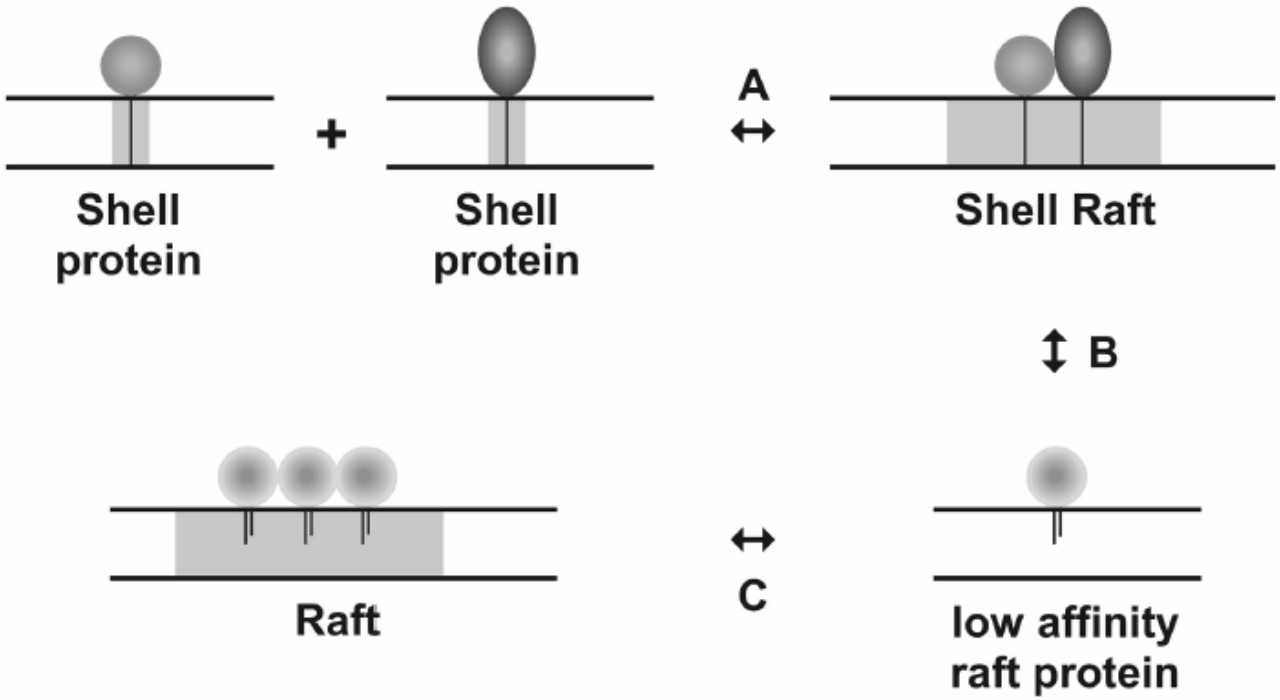
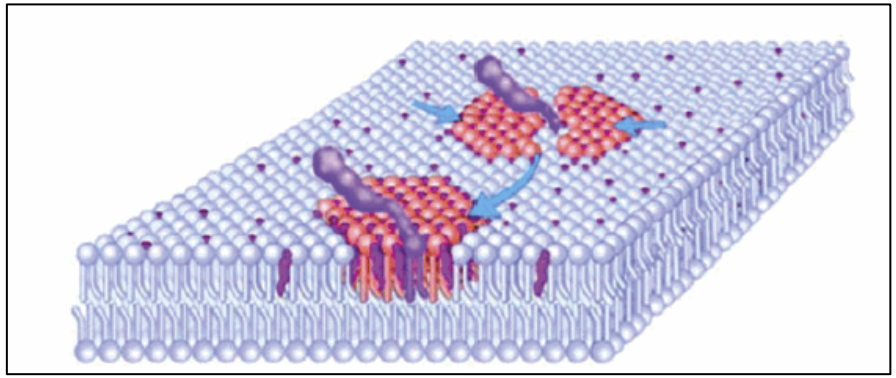


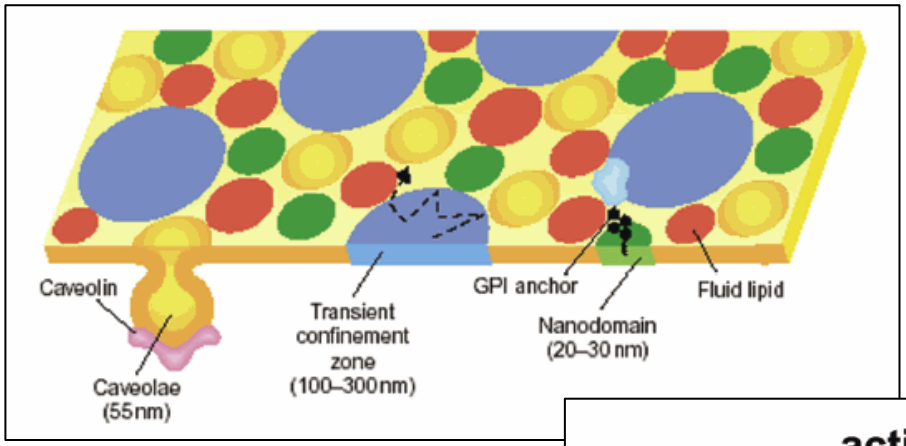
Figure 1: Lipid rafts. Proteins with a high affinity for selected lipids are suggested to form lipid shells (9). It remains to be determined whether shells can already represent a functional unit or whether larger (raft) structures are required to generate functional domains. Protein–protein interactions between shell proteins can create larger functional units called lipid rafts (A). Dual-acylated proteins (including GPI-anchored proteins) can associate with pre-existing lipid rafts based on their low affinity for raft lipids of the acyl moiety. This affinity can be enhanced by lipid-protein and/or protein–protein interactions (B). By oligomerization of low-affinity raft proteins, enough low affinity lipid-interacting moieties may be combined to stabilize a functional raft domain (C). Under these conditions the oligomerization process may create and stabilize raft domains.

Traffic 2004; 5: 247–254

Various views and hypotheses for rafts

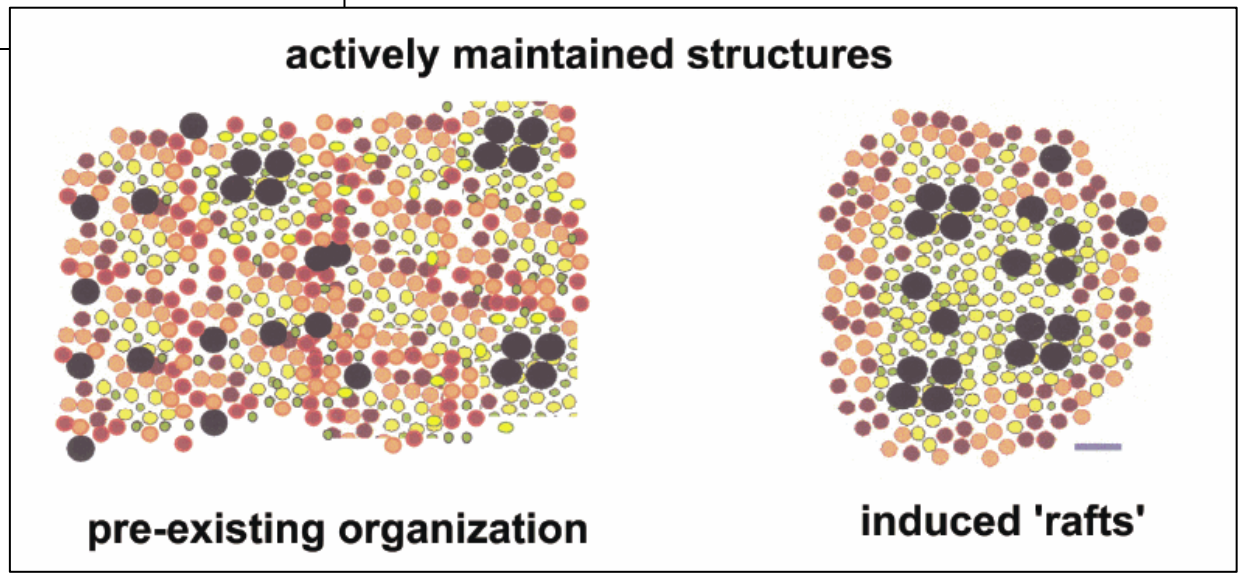
specific associations with "raft" proteins →





← mosaic of domains
(with cholesterol-driven partition)

oscillations from "monomeric" to "assembled" structures →



Mayor & Rao Traffic. 2004 Apr;5(4):231-40

Rafts and membrane movements

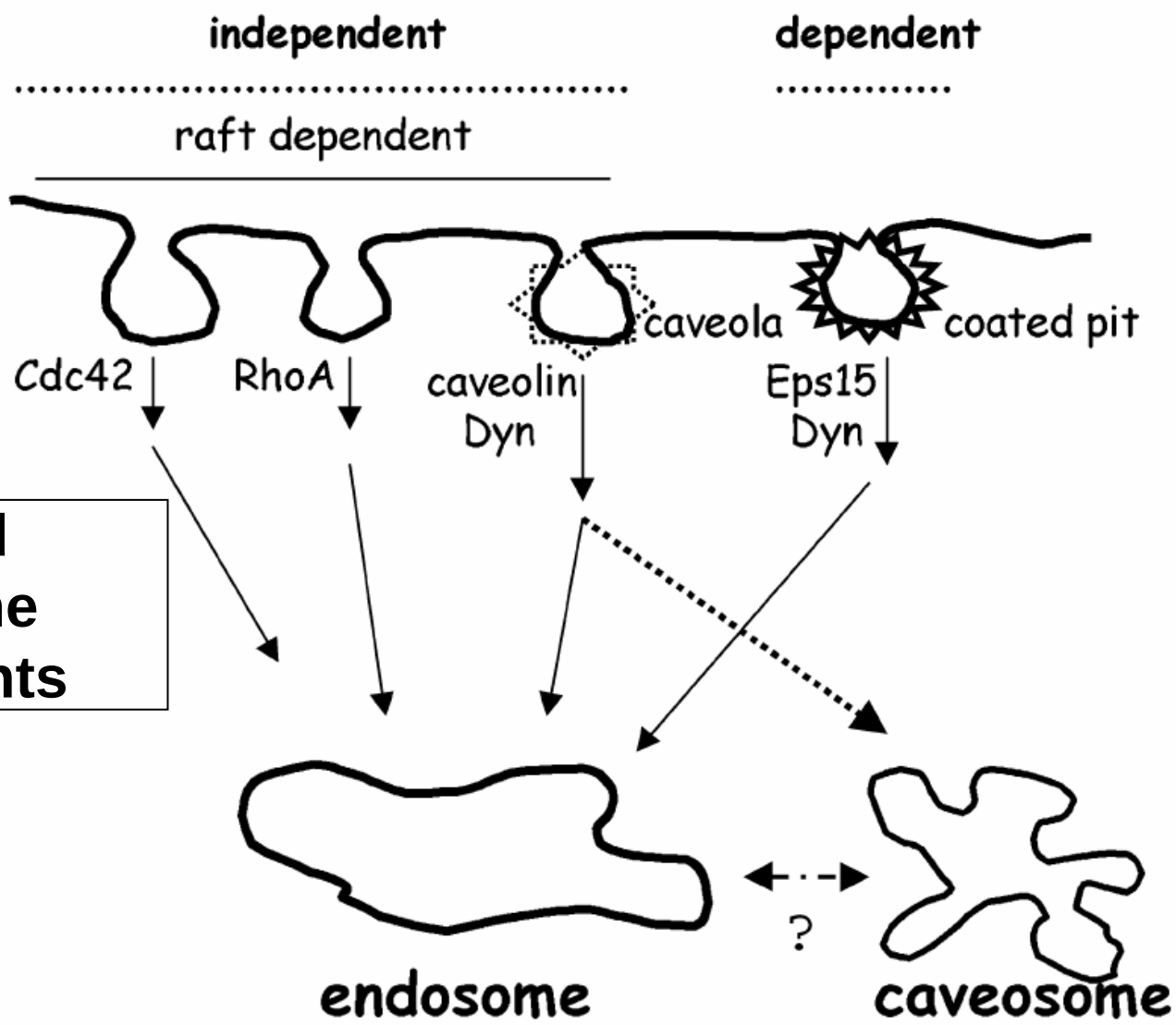


Figure 2: Multiple pathways of endocytosis. Different types of invaginations occur at the plasma membrane to mediate membrane endocytosis. Many surface receptors are internalized into coated pits by a clathrin-dependent pathway. Clathrin-independent, raft-dependent internalization pathways are distinct in caveolae and noncoated invaginations, but both are dynamin-independent and require different small GTPases. All pathways lead to the endosomal compartment, while caveolae fuse with another sorting compartment known as the caveosome. The relationship (if any) between the early endosomal compartment and the caveosome is not yet understood.

Helms & Zurzolo: Traffic. 2004 Apr;5(4):247-54.

Rafts and virus budding

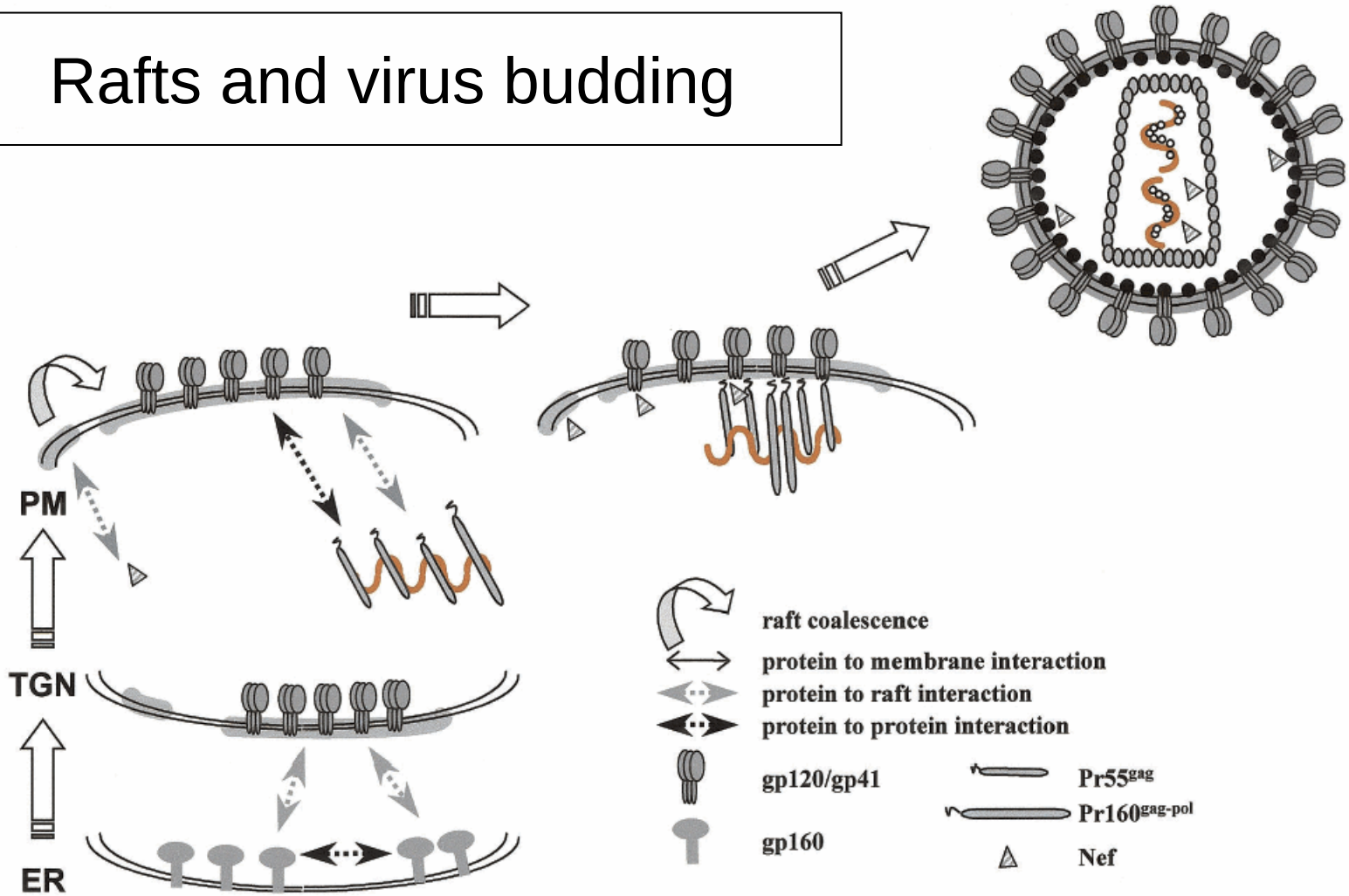
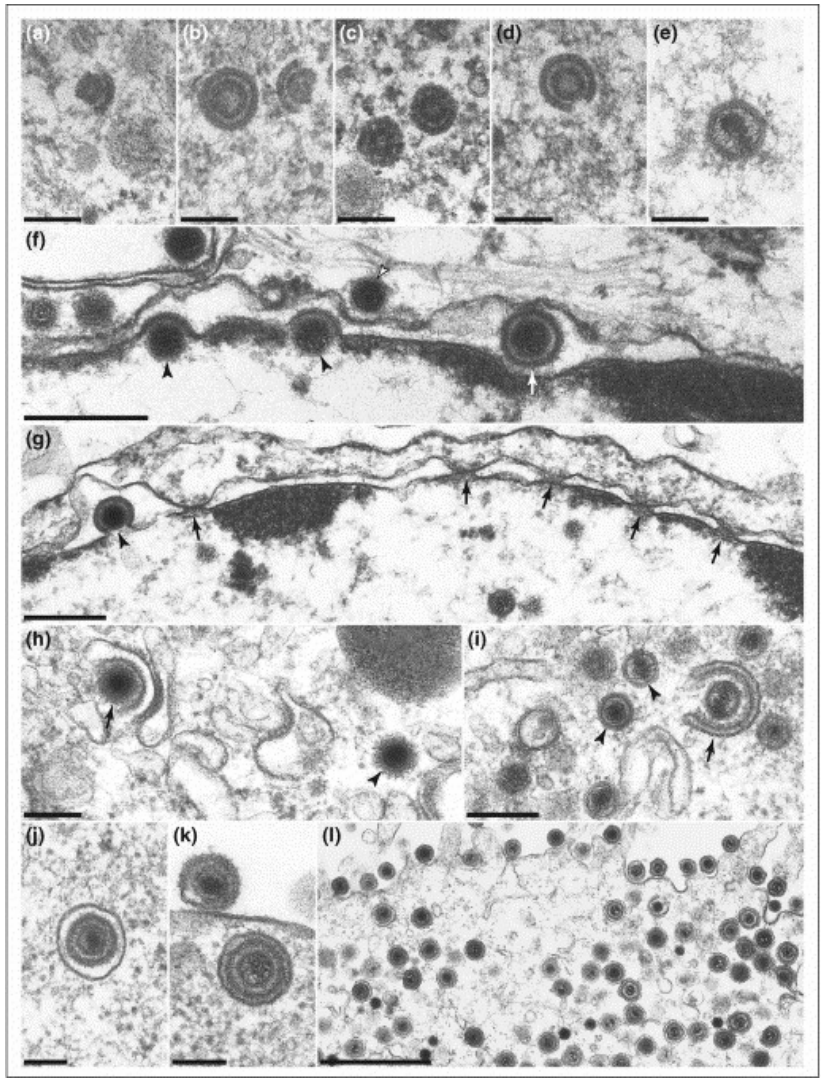
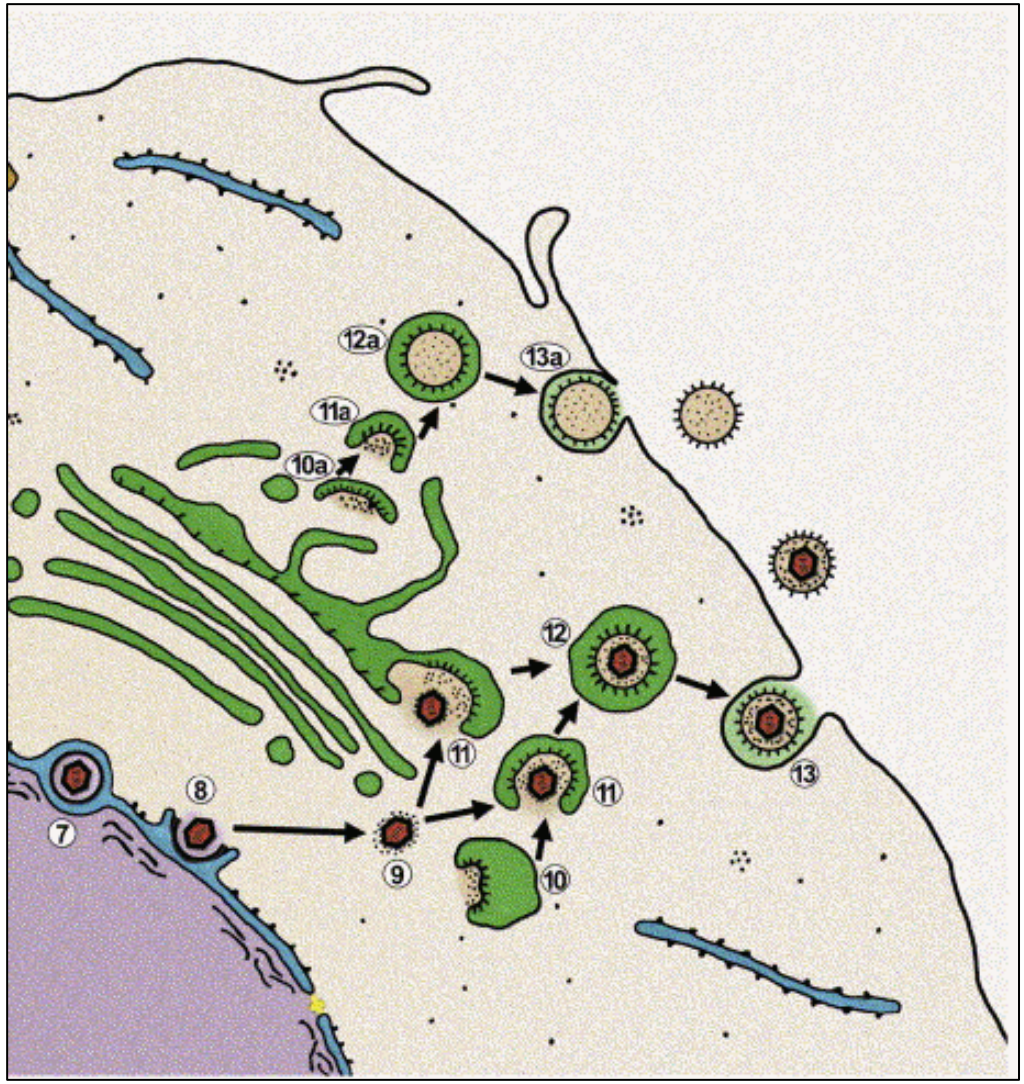


FIG. 3. Model of HIV-1 assembly and budding through membrane rafts. gp160 trimerizes within the ER and, on reaching the TGN, associates with rafts because of its affinity for lipid rafts. It then migrates to the plasma membrane. $Pr55^{gag}$ and $Pr160^{gag\text{-}pol}$ oligomerize around two genomic RNAs and associate simultaneously with plasma membrane rafts due to the anchoring myristate and intrinsic properties of the MA domain. This allows the binding of MA to the cytoplasmic tail of glycoproteins. The cytoplasmic Nef protein, after palmitoylation, associates with the inner leaflet of the plasma membrane raft. The raft coalescence results in Nef incorporation into HIV-1 particles and in the enrichment of the envelope in lipid rafts. Then HIV-1 matures (cleavage of Gag precursors in MA, CA, NC, p6, and enzymes) and buds from the plasma membrane rafts. Nef protein is initially bound to membrane rafts. When encapsidated into HIV-1 particles, Nef is partly cleaved off by the viral protease into a soluble domain, which is thought to bind to the RNP. Membrane rafts are represented as shaded grey regions within the lipid bilayer.

Chazal & Gerlier Microbiol Mol Biol Rev. 2003 Jun;67(2):226-37

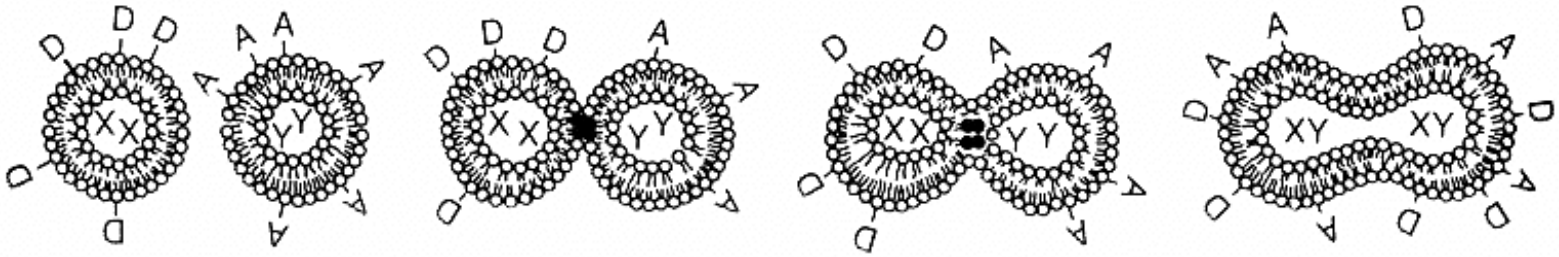
Membrane fusion: the role of lipids





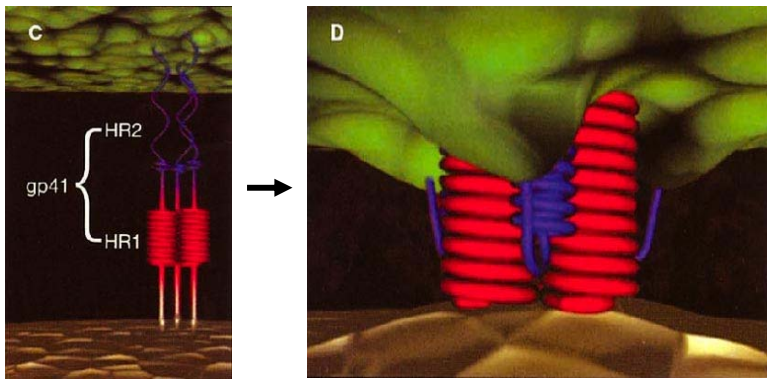
Mettenleiter et al. Current Opinion inn Microbiol. 2006 Aug;9(4):423-9.

Membrane fusion: mechanisms



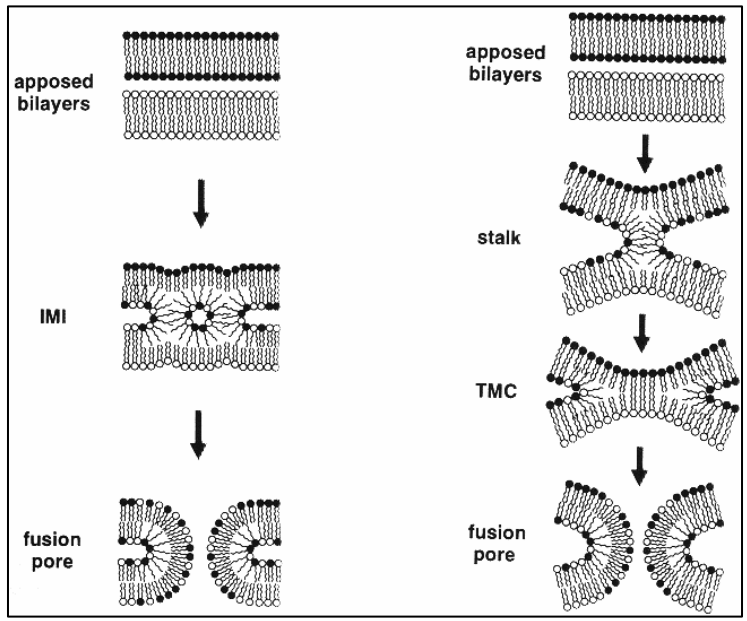
proteins play a key role in nearing membranes (viz. viruses)

but lipids do the job …

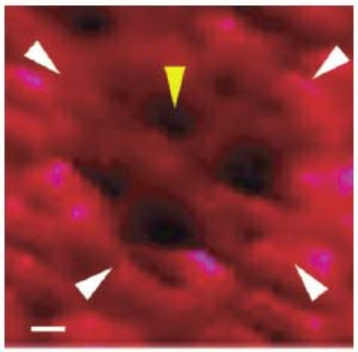


role of gp41 helical regions (HRs) to near viral and host cell membranes.

Cervia & Smith, Clinical Infectious Diseases 2003;37:1102-1106



Ultrastucture of cell secretion …



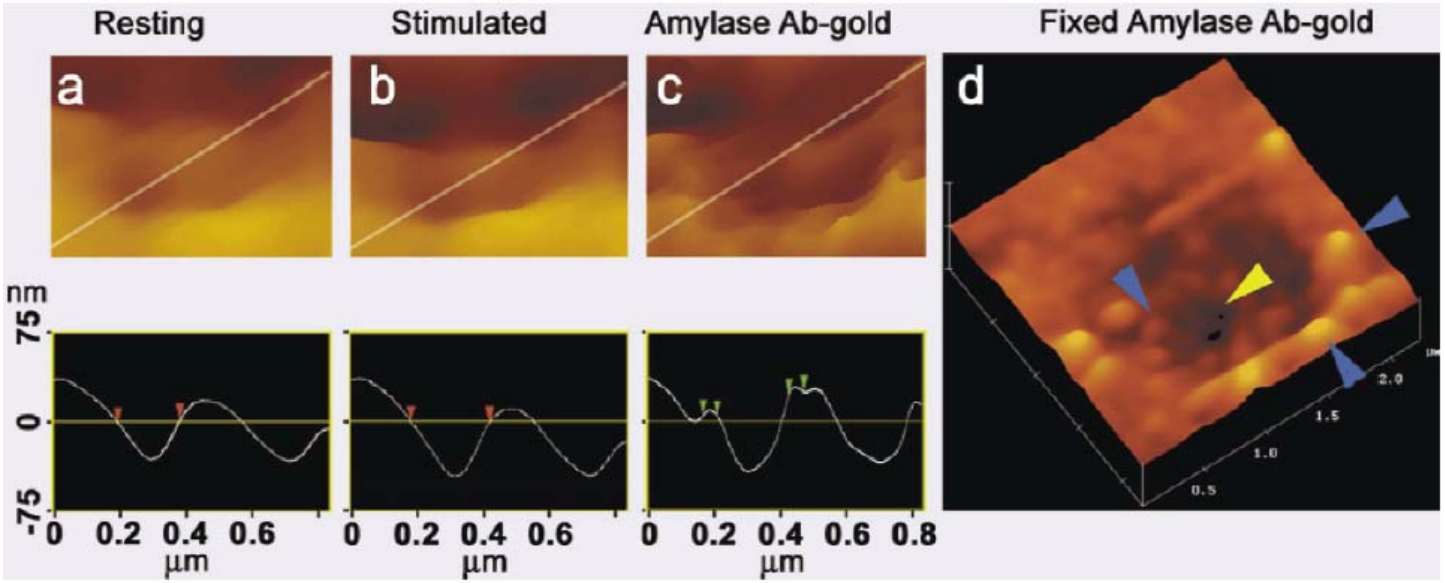


Fig. 2 AFM images (**a** and **b**) show dilation of the porosome to allow expulsion of vesicular contents. Section analysis through one of the porosomes in (**a**) and (**b**) show enlargement of the porosome following stimulation of secretion. (**c**) Exposure of live pancreatic cells to gold conjugated-amylase antibody, results in specific localization of gold to the edge of the porosome. (**d**) AFM micrograph of a stimulated, and fixed, pancreatic cell showing a pit (yellow arrowhead) with immunogold localization of amylase specific antibody (blue arrowhead) associated with the porosome [19]. *AFM images courtesy of Dr. Bhanu P. Jena.*

Allison & Drokticz: J Cell Mol Med. 2006 Oct-Dec;10(4):847-56.

The pharmacologist's and toxicologist's key question …

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  - the case of aminoglycosides, azithromycin, and lipoglycopeptides
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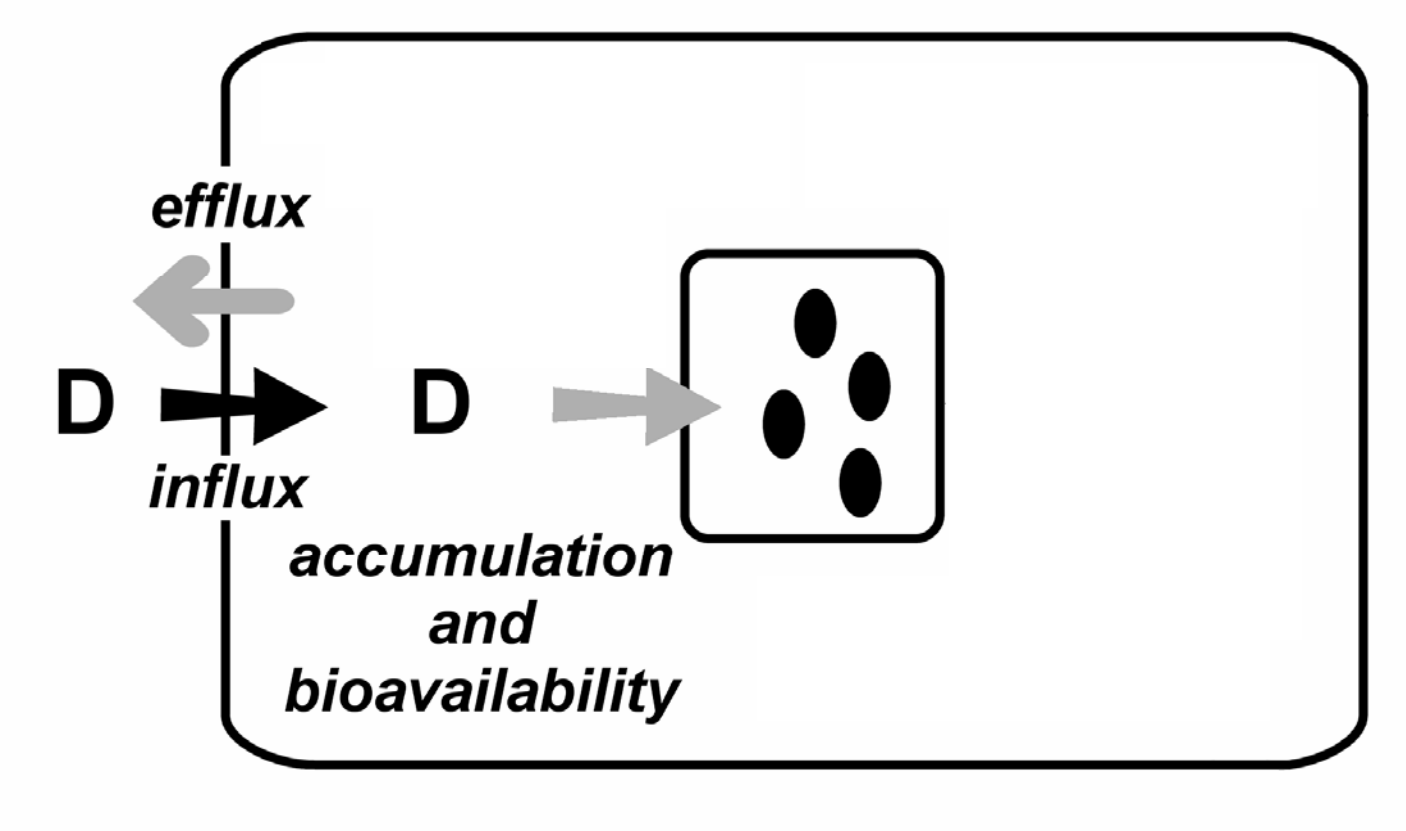
  - the case of new anti Gram (+) antibiotics (daptomcyin, telavancin, … and lantibiotics

Antibiotic acting intracellularly...



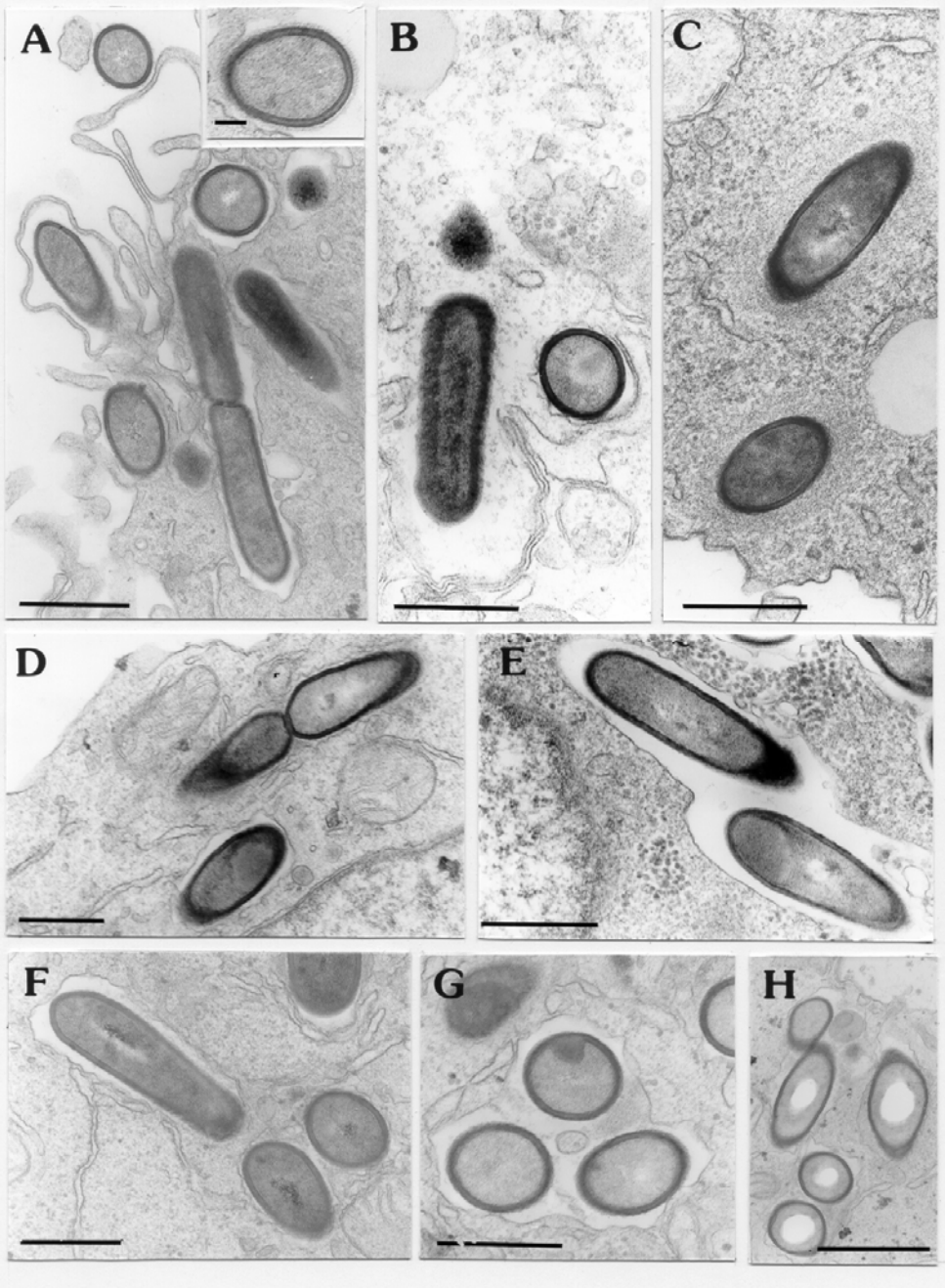
Question: wat is the importance of influx and efflux in overall activity

(modified from Tulkens, 1991; Carryn et al., 2003)

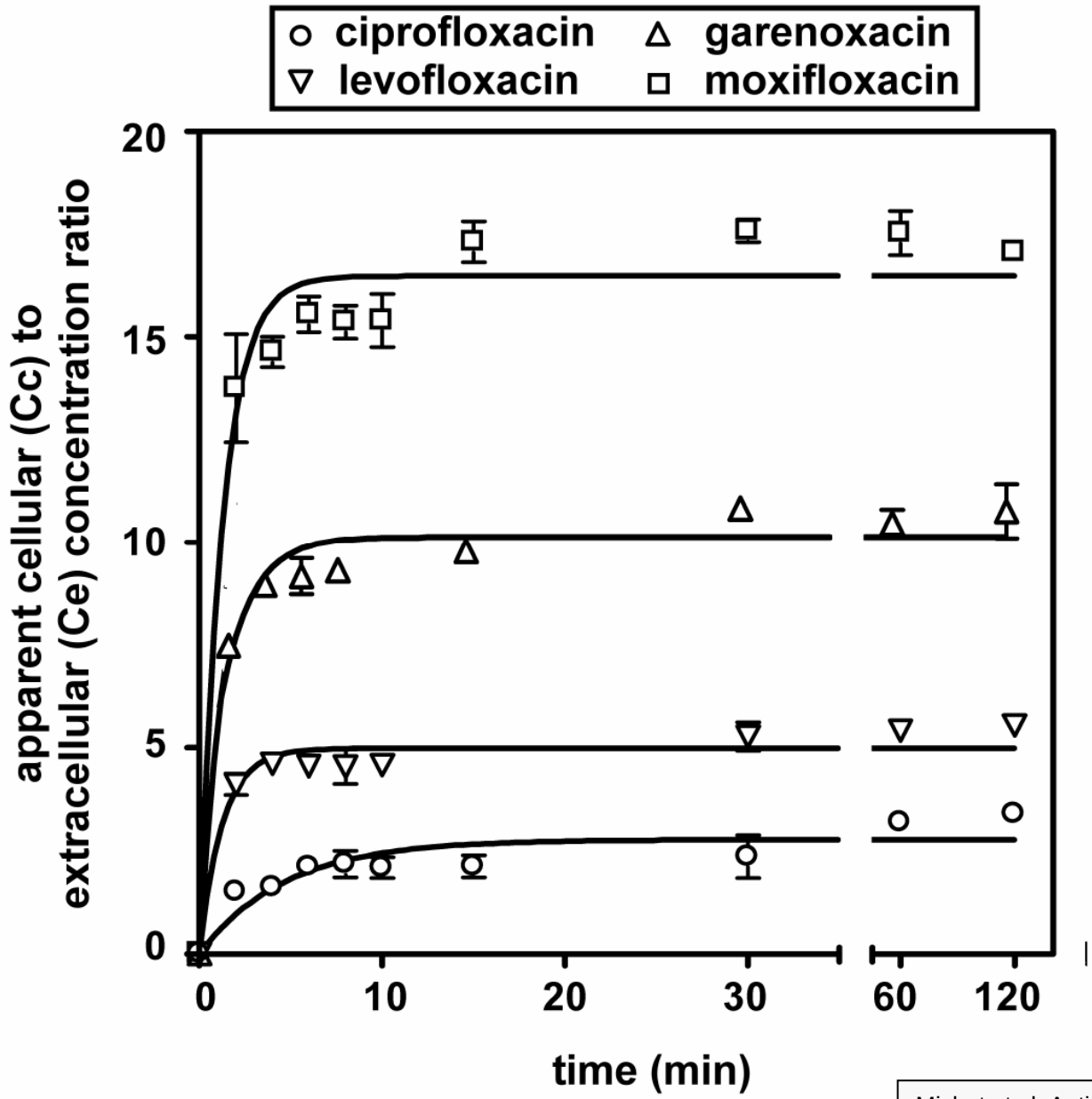
Example of intracellular bacteria

The intracellular pathway of *Listeria monocytogenes* ...

A-C: control
D-E: with gamma-interferon



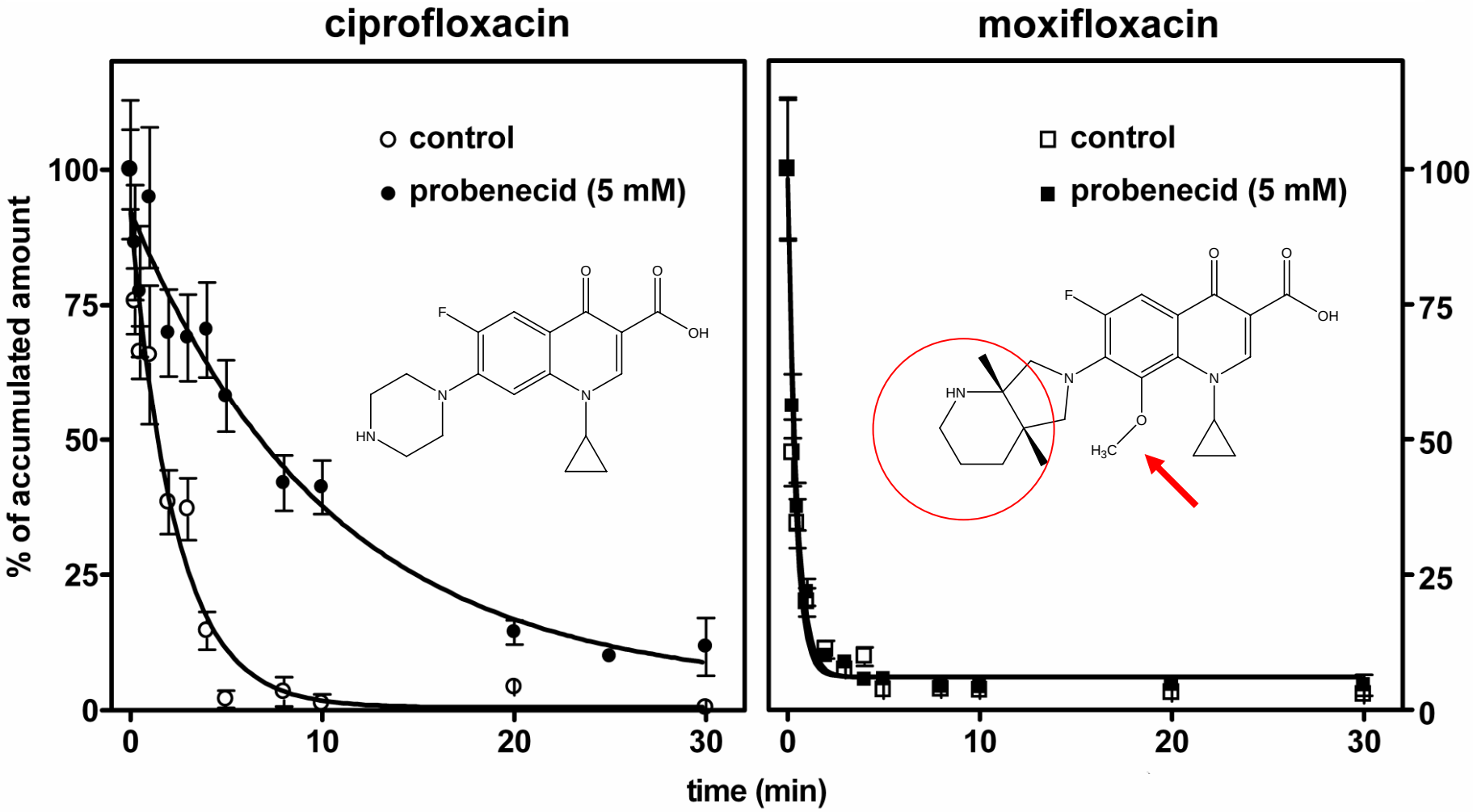
Ouadrhiri et al. Antimicrob. Agents Chemother. (1999) 43:1242-1251



"In and out" of closely related fluoroquinolones in macrophages

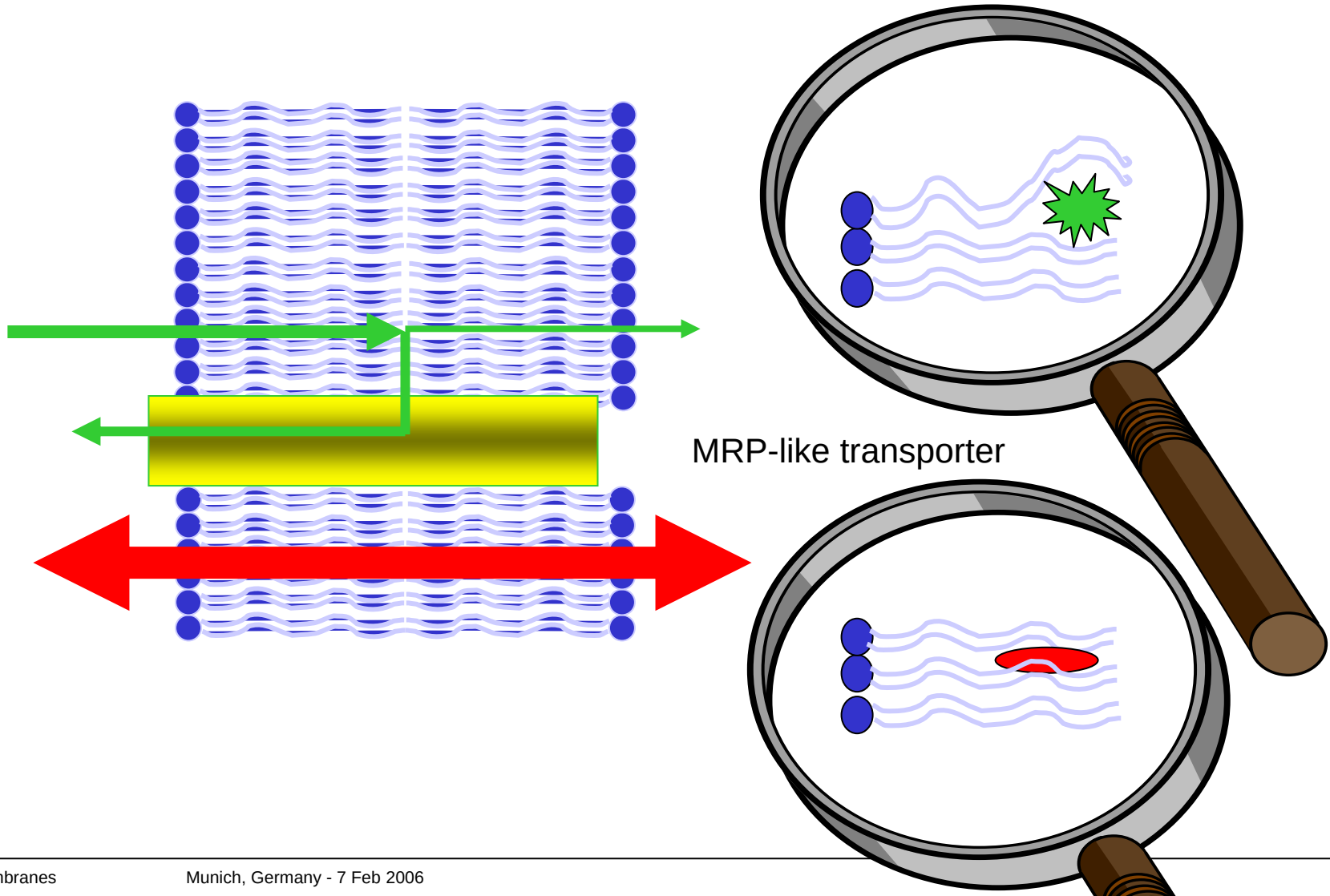
Michot et al. Antimicrob. Agents Chemother. 2005; 49:2429-2437

"In and out" of closely related fluoroquinolones in macrophages



Michot et al. Antimicrob. Agents Chemother. 2005; 49:2429-2437

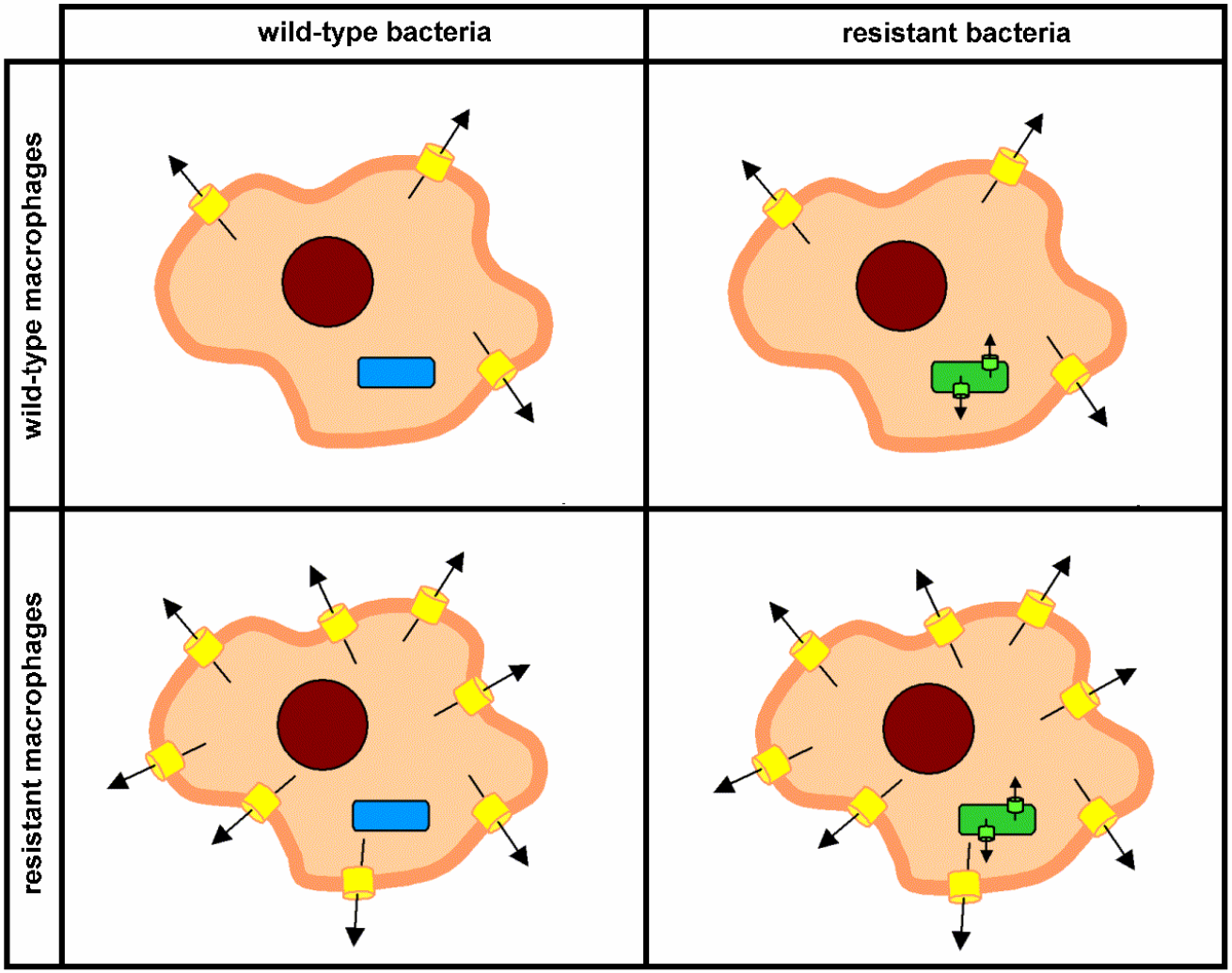
Fluoroquinolone model of penetration and efflux in macrophages



What if you diffuse faster
(and are not recognized by efflux transporters ?)

- You get a higher cellular accumulation
  - ↗ activity of moxifloxacin against intracellular *Listeria*, S*. aureus*, etc...
- Bacterial and eucaryotic efflux share many similarities
  - ↗ activity of moxifloxacin against pneumococci, *Listeria*, and all organisms with efflux-mediated resistance
- Combination of both ...

Cooperation between procaryotic and eucaryotic efflux pumps



Lismond *et al.* ICAAC 2006 – poster A1108

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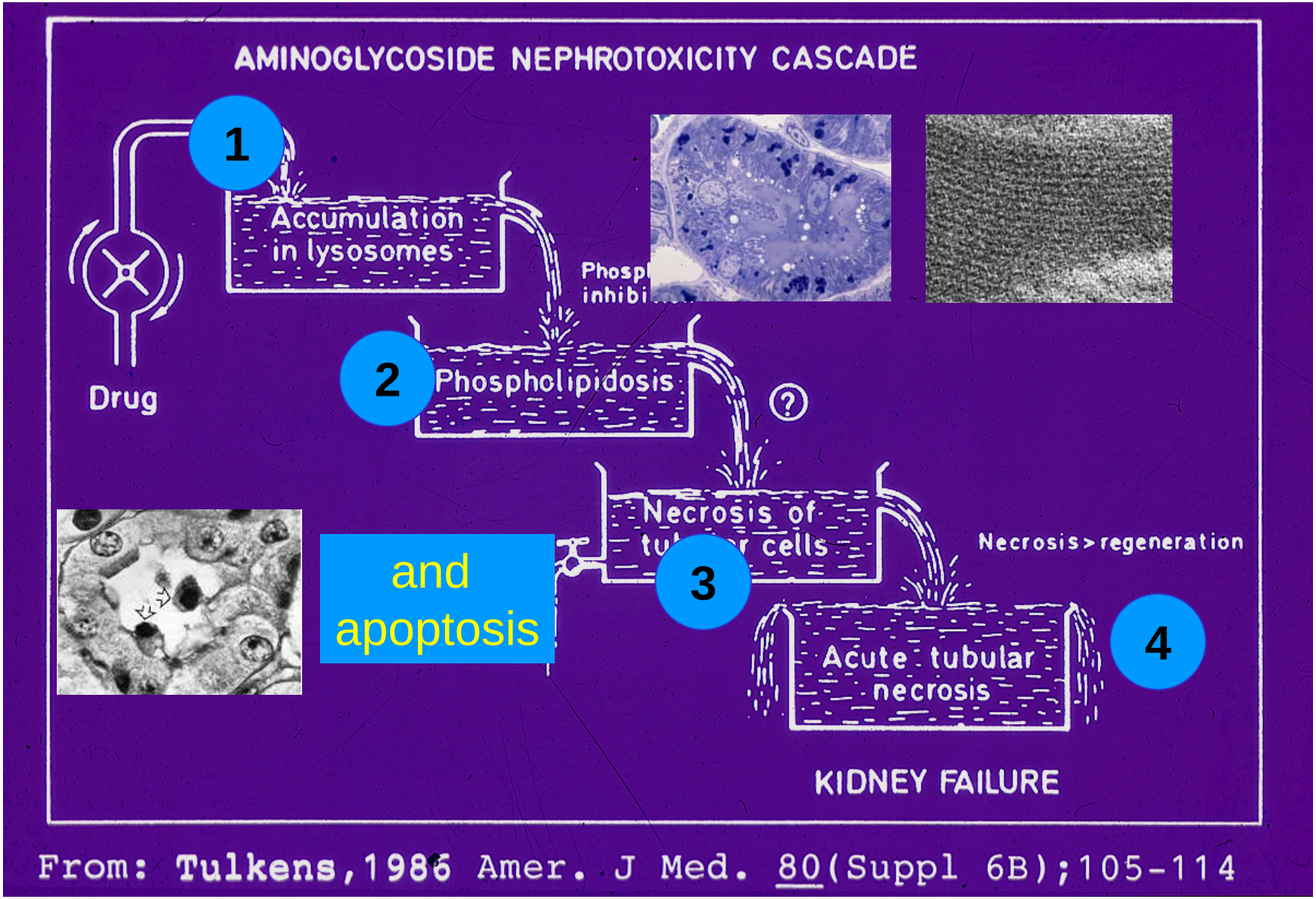
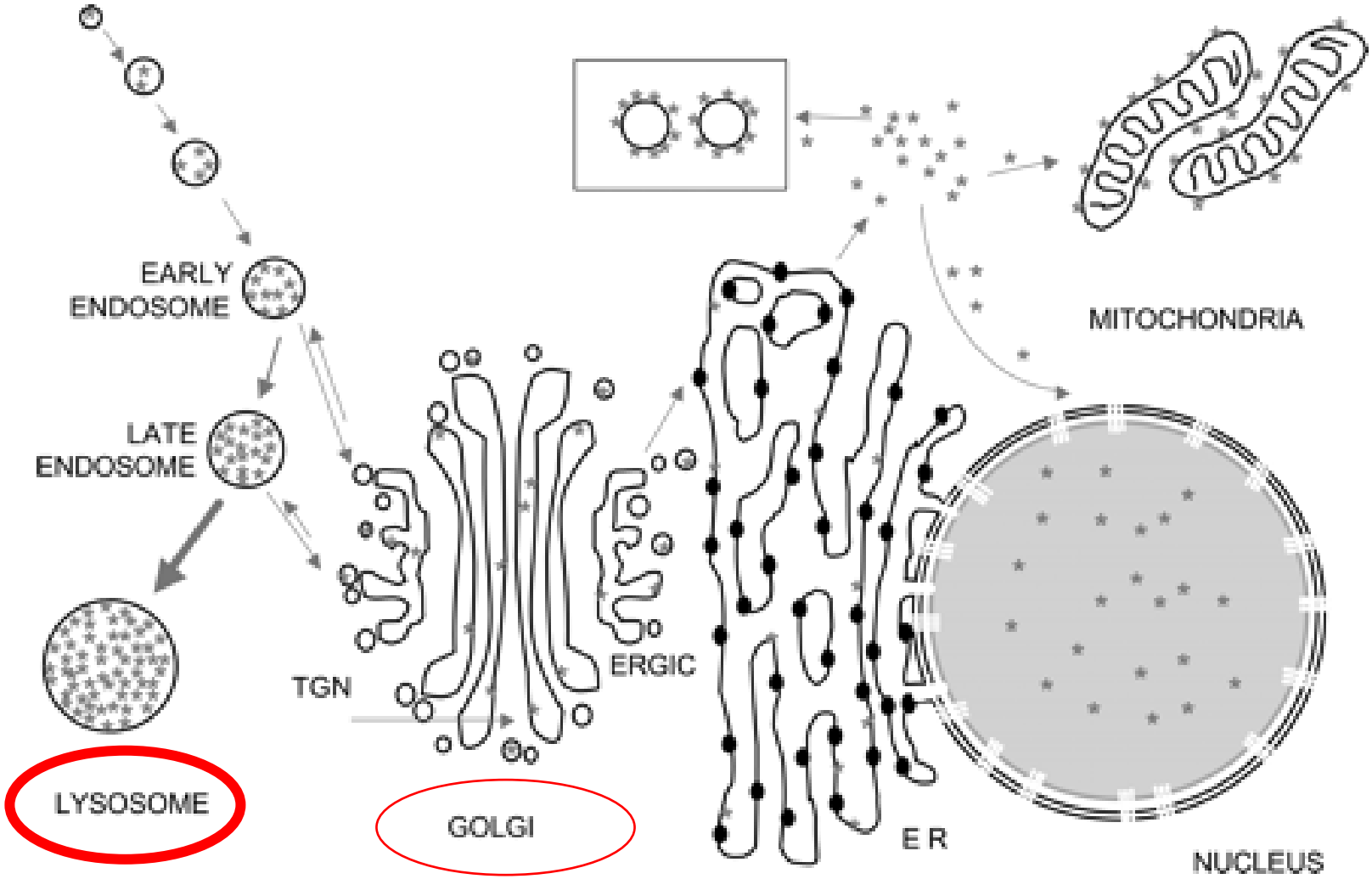
Aminoglycoside toxicity …

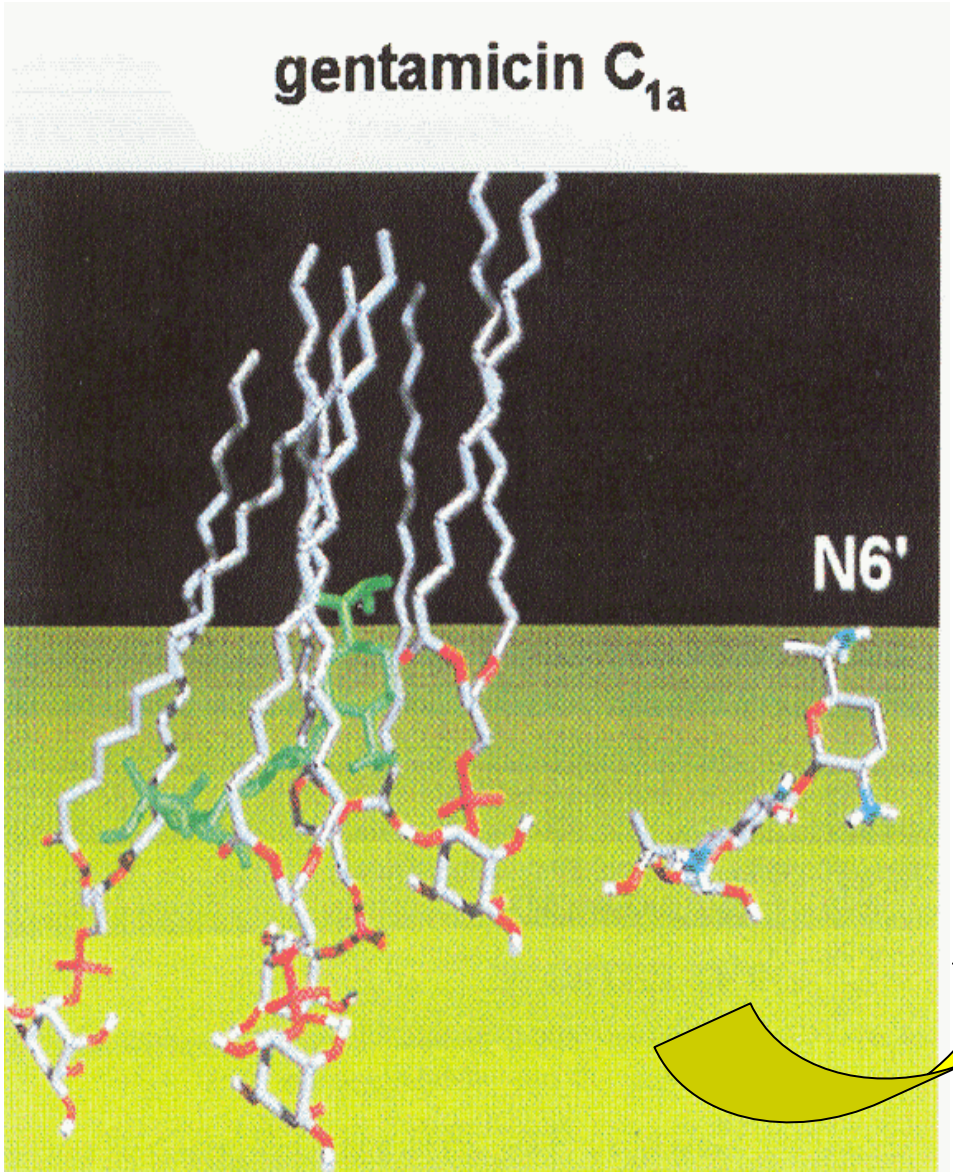


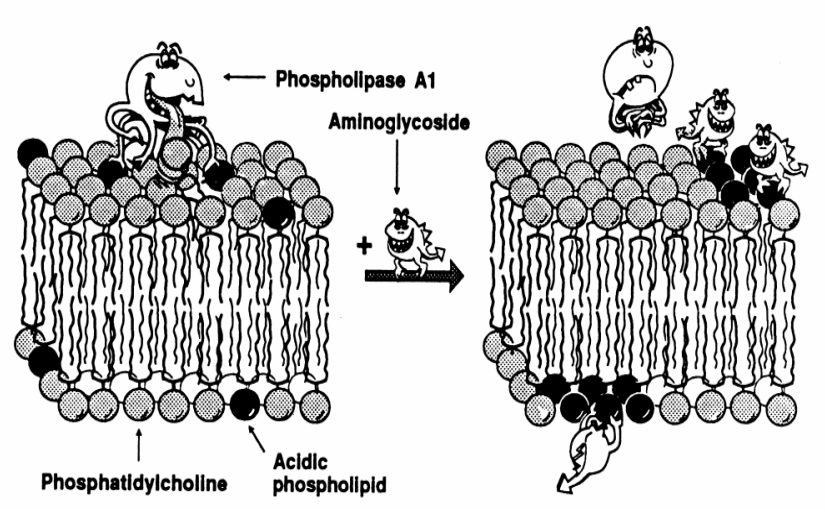
Aminoglycoside intracellular pathway …



Sandoval & Molitoris, Am J Physiol Renal Physiol 286: F617-F624, 2004

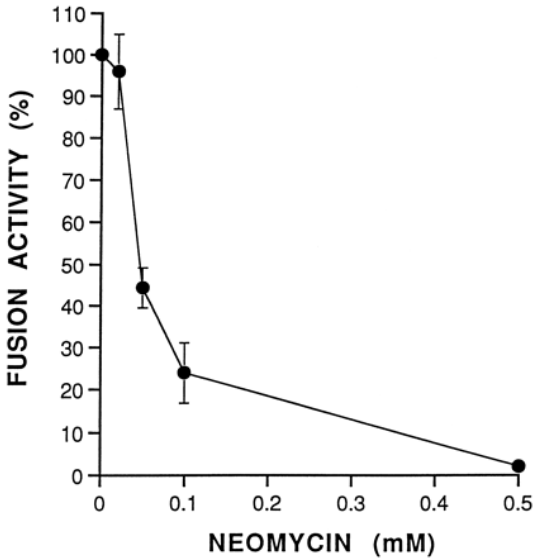
Aminoglycoside bind to lipid bilayers …





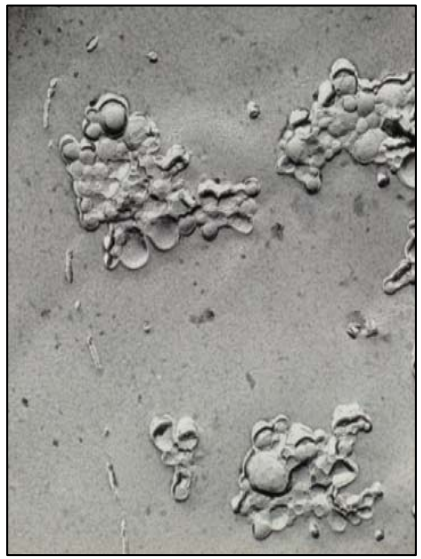
Mingeot-Leclercq &Tulkens, 1999, Antimicrob. Agents Chemother., 43: 1003-1012

Perturbation of intracelllular traffic by aminoglycoside binding to bilayers ...



Jones & Wessling-Resnick, 1998, J. Biol. Chem. 273: 25301-25309

Control, 37,500X



+ Gentamicin 37,500X

+ Gentamicin 85,500X

Mingeot-Leclercq et al, 1989, Biochem. Pharmacol.38: 729-741
Van Bambeke et al, 1995, Eur. J. Pharmacol.289:321-333

Intralysosomal gentamicin disrupts lysosomes...

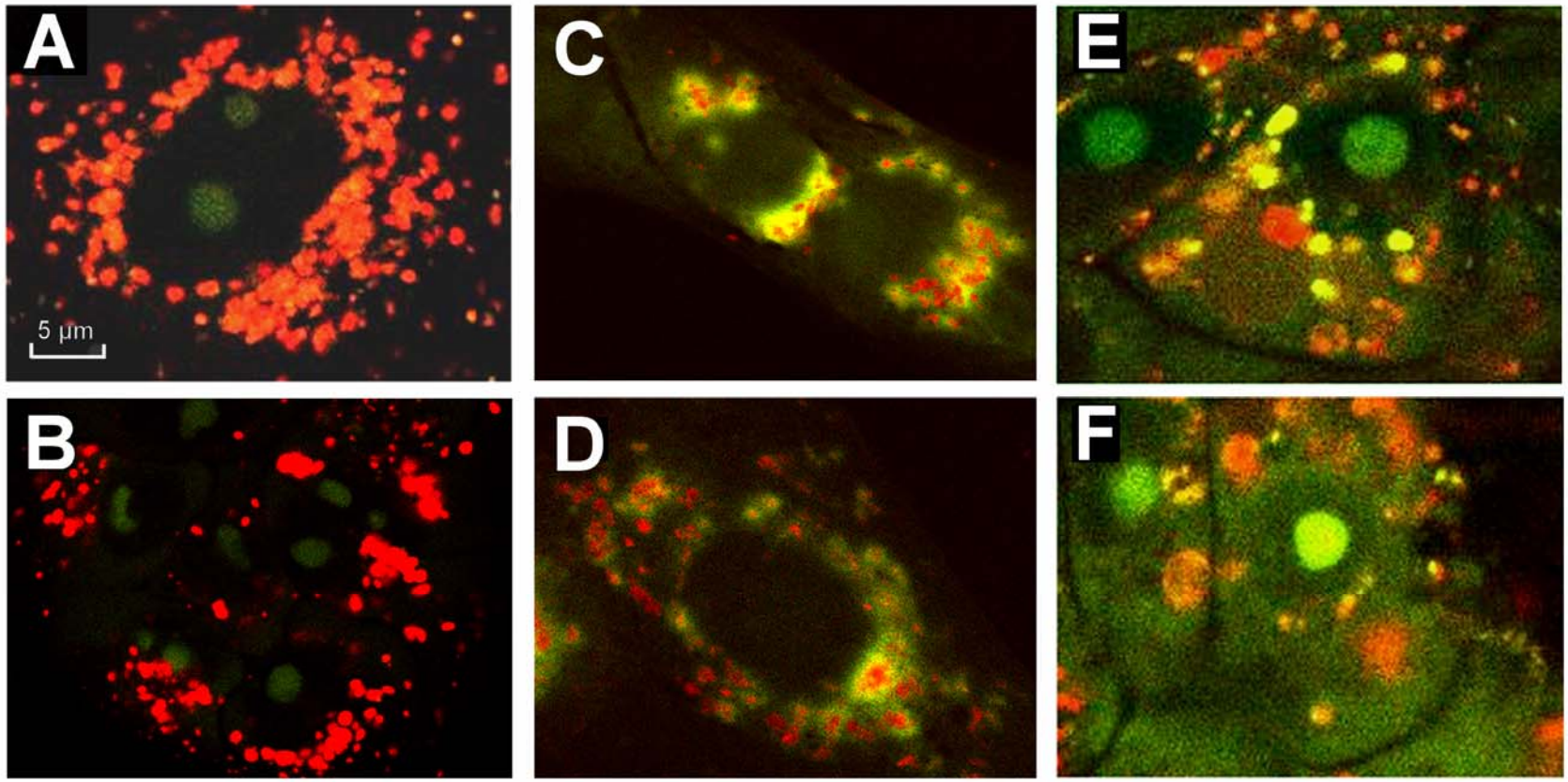
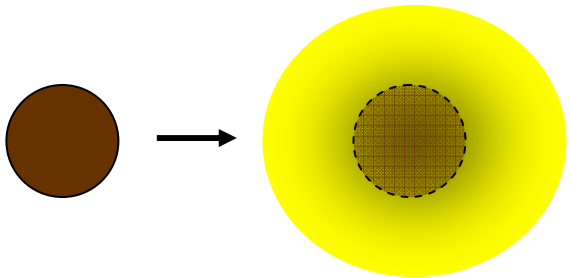


Fig. 4. Appearance of acridine orange-loaded LLC-PK1 cells in confocal microscopy. Cells were exposed to acridine orange (5 µg/ml) for 15 min and then returned to control medium for 3 h (A, B), or exposed to gentamicin (C and D, 3 mM, 3 h; E, 2 mM, 4 h) or MSDH (F, 25 µM, 3 h).

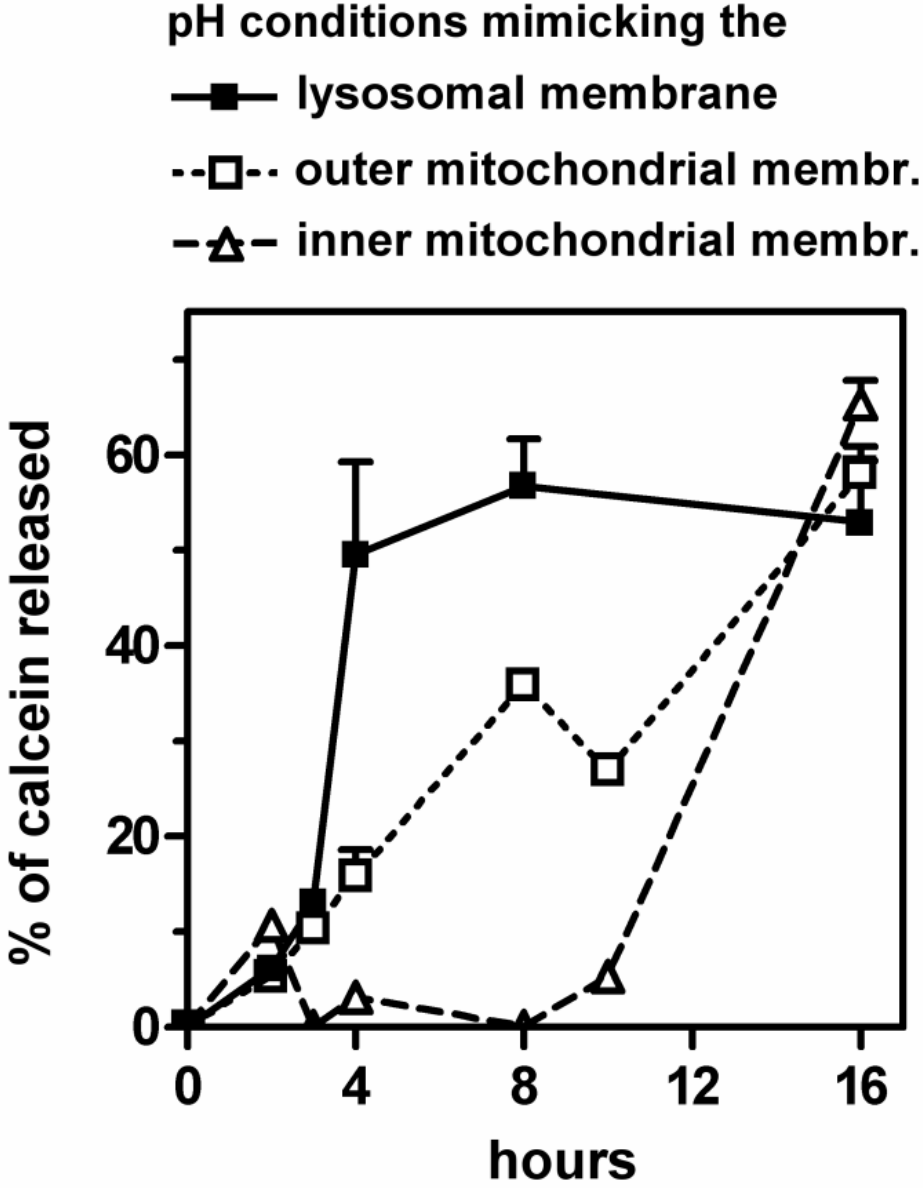
H. Servais et al. / Toxicology and Applied Pharmacology 206 (2005) 321–333

Is the lysosomal membrane specifically sensitive to gentamicin-induced disruption ?

Calcein-release experiments

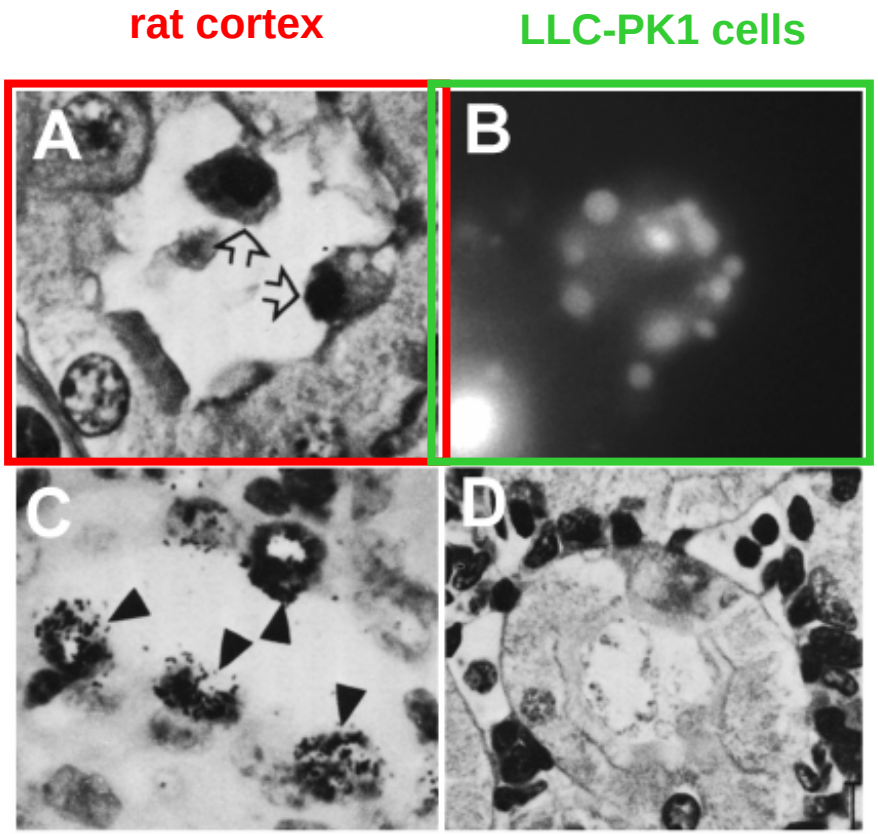


liposome composition and pH conditions mimicking the



H. Servais et al. / Toxicology and Applied Pharmacology 206 (2005) 321–333

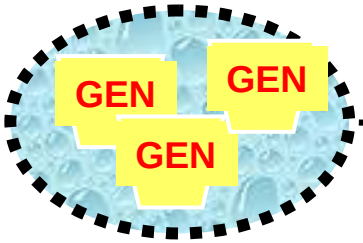
Aminoglycoside-induced lysosomal destabilization and perturbation of traffic causes apoptosis in kidney and renal cells …



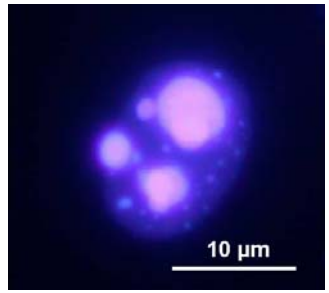
Morphological changes in rat renal cortex (A,C,D) upon treatment with gentamicin at low doses (10 mg/kg; 10 days) and in cultured LCC-PK1 renal cells (B) upon incubation with gentamicin (under conditions causing a drug accumulation similar to that observed in rat renal cortex of the animals treated as indicated in A, B, and C [approx. 10 µg/g;

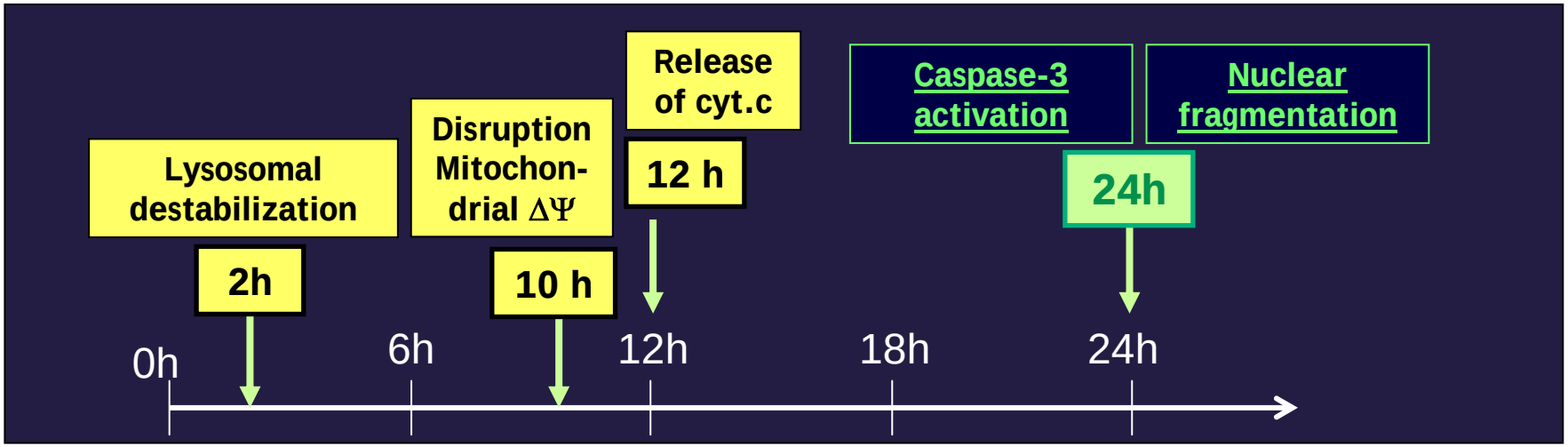
Servais et al. In: Toxicology of the Kidney (Target Organ Toxicology Series), 2004, chap. 16, pp 635-685,

Aminoglycoside-induced apoptosis …



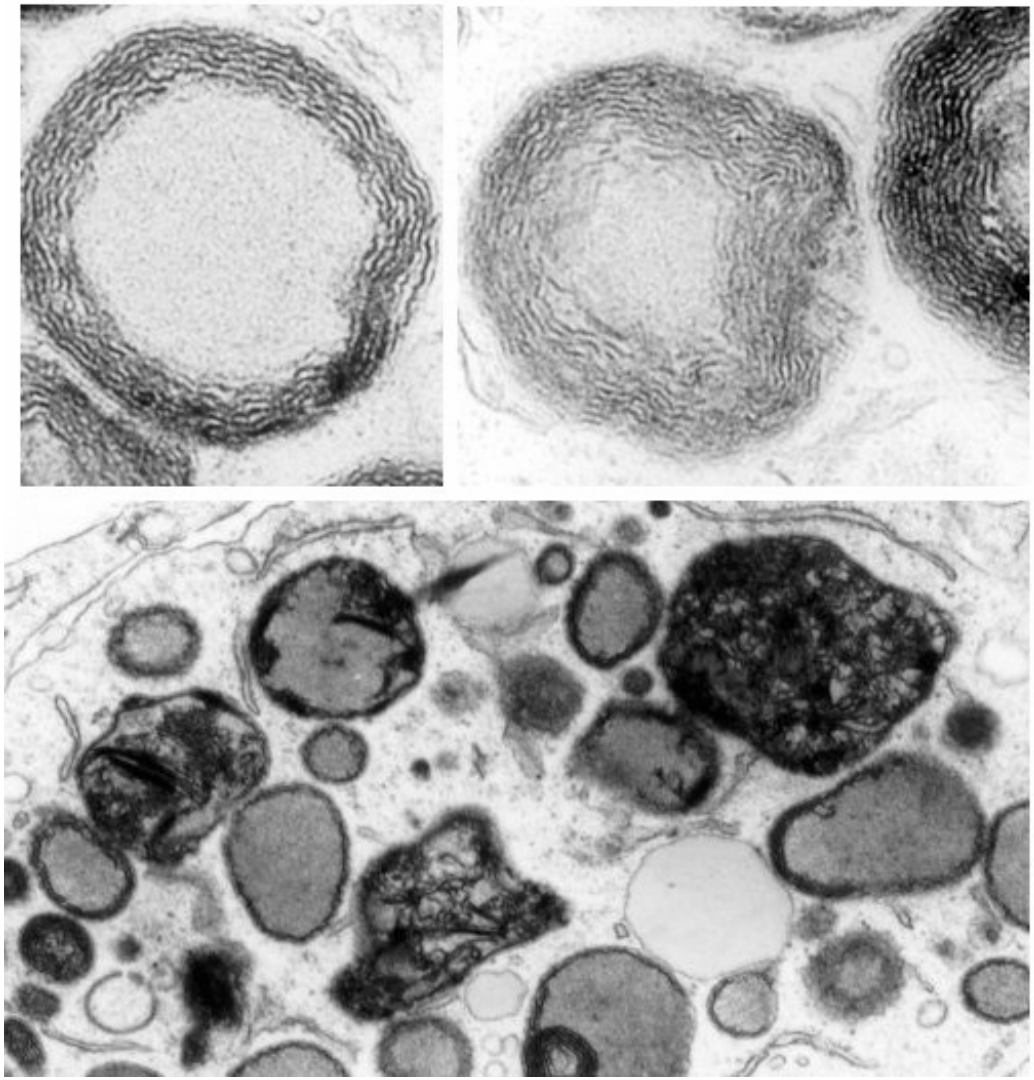
Brutal release of gentamicin in the cytosol





Servais et al. Toxicol. Appl. Pharmacol. 2005; 15:321-333 Antimicrob. Agents Chemother. 2006;50:1213-1221

Azithromycin-induced phospholipidosis



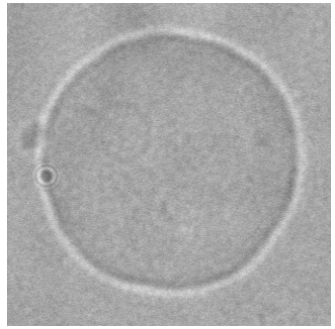
Ultrastuctural alterations observed in cultured fibroblats maintained with 0.03-0.1 mg/L of azithromycin for 7 to 16 days.

Bichemical studie show a predominant accumulation of phosphatidylcoline (no marked excees in cholesterol)

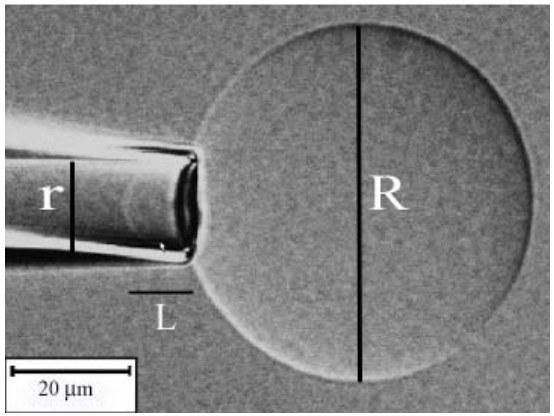
Van Bambeke et al., J. Antimicrob. Chemother. 42:761-767, 1968

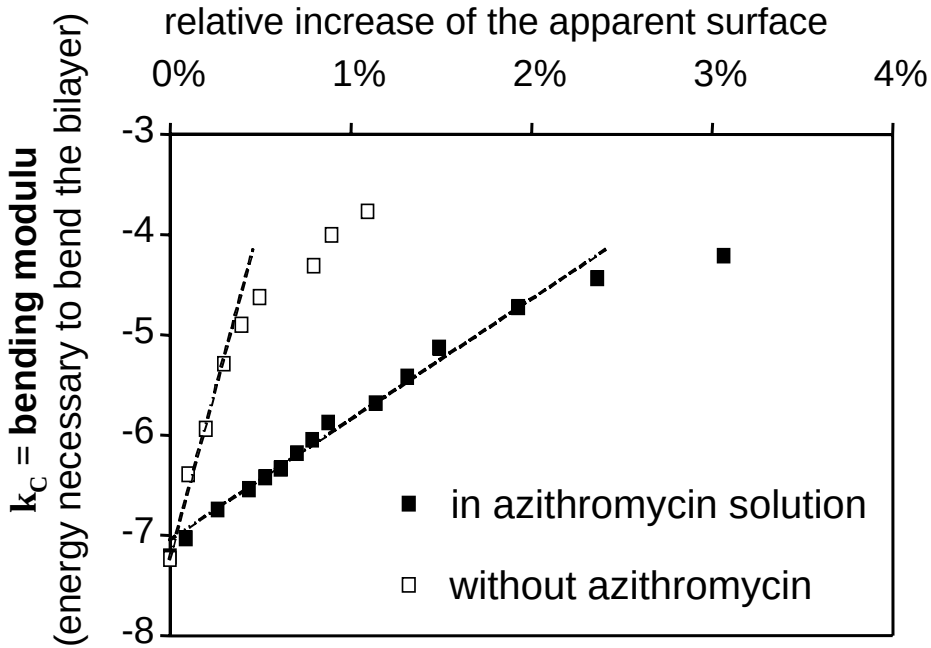
Azithromycin-induced modulation of membrane fluidity

Use of giant liposomes ⟶



Micropipet experiments :

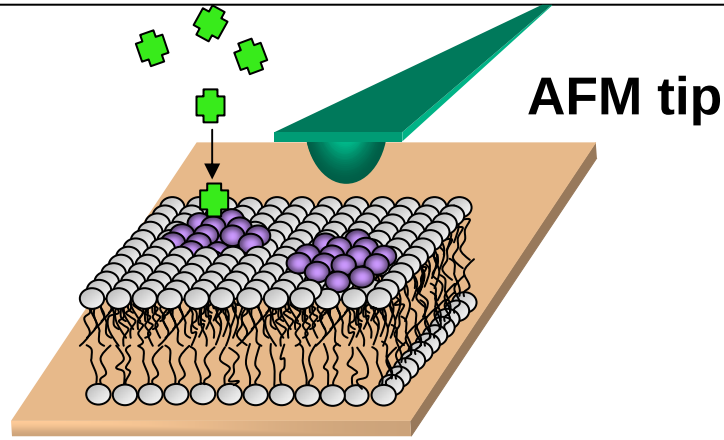




Fa et al. Chem Phys Lipids (2006) 144:108-16.
Fa et al. Biophysical Society 49th Annual Meeting, 2005

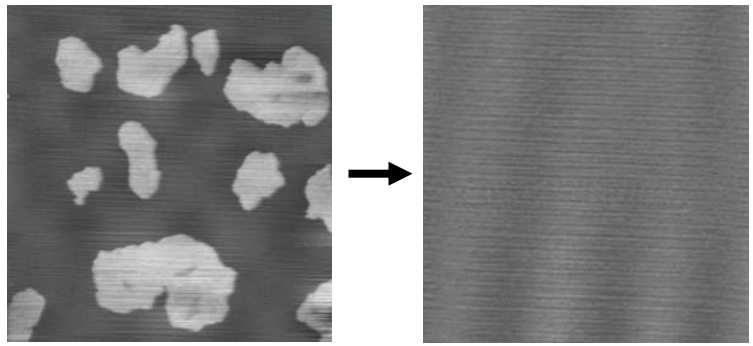
Azithromycin-induced modulation of membrane microstructure

Use of atomic force microscopy to detect changes in membrane surface



AFM on DOPC:DPPC 1:1 bilayers :

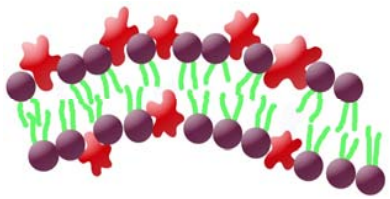
DPPC gel domains (white) in DOPC fluid matrix (dark); height difference: 1.10±0.05 nm



Addition of azithromycin + 60 mn : only one uniform fluid phase visible

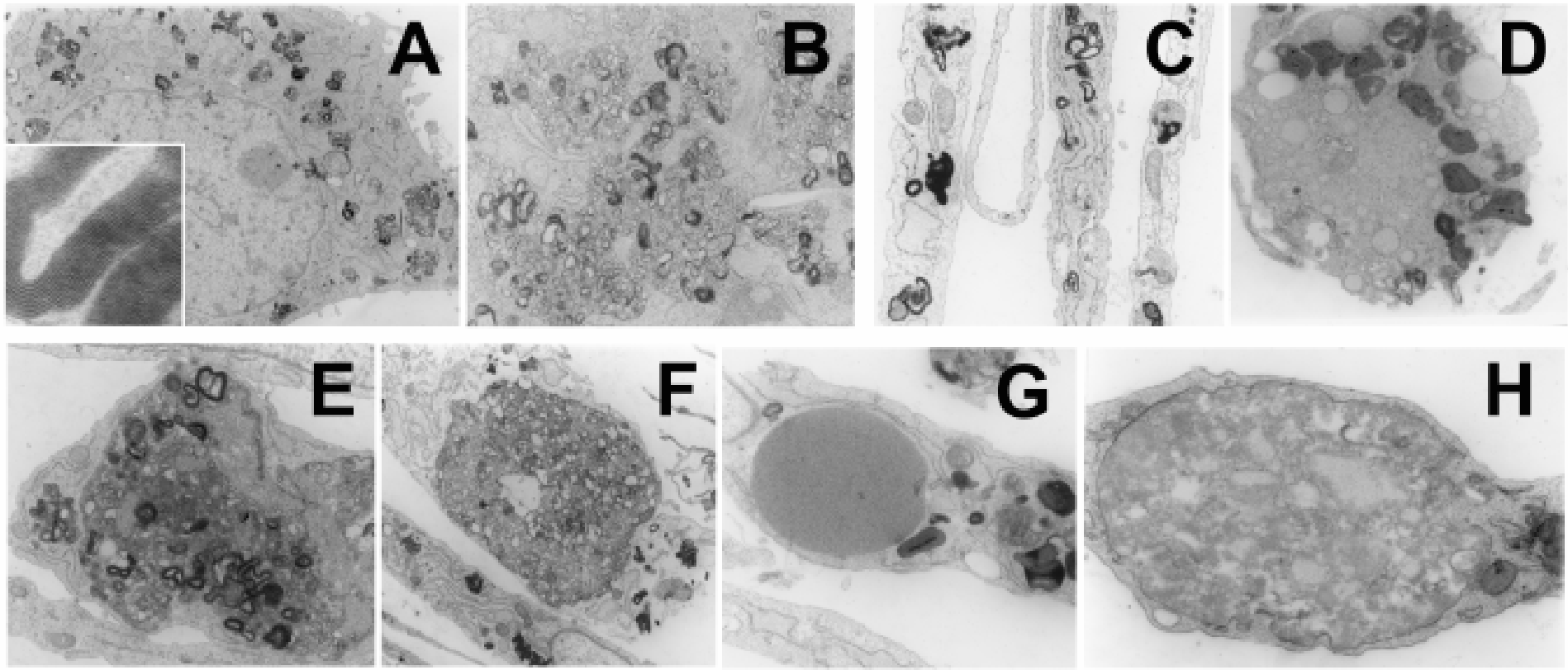
Current interpretation :

Azithromycin inserts in bilayers and increases lipid mixing



Fa et al. Chem Phys Lipids (2006) 144:108-16.
Fa et al. Biophysical Society 49th Annual Meeting, 2005

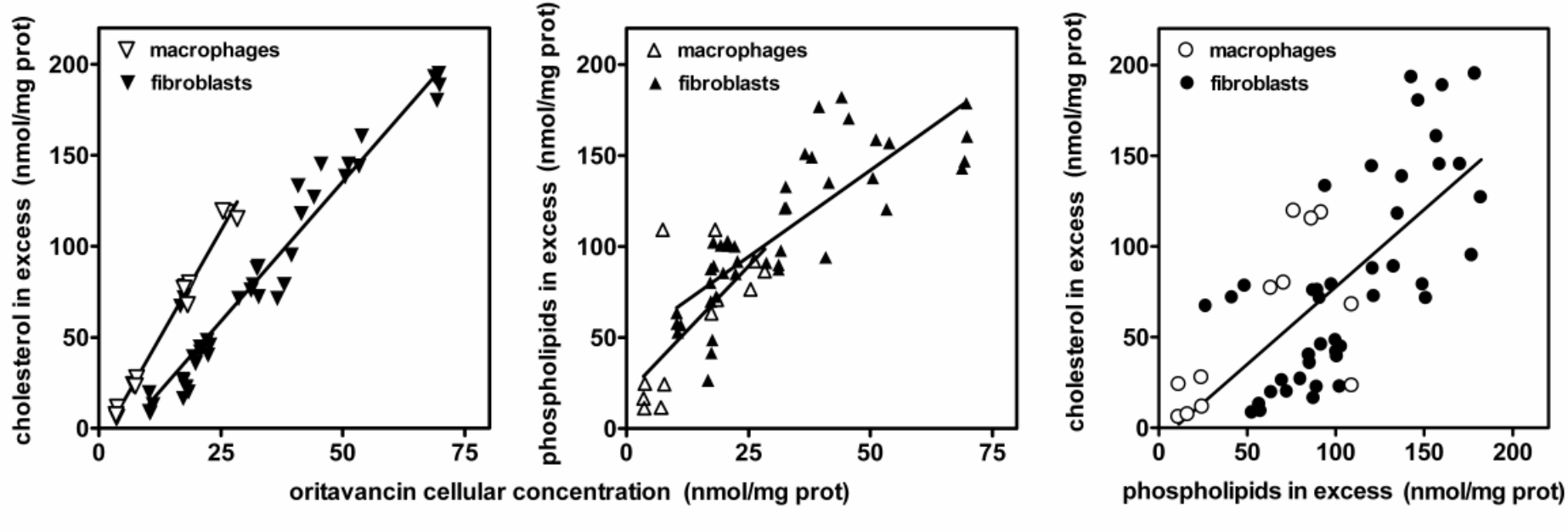
Oritavancin-induced lipid storage



cells incubated at clinically meaningful concentrations

Van Bambeke et al. Antimicrob. Agents Chemotherapy (2005) 49:1695-1700.

Oritavancin-induced lipid storage



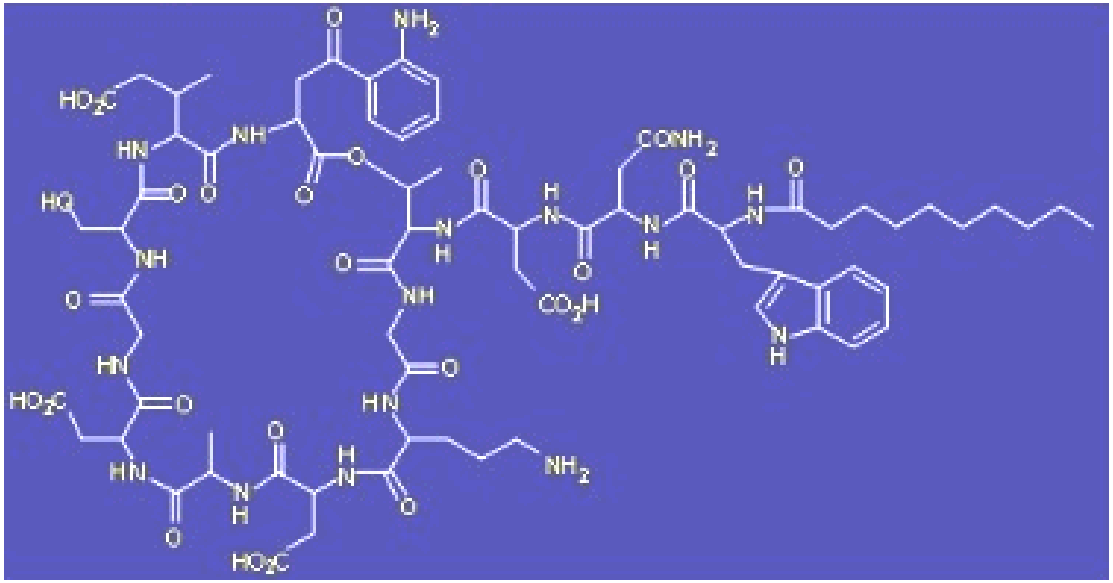
co-accumulation of phospholipids and cholesterol

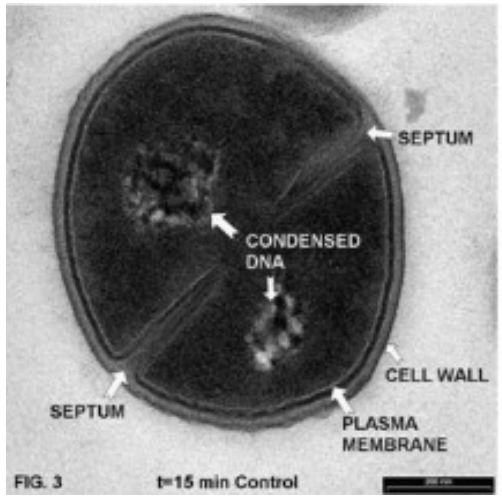
Van Bambeke et al. Antimicrob. Agents Chemotherapy (2005) 49:1695-1700.

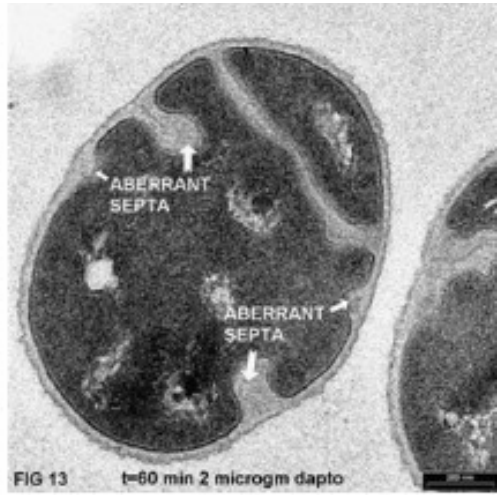
The pharmacologist's and toxicologist's key question …

- Wat do we know exactly about membranes (*vs.* proteins)
  - composition and lipid-distribution heterogeneity
  - structure and micro/macrostructure – influence from and on proteins
  - fusion and rupture
- Drug disposition: membranes and penetration/efflux
  - the case of two fluoroquinolones
- Drug toxicity toxicity
  - the case of aminoglycosides, azithromycin, and lipoglycopeptides
- **Drug activity activity**
  - the case of new anti Gram (+) antibiotics (daptomcyin, telavancin, … and lantibiotics)

Daptomycin...

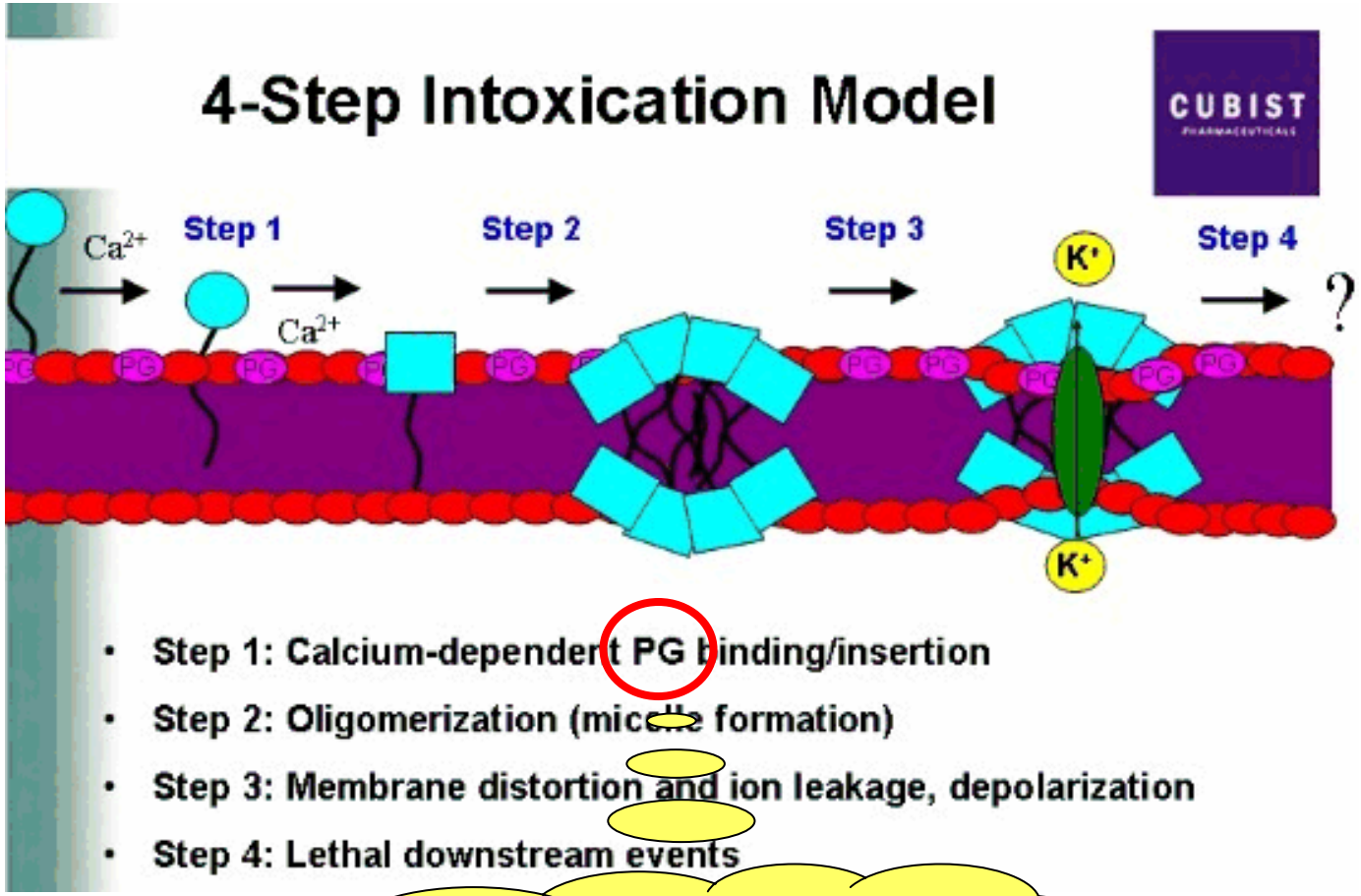






J. Silverman, 45thICAAC, 2005

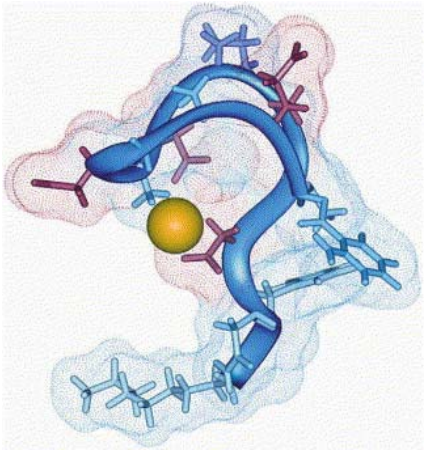
Daptomycin...

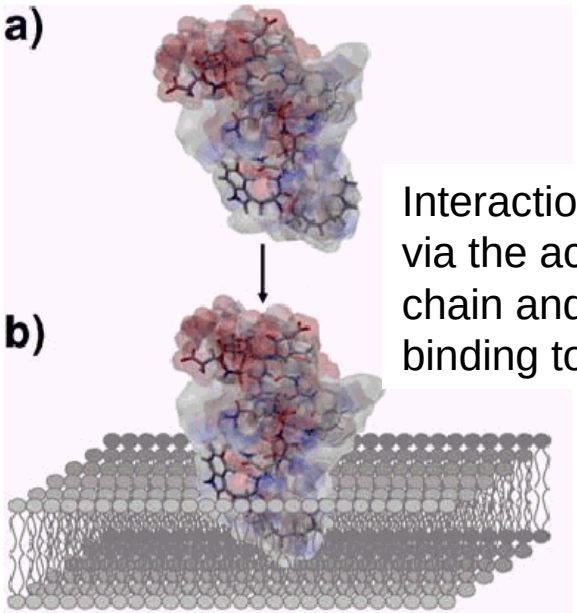


PG = phosphatidylglycerol
negatively-charged and
abundant in procaryotic cells

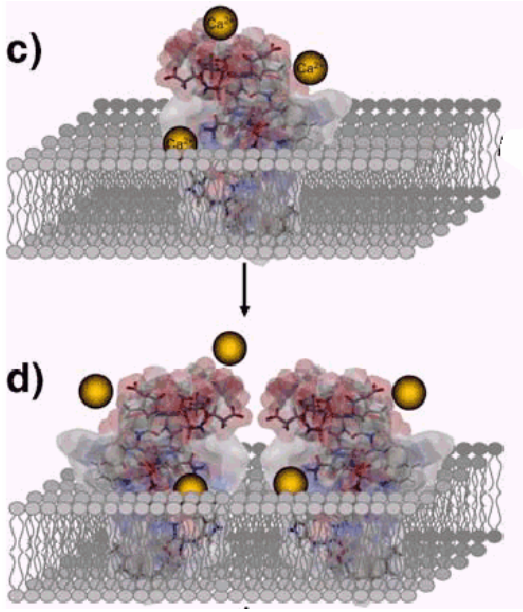
J. Silverman, 45thICAAC, 2005

Daptomycin: model 1



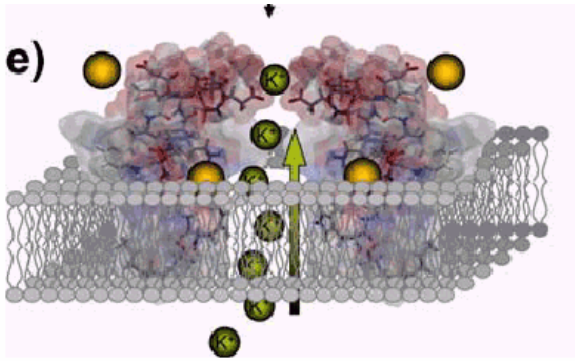


Interaction via the acyl chain and binding to PG



integration in membrane facilitated by Ca^{++}

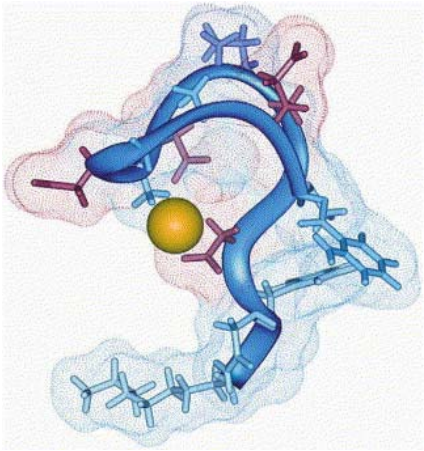
aggregation

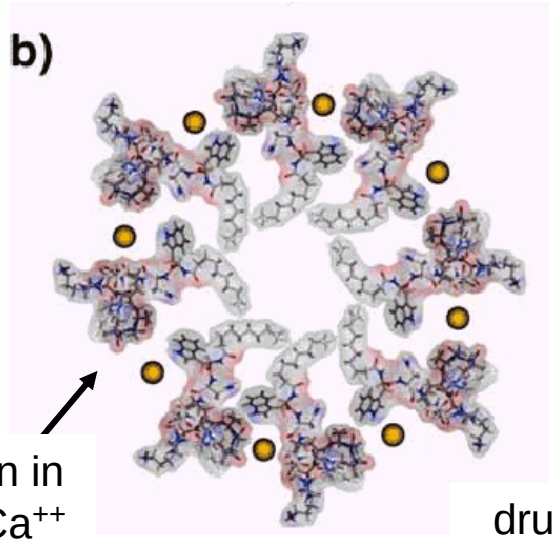


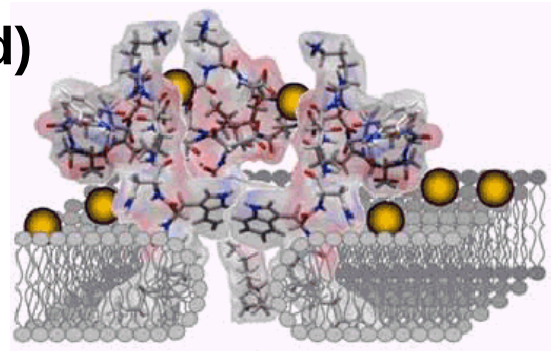
Pore formation and bacterial death

Model: Silverman et al. Antimicrob. Agents Chemother. 2003; 47: 2538-2544

Daptomycin: alternate model



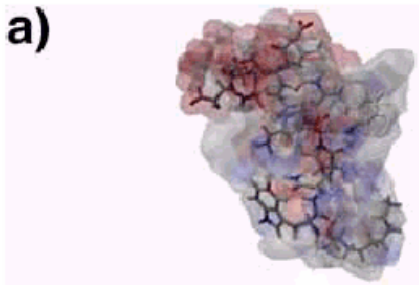


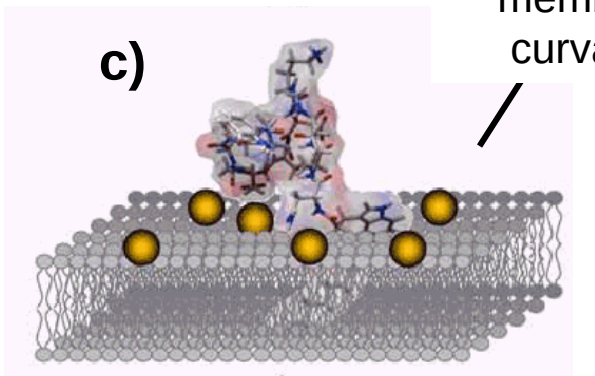


oligomerization in presence of Ca^{++}

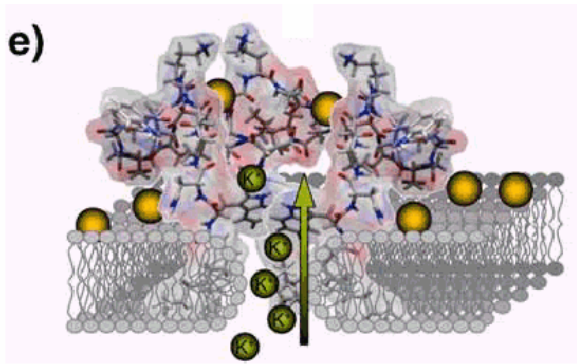
drug-induced change in membrane curvature

leakage and bacterial death





insertion in membrane

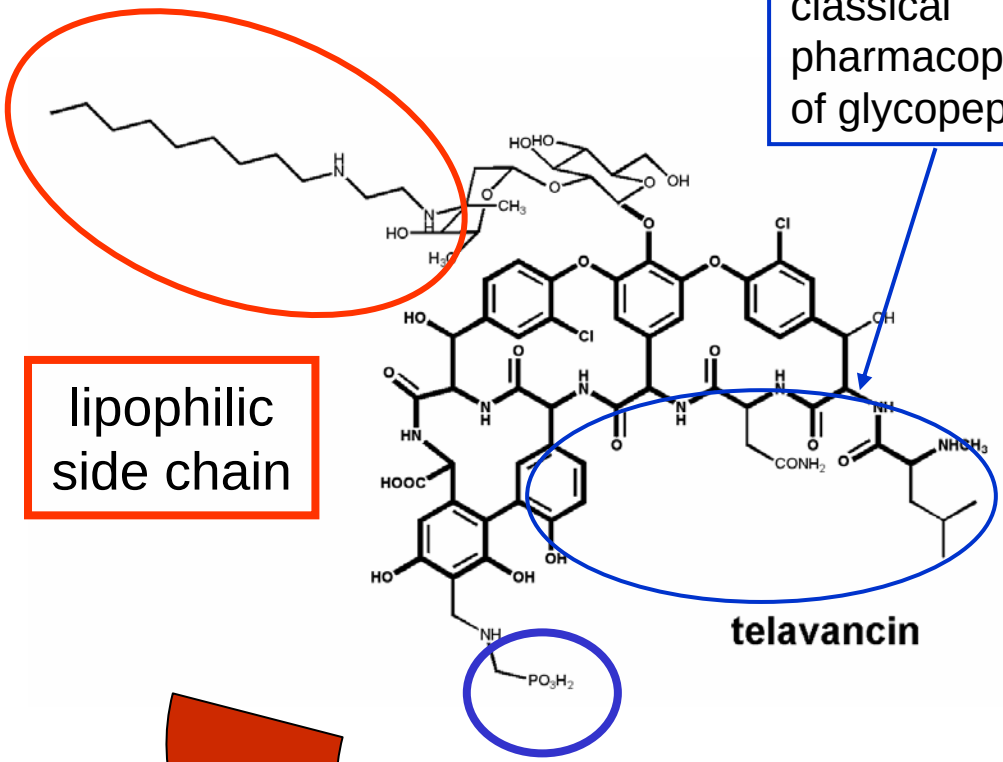


Strauss & Hancock, Biochim. Biophys. Acta 2006; 1758:1215-1223

Telavancin: a membrane destabilizing derivative of vancomycin

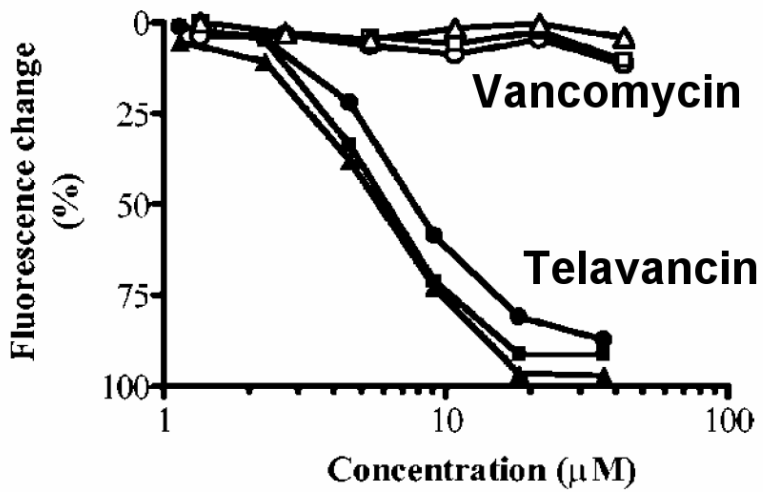
classical pharmacophore of glycopeptides

lipophilic side chain



polar group

membrane permeability assay



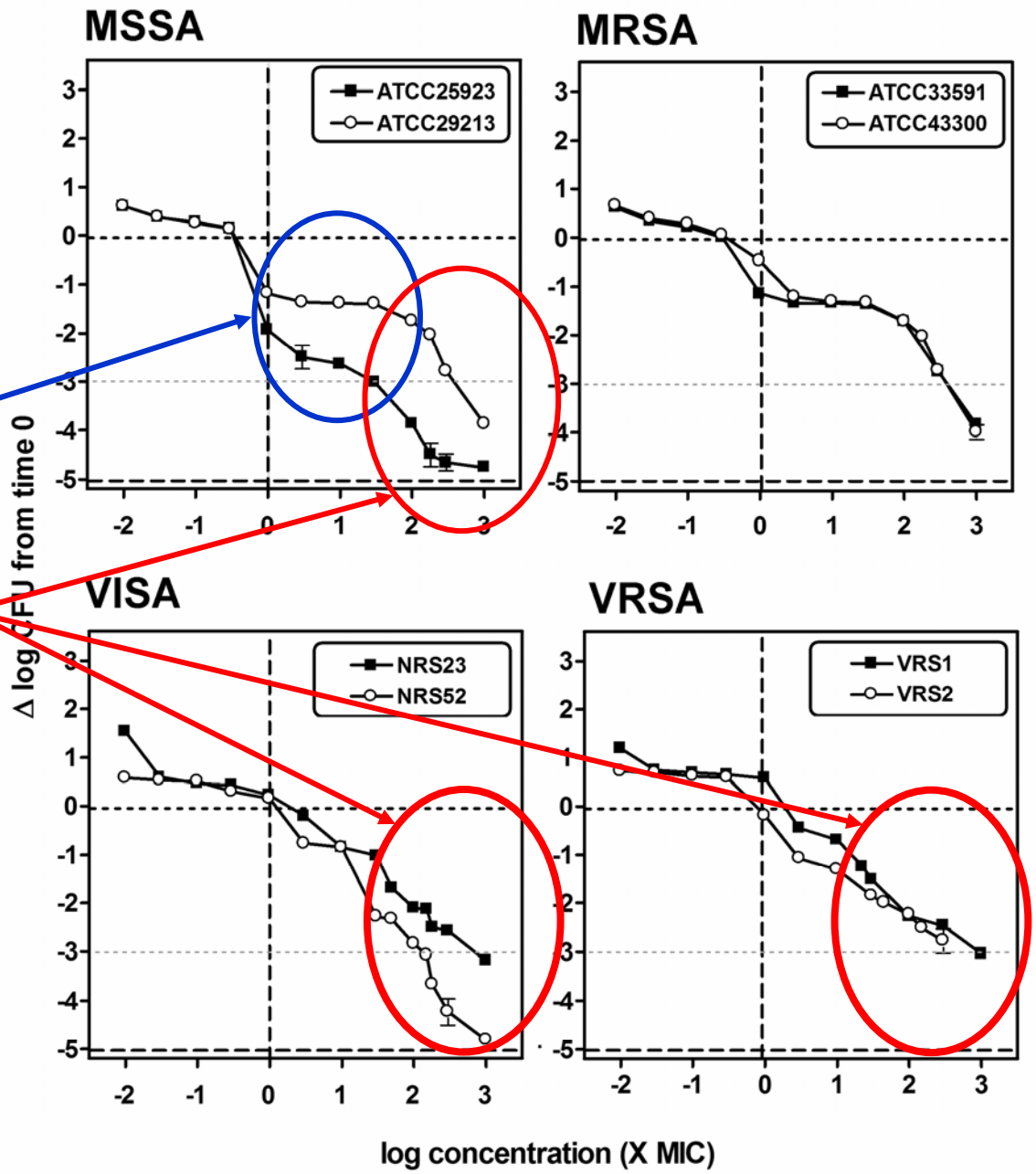
this causes increase in bacterial membrane permeability

Higgins *et al.*, Antimicrob Agents Chemother. (2005) 49:1127-34

Televancin dual mode of action ?

Classical mode of action

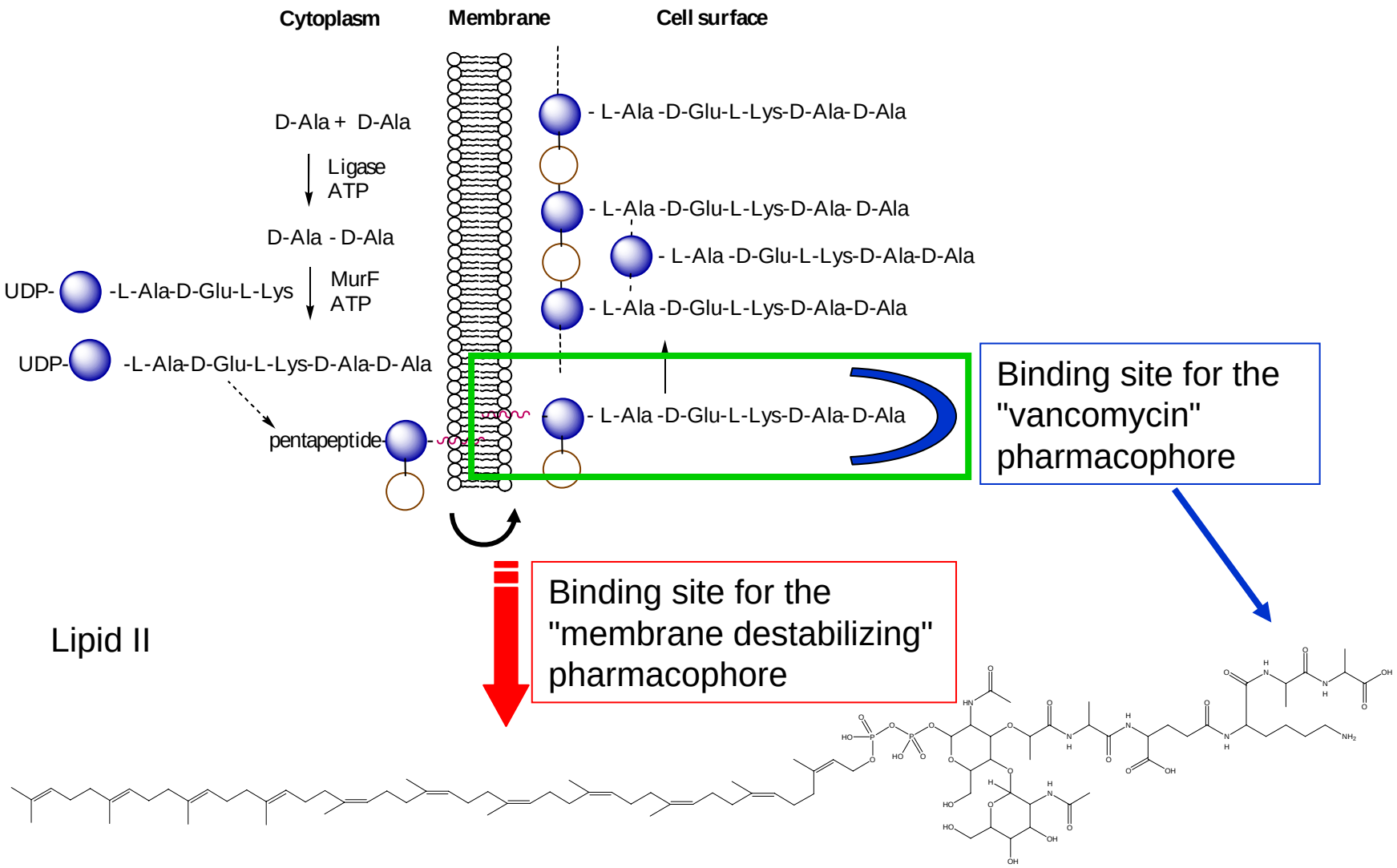
Membrane destabilizing mode of action



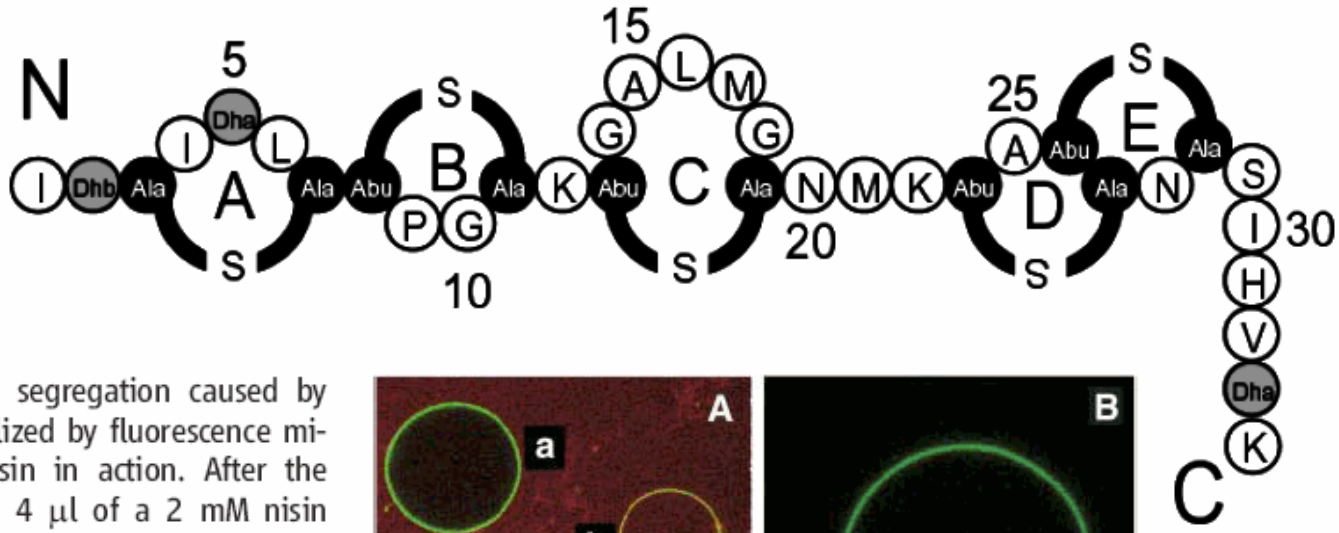
3 h kill curves extracellular bacteria

Barcia-Macay *et al.,* J Antimicrob. Chemother. 2006; 58(6):1177-84.

Why is telavancin specific of bacteria ?



Lantibiotics…



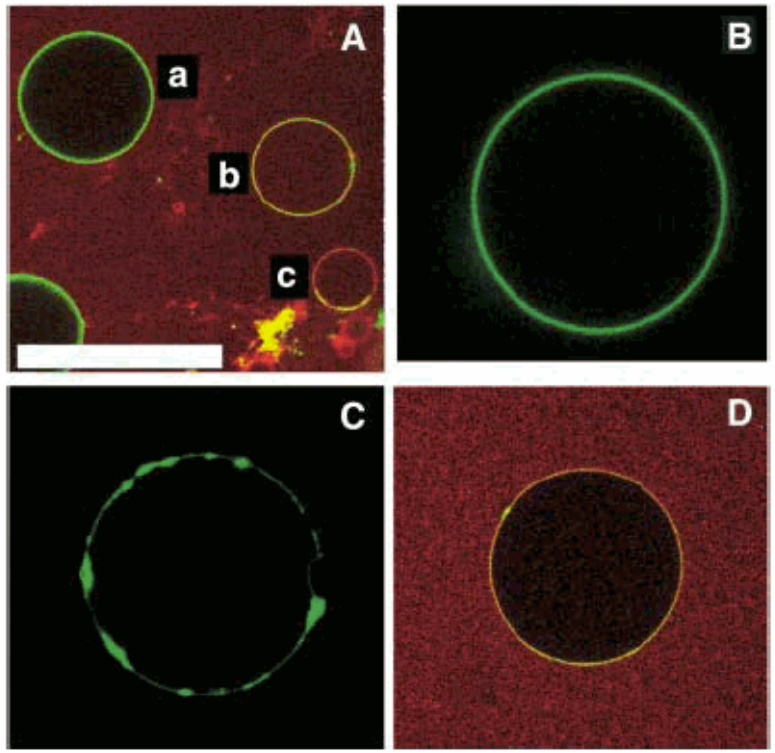


Fig. 1. Lipid II segregation caused by lantibiotics visualized by fluorescence microscopy. (**A**) Nisin in action. After the addition of 2 to 4 μl of a 2 mM nisin solution, the peptide started to diffuse into the field of view from the bottom right corner. This image is a snapshot of three vesicles that have either not yet encountered nisin (vesicle a), just encountered nisin (vesicle b), or already been exposed to nisin for some time (vesicle c). Scale bar, 60 μm. (**B**) GUV doped with NBD-labeled lipid II before treatment with mutacin 1140. (**C**) Mutacin 1140–induced segregation of NBD-labeled lipid II. (**D**) Snapshot of a GUV treated with mutacin 1140 just at the onset of the lipid II segregation; the interior remains black. The green fluorescence of the NBD-labeled lipid II and the red fluorescence of Texas Red were sequentially detected with the use of two lasers.

Hasper et al. Science (Wash.); 2006; 316:1336-37

Lantibiotics…

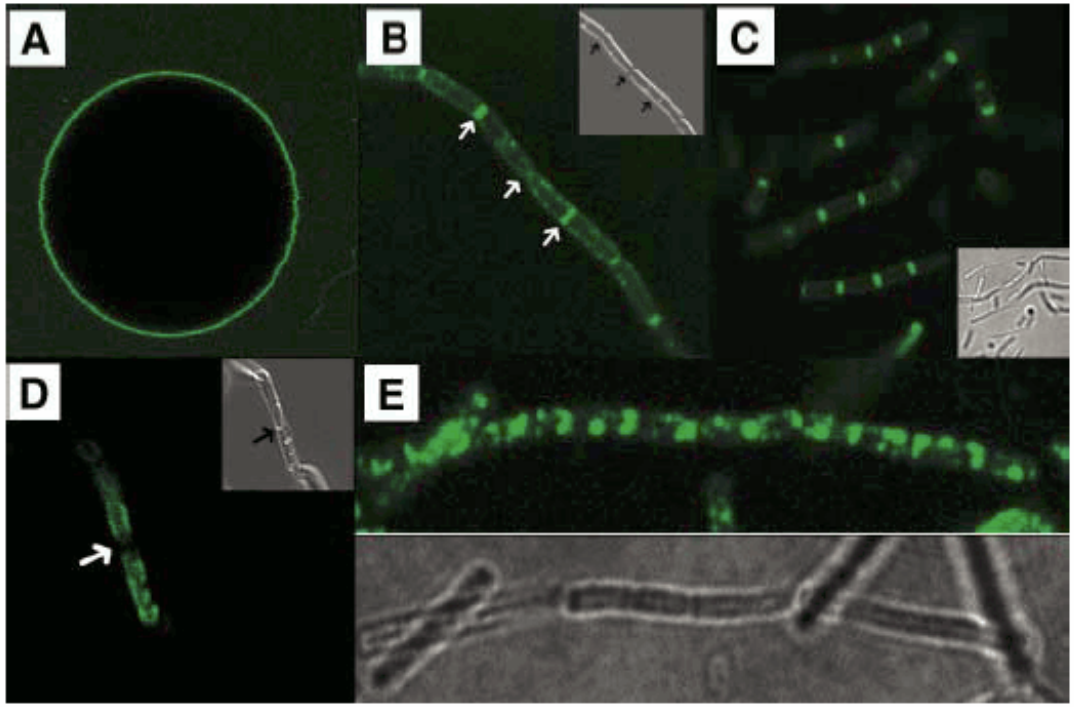
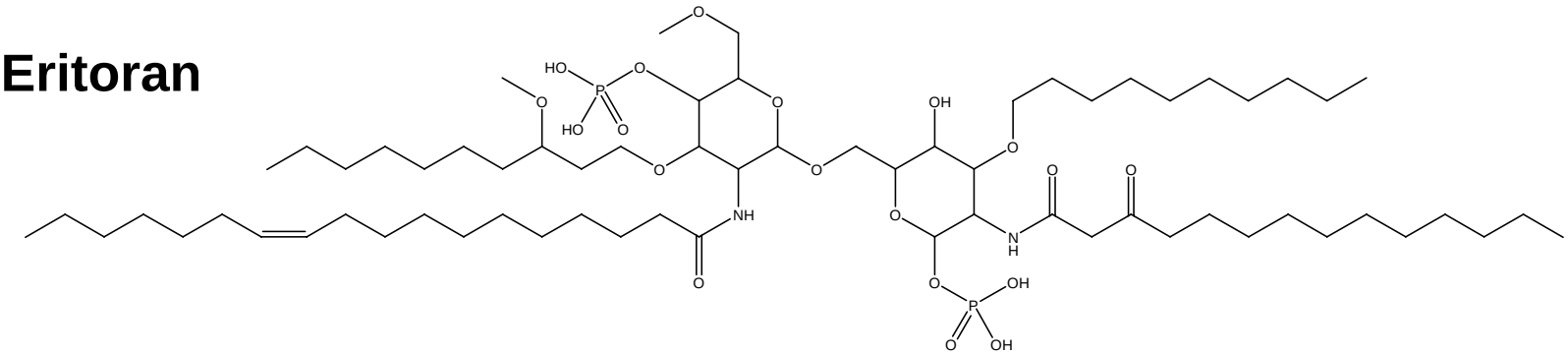


Fig. 2. Nisin segregates lipid II into nonphysiological domains in vivo. (**A**) GUV containing 0.5 mole percent wild-type lipid II 15 min after the addition of fluorescently labeled vancomycin. (**B**) *B. megaterium* cells that were incubated for 10 min with labeled vancomycin (2 μg/ml). The arrows point at newly formed division sites or older exemplars. (**C**) *B. subtilis* stained with fluorescent vancomycin (4 μg/ml). (**D**) *B. megaterium* cells after incubation for 10 min with fluorescein-labeled nisin (0.5 μg/ml). The arrow marks where the bacterium has already divided. (**E**) *B. subtilis* cells after incubation with fluorescein-labeled nisin (4 μg/ml). The bottom image in (E) and the insets in (B) to (D) show Nomarski images.

Hasper et al. Science (Wash.); 2006; 316:1336-37

One step ahead: targeting lipid A

Eritoran



- LPS inhibitory activity manifested via down-regulation of the intracellular generation of pro-inflammatory cytokines IL-6 and TNF-alpha in human monocytes.
  (Czeslick et al. Inflamm Res. 2006 Nov;55(11):511-5)

- inhibition of TLR4 with eritoran in an in situ murine model significantly reduces MI/R injury and markers of an inflammatory response.
  (Shimamoto et al. Circulation. 2006 Jul 4;114(1 Suppl):I270-4)

Another step ahead: targeting rafts

- Toxins, bacterial-, and viral-pathogens exploit cholesterol and/or lipid rafts to gain a foot hold in their target hosts…

- Statins cause lipid raft disruption by impairing cholesterol synthesis. Since lipid rafts have been implicated both in antigen internalization, antigen processing and presentation may be a selective target of statins.
  (Ghittoni et al Eur J Immunol. 2006 Nov;36(11):2885-93).

- Inhibition of sphingolipids biosynthesis may be a new approach to treatment of hepatitis C (since the virus replication complex resides in in rafts)
  (Sakamoto et al. Nat Chem Biol. 2005 Nov;1(6):333-7.)

- Association of the serotonin transporter with lipid rafts may represent a mechanism for regulating serotoninergic signaling in the central nervous system, through the modulation of the cholesterol content in the cell membrane…
  (Magani et al., J. Biol. Chem. 2004 Sep 10;279(37):38770-8)

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- Modulation of rafts to which P-glycoprotein is associated may also modulate resistance to P-gp-effluxed drugs
  (Hendrich & Michalak. Curr Drug Targets. 2003 Jan;4(1):23-30).

Conclusions

Lipids are potential drug targets …

1. for modulation of transport…
2. for toxicity …
3. for activity ...

Considerably more systematic research is warranted…