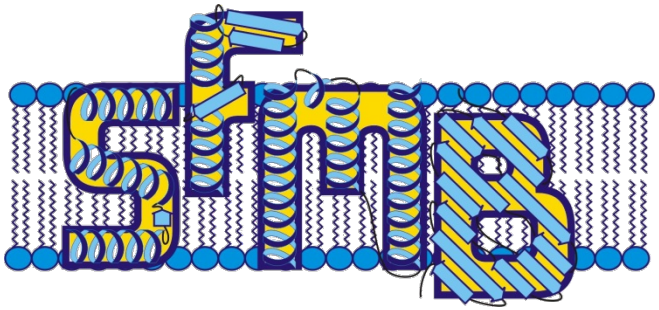
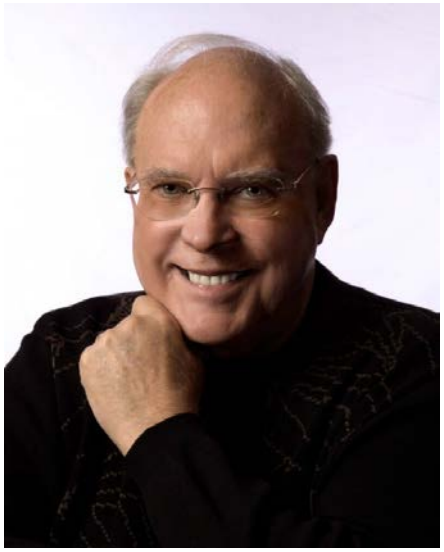
A NOVEL CROSS TALK BETWEEN MEMBRANE LIPIDS AND THE INNATE SYSTEM IS MEDIATED BY TOLL-LIKE RECEPTORS



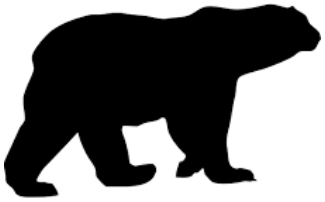
Laboratory for Structure and Function of Biological Membranes

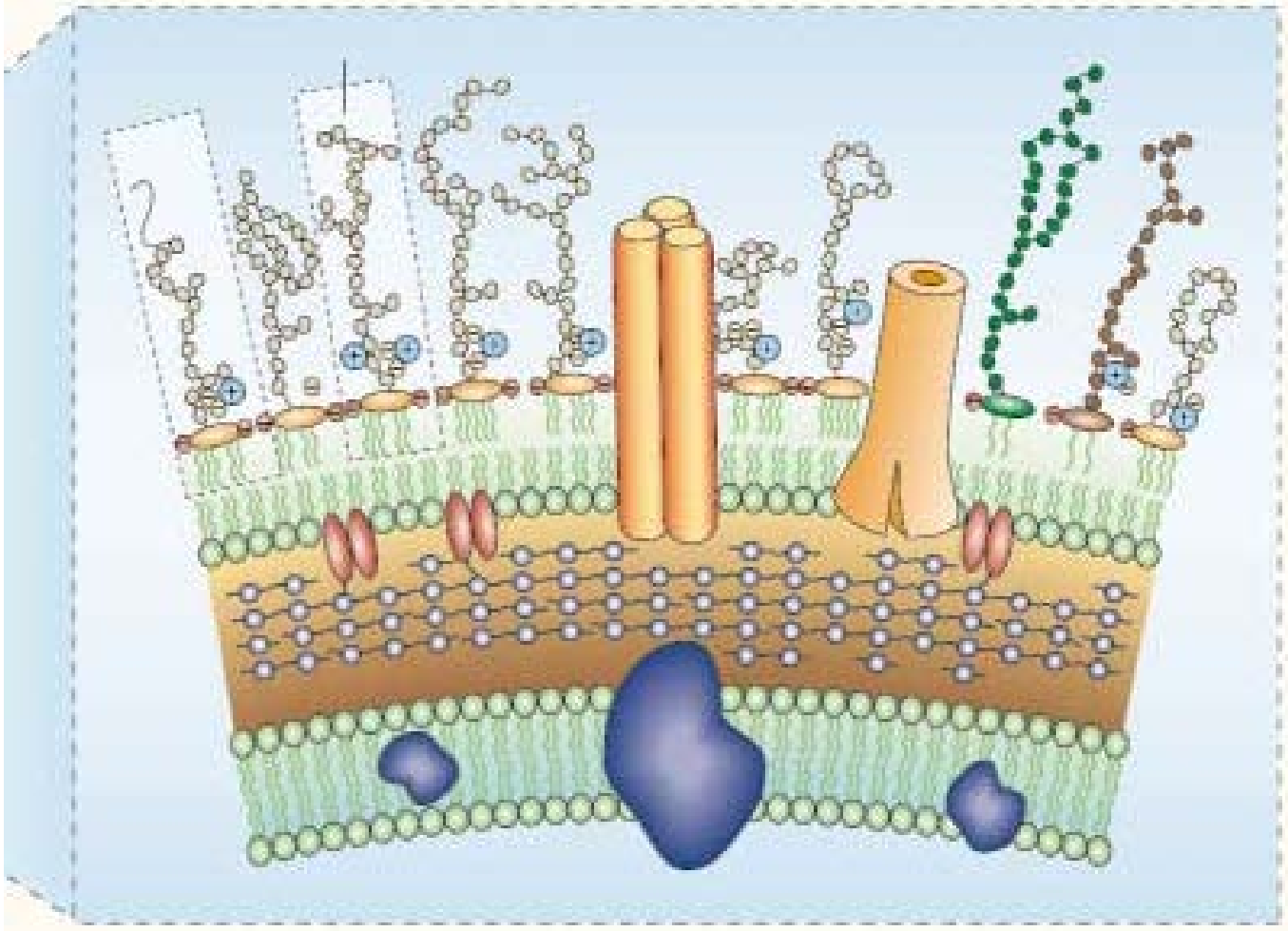
Universite Libre de Bruxelles (Belgium)

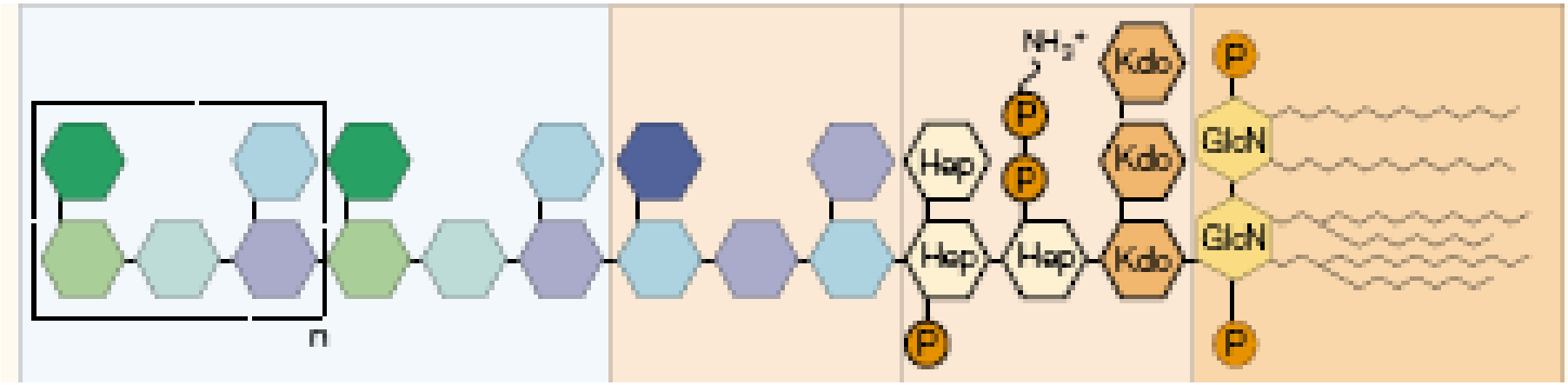
jmruyss@ulb.ac.be



Thanks Walt

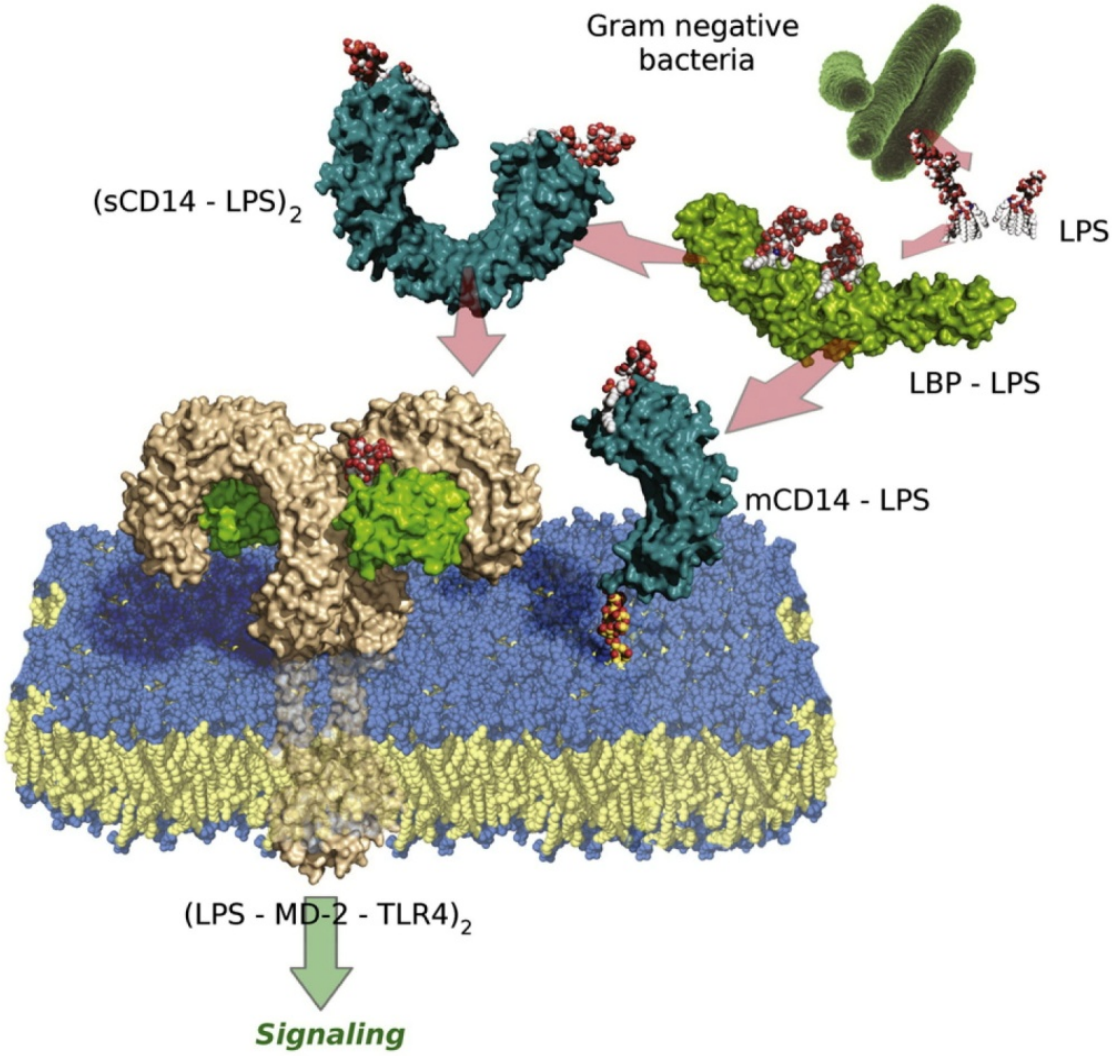






LPS(lipopolysaccharide)

The innate system as a first defense against bacterial and viral infection



LPS:Activator of the innate system

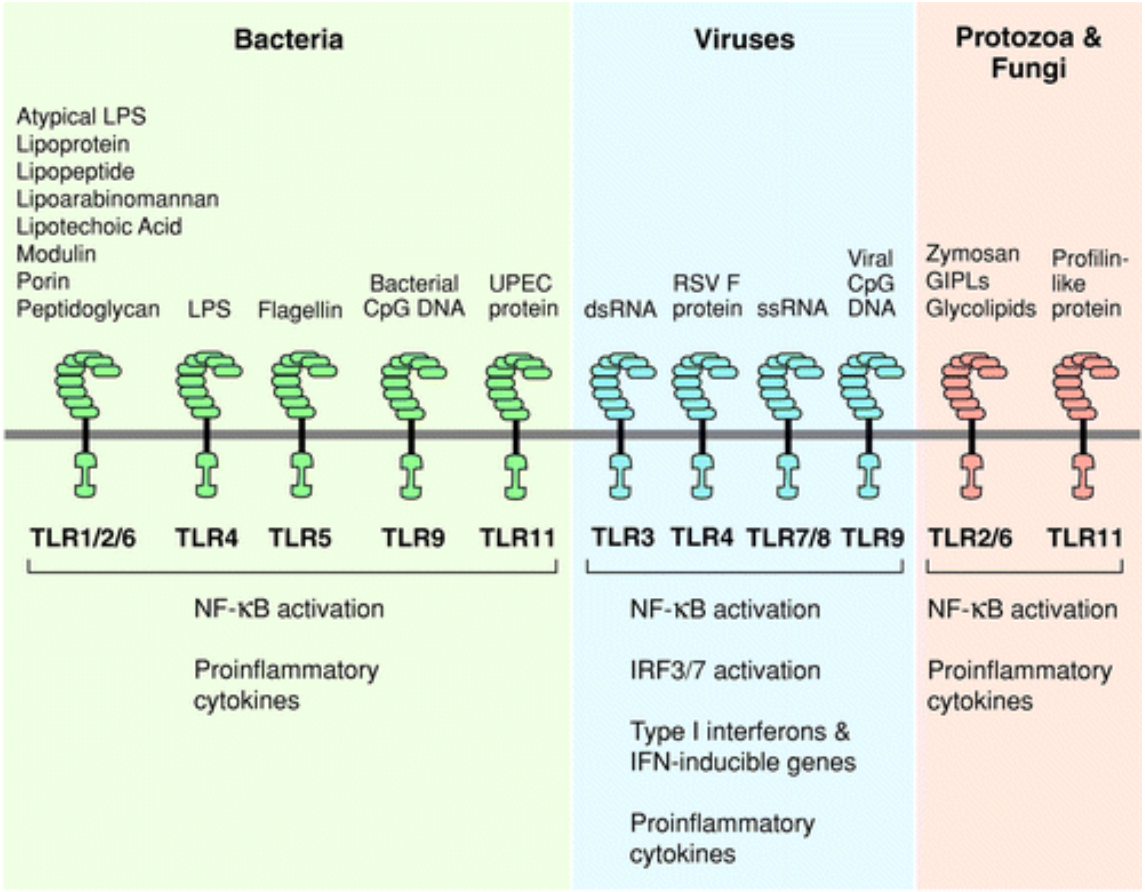
Toll-like receptors (*TLRs*) are proteins of the innate *system* that contribute to the first defense against bacterial and viral infection

Messages are then sent to specialised cells that will block the bacterial or viral attack

Toll-like receptors (TLRs) play an important role in the immune response by helping the body to recognise foreign molecules

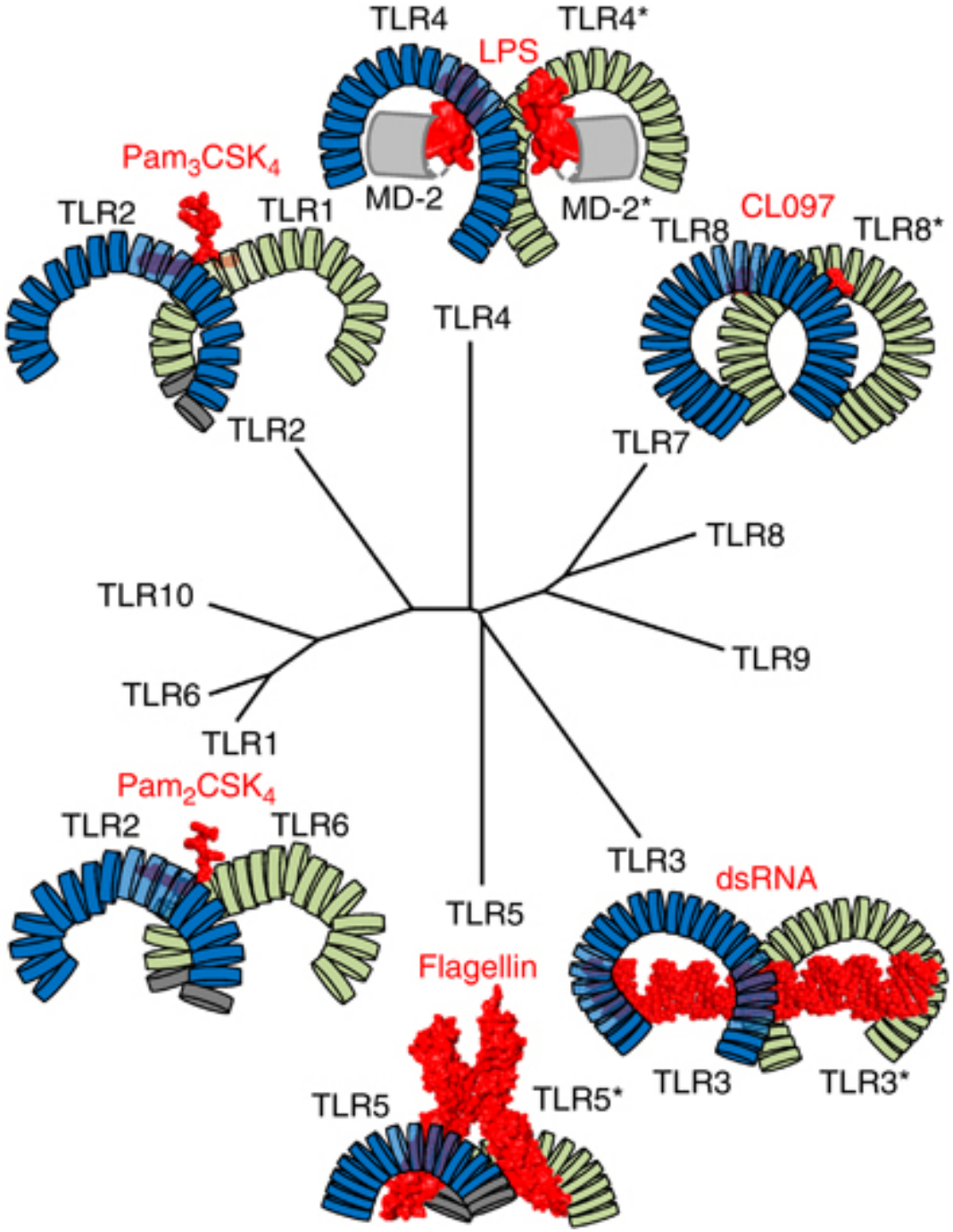
Toll-like receptors(TLRs)

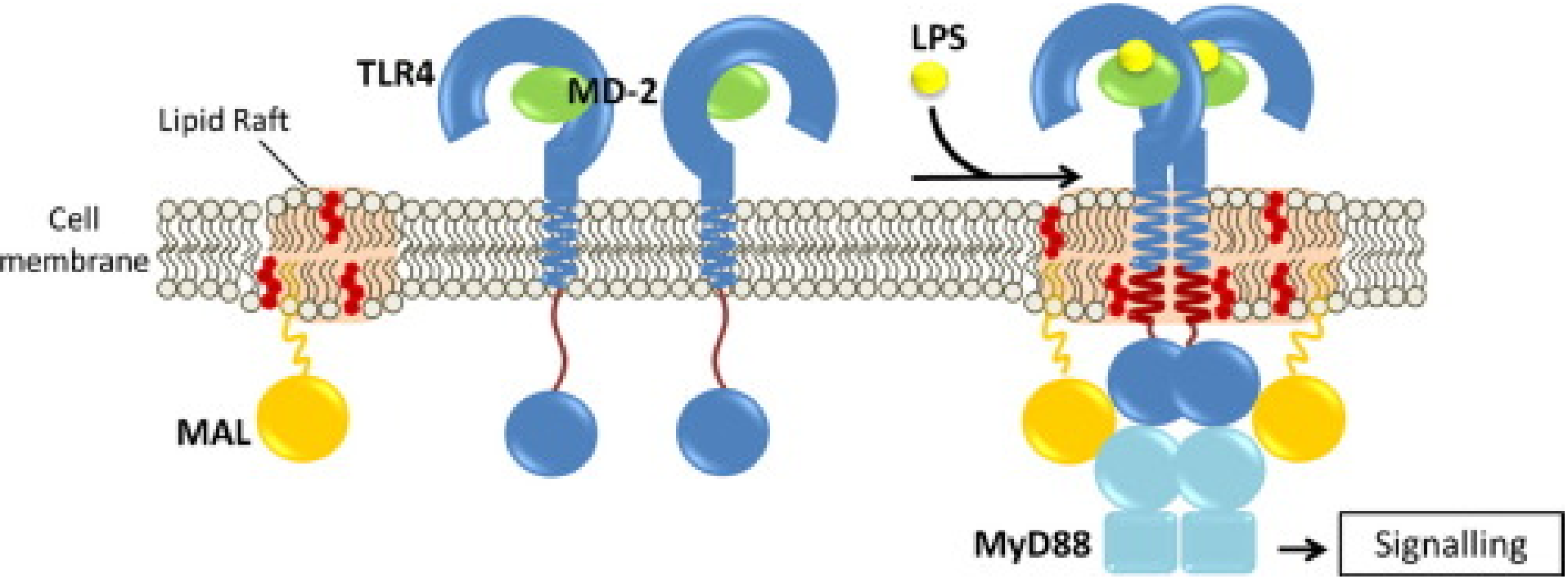
The Nobel Prize in **Physiology** or Medicine 2011
Bruce A. Beutler, Jules A. Hoffmann, Ralph M. Steinman



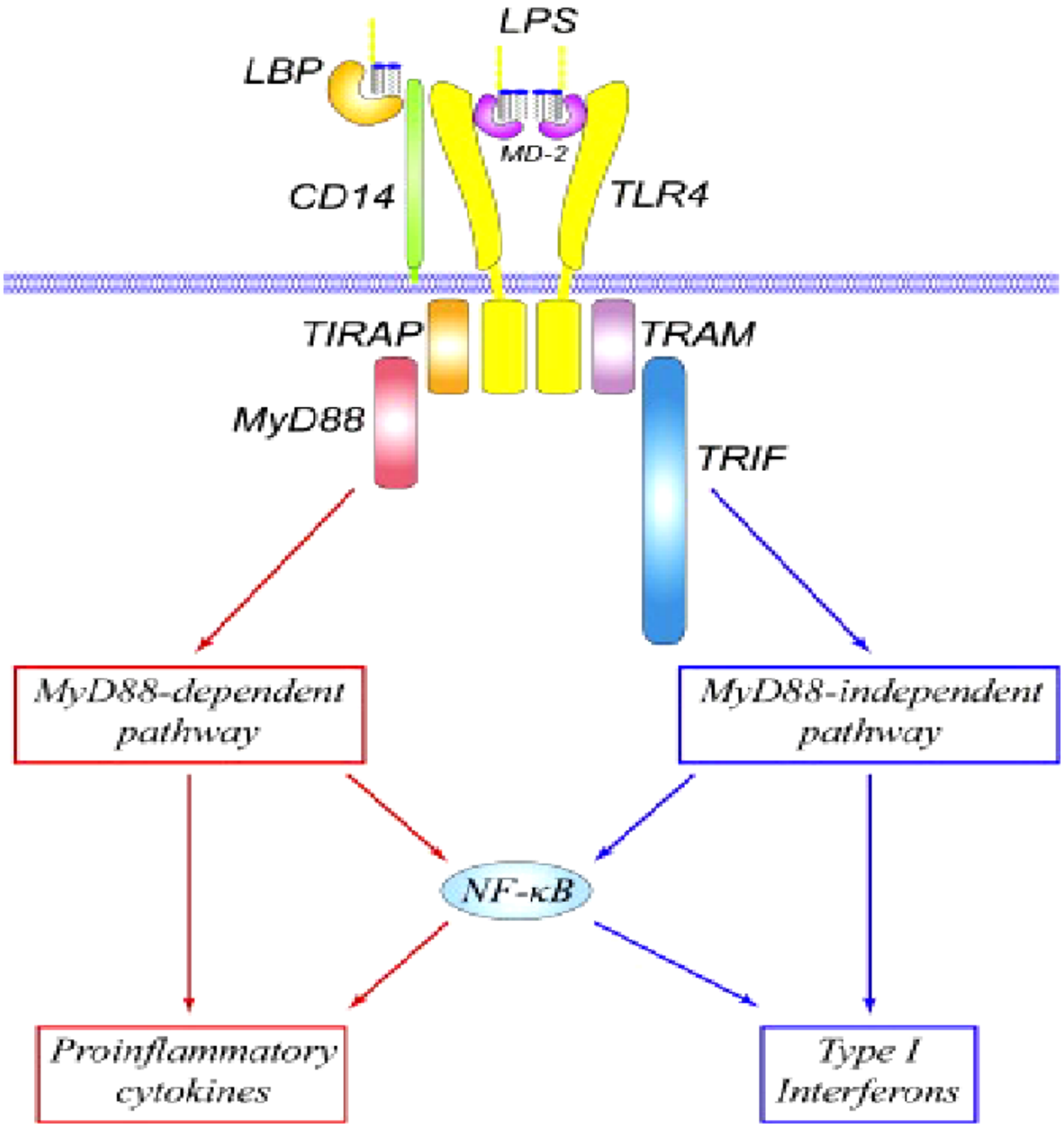
West AP, et al. 2006.
Annu. Rev. Cell Dev. Biol. 22:409–37

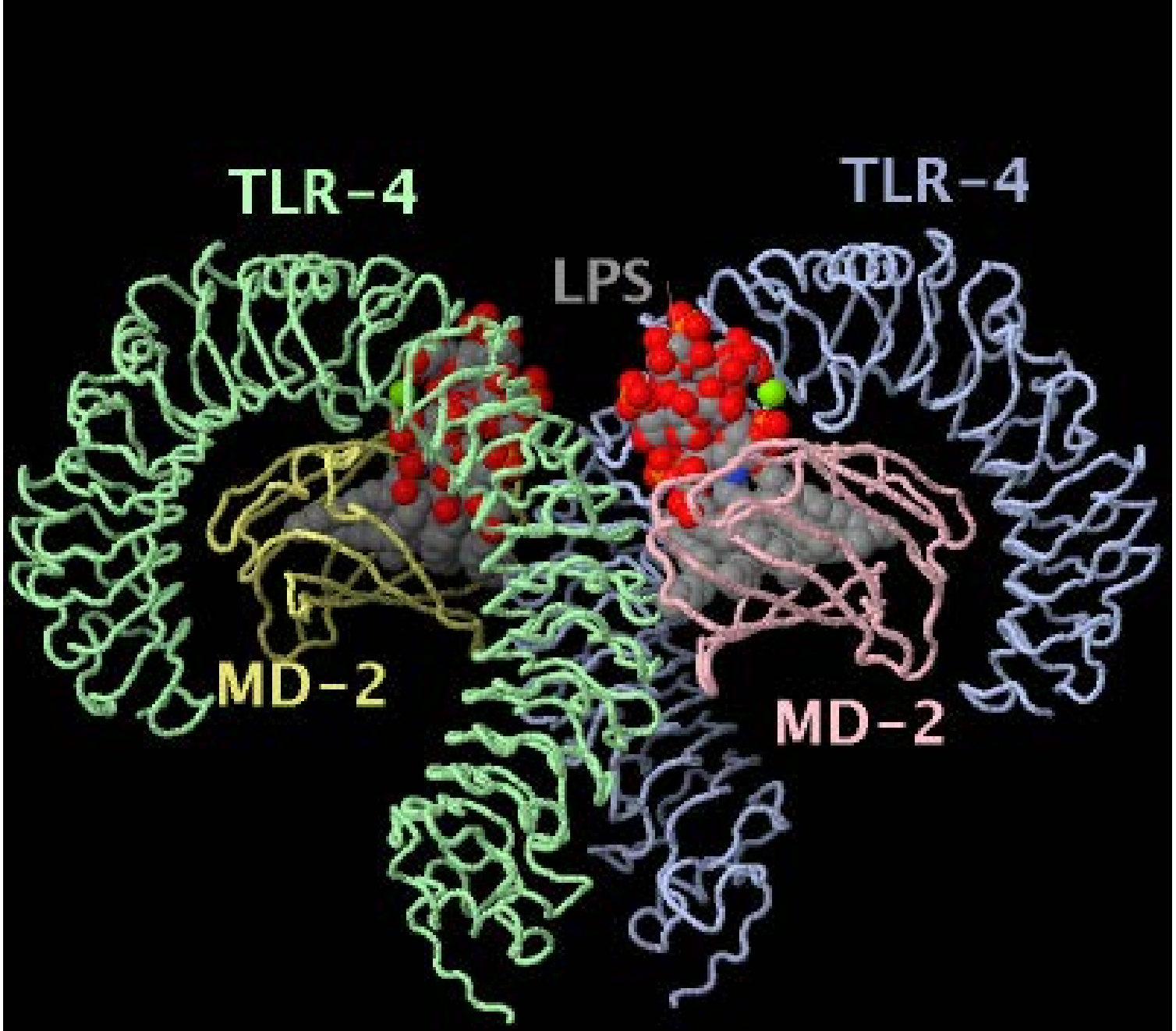
The extracellular domains consists of leucine rich repeats with horseshoe-like shapes


TLR4
TLR4*
LPS
MD-2
MD-2*
Pam3CSK4
TLR2
TLR1
CL097
TLR8
TLR8*
TLR4
TLR2
TLR7
TLR8
TLR10
TLR9
TLR6
TLR1
Pam2CSK4
TLR2
TLR6
TLR3
dsRNA
TLR5
Flagellin
TLR5
TLR5*
TLR3
TLR3*



Activation of TLR4 before and after stimulation by bacterial lipopolysaccharides (LPS).


LPS
LBP
MD-2
CD14
TLR4
TIRAP
TRAM
MyD88
TRIF
MyD88-dependent pathway
MyD88-independent pathway
NF-κB
Proinflammatory cytokines
Type I Interferons



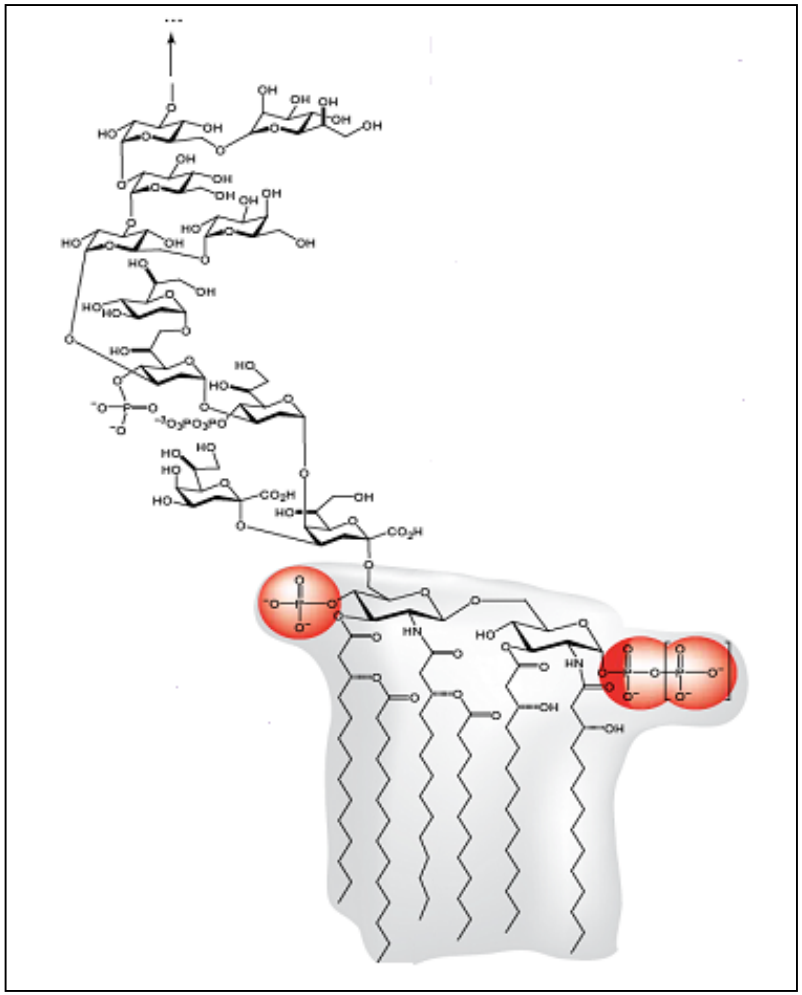
Park BS. et al. Nature. 2009. 458(7242):1191-5.

Numerous ligands of bacterial,viral origin are implicated as TLRs activator.This promiscuity raises questions concerning the manner in which molecules unrelated to microbial ligands might productively engage a signaling receptor

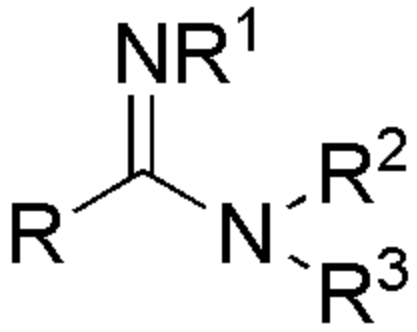
Bruce A. Beutler

Do TLRs recognize non bacterial ligands?

LPS:natural ligand

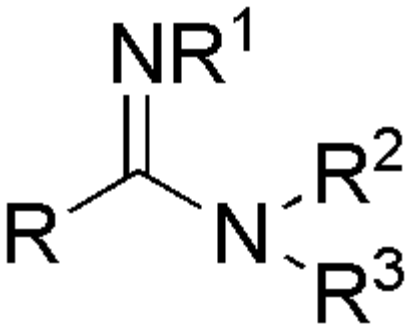


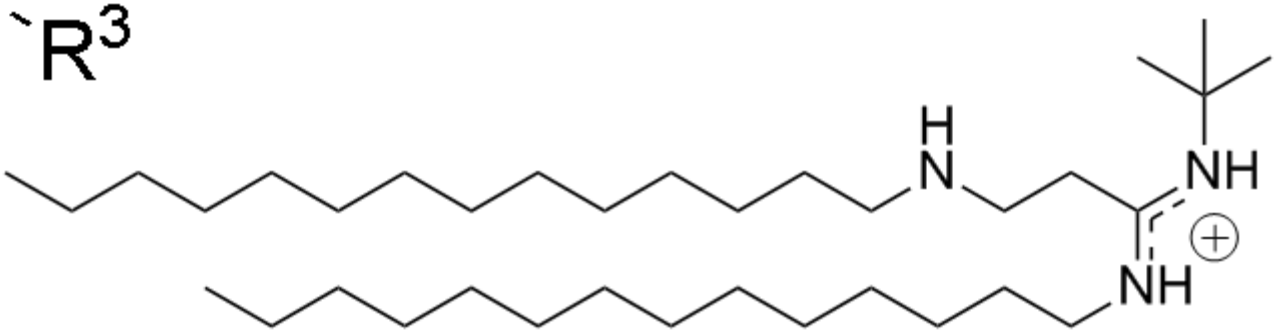
Robert Fuks

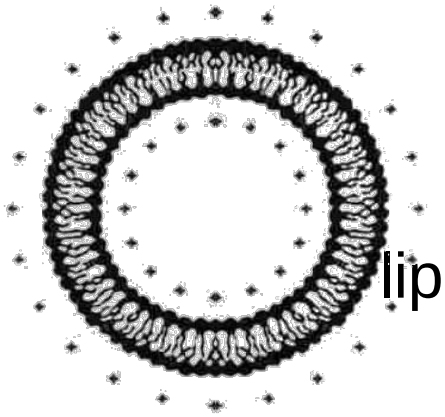


Amidine

N-t-butyl-N -tetradecyl-3-tetradecylamino-propionamidine (diC14-amidine)

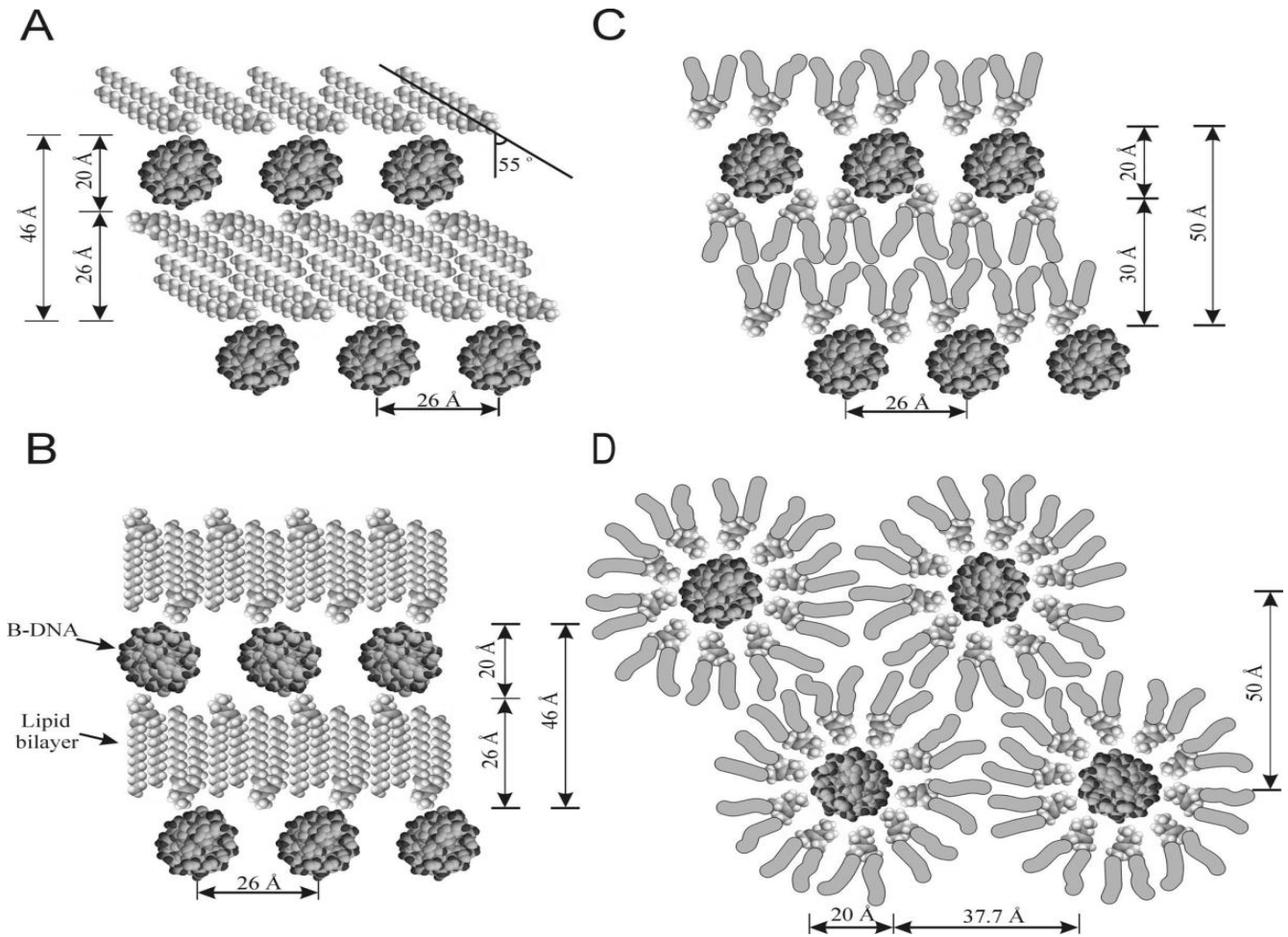






liposomes

diC14-amidine-Size 200nm-transition temperature:23C



Pector V. **Cherezov V, Qiu H, Pector V, Vandenbranden M, Ruysschaert JM, Caffrey M.** *et al.* 2000. J Biol Chem. 275:29533-8.

Molecular models of the diC14-amidine lipid/DNA complex. Two possible arrangements below the lipid chain melting transition temperature, 23°C, are shown in **A** and **B**.

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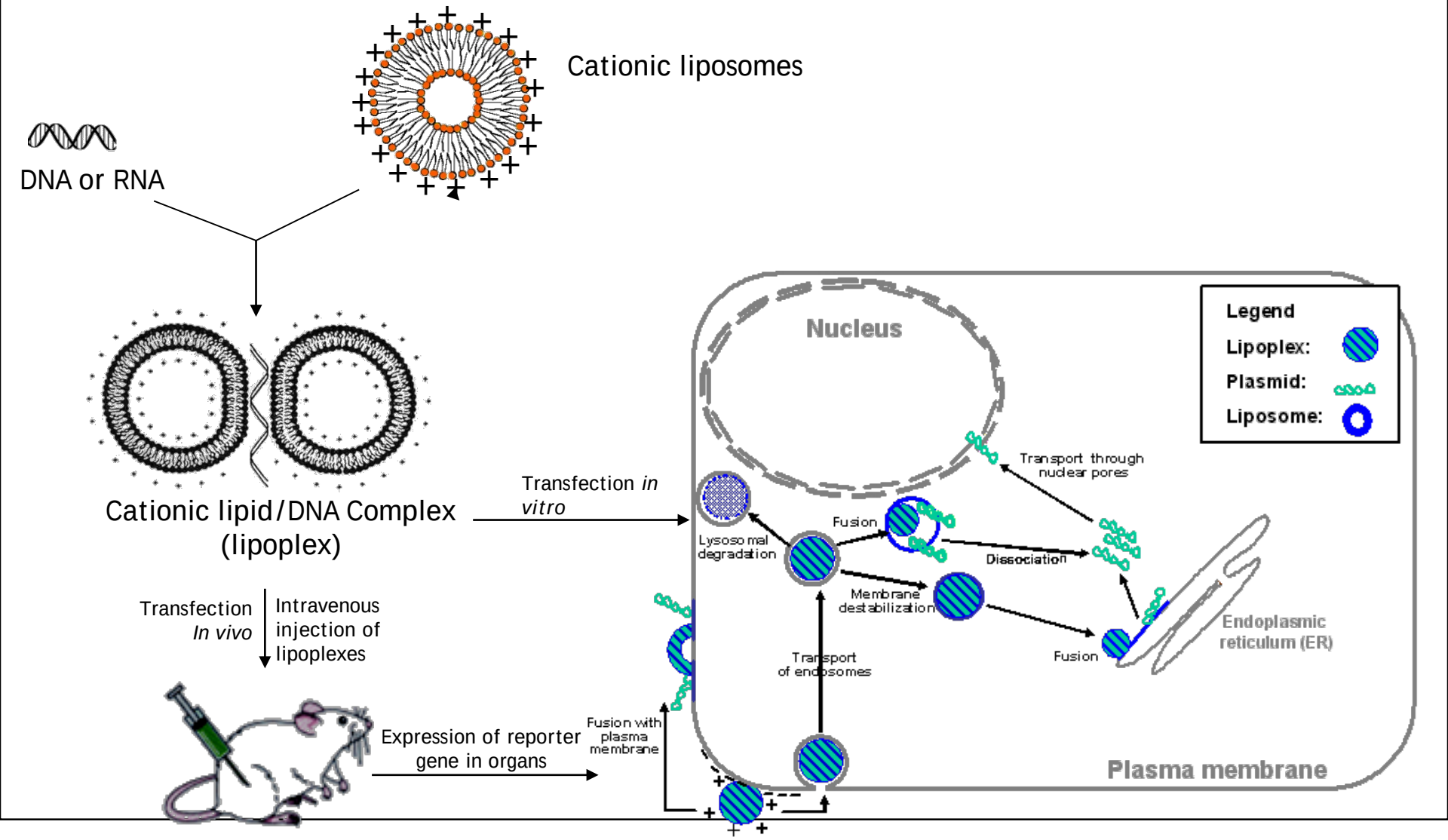
Cationic lipids as gene carriers



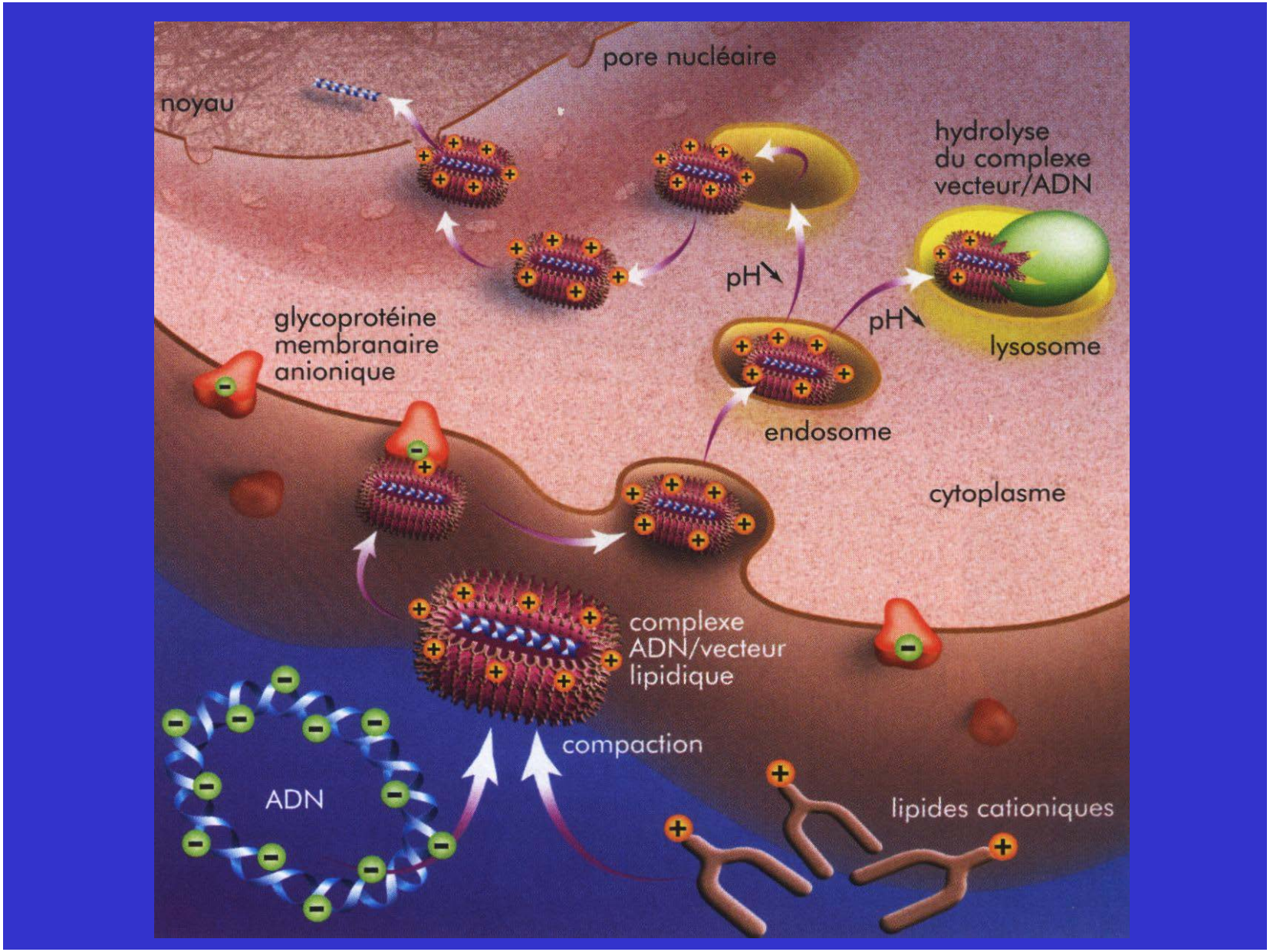
Elouahabi A.and Ruysschaert JM. 2005. Mol Ther. 11 : 336-47

1996:BioTech Tools

First steps of ASIT biotech on European stock exchange-EURONEXT-2016

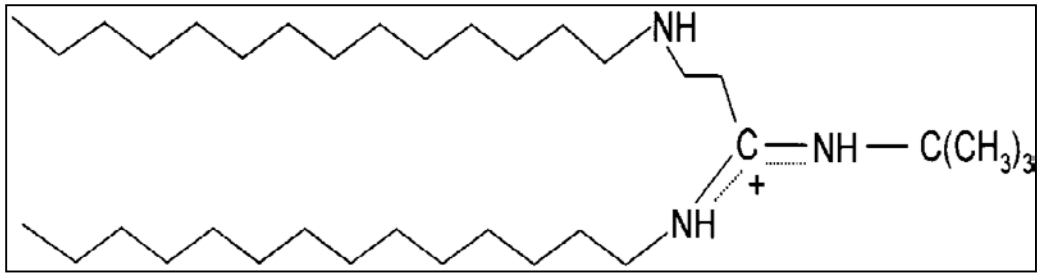
Do TLRs recognize non bacterial ligands?

PhD student did not inject in mice
the lipid –DNA complex
but just the lipid

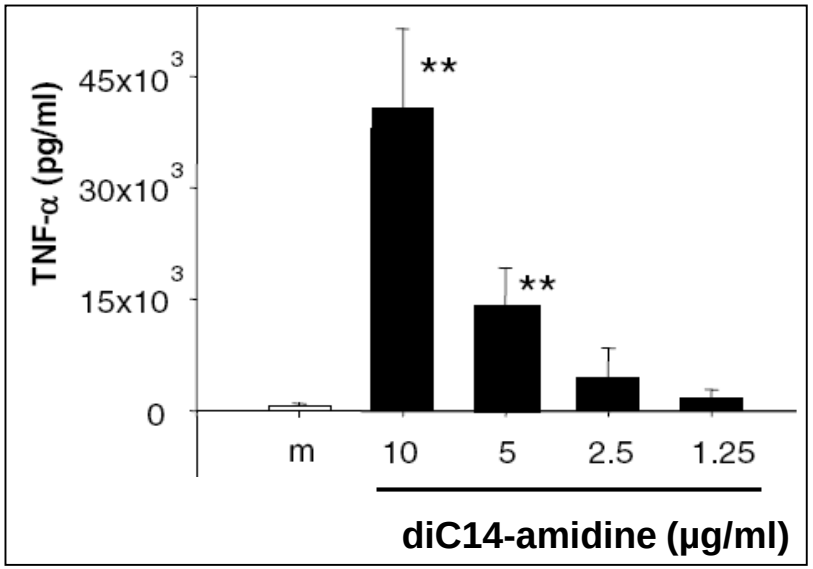


Elhouahabi,Ruysschaert-Molecular Therapy(2005) Review

When a gene carrier turns into a TLR4 agonist!



diC14-amidine



Jacquet A. *et al.* 2005. Mol Ther. 11(6):960-8.

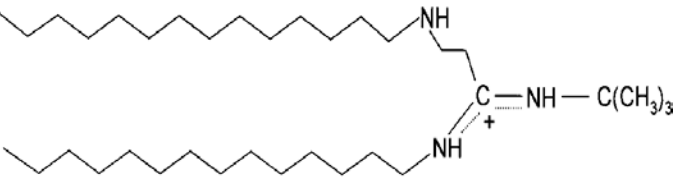
→ Th1 response
(characteristic of TLR signaling)

Tanaka T. *et al.* 2008. Eur J Immunol. 38(5):1351-7.

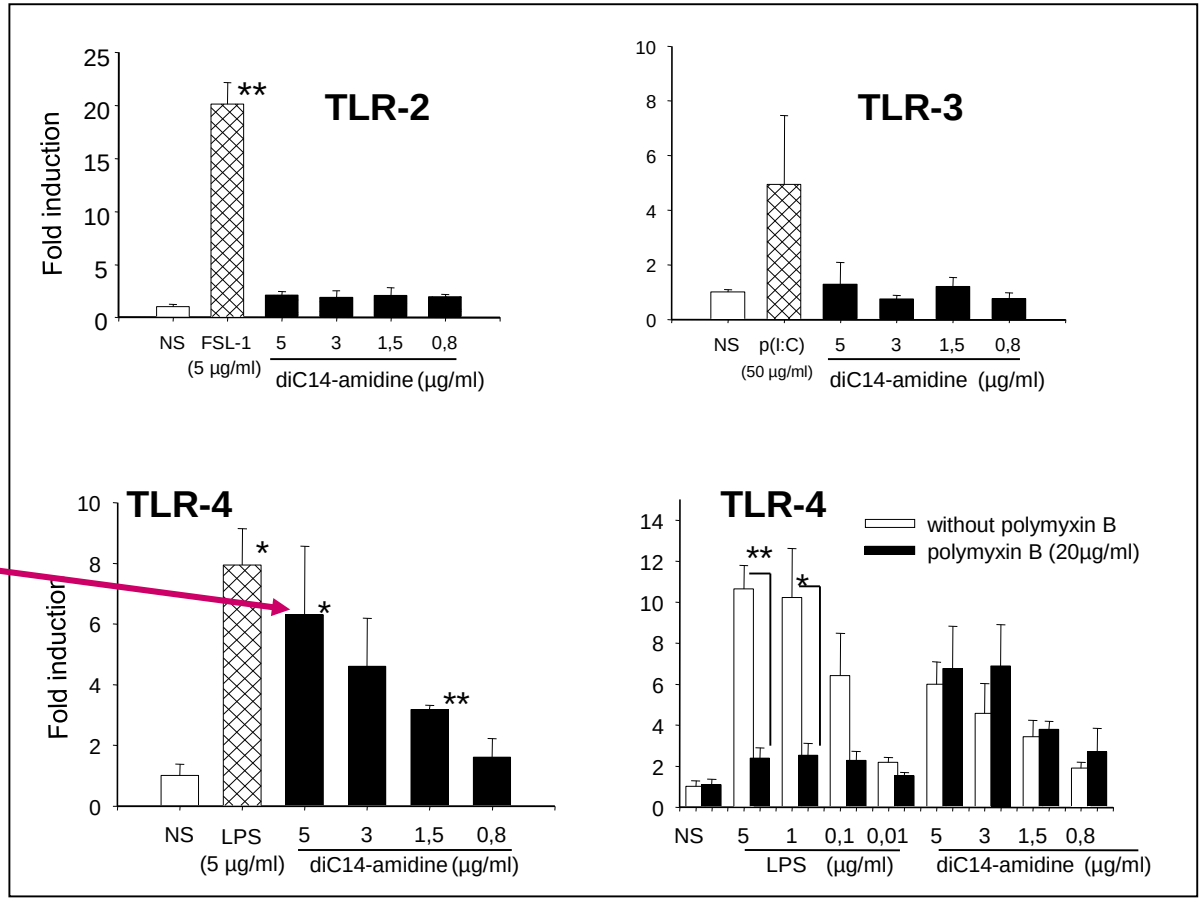
DiC14-amidine liposomes activate cytokine secretion.

Is Activation Toll-like receptor-dependent?

When a gene carrier turns into a TLR4 agonist!

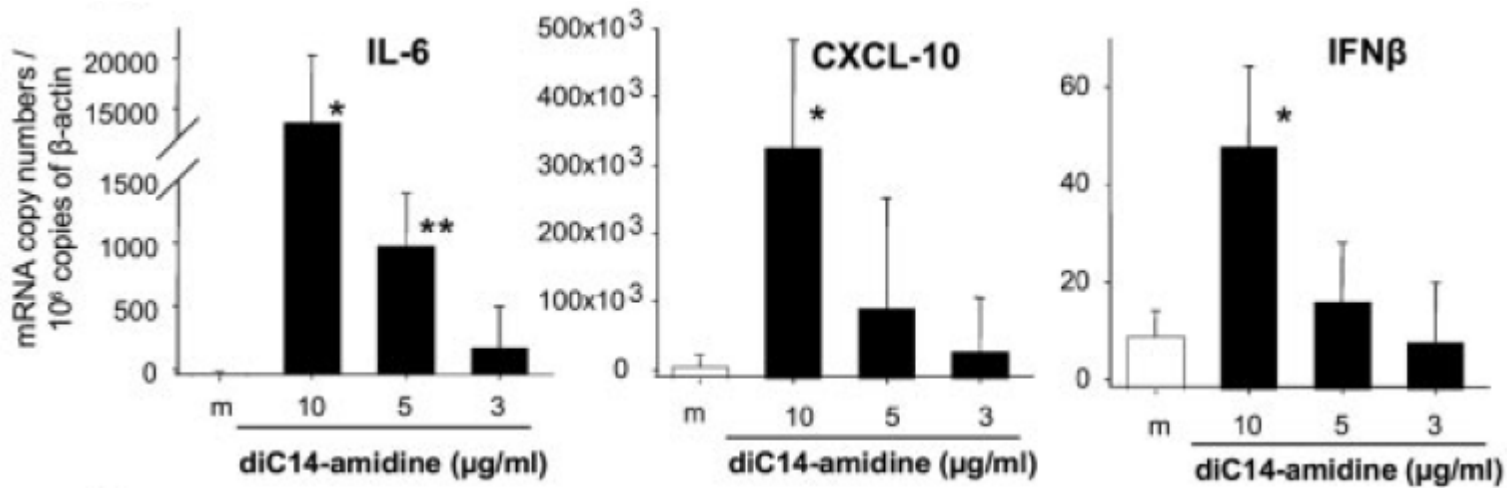


diC14-amidine liposomes activate NF-κB through **TLR-4**

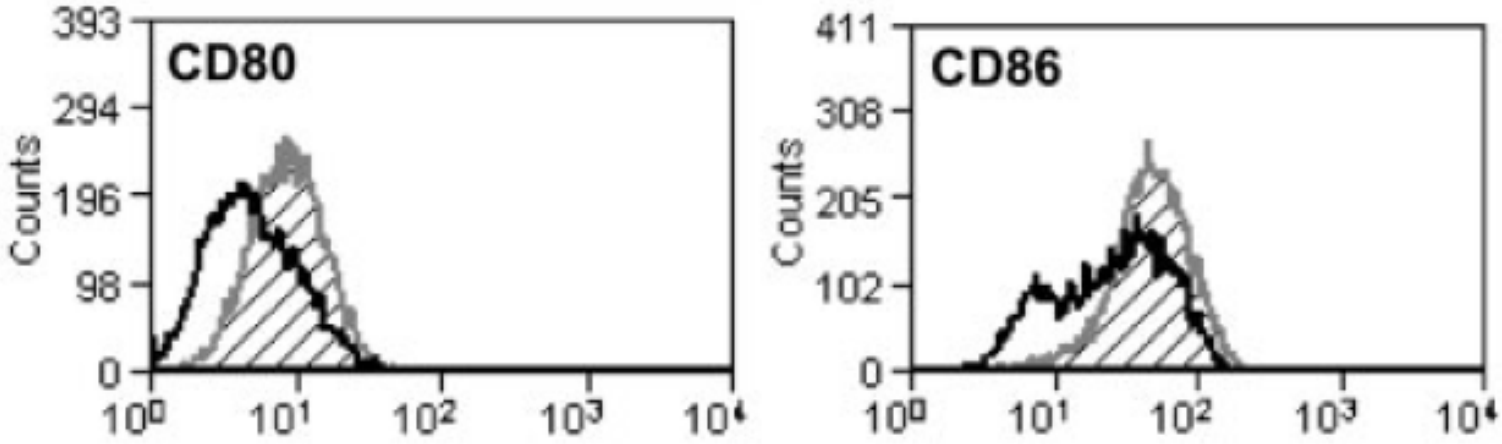


Tanaka T. *et al.* 2008. Eur J Immunol. 38(5):1351-7.

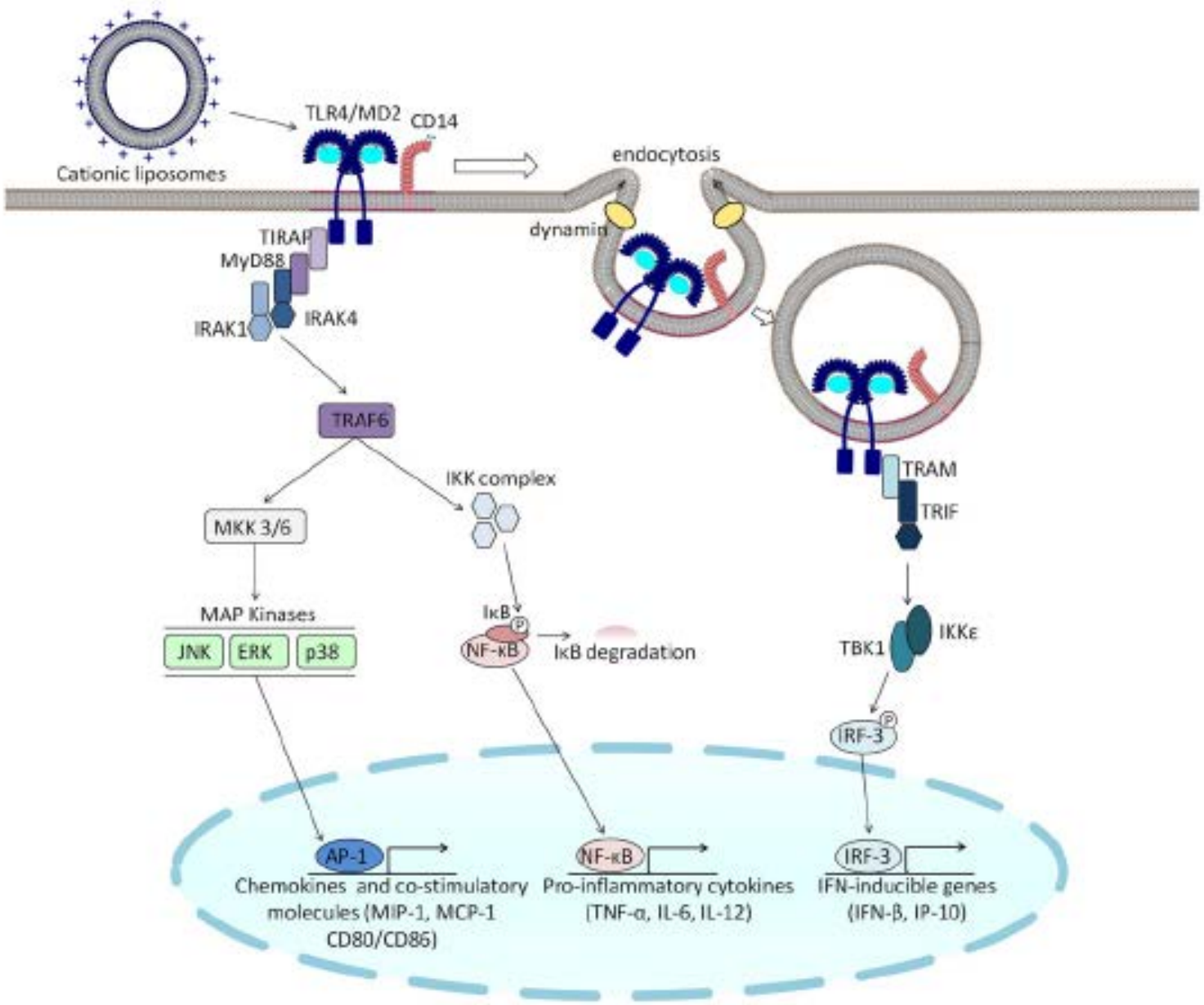
Cytokine secretion revealing activation of the innate system induced by a lipidic gene carrier



A gene carrier activates protein expression at the cell surface

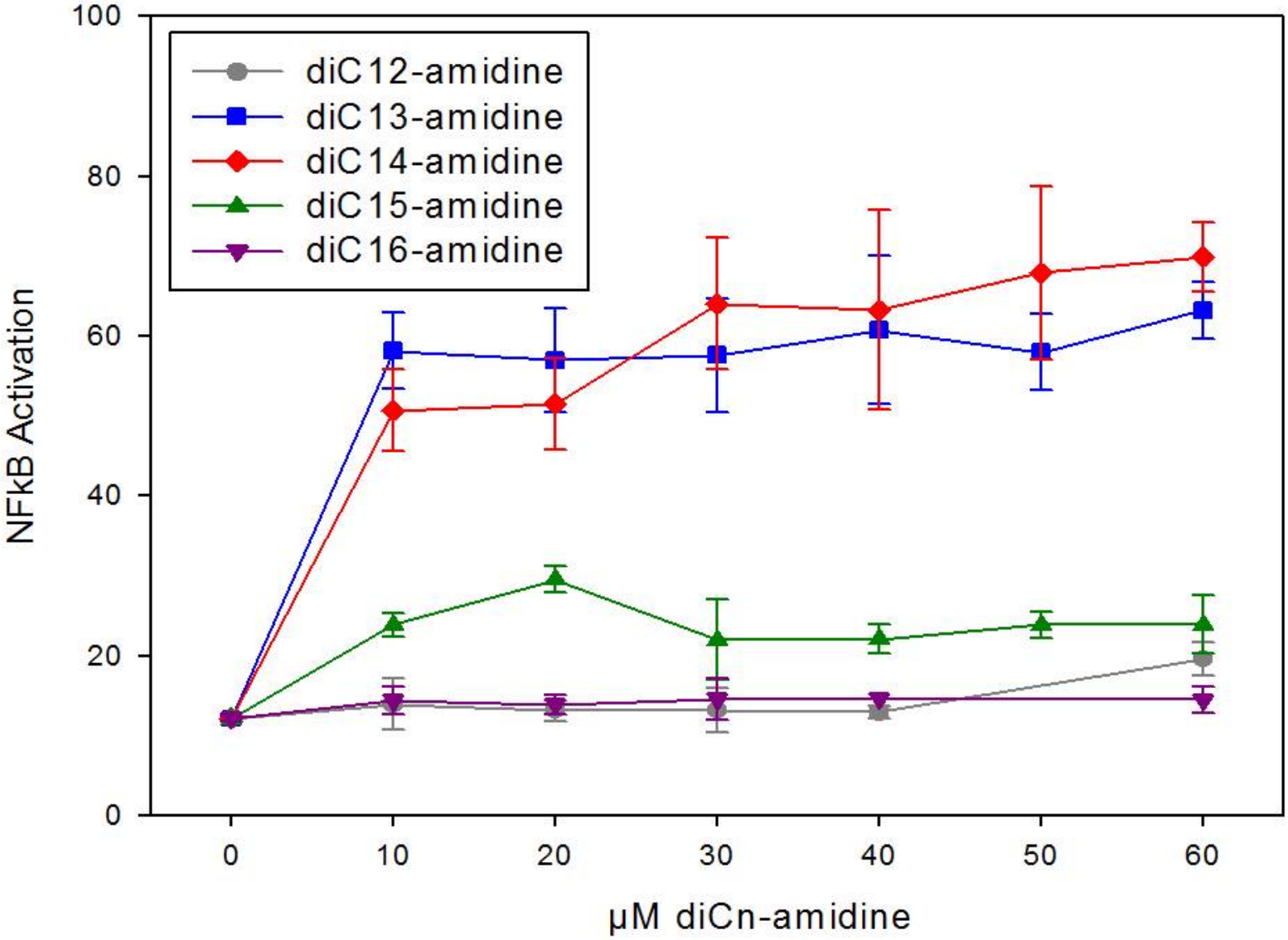


Cell surface expression of CD80 and CD86 as determined by flow cytometry in human dendritic cells in medium alone (black) and after incubation with amidine liposomes (grey) (5µgr/ml)



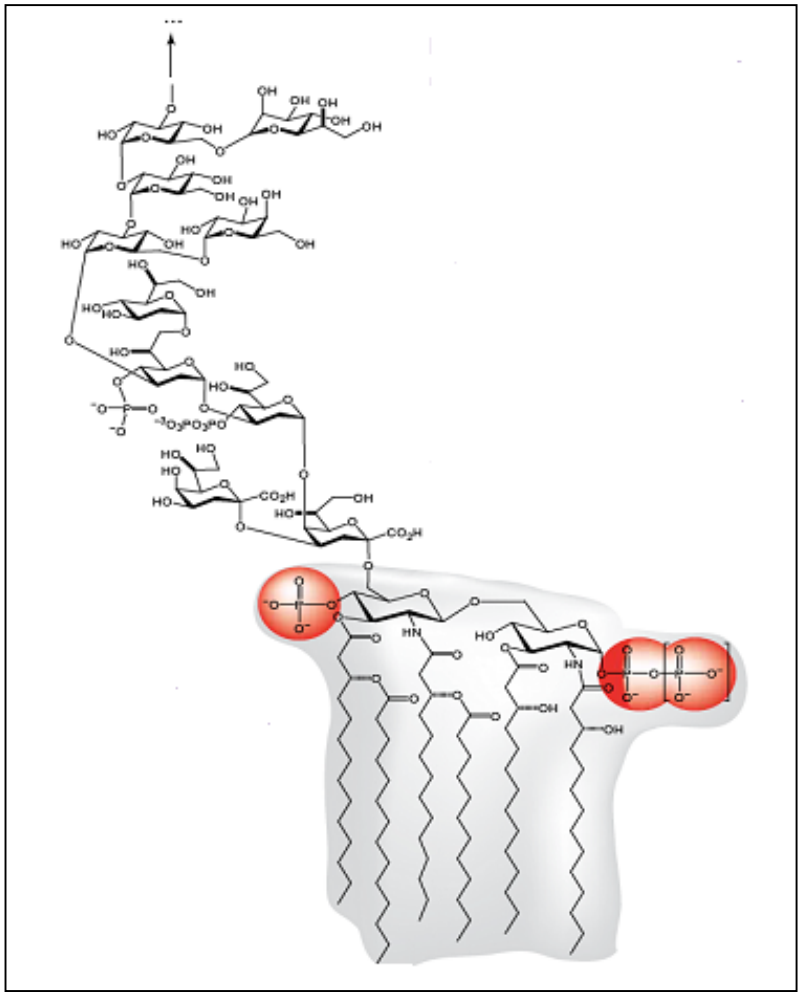
Lonez, C. Vandenbranden, M. Ruysschaert, J.M. Prog. Lipid Res-.2008, 47, 340-347
Lonez,C Vandenbranden, M. Ruysschaert, J.M Adv.Drug.Release- 2012,64,1749

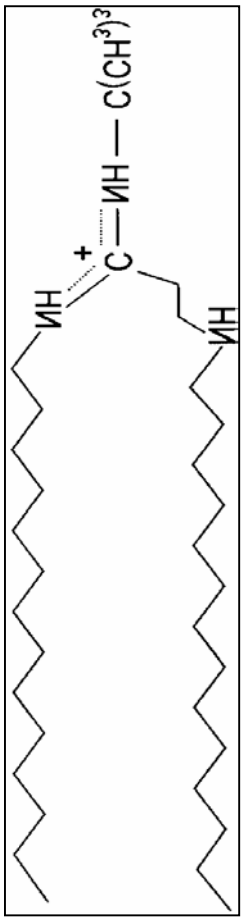
Specificity of diCn-amidine recognition by TLR4!



LPS:natural ligand

Cationic lipid:synthetic



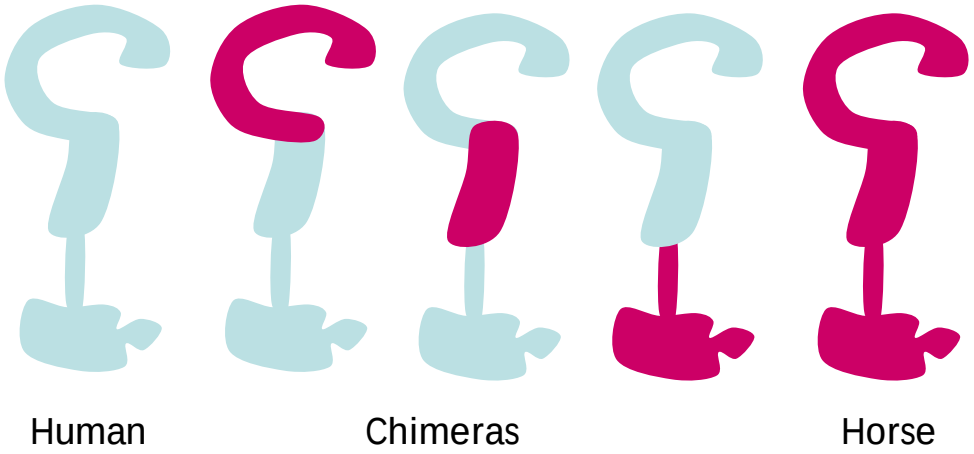


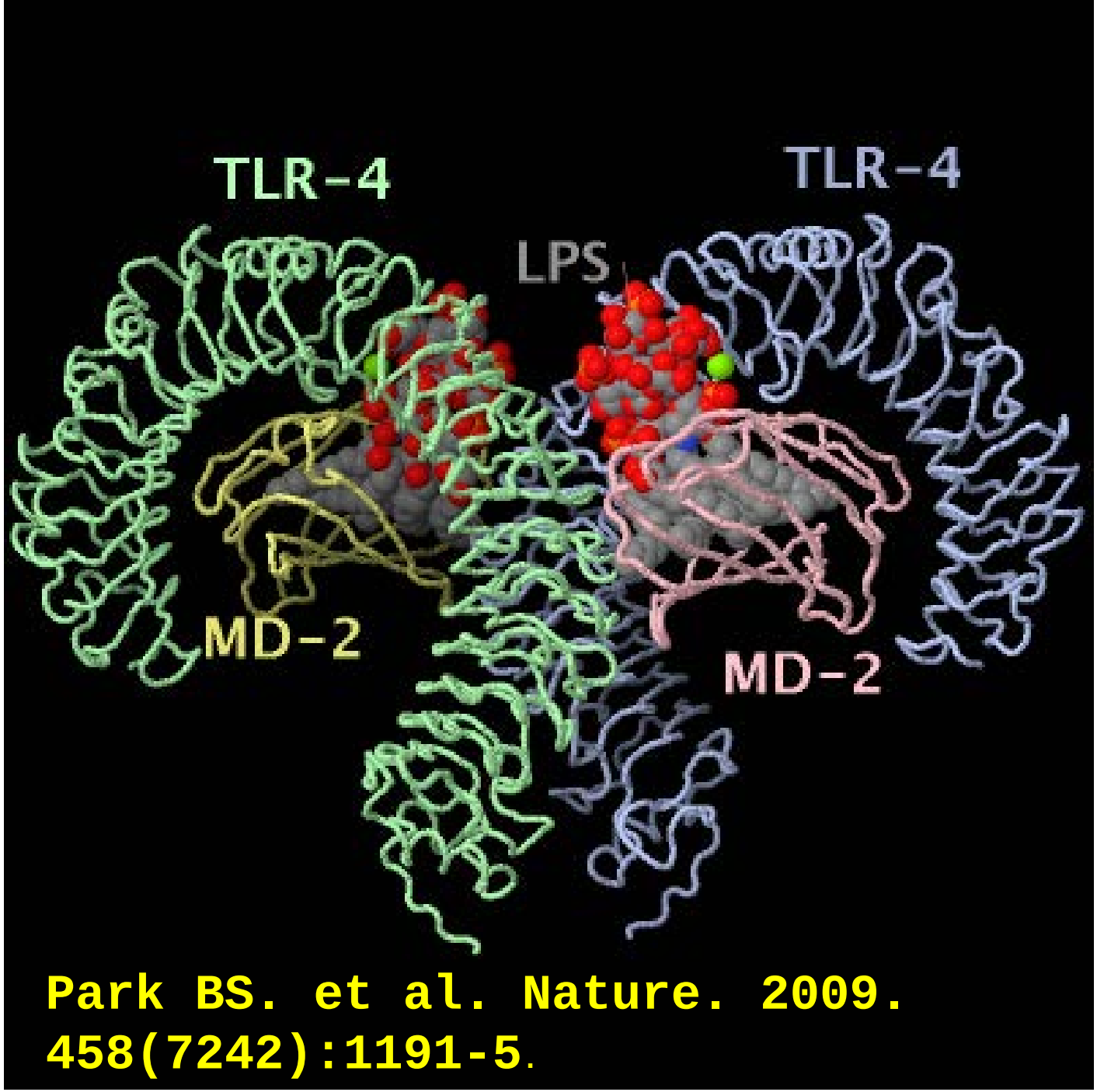
Non structurally-related ligands activate the same receptor !

LPS is recognized by human and horse TLR4 but amidine is recognized by human TLR4 not horse TLR4

Using chimeric constructs made from human and horse proteins, we identified the region in the human TLR4 that modulate the agonist activity of diC14-amidine. Interestingly, this region resides outside the previously identified LPS(natural ligand) binding domain.

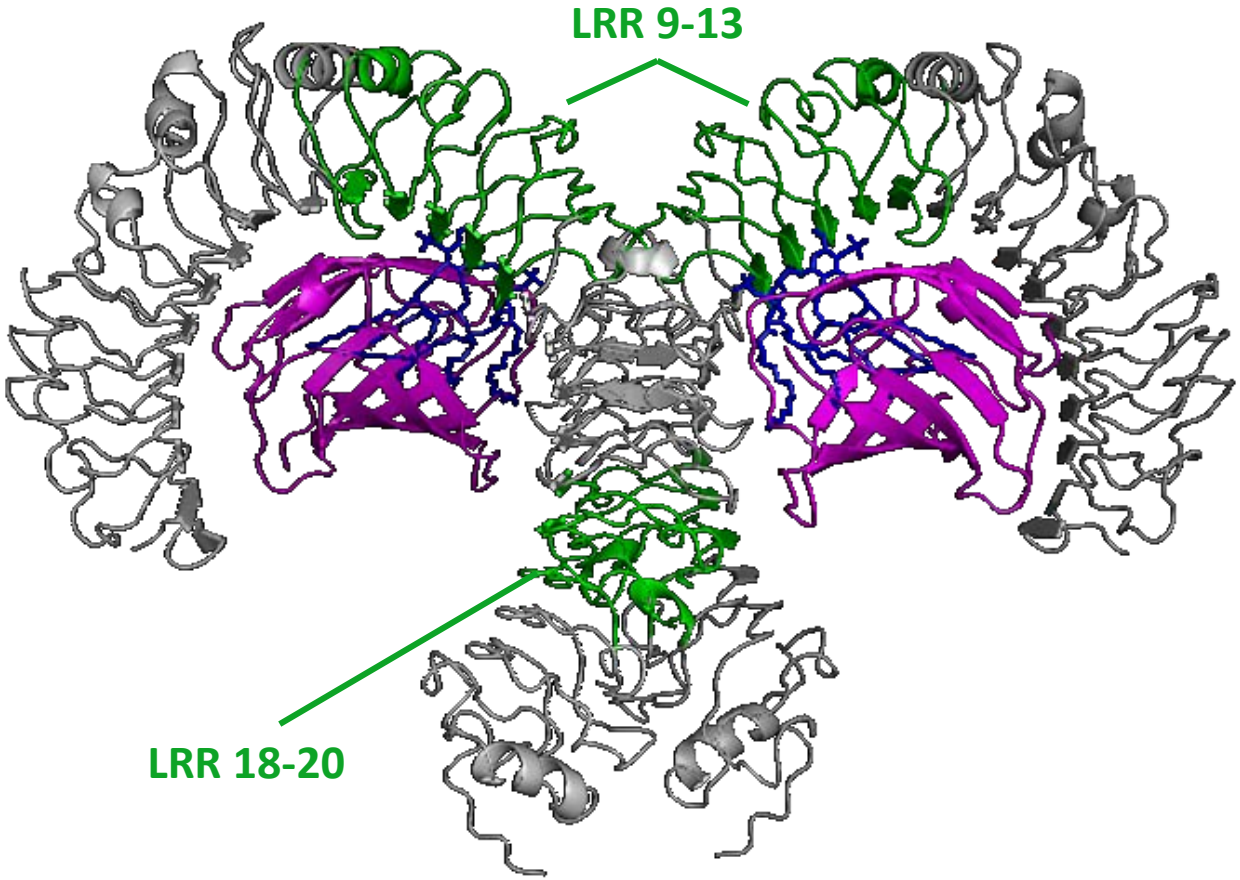
TLR4 chimeras & mutants





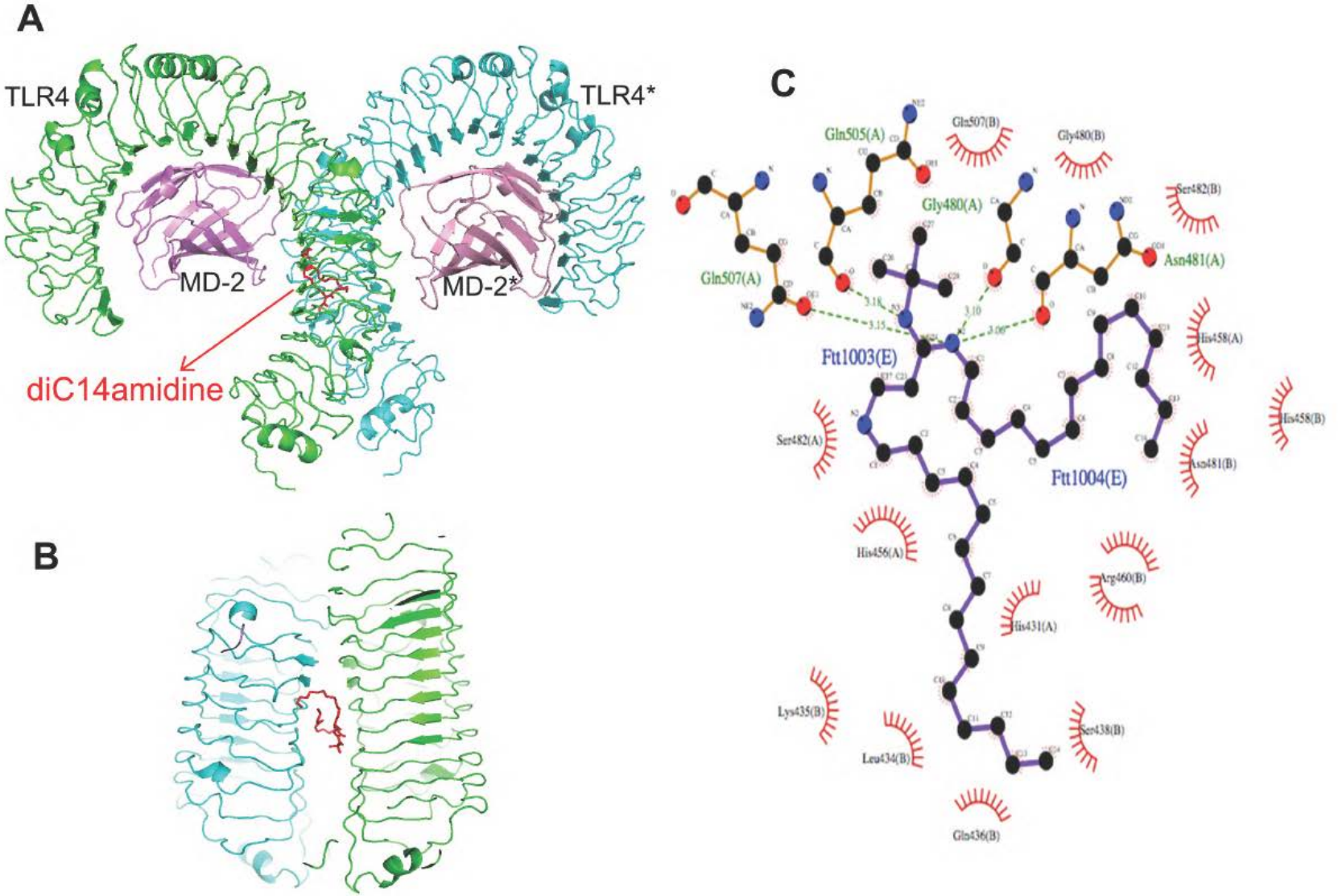
Park BS. et al. Nature. 2009. 458(7242):1191-5.

Interaction of diC14-amidine with TLR4



→Chimeras/mutant experiments suggest diC14-amidine interacts with a new binding site

Figure 5



Ruysschaert et al Immunity(2015)

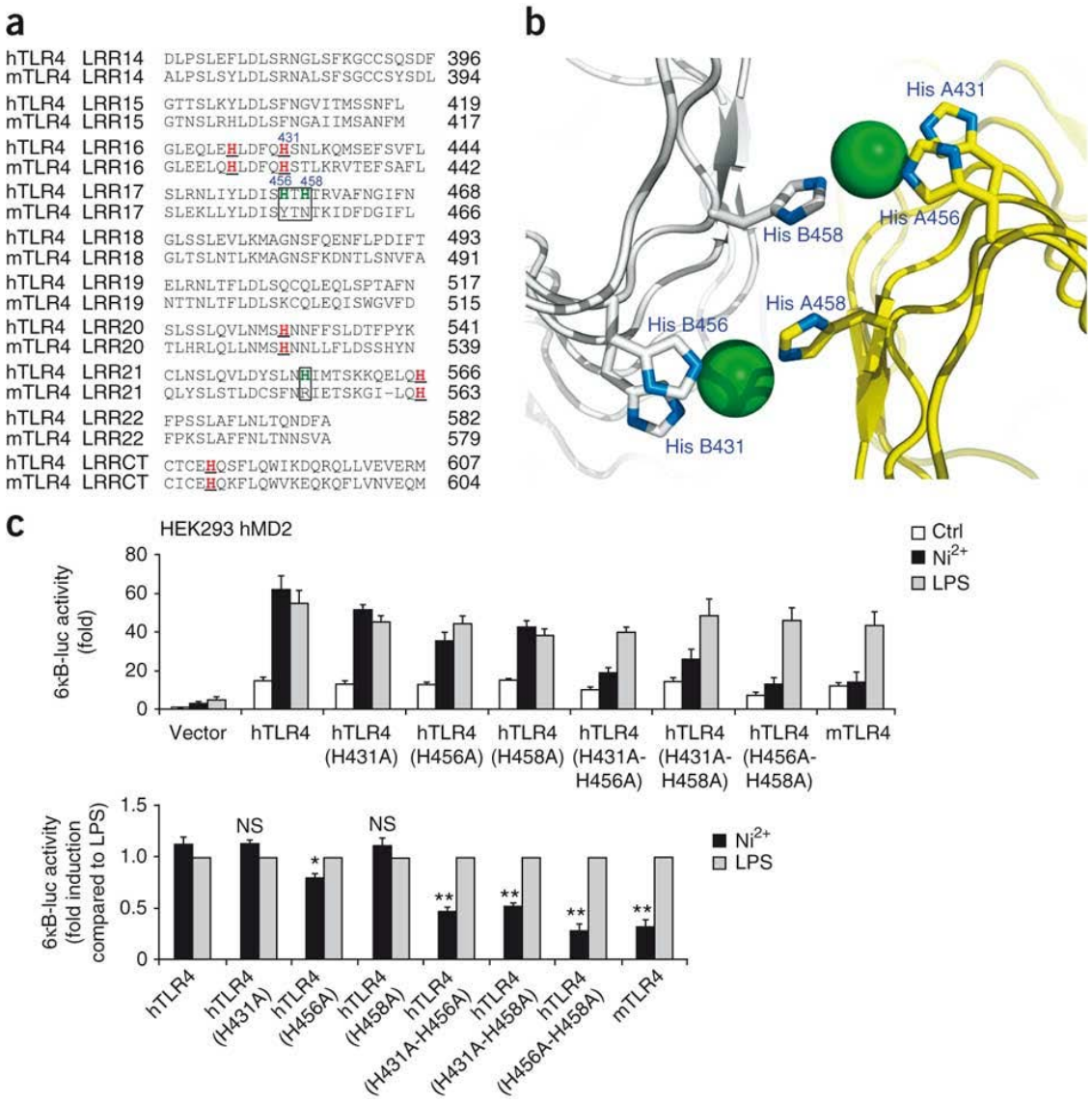
Two different binding sites!

Consequences:

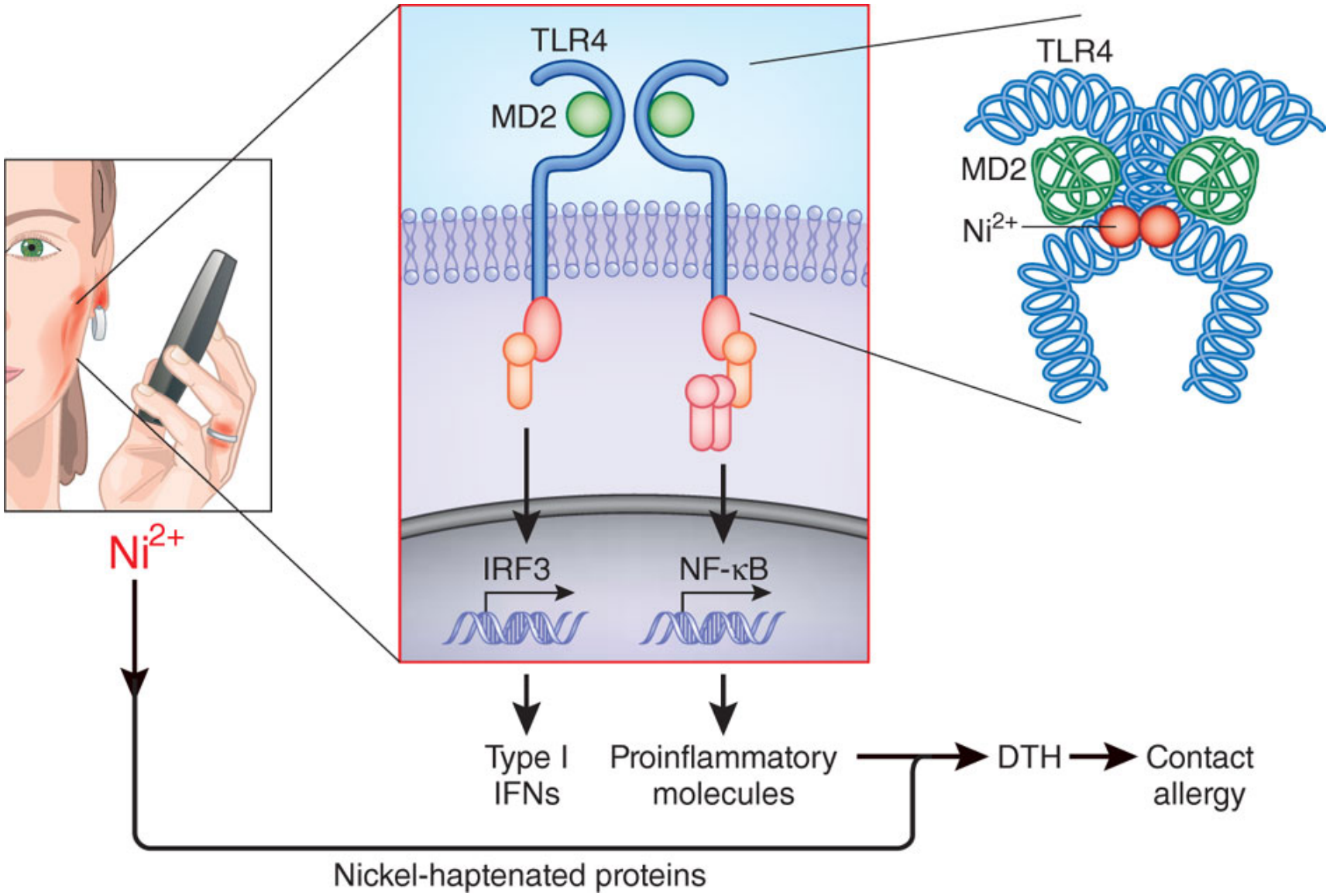
- Activation of new cascades in the innate sytem

-one can inhibit the innate activation without **suppressing the normal innate immunity function** which may be lethal on a long term basis

Nickel ions?Another activator of the innate cascades



Schmidt M et al Nat.Immunol 2010,814-819



Cationic lipid and nickel binding sites are identical

One can inhibit allergy without **suppressing the normal innate immunity function** which may be lethal on a long term basis

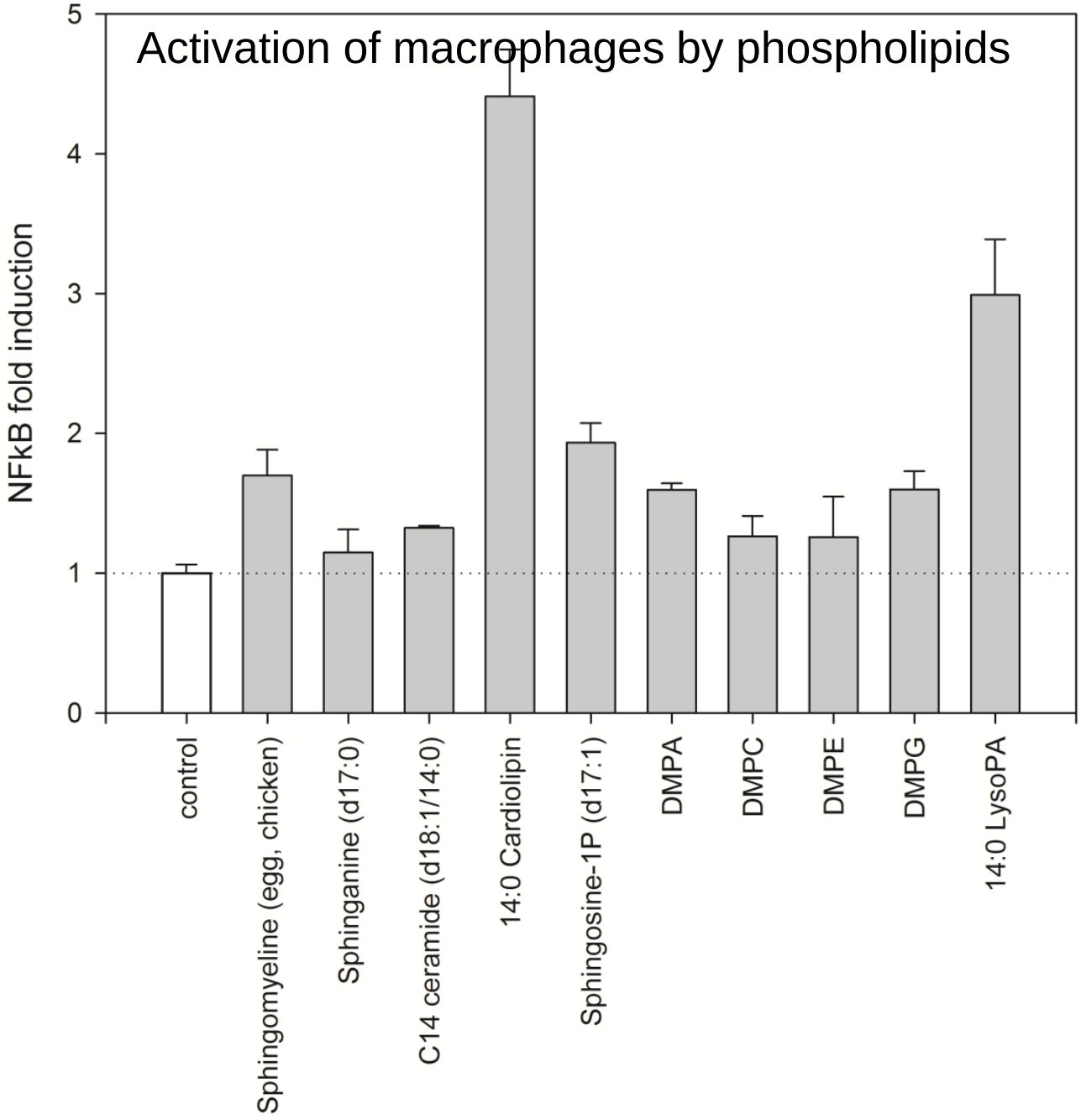
These inflammatory reactions can be desired (for vaccine development), **unwanted** (for delivery applications)

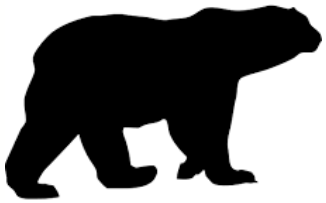
DiC14 amidine liposomes activate multiple recognition pathways of innate immune cells and is a novel adjuvant.

Physical–chemical study demonstrate that this molecular assembly is stable and easy-to-produce, which meet critical industrial and commercial purposes-ASIT-Biotech

Vaccine 30-, 414-424-2012

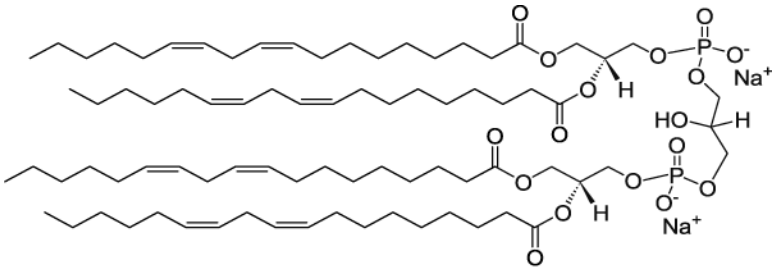
Endogenous lipids?


Activation of macrophages by phospholipids
NFkB fold induction
0
1
2
3
4
5
control
Sphingomyeline (egg, chicken)
Sphinganine (d17:0)
C14 ceramide (d18:1/14:0)
14:0 Cardiolipin
Sphingosine-1P (d17:1)
DMPA
DMPC
DMPE
DMPG
14:0 LysoPA

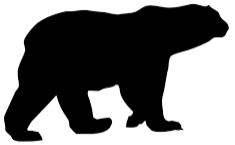


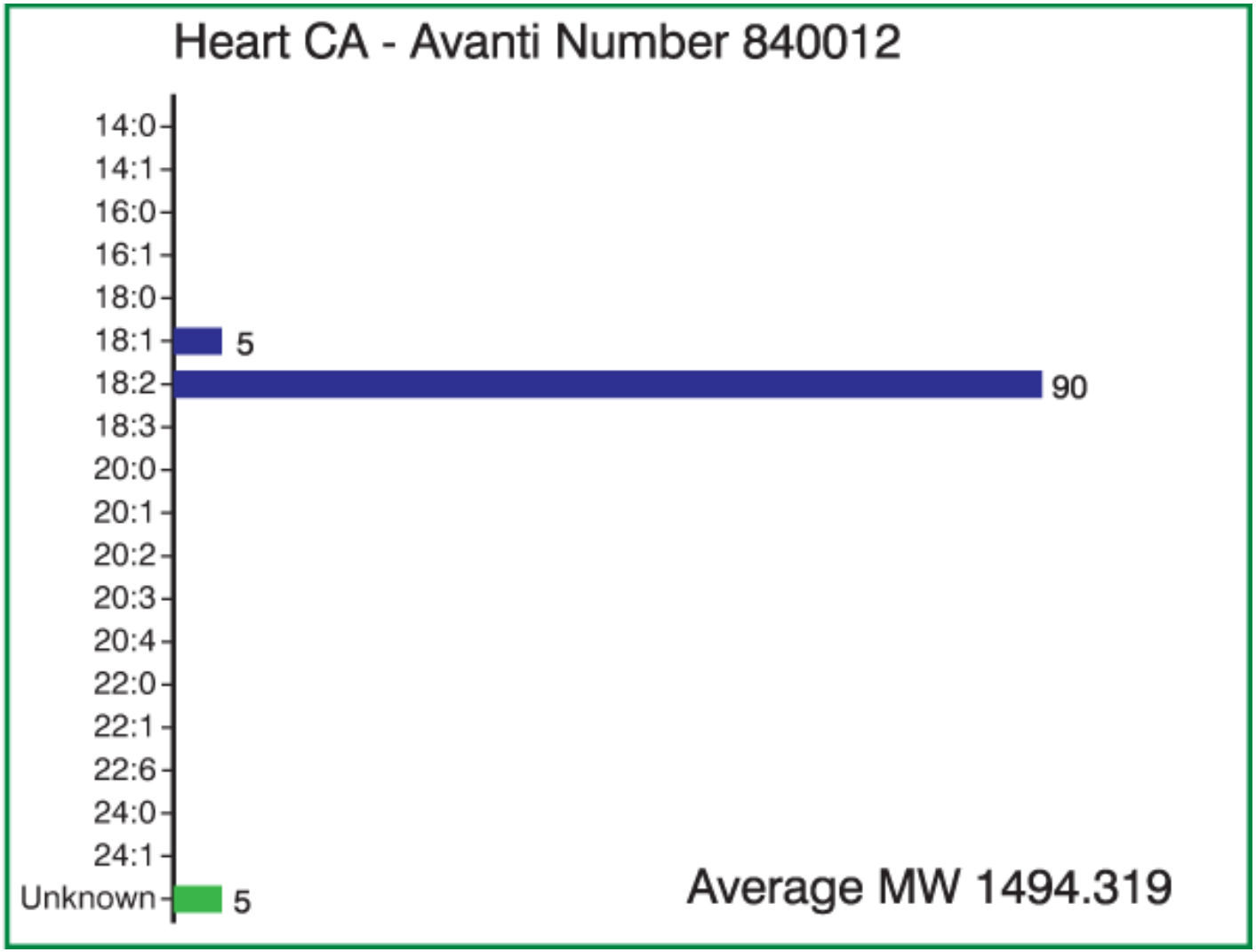
)

Heart cardiolipin

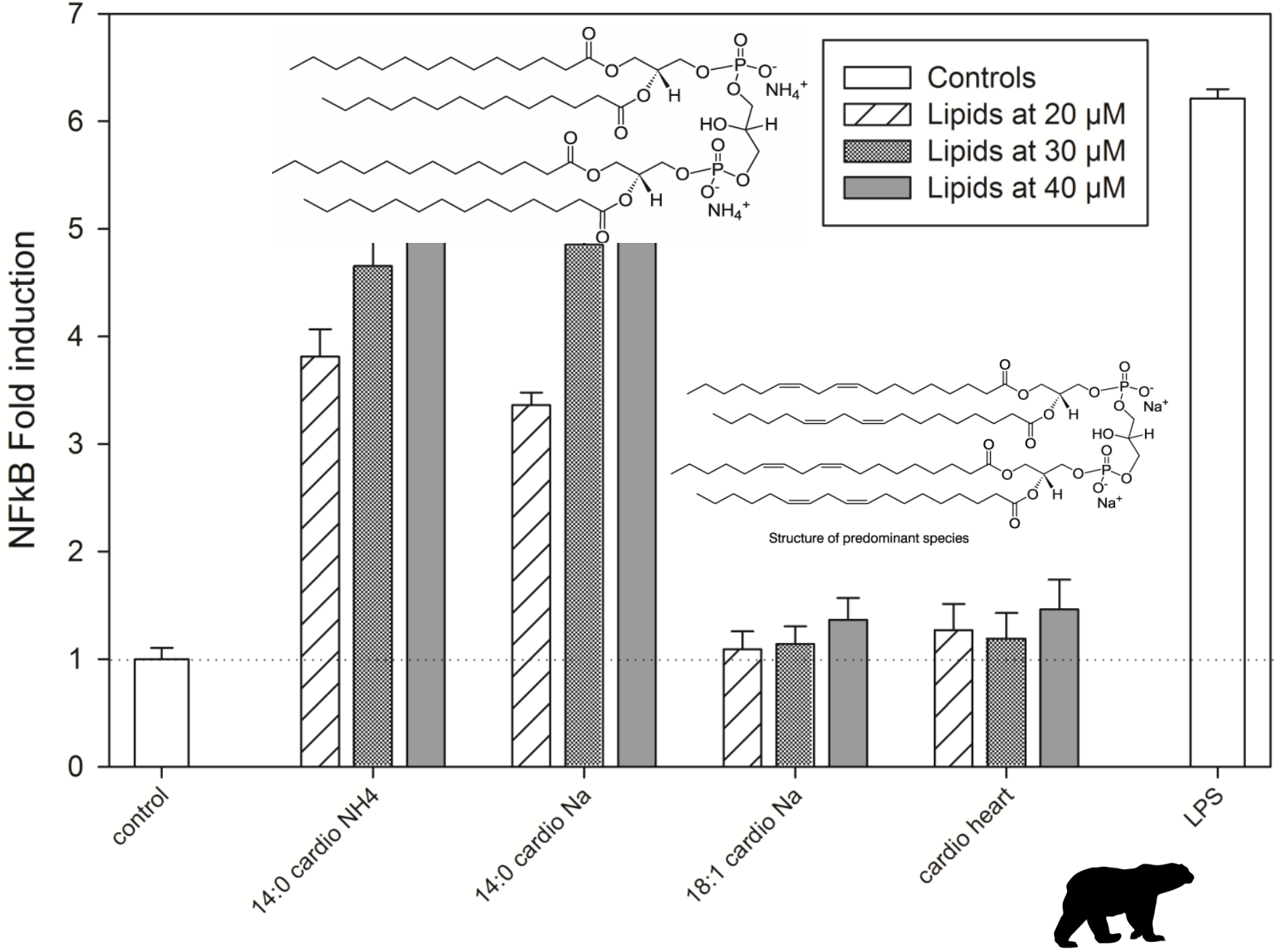


Structure of predominant species

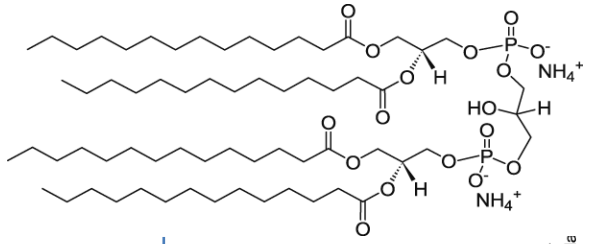


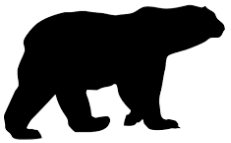


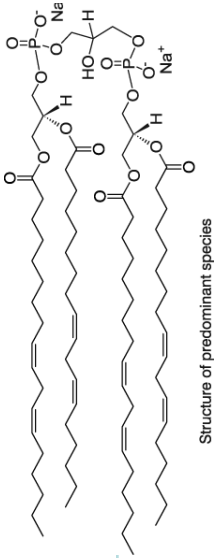
Activation of macrophages by saturated and unsaturated cardiolipin



Heart cardiolipin is a LPS antagonist

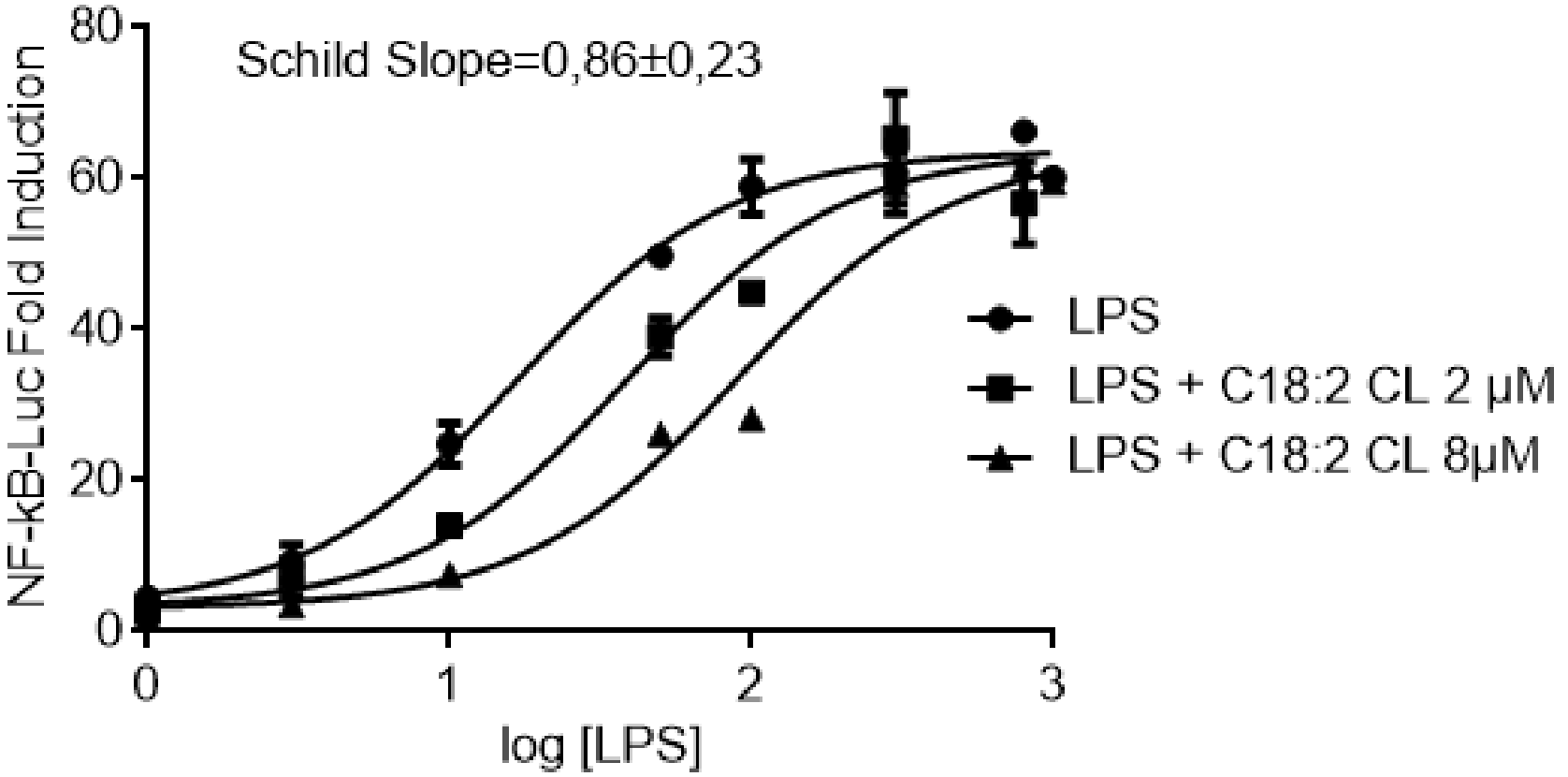






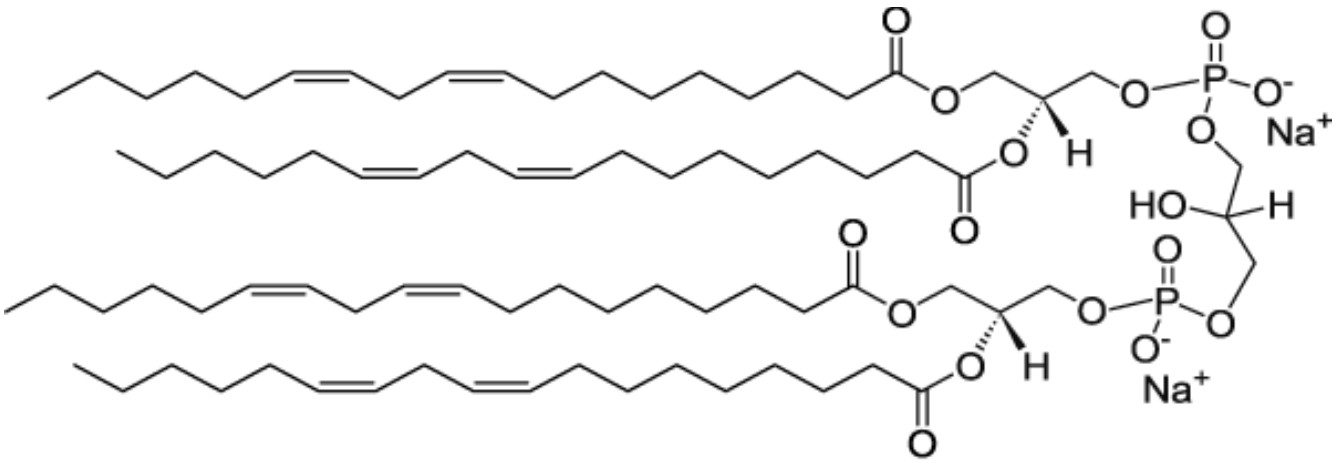
Murine macrophages Raw-Blue cells were stimulated for 16 hrs with LPS 100ng/mL alone (no antagonist) or co-incubated with C14:0 or heart cardiolipin .

Heart cardiolipin is an LPS competitive inhibitor



Unsaturated cardiolipins are able to inhibit the secretion of 2435pg/mL of TNF-alpha induced by 100ng/mL of LPS in THP-1 cells

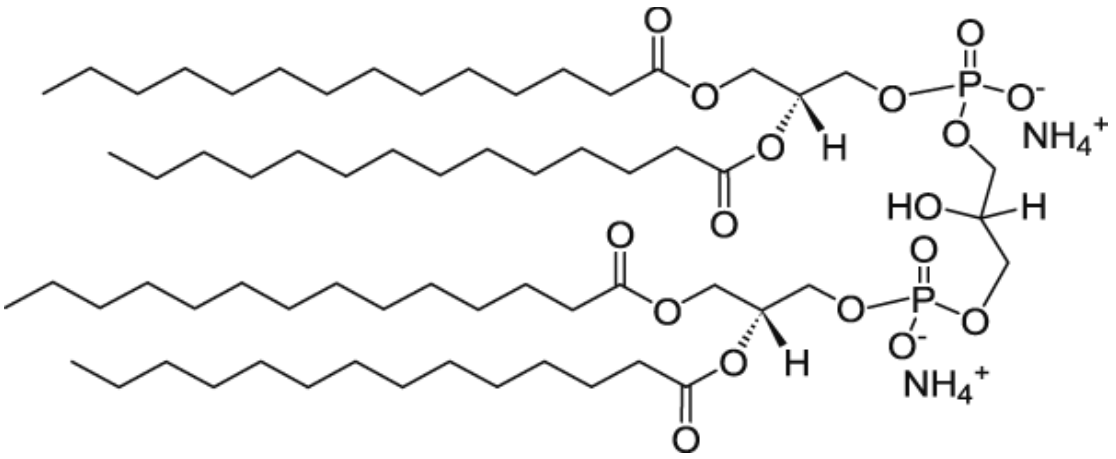
Unsaturated cardiolipin (heart) acts as a suppressor of TLR4-dependent immune response.



Structure of predominant species

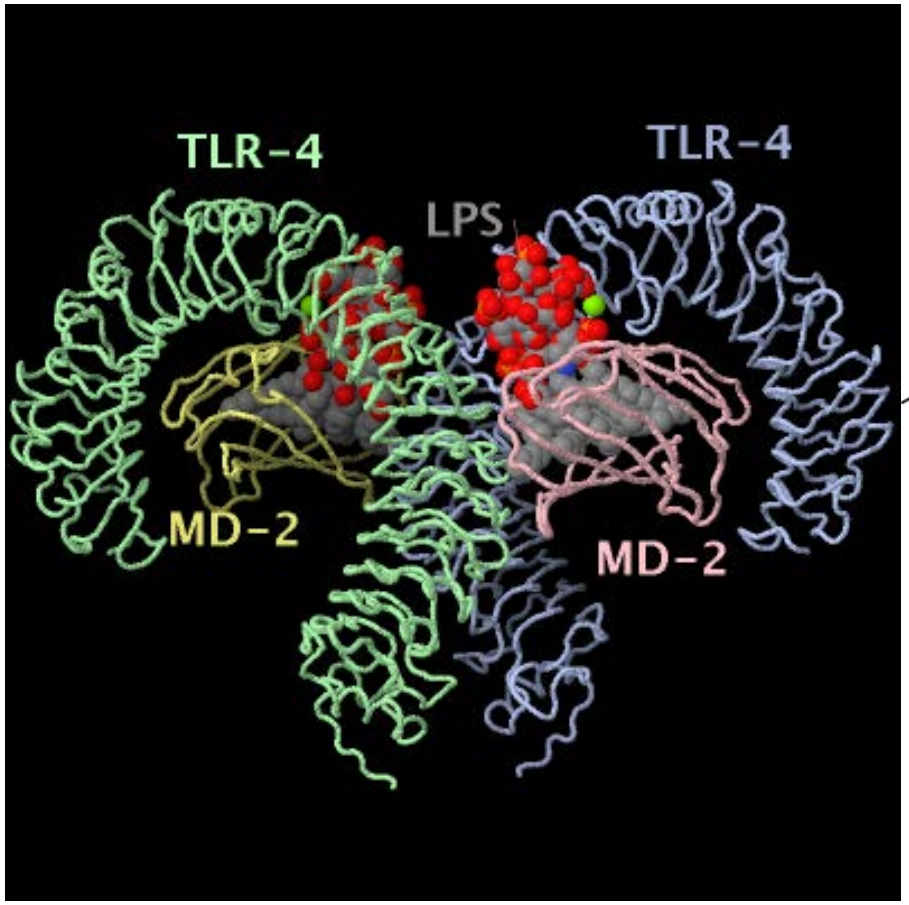
Our study extends the library of TLR4 ligands to molecules of easier synthesis, lower price and higher biocompatibility compared to the LPS-based structures.

-Saturated cardiolipin as an activator of the innate system like LPS

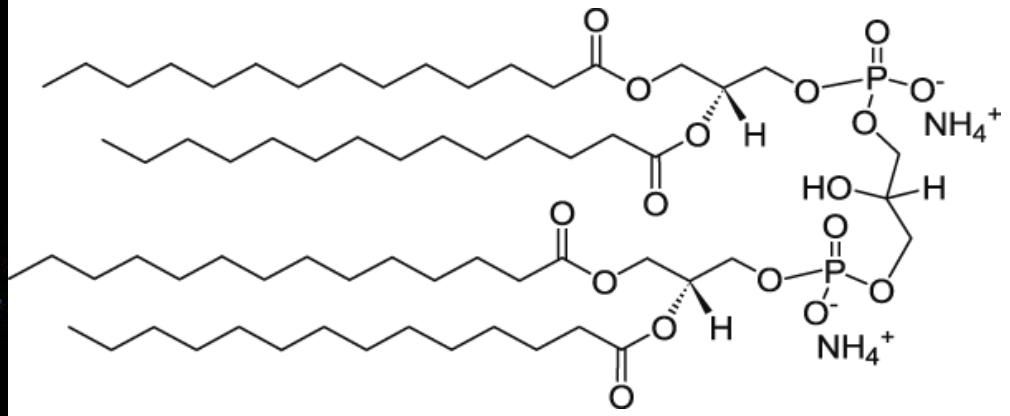


The cause of several diseases is a mutation in the enzyme that selects the fatty acids for the synthesis of cardiolipin. It results in a decrease of unsaturated CL synthesis and an increase of saturated one.These diseases are characterised by a severe inflammation state. Our results suggest that such cardiolipins act as inflammatory molecules in patients affected by this syndrome, giving more insights into the pathology of the disease

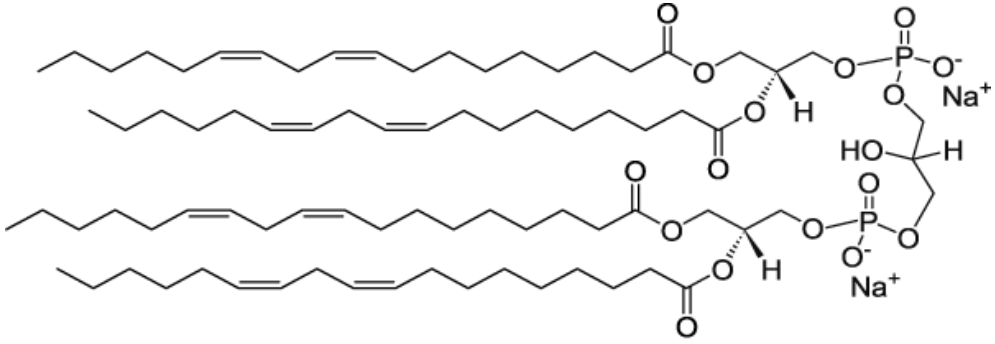
Cardiolipin from TLR4-antagonist to agonist, an unsaturation tale



AGONIST



ANTAGONIST



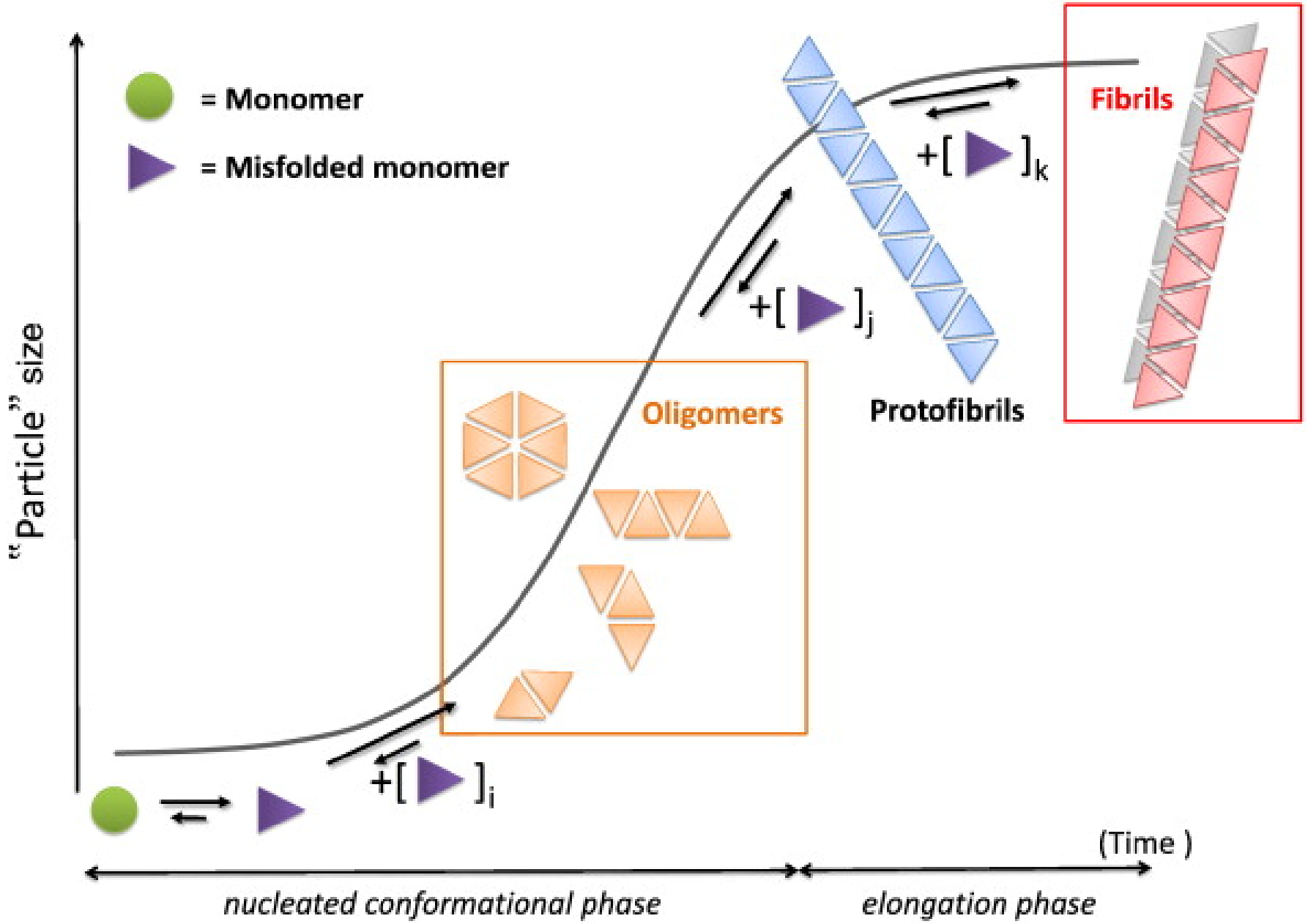
Structure of predominant species

Next?

X-Ray diffraction(in progress)

What about proteins aggregates?

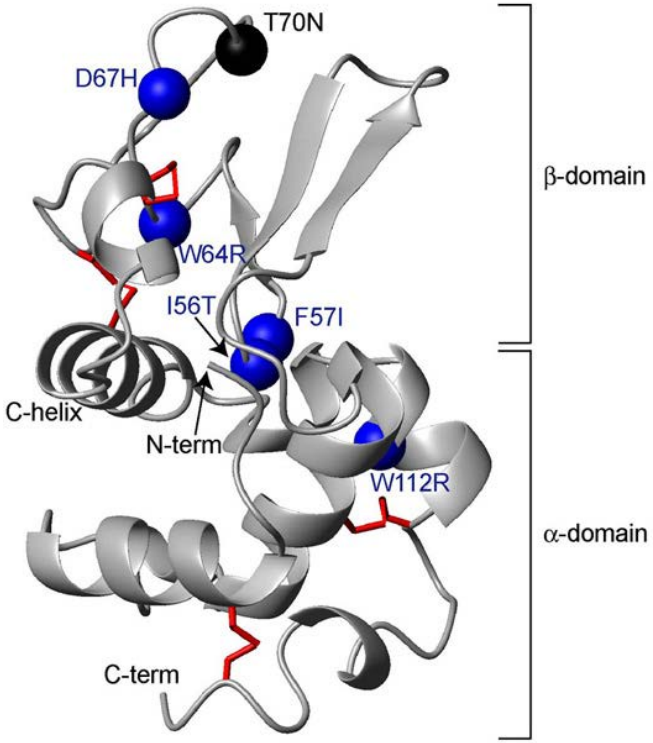
Do amyloid structures activate the innate system….???


= Monomer
= Misfolded monomer
Fibrils
+[ ]k
+[ ]j
Protofibrils
Oligomers
+[ ]i
"Particle" size
(Time)
nucleated conformational phase
elongation phase

Lysozyme systemic amyloidosis is a non-neuropathic hereditary disorder caused by the deposition of amyloid fibrils

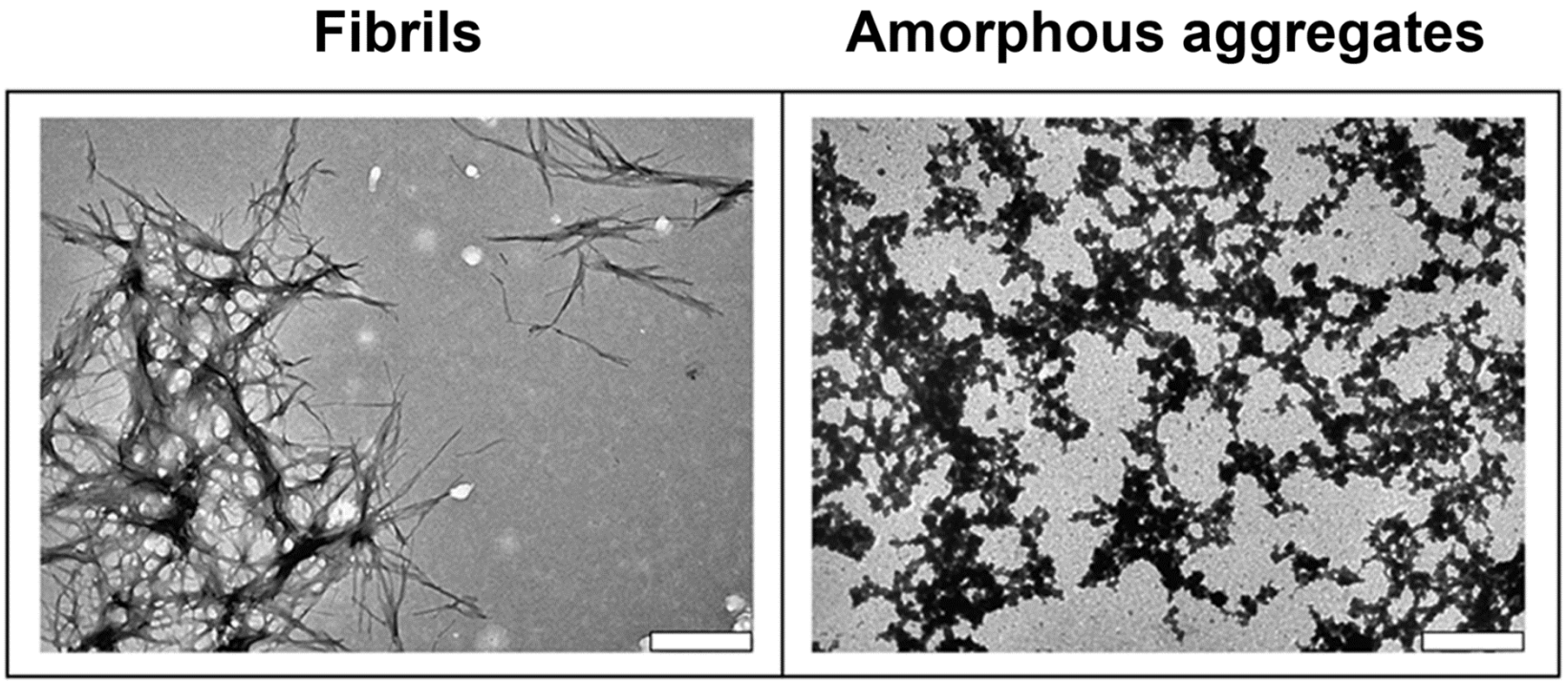
Dumoulin M, Kumita JR, Dobson CM (2006) Normal and aberrant biological self-assembly: insights from studies of human lysozyme and its amyloidogenic variants. Acc Chem Res 39:603–610

Human lysozyme



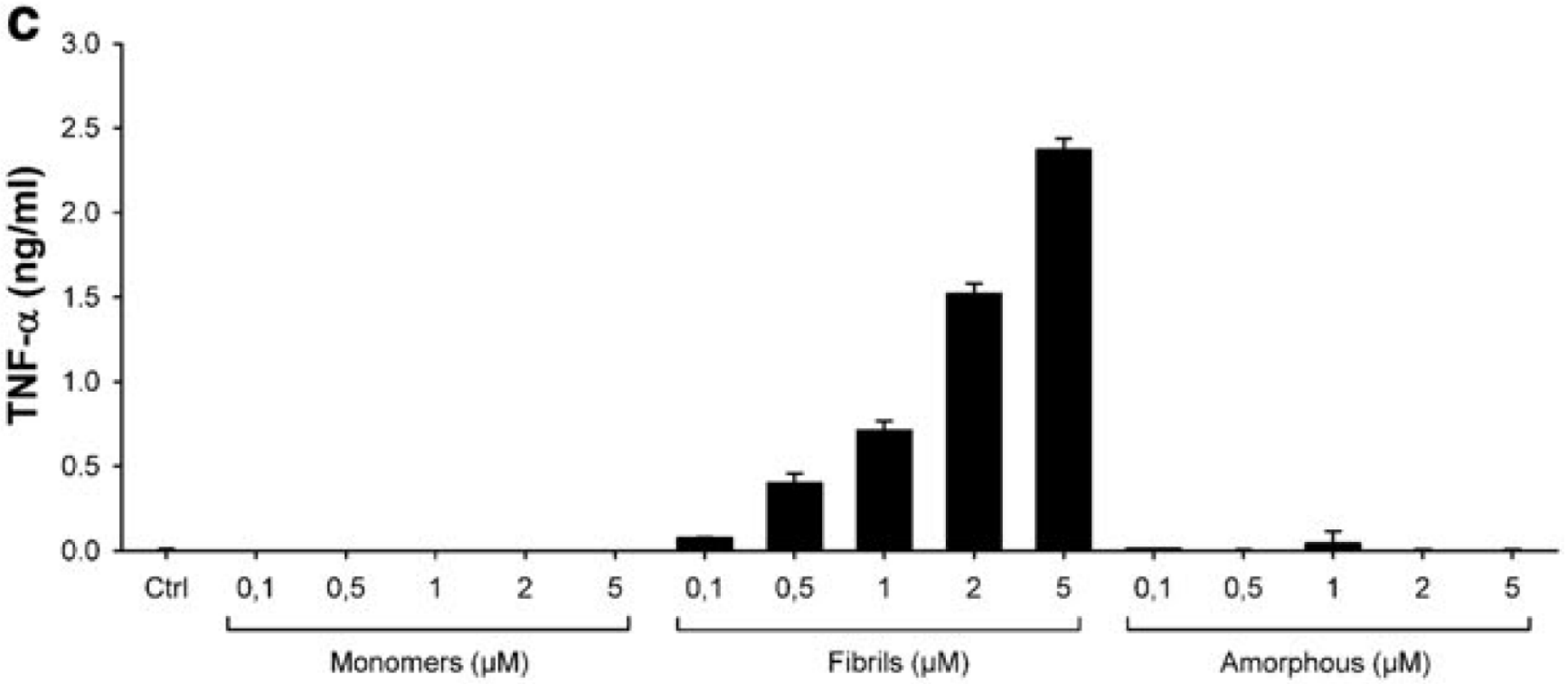
Structure of lysosyme in the different states(monomers,fibrils,aggregates)

Characterization of lysozyme species



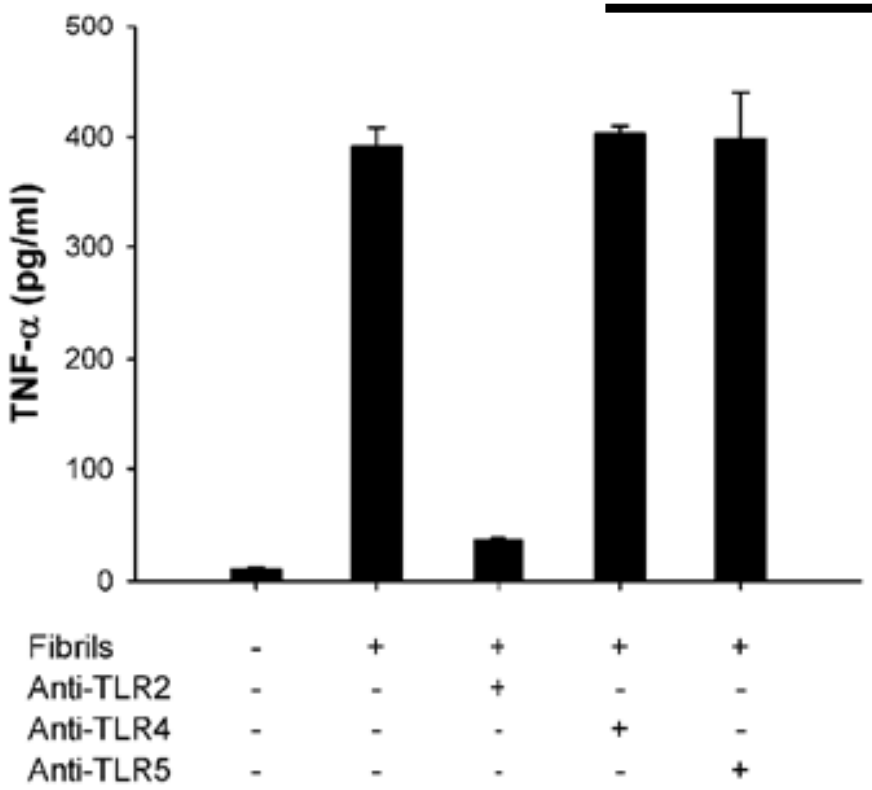
Negative stained TEM images of lysozyme fibrils (left) and amorphous aggregates (right). The scale bar represents 500 nm

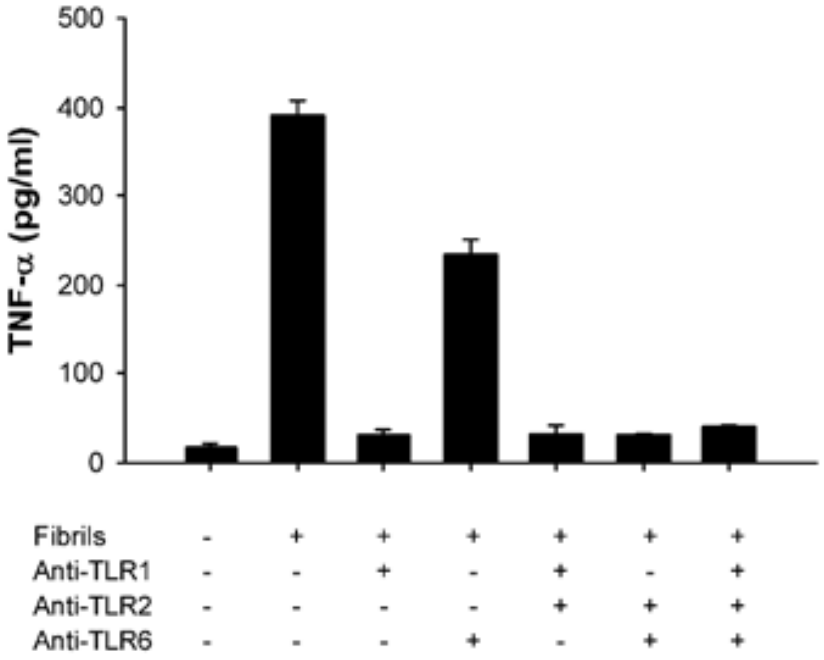
Lysozyme fibrils, but not amorphous aggregates, induce TNF secretion



THP1 cells were incubated for 6 hours with the indicated amounts of fibrils

Lysozyme fibrils activate TLR2/TLR1 heterodimer





THP1 cells were incubated for 6 h with 5 µM lysozyme fibrils in the presence or absence of 20 µg/ml anti-TLR2, anti-TLR4 or anti-TLR5 antibodies or 20 µg/ml anti-TLR2, anti-TLR1 or anti-TLR6 antibodies.

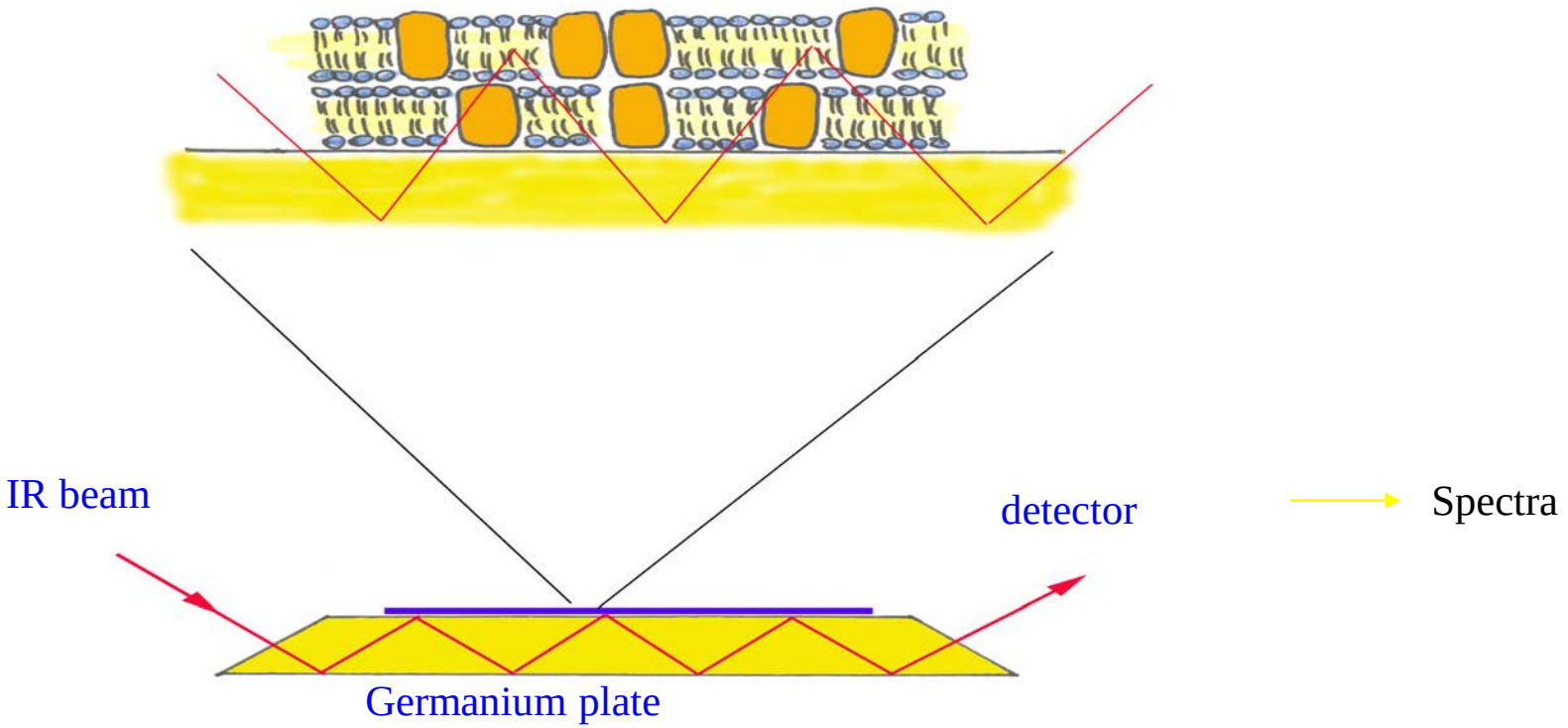
TNF-a was quantified in the cell supernatant by ELISA.

Is it a relationship between the activation of the innate system and the structure of the amyloid?

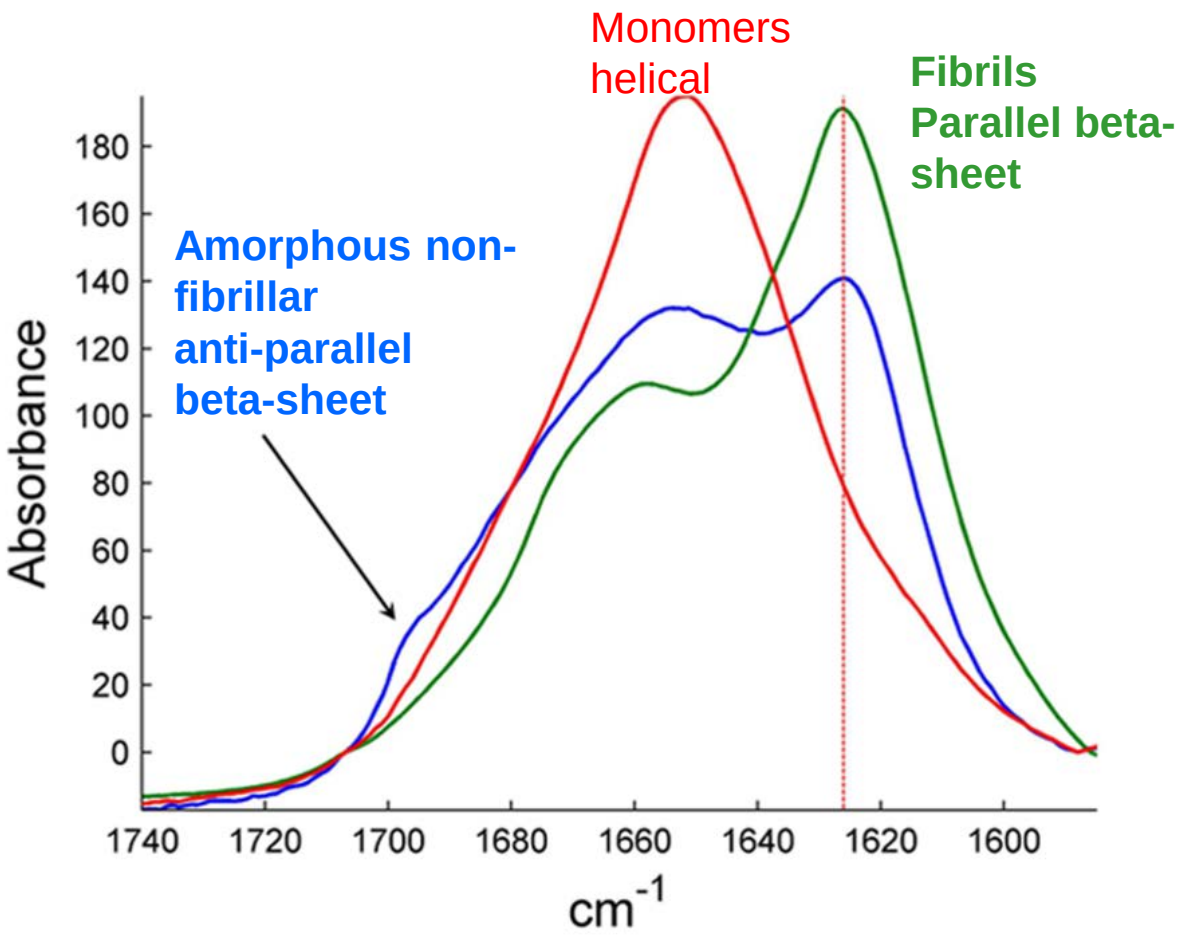
Attenuated Total Reflection IR Spectroscopy (ATR-IR) of proteins and lipids in biological membranes

- **Determination of secondary structures in a lipid environment (0.1 µg protein) and of protein aggregates**
  - **Fourier self-deconvolution**
  - **Curve fitting**
- **Tertiary conformational changes in membrane proteins. Hydrogen/deuterium exchange measurements.**
- **Orientation of the protein domains with respect to the lipid membrane. Polarised ATR-IR spectroscopy**
- **Reading of 2D-gels in terms of secondary structures.**

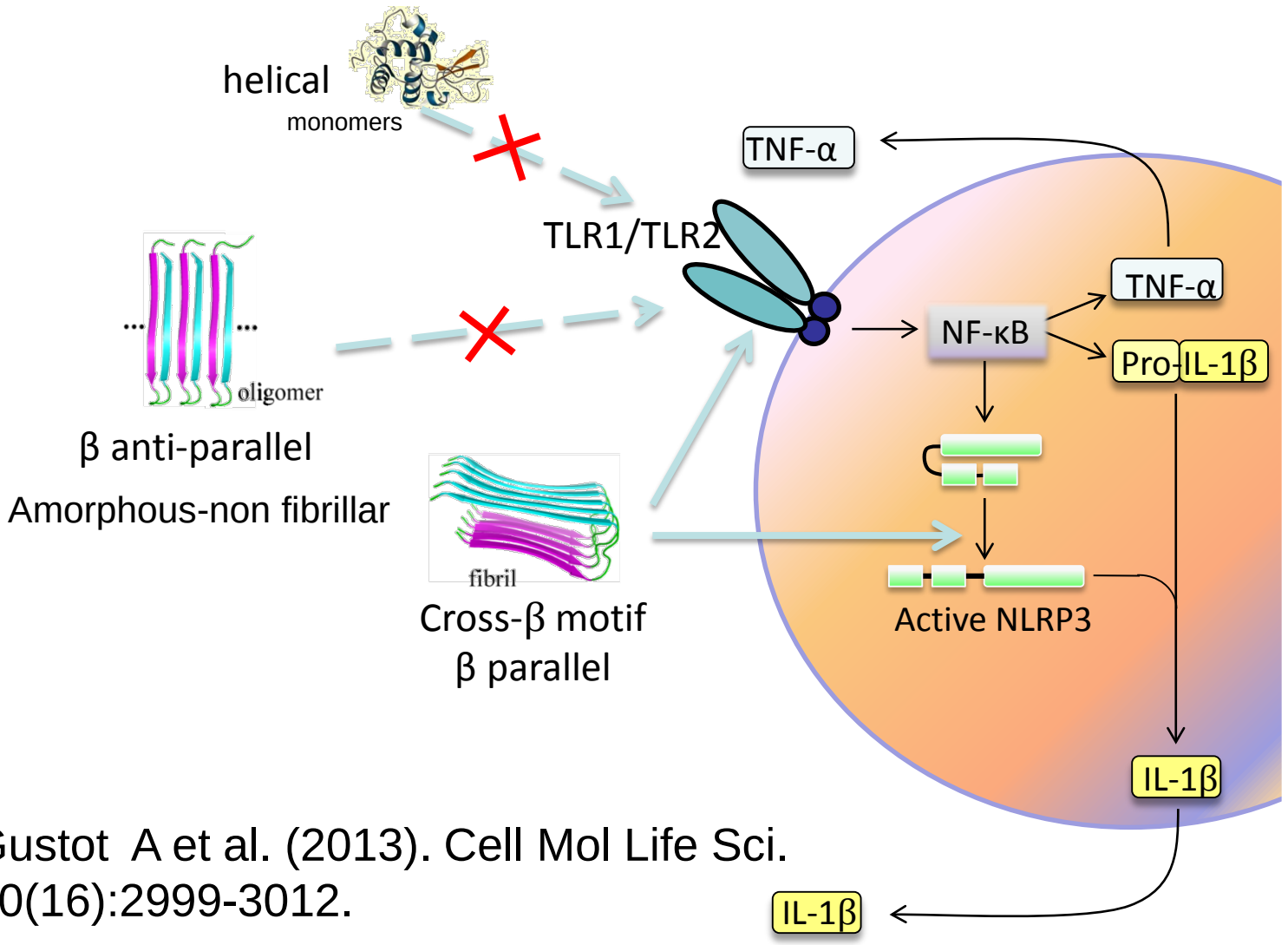
-Vigano C., Manciu L. and Ruysschaert J.-M., Acc. Chem. Res., 38(2): 117-126 Review (2005)
-Inda ME, Vandenbranden M, Fernández A, de Mendoza D, Ruysschaert JM, Cybulski L .Proc Natl Acad Sci U S A. 2014-111-3579-8
-Masureel M, Martens C, Stein RA, Mishra S,, Mchaourab HS, Govaerts C, Ruysschaert JM .Nat Chem Biol. 2014-149-55.



-Vigano C., Manciu .and Ruysschaert J.-M. Acc. Chem. Res 38-117-126 Review (2005)


Monomers
helical
Fibrils
Parallel beta-
sheet
Amorphous non-
fibrillar
anti-parallel
beta-sheet
Absorbance
180
160
140
120
100
80
60
40
20
0
1740
1720
1700
1680
1660
1640
1620
1600
cm-1

Recognition of cross-beta motifs by innate immune receptors

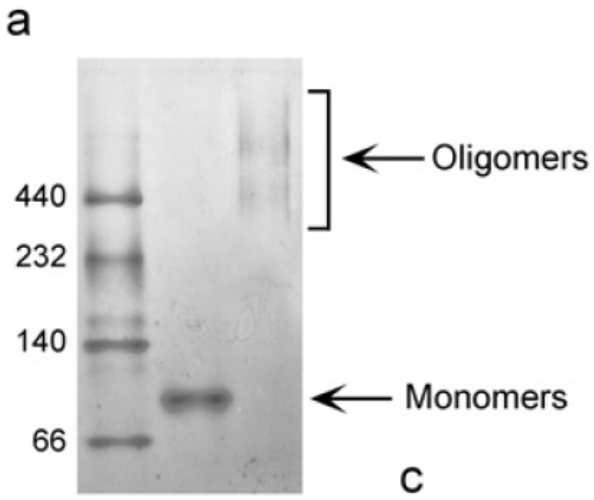


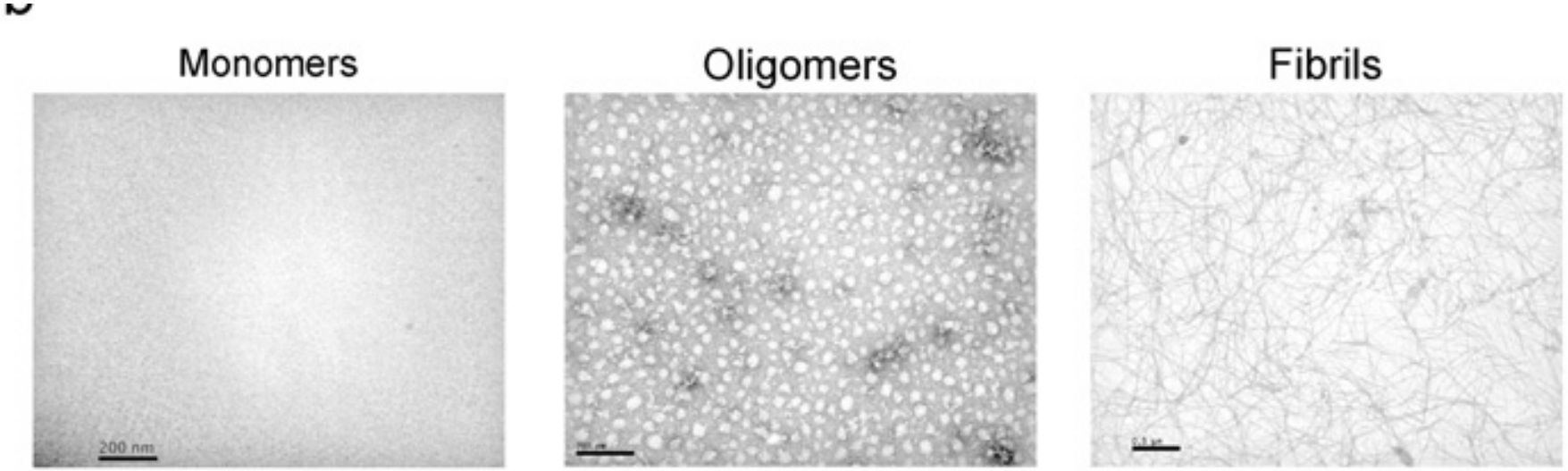
Gustot A et al. (2013). Cell Mol Life Sci. 70(16):2999-3012.

Parkinson disease

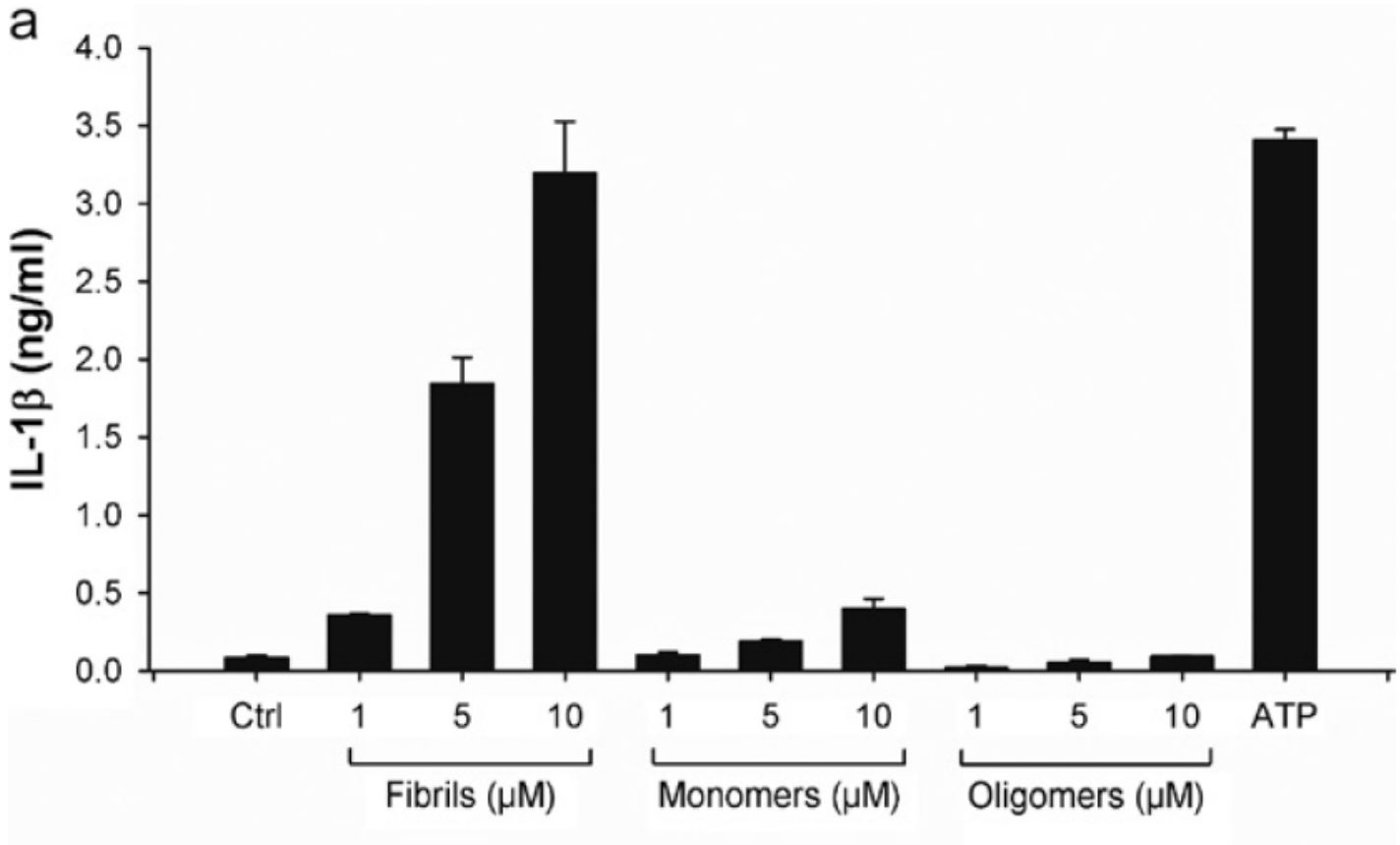
Gustot ,A et al Biochem.J-323-333-2015

Synuclein:Parkinson disease





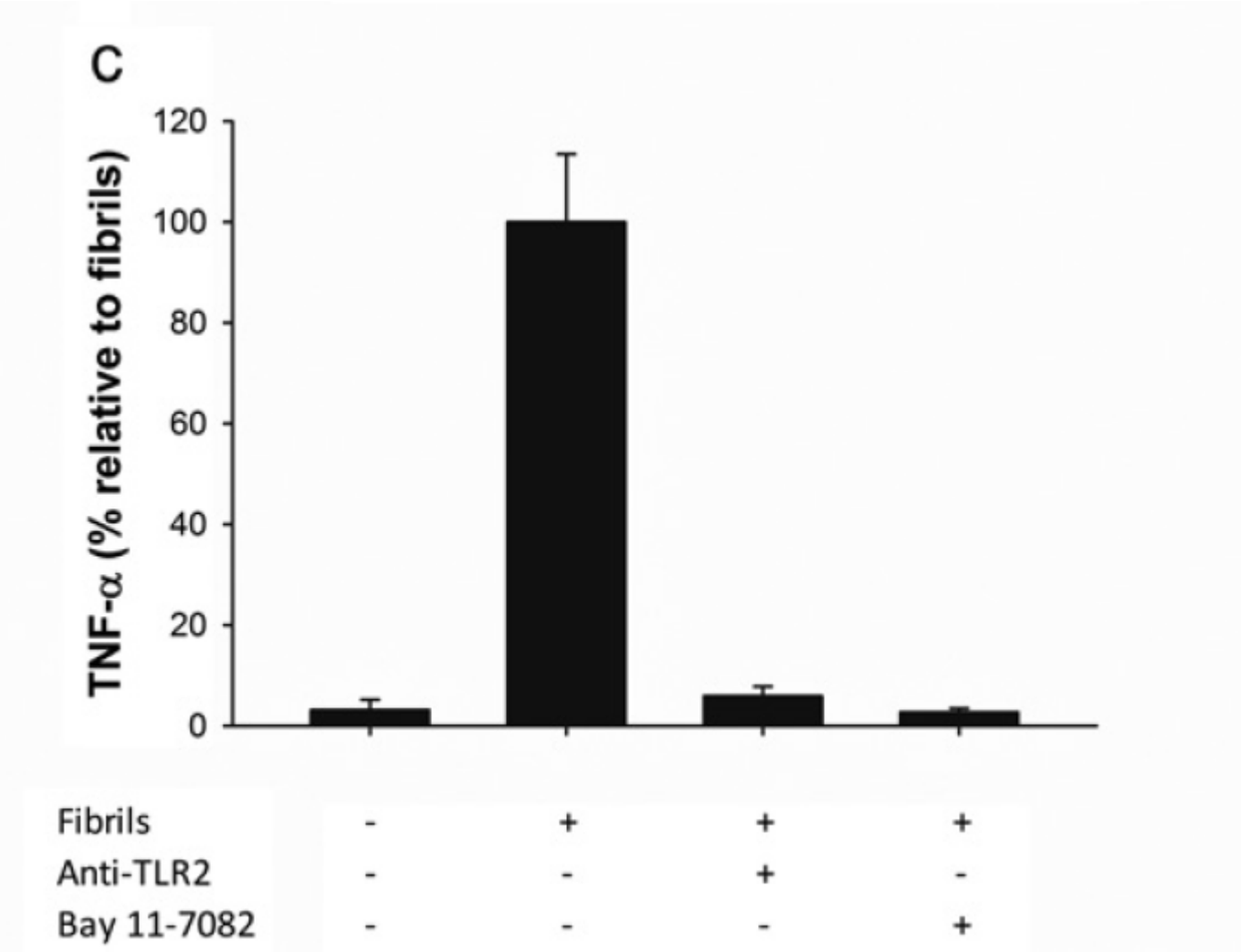
Induction of IL-1β secretion by α-syn requires the fibrillar state

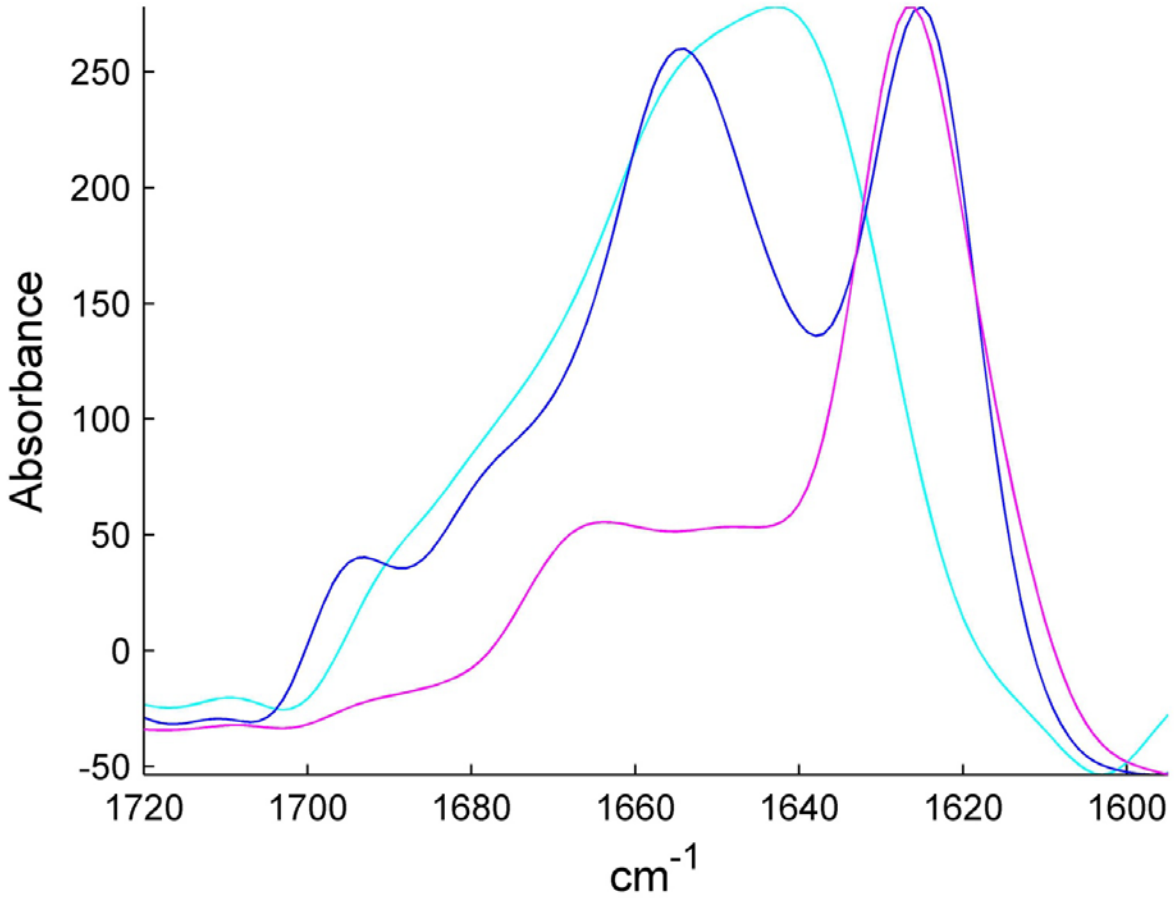


PMA-primed THP1 cells were incubated for 3 h with ATP (3 mM) or with the indicated amounts of α-syn fibrils, oligomers or monomers.

Gustot ,A et al Biochem.J-323-333-2015

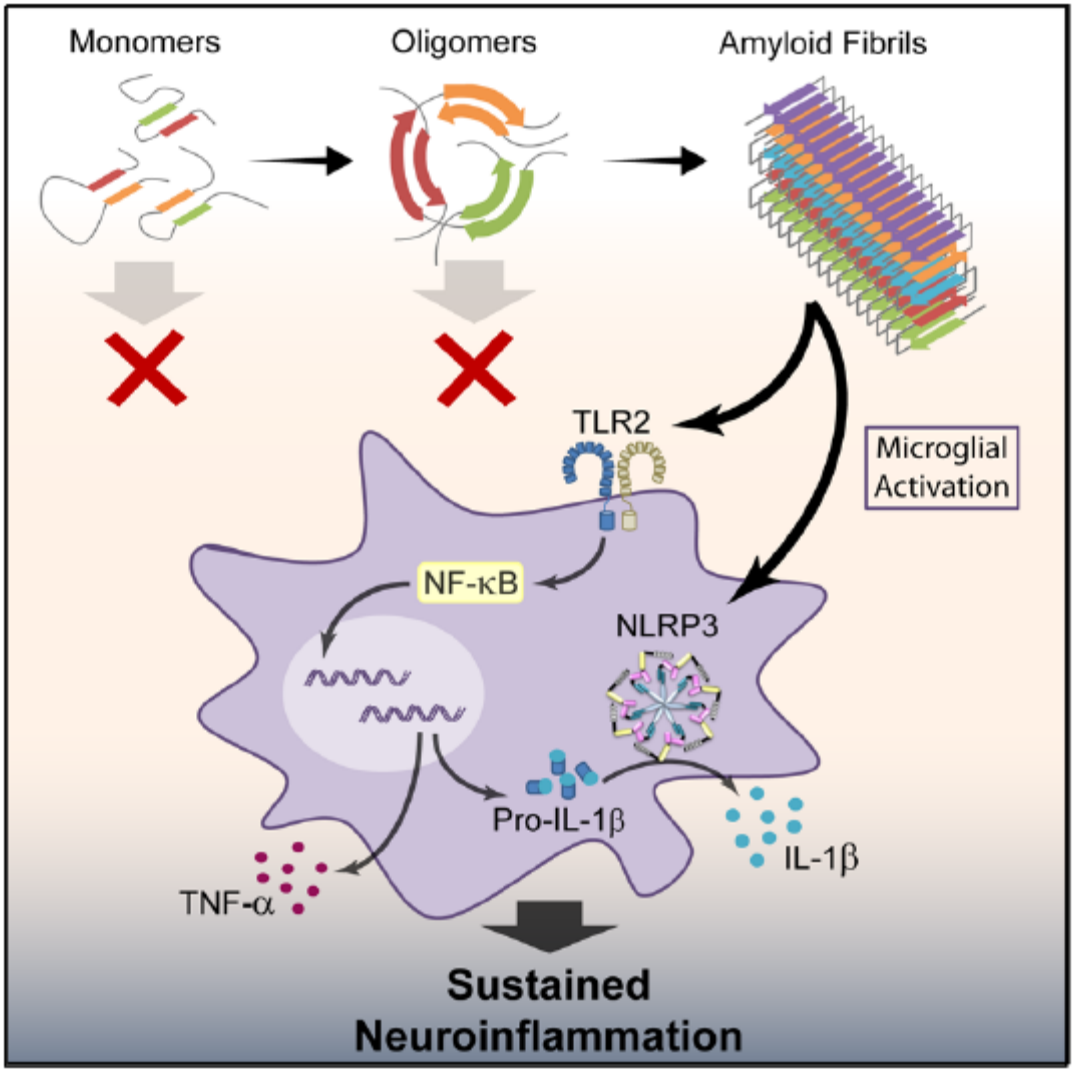
Induction of the NF-κB pathway by α-syn occurs through TLR2





IR spectra of *α*-syn monomers (light blue),oligomers (dark blue) and fibrils (red). Spectra were deconvoluted with a resolution enhancement factor K = 1.5 and scaled for identical amide I area (1711–1590 cm−1). The 1625 cm−1 peak is characteristic of *β*-sheets and the presence of an additional peak at 1695 cm−1 (arrow) is the spectral signature of antiparallel *β*-sheets

Synuclein aggregates-Parkinson disease



Gustot et al Biochem.J.2015

We show that induction of inflammatory responses by these amyloids is linked to their intrinsic structure not to a sequence

It is tempting to speculate that amyloid fibrils represent a new class of danger signals detected by the innate immune system, through sensing of their common cross-b structure that does not exist in any other proteins so far except in fibrils (neurodegenerative diseases,Parkinson,Alzheimer,…)

Importantly, persistent neuroinflammation, which is a well-defined feature of neurodegenerative diseases, actively contributes to disease progression.

Golenbock et al demonstrate strongly enhanced inflammatory activity in human brains of Alzheimer patients suggesting a role for the innate system in this neurodegenerative disease (Alzheimer,Parkinson..)

Understanding the molecular mechanisms by which amyloid deposits trigger inflammation might provide new clues to develop therapeutic strategies to combat these important diseases

Interestingly mice carrying mutations in inflammatory activation cascades components were largely protected from loss of spatial memory and other Alzheimer disease-associated symptoms.

NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice

Michael T. Heneka[1,2]*, Markus P. Kummer[1], Andrea Stutz[3], Andrea Delekate[4], Stephanie Schwartz[1], Ana Vieira-Saecker[1], Angelika Griep[1], Daisy Axt[1], Anita Remus[4], Te-Chen Tzeng[5], Ellen Gelpi[6], Annett Halle[7], Martin Korte[4,8], Eicke Latz[2,3,5]* & Douglas T. Golenbock[5]*

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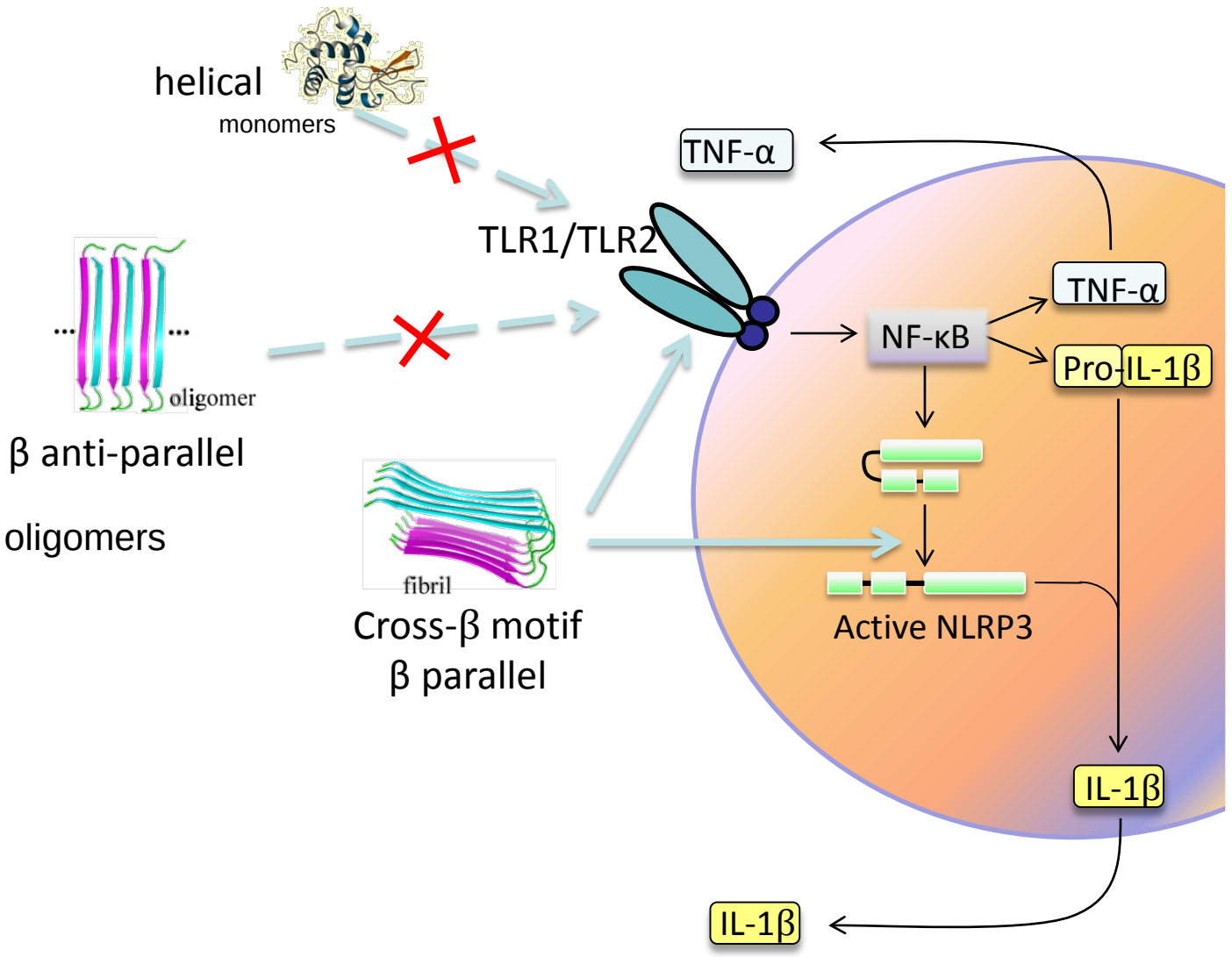
Recognition of cross-beta motifs by innate immune receptors



Gustot A et al. (2013). *Cell Mol Life Sci. 70(16):2999-3012.*

Message

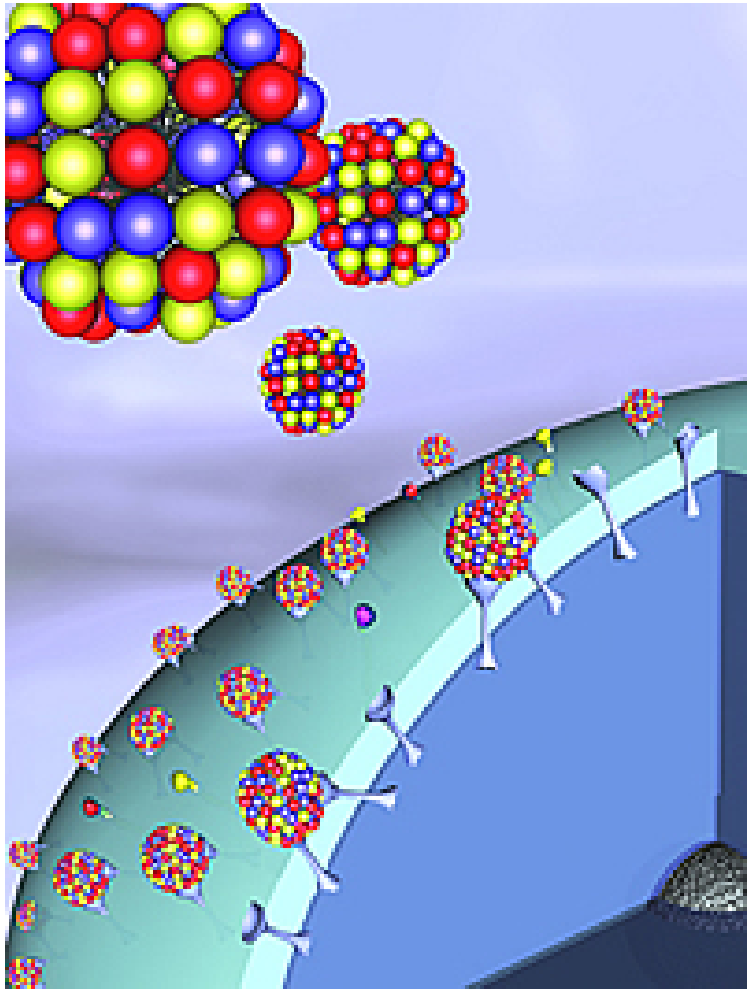
A therapeutic that blocks the activity of the inflammatory process might effectively interfere with the progression of Alzheimer disease

Non bacterial ligand can activate the innate immunity

These inflammatory reactions can be desired (for vaccine development), **unwanted** (for delivery applications) **or involved in the induction of non-infectious diseases** (cardiovascular, autoimmune, allergic diseases, cancer, diabetes, amyloidoses, prion-related diseases, or pneumoconioses). For that reason, **development of new molecules targeting or inhibiting these inflammatory responses may lead to therapeutic perspectives largely unintended until now**.

Is activation induced by molecules from bacterial,viral,fungal origine only?

TLR: the Swiss army knife of immunity



natural nanoparticles (silica particles, asbestosis, cholesterol crystals,amyloid aggregates)

engineered nanoparticles (fullerenes, gold nanoparticles, polymers, cationic liposomes)

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Georg Pabst
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END