

Forschungszentrum Borstel, Leibniz-  
Zentrum für Medizin und Biowissenschaften  
Biophysik



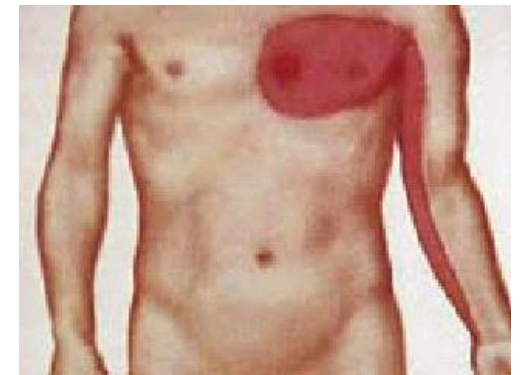
Martin-Luther-Universität  
Halle/Wittenberg  
Physikalische Chemie

# Die Geschichte mit dem rostigen Nagel und der Blutvergiftung...

## Lipopolysaccharide und das endotoxische Prinzip



Patrick Garidel  
und  
Klaus Brandenburg



# Erregerspektrum bei Sepsis

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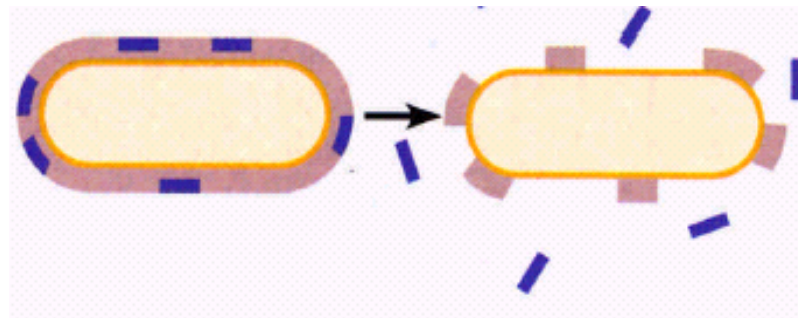
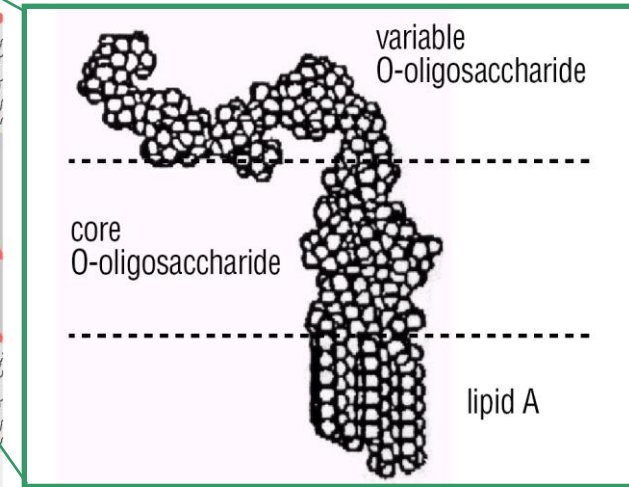
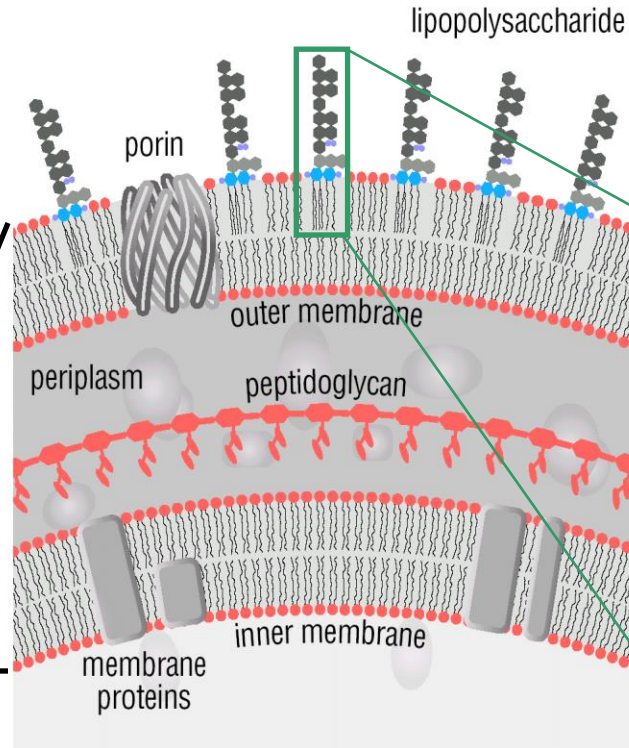
- Bakterien: gram + und gram -
- Pilze
- Viren
- Protozoen



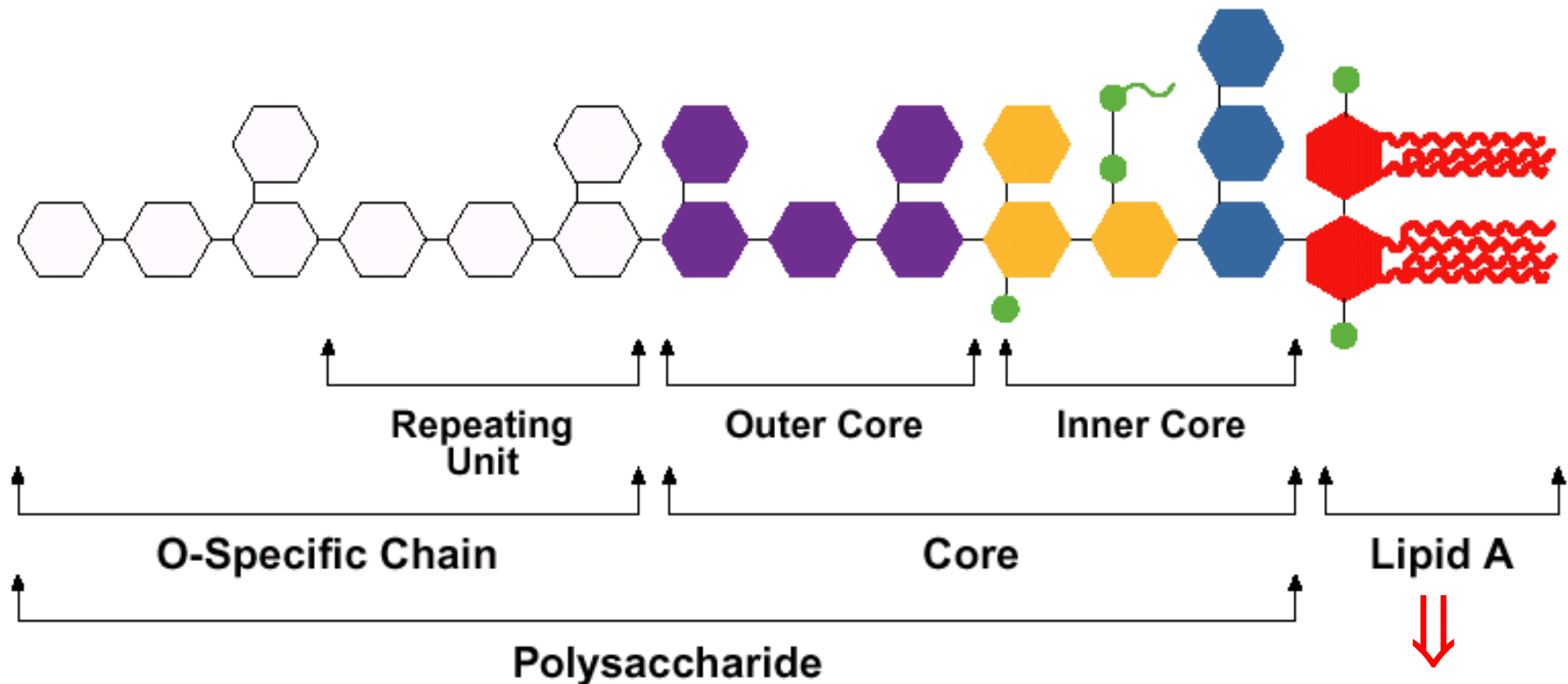
Drei gefürchtete Sepsis-Erreger. Von links: **Pseudomonas aeruginosa**, ein Gram-negatives Bakterium; **Streptococcus pneumoniae**, ein Gram-positives Bakterium; **Aspergillus fumigatus**, ein Pilz

# Bakterielle Endotoxine und LPS

## Gram-negativ Bakterien



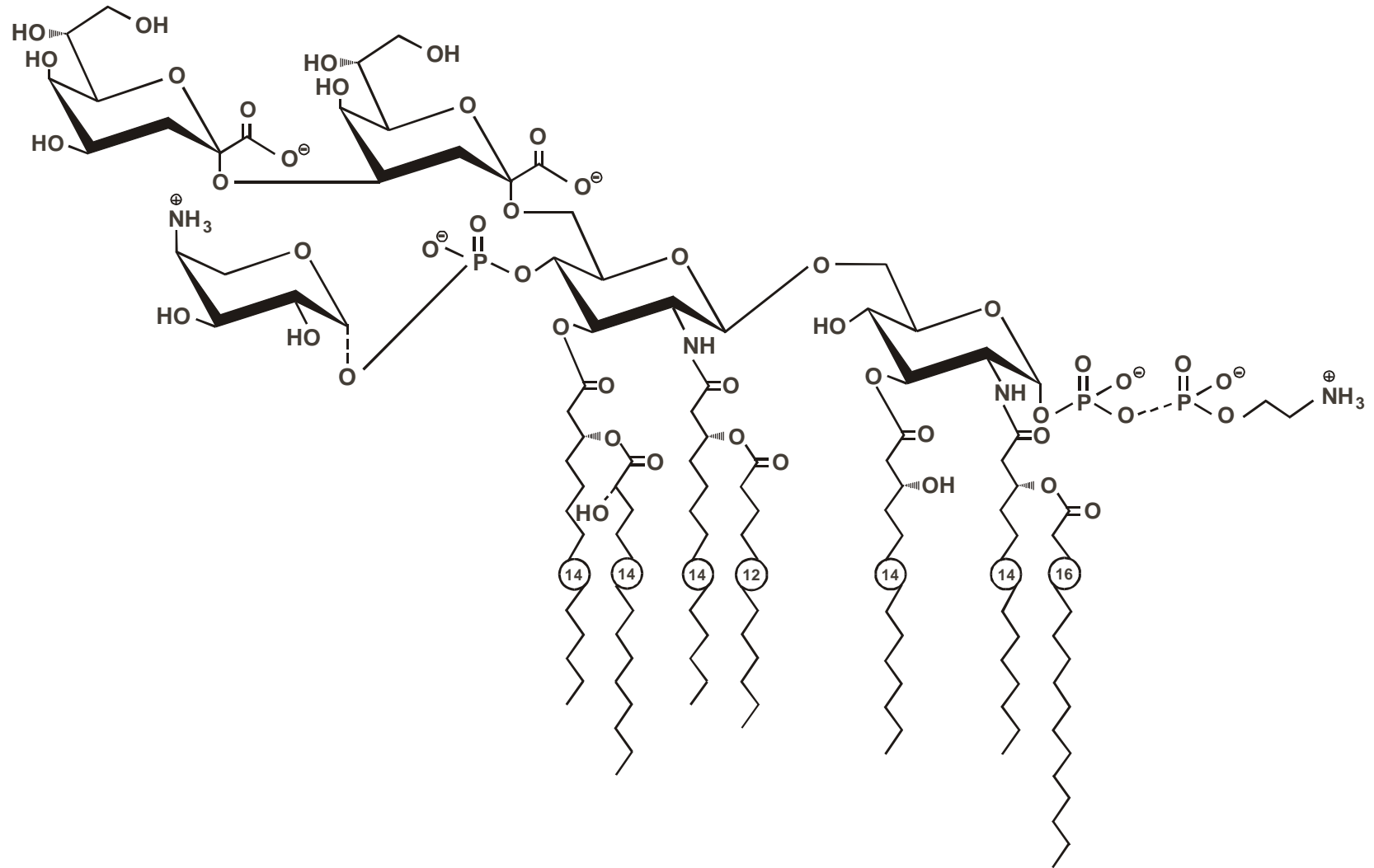
# Bakterielles Lipopolysaccharid (LPS)



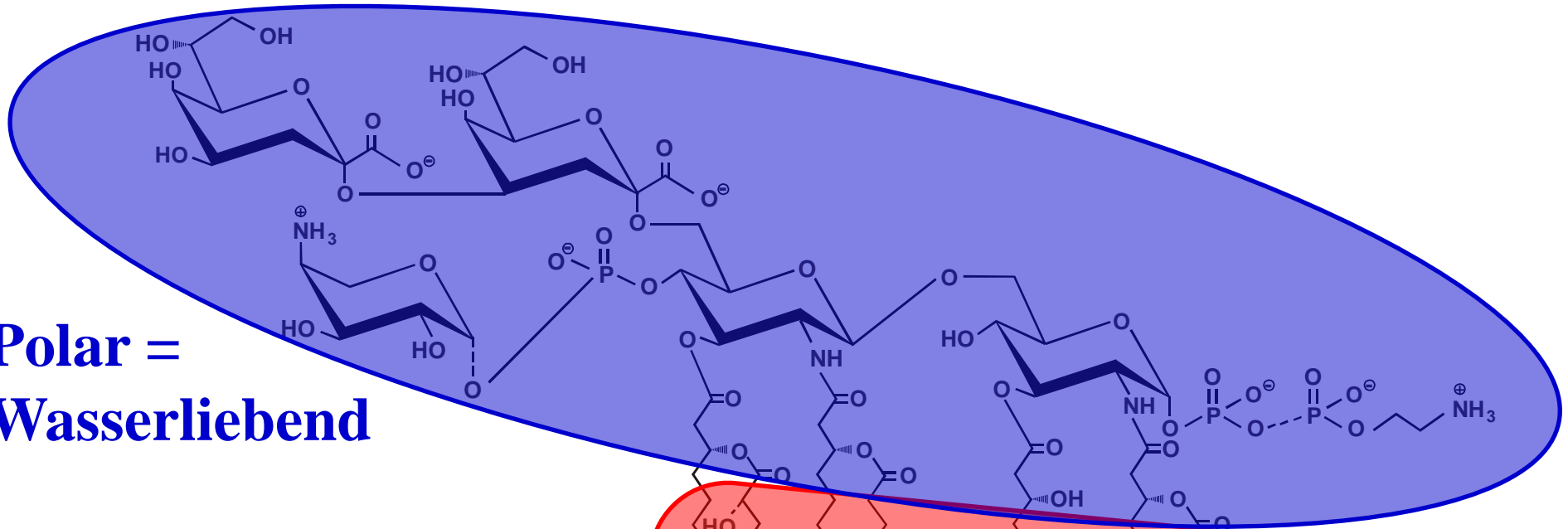
**Endotoxische Prinzip**

→ In der äusseren Zellwand von Gram-negativen Bakterien  
(Enterobacteriaceae und Pseudomonadaceae)

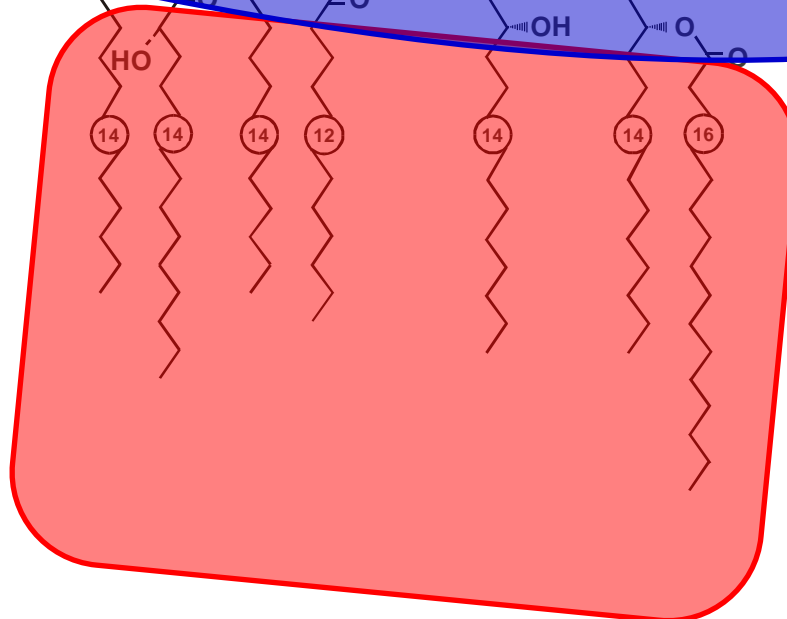
# LPS: *Salmonella minnesota*



# LPS: *Salmonella minnesota*



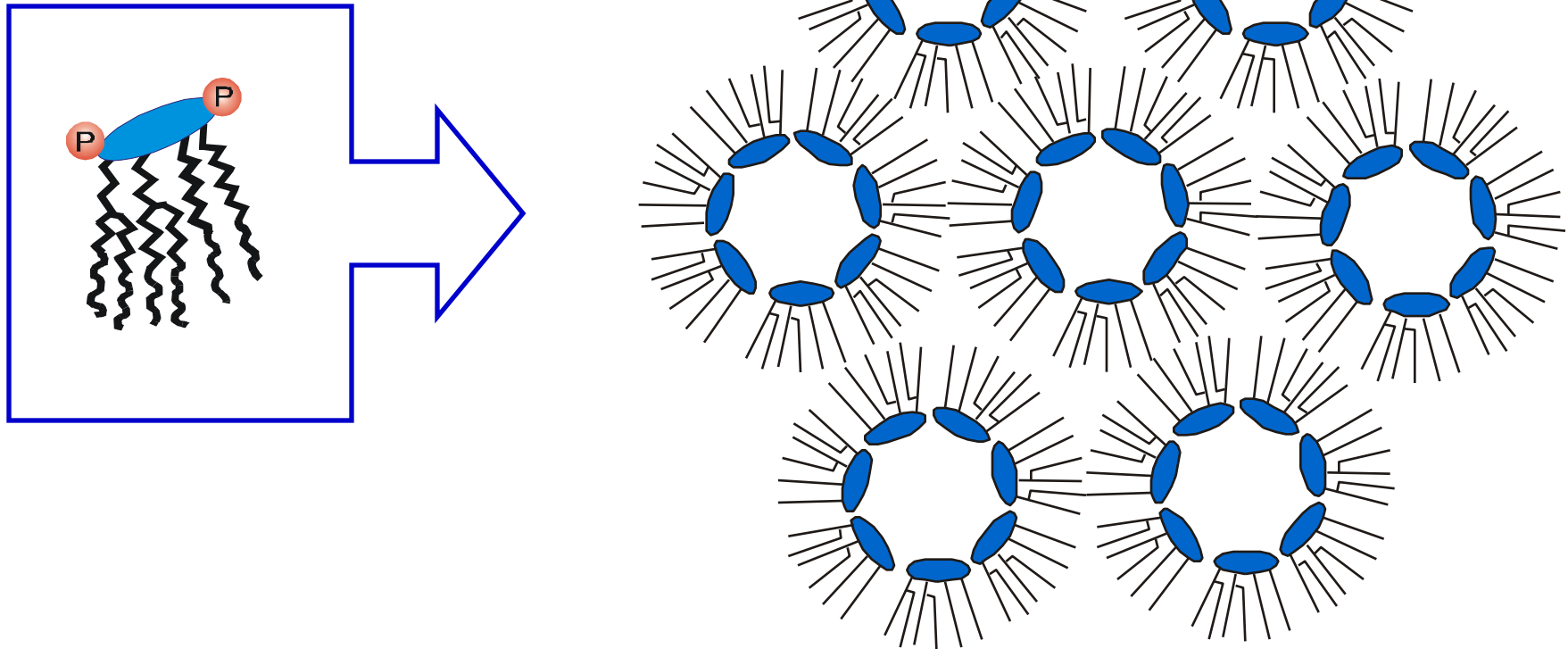
**Polar =  
Wasserliebend**



**Apolar =  
Wasserabweisend**

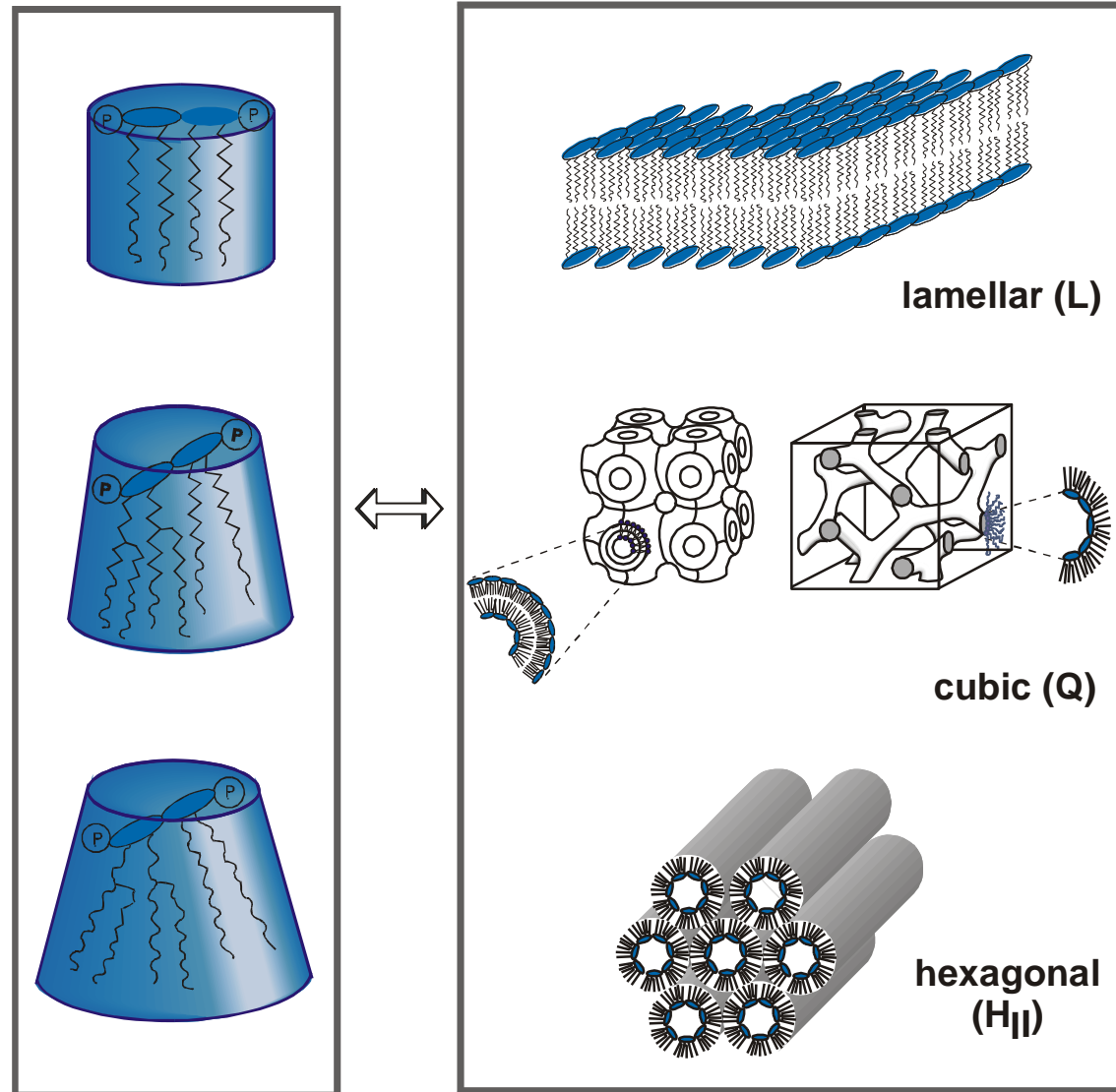
# Aggregatbildung

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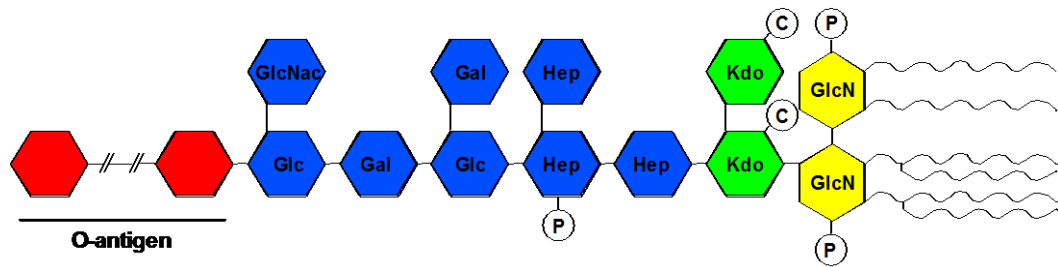


# Beziehung zwischen Molekülgeometrie und Supramolekularer Struktur

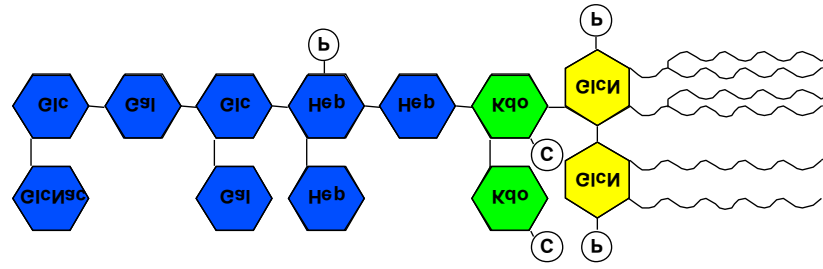


→ Packungskonzept nach Israelachvili

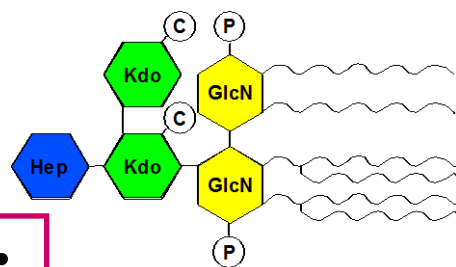




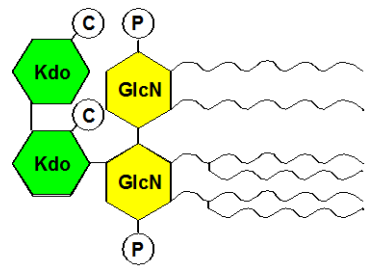
**LPS S-form / wild-type**



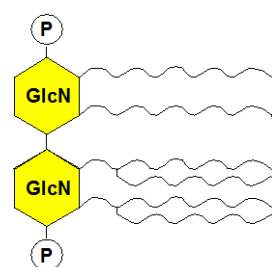
**LPS Ra / R60**



**LPS Rd2 / R4**



**LPS Re / R595**



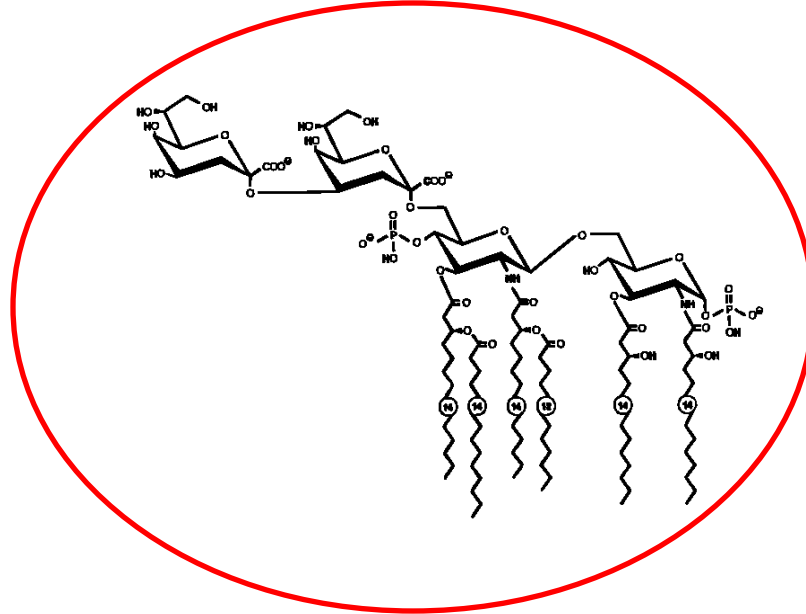
**Lipid A**

**Zusätzliche Variationen:**

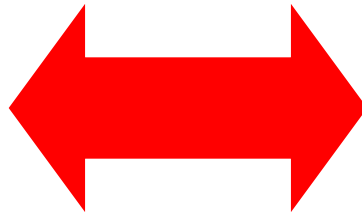
- Anzahl an KW-ketten
- Länge der KW-ketten
- Sättigungsgrad
- Verknüpfung
- Ladung (Phosphatgruppen)

# Beziehung Struktur versus Bioaktivität

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**Biophysikalische  
Untersuchungen**



**Biologische Assays  
(TNF- $\alpha$ , IL, Hämolyse,  
LAL etc)**

# Aggregatbildung und biologische Aktivität

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Untersuchungen an **synthetischen Lipid A Analoga**

→ Biophysikalische Charakterisierung

→ Beziehung zwischen Molekülgeometrie und Bio-Aktivität

# Biophysikalische Untersuchungen an LPS

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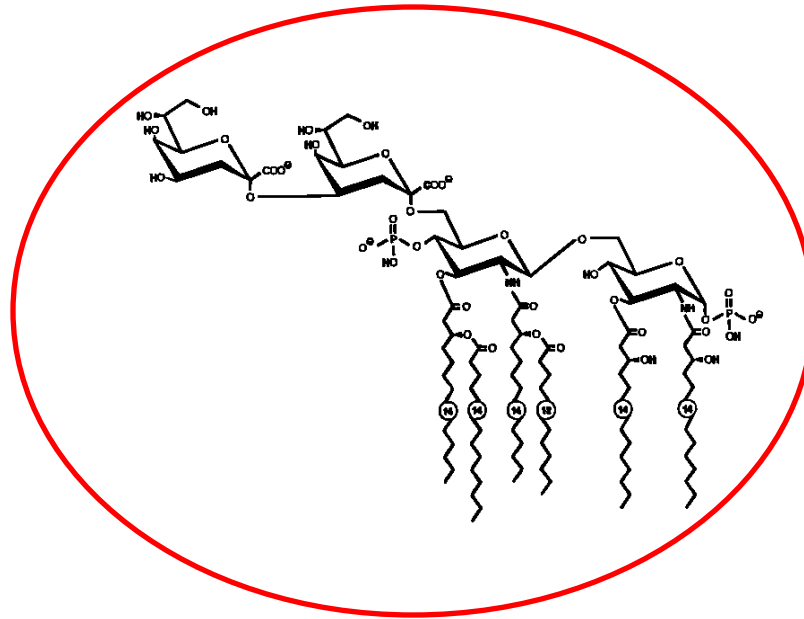
**Spektroskopie**  
(FTIR, CD, Fluoreszenz)

**Filmwaagetechneken**  
(FW, BAMS, Patch-Clamp)

**Morphologie**  
(AFM, FF-EM)

**Ladung**  
(Zeta-Potential)

**Kalorimetrie**  
(DSC, ITC)

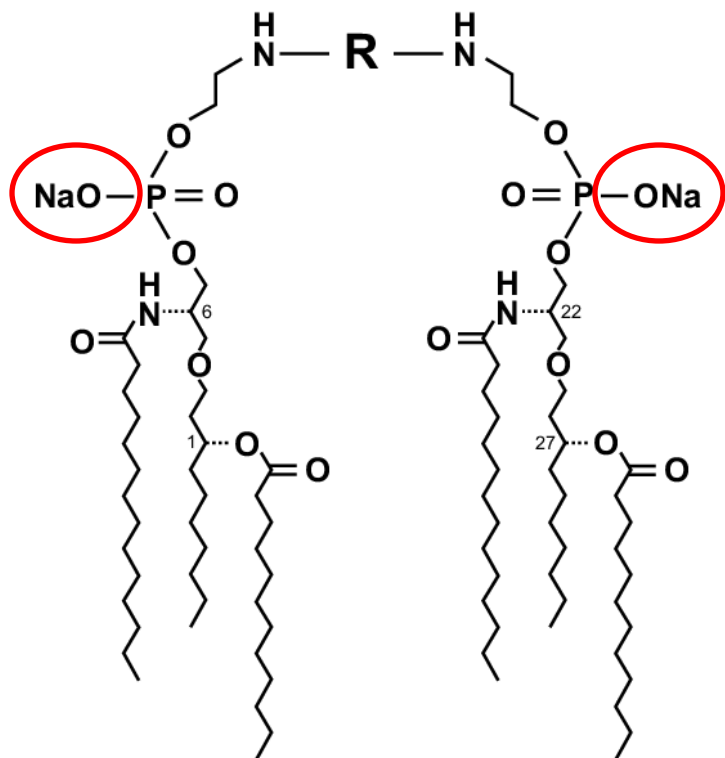


**MALDI-TOF**

**Streuung**  
(RALS, PCS)

**X-ray**  
(SAXS, WAXS)

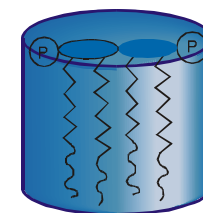
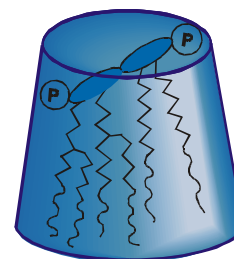
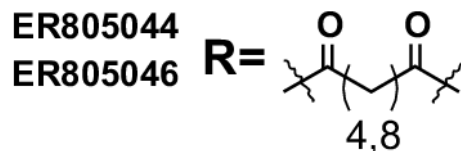
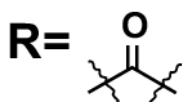
# Untersuchungen an synthetischen Lipid A Analoga



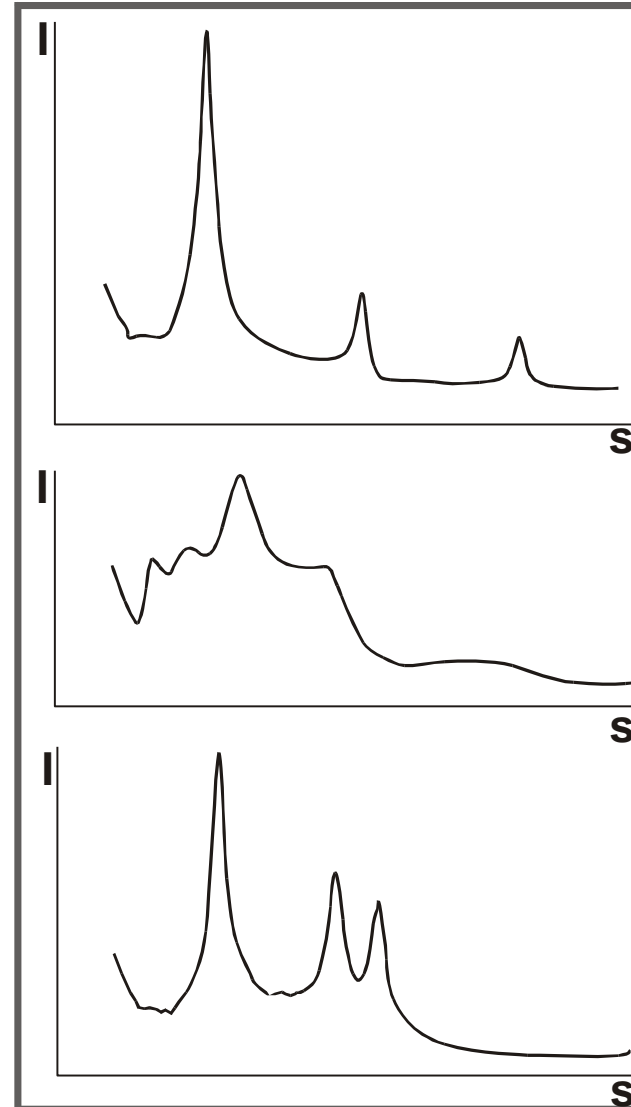
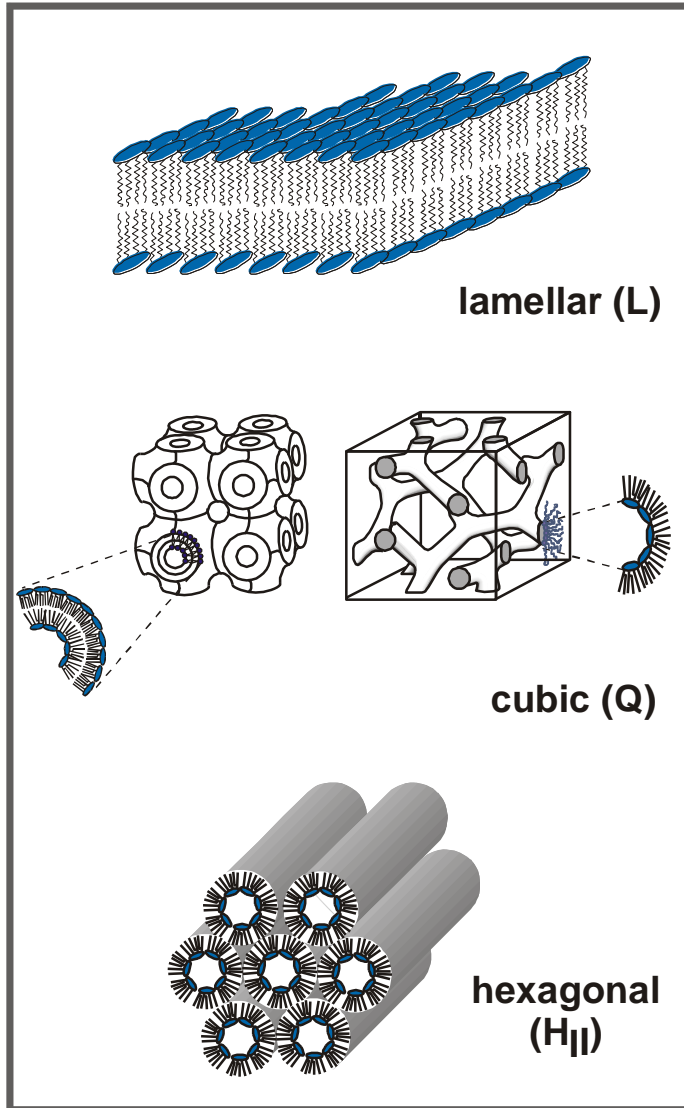
Compd.	<i>n</i>
ER-	<i>n</i>
805044	4
805046	8

→ Verschiedene Spacerlänge  
 → Verschiedene Stereochemie

ER803022  
 ER803732  
 ER805256  
 ER805259



# X-Ray (SAXS) und Supramolekulare Struktur



Verhältnisse  
der  
Reflexpositionen

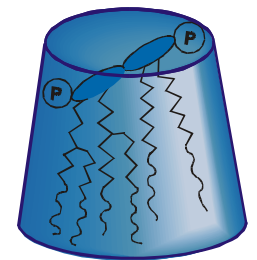
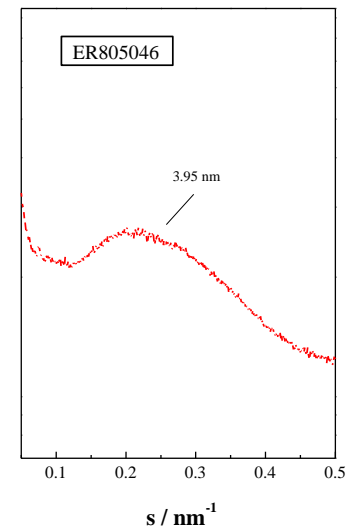
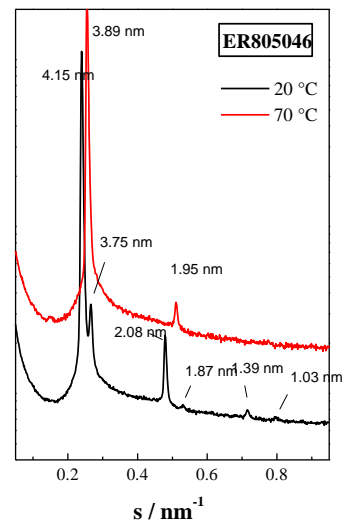
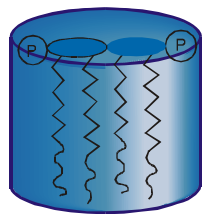
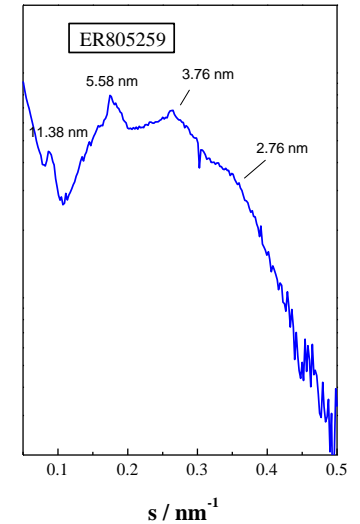
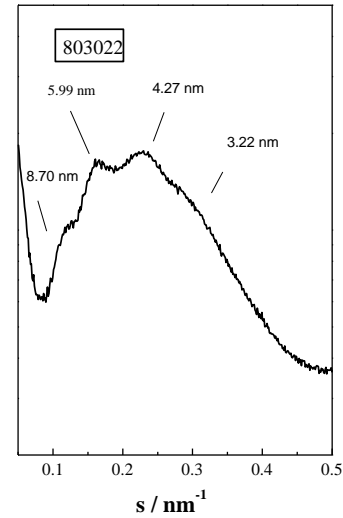
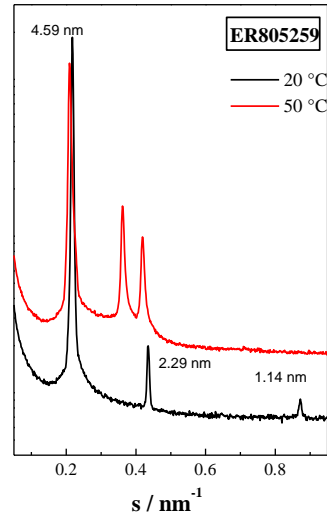
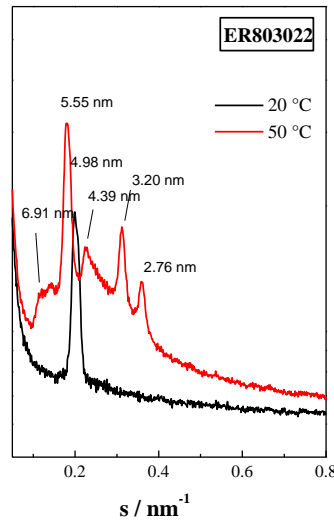
1, 2, 3, 4, 5, ...

1,  $\sqrt{2}$ ,  $\sqrt{3}$ , 2,  $\sqrt{5}$  ...

1,  $\sqrt{3}$ , 2,  $\sqrt{7}$ , 3 ...

# Untersuchungen an synthetischen Lipid A Analoga

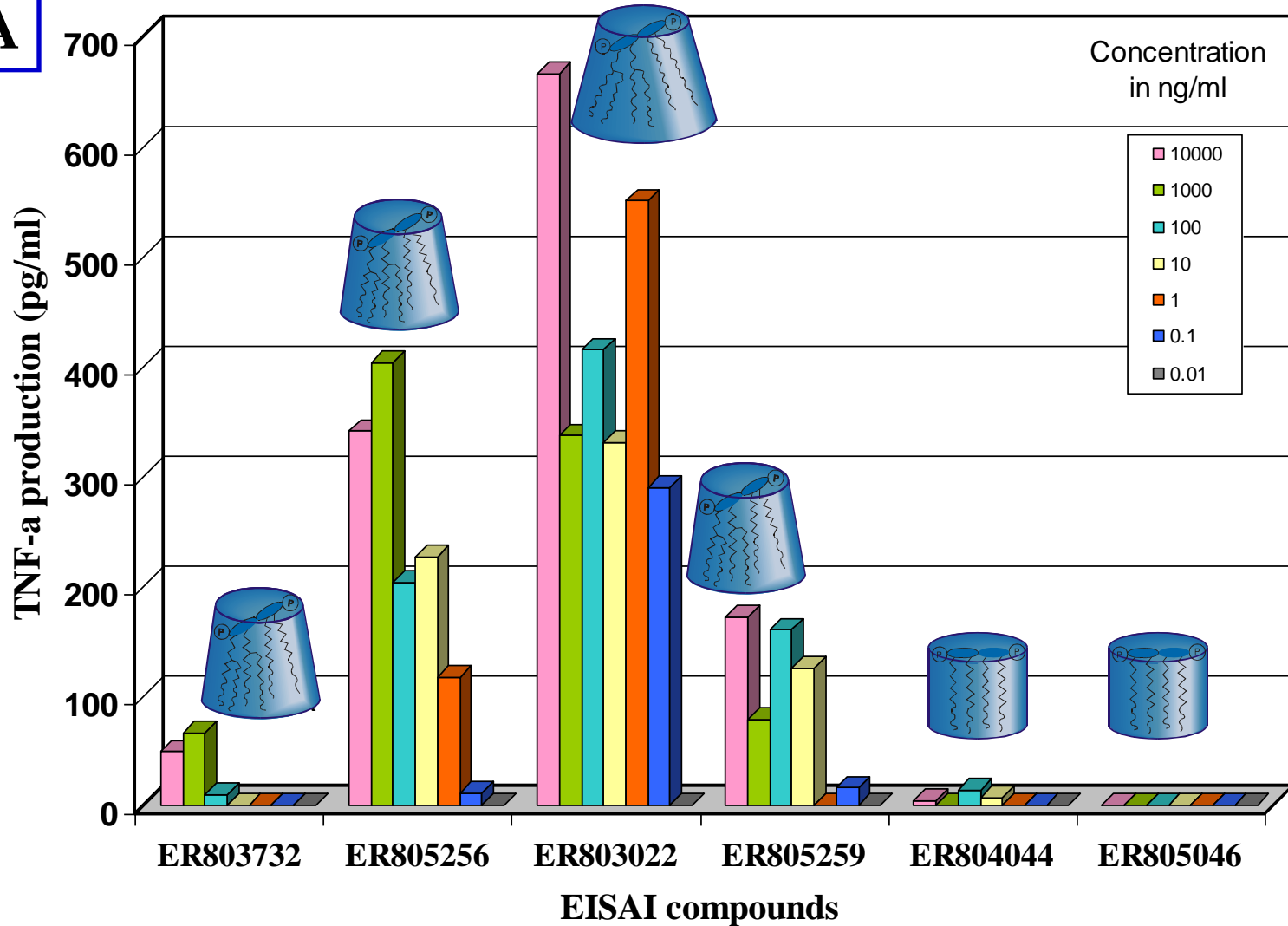
## SAXS





# Untersuchungen an synthetischen Lipid A Analoga

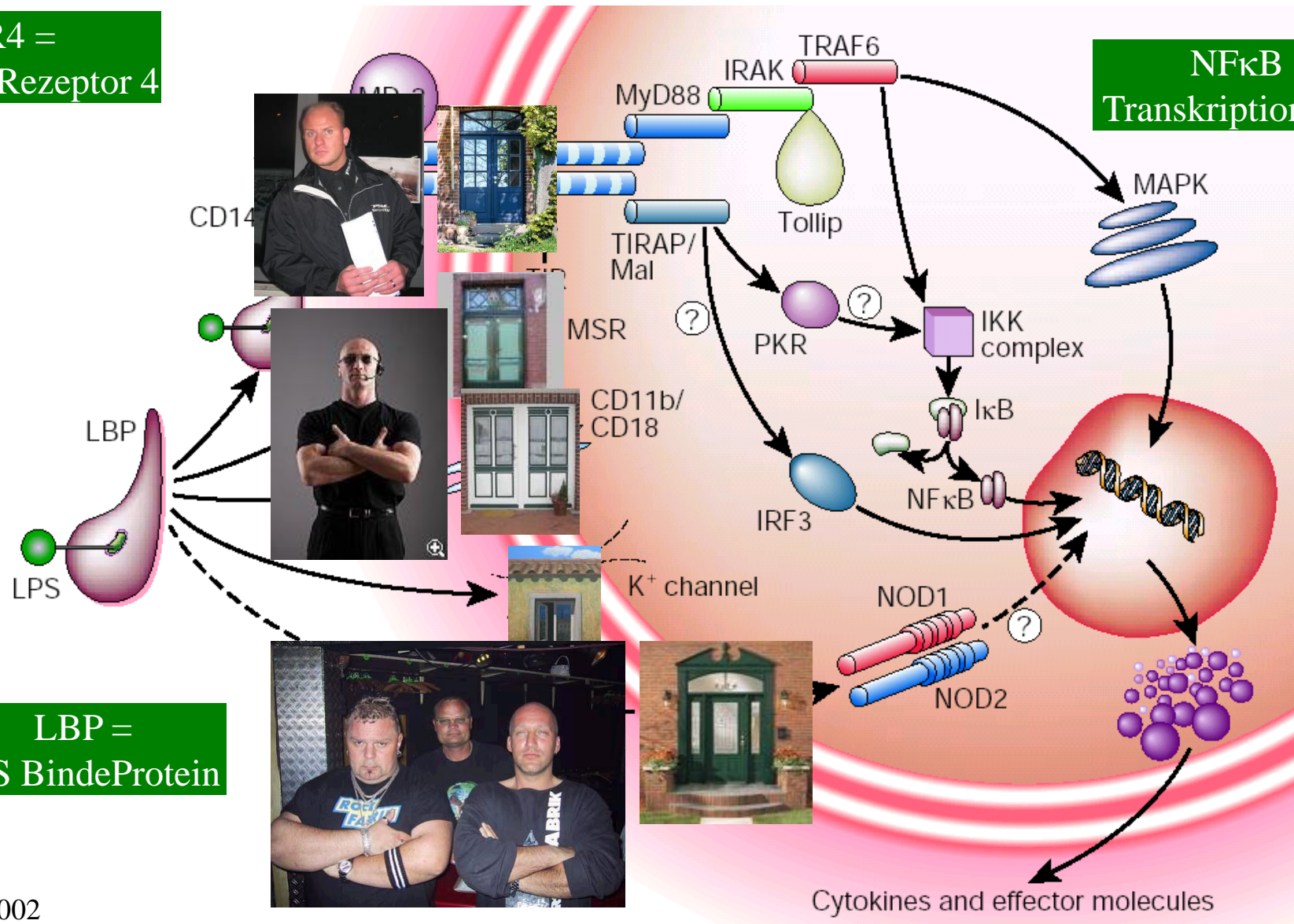
ELISA



# Endotoxin: Signalerkennung und -transduktion

TLR4 =  
Toll-Like-Rezeptor 4

NFκB =  
Transkriptionsfaktor



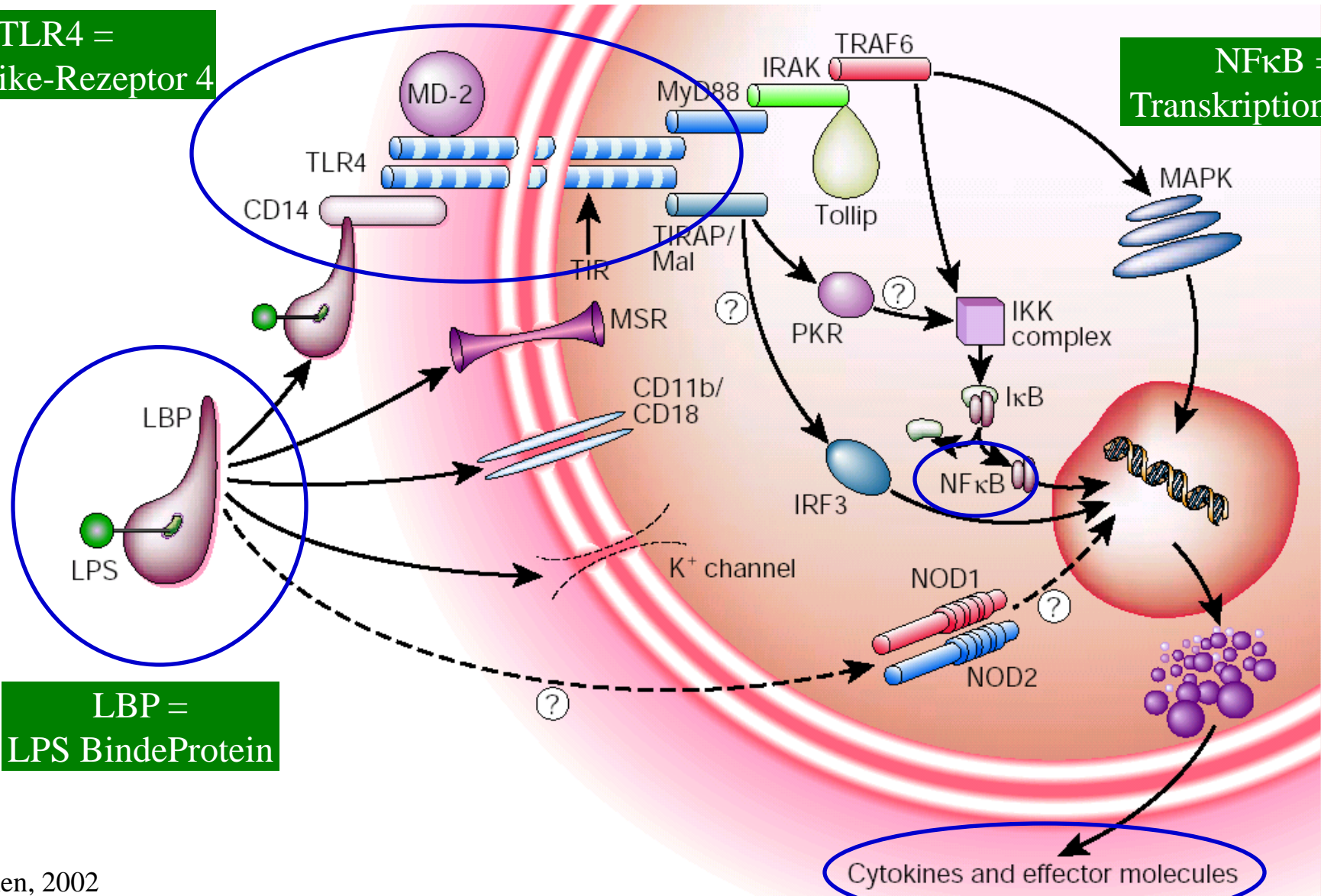
LBP =  
LPS BindeProtein



# Endotoxin: Signalerkennung und -transduktion

TLR4 =  
Toll-Like-Rezeptor 4

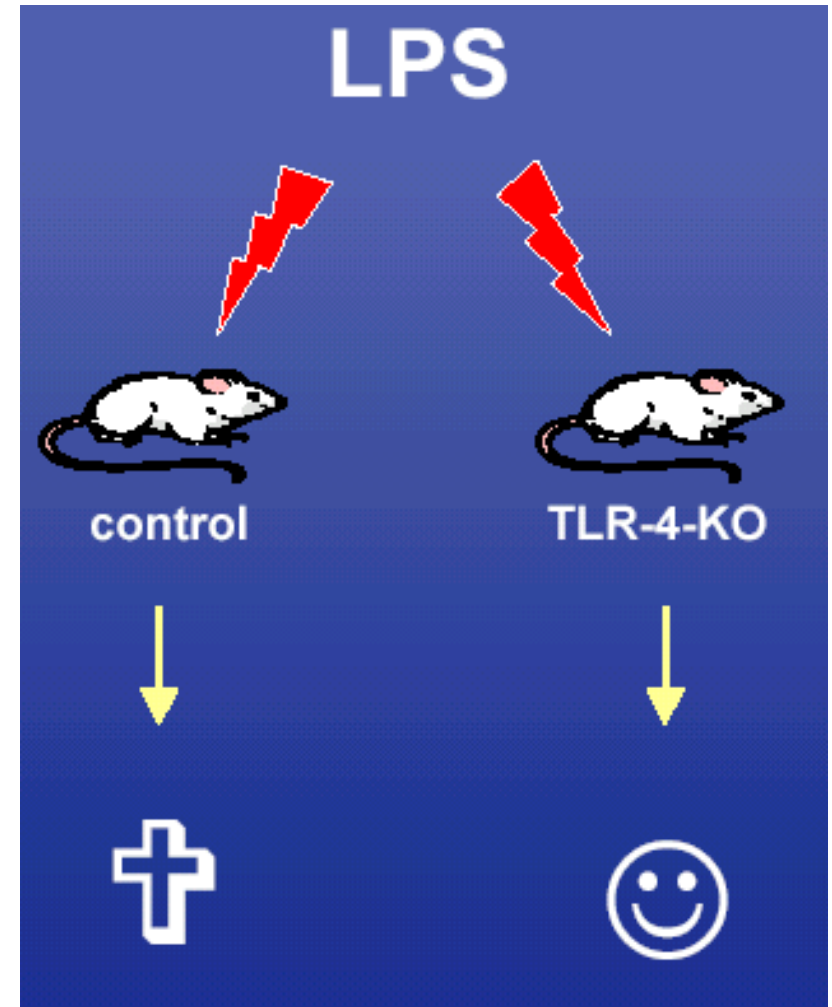
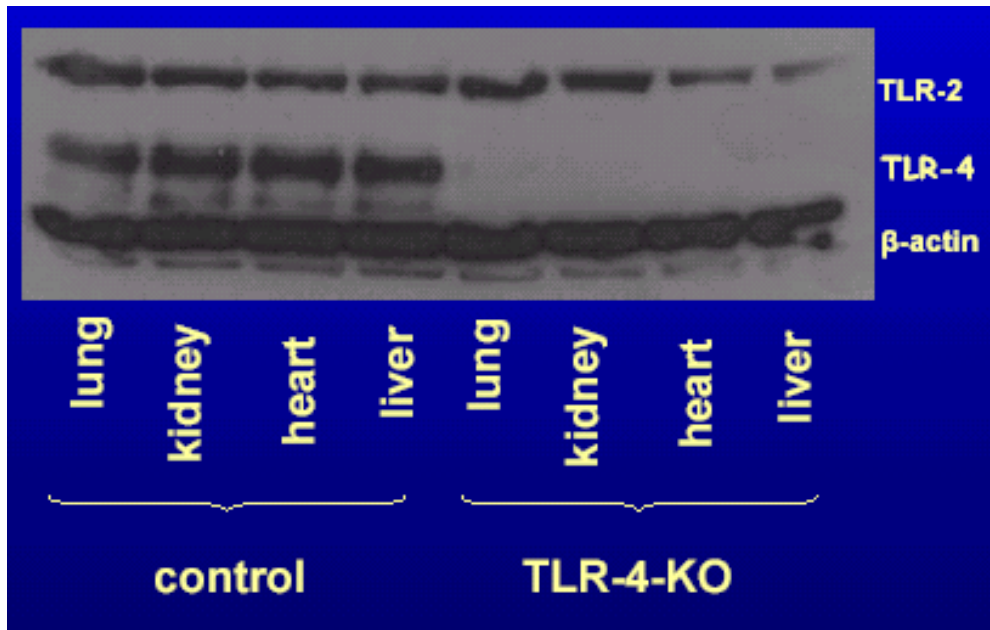
NFκB =  
Transkriptionsfaktor



LBP =  
LPS BindeProtein



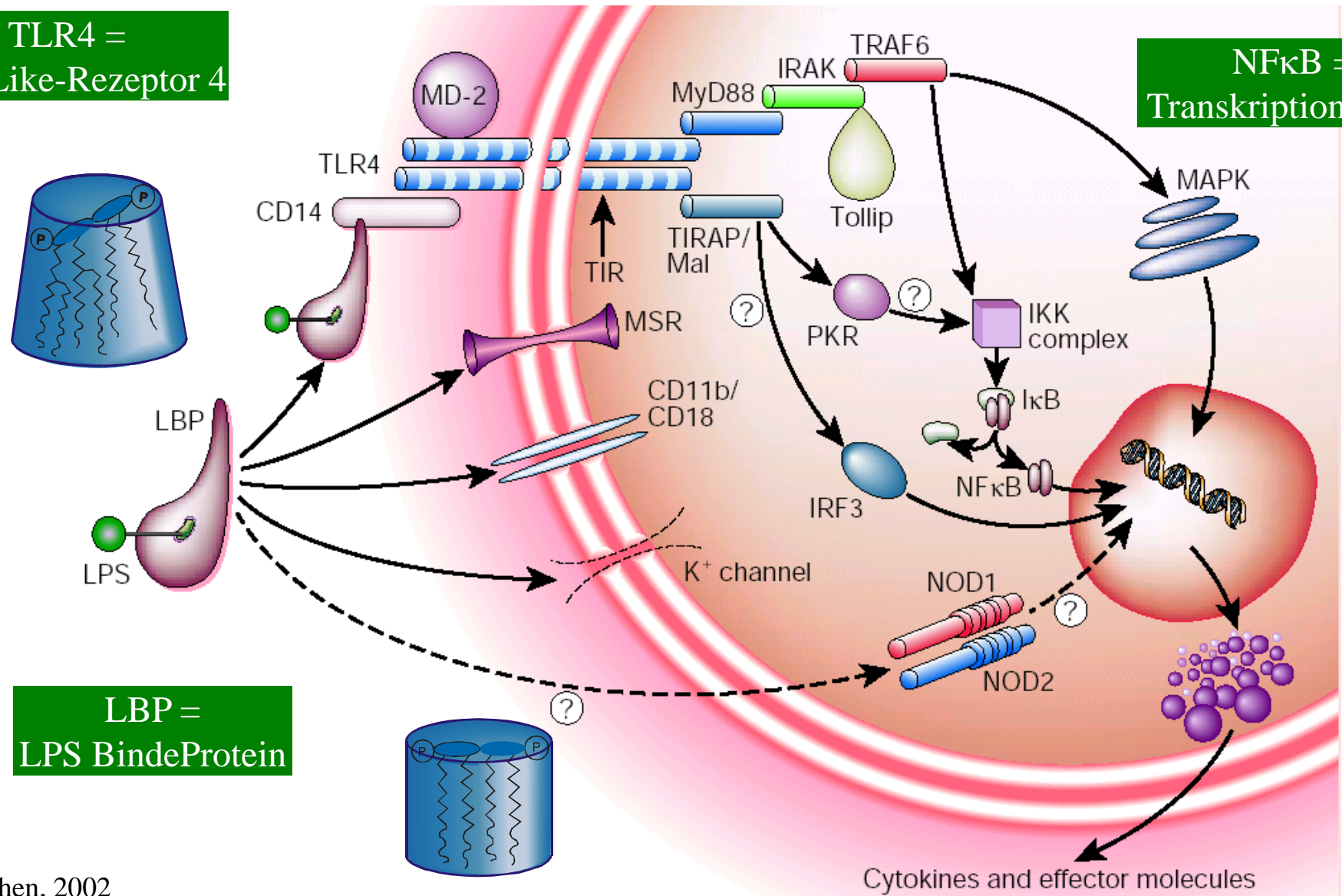
# TLR-4 & LPS



# Endotoxin: Signalerkennung und -transduktion

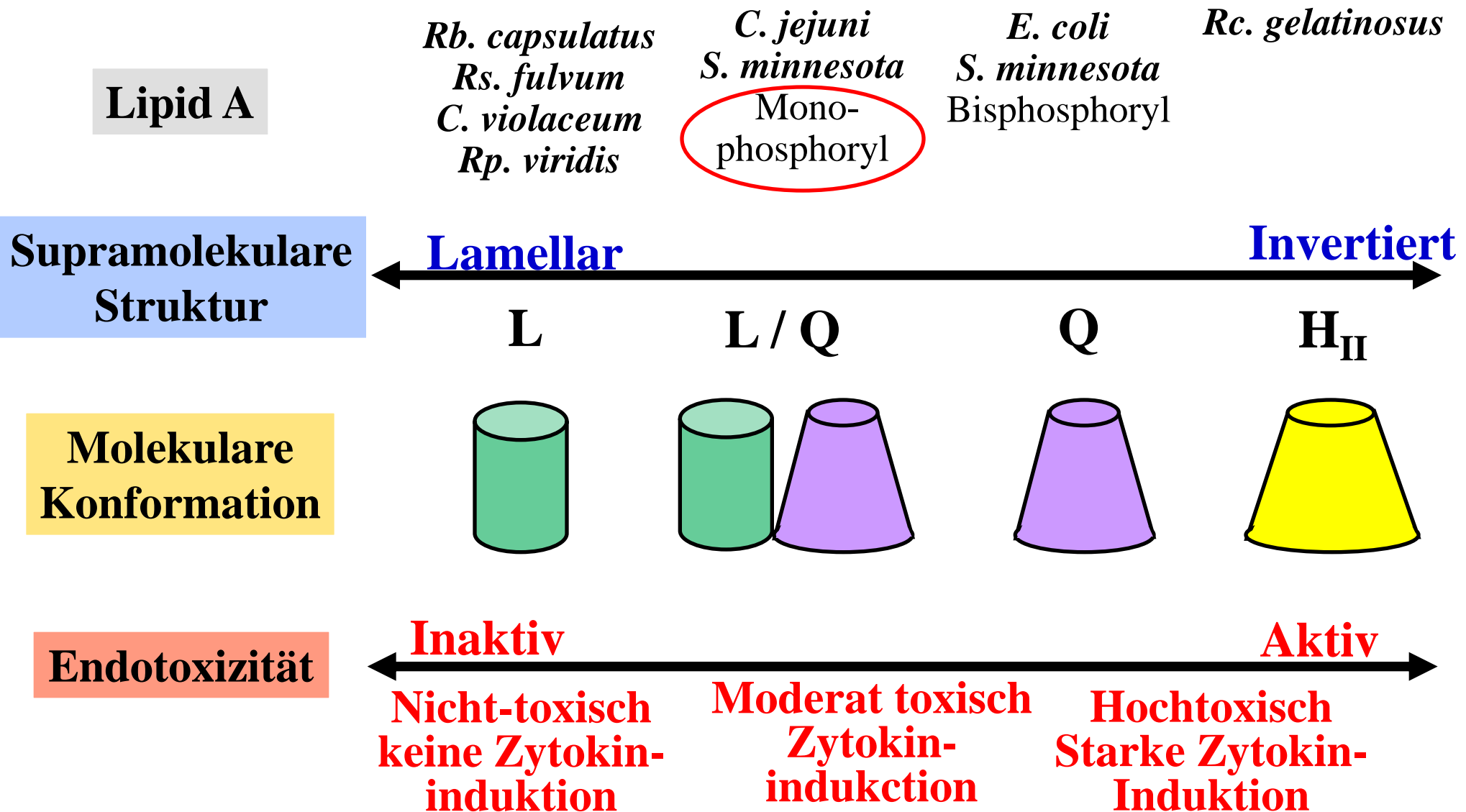
TLR4 =  
Toll-Like-Rezeptor 4

NFκB =  
Transkriptionsfaktor



LBP =  
LPS BindeProtein

# Korrelation zwischen der supramolekularen Struktur und der Bio-Aktivität



# Peptid Wechselwirkung mit Endotoxinen

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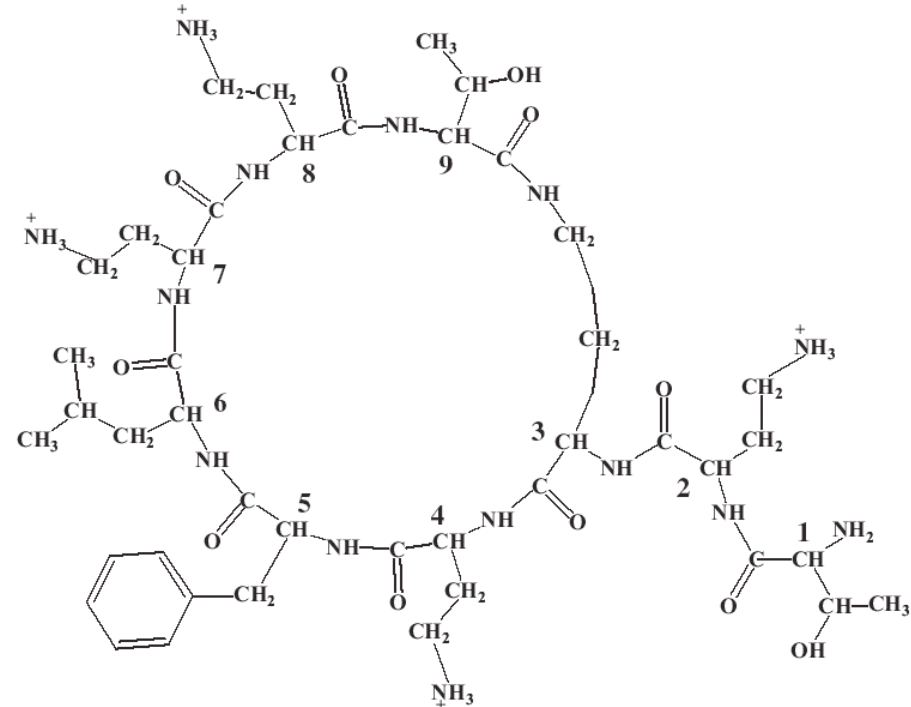
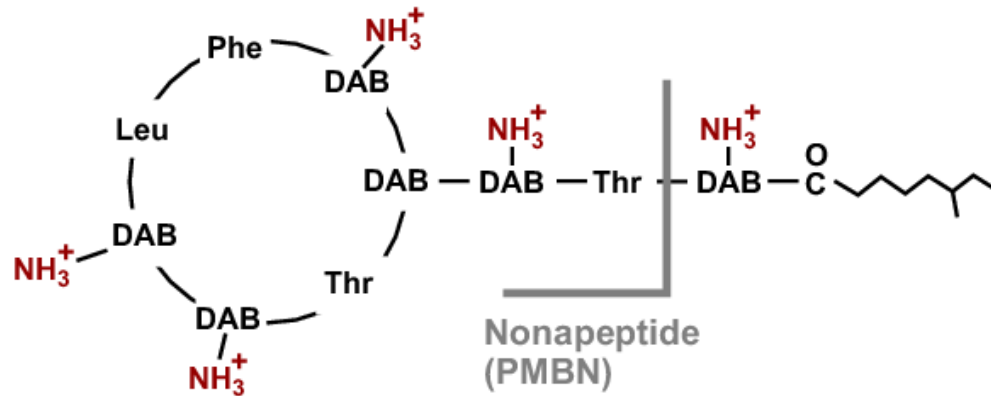
Bindungseigenschaften von **Polypeptid-Antibiotika**  
an Endotoxinen

→ Bindungsmechanismus

→ Peptid Optimierungsstrategien abzuleiten



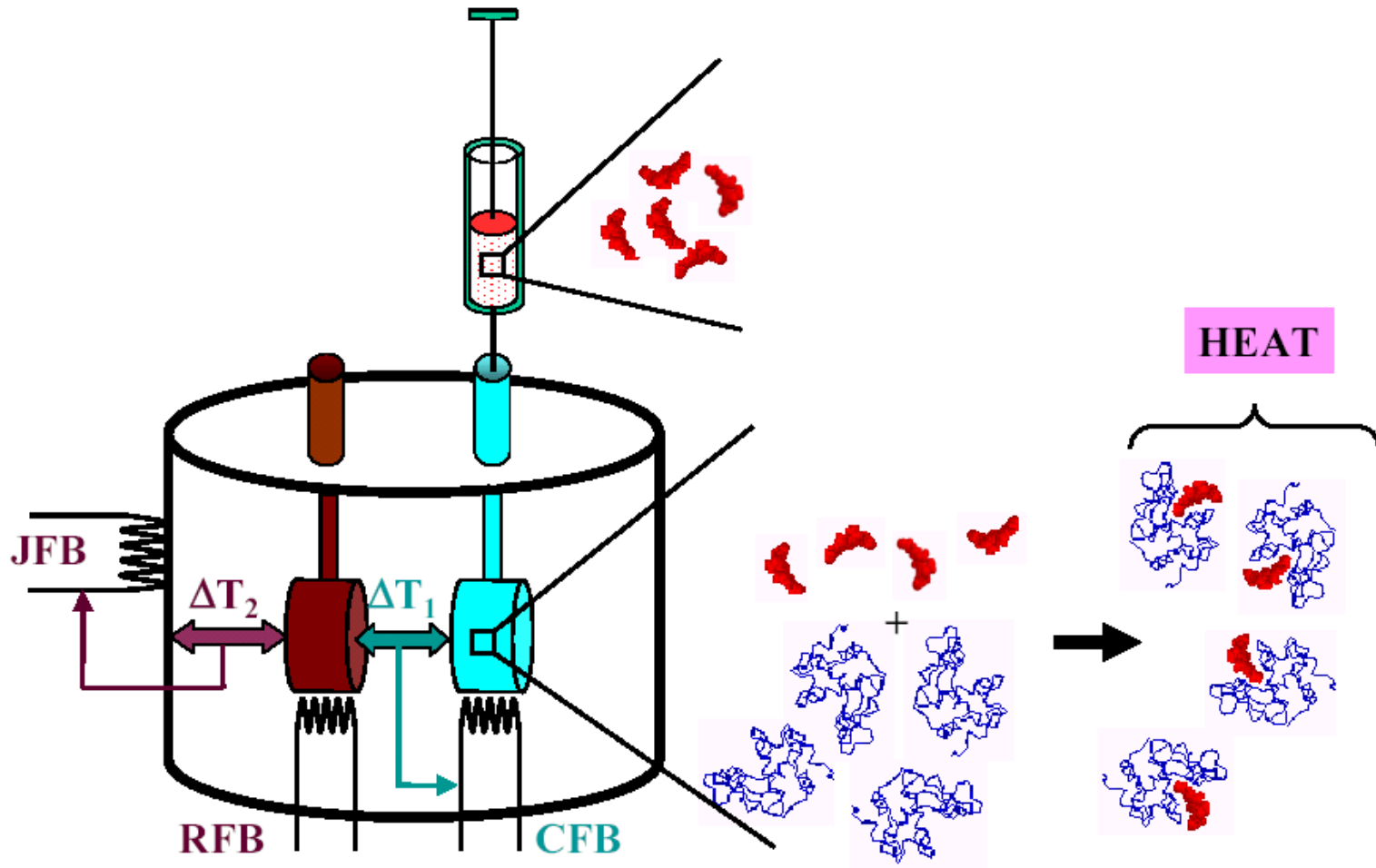
# Polymyxin B / Nonapeptide



Polymyxine (5+): Wichtigste Vertreter der **Polypeptid-Antibiotika**, deren charakterist. Bausteine L-2,4-Diaminobuttersäure (DAB), L-Threonin (Thr), D-Phenylalanin (Phe) sowie L- u. D-Leucin (Leu) sind.

PMB/PMBN: **Neutralisierungsagentien**  
Bei höheren Konzentrationen: toxisch

# Isothermal Titration Calorimetry



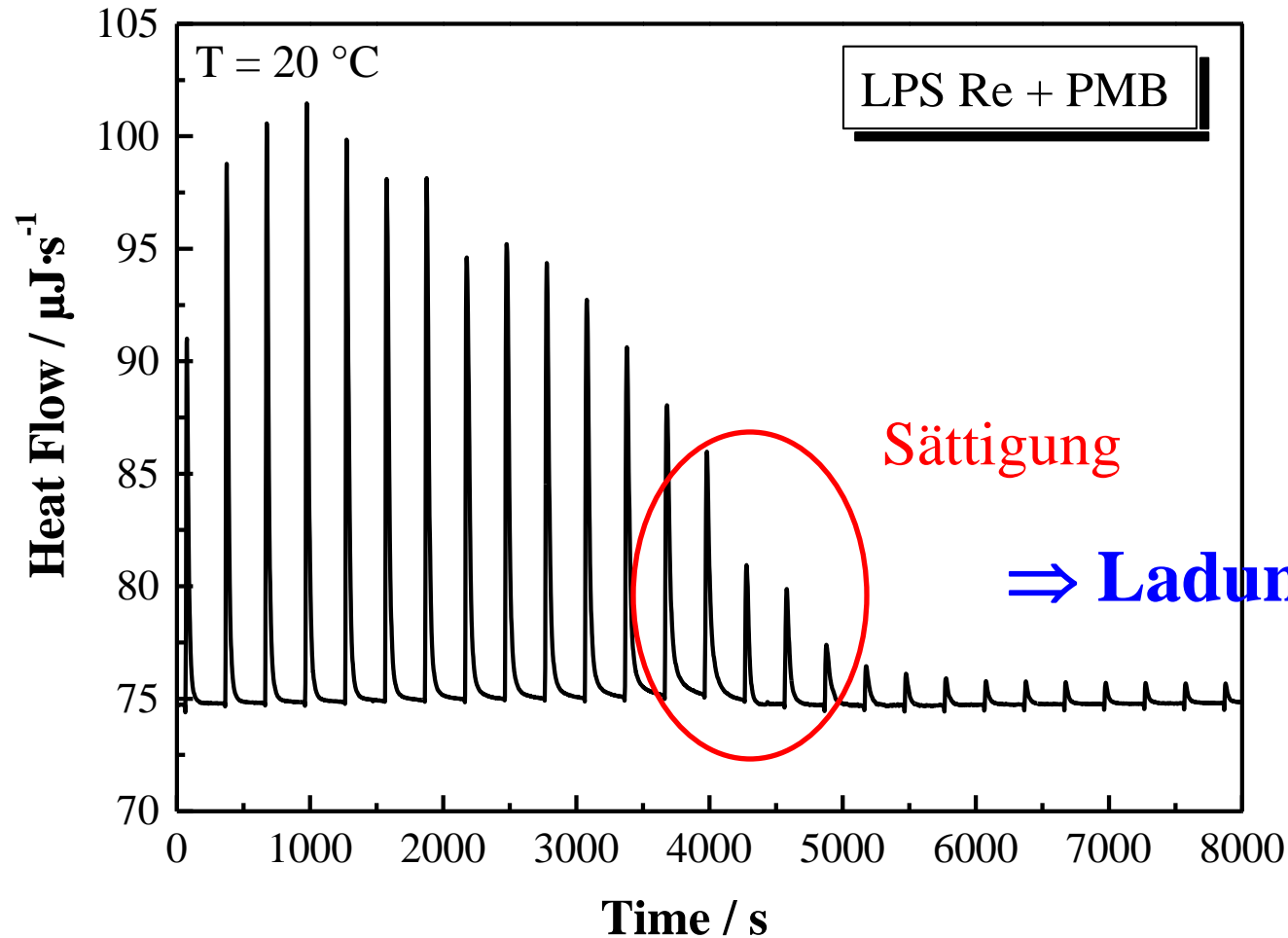
# PMB:LPS Re - Bindung

ITC

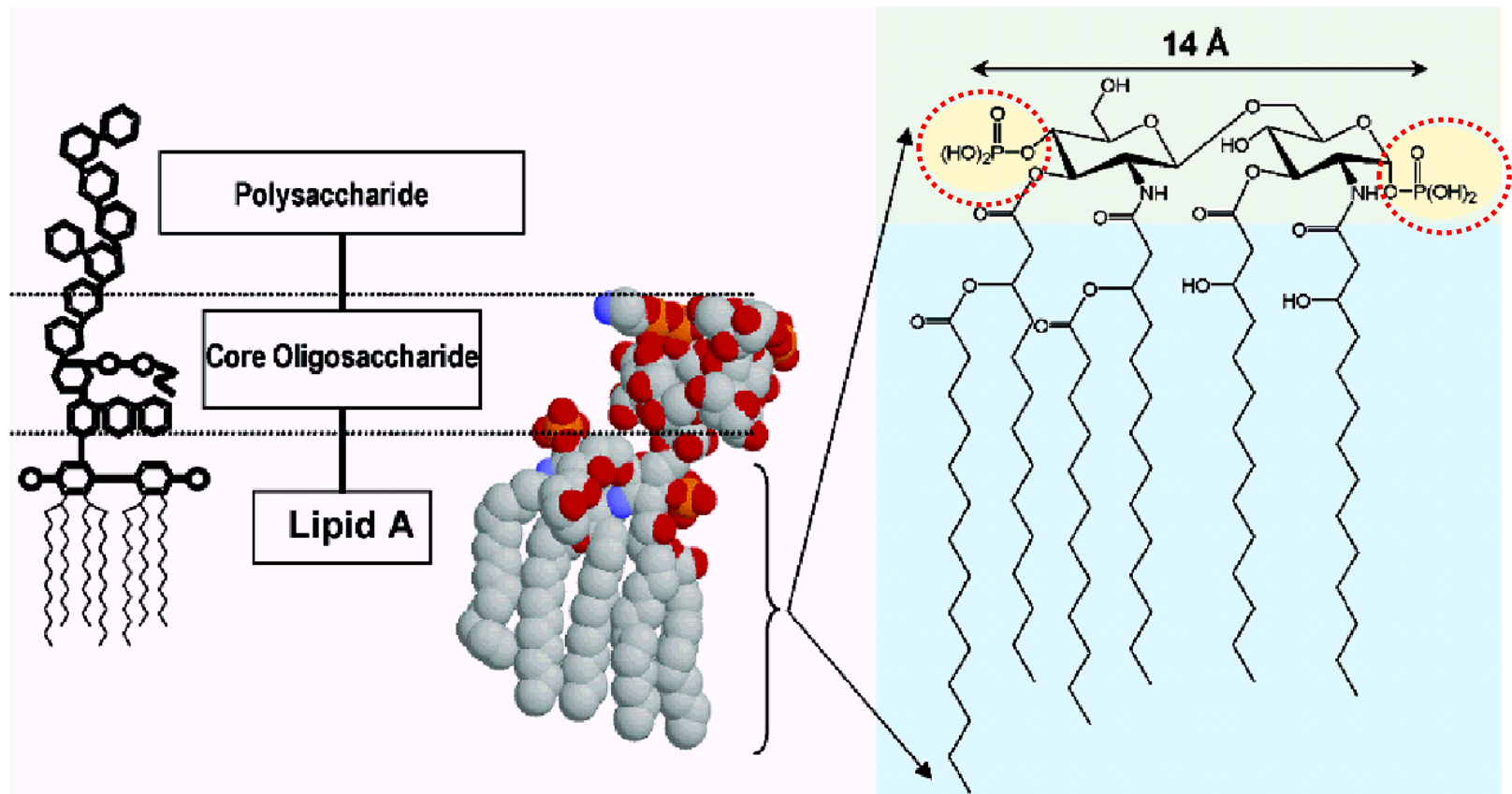
PMB → LPS Re

PMB: 5+

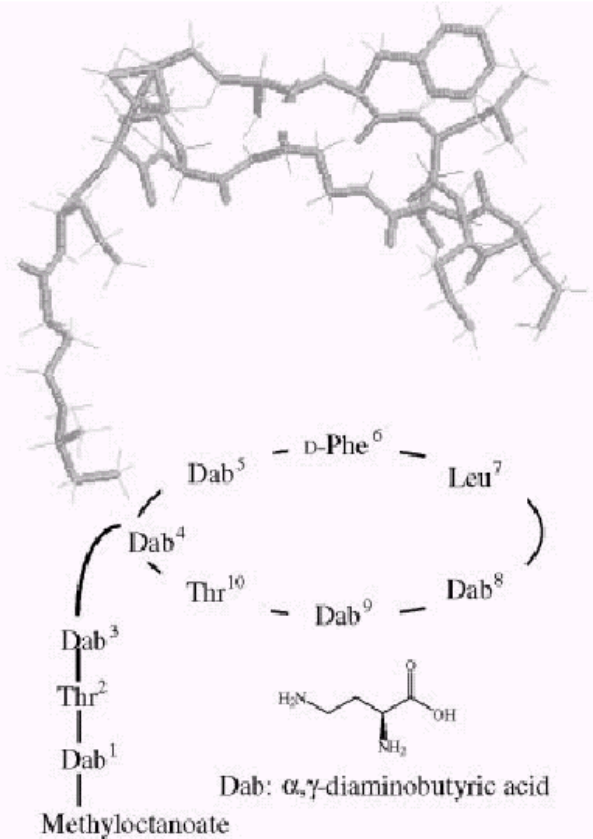
LPS Re: 4-



# LPS



# Polymyxin B

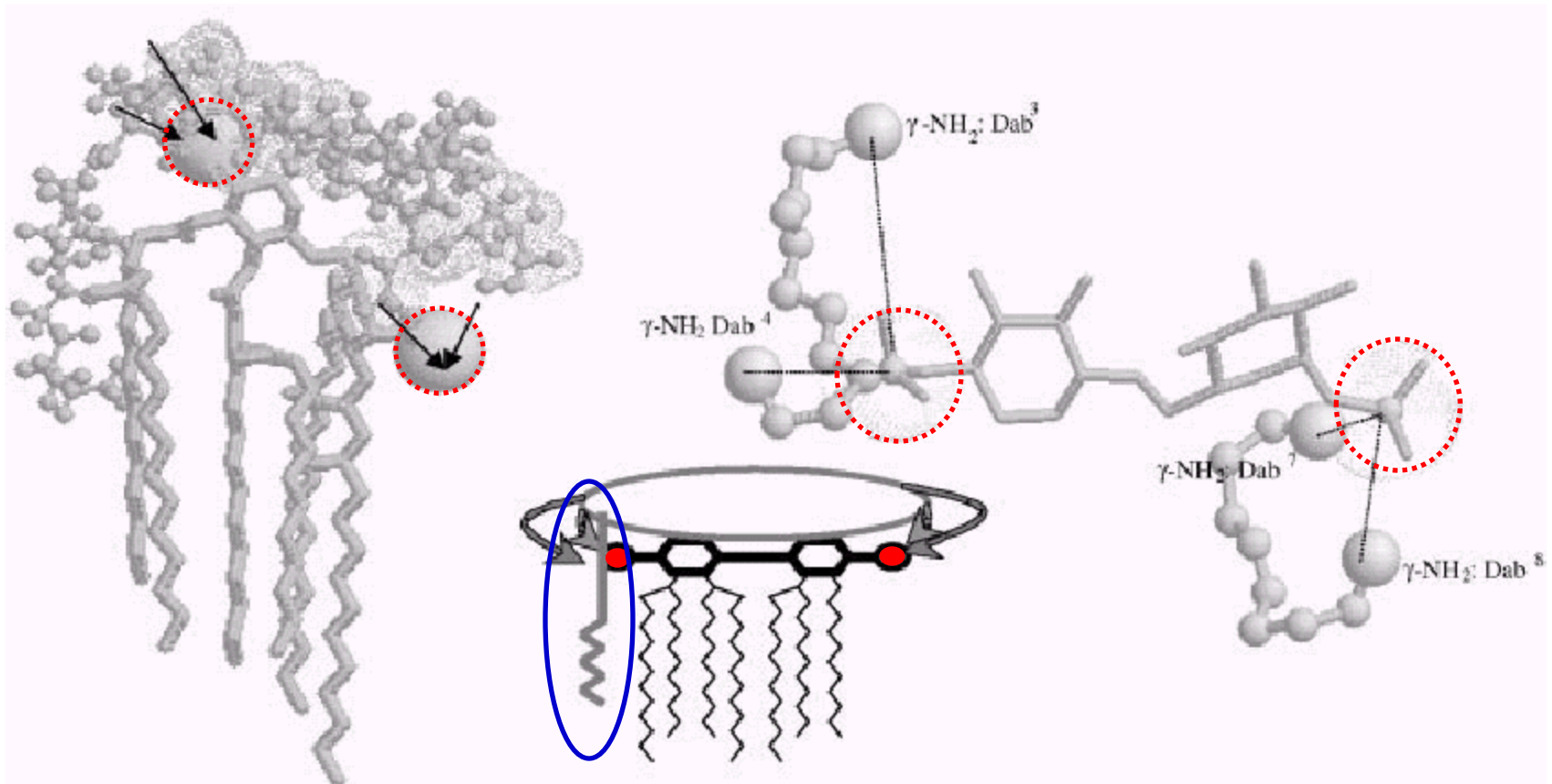


## Lösungs-struktur und Sequenz von PMB

David (2001) J Mol Recog 14: 370

Bhattacharjya et al. (1997) Biopolymers 41: 251

# Polymyxin B - Lipid A Bindung



Bindungsaffinität  $\approx \mu\text{M}$

# PMP/PMBN Bindung an Endotoxine

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## Bindungsmechanismus:

- Schritt 1: Elektrostatische Wechselwirkung zwischen Peptid und Endotoxin
- Schritt 2: Peptid Penetration/Einbau (hydrophobe KW-kette) in die Endotoxin Membran

⇒ **PMB > PMBN**

⇒ **Neue Strategieansätze zur Entwicklung von  
Therapeutischen Wirkstoffen zwecks Reduktion der Sepsis**

**Peptide von Interesse: BPI (Bactericidal/Permeability Increasing protein), CAP37 (Cationic Antimicrobial Protein), LALF (Limulus Anti-LPS Factor), lactoferrin/lactoferricin, NK (Natural Killer), ....**