Northern Nanotech
Copenhagen, September 23-25, 2008

NanoFOOD

- Antimicrobial peptides, fibrils, and biological response

Niels Chr. Nielsen
iNANO and inSPIN

www.nanofood.dk

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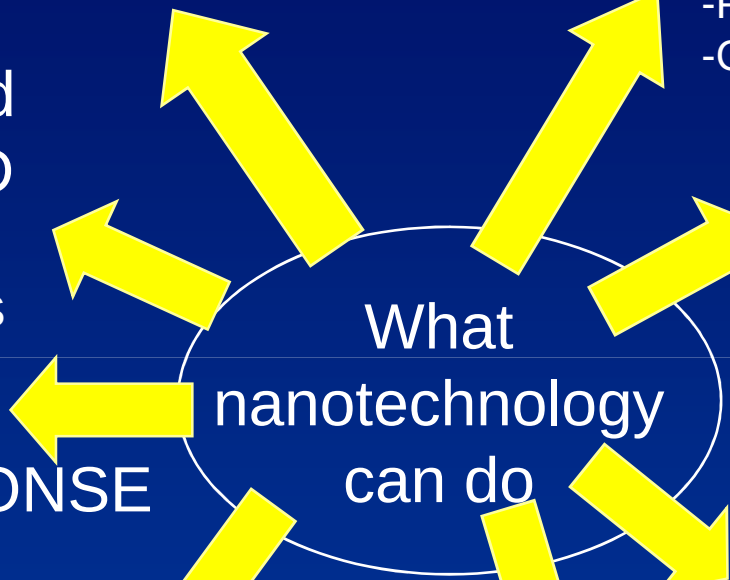
Nanotechnology in Food industry



FOOD FLAVOURS
-ENHANCEMENT
-SAFETY

NANO-SENSORS
-PACKAGE TRACKING
-CONTAMINATION CONTROL

DELIVERY and CONTROLLED RELEASE
-NEUTRACEUTICALS

SMART NANOMATERIALS
-RESPONSIVE TO ENVIRONMENT

FOOD RESPONSE
-NUTRIOMICS

IMPROVED PACKAGING
-STABLE TO UV LIGHT
-STRONGER BUT LIGHTER

SUSTANABLE PACKAGING
-ENVIRONMENTALLY FRIENDLY MATERIALS
-GREEN MANUFACTURING

FOOD SAFETY
-ANTI-BACTERIAL MATERIALS/COATINGS
-INFORMATION TRACKING

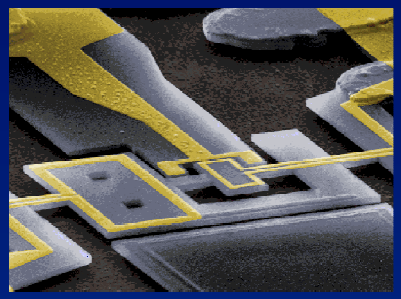
mm

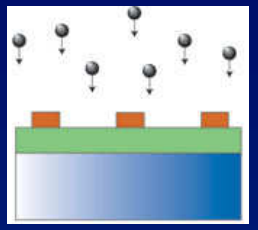
μm

nm

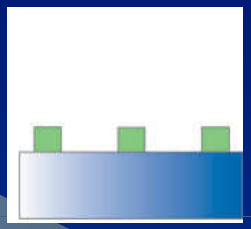
Nano approach

litography

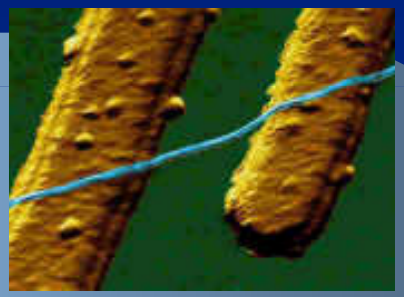


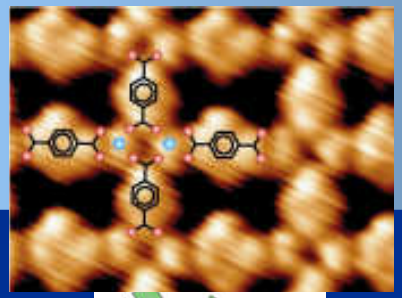


top-down

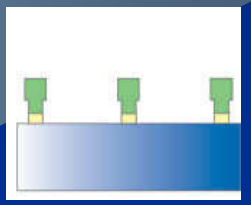


NANOTECHNOLOGy

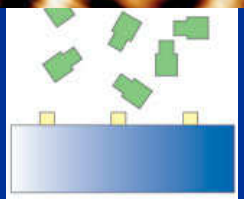




bottom-up



Self assembly



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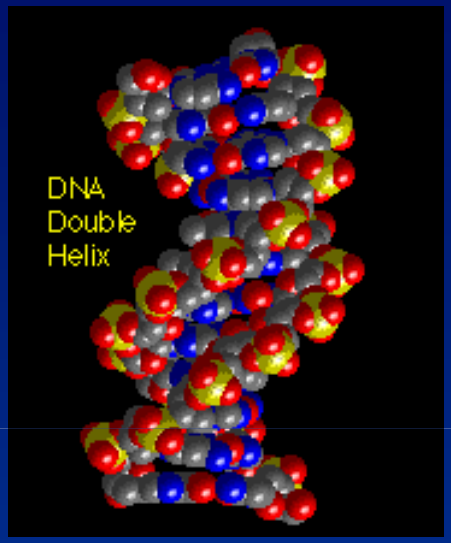
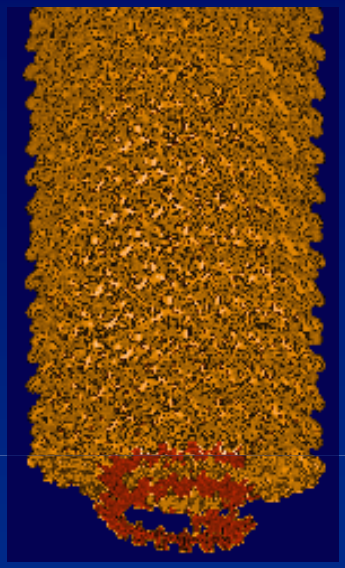
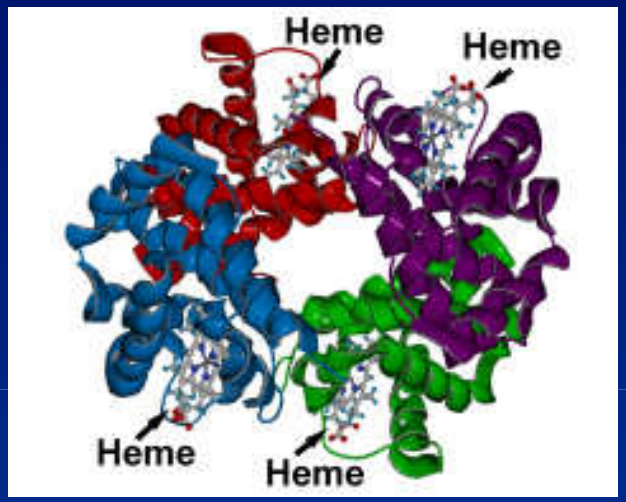
Nano: Inspiration from Nature



DNA



virus



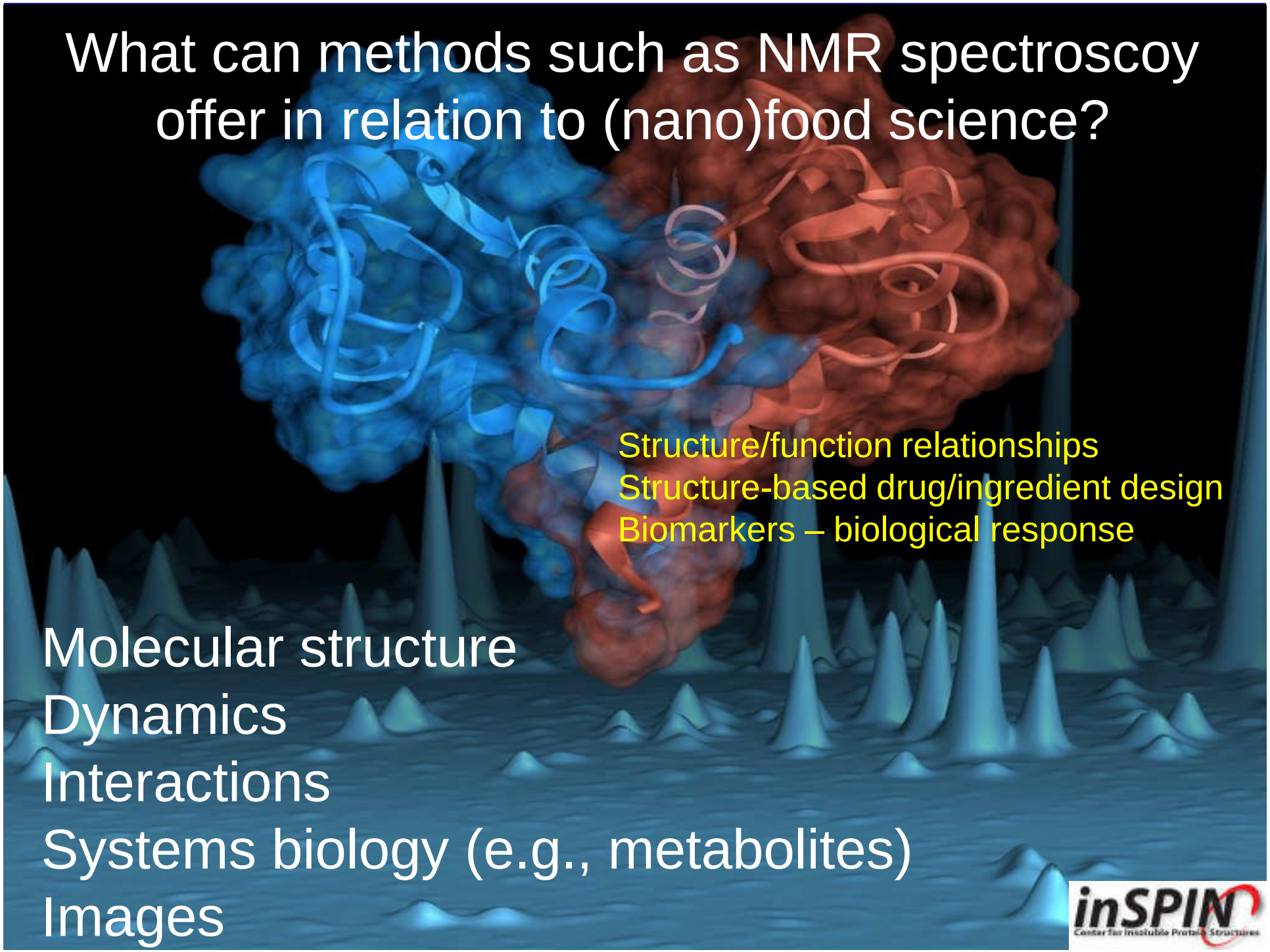
protein

- Non-covalent interactions:
  reversible, selective and oriented binding
- Assembly: recognition of „self“ and error correction

Inspiration from nature requires strong tools?

"If you can see it with that, it ain't nano."

What can methods such as NMR spectroscoy offer in relation to (nano)food science?



Structure/function relationships
Structure-based drug/ingredient design
Biomarkers – biological response

Molecular structure
Dynamics
Interactions
Systems biology (e.g., metabolites)
Images

inSPIN
Center for Insoluble Protein Structures

Four examples

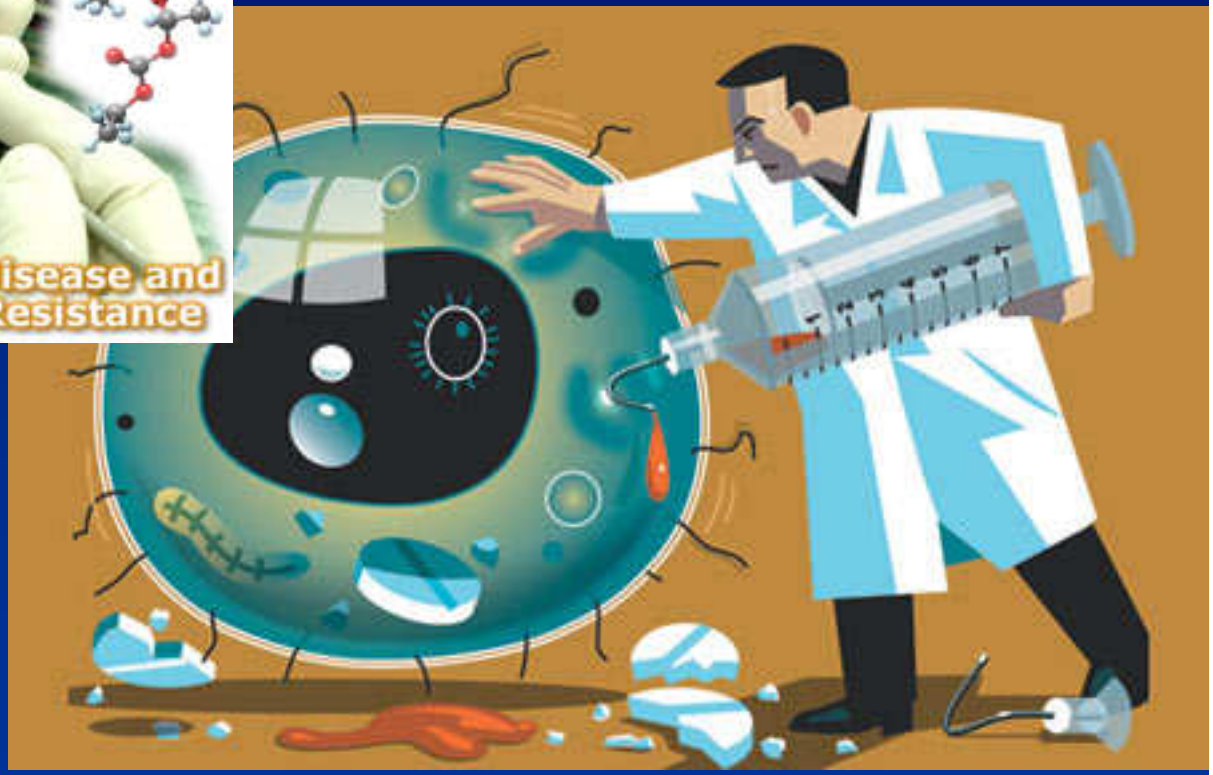
A. Antibiotic resistance - Antimicrobial peptides

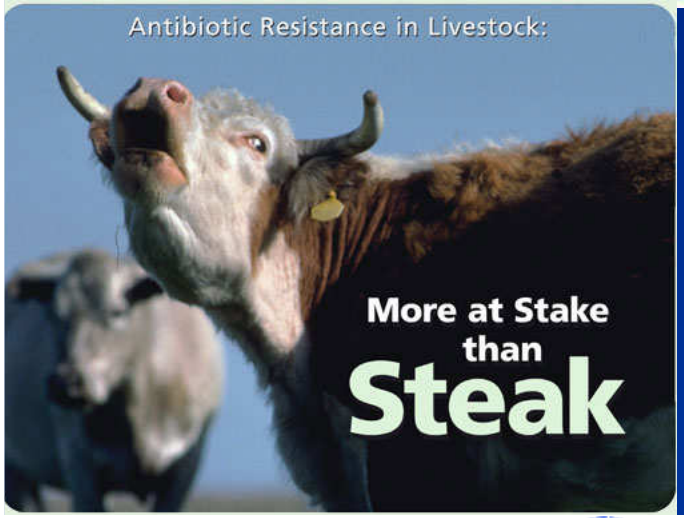
B. Biofilm formation - Protein fibrilation

C. Food response - NMR metabolomics

D. NMR for everyone - Development of low-field NMR for instrustrial applications

Small molecule antibiotic resistance – a major problem with relation to food production and food preservation





Antimicrobial Peptides

Increased interest due to growing bacterial resistance to small molecule antibiotics

Around 14 AMP´s currently in drug development (2006) and expected to rise

Owe their activity to either immune modulation or direct killing through membrane disruption

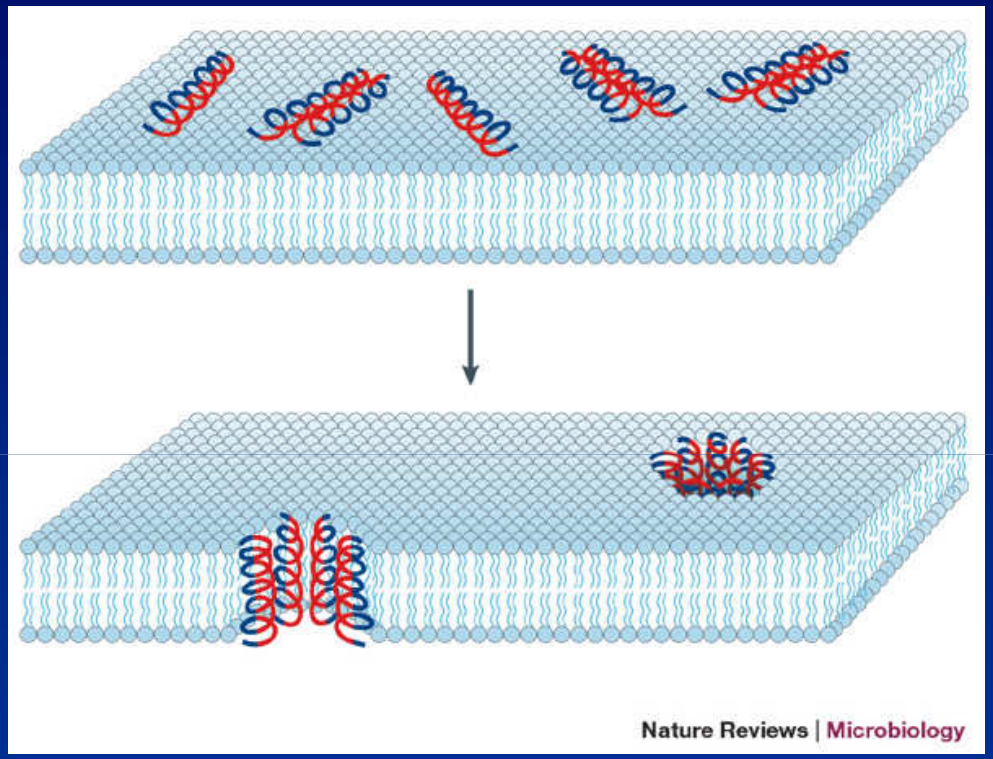
Are an important part of the host-defense mechanism in living organisms

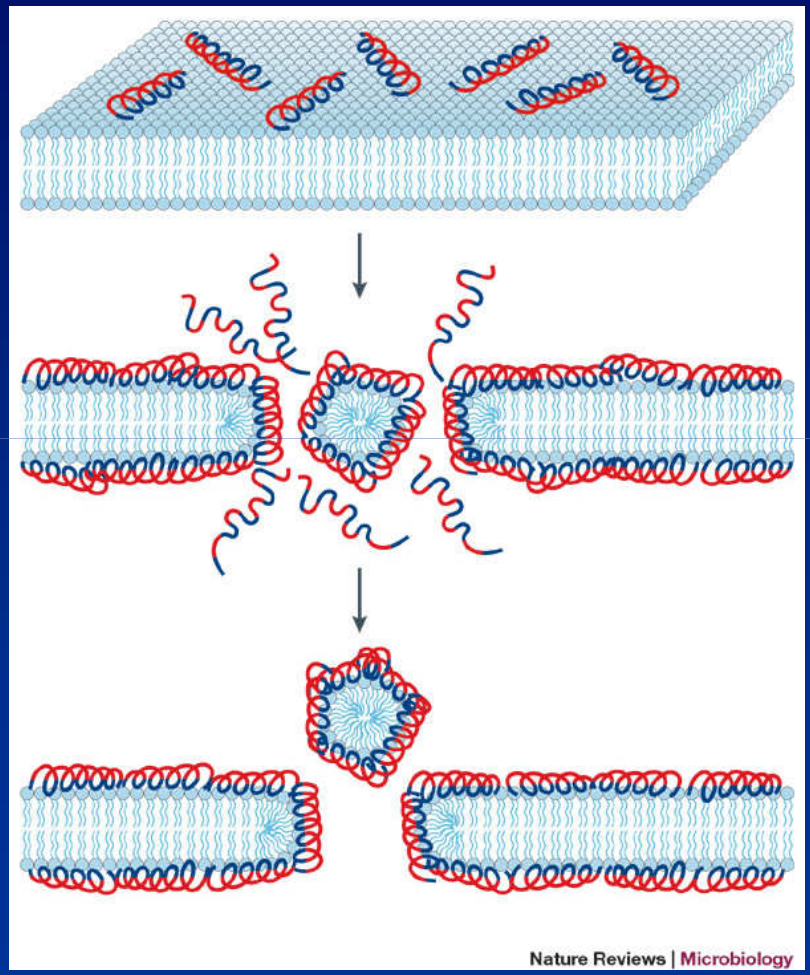
Nisin – so far the only FDA approved AMP

Alamethicin - fungi

Novicidin - sheep

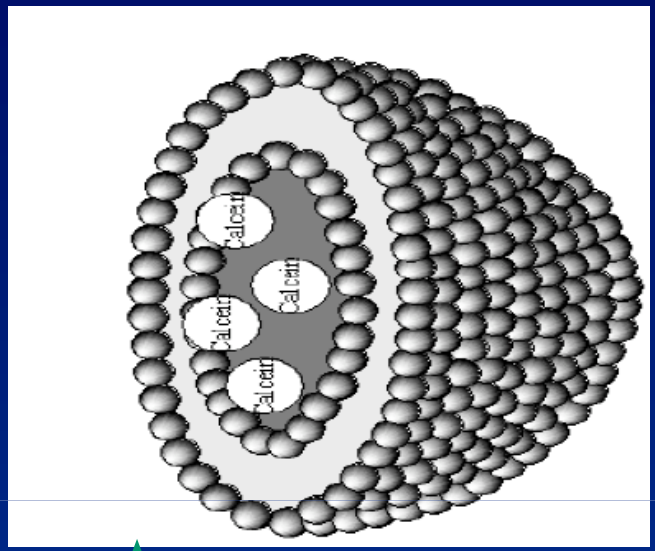
Antimicrobial Peptides
– mechanism of action?



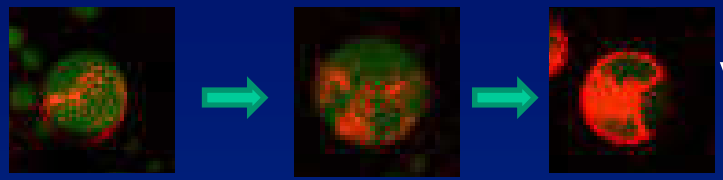


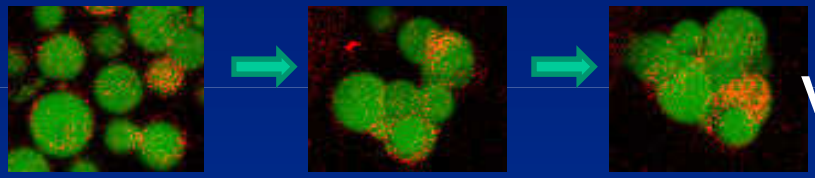
-Barrel-stave
-Worm hole
-Carpet

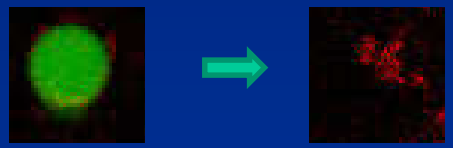
Action and perturbed action of novicidin
- optical detection with calcein



Action and perturbed action of novicidin
- optical detection with calcein

A  Vesicle leakage

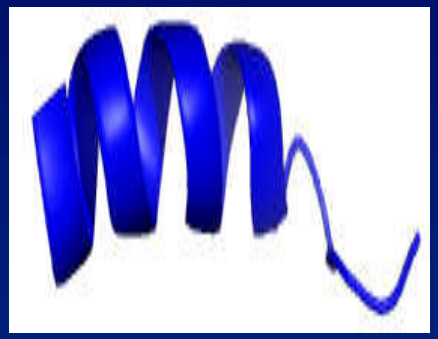
B  Vesicle fusion

C  Vesicle rupture

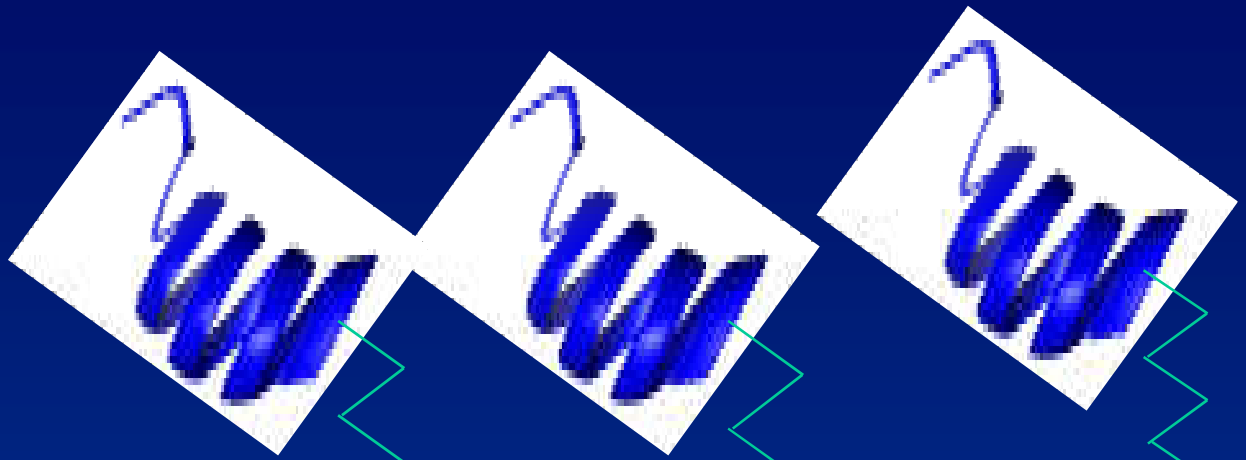
Water soluble probe

lipid soluble probe

Different action by chemically modified novicidin



Novicidin Wt



Novicidin C8

Novicidin C12

Novicidin C16

Vesicle leakage

Sample	DOPC vesicles	20% DOPG Vesicles
Novicidin	+	+++
Novicidin C8	-	-
Novicidin C12	-	-
Novicidin C16	+	++

Vesicle Fusion

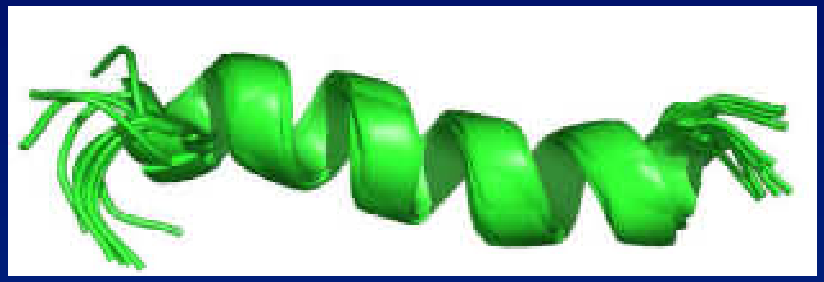
Sample	DOPC vesicles	20% DOPG Vesicles
Novicidin	+	+++
Novicidin C8	-	-
Novicidin C12	++	+
Novicidin C16	+++	++

Vesicle rupture

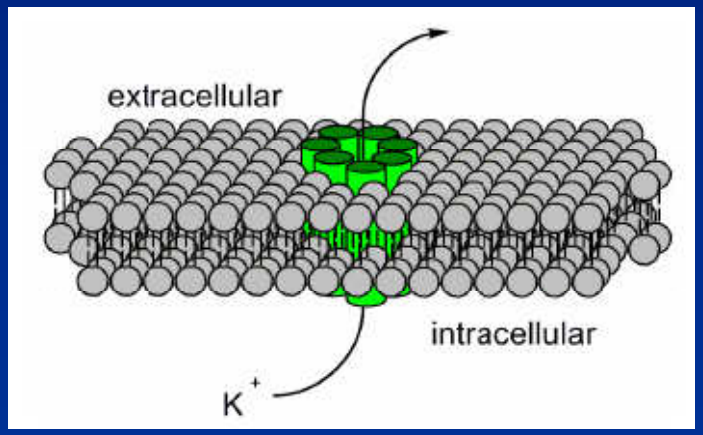
Sample	DOPC vesicles	20% DOPG Vesicles
Novicidin	++	+
Novicidin C8	+++	++
Novicidin C12	+++	+++
Novicidin C16	++	+

Alamethicin – a structural basis for antimicrobial peptides

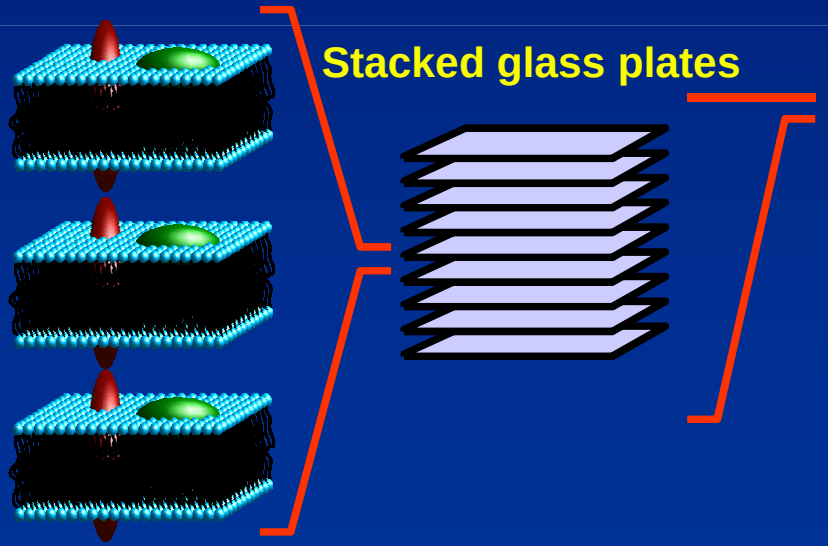
Liquid-state NMR



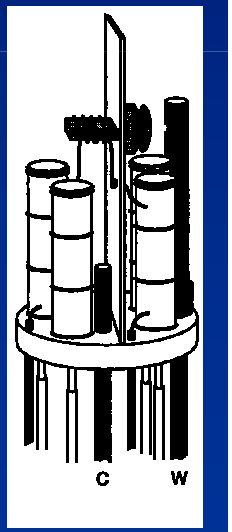
Solid-state NMR



Proposed channel structure

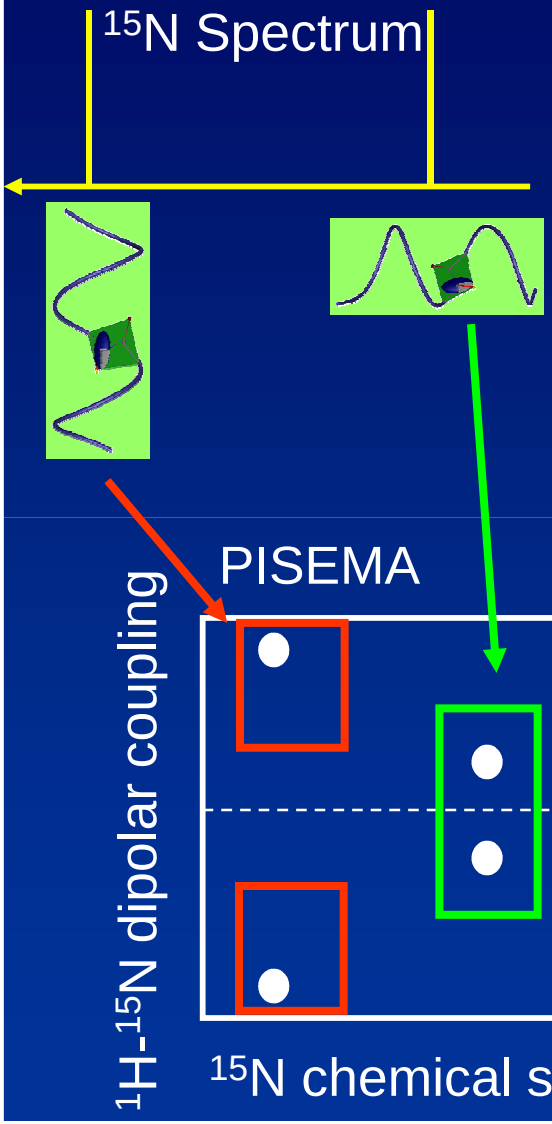


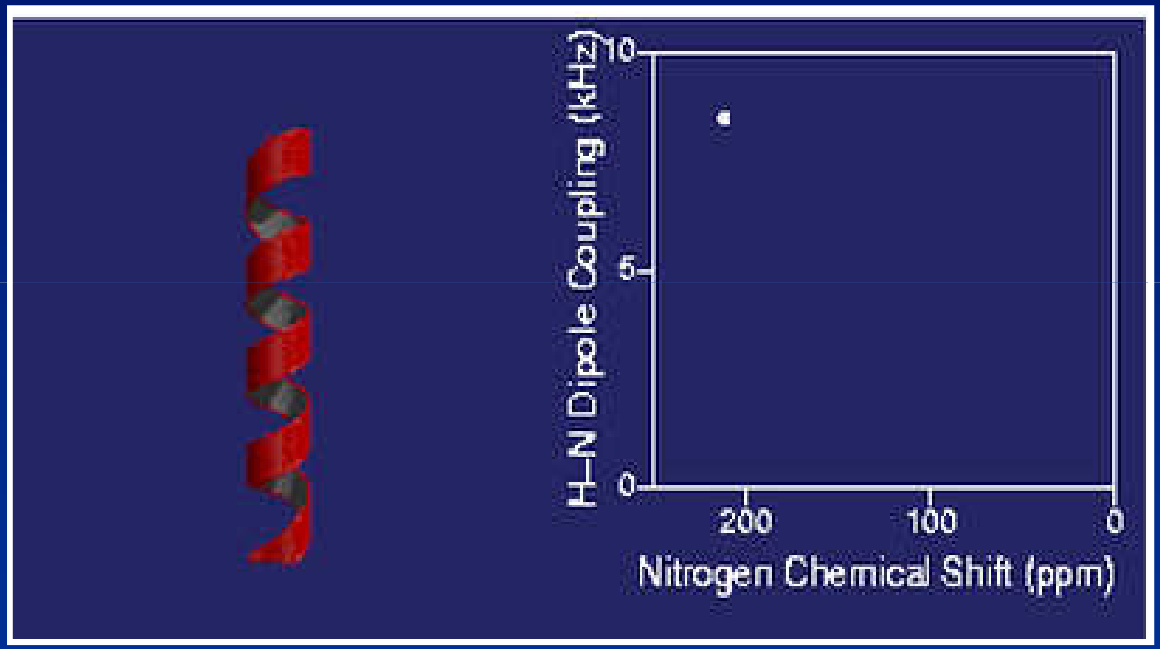
Protein in lipid bilayer



Flatcoil Probe

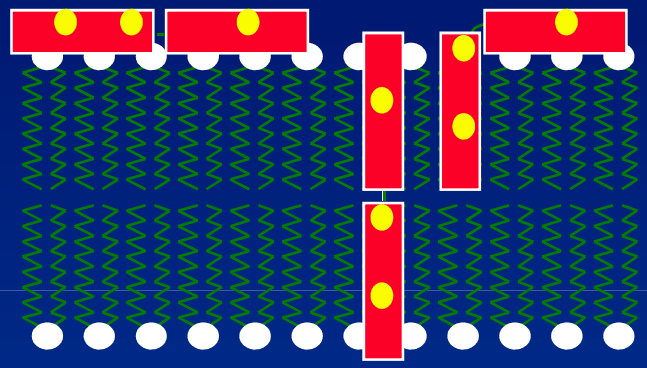
Conformation by solid-state NMR





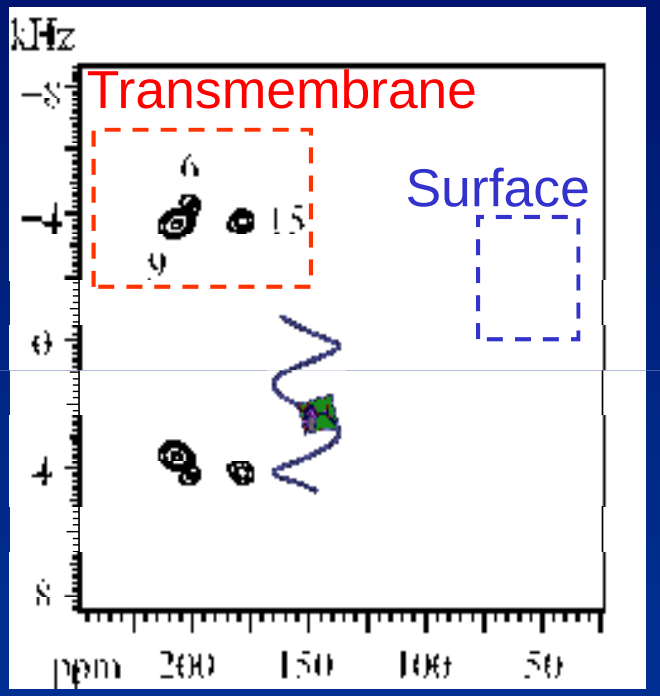
The alamethicin ionophore
- by solid-state NMR

Conformation?



Proline-14 kink?

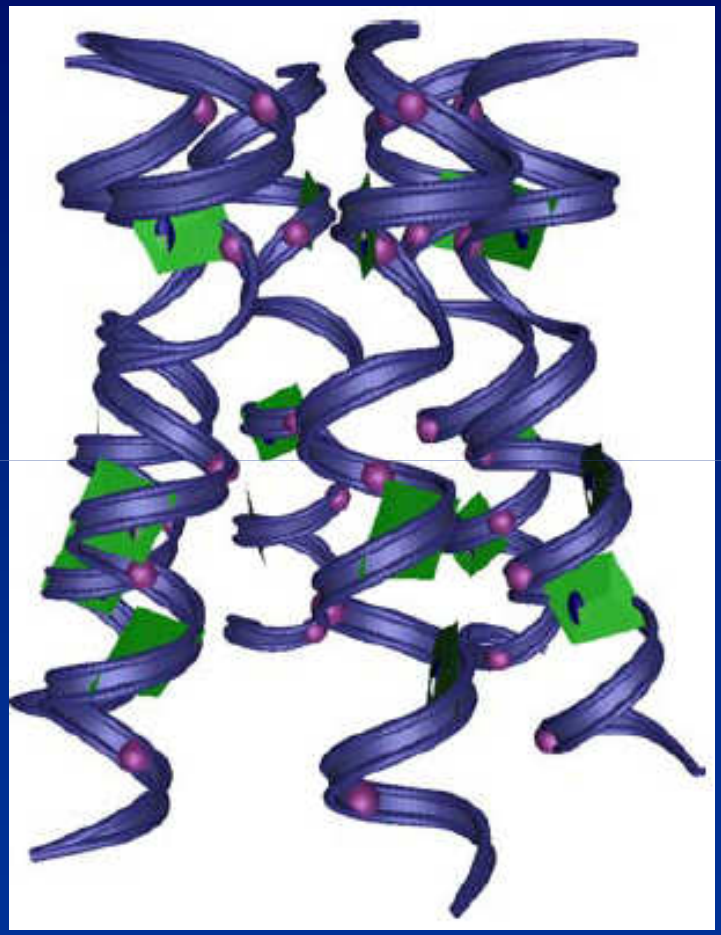
Multimerization?

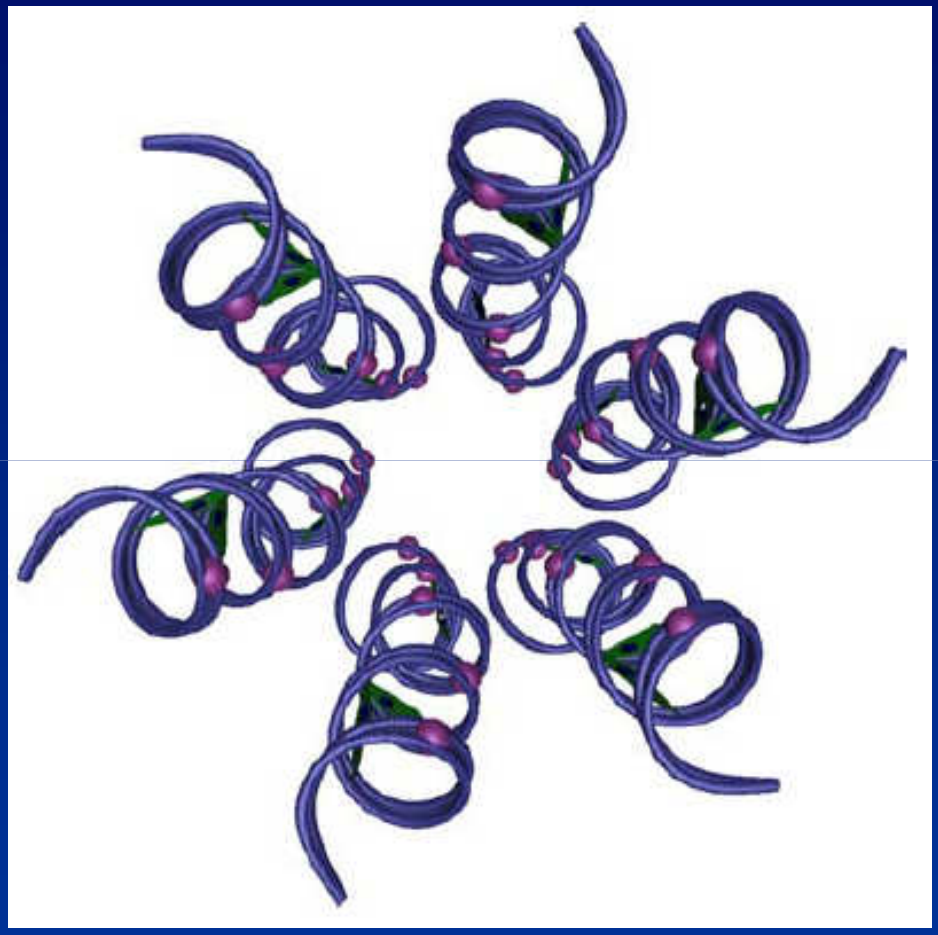


1 5 10 15 20

Ac*-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-*Phol

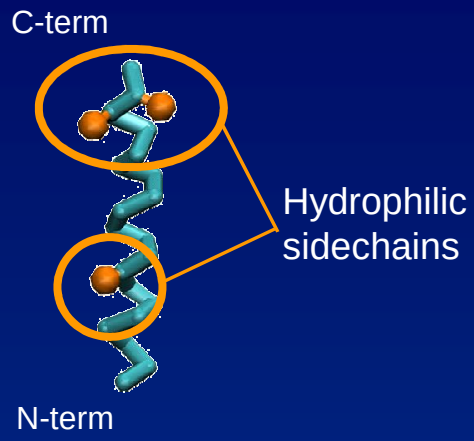
Alamethicin – membrane conformation



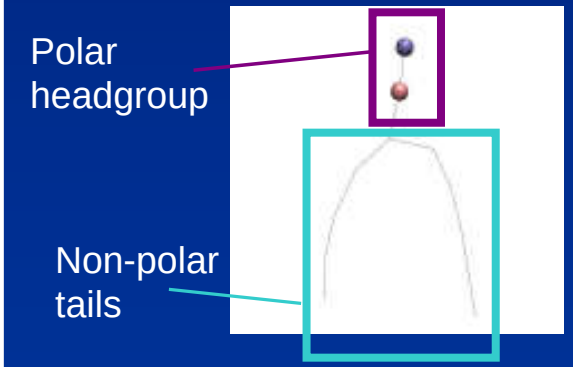


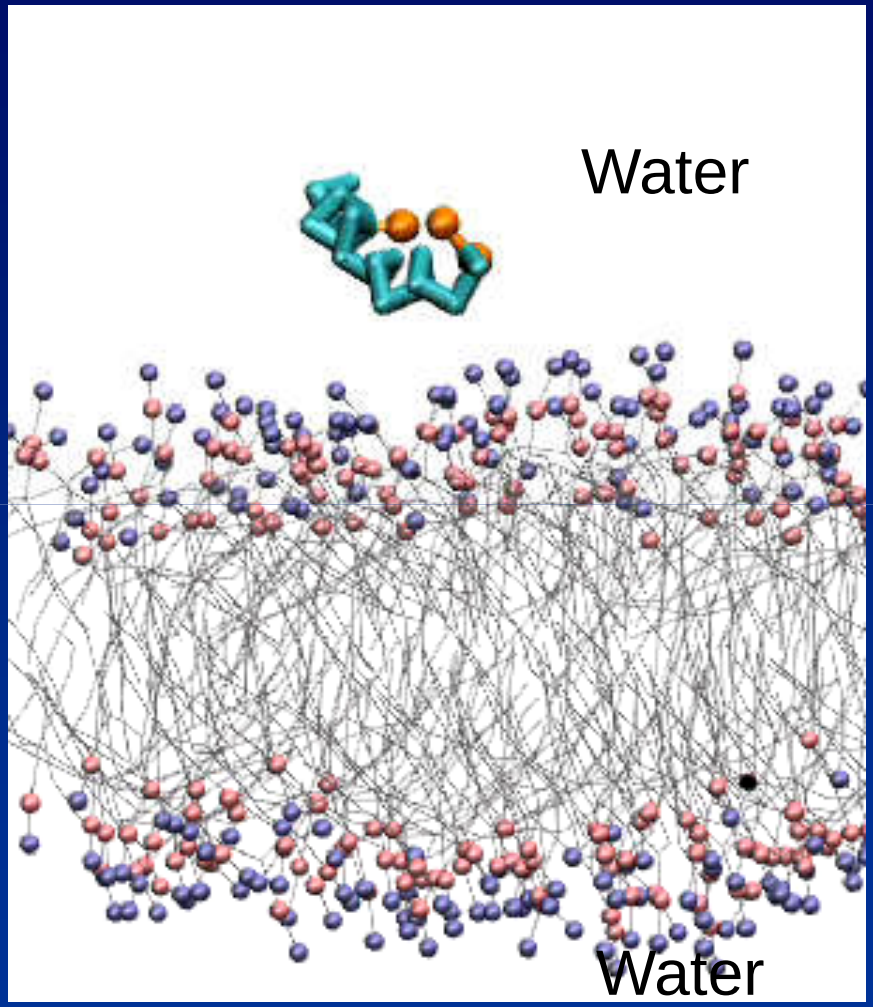
Establishment of contact

Alamethicin



Lipid

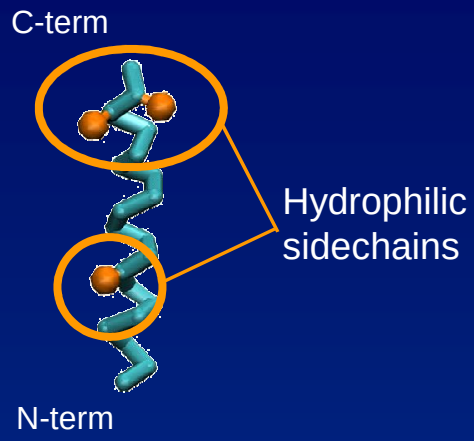




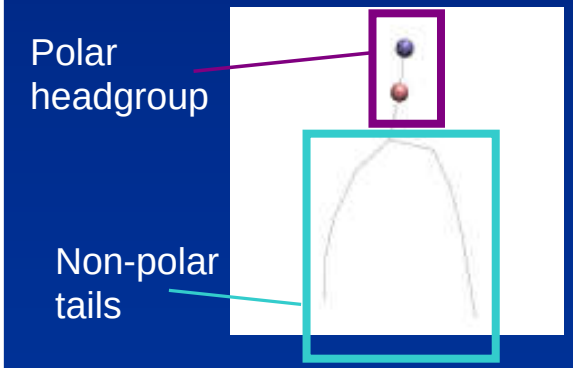
7ns

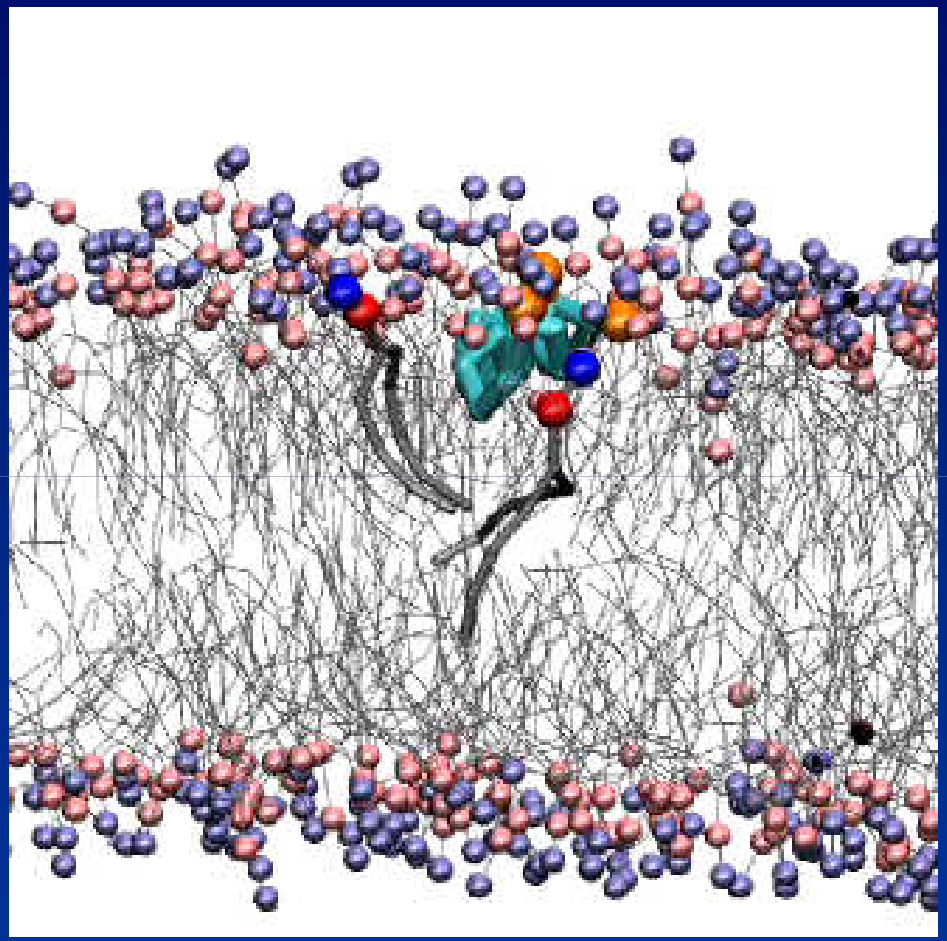
Penetration of the membrane

Alamethicin



Lipid

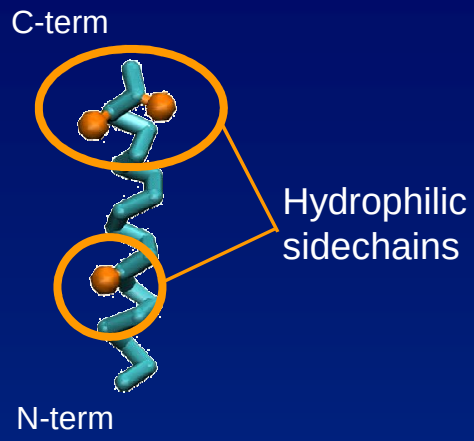




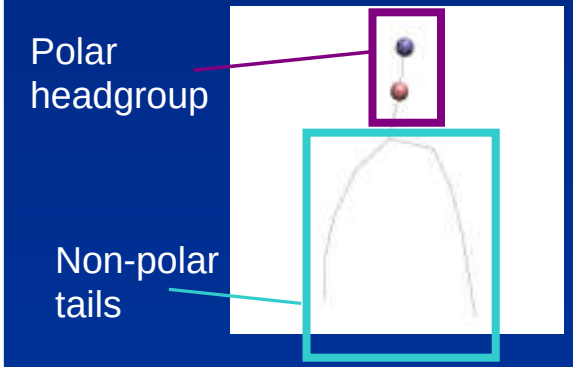
20ns

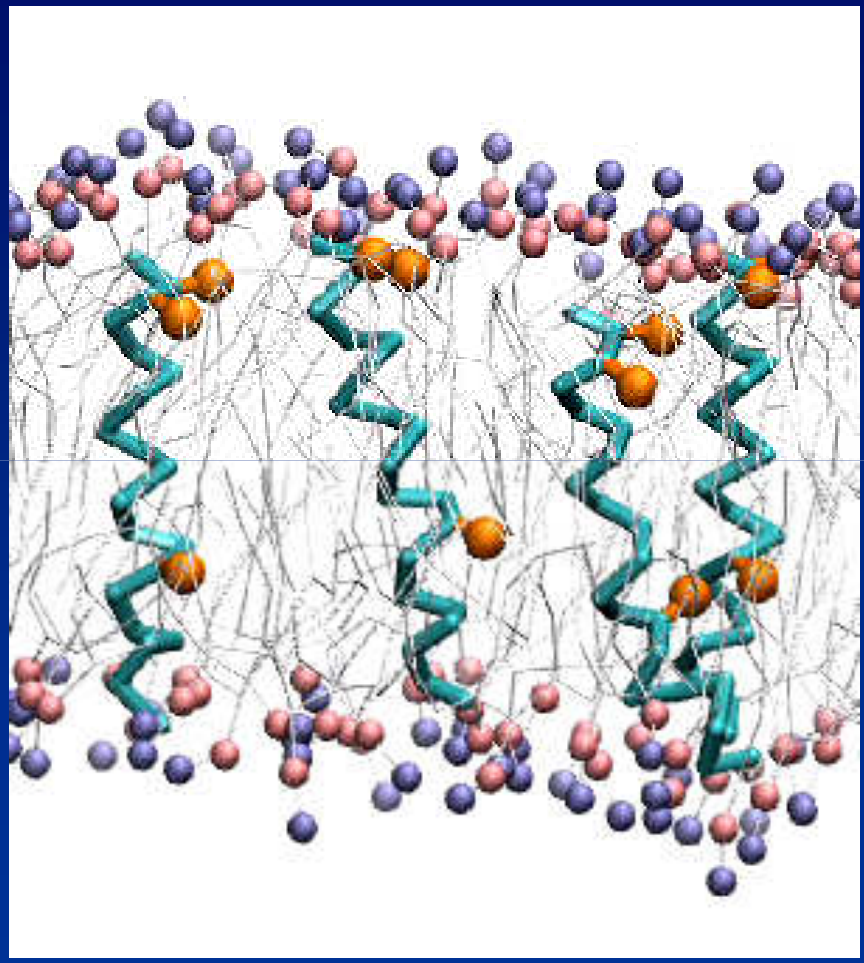
Assembly of the ion-channel

Alamethicin



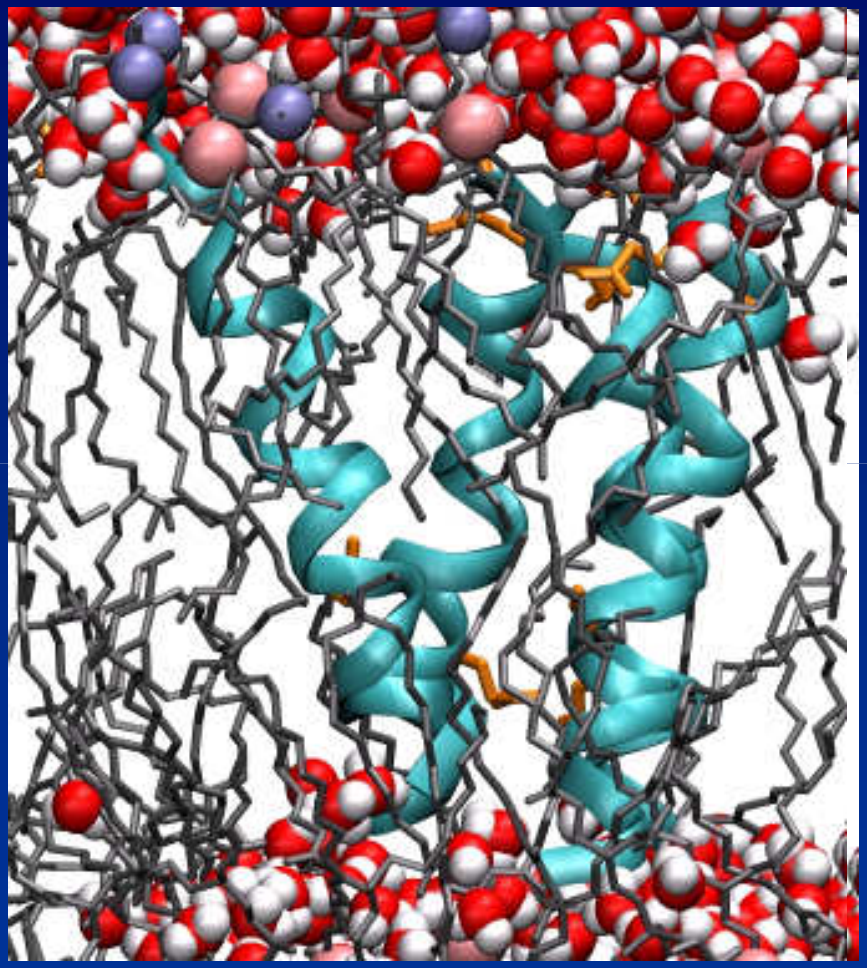
Lipid





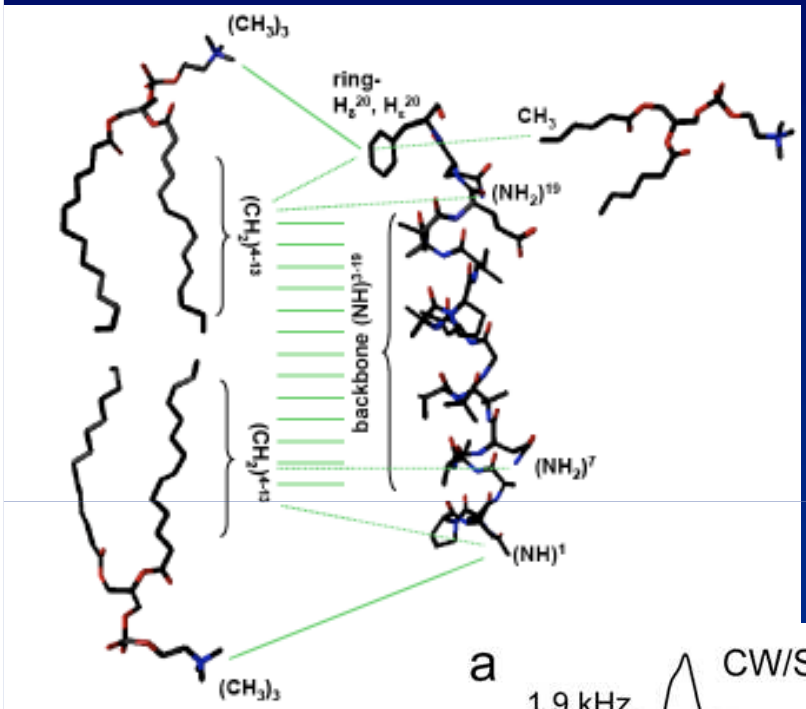
20ns

Lysing of the cell membrane

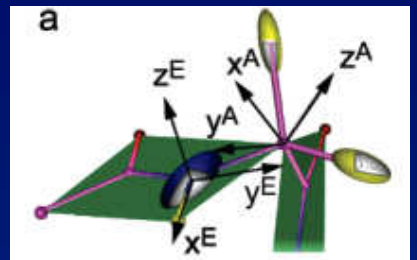


8ns

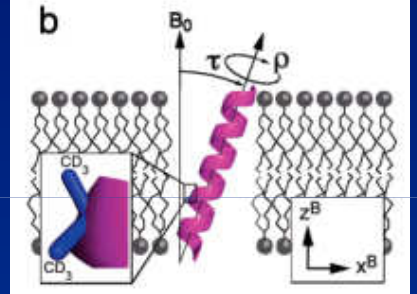
Exploring experimentally the details of **flexible ion channels in lipid bilayers**



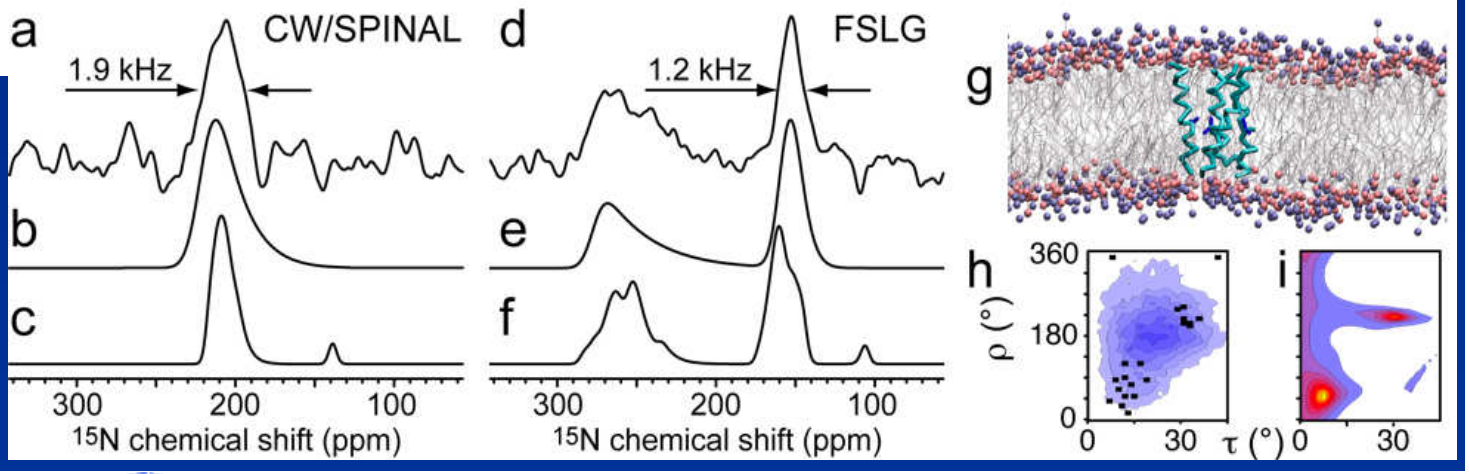
Lipid-protein Interactions -and the water channel



Dynamics of sidechains



The effect of Proteins Dynamics and Mosaic spread



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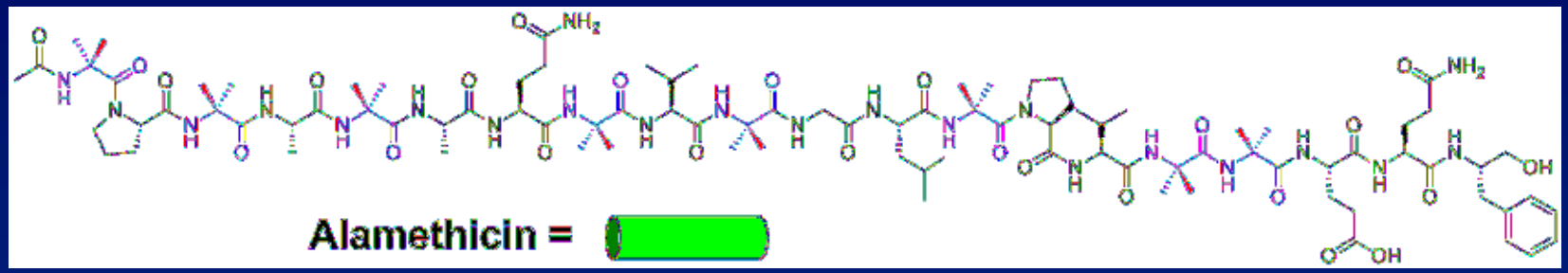
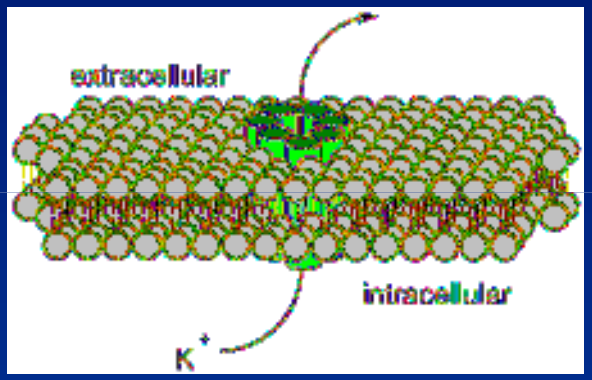
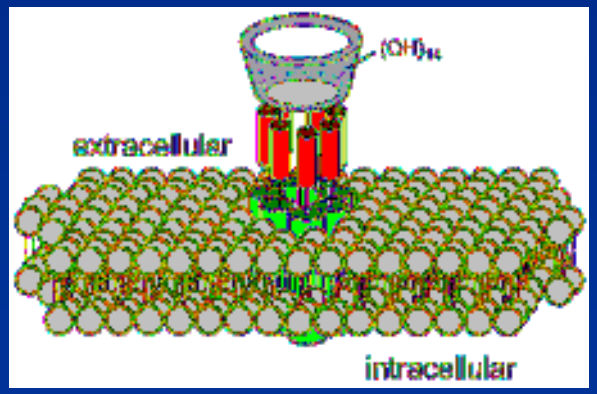
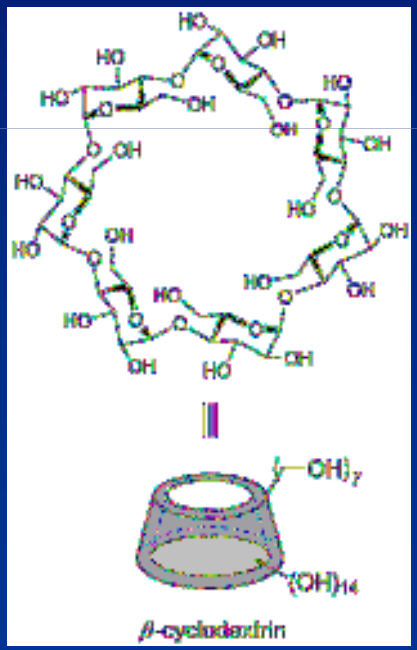
Nanoscience to control antimicrobial activity

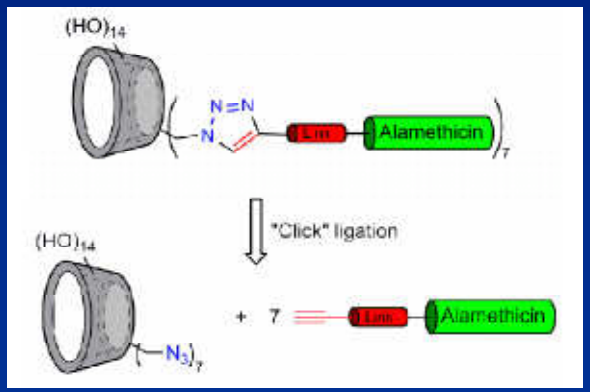
Artificial ion channels











Four examples

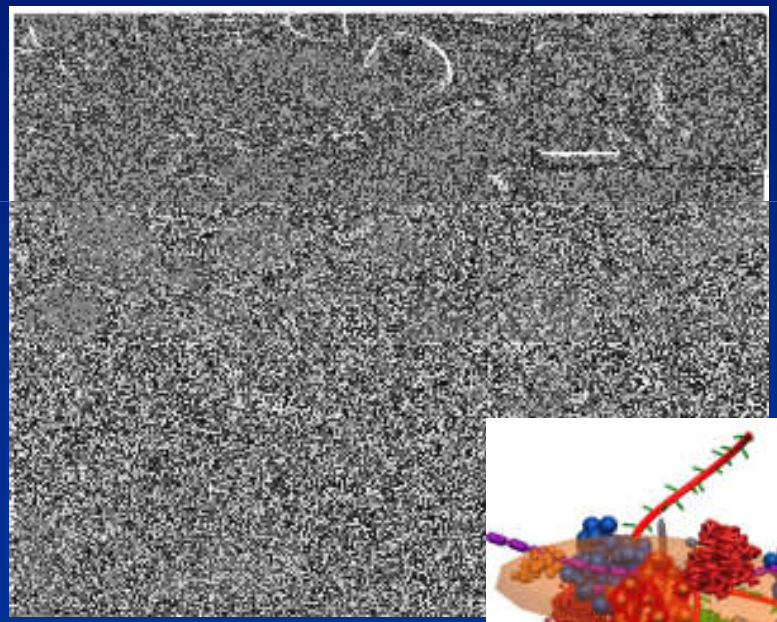
A. Antibiotic resistance - Antimicrobial peptides

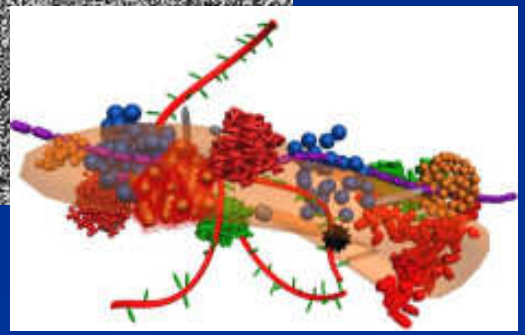
B. Biofilm formation - Protein fibrilation

C. Food response - NMR metabolomics

D. NMR for everyone - Development of low-field NMR for instrustrial applications

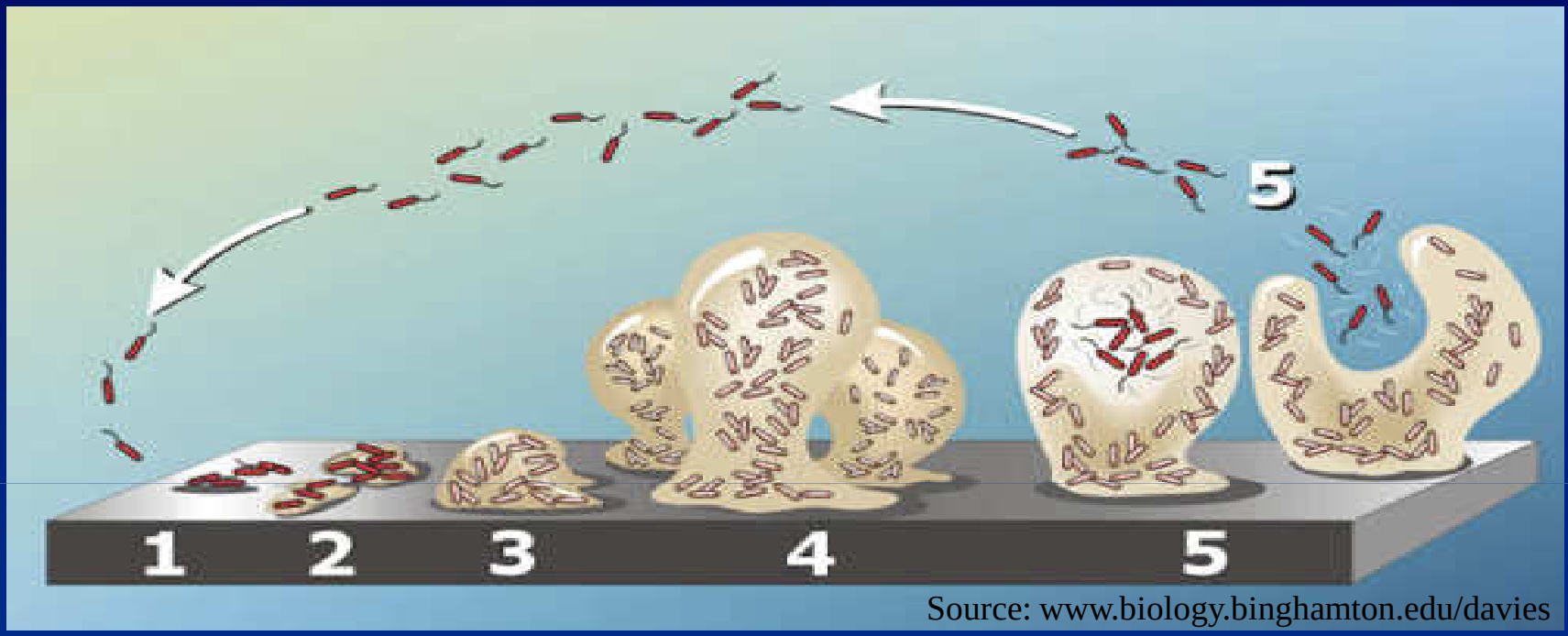
Why is this relevant for FOOD?





Industry: Bacteral-biofilm
E.Coli, Salmonella
Curli stabilizes biofilm

Biofilms – more than just bacteria

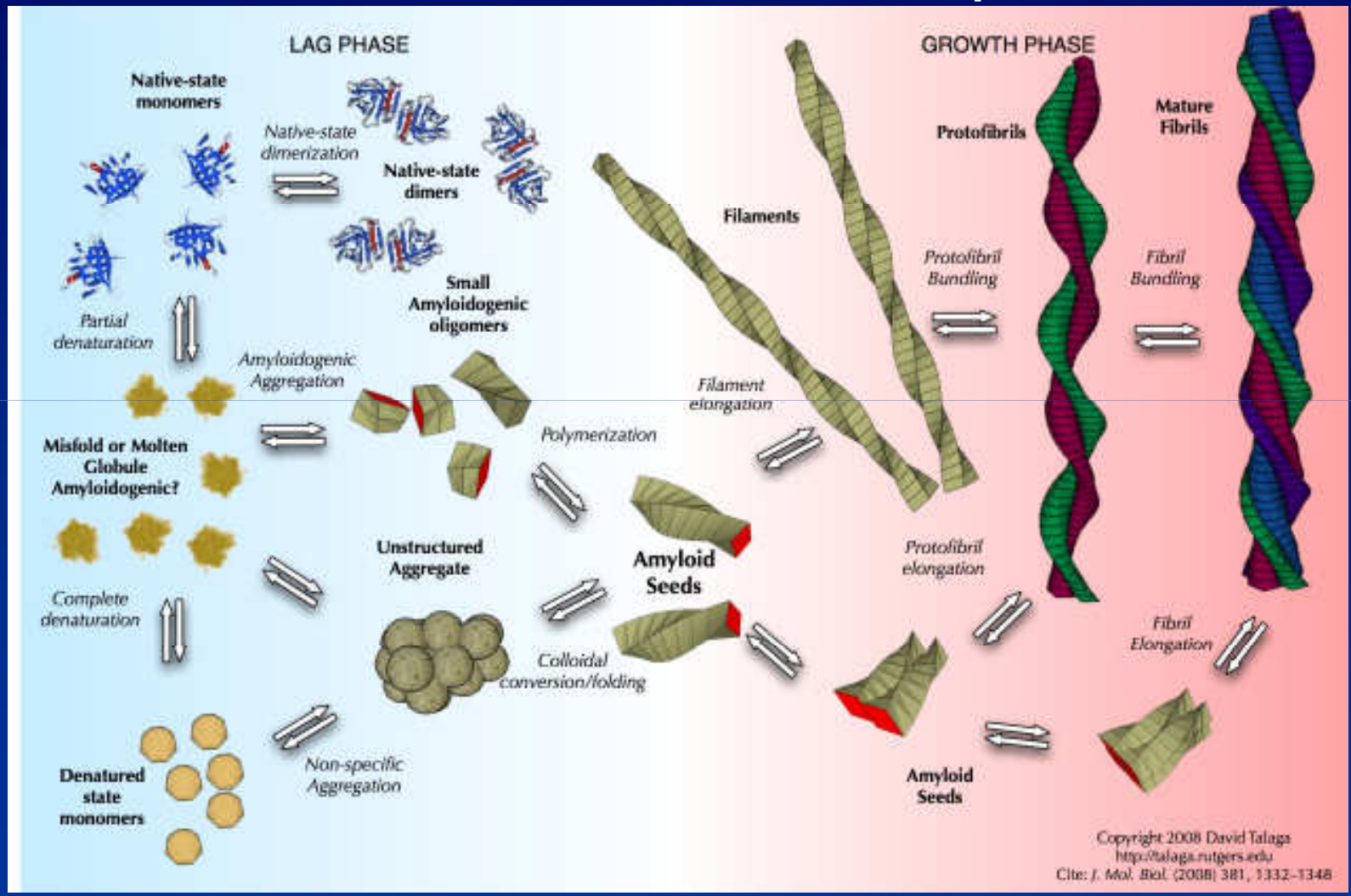


Source: www.biology.binghamton.edu/davies

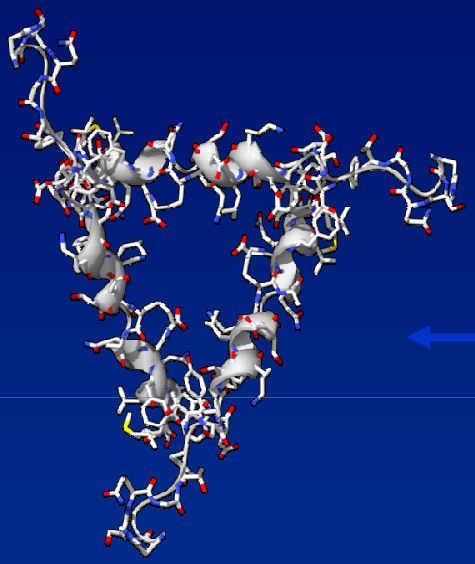
- Bacteria
- Polysaccharides
- Proteins
- Extracellular DNA

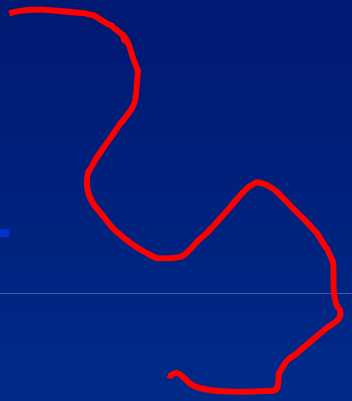
SOLUTIONS:
Antifouling surfaces
Biofilm hindring substrates

Fibril formation – can it be measured?
- can it be prevented?



Glucagon: a peptide hormon

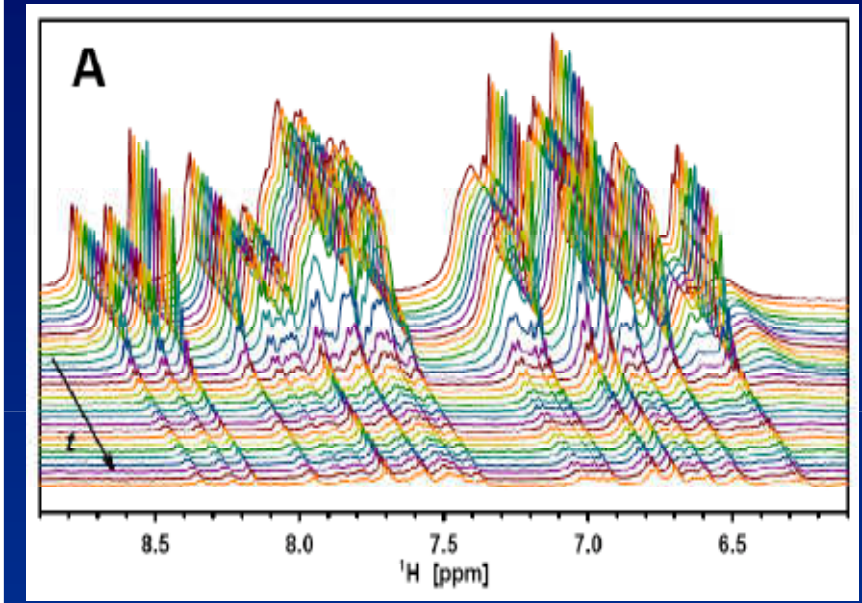


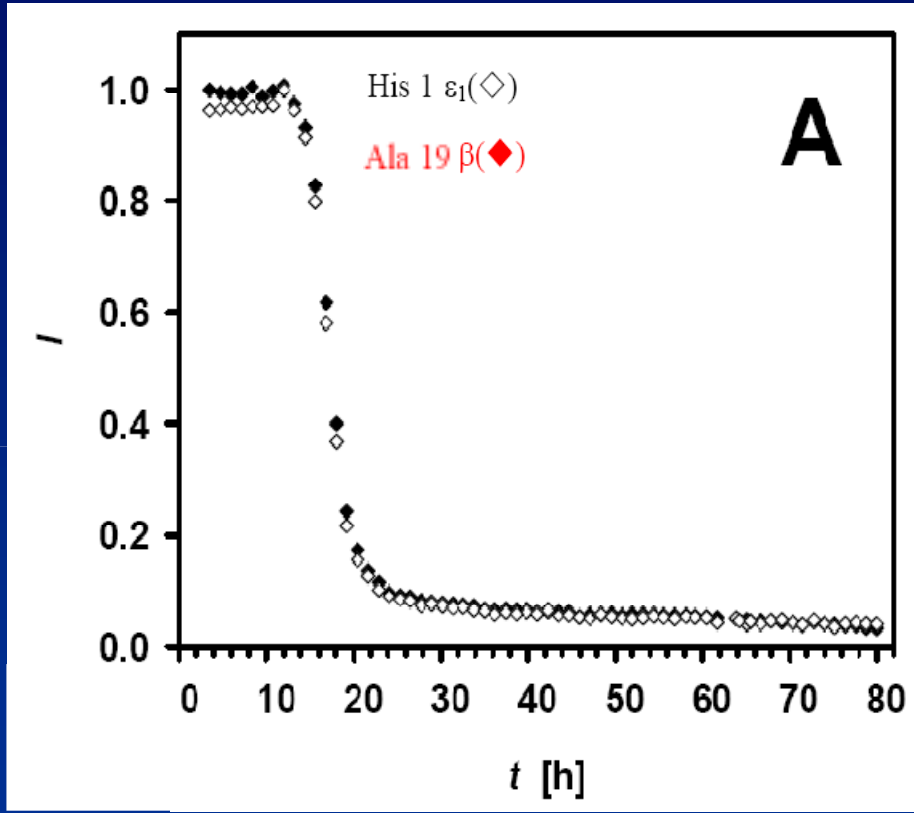


Fibrillar polymorphism incarnate

^{1}H NMR

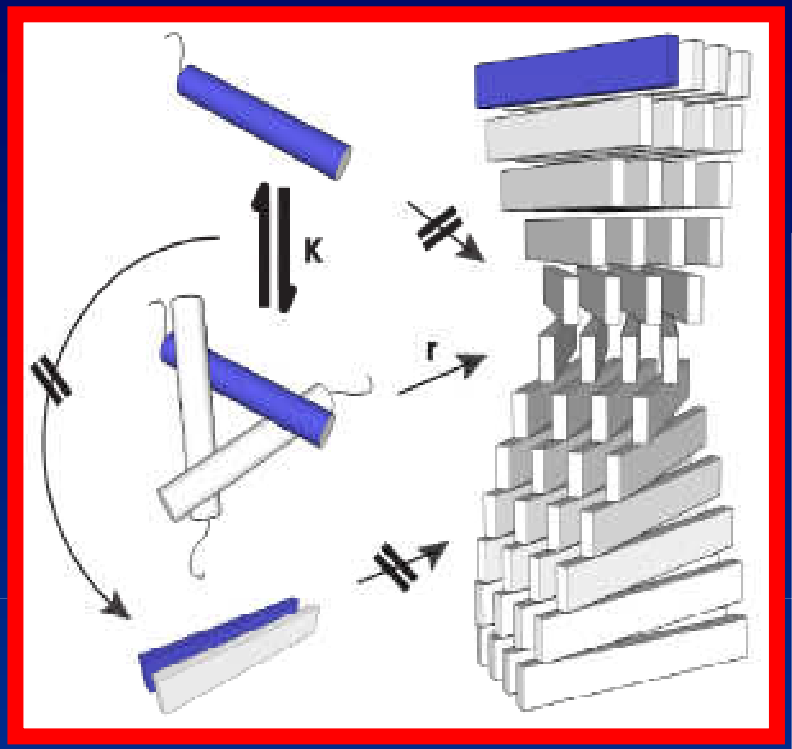
What does the signal disappearance tell us?

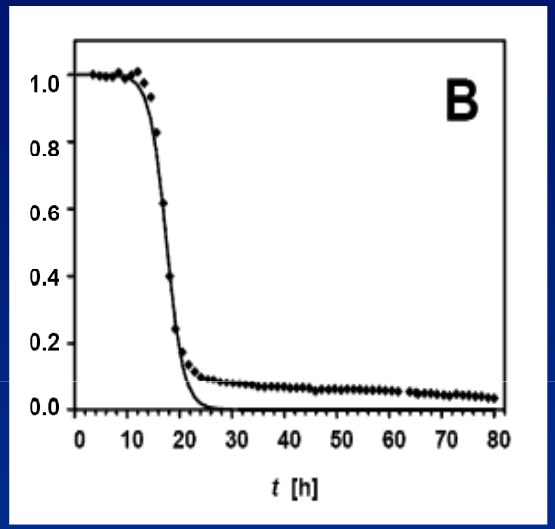




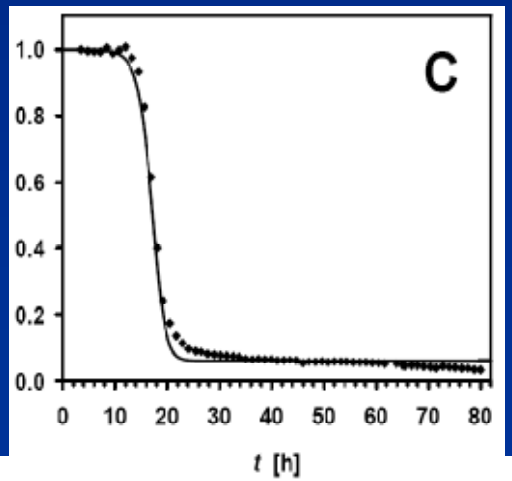
^{1}H NMR

The fibrils are generated from α-helical trimers!

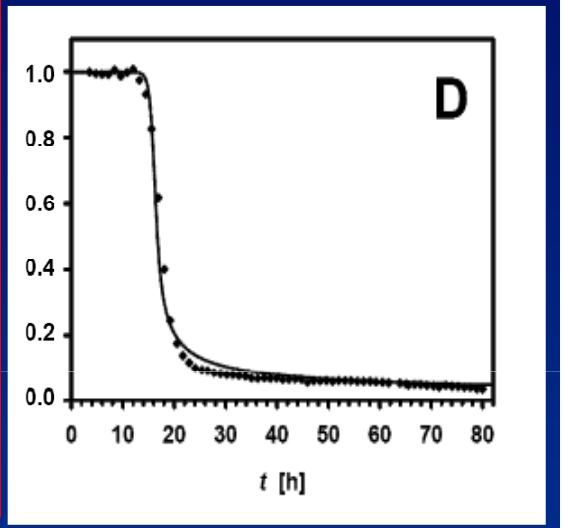




Monomer fibrillation

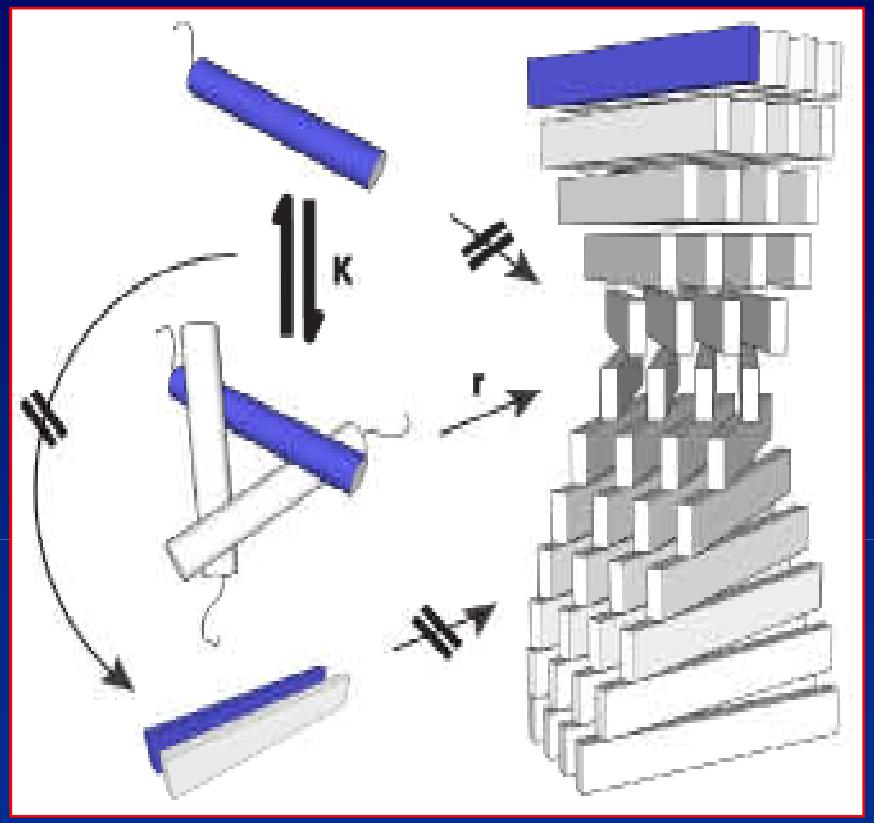


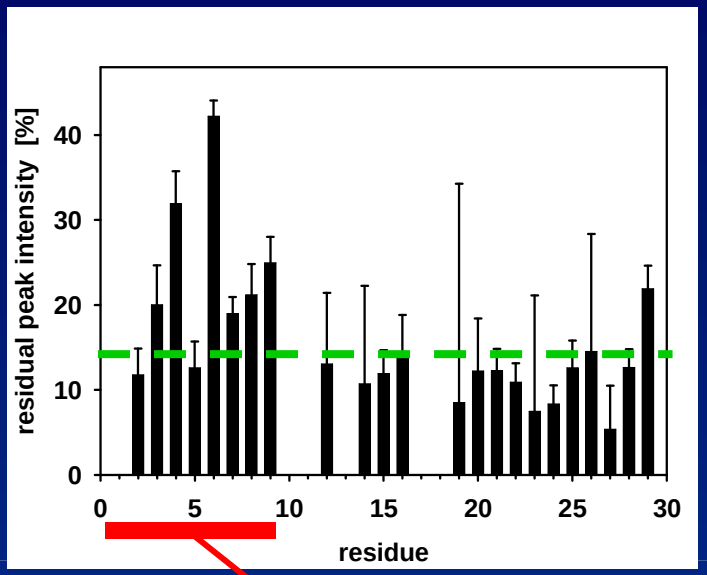
Monomer fibrillation with backtransfer

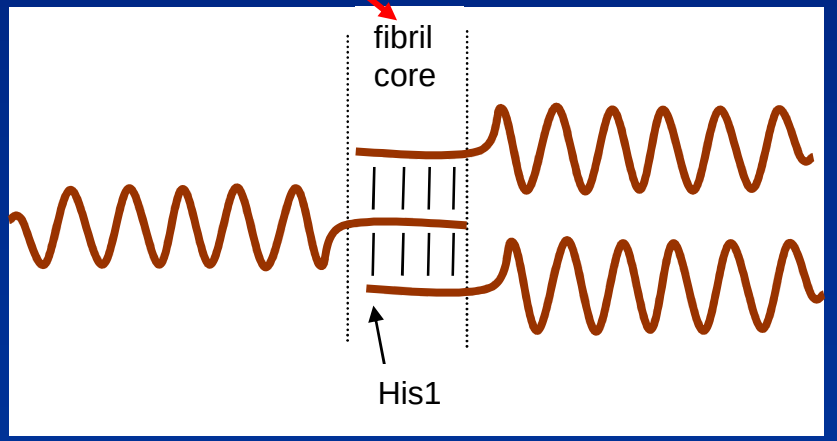


Trimer fibrillation in equilibrium with monomer-trimer exchange

Trimers with loose N-terminals makes the fibrils:
H/D exchange tells something about its role in the fibril core





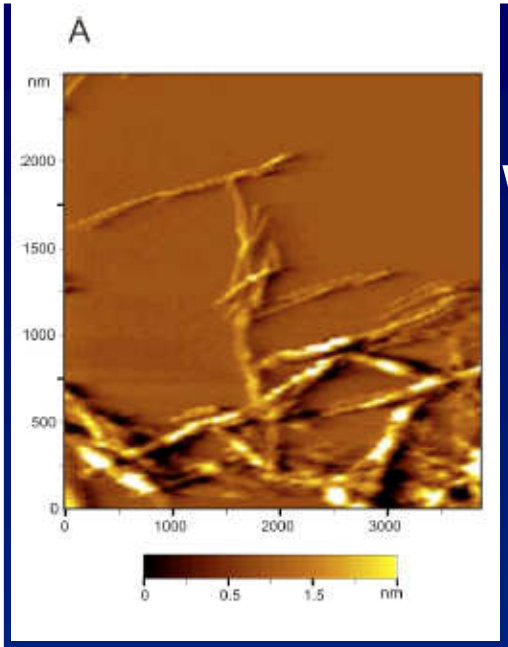


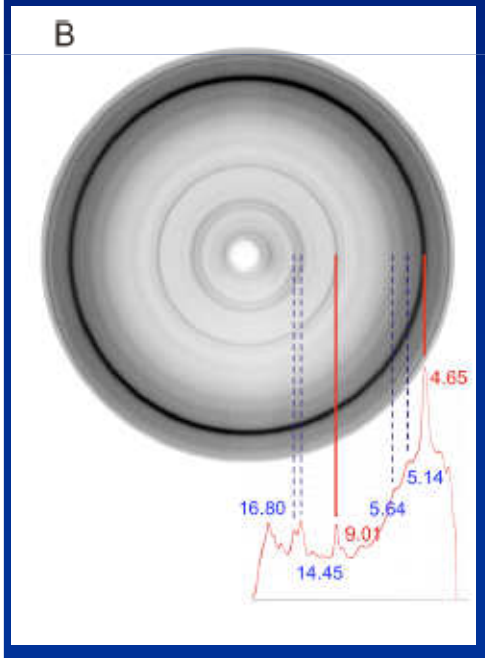
a) Fibrillate – freeze-dry
b) Add D buffer – shake 24 h
c) Resuspension in DMSO

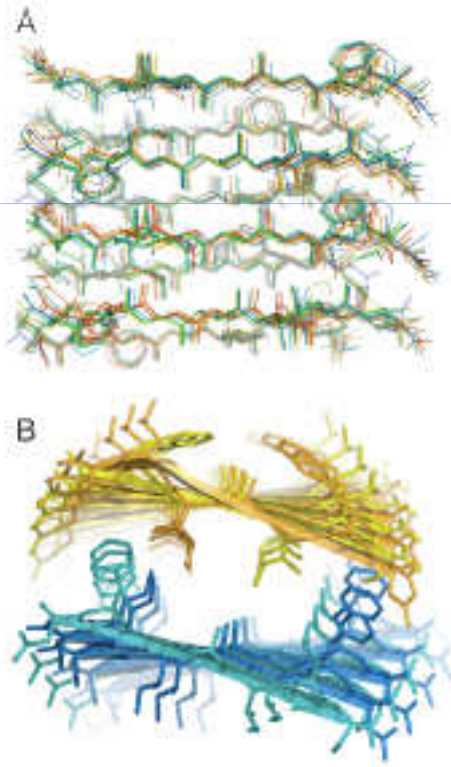
D in core low exchange – D in accessible parts high exchange

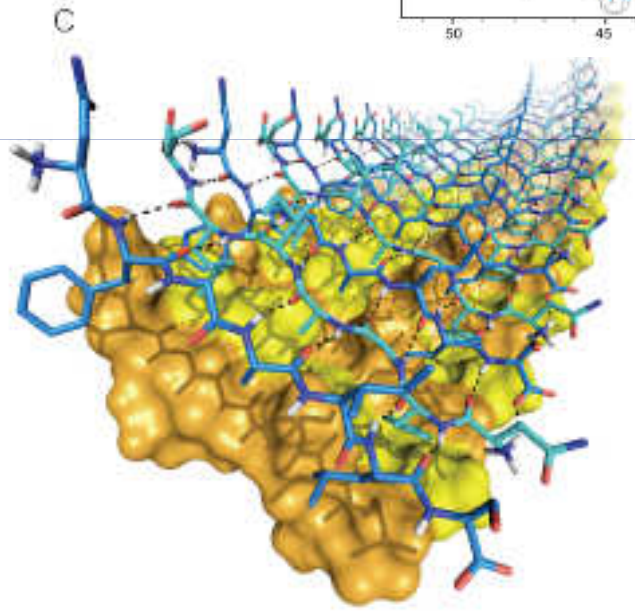
Fibril structures with 0.5 Å resolution

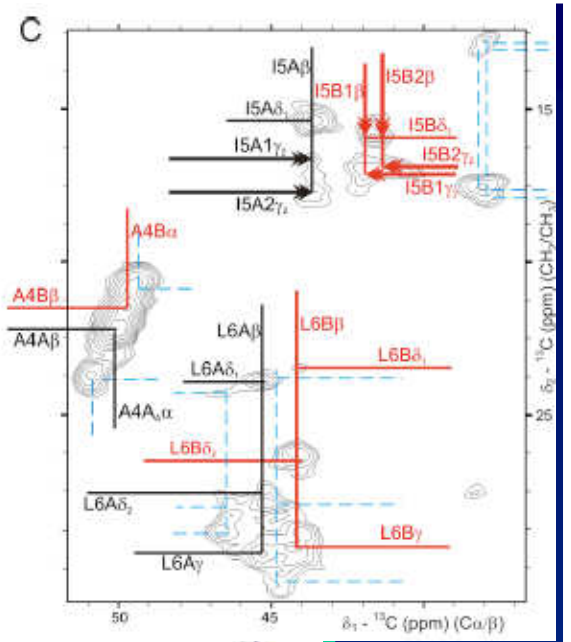
hIAPP – relevant for diabetes 2











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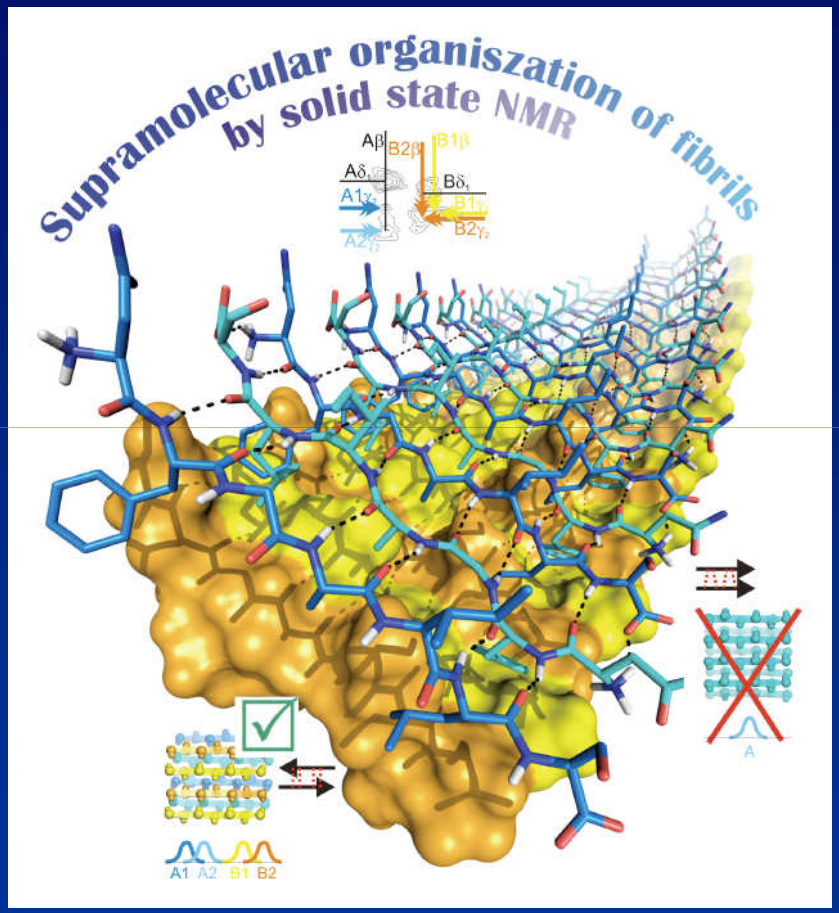
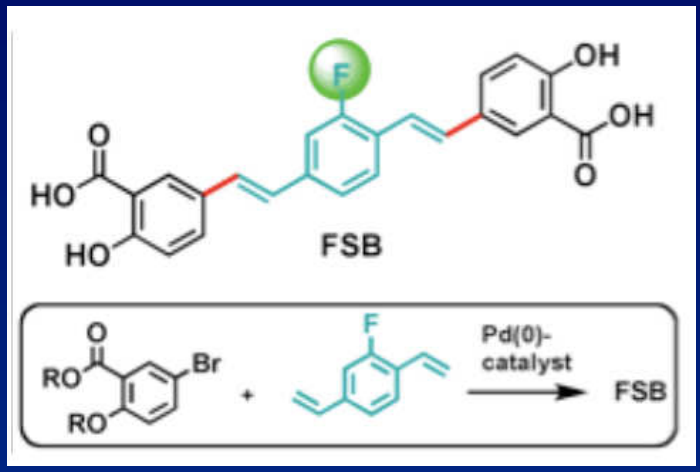
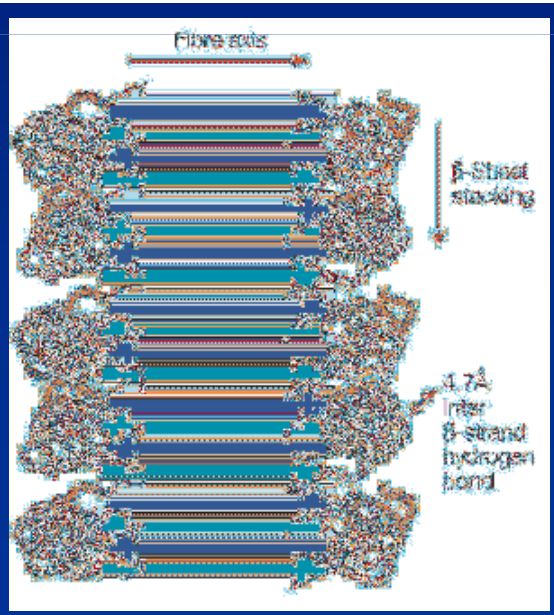
Fibril structures for rational design of inhibitors & contrast agents!







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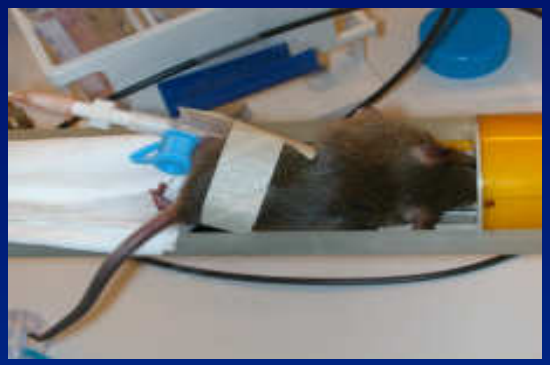
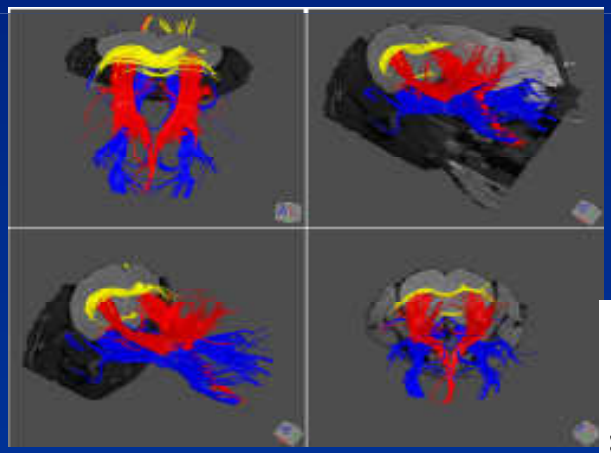
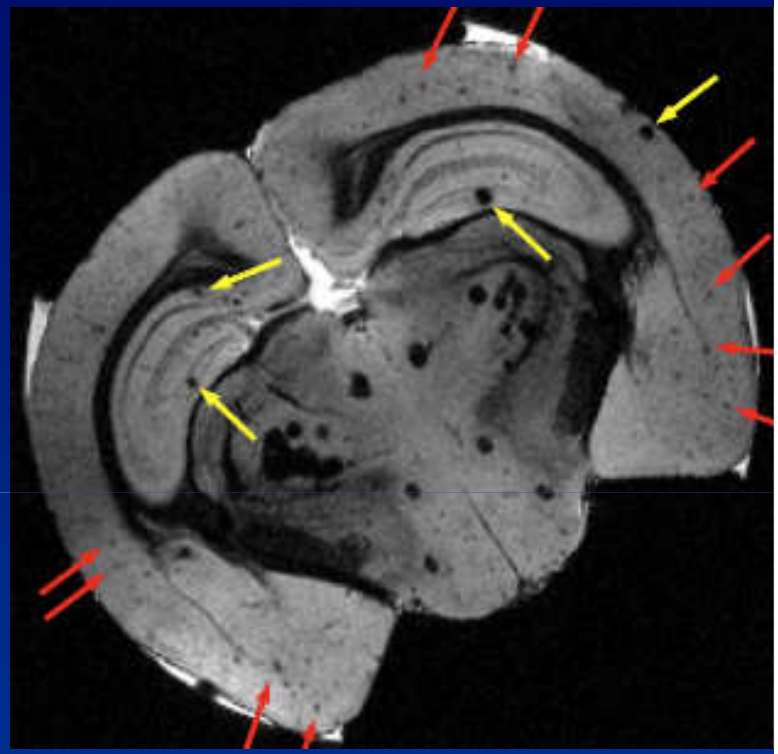
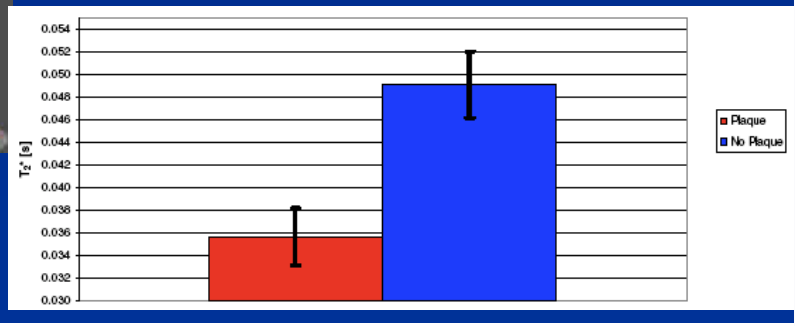
MRI on Aβ fibrils in Alzheimer Disease transgenic mice









17 T ultra-high field MRI

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Four examples

A. Antibiotic resistance - Antimicrobial peptides

B. Biofilm formation - Protein fibrilation

C. Food response - NMR metabolomics

D. NMR for everyone - Development of low-field NMR for instrustrial applications

Food functionality – nutriomics

Nanofood functionality

Compound selection

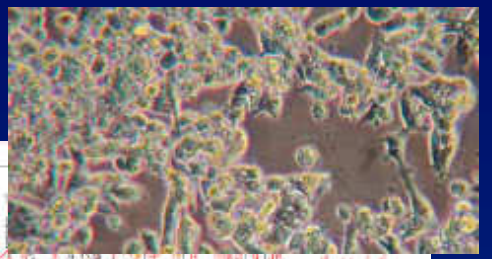
Health food factors
Lipids and Fatty acids
Bioactive Milk Proteins
Nano-encapsulated food ingredients
probiotics

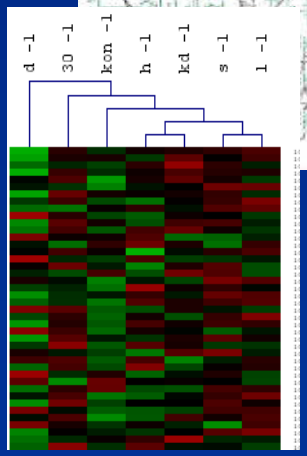
Food factors applied to model systems

Cell Culture
Liver cells
Caco-2 cells
Organ models
In vitro Liver model
In vitro Intestinal model
Whole animal Models

Biological response and biomarkers

Genomics
Chip array analysis
Proteomics
2D gel analysis, Mass spectrometry
Metabolomics
NMR
Comparison with other methods
Conventional "nutrition screening"





Probiotic consumation

Dairy products containing probiotic bacteria is a succesful category of functional food

.... probiotics has positive effects on chronic inflammatory diseases such as IBS (Irretable Bowel Syndrome) and IBD´s (Inflammatory Bowel Diseases), such as Crohn´s disease and ulcerative colitis.

Ex:

Actimel®: *Lactobacillus defensis immunitas (Danone)*

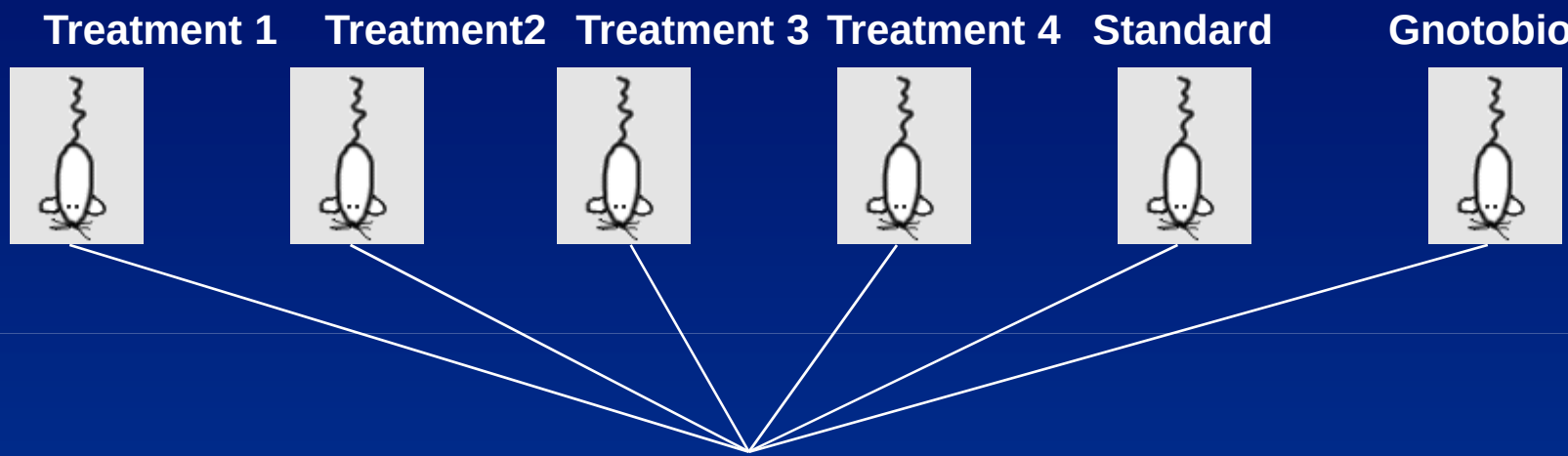
Yakult®: *Lactobacillus defensis shirota*

Cultura®: *Lactobacillus casei (Arla)*

Estimated retail sales of 1.2 billion Euro annually

Experimental setup

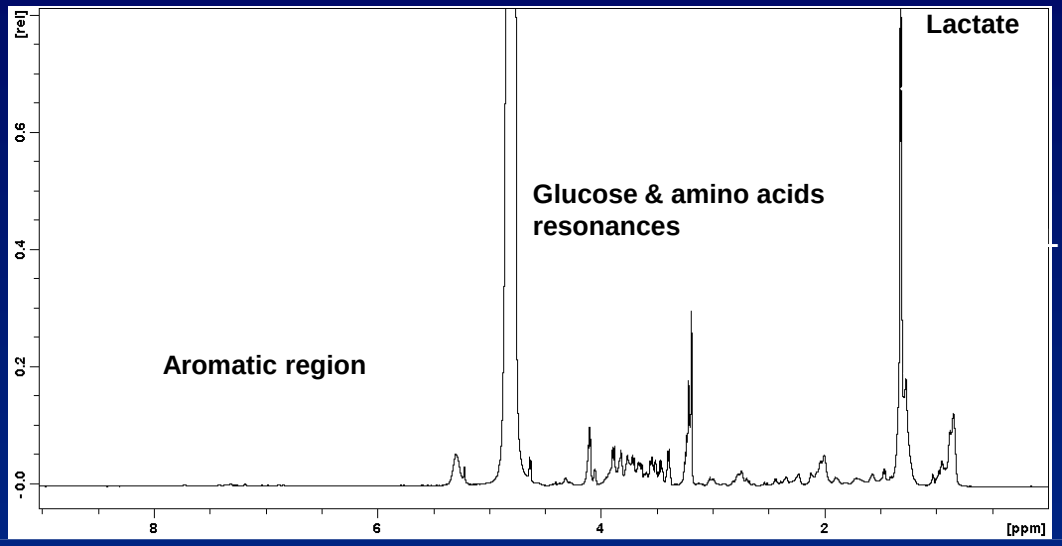
6 different treatments for 4 weeks



NMR on plasma samples

Multivariate dataanalysis

Representative 1D CPMG ^{1}H-NMR spectrum of plasma from mice treated with probiotic bacteria

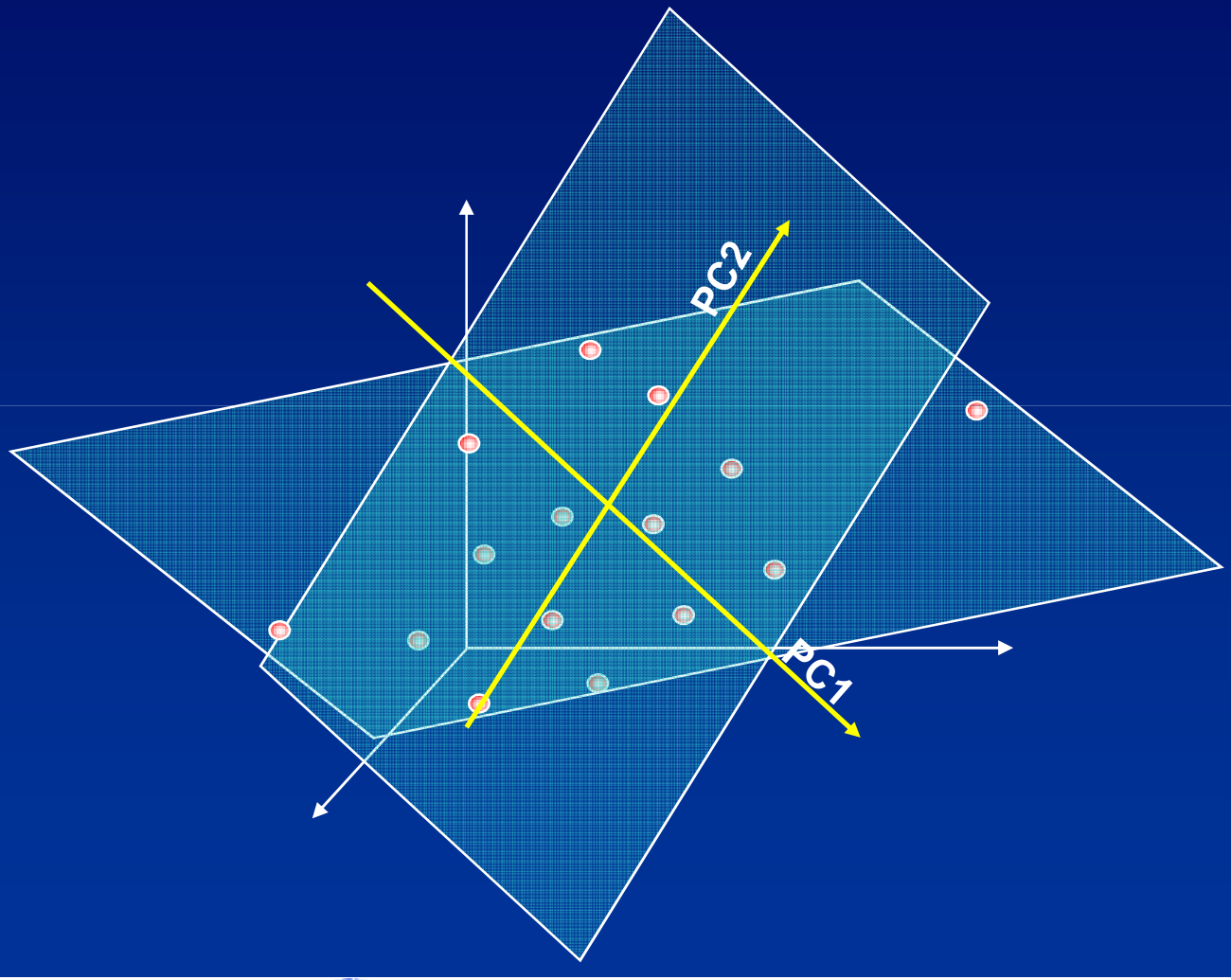


Processing of NMR data

- Each spectrum divided into bins of 0.01 ppm
- Normalisation of each spectra to total area
- Multivariate dataanalysis
- Assignment of metabolites in combination 2D TOCSY spectrum

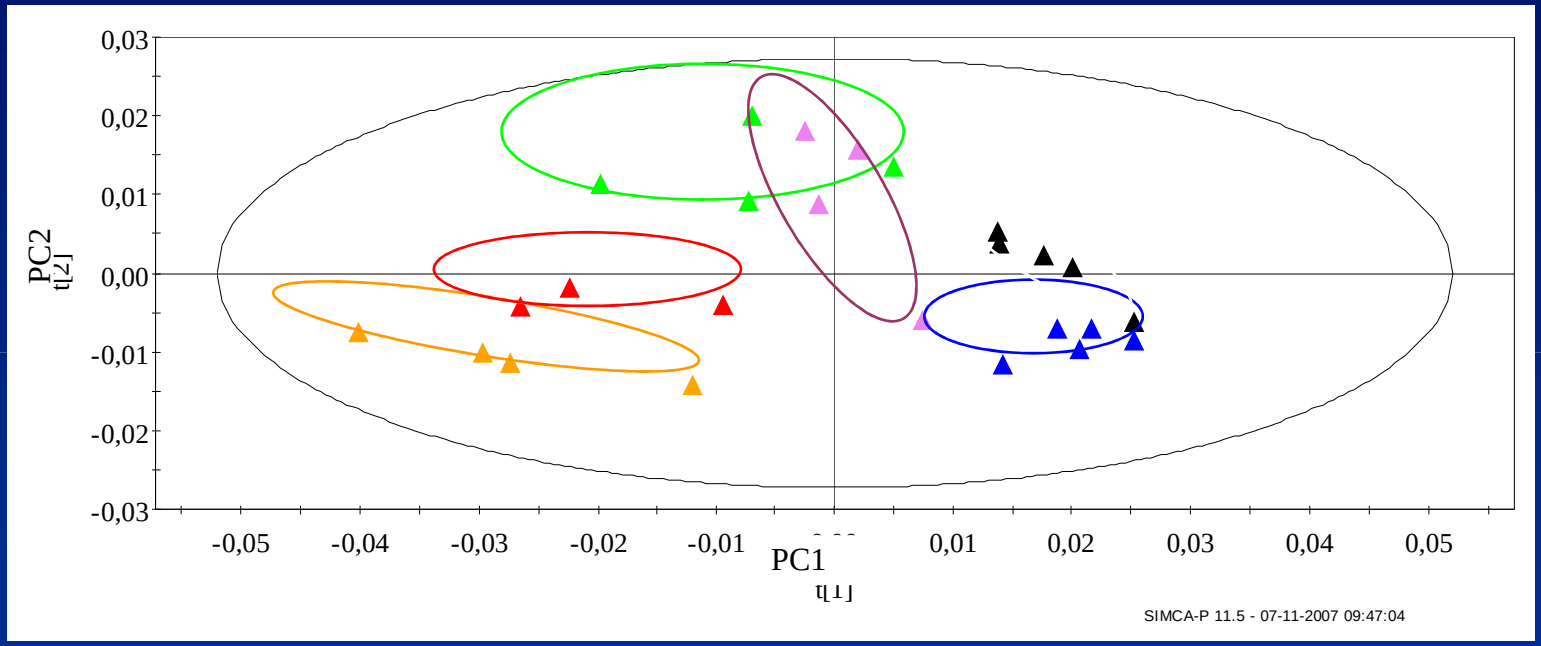
Multivariate dataanalysis (MVA)

Breaking complicated vast dataset down into easy interpretable data



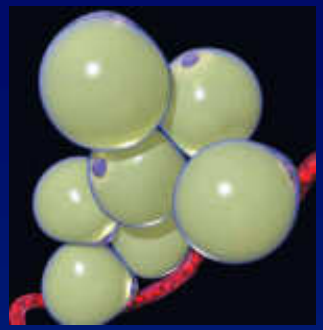
Principal Component Analysis (PCA)

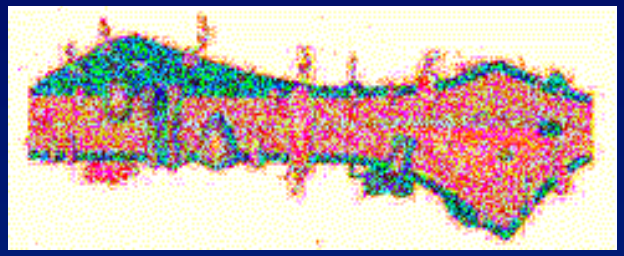
Unsupervised PCA of 1H-NMR spectral data from mice submitted to six different treatments over 4 weeks



Treatment 1 | Treatment 2 | Treatment 3 | Treatment 4 | Standard | Gnotobiotic

Fat – at good and bad

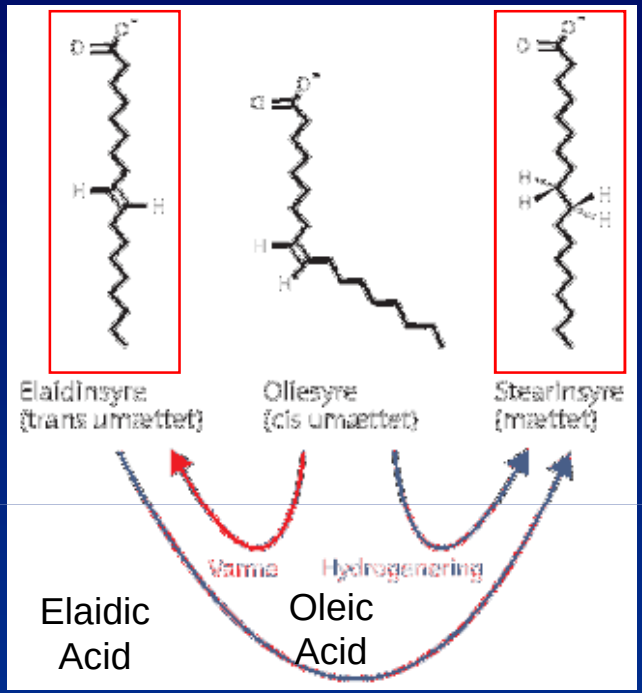




Cholesterol

Type Fatty Acid	LDL	HDL	
Mono- and poly unsaturated (Cis)	↓	↑	Good
Saturated	↑	-	Bad
Trans	↑	↓	Worse

Low and High Density Lipoproteins

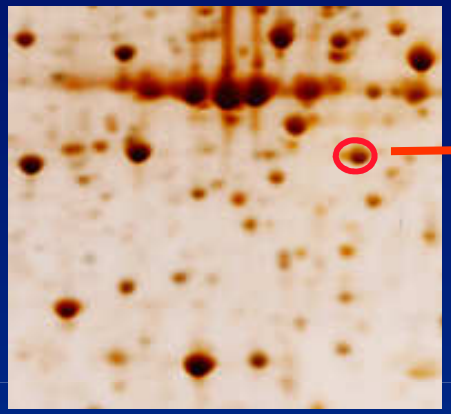


Produkt	Fedt total/g	Mættede/g	Monoumættede/g	Polyumættede/g
Smør	81,4	51,8	23,1	1,8
Stegemargarine, vegetabilsk	82,5	22,1	21,1	22,4
Rapsolie	100	6,4	55,4	33,2
Olivenolie	100	13	67,7	7,6
Vindruekerneolie	100	9,6	18,2	67,8

TFA can easily reach more than 20%

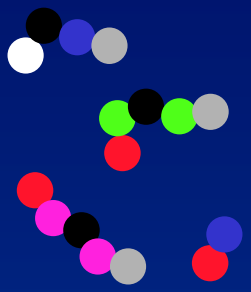
Regulation > 2%

Peptide mass fingerprinting by MALDI-MS



Coloring with chlorophore

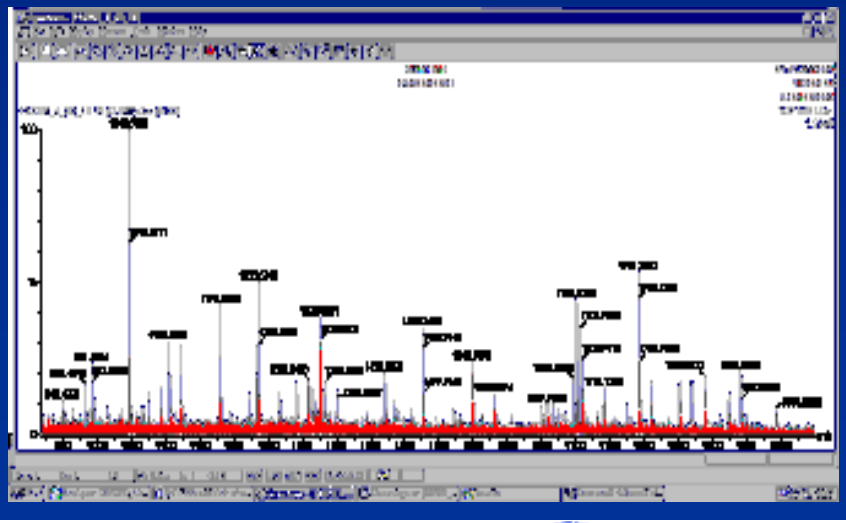
Trypsin digestion



Peptide extraction

Spot MALDI target

MALDI-MS



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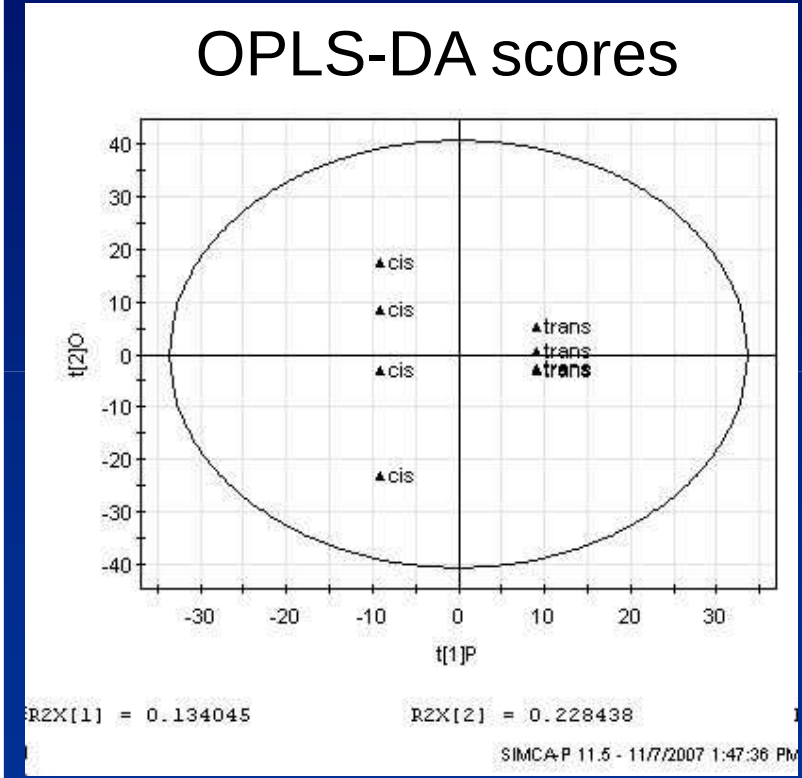
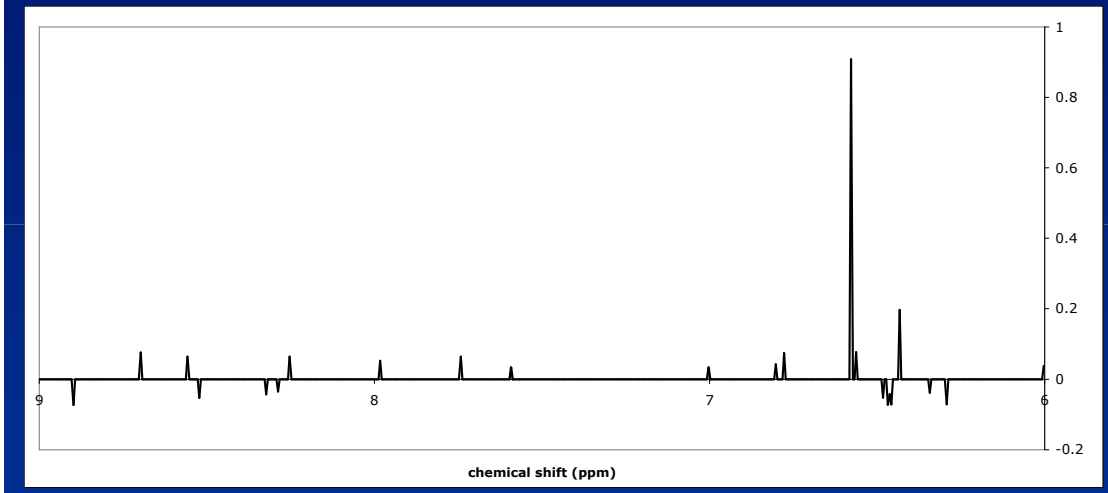
NMR metabonomics
cis vs. trans fatty acids

OPLS-DA scores



OPLS-DA loadings

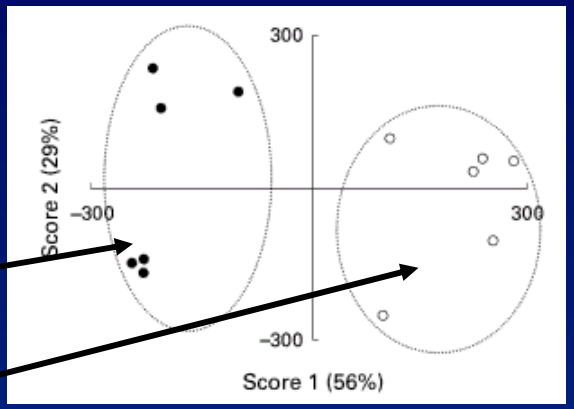


$Q^2 = 0.75$

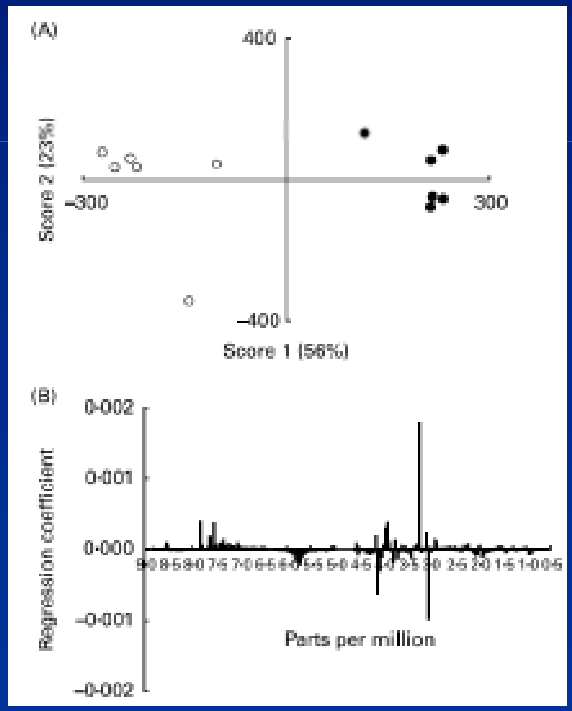
Food response: Effect of diets

Non-Whole-grain diet

Whole-grain diet



PCA



PLS

Table 2. Summary of the major differences between non-wholegrain diet (NWD) and wholegrain diet (WGD) samples*

NMR spectral region (ppm)	Assignment†	Plasma‡	Urine‡
approximately 1·2–1·4	$(CH_2)_n$ in fatty acids	↑	–
3·03	N-CH_3 in creatinine/creatine	–	↓
3·25	N-CH_3 in betaine	↑	↑
approximately 3·5–4·5	CH_1 in glucose and amino acids	↓	–
3·90–3·94	CH_2 in betaine	↑	↑
4·06–4·10	N-CH_2 in creatinine	–	↓
5·69–5·81	NH_2 in urea	–	↓
7·54	CH_3/CH_5 in hippurate	–	↑
7·83	CH_2/CH_6 in hippurate	–	↑

Collaboration with H. Bertram, H. J. Andersen, K.E.B. Knudsen et al, Foulum

Low-field, mobile NMR for production control

SpinCore
RadioProcessor
0-70MHz

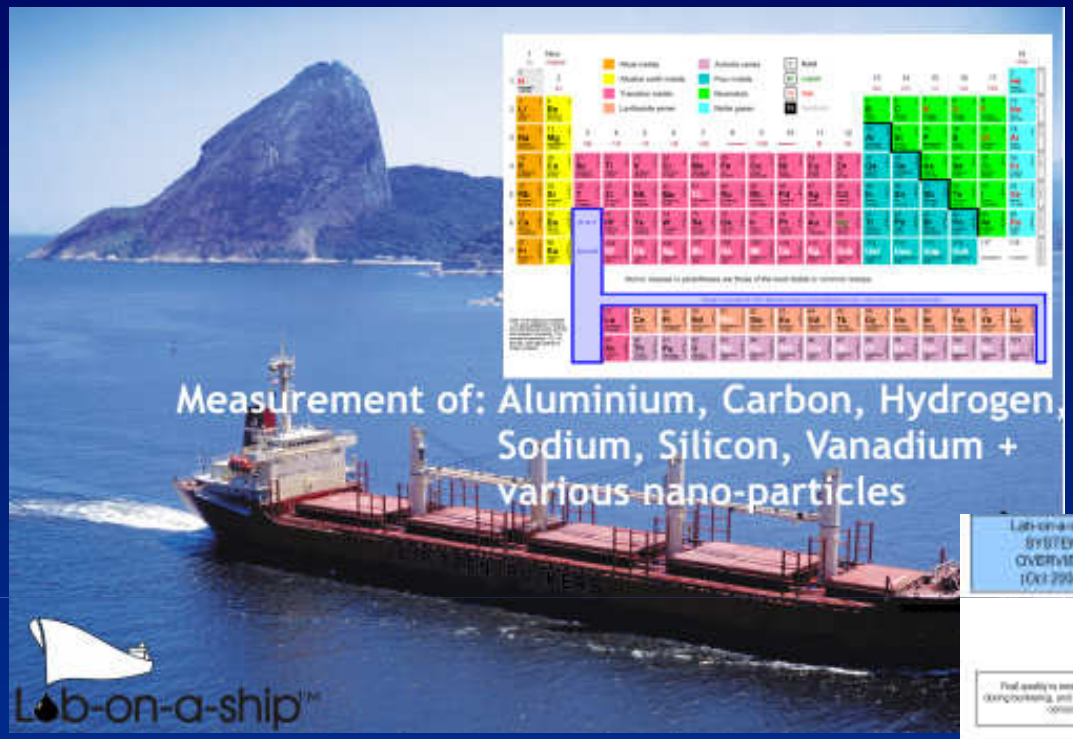
Tx/Rx Switch
and Preamplifier

PC
and
Software

Power Amplifier
500W

Permanent Magnet
B_0=0.36T
and NMR Probe

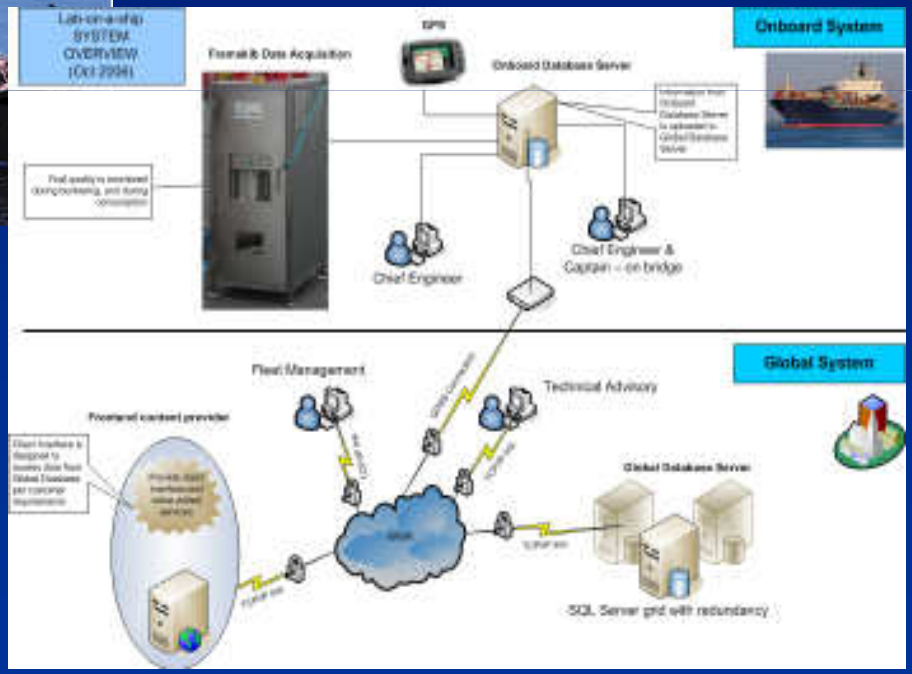
OnBoard NMR Spectroscopy



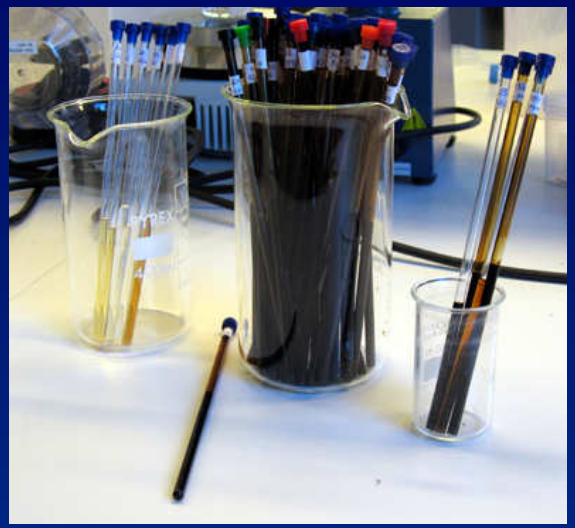
iNANO + NanoNord

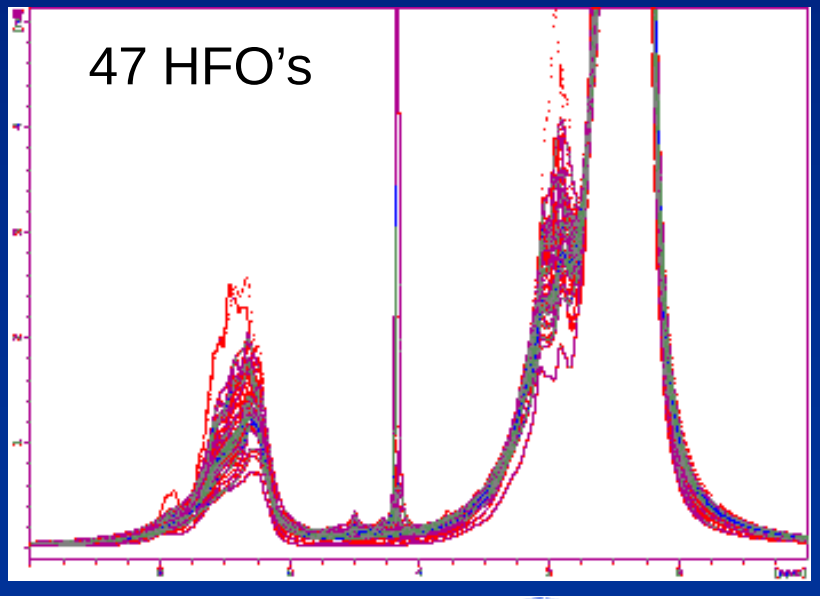
Mobile NMR Spectroscopy:

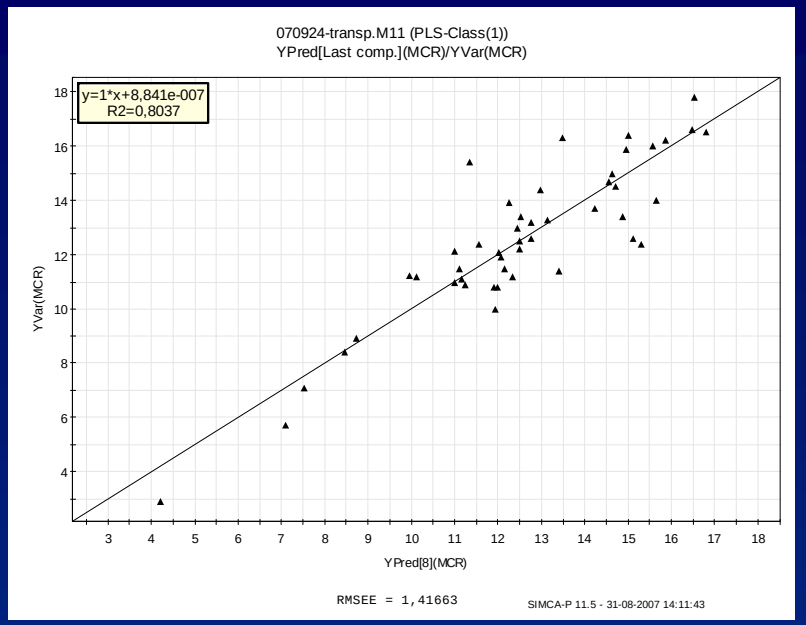
Development of low-cost NMR
Instrument +
New measuring methods

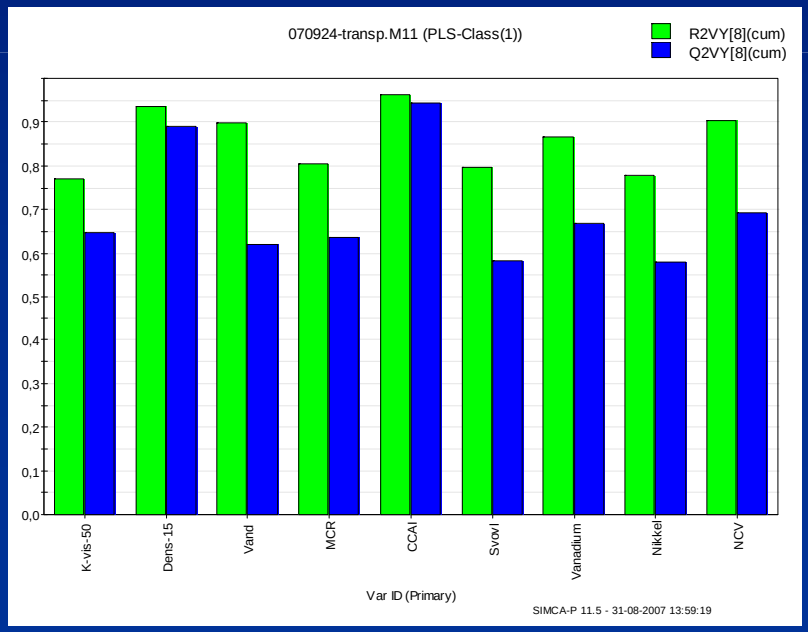


Oil analysis by PCA and PLS









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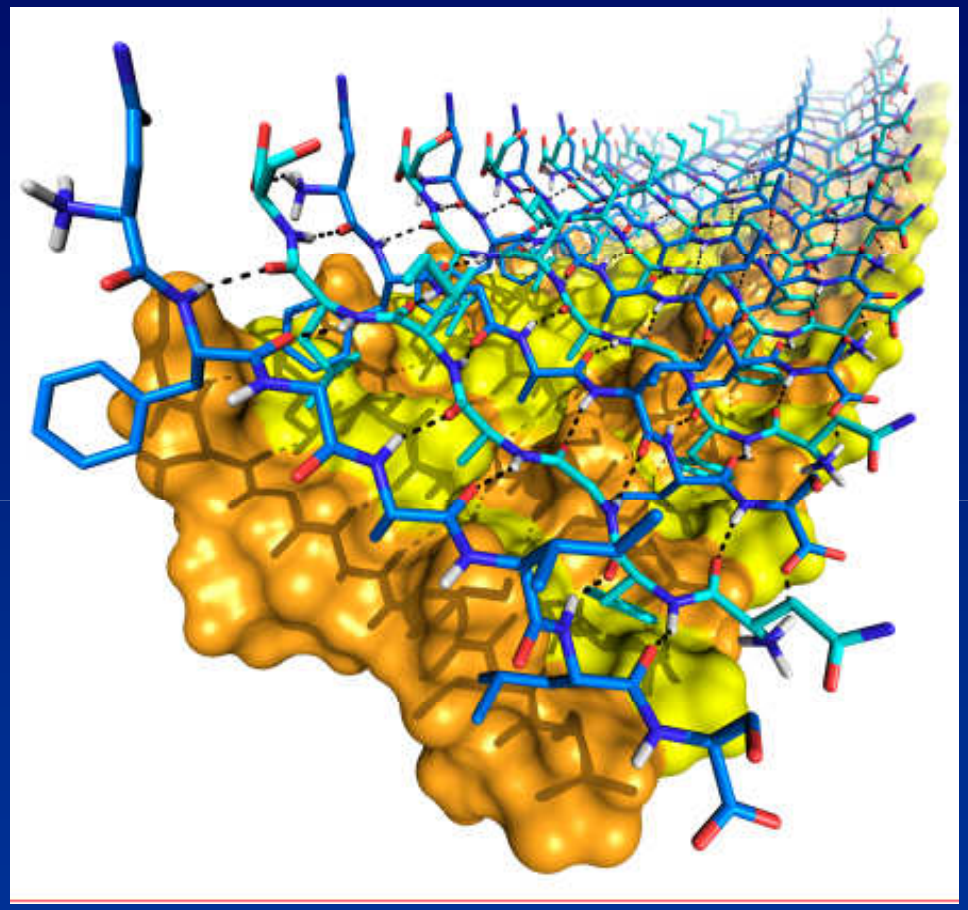
Ole Jensen, Nanonord
Emad Tajkhorshid, UICU



Thanks for listening

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