New insights into the morphological and molecular structure of the Stratum corneum

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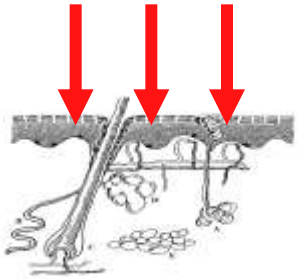
Content:

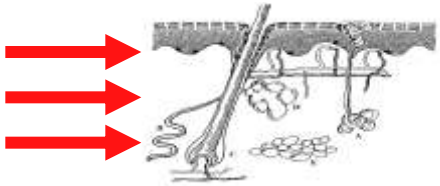
1. Introduction
2. Structure of the Stratum corneum (SC)
   - Morphology
   - Molecular architecture
   - Impact of neutron diffraction
3. Influence of different ceramides on the molecular structure
   - Impact of the ceramides [AP], [EOS], [NP]
   - New structure models of the SC bilayer
   - Impact of enhancer molecules
4. Conclusions

Principles of drug administration to the skin

1. **Effects at the surface of the skin**
   - Cleaning

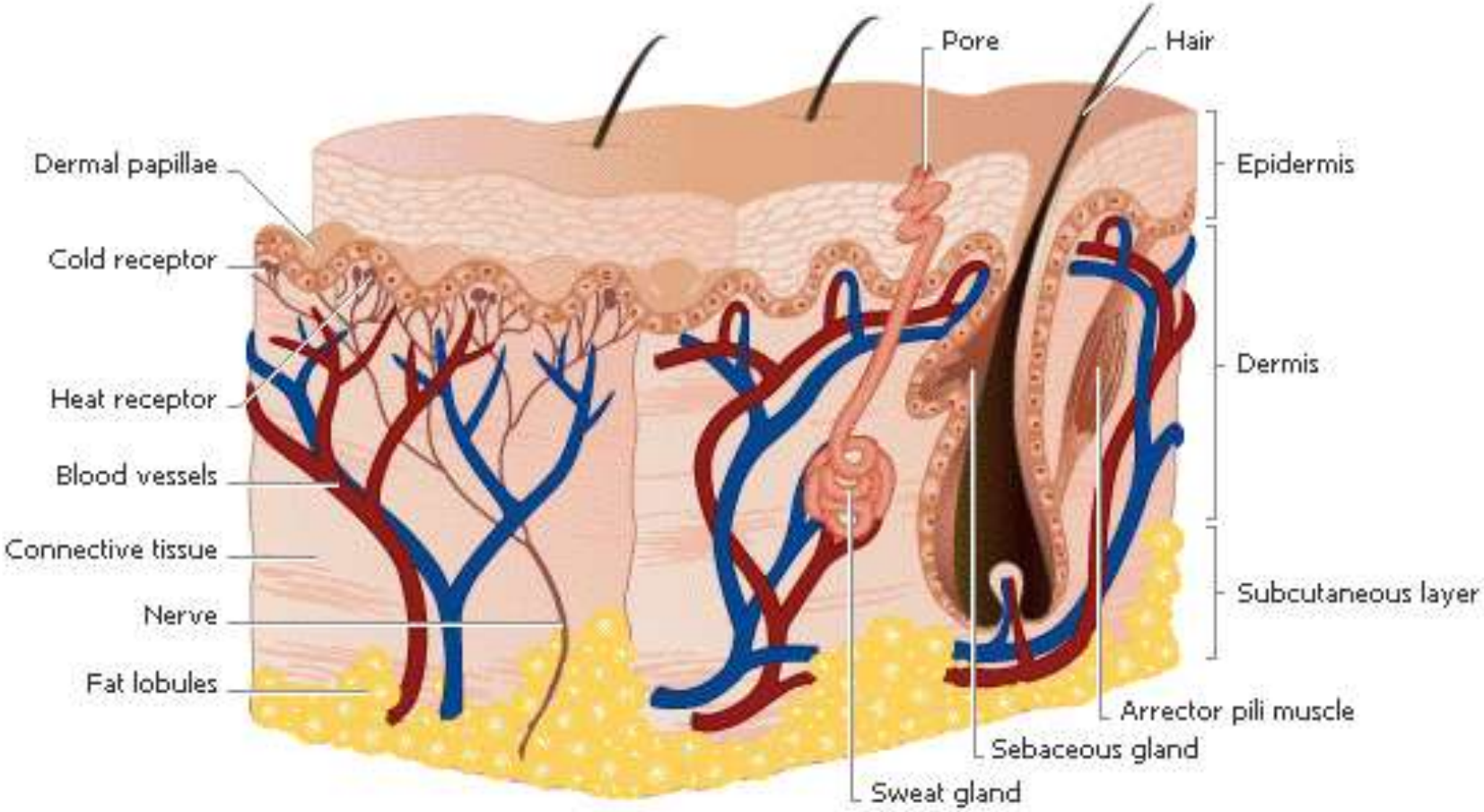


2. **Dermal therapy**
   - Effects in viable epidermis
     e.g. Psoriasis, Atopic dermatitis
   - Effects in the folliculae

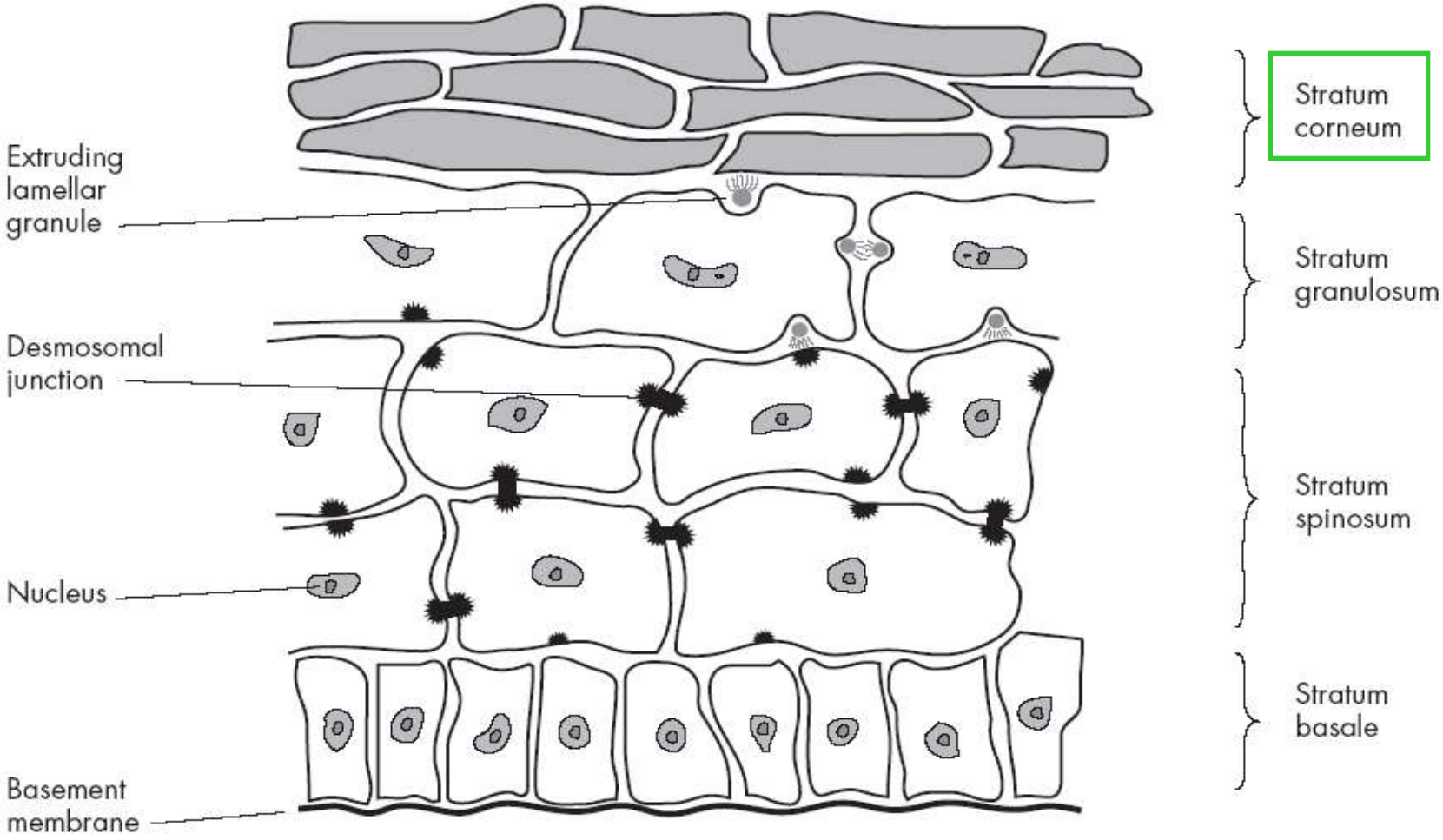


3. **Transdermal therapy**
   - Administration to the skin,
     systemic effects

Structure of the human skin



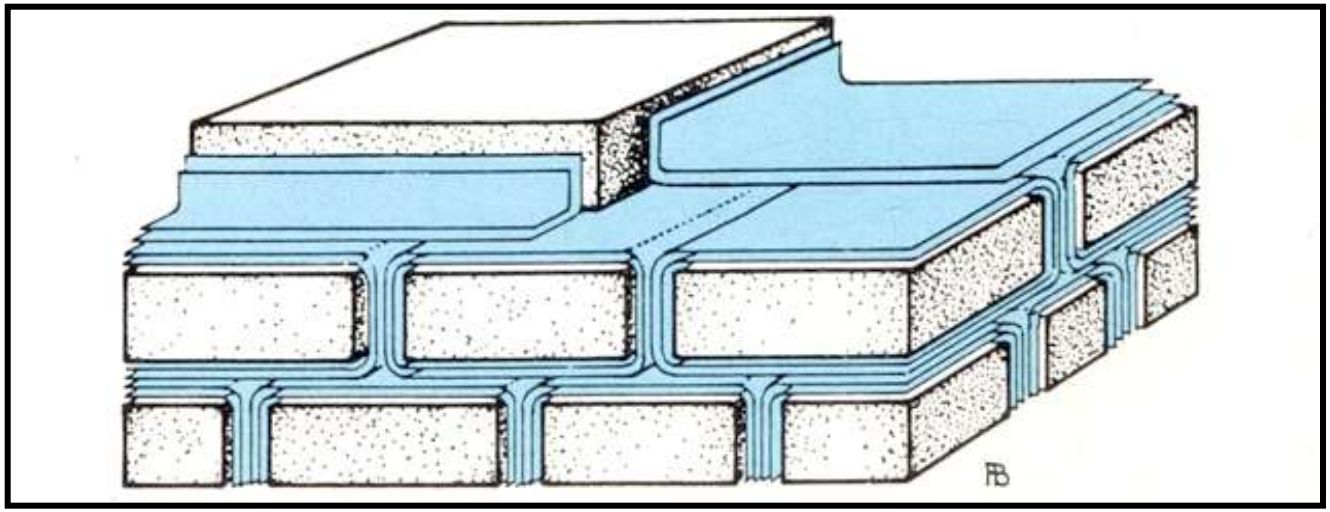
Main barrier of the skin: Stratum corneum (SC)



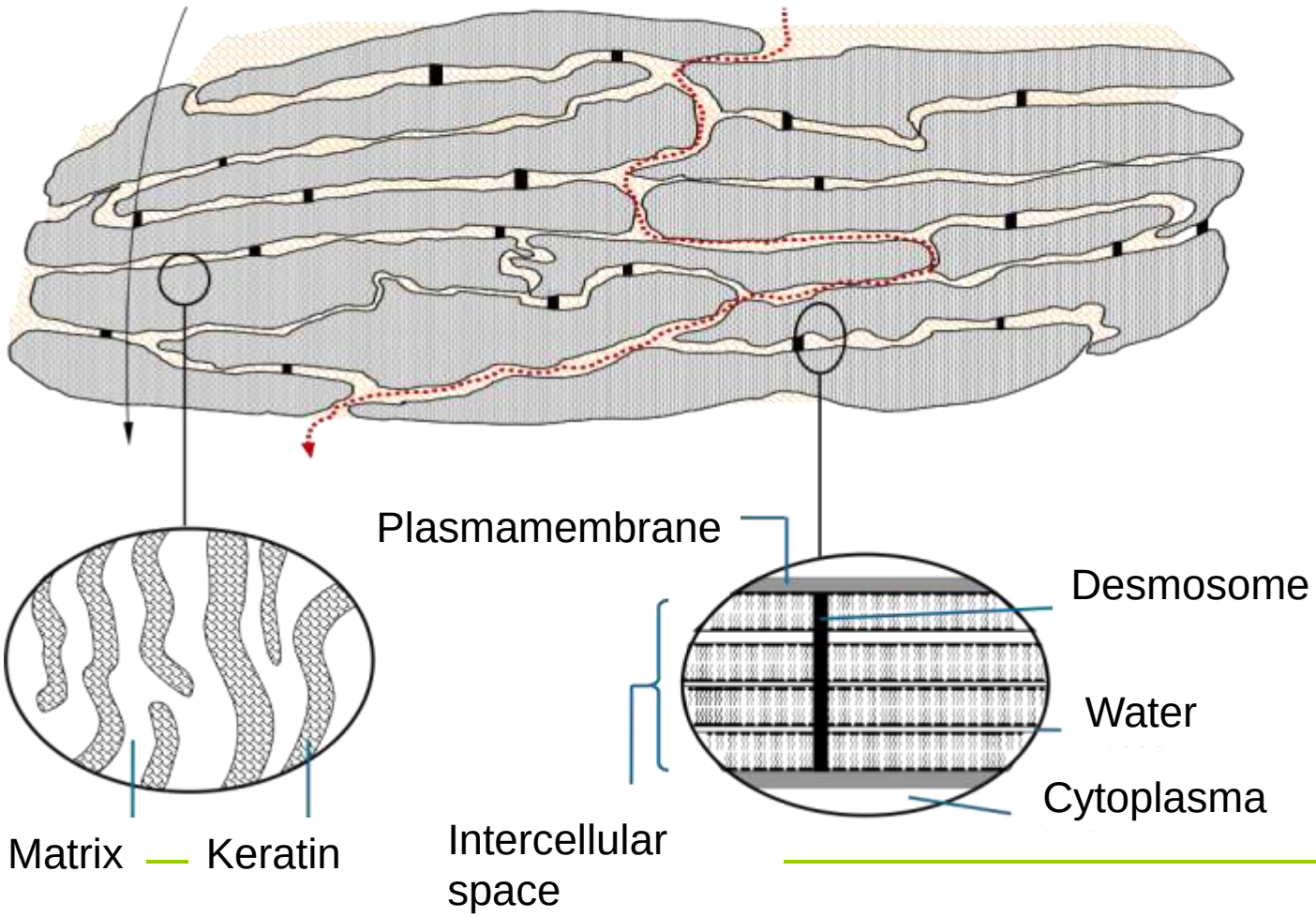
Main barrier of the skin: Stratum corneum (SC)

- „Brick and mortar“ model of the Stratum corneum (horny layer) according to **Elias** (1981), to understand barrier function:

Corneocytes: the „bricks“
Intercellular Lipids: the „mortar“

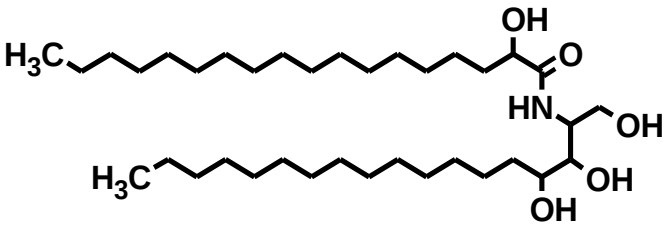


Penetration pathways into and through the Stratum corneum (SC)

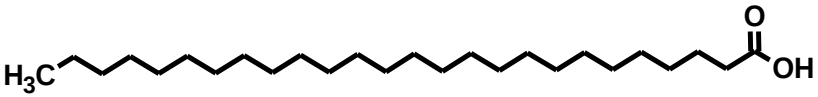


Stratum corneum Lipids

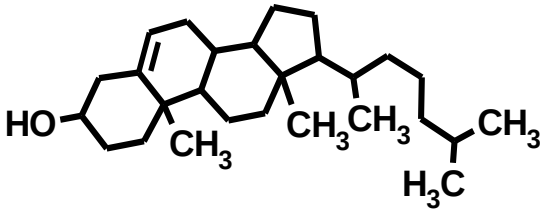
Ceramides (Sphingolipids) 30 %



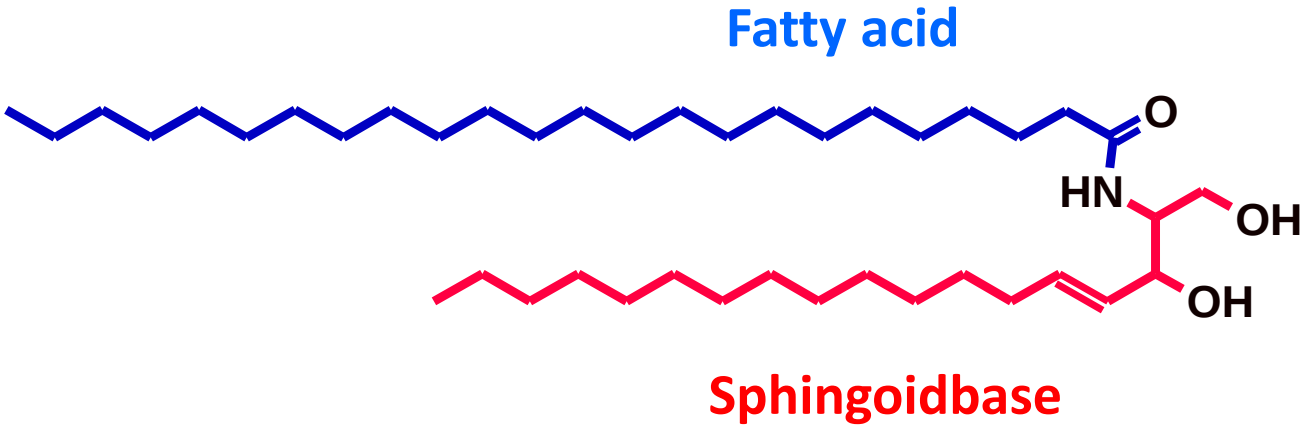
Free fatty acids 30 %



Cholesterol and Derivatives 30 %



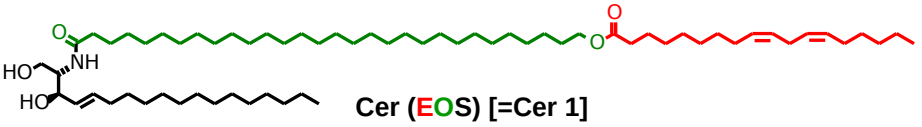
Ceramides of the Stratum corneum



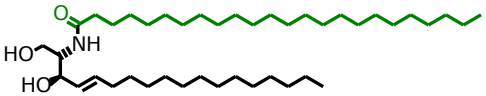
Fatty acid	α-hydroxylated (A)	Non α-hydroxylated (N)	ω-esterified (EO)
Sphingoid base	Sphingosine (S)	Phytosphingosine (P)	6-Hydroxy-Sphingosine (H)

Ceramides of the Stratum corneum

Free ceramides of human stratum corneum

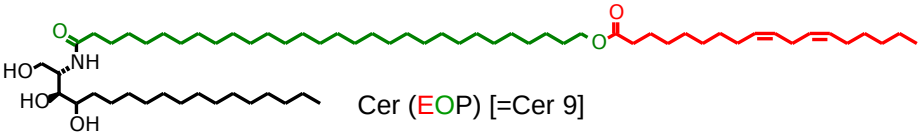


Cer (EOS) [=Cer 1]

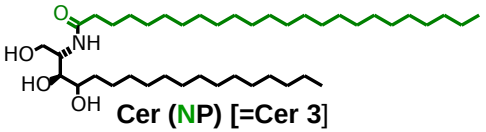


Cer (NS) [=Cer 2]

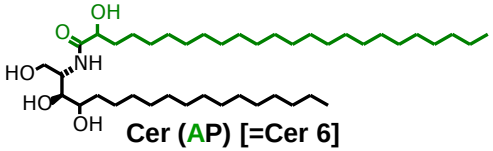
Cer (AS) [=Cer 5]



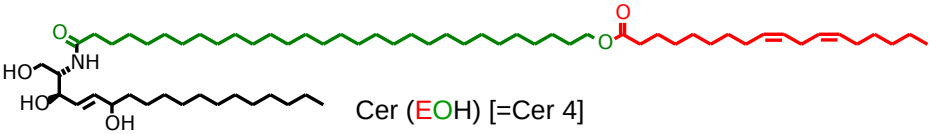
Cer (EOP) [=Cer 9]



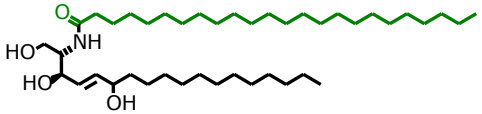
Cer (NP) [=Cer 3]



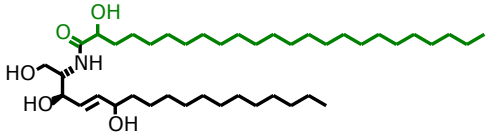
Cer (AP) [=Cer 6]



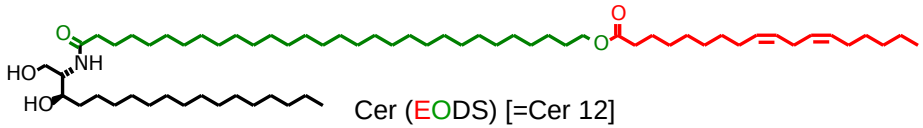
Cer (EOH) [=Cer 4]



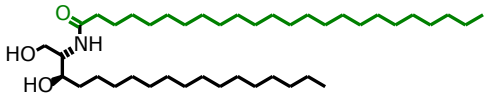
Cer (NH) [=Cer 8]



Cer (AH) [=Cer 7]



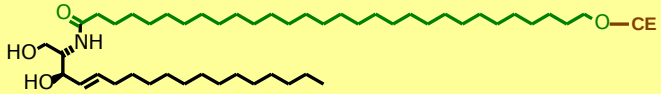
Cer (EODS) [=Cer 12]



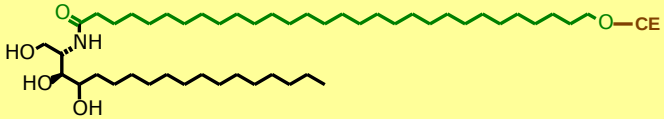
Cer (NDS) [=Cer 10]

Cer (ADS) [=Cer 11]

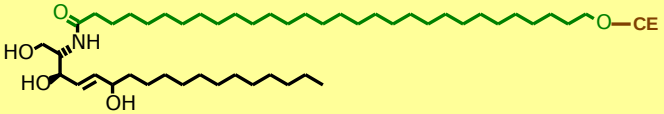
Ceramides covalently bound to the cornified envelope



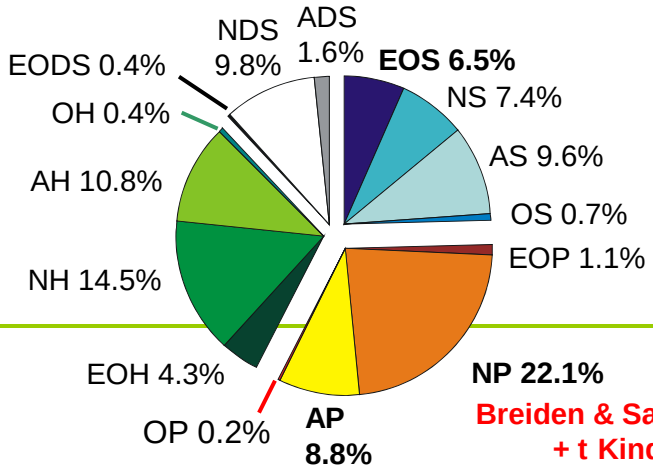
Cer (OS)



Cer (OP)



Cer (OH)



Breiden & Sandhoff, 2013
+ t Kindt, 2012

Research work of the recent years shows:

1. The „brick and mortar“ model has to be re-evaluated

2. The mechanical stability and the barrier function of the SC are realized by:

   A. **„Hook like“** structures of the corneocytes,

   B. The existence of the **corneodesmosomes** and

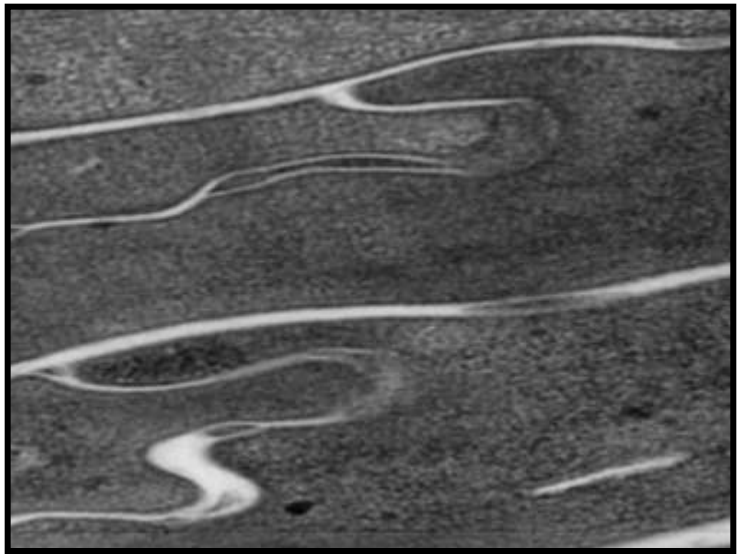
   C. **The bilayer structure** of the SC lipids.

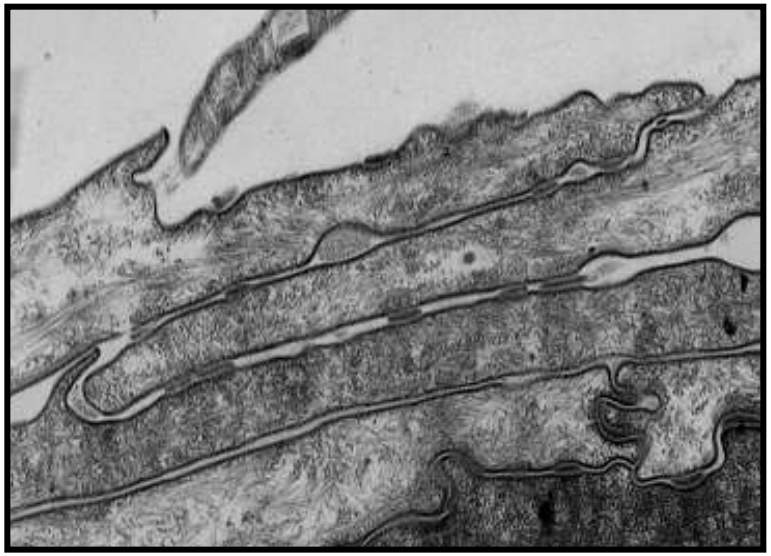
Neubert und Wepf, PZ 152, 1506-1513 (2007)

Research work of the recent years shows:

The mechanical stability (adhesion) of the SC (horny layer) is realised by

A. „Hook like“ structures of the corneocytes





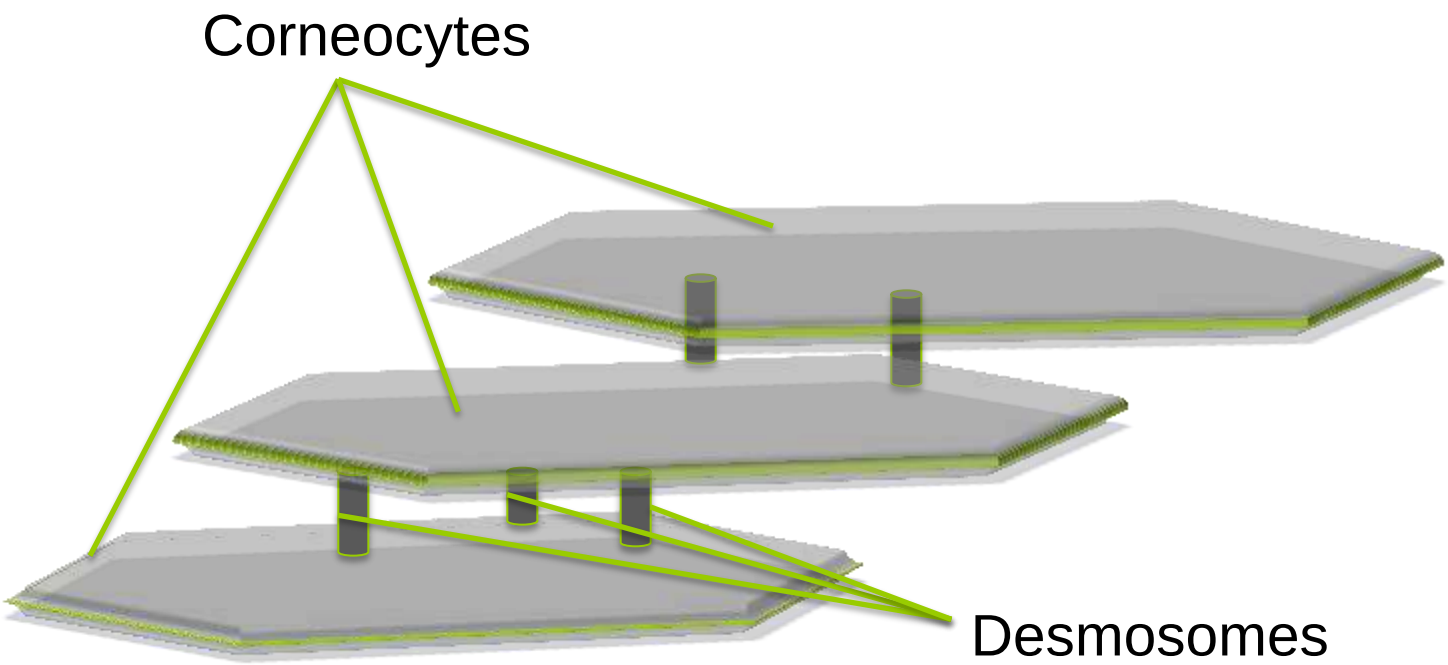
Kindly supplied by Dr. R. Wepf, ETH Zürich

Research work of the recent years shows:

The mechanical stability (adhesion) of the SC (horny layer) is realised by:

B. Corneodesmosomes: „Rivets“ consisting of a protein frame work:

1 corneodesmosome/μm^2 = 400-600 corneodesmosomes/corneocyte



Research work of the recent years shows:

The mechanical stability (adhesion) of the SC (horny layer) is realized by:

C. The **bilayer structure** of the Stratum corneum

Methods:

- New results using neutron scattering (NS) and SC lipid bilayers at a quartz surface show new insights.

Co-operation with JINR in Dubna, Russia
Helmholtz-Zentrum for Materials and Energy, Berlin
Institute Laue Langevin, Grenoble

Applied Methods

- Neutron diffraction
- Molecular simulations (MS)

↳ Internal membrane nanostructure can be analysed

↳ Direct localisation of deuterated label within membrane possible

↳ Comparison of MS model with experimental data

Why do we use model systems?

- Composition of the native lipid matrix to complex to receive detailed information about internal structural properties
  → so far 18 different ceramide species have been identified

- Specific influences of the main components, particularly of the ceramides, to the complete system have to be fundamentally characterised

- Simplistic composition enables a systematic investigation
  ⏩ **Structure-function relationship can be evaluated**

- Using synthetically derived lipids → variability of native lipids can be overcome

Advantages of Neutron Scattering:

Advantages of the Neutron scattering (NS) on SC lipid bilayer on a quartz surface:

- Calculation of neutron scattering length density profiles ⇨ Information about structural assembly of the lipids on a molecular level possible

❖ Coherent neutron scattering lengths b_{coh}:

Hydrogen (^1H): $b_{coh} = -0.374 \times 10^{-12}$ cm
Deuterium ($D/^2H$): $b_{coh} = 0.667 \times 10^{-12}$ cm

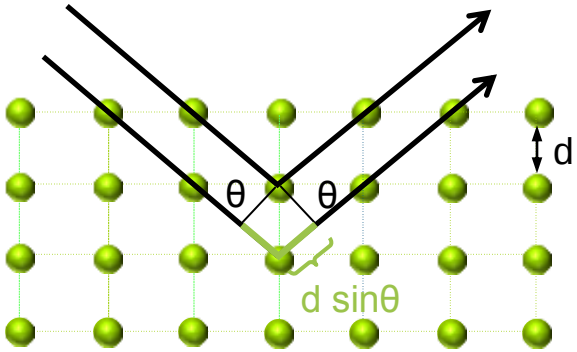
Enables differentiation of isotopes ⇨ "contrast variation"

Application of specifically deuterated lipid molecules (D-Labelling)

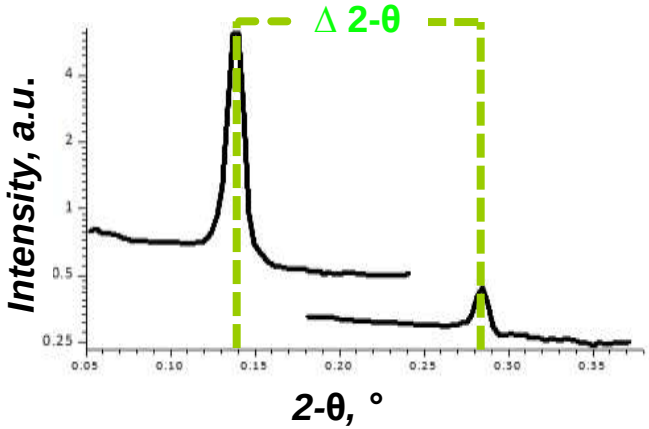
Localisation of labelled molecular group possible

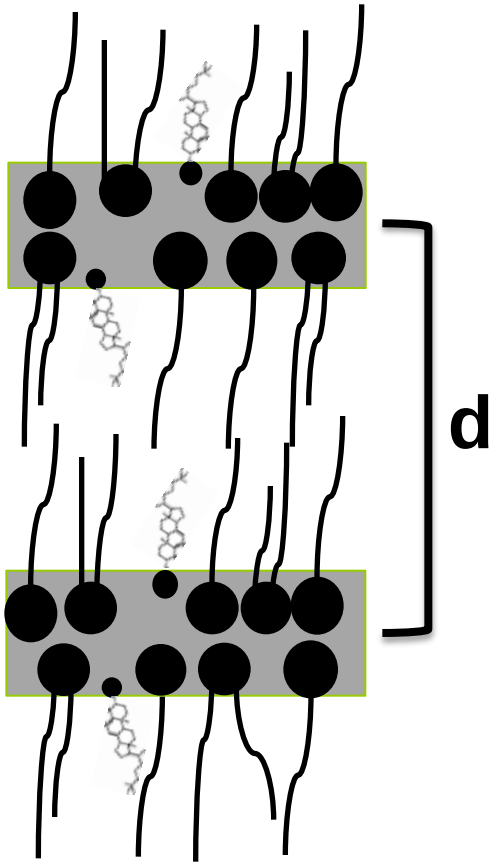
The Bilayer of the Stratum corneum:

New results using **Neutron scattering (NS)** and SC lipid bilayer on a quartz surface allow new insights at V1 Diffractometer, HZ Berlin.



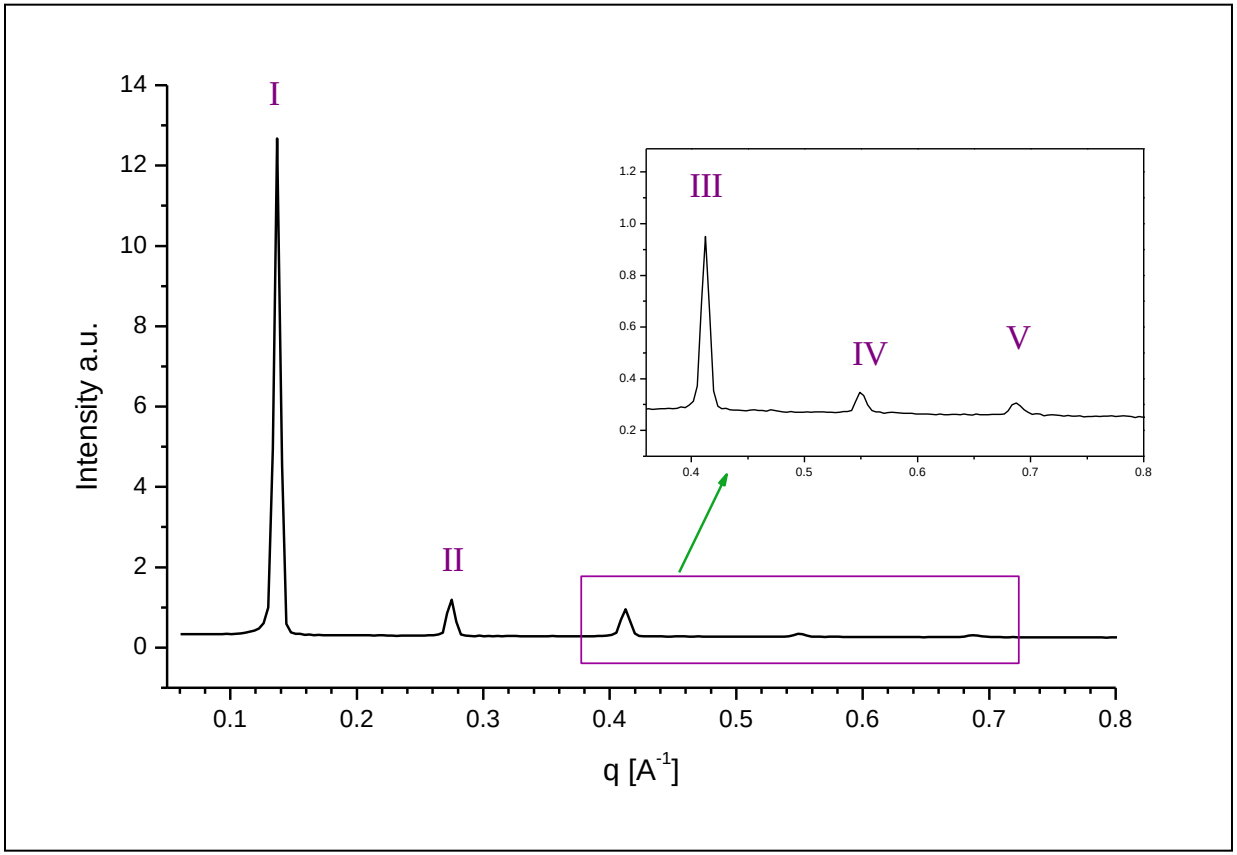
$$l = 2d \times \sin q$$





Stratum corneum Lipid Model Membranes

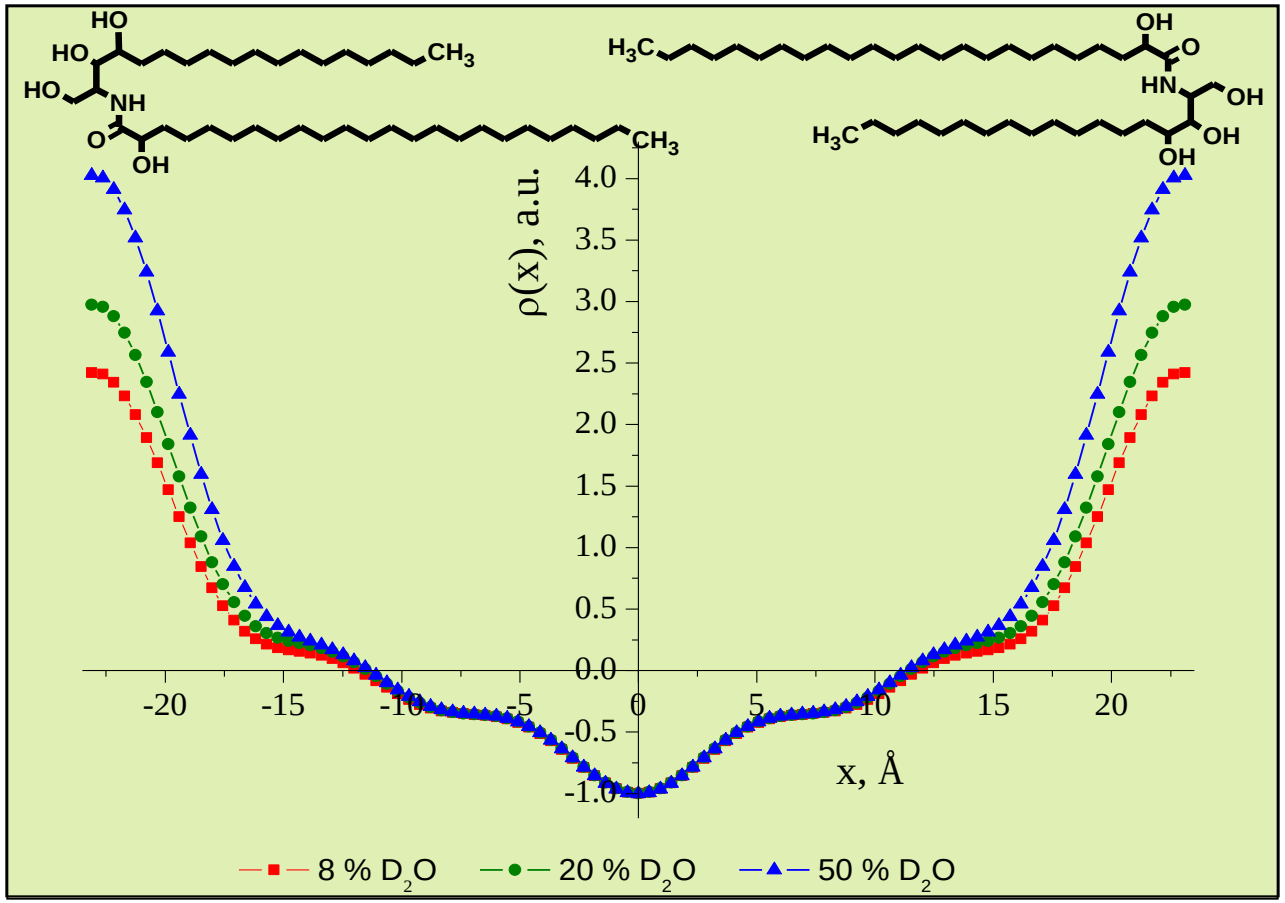
Diffractogram: **Five diffraction orders** detectable!



- **Ceramide AP/Cholesterol/Palmitic acid/Cholesterol sulfate**
- T=32 ^{o}C, 60 % relative humidity, 8 % D_2O

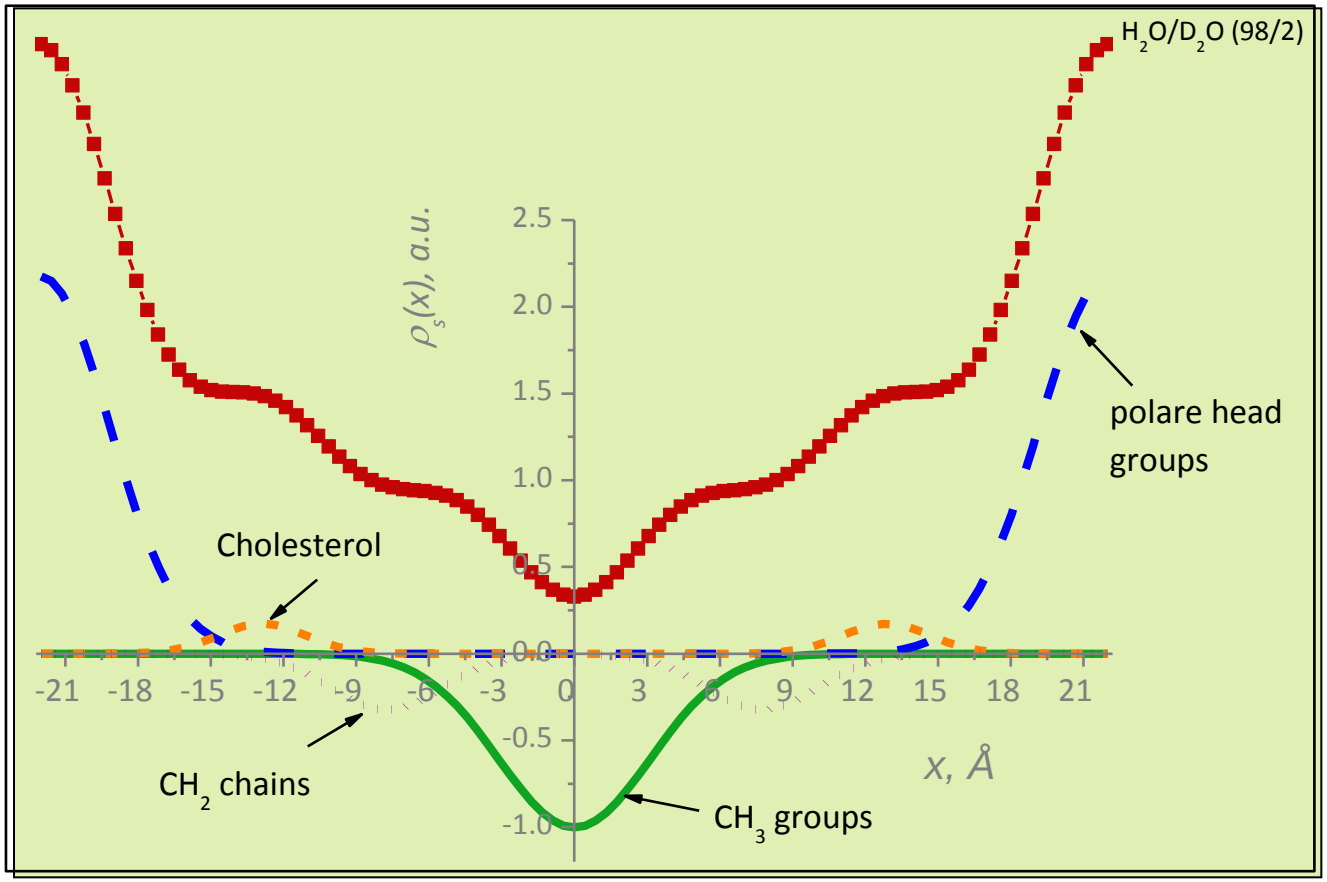
Stratum corneum Lipid Model Membranes

Neutron scattering length density (NSLD) profile: SC-lipid model system CER[AP]/Chol/SA/CholS, T = 32 °C, 60 % relative humidity



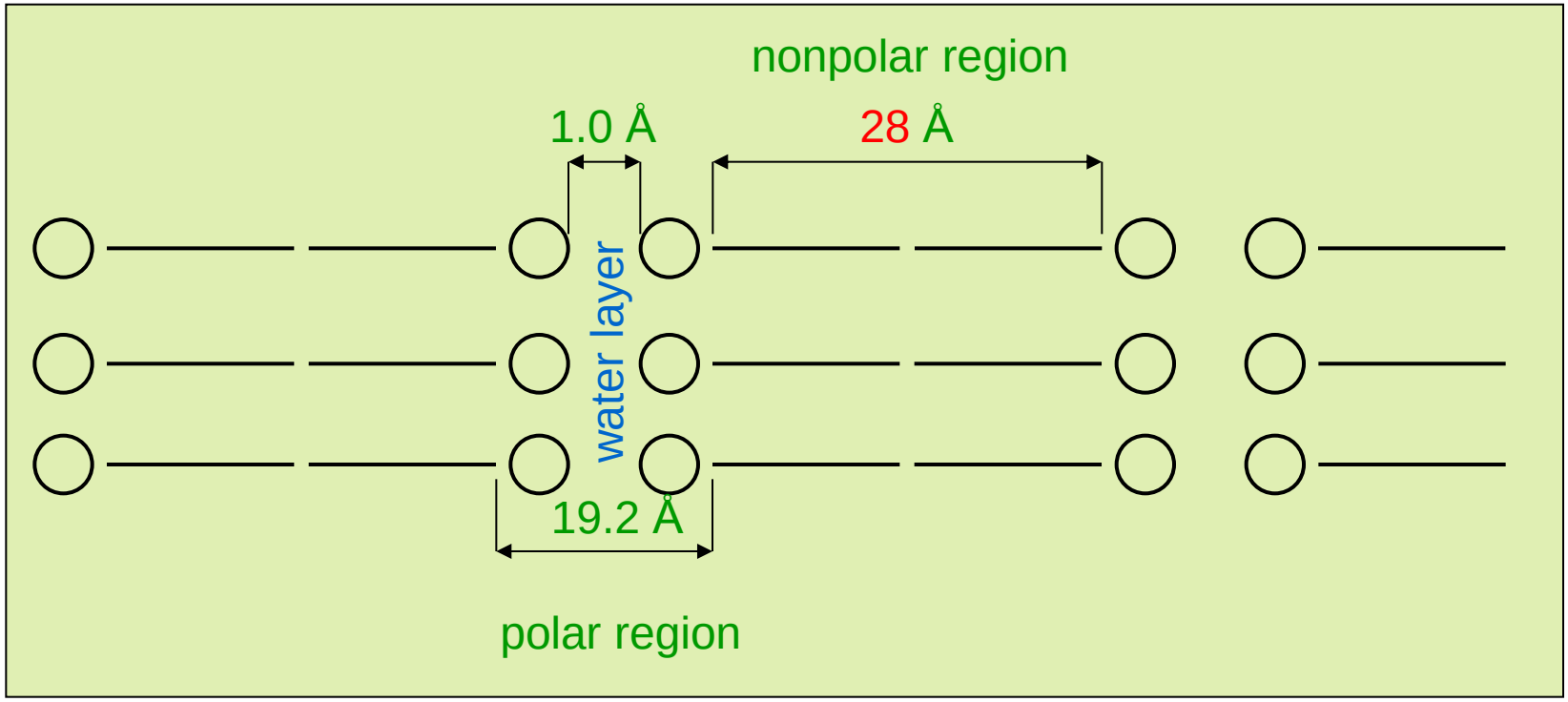
Stratum corneum Lipid Model Membranes

Model calculations of the neutron scattering length density of the model system (CER[AP], CHOL, Behenic acid and Cholesterol sulfate)



Stratum corneum Lipid Model Membranes

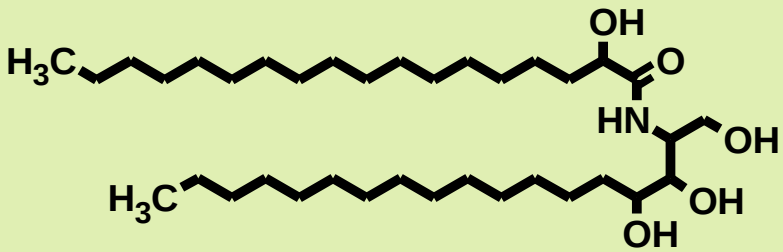
- **Neutron scattering** allows new insights into the molecular structure of the SC bilayer: First results



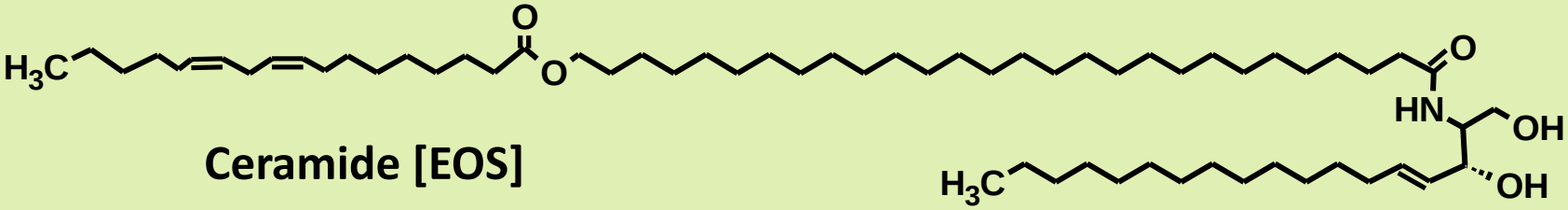
Kiselev, Neubert et al., Eur. Biophys. J. 34, 1030 (2005)

Stratum corneum Lipid Model Membranes

Influence of long-chain ceramides



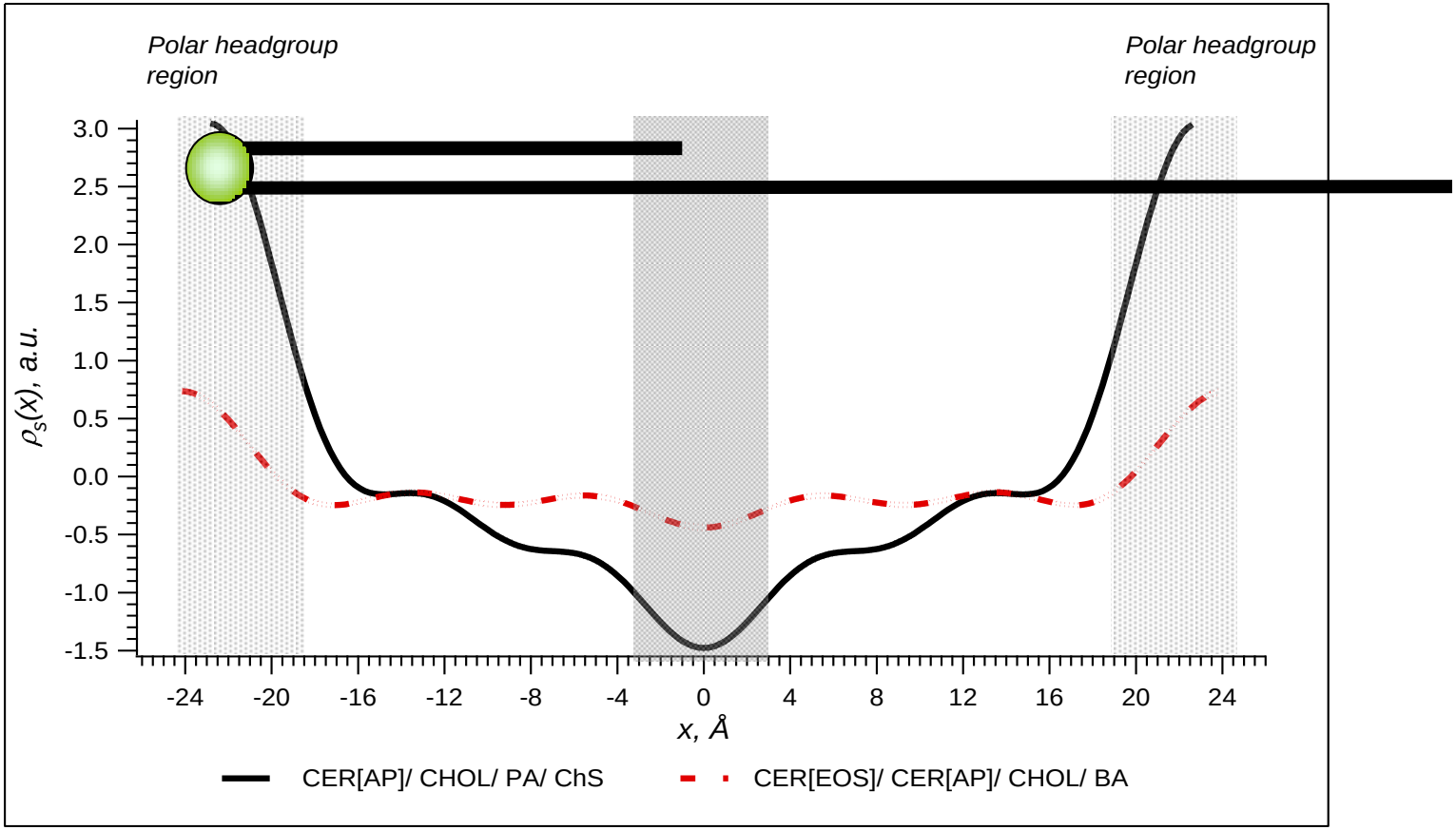
Ceramide [AP]



Ceramide [EOS]

15 % Fatty acid (C26), C(24), C(22), e.g. Cerotic acid

Stratum corneum Lipid Model Membranes
Influence of long-chain ceramides

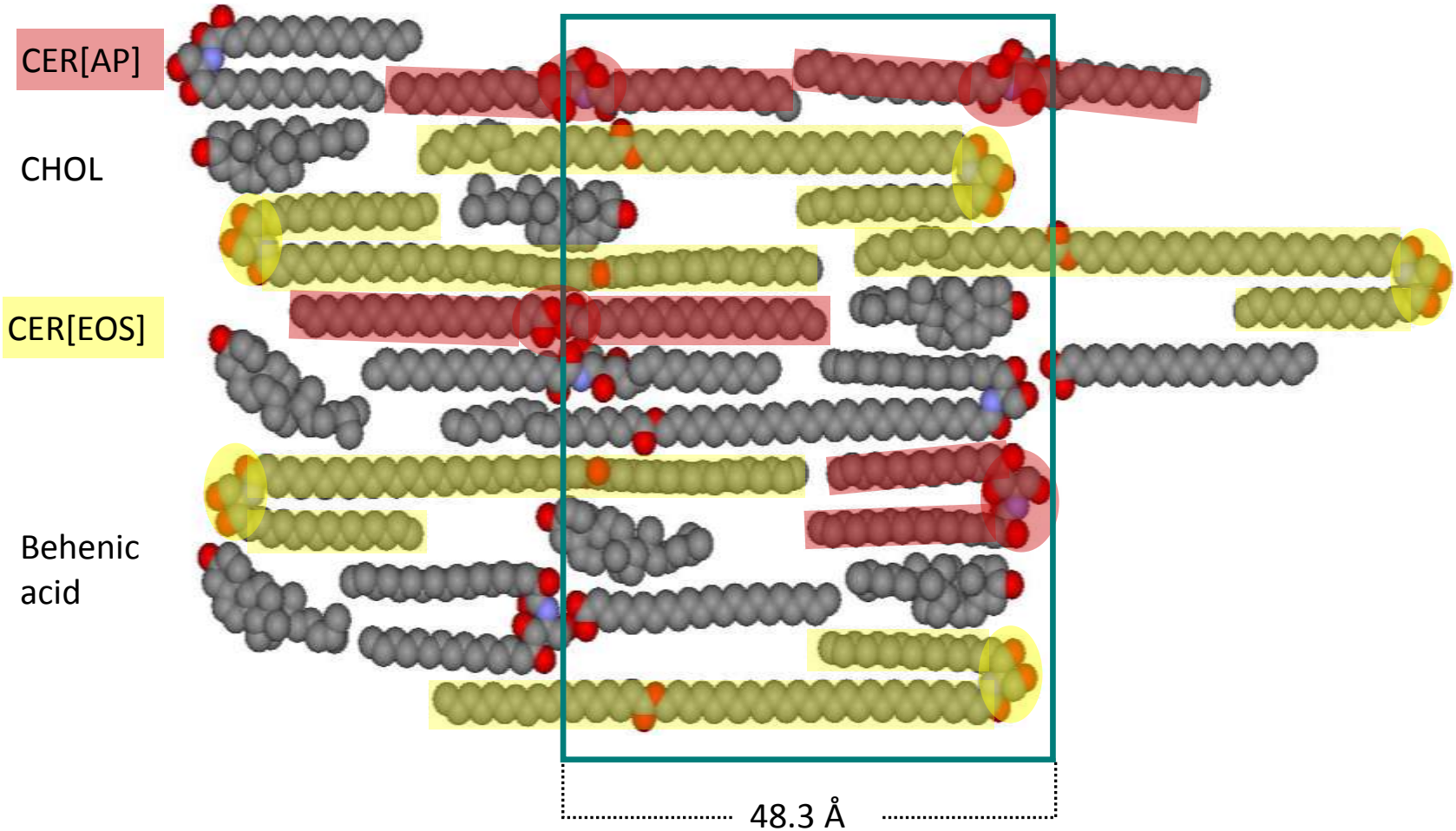


Schröter et al. 2009, Biophys J. 97

New structure model of the SC:
"Armature reinforcement model"

Composition	Membrane thickness
CER[AP]/CHOL/PA/ChS	**45.6 Å**
CER[NS]/CHOL/PA/ChS	57.0 Å
CER[EOS]/**CER[AP]/**CHOL	**45.2 Å**
CER[EOS]/CER[NS]/**CER[AP]/**CHOL	**44.0 Å**
CER[EOS]/**CER[AP]/**CHOL/PA	**45.0 Å**
CER[EOS]/**CER[AP]/**CHOL/BA	**46.5 Å**

New structure model of the SC:
"Armature reinforcement model"



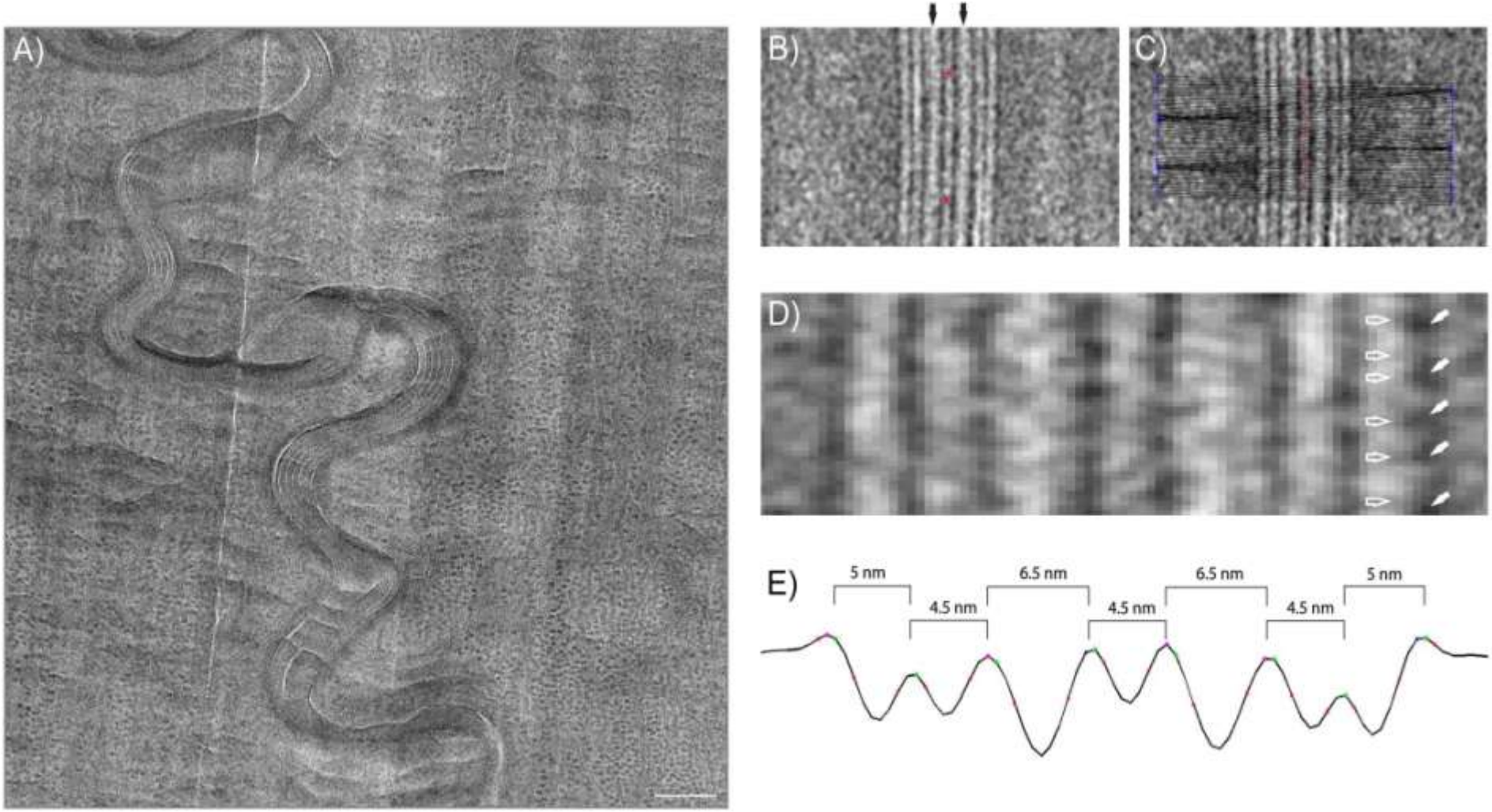
New model of the SC bilayer Structure
"Armature reinforcement model"

1. Ceramide [AP]: Most hydrophilic ceramide, four OH-groups, H-bridges stabilize the hair pin structure

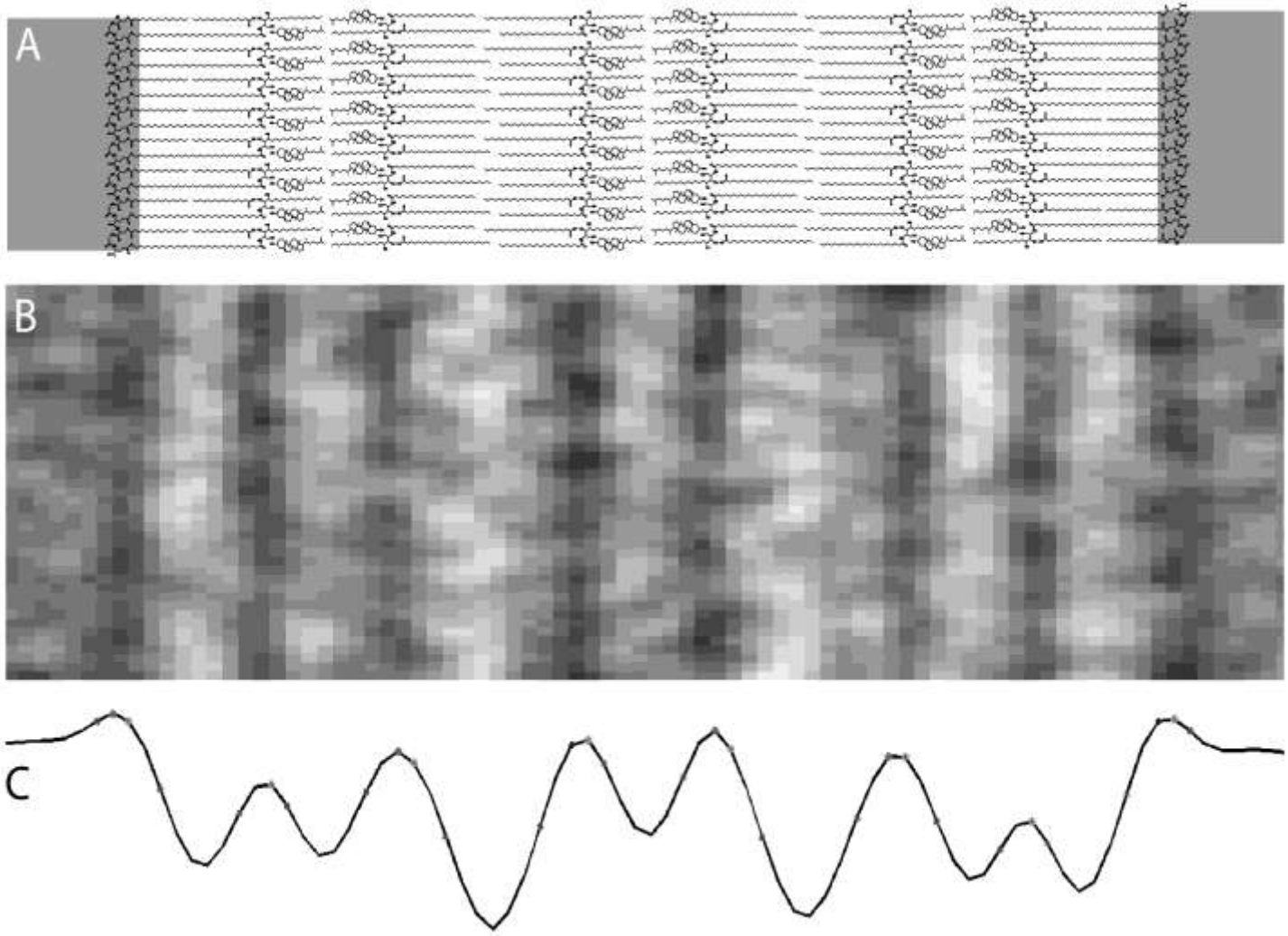
2. Ceramide [AP]: Fully extended conformation stabilizes the bilayer structure

3. Ceramide [EOS]: The long alcyl chain penetrates into the next bilayer

New “Asymmetry model” by L. Norlén



New “Asymmetry model” by L. Norlén



New model of the SC bilayer structure

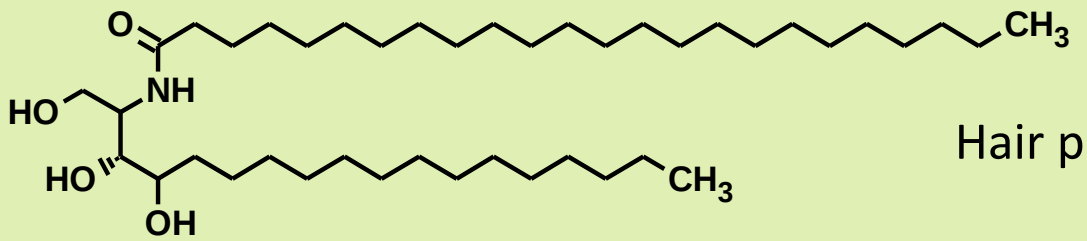
Asymmetry model according to L. Norlén:

1. *65 Å- Bilayer:* Consisting on the long chain of CER[NP] and of the free fatty acids (mainly: lignoceric acid, C24)

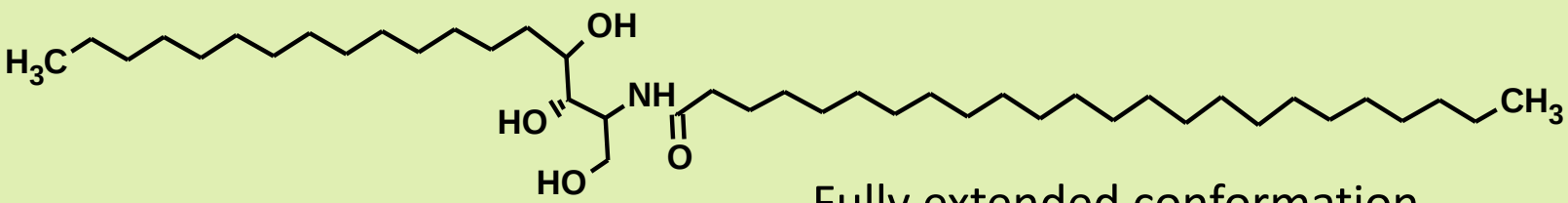
2. 45 Å- Bilayer: Consisting on the short chain of CER[NP] and of cholesterol

New Asymmetry model

Important question: Does **CER[NP] C18/C24** exists in the hairpin or fully extended conformation in model membranes?



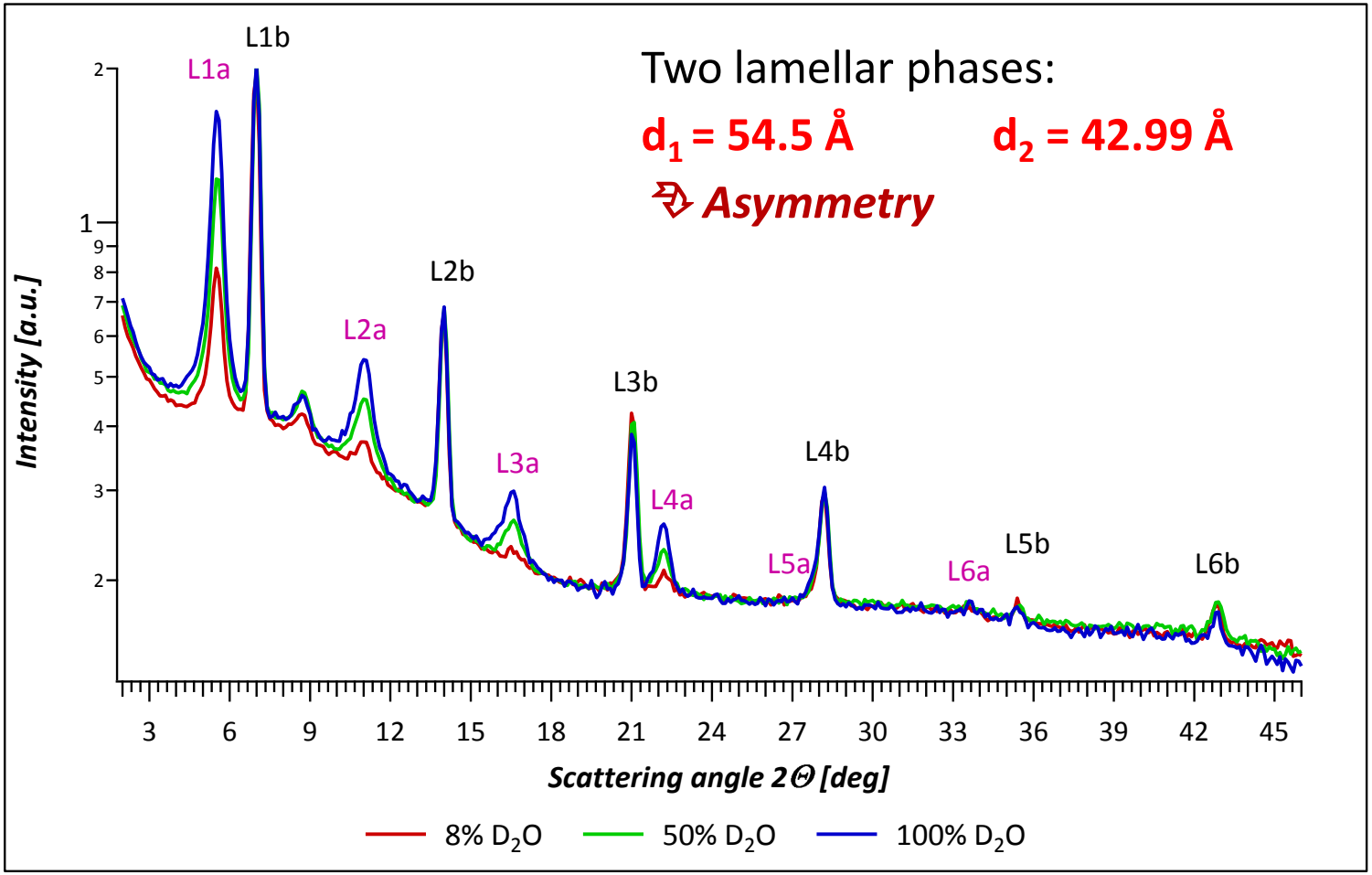
Hair pin conformation



Fully extended conformation

SC Lipid model membranes:
CER[NP]C18,C24 /CHOL /lignoceric acid

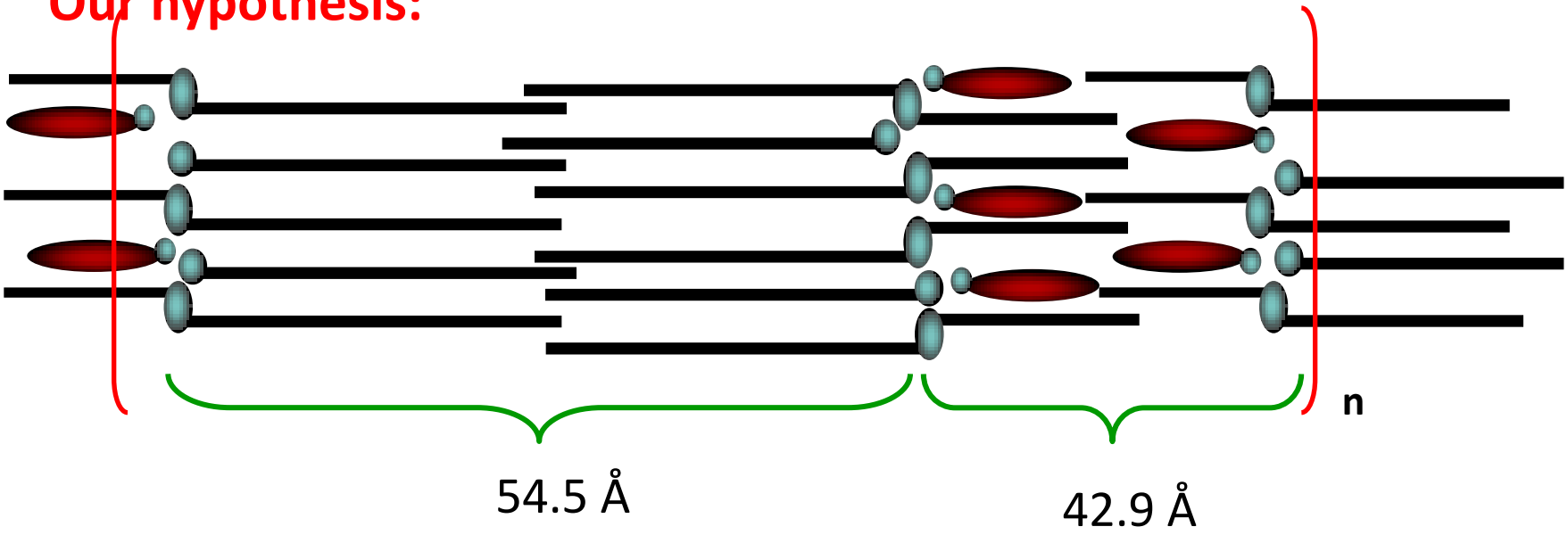
CER[NP] C18,C24/ **c**holesterol/ lignoceric acid (equimolar)



Structural architecture of the lipids:

CER[NP]C18,C24/ cholesterol/ lignoceric acid (C24)

Our hypothesis:

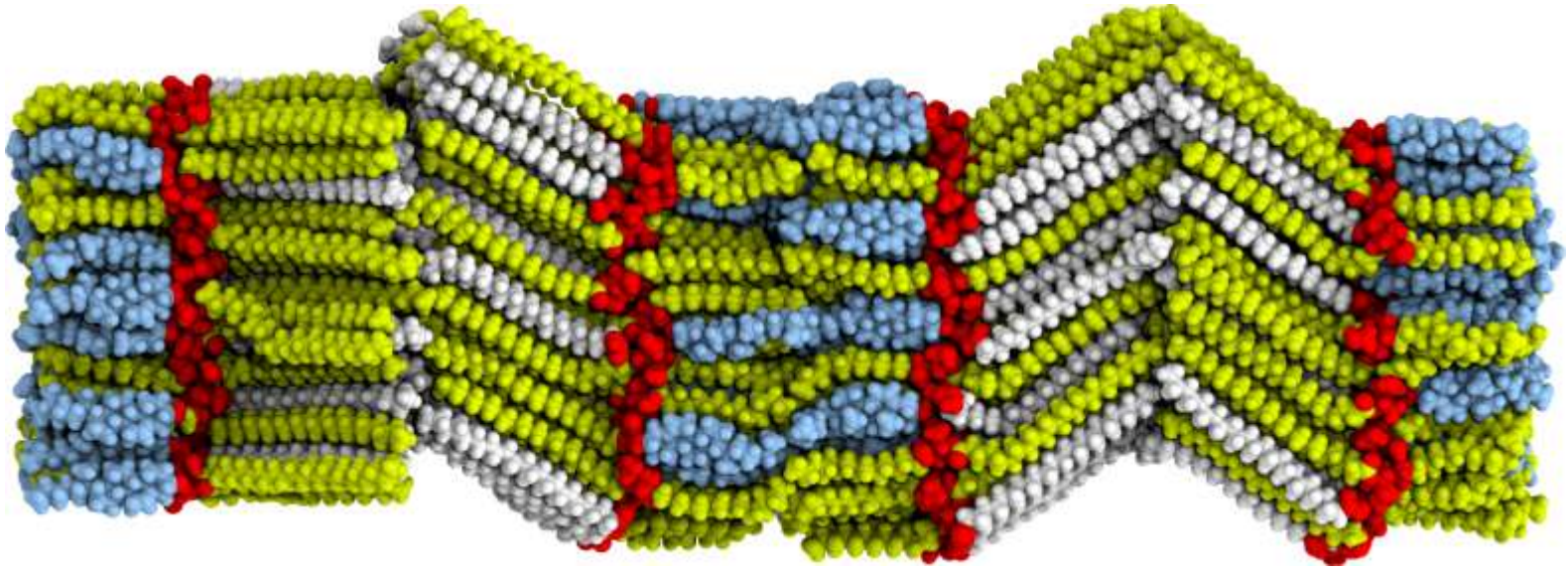


CER[NP] C18/C24-molecules present in the fully extended conformation !

The localisation of cholesterol and lignoceric acid has to be identified by applying deuterated derivatives.

CER[NP]: Asymmetric arrangement

Molecular dynamic simulations

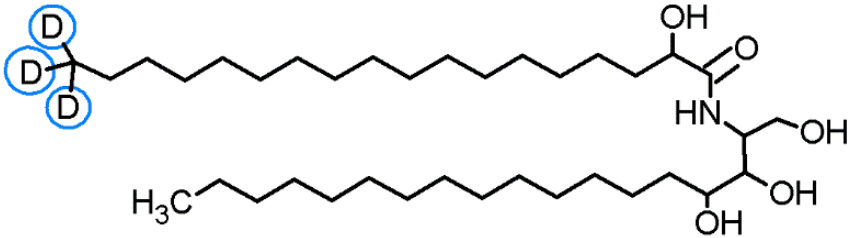


- Only model simulation with CER[NP]-C24 in fully extended conformation agrees with experimental obtained data
- Asymmetric distribution of lipids

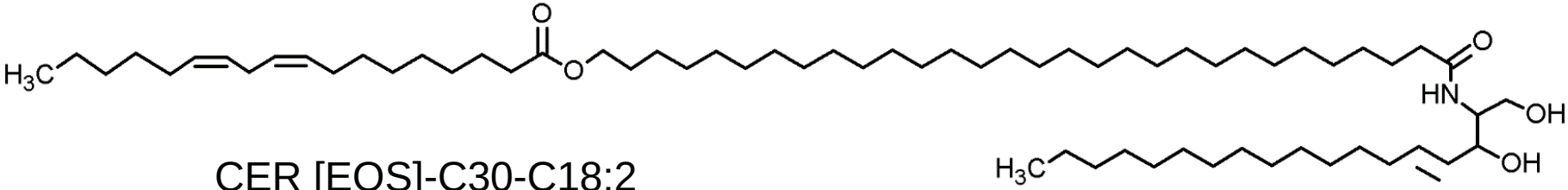
Molecular simulations conducted by A. Vogel, University of Leipzig

Specific deuterated ceramides

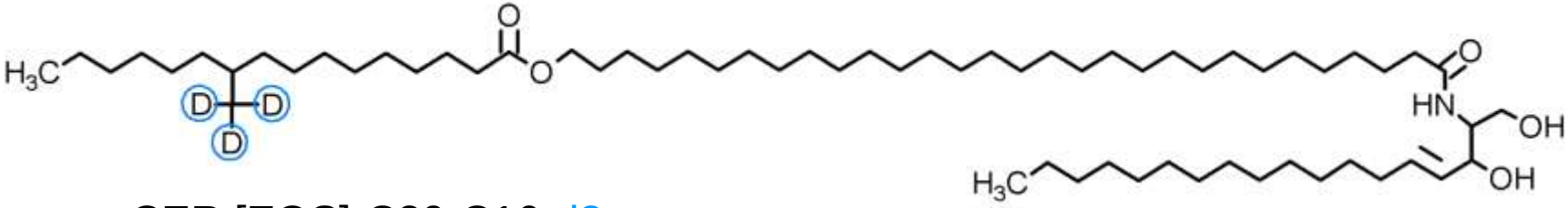
CER [AP]-C18-d3



CER [EOS]-C30-C18:2



CER [EOS]-C30-C16-d3



CER [AP] : CER [EOS] : CHOL : BA = 10 : 23 : 33 : 33 % wt

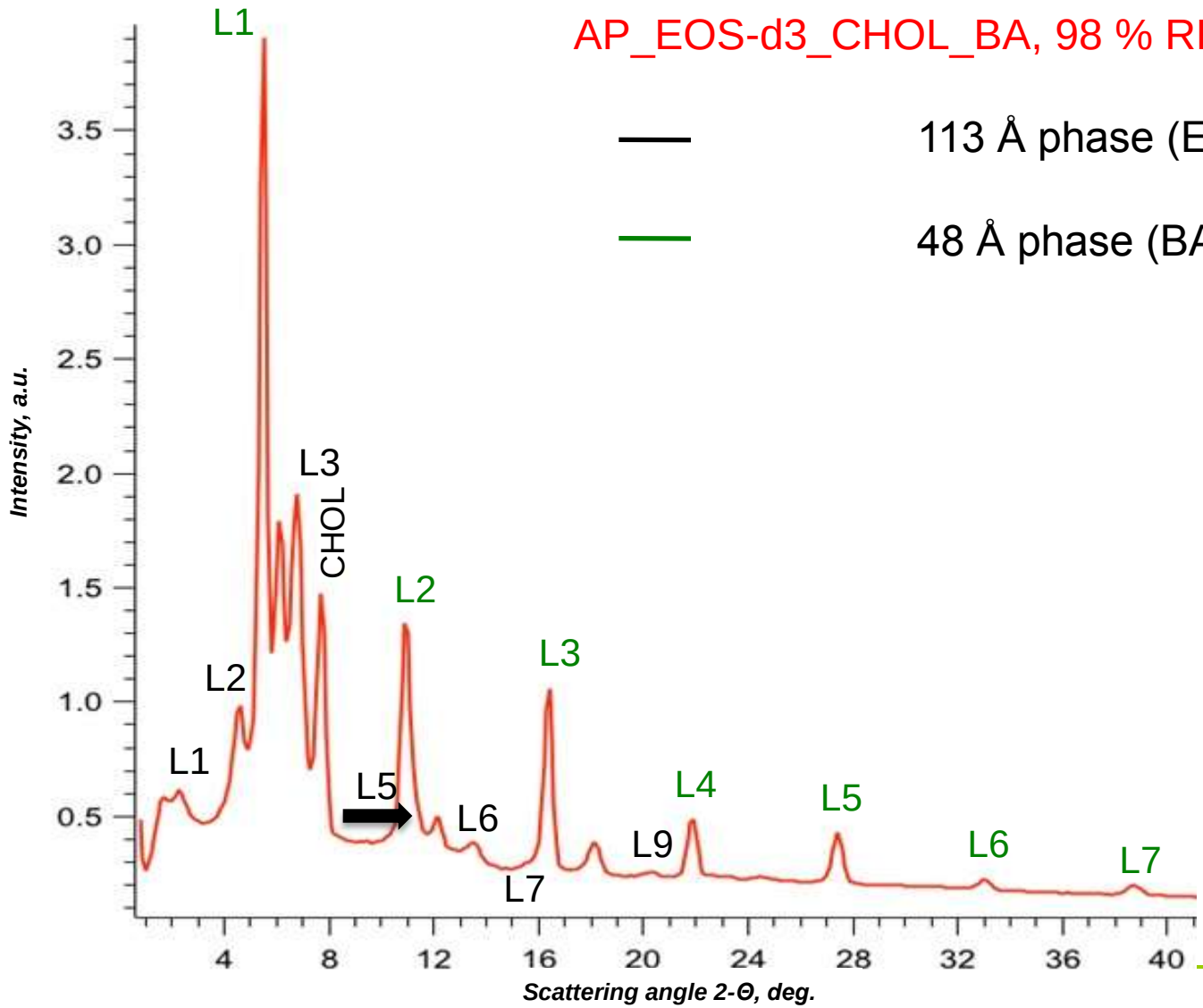
Results: Three phases

48 Å

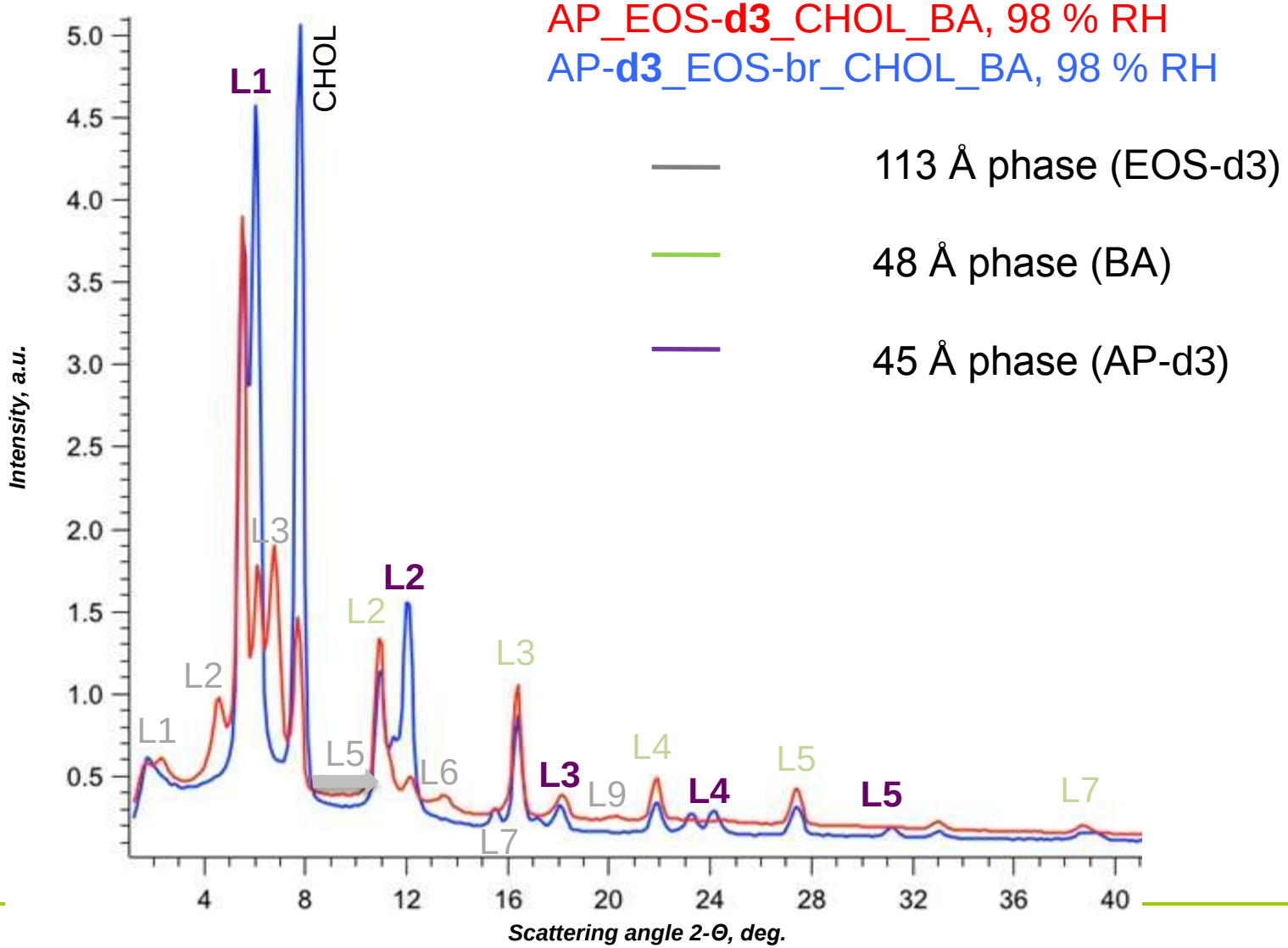
113 Å

45 Å

Scattering Profiles



Scattering Profiles



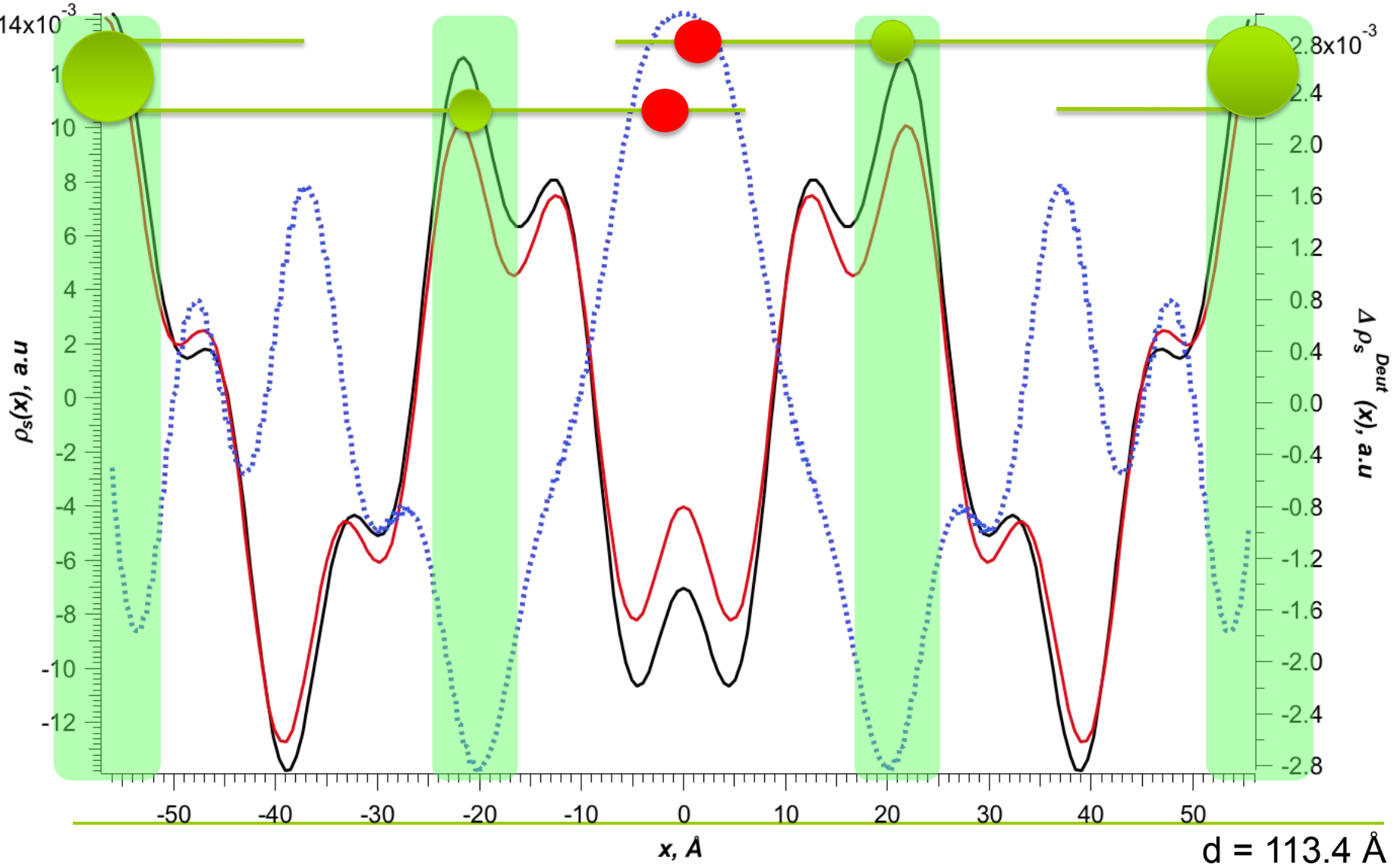
Results

48 Å

113 Å

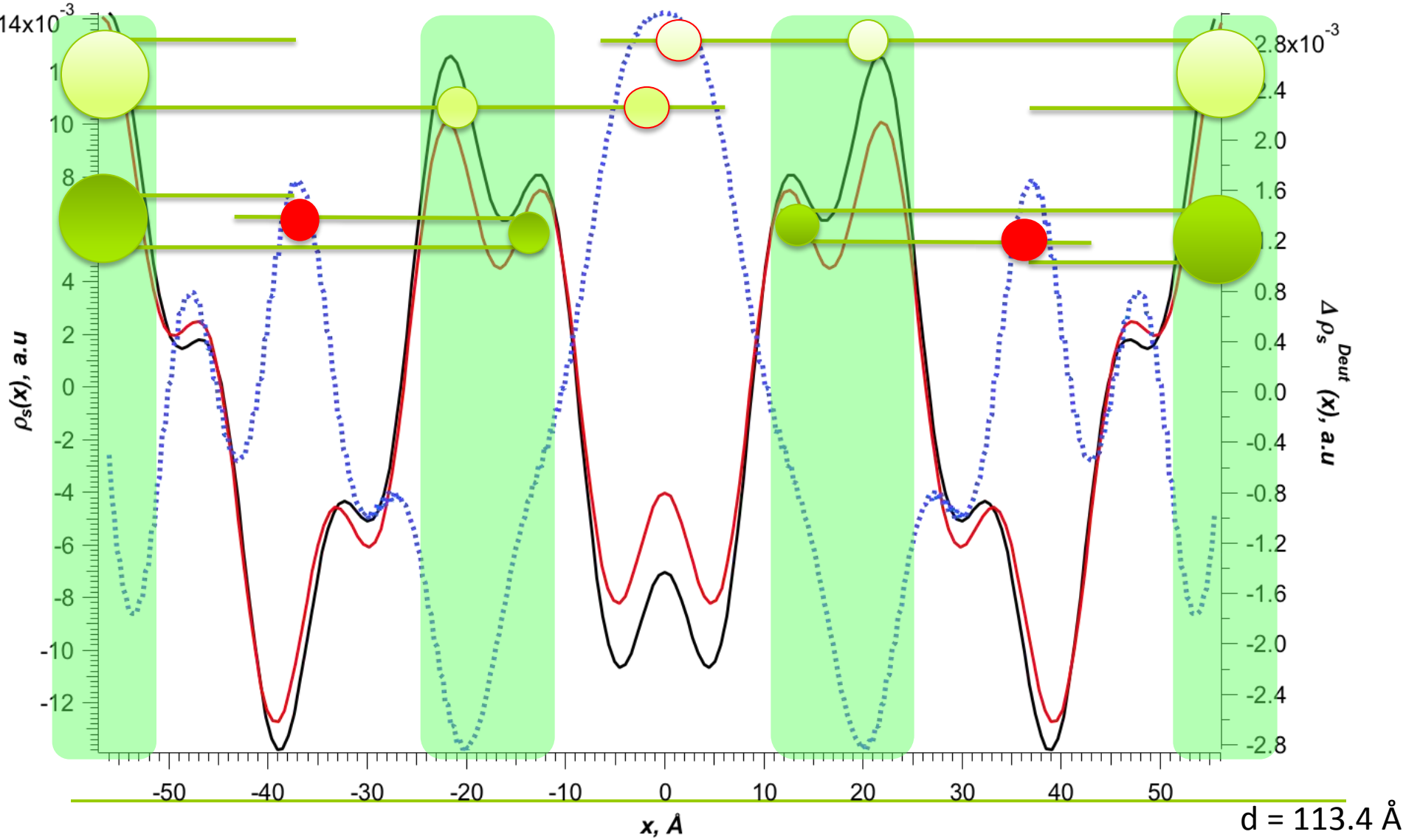
45 Å

NSLD-profile: 113 Å phase (EOS-d3)



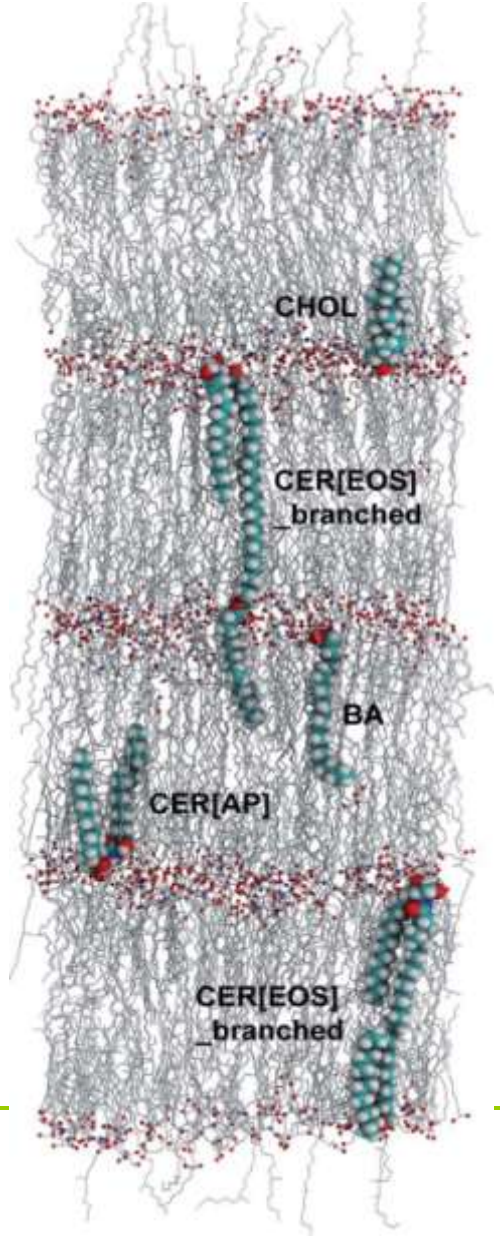
d = 113.4 Å

NSLD-profile: 113 Å phase (EOS-d3)



d = 113.4 Å

Molecular Dynamic Simulation



Engelbrecht T, et al.,
Soft Matter 7 (2011) 8998-9010

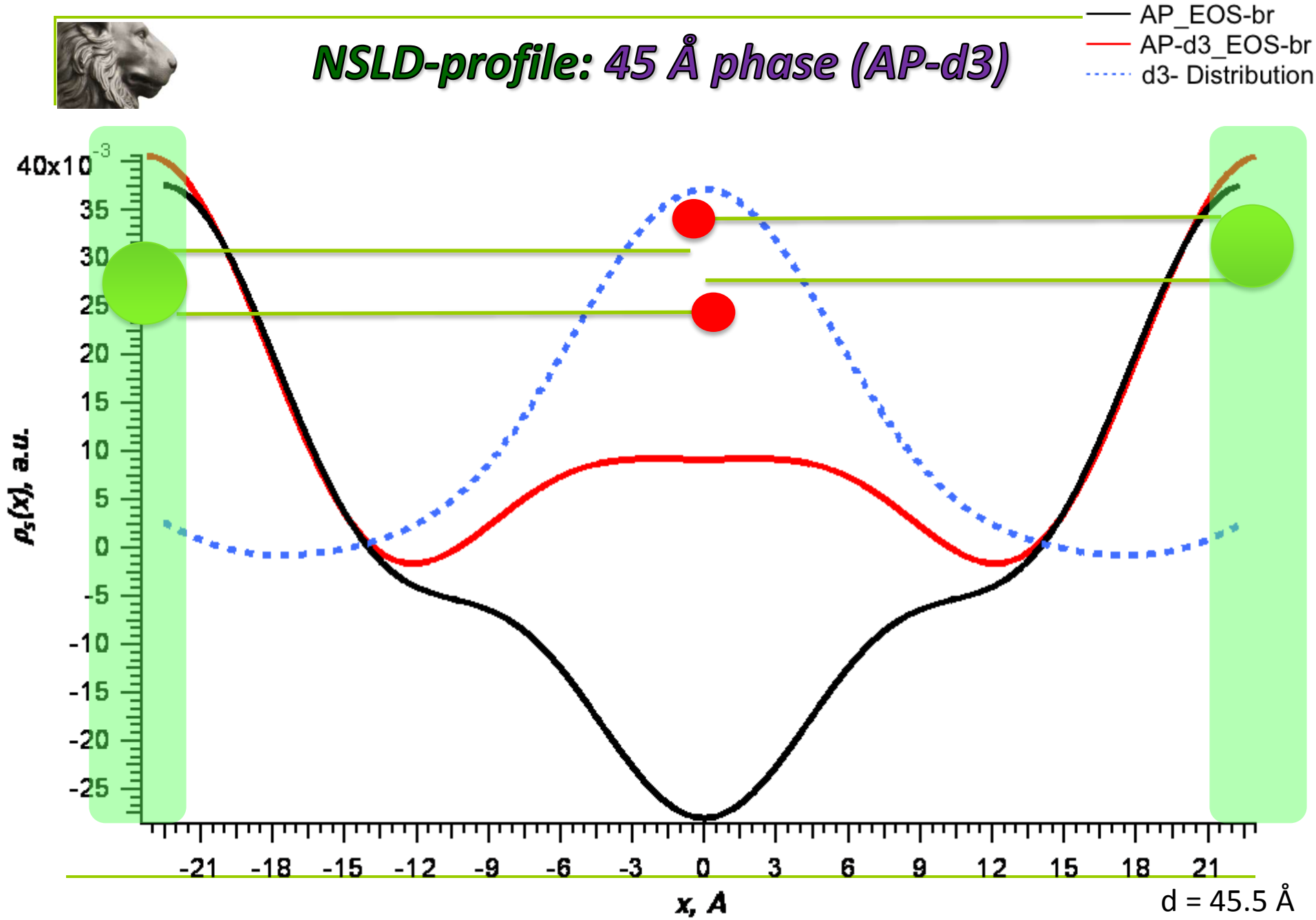
Results

48 Å

113 Å

45 Å

NSLD-profile: 45 Å phase (AP-d3)



d = 45.5 Å

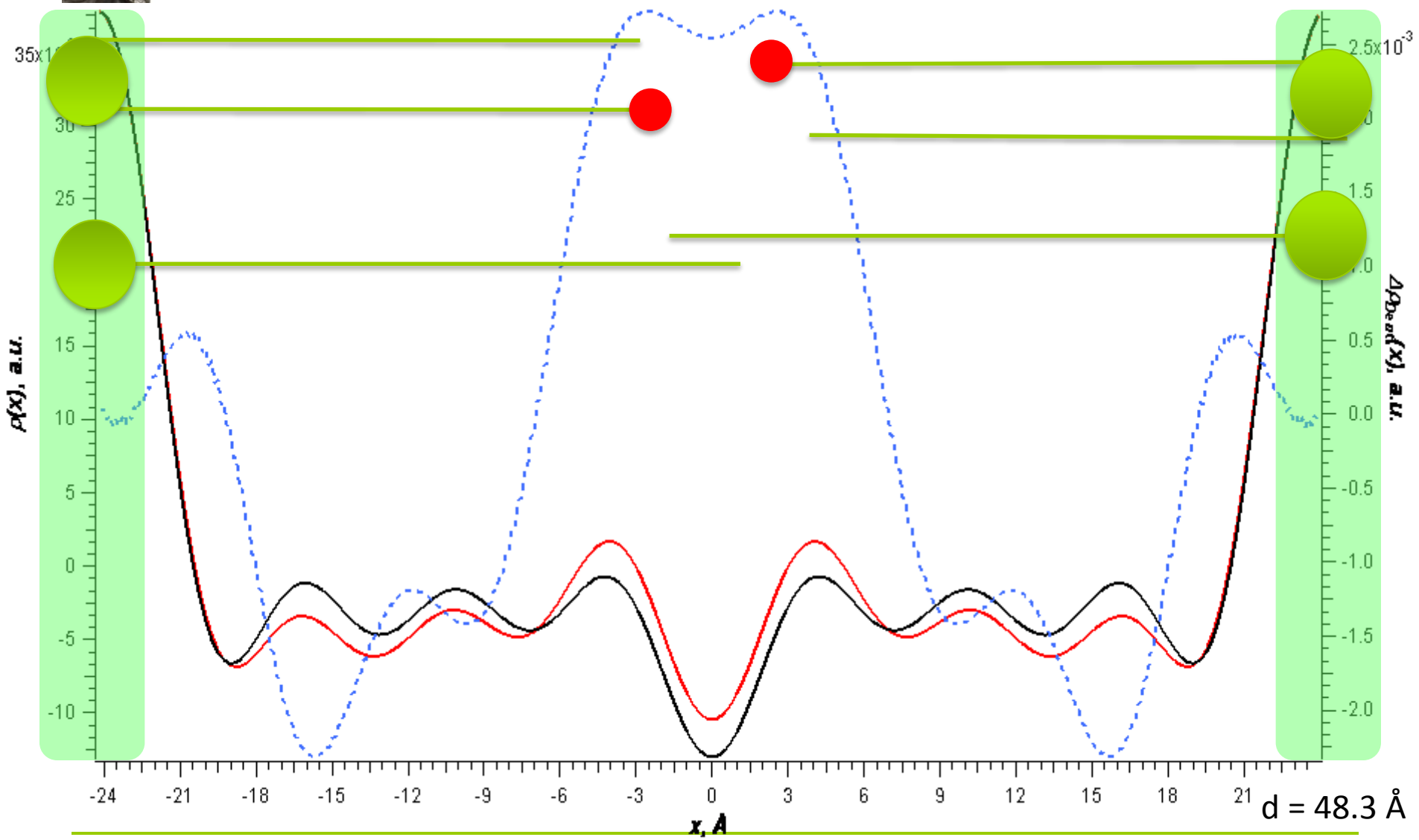
Results

48 Å

113 Å

45 Å

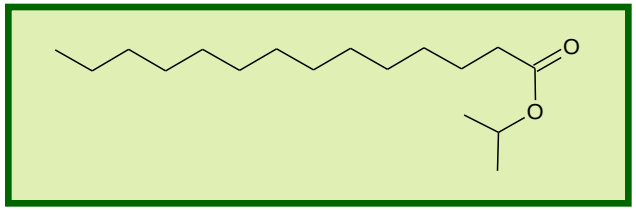
NSLD-profile: 48 Å phase



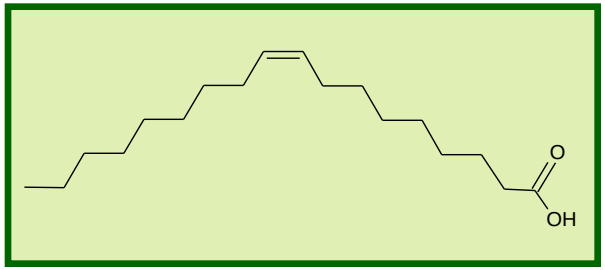
d = 48.3 Å

Effect of Penetration enhancers

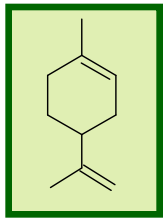
- Overcoming the skin barrier essential for transdermal drug administration
- Increased drug flux realized e.g. by PENETRATION ENHANCERS
- Mode of action not yet fully elucidated on molecular level
- Assumptions:
  - ❖ Fluidization of SC lipids
  - ❖ Increase of lamellar disorder
  - ❖ Induction of phase separation



Isopropyl myristate



Oleic acid

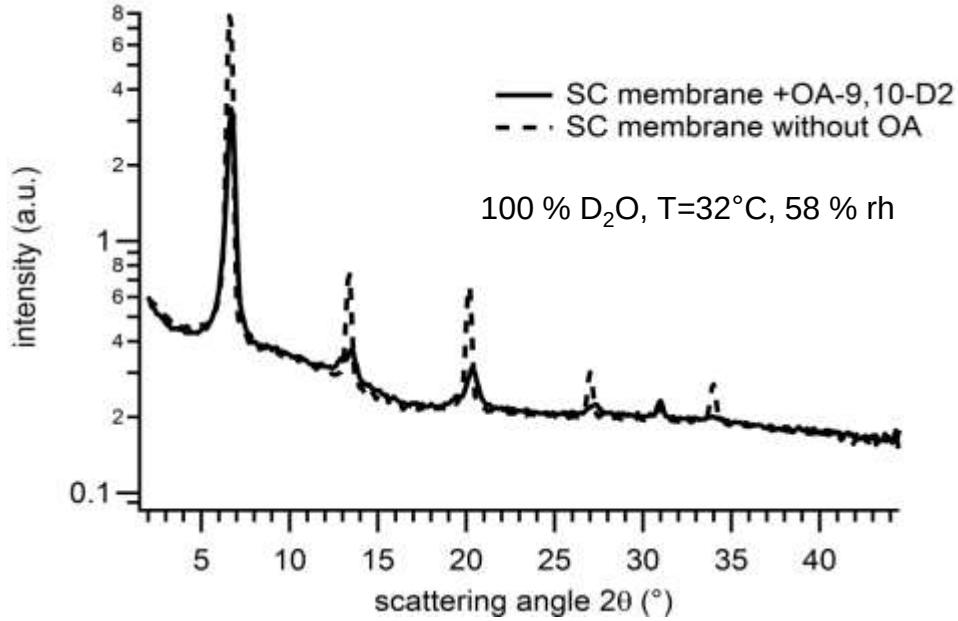


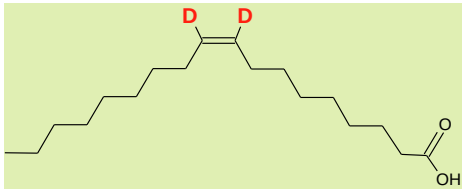
Limonene

Effect of Penetration enhancers – Oleic acid

- Model membrane (mass ratio): **CER[AP]/CHOL/PA/ChS** 55/25/15/5 (Quat. Basic Syst.)

+ 10 % **Oleic acid** (”Quat. Basic_**OA“**)
+ 10 **% Oleic acid-9,10-D2** (“Basic_**OA-D2“**)



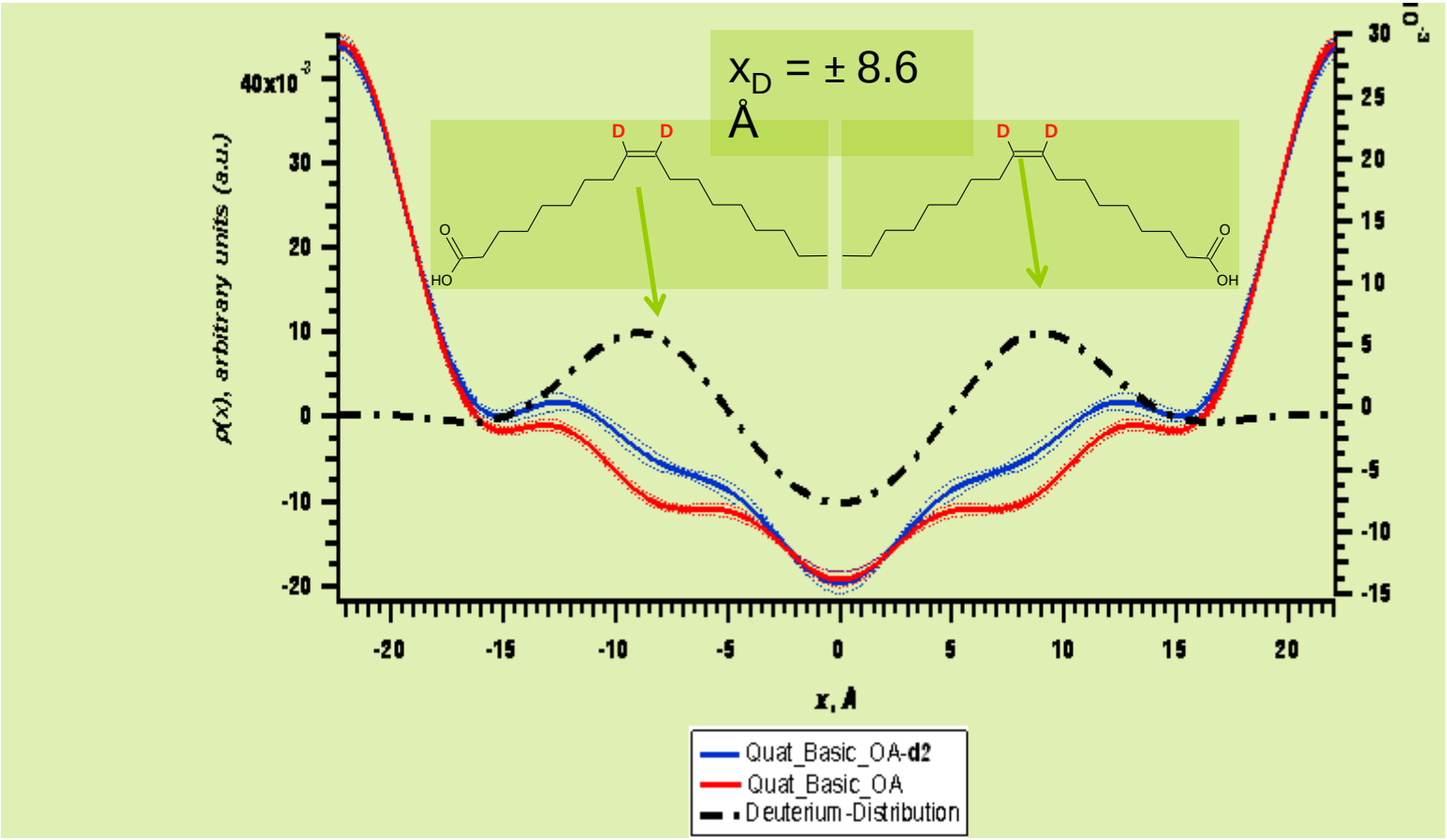


	Lamellar repeat distance *d*
Quat_ Basic_OA-d2	44.39 ± 0.08 Å

Effect of Penetration enhancers – Oleic acid

- Model membrane (mass ratio): **CER[AP]/CHOL/PA/ChS** 55/25/15/5

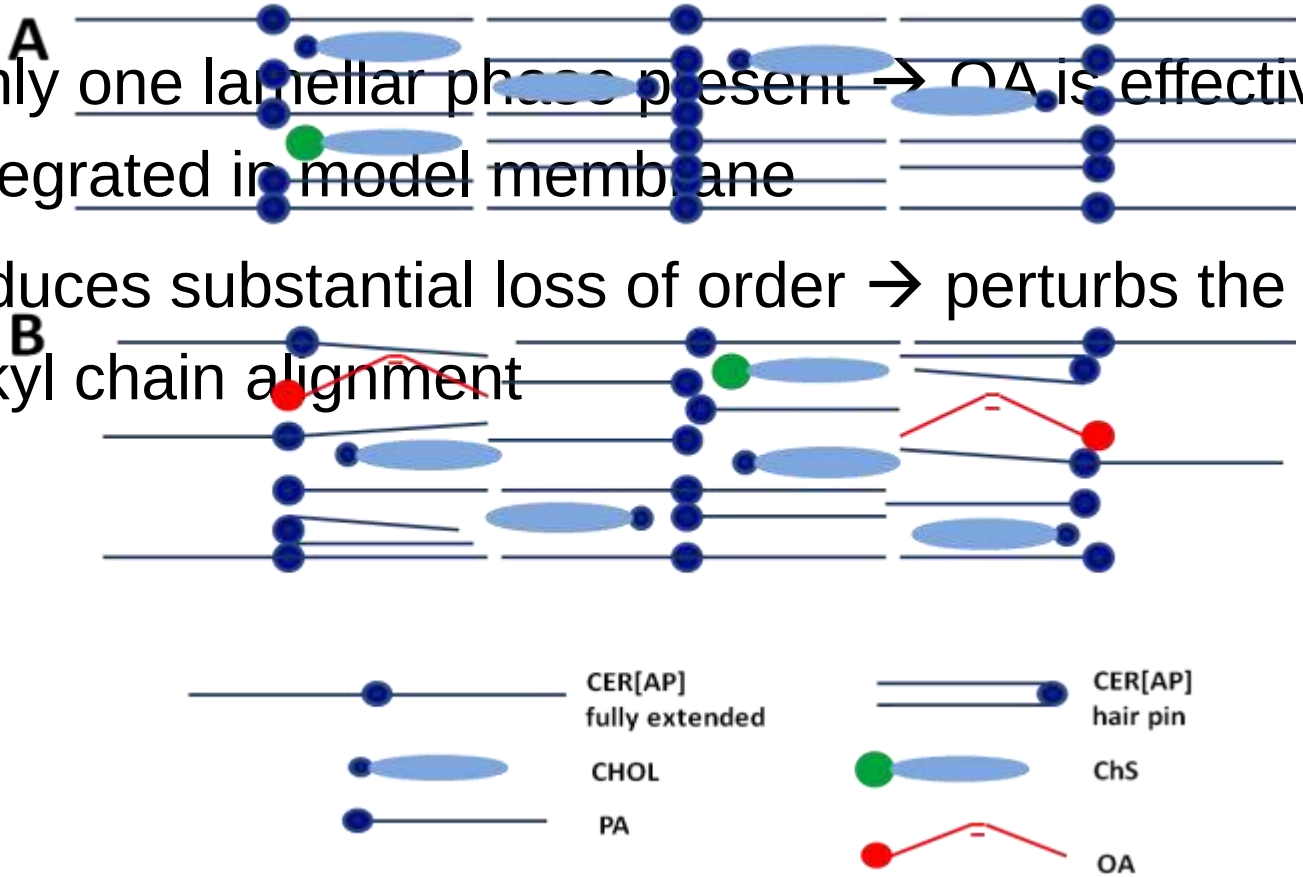
+ 10 % **Oleic acid** (”Quat_Basic_**OA**“)
+ 10 **% Oleic acid-9,10-D2** (“Basic_**OA-D2**“)



Influence of penetration enhancer

CER[AP]/ Cholesterol/ stearic acid + 10% (m/m) Oleic acid

- Only one lamellar phase present → OA is effectively integrated in model membrane
- Induces substantial loss of order → perturbs the proper alkyl chain alignment



Engelbrecht, et al., *BBA – Biomembranes* 1808 (2011) 2798-2808.

Conclusion I

- Mechanical stability of the SC realized at three levels:

  - „Hook“ like structures,
  - Corneodesmosmes and
  - Bilayers of the SC lipids.

- Neutron scattering allows new insights into the molecular architecture of the SC bilayers.

- A new model of the SC bilayer structure was created: The ***armature reinforcement model.***

Conclusion II

- **Ceramide [AP]:** Most hydrophilic ceramide, four OH-groups, H-bridges stabilize the **hair pin structure** conformation **stabilizes the bilayer structure.**

- **Ceramide [NP]:** This ceramide, three OH-groups, seems to force asymmetric bilayer structures in fully extended structure. Therefore ,the ***asymmetry model*** was created.

Conclusion III

- **Ceramide [EOS]**: **Long phase (113 Å)** is not a real LPP, no [AP]-d3 included. The long alkyl chain penetrates into the next bilayer and form angled structure.

- **Long phase (113 Å exists only under certain conditions (high RH), depending on concentration of CER [EOS]!**

- **Structure models:** Has to be confirmed using specifically deuterated ceramides (CER[AP], CER[NP].

Conclusion IV and Outlook

- **48 nm phase:** Behenic influence causes a d-spacing, CER [AP]-d3 is incorporated in this phase.
- **Penetration enhancers:** Can be studied on a molecular level.
- **Further neutron measurements necessary, studies are running!**
- **For that we need PhD student working at JINR in Dubna. Double degree is possible (JINR and MLU).**

Thanks to:

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Dr. Doreen Kessner, Department of Pharmacy, MLU

Dr. Tanja Engelbrecht, Department of Pharmacy, MLU

Adina Eichner, Department of Pharmacy, MLU

List of Publications – Research Articles

1. D. Kessner, M. Kiselev, S. Dante, T. Hauss, P. Lersch, S. Wartewig and R.H.H. Neubert, Eur Biophys J Biophy, 37, 6, 2008, 989-999.
2. D. Kessner, M.A. Kiselev, T. Hauss, S. Dante, S. Wartewig and R.H.H. Neubert, Eur Biophys J Biophy, 37, 6, 2008, 1051-1057.
3. D. Kessner, A. Ruettinger, M.A. Kiselev, S. Wartewig and R.H.H. Neubert, Skin Pharmacol Physiol, 21, 2, 2008, 58-74.
4. M.A. Kiselev, N.Y. Ryabova, A.M. Balagurov, S. Dante, T. Hauss, J. Zbytovska, S. Wartewig and R.H. H. Neubert, Eur Biophys J, 34, 8, 2005, 1030-40.
5. M.A. Kiselev, N.Y. Ryabova, A.M. Balagurov, D. Otto, S. Dante, T. Hauss, S. Wartewig and R.H.H. Neubert,, Poverchnost. X-ray, synchrotron and neutron investigations, 6, 2006, 30-37.
6. A. Ruettinger, M.A. Kiselev, T. Hauss, S. Dante, A.M. Balagurov and R.H.H. Neubert, Eur Biophys J, Volume 37, 6, 2008, 759-771.
7. A. Schroeter, M.A. Kiselev, T. Hauß, S. Dante and R.H.H. Neubert, Biochim Biophys Acta Biomembr, 1788, 10, 2009, 2203.
8. A. Schröter, D. Kessner, M.A. Kiselev, T. Hauß, S. Dante and R.H.H. Neubert, Biophys J, 97, 4, 2009, 1114.
9. Kiselev, M.A., Gutberlet, T., Hauss, T., Ollivon, M., Neubert, R.H.H. Chem. Phys. Lipids 133, 181-193 (2005)

List of Publications – Research Articles

10. Kessner D, Brezesinski G, Funari SS, Dobner B, Neubert RHH. 2010. Impact of the long chain omega-acyl ceramides on the Stratum corneum lipid nanostructure. Part 1: Thermotropic phase behaviour of CER[EOS] and CER[EOP] studied using X-ray powder diffraction and FT-Raman spectroscopy. Chem Phys Lipids 163: 42-50.
11. Engelbrecht T, Hauß T, Süß K, Vogel, A, Roark M, Feller SE, Neubert RHH, Dobner B. 2011. Charactreisation of a new ceramide EOS species: Synthesis, investigation of the thermotropicphase behaviour and influence on the bilayer architecture of stratum corneum lipid model membranes. Soft Mater 7: 8998-9010.
12. Engelbrecht T, Schroeter A, Hauß T, Neubert RHH. 2011. Lipophilic penetration enhancers and their impact to the bilayer structure of stratum corneum lipid model membranes: Neutron diffraction studies based on the example oleic acid. Biophys Acta Biomembr 1808: 2798-2808.
13. Engelbrecht T, Deme B, Dobner B, Neubert RHH. 2012. Study of the Influence of the Penetration Enhancer Isopropyl Myristate on the Nanostructure of Stratum Corneum Lipid Model Membranes Using Neutron Diffraction and Deuterium-Labeling. Skin Pharmacol Physiol, doi: 10.1159/000338538.
14. Engelbrecht T, Schröter A**,** Hauß T, Deme B, Scheidt H, Huster D, Neubert RHH. 2012. The impact of ceramides NP and AP to nanostructure of stratum corneum lipid model membranes. Part I: Neutron diffraction and 2H NMR studies on multilamellar models based on ceramides with symmetric alkyl chain length distribution. Soft Matter 8 (24),2599-2607

Thank you very much for your attention!

Available at:

http://pharmtech.pharmazie.uni-halle.de/downloads/newinsights.pdf