SPring-8ワークショップ

＜放射光利用によるヘルスケア製品の機能評価＞

2005年1月28日，コンファレンススクエアM＋，東京

ヘルスケア製品開発における皮膚・毛髪の分子レベルでの構造情報の重要性

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ヘルスケア製品の有効性の検証

分子レベルでの相互作用の検出:

- X線構造解析(SAXD, WAXD)
- 原子間力顕微鏡(AFM)
- 電子顕微鏡・電子線回折・SEM
- 分光学的な方法(ラマン散乱，FTIR，・・・）
- 磁気共鳴（電子スピン，核）
- 熱分析

細胞間脂質（水和脂質2層膜モデルと経皮吸収）

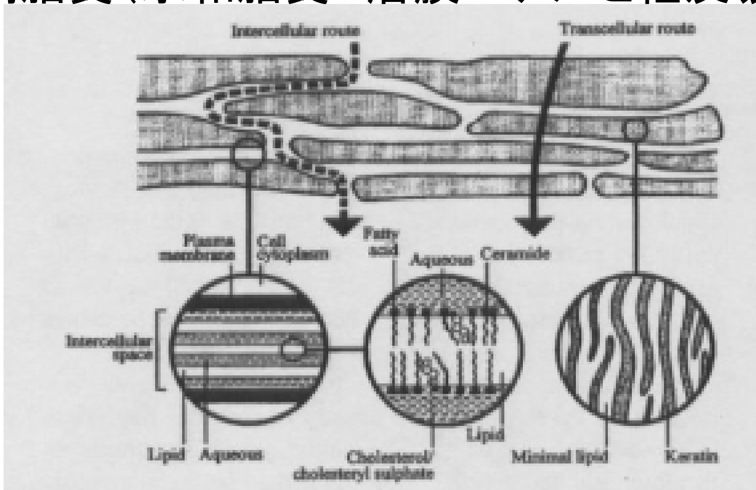


Fig. 2. Simplified diagram of stratum corneum and two microroutes of drug penetration.

・細胞間隙と細胞横断透過のモデル

B. W. Barry: Eur. J. Pharm. Sci. **14** (2001) 101-114

細胞間脂質（水和脂質2層膜モデル；経皮吸収）

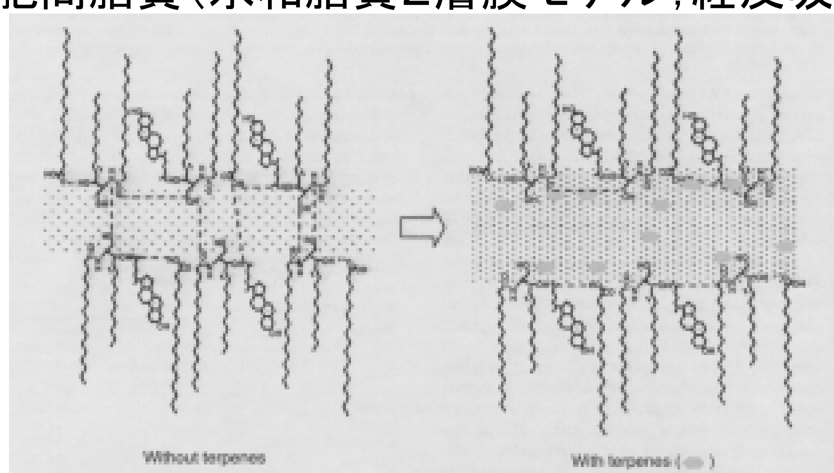


Fig. 3. Schematic illustration of SC lipid bilayer arrangement and effect of terpenes on it. Without terpenes, lipids are held together in lamellae by lateral and transverse hydrogen bonding. Terpenes break transverse hydrogen bonding leading to widening of aqueous region near head groups thereby increasing diffusivity of polar molecules. Note: Bond lengths and bond angles are not to be scaled.

・テルペンの振舞に関するモデル

S. Thomas, K. Marishetty and R. Panchagnula: J. Control. Release **95** (2004) 367-379.

細胞間脂質（水和脂質2層膜モデル）

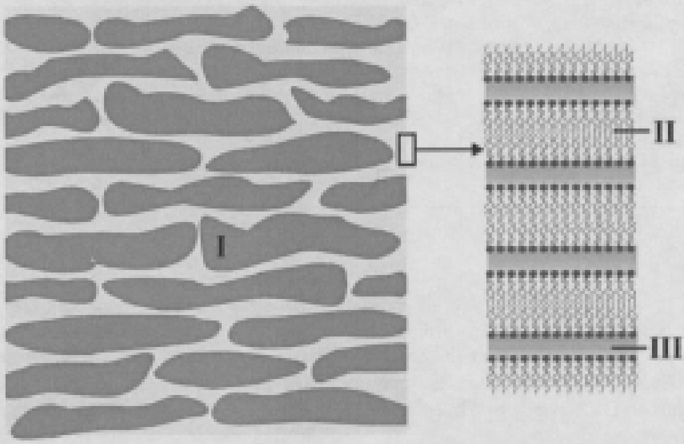
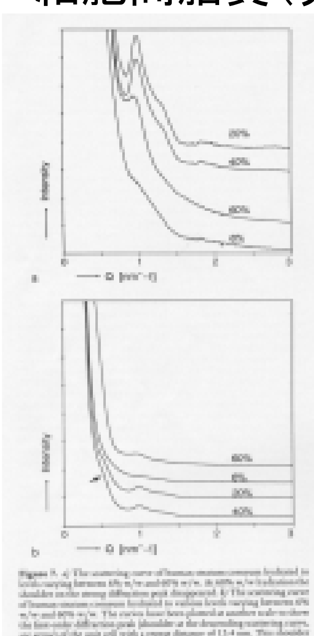


Fig. 1. The "brick and mortar" representation of the structure of stratum corneum is shown on the left side, i.e., corneocytes (dark gray) are surrounded by an extended lipid bilayer system (light gray). The right side gives an idealized picture of the hydrated lipid bilayer system. The labels correspond to (I) corneocytes, (II) lipid bilayer, and (III) hydration water phases A and B.

J. Pieper, G. Charalambopoulou, Th. Steriotis, V. Vasenkov, A. Desmedt and R. E. Lechner: Chem. Phys. **292** (2003) 465-476.

細胞間脂質（非水和長周期ラメラ構造）



・周期13 nmのラメラ構造は水分量により変化しない.

・角層中の水分量20% w/wで長周期ラメラ構造によるX線反射のピークが鋭くなる.

J. A. Bouwstra, G. S. Gooris, J. A. van der Spek and W. Bras: J. Invest. Derm. **97** (1991)1005-1012.

The position of the main diffraction peak at 13.4 nm does not change between 6 and 40% w/w hydration level. ･･･This indicates that the repeat distance does not change upon hydration and that no swelling of the bilayers occurs.

J. A. Bouwstra, G. S. Gooris, J. A. van der Spek and W. Bras: J. Invest. Derm. **97** (1991)1005-1012.

The hydration level was varied between 6 and 40% w/w. Upon hydration from 6 to 20% w/w the reflections at 0.378 and 0.417 nm became sharper but were not shifted. This can be explained based on ordering of the lateral packing of alkyl chains, but no lateral swelling took place. Between 20 and 40% w/w hydration no changes in the scattering pattern were observed. SAXD (Bouwstra *et al*., 1991) revealed that swelling of the bilayers did not occur upon hydration.

J. A. Bouwstra, G. S. Gooris, M. A. Salomons-de Vries, J. A. van der Spek and W. Bras: Intern. J. Pharm. **84** (1992) 205-216.

細胞間脂質（角層中の水の分布）

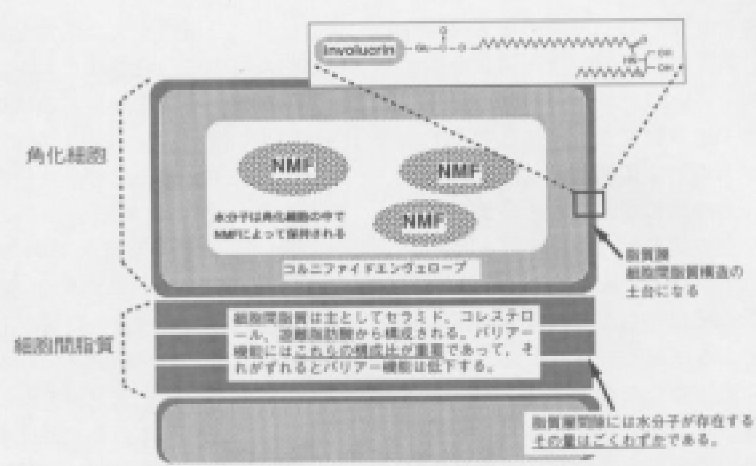
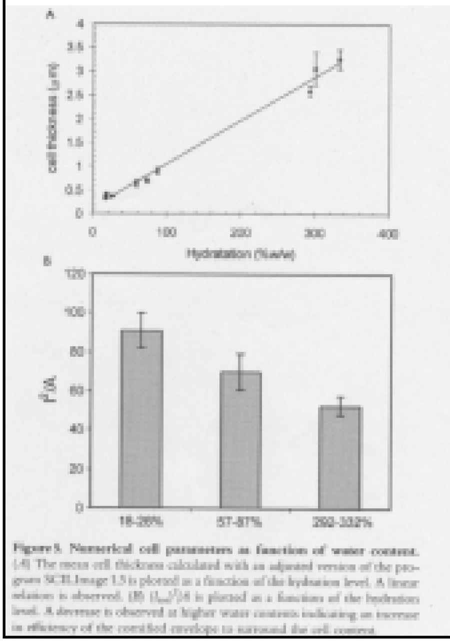


図2　皮膚角質層の微細構造とその役割

伝田光洋：FRAGRANCE JOURNAL **27** [10] (1999) 17-22.

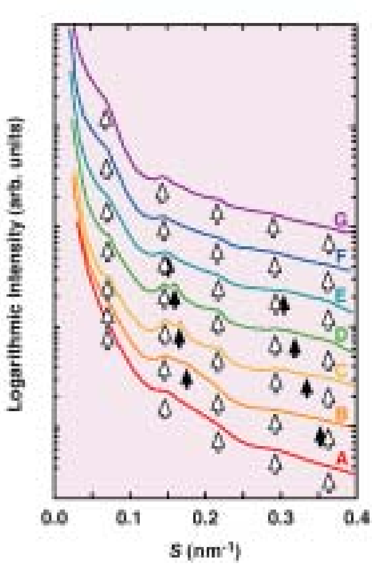
細胞間脂質（角層中の水の分布）



・自然の状態は20% w/w

J. A. Bouwstra, A. de Graaf, G. S. Gooris, J. Jaap, J. W. Wiechers and A. C. van Aelst: J. Inves. Dermatol. **120** (2003) 750-758.

細胞間脂質（長短ラメラ構造のX線回折像）

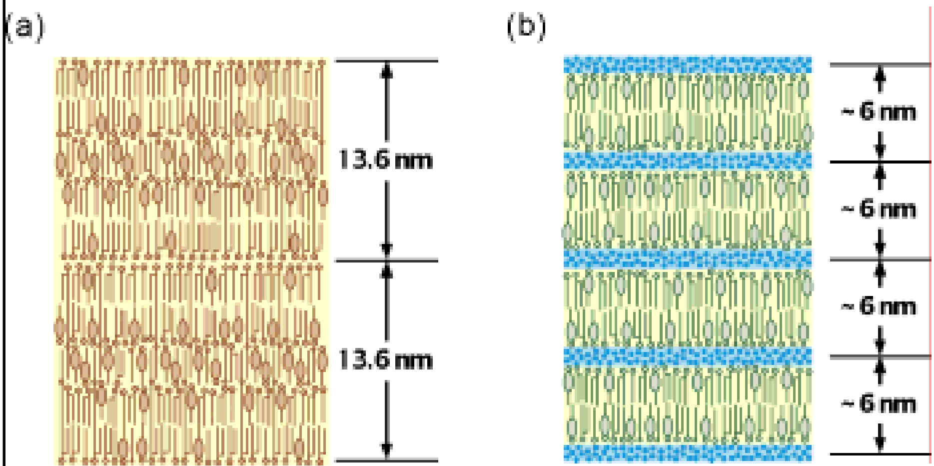


・白矢印:　　13 nm

・黒矢印:　 ～6 nm

N. Ohta, S. Ban, H. Tanaka, S. Nakata and I. Hatta: Chem. Phys. Lipids **123** (2003) 1-8.

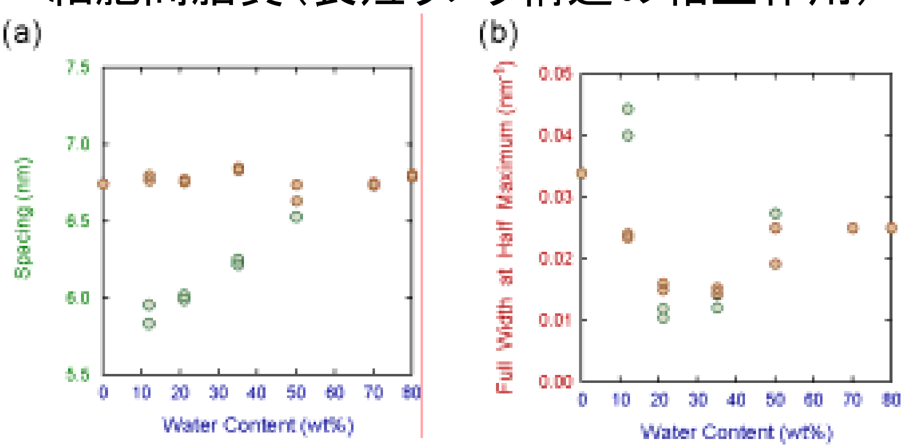
細胞間脂質（長短周期ラメラ構造のモデル）



・セラミド，コレステロール，脂肪酸から成っている

I. Hatta and N. Ohta: Photon Factory Activity Report 2003 A (2004) 49-50.

細胞間脂質（長短ラメラ構造の相互作用）



・濃い○：13 nm, 薄い○：～6 nm

N. Ohta, S. Ban, H. Tanaka, S. Nakata and I. Hatta: Chem. Phys. Lipids **123** (2003) 1-8.

細胞間脂質(透過経路)

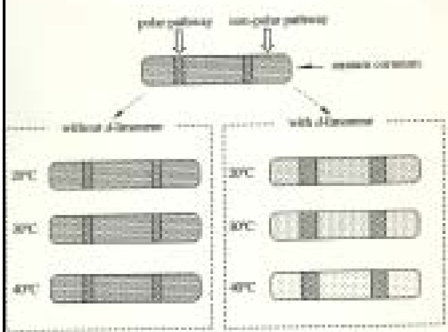
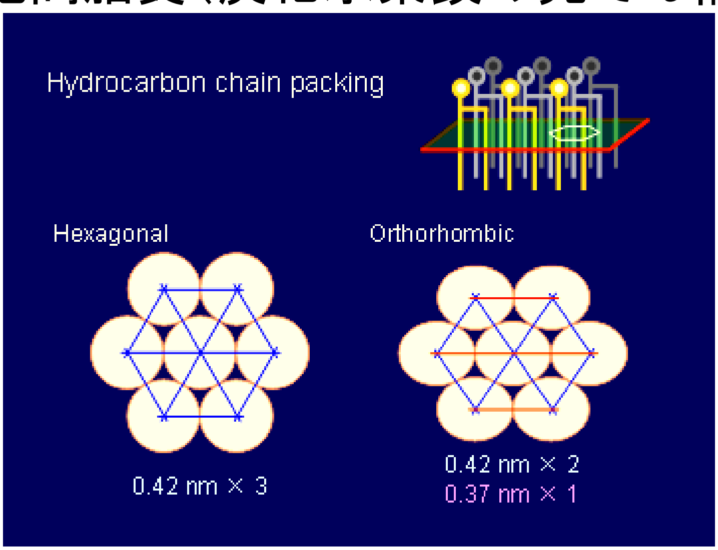


Fig. 16 Proposed mechanism of combined effect of d-limonene and temperature on polar and non-polar pathways in the skin. The structure of polar pathway could be changed with a pretreatment with d-limonene independently on applied heat, however, the non-polar pathway could be synergistically affected by the combination of d-limonene pretreatment with applied heat.

・親水性薬物と疎水性薬物の透過

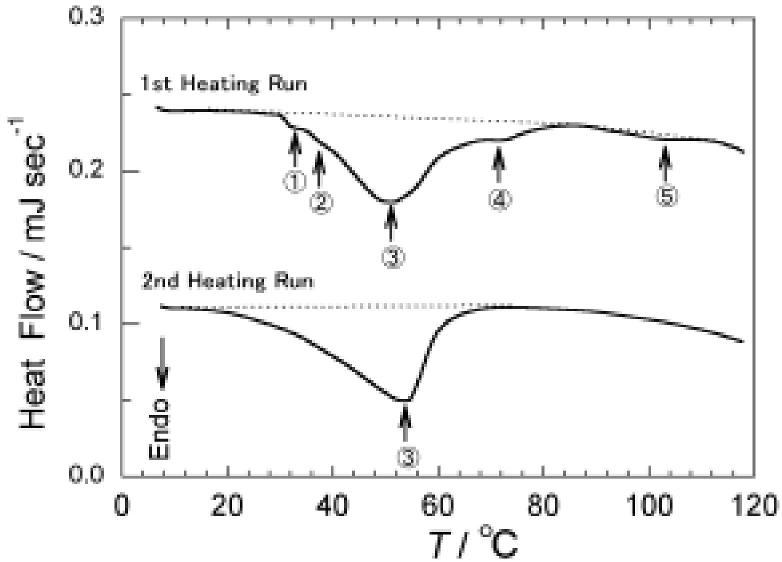
N. Ohara, K. Takayama and T. Nagai, Biol. Pharm. Bull.**18** (1995) 439-442.

細胞間脂質(炭化水素鎖の充てん構造)



六方晶(hexagonal)と斜方晶(orthorhombic)
格子定数0.42 nmが共通

細胞間脂質（構造相転移）



I.Hatta, K. Nakanishi and K. Ishikiriyama: Thermochim. Acta (2005) in press.

細胞間脂質(再構成脂質膜）

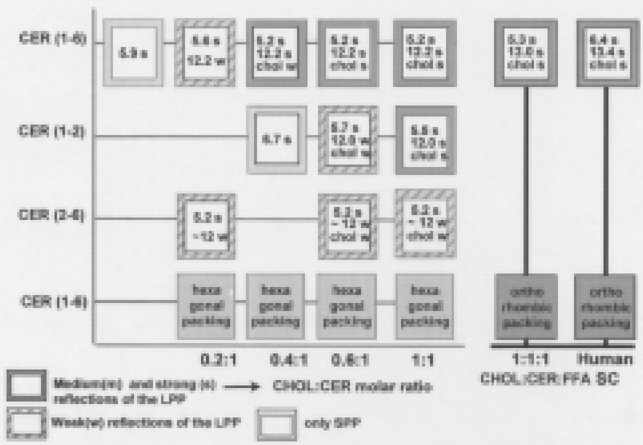
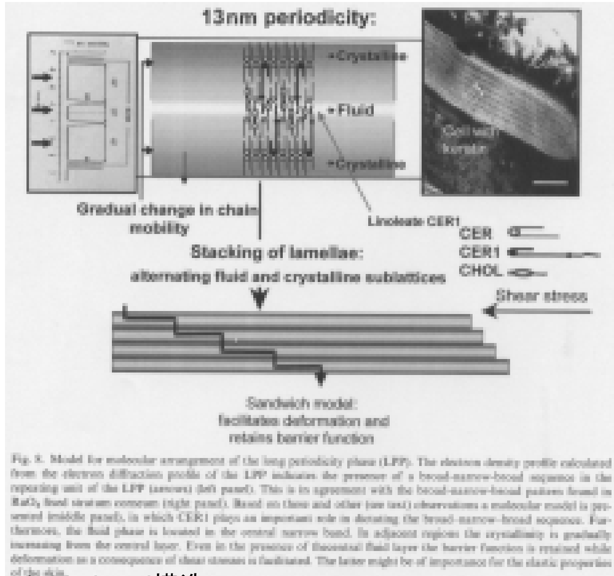


Fig. 7. Schematic presentation of the phase behavior of various CHOL:pigCER mixtures as function of CER composition and of CHOL:CER molar ratio. Mixtures were prepared with full spectrum of CER [CER(1-6)], with CER 1 and 2 [CER(1,2)], or with CER mixture in which CER1 is absent [CER(2-6)]. In addition, the phase behavior of the equimolar CHOL:CER:FFA mixture and human stratum corneum (SC) is depicted. The equimolar CHOL:CER(1-6):FFA mixture mimics most closely the phase behavior in human stratum corneum.

J. A. Bouwstra, P. L. Honeywell-Nguyen, G. S. Gooris and M. Ponec: Progr. Lipid Res. **42** (2003) 1-36.

細胞間脂質（長周期ラメラ構造）



Wide/narrow/wide band構造

J. A. Bouwstra, P. L. Honeywell-Nguyen, G. S. Gooris and M. Ponec: Progr. Lipid Res. **42** (2003) 1-36.

･･･one lamellar phase with a periodicity of approximately 6 nm (short periodicity phase; SPP), and the other phase with a periodicity of approximately 13 nm (long periodicity phase; LPP). *Since the LPP has been found to be present in all species examined until now, and has a very characteristic molecular organization, it has been suggested that the presence of this phase plays an important role in skin barrier function.*

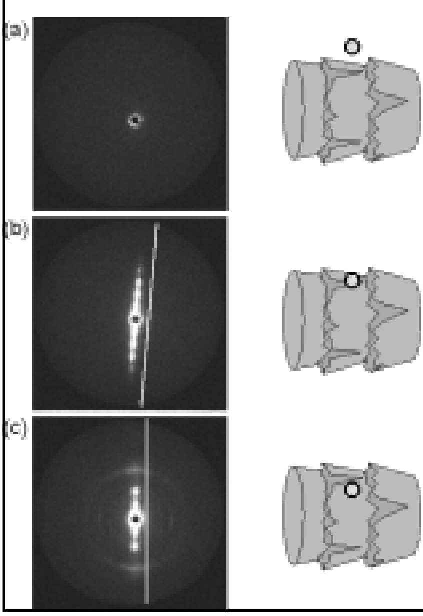
J. A. Bouwstra, P. L. Honeywell-Nguyen, G. S. Gooris and M. Ponec: Progr. Lipid Res. **42** (2003) 1-36.

G. S. K. Pilgram and J. A. Bouwstra: Basic and Clinical Dermatology **26** (2004) 107-152.

皮膚角層についてのまとめ

- 機能発現機構を検討する際に，類推に基づくモデルではなく，分子レベルでの証拠に基づくモデルで—要素還元主義に陥ることなく—
- 鋭いX線回折像が得られる条件下での測定からはじめることが重要性

毛髪キューティクル（X線回折実験）

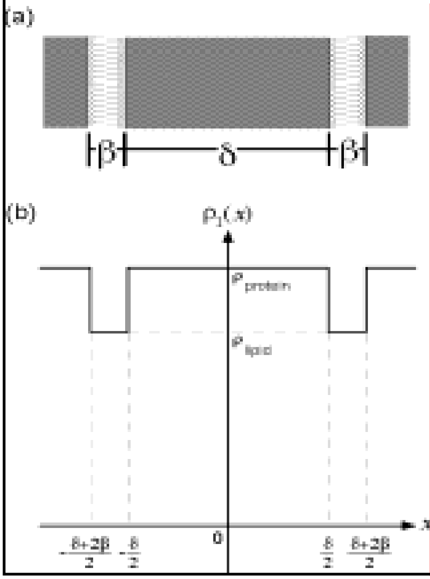


・X線ビーム位置と回折像の関係

(a) 真空
(b) キューティクル
(c) コルテックス

N. Ohta, T. Oka, K. Inoue, N. Yagi, S. Kato and I. Hatta: J. Appl. Cryst., in press.

毛髪キューティクル（CMCの電子密度分布）



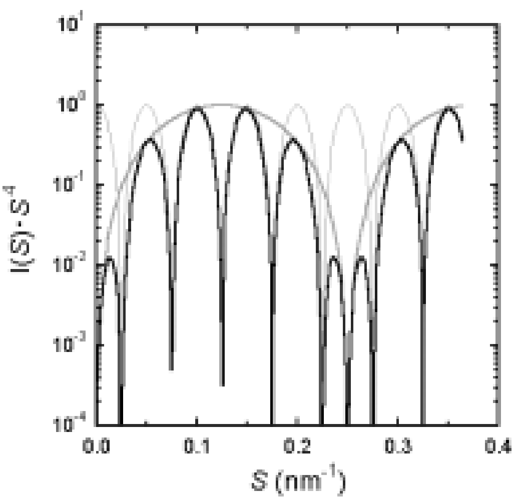
$\delta \pm \Delta\delta$

$\beta \pm \Delta\beta$

・CMC (cell membrane complex；細胞膜複合体)の構造：
upper β, δ, lowerβ layers

N. Ohta, T. Oka, K. Inoue, N. Yagi, S. Kato and I. Hatta: J. Appl. Cryst., in press.

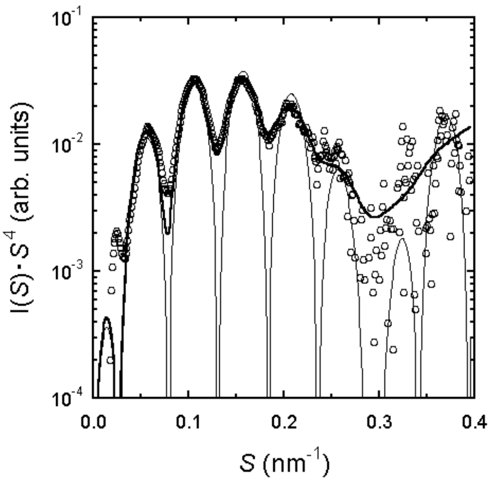
毛髪キューティクル（単層膜のX線回折像）



・CMC単層（β-δ-β）膜によるX線回折像

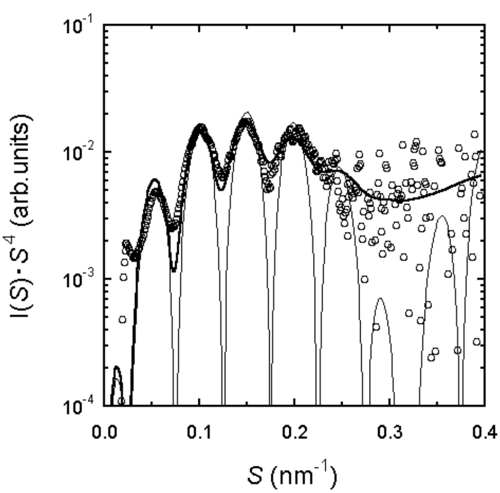
N. Ohta, T. Oka, K. Inoue, N. Yagi, S. Kato and I. Hatta: J. Appl. Cryst., in press.

毛髪キューティクル（rat whisker）



N. Ohta, T. Oka, K. Inoue, N. Yagi, S. Kato and I. Hatta: J. Appl. Cryst., in press.

毛髪キューティクル（human hair）



N. Ohta, T. Oka, K. Inoue, N. Yagi, S. Kato and I. Hatta: J. Appl. Cryst., in press.

毛髪についてのまとめ

- 外界から毛髪中へのイオン，分子の透過
- キューティクル(CMC)の構造変化
- ケラチンの構造変化