台大分子影像中心-電鏡分子影像核心實驗室

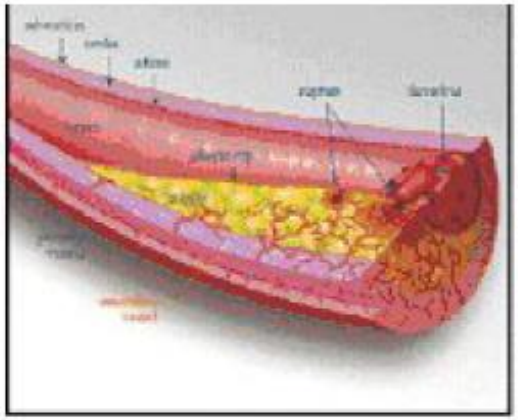
生物電子顯微術

PI： 趙治宇 教授

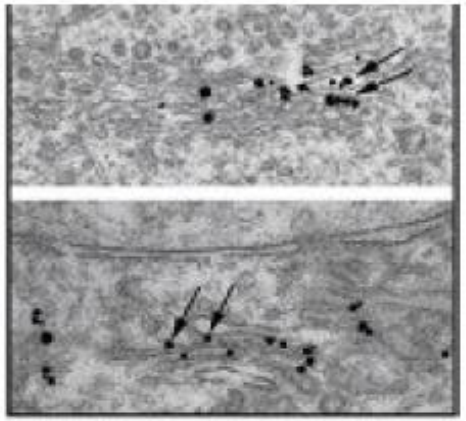
Correlative microscopy

Organs

Cells in tissue



Molecules in cells



In vivo imaging

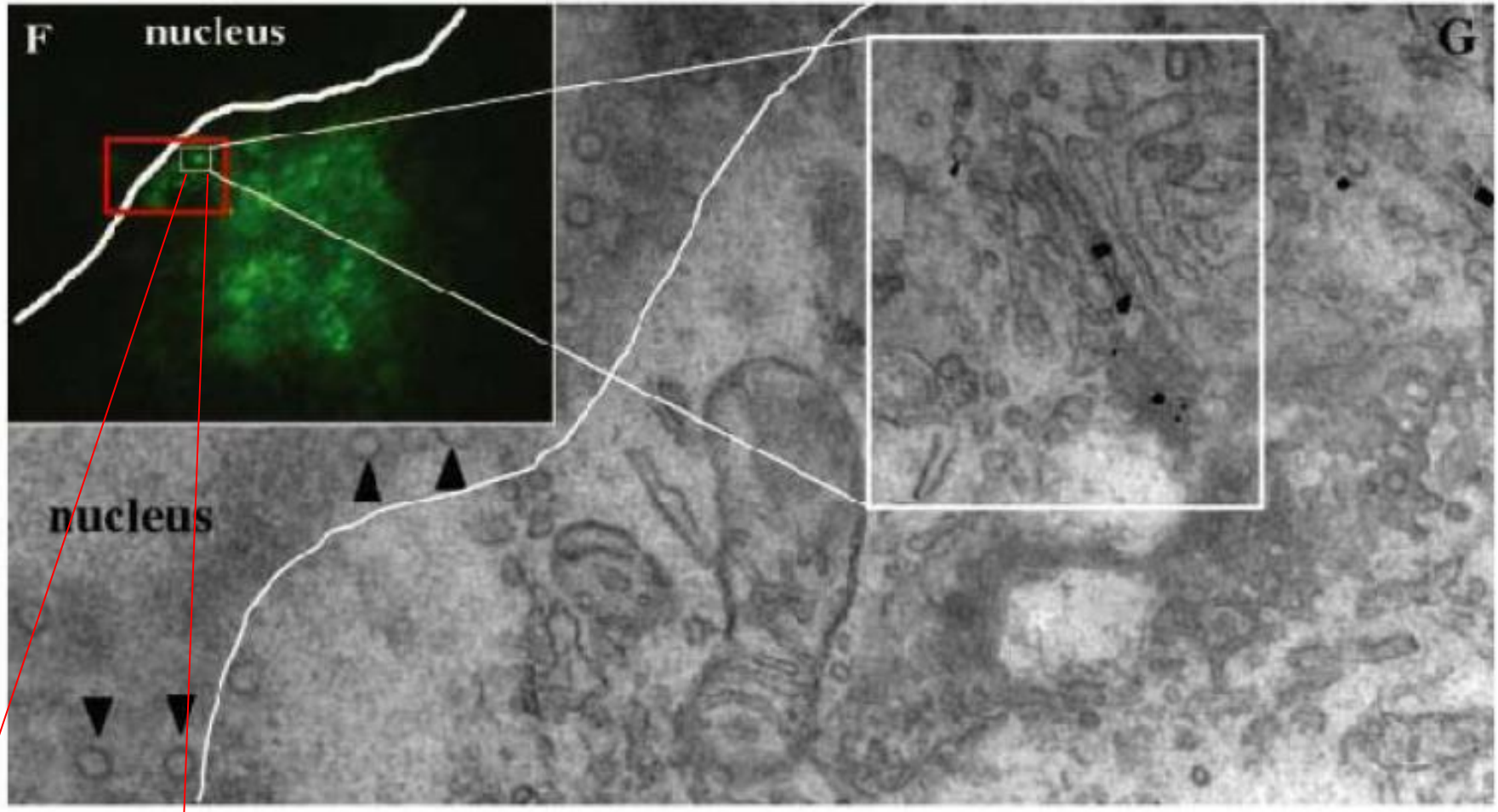
Light microscopy

Electron microscopy

Resolution - → Resolution +

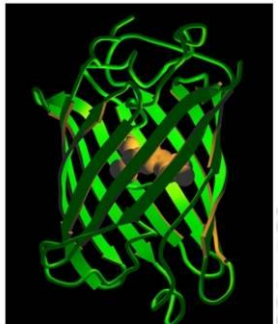
System Biology — Cellomics ⟷ Genomics/Proteomics

FEI



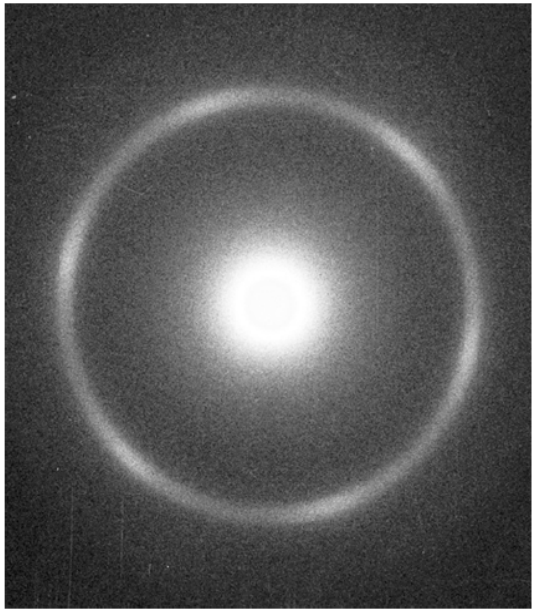
Mironov et al. Journal of Cell Biology, 2001, Vol 155, 1225-1238

VSVG-GFP



First Observation of Fully-Hydrated Biological samples in TEM

C. Y. Chao et al., Physical Review Letters 93, 247801, 2004.



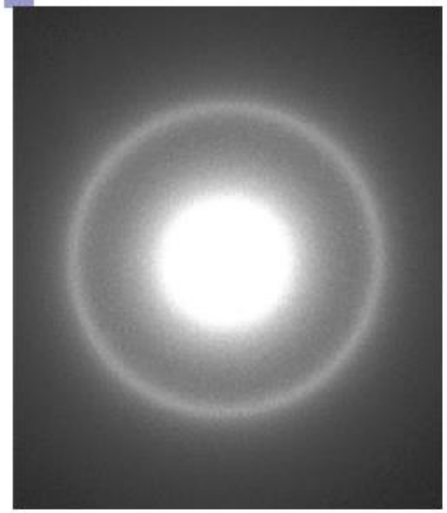
An Exotic Liquid Phase in a Biomembrane-Like System

One of the major recent advances in condensed matter physics is the prediction and subsequent experimental confirmation of the existence of an intermediate hexatic phase between the crystalline solid and the isotropic liquid in reduced dimensions. This phase has long-range bond-orientational order, and its positional correlations, while short-range, should be much stronger than those in an ordinary liquid. It has long been suspected but never demonstrated that lyotropic lamellar membrane systems, because of their intrinsic two-dimensional nature, should exhibit hexatic behavior.

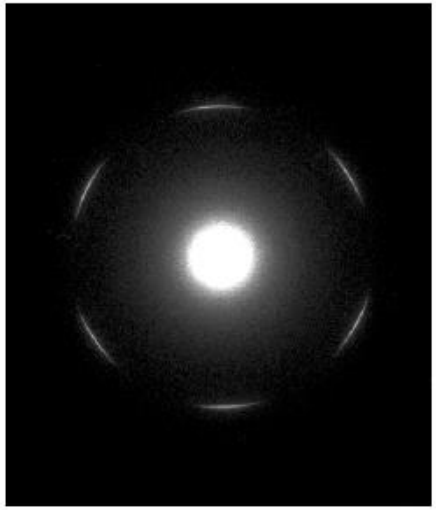
Our discovery is important in two respects. This is the first report of hexatic behavior in a lyotropic liquid crystal consisting of amphiphilic bilayers similar to those in biological membrane systems, and should have a broad interest beyond physics. Furthermore, this appears to be a new type of hexatic liquid phase, with the characteristic six-fold symmetry but only liquid-like nearest-neighbor positional correlations. Its existence brings into question our basic understanding of the fundamental mechanism for melting based on the introduction of defects.

Our discovery also represents the first report of in-plane hydration force in lyotropic lamellar bilayer systems. In addition, our experimental results also suggest that the water molecules possessing larger kinetic energy in between those bilayers could have the chance of passing through the membrane-like bilayer films via the osmosis process, which is directly related to the hydration force. Our results further suggest that the intercalated water molecules or the the plausible passage of water molecules through the membrane-like bilayers weakens the interaction force between the molecules of these bilayer films, and the resulting dynamic balance causes the surface of these lamellar bilayer films to exist in a '**state**' qualitatively similar but quantitatively different from the conventional 2D hexatic and 2D liquid states reported previously in many other LC films (*Liquid Crystals Today* **14**, 1, 2005).

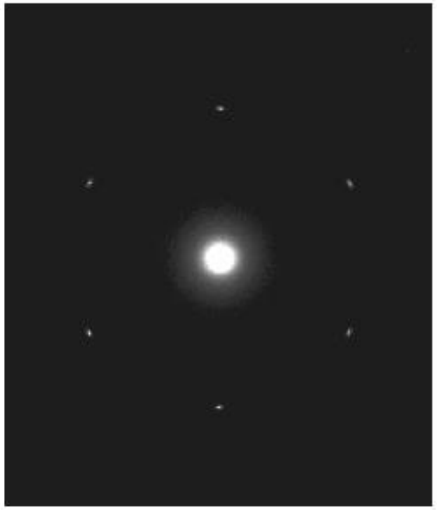
Our discovery not only reinforces the existence of surface hexatic liquid in nature, but also further provides important information about the composition and organization of bilayers which are model systems for biological membranes. Besides, it provides new food for thought in regard to the location of water molecules in these bilayer membrane films. Moreover, our unique environmental TEM can also open the door to new research areas linking the disciplines of physics, chemistry, biology, and medicine.



液態



液晶態



固態

酯膜結構

This new state forms due to the existence of hydration force in lipid-water system in biological native environment.

($[C_{12}H_{25}\text{-imH}]\cdot\ n\ H_2O$)

「酯膜–水」結構與液態、液晶態、和固態均不相同

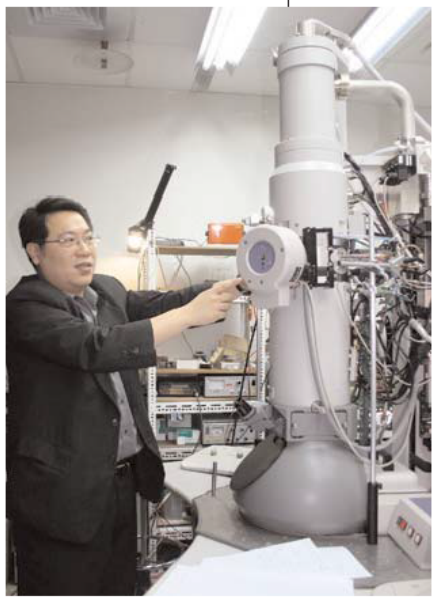
【生物物理】

與水共舞的脂膜分子

從脂膜分子與水的微妙律動，開創出醫學研究的嶄新視野。

撰文／邱淑慧

沒有水就沒有生命，生物體內的每個小小細胞都充滿了水，也都生存在水裡，但是，水分子是否能直接通過細胞膜，卻一直未有定論。而台灣大學物理系趙治宇，利用自己改造的全球第一部生物環境穿透式電子顯微鏡（Bio TEM），首度觀察到水與脂膜分子的共存狀態，是一種未曾發現的微妙動態平衡。這項研究成果發表於2004年12月10日《物理評論通訊》（*Physical Review Letters*），引起了國內外許多生物學家與醫學家的注意與重視。



趙治宇以他自行改裝的BioTEM，觀察到一種前所未見、由脂膜與水分子所形成的共存狀態。

細胞膜是一種雙層脂膜（lipid bilayer），膜外層為親水端，內部則為疏水端，因此離子與極性分子不易通過。1980年代以前普遍認為，當分子夠小、動能夠大時，分子可以經由滲透方式而通過細胞膜。1988年，阿格雷（Peter Agre）以實驗證實，水分子可經由膜蛋白構成的通道而進出細胞；1998年麥金農（Roderick MacKinnon）則利用X光繞射，確認了細胞膜上離子通道的蛋白質結構。阿格雷與麥金農也因此獲得2003年諾貝爾化學獎。然而，細胞膜的脂膜分子間並無鍵結，水分子真的不能穿越？

趙治宇用Bio TEM觀察離子型液晶分子「1-十二烷基咪唑」的硝酸鹽（化學式為$[C_{12}H_{25}\text{-}imH][NO_3]$），這種分子有個很長的烷基疏水端，又有個親水的含氮雜環，性質上與細胞膜分子非常類似，在水中也會形成像細胞膜般的雙層脂膜結構。在Bio TEM下，這種脂膜分子和水分子產生了一種從未見過的繞射圖案（見右頁上圖），趙治宇表示：「這個圖案具有六角方向性，顯示它不是液體；但這個圖形也非清晰的繞射點，可見分子具有運動，不是固體；此外，它的六個弧狀弧形不似液晶的清晰可辨，可見分子間關聯性極低，排除是液晶的可能。而且，這個由水與脂膜共存時所呈現的新狀態，還具有可逆的相變發生，不是一個混合態。那麼，它到底是什麼？」

經繞射圖形分析與位置關聯性的計算，趙治宇發現脂膜分子的關聯性與液態相同，且在脂膜分子間有約0.5奈米的空

Biological Environmental TEM

- **Physical Review Letters (PRL)**為第一作者/通訊作者的文章共**8**篇
  (IF=7.96 ; 5/85=6% Physics Multidisciplinary)

- 目前已取得和申請中與液態電子顯微鏡相關之世界各國專利共有**96**件

Electron Microscopy of Whole Cells and Biological Specimens in Liquid

☞ 生物環境式電子顯微鏡之技術來觀察液晶生物薄膜的相變，透過電子顯微鏡之繞射照片，我們發現了一個介於液體與液晶間可能的新狀態[1,2]，此狀態很有可能就是液晶酯膜與水共存時之狀態，並提供了我們一個對於瞭解水通過液晶脂膜之動態過程之新的看法[1]，此技術也已經申請了**世界各國專利共計96項**。**此項技術之終極目標，在於能在電子顯微鏡高真空腔體內觀察水溶液環境下活體細胞奈米等級之結構，而其可行性也已經由國外之團隊證明[3,4]，他們利用掃描穿透式環境生物電子顯微鏡(Bio-STEM)，成功的觀察用金粒子與表皮生長因子標定之成纖維細胞(厚度約10-15μm)，藉此發現表皮生長因子接收器之位置，目前解析度已達2-3nm，未來隨著奈米標定技術的進步其解析度可望提升到只有幾個原子的大小，同時IBM團隊也正全力投入積極研發liquid-(S)TEM的技術與其應用，目前已開使接受各項有潛力之國際合作案的申請[5]，同時IBM也邀請本人共同參與liquid membrane TEM技術與其相關應用的開發工作。**

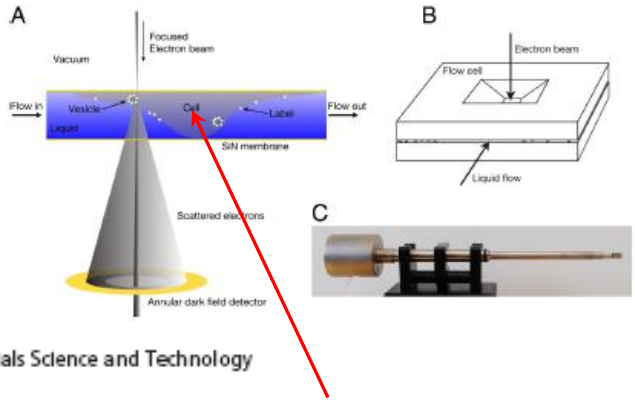
References:

1. C. Y. Chao *et al.*, Liquid Crystals Today **14(3)**, 1 (2005). **(Issue Cover)**
2. C. Y. Chao *et al.*, Phys. Rev. Lett. **93**, 247801 (2004).
3. N. de Jonge and F. M. Ross, **Electron microscopy of specimens in liquid**, Nature Nanotechnology **6**, 695 (2011).
4. N. de Jonge, D. B. Peckys, G. J. Kremers, and D. W. Piston, **Electron microscopy of whole cells in liquid with nanometer resolution**, PANS **106**, 2159 (2009).
5. 國科會內部資料: 其中**Development of liquid cell transmission electron microscopy**為NSC-IBM project中IBM所規劃的計畫之一.

Electron microscopy of whole cells in liquid with nanometer resolution

N. de Jonge[a,b,1], D. B. Peckys[b,c], G. J. Kremers[a], and D. W. Piston[a]

[a]Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN 37232-0615; [b]Materials Science and Technology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6064; and [c]University of Tennessee, Knoxville, TN 37996-2200



Resolution 4nm → now < 2nm

Sample thickness ~15um operating in the STEM mode

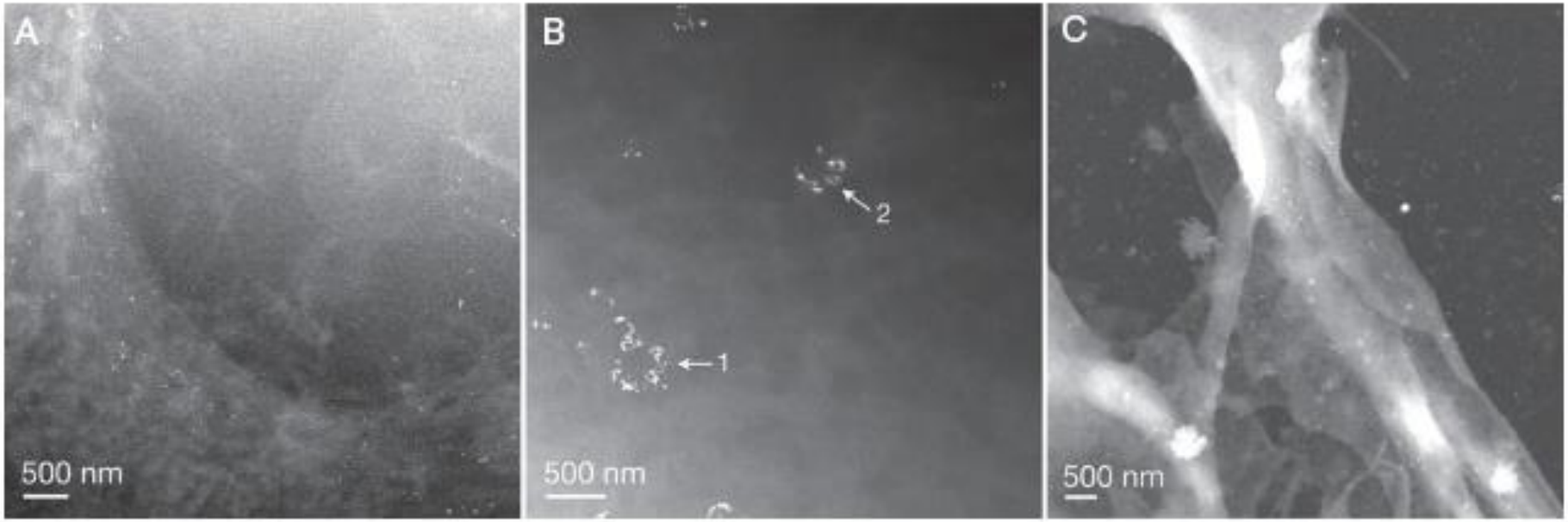


Fig. 2. Liquid STEM images of COS7 fibroblast cells labeled EGF-Au. (*A*) Image of the edge of a fixed COS7 cell after 5-min incubation with EGF-Au. The labels are visible as bright spots and the cellular material is shown as light-gray matter on a dark-gray background. The pixel size was 5.7 nm. (*B*) Image of a COS7 cell incubated with EGF-Au for 10 min and incubated in buffer (without EGF-Au) for an additional 15 min. The pixel size was 4.4 nm. (*C*) Image of the sample used in *A* recorded after the flow cell was opened and the sample was dried in air. The pixel size was 8.9 nm. Note that the salt of this sample was not removed.

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nature nanotechnology

REVIEW ARTICLE

PUBLISHED ONLINE: 23 OCTOBER 2011 | DOI: 10.1038/NNANO.2011.161

Electron microscopy of specimens in liquid

Niels de Jonge[1]* and Frances M. Ross[2]*

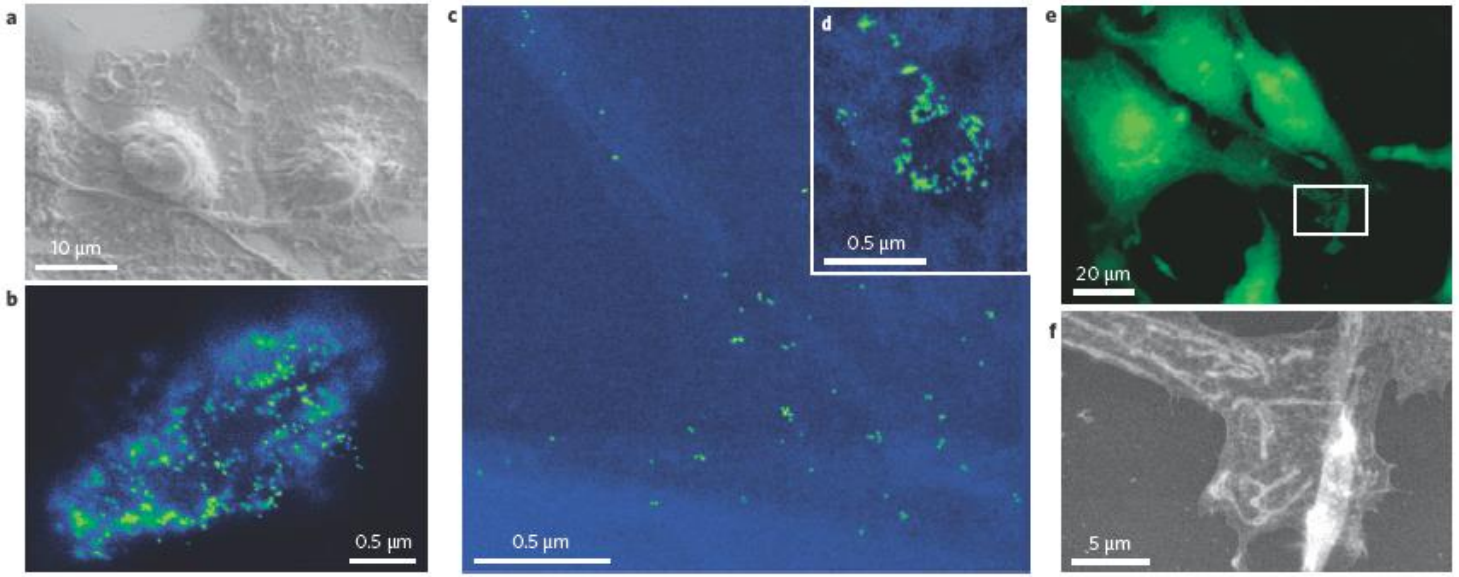


Figure 3 | Electron microscopy of biological samples. a, Human monocyte-derived macrophages imaged with SEM in a wet environment[39] at 4.9 torr and 7 °C. Samples were fixed in glutaraldehyde and rinsed with deionized water before imaging. **b**, SEM image of *H. pylori* bacterium fully immersed in liquid, imaged using a capsule with a thin window. Samples were incubated with complexed biotinylated gastrin on streptavidin-coated 20 nm Au particles, followed by glutaraldehyde fixation. **c**, STEM image of Au-labelled epidermal growth factor receptors on whole fixed COS7 fibroblast cells in liquid. The Au labels are visible as yellow spots on the light blue cellular material. The background shows in dark blue. **d**, Endocytotic vesicles were formed in a second sample after a longer incubation. **e**, Fluorescence microscopy image of COS7 cells[33]. The cells were fixed, labelled with a fluorescent dye, and stained for contrast in SEM. **f**, SEM image of the cellular material in the rectangle in **e** imaged under fully hydrated conditions[33]. Panels reproduced with permission from: **a**, ref. 39, © 2009 Wiley; **e**,**f**, ref. 33, © 2010 Elsevier. Panels modified with permission from: **b**, ref. 2, © 2004 National Academy of Sciences; **c**,**d**, ref. 4, © 2009 National Academy of Sciences.

World-Leading Technique

☞ **World-leading Biological TEM Technique (w/ humidity & hydration control at 1 atm pressure)**. 主持人曾多次受邀參與**IBM**規劃之**liquid (S)TEM液態電顯技術**開發工作與**liquid cell membrane TEM**相關應用的研發工作，**目前本技術已商品化，且國內已有電顯液體樣本之代工服務(Bio MA-tek公司)**。

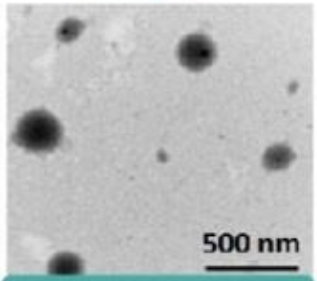
(http://www.bioma-tek.com/Services_B1-2.html)

Bio Ma-Tek Inc.

樣品應處於原液環境中觀察

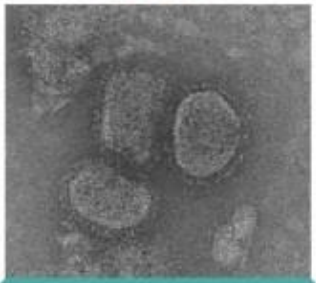
先進完整的樣品製備技術

PLGA 粒子



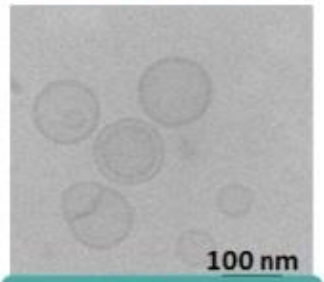
普通件
(乾燥、真空)

流感病毒



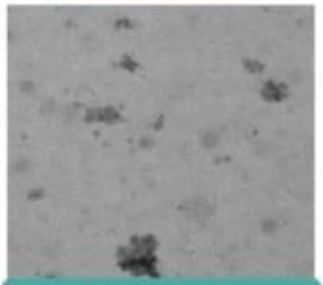
負染stained
(乾燥、真空)

微脂體 (Liposomes)



冷凍cryo
(冷凍、真空)

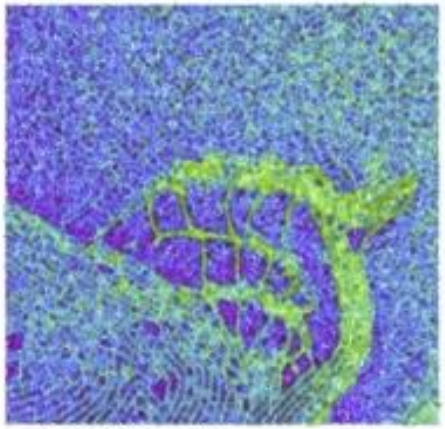
血液中的Resovist®



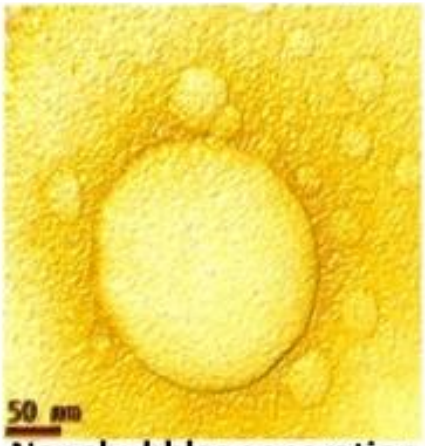
液態k-kit
(液態、原液)

SCIENTEK CORP. 科榮股份有限公司 TEMIC

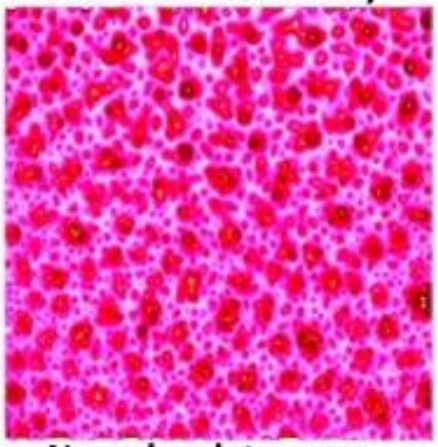
臨場電子顯微鏡新領域 - 液體、生物與軟物質觀察



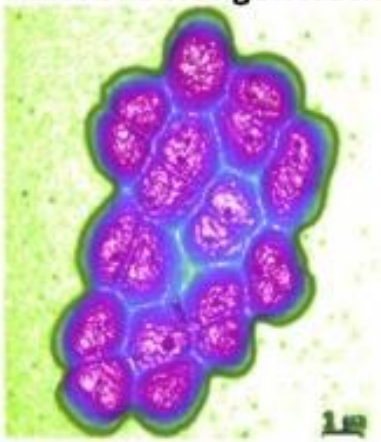
PDMS self-assembly



Nanobubbles generation

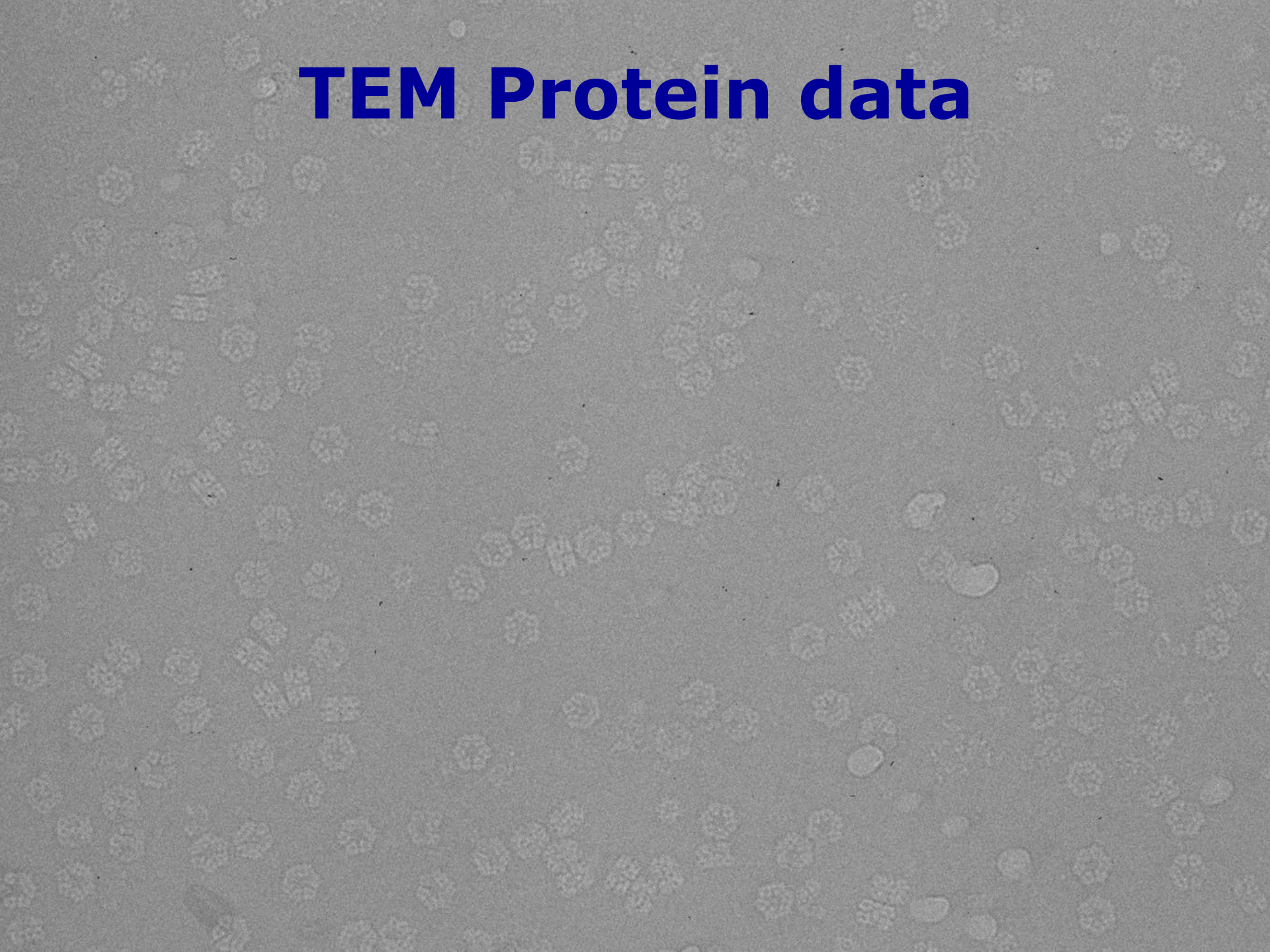


Nanodroplet merge

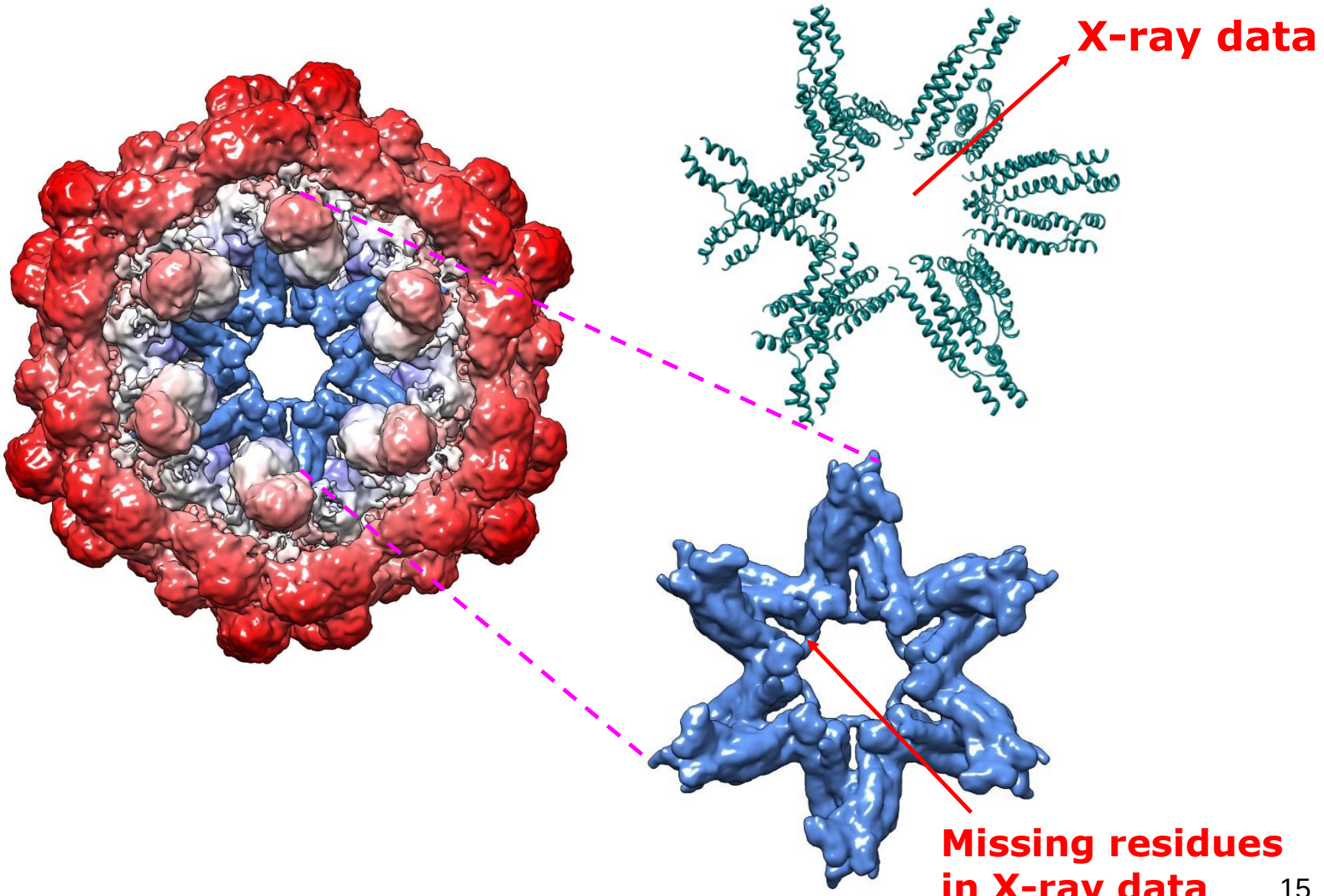


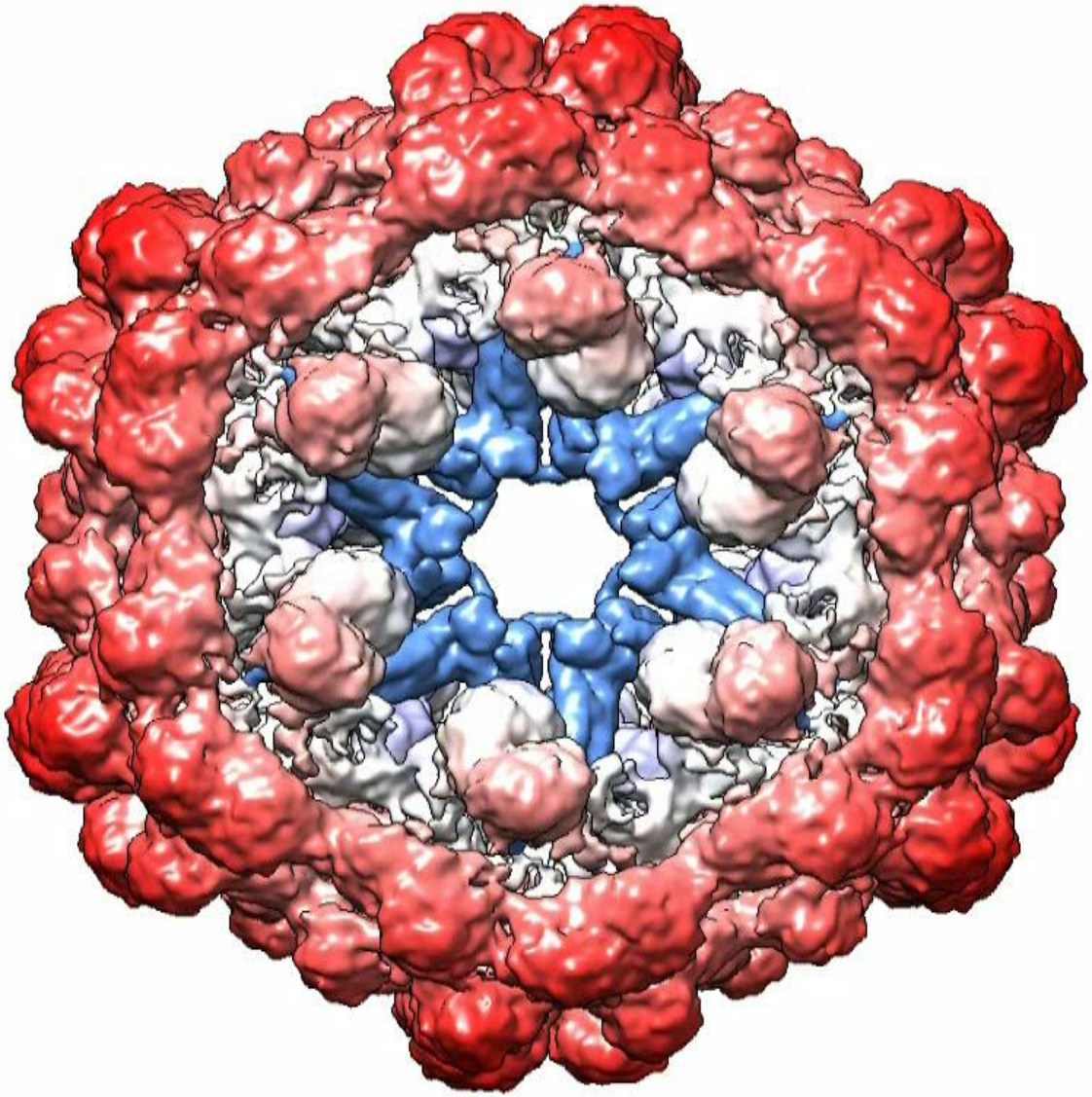
D. Radiodurons growth

TEM Protein data



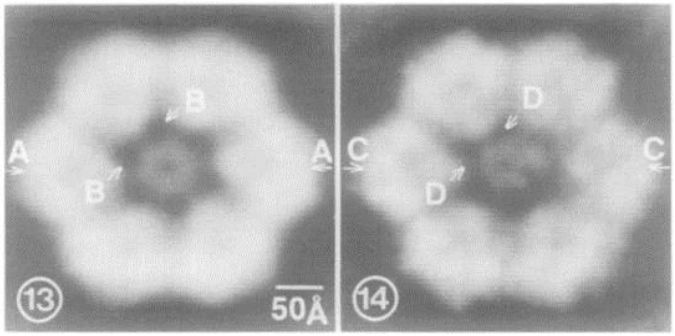
➢Extra density in the central linker





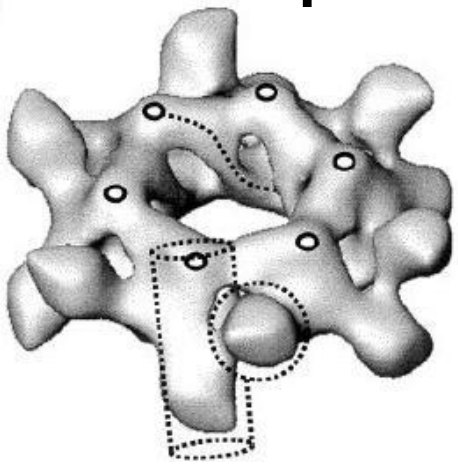
➢Central bracelet

✓ Central substructure



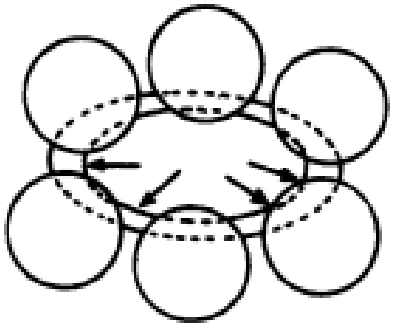
J. Ultrastruct. Res. **83,** 312-318 (1983).

✓ Sinusoidal pillars



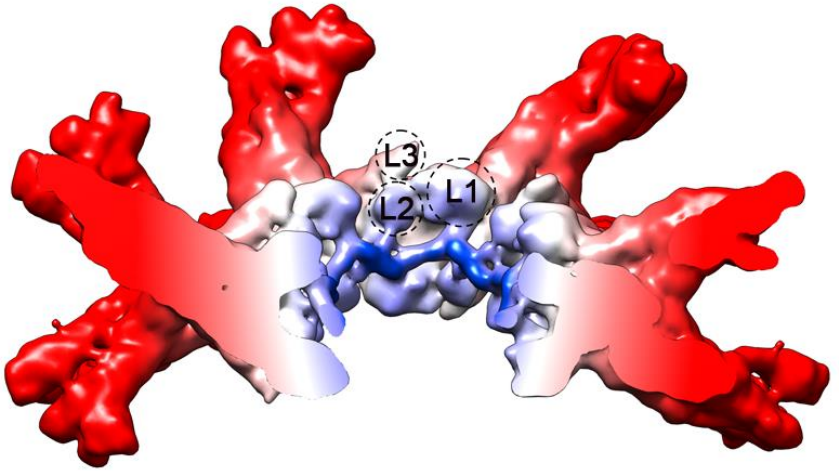
J. Struct. Biol. **133,** 176-192 (2001).

✓ Bracelet model

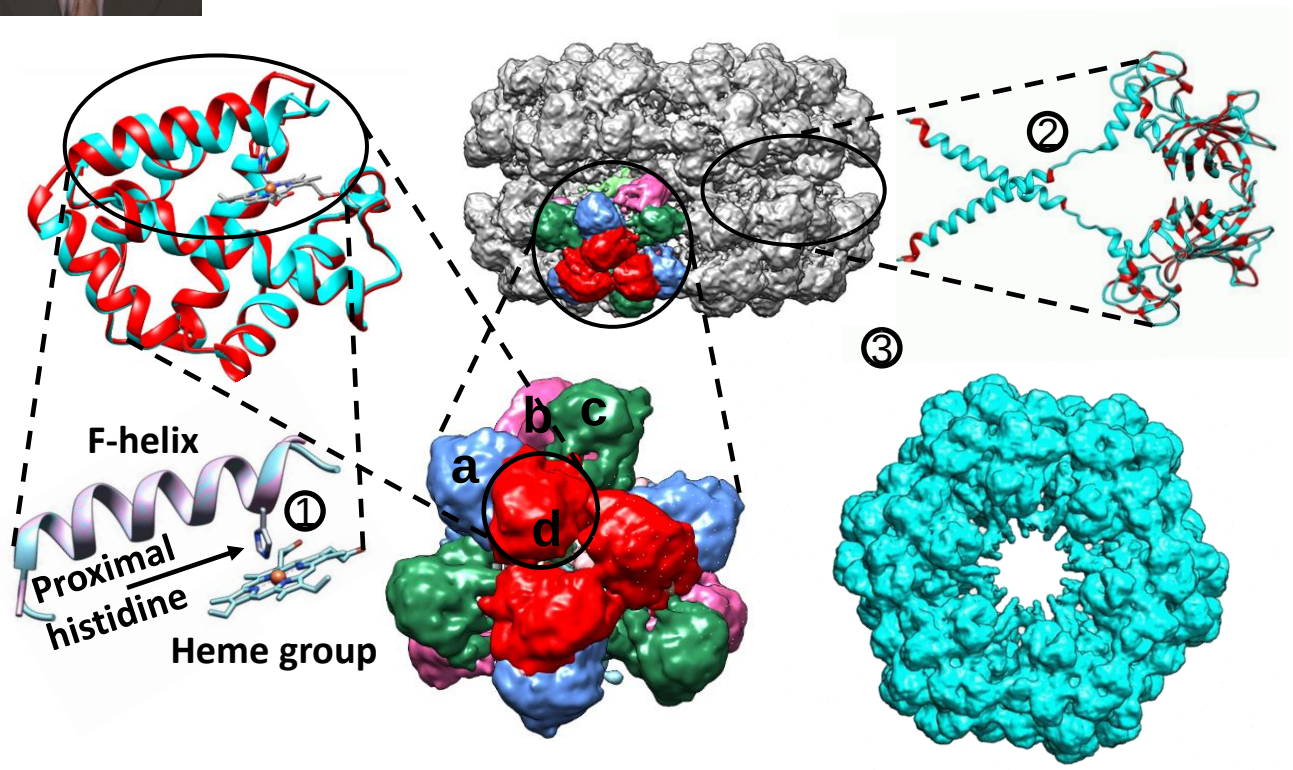


Proc. Natl Acad. Sci. USA, **83**, 8034 (1986).

✓ Direct observation of the sinusoidal bracelet



Cooperative oxygen binding mechanism of earthworm hemoglobin



① **Upon oxygen binding, the allosteric signal is transmitted through the heme iron to the proximal histidine, causing the tilting of helix-F.**

② **In the next step, the allosteric signal is transmitted to the linker, and the β barrel domain is pushed outward.**

③ **As a result, the oxygenation causes a radial expansion of the whole hemoglobin complex.**

1. 趙治宇教授率領台大團隊，利用生物電鏡首度發現蚯蚓血紅蛋白之動態攜氧協同機制及首度瞭解此蛋白其如何形成之原因，成果發表在Nature雜誌的Scientific Reports.
2. 趙教授團隊將進行人體局部急性重症之患者進行急救藥物之開發，因將此極高攜氧量之血紅素注入急性肺衰竭或急性腦中風/拴塞的患者，可使細胞不致於因太早缺氧而死亡，目前正與本校醫院神經內科、胸腔科和血液腫瘤科的醫師申請第一期phase1的人體試驗(IRB)，同時趙教授也正與兩家藥廠洽談進一步合作開發這類急救性藥物之可能性。

Thank you