**以自由電子雷射同調繞射影像術解析水溶液中攜帶藥物之微脂體影像**

微脂體是由脂質所組成，接近球形的囊泡，常被用於營養物質或藥物傳送。臨床上，許多廣泛性的抗癌藥物便是利用微脂體優異的輸送性質，進行輸送。進一步改變微脂體的大小及性質，對於醫療應用及社會貢獻度將具有相當價值。然而，要改進微脂體需要了解其結構及特性，因此則必須透過一些成像方法觀察。然而目前的成像方法在天然的液體環境中要評估脂質體的形狀及尺寸具有相當的難度。X光自由電子雷射術(X-FEL)的發展，對小於500微米物體結構的觀察，產生革命性的變化。該技術對單一粒子可以解析出許多詳細的訊息。尤其對於具有較大尺寸的金屬納米粒子和病毒的影像特別有幫助。本所胡宇光研究員及其團隊與台灣東洋製藥合作，並在日本北海道大學西野教授團隊的協助下，利用位於日本同步輻射中心X光自由電子雷射術(SACLA)及其偵測器成功取得於水相環境中的100nm微脂體顆粒及其所包覆藥物的衍射影像，儘管它們僅是由微弱散射的有機分子組成，但其經由X-FEL所產生的衍射信號，結合李定國所長團隊所改良的同調繞射影像術(CDI)進行重建，就足以產生可以定量的結構信息。

該研究團隊將包覆doxorubicin之微脂體經由照射X-FEL後，取得影像以 CDI進行演算及重組，重組影像與低溫電子顯微鏡（Cyro-EM）所得到的影像或經由X光小角度散射(SAXS)分析所得的散斑圖案結果一致。然而值得注意的是，低溫電子顯微鏡並無法觀測在一般液態環境中的微脂體。而常規的SAXS無法偵測在水溶液中偵測非均質性及大小不均勻的奈米棒狀樣品。特別的是這些搭載藥物的奈米顆粒其典型結構是具特異性的並且大小不均勻。這樣的特性也會影響其他成像技術：例如，動態光散射儀，其測得平均值遠超過許多顆粒大小，同時也無法檢測奈米顆粒內的藥物。該團隊展示了X-FEL結合CDI可以克服這些限制，並得到微脂體包覆藥物在水溶液中的影像。該方法也可以做為日後，藥廠對於同類型藥物於水溶液中型態分析之方法。詳細的研究結果已發表於Nanoscale。http://pubs.rsc.org/en/content/articlelanding/2018/nr/c7nr09395k#!divAbstract



圖一、微脂體顆粒之低溫電子顯微鏡影像。(a)微脂體。小黑點為接合在微脂體上之金奈米粒子。(b)包覆Doxorubicin之微脂體。(c)做為臨床治療藥物之包覆有Doxorubicin之微脂體，圖中可見其外觀大小具有相當之均勻度。圖中比例為200奈米。



圖二、同調繞射影像術重建四個不同帶有doxorubicin之微脂體影像。(a)-(d)帶doxorubicin之微脂體影像經自由電子雷射術所產生的繞射影像。(e)-(h)其經由同調繞射影像術所重建之影像。比例尺為200奈米。

**Free-Electron-Laser Coherent Diffraction Images Individual Drug-Carrying Liposome Particles in Solution**

Using the excellent performances of the SACLA (RIKEN/HARIMA, Japan) X-ray free electron laser (X-FEL), coherent diffraction imaging (CDI) was used to detect individual liposome particles in water, with or without inserted doxorubicin nanorods. This was possible because of the electron density differences between the carrier, the liposome, and the drug. The result is important since liposome nanocarriers dominate at present the drug delivery systems. In spite of the low cross section of the original ingredients, the diffracted intensity of drug-free liposomes was sufficient for spatial reconstruction yielding quantitative structural information. For particles containing doxorubicin, the structural parameters of the nanorods could be extracted from CDI. Furthermore, the measurement of the electron density of the solution enclosed in each liposome provides direct evidence of the incorporation of ammonium sulphate into the nanorods. Overall, ours is an important test for extending the X-FEL analysis of individual nanoparticles to low cross-section-systems in solution, and also for its potential use to optimize the manufacturing of drug nanocarriers. The complete results have been published in the Nanoscale.

http://pubs.rsc.org/en/content/articlelanding/2018/nr/c7nr09395k#!divAbstract



Fig. 1 Cryo-electron microscopy images of liposome particles. (a) blank and (b) doxorubicin-containing liposomes (the black dots in (a) are Au nanoparticles decorating one of the liposomes). (c) Processed doxorubicin-containing liposome compliant with the human therapy requirements, with more uniform size and shape. Scale bars = 200 nm.



Fig. 2 CDI patterns of four individual doxorubicin-containing liposomes. (a)-(d), diffraction patterns; (e)-(h), their corresponding reconstructed images. Bars = 200 nm.