

行政院國家科學委員會專題研究計畫 期末報告

發展同調 X 光散射影像實驗在生物樣品結構之研究

計畫類別：個別型
計畫編號：NSC 100-2119-M-009-001-
執行期間：100年08月01日至101年10月31日
執行單位：國立交通大學電子物理學系(所)

計畫主持人：梁耕三
共同主持人：胡宇光
計畫參與人員：博士班研究生-兼任助理人員：黃啟峰
 博士班研究生-兼任助理人員：洪誌彰

報告附件：出席國際會議研究心得報告及發表論文

公開資訊：本計畫可公開查詢

中華民國 102 年 03 月 06 日

中文摘要： 在本期計畫中，我們以 *Magnetospirillum magnetotacticum* 作為樣品，利用第三代同步輻射的光源進行同調 X 光散射的生物樣品的成像實驗，結果已成功得到繞射圖像，透過‘混合輸入輸出演算法’ (Hybrid input output algorithm) 初步解析其影像。對於 Nitric oxide synthase (NOS) protein 透過小角度 x 光散射的實驗解析其結構，並設計紫光 (400 nm) 激發白光 (525~750 nm) 探測時間解析光譜系統，用來解析電子在 NOS 中的傳遞機制。對於 HIV 病毒的 gp140 我們以穿透式電子顯微鏡發現了其數百奈米的結晶可以作為未來自由電子雷射的奈米晶體的結構解析的材料。

中文關鍵詞： 同步輻射 X 光散射，同調 X 光散射，X 光顯微術，自由電子雷射，膜蛋白

英文摘要：

英文關鍵詞： X-ray free electron laser, x-ray coherent diffractive imaging (CDI), single molecular imaging, X-ray nano protein crystallography, nitric oxide synthase, HIV-1 envelop protein, membrane protein

計畫類別：個別型計畫 整合型計畫

計畫編號： NSC 100-2119-M-009-001

執行期間： 2011年8月1日至 2012年10月31日

執行機構及系所：

計畫主持人：梁耕三

共同主持人：胡宇光

計畫參與人員：

博士班研究生 黃啟峰

博士班研究生 洪誌彰

本計畫除繳交成果報告外，另含下列出國報告，共 2 份：

移地研究心得報告

出席國際學術會議心得報告

國際合作研究計畫國外研究報告

處理方式：除列管計畫及下列情形者外，得立即公開查詢

涉及專利或其他智慧財產權，一年二年後可公開查詢

中 華 民 國 102 年 1 月 31 日

- 發展同調 X 光散射影像實驗在生物樣品結構之研究
Proposal #100-2119-M-009-001 (100.8 – 101.7)

The major progresses carried out under the period (100.8 -101.7) are summarized below in three research areas of the proposal: (1) Coherent X-ray diffractive imaging, (2) Dynamics of Nitric Oxide Synthase in solution, and (3) New initiative in X-ray free electron laser for nano protein crystallography: study of HIV-1 envelop proteins.

(1) Coherent X-ray Diffractive Imaging (Chih-Feng Huang/PhD student, K.S. Liang, NCTU; Y. Hwu, T.K. Lee, Academia Sinica; D.Y. Noh, GIST, Korea)

Imaging of large, unstained biological specimens by long penetration depth of X-ray is recognized as a possible way to solve thickness restrictions of electron microscopy. The X-ray coherent diffractive imaging (CDI) offer a way to determine the noncrystalline biological specimen image by measuring the diffraction pattern and using the oversampling theory to reconstruct the image. The biological specimens are low Z scatters so biological specimens need to exposure long time to collect the analyzable diffraction pattern. However, with the increasing of exposure time the radiation damage problem will greatly influence the status of sample and limit us to acquire the high resolution image. The high Z scatters, such as Au or Fe, are easier to acquire clear diffraction pattern. If the high Z scatters and low Z scatters are illumined at the same time, the diffraction from low Z scatters may be interference by diffraction from high Z scatters. Then the interferenced diffraction pattern may provide the diffraction with high intensity and low exposure time.

Magnetospirillum magnetotacticum's most interesting property is that it forms single-domain crystals of Fe_3O_4 . The crystals are covered by a membrane, which is called a magnetosome. The crystals are arranged in long chains keeping the north/south orientation (Fig 1.) so that the whole structure functions as a bar magnet and causes the organism to exhibit magnetotaxis. There is considerable interest in understanding both the mechanism by which the organism synthesizes magnetite and the crystalline structure of the magnetite product. Biologically it is studied as a possible model for the process of biomineralization and for its role in the evolution of the magnetotactic response in higher organisms. It is of geological interest for its contribution to the magnetization of sediments and for its potential as a geobiological tracer, since it leaves a detectable fossil remains. For our CDI studying the high Z scatters, Fe_3O_4 , may help us to improve the weak intensity of diffraction from a biological sample. The resolution of reconstructed image could also be improved by the diffraction pattern of strong intensity with high signal and noise ratio.

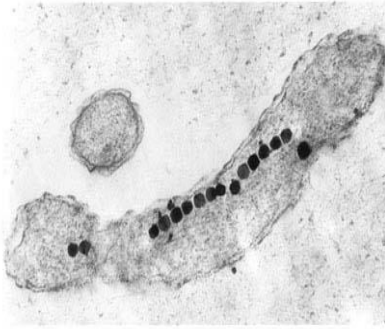


Fig 1.

We collect the diffraction pattern (Fig 2(a)) from *Magnetospirillum magnetotacticum*. The reconstruction (Fig 2(b)) of the pattern can not image *Magnetospirillum magnetotacticum* well and a little symmetry image is shown. This image may still result from the low scattering intensity of the sample. Even *Magnetospirillum magnetotacticum* has high Z scatters, Fe_3O_4 . The low intensity can not support enough information to make the reconstructed image converge. Maybe sample on a nonsymmetrical golden pattern is needed to support a asymmetry signal and help image converge. The nonsymmetrical golden pattern on sample holder can help us to increase the intensity and the signal of the biosample can be enhanced by golden pattern. The GHIO algorithm may also find the right position of the biosample with a reference of gold pattern. So, we will try to design a proper gold pattern on sample holder to improve the experiment result.

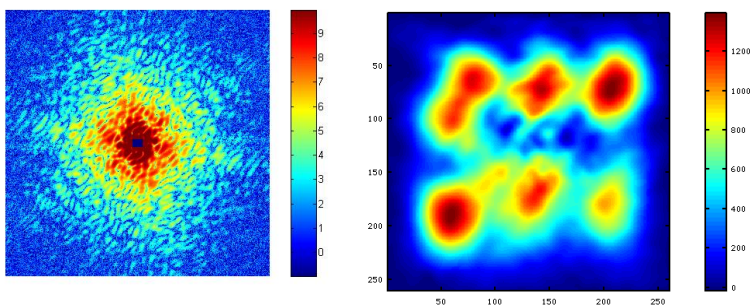


Fig 2.(a)

Fig 2.(b)

Reference

- [1] Sayre D. In *Imaging Processes and Coherence in Physics*. Springer Lecture Notes in Physics, ed Schlenker M (Springer, Berlin), 1980, 112, pp 229–235.
- [2] Miao J, Charalambous P, Kirz J, Sayre D. *Nature*, 1999, 400:342–344.
- [3] Robinson I K, Vartanyants I A, Williams G J, Piferfer M A, Pitney J A. *Phys Rev*

Lett 2001, 87:195505.

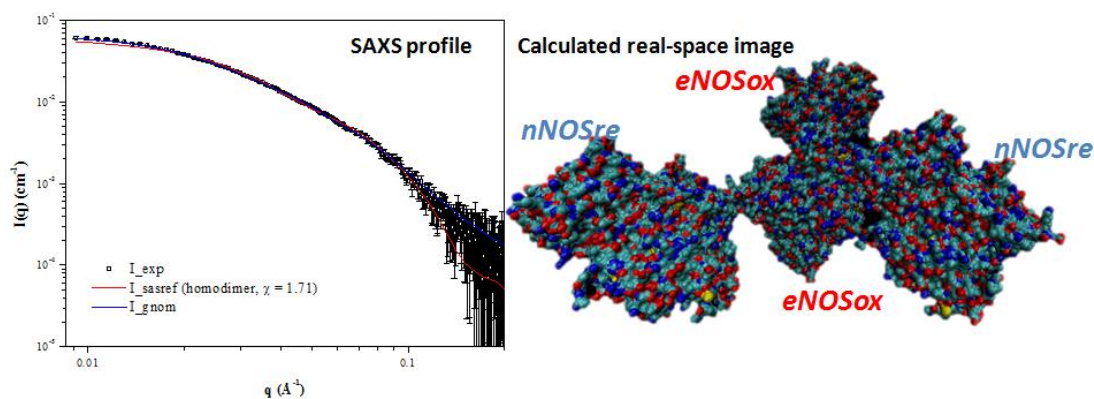
[4]Miao J, Ishikawa T, Johnson B, Anderson E H, Lai B, Hodgson K O. Phys Rev Lett 2002, 89:088303.

[5]J. C. Thrash, S. Ahmadi, T. Torok, and J. D. Coates, Applied and environmental microbiology 76, 4730 (2010).

[6] M. C. Kuo, H. F. Chen, J. J. Shyue, D. M. Bassani, and K. T. Wong, Chem Commun (Camb) 48, 8051 (2012).

(2) Dynamics of Nitric Oxide Synthase in Solution (Chih-Chang Hong/PhD student, T. Yabushita, K.S. Liang, NCTU; P.F. Chen, NHRI)

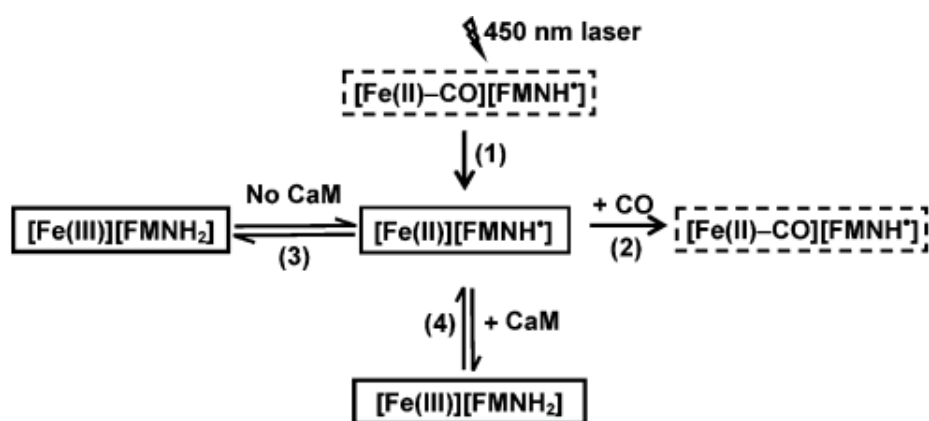
Nitric oxide synthase (NOS) produce nitric oxide, which participates in a variety of physiological processes such as neurotransmission, cardiovascular homeostasis, and immune modulation. The NOS family including the neuronal NOS (nNOS) and the inducible NOS (iNOS), the endothelial isoform (eNOS) consists of C-terminal reductase domain and N-terminal oxygenase domain. Full length NOSs are hard to form crystal structure, and only single oxygenase or reductase domains can form crystal structure with dimer form. Last year, eNOS in solution was studied by SAXS and a dimeric form of the envelope structure of this protein was concluded. (Fig. 3)



The catalytic reaction is more complicated. From biochemistry studies on eNOS, it shows that the reaction involves the adsorption of calmodulin and Ca^{+} to trigger a conformational change of e-NOS as illustrated schematically in [Fig. 4a]. The association of FMN domain is switched from FAD to heme for the electron transfer to take place. [Ref. 5] If so, such a conformational change of eNOS should be observed in the low- q region of the SAXS profile. SAXS measurements were carried out on eNOS solutions at different concentration of calmodulin and

Ca+, but failed to observe changes in the scattering profile.

Our study has turned to investigate the optical spectroscopy technique as reported in the literature. The chemical activity studies can be observed directly by the photolysis pump-probe technique which is based on the excitation of the Fe in the heme domain from Fe+2 to Fe+3 and watch for time decay. Such studies have been done only in the second scale time domain. We are setting up to do the photolysis experiments in the mini-sec to femto-sec domain to directly reveal the short time reaction kinetics. The laser pump-probe experiments will be carried at Prof. Luo's lab at the Electro-physics Department, NCTU.



^a Species in dashed boxes are CO-bound forms, whereas those in solid boxes are CO-free and participate in the FMN-heme IET.

Scheme 2. Summary of processes occurring upon CO photolysis^a

(C. Feng et al., "Direct measurement by laser flash photolysis of intraprotein electron transfer in a rat neuronal nitric oxide synthase", J. Am. Chem. Soc., 129, 5621-5629 (2007))

Ultrafast pump probe spectroscopy setup for NOS studies (Chi-Chang Hong)

實驗進度規劃如圖 a.所示，本實驗需要兩個超快時間解析光譜系統，一為紫光(400 nm) 激發白光(525~750 nm)探測時間解析光譜系統，用來解析電子在 NOS 中的傳遞機制；一為超短脈衝紫光激發探測系統(UV-10fs pump probe)，用來解析 NOS 的分子振動轉動等動態行為。目前紫光(400 nm) 激發白光(525~750 nm) 探測時間解析光譜系統已架設完成(如圖 b 所示)，並利用標準樣品(DCM)測試系統解系度為 220 fs。超短脈衝紫光激發探測系統目前正在建構當中預計明年二月前完成。圖 c 為利用超短脈衝紫光激發探測系統觀察 eNOS 的瞬態時間解析光譜，並獲得其時間常數為 247 fs。

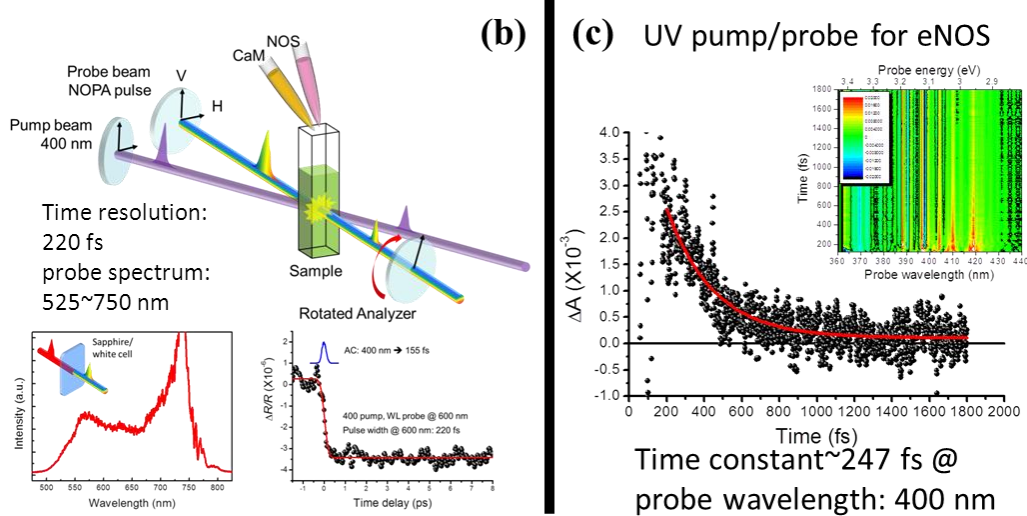
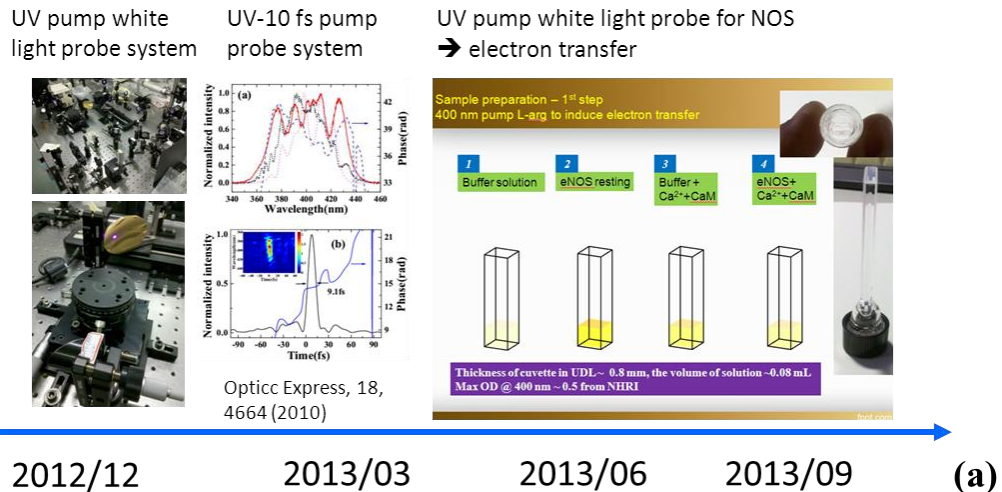


圖 4a. 近期實驗規劃。圖 b. 紫光(400 nm) 激發白光(525~750 nm)探測時間解析光譜系統設計與規格說明。圖 c. 利用超快紫光時間解析光譜量測 eNOS 樣品之實驗數據。

(3) New Initiative in X-ray Free Electron Laser for Nano Protein Crystallography: study of HIV-1 envelop proteins (Chi-Feng Huang/PhD student, K. Liang, NCTU; C. Ma, Y. Hwu, Academia Sinica; H. Naitow, N. Kunishima, RIKEN SPring-8 Center)

Characterization of membrane protein gp140

According to a statistical summary from UNAIDS, World Health Organization, as of 2010 there are 34 millions of people living with HIV, among which 2.7 millions are newly infected in 2010 and the deaths from AIDS in 2010 are 1.8 millions. The major surface envelope glycoprotein of HIV is gp160. It is not only responsible for

viral attachment to host cell surface receptor CD4 and co-receptor CCR5 and CXCR4, but also critical for membrane fusion between HIV virus and host cells. The high-resolution structure of the functional trimeric envelope glycoprotein from HIV has been viewed as one of the most difficult quest for structural biologists in the past 25 years, and traditional X-ray crystallographic approach has not been able to solve this problem. This is mainly due to the difficulty of preparing trimeric HIV envelope glycoprotein sample, one of the mostly glycosylated proteins, in a homogenous form suitable for crystallographic studies. We have recently successfully expressed and purified trimeric gp140, the soluble surface domain of gp160, using a suspension human cell culture system. Structural information of the trimeric gp140 will not only be critical for designing new therapeutics against HIV infection at the stages of viral attachment and membrane fusion, but also vital for developing a better HIV vaccine.

References:

1. Trimeric HIV-1 glycoprotein gp140 immunogens and native HIV-1 envelope glycoproteins display the same closed and open quaternary molecular architectures. *PNAS* (2011), 108, 11440-11445
2. Immunogenicity of HIV-1 envelope glycoprotein oligomers. *Curr. Opin. HIV AIDS* (2009), 4, 380-387.

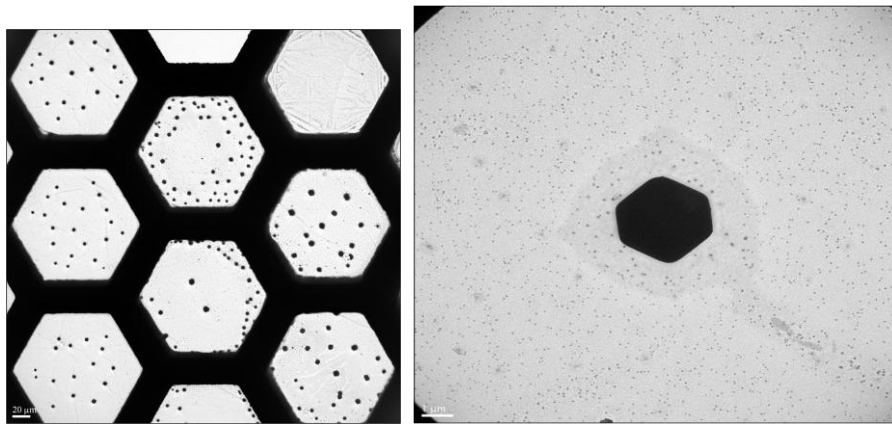


Fig. 5: (a) TEM images on dry samples reveal nano crystals. (b) A gp140 crystal of maximum size ~ 1μm

Trip Report (Proposal NSC 100-2119-M-009-001)

K. S. Liang 6/26/2012

Meeting: Coherence 2012, Fukuoka, June 18-21, 2012

The three day workshop provided the most recent progresses on phase retrieval and coherent scattering, an emerging field of research stimulated by the opening of user operation at LCLS at Stanford in October 2010 and SACLA at SPring-8 in March 2012. The workshop chair is T. Ishikawa, the Director of RIKEN SPring-8 Center. The conference is the 6th of the series with the 1st one held at Berkeley in 2000. The Fukuoka meeting had 112 attendance; from Asian region including 4 from Taiwan, 4 from Australia, and 2 from Korea. All reports were given in 20 min presentations so that all works in XFEL were “heard”. Our CDI work performed at Taiwan Beamline 12XU at SPring-8 was presented by our collaborator Prof. Do Young Noh of GIST.

Coherent Diffractive Imaging

The first x-ray CDI was demonstrated by John Miao in 1999. We heard many CDI reports in this conference in different areas. Studies on frozen hydrated cells led by Miao and collaborators reported a resolution of 5 to 10 nm range in 3D at SACLA and LCLS. For cell imaging at storage rings, the work of T. Salditt at DESY using cone beam full field imaging is worth of attention. The undulator beam is focused by elliptical mirrors to 200 nm and the beam is then coupled into a set of two orthogonally aligned x-ray waveguides. Our work used zone-plate optics to gain flux and use interferences of reference and sample to enhance the sensitivity. Imaging DNA chromatin and telomere, or polymerases is in the dream.

The Bragg CDI study of nano crystals is another area of many reports at the meeting. Most works show images of strain field in nano particles, a field lead by Ian Robinson. Studies include quantum dots, zeolite, colloidal particles, etc. But real scientific impacts of strain studies are yet to be seen.

There were 2 talks on electron CDI and I am glad to see our publication was quoted in a few talks. I feel that electron CDI has a chance for small particles and bio molecules and should be explored further. Keith Nugent's Coherence Center in Australia is doing

Ptychography

The ptychography is an EM technique developed in 1960 to increase the field of view by overlapping multiple illumination spots. Many report on x-ray ptychography at this meeting. I am mostly impressed by the work of Y. Takahashi of imaging weakly

scattering objects at the pixel resolution of better than 10 nm in a field of view larger than 5 μm x 5 μm , and observed dislocation singularities in crystals. M. Stockmar of ESRF reported an interesting approach for inline holographic phase retrieval.

Protein Nano Crystallography

A status report was given by A. Barty representing 18 institutions and more than 70 authors, reflecting the big stake of this game. “Experiments using sub-micron protein crystals have enabled structural determination to better than 2 Å” – that is the claim! We have submitted a proposal to SACLA and hope to get into this game in this fall. Angle correlation analysis of the powder patterns needs to be developed for analyzing the massive data from XFEL.

Photon Correlation Spectroscopy

A number of reports but are limited by short of coherence flux at storage rings and beam jittering at XFEL. The recent success of “seeding” by inline diamond crystals brings great hope of a stable x-ray beam.

Others

Besides Japan, the largest attendance is from German geared to the DESY’s European XFEL for 2016. Most theoretical work was done by Russian educated scientists. Ivan Vartanyants and his group members presented many talks, including Roman Dronyak, my former PhD student. Ivan and many other groups asked for postdoc or student candidates. NSRRC may take advantage of these opportunities in its SR graduate program to send students abroad.

Trip Report – NSC Proposal 100-2119-M-009-001

K. S. Liang

July 6 – 9, 2012

SSRL, SLAC, Stanford, California

In the proposal, “發展同調 X 光散射影像實驗在生物樣品結構之研究”, the time-resolved pump-probe experiment on the Fe edge of the heme center in Nitric Oxide Synthase proteins has been proposed to be carried out at SLAC in collaboration with Dr. Tsu-Chien Weng. My trip was to prepare for the experiments.

Background

At SLAC, two synchrotron facilities can run the real-time experiments at the Fe edge in the pico to femto second regime. The X-ray pulses at SSRL has pico-sec width when runs at alpha-focusing mode and at LCLS has ~10fs pulses as an X-ray laser. So the Stanford facilities are the best places of choice to carry out the proposed experiments.

Logistics

The experiments can only be executed under a User Proposal. On July 6, I had passed the Safety Training at SLAC, which include radiation safety and computer security safety. The training required Q&A and took 4 hours to complete. I have become a qualified user at SLAC.

Planning of the Experiment

I visited the beamline stations at both SSRL and LCLS. A number of technical issues for executing the experiments have been concluded with Dr. Tsu-Chien Weng, including the need of a VUV laser for pump, the design and construction of the flow-mixing sample cell, the candidates of NOS proteins for initial experiments, etc. We expect to begin the screening X-ray measurements next year.

國科會補助計畫衍生研發成果推廣資料表

日期:2013/03/06

國科會補助計畫	計畫名稱: 發展同調X光散射影像實驗在生物樣品結構之研究
	計畫主持人: 梁耕三
	計畫編號: 100-2119-M-009-001- 學門領域: 軟物質及生物物理－實驗
無研發成果推廣資料	

100 年度專題研究計畫研究成果彙整表

計畫主持人：梁耕三		計畫編號：100-2119-M-009-001-					
計畫名稱：發展同調 X 光散射影像實驗在生物樣品結構之研究							
成果項目		量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）	
		實際已達成數（被接受或已發表）	預期總達成數（含實際已達成數）	本計畫實際貢獻百分比			
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力 （本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	0	4	100%	篇	
		研究報告/技術報告	3	10	100%		
		研討會論文	1	5	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力 （外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

<p>其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)</p>	<p>註：計畫執行規畫三年完成</p>
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	成果項目	量化	名稱或內容性質簡述
科 教 處 計 畫 加 填 項 目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與(閱聽)人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

達成目標

未達成目標（請說明，以 100 字為限）

實驗失敗

因故實驗中斷

其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文： 已發表 未發表之文稿 撰寫中 無

專利： 已獲得 申請中 無

技轉： 已技轉 洽談中 無

其他：（以 100 字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以 500 字為限）

同調 X 光散射影像術具有對生物大分子的直接偵測，並達 sub-nanometer 解析度的可能性，配合 X 光自由電子雷射的出光，此領域是 X 光科學最前瞻之研究。本研究為台灣目前唯一的團隊在發展此技術。