

期中報告：

概述：

人類之cyclin I 與 securin 為無穩定構型蛋白質(Intrinsically disordered protein, IDP)其功能為蛋白質交互作用網絡之核心，並從而調控其他蛋白質之功能。然而其穩定予否則端賴其是否摺疊正確。為此intrinsically disordered protein 之摺疊反應是否會依循蛋白質摺疊似一階態轉變模型則為本計畫所擬探討之重點。在本年度內我們已成功的轉殖與表現cyclin I與 securin 蛋白質。藉由中間體摺疊結構之觀察我們發現cyclin I為具有helix二級結構之IDP。反之securin為不具二級結構之IDP。由於結構之差異其動態摺疊反應模型將是我們進一步探討之主題。其功能分析方面我們已初步建立securin交互作用網，俟後續成果完備後將撰寫為文，送國際期刊審查發表。除此之外，金屬硫蛋白(metallothionein, MT)為金屬鍵結另一種IDP之例子，我們成功的使MT摺疊形成銅鍵結MT, Cu-MT 並運用同步輻射小角度散射方式分析其結構。這一成果發表於極佳之生物物理國際期刊”Biophysical Journal” 上。蛋白質聚集(aggregation)研究方面我們探討熱休克蛋白HSP60 (heat shock protein 60)之可逆聚集反應，並研究其聚集模式這一成果發表於生物物理國際期刊”Biochem. Biophys. Res. Commun”上。此外運用生物巨分子自組織之特性我們亦成功的開發出世界上首見之室溫下具有負微分電阻特性之導電DNA此一成果發表於極佳之應用物理國際期刊”Applied Physics Letter” 上。其餘相關論文成果清單臚列於後。

2009年論文發表清單

1. Lin C.-Y., Huang Y.-S., Li C.-H., Hsieh Y.-T., He P.-J., Hsu W.-T., Yeh Y.-C., Chiang F.-H., Wu M.-S., **Chang C.-C.**, Liao K.-W.* (2009) Characterizing the polymeric status of *Helicobacter pylori* heat shock protein 60. *Biochem. Biophys. Res. Commun* **388**,283-289. (IF:2.648, 28/71)
2. Jangjian P.C., Liu T-F, Tsai C.-M., Li M.-Y., Tsai M.-S., Tseng S.-H., Cheng T.-M. and **Chang C.-C.*** (2009) DNA mismatch detection by metal ion enhanced impedance analysis. *Chinese J. Physics* **47**, 755-761. (IF:0.270, 63/68)
3. Li M, Huang Y-S, Jeng U-S*, Hsu I-J*, Wu Y-C-S, Lai Y-H, Su C-H, Lee J-F, Wang Y, and **Chang C-C** (2009) Resonant X-ray Scattering and Absorption for the Global and Local Structures of Cu-modified Metallothioneins in Solution. *Biophysical J.* **97**, 609-617. (IF:4.683, 9/71)
4. Jangjian P.-C., Liu T.-F., Li M.-Y., Tsai M.-S., **Chang C.-C.*** (2009) Room temperature negative differential resistance in DNA-based molecular devices. *Applied Physics Letters*. **94**, 043105. This paper has been selected for publication in **Virtual Journal of Nanoscale Science & Technology (2009) and** This paper has been selected in **Virtual Journal of Biological Physics Research (2009), too.** (IF:3.726, 10/95)

5. Liu C.-P., Wey M.-T., **Chang C.-C.***, Kan L.-S. (2009) Direct observation of single molecule conformational change of tight-turn paperclip DNA triplex in solution. *Applied Biochemistry and Biotechnology*. **159**, 261-269. (IF:1.040, 108/144)

Summary of research achievements

Folding of Human Intrinsic Disorder Proteins: Cyclin I and Securin

Unlike regular protein, an intrinsic disordered protein possesses its function without stable tertiary structure; human cyclin I (Ccn1) and securin are two cases of intrinsic disordered proteins. Both proteins were found in inclusion body of *E. coli*, a recombinant expression system. It is intriguing to know the common folding motif of these proteins. In this study, we found that the recombinant proteins can be folded continuously by an over critical folding process (on-path folding process). These proteins aggregated under off-path folding process. Differential scanning micro-calorimeter analysis indicated that both recombinant cyclin I and securin are intrinsic disordered proteins. However, the circular dichroism spectra denoted that cyclin I is a helical major protein; the securing contains no secondary structure at all. Functional analysis indicated that the folded cyclin I can directly bind with p21^{cip1/waf1} under the condition with calcium ion and securing can bind with p53. Fluorescence spectra of both proteins indicate that both proteins possess tight hydrophobic core which can not be completely unwound in denaturing environment. Meanwhile, fluorescence spectra of folding intermediates of both proteins indicate that these hydrophobic motifs can be restored by an over critical folding process. These results prove that we have folded these intrinsic disordered proteins into their active form. Meanwhile, the hydrophobic motif may be the only or most important motif of intrinsic disordered protein. (Will be submitted)

Resonant X-ray Scattering and Absorption for the Global and Local Structures of Cu-modified Metallothioneins in Solution

With Cd and Zn metal ions removed from the native rabbit-liver metallothionein upon unfolding, Cu-modified metallothioneins (Cu-MTs) were obtained during refolding in solutions containing Cu^I or Cu^{II} ions. X-ray absorption near-edge spectroscopic (XANES) results confirm the respectively assigned oxidation states of the copper ions in Cu^I-MT and Cu^{II}-MT. Global and local structures of the Cu-MTs were subsequently characterized by anomalous small angle X-ray scattering (ASAXS) and extended X-ray absorption fine structure (EXAFS). Energy-dependent ASAXS results indicate that the morphology of Cu^{II}-MT resembles that of the native MT whereas Cu^I-MT

forms oligomers with a higher copper content. Both dummy residue simulation and model-shape fitting of the ASAXS data reveal consistently rod-like morphology for Cu^{II}-MT. Clearly identified Cu-S, Cu-O, and Cu-Cu contributions in the EXAFS analysis indicate that both Cu^I and Cu^{II} ions are bonded with O and S atoms of nearby amino acids in a four-coordination environment, forming metal clusters smaller than metal thiolate clusters in the native MT. It is demonstrated that a combination of resonant X-ray scattering and X-ray absorption can be particularly useful in revealing complementary global and local structures of metalloproteins due to the atom specific characteristics of the two techniques. (Published in *Biophysical J* 2009)

Characterizing the polymeric status of *Helicobacter pylori* heat shock protein 60

Helicobacter pylori heat shock protein 60 (HpHsp60) was first identified as an adhesion molecule associating with *H. pylori* infection and has also been investigated as a potent immunogen to elicit host proinflammatory responses. Here we have analyzed the structure of HpHsp60 via amino acid blast, circular dichroism and electrophoresis and the results indicated most recombinant HpHsp60s form dimers or tetramers that are quite different than *E. coli* Hsp60. Furthermore, the activity of recombinant HpHsp60 for immunogen was also determined and the results showed that the HpHsp60 could up-regulate a panel of cytokine expressions including IL-1 α , IL-8, IL-10, IFN- γ , TNF- α , TGF- β , GRO and RANTES. Interestingly, an only cystein residue in the active recombinant HpHsp60 was carboxymethylated that switched the oligomeric status. In addition, the carboxymethylated HpHsp60 could further enhance NF- κ B-mediated IL-8 and TNF- α secretion in THP-1 cells comparing to intact HpHsp60s. This study suggested that HpHsp60s formed dimers or tetramers and their cystein residues involved in oligomeric status that could affect the activity to stimulating the proinflammatory cytokine release. (Published in *Biochem. Biophys. Res. Commun* 2009)

Room temperature negative differential resistance in DNA-based molecular devices

A molecular device fabricated from metallic deoxyribonucleic acid (M-DNA) exhibits a negative differential resistance (NDR) behavior. When two gold electrodes were connected by Ni²⁺-chelated DNA, which was converted from λ -DNA, not only was the conductivity of DNA improved, but a NDR device was formed as a full cyclic voltage sweep was applied to measure its current versus voltage characteristics at room temperature and in an ambient environment. Such electronic characteristics of an M-DNA device may have been caused by the redox reactions of Ni ions. This finding provides a simple way to construct electrical nanodevices from biological

molecules. (Published in *Applied Physics Letters* 2009)

計畫成果自評

本計畫執行進度與成效極佳，在無穩定構型蛋白質摺疊已有重要之突破，我們將會是世界上第一個成功研究出無穩定構型蛋白質摺疊反應機制之研究團隊。此外在其他無穩定構型蛋白質摺疊與聚集及相關研究方面已有多項成果發表於頂尖國際期刊上，這些成果除了學術價值外並將可供業界採用之處。