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無穩定構型蛋白質之摺疊、聚集與分子間交互作用

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中文摘要

人類之 cyclin I 與 securing 為無穩定構型蛋白質(Intrinsically disordered protein)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 securing 蛋白質。藉由不同摺疊路徑之熱反應與光譜特性分析發現，不同之反應路徑之蛋白質其結構與功能有異。此成果將撰寫為文送國際期刊審查發表。除了蛋白質摺疊外，我們運用生物巨分子自組織與自組裝之特性我們成功的將 DNA 轉變為導電分子。再結合單分子分析技術，我們成功的直接觀察與解析三螺旋 DNA 之結合與解離之變化。將我們摺疊完成之蛋白質與奈米粒子結合後可發展成受奈秒雷射誘導之奈米刀。這些成果均已發表於極佳之國際期刊如"Applied Physics Letters" 與 Nanotechnology 上。詳細論文清單臚列於後。

Abstract:

Human cyclin I and securing are intrinsically disordered proteins which do not possess stable tertiary structures. These unstable tertiary structures play the roles of adapters to bind with several different proteins and then to regulate their functions. It is intriguing to reveal the folding paths effect of these unique proteins both in structural and functional features. In this granted year, we have cloned and expression the proteins of cyclin I and securing from human cDNA. By analyzing their structural and functional properties, we find that the folding paths may affect the stability of their hydrophobic cores. Meanwhile, the functional analysis also indicates that different folding pathways may affect their functions vitally. In spite of protein folding, we utilized the intrinsic properties such as self-organized and self-assembled of bio-macro-molecule, we convert DNA into conducting polymer successfully. Moreover, combining the single molecular detecting approaches, we can observe triplex DNA formation and deformation in variant solvent environments directly. By linkage the refolded protein and nano-particles we can synthesis novel functional nanometrials which can be used as nanoknife by inducing with nano-second pulse Laser. These achievements have been submitted and published in some prestige international journals, such as "Applied Physics Letters" and Nanotechnology. The publication details are in the following.

Publications

1. Liu C.-P., Wey M.-T., **Chang C.-C.***,Kan L.-S*. (2008) Direct observation of single molecule conformational change of tight-turn paperclip DNA triplex in solution. Applied Biochemistry and Biotechnology. (Accepted) (IF: 1.643; Ranking 86/138)
2. **Chang C.-C.***,Chen P.-H.,Chu H.-L.,Lee T.-C., Chou C.-C., Chao J.-I., Su C.-Y., Chen J. S.,Tsai J.-S.,Tsai C.-M., Ho Y.-P., Sun K.-W., Cheng C.-L., Chen F.-R. (2008) Laser induced popcorn-like conformational transition of nano-diamond as a nano-knife. Applied Physics Letters. 93, 033905. This paper has been selected for publication in **Virtual Journal of Biological Physics Research (2008)** (IF: 3.596; Ranking 8/94)

3. JangJian P.-C., Liu T.-F., Tsai C.-M., Tsai M.-S., **Chang C.-C.*** (2008) Ni^{2+} doping DNA: a semiconducting biopolymer. Nanotechnology 19, 355703. (IF: 3.310; Ranking 2/67)
4. Liu K.-K., Chen M.-F., Chen P.-Y., Lee T. J.F., Cheng C.-L., **Chang C.-C.**, Ho Y.-P. ,Chao J.-I. (2008) Alpha-bungarotoxin binding to target cell in a developing visual system by carboxylated nanodiamond. Nanotechnology, **19** 205102. (IF: 3.310; Ranking 2/67)

The following are the digest of the manuscript of folding of intrinsically disordered proteins.

The Structures and Functions Analysis of Human Intrinsically Disordered Proteins, Cyclin I and Securin.

Intrinsic disordered proteins are characterized by its flexible and dynamic tertiary structure even secondary structure [1].In the recently years, the intrinsic disordered protein was found in cell signaling proteins and transcription factors [2], suggest that they plays an important role in their regulation capacity. Since these proteins are known to possess specific regions/domains that interact with specific co-regulatory proteins for their efficient functioning [3], the large flexible regions in this class of proteins may have an advantage over fully folded proteins that can allow them to make more efficient physical functional interactions with their target partners, which may represent a mechanism for regulation of cellular processes [3,4].

The biological functions of ID under physiological condition are interesting. Include the well-ordered conformation in a protein may no longer be required for functioning, and conformational flexibility in ID proteins may serve specific functions [5,6]. In some studies have suggested that the conformational flexibility in ID proteins under physiological conditions might allow them to interact with several different targets for specific functions [5,7]. The phenomenon indicates that the simple mechanism can regulate several cellular processes such as transcription and cell cycle control by the ID binding with other specific targets [8-10]. The intrinsic disordered proteins have an advantage in interacting with different target in response to inter and intra cellular environment to own different functions [11]. The structure characters are suitable for the environment in the different part of cell. Human securin [12] and cyclin I are intrinsic disordered protein and has been demonstrated the securin is a multifunction protein [13-17]. The functions of cyclin I protein are not clear expect of it can stabilize p21 protein to avoid cell apoptosis[18]. In this paper, we will discuss the function and structure relationship of securin and cyclin I protein, and demonstrate that the cyclin I stabilize p21 by directly binding.

Human securin protein (Pituitary tumor-transforming gene, PTTG1), PTTG1 overexpression has been reported in a variety of endocrine-related and nonendoncrine-related tumors, including pituitary, thyroid, breast, ovarian, and uterine tumors, central nervous system, pulmonary system, and gastrointestinal system[19-25]. The expression level of securin protein corresponded with tumor invasion[26]. In the cell cycle progress, securin can bind to separase and inhibit the separation of sister chromatin. Securin is the target of the anaphase promoting complex (APC) and would be degradation before entering anaphase by polyubiquitinated which depended on the destruction sequences[27]. Expect the cell cycle regulation, securin involved in DNA damage

repair[17], metabolism[28].

In the cell cycle, cyclins, are famous cell cycle regulation family. Cyclins are regulatory subunits of cyclin dependent kinases (CDKs)[29]. The Cyclin–CDK complexes regulate cell mitosis[30]. The cyclins would be degraded at the end of each cell cycle stage via the ubiquitin system[31]. Cyclin I (Ccni) contains a typical cyclin box at its N-terminal and it is similar to the ones of cyclin G and E. Meanwhile, it contains PEST sequences at its C-terminal. Unlike other cyclins, cyclin I's expression level is high in post-mitotic tissues, including heart, brain, and skeletal muscle, and it is expressed constantly during cell cycle progression. Therefore, the functions of cyclin I may be independent on the cell cycle[32]. However, cyclin I is expressed in human breast cancer and closely associated with VEGF and KDR expression[33] and protects podocytes to apoptosis by stabilization of p21[18]. It indicated that the function of cyclin I may be activated by interacting with other regulatory proteins

To understand the structure prosperity of intrinsic disordered protein, we use thermal equipment dialysis method [34-37] to refold recombinant securin and cyclin I protein. By thermal equipment dialysis, we can analysis the structure change during folding process by analysis the folding intermediate. The challenges of intrinsic disordered protein folding are low solubility and hard to define the protein in the native state.

In this study, we have demonstrated that the securin and cyclin I proteins are native intrinsic disordered proteins by DSC measurement. Meanwhile, the there is no secondary structure of securin protein but in the cyclin I is helix major, it indicates the disordered tertiary structure may compose of regular or non-regular secondary structure. Moreover, the binding of cyclin I and p21 need calcium ion also showed that the functions of cyclin I protein is environment depend in the cell, especially during early apoptosis signal present.

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quasi-static-like process: A first-order state transition. Physical Review E 66, 021903.

Attached is one of the reprint of Laser induced nanoknife which published in Applied Physics Letter. Meanwhile, this paper has been selected for publication in **Virtual Journal of Biological Physics Research (2008)**

計畫成果自評

本計畫執行進度佳且有多項相關成果發表，這些成果除了學術價值外並將可供業界採用之處。

Laser induced popcornlike conformational transition of nanodiamond as a nanoknife

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Nanodiamond (ND) is surrounded by layers of graphite on its surface. This unique structure feature creates unusual fluorescence spectra, which can be used as an indicator to monitor its surface modification. Meanwhile, the impurity, nitroso (C—N=O) inside the ND can be photolyzed by two-photon absorption, releasing NO to facilitate the formation of a sp^3 diamond structure in the core of ND and transforming it into a sp^2 graphite structure. Such a conformational transition enlarges the size of ND from 8 to 90 nm, resulting in a popcornlike structure. This transition reaction may be useful as nanoknives in biomedical application. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2955840]

Nanodiamond (ND) has been shown to be nontoxic and is considered one of the most important biocompatible nanomaterials.[1] Its unique fluorescence properties allow observation of its presence in variant wavelengths.[2,3] The surface of ND can be carboxylated; the nanoparticles so modified exhibit high affinity to proteins,[2,4] rendering conjugation possible with DNA,[3,5] lysozyme,[2] and cytochrome c.[6]

ND (average diameter 4–6 nm, Nanodiamond, TI, Switzerland) has been synthesized from the detonation of the mixture of trinitrotoluene and hexogen,[7] Its conformation and composition remain unclear. In this study, we studied the conformation using ultra high resolution field emission transmission electron microscopy (FE-TEM) (JEM-2010F, JEOL, Ltd. Tokyo, Japan), electron energy loss spectroscopy (EELS), field emission scanning electron microscopy (SEM) (Hitachi S-4300, Hitachi High-Technologies Corporation, Tokyo, Japan) and atomic force microscopy (AFM) (D3100, Veeco Instruments Inc, NY, USA) and Raman spectroscopy.

As shown in Fig. 1, both AFM and TEM images indicate that the size of the ND particles are uniform [Fig. 1(a)]. A magnified view inside red circle is shown as an inset in upper right corner, which indicates that the ND contains a 6 nm diamond core and around 1 nm thick graphite shell. The lattice spacing is about 2 Å which may correspond to {111} plane of diamond structure. The inset in the low right corner is a diffraction pattern from the NDs in the view. It shows a typical electron diffraction ring for diamond. The inset in the low left corner is an AFM image that shows the surface morphology of the ND. Figure 1(b) is a SEM image of ND after laser radiated. The average size of laser radiated ND is about 90 nm. Inset is a TEM image shows the magnified view of ND after laser radiated.

These observations are consistent with the Raman spectral analysis [Figs. 2(a) and 2(b)] and nanobeam EELS spectra analysis [Figs. 2(c)]. As indicated in Fig. 2(a) the ND contains both broaden diamond Raman absorption at 1324 cm^{-1} and a planar graphite (*G* band) spectrum at 1575 cm^{-1}. The broaden 1324 cm^{-1} peak may contain part of the absorption of distortion graphite (*D* band). This core-shell interface structure may create unique surface plasmonic mode and emit the unusual fluorescence spectra in variant wavelengths, as mentioned in previous studies.[2,3]

Elemental data provided by manufacturer showed that there is 9.3% of trace nitrogen inside the structure of the ND. However, previous surface modification study indicated that no nitrogen containing functional group could be observed on the ND surface.[7] We examined the ND with Raman spectroscopy in the range of 500–1100 cm^{-1}, and detected a very weak signal near 604 cm^{-1}. This signal was seen only after long (10 min) accumulation [Fig. 2(b)], and is attributable to nitrogen containing functional group nitroso (—C—N=O).[8] By systematic simulation of the nitroso containing molecules from one carbon, H_3C—N=O, to 30 carbons, Tri-adamantane-NO (R—C—N=O), with Gaussian 03 (B3LYP/6–31G (d) Opt(Raman), with scaling factor 0.89), we noted the Raman shift of the bending mode of

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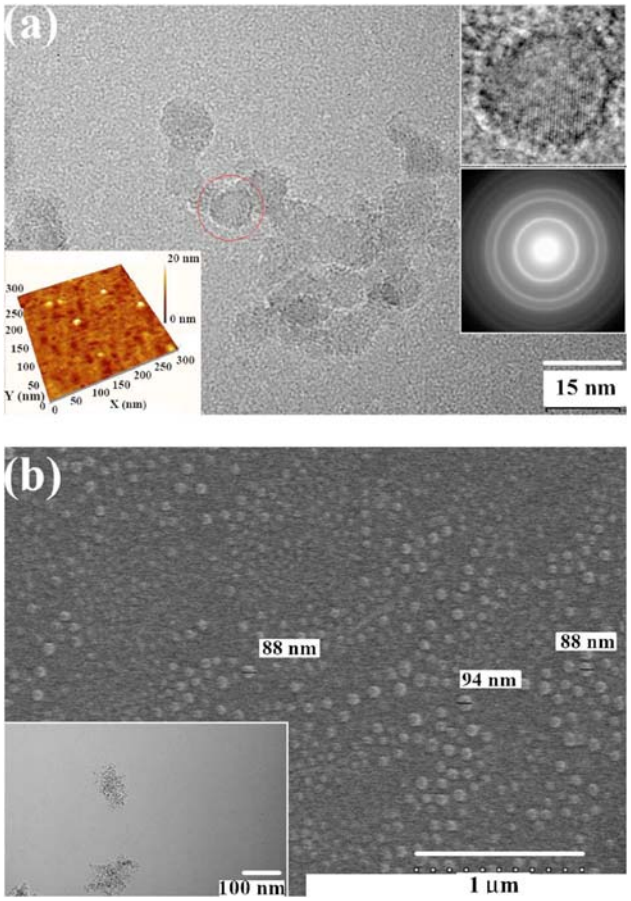

FIG. 1. (Color online) (a) TEM image of ND. A magnified view inside red circle is shown as an inset in upper right corner. The lattice spacing is about 2 Å which may correspond to {111} plane of diamond structure. The inset in the low right corner is a diffraction pattern from the NDs in the view. It shows a typical electron diffraction ring for diamond. The inset in the low left corner is an AFM image that shows the surface morphology of the ND. (b) SEM image of ND after laser radiated. The average size of laser radiated ND is about 90 nm. Inset is a TEM image shows the magnified view of ND after laser radiated.

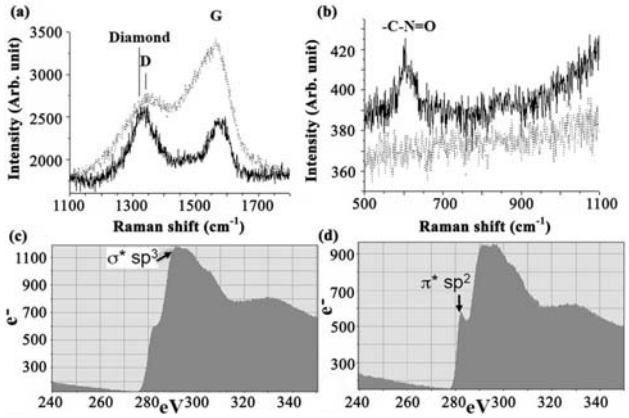

FIG. 2. (a) Raman spectra of the ND (solid line) and laser irradiated ND (dash line) at the range from 1100 to 1800 cm^{-1}. (b) The Raman range of 500–1100 cm^{-1}. (c) and (d) are EELS spectra of ND before and after laser radiation, respectively. (c) shows the very weak signal associated with the π^* bond, while (d) shows enhancement of the of π^* signal.

—C—N of nitroso increased from 504 cm^{-1} and plateau near 606 cm^{-1} when the carbon number was larger than ten. On this basis, we surmise that the Raman peak at 604 cm^{-1} is caused by the bending mode of —C—N=O of nitroso of ND.

Nitroso is an active functional group; dissociation of the C—N bond of nitroso (—C—N=O) is in the range of 225–270 nm of UV light,[8] where nitroso undergoes photolysis and releases nitro monoxide. However, we found that there was no photolysis taking place of the NDs, when irradiated with regular UV light. It is possible that the electronic band gap of ND is around 5.47 eV (or approximately 227.8 nm UV wavelength)[9] and the UV light was absorbed by the diamond structure.

In further test, the possible involvement of photolysis within the ND, we irradiated the ND solution for 30 s with 532 nm; 20 ns, full width at half maximum, Nd:YAG (yttrium aluminum garnet), pulse laser (LS2137U/2, Lotis TII Ltd., Minsk, Belarus); with 140 mW average power; 10 Hz repetition rate; 2 mm beam size. Interestingly, a popcornlike conformational change of ND was observed after the laser irradiation. The size of ND changed from 8 nm [Fig. 1(a)] to approximate 90 nm [Fig. 1(b)]. This observation is consistent with our previous observation.[10] These laser irradiated particles were fragile and no clear granule image could be observed by FE-TEM [Fig. 1(b)] when they were removed from glass substrate. Raman spectra [Figs. 2(a) and 2(b)] indicated that both peaks of nitroso group and diamond structure disappeared after the laser irradiation; instead, there were *D* (distortion) and *G* bands of graphite.[11] Similar observation was obtained by surface analysis of the FE-TEM. The transformation from diamond to graphite after laser irradiation is also confirmed from the EELS spectra. Fig. 2(c) and 2(d) are EELS spectra taken from ND before and after laser radiation, respectively. Figure 2(c) shows the very weak signal associated with the π^* bond (at 283 eV), while Fig. 2(d) shows enhancement of the π^* signal. That suggests that laser radiation may promote the transformation of sp^3 to sp^2 bonds for NDs

These results suggested that photolysis of nitroso and conformational change of this ND occurred concomitantly. As shown in previous studies,[7] nitroso groups are buried within the structure of ND which is consistent with the assumption of our previous study.[10] Their photolysis into nitro monoxide (NO) molecules may generate large internal pressure triggering conformational changes (explosion) in ND. This can be envisioned as a transformation of sp^3 tetrahedral diamond core [Fig. 1(a)] into a sp^2 planar graphite conformation, expanding its size, approximately 12 fold [Fig. 1(b)]. This is similar to our previous observation.[10]

Although the 225–270 nm UV light may induce photolysis of CNO group, the ND structure may absorb UV light and protect it. We have observed that ND is stable under both bright light and regular UV nm light irradiation; it does not absorb long wavelength 532 nm light. However, under ultrahigh intensity condition (around 2.2×10^7 W/cm^2), there is a nonlinear two-photon absorption effect which allows the CNO group of ND to absorb two incident photons of 532 nm. As a result, multiple ND photolyses occur simultaneously. We propose the molecular mechanism of diamond graphite transition of ND involves a popcornlike conformation transition which is a physical explosion reaction, with affecting distance is in submicron range (around 90 nm). Such is the case, ND may be used as a nanoknife in biosystems, in addition to other applications.

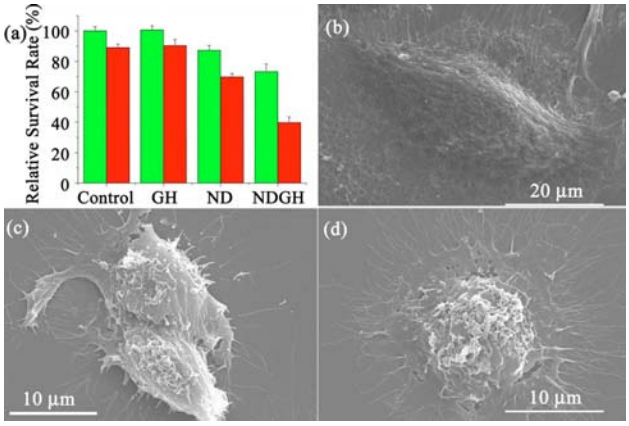

FIG. 3. (Color online) (a) Cell viability assay, (b) SEM images of the A549 cell lines, which were not treated with ND, (c) treated with ND and (d) irradiated with laser following ND treatment. The green bar denotes the cells without laser irradiation and the red bar denotes the cell after laser irradiation and incubation for 24 h.

The feasibility of ND as a nanoknife was indeed tested. We coupled ND to growth hormone (GH), one of the typical growth factors for certain normal tissues and carcinoma. It exerts regulatory functions in controlling metabolism, balanced growth, and differentiated cell expression by acting on specific receptors, GH receptor (GHR), in liver or on cartilage cell surface, triggering a phosphorylation cascade. Thus numerous signaling pathways are modulated and specific gene expression dictated.[12] GH has also been reported to stimulate melanoma cell growth.[12,13] The level of GH receptors is relatively higher in melanocytic tumorous than normal cutaneous cells.[12,13] Similar studies have reported for both mammary epithelial and colorectal cancer cells.[13] GHR is one of the general targets of cancer drug development, as blocking or inhibiting the function of GHR may be a basis for cancer therapy.

The lung cancer cell A549 was used as a model system to be treated with ND linked GH. The graphite surface of ND was first carboxylated by mixing ND with nitrate/sulfate (9:1) at 70 °C and stirred for 24 h. The excess acid was neutralized with 0.1*N* NaOH and washed with ddH_2O. The GH was prepared in our laboratory using recombinant techniques as described previously.[14,15] The carboxylated ND molecules were linked by peptide bonding with GH via the zero length cross linkers 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride and *N*-hydroxysuccinimide (Sulfo-NHS) (Pierce Chemical Comp., USA). The reaction was monitored by changes in the fluorescence spectra of the unique fluorescence of ND and autofluorescence of GH. This was followed by examination of MALDI-TOF (matrix-assisted laser desorption ionization-time of flight) mass spectra. Each ND particle was bound with two molecules of GH, forming a NDGH complex (data not shown). The NDGH complex (19 μM) was incubated with the A549 lung cancer cells in culture. After 8 h of incubation, the cells were washed with phosphate buffered saline to remove the nonspecific binding complex. The NDGH complex bound to A549 cell membrane avidly. This was consistent with our previous observation.[16] The cells were then irradiated with the same laser power mentioned above. The results show that approximately 60% of the cells died within 24 h after the irradiation [Fig. 3(a)]. In contrast, less than 10% of the controls cells were found dead under comparable treatment. As shown in Figs. 3(b)–3(d), the cell death can be attributed to the explosion of ND on the cell surface.

In summary, photolysis of nitroso plays a vital role of ND conformational transition. We have developed a NDGH complex, which preferentially kills tumor cells upon high energy pulse laser irradiation. The mechanism may involve the high affinity of NDGH to tumor cell membrane and ND explosion resulted in exposing the high energy locally within hundred nanometers, damaging the membrane leading to cell death. This popcornlike transition reaction of ND is potentially useful as a nanoknife in biomedical application.

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