Electronic Supplementary Material (ESI) for Analyst.
This journal is © The Royal Society of Chemistry 2015

Molecular "Light switch" $[Ru(phen)_2dppzidzo]^{2+}$ Monitoring the Aggregation of Tau

Xing Gao, Li Wang*, Hai-Liang Huang, Lin-Lin Wang, Jun-Liang Yao, Shuo Shi and Tian-Ming Yao*

Shanghai Key Lab of Chemical Assessment and Sustainability, Department of Chemistry, Tongji University, 1239 Siping Road, Shanghai, 200092, China.[1]

Table of contents

* Corresponding author.

E-mail: tmyao@tongji.edu.cn (Tian Ming Yao), wangli@tongji.edu.cn (Li Wang)

Experimental Details

Materials

The R3 peptide (R3= ^{306}VQIVY KPVDL SKVTS KCGSL GNIHH KPGGG Q^{336}) was synthesized using a solid-phase peptide synthesizer and purified by reverse-phase high performance liquid chromatography (HPLC) to 95.0% level. R2 peptide (^{275}VQIIN KKLDL SNVQS KCGSK DNIKH VPGGG S^{305}) and R2-C291A (^{275}VQIIN KKLDL SNVQS KAGSK DNIKH VPGGG S^{305}) peptide were also obtained using the same synthetic method. The purified peptides were lyophilized and stored at -20 °C before used. Fresh working solutions of peptides were prepared in Tris-HCl buffer (50 mM, pH 7.5) with a concentration of 1 mg/mL (300 μM). ThS and heparin were purchased from *Sigma-Aldrich*. $[Ru(phen)_2dppzidzo]^{2+}$ was synthesized and purified as described by Yao.[1] $[Ru(phen)_2dppz]^{2+}$ was synthesized and purified as described by Hartshorn et al.[2] The other chemicals were obtained from commercial sources and used without further purification unless otherwise noted.

Excitation and emission spectra of ThS in the presence and absence of R3 filaments

Excitation and emission spectra of ThS were carried out using a 2 mm quartz cell at 37 °C on Hitachi F-7000 fluorescence spectrophotometer. R3 filaments (the concentration of R3 peptide was adjusted to 15 μM) were obtained by two hour incubation with heparin (3.8 μM) at 37 °C. Then, ThS (10 μM) was added to the mixture, and vibrated for about ten to twenty seconds before excitation and emission spectra were measured. Similar experiments of ThS (10 μM) in Tris-HCl (50 mM, pH=7.5) buffer were also performed. Here, the excitation of emission spectra was set at 400 nm, to illustrate "red shift" of ThS fluorescence when interacted with tau filaments.

Excitation spectra of $[Ru(phen)_2dppzidzo]^{2+}$ in the presence R3 filaments

Excitation spectra of $[Ru(phen)_2dppzidzo]^{2+}$ (10 μM) was carried out only in the presence of R3 filaments. R3 filaments (the concentration of R3 peptide was adjusted to 15 μM) were obtained by two hour incubation with heparin (3.8 μM) at 37 °C. $[Ru(phen)_2dppzidzo]^{2+}$ (10

μM) was added to the mixture, and vibrated for about ten to twenty seconds before excitation and emission spectra were measured. The emission wavelength of $[Ru(phen)_2dppzidzo]^{2+}$ was set at 610 nm.

Kinetics of R3 aggregation monitored by time-continuous luminescence

The time-continuous luminescence experiments were performed on a Hitachi F-7000 fluorescence spectrophotometer with a 2 mm quartz cell at 37 °C (a circulating water bath was used). The R3 peptide was adjusted to a concentration of 15 μM in Tris-HCl buffer (50 mM, pH= 7.5), and 10 μM ThS dye or 10 μM $[Ru(phen)_2dppzidzo]^{2+}$ was added to the R3 solution prior to determination. The aggregation was induced by the addition of heparin (final concentration was 3.8 μM). The time scan experiment was immediately monitored by fluorescence spectrophotometer, with excitation at 440 nm and emission at 500 nm for ThS. For $[Ru(phen)_2dppzidzo]^{2+}$, excitation was set 404 nm and emission was set 610 nm. The data points were recorded every 5 seconds. The excitation and emission slit widths were both set at 10 nm. Similar experiment was carried out with 10 μM of $[Ru(phen)_2dppz]^{2+}$ with excitation at 440 nm and emission at 610 nm.

Dye-free R3 aggregation monitored by time-dependent luminescence

R3 peptide (15 μM) solution was firstly prepared in Tris-HCl buffer (50 mM, pH= 7.5) at 37 °C. Heparin (3.8 μM) was added to the solution to induce the aggregation of R3 peptide. The solution was then evenly divided into 30 tubes. At different time points, ThS (10 μM) or $[Ru(phen)_2dppzidzo]^{2+}$ (10 μM) were added into different R3 tubes. After vibrating for ten to twenty seconds, luminescence spectra of ThS or $[Ru(phen)_2dppzidzo]^{2+}$ was measured on a Hitachi F-7000 fluorescence spectrophotometer with a 2 mm quartz cell.

Impacts of $[Ru(phen)_2dppzidzo]^{2+}$ on R3 aggregation monitored by luminescence

Firstly, R3 peptide (15 μM) was prepared in the presence and absence of $[Ru(phen)_2dppzidzo]^{2+}$ (10 μM). Then, heparin (3.8 μM) was added to R3 solutions and triggered the aggregation. After incubating at 37 °C for two hours, the R3 solution with $[Ru(phen)_2dppzidzo]^{2+}$ was measured immediately with excitation at 404 nm. $[Ru(phen)_2dppzidzo]^{2+}$ (10 μM) was added to the dye-free R3 solution, and vibrated for about

ten to twenty seconds before the luminescence spectrum was measured.

Impacts of ThS on R3 aggregation monitored by luminescence

Firstly, R3 peptide (15 μM) was prepared in the presence and absence of ThS (10 μM). Then, heparin (3.8 μM) was added to R3 solutions and triggered the aggregation. After incubating at 37 °C for two hours, the R3 solution with ThS was measured immediately with excitation at 440 nm. Same amount of ThS was added to the ThS-free R3 solution, and vibrated for about ten to twenty seconds before the luminescence spectrum was measured.

Luminescence spectra of $[Ru(phen)_2dppzidzo]^{2+}$ corresponding to different amount of tau R3 filaments

R3 peptide concentration dependent luminescence spectra experiments were carried out as described below. R3 was adjusted gradient increased concentrations from 0 to 160 μM in the presence of fixed amount of heparin (3.8 μM). After incubating for two hours at 37 °C, $[Ru(phen)_2dppzidzo]^{2+}$ (10 μM) was added to the R3 filaments right before the luminescence measurement. The luminescence spectra of Δ and Λ-$[Ru(bpy)_2dppzidzo]^{2+}$ (10 μM) in the presence of R3 filaments (30 μM) and heparin (3.8 μM) were detected similarly.

Luminescence spectra of $[Ru(phen)_2dppzidzo]^{2+}$ corresponding to different types of Tau peptide

R2, R3 and R2-C291A peptides were adjusted to 15 μM in 50 μM Tris-HCl buffer. Heparin (3.8 μM) was added to all three samples to trigger the aggregation. After incubating at 37 °C for one hour, 10 μM of $[Ru(phen)_2dppzidzo]^{2+}$ were added to each sample and vibrated for ten to twenty seconds. Then luminescence measurement was carried out.

Luminescence life time measurement

The luminescence life time data of the dyes were collected on a PTI QM/TM/IM Time-resolved Fluorescence Spectro-fluorometer (USA/CAN photon technology international Int.) at room temperature. Utilizing the picosecond pulses the time delay spectra of dye-acetonitrile solutions were detected for lifetime measurements with an emission polarizer and depolarizer. The excitation of ThS was set at 400 nm and emission was set at 450 nm. The excitation of $[Ru(phen)_2dppzidzo]^{2+}$ was set at 404 nm and emission was set at 610 nm.

Transmission electron microscopy (TEM) experiments

Three R3 peptide (15 μM) samples were prepared in 50 μM Tris-HCl buffer in advance. 10 μM of ThS and 10 μM of $[Ru(phen)_2dppzidzo]^{2+}$ were each injected into one of the R3 solutions with heparin added to achieve a final concentration of 3.8 μM. After incubating at 37 °C for two hours, the solutions were painted on 600 mesh copper grids, and inspected under transmission electron microscope (JEOL JSM-1200EX II) using 2 % uranyl acetate for negatively-staining with an acceleration voltage of 80 kV.

Light Scattering Assay of Aggregation

Insoluble filament formation via the extension processes of tau R3 peptide was measured by 90° angle light scattering (LS) using Hitachi F-7000 fluorescence spectrophotometer, where the excitation and emission wavelengths were both set at 550 nm. The peptide was adjusted to a final concentration of 15 μM in 50 mM Tris-HCl buffer (pH = 7.5) and placed in a fluorometer cuvette with a 1.0 cm pathlength. The sample was maintained at 37C by a circulating water bath, and heparin was added to achieve a final reaction concentration of 3.8 μM. Experiments with the addition of ThS (10 μM) or $[Ru(phen)_2dppzidzo]^{2+}$ (10 μM) were also carried out to evaluate the impacts of the dyes on the tau R3 aggregation.

Molecular modelling

3D models of R3 PHF core sequence were built based on multiple-threading alignments by LOMETS.[3] Sequence of R3 (^{306}VQIVY KPVDL SKVTS KCGSL GNIHH KPGGG Q^{336}) was submitted to the LOMETS web site (http://zhanglab.ccmb.med.umich.edu/LOMETS/), and ten models was obtained (Figure S6). Model 10 was used to construct tau-tau PHF core protofibril, because of, *a*) the model could cover all 31 residues; *b*) model 10 was predicted using PPA-I method of LOMETS with high confidence, PPA-I was a profile-profile alignment approach, mainly considered query and template profiles and predicted secondary structure information; *c*) according to the circular dichroism research of Mizushima et al, [4] the R3 filament was consist of 10 percent of α- helix, 55 percent of β-structure and 35 percent of random coil. The sequence motif ^{306}VQIVYK^{311} was reported to form β-structures. [7] Among the ten models obtained by LOMETS, it is clear that model 10 is the closest to the experiment results. Here, another motif ^{330}HKPGG^{334} was also predicted as β-structures. The

obtained 3D model of R3 was used to form a dimer firstly, which was used to form a R3 oligomer aggregation. The structure of $[Ru(phen)_2dppzidzo]^{2+}$ was optimized using Gaussian 03 at B3LYP/6-311G**/SDD level. Autodock vina was used as the docking strategy.[5]

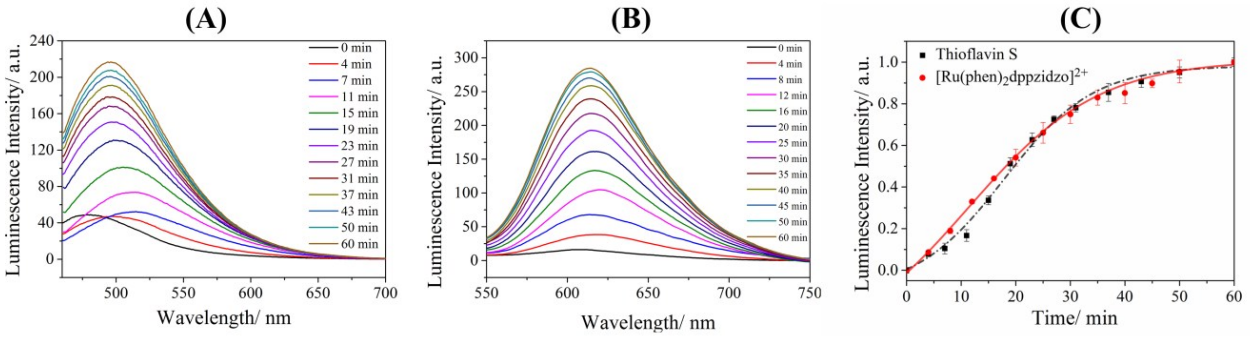

Figure S1 (A) Emission spectra (λ_{ex} = 440 nm) of ThS (10 μM) mixed with R3 peptide (15 μM) incubated for different time; (B) Emission spectra (λ_{ex} = 404 nm) of $[Ru(bpy)_2dppzidzo]^{2+}$ (10 μM) with R3 peptide (15 μM) incubated for different time; (C) Normalized time dependent luminescence intensity growth of ThS (10 μM) and $[Ru(phen)_2dppzidzo]^{2+}$ (10 μM) with the R3 peptide (15 μM) and heparin (3.8 μM) incubated without dyes.

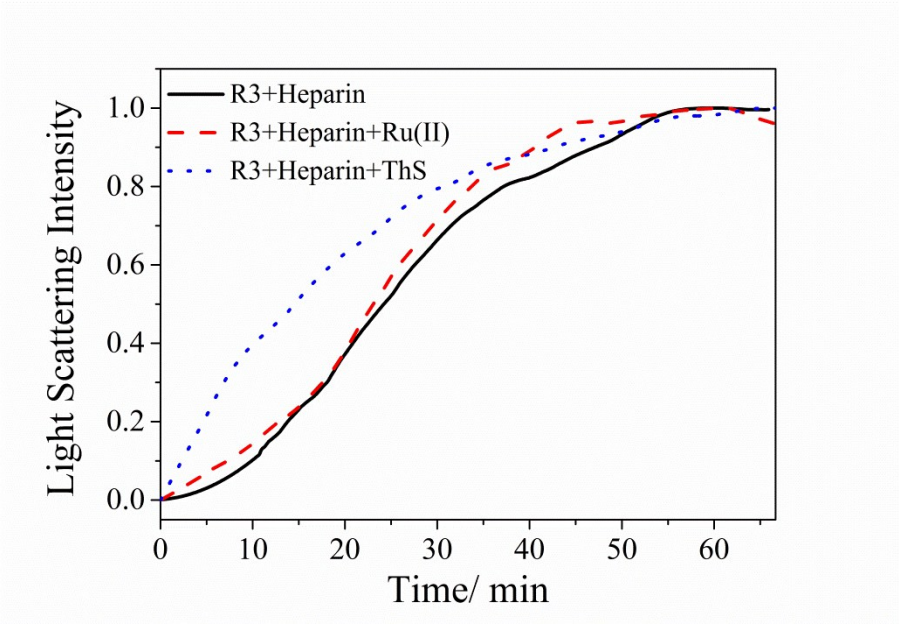

Figure S2 Normalized time dependent light scattering intensity growth of ThS (10 μM) and $[Ru(phen)_2dppzidzo]^{2+}$ (10 μM) with the R3 peptide (15 μM) and heparin (3.8 μM).

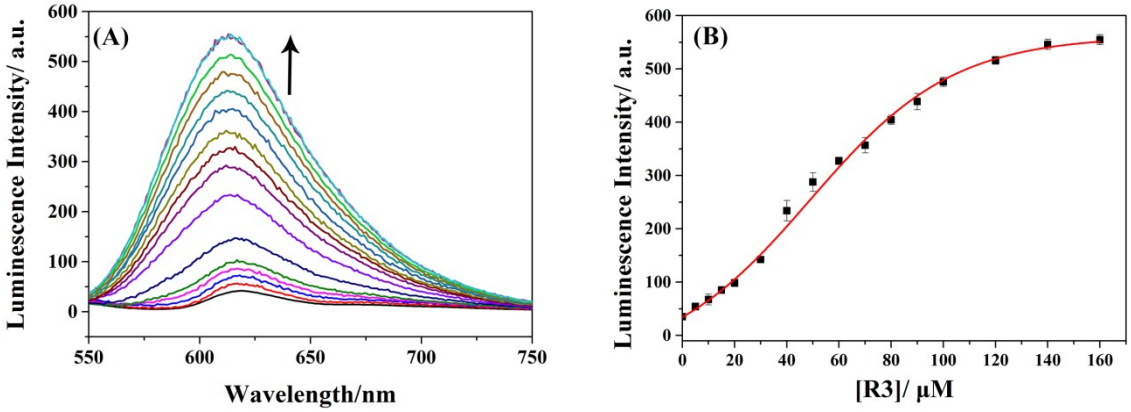

Figure S3 (A) Changes in the emission spectra (λ_{ex} = 404 nm) of 10 μM of $[Ru(bpy)_2dppzidzo]^{2+}$ with increasing concentrations of R3 filaments (0-160 μM); (B) Luminescence intensity changes at 610 nm of 10 μM of $[Ru(bpy)_2dppzidzo]^{2+}$ with increasing concentrations of R3 filaments (0-160 μM).

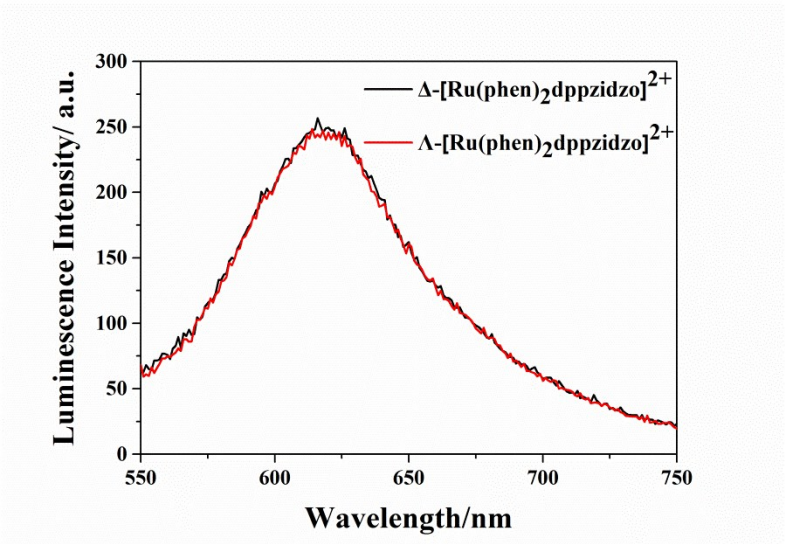

Figure S4 Emission spectra of 10 μM of Δ and Λ-$[Ru(bpy)_2dppzidzo]^{2+}$ with 30 μM of R3 filaments.

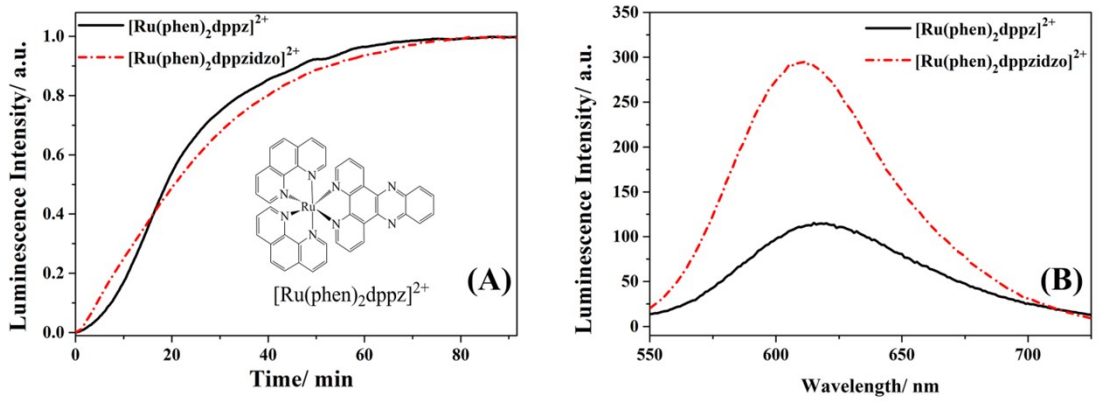

Figure S5 (A) Normalized time dependent luminescence intensity growth of $[Ru(phen)_2dppz]^{2+}$ (10 μM) and $[Ru(phen)_2dppzidzo]^{2+}$ (10 μM) with the R3 peptide (15 μM) and heparin (3.8 μM) incubated without dyes; (B) Emission spectra of $[Ru(phen)_2dppz]^{2+}$ (10 μM) and $[Ru(phen)_2dppzidzo]^{2+}$ (10 μM) with 30 μM of R3 filaments.

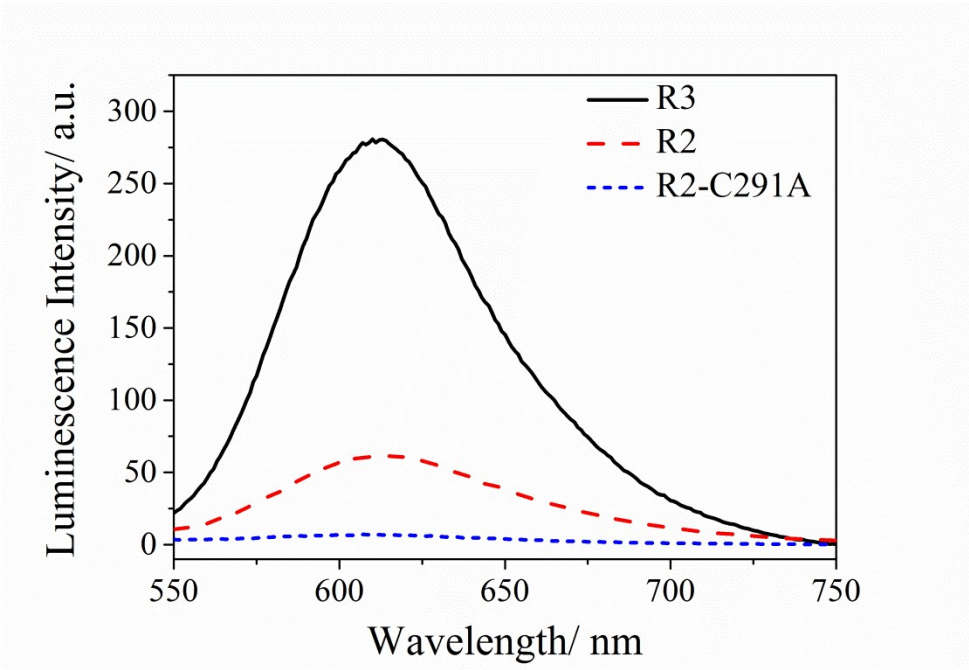

Figure S6 Emission spectra of $[Ru(phen)_2dppzidzo]^{2+}$ (10 μM) with 30 μM of R3, R2 and R2C291A peptide (incubated for 1 hour).

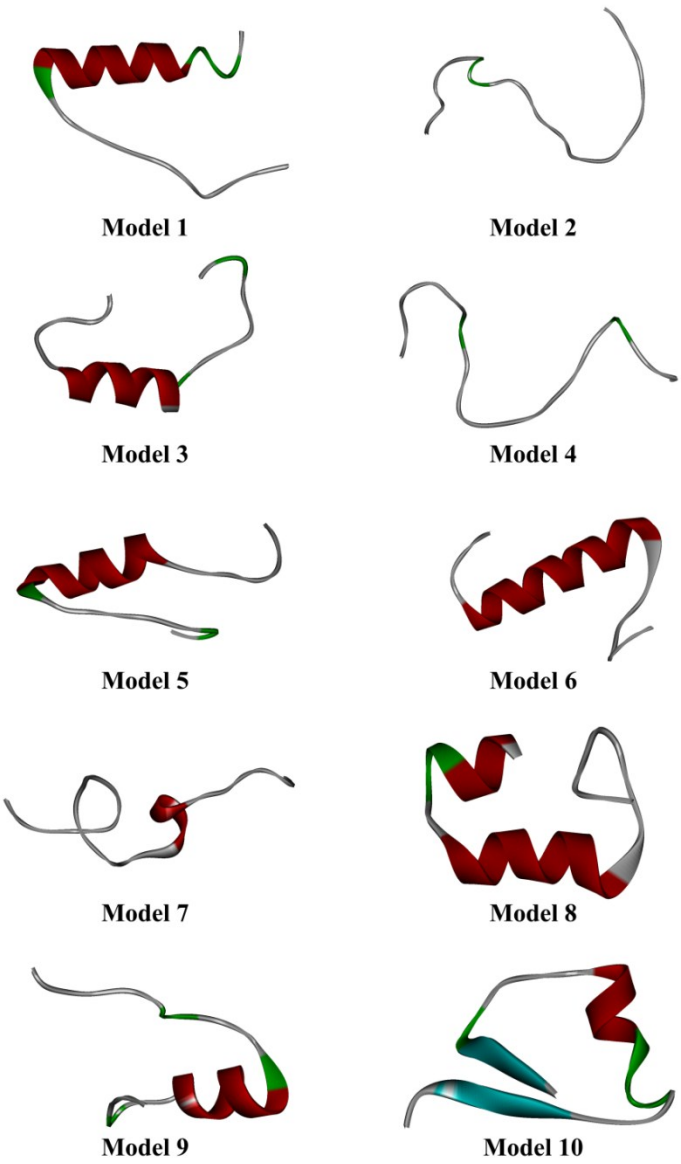

Figure S7 Ten R3 peptide model obtained by LOMETS. Sequence of R3 (^{306}VQIVY KPVDL SKVTS KCGSL GNIHH KPGGG Q^{336}) was submitted to the LOMETS web site (http://zhanglab.ccmb.med.umich.edu/LOMETS/), and ten models was obtained.

Refferences

(1) Yao, J. L.; Gao, X.; Sun, W.; Fan, X. Z.; Shi, S.; Yao, T. M. A naked-eye on-off-on molecular "light switch" based on a reversible "conformational switch" of G-quadruplex DNA. *Inorg Chem* **2012**, *51*, 12591-12593.

(2) Hartshorn, R. M.; Barton, J. K. Novel dipyridophenazine complexes of ruthenium(II): exploring luminescent reporters of DNA. *J Am Chem Soc* **1992**, *114*, 5919-5925.

(3) Zhang, Y. I-TASSER server for protein 3D structure prediction. *BMC Bioinformatics* **2008**, *9*, 40.

(4) Mizushima, F.; Minoura, K.; Tomoo, K.; Sumida, M.; Taniguchi, T.; Ishida, T. Fluorescence-coupled CD conformational monitoring of filament formation of tau microtubule-binding repeat domain. *Biochemical and biophysical research communications* **2006**, *343*, 712-718.

(5) Trott, O.; Olson, A. J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* **2010**, *31*, 455-461.