REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
05-06-2017	Final Report	15-Aug-2015 - 15-Nov-2016

4. TITLE AND SUBTITLE

Final Report: Acquisition of a Femtosecond Laser System for Materials Characterization and Education

5a. CONTRACT NUMBER
W911NF-15-1-0330

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611103

6. AUTHORS

Guillermo Bazan, Alexander Mikhailovsky, Thuc-Quyen Nguyen

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES

University of California - Santa Barbara
3227 Cheadle Hall
3rd floor, MC 2050
Santa Barbara, CA 93106 -2050

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)

U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
66886-LS-RIP.3

12. DISTRIBUTION AVAILIBILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

The funding provided by DURIP program was used to acquire an ultrafast laser spectroscopy system with extensive material characterization capabilities. The new system is based on Coherent Astrella Ti:Sapphire regenerative amplifier coupled to LightConversion TOPAS?Prime optical parametric amplifier and LightConversion NIRUVVIS optical mixer unit. The system is capable of producing ~120 fs laser pulses with wavelength continuously tunable in the range 220-2600 nm. Also, DURIP funding was used to acquire instrumentation necessary for the laser system operation (detectors, oscilloscope, computer interface cards, and

15. SUBJECT TERMS

Ultrafast spectroscopy, time-resolved spectroscopy, non-linear optical phenomena, femtosecond laser

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Guillermo Bazan
UU	UU	UU	UU		19b. TELEPHONE NUMBER 805-893-5538

Report Title

Final Report: Acquisition of a Femtosecond Laser System for Materials Characterization and Education

ABSTRACT

The funding provided by DURIP program was used to acquire an ultrafast laser
spectroscopy system with extensive material characterization capabilities. The new system is based on Coherent Astrella Ti:Sapphire regenerative amplifier coupled to LightConversion TOPAS?Prime optical parametric amplifier and LightConversion NIRUVVIS optical mixer unit. The system is capable of producing ~120 fs laser pulses with wavelength continuously tunable in the range 220-2600 nm. Also, DURIP funding was used to acquire instrumentation necessary for the laser system operation (detectors, oscilloscope, computer interface cards, and safety equipment). The laser system was integrated into UCSB Optical Characterization Facility to extend its capabilities and meet the needs of campus researchers. It has been used successfully in ultrafast pump-probe experiments, time-resolved studies of phosphorescent materials, laser-induced
activation of cancer treatment drugs prototypes. It has been instrumental in research projects funded by DoD (ARO and ONR), NSF, and collaborative interuniversity efforts.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
06/02/2017	1	Bing Wang, Ming Wang, Alexander Mikhailovsky, Shu Wang, Guillermo C. Bazan. A Membrane-Intercalating Conjugated Oligoelectrolyte with High-Efficiency Photodynamic Antimicrobial Activity, Angewandte Chemie International Edition, ( ): 5031. doi:
		1,042,960.00
TOTAL:	**1**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received		Paper
TOTAL:		

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received	Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Bazan, Guillermo	0.00	
Nguyen, Thuc-Quyen	0.00	
Mikhailovsky, Alexander	0.00	
FTE Equivalent:	**0.00**	
Total Number:	**3**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: 0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See Attachment.

Technology Transfer

Scientific Progress and Accomplishments

Description of the system acquired:

DURIP funding was used to acquire an ultrafast laser system with extended capabilities for spectroscopy of materials. The system is based on Coherent Astrella Ti:Sapphire regenerative amplifier producing 100 femtosecond (fs) laser pulses with center wavelength 800 nm, repetition rate of 5 kHz, and pulse energy of <1.2 mJ. Astrella is a single-box, low maintenance system which can be operated in a turn-key mode by users with minimal experience in advanced laser systems operations. This allows to minimize the time required for the laser tweaking and maintenance and allot more resources to research. 85% of the regenerative amplifier's output is coupled into TOPAS-Prime Optical Parametric Amplifier (OPA) by Light Conversion. The OPA provides continuously tunable infrared (IR) light in the range 1100-2600 nm with efficiency <25%. OPA's output can be upconverted further using Light Conversion NIRUVVIS optical harmonics and frequency mixing module. OPA and NIRUVVIS modules are fully computer-controlled and allow for virtually hands-free operation. The complete laser system provides ~100 fs pulses with wavelength continuously tunable in the spectral range 220-2600 nm. The maximum repetition rate of the laser system is 5 kHz but it can be scaled down to a single pulse operation, if required by an application. High repetition rate of the laser allows to improve signal/noise ratio in optical measurements, decrease data acquisition time, and lower exposure to the laser light for materials degrading under intense laser irradiation. The acquired laser system is insensitive to the environmental fluctuations (±2 ºC) and exhibits low long-term drift of the laser pulse energy and pulse-width (both ~1% over 24 hours). Use of an air-to-water laser chiller additionally increases the system stability by decreasing the laboratory heat load. Additional equipment acquired with DURIP funding includes beam steering and conditioning optics, digital oscilloscope, power meter, computer interface boards, detectors, pump laser for a standalone femtosecond oscillator, and safety gear required for the operation, maintenance, and alignment of the laser system.

The new laser system became a heart of the renovated pump-probe transient absorption (TA) experiment. TA setup is similar to the one developed by Klimov and McBranch [1] with some improvements (Figure 1, See Attachment). The output of the regenerative amplifier is split into pump and probe arms with pump beam being directed into the OPA/frequency mixer to produce photons with required wavelengths. The OPA/frequency mixer output beam is then directed through a computer-controlled optical delay line and focused onto the sample. The probe beam is attenuated to μJ level and is focused on a sapphire plate to produce fs supercontinuum capable of probing photoinduced absorption changes in the range 440-1400 nm. The supercontiuum is overlapped with the pump spot on the sample by a parabolic mirror at a small angle and routed through a monochromator onto a photodiode detector (Si pin-photodiode for the range 440-950 nm and InGaAs photodiode for the range 950-1400 nm). The pump beam is modulated by an optical chopper at the first subharmonic frequency of the laser repetition rate. The photodiode signal is fed through a transimpedance amplifier into a lock-in amplifier synchronized with the chopper frequency to measure pump-induced sample's optical transmission variation and into another lock-in amplifier synchronized with the full laser repetition rate to measure unperturbed optical transmission of the sample. Pump-probe delay and the monochromator's wavelength are controlled by a computer to collect single-wavelength TA kinetics and time-resolved spectra.

The setup is capable of measuring differential transmission signals down to 10^{-5} with sub-100 fs resolution and can correct for the probe light chirp on the go.

Additionally, DURIP funding allowed to improve OCF capabilities for the time-resolved fluorescence measurements by Time-Correlated Single Photon Counting (TCSPC) method [2]. The description of the setup can be found elsewhere [3]. The use of the upgraded pump laser enabled us to extend the wavelength tuning range of the femtosecond oscillator to 700-1000 nm (The old system was tunable in the range 720-920 nm) significantly extending the variety of chromophores which can be studied by the setup.

The laser system has been integrated into UCSB Optical Characterization Facility (OCF). OCF provides users with an access to the state-of-the-art optical instrumentation and techniques on the pay-per-use basis. The facility is overseen and operated by a full-time technical staff member, Dr. Alexander Mikhailovsky, with extensive knowledge and experience in the field of spectroscopy with the focus on the ultrafast time-resolved spectroscopy. The new laser system has been sought to improve the OCF capabilities in pump-probe and photoluminescence time-resolved experiments, laser processing of materials, and multiphoton absorption spectroscopy. The improvement stems from the extended tuning range of the laser system and higher repetition rate of the laser. The former enables one to interrogate a broad range of optical states and transitions in the materials studied, and the latter makes one capable to improve signal/noise ratio in variety of optical techniques and reduce the optical power used in experiments, thus useful for studies of materials with weak optical response or photodegrading under intense laser beams.

Applications of the Upgraded System and Important Results

The laser system has been already used in several projects which relied heavily on the unique capabilities of the new setup.

Mechanistic Investigation of Protein Translocation on DNA

Prof. Norbert Reich (UCSB)

Funding source: NSF CHE-1413722

The project utilizes the new laser system for irradiation of gold nanostructures, namely hollow gold nanoshells (HGNs) with ultrashort laser pulses with sub-mJ energy. The laser setup allows irradiation of HGNs at different repetitions rate, and wide wavelength tunability provided by the OPA and the optical mixer unit enable selective excitation of nanostructures with plasmon resonance wavelength tuned via particles' geometry adjustment.

A new platform technology is based on highly monodisperse HGNs that can be functionalized with thiol-linked protein or short interfering ribonucleic acid (siRNA) cargo**.** Following endocytosis facilitated by a cell penetrating peptide, such as transactivator of transcription, the plasmon-resonant HGN is activated by femtosecond NIR light pulses; the light energy is converted within picoseconds into "hot electrons" that cleave the thiol bonds linking the HGN to the protein or siRNA cargo. In the following nanoseconds, *the HGN dissipates its heat by*

vaporizing a minute amount of water surrounding the HGN, forming vapor nanobubbles that insulate both the cell and cargo from significant temperature changes. The nanobubbles rapidly expand and collapse, similar to cavitation bubbles in ultrasound, leaving no toxic chemical residue. Mechanical forces generated by the bubbles rupture the endosomes providing a unique method of endosome escape and fast, efficient, and spatio-temporally controlled protein or siRNA delivery to the cytoplasm, overcoming the ineffective endosomal escape that plagues other nanoparticle methods. The total energy input is similar to that used for two-photon imaging and results in negligible bulk heating and high cell viability (≥ 90). NIR light can penetrate through as much as 1 cm of tissue providing access to three-dimensional structures such as human embryonic stem cells spheroids. Highly efficient endosome escape via NIR light activated nanobubble formation means that we require only picomolar carrier concentrations and nanomolar protein or siRNA concentrations.

Ultrafast time-resolved studies on fluorescein for recognition strands architecture in amyloid fibrils (submitted for publication to JACS, manuscript ja-2017-05482h)

Piotr Hanczyc, Alexander Mikhailovsky, and Alan Heeger (UCSB), Michael R. Sawaya and David Eisenberg (UCLA)

Funding: Office of Naval Research Award No. N00014-14-1-0580 (UCSB), NIH grant AG054022 (UCLA)

This project studies excited state kinetics of fluorescein dye molecules bound to amyloid fibrils and relies heavily on the OCF capabilities provided by the newly acquired laser system, such as high sensitivity transient absorption and transient photoluminescence measurements. The samples studied exhibit inhomogeneous local environment which result in optical signals with a broad range of characteristic life-times ranging from few ps to tens of nanoseconds. The former was visualized using femtosecond pump-probe transient absorption experiments with near resonant optical excitation. The pump pulses were generated using OPA and the optical harmonics/mixer unit. The measurements were conducted at low excitation flux levels (~1 $\mu J/cm^2$) to avoid photodegradation of samples and observation of multiexciton annihilation effects. High repetition rate of the laser system enabled us to collect high quality data within a reasonable time. The photoluminescence transients were collected on a home-built time-correlated photon counting system with wavelength tuning range expanded with the help of the new pump laser with increased power and stability.

Protein aggregation is associated with numerous devastating diseases such as Alzheimer's, Parkinson's, and prion diseases. Development of therapeutics would benefit from the knowledge of the structural organization of protein molecules in these amyloid aggregates, particularly in their aqueous biological milieu. However, detailed structural studies to date have been mainly on the solid state, and have required large quantities of purified aggregate. Moreover, these conventional methods require the aggregated assembly to remain structurally stable over days or weeks required to perform the experiment, whereas the pathologically relevant species of in vivo aggregates may be shorter lived. Here, we show the organization of protein chains in dissolved amyloid aggregates can be readily determined spectroscopically using minute quantities of fluorescein-labeled protein segments in a matter of minutes. Specifically, we investigated the

possibility of using the ultrafast dynamics of fluorescein to distinguish among three categories of β-sheet geometry: **(1)** anti-parallel in-register, **(2)** parallel in-register or **(3)** anti-parallel out-of-register. Fluorescein, the most commonly used staining dye in biology and medicine, was covalently attached to the N-termini of peptide sequences selected from a library of known amyloid crystal structures. We investigated the aggregates in solution using steady-state and time-resolved absorption and fluorescence spectroscopy. We found that the dynamics of fluorescein relaxation from the excited state revealed amyloid structure-specific information. Time-resolved fluorescence spectroscopy indicated that fibril-bound dye molecules are isolated from the environment and have excited state kinetics completely different from that of the free chromophore (Figure 2, See Attachment). Kinetics of the fluorescein excited state related to the β-sheet geometry has been attributed to the cationic form of the molecule and visualized via transient absorption experiments for typical time scale of these processes is below the resolution of the time-resolved emission measurements (Figure 3, See Attachment). We demonstrate that time-resolved absorption is capable of differentiating strand organization in β-sheet aggregates when strong intermolecular coupling between chromophores occurs.

A Membrane-Intercalating Conjugated Oligoelectrolyte with High-Efficiency Photodynamic Antimicrobial Activity[4]

Bing Wang, Ming Wang, Alexander Mikhailovsky, Guillermo C. Bazan (UCSB), Shu Wang (Beijing National Laboratory of Molecular Science, PRC)

Funding source: Institute for Collaborative Biotechnologies through grant W911NF-09-0001 from the U.S. Army Research Office

A membrane intercalating conjugated oligoelectrolyte (COE), **PTTP**, was designed and synthesized with the goal of providing red-shifted absorption spectra compared to previously synthesized COE analogs. Specifically, electron rich and electron poor subunits were introduced in the conjugated backbone to modulate the bandgap. **PTTP** exhibits maxima of absorption at 507 nm and of emission at 725 nm. **PTTP** can also efficiently function to generate singlet oxygen in-situ (Φ_{Δ} = ~20 %) and has appropriate topology and dimensions to interact with lipid membranes. The resulting rapid membrane insertion and sensitizing ability provide **PTTP** with a highly efficient antibacterial capability under a low light dose (0.6 J/cm^2) toward Gram-negative bacteria *E. coli*, making it a remarkably efficient optically mediated antimicrobial agent (Figure 4, See Attachment).

Within the scope of this work, **PPTP** molecule underwent extensive photophysical characterization. The new instrumentation in the OCF has been used to investigate **PPTP** excited state kinetics, evaluate the time-scale of the processes affecting formation of the triplet excited state and production of the biologically-relevant singlet oxygen. The photoluminescence spectroscopy of **PPTP** and studies of the singlet oxygen emission in various solvents indicated that despite low singlet emission efficiency, the yield of the singlet oxygen generation is substantial. This means that the rate of the intersystem crossing is unusually high for this molecule. Interestingly, time-resolved PL and TA measurements tracking exclusively singlet excited state population show only minute differences in degassed and air-saturated solutions of **PPTP.** This is an indication that perhaps different routes of the excited state relaxation exist in

polar solvent, one of which is favoring intersystem crossing and another one involving stable singlet state. The nature of this branching is not clear and is a subject to further investigation.

References

1. Klimov, V.I.; McBranch, D.W., *Opt.Lett,*, **23**, 277-279 (1998).
2. W. Becker, Advanced time-correlated single-photon counting techniques. Springer, Berlin, Heidelberg, New York, 2005
3. Franco, L.P., *et al*, *Journal of Phys. Chem. A*, **118(51),** 12184-12191 (2014).
4. Wang, B.; Wang, M.; Mikhailovsky, A.; Wang, S.; Bazan, G.C.; *ANGEWANDTE CHEMIE-INTERNATIONAL EDITION*, **56(18)**, 5031-5034 (2017).

Attachment

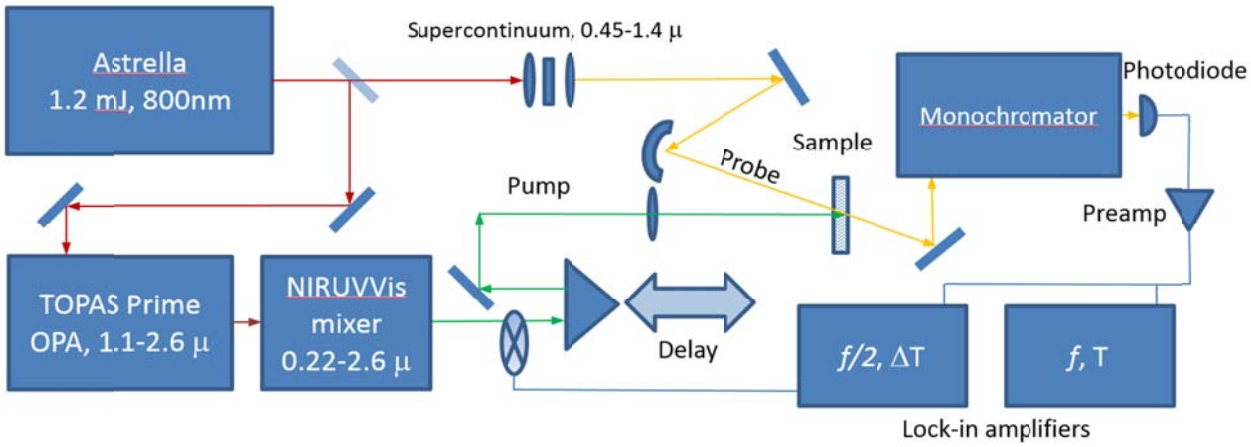

Figure 1. Layout of the TA setup

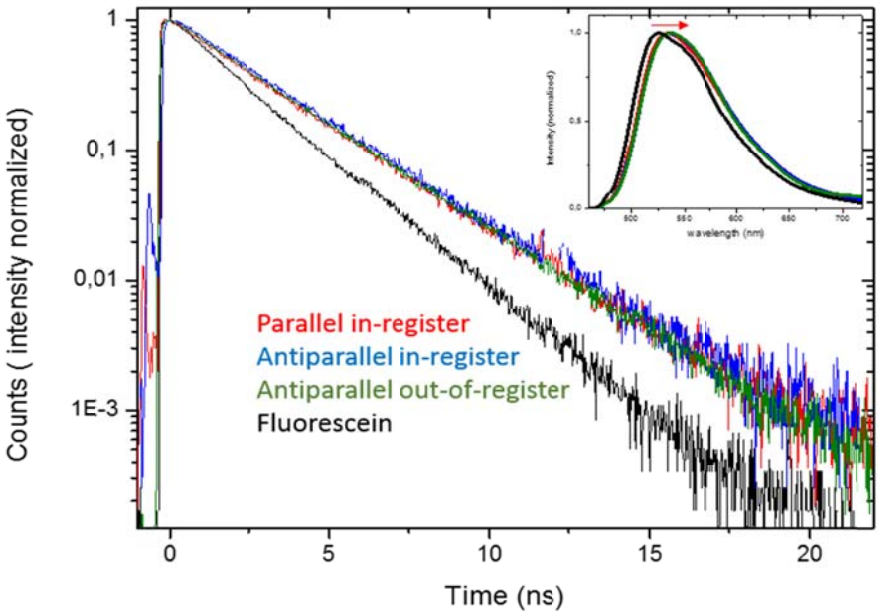

Figure 2. 4 Lifetime data of fluorescein recorded at 530 nm (black) and in conjugation with three different zipper structures (1) antiparallel in-register (blue), (2) parallel in-register (red), (3) antiparallel out-of-register (green), all samples measured at pH 2. The inset is the steady-state fluorescence spectra with arrow indicating red-shifting of the spectrum when fluorescein is in conjugation with peptides in the aggregated state. The representative lifetimes are shown for aggregated peptides: FGAILSS (1), LVEALYL (2) and ASLTVS (3) that had no aromatic amino acid in the sequence.

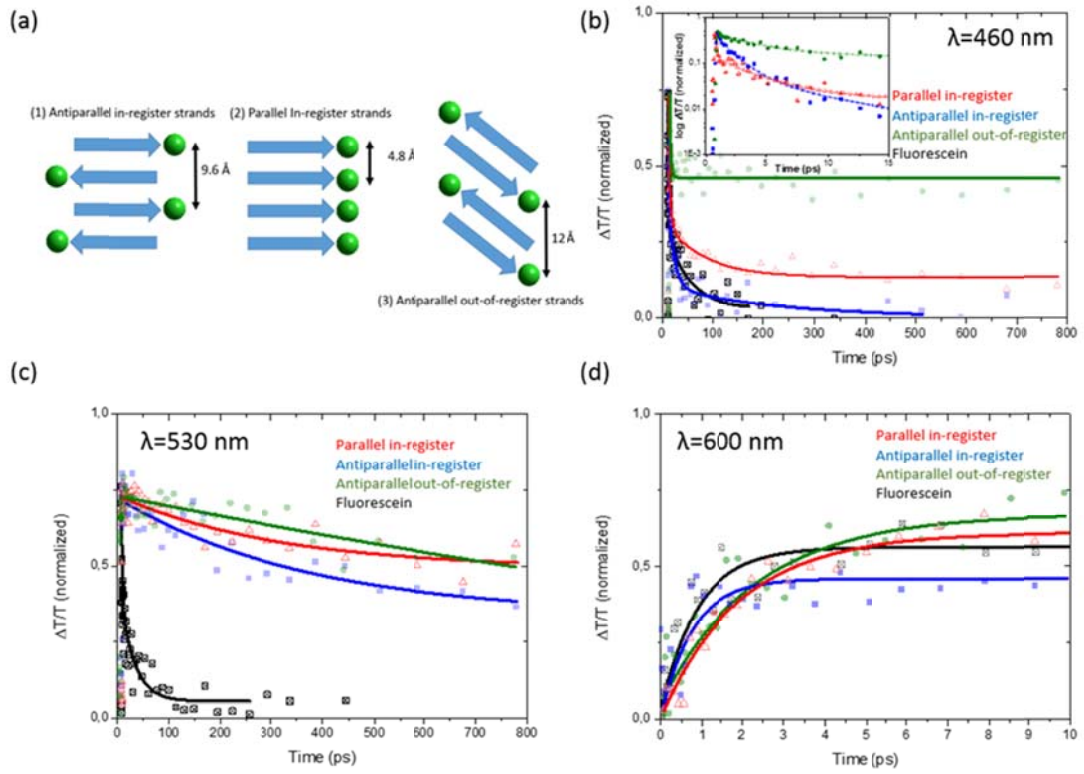

Figure 3 (a) Schematic drawing of strand architectures in the three β-sheet architectures with chromophore attached **(1)** antiparallel in-register, **(2)** parallel in-register, **(3)** antiparallel out-of-register. Dynamics of pristine fluorescein (black) and in conjugation with peptides forming different β-sheet architectures (blue, **1**) , (red, **2**), (, green, **3**) at (b) 460 nm. The inset of Fig. b represents time domain window >15 ps; (c) dynamics at 530 nm and (d) at 600 nm. Presented dynamics in the graphs are representative decays of fluorescein conjugated with: GYVLGSA **(1)**; NNQNTF **(2)** and VAVHVF **(3)**.

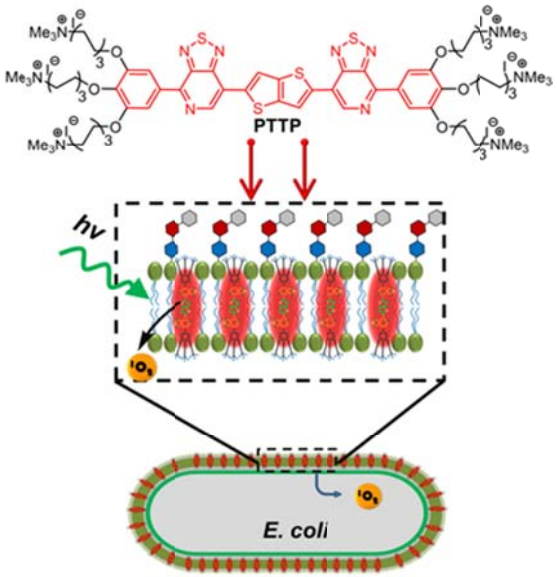

Figure 4. Chemical structure of **PTTP**, cartoon representation of the insertion into a cell membrane, and proposed mechanism of PDAT through photogeneration of $^1O_2$.