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HYBRID METAMATERIALS FOR SOLAR BIOFUEL GENERATION

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We are creating a novel multifunctional solar biofuel generating platform by coupling designed protein charge separation constructs with newly developed photonic metamaterials – metal/dielectric composites that control light in ways not possible with traditional optics. To accomplish this, we have assembled a team of laboratories who are each at the forefront of the new technologies necessary in its construction – de novo protein design (Koder), nanoplasmonic metamaterials (Crouse) and protein film voltammetry (Elliott). We start with bio-inspired artificial protein domains designed to generate biofuels when modularly connected to enzyme domains directly derived from nature. The designed protein domain serves as a self-assembling 'smart matrix' incorporating synthetic zinc phthalocyanine (ZnPC) cofactors and porphyrins tailored for photon absorption, charge separation and directional charge/hole transfer. The designed charge separation domain will be benignly expressed in bacteria as a chimera with naturally occurring protein domains which utilize the high energy electrons supplied by the charge separation domain to generate biofuels. This domain will be attached to a novel metamaterial light harvesting template which traps and concentrates >95% of the light of a given wavelength int

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Title: Hybrid metamaterials for solar biofuel generation

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Abstract:
Program Manager: Patrick Bradshaw – AFOSR Chemistry and Materials Directorate
We are creating a novel multifunctional solar biofuel generating platform by coupling designed protein charge separation constructs with newly developed photonic metamaterials – metal/dielectric composites that control light in ways not possible with traditional optics. To accomplish this, we have assembled a team of laboratories who are each at the forefront of the new technologies necessary in its construction – de novo protein design (Koder), nanoplasmonic metamaterials (Crouse) and protein film voltammetry (Elliott). We start with bio-inspired artificial protein domains designed to generate biofuels when modularly connected to enzyme domains directly derived from nature. The designed protein domain serves as a self-assembling 'smart matrix' incorporating synthetic zinc phthalocyanine (ZnPC) cofactors and porphyrins tailored for photon absorption, charge separation and directional charge/hole transfer. The designed charge separation domain will be benignly expressed in bacteria as a chimera with naturally occurring protein domains which utilize the high energy electrons supplied by the charge separation domain to generate biofuels. This domain will be attached to a novel metamaterial light harvesting template which traps and concentrates >95% of the light of a given wavelength into a cavity within a five wavelength radius. This solid state light harvesting device is thus *over three orders of magnitude more effective* than that found in green plants for light of 750 nm wavelength, the solar emission maximum. Furthermore, the properties of metamaterials open the door towards creating a multijunction device capable of separating white light into discrete wavelength ranges and trapping it efficiently into different, separately wired cavities. The protein-metamaterial contacts will be mediated by a novel click chemistry attachment strategy which utilizes *in vivo* incorporation of azidohomoalanine into the designed protein domain. These hybrid metamaterial-artificial protein based photonic energy conversion constructs represent a new direction in the design of solar-powered materials, combining *de novo* protein design, synthetic biology and the light-controlling abilities of metamaterials to create a biofuel-generating construct that is 'green', robust, and cost effective.

Original Research Plan

Project Narrative Koder, R.L., Crouse, D., Elliott, S.J.

Statement of Objectives:
These objectives describe the creation of a proof-of-principle light-driven NADH producing device which contains components which separately address each of the three principle elements of solar biofuel generation – a *de novo* designed charge separation domain, a nanoplasmonic light-harvesting crystal electrode, and a simplified flavoprotein reductase domain.

1**. Design, manufacture and analysis of a two junction, two wavelength metamaterial electrode (Crouse)** We will model and optimize a metamaterial which traps two non-overlapping spectral ranges of light (600-700 nm and 700-800 nm) into two spatially distinct sets of cavities with separate electrical contacts to each aperture type. The metamaterial composite in our device will serve three purposes: (1) to distribute light spatially by wavelength band and to channel individual wavelength bands to different cavities containing protein chimeras with different absorption characteristics, (2) to serve as an electrode, and (3) to serve as a heat sink to avoid the harmful effects of high temperatures. The prototype plasmonic crystal film and several simple control/calibration films will be fabricated at the Cornell Nanoscale Science and Technology Facility (CNF) and then be optically and electrically characterized in the Metamaterials Research Lab at CCNY.

2. Creating wavelength-optimized chimeras for use in the device. (Koder)

Two different charge separation domains will be developed which each contain different phthalocyanine cofactors with action spectra matching those of the two metamaterial cavities. We will further optimize the flavoprotein reductase-designed charge separation domain chimera for attachment to the metamaterial electrode by adding a third, donor B-type heme cofactor to the end of the designed bundle distal to the NADH-forming flavoprotein. This added cofactor will serve as an electron sink which can take up an electron from the electrode in the absence of light, limiting short-circuiting reactions and accelerating turnover under low light conditions. Laser flash activation will be used to monitor the lifetime of the charge separated state and if necessary it will be optimized by tuning the donor and acceptor cofactor reduction potentials. The designed protein domain and the full chimera will be further examined and optimized using protein film voltammetry as in Aim 3. This creates a helical bundle charge separation domain which is optimized to take in a low energy electron from the metamaterial electrode, add to it the energy of the photon, and pass it to the coupled natural protein domain, serving as the driving force for biofuel generation.

3. Optimizing a conductive protein-electrode interface (Elliott)

Protein film voltammetry is ideal for the characterization and optimization of this device as it allows the investigation of the electron transfer kinetics and thermodynamics in a multicofactor system, enabling us to optimize protein stability on the surface, spacer length and conductivity. Self-assembled monolayers (SAM) of designed proteins will be attached to gold electrode surfaces as follows: SAMs containing dilute alkyne-terminated alkane thiols will be assembled on the electrode surface. Proteins will be expressed using an *E. coli* strain which incorporates azidohomoalanine *in vivo* and the Sharpless click chemistry reaction will be initiated electrochemically, by poising the potential to reduce soluble Cu(II) to Cu(I) at the electrode surface. Protein film voltammetry will be used to determine electrode coverage, electrochemical reversibility, electron transfer rates and electrode robustness. These studies will be begun with artificial bi-cofactor four helix bundle proteins HF and HH. The larger, more complex domains and chimeras binding additional electron donors or acceptors will be characterized on electrodes as they are produced.

4. Assembly and testing of the full platform. (Crouse, Elliott, Koder)

The two optimized chimeric proteins from Aim 2 will be attached to their matching metamaterial cavities (Aim 1) using the optimized protein attachment SAM (Aim 3). First, absorbance and fluorescence of the hybrid metamaterial will be measured as a function of wavelength. The product NADH is fluorescent, allowing the rapid and sensitive detection of device turnover. The intensity and spacial localization of the product will be measured as a function of applied wavelength. Localization of the fluorescent signal at and near the cavity modes will demonstrate both light channeling and light-driven enzyme turnover. Catalytic wave voltammetry will be utilized to quantitate turnover rates as a function of light intensity and applied wavelength. The entire assembly will be spin coated with a stabilizing sol-gel matrix, and the sol-gel pore size optimized using the same characterization techniques.

Project Narrative:

1. Background and Significance

The Design Challenge. The carbon-neutral generation of chemical energy is among the greatest technical challenges of the 21st century.[1] Natural photosynthesis converts solar energy to chemical energy and in doing so supports all life on earth. The study of photosynthetic organisms has resulted in a molecular-level understanding of the design features allowing efficient, robust conversion of solar to chemical energy by antenna complexes and reaction center proteins embedded in a membrane.[2,3] However, although these protein complexes are evolutionarily optimized to generate chemical energy useful to the plant host, they are not optimized to create energy in a form useful to humankind. Biosolar energy research has begun to modify photosynthetic bacteria, algae, or plants to carry out more efficient production of desirable energy products such as H_2 or biomass. However, organisms can only be modified to a limited extent before they cease to be viable. Bio-inspired synthetic systems linking organic molecules have successfully recreated individual elements of the photosynthetic apparatus.[4,5] In particular, they have succeed in using the principles of electron transfer theory to assemble structures which use the energy of light to drive long distance charge separation producing a high yield of long lived ion pairs.[6] These have been attached to electrodes to create photovoltaic cells or placed in membranes, and set up to drive redox reactions such as the hydrolysis of

water. Unfortunately these systems are expensive, synthetically challenging, and their production is environmentally damaging.[7] For these reasons, they are unlikely to find commercial application. Artificial proteins offer an inexpensive, environmentally benign alternative.

Photosynthesis can be reduced to three *functional elements* which any biomimetic system must duplicate:

(1) *Light Harvesting* – the collection of photons over the solar emission spectra, the reduction of their energy to that of the reaction center action potential and the transfer of these photons to the reaction center. This is performed in green plants with a complex light harvesting complex composed of peptides and chlorophyll molecules.

(2)*Charge separation* – the transformation of photonic energy into a directional electronic driving force. Photosynthetic plants perform this function using chlorophyll cofactors.

(3)*Chemical coupling* – the utilization of the driving force derived from charge separation to create high energy chemical compounds. In photosynthesis, this takes the form of the creation of a transmembrane potential which is coupled to ATP synthesis in a process called photophosphorylation.

1.1 Plasmonic metamaterials (functional element 1). Metamaterials will serve as a solid state light harvesting component in our device. Anomalous optical transmission (AOT) through subwavelength apertures was first observed by T.W. Ebbesen in 1998 in his article in *Nature* on optical characteristics of metallic thin films perforated with subwavelength-sized apertures, an article that started a frenzy of research on metamaterials and anomalous transmission.[8] Ebbesen described transmission of light through apertures significantly smaller than the wavelength of light being studied.

Research into metamaterials (also called plasmonic crystals) has advanced greatly since 1998, with many novel and useful optical phenomena observed in these structures, including: anomalous optical transmission (AOT), negative index of refraction, light trapping and super focusing. One of us (Crouse) has 15 years experience in the area of metamaterials [9-18], and we have recently begun to perform preliminary research on how these metamaterials can increase the performance of green energy devices. The metamaterials that we are developing are single-layer metal/glass/semiconductor composites in which the various components (i.e., metal, glass, semiconductor) are arranged in highly organized, and often periodically repeating patterns, with pattern feature sizes on the scale of several hundred nanometers. The shapes of the patterns and the chemical composition of the component materials are chosen according to what kind of light-controlling behavior we want to achieve. Patterns can be simple grids or arrays of holes in a metal film or can take on more complex designs, such as concentric circles or ellipses. Fabrication of the metamaterial structures requires standard materials and process steps, all of which are routine within the semiconductor device industry. Minimum device feature sizes tend to be on the order of several hundred nanometers, a size regime that allows for use of routine photolithographic processing in widespread use in the in-house fabrication facilities of major optoelectronic device and computer chip manufacturers.

Metamaterials control light through selective excitation of optical/electromagnetic resonance modes that occur within patterned composites, including: surface plasmons, waveguide (cavity) modes and diffractive modes. The dimensions, patterns and composition of our composites determine which modes are excited within a given structure. Definitions of these various modes are as follows and representative nanostructures which form them are depicted Figure 2:

1. *Surface Plasmons:* Surface charge oscillations and associated electromagnetic fields at metal interface.
2. *Rayleigh Anomalies*: RAs occur when one diffraction order grazes the surface of the structure as it changes from an evanescently decaying surface-confined mode to a radiating diffracted mode while the wavelength of the incident beam is decreased.
3. *Cavity modes (CMs):* CMs are resonantly excited modes within the light-transmitting apertures of a structure and can be produced by waveguide modes for both polarization states of light.

Project Narrative Koder, R.L., Crouse, D., Elliott, S.J.

Metamaterials thus promise to perform admirably as a solid state light harvesting structure in artificial photosynthesis applications. Constructed to use cavity modes, they can be designed to absorb white light, separate it into discrete wavelength ranges and *channel all of the energy of different wavelength ranges* into different cavities. These cavities concentrate light from far away, trapping >95% of the light of a given wavelength into a cavity within a five wavelength radius. This solid state light harvesting device is thus over three orders of magnitude more effective that that found in green plants. This calculation of improvement does not include device lifetime. Unlike the natural light harvesting apparatus, metamaterials do not suffer from photodecomposition. If device lifetime is considered in the calculation, this factor of improvement becomes even higher.

1.2 De novo protein design: We will design and optimize an artificial charge separation protein domain which uses phthalocyanine cofactors as the site of light-activated electron transfer (functional element 2). De novo designed proteins containing bound cofactors were first created over 15 years ago.[19,20] These proteins

have been termed 'maquettes', small, simple, robust models of protein function.[21] While the first creations were molten globules, cofactor-containing proteins with a singular, native-like structure are now commonplace [22,23] and, as the recent report of a fully functional artificial neuroglobin by one of us (Koder) attests,[24] the technology has advanced to encompass the realization of sophisticated biological function. One of us (Koder) has developed a simple protein design algorithm which has found repeated success (See Figure 3):[21-28] An explicitly designed active site is created either computationally or by extracting consensus structures using bioinformatics, this active site is fit to a simple, robust fold such as a helical bundle or a TIM barrel, and the remainder of the amino acid side chains are selected using binary patterning[29-31] – the randomized placement of hydrophobic residues at buried positions and polar residues at exposed positions in the structure. Binary patterning of helices, for example, utilizes a simple alternating pattern of hydrophilic and hydrophobic helix-forming residues with the heptamer repeat sequence ●○○●●○○, where the first, third and fourth amino acids are non-polar (●) and the rest polar (○), imposes tetramerization via hydrophobic sequestration.[30,31] The only other selection criteria is that high helical propensity residues are used in helices and residues with high beta sheet propensities in beta sheets.

Our charge separation domains are composed of helical bundles with internal cofactor binding sites.[32] In the four-helix bundle scaffold that is the basis for our design, the histidines that bind cofactors between pairs of parallel helices must be separated by integral numbers of amino acid heptads.[19] Seven residues in an undistorted helix has a rise of 10.5 Å. Cofactors two heptads apart, with a His-His distance of 21 Å, fix the edge-to-edge cofactor separation at 10 Å, which is increased to 12 Å when the fact that the two cofactors are bound to different helical pairs is accounted for. An alternate binding mode, which entails the placing the ligand residues on diagonal antiparallel helices, allows a closer cofactor approach of 5 Å.[33]

Bioinspired photonic energy transduction. Electrons will move between cofactors within the construct by quantum mechanical electron tunneling, with kinetic rates governed by the tenets of Marcus theory.[34,35] Given a high-resolution structure, electron tunneling rates can be explicitly calculated.[36] For low-resolution models, observation of biological electron transfer in natural systems has led to an empirical expression known as the Moser-Dutton ruler, further modified by Crofts and Rose[37] which describes electron tunneling rates in proteins:[38]

$$\mathrm{Log}_{10}\, k_{et} = 15 - 0.6R - 4.23(\Delta G + \lambda)^2/\lambda \qquad (1)$$

where k_{et} is the rate of transfer, R is the edge-to-edge distance in Angstroms separating the cofactors, ΔG is the difference between the donor and acceptor reduction potential in volts, and λ is the reorganization

energy for both participating cofactors in volts. The important factors which determine electron tunneling kinetics are thus cofactor-to-cofactor distances and their relative reduction potentials. The final term in expression is derived from Marcus theory [34,39] and reflects the Gaussian dependence of tunneling kinetics on the driving force, or the difference in reduction potentials, between the electron donor and acceptor.

The basic three cofactor arrangement for long lived charge separation is depicted schematically in Figure 3:[6] upon photoexcitation (Figure 4A1) the site of charge separation or primary donor (PD, the chlorophyll 'special pair' in photosynthesis) transfers an electron (Figure 4A2) to an acceptor molecule (A), a redox cofactor. Before unproductive charge recombination via back electron transfer can occur, a donor molecule (D) transfers an electron to the primary donor, blocking the back reaction (Figure 4A2). The energy diagram for this process is depicted in Figure 4B. The fully charge-separated state principally relaxes back to the ground state by one of two mechanisms: direct long range tunneling between the donor and the acceptor molecules (Figure 4CI) or a two step recombination process that either consists of equilibration between the charge separated state and the D PD^+ A^- intermediate followed by tunneling from the acceptor to the ground state of the reaction center (called the 'thermal back reaction' because of the temperature dependence induced by the initial equilibration step)[40] or a three well single quantum tunneling event which involves the reaction center (called superexchange).[35]

Thus robust charge separation can be achieved by simply spacing the three cofactors of the domain at the correct separations: the acceptor molecule, which must be oxidized at the time of photoexcitation, must be either further in distance from the reaction center or higher in potential than the (prereduced) donor. If the donor and acceptor are of similar reduction potentials, eqn. 1 describes how a few Angstroms difference in distances readily allows the electron transfer to the donor outcompete charge recombination (electron transfer back to the accepter) by several orders of magnitude, creating the long lived, long distance charge separation necessary for the function of the domain.

2.5 Phthalocyanines and the search for 'Splendid Isolation'. Zinc phthalocyanine (ZnPC) will serve as the primary donor cofactor in the domain. Phthalocyanines have long been proposed as ideal molecules to act as primary donors in artificial light-powered devices,[41,42] and they have been used extensively in synthetic charge separation constructs. This is due to their chemical robustness (relative to chlorophyll and porphyrin derivatives), especially against photodecomposition, their ease of synthesis, and their long

wavelength action spectrum – their high molar absorptivity B bands, with maxima at near infrared wavelengths of 650nm and higher, are ideal for solar energy transversion. Simple modifications of the ZnPC macrocycle can move their action spectrum by several hundred nm.[43-45] This enables ZnPC-based charge separation

constructs to be constructed to work over a large fraction of the solar spectrum.

1.4Protein Film Voltammetry. We will use Protein Film Voltammetry (PFV) to assess and optimize the electronic connectivity between gold-based electrodes and the *de novo* designed PC-bearing proteins described above. One of us (Elliott) has over a decade of experience in this technique.[46-61] PFV is a general approach that reports on redox-active cofactors of immobilized molecules at an electrode surface.[59,62-64] Here, this analytical platform will be used to assess the thernodynamics and kinetics of charge transfer between electrode formations and redox-protein modules, including the flavo-protein domain reductase described below. PFV has singular strengths that are critical to this project: it can be used to simultaneously reveal the redox characteristics of multiple cofactors, interrogate electrocatalytic processes, and test the robustness of a protein-electrode material over time.[59,65] Further, as a technique PFV provides a platform for a series of electrochemical experiments, including the ability to assess changes of current for catalytic and photocatalytic processes. Our ultimate goal will be the demonstration of photocatalysis of a chimera protein which links a ZnPC-bearing helical bundle to a flavoprotein active site. Further, PFV will be used to assess the baseline electrochemical properties of other redox enzymes that could be tethered to ZnPC-bearing helical bundles in further applications of our solar-to-fuels strategy, such as soluble domains of hydrogenases, CO_2 sequestering CODH enzymes, and ammonia-producing reductases.

Figure 5 shows a theoretical voltammogram for a simple Oxidized ↔ Reduced interconversion of a redox-active species by a reversible, one-electron step (*Nonturnover*, in light gray). The simple signal is observed as a set of 'peaks' with positive and negative current, which result from the forward and backward scans of a cyclic voltammetry experiment. The area of such peaks corresponds to stoichiometric information about the surface coverage, whereas the peak shape (height and peak width) reveal information about the stoichiometry of the redox equilibrium itself. *Catalytic* PFV (Figure 5, bold) has been used in a remarkable enzymological technique that determines catalytic activity of electroactive enzymes, while simultaneously reporting upon the nature of the electron transfer chemistry that controls enzyme mechanism. In a catalytic PFV experiment, nonturnover 'peaks' transform into a sigmoidal 'wave' (bold), as an oxidized substrate is transformed into a reduced product, such as the conversion of NAD+ to NADH. In the experiments described below, PFV will be used to ascertain whether or not chimera proteins produced are electrochemically active at gold-based electrodes, to optimize the means by which the proteins are attached to electrode surface, as well as to quantify the hypothesized boost in catalytic currents that can be achieved by conducting experiments in the presence of light.

1.5 Protein stabilization using sol-gel encapsulation. Sol-gel encapsulation will be utilized to extend the lifetime of the natural and designed protein domains.[66-69] Sol-gels are nanoporous silica – polymerized in the presence of an enzyme they develop a biocompatible form-fitting mold which greatly stabilizes the protein to denaturation. These matrices possess chemical inertness, physical rigidity, negligible swelling in aqueous solution, tunable porosity, high photochemical and thermal stability, and optical transparency. Sol-gel encapsulation has been used extensively to stabilize proteins for biosensing applications, resulting in the extension of enzymatic lifetimes from days in solution to years in after encapsulation.[70] They have also been use to stabilize several enzymes critical to biofuel production.[71,72]

2. Preliminary Data

2.1.1Modeling a two cavity, multijunction metamaterial. The optimal protein-metamaterial hybrid structure will be able to utilize all portions of the solar spectrum by taking advantage of the metamaterial's capacity to behave as a wavelength dependent beam-splitter. Wavelength bands will be spatially separated and channeled to different cavities containing different protein chimeras with absorption spectra tailored to different parts of the solar spectrum. Using a variety of software packages including Ansoft's HFSS and Sonnet Software's CST Microwave Studios on the Metamaterials Laboratory's 10-node high performance computing cluster, we have computationally demonstrated that a two cavity metamaterial can be created which has the ability to split incoming white light into two wavelength bands – 550-650 nm and 670-770 nm. This structure is a multi-wavelength-selective periodically perforated film with strong angle-independent antireflecting properties across the 550nm-700nm spectrum.that spatially splits the incident light and transmits light within different spectral bands to two different cavities within each repeating unit cell of the periodic structure. As Figure 6 indicates, we calculate that this metamaterial prototype will be effective at two-color light distribution.

2.1.2Constructing metamaterial templates. The Metamaterials Laboratory has five current projects underway, funded by the NSF, NASA, MDA, and DARPA to develop metamaterials for a broad range of

applications from polarimetric sensors to solar cells. One of these projects (NASA) involves the develeopment of polarimetric focal plane arrays that use an array of micro-polarizers made using our metamaterials that eliminates crosstalk and significantly improves the performance of polarimetric, multi-wavelength focal plane arrays. The devices are being investigated for use in NASA's upcoming ACE, CLARREO and VIIRs missions as well as for many commercial applications. Figure 7 shows four oriented metamaterial polarizers/lenses without light illuminating the backside (Fig. 7A) and with light illuminating the backside and being transmited through the structure while being polarized and focused (Fig. 7B).

2.2 Development of a charge separation domain-flavoprotein reductase domain chimera.

2.2.1 Protein scaffold design using binary patterning. Native-like structure is necessary for the structural. analysis and subsequent improvement of designed proteins. We have designed an extensive series of four alpha helix bundles which each bind two hemes using bis-histidine ligation in concert with a molten globule to native-like phase change. The original member of the family, HP-1, was the first designed heme protein which had native-like structure as a holoprotein [22]. It was designed using the principles of binary patterning, with the ligand histidines placed at internal positions between parallel helical pairs to form the binding site. To increase chemical shift dispersion, the helical sequence was extensively modified in a stepwise manner, resulting in the homotetrameric protein HP-6. Keeping the helical sequences intact, but altering the connectivity, allowed the creation of the homodimeric helix-loop-helix variant HP-7, which was expressed isotopically labeled in *E. coli* as a fusion protein with a TEV protease cleavage site. Sequences are (with helical portions underlined):


```
HP-1: CGGGEIWKQHEEALKKFEEALKQFEELKKL                                    (2)
HP-6: CGGGEIWKQHEDALQKFEEALNQFEDLKQL
HP-7:    GEIWKQHEDALQKFEEALNQFEDLKQLGGSGCGSGGEIWKQHEDALQKFEEALNQFEDLKQL
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We have completely assigned HP-7 by NMR. This also allowed the demonstration of stepwise molten globule to native-like transitioning in this protein: each helix that donates a ligand to a heme cofactor becomes native-like (see Figure 8) This is also true for differently shaped cofactors such as tetraphenylporphyrins, bacteriochlorophylls and Heme A.[23] Despite the sequence alterations used in moving from HP-1 to HP7 (over 40% of the helical residues were mutated, all on the protein surface) each protein displayed the same molten globule to native-like transition upon the addition of cofactor. This data demonstrates that binary patterning, coupled with appropriate positioning of ligand residues, is sufficient to create native-like structure in proteins with bound cofactors. With the modifications delineated below, HP-7 will form the basis for the light-gating domain at the center of the full construct.

Figure 8. Stepwise ordering in a de novo designed heme protein. (A) The apoprotein HP-7 is a molten globule, as evinced by the lack of chemical shift dispersion in ^{15}N HSQC. (B) Addition of one equivalent of heme orders two of the four helices, while the two unligated helices remain in the MG state. (C) Addition of another equivalent lifts the rest of the protein into the native-like state.

2.2.2Directing cofactor redox potentials using the cofactor structure itself. It is critical that we have the ability to control cofactor reduction potentials as electron transfer rates in our construct will principally be modulated by varying the reduction potentials at each site. The simplicity of the binding sites in our designed helical bundles, strategically placed pairs of ligand histidines in a binary-patterned context, enables the binding of a large number of different porphyrin-based cofactors. This has allowed us to create complexes with a range of almost 0.4 V of reduction potentials within the same protein context. The two naturally occurring heme cofactors with the most positive and negative intrinsic reduction potentials, hemes A and B respectively [73], differ by 176 mV when bound to HP-1 (see Figure 8A). The use ofsynthetic cofactors greatly increases this range: ferric phthalocyanine and a B heme with the vinyl groups replaced by electron withdrawing cyano groups (a gift from Kevin Smith, U of LA Dept. of Chemistry) increase it by almost 200 mV. This lack of cofactor specificity is a strength of this design method. As synthetic porphyrins have been characterized with potentials ranging over more than a Volt [74], this grants us the ability to specify the reduction potential of a cofactor at any site we choose using the covalent structure of the cofactor, an impossibility in natural proteins without extensive active site re-engineering.

2.2.3Directing cofactor reduction potentials using protein electrostatics. The transition from HP-1 to HP-7, which involves only surface residues, decreased the net charge of the protein from nearly neutral to highly anionic – the calculated net charge of the HP-7 homodimer is -15.6 at pH 7.0. This causes the reduction potential of the heme complex to be 45 mV more negative than the HP-1-heme complex. To demonstrate the further modulation of cofactor reduction potentials electrostatically, we mutated the three anionic glutamate residues in HP-7 which are predicted to be buried in the hydrophobic core of the protein[22] to alanine (see Eqn.3, mutations in grey, helices underlined):

```
HP-7:  GEIWKQHEDALQKFEEALNQFEDLKQLGGSGCGSGGEIWKQHEDALQKFEEALNQFEDLKQL     (3)
CC-9:  GEIWKQHEDALQKFEEALNQFEDLKQLGGSGCGSGGEIWKQHADALQKFAEALNQFADLKQL
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A single heme binds to the H7F mutant of each protein between the second helices of the homodimer. The three Glu→Ala mutations generate a 101 mV increase in the bound heme reduction potential (see Figure 5B). This data demonstrates that we can significantly modulate cofactor E_m's using simple electrostatics.

2.2.4Binding a phthalocyanine primary donor cofactor. As the above data demonstrates, we can readily bind heme and porphyrin cofactors using binary patterned helical bundles with two ligand histidine residues placed at buried D positions in the helical core. ZnPCs have a preference for pentacoordinate binding – in other words a ZnPC binding site needs only one ligand histidine. The homodimeric protein HP-7 binds two ZnPC cofactors into its ZnPC binding site, stacked in a noncovalent dimer in which each ZnPC binds to a single position 42 histidine residue originating from one protein monomer. This can readily be seen by the exciton splitting in the B-band region of the protein complex (See Fig. 10A) and has been further confirmed in magnetic circular dichroism experiments[44] performed by our collaborator Martin Stillman (University of Western Ontario Dept. of Chemistry).

In order to make a two-cofactor binding protein with one bis-histidine heme binding site and a mono-histidine ZnPC binding site, we constructed a new four-helix single-chain protein by connecting the gene for HP-7 to the gene for HP-7 H7F separated with a GGSGSGSGG connecting loop. We call this protein HF. In biochemical titrations, HF tightly binds one equivalent of heme and then one equivalent of ZnPC as demonstrated optically (Fig. 10B) and by magnetic circular dichroism. NMR analysis of the heterocomplex demonstrated that it has native-like structure (not shown). Thus, we have successfully utilized de novo protein design to create a matrix which can hold a single phthalocyanine isolation.

2.2.5Phthalocyanine-based charge separation in HF (functional element 2). When exposed to light in the presence of the sacrificial electron donor EDTA, the ZnPC cofactor of HF photoreduces the heme cofactor in tens of minutes (Figure 11B-C). *Net reduction of the heme must occur via a charge separated state:* the photoexcited ZnPC donates an electron to the oxidized heme (charge separation) and before the electron returns the solution EDTA reduces the $ZnPC^+$ cation. Given the reduction potentials and separation of the two cofactors, this charge separated state likely lasts only tens of nanoseconds. A small fraction of the time the ZnPC radical cation is collisionally reduced by an EDTA molecule, resulting in a tens of minutes net build-up of reduced heme. This photoreduction experiment demonstrates that we can create cofactor pairs of fixed identity and separation capable of light-induced vectorial electron transfer and charge separation.

2.3 The addition of a reductase domain (functional element 3). For the purposes of our prototype, we are constructing a platform which will support light-driven NADH production. NADH is an expensive, difficult-to-store biochemical coenzyme, and the ability to generate NADH at the site of its use will be of utility in driving a number of enzymatic reactions, principle among them the conversion of CO_2 to methanol.[75] This reaction pathway requires three enzymes which all utilize NADH as the source of reducing equivalents, and all three have been shown to be active and long-lived in a sol-gel matrix.[71]

2.3.1 Assessing the suitability of the Phthalate dioxygenase reductase (PDR) flavin domain. PDR is the best choice for the reductase component of the construct because it has been well characterized biochemically,[76,77] it has been shown to be functional after proteolytic cleavage of its iron-sulfur domain,[78] it expresses very well in *E. coli*[79] and there are high resolution crystal structures of the full-length protein.[80] We re-engineered a truncated form of this gene which lacks the iron-sulfur domain, creating a TEV protease-cleavable his_6 tagged construct. As Figure 12A shows, expression, purification and cleavage of the his_6 tag results in a

folded flavoprotein capable of reacting with NADH (the significant semiquinone absorbance at 600 nm precludes the possibility that we are titrating free flavin). This is the simplified flavoprotein which we have added to the charge separation domain.

2.3.2 Optimizing the loop connection to PDR. Connecting the light gating domain to the flavoprotein domain of PDR requires the shortest possible loop connection between the two domains which still allows both to retain their structure and function. This will ensure the minimal separation between the flavin cofactor and the donor heme and therefore the maximum electron transfer rate. To screen for structural integrity we have developed a novel method for analyzing optimal loop length (Figure 12B-C): we have added a single copy (containing two of the four helices) of HP7 to our PDR construct with a variable loop in between. We then added an equimolar amount of HP7 monomer and templating ferric heme, then screened for dimer formation using native gel analysis. The loop with the darkest heterocomplex band is that which is the most structurally stable. The three loop sequences that we have screened are GATNGG, GATNTNGG and GATNTNARGG-three different lengths of the loop which originally attached the iron-sulfur domain. The second loop is the shortest that forms a stable heterocomplex. We used this linker to connect the truncated

Figure 12. Construction of a flavoprotein reductase-helical bundle chimera. (A) An expression vector for the flavin domain of phthalate dioxygenase reductase was constructed. This shows the absorbance of the flavin cofactor as NADH is added. (B-C) A novel method for screening loops is demonstrated: a number of chimeras with different sized loops, fusing half of the helical bundle to the flavin domain, were mixed with the other half of the bundle. A disulfide was used to trap the complex. (D) The shortest loop which allows full bundle formation was used in the creation of a single chain chimera of the flavoprotein domain (PDR) and the helical bundle domain (H4).

PDR domain to a derivative of HF called H4. H4 binds two hemes, and the chimera PDR-H4 tightly binds one FMN cofactor and two hemes (figure 12 D) while retaining a unique three dimensional structure as detected by NMR. This is the protein chimera which we will optimize for use in the device.

time, temperature and buffer conditions.

2.4 Protein Film Voltammetry. At present, the PFV methodology has been successfully employed by one of us (Ellitott) to examine many complex proteins, containing several redox cofactors. Figure 13 shows voltammetric data for cytochrome c_{554} a tetraheme cytochrome, which can be directly adsorbed onto either modified gold or graphite electrodes.[81] All four heme cofactors can be easily resolved from the voltammetric response, and the signals quantified for redox-cooperativity, as well as interfacial electron transfer rate. In a similar fashion, we will be able to assess the ability to investigate the multiple redox couples of a ZnPc- bearing helical bundle, and the chimera proteins described below. Interfacial electron transfer rates can be
readily determined from such data, and the long-term robustness of such protein-electrodes will be determined as a function of

This grant concerns a prototype platform for the development of electrocatalytic systems that are driven by light-activation. To this end, potential enzymatic candidates for light-coupled devices will also be assessed electrochemically, to ensure that they are suitable for the incorporation into a light-driven design. One such candidate is bacterial sulfite/nitrite reductase (SiR), an $[Fe_4S_4]$-cluster and heme-containing protein that is capable of the 6-electron reduction of nitrite to ammonia: an attractive process for the capture of energy from the sun in the form of ammonia. Figure 14 illustrates our initial success at interrogating electrocatalysis with this enzyme. Here, a variety of nitrogen compounds can be reduced to the level of ammonia by SiR, and each with slightly different catalytic reduction couples. This data shows that SiR is another potential target for the design of ZnPc-bearing chimera, allowing for trapping of solar energy.

3.**Research Plan**

3.1.Design and Construction of a Multijunction Metamaterial (functional element 1). The light-activated NADH-producing protein chimeras will be attached to a novel metamaterial light harvesting template that serves three purposes: (1) distributing light spatially by wavelength band and channeling individual wavelength bands to different cavities containing protein chimeras with different absorption characteristics (2) serving as an electrode, and (3) serving as a heat sink to avoid the harmful effects of high temperatures. These metamaterial cavities concentrate light from far away, trapping >95% of the light of a given wavelength into a cavity within a 5 wavelength radius. This solid state light harvesting device thus promises to be over three orders of magnitude more effective that that found in green plants. This calculation of improvement does not include device lifetime. Unlike the natural light harvesting apparatus, metamaterials do not suffer from photodecomposition. If device lifetime is considered in the calculation, this factor of improvement becomes even higher.

A preliminary schematic of the device that will be designed, fabricated, characterized and optimized in this project is shown in Fig. 15. Aspects of the device will certainly change as the project progresses and as we are able to optimize the shapes of the light absorbing cavities, the array configuration of the structure, the electrical contact configuration, etc. However the structure shown in Fig. 15 will exhibit many important properties:

1. *Large light absorption:* The cavities make up a large area of the surface of the structure.

This fact, coupled with the metamaterial phenomenon of anomalous light channeling (i.e., "light whirlpool modes"), will insure that all the incident light in the spectral bands of interest are channel to and into the cavities and subsequently absorbed by the proteins.

2. Spectral band splitting and discriminate absorption: The differently shaped sets of apertures (i.e., the red and blue cavities in Fig. 15) will channel light of different spectral bands (600-700 nm and 700-800 nm) into the sets of cavities.

3. Polarization Independent: Hole arrays like that shown in Figure 15 have the ability to act as a polarizer for linearly polarized light parallel to the hole array. We will use cavities which have an L-shape, fashioned to absorb incident light of any polarization equally.

4. Manufacturability: The device uses standard materials and semiconductor fabrication equipment, therefore it may be amenable to commercialization.

The development of the template is an iterative process, starting with the design using our high performance computing cluster, followed by the fabrication, characterization and analysis and repeated until the all the materials' constants are accurately determined and the fabrication process are fully developed. Below, we describe on iteration of this Design → Fabrication → Characterization → Analysis cycle.

3.1.1 Design of the two-cavity beam-splitting metamaterial. The design and modeling of the metamaterial template will be performed as in 2.1.1 using our 10-node high performance computing cluster (HPCC) and numerous software packages including Ansoft's HFSS, CST Microwave Studios and our proprietary rigorous coupled wave analysis software. Our HPCC system is optimized to run HFSS and CST quickly and has six access terminals that can be used by researchers simultaneously to design the template.

3.1.2 Fabricating a two-cavity beam-splitting metamaterial. The fabrication of the metamaterial template will be performed at the Cornell Nanoscale Science and Technology Center (CNF) using standard semiconductor fabrication techniques that have been developed by Dr. Crouse at the Metamaterials Laboratory over the past 5 years for projects ranging from the development of polarimetric detectors for NASA and the NSF, solar cell coatings for DARPA and active optics for the MDA. The fabrication techniques we will use, all standard CMOS fabrication techniques, are described in Fig. 16:

Bottom Electrode Deposition. Starting with a 4" fused silica insulating substrate, a 50 Å thick layer of Ti (an adhesion layer) is deposited, followed by the deposition of approximately 50nm of gold using thermal evaporation.

Aperture Patterning. The two different types of apertures will be patterned at the same time. For this, the metal layers will be patterned using optical photolithography (the deep UV photolithography system at the CNF). Reactive ion etching will be used with a polymerization component

to produce straight and smooth sidewalls of the apertures.

Electrical Contact Patterning. The gold film will be further patterned to define the two separate, interdigitated sets of electrical contacts. The electrical contacts will be fabricated using the same metal layer that defines the apertures. Isolation trenches will be fabricated to separate the interdigitated electrodes that connect the two different sets of light absorbing apertures. The electrode fingers will terminate at large bond pads.

3.1.3 Characterizing the Metamaterial Template. The metamaterial template will be characterized for its reflection/absorption properties using the Metamaterials Laboratory's optical characterization equipment. For

these tests, a broadband white light source is put through a computer-controlled monochrometer which selects different wavelengths of light that is then incident upon the sample. The incident light at these different wavelengths is either absorbed or reflected, the degree to which is determined by measuring the reflected component via a silicon photodetector (for the visible to near IR spectral bands).

Reflectance/Absorption Measurement of the Metamaterial Template: The reflectance and absorption of the metamaterial will be measured as a function of wavelength, angle of incidence and polarization and compared with the simulated data. Analysis of the experimental data will include fitting the data to the simulated results by adjusting the dielectric constants of the metal and sol-gel. Rounding of the corners of the apertures and sloping of the sidewalls will be assessed and modeled to insure an accurate comparison between simulated and experimental results. This device will then be ready for the deposition of the proteins and sol-gel encapsulation layer.

3.2.Optimizing the charge separation domain:reductase chimera for use in the device.

3.2.1Moving the charge separation action spectrum to longer wavelengths. The optimized ZnPC domain constructed in 2.2.5 will function in the 600-700 nm wavelength range, matching the light trapped in one of the two cavities. In order to create a charge separation domain which will absorb the longer (700-800 nm) wavelength range we will synthesize zinc (II)-naphthocyanine, a ZnPC derivative, in which the macrocycle conjugation is extended by the addition of benzene rings, using the method of Kobayashi.[86] This has been shown to increase the cofactor action spectrum by over 100 nm, ideal for use in the longer wavelength metamaterial cavities.[45] This cofactor will be bound to the full construct in place of the ZnPC cofactor, and the binding affinity, structural specificity, cofactor reduction potentials and ability to create a charge separated state will be assessed experimentally as above.

3.2.2 Adding a donor cofactor. We have described the creation of a primary donor-acceptor dyad protein, capable of transient charge separation which is made vectorial by the addition of a sacrificial electron donor (see Figure 11B). To this we have added a flavoprotein reductase domain using an optimized connecting loop, thus adding the domain responsible for chemical coupling. This simple dyad configuration, with the electrode acting as the donor site, has similarities to $Ru(bpy)_3$-sensitized nanocrystalline TiO_2 electrodes. In these constructs, Durrant *et al* have shown that the addition of an explicit donor site serves as an electron sink which can take up an electron from the electrode in the absence of light, limiting short-circuiting reactions and accelerating turnover under low light conditions.[82-84]

To similarly optimize this domain for attachment to the rest of the device, we will add a B-type heme donor site 5 Å from the central phthalocyanine, distal to the PDR domain.. This constrains the ZnPC at optimal distances from the donor and acceptor heme sites ensuring stable and long-lived directional charge separation, and furthermore constrains the donor and acceptor molecules at a sufficient distance (19 Å) that charge recombination by direct electron transfer will be slowed to rates of >1 min^{-1}. Residue identities in the helical extension will again be selected using binary patterning and the results of our heme binding site analysis in combination with our knowledge of the bicomplex structure.

As in the two cofactor bundle HF, the differing ligation motifs of the two cofactor types allows the targeting of each to its correct location: the hexacoordinate heme and/or ferric porphyrin cofactors bind solely to the bis-histidine site and the obligately pentacoordinate ZnPC binds solely to the mono-histidine site. Cofactor binding, structural specificity, and complex stability will all again be assessed using the methods used for the bicofactor bundle. Structural specificity will again be determined using NMR. If necessary, further rounds of iterative redesign will be used to optimize the new tricofactor complex.

The charge separated state ($D^-PD\ A^+$) in the central domain of Figure 1C must last sufficiently long that the second electron transfer to the flavin semiquinone radical to the donor cofactor outcompetes charge recombination. This second electron transfer is predicted based on the modeled FMN-to-donor distance to have a rate of 50-500 ns. This charge separation domain is designed to produce tens of μs charge separated state lifetimes. While the electrode will reduce the donor heme in the full device, this will be assessed at this

stage by adding a 1:1 mixture of heme B and dicyano heme B to the two heme binding sites. The solution potential will be lowered until the dicyano hemes will be reduced while the heme B cofactors will remain oxidized. The quarter of proteins which have a dicyano heme at the donor site and a heme B at the acceptor site will be capable of charge separation in the flash experiments described below, while the other variations will either be unreactive or will exhibit charge recombination on a timescale (<ns) too fast to be detected. Testing for stable charge separation at this point allows us examine the system while the system is still relatively simple, and redesign the protein if necessary to optimize the light-gating domain for its intended purpose.

3.2.3 Optimizing the reduction potentials for use in the device. Reduction potentials for the bound heme cofactors will be determined as in Figure 9 using potentiometry [85]. There is a large window in which the acceptor heme cofactor should be functional, as it needs to have a reduction potential lower than that of the flavin in PDR (-190 mV). Every helical bundle heme protein we have designed to date has reduction potentials below this value, but if this is not the case, we will modulate the cofactor reduction potentials either by altering the covalent structure of the bound hemes (for example, using monocyano heme B) or electrostatically by altering surface charges in the vicinity of the cofactor as in Figure 9B.

3.3Optimizing protein attachment to electrodes. As the ultimate goal is the generation of highly conductive protein-electrode devices, we must investigate and optimize the interface between the protein domain with the metamaterial surface. The resulting covalent assembly is an obligate part of such a device, and here we describe electrochemical and physical measurements that will be made in order to satisfy the following criteria: *facile conductivity*, *robustness*, and *flexibility*. Below, we describe the strategies that can be applied to generate highly conductive assemblies on the gold metamaterial electrode using Sharpless 'click chemistry' attachment strategies.

A general strategy will be employed to investigate the conductivity and robustness of the protein-electrode interaction, with initial experiments focusing on the heme-bearing HP7 and ZnPc/heme-containing HF constructs. To provide controls for the comparisons with the ultimate protein:metamaterial assembly, the enzymatic components will also be separately assessed to have detailed catalytic parameters (turnover rates, temperature and chemical robustness), *e.g.*, for the PDR flavin reductase's ability to produce NADH.

3.3.1Creating click compatible proteins. The experiments will optimize the protein-electrode interaction through 'click' chemistry between uniquely engineered azido-homoalanine in the helical bundle of the ZnPC or heme-bearing module, and surface-localized terminal alkynes. This strategy has been enormously successful in the generation of covalently tethered redox catalysts,[88-90] and has proven to result in a highly conductive triazole bridge.[91] One of us (Koder), in collaboration with Jamie Link at (Princeton University School of Engineering), has expressed HF mutants containing single methionine residues in an engineered strain of *E. coli* which incorporates one half of the 'click' chemistry pairing as an azido side chain, azidoalanine, at methionine positions with near 100% efficiency.[87] In HF, we are already using this to attach ferrocene molecules capable of acting as the donor molecule in a simplified version of the charge separation domain depicted in Figure 1C. Unique methionine residues will be inserted into loop sequences at the site where electrode attachment is desired, the proteins expressed in the azidohomoalanine *E. coli* strain, and the purified proteins will be attached to alkynated electrode surfaces.

3.3.2Protein attachment. Alkyne-bearing electrodes will be prepared by the conversion of the cognate alcohol into a thiol[94] and subsequent adsorption onto gold via Whiteside self-assembled monolayer chemistry. It is anticipated that control of surface coverage will be a requirement to prevent steric constraints that might occur: In particular, in the case of gold-based "clicked" proteins, it is presumed that appropriate diluting thiols will also need to be co-deposited upon the electrode surface to ensure that discrete (isolated) nature of the proteins. Such passivating molecules will be screened in parallel as a function of length and terminal functionality (hydrophobic, acidic or alcohol). Finally, with covalently tethered protein:electrode constructs in hand, we will compare the catalytic parameters determined in the presence of saturating concentrations of substrate (to minimize the diffusional constraints of concentration at the surface) to similar parameters for the same protein, but without tethering. Finally, while the previous studies can be viewed as "dark" reactions, in contrast to our goal of light-activated chemistry, catalytic currents will be determined in the presence of light, to quantify the extent of the light activation.

3.4 Assembling and testing the full device. Two sets of optimized protein chimeras will be prepared, one which utilizes ZnPC as the primary donor and one which uses Zinc(II)-naphthylcyanine. These will be attached, one at a time, to the appropriate metamaterial cavity using the optimal attachment strategy determined in section 3.2. It is now that the fact that the cavities are separately wired becomes important – while the first cavity is being coated with its matching protein chimera, a large bias current will be applied to the other, preventing protein attachment. This method has been used to nanopattern small molecules on electrodes using click chemistry.[95]

The hybrid protein-metamaterial construct will be assessed as follows:
Test 1: Intensity of the *reflected* and scattered light as a function of scattering angle and wavelength for normally incidence beam of light. This test will provide the absorption of the film for normal incidence light as a function of wavelength. Absorbed light is what gets trapped into the metamaterial cavities. This test allows us to quantitate the metamaterial performance when the cavity dielectric is altered by protein attachment.

Test 2: Ability of the device to support light-driven NADH production. The product NADH is fluorescent, allowing rapid and sensitive detection of any device turnover. The intensity and special localization of the product will be measured as a function of applied wavelength. Localization of the fluorescent signal at and near the cavities will demonstrate light channeling and light-driven enzyme turnover.

Anticipated Outcomes
The goal of this project is to optimize a simple, robust, oxygen insensitive light-induced charge separation domain which can be modularly attached to other designed or natural protein domains and act as a center for light activated electron extraction and/or injection. This is coupled with new advances in solid state photonics to create a new hybrid material which combines the best features of nanotechnology and solid state physics to create a multifunctional biofuel generating device. This device, and its various constructs, is an enabling technology which will be used by us and others in several areas: Our first generation construct, the focus of this proposal, is connected to an electrode as a light-driven source of reduced NADH, biology's version of molecular hydrogen. The next generation construct will be the connection of this same protein domain to a soluble hydrogenase, thus creating a simple, single chain protein capable of light-driven hydrogen evolution. Future plans include uses ranging CO_2 sequestration (using formate dehydrogenase (28)) to direct photocurrent generation.

These metamaterials can be designed to absorb white light and separate different wavelength ranges into different cavities. The long-range goal of this project is to coat each cavity with different proteins, each of which is designed to utilize the wavelength targeted to that cavity. On the end of each charge separation domain will be placed different catalysts, each of which drives a different reaction in the metabolic formation of a high value product. Those enzymes with high activation energies will be placed in the low wavelength (high energy) cavities, while lower activation energy catalysts will be coupled to their matching longer wavelength cavities. This is a metabolic version of a *multi-junction solar cell*, a long sought-after solar h can have efficiencies as high as 90% in theory (compared to the best solar panels now available, which approach 20% efficiency).

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Publications from Grant


Koder PLOS paper.pdf


Light localization, photon sorting, and e


Near field light localization and photc


Rational design of a phthalocyanine bindir

DURIP Award FA9550-12-1-0010

The PO does not have access to the grant document. The title of the grant is Laser Flash Transient Absorption and Fluorescence Spectrometer for Research and Education. There is not final report for this grant either. The grantee will not respond to requests for submission of his final report.

DURIP: Laser Flash Transient Absorption and Fluorescence Spectrometer for Research and Education

This proposal should be directed to:

Mathematics, Information and Life Sciences: Bioenergy Dr. Walt Kozumbo AFOSR/NL (703) 696-7720
DSN 426-7720 FAX (703) 696-8449
E-Mail: walter.kozumbo@afosr.af.mil

Abstract

We are requesting funds for the acquisition of a state-of-the-art LP920 laser flash-activated

nanosecond transient absorption spectrometer from Edinburgh Instruments. This newly available machine is the first complete device manufactured for this purpose – previously researchers had to build their own transient absorption spectrometers. **The system will dramatically enhance the activities in several interdisciplinary projects that have been funded by, or will be submitted to, the Department of Defense:** The PI of this proposal, Ronald Koder, has received funding as the lead PI on an AFOSR project that concerns the development of a series of de novo designed charge separation proteins, their attachment to biofuel-generating natural and designed enzymatic domains, and their deposition on a light harvesting metamaterial electrode. He is also academic PI on a DARPA phase I SBIR project centered on the creation of multi-junction dye-sensitized solar cells, PI on a pending grant with the Army regarding the creation of designed protein blood substitutes and preparing a proposal to the NRL on the creation of metamaterial biosensors. The co-PI of this project, David Crouse, is a co-PI on the AFOSR biofuels project and the academic PI of a phase II SBIR project funded by the MDA on the creation of a metamaterials-based infrared polarimetric sensor array. **In addition to supporting multiple Defense-related projects at the City College of New York (CCNY), the transient absorption spectrometer will be incorporated into the educational activities in the Department of Physics.** Transient absorption measurement of electron transfer events in complex biological and synthetic nanomaterials will be incorporated into both the Biological Physics course which the PI teaches and the new Solar Energy Physics course which the PI is developing. **Therefore, the funding of this DURIP proposal will significantly enhance both research and research-related education in the Department of Physics at CCNY.**

Budget

The full amount of the requested funds for the LP920 and the accessories necessary for its purpose, including a peltier temperature controlled sample holder and a tunable laser totals $US $326,962. Edinburgh Instruments is the only company in the world which sells a complete device with the required capabilities. The recent availability of a commercial transient absorption spectrophotomer, capable of taking complete time-dependent absorbance spectra with 5 nanosecond time resolution, has sparked a rapid increase in the research output of several photochemistry groups across the country. An itemized quotation is listed below. The quotation was provided by Nicholas Leeson, a sales engineer at Edinburgh Instruments. Details of the LP920 can be found at http://www.edinst.com/lp920.htm or by contacting Mr. Leeson by phone at +44 (0) 1506 425 300.

Description	Price ($)	Discount	Price with Discount ($)
LP920-KS (with Ext.Red ICCD, and PMT detector removed)	198,730	20%	158,984
Extended Range Photomultiplier Assembly	10,200	20%	8,160
Grating for Camera, 150 grooves/mm - 500nm	2,444	20%	1,955
Oscilloscope TDS3032C	12,750	20%	10,200
50W Halogen lamp	4,250	20%	3,400
Diffuse Reflectance Option	2,656	20%	2,125
Quasi Co-linear Optics	4,038	20%	3,230
TE Cooled Sample Holder - Extended Range	12,219	20%	9,775
Second Site User License of L900 software	1,275	20%	1,020
Stopped Flow Cell, computer controlled operation	26,563	20%	21,250
Additional Excitation Monochromator TMS300-X	12,963	20%	10,370
Vibrant 355 II	110,063	20%	88,050
Optical Breadboard with Frame	8,054	20%	6,443
Shipping, DDU New York.			2,000
Total ($, US Dollars)			$326,962

Supporting Information

Transient Absorption Spectroscopy

Transient absorption spectroscopy is the central experiment in the analysis of photochemical processes (*1*). The experimental consists of a short (<5 ns) intense excitation laser light pulse which

is absorbed by a photoactive molecule followed by the quantitation of the sample's response to this excitation by observing time-dependent changes in its absorption spectrum. This response may be, among others, a cascade of electron transfers in photosynthetic (*2*) or artificial photosynthetic systems (*3, 4*), conformational changes in stillbene-based synthetic chemical systems and flavin-based photosensing BLUF circadian rhythm proteins (*5*), and ligand detachment and reassembly in synthetic metal-ligand complexes and natural heme protein-ligand complexes such as those involved in oxygen transport (*6, 7*).

In the typical experimental setup, a fraction of molecules in a sample (ranging from 0.1% to tens of percent) is promoted to an electronically excited state by means of an excitation (or pump) pulse. A weak probe pulse (i.e. a pulse that has such a low intensity that multiphoton/multistep processes are avoided during probing) is sent through the sample and used to track changes in absorbance either as a function of time at a single wavelength or as a function of wavelength at a time delay τ. By changing both the time delay τ and the wavelength λ, the function $\Delta A(\lambda,\tau)$ is obtained. $\Delta A(\lambda,\tau)$ contains information on the dynamic processes that occur in the photoactive system under study, such as excited state energy migration, electron and/or proton transfer processes, isomerization, and intersystem crossing. In order to extract this information, global analysis procedures may be applied. One advantage of time-resolved absorption spectroscopy over time-resolved fluorescence is that with the former, the evolution of non-emissive states and dark states can be investigated. This is of particular importance in artificial photosynthesis where dark (nonemissive) states can play a number of vital roles.

In general, a ΔA spectrum contains contributions from various processes (*2*):

2. The first contribution is by **ground-state bleach**. As a fraction of the molecules has been promoted to the excited state through the action of the pump pulse, the number of molecules in the ground state has been decreased. Hence, the ground-state absorption in the excited sample is less than that in the non-excited sample. Consequently, a negative signal in the ΔA spectrum is observed in the wavelength region of ground state absorption.

3. The second contribution is by **stimulated emission**. Stimulated emission will occur only for optically allowed transitions and will have a spectral profile that (broadly speaking) follows the fluorescence spectrum of the excited chromophore, i.e., it is Stokes-shifted with respect to the ground-state bleach. During the physical process of stimulated emission, a photon from the probe pulse induces emission of another photon from the excited molecule, which returns to the ground state. The photon produced by stimulated emission is emitted in the exact same direction as the probe photon, and hence both will be detected. Note that the intensity of the probe pulse is so weak that the excited-state population is not affected appreciably by this process. Stimulated emission results in an increase of light intensity on the detector, corresponding to a negative ΔA signal. In many chromophores including bacteriochlorophyll (*8*), the Stokes shift may be so small that the stimulated emission band spectrally overlaps with ground-state bleach and merges into one band.

4. The third contribution is provided by **excited-state absorption**. Upon excitation with the pump beam, optically allowed transitions from the excited (populated) states of a chromophore to higher excited states may exist in certain wavelength regions, and absorption of the probe pulse at these wavelengths will occur. Consequently, a positive signal in the ΔA spectrum is observed in the wavelength region of excited-state absorption. Again, the intensity of the probe pulse is so weak that the excited-state population is not affected appreciably by the excited-state absorption process.