FINAL REPORT *DE-FG02-04ER46156***Title: Novel Photonic & Electronic BioMolecular Materials****Investigators: J.G. Saven, W.F. DeGrado, M.J. Therien & J.K. Blasie: University of Pennsylvania****I Project overview**

Cofactors, which can range in size from single atoms to small macromolecules, are compounds that confer function to many biological proteins. They can be either transiently or permanently associated with the protein, and in the latter case are referred to as prosthetic groups. Cofactors include metal atoms, flavins, quinones, metalloporphyrins, etc. Artificial protein modules, based on both α -helical bundle and β -sheet structural motifs, have been designed to incorporate biological cofactors, based on the “bio-inspiration” provided by the known high-resolution structures of the cofactor’s local environment within the generally much larger soluble protein or membrane protein. While such modules may be much less complex structurally and considerably more robust, they generally can, at best, only mimic the functions of the natural proteins. However, consideration of an artificial protein as a non-biological heteropolymer coupled with computational design can now lead to modules capable of incorporating synthetic non-biological cofactors, thereby creating peptide-based systems with novel properties not exhibited by Nature. For example, cofactors possessing extended π -electron systems can now be designed and tailored, with appropriate donors, acceptors and constituents, to exhibit selected nonlinear optical (NLO) responses and light-induced electron transfer (LIET) over large distances. Importantly, the interior of the artificial protein scaffold can be used to control the conformation, solubility, position, orientation, local environment, and indeed the properties of the cofactor within the peptide (Figure 1).

However, an ensemble of such artificial protein modules with these designed novel properties cannot form a material unless they can be ordered in one, two or three dimensions on macroscopic length scales (Figure 2). Gaining control over such intermolecular ordering would then result in a material whose macroscopic properties derive at a minimum from the incoherent superposition of the designed molecular properties of the ensemble, with the additional possibility of the ensemble generating coherent phenomena based on these properties. Again as importantly, the exterior of the artificial protein scaffold can be used to control the peptide's supramolecular assembly into sufficiently ordered nanophase materials whose macroscopic behavior arises from such novel properties (Figure 2). In addition, the protein scaffold can be employed to control the degree of interaction or non-interaction between neighboring cofactors in the ordered nanophase material. The latter (non-interaction) may be especially important to ensure that the designed microscopic properties at the molecular level of the peptide-cofactor complex translate into the desired macroscopic phenomena in the ordered material (Cacialli et al. 2002;

Swager 2002). For example, in nonlinear optics, the lack of control of the relative orientations and interactions between chromophores is thought to be responsible for the inability of chromophore ensembles to achieve their predicted optimal material properties (Dalton et al. 1997; Liakatas et al. 2000). In the case of light-induced electric charge separation, the inability to achieve purely uni-directional charge separation over large nano-scale distances is thought to contribute substantially to their relatively low efficiencies (Coakley et al. 2004).

Engineering the structure of such artificial peptide-cofactor modules requires careful design of peptide primary sequences, especially when non-biological cofactors are involved, because the “bio-inspiration” is absent. This is a key requirement for dipolar "push-pull" NLO cofactors and for "donor-bridge-acceptor" LIET cofactors, both based on linearly extended π -electron systems. For example, the desired target structure of a water-soluble four-helix bundle incorporating the "push-pull" NLO cofactor “**RuPZn**” in a pre-determined conformation is shown in

Figure 1, where the peptide backbone structure, metal coordination, and noncovalent peptide-cofactor interactions must each be optimized simultaneously.

As mentioned above, the structural motifs chosen for the artificial protein modules can have properties that are significantly more robust than those evident for natural functional proteins. However, the interior of the module's scaffold may not be so stable to the supramolecular assembly process required to form a sufficiently ordered ensemble material. Thus, it is important to monitor structurally the various stages of the self-assembly process starting from the designed peptide, e.g. a particular α -helical bundle, to the incorporation of the non-biological cofactor, through to the supramolecular assembly of the peptide-cofactor complexes to a material ordered on a macroscopic scale. Since the desired material properties need not necessarily require long-range periodic order, as opposed to orientational order, in one, two or three dimensions, structural determination in both the absence or presence of such “crystallinity” is essential.

II Nonlinear Optics (NLO):

II.A Introduction NLO:

Although nonlinear optical responses were first discovered in semiconductors and inorganic crystals, organic materials have been under steady development since the mid-1980's. Potential advantages of organic over inorganic materials include large and ultrafast NLO responses, low dielectric constants, processability, and enormous design flexibility allowed by molecular engineering. In particular, organic chromophores can be designed to possess highly polarizable, linearly extended π -electron systems that, in turn, can be tailored to optimize the NLO response, e.g., their molecular hyperpolarizability. For example, dipolar "push-pull" chromophores, coupling electron donor and acceptor components via a conjugated bridge, can exhibit exceptionally large molecular hyperpolarizabilities (Risser 1993; Kang 2007). Importantly, those possessing, for example, metal-porphyrin donors and metal-polypyridyl acceptors can exhibit large hyperpolarizabilities at the longer wavelengths (800-1500 nm) relevant to optical communications applications (Uyeda et al. 2002; Duncan et al. 2007). Dipolar chromophores exhibiting large molecular hyperpolarizabilities have the potential for a wide range of photonic device applications, including frequency doublers, frequency converters and electro-optic modulators, based on the macroscopic 2nd-order nonlinear response of suitably organized ensembles. However, such ensembles must be engineered to possess a non-centrosymmetric arrangement of the chromophores and minimize inter-chromophore interactions in order to optimize the macroscopic response (Dalton 2007). Traditionally, the former has been achieved via either incorporation of the chromophore into a polymer matrix and orienting the chromophores via an applied electric field (i.e., "poling") or via directed-

assembly techniques, for example by incorporating the chromophore either into or among amphiphilic molecules at an interface between polar and non-polar media followed by Langmuir-Blodgett deposition to form multilayer assemblies. Antiparallel dipolar interactions at high chromophore densities ultimately limit the macroscopic response, presumably by reducing the necessary acentric order within the ensemble (Harper 1998; Reyes-Esqueda 2001). More recently, the development of octopolar chromophores has become increasingly attractive, based on group theoretical and quantum mechanical calculations in the early 1990's suggesting their potential for exceptionally large molecular hyperpolarizabilities (Zyss 1993; Maury 2005; Claessens 2006; Duncan et al. 2008b). The vector part of the hyperpolarizability tensor vanishes in these chromophores leaving only the octopolar contribution, thereby providing several potential advantages. These include, for example, circumventing the electrostatic interactions that drive bulk-phase centrosymmetry at high chromophore concentrations, and effectively removing light polarization requirements in NLO device applications. Approaches to achieving such acentric ordering have included tailoring the chromophore's peripheral substituents to promote dendrimer formation in 2-3 dimensions, organization within a liquid crystal phase, or facilitation of their non-bonded self-assembly at interfaces to form thin films (Maury 2005; Claessens 2006).

II.B Progress Report

Here we summarize our research accomplishments. In the Progress Report below, we opt not to detail all studies from the previous funding period and present four major recent advances.

- Symmetric chromophores **RuPZnRu**, **PZnRuPZn**, and **PZnEnPZn** exhibit D_2 -symmetric or "twisted" sub-populations in isotropic solution. This octopolar symmetry of these chromophores yields large molecular hyperpolarizabilities.
- The "push-pull" **RuPZn** chromophore group can be employed as a building block to create octopolar chromophores exhibiting the largest octopolar molecular hyperpolarizabilities known.
- Artificial protein modules can be designed to incorporate vectorially the dipolar chromophore **RuPZn** and subsequently direct their organization into 2-D ensembles possessing the required acentric order and high in-plane chromophore densities resulting in a coherent macroscopic 2nd-order NLO response.
- A single four-helix protein has been computationally designed that encapsulates the **RuPZn** cofactor with near nanomolar affinity.
- Artificial proteins can be computationally designed to self-assemble into macroscopic 3-D periodic ensembles (crystals) possessing non-centrosymmetric P6 symmetry.

Following the listing of publications is a description of some of our current progress and studies near completion. Special emphasis is given to the unique NLO chromophores and their corresponding hyperpolarizable proteins designed, characterized, and studied by the group.

II.B.1 Publications [peer-reviewed, acknowledging DOE support, 2007-2010; P.I.'s and year in boldface]:

- 1) 3-D Structure and Dynamics via Molecular Dynamics Simulation of a *De Novo* Designed, Amphiphilic Heme Protein Maquette at Soft Interfaces. H. Zou, J. Strzalka, T. Xu, A. Tronin and J.K. **Blasie**. *Phys. Chem. B*, **2007**, 111: 1823-1833.
- 2) Molecular Engineering of Intensely Near-Infrared Absorbing Excited-States in Highly Conjugated Oligo(Porphinato)Zinc-(Polypyridyl)Metal(II) Supermolecules, T. V. Duncan, T. Ishizuka, and M. J. **Therien**, *J. Am. Chem. Soc.* **2007**, 129: 9691-9703.
- 3) *De Novo* Design of a Single Chain Diphenylporphyrin Metalloprotein, G. M. Bender, A. Lehmann, H. Zou, H. Cheng, H. C. Fry, D. Engel, M. J. **Therien**, J. K. **Blasie**, H. Roder, J. G. **Saven**, and W. F. **DeGrado**, *J. Am. Chem. Soc.* **2007**, 129: 10732-10740.

- 4) Computational Protein Design: Structure, Function and Combinatorial diversity. S-G. Kang and J. G. **Saven**, *Current Opinion in Chemical Biology*, **2007**, 11: 329-334.
- 5) Structure and Dynamics of an Extended Conjugated NLO Chromophore within an Amphiphilic 4-Helix Bundle Peptide by Molecular Dynamics Simulation, H. Zou, M. J. **Therien**, and J. K. **Blasie**, *J. Phys. Chem. B*, **2008**, 112, 1350-1357.
- 6) Molecular Symmetry and Solution Phase Structure Interrogated by Hyper-Raleigh Depolarization Measurements: Insights for Elaborating Highly Hyperpolarizable D_2 -Symmetric Chromophores, T. V. Duncan, K. Song, S.-T. Hung, I. Miloradovic, A. Persoons, T. Verbiest, M. J. **Therien**, and K. Clays, *Angew. Chemie, Int. Ed. Engl.*, **2008**, 47: 2978–2981.
- 7) Using alpha-Helical Coiled-Coils to Design Nanostructured Metalloporphyrin Arrays. K. McAllister, H. Zou, F. Cochran, G. Bender, A. Senes, H. Fry, V. Nanda, P. Keenan, J. Lear, J.G. **Saven**, M.J. **Therien**, J.K. **Blasie**, and W.F. **DeGrado**. *Journal of the American Chemical Society*, **2008**. 130: 11921-11927.
- 8) Computational Design of Four-Helix Bundle Proteins That Bind Nonbiological Cofactors, A. Lehmann and J.G. **Saven**, *Biotechnol. Prog.*, **2008**. 24: 74 -79.
- 9) How to Improve Your Image, M. J. **Therien**, *Nature (London)* **2009**, 458: 716-717.
- 10) Portable UV-Visible Spectrometer for Measuring Absorbance and Dichroism of Langmuir Monolayers at Air-Water Interfaces, A. Tronin, J. Strzalka, V. Krishnan, H. C. Fry, M. J. **Therien**, I. Kuzmenko, J. K. **Blasie**, *Rev. Sci. Instr.* **2009**, 80: 033102.
- 11) Predicting the Frequency Dispersion of Electronic Hyperpolarizabilities based on Absorption Data and Thomas-Kuhn Sum Rules, X. Hu, D. Xiao, S. Keinan, I. Asselberghs, M. J. **Therien**, K. Clays, W. Yang, and D. N. Beratan, *J. Phys. Chem. C*, **2010**, 114: 2349–2359.
- 12) Computational Design and Elaboration of a *De Novo* Heterotetrameric alpha-Helical Protein that Selectively Binds an Emissive Abiological (Porphinato)zinc Chromophore, H. C. Fry, A. Lehmann, J. G. **Saven**, W. F. **DeGrado**, and M. J. **Therien**, *J. Am. Chem. Soc.* **2010**, 132: 3997-4005.
- 13) Computational Protein Design: Advances in the Design and Redesign of Biomolecular Nanostructures. J. G. **Saven**, *Current Opinion in Colloid and Interface Science*, **2010**. 15: 13-17.
- 14) Interferometric Enhancement of X-ray Reflectivity from Unperturbed Langmuir Monolayers of Amphiphiles at the Liquid-Gas Interface. V. Krishnan, J. Strzalka, J. Liu, C. Liu, I. Kuzmenko, T. Gog and J.K. **Blasie**, *Phys. Rev. E* **2010**, 81: 021604-1-10.
- 15) Control of the Orientational Order and Nonlinear Optical Response of the “Push-Pull” Chromophore RuPZn via Specific Incorporation into Densely-Packed Monolayer Ensembles of an Amphiphilic 4-Helix Bundle Peptide: Part I - Characterization of the Cofactor-Peptide Complex in Monolayer Ensembles, V. Krishnan, A. Tronin, J. Strzalka, H. C. Fry, M. J. **Therien**, and J. K. **Blasie**, *J. Am. Chem. Soc.* **2010**, 132(32):11083-11092.
- 16) Control of the Orientational Order and Nonlinear Optical Response of the "Push-Pull" Chromophore RuPZn via Specific Incorporation into Densely-Packed Monolayer Ensembles of an Amphiphilic 4-Helix Bundle Peptide: Part II - Second Harmonic Generation at High Chromophore Densities, G. Gonella, H.-L. Dai, H. C. Fry, M. J. **Therien**, V. Krishnan, A. Tronin, and J. K. **Blasie**, *J. Am. Chem. Soc.* **2010**, 132 (28): 9693–9700.
- 18) The Roles of Molecular Structure and Effective Optical Symmetry in Evolving Dipolar Chromophoric Building Blocks to Potent Octopolar Nonlinear Optical Chromophores, T. Ishizuka, L. E. Sinks, K. Song, S.-T. Hung, A. Nayak, K. Clays, and M. J. **Therien**, *J. Am. Chem. Soc.* **2011**, 133: 2884-2896.

Other Publications [acknowledging DOE support, 2007-2010; P.I.'s and year in boldface]:

- 1) Computational aspects of protein design. J.G. **Saven**. Book chapter in Computational Structural Biology (eds. Manuel C. Peitsch and Torsten Schwede), **2008**.
- 2) Computational approaches to protein engineering. J.G. **Saven**. Book chapter in Protein Engineering Handbook, (ed. Stefan Lutz and Uwe Bornscheuer, John Wiley), **2008**.

- 3) Computational Protein Design. A. Lehmann, C.J. Lanci, T.J. Petty II, S.-G. Kang and J.G. **Saven**. Book chapter in Protein Folding and Misfolding (ed. V. Munoz; Royal Society of Chemistry), **2008**.

II.B.2 D₂-Symmetric NLO Chromophores for Novel 2- and 3-D Ordered Electro-Optic Materials.

Dynamic hyperpolarizabilities (β_i values) can depend sensitively upon molecular structure.(Beratan 1991; Prasad et al. 1991) Whether the nonlinear optical (NLO) chromophore is an electronically asymmetric, dipolar, donor-bridge-acceptor (D-Br-A) molecule, or an electronically symmetric, yet noncentrosymmetric, D-Br-D or A-Br-A octopolar structure, oscillator strength and the extent to which charge is redistributed in electronic transitions depend upon the coupling of D and A to the conjugated Br. In virtually all known NLO chromophores, the D, Br, and A orientations are not rigidly fixed; hence, the observed electronic coupling between these units results from a subset of the population of conformations in condensed-phases.(Chauchard et al. 1995; Marder et al. 1997; Verbiest et al. 1997; Dalton et al. 1999; Wolff et al. 1999) With octopolar chromophores, the required noncentrosymmetry restricts the spatial arrangement of D, Br, and A moieties: virtually all known NLO octopoles possess either D_{3h} or T_d symmetry.(Brédas et al. 1992; Zyss et al. 1993a; Zyss et al. 1993b; McDonagh et al. 1999; Le Bozec et al. 2001) D_2 and D_{2d} symmetries could be exploited in single-oscillator octopolar NLO chromophores,(Blanchard-Desce et al. 1999) or octopolar compounds featuring metal-to-ligand charge-transfer transitions.(Sénéchal et al. 2002; Maury et al. 2004) However, no interesting chromophore yet possess such symmetries.(Ostroverkhov et al. 2001) In this regard, the few established NLO octopoles having D_2 symmetry have weakly coupled oscillators in which charge redistribution is provided by through-space delocalization.(Bartholomew et al. 2002) We have shown recently that strongly coupled D_2 symmetric oscillators provide a motif for potent octopolar NLO chromophores, and we have demonstrated the utility of hyper-Rayleigh light scattering (HRS) measurements to interrogate chromophore conformeric populations having NLO-active D_2 and D_{2d} symmetries at ambient temperature in solution.(Duncan et al. 2008b)

HRS is intrinsically sensitive to symmetry, and at the molecular level, a hyperpolarizable molecule must be noncentrosymmetric. When a substantial HRS signal is observed from an isotropic solution of molecules, a fraction of these must possess noncentrosymmetric structures. This property has been utilized to characterize the electrooptic characteristics of nondipolar chromophores **RuR₂PZnRu**, **OsR₂PZnOs**, **OsPZnOs**, **PZnRuPZn**, and **PZnOsPZn** (Figure 3). These non-emissive species belong to a class of supermolecules in which (porphinato)zinc(II) (**PZn**) and metal(II)polypyridyl (**M**) units are connected via an ethyne bridge that mixes effectively PZn-based and metal polypyridyl charge-resonance absorption oscillator strength. The respective charge transfer transition dipoles of these chromophores are aligned along the highly conjugated molecular axis.(Uyeda et al. 2002; Duncan et al. 2004) These structures possess extensive excited-state interpigment electronic interactions and possess high oscillator strength excited-state absorptions over broad spectral windows of the near IR (NIR).(Duncan et al. 2004) Related chromophores such as **RuPZn** (

Figure 1) and **RuPZnA** (Figure 3) manifest extraordinarily large β_i values at telecommunication relevant wavelengths.(Uyeda et al. 2002) Interestingly, **RuR₂PZnRu**, **OsR₂PZnOs**, and **OsPZnOs** possess nonzero dynamic hyperpolarizabilities.(Duncan et al. 2004) Table 1 highlights NLO responses observed for these compounds at an incident irradiation wavelength (λ_{inc}) of 800 nm. Frequency-resolved fs HRS measurements demonstrate that multiphoton fluorescence does not contribute to these observed HRS signals.(Olbrechts et al. 1998) The hyperpolarizabilities greatly exceed the values expected from solvent electric-field fluctuations at a centrosymmetric molecule.(Kielich et al. 1971; Yaliraki et al. 1999)

These compounds in solution must therefore feature a conformeric subpopulation having dipolar or octopolar symmetry. HRS depolarization ratios (ρ values) (Heesink et al. 1993; Kaatz et al. 1996) indicate

the nature of the NLO response: for a purely octopolar chromophore, the lower limit of $\rho = 1.5$ is expected, while for an ideal dipolar ($C_{\infty v}$) molecule, $\rho = 5$. (Cyvin et al. 1965; Clays et al. 2000) This latter ρ value is attained only in the limit of an HRS experiment carried out using an infinitely small numerical aperture for a hypothetical chromophore that possesses no finite off-diagonal tensor contributions to β_{λ} . HRS depolarization ratios for classic octopolar chromophores such as the tricyanomethanide anion (*i.e.*, crystal violet, **CV**), and 1,3,5-trihydroxy-2,4,6-trinitrobenzene have been determined to be $\rho = 1.5$; (Verbiest et al. 1993; Verbiest et al. 1994) in contrast, disperse red 1 (**DR1**), a benchmark dipolar chromophore, $\rho = 3.4$ is observed under identical experimental conditions (Table 1).

Compound ^b	β_{HRS}^c	β_{λ}^d	ρ
CV	208	340	1.4 ± 0.1
DR1	22^e	54^e	3.4 ± 0.3^e
Ru-PZn-A	90 ± 10	220 ± 20	2.9 ± 0.3
Ru-R ₄ PZn-Ru	165 ± 20	90 ± 10	1.7 ± 0.5
Os-R ₄ PZn-Os	200 ± 20	110 ± 10	1.9 ± 0.4
Os-PZn-Os	240 ± 20	130 ± 10	1.8 ± 0.4
PZn-Ru-PZn	123	550	1.3
PZn-Os-PZn	36 ± 3	160 ± 10	1.4

Table 1. Dynamic Hyperpolarizabilities [β_{λ} Values, ($\times 10^{-30}$ esu)] and Depolarization Ratios (ρ Values) Determined via Hyper Rayleigh Light Scattering Experiments at $\lambda_{\text{inc}} = 800$ nm.^a

^aConditions: T = 20 °C, solvent = CH₃CN. β_{λ} values are independent of frequency modulation of the fundamental beam. ^bCV = crystal violet (purely octopolar); DR1 = disperse red 1 (largely dipolar). ^cDefined as [$\langle \beta_{\text{ZZZ}}^2 \rangle + \langle \beta_{\text{YYZ}}^2 \rangle$]^{1/2}. ^dDefined as β_{yyy} for CV, as β_{zzz} for $\rho > 2.5$, as $\beta_{\text{xyz}} = \beta_{\text{xzy}} = \beta_{\text{yxz}} = \beta_{\text{yzx}} = \beta_{\text{zxy}} = \beta_{\text{zyx}}$ for $\rho < 2.5$. The relations between β_{HRS} and β_{λ} are $\beta_{\text{HRS}}^2 = [\langle \beta_{\text{ZZZ}}^2 \rangle + \langle \beta_{\text{YYZ}}^2 \rangle] = (8/21)\beta_{\text{yyy}}^2$ for CV (D_{3h}); $\beta_{\text{HRS}}^2 = [\langle \beta_{\text{ZZZ}}^2 \rangle + \langle \beta_{\text{YYZ}}^2 \rangle] = (6/35)\beta_{\text{zzz}}^2$ for dipolar compounds, and $\beta_{\text{HRS}}^2 = [\langle \beta_{\text{ZZZ}}^2 \rangle + \langle \beta_{\text{YYZ}}^2 \rangle] = (120/35)\beta_{\text{xyz}}^2$ for octopolar (T_d , D_2 , or D_{2d}) compounds. ^eReference values for HRS experiments at 1300 nm, as DR1 shows multiphoton fluorescence at $\lambda_{\text{inc}} = 800$ nm.

Figure 3. D_2 -Symmetric chromophores based on **MPZnM** and **PZnMPZn** structural motifs, and the dipolar **RuPZnA** chromophore benchmark.

M = Ru [RuPZnA]
R = C₃F₇, M = Ru: [RuR₄PZnRu]
R = C₃F₇, M = Os: [OsR₄PZnOs]
R = 2,6-bis(3,3-dimethylbutyloxy)phenyl, M = Os: [OsPZnOs]
R = 2,6-bis(3,3-dimethylbutyloxy)phenyl, M = Ru: [PZnRuPZn]
R = 2,6-bis(3,3-dimethylbutyloxy)phenyl, M = Os: [PZnOsPZn]

RuR_fPZnRu, **OsR_fPZnOs**, and **OsPZnOs** have depolarization ratios near 1.5. The chemical topologies of these structures (see Figure 3) exclude the possibilities of dipolar (*i.e.*, C_{2v} symmetry with $\beta_{zxx} = -\beta_{zzz}$) or octopolar solution-phase symmetries of T_d or D_{3h} . Therefore the HRS response derives from structural conformers having D_2 and D_{2d} symmetries, **MPZnM** conformers where $\theta \sim -\phi$, and $\theta = -\phi = |45^\circ|$, respectively (Figure 4). These angles refer to torsion involving the terpyridyl (trpy) units and the PZn least-squares plane. As a D_{2h} structure is centrosymmetric ($\theta = \phi = 0^\circ$), and the experimentally determined ρ values indicate that dipolar conformers ($\theta \neq -\phi$) contribute little to the measured HRS signal. If a Boltzmann-weighted distribution of torsional angles is assumed, (Rubtsov 2003) only a few percent of the **MPZnM** conformers have $\theta \sim -\phi$. The nonlinear optical response for **MPZnM** derives from a small population of conformers with octopolar D_2 and/or D_{2d} structures at ambient temperatures.

PZnRuPZn and **PZnOsPZn** exhibit nearly homogeneous conformeric populations manifesting D_{2d} pseudo symmetry due to the orthogonal configuration of the central terpyridyl units (Figure 3). As a result, the electronic coupling between the terminal PZn units of **PZnRuPZn** and **PZnOsPZn** is diminished with respect to that which exists between the terminal M units of their **MPZnM** analogs. The dynamic hyperpolarizabilities and depolarization ratios for **PZnRuPZn** and **PZnOsPZn** (Table 1) are consistent with an NLO response that derives almost exclusively from noncentrosymmetric octopolar structures.

While the hyperpolarizability values for **RuR_fPZnRu**, **OsR_fPZnOs**, and **OsPZnOs** derive from D_2/D_{2d} conformeric subpopulations, the β_{HRS} values measured for pseudo D_{2d} -symmetric **PZnRuPZn** and **PZnOsPZn** stem from essentially all of the chromophores probed in the HRS experiment. Given the differences in the solution-phase HRS-active conformeric populations between the **MR_fPZnM/MPZnM** and **PZnMPZn** chromophore families, other key factors can control the relative magnitudes of the measured β_{HRS} values. Note that in addition to the disparate degrees of electronic communication between the terminal chromophoric components noted above, resonance enhancement effects at $\lambda_{\text{inc}} = 800$ nm for **PZnRuPZn** and **PZnOsPZn** are significantly less than those apparent for **RuR_fPZnRu**, **OsR_fPZnOs**, and **OsPZnOs** species. (Duncan et al. 2008b)

The impact that an octopolar D_2/D_{2d} conformeric subpopulation has upon measured solution phase β_{HRS} values can be seen also in chromophores **PZnEPZn** and **PZnE₂PZn** (Figure 5). **PZnEPZn** and **PZnE₂PZn** lack the oscillator strength and excited-state charge redistribution contributions driven by the central **M** unit of the **PZnMPZn** structures but feature strong PZn-PZn electronic coupling.(Anderson 1994; Lin et al. 1994; Angiolillo et al. 1995; Lin et al. 1995; Anderson 1999; Rubtsov 2003; Winters et al. 2007) Ethyne-bridged **PZnEPZn** exhibits a modest PZn-PZn torsional angle distribution in solution.(Lin et al. 1994; Angiolillo et al. 1995; Lin et al. 1995; Rubtsov 2003) **PZnE₂PZn** (Anderson 1994; Anderson 1999; Winters et al. 2007) features diminished electronic coupling between its PZn units relative to **PZnEPZn**.(Lin et al. 1994; Lin et al. 1995; Rubtsov 2003) Both **PZnEPZn** and **PZnE₂PZn** conformeric distributions are centered about a coplanar geometry, and the **PZnE₂PZn** torsional angle distribution would be expected to feature larger populations of HRS active D_2 - and D_{2d} -symmetric conformers.

Dynamic hyperpolarizabilities and depolarization ratios determined for the nondipolar chromophores **PZnEPZn** and **PZnE₂PZn** (Table 2) suggest that HRS-active, noncentrosymmetric conformeric populations must be present in solution. Depolarization ratio data arise from conformeric sub-populations that feature octopolar D_2/D_{2d} symmetry. A dipolar **PZnE** benchmark compound (Table 2) possesses only a modest β_{HRS} value. The dynamic hyperpolarizability of **PZnE₂PZn** is nearly twice that observed for **PZnEPZn**. **PZnEPZn** and **PZnE₂PZn** possess closely related electronic absorption spectra,(Anderson 1994; Lin et al. 1994; Angiolillo et al. 1995; Lin et al. 1995; Anderson 1999; Rubtsov 2003; Winters et al. 2007), and **PZnE₂PZn**'s two-fold greater HRS response reflects the greater degree of structural inhomogeneity of this species in solution relative to **PZnEPZn**, resulting in a larger concentration of HRS-active D_2 or D_{2d} conformers in solution. These data for **PZnEPZn** and **PZnE₂PZn** illustrate how HRS hyperpolarizability and depolarization ratio measurements can be used in tandem to probe the structural inhomogeneity of related chromophores in the solution and its impact on NLO activity.

Compound ^b	β_{HRS} ^c	β_{v} ^d	ρ
CV	208	340	1.4 ± 0.1
DR1	22	54	3.4 ± 0.3
PZnEPZn	220 ± 30	1000 ± 150	1.33
PZnE₂PZn	440 ± 50	2000 ± 250	1.46

Table 2. Dynamic Hyperpolarizabilities [β Values, ($\times 10^{-30}$ esu)] and Depolarization Ratios (ρ) Determined via Hyper Rayleigh Light Scattering Experiments at $\lambda_{\text{inc}} = 1300 \text{ nm}^a$ for **PZnEPZn** and **PZnE₂PZn**. (See footnotes of Table 1.)

These pioneering studies show that strongly coupled D_2 symmetric oscillators provide potent octopolar NLO chromophores.(Duncan et al. 2008b) Further, these HRS depolarization experiments show that the measured hyperpolarizability in **MPZnM** structures derives predominantly from **MPZnM** conformers where the torsional angles between the trpy units and the PZn plane are approximately equivalent in magnitude and opposite in sign, indicating that this small solution-phase structural subpopulation possess exceptional hyperpolarizabilities. Finally, the NLO responses of ethyne- and butadiyne-bridged bis(porphinato)zinc(II) chromophores **PZnEPZn** and **PZnE₂PZn** not only underscore that HRS signal intensity is correlated with the relative population of D_2/D_{2d} -symmetric conformers present in solution: **MPZnM**, **PZnEPZn**, and related chromophores clearly define potential hyperpolarizable octopolar building blocks for new classes of nonpolar chiral electrooptic materials.(Anderson 1994; Lin et al. 1994; Angiolillo et al. 1995; Lin et al. 1995; Anderson 1999; Rubtsov 2003; Winters et al. 2007) In this regard, it is important to emphasize that octopolar chromophores possess omnidirectional hyperpolarizabilities (β_{xyz} s) instead of unidirectional hyperpolarizabilities (β_{zzz} s), which make such species potentially superior active components of electrooptic devices. A similar problem, however, exists with respect to elaborating these classes of electrooptically active molecules into functional materials: dipolar NLO chromophores require a host matrix that enforces long-range dipolar order in the bulk phase, while octopolar NLO chromophores

require media that drive bulk phase octopolar order. Research to date that has focused on organizing NLO octopoles in the bulk phase has relied upon the synthesis of high generation dendritic octopoles,(Le Bozec et al. 2001) spontaneous organization within an appropriate liquid-crystalline phase,(Hennrich et al. 2006) or all-optical poling;(Nunzi et al. 1994; Fiorini et al. 1995) all of these approaches have met with only limited success. We propose to confer macroscopic order cofactors by leveraging the directed and self assembly capabilities of designed protein-chromophore complexes (see Section **Error! Reference source not found.**).

II.B.3 Octopolar Ru-PZn-Based Chromophores that Possess High Effective Optical Symmetry: Design, Spectroscopy and Molecular NLO Response.

Large first hyperpolarizabilities have been realized for several dipolar, D-Br-A chromophores over the 1300-1500 nm spectral domain.(Marder et al. 1994; Clays et al. 2000; Ma et al. 2002; Uyeda et al. 2002; Zheng et al. 2005) The development of NLO materials based on these chromophores requires macroscopic, non-centrosymmetric ordering. For dipolar molecules, electrostatic interactions drive detrimental, centrosymmetric aggregation. As mentioned above, strategies exist to align chromophoric guests in polymeric hosts to produce processable materials, but the net bulk-phase dipolar order realized in such NLO materials rarely, if ever, exceeds several percent.(Dalton et al. 1997; Dalton et al. 1999; Robinson et al. 1999; Steier et al. 1999; Liakatas et al. 2000; Dalton 2002; Ma et al. 2002; Luo et al. 2004) The protein-based routes for achieving dipolar order discussed in this proposal provide a promising route to achieve long-range orientational ordering of dipolar NLO chromophores; furthermore, this approach provides as well a pathway to organize hyperpolarizable molecules having higher order symmetry. A key first step in this latter aim requires the design of octopolar chromophores having exceptional hyperpolarizabilities; our chromophore design efforts summarized below provide octopolar supermolecules that possess the largest off-diagonal octopolar first hyperpolarizability tensor components ever reported.

Evolution of NLO octopoles from dipolar chromophoric archetypes has been demonstrated in molecules having modest hyperpolarizabilities.(Lambert et al. 1998; Wolff et al. 1999; Cho et al. 2001; Hennrich et al. 2004; Le Floc'h et al. 2005; Hennrich et al. 2006) We have shown that ruthenium(II) [5-(4'-ethynyl-(2,2';6',2''-terpyridinyl))-10,20-bis(2',6'-bis(3,3-dimethyl-1-butyloxy)phenyl)porphinato]zinc(II)-(2,2';6',2''-terpyridine)²⁺ bis-hexafluorophosphate (**RuPZn**) provides a robust, benchmark dipolar chromophore that possesses an extraordinary β_{λ} value at a telecommunication relevant wavelength ($\beta_{1300} = 5100 \times 10^{-30}$ esu).(Uyeda et al. 2002) **RuPZn** can serve as a motif for the development of octopolar NLO chromophores (Figure 7); **MPZn**-based supermolecules that couple multiple charge-transfer oscillators are used to evolve the effective chromophore optical symmetry from purely dipolar to octopolar (Figure 7), yielding NLO chromophores having impressive β_{HRS} values.

Figure 6. Reaction scheme outlining the evolution of NLO octopoles from dipolar chromophoric building blocks. The species in Figure 7 were synthesized in high yield from appropriately ethynylated porphyrinic and halogenated ruthenium bis(tpy) precursor molecules (Figure 6). (Heck 1979; Kumada 1980; Takahashi et al. 1980; Negishi et al. 1982; Stille 1986; DiMugno et al. 1993b; DiMugno et al. 1993a; Lin et al. 1994; Uyeda et al. 2002)

The Figure 8 spectra indicate that multiple PZn moieties linked via ethynes to a $[\text{Ru}(\text{tpy})_2]^{2+}$ core do not have appreciable electronic coupling between PZn units (compounds **3-9**). The absorption oscillator strengths of the porphyrin-dominated bands scale roughly with the number of macrocycles (Table 3), though the strong mixing of MLCT, tpy ligand, Soret, and Q electronic states makes rigorous quantification difficult. There is no evidence of strong coupling in these multi-porphyrin systems (*e.g.*, dramatic transfer of oscillator strength from the B- band to the Q-band, and strong Q-band red-shifting), (Lin et al. 1994; Angiolillo et al. 1995; Lin et al. 1995; Angiolillo et al. 2001; Fletcher et al. 2002b; Fletcher et al. 2002a; Screen et al. 2002; Susumu et al. 2002; Ostrowski et al. 2003; Angiolillo et al. 2004; Drobizhev et al. 2005; Susumu et al. 2005; Duncan et al. 2006a; Duncan et al. 2007; Frail et al. 2007; Thorley et al. 2008; Fisher et al. 2009), and the observed spectral properties of these supermolecules derives from a simple composite of multiple RuPZn oscillators.

Figure 8. Comparative electronic absorption spectra of: (A) PZn-(4'Ru) (**1**) and PZn-(4Ru) (**2**); (B) PZn-(4'Ru4')-PZn (**3**), PZn-(4Ru4')-PZn (**4**), PZn-(4Ru4)-PZn (**5**), and (PZn)₂-(4,4''Ru) (**6**); (C) PZn-(4'Ru4,4'')-(PZn)₂ (**7**), PZn-(4Ru4,4'')-(PZn)₂ (**8**), and (PZn)₂-(4,4''Ru4,4'')-(PZn)₂ (**9**) in CH₃CN solvent.

Figure 9. Magic angle pump-probe transient absorption spectra of compounds **2** (A) and **7** (B), recorded at several time delays illustrating the general transient features of chromophores **1-9**. Note that the NIR absorption manifold is strongly established at a time delay of 250 fs in compound **7** (as well as in chromophores **1**, **3-6**, and **8-9**), but not in compound **2**. The laser line from the pump pulse at 605 nm is observed in both spectra. Experimental conditions: solvent = acetonitrile, ambient temperature, $\lambda_{\text{exc}} = 605$ nm.

Table 3. Electronic Spectral Data for Ru-PZn Derivatives.

	Abs band maxima [nm] ($\epsilon \times 10^{-5} [\text{M}^{-1} \text{cm}^{-1}]$)				Oscillator strength		
	B-band ^a	¹ MLCT ^a	Q-bands ^a		B-band region ^{a,b}	Q-band region ^{a,c}	total ^d
PZn-(4'Ru) (1)	428 (1.07)	506 (0.56)	567 (0.15)	639 (0.51)	2.10	0.27	2.37
PZn-(4Ru) (2)	445 (1.18)	—	565 (0.14)	636 (0.35)	1.90	0.25	2.15
PZn-(4'Ru4')-PZn (3)	426 (1.95))	521 (0.89)	565 (0.32)	648 (1.13)	3.55	0.67	4.22
PZn-(4Ru4')-PZn (4)	445 (2.44)	509 (0.65)	567 (0.32)	642 (0.95)	4.28	0.60	4.88
PZn-(4Ru4)-PZn (5)	446 (2.23)	—	566 (0.27)	639 (0.75)	3.62	0.46	4.08
(PZn) ₂ -(4,4''Ru) (6)	447 (2.17)	—	564 (0.30)	644 (0.87)	3.55	0.57	4.12
PZn-(4'Ru4,4'')-(PZn) ₂ (7)	445 (2.89)	517 (0.80)	565 (0.42)	646 (1.43)	5.23	0.81	6.07
PZn-(4Ru4,4'')-(PZn) ₂ (8)	446 (2.96)	—	565 (0.39)	644 (1.16)	4.96	0.72	5.68
(PZn) ₂ -(4,4''Ru4,4'')-(PZn) ₂ (9)	446 (3.54)	—	566 (0.49)	647 (1.48)	6.12	0.96	7.09

^aTransition labels denote the dominant contributor to the absorption oscillator; ^bOscillator strengths calculated over the 360→555 wavelength domain; ^cOscillator strengths calculated over the 555→850 wavelength domain; ^dOscillator strengths calculated over the 360→850 wavelength domain.

Pump-probe transient absorption spectroscopic studies of this entire class of compounds (chromophores **1-9**) demonstrate that all of these species exhibit excited-state dynamical characteristics and transient spectral features similar to those reported for **PZn-(4'Ru) (1)**, (Duncan et al. 2004; Duncan et al. 2007). Congruent with insights derived from electronic spectral data, these transient absorption spectral studies further support the notion that the individual RuPZn fragments of chromophores **3-9** exhibit, at best, only modest excited-state electronic interactions that derive from factors other than the dipolar interactions of the component RuPZn oscillators. Because the nature of the chromophore electronically excited states present at early and long delay times are reminiscent of that previously established for **PZn-(4'Ru) (1)**, (Duncan et al. 2004; Duncan et al. 2007) the electronically excited states of supermolecules **3-9** can thus be approximated as a collection of interacting RuPZn transition dipoles that to first order behave as independent oscillators. While a detailed description would certainly need to consider excitation delocalization via energy transfer and the impact of conformational dynamics upon these excited states, the electronic structural data for **1-9** that emerge from these pump-probe transient absorption experiments indicate fundamentally sound design principles. Because the nature of the instantaneously prepared excited states determine the virtual states that govern the NLO response, the approximately independent character of the component RuPZn oscillators for **3-9** suggest nonlinear optical responses for these supermolecules consistent with those defined for multipolar chromophores (*vide infra*).

Table 4 lists dynamic hyperpolarizability (β) values and depolarization ratios (ρ) determined from hyper-Rayleigh light scattering (HRS) measurements carried out at an incident irradiation wavelength (λ_{inc}) of 1300 nm in CH₃CN solvent. The depolarization ratio provides an experimental measure of chromophore optical symmetry and also an important experimental validation of the evolution of the effective chromophore symmetry from purely dipolar to octopolar. For the purpose of analyzing the depolarization ratio data, these species may be viewed as assemblies of RuPZn dipoles. Insight into the nature of excited-state electron density distributions for **3-9** is thus gleaned through vector addition of these RuPZn dipoles. The PZn substitution pattern about the Ru(tpy)₂ core determines the extent to which these dipoles add or cancel. Some of the supermolecular chromophores do not, in fact, possess high molecular symmetry, due to the fact that the tpy 4 and 4' (4'') positions are not electronically or chemically equivalent. For **3-9** where these strong RuPZn oscillators exhibit significantly independent character, however, depolarization ratio measurements reveal the effective chromophore optical symmetry. This effective optical symmetry reflects the spatial arrangement of the RuPZn oscillators about the Ru(tpy)₂ core, that is, an approximate molecular symmetry that would exist if all points of connectivity to this unit were equivalent.

Table 4. Dynamic Hyperpolarizabilities [β_{ijk} , (x 10 ⁻³⁰ esu)] and Depolarization Ratios (ρ) Determined by Hyper-Rayleigh Experiments ^a			
	$\beta_{\text{ijk}}^{\text{b}}$	β_{HRS}	ρ^{c}
PZn-(4'Ru)(1)	5100 ^d	2100	3.8 ± 0.1
PZn-(4Ru)(2)	3100 ± 50	1280 ± 20	3.8 ± 0.1
PZn-(4'Ru4')-PZn (3)	1920 ± 70	800 ± 30	3.95 ± 0.1
PZn-(4Ru4')-PZn (4)		880 ± 30	2.2 ± 0.1
PZn-(4Ru4)-PZn (5)		960 ± 30	2.1 ± 0.1
(PZn) ₂ -(4,4''Ru) (6)		1080 ± 40	3.4 ± 0.1
PZn-(4'Ru4,4'')-(PZn) ₂ (7)		750	2.1 ± 0.2
PZn-(4Ru4,4'')-(PZn) ₂ (8)		894	2.1 ± 0.2
(PZn) ₂ -(4,4''Ru4,4'')-(PZn) ₂ (9)	560 ± 100	1040 ± 200	1.46 ± 0.1

^a $\lambda_{\text{inc}} = 1300 \text{ nm}$; ^b $\beta_{\text{ijk}} = \beta_{\text{zzz}}$ for upper 3 entries with dipolar molecular symmetry ($\rho \sim 4$) and $\beta_{\text{ijk}} = \beta_{\text{xyz}}$ for

last entry (**9**) having effective octopolar symmetry ($\rho = 1.5$); $\beta_{\text{HRS}}^2 = [\langle \beta_{\text{ZZZ}}^2 \rangle + \langle \beta_{\text{YYZ}}^2 \rangle] = 6/35 \beta_{\text{ZZZ}}^2$ for dipolar compounds, and $\beta_{\text{HRS}}^2 = [\langle \beta_{\text{ZZZ}}^2 \rangle + \langle \beta_{\text{YYZ}}^2 \rangle] = 120/35 \beta_{\text{XYZ}}^2$ for octopolar (T_d , D_2 or D_{2d}) symmetries; $^c\rho = \langle \beta_{\text{ZZZ}}^2 \rangle / \langle \beta_{\text{YYZ}}^2 \rangle$; ^{d} value taken from ref.(Uyeda et al. 2002)

Depolarization ratio data compiled in Table 4 shows evolution of ρ values from dipolar ($\rho \sim 4$, chromophores **1-3**) to octopolar ($\rho = 1.46$, chromophore **9**). Given the lower approximate molecular symmetries of supermolecules **4-8** (Figure 7), intermediate values of ρ are manifest. Note that these ρ values are neither consistent with dipolar nor octopolar optical symmetry but reflect the spatial arrangement of the component RuPZn oscillators of these species and the corresponding vector sums of these dipoles. Congruent with this, the small measured difference in the depolarization ratios for supermolecules **4** and **5**, which possess a similar relative spatial arrangement of their respective component RuPZn oscillators yet different linkage topologies, illustrates the utility of the notion of approximate molecular symmetry in rationalizing the relative magnitudes of ρ for compounds **4-9**. Given the electronic structural characteristics of **1-9** and the substantial RuPZn oscillator strength, the absolute nature of PZn-tpy connectivity (4, 4', or 4'') has little impact upon the chromophore's effective optical symmetry.

Consistent with the established NLO properties of **RuPZn**(Uyeda et al. 2002; Duncan et al. 2008b; Hu et al. 2010) and the dependence of chromophore effective optical symmetry upon structure, supermolecules **1-9** exhibit impressive second-order nonlinear optical responses at 1300 nm. In Table 4, note that $\beta_{\text{HRS}}^2 = [\langle \beta_{\text{ZZZ}}^2 \rangle + \langle \beta_{\text{YYZ}}^2 \rangle]$ reflects the total HRS intensity, providing an average hyperpolarizability value that is independent of the actual molecular symmetry.(Cyvin et al. 1965)

For the simple dipolar compounds, note that β_{1300} value determined for **PZn-(4'Ru)** (**1**, $\beta_{\text{HRS}} = 2100 \times 10^{-30}$ esu) exceeds that of **PZn-(4Ru)** (**2**, $\beta_{\text{HRS}} = 1280 \times 10^{-30}$ esu); this result is consistent with the fact that 4'-tpy attachment facilitates the strongest mixing of the PZn B_x and metal polypyridyl charge-resonance states (Figure 8). In supermolecules **3-9**, which feature multiple RuPZn oscillators, the magnitude of β_{HRS} does not reflect such a dramatic difference between 4' or 4 (4'') points of connectivity for the 5-ethynyl(porphinato)zinc units.

With respect to the magnitude of the measured hyperpolarizability, the coupling of multiple RuPZn oscillators in structures **3-9** evolves chromophore symmetry from dipolar to octopolar (Table 4). This approach to chromophore design yields octopolar supermolecules that possess by far the largest off-diagonal octopolar first hyperpolarizability tensor components ever reported. **(PZn)₂-(4,4''Ru₄,4'')**-**(PZn)₂** (**9**) which features a 5-ethynyl(porphinato)zinc substitution pattern compatible with D_{2d} symmetry, shows complete cancellation of the vectorial dipolar contributions to the NLO response associated with its 4 component RuPZn oscillator fragments, and manifests a ρ value of 1.5, as expected for an ideal octopole. Note that for chromophores **3-8**, which exhibit substantial variation in the magnitude of the measured depolarization ratio, display averaged hyperpolarizabilities (β_{HRS} values) that remain extremely large, independent of structure. These values range from $750\text{-}1080 \times 10^{-30}$ esu, similar in magnitude to that determined for the octopolar supermolecule **9** ($\beta_{\text{HRS}} = 1040 \times 10^{-30}$ esu).

Because variations in molecular structure determine chromophore effective optical symmetry and lead to different non-zero hyperpolarizability tensor components contributing to the overall HRS response, the simplest comparative metric is the averaged hyperpolarizability, as discussed above. However, in order to contrast the HRS responses of these systems with key literature chromophoric benchmarks, the various tensor elements that contribute to the HRS response must be evaluated; such tensor-element-specific HRS responses for molecules of different symmetries can be compared directly. This approach is exemplified for **(PZn)₂-(4,4''Ru₄,4'')**-**(PZn)₂** (**9**), where $\beta_{\text{HRS}} = 1040 \times 10^{-30}$ esu and

$\beta_{xyz} = 560 \times 10^{-30}$ esu for the 6 contributing non-zero off-diagonal hyperpolarizability tensor components. Supermolecule **9** exhibits the largest octopolar response reported, irrespective of symmetry or dispersion (frequency dependence of the response) considerations. Analyzing compound **9** NLO data within the context of effective D_{2d} symmetry, there are 6 non-zero tensor hyperpolarizability tensor elements β_{ijk} , $i \neq j \neq k$ that contribute to the total signal. With respect to comparisons of tensor-element-specific HRS responses, note that the relations between the observables are given by the following equations: $\beta_{HRS}^2 = [\langle \beta_{zzz}^2 \rangle + \langle \beta_{yzz}^2 \rangle] = 6/35 \beta_{zzz}^2$ for dipolar compounds, $\beta_{HRS}^2 = [\langle \beta_{zzz}^2 \rangle + \langle \beta_{yzz}^2 \rangle] = 8/21 \beta_{xxx}^2$ for octopolar D_{3h} symmetry, and $\beta_{HRS}^2 = [\langle \beta_{zzz}^2 \rangle + \langle \beta_{yzz}^2 \rangle] = 120/35 \beta_{xyz}^2$ for octopolar T_d , D_2 , or D_{2d} symmetries. This difference in prefactors for dipolar and octopolar symmetries (6/35 vs. 8/21 or 120/35) results in a seemingly small octopolar response (β_{HRS} value) for compound **9** compared to that determined for dipolar chromophores **1** and **2**. Calculation of the tensor elements for an average $\beta_{HRS} = 1040 \times 10^{-30}$ esu for structures of different symmetries, it is easy to appreciate the extraordinary response of octopolar chromophore **9**. For a dipolar compound, a $\beta_{zzz} = 2500 \times 10^{-30}$ esu would be needed to generate a $\beta_{HRS} = 1040 \times 10^{-30}$ esu; similarly, an octopolar, but D_{3h} molecule would require $\beta_{xxx} = 1700 \times 10^{-30}$ esu in order to provide a β_{HRS} value exceeding 1000×10^{-30} esu. (Cyvin et al. 1965) This analysis provides a yardstick by which to compare molecules of different optical symmetries benchmarked to a specific second order response. Note that octopolar **(PZn)₂-(4,4''Ru4,4'')-(PZn)₂** gives rise to β_{HRS} value approximately equivalent to that for dipolar **PZn-(4Ru)** ($\beta_{zzz} = 3100 \times 10^{-30}$ esu), showing that it is possible to retain large NLO responses as the nature of the optical symmetry is modulated.

These considerations are important in the development of nonlinear optical materials based upon hyperpolarizable chromophores. The omnidirectional averaged response for molecules with high symmetry (yet non-centrosymmetry) results in a large nonlinear effect, as is observed the 6 non-zero β_{ijk} , $i \neq j \neq k$ tensor components that contribute to the total average HRS signal observed for **9** ($\beta_{HRS} = 1040 \times 10^{-30}$ esu). A large off-diagonal β_{xyz} hyperpolarizability tensor component translates into an *omnidirectional* NLO response in a bulk materials and thin films, resulting in polarization insensitive devices that can be utilized as frequency-converters or electro-optic modulators.

It is important to underscore the magnitude of the off-diagonal hyperpolarizability tensor component β_{xyz} for octopolar chromophore **9** (560×10^{-30} esu) was determined for a technologically important incident irradiation wavelength of 1300 nm. Pump-probe transient optical data evince that **1-9** are non-emissive; furthermore the molecular hyperpolarizabilities determined for these species were acquired under experimental conditions that utilized classic fluorescence demodulation methods. (Clays et al. 1991; Zyss 1991; Brédas et al. 1992; Zyss et al. 1993a; Zyss et al. 1993b; Verbiest et al. 1994; Lambert et al. 1998; McDonagh et al. 1999; Wolff et al. 1999; Cho et al. 2001; Le Bozec et al. 2001; Hennrich et al. 2004; Le Floch et al. 2005; Maury 2005; Hennrich et al. 2006; Kim et al. 2009) The large β_{HRS} values reported herein are uncontaminated by multi-photon fluorescence contributions. The largest octopolar β_{1300} value reported to date corresponds to structural analog of the $[\text{Ru}(\text{bpy})_3]^{2+}$. (Dhenaut et al. 1995; Morrison et al. 1996) As measurements at this irradiation wavelength are known to be heavily contaminated by multi-photon fluorescence, in order to provide a more accurate benchmark with which to compare to **9**, we note β_{xxx} for this particular $[\text{Ru}(\text{bpy})_3]^{2+}$ derivative determined at $\lambda_{\text{inc}} = 1900$ nm corresponds to 320×10^{-30} esu. (Le Bozec et al. 2001) While multi-fluorescence contamination is always an issue when determining the hyperpolarizabilities of emissive chromophores, these concerns are somewhat mitigated at long irradiation wavelength. Projecting this β_{1900} value back to 1300 nm using the conventional dispersion relation, gives $\beta_{xxx} = 500 \times 10^{-30}$ esu, and a corresponding averaged nonlinear response $\beta_{HRS} = 310 \times 10^{-30}$ esu. The supermolecular chromophore **(PZn)₂-(4,4''Ru4,4'')-(PZn)₂** thus possesses a β_{HRS} value at 1300 nm that is more than a factor of three larger than that determined for any chromophore having octopolar symmetry examined to date.

These studies, which demonstrate that appropriate coupling of multiple, related charge-transfer oscillators can be utilized to evolve the effective chromophore symmetry in RuPZn-based supermolecules from purely dipolar to octopolar, and generate octopolar NLO chromophores having impressive β_{HRS} values at 1300 nm ($\beta_{\text{HRS}} > 1000 \times 10^{-30}$ esu), motivate next-generation protein-design challenges: because NLO octopoles possess omnidirectional NLO responses, the advent of these octopolar NLO chromophores having vastly superior β_{HRS} values at technologically important frequencies underscores the importance of *de novo* designed protein-based biomolecular assemblies as a new experimental approach to achieve acentric order in both bulk-phase and thin film structures (see p. **Error! Bookmark not defined.** and p. **Error! Bookmark not defined.**).

II.B.4 Dipolar "push-pull" RuPZn chromophore incorporated into the heptad-based, amphiphilic 4-helix bundle peptide AP0

As discussed, a series of "push-pull" NLO chromophores have been developed by the group that are based on a zinc-porphyrin donor and a metal (Ru, Os)-polypyridyl acceptor(s) coupled via an ethyne bridge (Duncan et al. 2007). These have been shown in isotropic solution to exhibit exceptionally large molecular hyperpolarizabilities, especially at the longer wavelengths more relevant for optical communications applications, via hyper-Rayleigh scattering (Uyeda et al. 2002). One of these designated **RuPZn** (

Figure 1, Figure 7) was incorporated with high specificity via axial histidyl ligation of the porphyrin's Zn-atom into the non-polar core of the hydrophilic domain of an amphiphilic 4-helix bundle peptide designated AP0 (Ye 2004; Xu et al. 2006). The latter was non-computationally designed based on the heptad repeat approximation for the primary sequence of the α -helices comprising the 4-helix bundle (i.e., utilizing 3.5 residues/turn instead of 3.6 typical of biological α -helices). The designed amphiphilicity of the exterior of the bundle, i.e., possessing distinct hydrophilic and hydrophobic domains over its length, facilitated the vectorial orientation of the bundle in Langmuir monolayers at the water-gas interface upon compression (Ye 2004; Strzalka et al. 2006). In this more recent work, *in situ* absorption spectroscopy was utilized to establish the chromophore/helix stoichiometry in the compressed monolayer. Synchrotron radiation based x-ray reflectivity was utilized to establish that the bundle's long-axis was oriented perpendicular to the interface and the chromophore was indeed located with its long-axis along the core of the bundle's hydrophilic domain, following compression. Similarly, grazing-incidence x-ray diffraction demonstrated 4-helix bundle formation and established that the dense 2-D positional ordering of the complex in the monolayer ensemble was nevertheless glass-like.

The uppermost surface of the compressed Langmuir monolayer of the RuPZn/AP0 complex exposed a -SH residue at the end of the peptide's hydrophobic domain by design. This facilitated the covalent attachment of the ordered 2-D ensemble of the complex to an alkylated planar surface of an inorganic substrate, e.g., silicon oxide, the alkylating chains possessing suitable endgroups to effect the necessary chemistry. Si-Ni-Si multilayer substrates were utilized in order to employ x-ray interferometry to confirm the subsequent transfer of the monolayer ensemble of the RuPZn/AP0 complex from the water-gas interface onto the surface of the inorganic substrate [Figure 10]. Similarly alkylated quartz and fused silica substrates were utilized *in parallel* so that the chromophore/helix stoichiometry in the transferred monolayer could be confirmed via linear absorption spectroscopy and the nonlinear optical response of the monolayer ensemble could be investigated via polarized second-harmonic generation (SHG) [Figure 11], respectively. The magnitude of the macroscopic nonlinear response of these monolayer ensembles as a function of chromophore in-plane density demonstrated that the molecular hyperpolarizability of the chromophore β_1 was preserved as the coherent sum in the macroscopic response $\chi^{(2)}$, thereby firmly establishing that the desired non-centrosymmetric vectorial orientation of the chromophores throughout the ensemble had been achieved [Figure 12]. The order parameter from

analysis of the polarization dependence of the macroscopic nonlinear response demonstrated that while the average orientation of the chromophore's long-axis was normal to the plane of the monolayer ensemble, the distribution of tilt-angles of the long-axis with respect to the normal was broad [Figure 13, Figure 14]. These RuPZn/AP0 monolayer ensembles have proven to be reasonably robust for an organic material and thereby potentially suitable for nonlinear optical device applications, as they are stable in air indefinitely and capable of withstanding relatively intense laser irradiation (e.g., peak power per unit surface of $\sim 100 \text{ MW/cm}^2$), although some degradation in the SHG signal was observed under such continuous irradiation over the time-scale of a hours.

It should be noted that as described above, the reactivity of both the hydrophilic vs. hydrophobic ends of the bundle can be easily controlled (e.g., as in Figure 10 vs. **Error! Reference source not found.**), and can made orthogonal if necessary. Thus, with this directed-assembly approach employing amphiphilic bundles vectorially incorporating the dipolar NLO chromophore, covalent multilayer structures can be assembled layer-by-layer, thereby preserving the essential acentric ordering present in each monolayer throughout the multilayer structure.

II.B.5 "Push-pull" RuPZn chromophore incorporation into computationally-designed single-chain soluble proteins

The amphiphilic AP0 tetrahelical bundles discussed in page 18 are versatile but bind cofactors nonspecifically; these systems associate to bind the cofactors, and metal coordination is largely responsible for cofactor affinity. To obtain higher affinities and more control over the chromophore's local environment, we have designed protein complexes that encapsulate nonbiological Fe- and Zn-porphyrin cofactors, (Cochran et al. 2005; McAllister et al. 2008; Fry et al. 2010). We have extended this approach to design a fully asymmetric helical-bundle, single-chain protein that has been tailored to encapsulate the RuPZn cofactor (manuscript in preparation). In this protein, three loops connect the four helices. The template structure of the 108-residue single chain protein, was derived from a model of the corresponding alpha helical homotetramer. Interhelical loops with favorable conformations and sequences were introduced into the structure by grafting known interhelical backbone structures onto the model. The precise conformation of the loops in the structure were obtained by: (1) superimposing helical hairpins from proteins of known structure onto the helices; (2) selecting hairpin loops whose

helical segments closely coincide (i.e., have low root mean square distances between equivalent backbone atoms) with the terminal residues of the four model helices; and (3) selecting structures that did not interfere with cofactor binding (see Figure 16). Residues directly involved in cofactor ligation or second-shell interactions were predefined (one His and one Thr), while the remaining positions of the protein were specified using the computational design methodology. (Calhoun et al. 2003; Slovic et al. 2004; Cochran et al. 2005; North et al. 2006; Swift et al. 2006; Bender et al. 2007; McAllister et al. 2008; Fry et al. 2010) The resulting designed protein folds to present a polar exterior and complementary, asymmetric hydrophobic interactions within the interior that are difficult to realize in symmetric bundles, particularly for such an asymmetric cofactor. Two designed sequences express well in *E. coli* and are highly helical in both the apo (no cofactor) and holo (bound to cofactor) forms. Each binds the cofactor with the desired stoichiometry. The CD data monitored as a function of temperature suggest the designed proteins are highly stable, as cooperative unfolding is not observed over the temperature range of 0-95 °C. UV/vis spectral studies are consistent with tight binding of the cofactor to the protein in the desired stoichiometry and near nanomolar affinity for the cofactor (see Figure 17). As monitored by femtosecond and nanosecond pump-probe spectroscopies, the encapsulated cofactor possesses photophysical properties comparable to those of the cofactor alone in organic solvents, e.g., a triplet lifetime of 25 microseconds. As determined by hyper-Raleigh scattering (HRS), the protein retains the molecular hyperpolarizability it has at telecommunications wavelengths ($\beta_{1340} = 3100 \times 10^{-30}$ esu at 1340 nm). This work represent the first encapsulation of a nonbiological cofactor having a significant nonlinear optical response within a protein. A thiol containing variant of the designed protein is immobilized at a silicon surfaces, and x-ray reflectivity studies reveal that the cofactor is re-oriented parallel to the surface. (A manuscript detailing this work is presently in preparation.) In future work, we will go beyond a single thiol linkage to further control the orientation of the protein and hence the cofactor.

II.B.6 Experimental characterization of complexes with D_2 symmetric cofactors.

The heptad-based design of the amphiphilic 4-helix bundle peptide AP0 results in a coiled-coil structure for the bundle, verified experimentally by grazing-incidence x-ray diffraction from compressed Langmuir monolayer ensembles (Ye 2004; Strzalka et al. 2006). Molecular dynamics simulations have indicated that this coiled-coil structure tightly enforces a twisted D_2 symmetric conformation of the butadiyne-bridged zinc-porphyrin dimer chromophore, designated **PZnE₂PZn**, located within the core of the bundle [Figure 5, Figure 18] (Zou 2008). The chromophore's position along the core depends on the position of the histidine residue in the primary sequence of the helices that binds only one of the two zinc-porphyrins in the core via axial ligation of the porphyrin's zinc atom (Strzalka et al. 2006). Since this ligation could also induce some weak asymmetry in the electronic structure of the chromophore, we have designed a

new version "L6H13L20" of the AP0 peptide that has been shown capable of incorporating a PZn trimer chromophore (Susumu et al. 2002), designated **PZn₃**, into the non-polar core of its hydrophilic domain [Figure 19, **Error! Reference source not found.**]. The position of the H13 histidine residue in the primary sequence of the helices ensures axial ligation of only the central PZn of the trimer, the length of the trimer being comparable to that of the bundle's hydrophilic domain, thereby making the ligation far less likely to induce any asymmetry in the chromophore's electronic structure. Complexes of this new AP0 peptide with the PZn trimer within compressed Langmuir monolayers at the water-gas interface have been characterized via *in situ* linear absorption spectroscopy and both x-ray reflectivity and grazing-incidence x-ray diffraction. This characterization has shown that the chromophore is located as expected within the non-polar core of the bundle's hydrophilic domain and the chromophore's long-axis is coincident with that of the bundle normal to the plane of the monolayer ensemble, for chromophore/helix stoichiometries of from 1:4 to 1:2 [Figure 21], while maintaining the 4-helix bundle's coiled-coil structure [Figure 22]. Hyper-Rayleigh scattering experiments on isotropic detergent solutions of these complexes to establish the symmetry of this chromophore within the ensemble are in progress at the time of writing this proposal.

Figure 19. Linear absorption spectra showing the red-shift of the B, MLCT and Q bands of the PZn trimer chromophore upon axial ligation of its central zinc-porphyrin within the non-polar core of the hydrophilic domain of the amphiphilic 4-helix bundle peptide L6H13L20 AP0.

Figure 21. Upper panel: Electron density profiles for the compressed Langmuir monolayers of the amphiphilic 4-helix bundle peptide AP0 containing varying stoichiometries of the specifically bound PZn trimer chromophore. The helices become oriented perpendicular to the water-gas interface at higher surface pressures, extending over the $-60\text{Å} < z < 0\text{Å}$ region of the monolayer profiles, the aqueous subphase shown to the left and helium gas to the right of that region.

Lower panel: Difference monolayer electron density profile between two different chromophore/helix stoichiometries. The difference profile for the 2:1 minus the 1:1 stoichiometry is more relevant, since the perturbing chromophore is present in both, unlike comparisons of a holo-peptide with the apo-peptide. This profile has a broad maximum centered at $z \sim 40\text{Å}$, halfway along the length of the bundle's hydrophilic domain, consistent with the axial ligation of the zinc atom of the central PZn unit of the PZn trimer chromophore by the H13 residue in the helices' sequence.

Figure 22. Grazing-incidence x-ray diffraction for photon momentum transfer parallel to the plane of the compressed Langmuir monolayers of the amphiphilic 4-helix bundle peptide containing varying stoichiometries of the specifically bound PZn trimer chromophore. The maximum as a function of Q_{xy} arises from inter-helix interference within the bundles and indicates that chromophore incorporation does not greatly disturb the bundle's 4-helix structure.

II.B.7 2-D and 3-D periodic peptide ensembles via self-assembly

Crystallization achieves atomically precise ordering of molecules via self-assembly (Hollingsworth 2002), and this self-organization can be leveraged in the design of crystal structures that have many applications including NLO materials. Weak intermolecular forces stabilize crystalline ordering, and as a result, quantitative, predictive approaches to molecular crystal design remain challenging even when using small molecules. Though protein crystallization is well known and a crucial part of efforts in structural biology, such crystals have not been designed *de novo*. We have computationally redesigned

the exterior of a helical protein, a parallel homotrimer, so as to achieve a crystal possessing an acentric, layered P6 space group that exhibits a rare parallel orientation of the helices throughout (see Figure 23). In designing the protein, the geometry of interprotein orientations is varied so as to create a large number (10^4) of crystalline structures (unit cells) consistent with the P6 space group. High-energy configurations as determined using a molecular force field are discarded, and the sequence-structure energy landscape is energetically characterized using computational protein design. Candidate proteins are identified from minima on this modeled energy landscape. Five such proteins were experimentally realized and screened for crystallization. Three of the proteins yielded crystals within hours, and x-ray crystallographic studies reveal that one of these exhibits the targeted crystalline order. The experimental structure is in excellent agreement with the computationally designed model (see Figure 24). The results suggest principles for the design of protein crystals based on α -helical structures. This work presents a first step in the *de novo* design of ordered, acentric protein crystals for the realization of atomically precise ordering within biomolecular crystalline materials; such order is essential for NLO and vectorial electron transfer applications. (A manuscript detailing this work recently appeared in *PNAS*.)

	Figure 23. Experimentally determined crystal structure of computationally designed crystalline array of three helix bundle proteins. The orientation of points of closest approach between neighboring three helix bundles (magenta) highlight the parallel, acentric order of the designed crystal.
	Figure 24. Superposition of the alpha-carbon coordinates of neighboring three-helix bundles. Computational model identified as a low energy minimum used to design the sequence (gray); experimentally determined crystal structure (cyan).