

**SELF-ASSEMBLY OF PI-CONJUGATED PEPTIDES IN AQUEOUS ENVIRONMENTS LEADING TO  
ENERGY-TRANSPORTING BIOELECTRONIC NANOSTRUCTURES**

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**Project Objectives:** The realization of new supramolecular pi-conjugated organic structures inspired and driven by peptide-based self-assembly will offer a new approach to interface with the biotic environment in a way that will help to meet many DOE-recognized grand challenges. Previously, we developed pi-conjugated peptides that undergo supramolecular self-assembly into one-dimensional (1-D) organic electronic nanomaterials under benign aqueous conditions. The intermolecular interactions among the pi-conjugated organic segments within these nanomaterials lead to defined perturbations of their optoelectronic properties and yield nanoscale conduits that support energy transport within individual nanostructures and throughout bulk macroscopic collections of nanomaterials. Our objectives for future research are to construct and study biomimetic electronic materials for energy-related technology optimized for harsher non-biological environments where peptide-driven self-assembly enhances pi-stacking within nanostructured biomaterials, as detailed in the following specific tasks: (1) **synthesis and detailed optoelectronic characterization** of new pi-electron units to embed within homogeneous self-assembling peptides, (2) **molecular and data-driven modeling** of the nanomaterial aggregates and their higher-order assemblies, and (3) development of new hierarchical assembly paradigms to organize multiple electronic subunits within the nanomaterials leading to **heterogeneous electronic properties** (*i.e.* gradients and localized electric fields). These intertwined research tasks will lead to the continued development and fundamental mechanistic understanding of a powerful bioinspired materials set capable

of making connections between nanoscale electronic materials and macroscopic bulk interfaces, be they those of a cell, a protein or a device.

## RESULTS FROM THE DOE-BES PROJECT

The past six year funding period supported research efforts relating to the development of new synthetic approaches to functional pi-conjugated peptides and their subsequent optoelectronic characterization. They furthermore laid a strong foundation for continuing work dealing with **molecular and nanoscale materials** properties centered at JHU and led to the initiation of parallel efforts in the context of **mesoscale materials** led through an independent collaborative program centered at UIUC.

- 1) New synthetic methods. We have developed two parallel synthesis programs to more easily diversify the nature of the pi-electron materials embedded within the self-assembling material platform. The first entails the use of Pd-catalyzed chemistry to construct requisite oligomeric pi-systems directly during the peptide synthesis, where a portion of the oligomeric unit is covalently incorporated into the peptide backbone, and the complement is then added while the substrate is immobilized on the synthesis support. This method eliminated the need to prepare a complex pi-conjugated amino acid prior to peptide synthesis work. The second method capitalizes on our discovery of relevant site-site reactivity propensities on commercial synthesis supports (Chem Comm 2010) but now also using similar Pd-mediated chemistry (ACS Macro Letters 2012). These approaches have allowed for the synthesis of what to our knowledge are the longest pi-electron segments to be embedded within peptide constructs that allow for semiconductor behavior. We are therefore very confident in the success of incorporating more exotic pi-electron units into the peptide platforms for other types of optoelectronic responses as described below.
- 2) Diacetylene polymerizations. Using our established on-resin dimerization strategies (Chem Comm 2010), we successfully incorporated diacetylene motifs that were subjected to photochemical topochemical polymerization into nanoscale polydiacetylene wires (JACS 2012). Responsive chromic properties were discovered that will be pursued in this present application in the context of monitoring biological recognition with the pi-conjugated polymeric nanostructures.
- 3) Mesoscale “noodle” fabrication. We have found simple solution processing techniques that lead to global alignment of peptide nanostructures within macroscale regimes (Adv Mater 2011). These noodle-like structures have optoelectronic properties distinct from random collections of the component nanomaterials in isolation and will be subjected to continued study. This research activity has also sparked a parallel and independent effort to prepare and further characterize **mesoscale electronic materials** using microfluidic technologies under the leadership of a collaborative UIUC-centered team led by William Wilson (Materials Research Laboratory) and Charles Schroeder (Chemical Engineering). This parallel aspect will fit well with emerging DOE interests detailed in the September 2012 report *From Quanta to the Continuum: Opportunities for Mesoscale Science*.<sup>79</sup> PIs Tovar and Ferguson are no-effort collaborators to provide material and computational support, respectively, independent of the work proposed herein.
- 4) Supramolecular chromophore “polymorphism.” The ease of the on-resin dimerization approaches has allowed us to undertake systematic variations in peptide composition that have direct impact on the observed optoelectronic properties of the nanoscale peptide aggregates. Steric and hydrophobic properties of component amino acid residues lead to variances in exciton coupling among a given pi-conjugated chromophore and thus in the observed electronic properties (manuscript in final preparation stage). These subtle differences will be exploited in continuance in the context of nanoscale heterostructure synthesis whereby the resulting nanomaterials will present spatially graded electronic properties.
- 5) Electrical device characterization. We have characterized the electrical properties relevant for organic semiconductivity both in terms of visualizing the polarizability of the nanostructures under an applied electrical bias (JACS 2012) as well as within the active layer of field-effect transistors (Adv Mater 2011). These capabilities will remain available in collaborative efforts to our team.

- 6) Synthesis of new peptide- $\pi$ -electron conjugates. A major effort in this program over the past years has been the development of new synthetic methods through which we may embed  $\pi$ -electron function into linear self-assembling peptides. We are just now finalizing a new report whereby we can attach peptide units onto 2-D conjugated structures thus providing multivalent peptide presentation. Among the chromophores amenable to this multivalent presentation are hexabenzocoronenes (graphene subunits) and porphyrins. We are therefore very confident in the success of incorporating more exotic linear (1-D) and 2-D  $\pi$ -electron units into the peptide platforms for other types of optoelectronic responses as described in Task 1 below.
- 7) Supramolecular “polymorphism.” The ease of the on-resin dimerization approaches has allowed us to undertake systematic variations in peptide composition that have direct impact on the observed optoelectronic properties of the nanoscale peptide aggregates. We have shown how steric and hydrophobic properties of component amino acid residues lead to variances in exciton coupling among  $\pi$ -conjugated fluorophores (Langmuir 2014) and in electrical transport through embedded semiconductors (J. Mater. Chem. C 2015, ACS Nano 2015). These subtle differences will be exploited in the renewal project in the context of nanoscale heterostructure synthesis whereby the resulting nanomaterials will present spatially graded electronic properties. We describe other prospects for sequence variation in Tasks 2 and 3 below.
- 8) Computational interrogation of fundamental assembly mechanisms. Using all-atom molecular dynamics simulations, we quantified the morphology, thermodynamic stability, and assembly mechanism of short oligomeric aggregates that form during the early stages of self-assembly (Langmuir 2014). We have resolved a hierarchical assembly mechanism in which peptides coalesce into small clusters over tens of nanoseconds followed by structural ripening and diffusion limited aggregation on longer time scales. Oligomers form well-aligned peptide stacks driven by dispersion interactions between the  $\pi$ -conjugated cores, hydrogen bonding between the peptide wings, and the “hydrophobic effect” resulting in a strongly favorable driving force for aggregation of  $\Delta F \approx -15$  kcal/mol (Mol. Sim. 2015).
- 9) Energy migration and electric field creation. We revealed several critical aspects of energy flow within the peptide nanomaterials, spanning the observation of photonic energy migration in donor-acceptor heterostructures (Chem. Sci. 2015), the transmission of gate voltages through peptide nanomaterials in field-effect transistors (ACS Nano 2015), and the creation of charge separated states via photoinduced electron transfer (under review). This work provides the foundation for continued work outlined in Task 3.

#### Publications acknowledging DOE support:

[22] W. G. M. Liyanage and J. D. Tovar, “Cross-linking approaches to tune the mechanical properties of peptide- $\pi$ -electron based hydrogels,” invited by *Bioconjugate Chemistry*, *submitted*.

[21] A. M. Sanders, T. S. Kale, H. E. Katz and J. D. Tovar, “Self-assembling multivalent  $\pi$ -conjugated peptide nanomaterials,” *submitted*.

[20] T. S. Kale and J. D. Tovar, “Synthesis and Evaluation of Self—Assembled Nanostructures of Peptide- $\pi$  chromophore Conjugates,” invited by *Methods in Molecular Biology*, *in press*.

[19] T. S. Kale and J. D. Tovar, “Regulation of Peptide- $\pi$ -Peptide Nanostructure Bundling: The Impact of “Cruciform”  $\pi$ -Electron Segments,” invited as part of a Symposium-in-Print in honor of the retirement of Professor Gary H. Posner, *Tetrahedron*, 2016 (72) 6084-6090. (DOI:10.1016/j.tet.2016.07.064)

[18] A. M. Sanders, T. J. Magnanelli, A. E. Bragg and J. D. Tovar, “Photoinduced electron transfer within supramolecular donor-acceptor peptide nanostructures under aqueous conditions,” in the *Journal of the American Chemical Society*, 2016 (138) 3362-3370. (DOI: 10.1021/jacs.5b12001)

- [17] B. A. Thurston, J. D. Tovar and A. L. Ferguson, "Thermodynamics, morphology, and kinetics of early-stage self-assembly of  $\pi$ -conjugated oligopeptides," in *Molecular Simulations*, 2016 (42) 955-975. (DOI: 10.1080/08927022.2015.1125997)
- [16] K. Besar, H. A. M. Ardoña, J. D. Tovar and H. E. Katz, "Demonstration of hole transport and voltage equilibration in self-assembled pi-conjugated peptide nanostructures using field-effect transistor architectures," in *ACS Nano* 2015 (9) 12401-12409. (DOI: 10.1021/acsnano.5b05752)
- [15] H. A. M. Ardoña and J. D. Tovar, "Peptide pi-electron conjugates: organic electronics for biology?" invited cover article by *Bioconjugate Chemistry* 2015 (26) 2290-2302. (DOI: 10.1021/acs.bioconjchem.5b00497)
- [14] H. A. M. Ardoña, K. Besar, **M. Togninalli**, H. E. Katz and J. D. Tovar, "Sequence-dependent mechanical, photophysical and electrical properties of pi-conjugated peptide hydrogelators," in the *Journal of Materials Chemistry C* 2015 (3) 6505-6514 (part of a special web-themed issue on "Bioelectronics"). (DOI: 10.1039/C5TC00100E)
- [13] J. D. Tovar, "Peptide nanostructures with pi-ways: photophysical consequences of peptide/pi-electron molecular self-assembly," invited by the *Israel Journal of Chemistry* 2015 (55) 622-627 (part of a special issue on Functional Peptide and Protein Nanostructures). (DOI: 10.1002/ijch.201400161)
- [12] H. A. M. Ardoña and J. D. Tovar, "Energy transfer within responsive pi-conjugated peptide-based coassembled nanostructures in aqueous environments," in *Chemical Science*, 2015 (6) 1474-1484 (DOI: 10.1039/C4SC03122A)
- [11] B. D. Wall, **Y. Zhou**, **S. Mei**, H. A. M. Ardoña, A. L. Ferguson and J. D. Tovar, "Variation of formal hydrogen bonding networks within electronically delocalized pi-conjugated oligopeptide nanostructures," in *Langmuir*, 2014 (30) 11375-11385. (DOI: 10.1021/la501999g)
- [10] B. D. Wall, A. E. Zacca, A. M. Sanders, W. L. Wilson, A. L. Ferguson and J. D. Tovar, "Supramolecular polymorphism: Tunable electronic interactions within pi-conjugated peptide nanostructures dictated by primary amino acid sequence," in *Langmuir*, 2014 (30) 5946-5956. (DOI: 10.1021/la500222y)
- [9] A. M. Sanders and J. D. Tovar, "Solid-phase Pd-catalyzed cross-coupling methods for the construction of pi-conjugated peptide nanomaterials," in *Supramolecular Chemistry*, 2014 (26) 259-266. (DOI: 10.1080/10610278.2013.852675)
- [8] A. B. Marciel, M. Tanyeri, B. D. Wall, J. D. Tovar, C. M. Schroeder, W. L. Wilson, "Fluidic-directed assembly of aligned oligopeptides with  $\pi$ -conjugated cores," in *Advanced Materials*, 2013 (25) 6398-6404. (DOI: 10.1002/adma.201302496)
- [7] S. R. Diegelmann and J. D. Tovar, "Polydiacetylene-peptide one-dimensional nanomaterials," invited as a Feature Article by *Macromolecular Rapid Communications*, 2013 (34) 1343-1350. (DOI: 10.1002/marc.201300423)
- [6] J. D. Tovar, "Supramolecular construction of optoelectronic biomaterials," invited by *Accounts of Chemical Research*, 2013 (46) 1527-1537. (DOI: 10.1021/ar3002969)

[5] A. M. Sanders, T. J. Dawidczyk, H. E. Katz and J. D. Tovar, "Peptide-based supramolecular semiconductor nanomaterials via Pd-catalyzed solid-phase 'dimerizations'," in **ACS Macro Letters**, 2012 (1) 1326-1329. (DOI: 10.1021/mz3004665)

NOTE: correction posted 3 December 2012 (DOI: 10.1021/mz300622w)

[4] B. D. Wall and J. D. Tovar, "Synthesis and characterization of pi-conjugated peptide-based supramolecular materials," invited by **Pure and Applied Chemistry** (selected conference papers from the 14<sup>th</sup> International Symposium on Novel Aromatic Compounds, Eugene, OR, July 2011), 2012 (84) 1039-1045. (DOI:10.1351/PAC-CON-11-10-24)

[3] S. R. Diegelmann, N. Hartman, N. Markovic and J. D. Tovar, "Synthesis and alignment of discrete polydiacetylene-peptide nanostructures," in the **Journal of the American Chemical Society**, 2012 (134) 2028-2031. (DOI: 10.1021/ja211539j)

[2] B. D. Wall,\* S. R. Diegelmann,\* S. Zhang,\* T. J. Dawidczyk, W. L. Wilson, H. E. Katz, H.-Q. Mao and J. D. Tovar, "Aligned macroscopic domains of optoelectronic nanostructures prepared via the shear flow assembly of peptide hydrogels," cover article in **Advanced Materials**, 2011 (23) 5009-5014. (DOI: 10.1002/adma.201102963) (\* contributed equally)

[1] J. D. Tovar, "Organic halogenation chemistry as a vital tool for the construction of functional pi-conjugated materials" invited by **Synthesis** (special issue on "organic halogenation," Erick M. Carreira, editor), 2011, 2387-2391. (DOI: 10.1055/s-0030-1260086)

#### **Patents issued or filed based on DOE-supported research**

[3] J. D. Tovar, H. E. Katz, H. A. M. Ardoña, A. M. Sanders, K. Besar, "Energy transfer within pi-conjugated peptide heterostructures in aqueous environments, provisional patent filed 11/24/14.

[2] J. D. Tovar and A. M. Sanders, "Palladium catalyzed reactions executed on solid-phase peptide synthesis supports including on-resin dimerization for the production of self-assembling peptides embedded with complex organic electronic subunits," U.S. Patent No. 9,234,003 (issued 1/12/2016).

[1] J. D. Tovar and A. M. Sanders, "Palladium catalyzed reactions executed on solid-phase peptide synthesis supports including on-resin dimerization for the production of self-assembling peptides embedded with complex organic electronic subunits," U.S. Patent No. 8,871,903 (issued 10/22/2014).