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14. ABSTRACT The main focus of current activities was on synthesis of new branched and peptide-containing molecules and further development of sophisticated freely standing membranes with micropatterned structure. We synthesized several new amphiphilic hyperbranched molecules and silver-binding peptide for further incorporation into membrane film. Freely-standing arrays of carbon nanotubes and gold nanoparticles were fabricated as well and their properties tested with micromechanical studies and Raman spectroscopy. Specifically we studies: flexible nanomembranes with encapsulated silver nanowires and semiconducting quantum dots display outstanding micromechanical, fluorescence, and conducting properties; quantum dot nanomembranes suspended over optical cavities show exceptional backlight-enhanced fluorescence intensity; multifunctional hyperbranched molecules control monodisperse silver nanoparticle monolayer growth at the air-water interface. Silver-reducing peptides were encapsulated into ultrathin polymer film and formation of silver nanoparticles was demonstrated.					
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(Transferred from Iowa State to Georgia Tech in 2006)
GRANT FA9550-05-1-0209**

FLEX BIOHYBRID NANOMEMBRANES AS A PLATFORM FOR MULTIFUNCTIONAL SENSORS

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Project objectives

The study proposed here is focused on the introduction and development of a novel class of robust, freely suspended, lightweight, organic-inorganic *flex nanomembranes* with an effective thickness of 30-100 nm exhibiting outstanding sensitive properties. A clear understanding of the fundamentals of nanoscale interactions and structures important for the formation, responsive behavior, and functioning will be crucial for the successful assembly of highly sensitive and ultrathin nanomembranes.

DoD potential enhancement: Prospective ultra-sensing nanotechnology (thermal, acoustic, chemical) based upon flexible suspended nanostructures leading to dramatic miniaturization and superior sensitivity of lightweight hybrid sensor arrays for acoustic, infrared, and photothermal detection.

Major focus areas

- Implementing directed assembly of multilayered organic-inorganic flexible nanomembranes and the deposition of such membranes on micro-perforated solid substrates in free-suspended state;
- understanding of hierarchical nanostructural organization and multi-length scale molecular interactions governing the nano-mechanical behavior of these membranes under variable environmental conditions;
- designing the outermost polymer multilayers as an effective elastic nanoscale matrix for free standing membranes as well as a tunable barrier for controlling interactions between the central sensing layer and the environment;
- designing the central sensing layer via principles of biomineralization and exploring their ability to assemble into organized arrays in a fashion suitable for stimuli responsive nanoparticle behavior;
- searching for the integration of flex nanomembranes in microfabricated arrays with feasible read-out (optical, electrical) schemes.

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Major accomplishments

The main focus of current activities was on synthesis of new branched and peptide-containing molecules and further development of sophisticated freely standing membranes with micropatterned structure. We synthesized several new amphiphilic hyperbranched molecules and silver-binding peptide for further incorporation into membrane film. Freely-standing arrays of carbon nanotubes and gold nanoparticles were fabricated as well and their properties tested with micromechanical studies and Raman spectroscopy.

Specifically, we studies:

- flexible nanomembranes with encapsulated silver nanowires and semiconducting quantum dots display outstanding micromechanical, fluorescence, and conducting properties;
- quantum dot nanomembranes suspended over optical cavities show exceptional backlight-enhanced fluorescence intensity;
- multifunctional hyperbranched molecules control monodisperse silver nanoparticle monolayer growth at the air-water interface
- Silver-reducing peptides were encapsulated into ultrathin polymer film and formation of silver nanoparticles was demonstrated
- Silk fibroin molecules have been stretched and multistage unfolding dynamics was correlated with domain composition of these proteins
- Free-standing films from silk material has been fabricated and outstanding strength has been measured

Examples of studies conducted

Design on new functional materials for biomineralization of central layer: silver peptide synthesis.

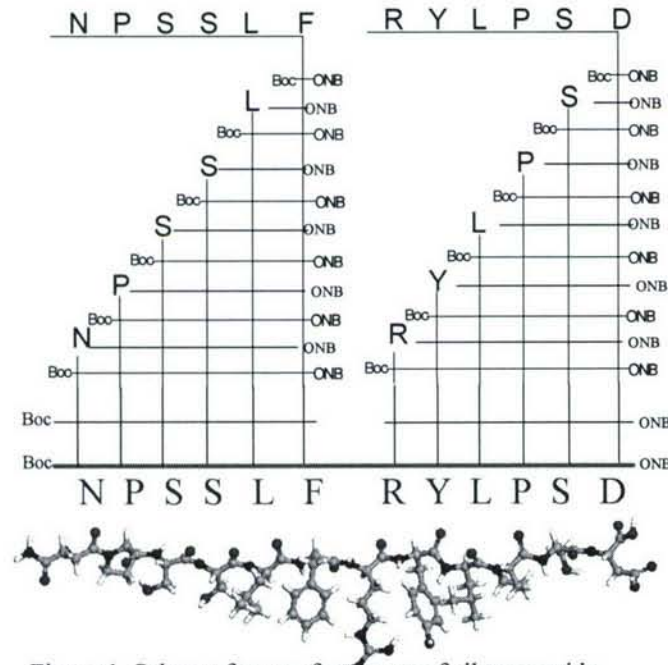


Figure 1. Scheme for two fragments of silver peptide AG4, and chemical structure of completed peptide.

We started synthesis of silver-precipitating peptide sequence reported by Dr. Stone and coworkers. Previously AG4 peptide has been obtained via gene engineering methods, and also it was obtained by solid phase synthesis. Each of these techniques results in unprotected peptides, which are not suitable for controlled attachment to polymer substrates; also quantities of the peptide are very little. In order to obtain the amount of silver-precipitating peptide sufficient for polymer

modification, liquid phase-peptide synthesis method was utilized. Technical simplicity and scalability of the liquid-phase peptide synthesis method renders it particularly attractive for large-scale preparation of peptides. Because of its size, the AG4 peptide can be obtained via synthesis of small segments or fragments that are subsequently assembled to give the final sequence.

Synthesized two half peptides, 6 amino acids each are shown in figure 1. Each short peptide sequence was synthesized by stepwise addition of each amino acid in the sequence until the entire sequence has been assembled, using Repetitive Excess Mixed Anhydride (REMA) synthesis. Synthesis allows omitting tedious purification during intermediate stages and use crude extraction or precipitation instead.

Synthesis of new amphiphilic hyperbranched polymers for central layer.

Polymer, 2006, 47, 8137)COOH and NH₂. The latest groups are essential for attachment peptide groups to the hyperbranched polymer.

Synthesis of aliphatic esters was performed via reaction with palmitoyl chloride. Synthesis of HBP with NH₂ terminal groups was done following the common DCC coupling procedure. First boc-amino-hexanoic acid was attached to the alkyl modified HBP. Then boc protective group was removed by acidic hydrolysis. Synthesis of HBP with -COOH terminal group was accomplished via esterification of the alkyl modified HBP with succinic anhydride. Every step of the synthesis was followed by purification of the product using column chromatography, and dialysis. Purity of the product was confirmed using GPC analysis. Composition of synthesized polymers was estimated from ¹H and ¹³C NMR. Resulting chemical structures build in ChemDraw give estimated molecular weights of the polymers.

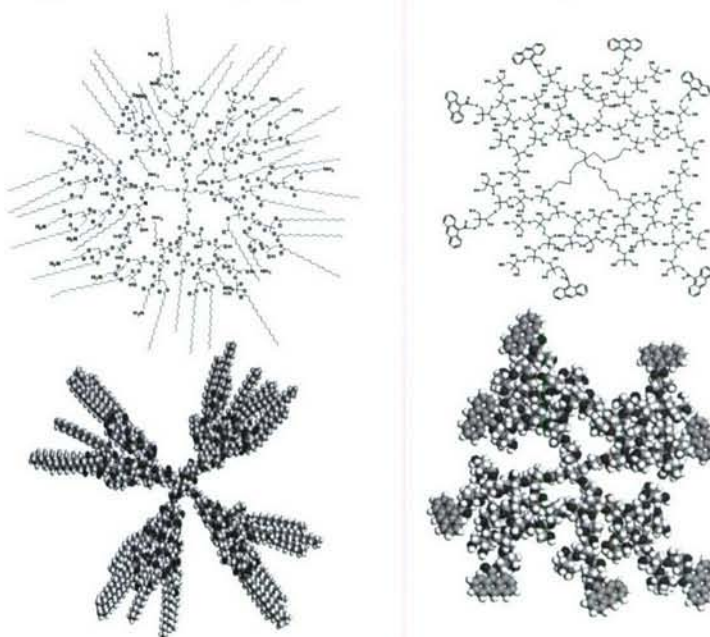


Table 1. Composition of new hyperbranched polymers with aliphatic and ionic groups:

Polymer	Number of palmitic groups	Ionic group	Number of ionic groups	Calc. MW

P30Suc	33	-COOH	29	18,067
MO55	13	-COOH	36	13,999
D3PN	38	-NH ₂	9	17,376
MO56	19	-NH ₂	16	13,638

Freely standing nanomembranes with micropatterned interior.

We demonstrated the fabrication of carbon nanotube arrays with microscopic spacing

encapsulated into freely suspended flexible films with a nanoscale thickness

(*Adv. Mater.*, **2005**, *17*, 2127).

The patterned array of monolayered carbon nanotubes with a thickness of 5 nm was sandwiched

between two ultrathin polymer multilayers with the thickness of about 19 nm each. A key

element of the microfabrication routine introduced here is the exploitation of a polymer sacrificial micropattern as a template for assembly of carbon nanotubes followed by its removal and further derivatization of the exposed surface areas with a topmost polyelectrolyte multilayer to complete encapsulation. By using confocal Raman imaging, we demonstrated that the carbon nanotube array embedded into the polymer film generates highly contrasted resonance Raman grating which might be considered for prospective sensing applications (Figure 2).

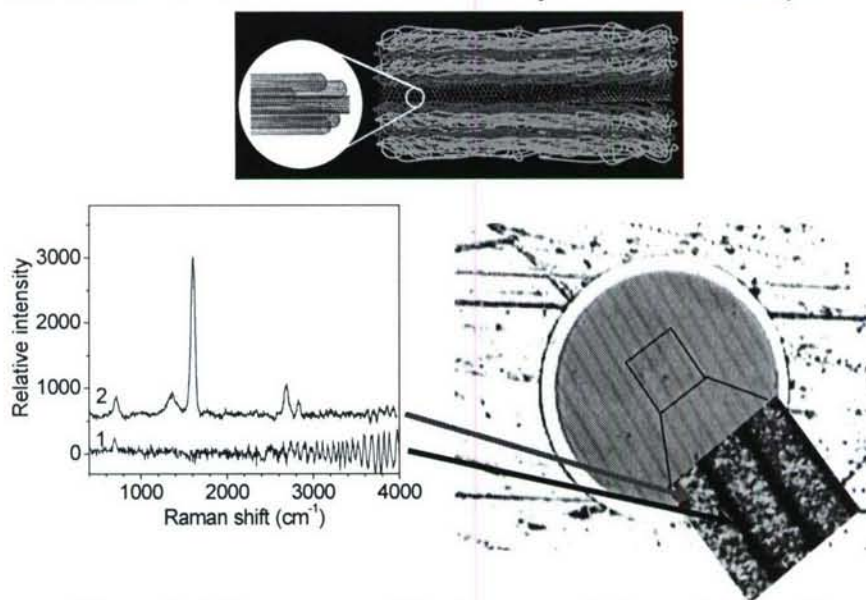


Figure 2. Micropatterned freely suspended membrane with encapsulated carbon nanotubes and its Raman appearance.

We fabricated freely suspended gold nanoparticle arrays encapsulated into multilayered LbL nanomembranes (Figure 3) (*Adv. Mater.* **2005**, *17*, 1669). Such micropatterned nanomembranes preserved outstanding micromechanical properties observed for continuous nanomembranes and possessed an anisotropic mechanical response caused by different composite moduli along and perpendicular to the nanoparticles-containing stripes. The periodic variation of the Raman scattering across the micropatterned array (*microscopic SERS grating*) was found for these micropatterned nanomembranes.

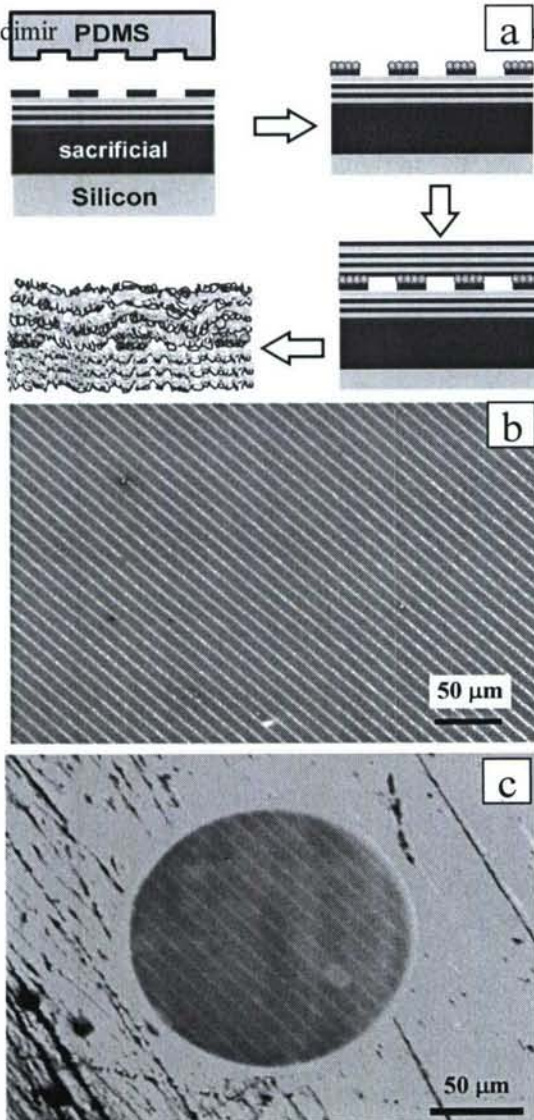


Figure 3. Micropatterned freely suspended membrane with encapsulated gold-nanoparticulate array and its SERS Raman spectra.

statistics and found modest increase of the elastic modulus with decreasing membrane diameter. And, finally, we observed the scale-dependent elastic behavior with slow, logarithmic increase of the bending rigidity with decreasing diameter of the membranes in the microscopic range of diameters. This behavior is satisfactory described by the model of the bending elastic membrane under the point-load with realistic loading characteristics.

The molecular chain reorganization in freely-standing polymeric-nanoparticle membranes with encapsulated gold nanoparticles was studied with SERS which was enabled by the designing of gold nanoparticle forming long chain-like

We assembled freely-suspended ultrathin LbL nanomembranes on the 4×4 array with constant and gradient opening diameters and demonstrated its applicability for *concurrent monitoring* of collective deflection behavior of circular nanomembranes under external hydrostatic pressure (Figure 4) (*Adv. Funct. Mater.*, **2006**, *16*, 27). The

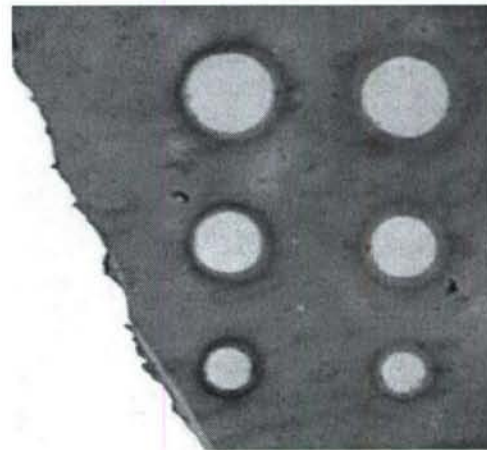


Figure 4. Membrane covering several openings.

gradient array designed with a variable diameter of openings in one direction provided a means for efficient and fast screening of scale-dependent membrane properties. By using this approach, we significantly improved experimental

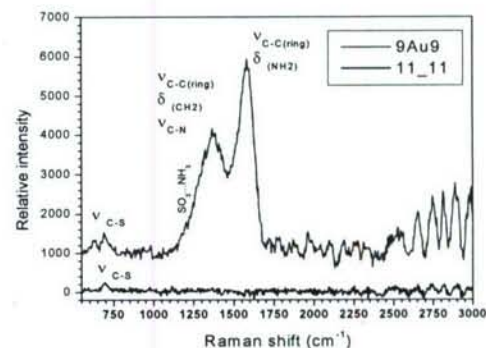


Fig. 5. Raman spectra of 35 nm LbL film (black) and after

aggregates thus, creating an exceptional ability for *in-situ* monitoring of the femtogram amount of material (Fig. 5) (*Phys. Rev. Lett.* **2005**, *95*, 115503). We observed small deformations (strain below 0.05%) resulted in the radial orientation of side phenyl rings of polymer backbones while larger deflections led to the polymer chains bridging adjacent nanoparticles within one-dimensional aggregates. We suggested that this phenomenon is responsible for the outstanding robustness and self-recovery of these nanomembranes critical for their unsurpassed performance as sensitive thermal and pressure microsensors.

Indeed, we observed the photothermal deflection of these flexible, freely suspended nanoscale polymeric membranes with encapsulated gold nanoparticles which seal microfabricated cavities (*Chem. Mater.*, **2006**, *18*, 2632).

We demonstrated that the photothermal behavior can be scaled up to a large (64x64) array of the microscopic cavities thus creating efficient microscopic thermal imaging array operating at room temperature (Fig. 6). Multiple transitions from convex to concave shape of the flexible nanomembranes caused by air thermal expansion and contraction within sealed cells which was used for tracking a NIR laser beam footprint (Fig. 6).

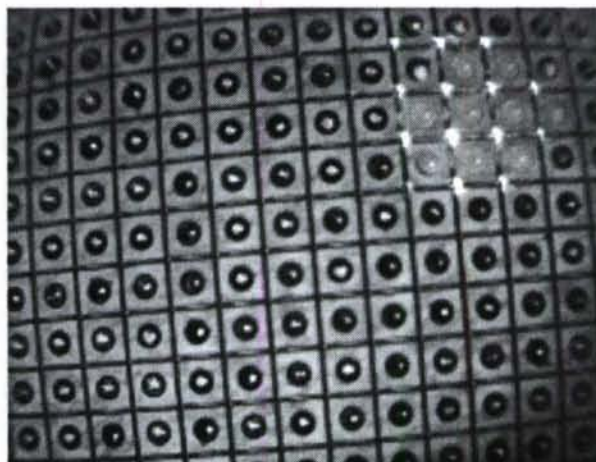


Fig. 6. Tracing NIR laser beam (top-right) with an array of microfabricated cavities sealed with flexible membranes (each cell is 80 μm).

Multi-scale force spectroscopy was applied to measure unfolding properties and the internal domain structure of *Bombyx mori* silk fibroin (*Polymer*, **2006**, *47*, 8137) (Fig. 7). We demonstrated that the complex multi-domain sequence and block design in this protein can be directly linked to multi-stage unfolding behavior of the specific regions through the use of single molecule force extension measurements (Fig. 7).

We observed multiple consequential unfolding of hydrophilic and hydrophobic domains with characteristics that can be directly related to known molecular dimensions of the protein backbones. The overall interpretation made on the basis of analysis of individual statistics for spacings and forces derived from the model of block sizes and distributions in this protein suggested the multilength unfolding pattern (Fig. 7). This behavior reflects a complex character with multiple events of unfolding of 12 hydrophilic and 12 hydrophobic domains before the ultimate stretching.

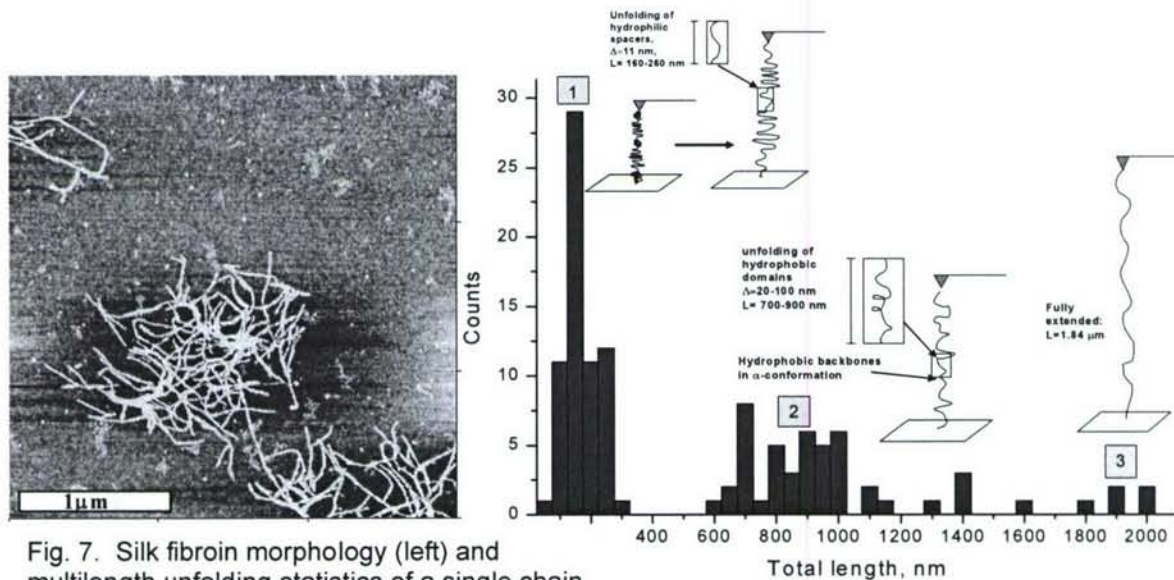


Fig. 7. Silk fibroin morphology (left) and multilength unfolding statistics of a single chain (right).

Robust ultrathin multilayer films of silk fibroin were fabricated with spin coating and spin-assisted LbL assembly and their mechanical properties were studied both in tensile and compression modes (*Adv. Funct. Mater.* **2007**, *17*, 2229). The ultrathin films were characterized by a high elastic modulus of 6-8 GPa and the ultimate tensile strength reaching 100 MPa (Fig. 8).

The outstanding toughness is also manifold higher than that usually observed for conventional polymer composites (328 kJ/m^3). These outstanding properties are suggested to be caused by the gradual

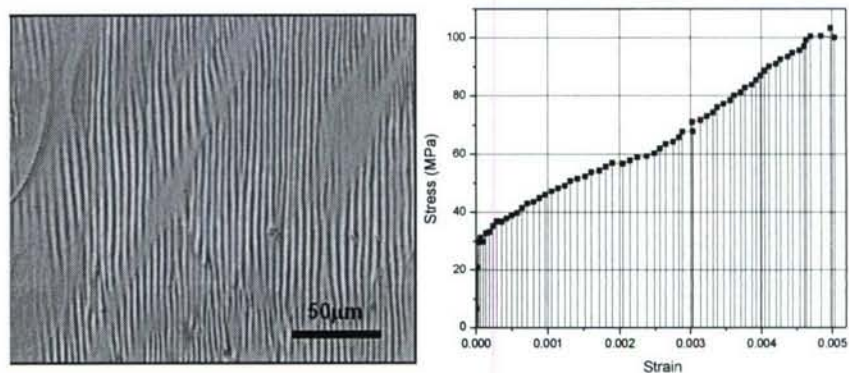


Fig. 8. Ultrathin silk LbL film (60 nm) under compression (left, buckling pattern) and tensile deformation (right).

development of the self-reinforcing microstructure of highly crystalline β -sheets, serving as reinforcing fillers and physical crosslinks, a process well known for the bulk silk materials but demonstrated to be efficient for ultrathin films as well. However, the confined state within films thinner than the length of the extended domains (<10 nm) causes the reduced elasticity which should be considered in design of the nanoscale films from silk materials.

Personnel trained and dissemination

Five students who participated in the project have been graduated and continue their careers in different venues (2-industry, 3-academia): MSE graduate students, Ms. M. Ornatska (PhD, 2006, moved to Clarkson U), Ms. B. Rybak (MS, 2005, Lockheed-Martin), Mr. M. Lemieux (PhD, 2006, CIA post-doctoral Fellow, Stanford), Ms. Y.H. Lin (PhD, 2007, Whirlpool). Dr. C. Jiang, a post-doctoral researcher became a faculty member at U South Dakota.

Current project participants, M. McConney, K. Bergman, and Dr. E. Kharlampieva are deeply involved in collaboration with AFRL researchers (two summer internships).

Total, 20 refereed publications resulted from this support in 2005-2007 (see a list of selected papers below). *Front Cover* of May 2005 issue of *Advanced Functional Materials* featured our nanomembranes, June 2005 issue of *MRS Bull.* included *Research News* on our micropatterning, and *Front Cover* of May 2006 issue of *Advanced Materials* displayed our microcantilever sensors (PI's AFOSR-STTR project).

Finally, an invited review on freely standing nanostructures has been published in March 2006 and another invited review on microcantilever sensors will be published soon in *Advanced Materials*. One patent is pending and an IP disclosure is filed with AFRL researchers.

Peer-reviewed publications with AFOSR support, 2005 – 2007:

1. C. Jiang, X. Wang, R. Gunawidjaja, Y.-H. Lin, M. K. Gupta, D. L. Kaplan, R. R. Naik, V. V. Tsukruk, Mechanical Properties of Robust Ultrathin Silk Fibroin Films, *Adv. Funct. Mater.* **2007**, *17*, 2229
2. S. Singamaneni, M. C. LeMieux, H. Jiang, T. J. Bunning, V. V. Tsukruk, Negative Thermal Expansion in Ultrathin Plasma Polymerized Films, *Chem. Mater.*, **2007**, *19*, 129.
3. S. Singamaneni, C. Jiang, E. Merrick, D. Kommireddy, V. V. Tsukruk, Robust Fluorescent Response of Micropatterned Multilayered Films, *J. Macromol. Sci., B: Phys.* **2007**, *46*, 7.
4. Y. H. Lin, C. Jiang, J. Xu, Z. Lin, V. V. Tsukruk, Robust, Fluorescent, and Nanoscale Freestanding LbL Conjugated Films, *Soft Matter.*, **2007**, *3*, 432.
5. C. Jiang, V. V. Tsukruk, Free Standing Nanostructures via Layer-by-Layer Assembly, *Adv. Mater.* **2006**, *18*, 829.

6. M. C. LeMieux, M. McConney, Y.-H. Lin, S. Singamaneni, H. Jiang, T.J. Bunning, V. V. Tsukruk: Polymeric Nanolayers as Actuators for Ultra- Sensitive Thermal Bimorphs, *Nano Lett.*, **2006**, 6, 730.
7. Y-H. Lin, M. McConney, M. LeMieux, S. Peleshanko, S. Singamaneni, V. V Tsukruk, Trilayered ceramic-metal-polymer microcantilevers with dramatically enhanced thermal sensitivity, *Adv. Mater.* **2006**, 18, 1157-1161.
8. C. Jiang, D. S. Kommireddy, V. V. Tsukruk, Gradient array of freely suspended nanomembranes, *Adv. Funct. Mater.*, **2006**, 16, 27-32.
9. B. M. Rybak, K. N. Bergman, M. Ornatska, K. L. Genson, V. V. Tsukruk, The formation of silver nanoparticles at the air-water interface mediated by the monolayer of functionalized hyperbranched molecules, *Langmuir*, **2006**, 22, 1027-1037.
10. C. Jiang, M. E. McConney, S. Singamaneni, E. Merrick, Y. Chen, J. Zhao, L. Zhang, V. V. Tsukruk, Thermo-optical Arrays of Flexible Nanomembranes Freely Suspended over Microfabricated Cavities as IR Microimagers, *Chem. Mater.*, **2006**, 18, 2632-2634.
11. C. Jiang, W. Y. Lio, V. V. Tsukruk, Surface Enhanced Raman Scattering Monitoring of Chain Alignment in Freely Suspended Nanomembranes, *Phys. Rev. Lett.*, **2005**, 95, 115503.
12. C. Jiang, S. Markutsya, H. Shulha, V. V. Tsukruk, Freely Suspended Gold Nanoparticles Arrays, *Adv. Mater.* **2005**, 17, 1669-1673.
13. C. Jiang, H. Ko, V. V. Tsukruk, Strain Sensitive Raman Modes of Carbon Nanotubes in Deflecting Freely Suspended Nanomembranes, *Adv. Mater.*, **2005**, 17, 2127-2131.
14. C. Jiang, V. V. Tsukruk, Organized Arrays of nanostructures in freely suspended nanomembranes, *Soft Matter*, **2005**, 1, 334-337.
15. C. Jiang, B. M. Rybak, S. Markutsya, P. E. Kladitis, V. V. Tsukruk, Self-recovery of Nanocomposite Nanomembranes, *Appl. Phys. Lett.*, **2005**, 86, 121912.
16. S. Markutsya, C. Jiang, Y. Pikus, V. V. Tsukruk, Free-standing multilayered nanocomposites films as highly sensitive nanomembranes, *Adv. Funct. Mater.*, **2005**, 15, 771-780.
17. H. Ko, C. Jiang, H. Shulha, V. V. Tsukruk Carbon nanotube arrays encapsulated into freely suspended flexible films, *Chem. Mater.*, **2005**, 17, 2490-2493.
18. H. Ko, C. Jiang, V. V. Tsukruk, Encapsulating nanoparticle arrays into layer-by-layer multilayers by capillary transfer lithography, *Chem. Mater.* **2005**, 17, 5489-5497.

25 presentations have been delivered in USA and Europe by the PI and his students at professional conferences and seminars

Related developments

Collaboration with AFRL researchers

The study relies heavily on intense collaboration with AFRL researchers facilitated by six mutual visits of PI and three researchers (Dr. M. Stone, Dr. R. Naik, and Dr. T. Bunning) and eight mutual student visits including two summer research of the PI students at AFRL. The collaboration included biomolecules supply (peptides and silk) from Dr. R. Naik (two joint refereed publications) and

ultrathin film fabrication and characterization at Dr. T. Bunning group (three joint refereed publications and one joint IP disclosure).

Discoveries

Patent application pending: *Compliant, nanoscale free-standing multilayer films*, September 30 2004, **US Pat. Appl. 20055175507, 08/11/2005.**

IP disclosure: *Negative Thermal Expansion Polymeric Material Prepared by PECVD*, AF Invention AFD 947, March 2007 (via AFRL)

Honors, fellowships, services

Students

- M. Lemieux, CIA Post-doctoral Fellowship, Stanford, 2007
- K. Bergman, AFRL Summer Internship, 2007
- M. McConney, AFRL Summer Internship, 2007
- K. Bergman, NSF-GAAN Fellowship, 2006

PI:

- co-organized symposium on highly branched molecules (ACS, Spring 2006)
- a member of Advisory Board, *Polymer*, 2006-present
- a member of Advisory Board, *Cur. Chem. Biology*, 2006-present
- visiting professorship at MIT, 2005