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FINAL REPORT

CONTRACT NUMBER: N00014-98-C-0279

CONTRACT TITLE: Production of Elastomeric Polypeptides for Materials Characterizations

PRINCIPAL INVESTIGATOR: Jie Xu, Ph.D. (e-mail:brl@oadi.uab.edu)

PI INSTITUTION: Bioelastics Research, Ltd.

AWARD PERIOD: 15 June 1998 - 14 June 2000

LONG-TERM RESEARCH OBJECTIVE: The long term research objective is to develop the full capacity of the hydrophobic folding and assembly transition of elastic protein-based (amphiphilic) polymers in an aqueous milieu for catalyzing the diverse energy conversions of biology.

S&T OBJECTIVES: To prepare a family of designed elastic protein-based polymers for free energy transduction. Specifically, this effort is to provide 100 gram quantities of six and 10 gram quantities of an additional nine elastomeric polypeptides for conventional and specialized materials characterizations and for further development toward specific applications of potential use to the Navy.

APPROACH: By using genetic engineering approaches, the genes encoding various elastomeric polypeptides were constructed and engineered into *E coli* expression hosts. By having *E coli* expressing those gene products at adequate levels, it is possible to synthesize reproducible polymer materials in large quantities through a single fermentation run. The 500 or 28 liter fermentors are used to cultivate the cells and the materials are isolated from the cells employing their unique inverse temperature transitional (phase separation) properties.

ACCOMPLISHMENTS: Polymer production and cross-linking efforts.

The full set of polymers that were produced is listed below. They were produced at the desired quantities and transferred to University of Minnesota (UMN) for their studies. They were finished ahead of schedule.

| | | | |
|---------|--------|---|----------|
| Polymer | *I: | (GVGVP) ₂₅₁ | |
| Polymer | *II: | (GVGIP) ₂₆₀ | |
| Polymer | *III: | (GVGVP GVGFP GEGFP GVGVP GVGFP GFGFP) _n (GVGVP) | E/5F |
| Polymer | *IV: | (GVGVP GVGFP GEGFP GVGVP GVGFP GVGFP) _n (GVGVP) | E/4F |
| Polymer | *V: | (GVGVP GVGFP GEGFP GVGVP GVGFP GFGFP) _n (GVGVP) | E/3F |
| Polymer | *VI: | (GVGVP GVGFP GEGFP GVGVP GVGVP GVGVP) _n (GVGVP) | E/2F |
| Polymer | *VII: | (GVGVP GVGFP GEGFP GVGVP GVGVP GVGVP) _n (GVGVP) | E/0F |
| Polymer | *VIII: | (GVGIP GFGEP GEGFP GVGVP GFGFP GFGIP GVGIP GFGEP GEGFP GVGVP GFGFP GFGIP) _n (GVGVP) | 2E/5F/2I |
| Polymer | *IX: | (GVGVP GVGFP GKGFVP GVGVP GVGFP GFGFP) _n (GVGVP) | K/5F |
| Polymer | *X: | (GVGVP GVGFP GKGFVP GVGVP GVGFP GVGFP) _n (GVGVP) | K/4F |
| Polymer | *XI: | (GVGVP GVGFP GKGFVP GVGVP GVGFP GFGFP) _n (GVGVP) | K/3F |
| Polymer | *XII: | (GVGVP GVGFP GKGFVP GVGVP GVGVP GVGVP) _n (GVGVP) | K/2F |
| Polymer | *XIII: | (GVGVP GVGFP GKGFVP GVGVP GVGVP GVGVP) _n (GVGVP) | K/0F |
| Polymer | *XIV: | (GVGVP GVGFP GEGFP GVGVP GVGFP GKGFVP) _n (GVGVP) | E/K/3F |
| Polymer | *XV: | (GVGVP GVGFP GEGFP GVGVP GVGVP GKGFVP) _n (GVGVP) | E/K/2F |

*Polymers supplied at 100 gram amounts. °Polymers supplied at 10 gram amounts. The polymers were completed by the end of September, which is months ahead of the revised and shortened timelines.

Large sheets of polymer I, II, III and VII were exposed to 20 Mrad of gamma-irradiation to form insoluble cross-linked matrixes and supplied to UMN.

A series of polymers, I, II, III, IV, VII, VIII and XIII, were cross-linked at varying doses of gamma irradiation. The compositions were exposed to 10, 14, 18, 22, 26, 30 and 34 Mrad for a total of seven cross-linked matrixes each. These were then supplied to UMN for their studies.

Also, additional fermentation runs were performed to produce materials to aid purification efforts by UMN and to demonstrate BRL levels of polymer expression. And a BRL scientist also visited UMN to assist fermentation efforts by UMN.

This set the stage for a more complete understanding of the physical basis for hydrophobic-induced shift in properties of functional groups.

CONCLUSIONS: All the polymers and materials proposed in the contract were made and delivered to the University of Minnesota. Additional and varied constructs were also prepared and supplied, once the need demonstrated. This was accomplished ahead of schedule so that UMN could complete their studies under the ONR contract number N00014-98-1-0656 which was running concurrently.

SIGNIFICANCE: There are numerous naval applications where free energy transduction is fundamental. One example would be in the development of MEMS (micro-electro-mechanical systems), which is directly related to stretch-induced free energy transductions.

TECHNOLOGY TRANSFER: The corporate entity, Bioelastics Research Ltd. (BRL), is already in place for the technology transfer. BRL holds basic patents to the polymer compositions and their use in a number of free energy transduction applications. An agreement between BRL and UMN governs the technology developed at UMN wherein BRL has exclusive rights for the technology derived during this effort.

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PUBLICATIONS ARISING FROM THE PRODUCTION OF POLYMERS FOR UMN

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2. Jonghwi Lee, Christopher W. Macosko, and Dan W. Urry, "Elastomeric Polypentapeptides Cross-Linked into Matrixes and Fibers," *Biomacromolecules* **2**, 170-179, 2001.
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