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as of 04-Nov-2021

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**Training Opportunities:** Nothing to Report

**Results Dissemination:** Huang, W. S. Ling, C. Li, F. G. Omenetto and D. L. Kaplan, Silkworm Silk-based Materials and Devices generated using Bio-nanotechnology, *Chemical Society Reviews*, (2018) 47(17): 6486 6504. doi: 10.1039/c8cs00187a, PMID: PMC6113080

Huang Y, Fitzpatrick V, Zheng N, Cheng R, Huang H, Ghezzi C, Kaplan DL, Yang C. Self-folding 3D silk biomaterial rolls to facilitate axon and bone regeneration. *Advanced Healthcare Materials* Aug 31:e2000520, ahead of print (2020) [PMID: 32864866]

Narayan OP, Mu X, Hasturk O, Kaplan DL. Dynamically tunable light responsive silk-elastin-like proteins. *Acta Biomaterialia*, 121:214-223 (2021) PMID: 33326881.

Parker RN, Wenyao A. Wu, Tina B. McKay, Qiaobing Xu, David L. Kaplan. Design of Silk-Elastin-Like Protein Nanoparticle Systems with Mucoadhesive Properties. *J Funct Biomater*. 2019 Dec; 10(4): 49. Published online (2019) Nov 12. doi: 10.3390/jfb10040049, PMID: PMC6963467

Parker RN, Cairns DM, Wu WA, Jordan K, Guo C, Huang W, Martin-Moldes Z, Kaplan DL. Smart materials hydrogel transfer devices fabricated with stimuli-responsive silk-elastin-like proteins. *Advanced Healthcare Materials* Apr 27:e2000266, ahead of print (2020) PMID: 32338463

Wang Y, Huang W, Wang Y, Mu X, Ling S, Yu H. Chen W, Guo C, Watson MC, Yu Y, Black LD, Li M, Omenetto FG, Li C, Kaplan DL. Stimuli-responsive composite biopolymer actuators with selective spatial deformation behavior. *Proceedings National Academy of Science* 117(25):14602-14608 (2020) PMID 32522869.

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**Participant Type:** Graduate Student (research assistant)

**Participant:** Onur Hasturk

**Person Months Worked:** 15.00

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**Participant Type:** Postdoctoral (scholar, fellow or other postdoctoral position)

**Participant:** Fredrik Backlund

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**Participant:** Om Prakash Narayan

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**Article Title:** Silk worm silk-based materials and devices generated using bio-nanotechnology

**Authors:** Wenwen Huang, Shengjie Ling, Chunmei Li, Fiorenzo G. Omenetto, David L. Kaplan

**Keywords:** silk, materials, devices, review, nanotechnology

**Abstract:** The versatility and sustainability of silk-based materials provides an impressive toolbox for tailoring materials to meet specific applications via eco-friendly approaches. Historically, silk worm silk has been used by the textile industry for thousands of years due to its excellent physical properties, such as lightweight, high mechanical strength, flexibility, and luster. Recently, due to these properties, along with its biocompatibility, biodegradability and non-immunogenicity, silk worm silk has become a candidate for biomedical utility. Further, the FDA has approved silk medical devices for sutures and as a support structure during reconstructive surgery. With increasing needs for implantable and degradable devices, silk worm silk has attracted interest for electronics, photonics for implantable yet degradable medical devices, along with a broader range of utility in different device applications.

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**Article Title:** Self-Folding 3D Silk Biomaterial Rolls to Facilitate Axon and Bone Regeneration

**Authors:** Yimin Huang, Vincent Fitzpatrick, Nan Zheng, Ran Cheng, Heyu Huang, Chiara Ghezzi, David L. Kaplan

**Keywords:** 3D cell cultures; heterogeneous cell cultures; neuron regeneration; osteogenesis; self-folding biomaterials.

**Abstract:** Three-dimensional organoid tissue culture models are a promising approach for the study of biological processes including diseases. Advances in these tissue culture technologies improve in vitro analysis compared to standard two-dimensional cellular approaches that are more representative of the physiological environment. However, a major challenge associated with organoid systems stems from the laborious processing involved in the analysis of large numbers of organoids. Here we present the design, characterization and application of silk-elastin-like protein-based smart carrier arrays for processing organoids. Fabrication of hydrogel-based carrier systems at room temperature resulted in organized arrays of organoids that maintained tissue culture plate orientation and could be processed simultaneously for histology.

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**Article Title:** Dynamically tunable light responsive silk-elastin-like proteins

**Authors:** Om Prakash Narayan, Xuan Mu, Onur Hasturk, David L. Kaplan

**Keywords:** silk, elastin, stimuli-responsive, biomaterials, silk-elastin-like proteins, bioengineering

**Abstract:** Three-dimensional organoid tissue culture models are a promising approach for the study of biological processes including diseases. Advances in these tissue culture technologies improve in vitro analysis compared to standard two-dimensional cellular approaches that are more representative of the physiological environment. However, a major challenge associated with organoid systems stems from the laborious processing involved in the analysis of large numbers of organoids. Here we present the design, characterization and application of silk-elastin-like protein-based smart carrier arrays for processing organoids. Fabrication of hydrogel-based carrier systems at room temperature resulted in organized arrays of organoids that maintained tissue culture plate orientation and could be processed simultaneously for histology.

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**Article Title:** Design of Silk-Elastin-Like Protein Nanoparticle Systems with Mucoadhesive Properties

**Authors:** Rachael N. Parker, Wenyao A. Wu, Tina B. McKay, Qiaobing Xu, and David L. Kaplan

**Keywords:** Silk-Elastin-Like Protein; Mucoadhesion; Transmucosal Drug Delivery, Nanoparticles

**Abstract:** Three-dimensional organoid tissue culture models are a promising approach for the study of biological processes including diseases. Advances in these tissue culture technologies improve in vitro analysis compared to standard two-dimensional cellular approaches that are more representative of the physiological environment. However, a major challenge associated with organoid systems stems from the laborious processing involved in the analysis of large numbers of organoids. Here we present the design, characterization and application of silk-elastin-like protein-based smart carrier arrays for processing organoids. Fabrication of hydrogel-based carrier systems at room temperature resulted in organized arrays of organoids that maintained tissue culture plate orientation and could be processed simultaneously for histology.

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**Article Title:** Smart Material Hydrogel Transfer Devices Fabricated with Stimuli-Responsive Silk-Elastin-Like Proteins

**Authors:** Rachael N. Parker, Dana M. Cairns, Wenyao A. Wu, Kathryn Jordan, Chengchen Guo, Wenwen Huang

**Keywords:** silk-elastin-like protein; organoids; histology; hydrogels

**Abstract:** Three-dimensional organoid tissue culture models are a promising approach for the study of biological processes including diseases. Advances in these tissue culture technologies improve in vitro analysis compared to standard two-dimensional cellular approaches that are more representative of the physiological environment. However, a major challenge associated with organoid systems stems from the laborious processing involved in the analysis of large numbers of organoids. Here we present the design, characterization and application of silk-elastin-like protein-based smart carrier arrays for processing organoids. Fabrication of hydrogel-based carrier systems at room temperature resulted in organized arrays of organoids that maintained tissue culture plate orientation and could be processed simultaneously for histology.

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**Article Title:** Stimuli-responsive composite biopolymer actuators with selective spatial deformation behavior

**Authors:** Yushu Wang, Wenwen Huang, Yu Wang, Xuan Mu, Shengjie Ling, Haipeng Yu, Wenshuai Chen, Cheng

**Keywords:** bionic; biopolymers; stimuli-response; reversible; actuation

**Abstract:** Bio-inspired actuators with stimuli-responsive and deformable properties are being pursued in fields such as artificial tissues, medical devices and diagnostics, and intelligent biosensors. These applications require that actuator systems have biocompatibility, controlled deformability, biodegradability, mechanical durability and stable reversibility. Herein, we report a new bionic actuator system consisting of stimuli-responsive genetically engineered silk–elastin-like protein (SELP) hydrogels and wood-derived cellulose nanofibers (CNF), which respond to temperature and ionic strength underwater by eco-friendly methods. Programmed site-selective actuation can be predicted and folded into 3D origami-like shapes. The reversible deformation performance of the SELP/CNF actuators was quantified, and complex spatial transformations of multilayer actuators were demonstrated, including a biomimetic flower design with selective pedal movements. Such actuators consisting entirely of biocompatib

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### Partners

I certify that the information in the report is complete and accurate:

Signature: David Kaplan

Signature Date: 10/29/21 10:04AM

## ARO – Final Report 2021: Dynamic materials based on bioengineered fibrous proteins

### A. Contributing Personnel:

Zaira Martín-Moldes, Rachael Parker, Fredrik Backlund, Yushu Wang, David L. Kaplan, Tufts University

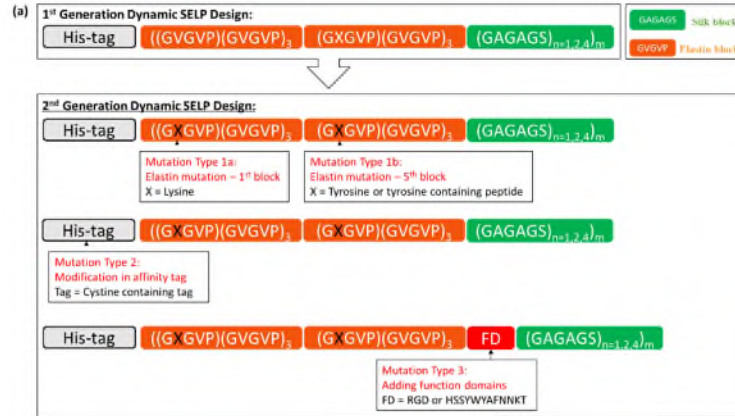
### B1. Accomplishments

**Project Summary** – Tailored biomaterials with tunable functional properties are crucial for a variety of task-specific applications ranging from health care to sustainable novel devices. To generate polymeric materials with predictive functional outcomes, exploiting designs from nature, while morphing them towards non-natural systems offers an important strategy. The *main goal* of the proposed research is to utilize new and efficient strategies to generate functional biomaterials that are dynamically responsive to specific biomolecular recognition events.

- **Task 1** - previously summarized in our early report from the project – it is included here for completeness for the Final Report
- **Task 2** - updated with the directions pursued since the early report and the rest of the project
- **Task 3** - was not funded but mentioned here for complete documentation for the Final Report

#### **Task 1 – Biomaterial inputs - expanded library of protein building blocks and protein size**

Fibrous proteins are Nature's building blocks, conferring structure and function to biological materials. Due to the genetic control of encoded protein sequence, mechanical properties, biocompatibility, and biodegradability, these proteins have been selected as a prototype model to emulate for the tunable designs of biomaterial systems. The *objective* of task 1 is to expand current responsive protein designs and identify the lead protein polymers for dynamically responsive biomaterials. To address this need, we further refined current silk-elastin-like protein sequences to provide further chemistry control. Additional functional domains were incorporated into the current library to expand material function. The protein sizes of silk-elastin-like proteins were also increased to a higher molecular weight (up to 110 kDa) for tunable responsive properties. The research progress of task 1 is summarized in **Figure 1**. A biomimetic design was carried out by combining the silk domain with the stimuli responsive elastin domains for a broader variety of dynamic protein polymers. The silk domain (GAGAGS), adopted as the representative sequence of the *Bombyx mori* silk heavy chain, tends to self-assemble into insoluble tightly packed secondary structures, beta sheets, to provide mechanical tunability and physical crosslinking sites to the SELP polymeric systems. The elastin domain (GVGVP) undergoes reversible structural transition upon exposure to environmental stimuli, endows SELPs with the dynamic function. To expand the existing protein library for developing dynamic materials, wherein input signals from environmental stimuli or biomolecular recognition (e.g., pH, chemical, optical, electrical, mechanical) drive biomolecular regulation towards a dynamic material response, the chemistry of the elastin domain was further refined by mutating the key amino acid into tyrosine and tyrosine containing peptide for diazonium coupling reaction. An additional lysine residue was also incorporated in the elastin domain for reactive oxygen species (ROS) responsive chemical modification. The cysteine residue was genetically engineered into protein affinity tags for binding of nanofillers (e.g. gold nanoparticles and gold nanorods) for light responsive biomaterials. These mutations in elastin domains and in affinity tags significantly changed the side chain chemistry of SELP molecules and therefore expanded the existing protein library with more diverse responsive properties. New function domains, such as a graphene binding domain, were genetically engineered into SELP sequences as a separate functional block for conductive dynamic biomaterials. New charged peptides were also incorporated into the sequences as a separate block/domain for electrically responsive biomaterials. The *outcomes* of task 1 are summarized in **Table 1**. The initial sequence designs were selected based on each of the target biomolecular recognition events listed, with subsequent efforts to increase the sizes of the proteins to as high as possible. These new constructs provide a broader suite of chemistries to work from, and higher molecular weight for diverse responsive properties.



**Figure 1.** Genetic design of 2nd generation dynamic materials based on bioengineered SELP proteins. Three different mutation strategies were used to expand SELP libraries. Mutation type 1 to provide better control of chemistry in the elastin block, mutation type 2 to provide affinity with gold nanofillers and also to include tyrosine crosslink sites, and mutation type 3 to provide additional functions.

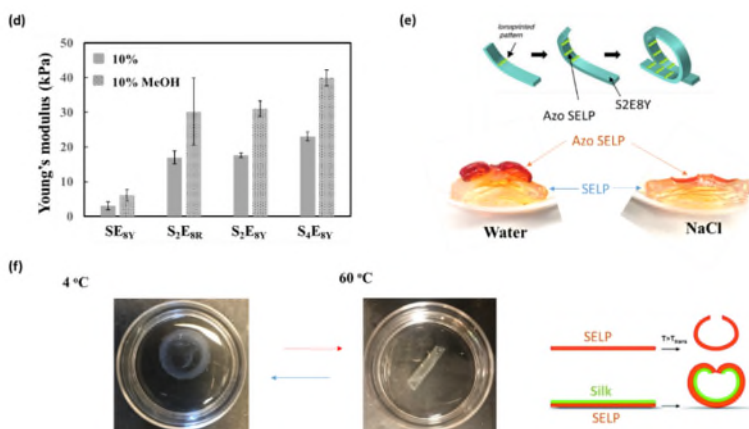
**Table 1.** New SELP variants for expanded library

<b>Mutation Type 1 – Elastin Mutation</b>			
Mutation location	Sequence variant	Stimuli	Function
1 <sup>st</sup> Elastin block	<u>G</u> KGVVP	pH, ionic strength	Increase charge density for E-field response, ROS responsive chemical modification
5 <sup>th</sup> Elastin block	G <u>Y</u> GVVP	Temperature, ionic strength	Di-tyrosine crosslinking, diazonium coupling reaction
5 <sup>th</sup> Elastin block	<u>G R G Y S L G</u> GVVP	Temperature, ionic strength, phosphorylation	Di-tyrosine crosslinking, diazonium coupling reaction, phosphorylation
<b>Mutation Type 2 – Tag Mutation</b>			
Mutation location	Sequence variant	Stimuli	Function
His-tag	<u>C</u>	Light	Attach nanofillers (e.g. gold nanorods)
<b>Mutation Type 3 – Functional Domain</b>			
Mutation location	Sequence variant	Stimuli	Function
After Elastin block	<u>G S R G D</u>	Temperature, ionic strength	Cell attachment
After Elastin block	<u>H S S Y W Y A F N N K T</u>	Temperature, ionic strength, electric field	Attach nanofillers (e.g. graphene)

### Task 2 – Select and characterize targeted materials to match a biomolecular recognition target

Stimuli-responsive protein polymers have exciting prospects for a variety of biomedical and biotechnology applications in protective materials, drug delivery, bio-sensing, regenerative medicine, soft robotics, artificial muscle and wearable devices. The *objective* of task 2 is to demonstrate the lead protein polymers developed in Task 1 exhibit stimuli-responsive features (responsive to pH, chemical, optical, electrical, mechanical force). To address this need, the SELPs were characterized for structure, thermal, morphological and dynamic/responsive functional features driven by environmental triggers and biomolecular recognition. The best SELP candidates that respond to environmental triggers were also fabricated into stimuli responsive hydrogel devices utilizing elastin domains that form enzymatic tyrosine-tyrosine crosslinks. The research progress of task 2 is summarized in **Figure 2**. A range of analytical techniques were used to characterize the physical properties of SELP molecules. The inverse transition

temperature of SELP were characterized using differential scanning calorimetry (DSC). The secondary structural changes of SELPs upon environmental triggers was characterized by circular dichroism (CD). The results suggested that SELPs with tyrosine mutations in the elastin domain exhibit thermal and ionic strength responsive features. In order to form dynamic hydrogel materials, an enzymatic crosslinking method based on horseradish peroxidase (HRP)-mediated redox reaction was further developed to form di-tyrosine networks for the hydrogel formation. Chemical and physical modifications were also applied to add additional functional features in SELP hydrogels. The viscoelasticity of SELP hydrogels was assessed by dynamic mechanical analysis (DMA) under different temperatures to verify the reversible stimuli responsive changes at the macroscopic scale. Tensile tests were performed to evaluate the stiffness based on the Young's modulus from stress-strain curves for SELP biomaterials before and after chemical modifications. The SELP hydrogels were also integrated with silk nanofiber films to form a bilayer structures. These bilayer structures can self-fold and self-expand upon thermal triggering, with potential for the design and fabrication of protein-based multifunctional soft robotics that can self-propel, navigate, and perform grabbing and release functions, and with potential biomedical applications in drug delivery, bio-sensing and energy harvesting. The *outcome* of task 2 is that a family of stimuli-responsive SELP were characterized and fabricated into hydrogel devices that reversibly respond selectively to environmental triggers. By integrating stimuli-responsive SELP with static protein polymers (e.g. silk), self-folding devices that can that can perform highly-specific, pre-programmed functions were fabricated with potential in bio-sensing, remote deployment, mechanical actuation and energy harvesting.



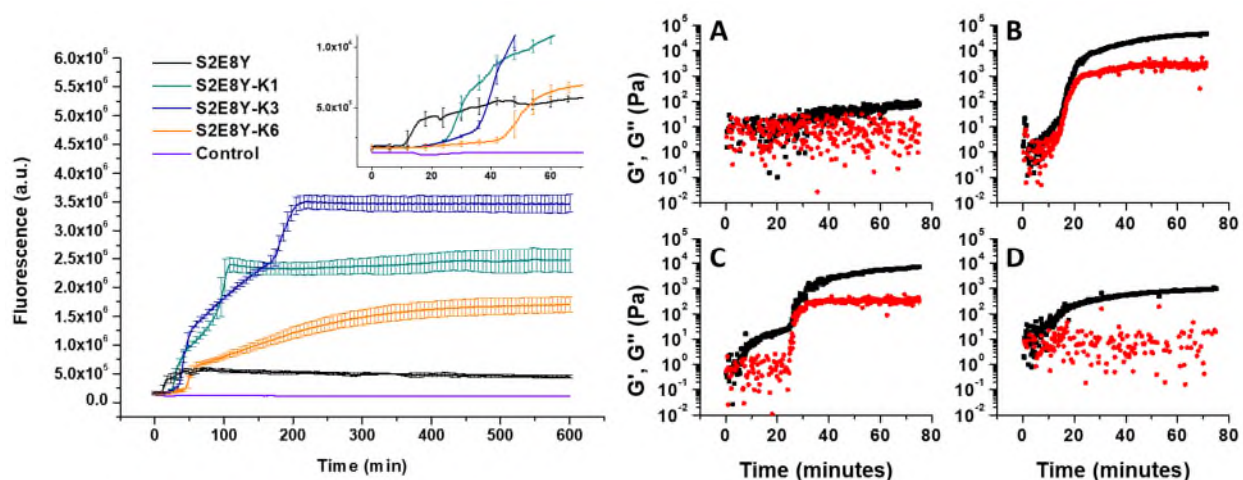
**Figure 2.** Dynamic materials based on bioengineered SELP proteins. (a) The inverse transition temperature ( $T_i$ ) was characterized by nanoDSC and the SELP with tyrosine in elastin block (SE8Y) exhibit  $T_i$  at 287°C. (b) The secondary structure transition of SE8Y measured in situ by CD. Ordered structure increased and disordered structure decreased while applying thermal triggers. (c) SELPs with tyrosine in the elastin block (S2E8R) were fabricated in to hydrogels via enzymatic crosslinking. The resulting hydrogels exhibited ionic strength responsive properties at ambient conditions. (d) SELP hydrogels were further modified by exposed to MeOH. The Young's modulus increased after the modification due to beta sheet formation in hydrogels. (e) Diazonium coupling were used to modify SELPs. The modified hydrogel, Azo SELP, exhibit significant swelling in water and deswelling in 5M NaCl, with potential in self-folding actuators. (f) SELP/silk bilayers exhibited self-folding and self-expanding properties for soft robotics.

**Charge-modulated activity of tyrosine-containing silk-elastin copolymers** - The modulation of reaction kinetics with horseradish peroxidase (HRP)-catalyzed crosslinking of proteins is a strategy to modulate the kinetics and properties of hydrogels formed from SELPs. Here, we demonstrate that the presence and location of positively charged lysines in SELPs modulates the reactivity of the tyrosines in the primary sequence and impacts the resulting thermal transition temperature of these polymers (**Table 2, Figure 3**). The positively charged lysine side chains decreased pi-pi interactions among tyrosines and reduced the rate of formation and number of HRP-mediated dityrosines bonds, dependent on the location of the charged

group in the protein. The results suggest that the location of repulsive charges can be used to tailor the reaction kinetics for enzymatic crosslinking, providing further control of gelation rates for in situ gel formation, as well as the resulting protein-based gel characteristics. This systematic study was used to modulate three aspects of the assembly or properties of genetically engineered SELPs: (i) pi-pi interactions, (ii) HRP-mediated crosslinking kinetics and (iii) mechanics of the gels, as summarized in Figure 3. Such control could be utilized to regulate the release of a bioactive peptide sequences or modulate the durability or lifetime of the protein materials.

**Table 2.** Amino acid composition of the SELPS used in this work.

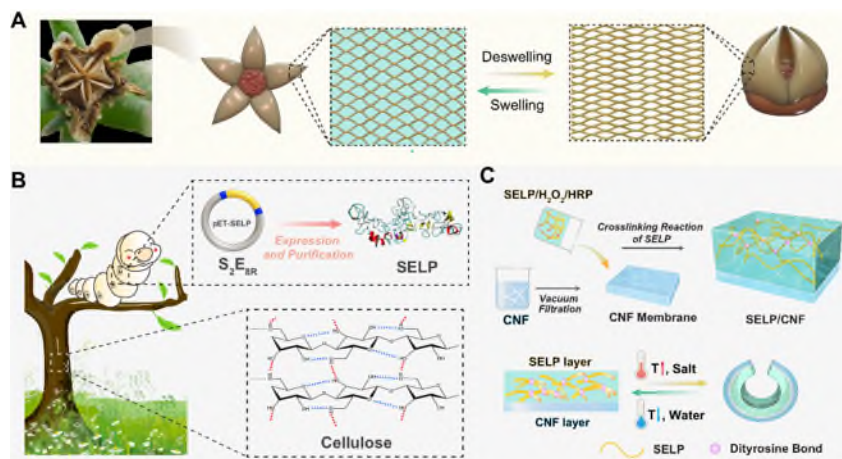
SELP	AMINO ACID COMPOSITION	M <sub>w</sub> (kDa)	pI
S2E8	[(GAGAGS) <sub>2</sub> (GVGVVP) <sub>8</sub> ] <sub>8</sub>	32.63	5.52
S3E8Y	[(GAGAGS) <sub>2</sub> (GVGVVP)(GVGVVP)(GVGVVP)(GVGVVP)(GYGVVP)(GVGVVP) <sub>3</sub> ] <sub>8</sub>	33.14	5.52
S3E8Y-K1	[(GAGAGS) <sub>2</sub> <b>K</b> (GVGVVP)(GVGVVP)(GVGVVP)(GVGVVP)(GYGVVP)(GVGVVP) <sub>3</sub> ] <sub>8</sub>	34.17	9.89
S3E8Y-K3	[(GAGAGS) <sub>2</sub> (GVGVVP)(GVGVVP) <b>K</b> (GVGVVP)(GVGVVP)(GYGVVP)(GVGVVP) <sub>3</sub> ] <sub>8</sub>	34.17	9.89
S3E8Y-K6	[(GAGAGS) <sub>2</sub> (GVGVVP)(GVGVVP)(GVGVVP)(GVGVVP)(GYGVVP) <b>K</b> (GVGVVP) <sub>3</sub> ] <sub>8</sub>	34.17	9.89



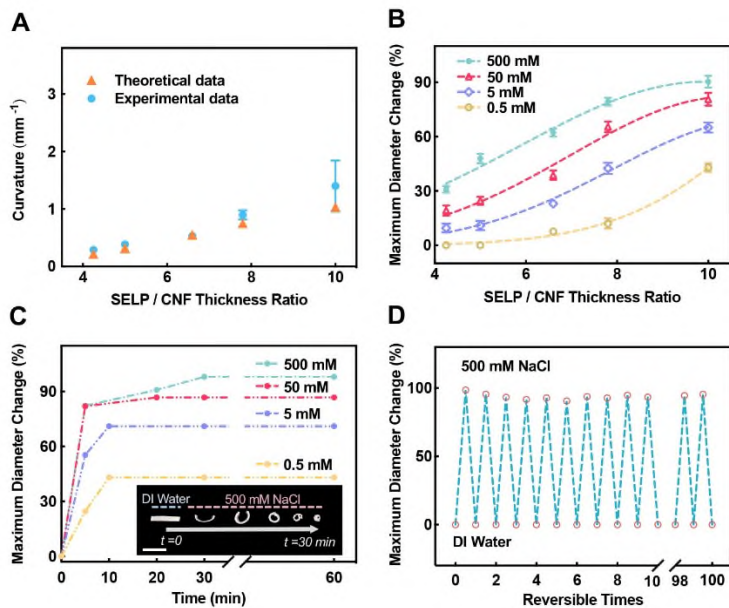
**Figure 3.** (Left) Fluorometric measurements of HRP-mediated tyrosine crosslinking for the 4 SELPs and their control (purple line). (Right) Rheological measurements of the HRP-mediated gelation of the (A) S2E8Y, (B) S2E8Y-K1, (C) S2E8Y-K3 and (D) S2E8Y-K6.

**Stimuli-responsive composite biopolymer actuators with selective spatial deformation behavior** - Bio-inspired actuators with stimuli-responsive and deformable properties are being pursued in fields such as artificial tissues, medical devices and diagnostics, and intelligent biosensors. These applications require actuator systems with controlled deformability, biodegradability, mechanical durability and stable reversibility. We reported bionic actuator systems consisting of stimuli-responsive genetically engineered SELP hydrogels and cellulose nanofibers (CNF), which respond to temperature and ionic strength in a dynamic fashion. Programmed site-selective actuation can be utilized to predict and fold the materials into 3D origami-like shapes. Stimuli-responsive genetically engineered SELP and CNF were combined to fabricate these actuator systems (**Figure 4**). Programmable and reversible deformations in response to the stimuli were achieved, where the SELP/CNF actuators take advantage of the characteristic of the SELP hydrogels which can respond to the changes in temperature and ionic strength, supporting intricate morphological transformations *via* prepatterned control of the materials assembly. Quantitative analysis of the final geometry allowed for predictions of deformation when triggered with stimuli, thus the ability to fine

tune actuators and shape-changes of the structures (**Figure 5**). Stable reversible deformations and durability of SELP/CNF actuators were demonstrated. Moreover, inspired by the motions from living organisms, biomimetic actuator movements (e.g., multilayer petals blooming and their closure) were achieved. We anticipate that such biocompatible actuators may provide a new avenue to design actuators with customized responsiveness towards for soft robotics and bionic devices.

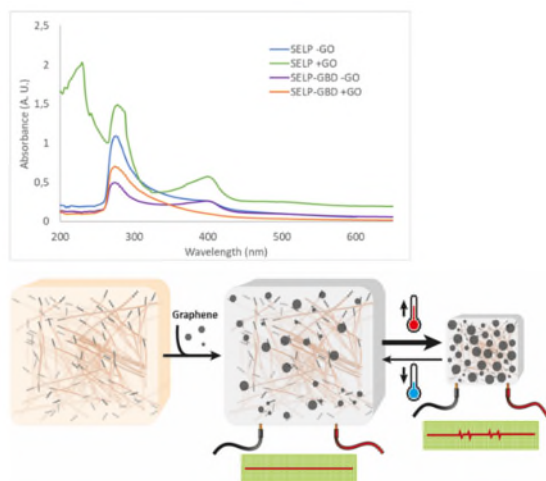


**Figure 4.** Design of the bionic actuators. (A) Inspiration from the hydration-dependent actuation of ice plant seed capsules. (B) Illustration of the genetically engineered SELP and wood-derived cellulose and their molecular structure. (C) Schematic diagram of the assembly process of the bilayer SELP/CNF actuators, and the reversible deformation of the actuator under various stimulations.



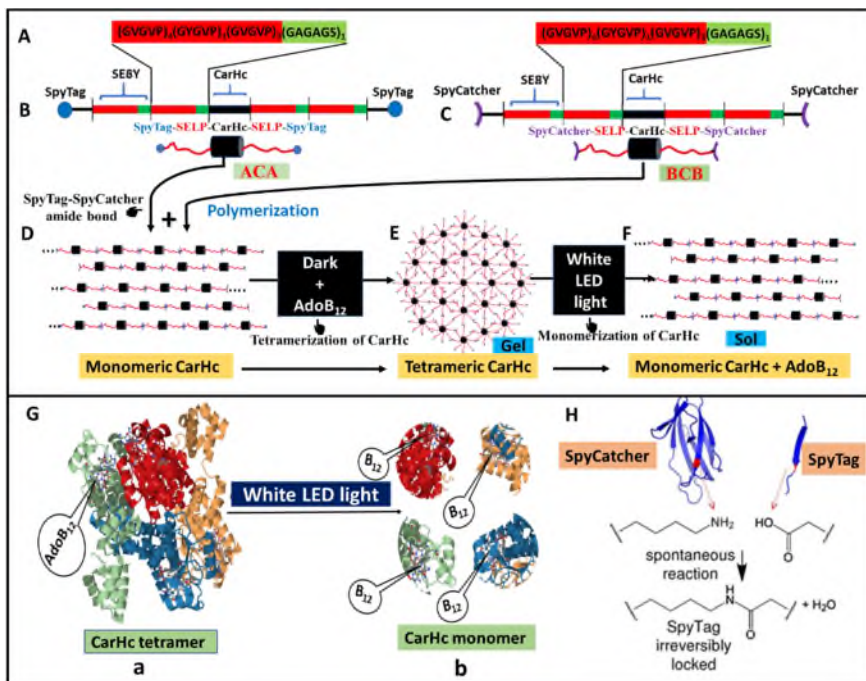
**Figure 5.** Quantitative analysis of the stimuli-responsive SELP/CNF bilayer actuators. (A) Bending curves for the bilayer strip actuators depending on the thickness ratio of the SELP to the CNF layer in 0.5 M NaCl solutions at room temperature. (B) MDC curves of the bilayer strip actuators with various thickness ratios, and (C) MDC versus time curves of bilayer strip actuators with thickness ratios of 10 in response to 0.5 mM, 5 mM, 50 mM and 500 mM NaCl solutions at room temperature, respectively. The insets are photographs of bending strip actuators at the corresponding time points. Scale bar = 5 mm. (D) Cyclic stability of the MDC of the bilayer strip actuators with thickness ratios of 10 under stimuli changes from 4°C DI water to NaCl solutions at room temperature.

**Graphene-SELP composites for electrical switches** - SELP-based materials bioengineered to include graphene binding domains could serve as interfaces to control conductivity (switches) (**Figure 6**). Graphene provides a delocalized electron network for distinctive electronic properties, as the pi orbitals facilitate conductivity and is remarkably mechanically robust, biocompatible with superior electronic performance. The goal was to design and generate SELP stimuli-responsive materials where electrical conductivity is modulated (as in a switch) through specific environmental stimuli – here by temperature. Increased temperature would drive SELP coalescence, thereby generating a conductive path in the material, whereas at low temperature there is insufficient percolation of the graphene component for conductivity. Bioengineered SELPs were designed as synthetic monomers containing the SELP structural and stimuli-responsive domains. Two versions were analyzed: SELP (12 monomers; ~55 KDa) and SELP-GBD (12 monomers; ~60 KDa), containing a graphene binding domain (GBD) with a linker inserted at the C-terminal region to examine impact of a GBD in the function (conductivity). After expression of the fusion proteins, purification was performed by inverse transition temperature (ITC). The fusion proteins were dissolved in water and the graphene added to the protein solution. Functionalized proteins mixed with graphene oxide (GO) were formed into hydrogels via enzymatic crosslinking to analyze dynamic shape changes with temperature and the impact on conductivity. The gels were characterized for mechanical properties, swelling, structure and rheology. To analyze the conductivity/resistance of the hydrogels after temperature increase or decrease to shrink and swell the hydrogels, a multimeter was used. The GO hydrogels, at 37-40°C contracted due to the coalescence of the SELP domains, thus, reducing gel size and allowing the graphene molecules to make continuous contact and to now be conductive. At lower temperature, the hydrogels were in the swollen state, thus, not conductive. The addition of GO did not have a direct impact on the secondary structure or in the mechanical properties of the material. After successive heating-cooling cycles, the SELP-GBD gels containing GO maintained conductivity, as opposed to the SELP gels containing GO added (but without the selective binding domain). This observation was supported by the analysis of the gel storage in water after 10 heating-cooling cycles, where GO was detected in SELP+GO samples but not in the SELP-GBD+GO. This SELP-designed thermal switch based on GO content and selective incorporation of a GO binding domain provides new options for bioengineered electronic devices that are switchable, dynamic, degradable and biocompatible. This manuscript is in preparation.



**Figure 6.** Recombinant SELP-graphene composites for electrical features. (Top) Absorbance spectra of supernatants collected after 10 heating-cooling cycles from SELP (SELP -GO), SELP-GO (SELP +GO), SELP-GBD (SELP-GBD -GO) and SELP-GBD-GO (SELP-GBD +GO) hydrogels. (Bottom) Proposed mechanism for electronic properties of SELP-GO biocomposite where control of conductivity of electrical fields is achieved by changing the hydrogel size - shrinking would produce the switch on and the swelling the switch off.

**Dynamically Tunable Light Responsive Silk-Elastin-Like Proteins** - The synthesis of genetically engineered SELP photoresponsive hydrogels was also pursued. Polymerization of the SELPs and monomeric adenosylcobalamin (AdoB<sub>12</sub>)-dependent photoreceptor C-terminal adenosylcobalamin binding domain (CarHc) was achieved using genetically encoded SpyTag-SpyCatcher peptide-protein pairs under mild physiological conditions (**Figure 7**). The hydrogels exhibited a rapid gel to sol phase transition upon exposure to visible light. The materials were also evaluated for cytotoxicity and the encapsulation and release of L929 murine fibroblasts from 3D cultures. Recombinant protein-based light-sensitive SELP-CarHc hydrogels were synthesized using SpyTag-SpyCatcher chemistry and were successfully demonstrated to undergo a dynamic phase transition upon exposure to white light. AdoB<sub>12</sub>-dependent CarHc tetramerization was essential for the formation of the hydrogels in the dark, while upon exposure to white light a gel to sol phase transition was demonstrated due to the conversion of CarHc tetramer back to monomer. This photo-induced hydrogel dissociation supported the facile release of encapsulated L929 murine fibroblasts from 3D cultures. The design of these photo-responsive proteins provides new stimuli-responsive SELP-CarHc hydrogels for dynamically tunable protein-based materials.



**Figure 7.** Diagrammatic representation of the assembly of recombinant SELPs incorporated with photoresponsive CarHc peptide. The recombinant protein (SELPs with CarHc) assembled into a molecular network through adenosylcobalamin (AdoB<sub>12</sub>) induced CarHc tetramerization in the dark and disassembled upon exposure to light (white light). Color code; Black block- CarHc domain; Red-green blocks- SELP peptide. **A:** Block diagram of SELP monomer **B:** Diagrammatic representation of domain arrangement in the recombinant fusion protein ACA. Red color: elastin domain; green color: silk domain; black color: CarHc domain. At both ends SpyTag is attached (blue color). **C:** Diagrammatic representation of domain arrangement in the recombinant fusion proteins BCB. Red color: elastin domain; green color: silk domain; black color: CarHc domain. At both ends, SpyCatcher is attached (purple color). **D:** Diagrammatic representation of proteins oligomerized/polymerized through SpyTag-SpyCatcher chemistry. The resulting polymers can further be assembled into a molecular network. Red color indicates separate silk and elastin proteins incorporated with photoresponsive CarHc protein in between. **E:** The resulting polymers can further be assembled into a molecular network through AdoB<sub>12</sub>-induced CarHc tetramerization in the dark **F:** Dissociation of CarHc tetramer into monomers resulting in a gel sol transformation under white light exposure **G:** Structures of CarHc protein. (a) CarHc dark state tetramer, showing the four identical subunits in four colors, and four molecules of AdoB<sub>12</sub> one bound to each subunit. (b) CarHc light-state monomer with AdoB<sub>12</sub> one bound to each subunit. Light exposure disassembles tetrameric CarHc accompanied by the degradation of AdoB<sub>12</sub>. Panel H adapted and modified from

Figure 4 in reference [49]. **H:** SpyTag-SpyCatcher chemistry. The amide bond formation between SpyTag and SpyCatcher peptides.

### **Task 3 – Devices and automation - higher throughput screening systems**

Task 3 was not funded in the original proposal but is included here for background. Biomaterial design strategies have historically focused on the rationale design and synthesis of polymers followed by the characterization of these new or modified polymers for structure-function features to match specific goals. This strategy is conducted in an iterative fashion, leading to trial-and-error outcomes and relatively long time frames to achieve specific functional goals for the new polymeric material. There remains an unmet need for a more robust high throughput strategy to address the need for new biomaterials with specific targeted functional features. The *objective* of task 3 is to develop microtiter plate systems to synthesize and screen the functional proteins generated from the libraries in Tasks 1 and 2 in a high throughput manner. To address this goal, robotics platforms combining 96-well plate reader and liquid handler are integrated.

### **Publications**

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