REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
16-01-2023	Final Report	1-Jul-2019 - 30-Jun-2022

4. TITLE AND SUBTITLE
Final Report: Soft, reconfigurable photonic systems inspired by cephalopod chromatophores: A platform to study dispersed light sensing in squid

5a. CONTRACT NUMBER
W911NF-19-2-0204

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

6. AUTHORS

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
74688-SM.6

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Leila Deravi
UU	UU	UU	UU		19b. TELEPHONE NUMBER
					617-373-3483

Standard Form 298 (Rev 8/98)
Prescribed by ANSI Std. Z39.18

RPPR Final Report
as of 23-Jan-2023

Agency Code: 21XD

Proposal Number: 74688SM
Agreement Number: W911NF-19-2-0204

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DUNS Number: 001423631
EIN: 104167998
Report Date: 30-Jun-2022
Date Received: 16-Jan-2023
Final Report for Period Beginning 01-Jul-2019 and Ending 30-Jun-2022
Title: Soft, reconfigurable photonic systems inspired by cephalopod chromatophores: A platform to study dispersed light sensing in squid
Begin Performance Period: 01-Jul-2019
End Performance Period: 30-Jun-2022
Report Term: 0-Other

Submitted By: Leila Deravi
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Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 0 **STEM Participants:** 5

Major Goals: The major goal of this proposal is to develop a better understanding of the mechanism responsible for dispersed light sensing originating from the cephalopod chromatophore, which in turn will facilitate the hierarchical design and assembly of reconfigurable optical materials that simultaneously achieves all the following conceptual advantages: low cost, low-energy, design simplicity, experimental feasibility, and scalability. The working hypothesis is that that the chromatophores are more than just pigment-based color filters; they contain structural color elements that aid in the amplification of reflected light during actuation. Specifically, we believe that the pigmented nanostructures localized within cephalopod organs behave as distributed light sensors that aid in the extraction and absorption of light, contributing to the rapid changes to skin patterning and coloration. Elucidating the structure-function relationships of these granules may not only provide insight into how cephalopods camouflage but also inform and accelerate the development of next-generation flexible displays, dye-sensitized solar cells, or light-sensitive textiles capable of absorbing and/or reflecting all wavelengths of visible light.

Specific goals of this proposal are:

(1) Build artificial cephalopod chromatophores. In this goal, we will design and develop biomimetic colloidal photonic crystals (CPCs) containing squid pigment granules and/or granule mimetics to recapitulate the structural and optical features of native chromatophores and determine how granule spacing and layering within the micro-structured sacs impact the photophysical properties (extinction and scattering efficiencies) of the network.
(2) Build synthetic squid tissue using artificial chromatophores (from goal 1). The CPCs from goal #1 will be incorporated as mechanically responsive free-standing films (replicating dermal tissue layer in squid) and evaluate whether tunable structural color inherent to the CPC can be used to amplify pigmentary color of the nanostructured chromatophore granules within the bulk material both with and without the presence of applied mechanical load.

This work will contribute directly to the objectives of the Materials Science Division of the Army Research Office by enabling the design and fabrication of advanced optical materials capable of absorbing and scattering all wavelengths of visible light while seeking to understand the complex mechanism of adaptive coloration in cephalopods. The results from our study will establish fundamental relationships that link the chemical composition and microstructure of the chromatophore that combine to contribute to the changes in skin patterning and coloration within the cephalopod skin, which will be important when considering the design of future color-changing materials. If successful, our findings will have broad implications for the field of wearable or flexible electronics, where photovoltaic, OLED, and solar energy conversion devices may benefit from processable cephalopod pigment derivatives.

Accomplishments: A PDF document summarizing all major accomplishments during the project period has been uploaded.

To summarize, our team investigated the role of nanostructure on the perceived visible color s of natural chromophores like xanthommatin (Xa), found in squid skin. To test this, we fabricated amorphous photonic assemblies containing Xa chemically coupled to and, in some cases, blended with polystyrene nanoparticles of varying sizes. We observed structural colors comprising these bidispersed colloidal assemblies that were tuned by the particle size of PS nanoparticles, the concentration of XA, the local environment, and the method of assembly. In all cases, the addition of XA on nanoparticles regulated the color hue and contrast of the resultant assemblies by increasing light absorption while minimizing incoherent light scattering. While our findings, on their own, introduce a new application space for this class of compounds in modulating visible color of photonic materials, we also draw important insights into why these molecules are often found packaged as heterogenous distributions of nanoparticles in natural systems. That is that the pigment/particle combination contributes to diversification of visible colors originating from the same biomolecule. Future experiments will expand on these preliminary findings, as we also explore optical simulations to better understand the performance properties of such Xa-based photonic materials.

We next translated the major learnings from the amorphous photonic assemblies work to build an artificial chromatophore system that utilizes an osmosis-controlled color switching platform to provide the reversible selection between two fundamentally different mechanisms of coloration. To achieve this function, we used microfluidics to prepare double emulsion droplets from polystyrene nanoparticles functionalized with Xa. In this system, the particle distribution and pigment loading density enable controlled oscillation between pigmentary color and angle-independent structural color upon application or release of osmotic stress, which is used to manipulate the physical structure of the artificial chromatophore system. We are actively investigating the synergistic relationship between these two coloration mechanisms by comparing the reflectance spectra of photonic capsules prepared with and without a pigmentary contribution. Our results will highlight a unique and potentially scalable approach for fabricating color-changing materials that provide access to a broad range of tunable visible colors.

Training Opportunities: - Current postdoctoral research assistants Ji-Young Lee and JaeHyun Kim actively work together, where Ji-Young traveled to UPenn for 2 days to learn the double emulsion system.
- Previous postdoctoral research assistants Jason (Zhaungsheng) Lin (Deravi Lab) and Zhe Gong (Lee Lab) have led the collaboration across both labs throughout the year. They and facilitated materials transferred between Northeastern and CCDC-SC (Natick, MA) to run experiments with collaborator, Richard Osgood in the Nanomaterials team and with each other across both institutions. This has given both Jason and Zhe the opportunity to work directly with and train with members at both the army labs and other institutions for the development of his project.

Results Dissemination: Outreach: Deravi engaged with first year undergraduate Chemistry majors and prospective graduate students in the Department of Chemistry and Chemical Biology at Northeastern to discuss the benefits of bio-inspired research for materials design.

Undergraduate researchers advised on this work:
- Mia Wasilewski 2022
- Amanda O'Brien, 2022

Graduate researchers advised on this work:
- Duncan Bower
- Mikey Bergman

Postdoctoral scholars advised on this work:
- Jason Lin 2020-2021
- Ji-Young Lee (2022- present)

In addition to the presentations given in 2020-2021, throughout 2022, PI Leila Deravi presented the following seminars on topics covered by this award. These include:
- November 2022 Materials Research Symposium, Boston, MA.
- October 2022 Electrochemical Society Conference, Atlanta, GA.
- September 2022 NC State University, Raleigh, NC.
- September 2022 Harvard SEAS Squishy Physics, Cambridge, MA.
- July 2022 Optica Novel Optical Materials and Applications (NOMA) Conference, Netherlands
- April 2022 BIG (bio-inspired green) Science & Technology Symposium, New York, NY
- 38. April 2022 American Chemical Society, San Diego, CA.
- April 2022 Baylor University, Waco, TX.
- March 2022 90th quarterly New England Complex Fluids Workshop
- January 2022 Western Washington University, virtual seminar

Honors and Awards: PI was recognized as:

- Faculty Undergraduate Research Mentorship Award in Chemistry, Chemical Biology, 2022
- ACS Polymer Materials Science and Engineering Young Investigator Award, 2021

Protocol Activity Status:

Technology Transfer: The following provisional patents were filed:

- Daniel Wilson, Leila F. Deravi. "Wearable Light Sensors Based on Unique Features of a Natural Biochrome" Provisional Patent Application No.: 63/236,231, 2021.
- Leila F. Deravi, Zhuangsheng Lin, Patrick Sullivan, Cassandra L. Martin. "A naturally-derived and edible photonic crystal color system," U.S. Provisional Patent Application No.: 63/050,728, 2021.

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Participant: Leila Deravi
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Co PD/PI
Participant: Daeyeon Lee
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: JaeHyun Kim
Person Months Worked: 5.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Duncan Bower
Person Months Worked: 5.00 **Funding Support:**
Project Contribution:
National Academy Member: N

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Participant: Zhaungsheng Lin
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
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Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Michael Bergman
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
National Academy Member: N

ARTICLES:

RPPR Final Report
as of 23-Jan-2023

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Matter
Publication Identifier Type: DOI Publication Identifier: 10.1016/j.matt.2021.06.011
Volume: 4 Issue: 8 First Page #: 2639
Date Submitted: 8/11/21 12:00AM Date Published: 8/1/21 4:00AM
Publication Location:
Article Title: Artificial cephalopod organs for bio-inspired display: Progress in emulating nature
Authors: Daniel J. Wilson, Leila F. Deravi
Keywords: color, chromatophore, review, adaptive technologies
Abstract: Cephalopods have inspired the development of many bio-inspired technologies. But how accurately can researchers model the optical features and functions of cephalopod skin without sacrificing speed or design? In ACS Applied Materials & Interfaces, Han et al. demonstrate one approach to tackle these challenges.
Distribution Statement: 2-Distribution Limited to U.S. Government agencies only; report contains proprietary info
Acknowledged Federal Support: **Y**

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Matter
Publication Identifier Type: DOI Publication Identifier: 10.1016/j.matt.2021.05.021
Volume: 4 Issue: 7 First Page #: 2163
Date Submitted: 8/11/21 12:00AM Date Published: 7/1/21 4:00AM
Publication Location:
Article Title: Engineering color, pattern, and texture: From nature to materials
Authors: Daniel J. Wilson, Zhuangsheng Lin, Duncan Q. Bower, Leila F. Deravi
Keywords: color, cephalopods, nature, adaptive
Abstract: While most animals retain the same visual appearance over their lifetime, some species feature adaptive systems that enable changes in color, pattern, or texture for defense, signaling, temperature regulation, or reproduction. Many of these features have inspired the development of materials and devices with tunable optical and mechanical properties. However, current bio-inspired color-changing systems are often limited to controlling single facets of visual perception independently, necessitating new materials or composites that can perform multiple optical, electrical, or mechanical functions simultaneously under the control of integrated hardware with practical energy requirements. In this Perspective, we examine color-changing systems in nature and discuss the current state of materials and devices derived from or inspired by these systems. Additionally, we discuss important design and performance criteria for new multifunctional materials or devices that may sense and display both
Distribution Statement: 2-Distribution Limited to U.S. Government agencies only; report contains proprietary info
Acknowledged Federal Support: **Y**

RPPR Final Report
as of 23-Jan-2023

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: ACS Applied Bio Materials
Publication Identifier Type: DOI Publication Identifier: 10.1021/acsabm.0c01034
Volume: 4 Issue: 1 First Page #: 507
Date Submitted: 8/11/21 12:00AM Date Published: 12/1/20 5:00AM
Publication Location:
Article Title: Biomimetic Colorants and Coatings Designed with Cephalopod-Inspired Nanocomposites
Authors: Camille A. Martin, Zhuangsheng Lin, Amrita Kumar, Sean R. Dinneen, Richard M. Osgood, Leila F. Dera
Keywords: colorants coatings xanthommatin bioinspired nanoparticles silica
Abstract: Brilliant and dynamic colors in nature have stimulated the design of dyes and pigments with broad applications ranging from electronic displays to apparel. Inspired by the nanostructured pigment granules present in cephalopod chromatophore organs, we describe the design and fabrication of biohybrid colorants containing the cephalopod-specific pigment, xanthommatin (Xa), encased within silica-based nanostructures. We employed a biomimetic approach to encapsulate Xa with amine-terminated polyamidoamine (PAMAM) dendrimer templates, which helped stabilize the pigment during encapsulation. Depending on the concentration of Xa used in the reaction, the resultant biohybrid nanomaterials generated a range of neutral colors of differing hues. When applied as coatings, these colorants can be triggered to change color from yellow/gold to red in the presence of a chemical reducing agent, as we leverage the natural redox-dependent color change of Xa. Altogether, these capabilities demonstrated the
Distribution Statement: 2-Distribution Limited to U.S. Government agencies only; report contains proprietary info
Acknowledged Federal Support: **Y**

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: ACS Sensors
Publication Identifier Type: DOI Publication Identifier: 10.1021/acssensors.1c02342
Volume: 7 Issue: 2 First Page #: 523
Date Submitted: 1/16/23 12:00AM Date Published: 2/1/22 5:00AM
Publication Location:
Article Title: Wearable Light Sensors Based on Unique Features of a Natural Biochrome
Authors: Daniel J. Wilson, Francisco J. Martín-Martínez, Leila F. Deravi
Keywords: bioinspired sensor paper-based wearable microfluidics DFT
Abstract: Overexposure to complete solar radiation (combined ultraviolet, visible, and infrared) is correlated with several harmful biological consequences including hyperpigmentation, skin cancer, eye damage, and immune suppression. With limited effective therapeutic options available for these conditions, significant efforts have been directed toward promoting preventative habits. Recently, wearable solar radiometers have emerged as practical tools for managing personal exposure to sunlight. However, designing simple and inexpensive sensors that can measure energy across multiple spectral regions without incorporating electronic components remains challenging, largely due to inherent spectral limitations of photoresponsive indicators. In this work, we report the design, fabrication, and characterization of wearable radiation sensors that leverage an unexpected feature of a natural biochrome, xanthommatin?its innate sensitivity to both ultraviolet and visible through near-infrared radiation. We
Distribution Statement: 1-Approved for public release; distribution is unlimited.
Acknowledged Federal Support: **Y**

RPPR Final Report
as of 23-Jan-2023

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Advanced Optical Materials
Publication Identifier Type: DOI Publication Identifier: 10.1002/adom.202100416
Volume: 9 Issue: 24 First Page #: 2100416
Date Submitted: 1/16/23 12:00AM Date Published: 9/1/21 4:00AM
Publication Location:
Article Title: Bidispersed Colloidal Assemblies Containing Xanthommatin Produce Angle?Independent Photonic Structures
Authors: Zhuangsheng Lin, Zhe Gong, Duncan Q. Bower, Daeyeon Lee, Leila F. Deravi
Keywords: bio-inspired, photonic, assemblies
Abstract: The biological chromophore xanthommatin (Xa) contributes to the yellow, red, and brown colors and hues in cephalopods and arthropods. In many cases, Xa is also present as part of or coupled to supramolecular nanostructures, whose function has yet to be fully explored. To investigate how such structural elements impact the perceived color of these natural chromophores, amorphous photonic assemblies containing Xa chemically coupled to 100 nm polystyrene nanoparticles (PS100-XA) are fabricated, and blended with pure polystyrene (PS) nanoparticles of varying sizes. Structural colors are observed comprising these bidispersed colloidal assemblies that are tuned by the particle size of PS nanoparticles, the concentration of PS100-XA, the local environment, and the method of assembly. In all cases, the addition of PS100-XA regulates the color hue and contrast of the resultant assemblies by increasing light absorption while minimizing incoherent light scattering. Taken together, the results dem
Distribution Statement: 1-Approved for public release; distribution is unlimited.
Acknowledged Federal Support: **Y**

Partners

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I certify that the information in the report is complete and accurate:
Signature: Leila Deravi
Signature Date: 1/16/23 4:28PM

Soft, reconfigurable photonic systems inspired by cephalopod chromatophores: A platform to study dispersed light sensing in squid

ARO Grant# W911NF1610455

Leila Deravi, Daeyeon Lee
Northeastern University and University of Pennsylvania

Executive Summary. Cephalopods including squid, cuttlefish, and octopus are arguably one of the most photonically sophisticated classes in nature. They represent a biological archetype for the design of flexible displays and wearable technologies that approach or exceed the performance of synthetic technologies on most key metrics such as flexibility, actuation, and adaptability to environmental cues. Dermal pigment-based chromatophore organs that populate cephalopod skin facilitate the rapid (~120 msec) and adaptive changes in skin color and patterning during actuation; however, the mechanism potentiating color change across multiple spatial scales (from molecular to meso-scale) is not well understood. Specifically, the extent to which variations in light (hue and intensity) from the surrounding oceanic atmosphere trigger the neuromuscular signaling cascade necessary to initiate color change and skin patterning remain largely unknown. What is known is that adaptive coloration is enhanced by the anatomical arrangement of pigment granules (average diameter of 528 ± 68 nm), thus should be included to accurately describe the dynamic range and speed of visible color displayed by the animal as they camouflage. The goal of this proposal is to investigate the contribution of these pigment granules in light sensing and amplification within the chromatophore. Specifically, we will develop a comprehensive understanding of the hierarchically assembled granules in the extraction and dissipation of light that contribute to dynamic changes in body patterning during camouflage, where we will focus on establishing how and to what extent features associated with structural coloration amplify the absorption and scattering of light using soft, reconfigurable chromatophore mimetic structures. To accomplish these research goals, the team will:

(1) Design and develop biomimetic colloidal photonic crystals (CPCs) as chromatophore mimetics. The CPCs will contain squid pigment granules and granule mimetics (synthesized de novo in the laboratory) that offer a tunable platform to study how granule spacing and layering within the micro-structured sacs impact light sensing (e.g. absorption) and dissipation (e.g. extinction and scattering efficiencies) of the network.

(2) Build a network of CPCs within a flexible, mechanically responsive free-standing film (replicating dermal tissue layer in squid). These films will be used to directly evaluate whether tunable structural color inherent to the CPC can be used to amplify pigmentary color of the nanostructured chromatophore granules within the bulk material both with and without the presence of applied mechanical load.

This work will contribute directly to the objectives of the Materials Science Division of the Army Research Office by enabling a deep understanding of the mechanism responsible for dispersed light sensing originating from the cephalopod chromatophore, which in turn will facilitate the hierarchical design and assembly of reconfigurable optical materials. The results from our study will establish fundamental relationships that link the chemical composition and microstructure of the chromatophore, which will be important when considering the design and development of next-generation flexible displays, dye-sensitized solar cells, or light-sensitive textiles capable of absorbing and/or reflecting all wavelengths of visible light.

List of publications and patents:

- Daniel J. Wilson, Francisco J. Martín-Martínez, Leila F. Deravi. "Wearable Light Sensors Based on Unique Features of a Natural Biochrome," **2022.**
- Daniel J. Wilson, Leila F. Deravi. "Artificial cephalopod organs for bio-inspired display: Progress and challenges in emulating nature," *Matter,* **2021.**
- Zhuangsheng Lin, Zhe Gong, Duncan Bower, Daeyeon Lee, Leila F. Deravi. "Tunable photonic glass made from bidispersed colloidal assemblies containing xanthommatin," *Advanced Optical Materials,* **2021.**
- Daniel J. Wilson, Zhuangsheng Lin, Duncan Bower, Leila F. Deravi. "Engineering Color, Pattern, and Texture: From Nature to Materials," *Matter,* **2021** 4 (7), 2163-2171**.**
- Camille A. Martin, Zhaungsheng Lin, Amrita Kumar, Sean R. Dinneen, Richard M. Osgood III, Leila F. Deravi. "Biomimetic colorants and coatings designed with cephalopod-inspired nanocomposites," *ACS Applied Biomaterials*, **2021**, 4(1): 507–513.

- Daniel Wilson, Leila F. Deravi. "Wearable Light Sensors Based on Unique Features of a Natural Biochrome" Provisional Patent Application No.: 63/236,231, **2021**.
- Leila F. Deravi, Zhuangsheng Lin, Patrick Sullivan, Cassandra L. Martin. "A naturally-derived and edible photonic crystal color system," U.S. Provisional Patent Application No.: 63/050,728, **2021**.

Technical Approach:

1- Build artificial cephalopod chromatophores

- Design biomimetic chromatophores

Synthesis of Xa pigment. Xa was synthesized via the cyclization of 3-hydroxykynurenine according to previous procedures but with modifications.[2, 20] Briefly, a 17.8 mM 3-hydroxykynurenine solution was prepared in 0.1M potassium phosphate buffer (pH 7.0). Next, potassium ferrocyanide (78.1 mM) was added dropwise into the 3-hydroxykynurenine solution then stirred at room temperature for 2.5 hours. The product was precipitated using 1N hydrochloric acid, washed 3 times in 0.2 N hydrochloric acid, dried in a fume hood overnight, and stored at 4°Cuntil further use. Proton NMR spectrum of the pigment was collected in DMSO-d_6 containing 4% trifluoroacetic acid using a 500 MHz Nuclear Magnetic Resonance (NMR) spectrometer (Varian, Palo Alto, Ca) (Figure S7). The product, Xa, displayed characteristic peaks at 8.4 ppm, 8.0 ppm, 7.8 ppm, 7.7 ppm, 6.7 ppm, 4.6 ppm, 3.9 ppm, which were in agreement with our previous reports.[2, 21] The molecular weight of the product was analyzed using a liquid chromatography – mass spectrometry (LC-MS) (Thermo LTQ Orbitrap XL, Thermo Fisher Scientific, Waltham, MA) (Figure S8). The m/z value of the major peak in the chromatograph was determined to be 424.078, in agreement with the molecular weight of Xa.

Synthesis of Xa functionalized nanoparticles. Xa functionalized nanoparticles were synthesized via EDC coupling of Xa onto carboxylic acid functionalized PS nanoparticles (Figure 1A). Briefly, EDC (5 mg/mL) was added into a 2.5 wt % 100 nm carboxylic acid-functionalized polystyrene (PS100) in 0.05 % SDS suspension, and the mixture was stirred at room temperature for 30 min. Then, Xa (2 mg/mL) in 0.1M MES buffer (pH 5.5) was added into the mixture and stirred for another 2.5 hours to tether the Xa pigment onto PS100. After the reaction was completed, the Xa functionalized polystyrene particles (PS100-XA) were cleaned by dialyzing against DI water. The dialyzed PS100-XA particle suspension was further centrifuged at 3000 g for 15 min, washed with DI water three times, then stored in 5 mL of 0.05 % SDS. Particle sizes were analyzed using a scanning electron microscopy (SEM) (Hitachi S4800, Tokyo, Japan). The surface charge of the particles during the reaction was analyzed using zeta-potential analysis in a Malvern Nano-ZS90 zetasizer (Malvern, United Kingdoms). The surface chemistry of the particles before and after Xa functionalization was analyzed using Attenuated Total Reflectance – Fourier-transform infrared spectroscopy (ATR-FITR) spectrometry (Bruker Alpha, Bruker Corporation, Billerica, MA) at a resolution of 4 cm^{-1} (32 scans) backgrounded against air.

Preparation of the artificial chromatophore systems. Photonic microcapsules containing bare PS nanoparticles and Xa-functionalized PS particles were fabricated using a glass capillary-based microfluidic device. We prepared water-in-oil-in-water (W/O/W) double emulsions according to a previously described method[11] using injection and collection capillaries with 120 µm and 200 µm diameters, respectively. To generate the W/O/W double emulsions, an aqueous suspension of polystyrene nanoparticles (5–10 wt %) was injected through an oil phase into water. The middle oil phase consisted of either: (i) 75 wt % part A of a two-part silicone rubber (Rubber Glass II, from Smooth-On), 23 wt % PDMS oil (1cSt, Clearco Products Co.) and 2 wt % Dow Corning 749 surfactant, or (ii) segregated hydrofluoroether oil (HFE 7500, 3M) containing 1 wt% surfactant (Krytox 157FSL, DuPont). For the silicone oil-based middle phase (i), the outer aqueous bulk fluid contained 2 wt % poly(vinyl alcohol) (PVA, 87-89% hydrolyzed, average *Mw* = 13,000–23,000, Sigma Aldrich) and 10 wt % glycerol (Sigma Aldrich) aqueous solution. For the fluorocarbon oil-based middle phase (ii), the outer aqueous bulk fluid contained 2 wt% PVA. The flow rates for the inner (i.e., aqueous suspension of polystyrene nanoparticles), middle (i.e., silicone or fluorocarbon oils), and outer (i.e., aqueous PVA solutions) fluids were set at 2 mL/h, 2 mL/h, and 40 mL/h, respectively. Formation of double emulsion capsules was monitored and recorded using an inverted microscope (Nikon Diaphot 300) with a high-speed camera (Phantom V7.1). Double emulsions capsules were collected in aqueous NaCl solutions with controlled osmolarity to induce osmotic annealing.

- Establish design rules to control color (reversible switching).

To induce osmotic compression, double emulsion capsules were collected in a concentrated aqueous NaCl solution and left undisturbed. Depending on the osmotic pressure across the semi-permeable oil shell of the capsules, we observed annealing times ranging from 1 hour to several hours (i.e., overnight) required for PS nanoparticles to pack and maintain their structural coloration. For osmotic expansion, the initial NaCl solution was carefully removed by pipetting and replaced by a 2 wt% PVA solution to create a hypotonic environment. To monitor cycling between osmotic compression and expansion, capsules were imaged in hypertonic or hypotonic environments using an optical microscope every 20 mins until their size remained constant, signifying a final equilibrium geometry. Once the capsules achieved the equilibrium state, the surrounding bulk solution was replaced to shift the central droplet toward the appropriate nanoparticle configuration.

2- Build synthetic squid tissue

- Incorporate double emulsions in mechanically responsive free-standing films

To fabricate the synthetic squid tissue, we used Poly(ethylene glycol) diacrylate (PEGDA, M_n700) as polymer matrix to embed the chromatophore emulsions. The double emulsions are loaded in the premixed PEGDA 20wt.%, and UV-crosslinker (Lithium phenyl-2,4,6-trimethylbenzoylphosphinate, 17mM) aqueous solution. After gently mixing, the emulsion loaded prepolymer solution 120µl is injected to 1cm x 2cm x 0.4mm mold. The injected prepolymer solution is UV cured (Omnicure, SERIES 1500) for 20seconds.

- Evaluate optical performance (static, dynamic)

The International Commission on Illumination L*a*b (CIELAB) coordinates[33] of the photonic films was measured using an Ocean Optics UV-Vis-shortwave NIR spectrophotometer (200-800 nm), equipped with a 45° diffuse reflectance probe, a certified diffuse reflectance standard (Labsphere), and a standard illuminant A. The CIELAB coordinate values were collected against a white standard reference (WS-1). All reflectance spectra were collected using an Evolution 220 spectrophotometer (Thermo Scientific, Waltham, MA) equipped with an ISA-220 integrated sphere accessory. Multi-angle reflectivity measurements were conducted using 15°, 30°, 45°, and 60° fixed angle accessories, wherein each sample was manually placed on the calibrated accessory prior to data acquisition. In this configuration, incident beams were cast onto the surface of the photonic films at each angle, and the specular reflectance of the corresponding angles were collected. All reflectance spectra were collected at a bandwidth of 2 nm with a spectral resolution of 1 nm.

Major Findings:

Understanding the role of xanthommatin only on light sensing

Because previous evaluations of photolabile color from ommochromes have highlighted sensitivities to acidic[33] or ionic[31] environments, we first investigated how xanthommatin dissolved in MES (2-(N-morpholino)ethanesulfonic acid) buffered to pH 6 or prepared as an acidified, unbuffered solution (pH < 1) responded to solar radiation. We did not evaluate basic conditions, which are known to destabilize ommatins.[36] After irradiating these solutions with simulated solar light, we observed that the absorbance maximum of oxidized pigment, occurring at 444 nm, was attenuated in both conditions. The visible color of pigment in buffered solutions shifted from a characteristic yellow to dark brown, and acidified solutions turned a deep red color representative of reduced xanthommatin,[37] suggesting that more dramatic colorimetric responses occur in acidified, unbuffered conditions.

While this color shift was visibly obvious, we explored the possibility of incorporating additives that could form reducing agents upon irradiation as a means of enhancing the accessible color range and analytical performance of xanthommatin-based radiation sensors. Recent characterizations of UV-induced protein degradation have revealed that tryptophan can act as a photosensitizer to facilitate radiative cleavage of disulfide bonds.[38] We asked whether xanthommatin, a tryptophan derivative,[39] could enable a similar conversion of disulfide bonds to free thiols, resulting in further reduction of the pigment and enrichment of the red color. In acidic solutions containing equimolar pigment and cystine, we did not observe color enhancement. However, irradiation of pigment and excess cystine (1:100) resulted in complete attenuation of absorbance at 444 nm, concurrent with precipitation of a red solid characteristic of xanthommatin treated with free cysteine. We solubilized this material using acidic methanol and compared its absorbance spectrum to that of xanthommatin reduced with ascorbic acid (1:1),[30] and observed that both solutions had absorbance peaks characteristic of reduced xanthommatin around 480 nm. This precipitate was not observed in the control, cystine-containing samples stored in darkness. To determine whether precipitation of reduced xanthommatin was caused by formation of free cysteine in response to light, we used 4,4'-dithiopyridine (4,4'-DPS)[40] to measure the concentration of free thiols in irradiated solutions containing cystine only. No free thiols were detected in the absence of xanthommatin, suggesting that this tryptophan-derived pigment acted as a photosensitizing agent for disulfide bonds and was reduced by free thiols upon formation of cysteine.

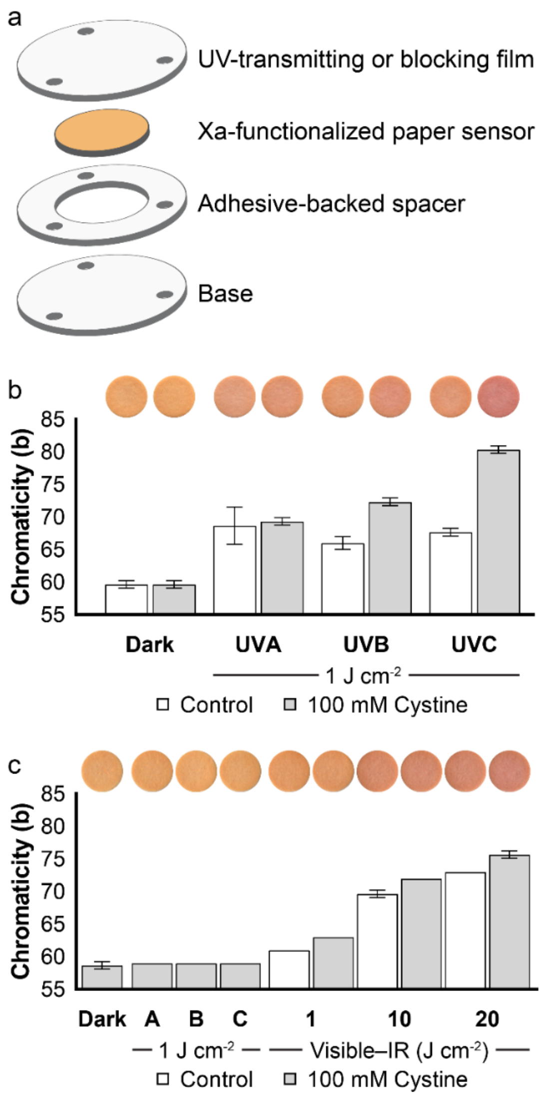

Figure 1 Xanthommatin-based light sensors. (A) Xanthommatin-functionalized paper substrates are sealed between UV-transmitting or UV-blocking films to control the energy received by the sensor while mitigating evaporation during irradiation. (B) Sensors are responsive to UV radiation, with signals in UVB and UVC irradiated devices enhanced by the incorporation of excess cystine. (C) Sensors laminated with UV-blocking films are not responsive to UV radiation but show dose-dependent photoreduction in response to visible–near IR radiation (400–1100 nm). Error bars represent standard deviation of n = 3 replicates per condition.

In our spectrophotometric analysis of xanthommatin photoreduction in solution, we found that colorimetric responses to sunlight were enhanced by radiation-induced formation of cysteine in acidic environment. To create miniature, processable sensors, we used carbodiimide chemistry to create crosslinked networks of xanthommatin throughout the porous architecture of round punches of chromatography paper. This established strategy for immobilizing colorimetric reagents[41] in an inexpensive, patternable substrate[42, 43] provided planar sensors functionalized with fully oxidized pigment. We hydrated these paper substrates with a 100 mM solution of cystine in acidified MES (pH 0.4) and laminated them between adhesive-backed plastic films to mitigate evaporation during irradiation. The top layer of each laminated assembly was either a UV-transmitting ethylene tetrafluoroethylene (ETFE) film or a UV-blocking polyester film to include or exclude energy from ultraviolet wavelengths, respectively (**Figure 1A**). We subjected these sensors to controlled doses of UVA (365 nm), UVB (302 nm), UVC (254 nm), and visible–near IR (400–1100 nm) radiation. Sensors stored in darkness remained yellow, while irradiated sensors shifted to a red color with increasing irradiation energy. At a cumulative dose of 1 J cm^{-2} for each UV wavelength, sensors showed increasing red color and decreasing measured values of yellow chromaticity (see Experimental Details) with higher-energy wavelengths. Interestingly, cystine-containing sensors showed enhanced color shifts over sensors treated with acidified MES solution only for irradiations performed with UVB and UVC exposure, but we observed no such enhancement in devices irradiated with UVA (**Figure 1B**). Sensors sealed with a UV-blocking film showed no response to equivalent doses of ultraviolet light but shifted up to 17 chromaticity units as the sensors became visibly red upon irradiation with 20 J cm^{-2} of 400-1100 nm solar simulated light, highlighting the multispectral performance of xanthommatin as photosensitive indicator (**Figure 1C**).

<u>*Understanding the role of the nanostructure on color*</u>

One primary goal of this project was to reproducibly fabricate biomimetic chromatophore granules that can be used/encapsulated within the double emulsion systems designed in Lee's laboratory. We opted to focus on optimizing synthetic granules – and not use the natural squid extracted granules – due to their minimal aggregation in solution (a necessary criteria in building the double emulsions). In our protocol, we initially started by coupling a synthetic form of the squid pigment xanthommatin (Xa) onto PS nanoparticles with 100 nm diameters (PS100-XA). These particles displayed a dark brown color, which differed significantly from the bright white unfunctionalized control (PS100, **Figure 2A**). Pigment incorporation was also supported with ATR-FTIR spectra (**Figure 2B**), which showed a broad transmittance band at 3400 cm^{-1} (Figure **2B, band A**) indicative of N-H stretching from amide groups between Xa and the carboxylic acid functionalized particles as seen in previous reports.[1, 2] The transmittance bands at 1320 cm^{-1} and 1650 cm^{-1} further supported the presence amide bonds, suggesting that Xa had successfully coupled onto the PS100 nanoparticles. This finding was also reinforced by the reduction of available carboxylic acid groups on the PS100 nanoparticles calculated from before and after EDC coupling using zeta potential measurements. This extrapolated density change in surface-accessible carboxylic acid groups was also utilized to approximate the Xa loading density on the nanoparticles,[3] which was estimated to be 4.0 ± 0.4 nmol/µg nanoparticles (N=3). Based on these values, we calculated that ~63 wt % of Xa had successfully coupled onto each particle.

Next, the visible color of the particles drop-casted as thin films was analyzed (**Figure 2C**). Not surprisingly, the control PS100 films displayed a white color, reflecting ~85% of visible light; whereas, the PS100-XA film displayed a brown color with a relatively low reflectance (~10 %) at 400-500 nm region with an inflection of 23.6 % from 600-700 nm. The reflectance profile of the PS100-XA was attributed to the absorption feature of Xa pigment, which has a characteristic absorption maximum of 430 nm in its oxidized form.[2, 4] The particle size of the PS100-XA nanostructures was determined to 93.2 ± 4.9 nm using SEM analysis (**Figure 2D**, N = 20 particles), indicating that functionalization did not significantly alter the template size.

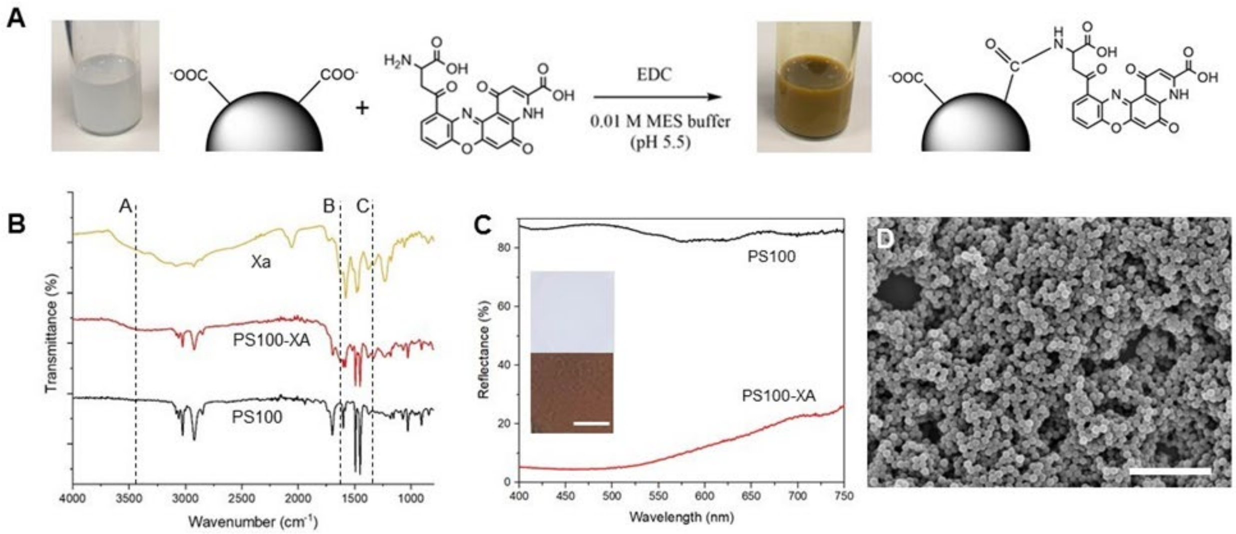

Figure 2. Xanthommatin functionalized nanoparticle synthesis on 100 nm polystyrene beads (PS100-XA): (A) The synthesis scheme including xanthommatin in its non-ionized form; (B) ATR-FTIR spectra of pure Xa, PS100 and PS100-XA; (C) reflectance spectra of PS100-XA (insert: photographic images of drop-casted PS100 (top) and PS100-XA (bottom) films (scale bar = 1 cm); (D) representative SEM micrograph of PS100-XA (scale bar = 1 μm).

We asked whether these pigmented particles could be used to create photonic assemblies with tunable visible colors and enhanced color contrast. To test this, we first created a bidispersed colloidal assembly containing mixtures of PS100-XA and pure PS nanoparticles of variable sizes, then used rapid centrifugation to sediment all particles to the bottom of the centrifuge tube (illustrated in **Figure 3A**). The subsequent materials demonstrated notable structural colors, where the observed hue was modulated by the particle size of the pure, un-functionalized PS nanoparticles (**Figure 3B**). The photonic assemblies retained the structural color when ground into powder (**Figure 3C**), suggesting the structures remained intact at the nanoscale. The mixed nanoparticles formed an amorphous array without any long-range order and periodicity **(Figure 3D**), which was consistent with photonic glasses shown in other studies.[5, 6] Blending two particles of different sizes, in fact, is a well-established method of producing glassy assemblies.[7-9] These structures differed from the three-dimensional photonic crystals formed by the pure monodispersed PS particles.[10]

To test the how the surrounding environment and production method impacted the assembly and subsequent photonic properties of the Xa-based materials, the PS100-XA:PS particles were assembled in W/O/W double emulsion droplets using osmotic annealing, with pure monodispersed polystyrene nanoparticles as controls. This method has been previously used to produce photonic crystals or glasses from a suspension of uniform colloids, leading to production of photonic balls exhibiting a wide range of colors.[11-13] Double emulsion droplets containing PS100-XA:PS particle mixtures in the inner aqueous phase were fabricated in a glass capillary based microfluidic device, collected in 1M NaCl, then slowly annealed over 4-5 hrs to enable the formation of amorphous photonic assemblies.[11] In this configuration, the outflux of the aqueous phase during osmotic annealing reduced the volume of the inner phase of the droplet and allowed the charged heterogenous particles to assemble. The resultant products prepared using both pure PS

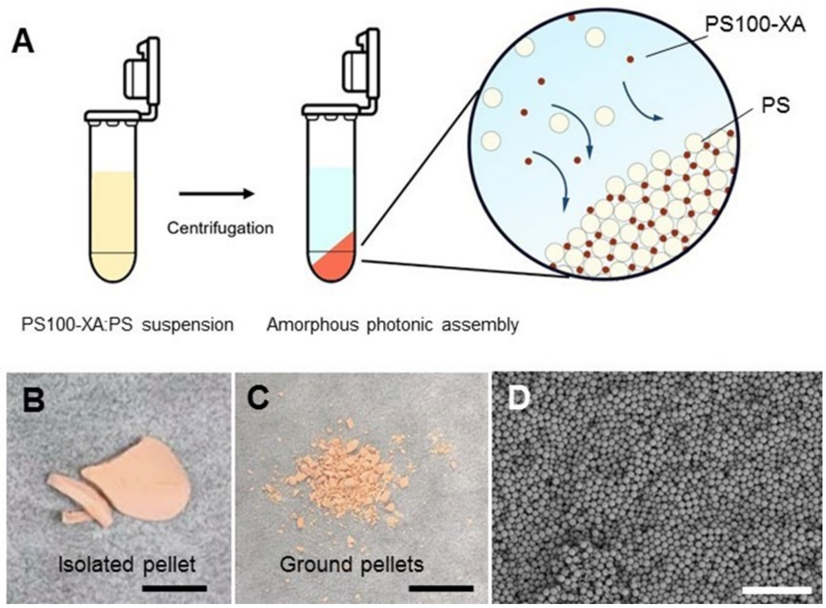

Figure 3. Assembly of the amorphous photonic materials: (A) Illustrating the preparation via centrifuging PS100-XA:PS mixtures; (B) photographic images of 5% PS100-XA:PS290 pellets (scale bar = 2 mm) and (C) powder (scale bar = 2 mm); (D) representative SEM micrograph of 5% PS100-XA/PS290 photonic assemblies (Scale bar = 250 μm).

nanoparticles and PS100-XA:PS particle mixtures (5 wt % PS100-XA:PS) reflected different colors, where the addition of PS100-XA changed the color of the pure PS photonic assemblies from green to blue and pink to yellow/green for the PS190 and PS220 particles, respectively. Interestingly, the control PS190 and PS220 photonic assemblies displayed colors that differed from the controls prepared by the centrifugation methods in air.

These particles did not crystalize in confined droplets but instead formed amorphous photonic assemblies with angle-independent reflectance peaks at 550 nm and 610 nm for the PS190 and PS220 nanoparticles, respectively. This red-shift of the reflected light was likely caused by the larger interparticle distances and the presence of water between particles –both of which would increase the effective refractive index of the medium as predicted by the single scattering model of Magkiriadou et al,[8, 14] where $n_{eff/water}$=1.49 and $n_{eff/air}$ =1.42. These trends reversed when the PS100-XA nanoparticles were incorporated at a 5 wt % density. We observed that the PS190 and PS220 photonic assemblies contributed to a blue-shift in color space, rather than the red-shift observed using the centrifugation methods (Figure 3C, D). This phenomenon is likely also caused by the change in packing configurations from the bidispersed system due to a reduction in the volume fraction of the larger PS particles when the smaller PS100-XA particles were added.[15]

Similar trends have also been observed when doping small-sized nanoparticles in bidispersed SiO_2 nanoparticle colloidal photonic crystals.[16] Despite these differences, the results showed that the coloration effects from configuration changes were again dominated by the pigmentary contribution of the PS100-XA.

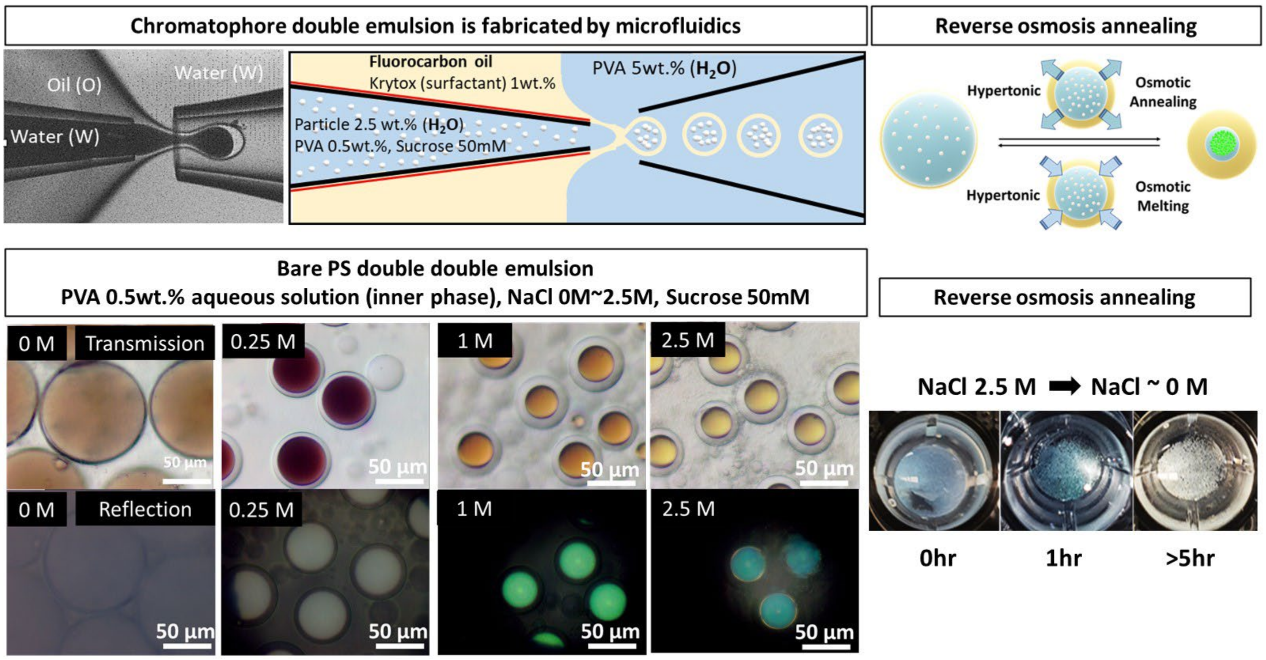

Figure 4. Assembly of the artificial chromatophore systems. (top) Illustration of the preparation of double emulsions containing pure XA-PS nanoparticles and the process reversible annealing; (bottom) photographic images of artificial chromatophores annealed at different salt conditions and the process to reverse the annealing to vary the visible colors.

Understanding the roles of pigmentary vs structural units on perceived colors

While more work, including the application of high-resolution imaging to resolve specific interparticle distances within the double emulsions, is needed to fully understand these interesting findings, for now we can agree that the presence of PS100-XA nanoparticles is able to impact the coloration of colloidal photonic assemblies in both powdered and aqueous medium, introducing a new method to generate angle-independent structural colors in multiple environments.

In cephalopod skin, cooperative display of both pigmentary and structural coloration elements enables rapid, precise changes in color, pattern, and visible appearance over spatial scales orders of magnitude larger than chromatophores and underlying structural reflectors. A major obstacle in creating engineered systems that approach the impressive capabilities of these natural systems is fabrication of selectively actuated pigmentary and structural display features that can be packaged or arrayed together to offer synergistic optical functions. We have exciting preliminary data that suggests it is possible to present manufacturable microcapsules that can be osmotically reconfigured to display pigmentary or structural color on-demand. In expanded double emulsion microcapsules, pigment-functionalized particles are uniformly dispersed throughout the central aqueous phase to provide uniform absorbance-based color. Depending on the rate of osmotic annealing induced by altering the salt concentration of the surrounding medium, compression of the central aqueous phase provides crystalline or amorphous organization of the geometrically constrained nanoparticles, resulting in angle-dependent or angle-independent color, respectively (**Figure 4**). As nanoparticles are physically rearranged to provide structural color, concentration of absorptive pigment molecules presented on the surfaces of the nanoparticles modulates the hue and saturation of the resulting colloidal architecture. In all cases, osmotic-induced actuation of the capsules was reversible, where—in the case of the pigment-containing system—we were able to controllably switch between pigmentary and structural coloration mechanisms using a common stimulus While these microcapsules are currently actuated via global changes in the surrounding environment, fabrication of independently addressable "pixels" capable of providing multiple forms of pigmentary and structural color presents new and unique opportunities for investigations of cooperative interactions between these fundamentally different coloration mechanisms, as well as development of future adaptive display technologies. When taken together, our findings represent an important step toward approximating the comprehensive function of coloration organs in cephalopod skin using engineered systems (**Figure 4 and 5**).

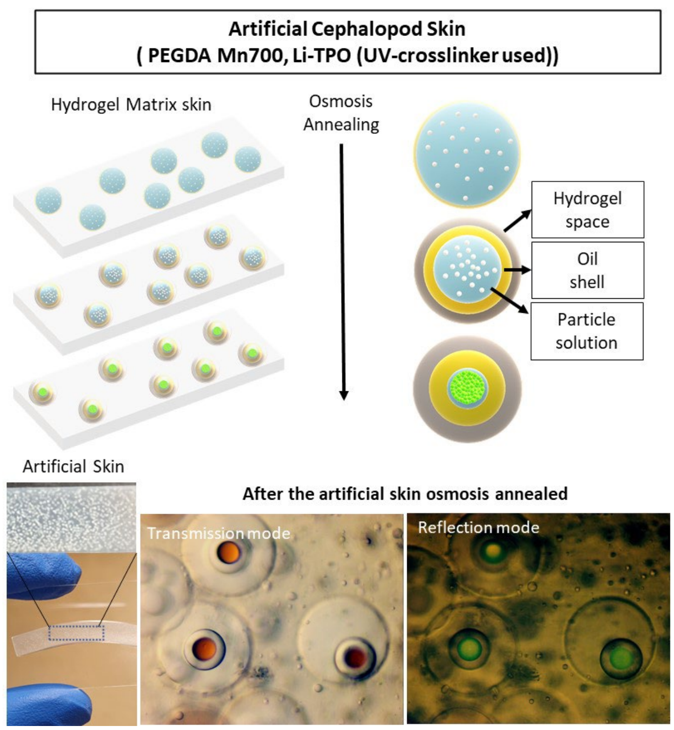

Figure 5. Assembly of the artificial squid skin by incorporating accessible double emulsions into a soft hydrogel system.

Impact and Next Steps: Despite decades of research, the mechanisms that regulate sensing and signaling events preceding color change in cephalopods are not well understood. The work enabled through this award tackled this challenge using biomimetic systems inspired by and built from component parts of the cephalopod chromatophore organ. From our work on xanthommatin light sensors, we developed an understanding of the intrinsic photochemical properties of xanthommatin and supplementing radiation-driven color changes using reducing agents generated in response to light. These fundamental science discoveries enabled the development of wearable sensors for personal radiation monitoring to quantify independent or combined doses from wavelengths across the UV and visible–near IR using a single colorimetric reporter, without requirements for photodiodes or other electronic components. By incorporating these sensors into user-friendly microfluidic devices, we have taken also demonstrated important first steps toward packaging the photochemical properties of xanthommatin into deployable systems that could be used to measure radiation exposure on-demand. However, it is important to note that in these devices, hydration of the paper-based sensor is dependent on gas-permeable ports at the beginning of the microfluidic channel and outside of the sensor area—fully closing either orifice in the device will halt flow by (i) creating a vacuum behind the fill port as capillary action draws fluid into the sensor, or (ii) creating pressure in the void volume surrounding the sensor because there is no path for air to escape as it is displaced from the paper during filling. This requirement for air to enter and escape the microfluidic network means that these devices are susceptible to evaporation during storage in ambient conditions, which could impact the amount of pressure required to activate the device or eventually render the device unusable. However, this shortcoming could be mitigated by humidifying the devices during storage or incorporating a destructible seal over the device ports that prohibits evaporation during storage but is crushed and permeabilized when the device is activated. While there are some challenges that currently preclude consideration of these self-contained devices as shelf-stable products, our initial evaluations of xanthommatin-based sensors for complete solar light and germicidal doses of ultraviolet radiation highlight the utility of this unique material for the development of multispectral colorimetric sensors.

Our work on the artificial chromatophores (double emulsion systems) also provided a number of new fundamental insights to help explain why biochromes like xanthommatin are often observed as part of heterogenous supramolecular structures in nature. Specifically, we observed structural colors comprising these bidispersed colloidal assemblies that were tuned by the particle size of PS nanoparticles, the concentration of PS100-XA, the local environment, and the method of assembly. In all cases, the addition of PS100-XA regulated the color hue and contrast of the resultant assemblies by increasing light absorption while minimizing incoherent light scattering. While our findings, on their own, introduce a new application space for this class of compounds in modulating visible color of photonic materials, we also draw important insights into why these molecules are often found packaged as heterogenous distributions of nanoparticles in natural systems. That is that the pigment/particle combination contributes to diversification of visible colors originating from the same biomolecule. Future experiments will expand on these preliminary findings, as we also explore optical simulations to better understand the performance properties of such Xa-based photonic materials.