An Autonomous Chemically Fueled Artificial Protein Muscle

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1. Introduction

Nature’s processes of life depend on material systems exerting dynamic movements via nonequilibrium states dissipating chemical energy. Muscular systems and molecular motors of nature, for example, the actin, kinesin, titin, or dynein muscle/motor system, enable autonomous movements utilizing chemical fuel, mainly adenosine triphosphate (ATP)\(^1\). The high efficiency, scalability, and adaptability of biological muscles systems have inspired muscle mimicry for a vast variety of applications ranging from soft robotics, nanotechnology, pharmacy, and biomedicine.\(^2\)

Bioinspired mimicry of muscular systems ranges from stimuli/C0-responsive gels, dielectric elastomer actuators, liquid crystal elastomers, and hydrogels\(^2,3\) over natural or modified biomacromolecules\(^4\) and cell-based biohybrid muscles\(^5\) to autonomously self-oscillating polymers and sol—gel transitions of chemical polymers mimicking movement of living organisms pioneered by the Yoshida group.\(^6\)

However, besides the implementation of nature’s proteins dynein, kinesin, and myosin as nanotechnological motors in microscopic systems\(^7\) and polymer hybrid muscles,\(^4b\) no protein muscles with mechanoactive protein components are realized. Biomimetic bio-based muscle systems allow to combine the potential of nature’s high-performance proteins, for example, silk, resilin, elastin, or titin, with novel adaptive and functional properties and sustainable biotechnological production. While biological protein motors and muscles are powered by the hydrolysis of adenosine triphosphate, no synthetic bio-based muscles are described, operating autonomously using chemical energy to exert directional movements. Herein, an artificial protein muscle is introduced, exerting rhythmic autonomous movements via nonequilibrium states driven by chemically fueled pH oscillation reactions. Key to the design are recombinantly produced human matrix proteins selectively reengineered to respond to different stimuli. The results also show how directional movements can be independently triggered by changes in pH and temperature including a selective on-switch and a combination of nonequilibrium states enabling “learning and oblivion”-like material effects. This paves the road for the next generation of autonomous materials in pharmacy, soft robotics and living matter.
components were realized. Interestingly, polymer muscles fueled by glucose were recently realized.⁴⁸ Artificial muscles and motors span a wide range of mimicry,⁹ for example, fast temperature-responsive polymer bicelles with dimensions spanning three orders of magnitude¹⁰ and molecular motors¹¹ including autonomous chemically fueled small-molecule motors based on catenanes and their chemical modification “fueling” their directional movement.¹² Biomolecular motors utilized by nature range from the molecular nanoscale (e.g., muscular myosin or kinesin)¹³ to micro- or millimeter scales (exemplified in locomotion).¹⁴ They strongly depend on filament assembly and orientation, currently limiting their macroscopic application above the millimeter scale.¹⁵ Still, artificial muscles are of great interest for soft robotics and biomedical applications.¹⁶ New materials, able to bridge the gap between body-own materials and polymer-based muscle mimicry used in soft robotics and biomedical technology, are still missing. Artificial muscles solely based on proteins, ideally based on human sequence motives, exerting muscle-like movements such as bending, would bridge this gap. Despite numerous polymer-based artificial muscles, only a small number of artificial muscles with bio-based peptide components has been described so far comprising viologen-modified peptides¹⁷ or viologen incorporated into poly-L-lysine-based hydrogels.¹⁸ Protein motors using an internal “fuel” enabling the self-propelled movement, for example, squid-derived proteins isolated from the tentacles from Linaria vulgaris utilizing the chemical Marangoni effect (based on a gradient of surface tension created by mass transfer along an interface between two fluids) via the release of hexafluoroisopropanol (HFIP), have been described from the Sitti group.¹⁹ Besides their interesting performance, they are limited by their internal “fuel” reservoir and the restriction to media where they can create surface tension gradients. Stimulus-responsive biomolecules ranging from carbohydrates over DNA to peptides and proteins are interesting candidates for artificial muscles but are currently rather limited in the case of proteins with just a few mainly chemically modified examples.⁴⁶ The current systems are controlled or respectively respond to various signals or stimuli ranging from chemical to electrical cues.

Thus, we envisioned the design of a protein-based artificial muscle exerting autonomous directional contraction/bending movements by adopting nonequilibrium material states driven by a chemical energy-dissipating oscillating reaction consuming chemical fuel, with additional temperature-based on/off switch characteristics (Figure 1).

With regard to our system, the terms “autonomous and directional movements” refer to the decoupling of the bilayer motion from the overall environmental status, which is achieved by the coupling of the pH-sensitive protein bilayer to a chemically fueled pH oscillation which perpetuates autonomously when fueled. In addition, the resulting bending is a directional process, which in turn is the basis for more complex and sophisticated motions that could be predetermined by the overall structure of bilayered objects.

The protein-based artificial muscle shall exert autonomous periodic and directional movements without the need for external control of the cycles (Figure 1). The muscle shall be based on a chemoselectively crosslinked covalent protein network, enabling the preparation of a 3D muscle, scalable in size and shape with material inherent stimulus responsiveness to design a system able to run under biological as well as technical conditions. The use of complex chemical material modifications or sensitive enzymes shall be avoided to enable efficient access to the system and enable system stability under various conditions for artificial protein muscle design, fabrication, and application. a) Natural muscle characteristics. Inspired by nature’s high-performance materials elastin and resilin. b) Novel protein genes are designed to biotechnologically produce the corresponding recombinant proteins. c) Yielding a pH-responsive protein (DSY)¹⁶ d) upper sequence in red) and a temperature-responsive protein (VRY)¹⁶ (d) lower sequence in gray/black). e) Photochemical protein network formation enables the fabrication of layered 3D muscles with variable shapes. f) Using a pH-oscillating reaction based on the Landolt reaction (lower box) allows for a chemically fueled semibatch reaction driving autonomous protein muscle movements in a specially designed and g) 3D-printed reaction setup.
environmental conditions. The protein system shall further fulfill the following requirements: The autonomous protein muscle movement should be driven by an oscillating chemical reaction and allow to permanently renew the chemical fuel by external addition. The implementation of an oscillating chemical reaction relying on a redox system has the potential to be replaced with different chemical/biological redox pairs autonomously driving periodic pH changes as muscle stimulus. pH changes were chosen as the response to pH changes as well as the proton conductivity within a material network is one of the most effective and fastest possible, besides electrically conductive or optical systems, while the latter is largely limited to thin and/or transparent materials. We selected a variant of the Landolt reaction, a chemical clock with oscillating pH,[21] Edblom, Orban, and Epstein (EOE) found remarkable oscillatory behavior in the iodate oxidation of sulfite in a stirred flow reactor (CSTR) in the presence of ferrocyanide,[22] where iodate can be substituted by bromate. The nonequilibrium states of the pH oscillations of the Landolt reaction we used were based on a bromate/sulfite/ferrocyanide reaction system.[23] Performing the Landolt pH oscillation in a semibatch format similar to Liedl et al.[24] at 23 °C generates pH oscillations between pH 4.0 and pH 7.9 with an average period of about 20 min. We designed and built a semibatch reactor, which was 3D printed via fused filament fabrication (FFF). This setup allows to perform a semibatch Landolt reaction with controlled fuel supply, simultaneous monitoring pH and visualization/record the resulting protein muscle contraction and expansion over a period of several hours. While the frequency and amplitude of the pH oscillation reaction could be manipulated via the Landolt reaction temperature and the amount of chemical fuel (Na2SO3), the design and nature of the covalently photocrosslinkable protein allow us to regulate the responsiveness of the protein muscle on the molecular level (Figure 1, S1, and S2, Supporting Information). The manufacturing and processing of the protein muscles from the micro- and millimeter to the centimeter scale enable us to adjust and direct the transformation of chemical and thermal energy into directed motion, similar to living matter at scalable dimensions. The visualized contractions of these muscles are comparable with certain results on the autonomously self-oscillating polymer PNIPAM (poly[NIPAAm]) described by the Yoshida group,[26] which was driven by the Belousov–Zhabotinsky (BZ) reaction (Figure 1). Even though of different functionality as Yoshida’s redox gels, stimuli–responsive resilin-like elastin-like proteins (ELPs) now enable the development of complex composite protein muscle actuators.

Inspired by nature’s high-performance mechanical proteins resilin and resilin, providing stimulus responsive properties, we designed protein-based muscle mimicry shown in Figure 1. We combine the programmable genetic adjustability and environmental stimulus responsivity (e.g., pH and temperature) of ELPs with the tyrosine–tyrosine crosslinking scheme of resilin to adopt its tunable elasticity. Thus, we termed this class protein resilin-like ELPs. This allows to transfer the molecular characteristics of single ELP molecules to a covalently crosslinked proteinaceous hydrogel network with tunable elasticity, susceptibility, and adjustable response to environmental changes and high resilience properties, yielding durable and scalable muscle devices with scalable and flexible 3D morphology and high environmental stability. Even physical assemblies of block-domain ELPs constituting lifelike systems provide ultra-high environmental and chemical stability.[25] The novel class of the protein network design is based on natural matrix protein sequences, redesigned by a special sequence domain assembly method, the One Vector ToolBox Platform (OVTP),[26] enabling the design of highly repetitive and asymmetric protein sequences combining different natural stimulus–response patterns. First, we adopt the modular ELP pentapeptide basic structural element[27] derived from the human tropoelastin sequence and second, we complement this with the spatial distribution pattern of the crosslinkable amino acid (aa) tyrosine (Y) of the recombinant resilin peptide sequence that originated from drosophila.[28] The choice of the aa at the fourth position of the pentapeptide sequence modulates the molecular responsiveness to environmental changes and tunes its intrinsic properties toward extrinsic factors, for example, ion concentration, temperature, and pH. ELPs are able to undergo reversible structural changes on the molecular level in response to environmental triggers at a defined transition temperature.[29] The aa composition defines its individual, sequence-dependent responsiveness, and character.[27,30] Furthermore, resilin-like ELPs belonging to the class of intrinsically disordered proteins (IDP) with interesting aspects of materials hysteresis and nonequilibrium phase behavior on the molecular level enable “learning and oblivion”–like material behavior as demonstrated later. Photocrosslinking of these molecules creates macroscopic protein networks and transfers the molecular characteristics from the molecular level to the protein network level from the nanoscale to the macroscale. The precise embodiment of the resulting protein networks conducted by form shaping during the photocrosslinking process enables us to direct the process of chemical to mechanical energy transformation in muscle applications. Moreover, using a genetically programmed protein material as a muscle-like actuator allows to encode different responsivities and response times into an identical class of biomacromolecules (proteins) that only diverge by its precise composition of amino acids. These artificial protein muscles can be programmed for defined responses on the material level as well as on the structural level, given by molded crosslinking and combination of proteins, and can be thermochemically fueled for actuation by different types of gradients (e.g., pH, temperature, salt concentration) encoded within the composition of the constituting proteins. In contrast to chemically synthesized polymers, these muscle-like proteins would fit perfectly within each biomedical context as potentially fueled protein machines and advanced stimulus–responsive drug delivery systems because of their biological origin, biocompatibility, and lack of immunogenicity of the components and their adjustable degradability to amino acids in the biological context.

Combining the regular pattern of crosslinkable tyrosine (Y) with regularly distributed glutamate (D) at the guest residue position, we developed a pH-responsive resilin-like ELP that can be photocrosslinked with Ru(bpy)32+/APS or bio compatible phosphoriboflavin/APS as photocatalyst[31] to a pH-responsive protein hydrogel that is demonstrated to be suitable to transform the inorganic chemical nonequilibrium Landolt pH oscillation reaction into motion of the pH-susceptible protein hydrogel (Figure 1 and Figure S1, Supporting Information).
To drive protein motion autonomously with chemical fuel or thermal energy, we perform nonequilibrium Landolt pH oscillation in a newly designed and optimized semibatch reactor (Figure S3, Supporting Information). This semibatch reaction chamber was individually designed to run the modified Landolt reaction and to hold the protein muscle inside the chamber to allow for its photo- & video-documentation and analysis, documenting the transformation of chemical and thermal energy into protein motion and muscle-like contraction movements.

2. pH Oscillations Coupled to Nonequilibrium States of Protein Pad Contractions

The nonequilibrium Landolt pH oscillation reaction exhibits several functions for the described approach, stimulating the responsive proteins under physiological temperature conditions: a large pH oscillation amplitude between room temperature and 45°C with an extended period of time at high pH provides sufficient time for adaptation of the (DSY)₁₆ resilin-like ELP pad. The complex reaction scheme can be simplified to a first 1) proton-serving process that lowers the pH and a second 2) proton-consuming process that serves as a negative feedback loop enabling between 10 and 20 oscillations in the semibatch reactor format[32] (see Figure 1).

\[
\text{BrO}_3^- + 3\text{HSO}_3^- + H^+ \rightarrow \text{Br}^- + 3\text{SO}_4^{2-} + 4H^+ \\
\text{BrO}_3^- + 6\text{Fe(CN)}_6^{4-} + 6H^+ \rightarrow \text{Br}^- + 6\text{Fe(CN)}_6^{3-} + 3H_2O
\]

We performed this chemical fuel-driven pH oscillation reaction based on sodium bromate, sodium sulfate, and ferrocyanide at different temperatures between 23 and 45°C, generating varying oscillation periods and frequencies, and modulated them between 10 and 30 min and a range of pH 4.0–7.9. This nonequilibrium system has been used to drive autonomous periodic oscillations transformed into muscle-like contractions with different frequencies and periodicities (Figure S5, Supporting Information) of a (DSY)₁₆ resilin-like ELP pad, also exerting a directed oscillating motion of a composite (DSY)₁₆/(VRY)₁₆ protein muscle in a pH-responsive manner.

The protein pads were placed at the presentation platform of the reaction chamber to allow to monitor the pH-responsive autonomous oscillations of the (DSY)₁₆ resilin-like ELP pad. The system demonstrates the conversion of chemical energy into motion of the protein hydrogel pad, exhibiting autonomous contraction behavior of the protein pads shown in Figure 2 and Movie S1, Supporting Information.

3. Autonomous Chemical Fuel Converting Artificial Protein Muscle

While the pure (DSY)₁₆ protein pad exhibits a muscle-like contraction behavior, we next combined two resilin-like ELPs with different pH and thermoresponse characteristics. We fabricated a composite protein stripe composed of a first layer of photocrosslinked (VRY)₁₆ and a second photocrosslinked (DSY)₁₆ protein layer both covalently attached to each other. Because the (DSY)₁₆ protein demonstrates strong shrinking behavior at low pH and the (VRY)₁₆ protein does not or tends to show reverse behavior (compare Figure S7, Supporting Information), such a composite protein stripe shows rationally tunable (via its shape and composition) muscle-like contractions, leading to a bending motion upon the oscillating Landolt reaction transforming chemical energy into directed motion via nonequilibrium states of the protein muscle shown in Figure 3 (see also Movie S2, Supporting Information).

![Figure 2](image-url) Periodic pH oscillations trigger autonomous contractions of a protein pad. Compare with Movie S1, Supporting Information. a) Graphical representation of the periodic pH oscillations (between pH 4.0 and 7.9) triggering the autonomous shape changes of a (DSY)₁₆ resilin-like ELP pad analyzed with automated shape tracking shown as change in the area (see also Figure S8, Supporting Information). The orange curve reveals the different measured area values recorded by automated shape tracking and exemplarily shown by representative images in Figure 2 b, c and d. Two images used for visualization and quantification purposes at a high- (I./III. gray arrow for pH max and orange arrow for corresponding area max in (a)) and a low-pH/pad area (II./IV. white arrow for pH min and orange arrow for corresponding area min (a)).
The timescale of protein muscle motion is limited by the diffusion of water out of or into the deswelling or swelling layers of the bilayered protein muscles. This process is directly dependent on the volume of the overall object and the volume of each protein layer, as well as the defined responsiveness of the constituting proteins with gradationally reversed behavior.

Figure 3. Autonomous chemically fueled periodic contractions of an artificial protein muscle. pH-dependent motion (bending) of a photocrosslinked (VRY)$_{16}$/(DSY)$_{16}$ composite protein muscle and visualization of its motion in $z$-direction, shown from the top. Orange layer represents the photocrosslinked (VRY)$_{16}$ resilin-like ELP protein layer covalently photocrosslinked on top of a photocrosslinked protein layer of (DSY)$_{16}$ resilin-like ELP (gray). Above each image, the position of the oscillation is shown as a function of time and pH and the movement of the protein bilayer in $z$-direction (green curve). On the left of each image the movement of the protein muscle is schematically shown in the side-view perspective (further details in Table S1, Supporting Information). The left images show higher muscle positions at pH 7, and the right images show the lower bilayer positions at pH 4 and 5.
The motion response can span a time window from a few seconds to minutes, depending on the volume and surface of the object.

4. Switchable Autonomous Protein Muscle Machine

The robust autonomous protein-muscle contraction running for hours can be further regulated and controlled, taking advantage of the temperature sensitiveness of both, the individual protein materials and the oscillating Landolt reaction itself (Figure 4, Movie S3 and S4, Supporting Information). Setting the whole protein muscle system in its Landolt reaction bath to 3 °C, no pH oscillation-triggered contraction occurs. Raising the temperature to 25 °C switches the muscle-like contraction to run autonomously for hours (Figure 4, Movie S5, Supporting Information).

5. Additive Effects of Learning-like Motion

IDPs as represented by ELPs can access a variety of nonequilibrium, hysteretic phase behaviors, which are tunable by their aa composition and order.[33] As nonequilibrium hysteretic phase behaviors are common to protein assemblies in biological systems,[34] we concluded that resilin-like ELP materials can be used to access various nonequilibrium states upon environmentally induced structural switches. They can include hysteresis and nonequilibrium material behavior, both of which are prerequisites for materials to manifest “learning processes” subsequently reaching new states that persist in the altered environment for a certain period of time. In Figure 4 and S9 (and Movie S6) Supporting Information, “additive motions” of the artificial protein muscle which are induced by nonequilibrium states via a series of pH oscillations (Figure 4 and S9, Supporting Information, development of “protein muscle positions” indicated by the green graph) led to learning-like behavior via the accumulation of muscle positions with slightly delayed volume changes of the bilayered protein muscle compared with actual changes in pH, hysteresis effects, and differing total time for volume changes of the protein muscle and pH oscillation duration. Accordingly, this class of the responsive protein material comprises the potential of adapting material states connectable to learning in response to external stimuli with oblivion via resetting learnt material states by another external trigger (e.g., thermal changes, pH reversion) (Figure S9, Supporting Information). The responsiveness of our artificial protein muscle shows a rapid response to changes in pH, and to temperatures exceeding the physiological range (45 °C shown in Figure S9 and Movie S6, Supporting Information), enabling us to reset the previously built protein muscle state.

6. Conclusion

Driving autonomous muscle movements by adopting nonequilibrium states responsive to changes in pH, temperature, and other stimuli can now be realized by artificial protein muscles. They are fueled by an oscillating chemical reaction consuming Na₂SO₃ as chemical fuel likely to be changed to other sources, for example, biomass-derived malate as an isotropic chemical energy source.

In contrast to impressive artificial synthetic smart polymer systems, as shown in particular by the Yoshida group, the usage of body-own or body-derived molecules, especially proteins, opens up completely new possibilities and fields of research. In addition, a sustainable and scalable access to renewable soft materials with great durability for technical applications such as soft robotics is the first step in future applications for biomedical and pharmaceutical applications. Using these composite materials in tissue regeneration via the reversible stimulus responsiveness will allow to instruct cellular behavior in tissue substitution and regeneration. The structural responsiveness of two rather independently acting protein materials is a very fundamental aspect for these applications, together with the design of “block domain” proteins exemplified by the two proteins developed for the artificial muscle application shown here. These repetitive structural proteins are by far the class of molecules with the highest degree of designability and can be biotechnologically synthesized with atomic precision using a variable set of 20(22) cotranslationally introducible building blocks (aa/monomers), thereby allowing to adjust not only pH responsiveness but also the responsiveness to other stimuli such as temperature or salt concentrations or combinations thereof within a single class of molecules, proteins. In classical polymer chemistry, the defined sequential incorporation of several different monomers into polymers poses a major challenge to the polymerization mechanism, although precise control over the sequence of the monomers to be incorporated into the polymer has not yet been achieved, especially when it comes to large polymers and more than 3–5 monomers. This still unsolved fundamental problem of the precise sequence control of polymer synthesis can be overcome utilizing proteins as biologically derived molecules with exact sequence control to access intelligent polymers responsive to multiple stimuli.

The high biocompatibility of the pure protein actuator and the possibility to match the mechanical properties of the material by varying the precise aa composition and degree of crosslinking of constituents to certain tissues will further increase the field of future applications in reconstructive medicine, pharmacy, and the design of exoprosthetics besides advanced soft robotic systems. Our protein muscle is easily shaped and programmed for stimulus—responsiveness constituting intelligent materials for soft robotics, reproducing the motion of living organisms with the potential to replace conventional power systems using chemical energy. The possibility to even switch/start the periodic muscle contraction by temperature, running autonomously afterward, enables delayed autonomous artificial muscle functions in complex soft robotic applications.

Linking this protein motor and artificial protein muscle to other stimuli as well as the type of stimulus—responsiveness, for example, electrical potential gradients, opens up interesting perspectives for applications in soft robotics, biomedicine, and may even enable the transformation of various types of energies in a bio-based fashion. The designability, adjustability, and programmability of the presented protein muscles offer a wide range of possibilities toward autonomously acting bio-based and biocompatible biorobots acting as quasiliving machines.
7. Experimental Section

Nonequilibrium Landolt pH Oscillation Reaction: Various Landolt pH oscillator systems were described within the last decades. We adopt a bromate/sulfite/ferrocyanide reaction system originally based on the work of Edblom et al.\textsuperscript{[23]} and originally developed for a continuous stirred tank reactor (CSTR). We modified this system for our nonequilibrium energy conversion approach to an individually designed semibatch reactor.

Figure 4. Complex protein muscle behavior and switchable oscillating muscle contractions. Stimulus—responsiveness of resilin-like ELPs: a) (VRY)\textsubscript{16} with strong temperature responsiveness and weak pH responsiveness. b) (DSY)\textsubscript{16} reveals strong pH responsiveness but very weak temperature responsiveness. c) pH responsiveness of resilin-like ELP composite muscle ((DSY)\textsubscript{16}/(VRY)\textsubscript{16}) at 23 and 37 °C, indicating a dramatic change in periodicity and total running time. d) "On switch" of the protein muscle oscillations: at low temperature (see gray graph in the middle, indicating the temperature profile), no change in contraction upon changes in pH is observed (upper scheme in image d); raising the temperature to 25 °C enables pH oscillation-triggered adoption of nonequilibrium material states driving autonomous muscle contractions leading to bending motions (Movie S5, Supporting Information).
because of its reliable pH range, the physiological temperature range, and the pH extremes well fitting for our developed protein hydrogels to be acti-
ated from this reaction.\textsuperscript{13}\textsuperscript{3} The varying parameters we used for the differ-
ent pH oscillating reactions are mentioned in the Supplementary Information section “Experimental details of the experiment presented in Figure 2” and Figure S5, Supporting Information.

Design and Production of Resilin-like Proteins: The resilin-like ELP DNA template modules were designed and cloned as described by Huber et al.\textsuperscript{26} The DNA templates for resilin-like ELPs (DSY)\textsubscript{16} ((VPGDG)(VPGSG)(VPGYG))\textsubscript{16} (with the negatively charged aa Asp/D at every 15th aa position) as well as for (VRY)\textsubscript{16} ((VPGCV)(VPCRG)(VPGYG))\textsubscript{16} (with the positively charged aa Arg/R at every 15th aa position) were assembled into the high-expressing pET28-NMBL-vector in analogy, as described for the OVTP mentioned before. Proteins were expressed in Escherichia coli BL21(DE3) cells, as described in Table S1, Figure S6, and S7, Supporting Information.

Photocrosslinking of (DSY)\textsubscript{16} and (VRY)\textsubscript{16} Proteins: Lyophilized or precipitated protein aliquots with known protein concentrations were dis-
olved in MilliQ H\textsubscript{2}O or 4 \textmu{}m urea, depending on the respective protein. In case of protein hydrogel pads, usually 4 \textmu{}m urea is used for solubilization.

As soon as the protein was dissolved homogeneously, riboflavin-photophor (stock solution: 50 mM) or tris(bipyridine)ruthenium(II) chloride (Ru(II)bpy; stock solution: 10 mM) was added to a final concentration of 2.5 mM riboflavin or 0.1 mM Ru(II)bpy, followed by brief vortexing and centrifugation; then, ammonium peroxodisulfate (APS) (stock solu-
tion: 1 M) was added to a final concentration of 30 mM, again followed by vortexing and centrifugation. This solution was subsequently cross-
linked with Prizmatix/Mountain Photonics UHP-T-DI LED, a high-power light-emitting diode (LED) light source with a power of 5.5 W, and a colli-
mat light beam of 460 nm wavelength connected to a power control element to adjust the desired intensity. Exposure time was normally 2 \times 2 min (2 min on every side) with a distance of 8 cm to the light source (Figure S4, Supporting Information). Protein solutions for resilin-like ELP photocrosslinking had a final protein concentration of about 20\% w/v. For the composite (DSY)\textsubscript{16}(VRY)\textsubscript{16} protein artificial muscle, a first layer of protein with 0.3 mm thickness was photocrosslinked for 1 min, followed by an additional layer of the second protein of 0.2 mm thickness, that was subsequently photocrosslinked for additional 2 \times 2 min to covalently connect onto the first layer of the crosslinked protein.

Experimental Setup and Development of Reaction and Monitoring Chamber: The nonequilibrium Landolt pH oscillations were performed in a semibatch reactor individually designed and manufactured in a custom automated manufacturing with a 3D filament printer (Prusa i3 MK3S), as shown in Figure S3, Supporting Information, to allow optimal process manage-
ment, visualization and analysis of recorded pH measurements, and protein oscillation images.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This research was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany’s Excellence Strategy – EXC-2193/1 – 390951807. The authors also gratefully acknowl-
edge the support of the BMBF (31A490 and Research Prize Next Generation of Biotechnological Processes 2014 Biotechnology 2020-1, 031A550), the Baden-Württemberg Stiftung Project BioUltraSpring, the Zentrum für Biosystem Analyse (ZBSA), the Freiburg Institute for Advanced Studies (FRIAS), the Institute for Macromolecular Chemistry, the Institute for Micro System Engineering (IMTEK), and EXC 294 BIOSS Centre for Biological Signalling Studies and the Rectorate of the University of Freiburg.

Open Access funding enabled and organized by Projekt DEAL.

[Correction added on April 19, 2022, after first online publication: Projekt DEAL funding statement has been added.]

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

S.M.S. and M.C.H. conceived the project. M.C.H. designed and cloned the protein constructs and planned and performed the pH oscillation experi-
ments. U.J. executed 3D printing and additive manufacturing of the reac-
tion chamber and analyzed the experimental results. M.C.H. and S.M.S. conceptualized the work and wrote the publication. All authors commen-
ted and discussed on the manuscript.

Data Availability Statement

The data that support the findings of this study are available in the sup-
plementary material of this article.

Keywords

artificial muscles, autonomous movements, nonequilibrium materials, protein soft robotics

Received: September 24, 2021
Revised: December 11, 2021
Published online: January 13, 2022
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