# MAPWORMS

## Mimicking Adaptation and Plasticity in WORMS

# D4.1 DELIVERY OF AVAILABLE SMART RESPONSIVE HYDROGELS FOR PRELIMINARY MODELLING AND FOR DEVISING INTEGRATION POSSIBILITIES

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## INTRODUCTION

**WP4 - *Smart stimuli-responsive hydrogels* aims to design and characterize stimuli-responsive DNA-based hydrogels exhibiting switchable stiffness, leading to controllable shape-memory, self-healing and molecules-release. The hydrogels stiffness change can be triggered by auxiliary biocatalytic/metabolic-stimuli, pH, heat, light, electrical, and redox stimuli.** These hydrogels, possibly combined with passive materials, such as metallic layers or polymeric membranes, will act as the building blocks for the future actuation units and reactive machines, as in Fig 1. **This report summarizes the research results of the first 12-month period of WP4 (M1-M15, M19-M45).**
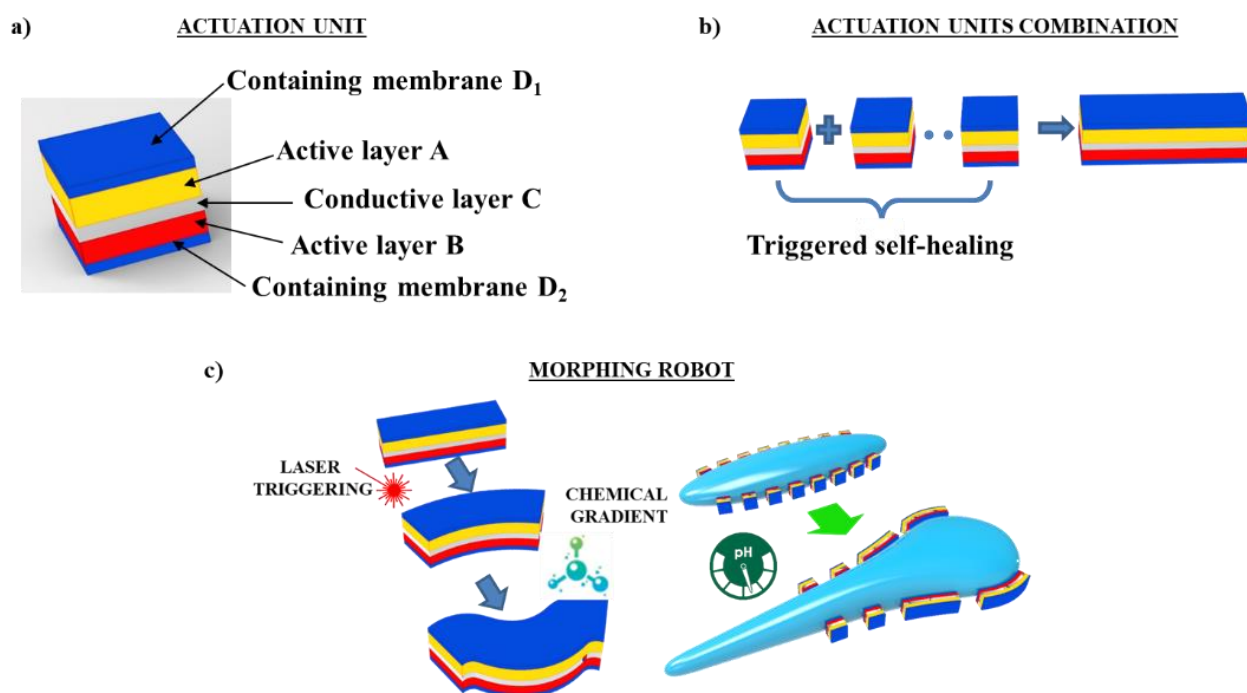


**Figure 1. MAPWORMS concept of shaped autonomous morphing robot. (a) Proposed actuation unit structure. (b, c) Proposed combinations of actuation units towards shape morphing robots.**

WP4 leader, Prof. Itamar Willner, has extensive background in developing stimuli-responsive hydrogel formulations (particularly, DNA-based hydrogels), responding to signals such as redox, light, heat, chemical agents, pH, thermoplasmonic nanoparticles and enzyme/metabolites as triggers. This background was recently reviewed by the WP leader.[1]

The deliverables of WP 4 for this period include the design, synthesis, and characterization of "smart" stimuli-responsive switchable hydrogels, revealing preliminary functional features for modeling soft robotic/mechanical applications while devising integration principles and stimuli-responsiveness. During the first 12 months of the project, HUJI focused on:

(i). Developing new types of DNA-based hydrogel formulations (cryogels) revealing pH and enzyme/metabolic reversible stiffness-responsiveness and their application for dynamic morphing and actuation.

(ii). The development of surface-confined pH-responsive hydrogel films revealing electrochemically-stimulated biocatalytic/metabolic stiffness changes for controlled-release.

(iii). Preliminary studies to develop transient, dissipative DNA-based hydrogels, as bulk material, or as transient functional materials on electrode surfaces.

The research results will be summarized in two comprehensive research papers to be submitted in May and June 2023, one added as addendum to the Deliverable:

1. Stiffness-Switchable Biocatalytic pH-Responsive DNA-Functionalized Polyacrylamide Cryogels and Their Mechanical Applications.
   Davison-Rozenfeld Gilad, Xhinhua Chen, Qin Yunlong, Yu Oyang, Sohn Yang Sung, and Itamar Willner.
   To be submitted to *Advanced Functional Materials* journal in June.

2. Stimuli-Responsive DNA-Based Hydrogels On Surfaces for Switchable Bioelectrocatalysis and Controlled Release of Loads.
   Fadeev Michael, Davidson-Rozenfeld Gilad, and Itamar Willner.
   To be submitted to *ACS Applied Materials & Interfaces* journal in May.
   (addendum link)

In the present Deliverable D1.4, the following topics were addressed:

**1.1** **<u>Stimuli-responsive DNA-based hydrogels and cryogels.</u>**
In this section, a versatile approach to design stimuli-responsive hydrogels and cryogels is described. Particularly, the preparation of pH- and glucose oxidase (GOx)/glucose (enzyme/metabolite)-responsive matrices are introduced.

**1.2** **<u>Characterization and application of stimuli-responsive DNA-based hydrogels/cryogels.</u>**
**a)** The characterization of the switchable stimuli-responsive stiffness properties of the hydrogels/cryogels by rheometry and Young's moduli (microindentation) is introduced.
**b)** The porosity of the hydrogels/cryogels is characterized by high-resolution SEM imaging and confocal microscopy imaging.
**c)** The dynamic, temporal control over stiffness and shapes of the hydrogels/cryogels upon guided mechanical transitions are characterized.

**1.3** **<u>Stimuli-responsive DNA-based hydrogels on surfaces</u>.**
**a)** A general methodology inducing stimuli-responsiveness of DNA-based hydrogels on surfaces is introduced.
**b)** A method to assemble pH-responsive hydrogels and enzyme-loaded, pH-responsive hydrogels on surface is introduced.
**c)** A method to assemble an enzyme loaded, $K^+$-ion/crown-ether responsive, G-quadruplex functionalized, hydrogel-modified electrode is introduced.

**1.4** **<u>Characterization of the stimuli-responsive hydrogel-modified surfaces.</u>**
**a)** The pH-responsive switchable-stiffness properties of the hydrogels were characterized by microindentations (Young's moduli), and Faradaic impedance spectroscopy.
**b)** The GOx-stimulated bioelectrocatalyzed oxidation of glucose and accompanying pH-changes were applied to control the stiffness of the hydrogel films and to release hydrogel-embedded loads (e.g., Insulin).
**c)** The $K^+$-ion/crown-ether switchable control of the stiffness of the electrode-modified hydrogels were measured by microindentation and electrochemical means (chronopotentiometry).
**d)** The $K^+$-ion/crown-ether switchable-stiffness functions of the hydrogel matrices were used to control (switch) the bioelectrocatalytic oxidation of glucose.

## 1.1 STIMULI-RESPONSIVE DNA-BASED HYDROGELS AND CRYOGELS

While versatile methods, formulations, and applications of stimuli-responsive DNA-based hydrogels have been demonstrated, they are still limited by their long-time response (in the order of hours) to auxiliary triggers, due to the lack of interconnected macropores. While interconnected macropores allow for convectional flow of solvent and solutes, the non-interconnected microporous structure of hydrogels restrain the displacement of particles in the matrix, resulting in slow diffusional based processes. An important class of gel materials includes cryogels. These cryogels are prepared under freezing temperatures, through analogue protocols to standard hydrogels synthesis. During cryo-polymerization, crystalline solvent domains coated by concentrated crosslinked polymer matrices are formed. This frozen structure leads, upon thawing of the frozen matrix, to permanent structures with interconnected macropores and macrochannels, embedded in the crosslinked micropores of the polymeric backbone. Important structural and functional features arise from the interconnected macropores and the high-polymer content of the boundaries, as compared to analogue hydrogels matrices, composed of nano/micro solute containments and relatively homogenous polymeric-content distribution. These differences are reflected by higher mechanical strength and higher compressibility of the cryogels originating from the higher polymer content in the framework boundaries, and the solute squeezability from the interconnected macropores of the cryogels. Importantly, the large interconnected solute macropores allow convectional transport of solutes in the cryogels, as compared to diffusionally-hindered transport in hydrogels, leading to enhanced mass transport between the solute and the polymer framework, and eventually, to enhanced responsiveness.[2] While enhanced responsiveness of DNA-based cryogels was recently reported,[3,4] the design of stimuli-responsive, reconfigurable nucleic acid-functionalized cryogels, and their reversible reconfiguration effect on the cryogel stiffness, are at present unreported, and could provide means to control cryogels properties, functions and possible applications. Furthermore, the incorporation of enzyme into the cryogel structure to facilitate biocatalyzed temporal stiffness-changes of DNA-based cryogels, as biomimetic approach to produce autonomous, mechanical actuation is unprecedented, and could provide means for modelling autonomous, fast-responding smart DNA-based soft-actuators.

Toward this aim, pH-responsive hydrogels or cryogels (hydrogel/cryogel) were prepared by radical-polymerization of acrylamide, pH-responsive Cytosine (C)-rich acrydite, complementary Guanosine (G)-rich acrydite monomer, and bis-acrylamide. The resulting hydrogel/cryogel in state A, was crosslinked by bis -acrylamide and (C)-rich/(G)-rich duplex units to form the higher-stiffness hydrogel. Treatment of the hydrogel/cryogel at pH 5.5 separates the duplex bridges through the formation of the i-motif structures to yield the lower-stiffness hydrogel/cryogel, state B (hydrogels are prepared at 4 $^0$C;cryogels are prepared at -18 $^0$C). By subjecting the hydrogel/cryogel to pH=7.4 and pH=5.5, the hydrogel

was cycled between high and low stiffness states. By integration of GOx into the hydrogel/cryogel matrices, the aerobic biocatalyzed oxidation of glucose led to the formation of gluconic acid, acidification of the gel matrices, and control over their stiffness, **Fig. 1.1 (A).** The main results obtained in this direction can be summarize as follows:

(a) The convectional mass transport features of the cryogel resulted in rapid stiffness-change (G'=120 Pa, at pH=7.4 → G'=55 Pa at pH=5.5 within 140 min). No significant stiffness-changes of the hydrogel were observed within this time-interval. The stiffness-changes were reversible, **Fig 1.1 (B)**.

(b) For the GOx-loaded hydrogels/cryogels, the aerobic biocatalyzed oxidation of glucose acidified the matrices, resulting in the pH-stimulated stiffness-changes.

- The biocatalytic aerobic oxidation of glucose in the cryogels proceeded under convection, led to rapid stiffness-changes, whereas the diffusional biocatalytic transformation reaction in the hydrogel led to slow stiffness-change. The stiffness-changes, driven by the biocatalytic processes were controlled by the concentration of glucose, **Fig 1.1 (C)**.

- The biocatalyzed stiffness-changes of the hydrogels/cryogels are reversible, **Fig 1.1 (D)**.
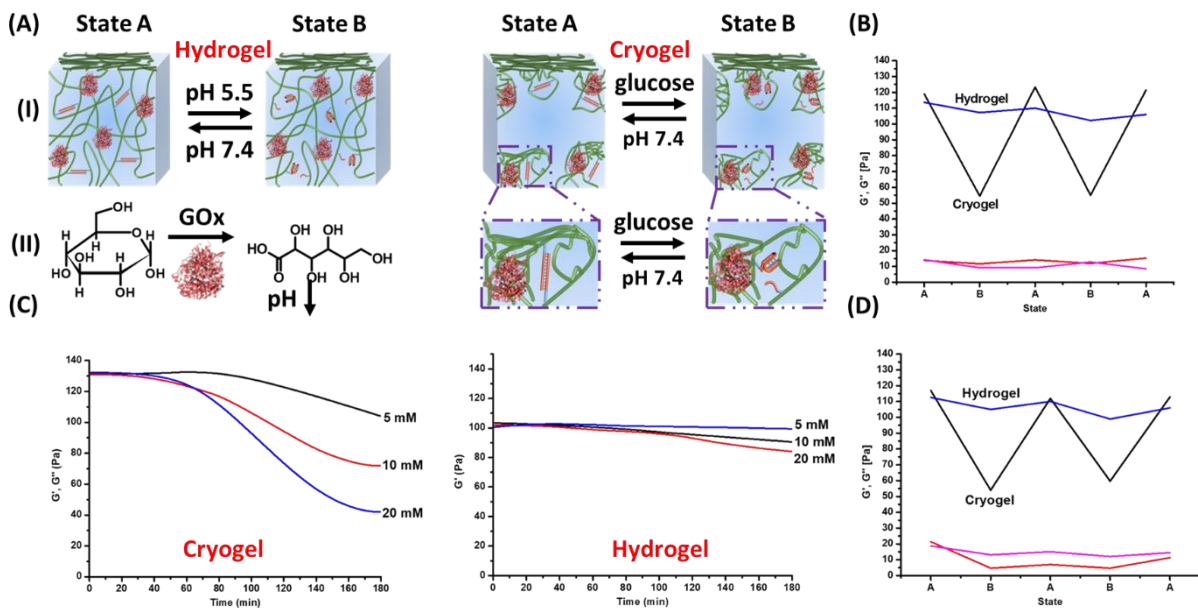


Figure 1.1. pH and glucose responsive hydrogels/cryogels. (A) Schematic representation of pH-stimulated stiffness-responsiveness, panel I, and acidification by GOx biocatalytic transformation of glucose, panel II. (B) pH-stimulated reversible stiffness-switch. (C) Control over stiffness by glucose concentrations. (D) GOx/glucose stimulated reversible stiffness changes.

## 1.2 CHARACTERIZATION AND APPLICATIONS OF STIMULI-RESPONSIVE DNA-BASED HYDROGELS/CRYOGELS

The temporal and switchable stiffness properties of the pH-responsive hydrogel/cryogel materials, using auxiliary pH-changes, and GOx/glucose-stimulated pH-changes, were characterized by rheometry and the results are summarized in **Figure 1.1**. In addition, high-resolution SEM and fluorescence confocal microscopy imaging demonstrated the structural differences between the hydrogel and the cryogel matrices.

**Figure 1.2 (A)** depicts the interconnected macropores and solute channels of the cryogel (panel I) and its pore size distribution, as compared to the microporous structure of the hydrogel (panel II) and the measured pore size. To further confirm the structural differences, **Fig 1.2 (B)** depicts the fluorophore-stained fluorescent confocal microscopy images of the hydrogel (panel I) and the cryogel matrices (panel II). While the hydrogel depicts homogenous distribution of fluorophore, consistent with homogenous polymeric content distribution, the cryogel fluorescence reveals concentrated polymeric content (red emission), that surround macro-contaminants of solute (non-fluorescent regions). The interconnected macroporous channels present in the cryogel, account for the convectional solute flow and effective mass transportation, and the rapid stiffness-changes observed for the cryogels.
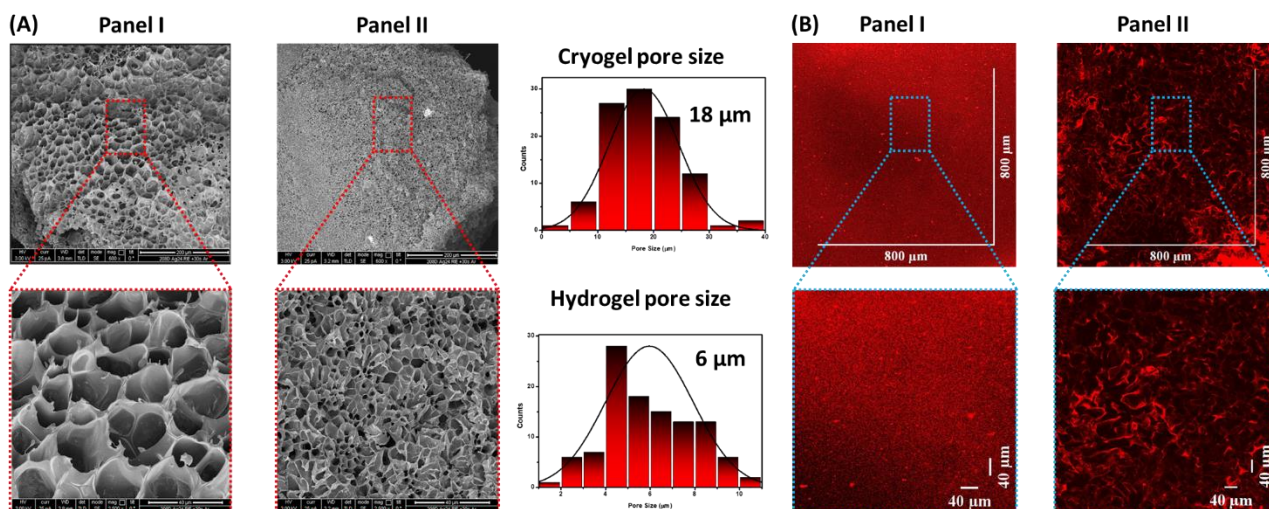


Figure 1.2. Hydrogels/cryogels microscopy and pore size distribution characterization. (A) SEM of macroporous cryogel, panel I, and of microporous hydrogel, panel II, Scale bars are 200 μm (up) and 40 μm (bottom). (B) fluorophore-stained fluorescent confocal microscopy of macroporous cryogel, panel I, and of microporous hydrogel, panel II, Scale bar 800 μm (up) and 40 μm (bottom).

For utilization of the enhanced properties of DNA-based cryogels and their demonstration as biomimetic model for autonomous, fast-responding soft-actuators, the pH-stimulated control over the stiffness properties of the acrylamide hydrogel/cryogel matrices was applied to assemble soft bending actuators. Toward this goal, bilayer matrices constructs consisting of a thermoresponsive poly-N-isopropylacrylamide (pNIPAM) gel layer and a pH-

sensitive polyacrylamide (pAAm) layer were constructed. The pH-responsive pAAm layer was activated by auxiliary pH-changes, as whereby the GOx-catalyzed pH-changes stimulated by the biocatalyzed oxidation of glucose through the GOx embedded in the pAAm layer occurs. The pNIPAM layer consisted always of a cryogel matrix that undergoes rapid convectional thermoresponsive compression duo to the dense polymers that results in high-stiffness differences between the pNIPAM layer and the pH-sensitive pAAm layer, leading to mechanical bending of the device. The selected formulation of unfunctionalized-pNIPAM and nucleic acids-functionalized pAAm allows for non-overlapping, dually triggered, stiffness changes that promotes bending. In addition, it allows for simple design due to utilization of only one stimuli-responsive reconfigurable DNA-complex. The fabrication of the bilayer constructs, and the mechanical, stiffness-controlled bending are depicted in **Figure 1.2.1 (A)**. Bilayer constructs consisting of pNIPAM (cryogel)/pAAm (cryogel) and pNIPAM (cryogel)/pAAm (hydrogel) were constructed and the dynamics of the bending of the device were examined under auxiliary pH-triggering or upon the GOx-catalyzed pH-stimulated changes in the pAAm layer. The main phenomena analysed can be summarized as follows:

- The reversibility of the mechanical bending functions of the different bilayer devices were examined.
- The temporal time-dependant rates of bending of the pNIPAM (cryogel)/pAAm (cryogel) Vs pNIPAM (cryogel)/pAAm (hydrogel) were examined.
- The effect of glucose concentrations on the GOx metabolic bending rates were evaluated.

A detailed description of all these results will be presented in a paper entitled "*Stiffness-Switchable Biocatalytic pH-Responsive DNA-Functionalized Polyacrylamide Cryogels and Their Mechanical Applications*" to be submitted to *Advanced Functional Materials* journal in June. Representative results are presented here.

**Figure 1.2.1 (B)**, panel I depicts the reversible dynamic pH-stimulated bending of the bilayer pNIPAM (cryogel)/pAAm (cryogel). The reversibility and Young's moduli of this bilayer system are displayed in panel II.

**Figure 1.2.1 (C)**, panel I presents the time-dependent temporal stepwise bending steps of the pNIPAM (cryogel)/ pAAm (cryogel) as a function of glucose concentrations. The dynamic bending is reversible, and as the glucose concentration is higher, the temporal bending events are enhanced, panel II.

**Figure 1.2.1 (D)**, panel I depicts the temporal bending transitions of the bilayer pNIPAM (cryogel)/ pAAm (hydrogel). The bending is reversible, yet demonstrating substantially slower bending time intervals, panel II, originating from the lower mass-transport, diffusional-controlled, pH-changes in the hydrogel matrix.
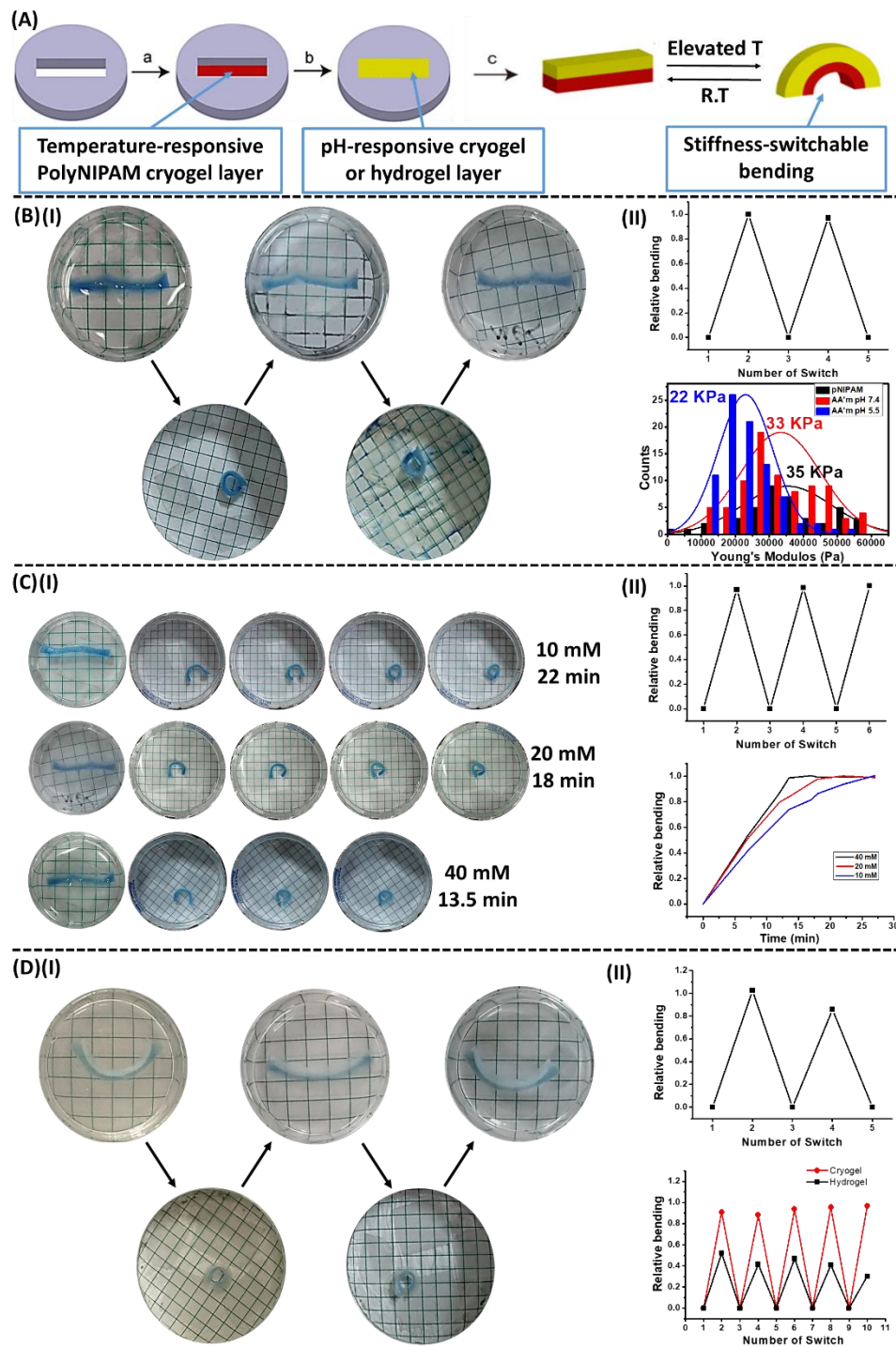
**Figure 1.2.1. Mechanical bending of bilayer constructs. (A)** Schematic representation of the synthetic procedure and bilayer construct bending in response to heat. **(B)** Dynamic bending of bilayer cryogel/cryogel construct in response to pH changes, panel I, and the reversible bending with the corresponding Young's moduli, panel II. **(C)** Time-dependent, glucose stimulated, temporal stepwise bending steps of bilayer cryogel/cryogel construct, panel I, and the reversible bending with the corresponding time-dependent relative bending curves, panel II. **(D)** Temporal bending transitions of the bilayer cryogel/hydrogel, panel I, and it's reversible bending with the slow relative bending, compared to the cryogel/cryogel construct, panel II.

The bilayer systems were characterized by high-resolution SEM and fluorescence confocal

microscopy images.

> The results demonstrate a biomimetic approach whereby "metabolic" processes control the stiffness and mechanical properties of soft materials, (cryo/hydro-gels) in analogy to native processes in "WORM" specimens.
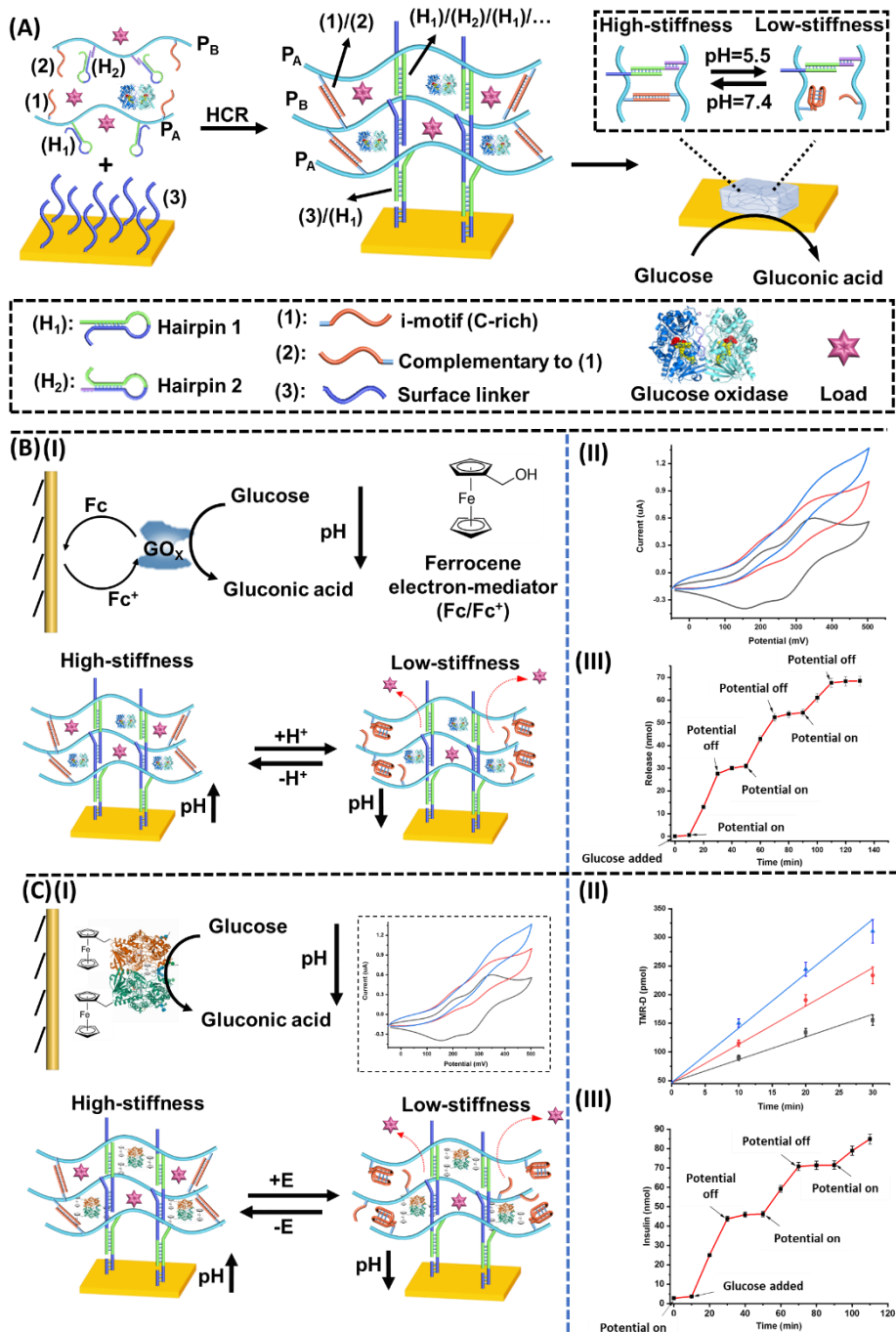
## 1.3 STIMULI-RESPONSIVE DNA-BASED HYDROGELS ON SURFACES

A general method to assemble stimuli-responsive DNA-based polyacrylamide hydrogel films on surfaces, and particularly on electrode surfaces, was developed for electrobiocatalyzed controlled load-release. This is exemplified in **Figure 1.3** with the assembly of pH-responsive hydrogels on an Au-coated surface. The method involves the deposition and hybridization chain reaction (HCR) stimulated crosslinking of hairpin-functionalized ($H_1$, $H_2$) polyacrylamide chains, that are modified with additional complementary tethers (**1**) and (**2**), using a promoter strand (**3**) as HCR activator. The resulting hydrogel is cooperatively crosslinked by the $H_1/H_2$ duplex chains, and the (**1**)/(**2**) duplexes, where the strand (**1**) is Cytosine-rich (C)-rich that assemble at pH 5.5 into an i-motif structure. **Figure 1.3 (A)** depicts the assembly, and the pH-stimulated control over the stiffness of the hydrogel films on surface. While at pH=7.4 the hydrogel reveals higher stiffness due to the cooperative crosslinking by the $H_1/H_2$ and (**1**)/(**2**) duplexes, the pH-triggered separation of the duplex (**1**)/(**2**) through reconfiguration of strand (**1**) into the i-motif structure, leads to a lower stiffness hydrogel.

The cyclic transitions of the hydrogel between states of higher stiffness and lower stiffness is affected by auxiliary pH-changes. Alternatively, the hydrogel was loaded with glucose oxidase (GOx) within the course of synthesis of the hydrogel. The aerobic GOx-catalyzed oxidation of glucose to gluconic acid results in acidification of the hydrogel films, thus, lowering their stiffness. By integration of loads (e.g., Tetramethylrhodamine dextran, TMR-D, or coumarine-labelled Insulin) into the GOx-loaded hydrogel, the pH-stimulated control of the hydrogel stiffness allows the pH-stimulated release of the loads (TMR-D or coumarine Insulin). While the high-stiffness state of the hydrogel leads to blocked release of the load, the reconfiguration of the nucleic acids into i-motif structures leads to separation of the crosslinking units, thus to increased pore size and release of load.

By exposing the GOx modified hydrogel electrode to the ferrocene electron mediator and glucose, bioelectrocatalyzed oxidation of glucose is stimulated under anaerobic conditions, leading to the acidification of the hydrogel, resulting in the release of the loads, **Figure 1.3 (B**), panel I, II, and III. By chemical modification of GOx with ferrocene electron mediator units, the electrical wiring of the integrated ferrocene/GOx hydrogel electrode was demonstrated. The bioelectrocatalyzed oxidation of glucose by the integrated, electrically-wired electrode, and the resulting acidification of the hydrogel and the release of dye-labelled insulin load, are depicted in **Figure 1.3.1 (C)**, panel I and II.

An analog K⁺-ion/crown-ether responsive, GOx-loaded hydrogel was deposited on an electrode surface. The switchable, stiffness-controlled bioelectrocatalyzed oxidation of glucose by the enzyme functionalized electrode were demonstrated.



Figure 1.3. Assembly of stimuli-responsive DNA-based hydrogel on electrodes and the auxiliary, triggered pH changes, using glucose and electrochemical triggers that lead to the stiffness-switch, and it's application for controlled drug-release. (A) Schematic representation of HCR-based, GOx and dye loaded, hydrogel assembly on an Au-electrode and the mechanism for the control over it's stiffness. (B) Ferrocene electron mediated bioelectrocatalyzed oxidation of glucose by GOx, panel I, and the corresponding cyclic voltamograms, panel II, resulting in the bioelectrochemical control over load release, panel III. (C) Bioelectrocatalyzed tranformation of glucose by electrically wired GOx, panel I, and load release rates that are dependent on glucose concentrations, panel II. Reversible bioelectrochemical switching of load release, panel III.

Characterization of the stimuli-responsive stiffness change of the hydrogel films on surfaces employed several techniques including microindentation for the evaluation of Yung's moduli of the hydrogels in the different stiffness states, evaluation of the electron transfer rates to redox labels in the presence of the different stiffness states of the hydrogels. In addition, the effects of pH-changes due to different glucose concentrations on the stiffness of the hydrogels, and the potential induced switchable-stiffness, as a result of the bioelectrocatalytic transformations, were evaluated. These characterization provides the stiffness of the hydrogel films and electrochemical and chemical properties corelation of the hydrogel-loaded electrode, both effecting the load-release properties. In addition, the characterized electron-transfer properties could provide further information for modelling surface-modified actuators. **Figure 1.4** exemplifies Young's moduli of the pH-responsive hydrogels shown in **Figure 1.4** (panel I) and the switchable-stiffness values of the hydrogel. **Figure 1.4**, panel II depicts the application of Faradaic impedance spectroscopy to follow the switchable-stiffness of the hydrogel. While high electron-transfer resistance (Ret$\cong$ 2.2 k$\Omega$) correlates to the high-stiffness, curve (a), the low-stiffness hydrogel reveal lower electron-transfer resistance (Ret$\cong$ 1.6 k$\Omega$), curve (b). In addition, SEM images of the surface activated hydrogel in the higher/lower stiffness states was applied to further support the differences between higher/lower stiffness states.
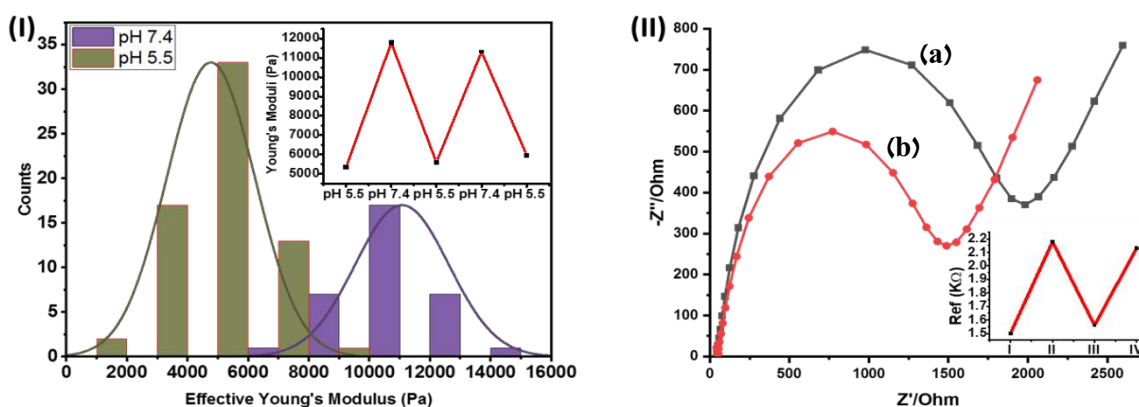


Figure 1.4. Characterization of GOx loaded DNA-based hydrogel on electrode. Young's moduli of the hydrogel on surface, meassured by microintentations, corresponding to reversible states of low-stiffness at pH 5.5, and high-stiffness at pH 7.4, panel I. Faradaic impedance spectroscopy revealing reversible, high-electron-transfer resistance at pH 7.4 (high-stiffness) and low electron-transfer resistance at pH 5.5 (low-stiffness), panel II.

The detailed results summarized in sections 1.3 and 1.4 are presented in the comprehensive paper "*Stimuli-Responsive DNA-Based Hydrogels On Surface for Switchable*

*Bioelectrocatalysis and Controlled-Release of Loads*", introduced as addendum to the report ([link](#)).

> The results reported in sections 1.3 and 1.4 demonstrate the electrochemically triggered control over the stiffness of DNA-based hydrogel material on surfaces by enzyme-driven transformations, thus providing biomimetic models to biological specimens. Beyond the relevance for the MAPWORMS concept, the system provided broader possible applications for controlled drug-release and particularly, dose controlled, glucose dictated, release of insulin from "artificial pancreas".

## FUTURE RESEARCH ACTIVITIES

The future research activities will follow the plans of WP4 indicated in the original MAPWORMS project. Specific efforts that will be addressed in the next 12 month-period include:

- Design of thermoresponsive hydrogels driven by light using thermoplasmonic nanoparticles and nanorods, and diverse hydrogel frameworks along with different thermoplasmonic substances and applications. In addition, different wavelength excitations toward optimal light-stimuli will be evaluated, using a novel vertical-external-cavity surface-emitting-laser (VECSEL) system with wavelength-tunability features over the wavelength ranges between 360 – 380 nm, 420 – 460 nm, and 780-820 nm, developed by Vexlum. The thermoplasmonic control over the stiffness of hydrogels will be evaluated and experiments directed toward thermoplasmonic dynamic morphing of hydrogels will be designed.

- Development of loaded electrically-wired enzymes integrated within DNA-based cryogels on surfaces, and application of the unique convectional mass transport features of cryogels for enhanced bioelectrocatalysis and dynamic stiffness-regulation of the cryogels.

- Efforts to design DNA hydrogels exhibiting dissipative, transient, stiffness properties will be undertaken. These soft materials will be examined as dynamic matrices for controlled load-release. Within the different activities, efforts to apply redox-triggers, and enzyme embedded in the dissipative hydrogels will be examined.

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