Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Programmed assembly of bespoke prototissues on a microfluidic platform

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1. Supplementary Videos

To acquire the videos, the microfluidic device was run for 25 min. Videos were captured at a frame rate of 7,500 fps, with a resolution of 1920x1080. Videos are played at 30 fps.

Video S1 – Fabrication of mono-compartmental w/o/w droplets. The video shows the encapsulation of a single protocell on the microfluidic device. The outer phase was set to 350 μL min^{-1}, the middle phase was set to 6 μL min^{-1} and both inner phases were set to 1 μL min^{-1}.

Video S2 – Fabrication of 1a,1b bi-compartmental w/o/w droplets. The video shows the encapsulation of 2 protocells on the microfluidic device. The outer phase was set to 330 μL min^{-1}, the middle phase was set to 6 μL min^{-1} and both inner phases were set to 1 μL min^{-1}.

Video S3 – Fabrication of 2a,2b tetra-compartmental w/o/w droplets. The video shows the encapsulation of 4 protocells on the microfluidic device. The outer phase was set to 310 μL min^{-1}, the middle phase was set to 6 μL min^{-1} and both inner phases were set to 1 μL min^{-1}.

Video S4 – Fabrication of 3a,3b hexa-compartmental w/o/w droplets. The video shows the encapsulation of 6 protocells on the microfluidic device. The outer phase was set to 290 μL min^{-1}, the middle phase was set to 6 μL min^{-1} and both inner phases were set to 1 μL min^{-1}.

Video S5 – Fabrication of 4a,4b octa-compartmental w/o/w droplets. The video shows the encapsulation of 8 protocells on the microfluidic device. The outer phase was set to 260 μL min^{-1}, the middle phase was set to 6 μL min^{-1} and both inner phases were set to 1 μL min^{-1}.

Video S6 – Fabrication of 5a,5b deca-compartmental w/o/w droplets. The video shows the encapsulation of 10 protocells on the microfluidic device. The outer phase was set to 240 μL min^{-1}, the middle phase was set to 6 μL min^{-1} and both inner phases were set to 1 μL min^{-1}.

Video S7 – Overview of working microfluidic device. The video shows a zoomed-out overview of the working microfluidic device. On the left-hand side it is possible to see the first two parallel flow focusing junctions forming both types of protocells. In the centre it is possible to see the third flow focusing junction for encapsulation of pre-determined numbers of protocells into multicompartmental w/o/w droplets. This video shows the encapsulation of individual azide- or BCN-functionalised protocells into an outer unreactive membrane of BSA/PNIPAM-co-MAA nanoconjugate. The outer phase was set to 350 μL min^{-1}, the middle phase was set to 6 μL min^{-1} and both inner phases were set to 1 μL min^{-1}.

Video S8 – Reorganisation of protocells within multicompartmental w/o/w droplets. In this video the free movement of protocells within multicompartmental w/o/w droplets can be visualised. The outer phase was set to 200 μL min^{-1}, the middle phase was set to 6 μL min^{-1} and both inner phases were set to 1 μL min^{-1}. Protocells enclosed within the outer membrane of the droplet move according to well-known flow recirculation effects. Due to the symmetry of the inner droplet flux they do not mix, hence maintaining the predetermined Janus-like organisation. The same effect was observed for every type of multicompartmental w/o/w droplet generated with our microfluidic device.

Video S9 – Thermally induced contraction of 2a,12b tetradeca-prototissue. In this video temperature was varied from 26 °C to 47 °C at a rate of 5 °C/min using a temperature stand. Time-dependent images were captured using confocal microscopy as described in the Materials and Methods section of the paper.

2. Supplementary Figures

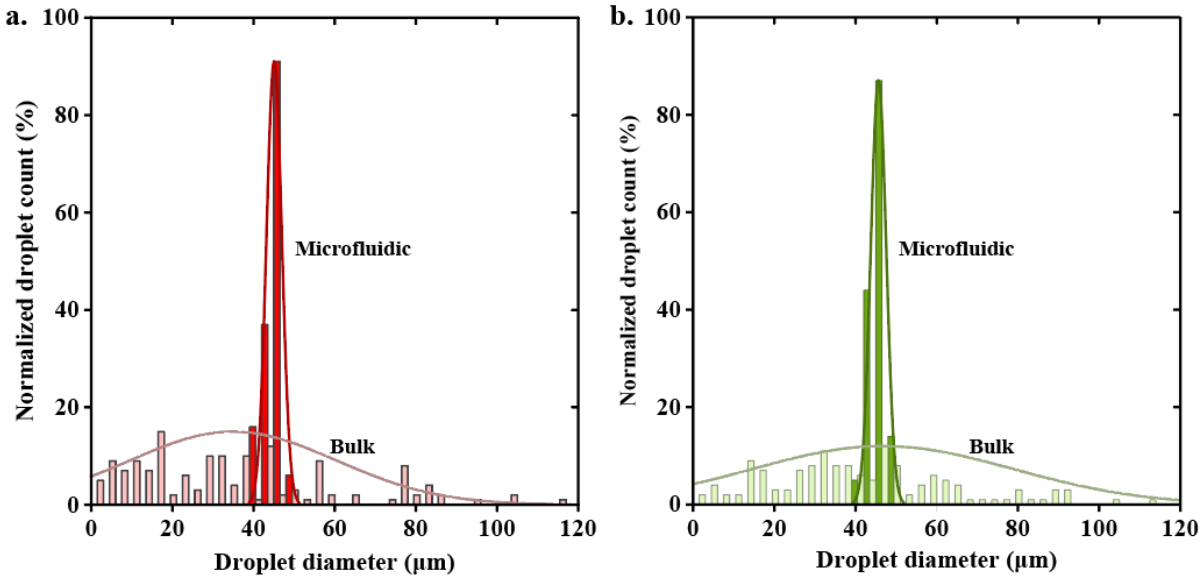

Figure S1. Size distribution of droplets created on-chip versus the traditional off-chip method. a) Graph comparing the size distributions of w/o droplets stabilised by azide-functionalised BSA/PNIPAM-co-MAA nanoconjugates produced in the microfluidic device (dark red) with the same droplets formed by manually shaking a vial (bulk method, light red). **b)** Graph comparing the size distributions of w/o droplets stabilised by BCN-functionalised BSA/PNIPAM-co-MAA nanoconjugates produced in the microfluidic device (dark green) with the same droplets formed using the bulk method (light green). For both graphs, each plot contains at least 150 data values, and the curves represent the fitted Gaussian distributions.

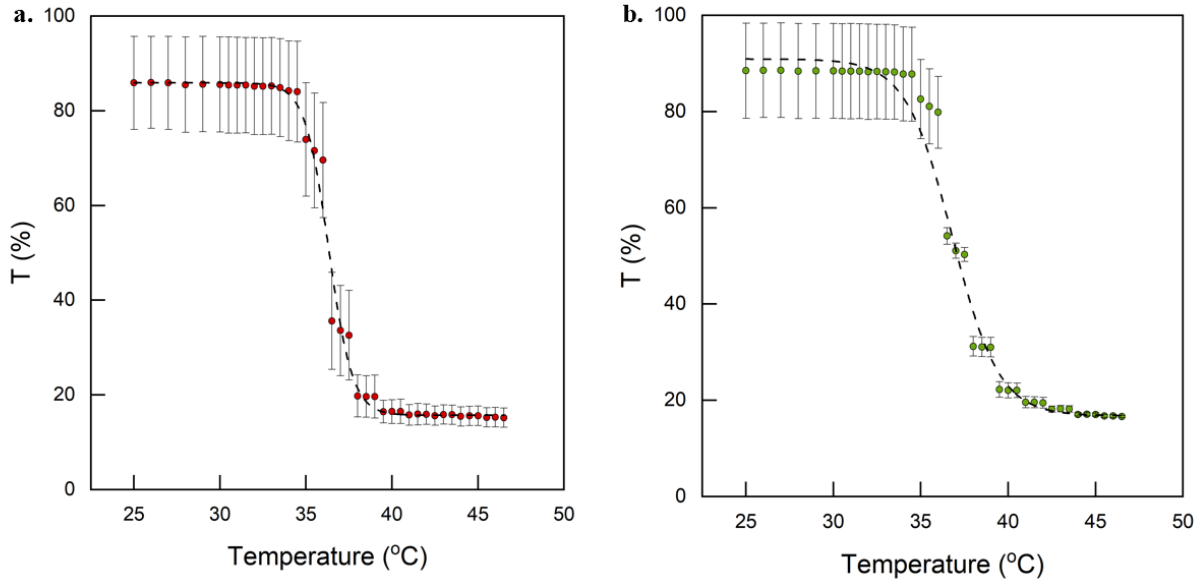

Figure S2. Estimation of the lower critical solution temperature (LCST) of protein-polymer nanoconjugates. a) LCST for the azide-functionalised BSA/PNIPAM-co-MAA nanoconjugate. **b)** LCST for the BCN-functionalised BSA/PNIPAM-co-MAA nanoconjugate. The LCST was estimated by the cloud point temperature (T_{cp}) of the compounds, which were determined by measuring the transmittance between 400 and 500 nm for a protein-polymer nanoconjugate solution in MilliQ water at a concentration of 1 mg mL^{-1}. The T_{cp}, taken as 50% of the initial transmittance value, was determined to be 36.3±0.1 °C for the azide-functionalised BSA/PNIPAM-co-MAA nanoconjugate, and 36.8±0.2 °C for the BCN-functionalised BSA/PNIPAM-co-MAA nanoconjugate.

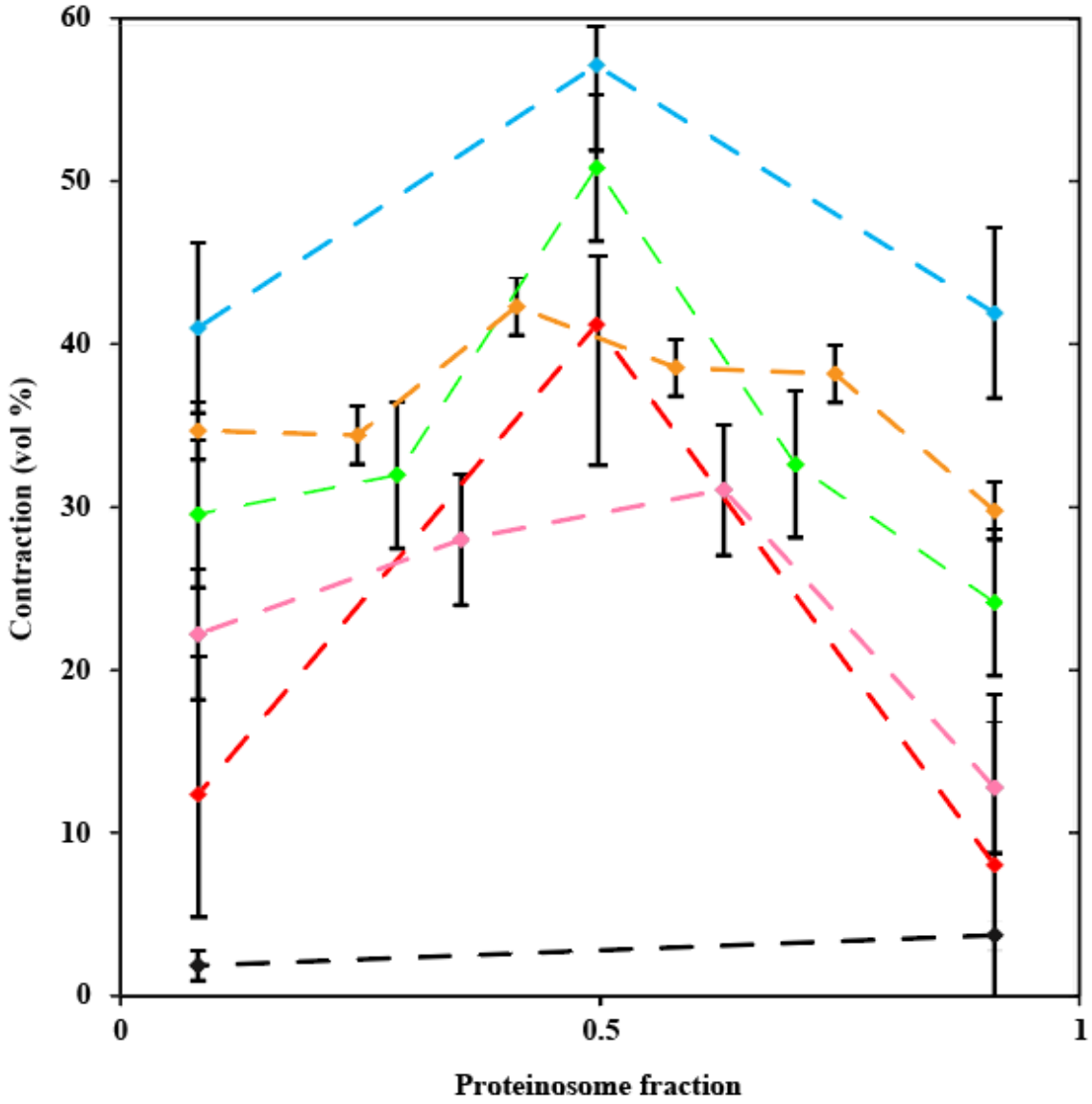

Figure S3. Statistical analysis of thermoresponsive reversible contractile behaviour of the prototissues. Graph showing the percent volume contraction in prototissues of different composition when the temperature is varied from 26 °C to 47 °C. Black plot: individual azide- and BCN-functionalised protocells enclosed in an BSA/PNIPAM-co-MAA outer membrane; red plot: bi-compartmental prototissues; pink plot: tri-compartmental prototissues; green plot: tetra-compartmental prototissues; yellow plot: penta-compartmental prototissues; and blue plot deca-compartmental prototissues. Error bars show the standard deviation. The same data are reported in Figure 3a.

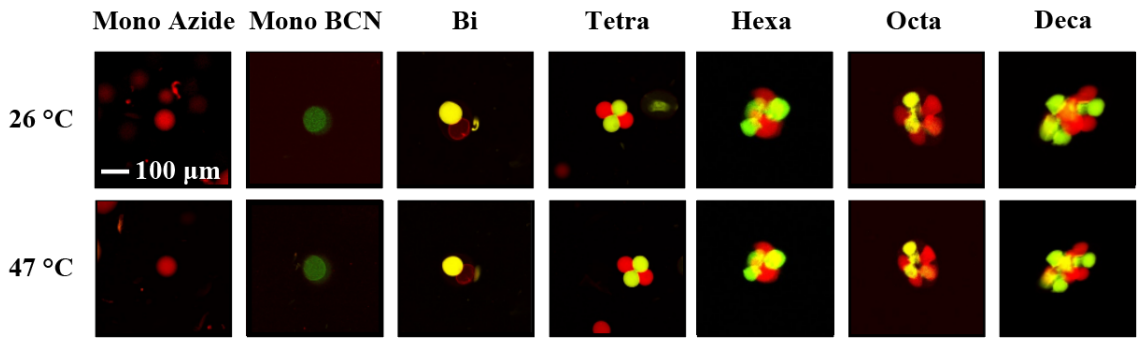

Figure S4. Collective thermoresponsive reversible contractile behaviour of bespoke prototissues. Confocal fluorescence microscopy images showing the thermally induced contraction of 1a,1b bi-, 2a,2b tetra-, 3a,3b hexa-, 4a,4b octa-, 5a,5b deca-compartmental prototissues in water when the temperature was varied from 26 °C to 47 °C. The "Mono Azide" label refers to a single azide-functionalised protocell enclosed within an unreactive BSA/PNIPAM-co-MAA outer membrane. The "Mono BCN" label refers to a single BCN-functionalised protocell enclosed within an unreactive BSA/PNIPAM-co-MAA outer membrane. The scale bar applies to all images.

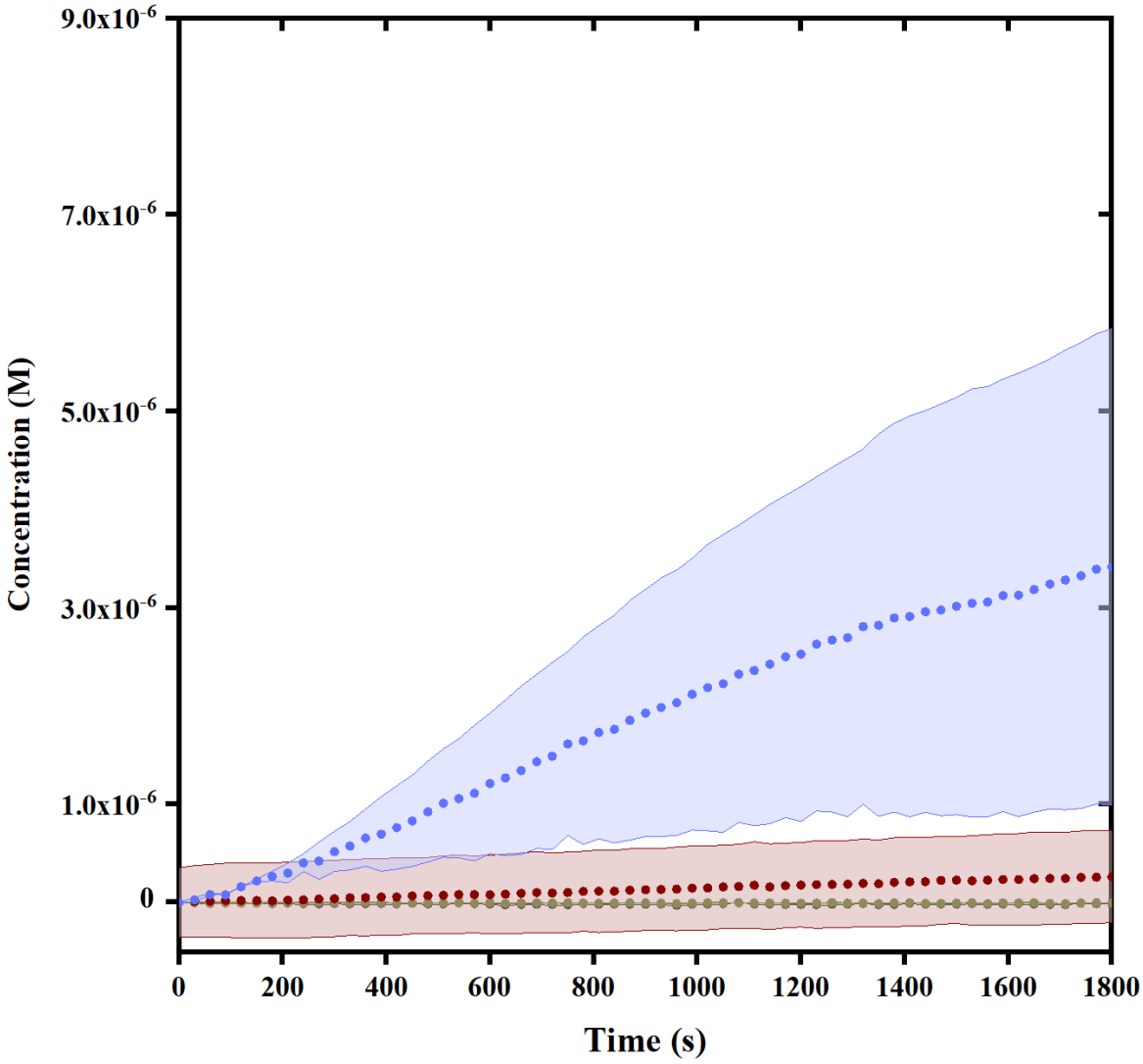

Figure S5. Control data for the GOx/HRP enzyme cascade reaction hosted within a 1a(HRP),9b(GOx) deca-compartmental prototissue. The red plot represents a control experiment where only Amplex Red was used (no glucose), the green plot represents a control experiment where only glucose was used (no Amplex Red), the black plot (obscured behind the green data) represents a control experiment in the absence of substrates, and the blue plot reports the experiment where both substrates were present (as shown in Figure 4b). Errors for black and green data are too small to appear on the graph and are comparable to those shown in Figures S5 and S6.

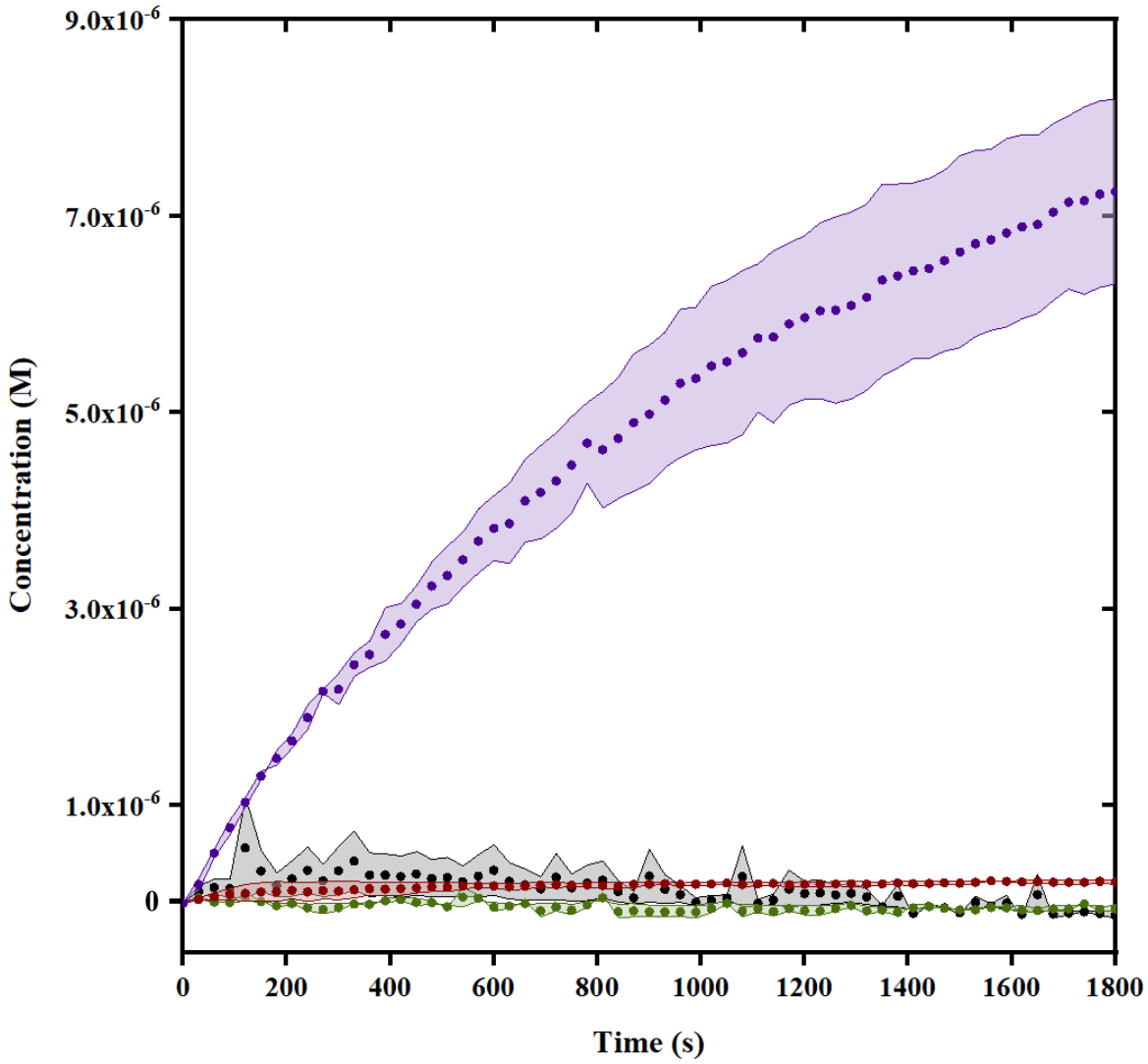

Figure S6. Control data for the GOx/HRP enzyme cascade reaction hosted within a 5a(HRP),5b(GOx) deca-compartmental prototissue. The red plot represents a control experiment where only Amplex Red was used (no glucose), the green plot represents a control experiment where only glucose was used (no Amplex Red), the black plot represents a control experiment in the absence of substrates, and the purple plot reports the experiment where both substrates were present (as shown in Figure 4b).

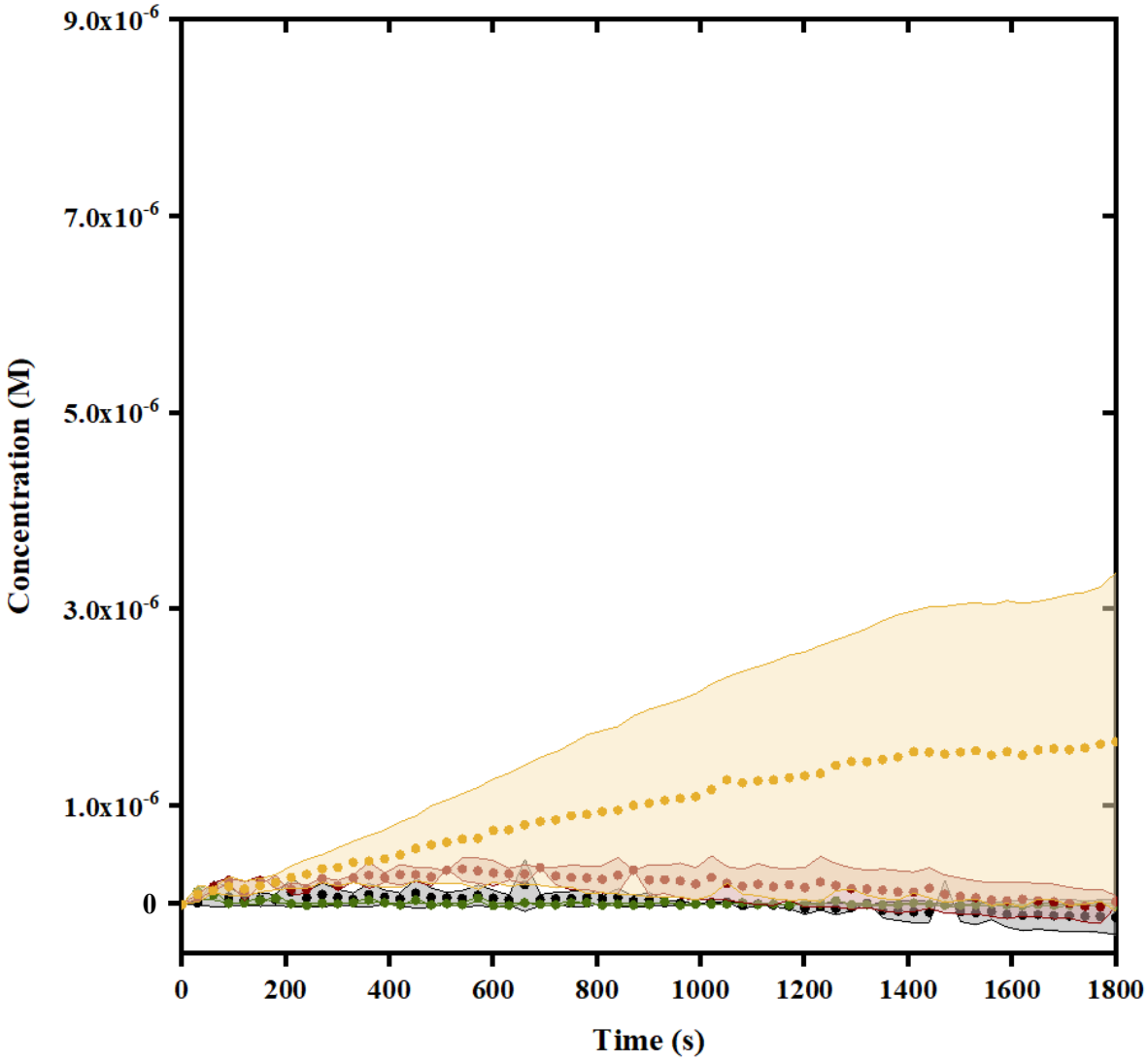

Figure S7. Control data for the GOx/HRP enzyme cascade reaction hosted within a 9a(HRP),1b(GOx) deca-compartmental prototissue. The red plot represents a control experiment where only Amplex Red was used (no glucose), the green plot represents a control experiment where only glucose was used (no Amplex Red), the black plot represents a control experiment in the absence of substrates, and the yellow plot reports the experiment where both substrates were present (as shown in Figure 4b).