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Supporting Information for the communication:

"Temporal and spatial programming in soft composite hydrogel objects"

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Experimental methods

Materials: Alginic acid sodium salt (*syn*. sodium alginate) from brown algae, urease from *Canavalia ensiformis* (Jack bean) type IX powder, 500,000 units/g solid, calcium chloride hexahydrate (98%), oil blue N (96%), oil red O and urea (powdered, ≥ 98%) was purchased from Sigma Aldrich. Ethylenediaminetetraacetic acid (EDTA, 99%), ethanol (absolute, analytical grade) and hydrochloric acid (37%) was purchased from VWR international. Vegetable oil was purchased from Costcutters Ltd. Bromothymol blue, ACS reagent, was purchased from Fisher Scientific. Oleoresin paprika oil 80,000 NS was purchased from Kalsec. Span 80 was purchased from Fluka.

Gel templates: Moulds corresponding to the desired hydrogel shapes were created in the software package *123 Design*. These were uploaded into the software package *PreForm* and printed into 3D polymer objects using the FormLab 2 SLA printer (grey resin GPGR03). Polymer moulds were washed in ethanol for 20 minutes to remove residual monomer, then post-cured under a UV lamp (365 nm) for 30 minutes. The bottom faces of the gel templates were coated with a thin layer of silicone grease (to keep the template water tight) and placed onto a flat silicone surface.

Pre-gel formation: Solutions of sodium alginate were prepared by dissolving dry powders of alginic acid sodium salt into deionised water at either 1 or 5 wt. %. Active regions of the material were created by dissolving the enzyme urease into 1 % sodium alginate solutions at a concentration of either 5, 10 or 20 mg mL^{-1}. Coloured regions of the gel were created by emulsifying an enzyme loaded 1 wt. % alginate solution with vegetable oil (containing 1 mg mL^{-1} of either oil blue N, oil red O or paprika oil), using 1 mg mL^{-1} of the surfactant Span 80 (enough to stabilise the emulsion for *ca*. 10 minutes). In the case of the pH indicator experiments, the 'pre gel' solutions contained 1 mg mL^{-1} of the dye bromothymol blue. These 'pre-gels' are loaded into the templates in the desired combination prior to gelation.

Gel cross-linking: Pre-gel loaded templates are exposed to a fine jet spray of 0.1 mol dm^{-3} calcium chloride solution to induce ionic cross-linking of the alginate polymer chains by calcium ions. After several jet sprays, the gel surface is covered in a further 2 mL of 0.1 mol dm^{-3} calcium chloride and left to fully cross-link for 20 minutes. Following this, the template was removed and the object rinsed with deionised water to remove residual calcium chloride.

Object behaviour: The composite gel objects was placed into a Petri dish containing 20 mL of 0.1 mol dm^{-3} EDTA and 2 mL of 5 mol dm^{-3} urea solution. Their behaviour was filmed using a Nikon D5100 camera equipped with a AF-S Micro Nikkor 40 mm 1:2.8 G lens. Still images are taken from these videos where appropriate.

Colour quantification: The hydrogel leaf on the far left of figure 2 was analysed using *ImageJ (Fiji)* to collect an RGB histogram of the pixels that make up a line scan across its width. These RGB coordinates were converted into HSL values, from which the reported hue angles were taken.[1]

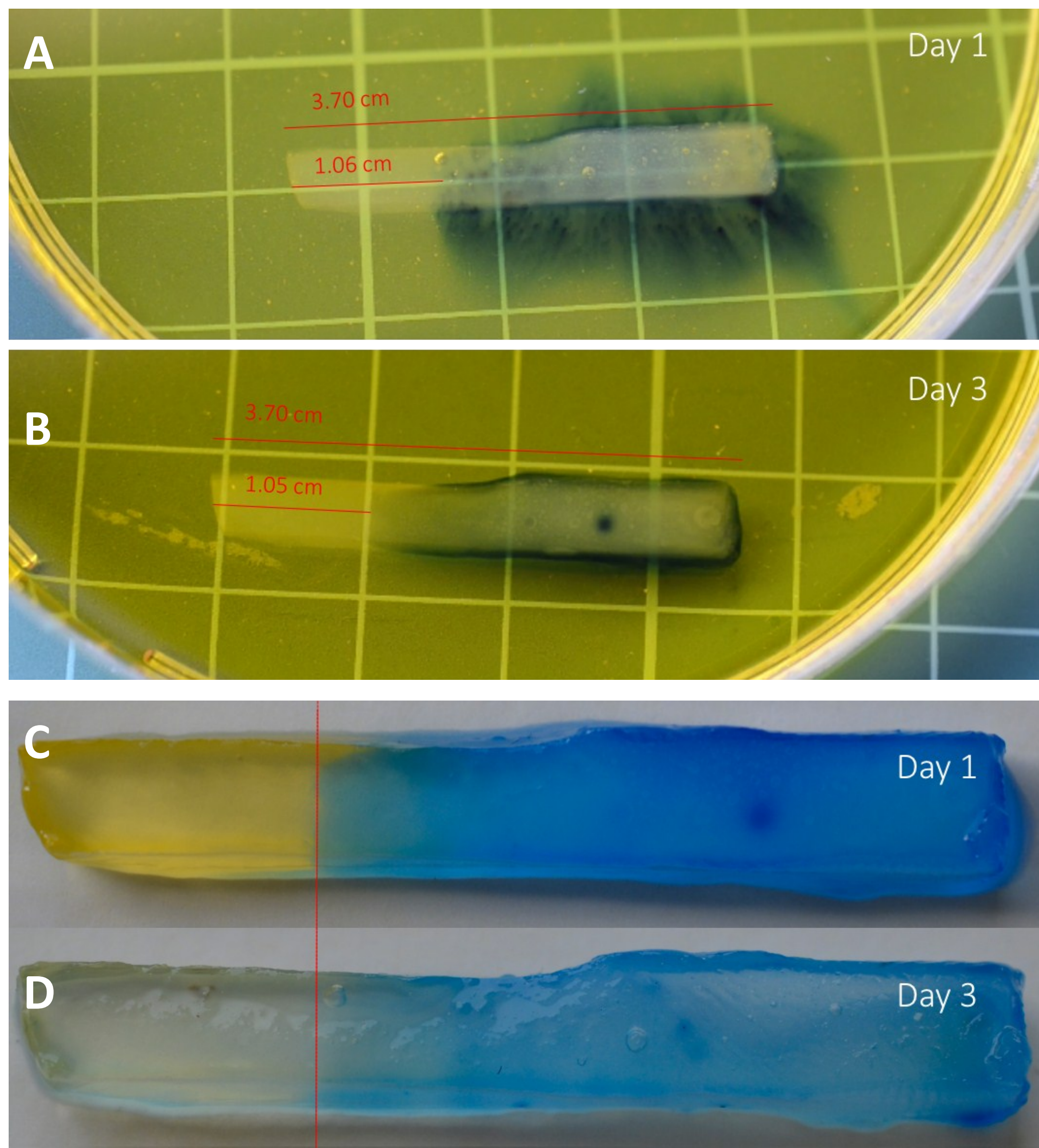

Figure 1 Photographs of 1 wt. % calcium cross-linked alginate hydrogel objects containing urease at a concentration of 1 g L^{-1} along approximately three quarters of its length. Photographs A and B show the hydrogel object, immersed in a saturated solution of the pH indicator bromothymol blue and 0.1 mol dm^{-3} urea, immediately after synthesis and 3 days later, respectively. A transition from yellow to blue indicates a pH higher than 7.6, and is therefore indicative of the location of urease (that is converting urea into ammonia and carbon dioxide). As images A and B show, the location of the urease appears to be approximately the same after 3 days, suggesting that the enzyme does not migrate through the hydrogel matrix. Images C and D are of the hydrogel objects removed from each of the solutions pictured in images A and B, respectively.

1. A. Hanbury, Constructing cylindrical coordinate colour spaces, *Pattern Recognition Letters,* **29**, 2008