Supplementary information

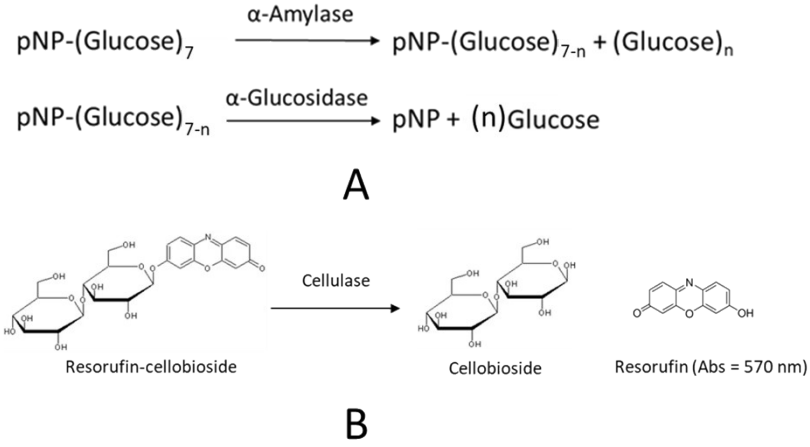


Fig. S1. Schematic representation of enzymatic reactions for α-amylase (A) and cellulase (B). Amylase breaks down pNP-G7, and α-glucosidase further processes the product, releasing pNP for a colorimetric signal at 405 nm. Cellulase hydrolyzes resorufin-β-D-cellobioside, releasing resorufin, a fluorescent product with absorbance at 570 nm.

Calculations for the accelerated aging time

The Accelerated Aging Factor (AAF) is calculated using the following equation:

$$AAF = Q_{10}^{((T_{aa} - T_{rt})/10)}$$

where:

T_{aa} = accelerated aging temperature (°C)

T_{rt} = real-time (ambient) temperature (°C)

Q_{10} equal to 2 is a common and conservative means of calculating an aging factor.

The Accelerated Aging Time (AAT) needed to simulate real-time aging is calculated as:

AAT = RT / AAF

Using an accelerated aging temperature of 37 °C and assuming a Q_{10} value of 2, the Accelerated Aging Factor (AAF) was calculated for two storage temperatures: 4 °C and 23 °C.

For 4 °C, the AAF is 9.85, meaning that:

- 1 month of real-time aging ≈ 3 days at 37 °C
- 3 months ≈ 9 days
- 6 months ≈ 18 days

For 23 °C, the AAF is 2.64, meaning that:

- 1 month of real-time aging ≈ 11 days at 37 °C
- 3 months ≈ 34 days

- 6 months ≈ 68 days

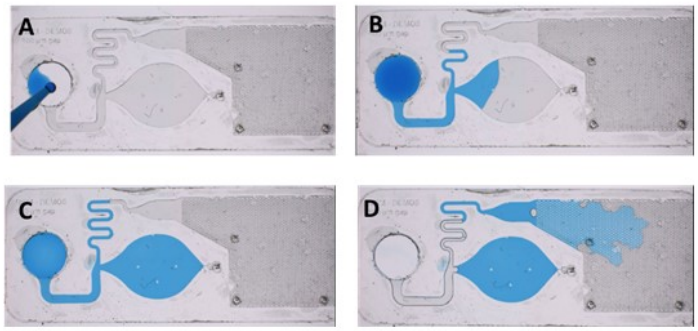


Fig. S2. Chip filling process: User add a drop of sample (A). The chip is self-filled ny capillary action (B). The sample reaches the reaction chamber (C). The hydrophilic pump remove liquid excess isolating the reaction chamber (D).

Table S1. Device specification

Device specifications		
Light source	405 nm	570 nm
Spectral Bandwidth at 50% (Δλ)	2.5 nm	20 nm
Photometric range	3.5 A	
W×D×H	6.4 × 7.5 × 3 cm	
Weight	135 g	
Battery life	200 h	
Charging Time	3 h (via USB, 1.3A charging port)	
Communication	Low energy BT	

Calculations for Amylase activity

The amylase activity is calculated as followed:

- Measure absorbance at 405 nm in a kinetic mode, every 1 min at RT protected from light.

- Amylase activity is calculated as:
  $\Delta Abs_{405nm} = A_2 - A_1$

  Where:
  A_1 is the sample at time T1
  A_2 is the sample at time T2

- Use the ΔAbs_{405nm} to obtain B, which means nmol of Nitrophenol generated by amylase during the reaction time ($\Delta T=T2-T1$). B is calculated by interpolating ΔAbs_{405nm} in the calibration curve (Fig. S3.). The calibration curve is measured by solving different pNP concentrations in the amylase buffer and measured with the portable reader in the reaction chamber of the chip.

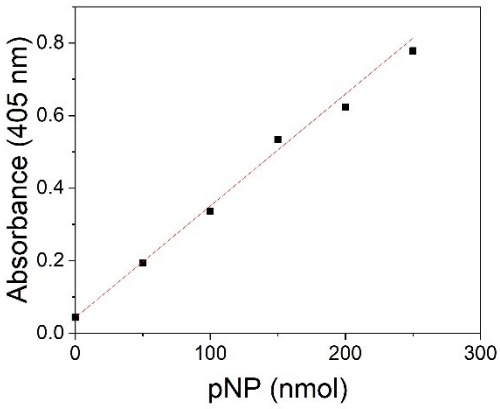


Fig. S3. pNP calibration curve

- Amylase activity =((B/(ΔTxV)xD)/m

Where:

B= nmol of Nitrophenol generated by amylase during the reaction time
ΔT= T2-T1
V = 0.012 mL (volume of the reaction chamber of the chip)
D=volume of extractant (distilled water)
m= mass of solid biodetergent =

Calculations cellulase activity

The cellulase activity is calculated as followed:

- Measure absorbance at 570 nm in a kinetic mode, every 1 min at RT protected from light.

- Cellulase activity is calculated as:
  $\Delta Abs_{570nm} = A_2 - A_1$

  Where:
  A_1 is the sample at time T1
  A_2 is the sample at time T2

- Use the ΔAbs_{570nm} to obtain B nmol of Resorufin generated by cellulase during the reaction time ($\Delta T=T2-T1$). B is calculated by interpolating ΔAbs_{570nm} in the calibration curve (Fig. S4). The calibration curve is measured by solving different resorufin

concentrations in the cellulase buffer and measured with the portable reader in the reaction chamber of the chip.

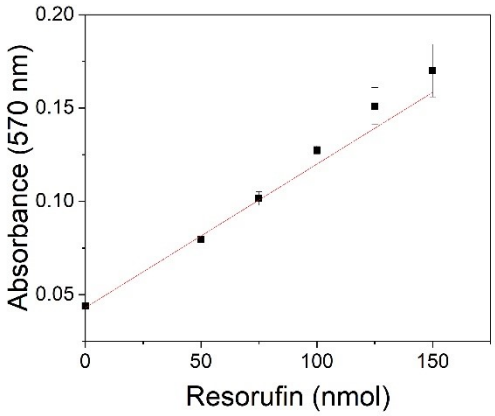


Fig. S4. Resorufin calibration curve

- Cellulase activity =((B/(ΔTxV)xD)/m

Where:
B= nmol
ΔT= T2-T1
V = 0.012 mL (volume of the reaction chamber of the chip)
D=volume of extractant (distilled water) = 15 mL
m= mass of solid biodetergent = 5 g

Capillary pump with irregular hexagonal geometry

One of the prototypes for the capillary pump design had a hexagonal geometry. In Fig. S5A. it is shown a simulation model of the filling of the pump. In Fig. S5B. it is showed a SEM image of the capillary pump with hexagonal geometry. The hexagons had equal sides in pairs with a length of 170, 630 and 720 µm. Some experiments were carried out to test the performance of the pump. In Fig. S5C. can be seen how the fluid flow stopped at the channel branching points. The pump had not enough strength to break the meniscus in the inlet and the reaction chamber could not be isolated. If the pump had worked well, the liquid would have filled the pump following the red line.

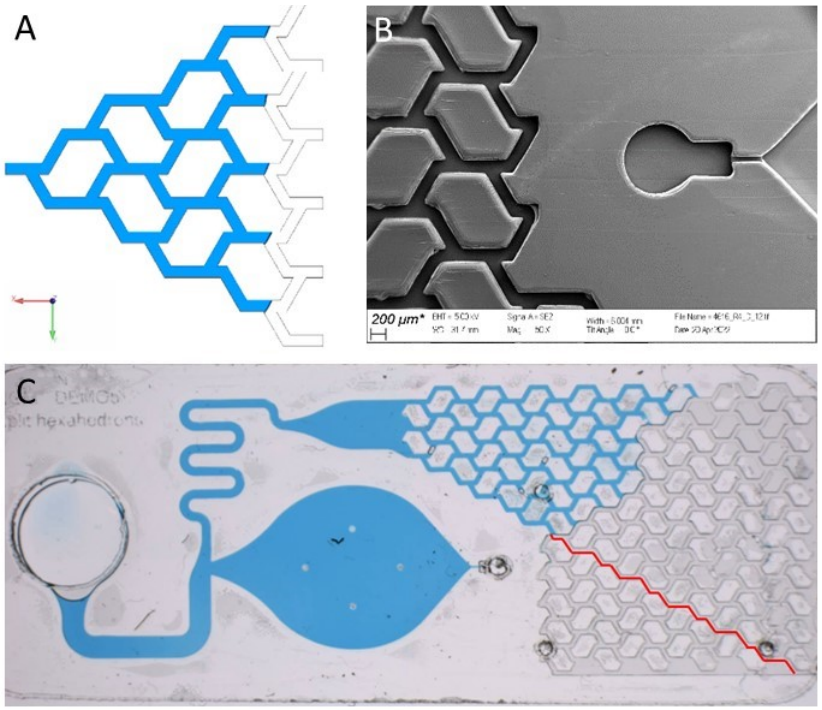


Fig. S5. 3D Simulation model (A) and SEM image (B) of the capillary pump with hexagonal structure. Chip with capillary pump with hexagonal structure filled (C).

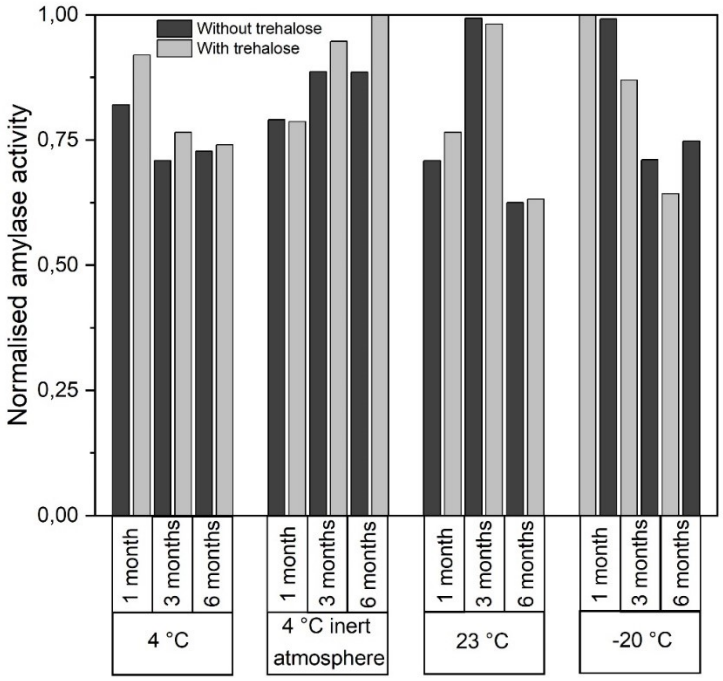


Fig. S6. Normalized α-amylase activity under various storage conditions: 4 °C, 4 °C under inert atmosphere, and 23 °C under accelerated conditions, as well as -20 °C in real-time storage, evaluated over 1, 3, and 6 months (1M, 3M, 6M), with and without the addition of trehalose when pNP-G7 is stored alone.

Table S2. Results of the chip self-filling with MOPSO buffer and different percentages of detergents (TWEEN-20 and TRITON X-100).

Buffer	Detergent	Chamber filling speed	Image 10 min after filling	Comments
MOPSO	0.1 % (v/v) TWEEN-20	30 s		The filling of the chip is too slow and sometimes the flow stops
MOPSO	0.2 % (v/v) TWEEN-20	12 s		The liquid flows outside the reaction chamber
MOPSO	0.3 % (v/v) TWEEN-20	10 s		The liquid flows outside the reaction chamber
MOPSO	0.1 % (v/v) TRITON-X100	10 s		Filling time adequate and the liquid do not flow outside the reaction chamber
MOPSO	0.2 % (v/v) TRITON-X100	4 s		The liquid flows outside the reaction chamber
MOPSO	0.3 % (v/v) TRITON-X100	2 s		The liquid flows outside the reaction chamber

Resorufin-β-D-cellobioside solubilization in LoC after drop-casting followed by freeze drying

For resorufin-β-D-cellobioside integration in the LoC, the initial efforts were focused on drop casting and freeze drying. The figure below shows the chip with the substrate lyophilized in the reaction chamber of the chip, and an image of the chip ten minutes after the introduction of the optimised cellulase self-filling buffer:

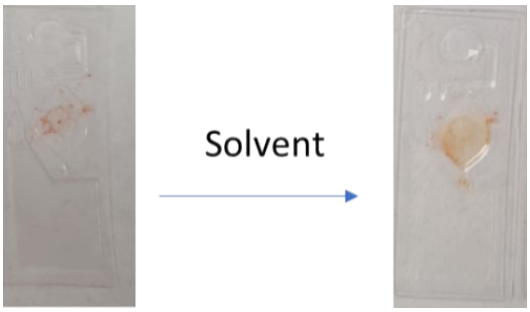


Fig. S7. Drop-casted and freeze-dried resorufin-β-D-cellobioside before (left) and after (right) adding a solution containing cellulase in the optimized reaction buffer

It can be observed that the lyophilized substrate is not homogeneous (left). The liquid in the reaction chamber is orange due to the partial solubilization of the substrate. However, it is not completely solved (right). In order to increase the solubility of the freeze-dried substrate a soluble carrier is added during lyophilization, see figure below:.

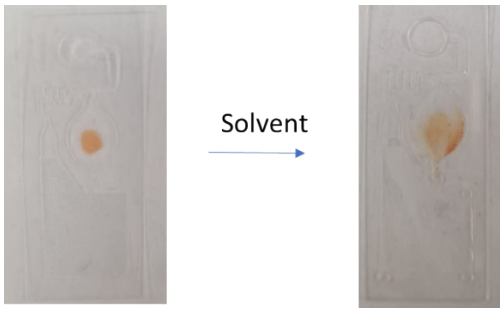
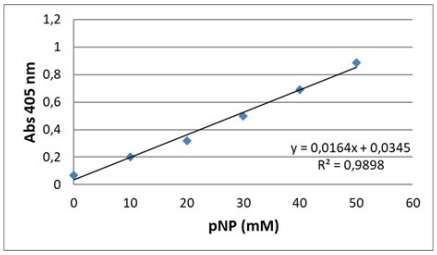


Fig. S8. Drop-casted and freeze-dried resorufin-β-D-cellobioside with a soluble carrier before (left) and after (right) adding a solution containing cellulase in the optimized reaction buffer

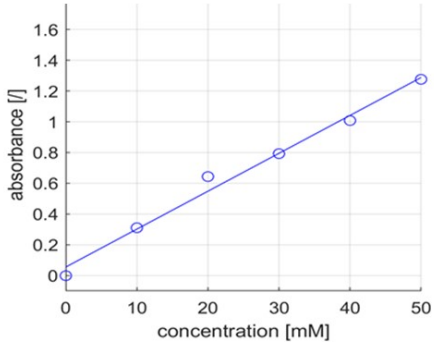
Even if the addition of the soluble carrier slightly improves the homogeneity of the lyophilized substrate (left) and the solubility of the substrate, complete dissolution of the substrate was not achieved (right). The solubility of the substrate in organic solvents hinders the solubilization after drop casting and freeze drying in aqueous solutions. Thus, spotting/air drying methodology was tested.

pNP standard curve measured on commercial microplate reader



LoC
R^2 = 0.9898
y = 0.0164x + 0.0345

pNP standard curve measured on Photogent reader



LoC
R^2 = 0.9874
y = 0.047x + 0.0550

Fig. S9. Calibration curve of pNP in PBS measured using the microfluidic chip with both a commercial microplate reader and the portable Photogent reader.

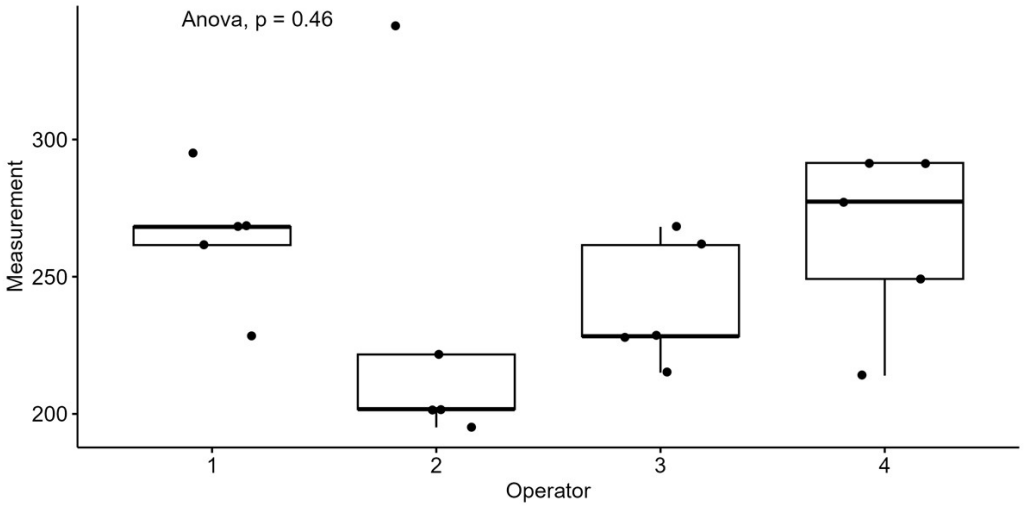


Fig. S10. Box plot showing enzymatic activity from five consecutive extractions performed by four operators. One-way ANOVA showed no significant differences among operators (p = 0.46), indicating reproducibility of the extraction protocol.

Cross-reactivity control experiments:

- **Control 1:** Extract rich in cellulase (S3. Cellulase) (40 µL) was incubated with pNP-G7 (45 µL, 100 mM) and monitored at 405 nm. No color change was expected because the substrate is specific for glucosidase, not cellulase.
- **Control 2:** Extract rich in cellulase (S3. Cellulase) (40 µL) was incubated with pNP-G7 (45 µL, 100 mM) in the presence of added glucosidase (30 µL, 10 mg/mL) and measured at 405 nm. Again, no color change was expected from the cellulase alone, confirming that the presence of cellulase does not interfere with the glucosidase reaction.
- **Control 3:** Extract rich in amylase was incubated (S3. Amylase) (40 µL) with pNP-G7 (45 µL, 100 mM) without added glucosidase (30 µL, 10 mg/mL) and monitored at 405 nm. No color change was expected since amylase does not act on this substrate.
- **Control 4:** Extract rich in amylase (S3. Amylase) (40 µL) was incubated with resorufin-β-D-cellobioside (5 µL, 10 mM) and measured at 570 nm. No color change was expected because the substrate is specific for cellulase, not amylase.

  These assays were carried out in a 96-well microplate, with a final assay volume of 100 µL. The remaining volume needed to reach 100 µL in each assay was adjusted with the optimized buffer for each enzymatic reaction.

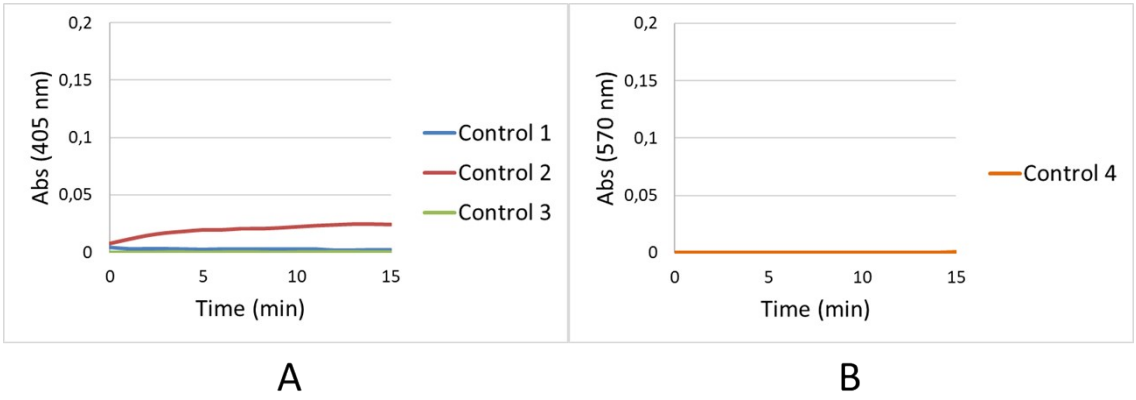


Fig. S11. Kinetic measurements of cross-reactivity control experiments. (A) Absorbance at 405 nm for the first three controls: cellulase extract with pNP-G7 (Control 1), cellulase extract with pNP-G7 and glucosidase (Control 2), and amylase extract with pNP-G7 (Control 3). (B) Absorbance at 570 nm for Control 4: amylase extract with resorufin-β-D-cellobioside.

No significant increase in absorbance was observed in any of the controls, except for a slight increase in control 2, which could be due to the fact that not all pNP-G7 chains contain seven glucose units (some may have fewer) leading to a very small increase in absorbance due to the presence of amylase. These results indicate there was no cross-reactivity between the enzymes and substrates.