Supplementary Information for

Responsive Capsules that Enable Hermetic Encapsulation of Contents and their Thermally Triggered Burst-Release

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Materials and Methods

Materials
Alkanes and fatty acids were purchased from Alfa Aesar; Sylgard 184 polydimethylsiloxane (PDMS) monomer and crosslinker from Ellsworth Adhesives; all other chemicals from Sigma; nylon mesh sieves from Component Supply Co.; and well plates from Greiner. A Formlabs Form2 printer was used for stereolithographic 3D printing with ≤ 0.05 mm layer height.

Fatty Acid Characterization
Fatty acids were characterized for their ability to act as phase-change partitions in a similar manner to the purified alkanes reported previously.15,16 To determine the optimal temperature for rapid layer melting, 50 µL of the respective fatty acid was layered above 50 µL of 3 µM tetramethylrhodamine (TAMRA) (Figure S1a and b). The pH sensitivity of fluorescein amidite (FAM) was leveraged: at high pH, fluorescein displays strong fluorescence but is almost completely quenched at low pH (Figure S1c and d). 50 µL of fatty acid was layered between 50 µL of a pH 8 fluorescein solution and 10 µL of a 1 M HCl solution. Increasing the temperature melted the fatty acid barrier, allowing the fluorescein solution to sink through and mix with the HCl layer, resulting in the expected fluorescence quenching. To demonstrate independent actuation of different barriers, tubes were filled with 50 µL octadecanoic (stearic) acid, then 10 µL brilliant blue dye, then 50 µL of the indicated fatty acid, then 50 µL thioflavin T dye (Figure S1e). All tubes were incubated for 5 min at the indicated temperatures, then allowed to cool to room temperature before being photographed.

Capsule Synthesis (Method I)
Wax-shelled capsule synthesis according to Method I is illustrated in Figure 2A. In the case of liquid cores, deionized (DI) water or an aqueous solution of 2% sodium alginate was used. In the case of gel cores, these were prepared by extruding a 2% sodium alginate solution into 0.5 M CaCl2 through a blunt 22G needle and incubating for 20 min. The Ca2+ ions crosslink the alginate chains, thus converting droplets into gelled beads (diameter 4-5 mm), which were then washed and used. Solutions or gel beads from the above step were cooled to 5°C and subsequently dropped into a reservoir of molten wax held at a temperature above its Tm (but within about 10°C). For example, in the case of paraffin wax (Tm ~ 57°C), the molten wax was heated to 65°C. A thin wax layer forms around the cold droplet/bead within seconds. After 5 – 20 s, the wax-shelled capsule was removed with tweezers. If the incubation time is longer or if the wax temperature was higher than Tm + 10, a stable shell does not form, as shown by Figure S2. For capsules with a shell of eicosane or other low-melting waxes, a second sealing step was needed to ensure...
a leak-proof shell. For this, the capsules were immersed for 10 – 30 s into octadecane at 34°C. To prepare wax-coated gels in specific shapes (Figure S7), a 2% solution of agar containing 5 mM of fluorescein was heated to 90°C, then cooled to room temperature to form a gel. The stiff agar gel was cut into specific shapes using a stencil and a blade. The gels were then cooled and dropped into hot paraffin wax to form the wax layer around them.

**Capsule Synthesis (Method II)**

Capsule synthesis by Method II is illustrated in Figure 2B. Capsules were designed to have a 10 µL hemispherical core with a 15 µL cylindrical headspace. Molds were made by 3D printing, either directly or via a master template. The master was printed with Formlabs High Temp Resin and coated in mold-release (Ease Release 200, Mann Release Technologies). An elastomer mold was made by filling the master with PDMS (prepared at a 10:1 ratio), curing in an 80°C oven for 30 min, removing the partially-cured mold, and replacing it in the oven for an additional 30 min. Molds were also directly 3D-printed in Formlabs Flexible Resin. A stamp, used to produce the hollow core of the phase-change cups during casting, was 3D-printed in High Temp Resin. Molds and stamps were coated with mold-release prior to each use. PDMS molds were cheaper and easier to produce in quantity than directly-printed molds, but slight (~1%) deformation during curing led to mis-alignment of the post array and produced many failed capsules with discontinuous walls. The directly-printed mold gave a higher yield, but imprecisions in the printing process still led to some failures.

Shell material was melted and held at 200°C, then poured into the cup-shaped molds. This high of a temperature was found to be necessary to delay solidification of the poured material to allow for positioning of mold components. The 3D-printed stamp consisting of an array of posts was then inserted into the molten material to form cup-shaped hollow cavities. The stamp was secured with heavy-duty binder clips. Air was allowed to circulate underneath the casting assembly during cooling. Once cooled, the mold was peeled away. The wax forms a shell of thickness 0.5 or 1 mm depending on the mold size used. Often, the resulting cups remained lightly trapped to stamp posts and were removed with a razor blade. After filling with the desired core material, the cups were capped with additional 200°C shell material. First, 15 µL was deposited and allowed to solidify, followed by an additional 5 µL. This was necessary because the high thermal gradients experienced by the small amount of wax often led to formation of a visible pore through the center of the solidified layer; the second shell addition adequately sealed this pore. The cap is ultimately much thicker than the molded cup walls, so the nominal shell thickness should be interpreted as the maximum thickness of the thinnest portion of the resulting capsule. For capsules with a shell of eicosane or other low-melting waxes, a second sealing step (immersion into octadecane at 34°C for 10 – 30 s) was implemented to achieve the final capsule.

**Capsule Encapsulation Tests**

For the tests shown in Figure 3, the following procedure was employed. 1 mM fluorescein in 90-10 glycerol-water was encapsulated in paraffin-wax-shelled capsules (diameter ~ 4 mm) using Method I. Each capsule was then placed into a separate vial containing 10 mL of deionized water. Over six weeks, the absorbances of the surrounding solutions in the vials were periodically measured by a UV-Vis spectrometer (Cary 50). The absorbance peak of fluorescein was at 490 nm. After 6 weeks, the capsules were melted at 65°C to release their contents into solution and the absorbances of the solutions were again measured.
For the tests shown in Figure S5, the following procedure was employed. Eicosane-shelled capsules (0.5 mm shell) were prepared using Method II, and these were filled with 10 µL 100 µM resorufin in 50 mM Tris-HCl, 320 mM NH₄OAc, pH 8.7. The capsules were then immersed in individual wells of a 96-well plate containing 200 µL de-ionized water (DI). At the indicated time points, 50 µL was removed from each well and placed in a black-walled half-area plate for fluorescent analysis in a Spectramax m5 plate reader. A reading ten-fold above the noise floor of the instrument (corresponding to ~0.1% leakage) was considered a failure. The solutions were then replaced with the capsules in their original wells. The test was continued for 5 days.

For the experiment in Figure S4A, the following procedure was employed. An alginate gel bead, prepared as described above, was immersed in 2 M NaOH for 30 min. This gel bead was cooled and encapsulated in a capsule with a paraffin-wax shell using Method I. The capsule was placed in a solution of 10 mM phenolphthalein in 70-30 water-ethanol at room temperature. After confirming that there was no leakage of base under these conditions, the capsule was heated to 65°C to melt the wax shell, and this process was recorded as a movie. The images shown in the figure are stills from this movie.

For the experiment in Figure S4B, the following procedure was employed. An alginate gel bead, prepared as described above, was immersed in glacial acetic acid for 30 min. This gel bead was cooled and encapsulated in a capsule with a paraffin-wax shell using Method I. The capsule was placed in a solution of 10 mM methyl red in ethanol at room temperature. After confirming that there was no leakage of acid under these conditions, the capsule was heated to 65°C to melt the wax shell, and this process was recorded as a movie. The images shown in the figure are stills from this movie.

For the experiment in Figure S4C, the following procedure was employed. An alginate gel bead, prepared as described above, was cooled and encapsulated in a capsule with a paraffin-wax shell using Method I. The capsule was placed in a solution of 10 mM methylene blue in water at room temperature for 3 days. Thereafter, the capsule was extracted from the vial using tweezers, rinsed with water, and then examined.

**Controlled Release of H₂O₂**

Sodium percarbonate was sieved to obtain granules approximately 1-2 mm in diameter, placed in eicosane-shelled capsules (0.5 mm shell, two per capsule), and capped. 1-2 mm alginate beads were prepared by extrusion of a 1.5% sodium alginate solution into 1 M CaCl₂ through a blunt 25G needle. Beads were allowed to crosslink for 1 h, vacuum-drained, immersed in 30% H₂O₂ for 1 h, vacuum-drained again, placed in eicosane-shelled capsules (0.5 mm shell, two per capsule), and capped. Capsules with no core material were also prepared. Capsules were placed in 200 µL PCR tubes with 100 µL 10 µM DCF-DA, then analyzed with a BioRad MiniOpticon thermocycler for 30 min at 25°C followed by 30 min at 45°C.

**Blue-Violet Test for Nitrates**

Test reagent was prepared by mixing 6 mg diphenylamine into 1.2 mL 3 M H₂SO₄, then adding 300 µL 18 M H₂SO₄ (this two-step process is necessary: the heat evolved during the addition of pure sulfuric acid allows the diphenylamine to fully dissolve). The traditional test is performed by mixing sample solution, test reagent, and pure sulfuric acid sequentially at a 3:3:10 ratio, producing a deep blue color in the presence of nitrate (improper order slows color development). Eicosane-shelled capsules with 0.5 mm shells containing 10 µL test reagent or 18 M H₂SO₄. 10 µL sample solution was placed in a 0.6 mL centrifuge tube with one test reagent capsule and three pure sulfuric acid capsules then immersed in near-boiling water.
Supplementary Figures

Figure S1. Characterization of fatty acids as phase-change partitions. A) Melting behaviour was characterized by placing a layer of the respective fatty acid on top of a fluorescent solution. B) The rate of change in observed fluorescence is indicative of the rate of melting at the given temperature. Fatty acids displayed well-defined melting points separated by several degrees Celsius. C) The pH sensitivity of fluorescein amidite (FAM) allowed quantification of the “breakthrough” point for each fatty acid. D) The fatty acids displayed well-defined breakthrough curves as the FAM solution mixed with the HCl solution, quenching the fluorescence. E) Stepwise increases in temperature melted (a) decanoic (“capric”) acid, (b) dodecanoic (“lauric”) acid, (c) tetradecanoic (“myristic”) acid, and (d) hexadecanoic (“palmitic”) acid sequentially, allowing solutions of yellow and blue dye to mix in a discrete, predictable manner. All tubes pictured were brought to the indicated temperature then allowed to cool to room temperature before being photographed.
Figure S2. Considerations during synthesis of wax-shelled capsules by Method I. The importance of wax temperature and time of incubation in the molten wax are shown by the images. The molten (liquid) wax must be above its $T_m$, but not much higher. If the liquid is too hot ($T >> T_m$) or if the droplet/gel is incubated in the liquid for too long (> 30 s), the wax shell formed initially will melt and disintegrate.
Figure S3. Capsules with different wax shells showing responsive hermetic behavior. Capsules are created with aqueous cores having solutions of different food dyes and with different wax shells (each with a defined melting temperature $T_m$), as indicated. In all cases, the dye is hermetically sealed in the capsule core at room temperature and there is no leakage into the surrounding water (top images). Heating above $T_m$ melts the shell and releases the dyes into water (bottom images). The image for the eicosane-shelled capsule is the same as in Figure 1.
**Figure S4. Wax-shelled capsules showing hermetic encapsulation and burst solute release.** In all cases, the shell is made of paraffin wax. (A) Hermetic encapsulation of strong base (NaOH) at room temperature and its burst release at 65°C. (B) Hermetic encapsulation of strong acid (glacial acetic acid) at room temperature and its burst release at 65°C. (C) External solute (methylene blue dye) is unable to penetrate through the wax shell at room temperature. After 3 days of incubation, the capsule when removed from solution still looks clear.

### A. Encapsulation and release of strong base

1. Paraffin-wax-shelled capsule with NaOH in gel core, at room temp in phenolphthalein (pH) solution.
2. Wax shell melts at 65°C, releasing NaOH into the solution. Streaks of pink as base contacts pH solution.
3. NaOH continues to diffuse into solution, and pH solution also diffuses into the gel core. Both turn pink.

### B. Encapsulation and release of strong acid

1. Paraffin-wax-shelled capsule with acetic acid in gel core, at room temp in methyl red solution (yellow color at pH 7).
2. Wax shell melts at 65°C, releasing acetic acid into the solution. Streaks of orange/red as acid contacts methyl red.
3. Acid continues to diffuse into solution, and the indicator also diffuses into the gel core. Both turn a deep red.

### C. Impermeability of shell to external solutes

1. Paraffin-wax-shelled capsule with gel core, at room temp in 10 mM methylene blue (MB) solution.
2. When removed after 3 days, dye does not adhere to wax shell.
3. Capsule is colorless after rinsing. This shows that the dye has not penetrated through the shell.
Figure S5. Eicosane-shelled capsules need to be ‘sealed’ to protect their cargo against leakage. Eicosane capsules are loaded with the fluorescent solute, resorufin and then sealed by briefly dipping into octadecane. Sealed capsules exhibit no leakage over five days, whereas one unsealed capsule failed in this time.

Figure S6. Negative results in nitrate assay for control sample. This is a control experiment to accompany Figure 4b in the main paper. The capsule-based assay is shown to yield no color change in the absence of nitrates in the test solution (bottom of the tube).
**Figure S7. Wax-coated gels in specific shapes.** In the top panel, agar gels in specific shapes (star, cube, doughnut) are shown on the left, and their counterparts with a layer of paraffin wax (formed by Method I) are shown on the right. As expected, the uncoated gels release their internal solute (fluorescein) rapidly (within an hour) when placed in water (bottom panel, vial on the left). However, no solute is released from the wax-coated gels placed in water (vial on the right).