Supporting information for

Lipase is essential for the study of *in vitro* release kinetics from organogels

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Contents

1. Synthesis of organogelators

1.1 Synthesis of behenoyl L-tyrosine methyl ester (BTyrOMe)

Freshly distilled triethylamine (8.5 mL, 2.2 eq.) was added under nitrogen atmosphere to a chloroform solution (140 mL) of L-tyrosine methyl ester hydrochloride (7.1 g, 1.1 eq.). The mixture was cooled to 4 ºC in an ice bath and stirred for 15 min. Then, behenoyl chloride (10 g, 1 eq.) was added drop-wise as a solution in 20 mL chloroform under nitrogen atmosphere. The mixture was heated to 45 ºC and stirred overnight. The solution was diluted with chloroform to 300 mL and successively washed with 150 mL of water, 10% HCl, aqueous saturated $NaHCO_3$, and brine. The organic phase was warmed to 60 ºC between extractions to avoid precipitation of the final product. The organic phase (warm) was dried over $MgSO_4$ and the product precipitated from the chloroform solution by cooling to 4 ºC to yield a white powder. Recovered yield: 92%. ^{1}H NMR δ (ppm): 6.71–9.95 (4 H, m, C_6H_4), 6.17 (1 H, s, C_6H_4OH), 5.92 (1 H, d, NH), 4.87 (1 H, q, CH_2CH(NH)CO), 3.73 (3 H, s, $COOCH_3$), 2.96–3.11 (2 H, m, $C_6H_4CH_2CH$), 2.17 (2 H, t, $COCH_2CH_2$), 1.58 (2 H, m, $CH_2CH_2(CH_2)_{18}$), 1.25 (36 H, m, $CH_2(CH_2)_{18}CH_3$), 0.88 (3 H, t, $(CH_2)_{18}CH_3$).

1.2 Synthesis of stearoyl L-tyrosine methyl ester (STyrOMe)

Freshly distilled triethylamine (5.1 mL, 2.2 eq.) was added under nitrogen atmosphere to a chloroform solution (140 mL) of L-tyrosine methyl ester hydrochloride (4.2 g, 1.1 eq.). The mixture was cooled to 4 ºC in an ice bath and stirred for 15 min. Then, stearoyl chloride (5.0 g, 1 eq.) was added drop-wise as a solution in 20 mL chloroform under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred overnight. The solution was then diluted with dichloromethane and washed with 10% HCl. The organic phase was dried over $MgSO_4$ and the solvent evaporated. The product was purified by silica gel chromatography (ethyl acetate/n-hexane 1/1 *vol*/*vol*) and re-crystallized from ethyl acetate/n-hexane (4/1 *vol*/*vol*) to yield white crystals. Recovered yield: 86%. ^{1}H NMR δ (ppm): 6.71–9.95 (4 H, m, C_6H_4), 6.26 (1 H, s, C_6H_4OH), 5.92 (1 H, d, NH), 4.88 (1 H, q, CH_2CH(NH)CO), 3.73 (3 H, s, $COOCH_3$), 2.95–3.11 (2 H, m, $C_6H_4CH_2CH$), 2.17 (2 H, t, $COCH_2CH_2$), 1.58 (2 H, m, $CH_2CH_2(CH_2)_{14}$), 1.25 (28 H, m, $CH_2(CH_2)_{14}CH_3$), 0.88 (3 H, t, $(CH_2)_{14}CH_3$).

1.3 Synthesis of lauroyl L-tyrosine methyl ester (LTyrOMe)

Freshly distilled triethylamine (6.0 mL, 2 eq.) was added under nitrogen atmosphere to a chloroform solution (140 mL) of L-tyrosine methyl ester hydrochloride (5.0 g, 1.1 eq.). The mixture was cooled to 4 ºC in an ice bath and stirred for 15 min. Then, lauroyl chloride (4.3 g, 1 eq.) was added drop-wise as a solution in 20 mL chloroform under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred overnight. The solution was then diluted with dichloromethane and washed with 10% HCl. The organic phase was dried

over $MgSO_4$ and the solvent evaporated. The product was further purified by silica gel chromatography (ethyl acetate/n-hexane 4/6 *vol*/*vol* supplemented with 1 vol % NMP) and re-crystallized from ethyl acetate/n-hexane (4/1 *vol*/*vol*) to yield white crystals. Recovered yield: 81%. ^{1}H NMR δ (ppm): 6.71–9.95 (4 H, m, C_6H_4), 6.29 (1 H, s, C_6H_4OH), 5.93 (1 H, d, N*H*), 4.88 (1 H, q, CH_2CH(NH)CO), 3.73 (3 H, s, $COOCH_3$), 2.95–3.11 (2 H, m, $C_6H_4CH_2CH$), 2.17 (2 H, t, $COCH_2CH_2$), 1.58 (2 H, m, $CH_2CH_2(CH_2)_8$), 1.25 (28 H, m, $CH_2(CH_2)_8CH_3$), 0.88 (3 H, t, $(CH_2)_8CH_3$).

1.4 Synthesis of stearoyl L-alanine methyl ester (SAlaOMe)

Freshly distilled triethylamine (7.0 mL, 2.2 eq.) was added under nitrogen atmosphere to a chloroform solution (140 mL) of L-alanine methyl ester hydrochloride (3.5 g, 1.1 eq.). The mixture was cooled to 4 ºC in an ice bath and stirred for 15 min. Then, stearoyl chloride (6.9 g, 1 eq.) was added drop-wise as a solution in 20 mL chloroform under nitrogen atmosphere. The mixture was allowed to reach room temperature and stirred overnight. The solution was then diluted with dichloromethane and washed with 10% HCl. The organic phase was dried over $MgSO_4$ and the solvent evaporated. The product was purified by silica gel chromatography (ethyl acetate/n-hexane 4/6 *vol*/*vol* supplemented with 1 vol % NMP) and re-crystallized from ethyl acetate/n-hexane (4/1 *vol*/*vol*) to yield white crystals. Recovered yield: 81%. ^{1}H NMR δ (ppm): 5.98 (1 H, d, N*H*), 4.61 (1 H, m, CH_3CH(NH)CO), 3.75 (3 H, s, $COOCH_3$), 2.20 (2 H, t, $COCH_2CH_2$), 1.62 (2 H, m, $CH_2CH_2(CH_2)_{14}$), 1.39 (3 H, d, CH_3CH), 1.25 (28 H, m, $CH_2(CH_2)_{14}CH_3$), 0.87 (3 H, t, $(CH_2)_{14}CH_3$).

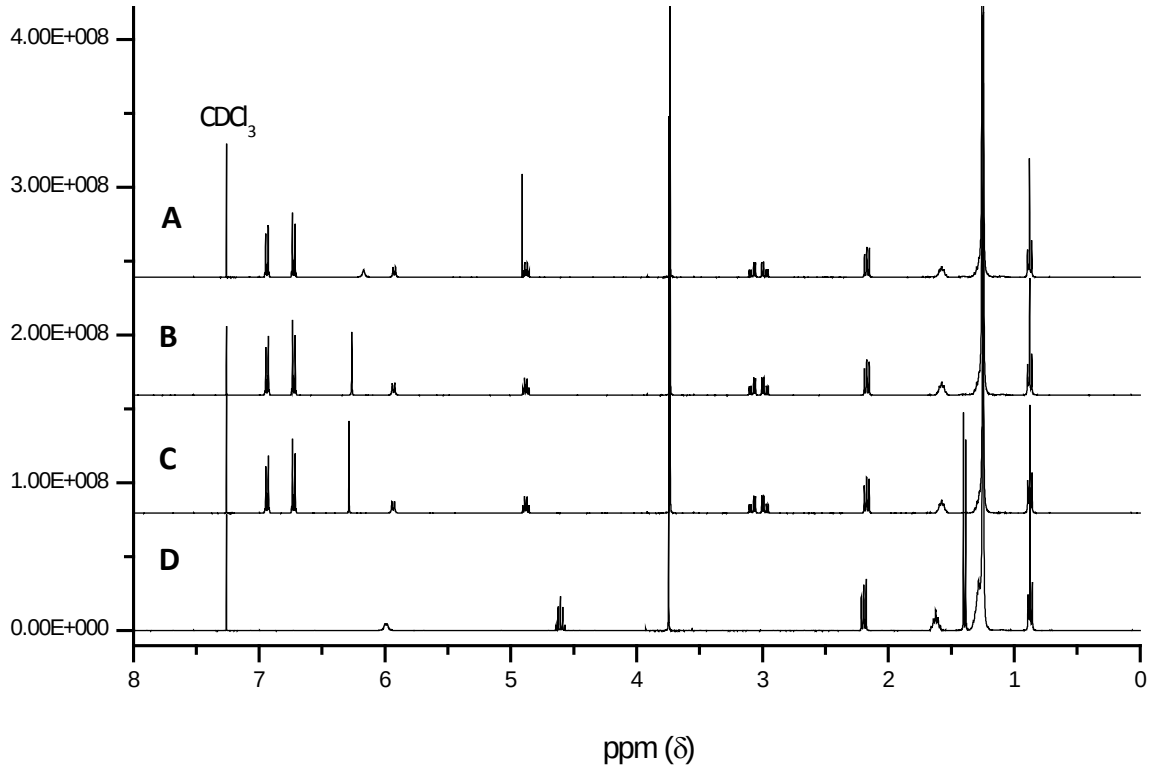

Figure S1. ^{1}H NMR spectra of (A) BTyrOMe, (B) STyrOMe, (C) LTyrOMe, and (D) SAlaOMe. The spectra were recorded in deuterated chloroform.

2. Custom device for measuring drug release

A custom drug release device was designed to overcome problems associated with analysis in dialysis bags or in glass flasks. The device, shown in Figure S2, is composed of a metal mesh basket that plunges to a fixed depth in a sealed 50 mL receptacle. This ensures that all surfaces of the gel are in contact with the release medium. In addition, the system is hermetically sealed to minimize evaporation of the release medium. Sampling of the latter is achieved from an opening at top of the bottle and is done without disturbing the gel. The volume of the release medium was 50 mL, which was sufficient to ensure sink conditions for 400 µL organogels loaded with as much as 15 wt % ceftiofur·Na.

Figure S2. Drug release system designed in-house. The gel is loaded in the basket and the basket immersed in the release medium. A hole at the center of the beige cap (hole not seen on figure) makes sampling possible using a syringe fitted with a needle.

3. Macroscopic observation of gel disintegration as a function of lipase activity

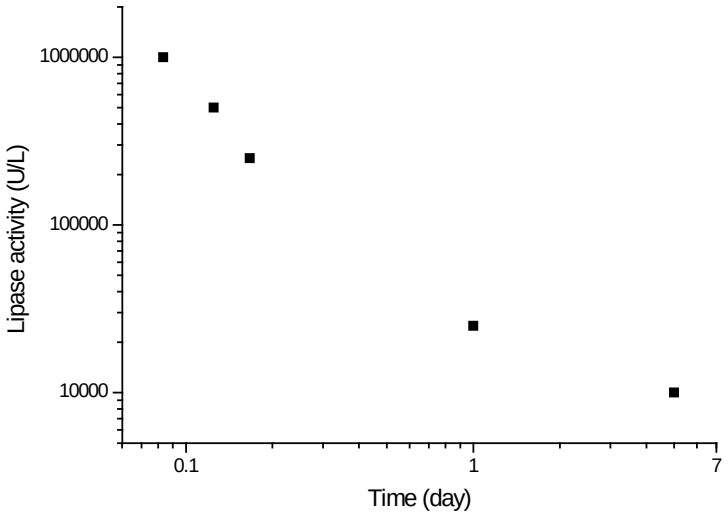

Figure S3. Time for complete disintegration of 400 µL BTyrOMe organogels. The organogels were incubated at 37 °C in PBS pH 7.4 in presence of increasing concentrations of lipase. The organogels were composed of 0.01 mmol BTyrOMe and 0.03 mmol NMP per gram of safflower oil.

4. Stability of ceftiofur in the organogels

4.1 Stability of ceftiofur in "dry" organogels

As a representative example, BTyrOMe gels (0.1 mmol.g^{-1}) were loaded with either 10 or 15 wt % ceftiofur·Na and gels were at 4 ºC. Aliquots were sampled after 3 months and the drug extracted from the gels using the method described in the main manuscript (Section 2.7). The relative amount of intact ceftiofur·Na was determined by HPLC as described in the main manuscript (Section 2.9). The results presented in Figure S4 show that the drug remained >98% intact during this period.

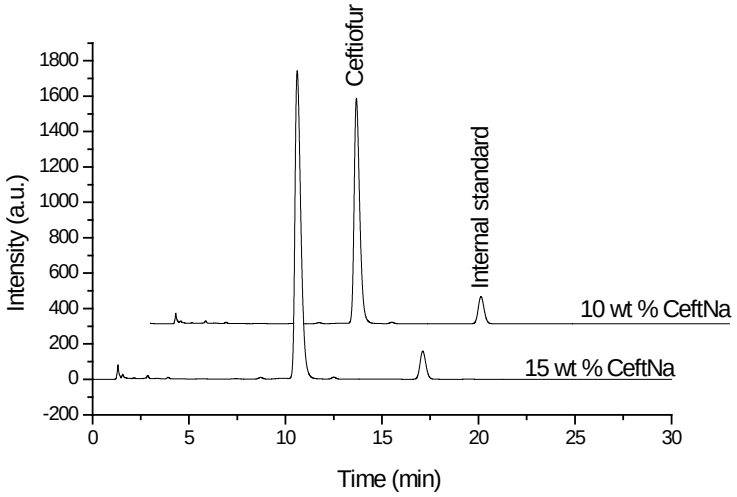

Figure S4. Chromatograms of ceftiofur·Na loaded in BTyrOMe gels and stored for 3 months at 4 ºC. Gels were loaded with either 10 or 15 wt % drug.

4.2 Stability of ceftiofur loaded in organogels after immersion in release buffer

Four hundred µL of drug-loaded organogel (10 wt % ceftiofur·Na, 0.1 mmol.g^{-1} BTyrOMe) were deposited at the bottom of a meshed metal basket and the basket immersed into 50 mL of PBS pH 7.4. The system was agitated at 100 rpm using an orbital agitator and maintained at 37 ºC in an incubator. After 48 h, the basket was removed from the release medium, patted dried, and immersed into 4 mL of NMP. One mL of this solution was mixed with 9 mL PBS and the drug extracted as described in the main manuscript. The integrity of the drug was evaluated by liquid chromatography using a mass spectrometry detector (LC/MS). As shown in Figure S5, only one peak, corresponding to intact ceftiofur, was detected, indicating that the drug remained stable in the immersed gel.

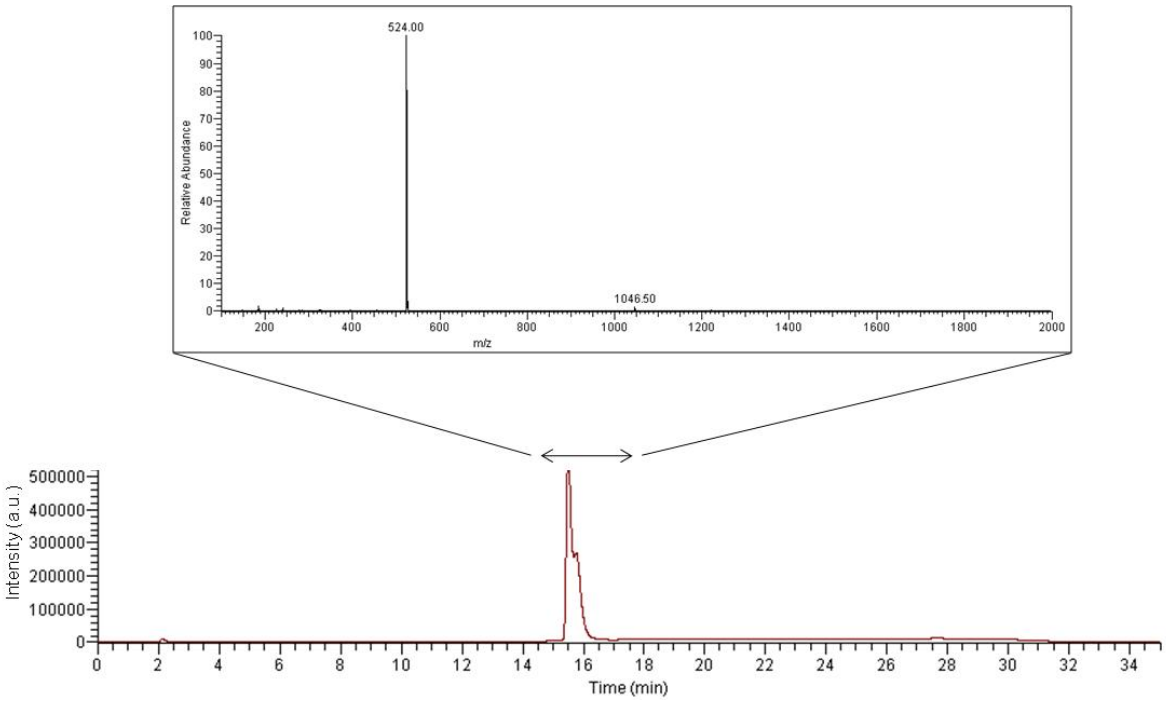

Figure S5. LC/MS analysis of ceftiofur after immersion of a drug-loaded organogel in PBS for 48 h. The bottom part of the figure shows the chromatogram of the extracted ceftiofur·Na while the inset shows the combined mass spectrometry data of the region spanning 14–17.5 min.

5. Comparison of drug release profiles – f_2 values

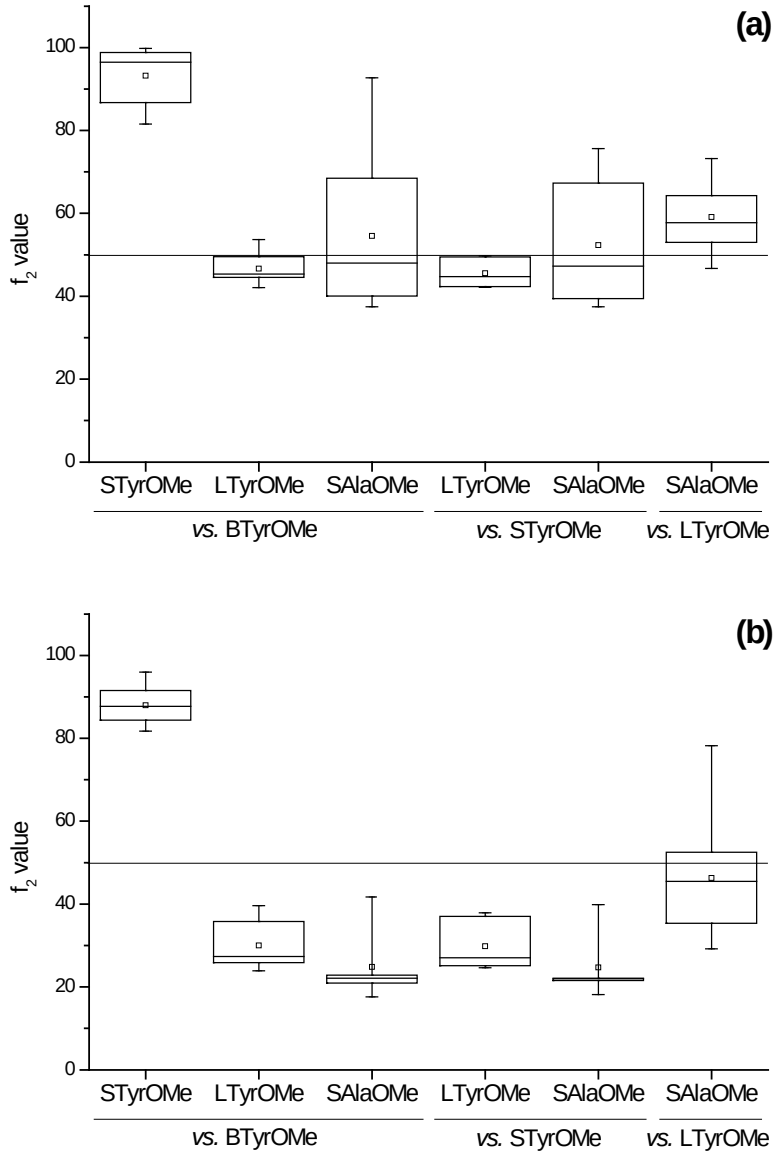

Figure S6. Distribution of the f_2 values calculated by comparing the individual replicates of the drug release curves shown in Figure 3 of the main text. The release studies were carried out in PBS (a) and in PBS containing lipase (b) at 37 °C. The organogels were composed of 0.1 mmol.g^{-1} organogelator, 0.3 mmol.g^{-1} NMP, and 10 wt % ceftiofur·Na in safflower oil.

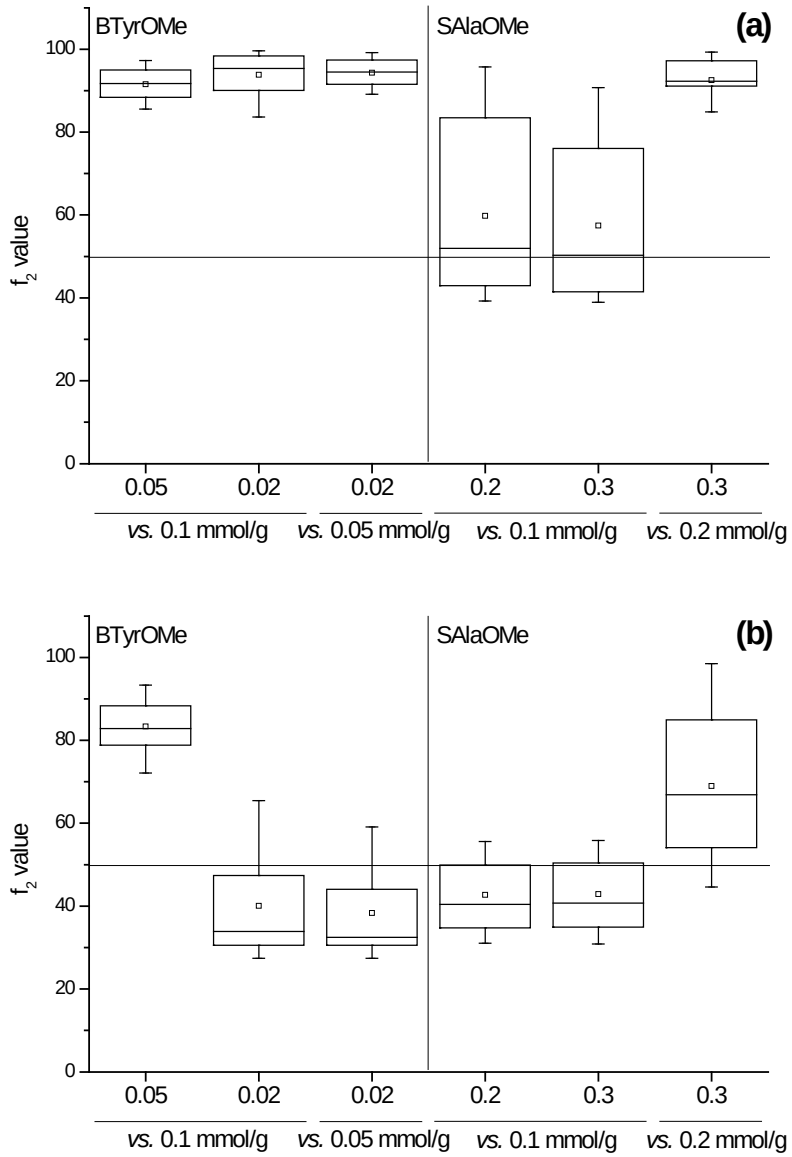

Figure S7. Distribution of the f_2 values calculated by comparing the individual replicates of the drug release curves shown in Figure 4 of the main text. The release studies were carried out in PBS (a) and in PBS containing lipase (b) at 37 °C. The organogels were composed of increasing concentrations of either BTyrOMe or SAlaOMe, 3 molar equivalents of NMP, and 10 wt % ceftiofur·Na in safflower oil.

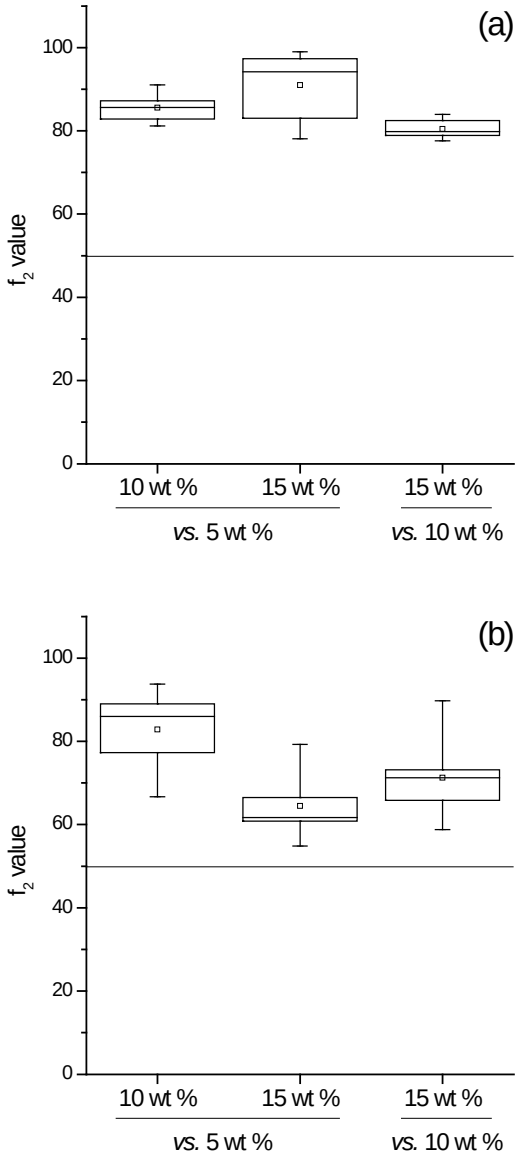

Figure S8. Distribution of the f_2 values calculated by comparing the individual replicates of the drug release curves shown in Figure 5 of the main text. The release studies were carried out in PBS (a) and in PBS containing lipase (b) at 37 °C. The organogels were composed of 0.1 mmol.g^{-1} BTyrOMe, 0.3 mmol.g^{-1} NMP, and increasing amounts of ceftiofur·Na in safflower oil.

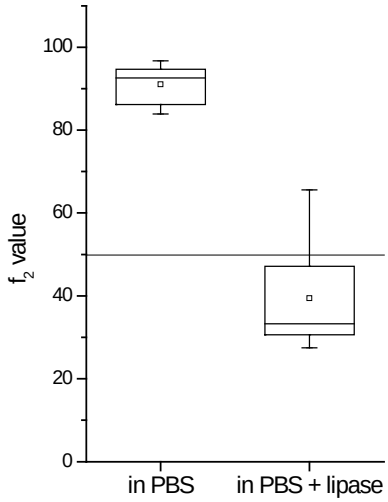

Figure S9. Distribution of the f_2 values calculated by comparing the individual replicates of the drug release curves obtained with organogels of different volume (400 µL versus 800 µL gels – curves shown in Figure 6 of the main text). The release studies were carried out in PBS and in PBS containing lipase at 37 °C. The organogels were composed of 0.02 mmol.g^{-1} BTyrOMe, 0.06 mmol.g^{-1} NMP, and either 5 or 10 wt % ceftiofur·Na for 800 and 400 µL gels, respectively, in safflower oil.

6. Mechanical properties of organogels

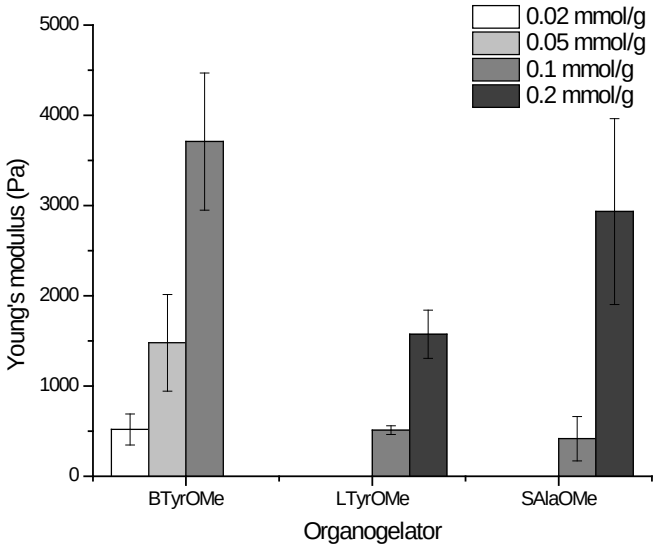

Figure S10. Effect of organogelator concentration on the Young's modulus of organogels. The organogels were composed of increasing concentrations of either BTyrOMe, LTyrOMe or SAlaOMe, 3 molar equivalents of NMP, and 10 wt % ceftiofur·Na in safflower oil. The NMP was allowed to diffuse out of the gels for 24 h at 37 °C prior to mechanical testing. Mean ± SD (n = 2–6). Organogelator concentration led to a statistically significant change of mechanical properties within samples prepared with a given organogelator ($p < 0.05$).

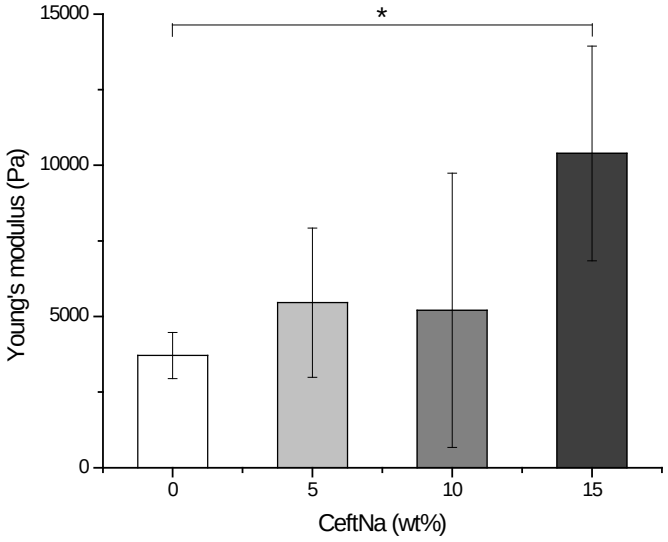

Figure S11. Effect of ceftiofur·Na loading on the Young's modulus of organogels. The organogels were composed of 0.1 mmol.g^{-1} BTyrOMe, 0.3 mmol.g^{-1} NMP, and increasing amounts of ceftiofur·Na in safflower oil. The NMP was allowed to diffuse out of the gels for 24 h at 37 °C prior to mechanical testing. Mean ± SD (n = 2–6). *$p < 0.05$

7. Correlation between release and mechanical properties of the gels

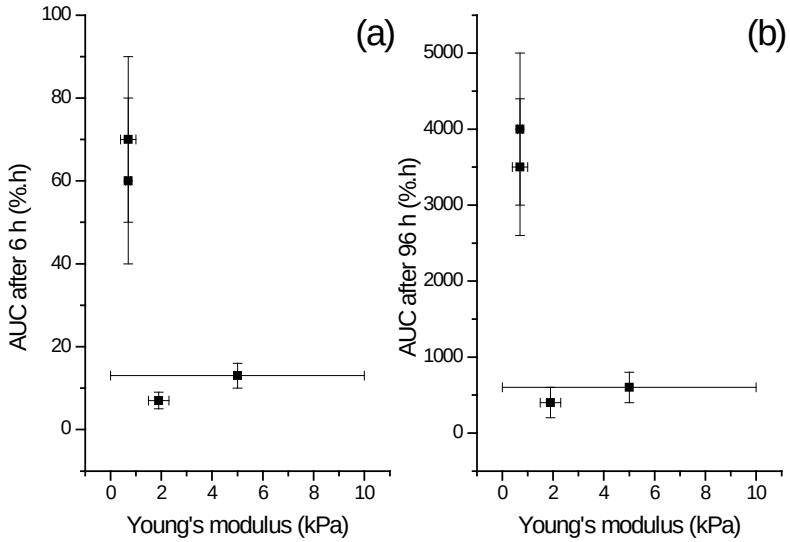

Figure S12. Correlation between mechanical properties and release of ceftiofur (*i.e.* AUC) at 6 (a) and 96 h (b). The organogels were composed of 0.1 mmol.g^{-1} organogelator (BTyrOMe, STyrOMe, LTyrOMe, SAlaOMe), 0.3 mmol.g^{-1} NMP, and 10 wt % ceftiofur·Na. No obvious trend is visible.