Supplementary Information for Drug Catalyzed Polymerization Yields One Pot Nanomedicines

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Supplementary Discussion

The Nature of Chlorhexidine conjugation to PLLA:

Regarding the conjugation of PLLA to chlorhexidine, questions remain regarding the nature of the modification. The loss of an equivalent of water (18 mass units) led us to hypothesize that intramolecular cyclization occurs via substitution of a terminal -OH with another -OH or guanidine =NH, particularly since only a loss of 18 is observed regardless of the degree of polymerization, rather than multiple units of water. The size of the resulting macrocycle would contain 11 + r6 members, where r is the number of L-lactide monomer units in the macrocycle. This large ring size would be free of enthalpic ring strain. However, it remains experimentally difficult to prove cyclization due to the presence of the bisguanidine backbone making a large segment of the cycle. NMR paired with both 1D and 2D proton and carbon studies could deduce if cyclization really occurs, but due to the low abundance of ^{15}N, synthesis of a ^{15}N doped chlorhexidine would be necessary, which is beyond the scope of this study. However, it should be noted that cyclization can occur with PLLA when N-heterocyclic carbene catalysts are used, although in this case, the carbenes merely facilitate cyclization by participating as an intermediate species in cyclization.[1,2]

Drug Solubility and Drug Loading:

Chlorhexidine and trimethoprim (both in the free base form) are not soluble in toluene. Upon addition of mPEG and L-lactide, the solubility of chlorhexidine and trimethoprim increased. However, in the case of trimethoprim, full solubility could not be reached without adding a small amount (~5%) of DMSO. For these reasons, we expect that the polymer assists in the drug loading of both chlorhexidine and trimethoprim. Chlorhexidine is conjugated to PLLA achieving 100% conjugation efficiency (Figure S8). On the other hand, trimethoprim is not conjugated to PLLA. Encapsulation efficiency studies were not conducted in the native solution as the peaks from toluene and the polymer would interfere with those associated with trimethoprim in the UV/Vis range. Rather, the encapsulation efficiency of trimethoprim in the polymer was conducted after resuspending the freeze-dried mixture in water. The encapsulation efficiency for **6-T** was experimentally determined to be about 82% (average of four runs) by UV/Vis.

Supplementary Tables:

Table S1: Acylation control experiments: Run 1 is the condition discussed in the main text.

Run	Vinyl acetate to CHX Ratio	Conditions	Conversion
1	20:1	2.4 mL DCM 0.3 mL DMSO	10%
2	20:1	2.7 mL DCM 0.05 m2PEG	8.3%
3	10:1	2.7 mL DCM 0.05 m2PEG + 35 °C	20%
4	10:1	2.7 mL DCM	<1%

Table S2: Synthetic conditions for chlorhexidine based ROPI-CDSA

Sample ID	Solids w/w %	L-lactide (mg)	mPEG (mg)	Chlorhex. (mg)	Toluene (mL)
1-C	10	259	80	45.5	4.00
2-C	10	389	80	68.2	5.58
3-C	20	195	80	34.1	1.61
4-C	20	259	80	45.5	1.78
5-C	20	389	80	68.2	2.48

Table S3: Synthetic conditions for chlorhexidine based ROPI-CDSA

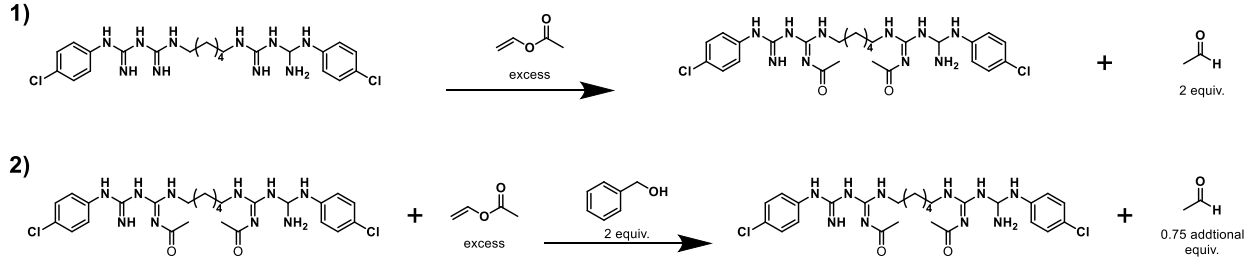
Sample ID	Solids w/w %	L-lactide (mg)	mPEG (mg)	Trimethop. (mg)	Toluene (mL)	DMSO (mL)
6-T	20	259	160	13.2	2	0.1
7-T	20	389	120	19.5	2.43	0.15

Table S4: All MIC studies including an extracted sample.

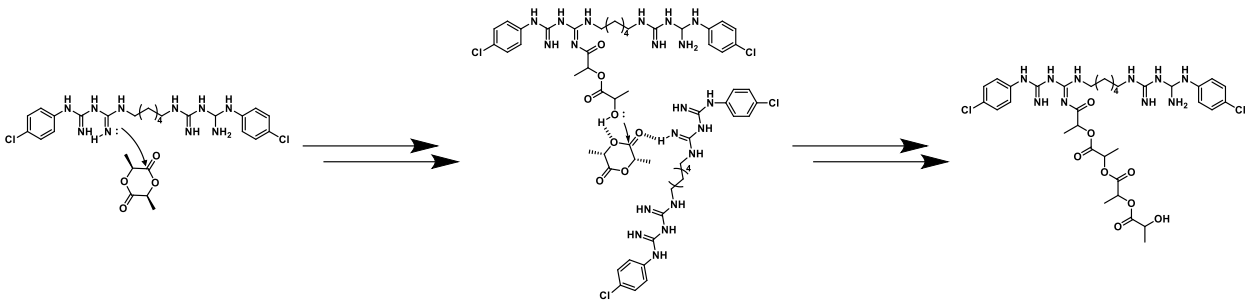
Bacteria	Free[1] CHX	Free CHX t[2]	**4-C** RL	**4-C** EX	Free TMP	**6-T** RL
B. subtilis	0.25	0.25	2	2	0.25	0.5
S. epi.	0.125	0.25	1	2	0.5	1
E. coli	0.25	0.25	2	2	0.25	1

1. Free Drug is the drug in its freebase form
2. Sample contains trace toluene identical to 4-D EX
3. RL= resuspension following lyophilization, EX=extracted and diluted from toluene into water

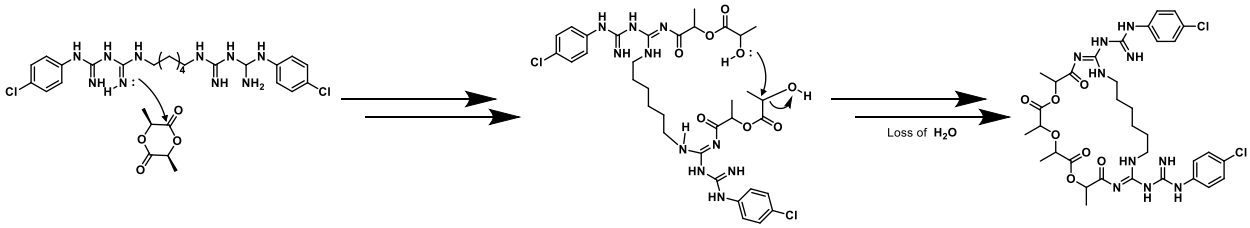
Supplementary Schemes:



Scheme S1: 1) Reaction of excess vinyl acetate with chlorhexidine leads to two equivalents of acylation as noted by the production of two equivalents of aldehyde. 2) The acylation is partially reversible: when 2 equivalents of benzyl alcohol are added, some trans-acylation occurs as noted by the production of an additional 0.75 equivalents of aldehyde.



Scheme S2: Proposed mechanism for the synthesis of chlorhexidine-PLLA. In the first step, chlorhexidine acts as an initiator by ring-opening L-lactide through acylation. The second step shows the propagation of L-lactide off the active -OH end. This polymerization can undergo transesterication or a termination event as shown in Scheme S3.



Scheme S3: The proposed mechanism for intramolecular cyclization which results in the loss of one equivalent of water (18 mass units). Following the initiation in step 1, the -OH end can attack the other growing chain end on the carbon alpha to the alcohol resulting in an irreversible intramolecular cyclization.

Supplementary Figures:

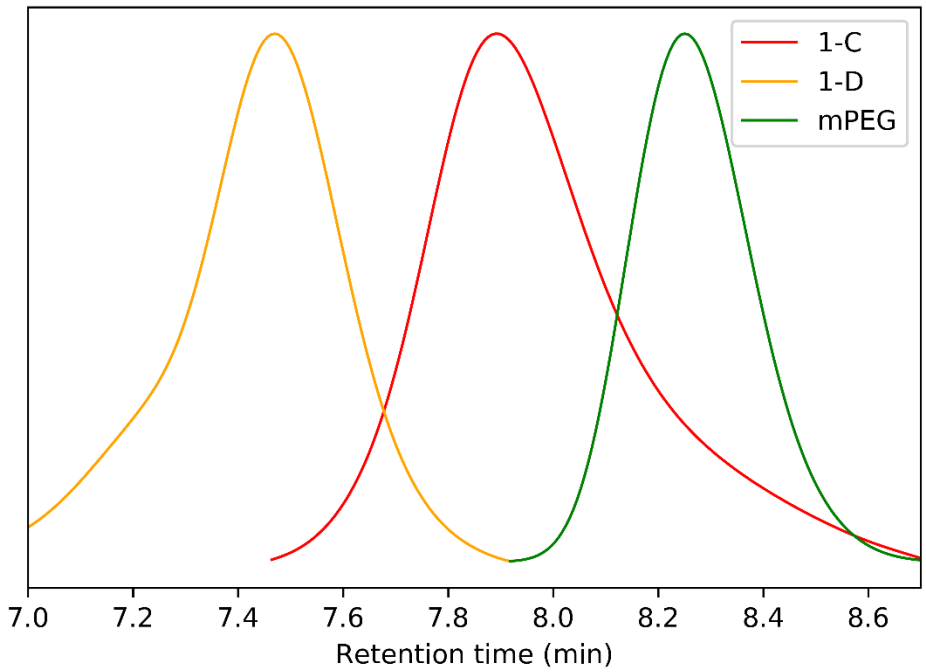


Figure S1: GPC trace showing that **1-C** has a longer retention time than that of **1-D**, a sample identical to **1-C** in setup except that chlorhexidine is swapped for DBU.

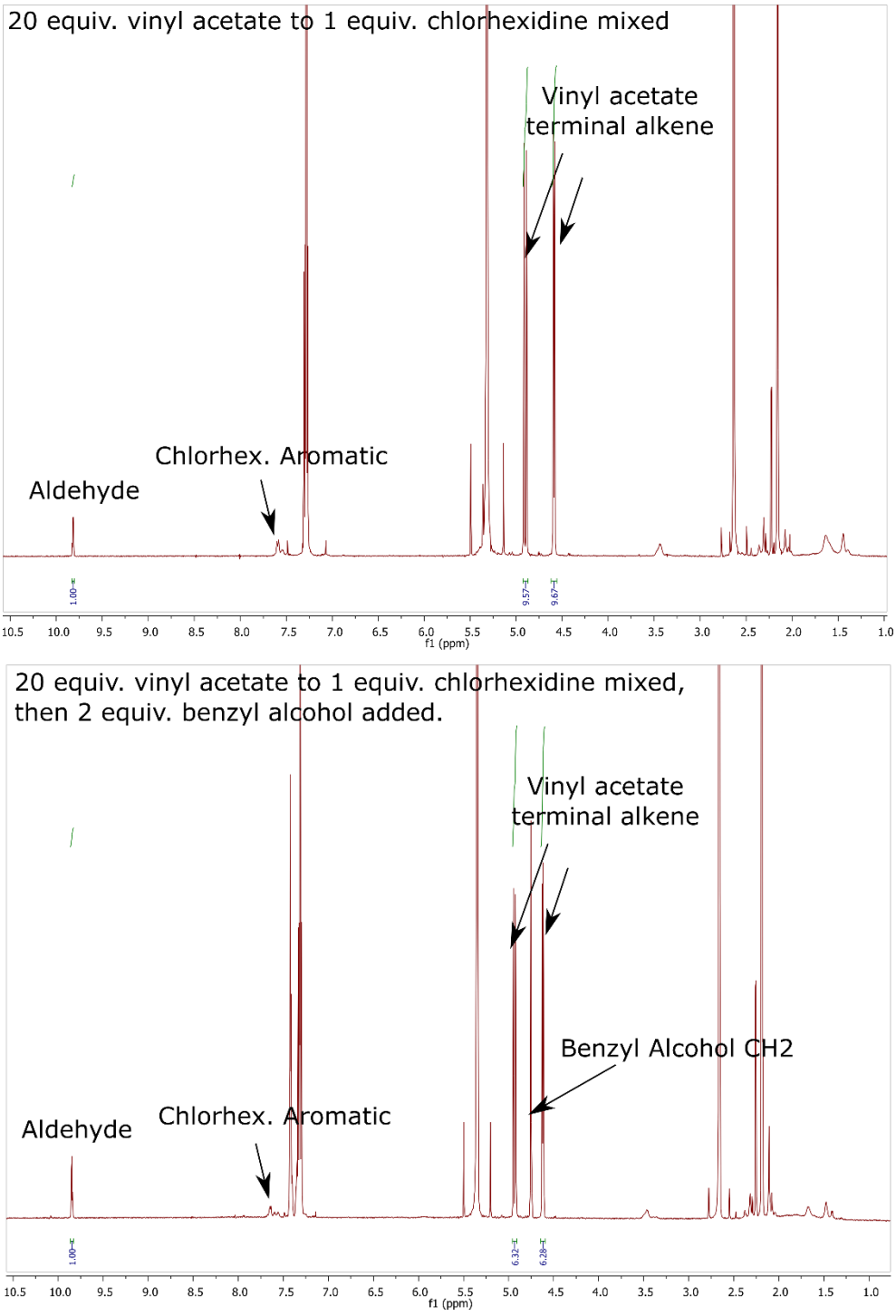


Figure S2: Acylation ^{1}H NMR

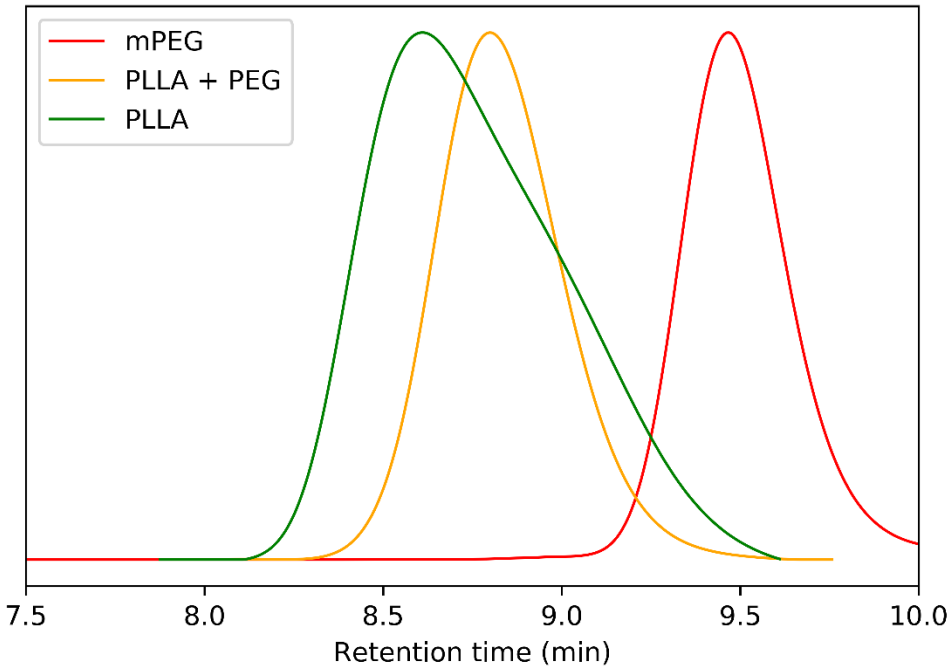


Figure S3: Homopolymer GPC results

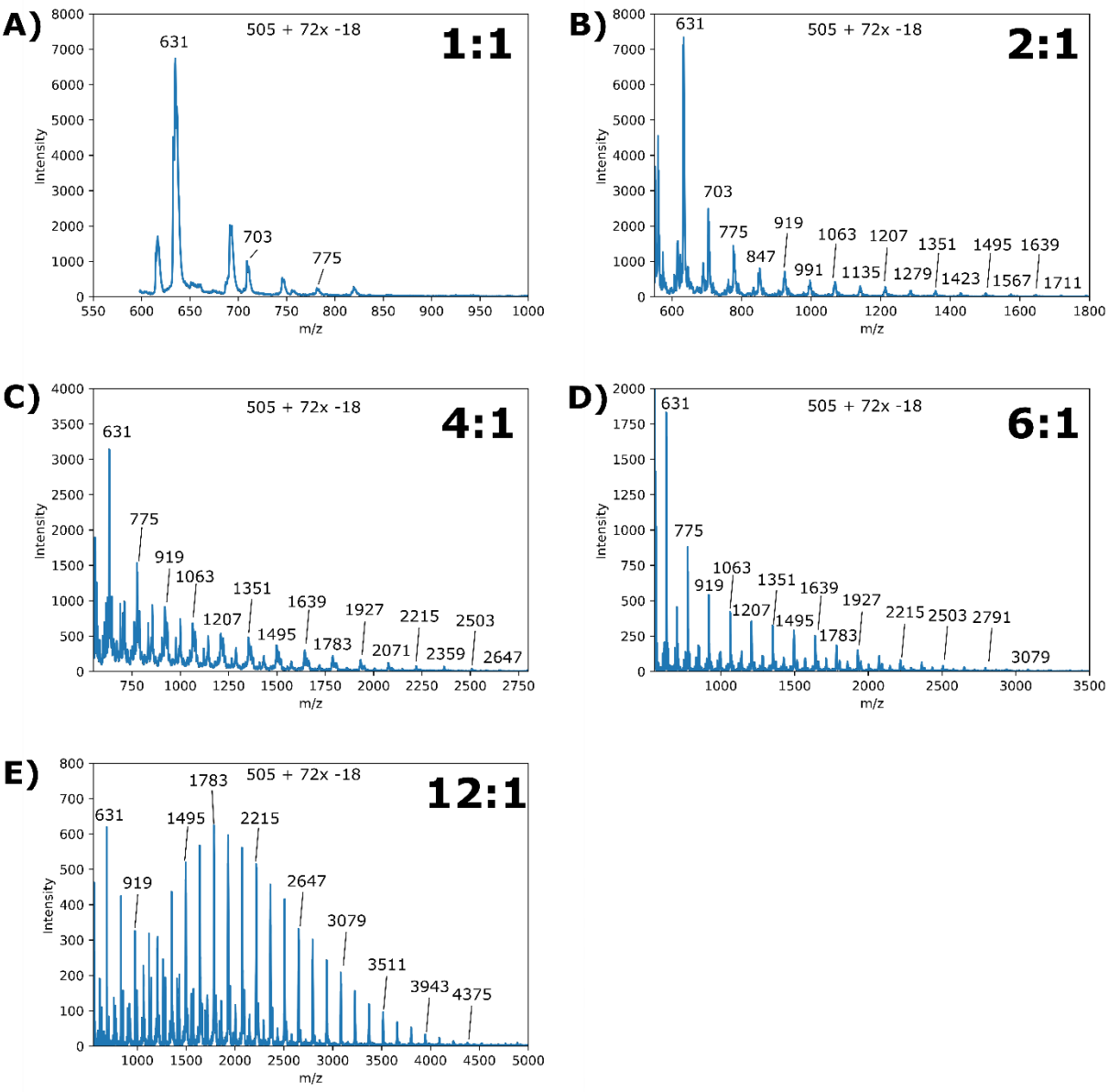


Figure S4: MALDI spectra of chlorhexidine initiated PLLA homopolymers. The ratio at the top right of each spectrum represents the ratio of L-lactide to chlorhexidine in each reaction. Peaks in the spectra are approximately 144 or 72 Da (mass unit) apart. The 72 Da is from transesterification. The lowest mass polymer peaks appear at 631 or 559 m/z corresponding to a loss of water (approximately 18 mass units) as chlorhexidine is approximately 505 Da.

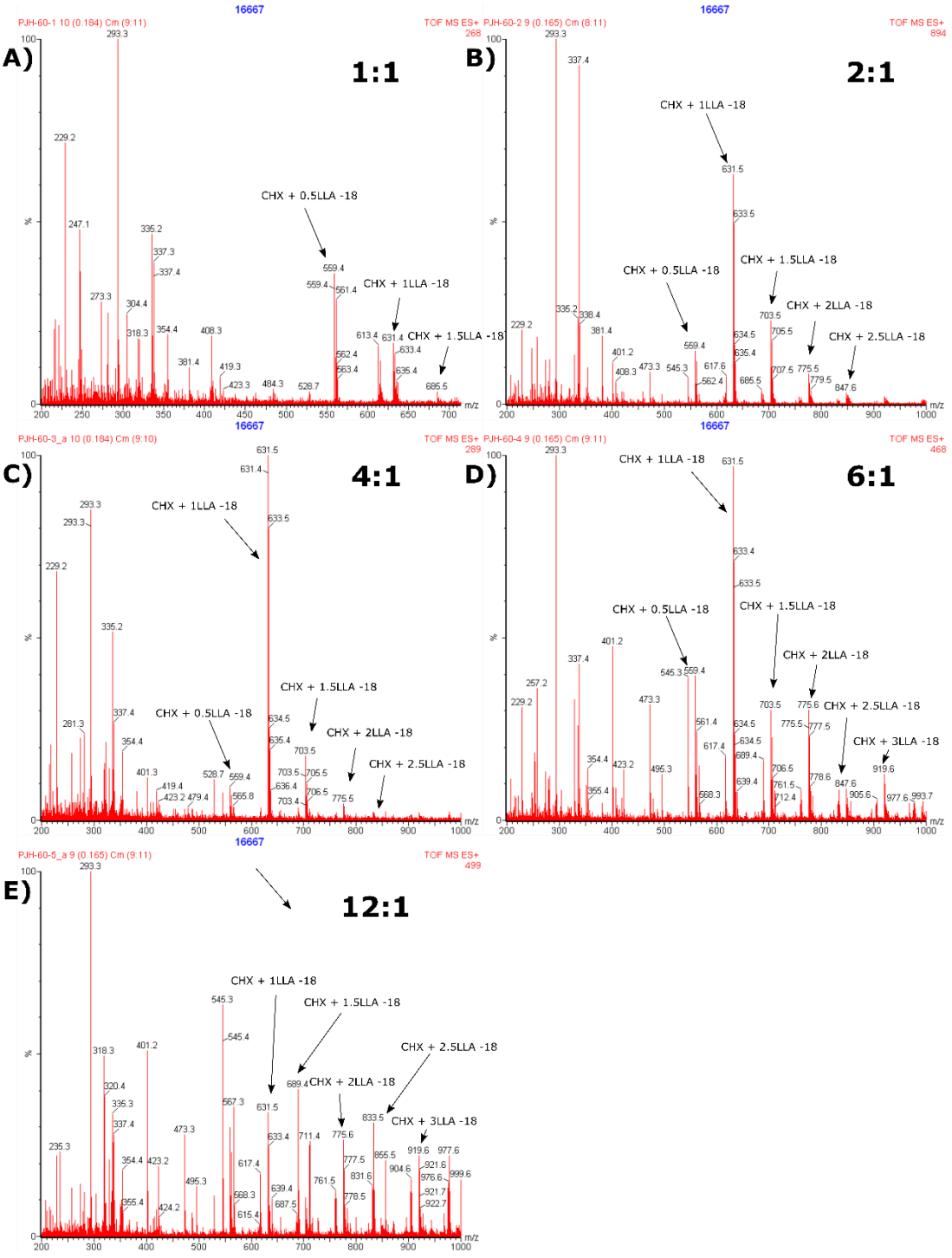


Figure S5: ESI spectra of chlorhexidine initiated PLLA homopolymers. The ratio at the top right of each spectrum represents the ratio of L-lactide to chlorhexidine in each reaction. Peaks in the spectra are approximately 144 or 72 Da apart. The 72 Da is from transesterification. The lowest mass polymer peaks are 631 or 559 m/z corresponding to a loss of water (approx.. 18 mass units) as chlorhexidine is approximately 505 Da.

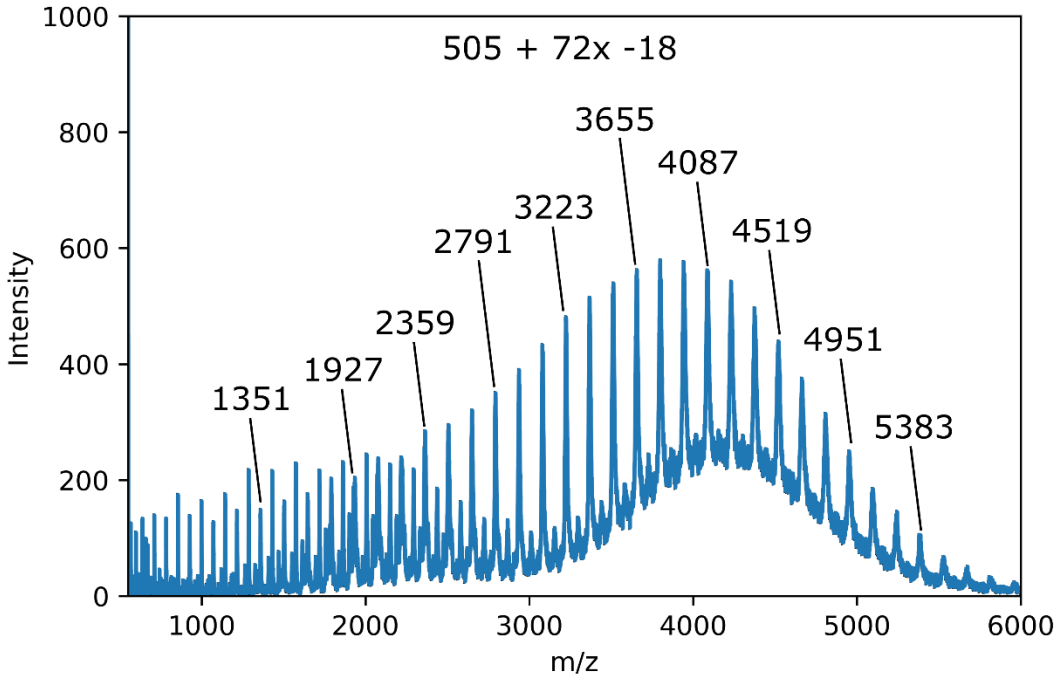


Figure S6: Representative MALDI spectrum for drug catalyzed ROPI-CDSA (**4-C**). This MALDI spectrum shows the chlorhexidine initiated PLLA homopolymer. PLLA-b-PEG was not detectable with our protocol as a MALDI run of a pure block copolymer yielded a blank signal.

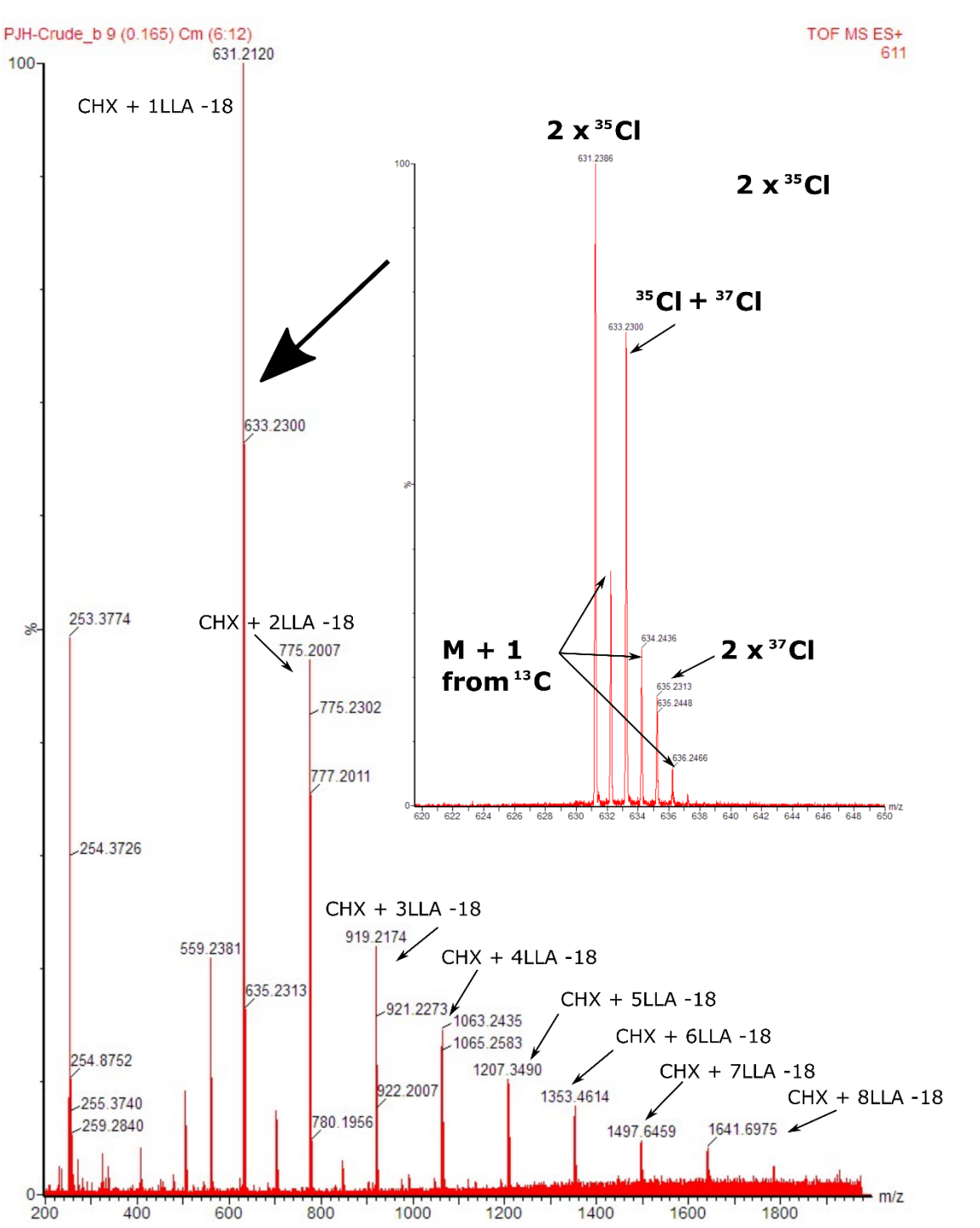


Figure S7: Representative ESI spectrum for drug catalyzed ROPI-CDSA (**4-C**). This ESI spectrum shows the chlorhexidine initiated PLLA homopolymer. PLLA-b-PEG was not detectable with our protocol as an ESI run of a pure block copolymer yielded a blank signal. An inset in the top right corner shows zoomed in section of 631 Da. The splitting pattern is consistent with an organic molecule that has 2 chlorine atoms.

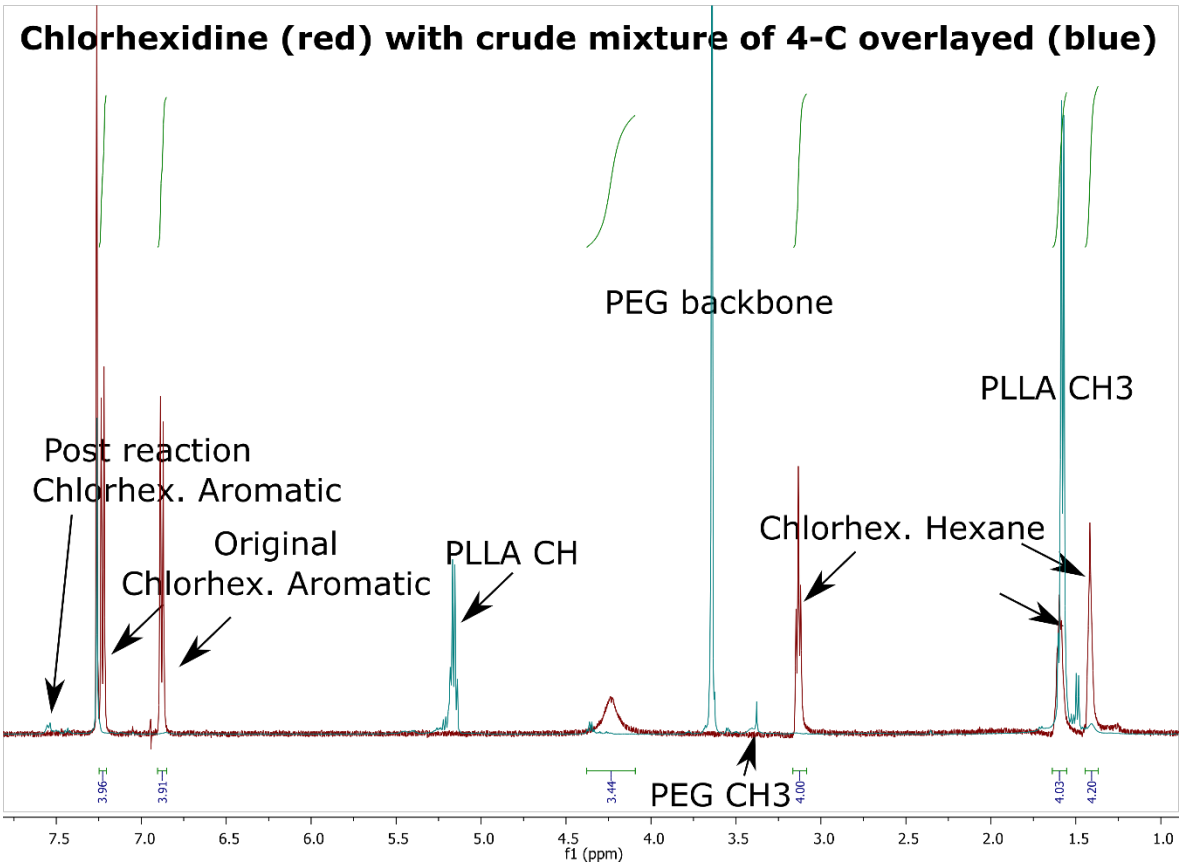


Figure S8: Chlorhexidine overlayed ^{1}H NMR for a crude mixture of **4-C**. Note that all chlorhexidine peaks disappear from the crude mixture indicating chemical modification of chlorhexidine.

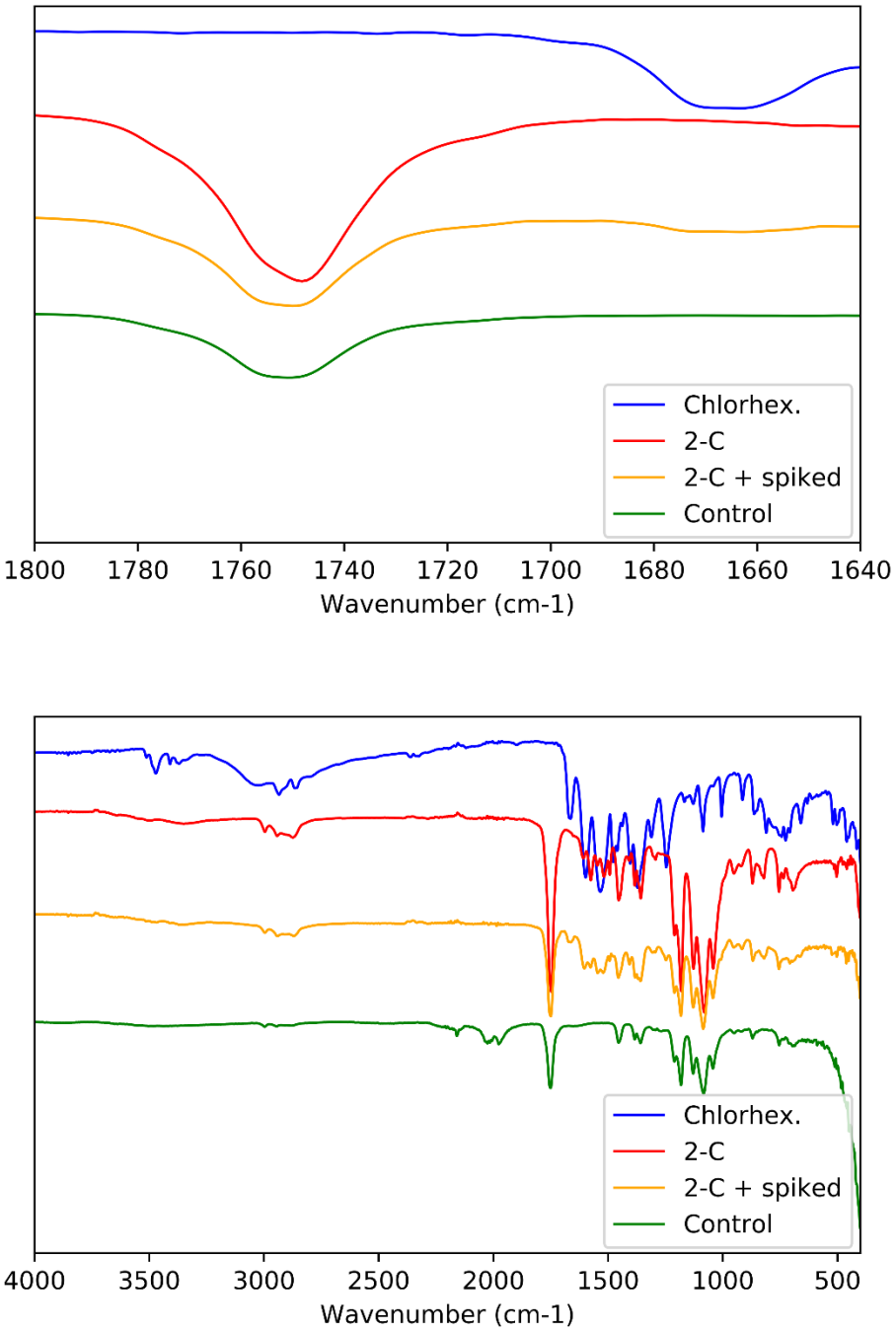


Figure S9: FTIR controls. FTIR spectra of chlorhexidine (blue), chlorhexidine-catalyzed polymer **2-C**, **2-C** spiked with free chlorhexidine (yellow), and a control PLLA-*b*-PEG purified polymer. **2-C** with spiking contains chlorhexidine peaks (e.g. peak around 1600 cm^{-1}). The lack of chlorhexidine peaks in **2-C** supports our conclusion that chlorhexidine was incorporated into the structure.

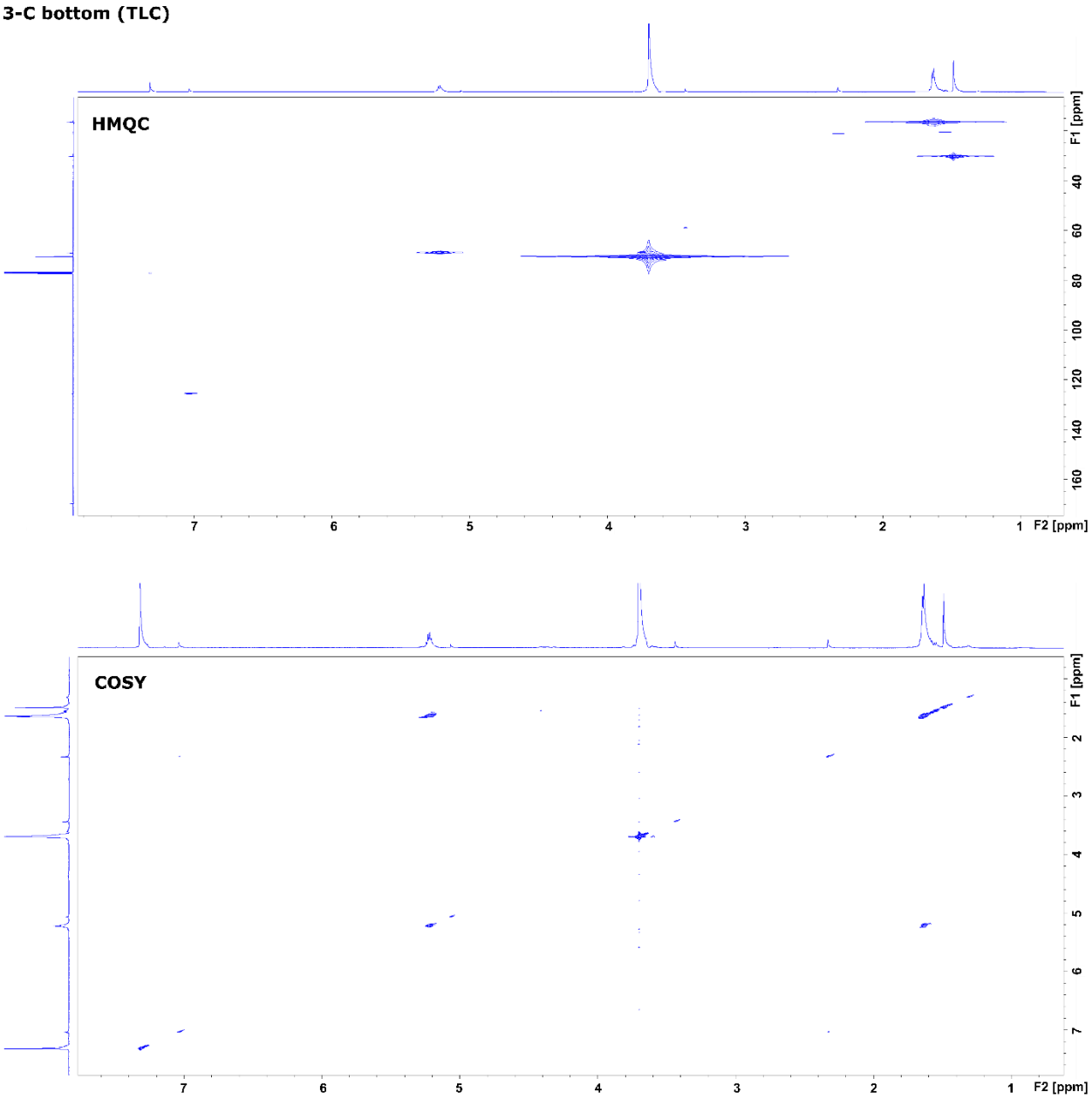


Figure S10: 2-D NMR of **3-C** bottom TLC spot. The top shows HMQC and the bottom shows COSY. Note the mPEG backbone peak around 3.5 ppm. Note the absence of aromatic peaks around 7.5 ppm.

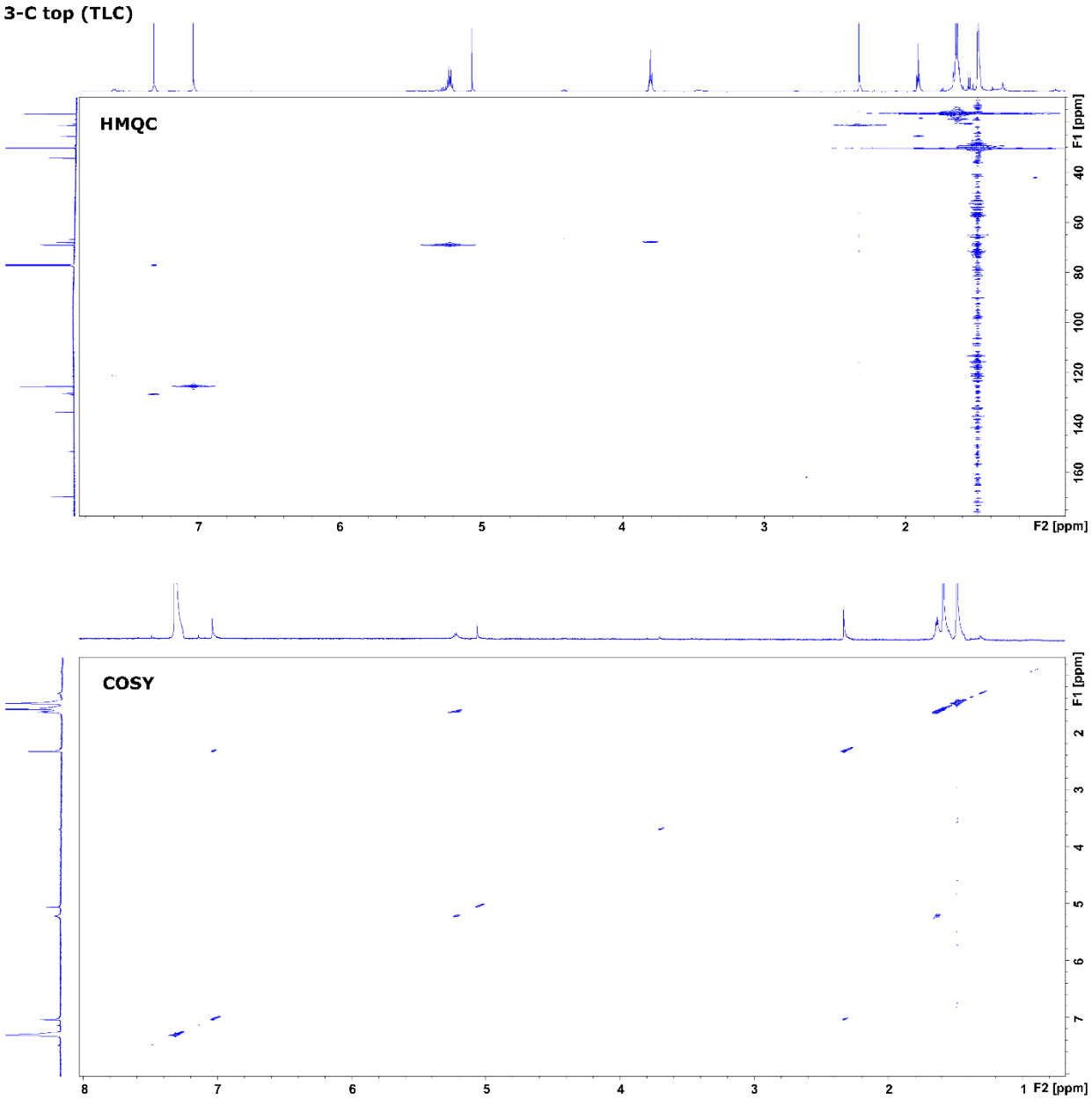


Figure S11: 2-D NMR **3-C** top TLC spot. The top shows HMQC and the bottom show COSY. Note the aromatic peaks around 7.5 ppm signifying chlorhexidine. Note the absence of a mPEG backbone peak around 3.5 ppm.

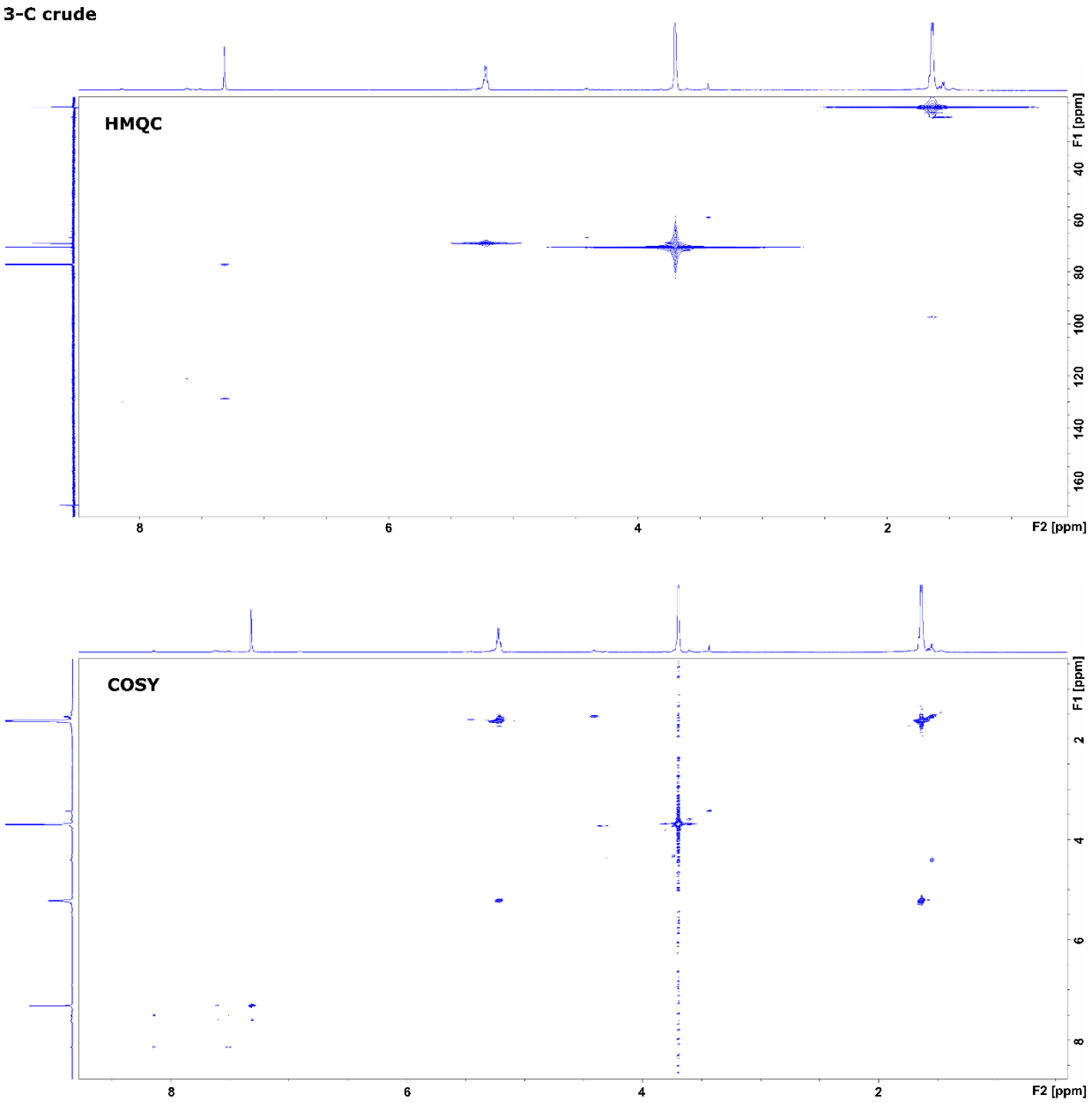


Figure S12: 2-D NMR **3-C** crude. The top shows HMQC and the bottom shows COSY.

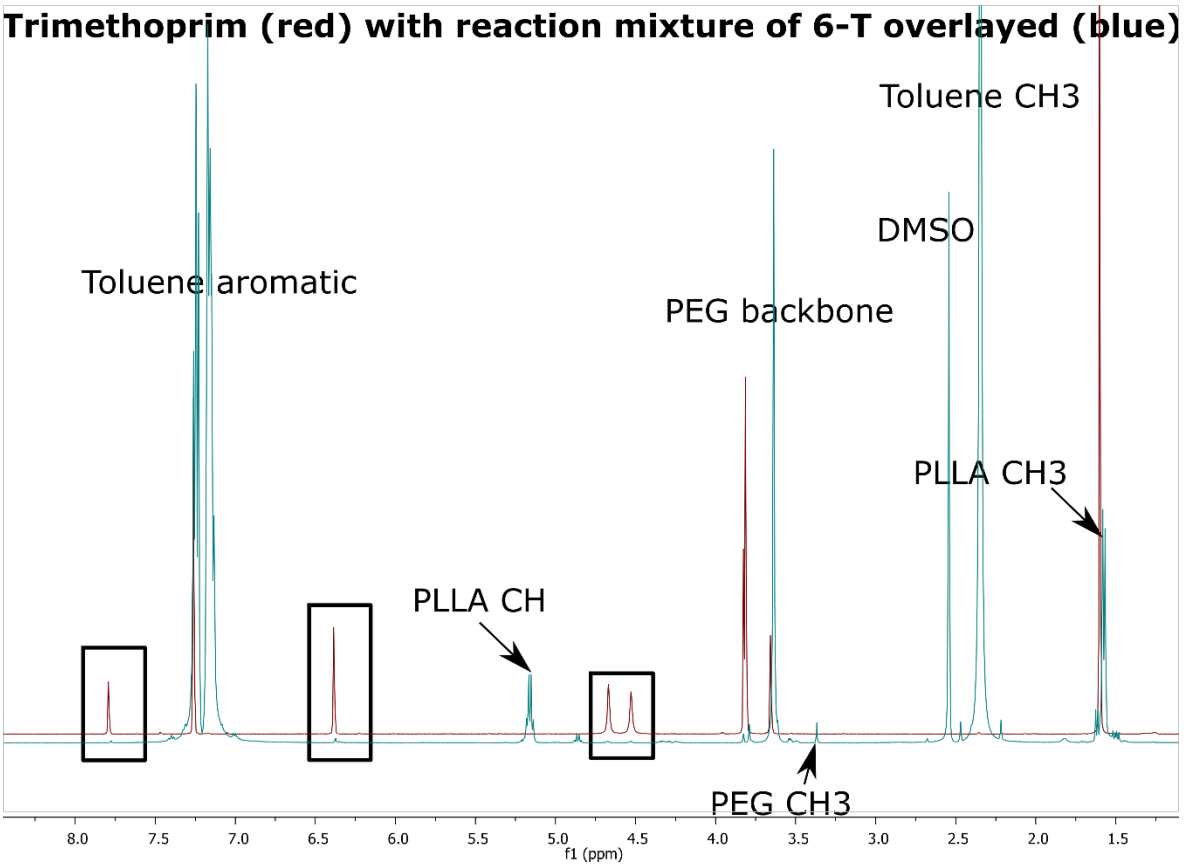


Figure S13: Trimethoprim ^{1}H NMR. Boxes show non-overlapping trimethoprim peaks suggesting that there is no shift in trimethoprim peaks in a trimethoprim sample (red) with a reaction mixture (**6-T**) using 2.5 mol% trimethoprim.

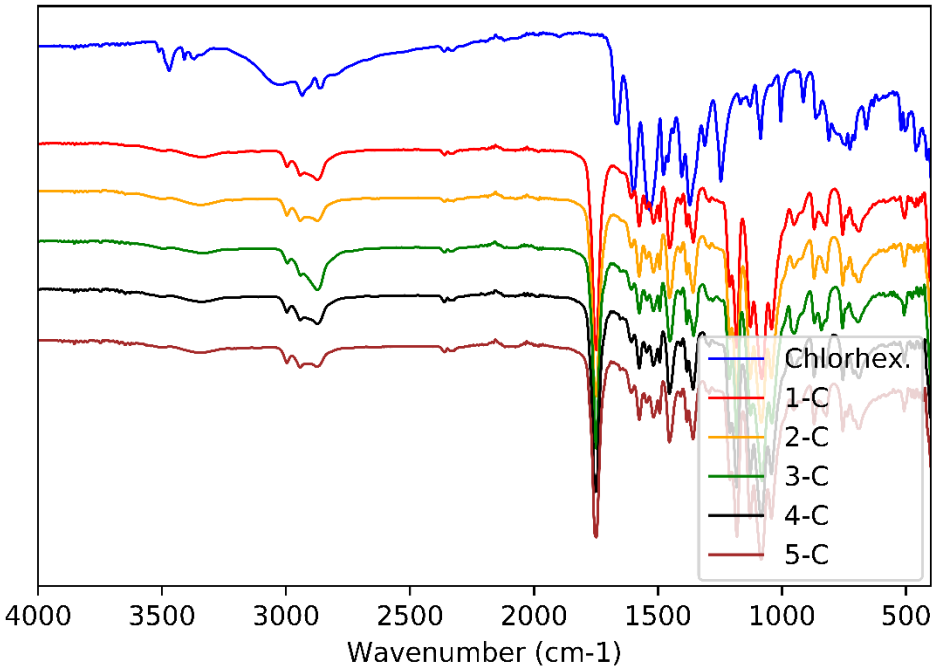


Figure S14: FTIR spectra of chlorhexidine and chlorhexidine catalyzed ROPI-CDSA polymers.

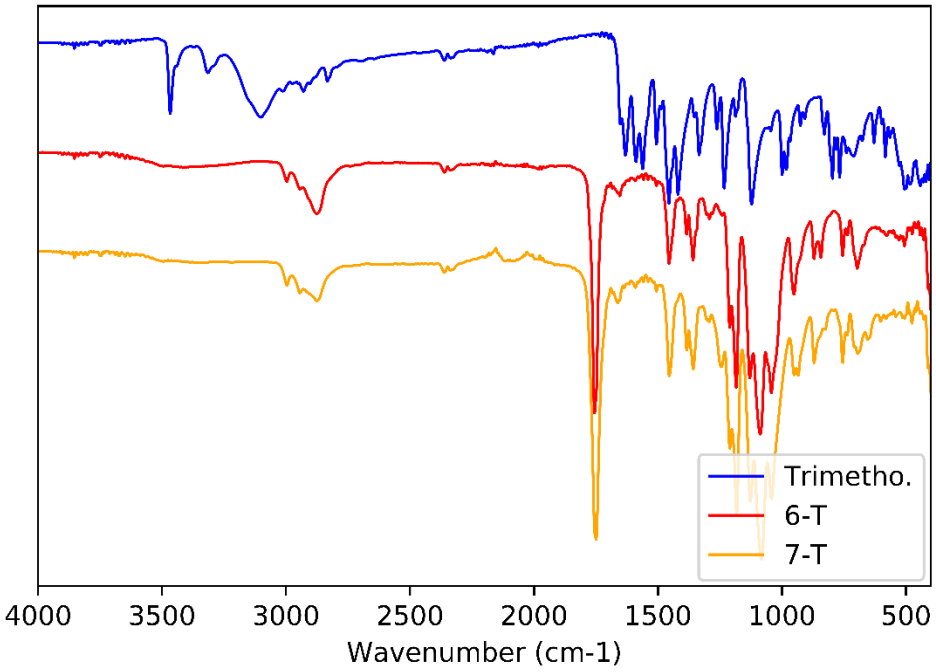


Figure S15: FTIR spectra of trimethoprim and trimethoprim catalyzed ROPI-CDSA polymers.

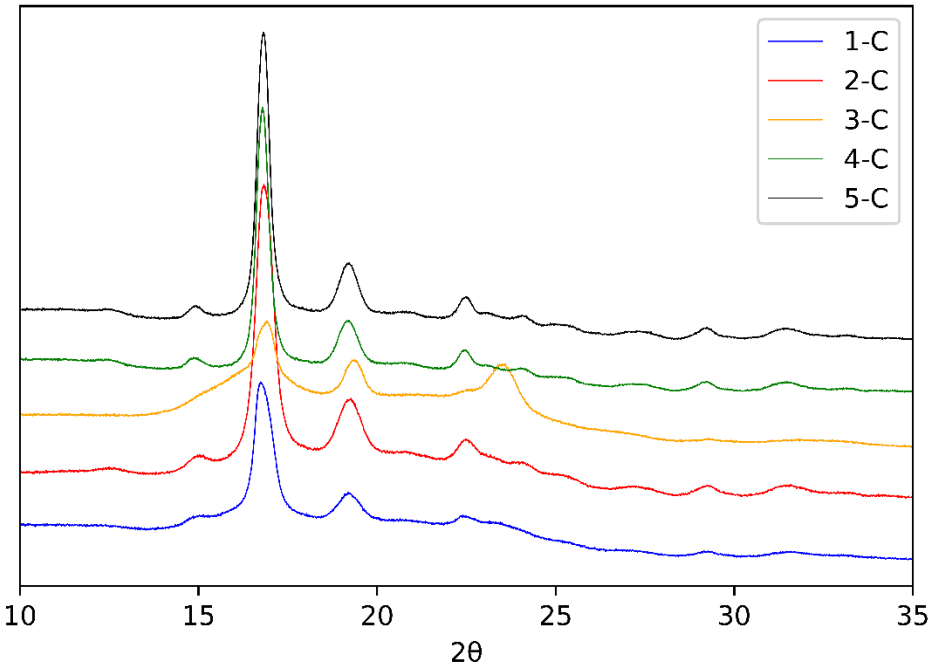


Figure S16: WAXS patterns of chlorhexidine catalyzed ROPI-CDSA polymers.

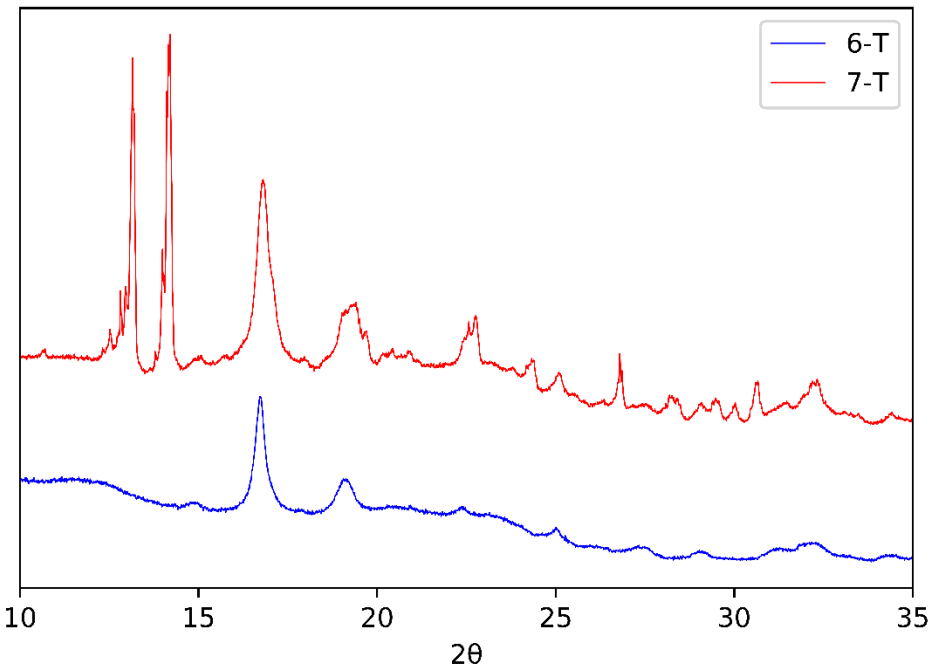


Figure S17: WAXS patterns of trimethoprim catalyzed ROPI-CDSA polymers. Note in **7-T** that there are some sharper peaks, likely L-lactide due to the relatively low conversion of **7-T** compared to **6-T**.

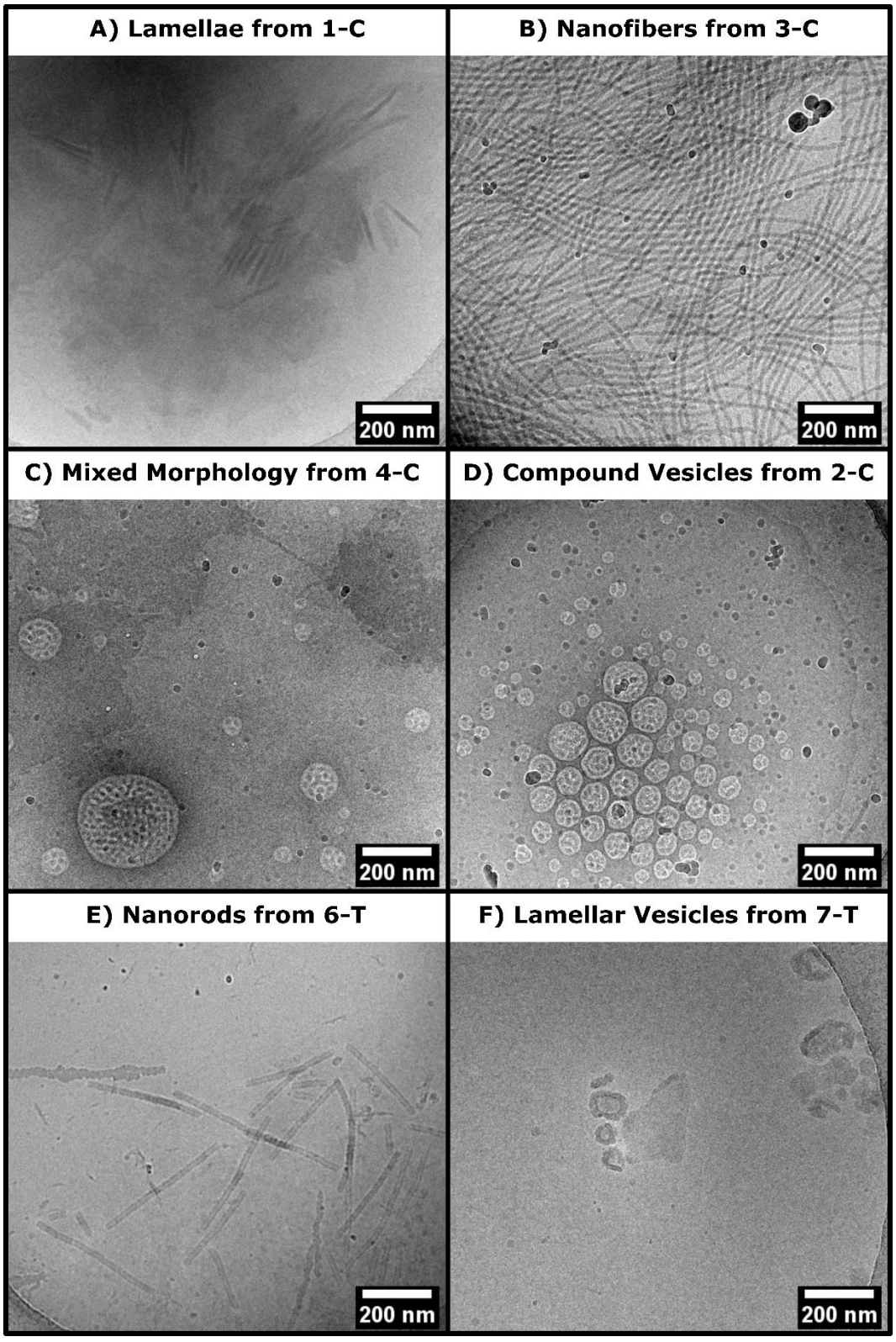


Figure S18: Additional cryoEM images of drug catalyzed ROPI-CDSA samples. Each image is labelled with its corresponding polymer sample.

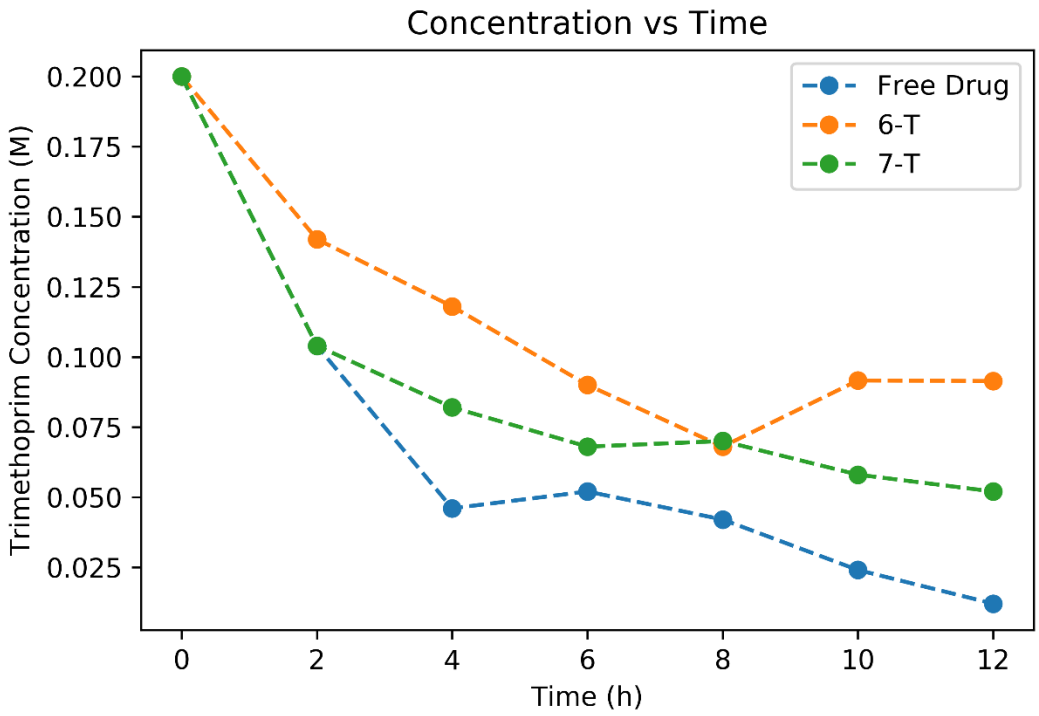


Figure S19: Release Profile of Trimethoprim during dialysis into aqueous solution (95% water, 5% DMSO). The free drug control (blue) shows a faster release of trimethoprim than **6-T** (orange) or **7-TB** (green).

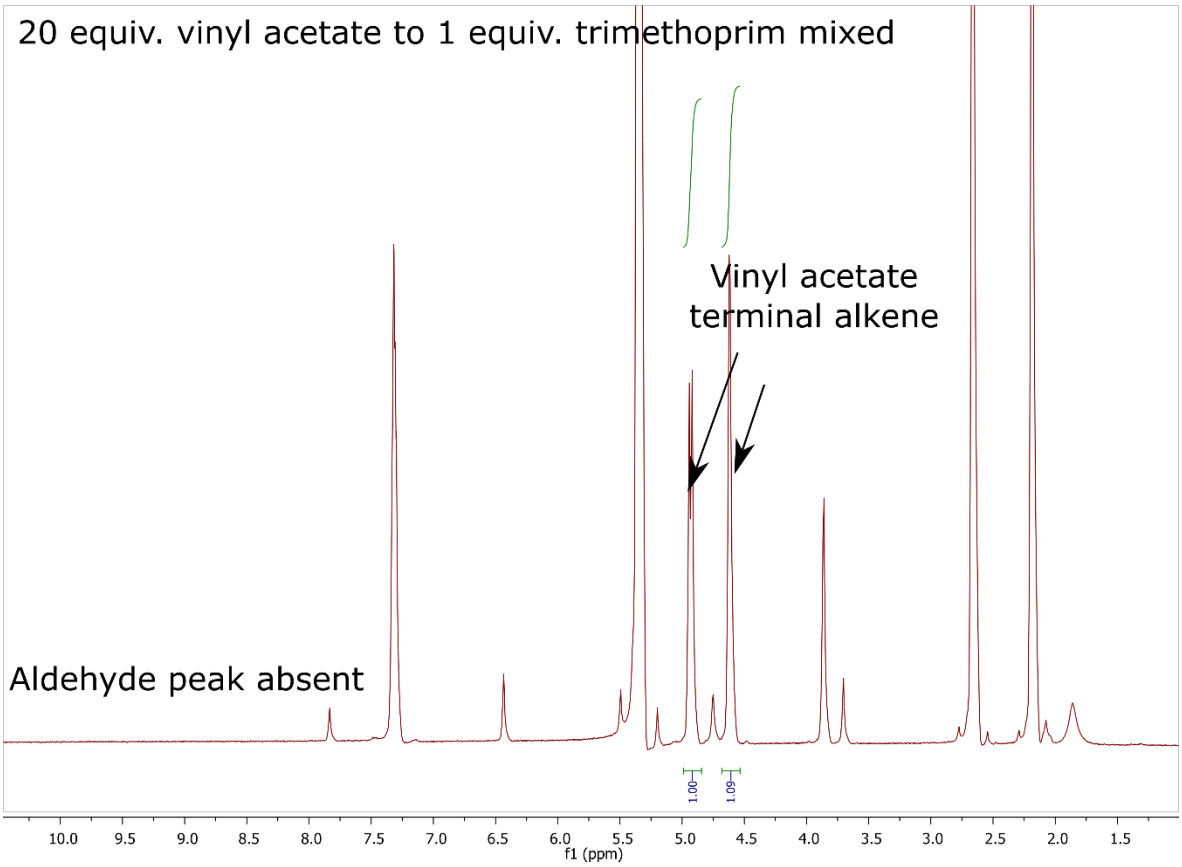


Figure S20: Attempted trimethoprim acylation ^{1}H NMR data.

References

1D. A. Culkin, W. Jeong, S. Csihony, E. D. Gomez, N. P. Balsara, J. L. Hedrick and R. M. Waymouth, *Angewandte Chemie*, 2007, **119**, 2681–2684.

2H. A. Brown and R. M. Waymouth, *Acc. Chem. Res.*, 2013, **46**, 2585–2596.