Supplemental Information to:

Exploiting Sequence to Control the Hydrolysis Behavior of Biodegradable PLGA Copolymers

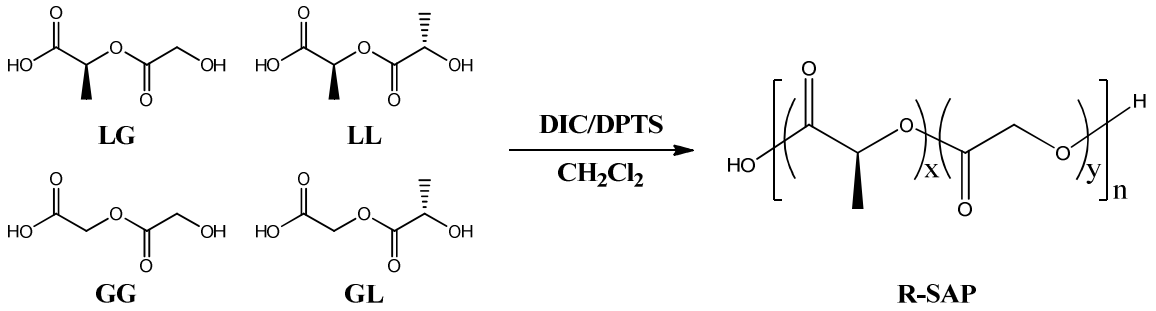
*Jian Li, Ryan M. Stayshich and Tara Y. Meyer**

Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260

Corresponding author: tmeyer@pitt.edu

Preparation of PLGA copolymers by segmer assembly polymerization

The copolymers **Poly LG(16k)**, **Poly LG(26k)** were prepared as previously reported.[1] **R-SAP** (Scheme S1) was prepared as follows. Segmers LL, LG, GL and GG were prepared as previously described.[1] Equivalent weights of each of the four segmers (0.34 g each, 2.26 mmol) were combined with (DPTS, 0.53 g, 1.8 mmol) and dissolved in CH_2Cl_2. After cooling the soln. to 0 ^oC, 1,3 diisopropylcarbodiimide (DIC, 1.71 g, 13.6 mmol) was added dropwise and the solution was stirred at RT for 2 h. The product was precipitated from methanol (150 ml, 2x), filtered and dried under vacuum to give a white solid (1.1 g, 90% yield). ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) see figure S1; SEC (THF) M_n – 27.4 kDa, M_w – 36.4 kDa, PDI - 1.33.



Scheme S1: Preparation of **R-SAP** copolymer.

Preparation of microparticles

Microparticles of all PLGAs were prepared by a standard emulsion method.[2] A solution of PLGA polymer (0.2 g) in CH_2Cl_2 (4 ml) was emulsified in a poly(vinyl alcohol) (PVA) solution (60 ml, 2 % w/v) using a homogenizer (10,000 rpm) for 1 min. The emulsion was then poured into another PVA solution (80 ml, 1% w/v) and stirred for 3 h at room temperature to evaporate the methylene chloride. The microparticles were centrifuged and washed with deionized water 3x and freeze-dried overnight to remove the residual solvent. The microparticles were stored in a desiccator.

***In vitro* degradation study**

Microparticles for each polymer (20 mg) were dispersed in phosphate buffer solution (1 ml, pH = 7.4) in multiple microcentrifuge tubes (2 samples for each time period). All tubes were incubated at 37 ^{o}C. The buffer was exchanged every two days by centrifugation followed by the decanting of the supernatant. At designated intervals, all microparticles from an individual microcentrifuge tube were collected, washed and freeze-dried.

Size exclusion chromatography (SEC)

Molecular weight data were determined by SEC using a Waters 515 HPLC pump with phenogel 10^4 and 500 Å columns and a Waters 2414 refractive index detector. THF was used as the mobile phase of the SEC with a flow rate of 0.5 ml/min. The sample was dissolved in THF, filtered and then injected into a 20 μL loop. The number and weight average molecular weight were determined from the SEC curve by calibration with polystyrene standards.

Differential scanning calorimetry (DSC)

The thermal properties of the microparticles were determined by DSC measurements. Microparticle samples (~ 5 mg) were placed in aluminum pans and analyzed with a Perkin-Elmer DSC 6 instrument by scanning from 10 ^{o}C to 200 ^{o}C with a heating rate of 10 ^{o}C / min. Reported transitions were obtained from the first heating cycle. The glass transition temperature (T_g) data obtained from the second heating cycle were given in the Table S1.

Nuclear magnetic resonance spectroscopy (NMR)

The composition of RSC PLGAs and random PLGAs were determined by ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ using Bruker NMR spectrometers. The chemical shifts were calibrated to the residual solvent peaks (δ 7.24 and δ 77.0, respectively) and are reported in δ units relative to Me_4Si (TMS).

Microstructural comparison of R-ROP and R-SAP by ^{13}C NMR spectroscopy.

A comparison of the glycolyl carbonyl region of the **R-ROP** and **R-SAP** random copolymers highlights differences in their microstructures. Although both polymers exhibit several overlapping resonances near δ 166.4,[3] the relative ratios of the individual peaks differ significantly. For example, the resonance at δ 166.33 in **R-ROP** is more intense than that at δ 166.42. The spectrum of **R-SAP** shows both peaks are similar in intensity (Figure S3). Previous studies[4,5] have suggested that the δ 166.33 resonance is associated with the glycolyl carbonyls of units located in the center of pure G blocks while that at δ 166.42 is characteristic of a glycolyl carbonyls with nearby L units. The relative ratios of these peaks are consistent the microstructural trends that are expected from the two distinct synthetic approaches. The **R-ROP** polymer**,** prepared by ring-opening of a 50:50 mixture of glycolide and lactide, are known to possess blocks of pure G.[6] In contrast the **R-SAP** copolymer, produced by the condensation of four dimers, should give statistically fewer runs of pure G unless the reaction rate of GG with other GG units is significantly higher than that with other units. Based on our extensive experience in handling these dimers, differences in reaction rates between dimers are not large.

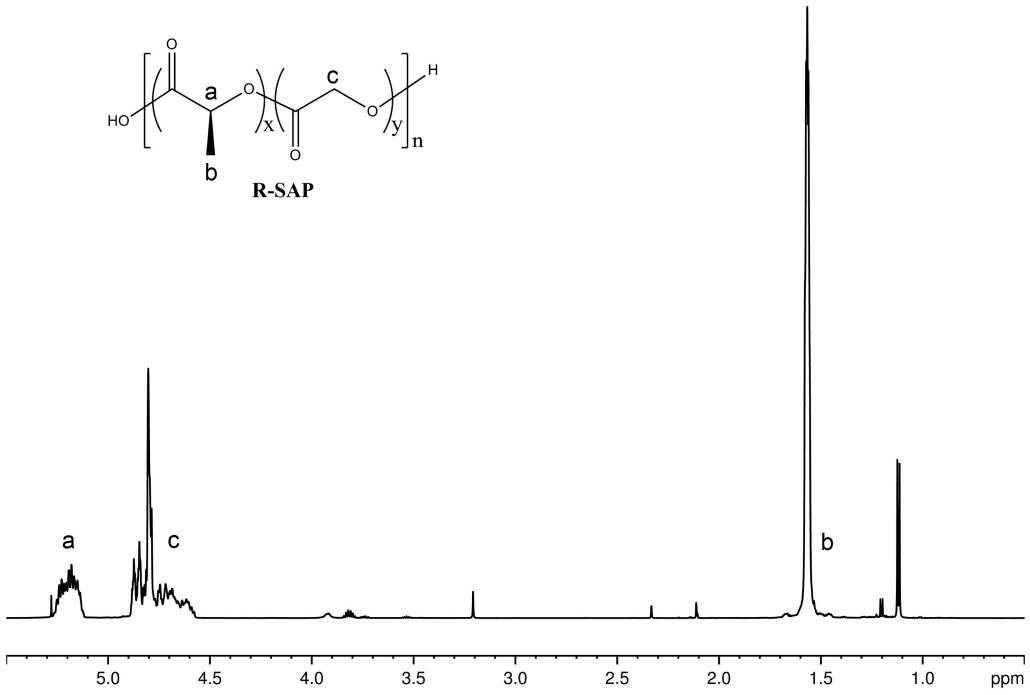


Figure S1. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of R-SAP.

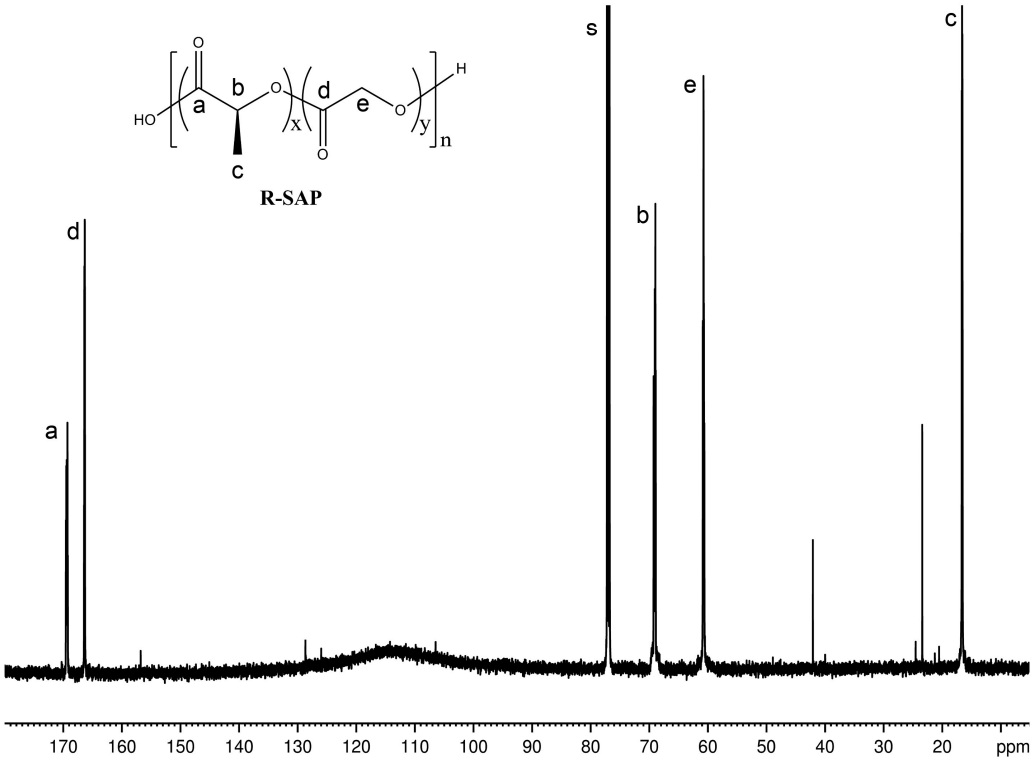


Figure S2. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of R-SAP.

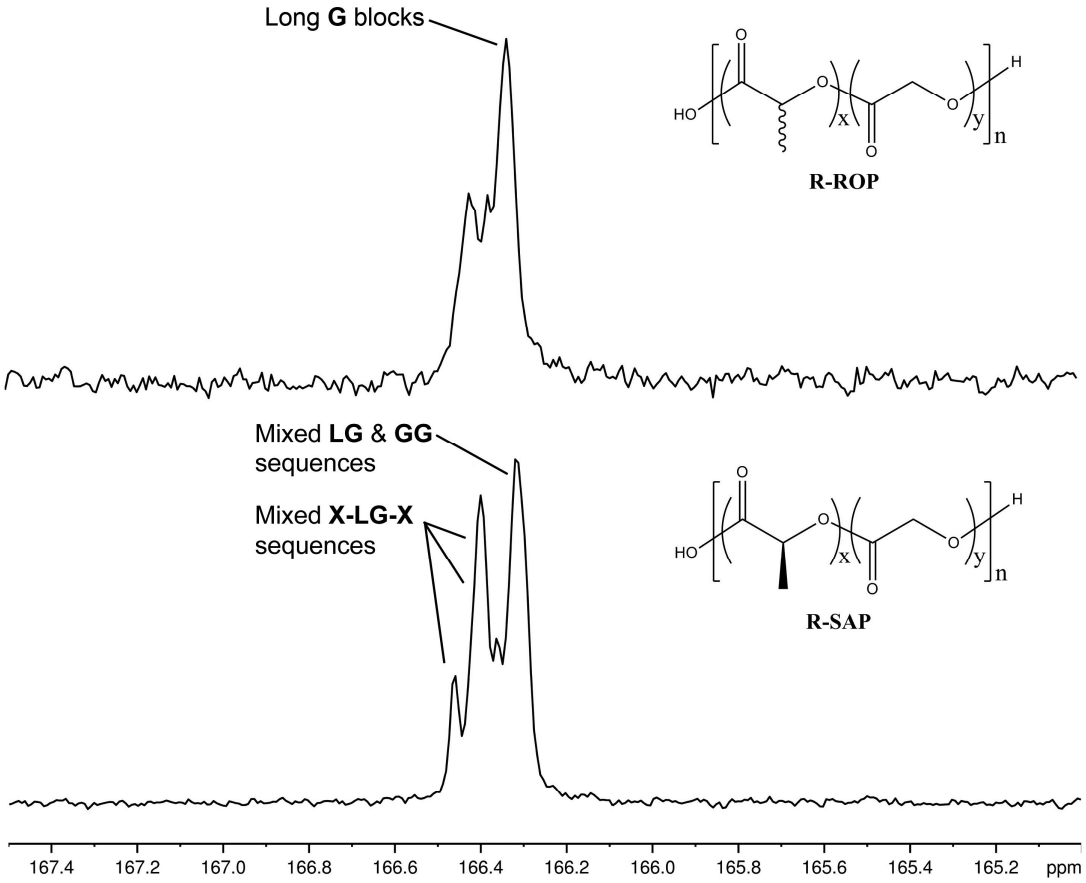


Figure S3. Comparison of glycolyl carbonyl resonances for **R-ROP** (top) and **R-SAP** (bottom). The X represents either L or G units.

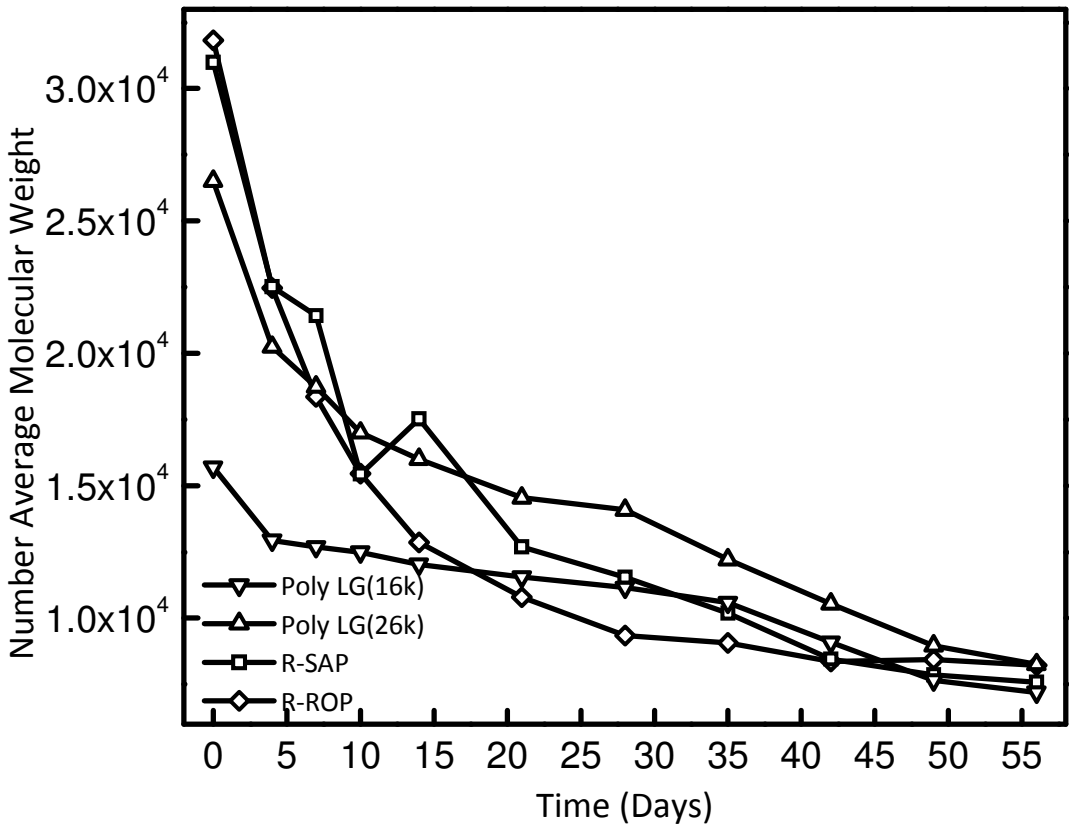


Figure S4. The number average molecular weight (M_n) decrease as a function of degradation time. The figure in the main body of the paper has been normalized relative to initial molecular weight.

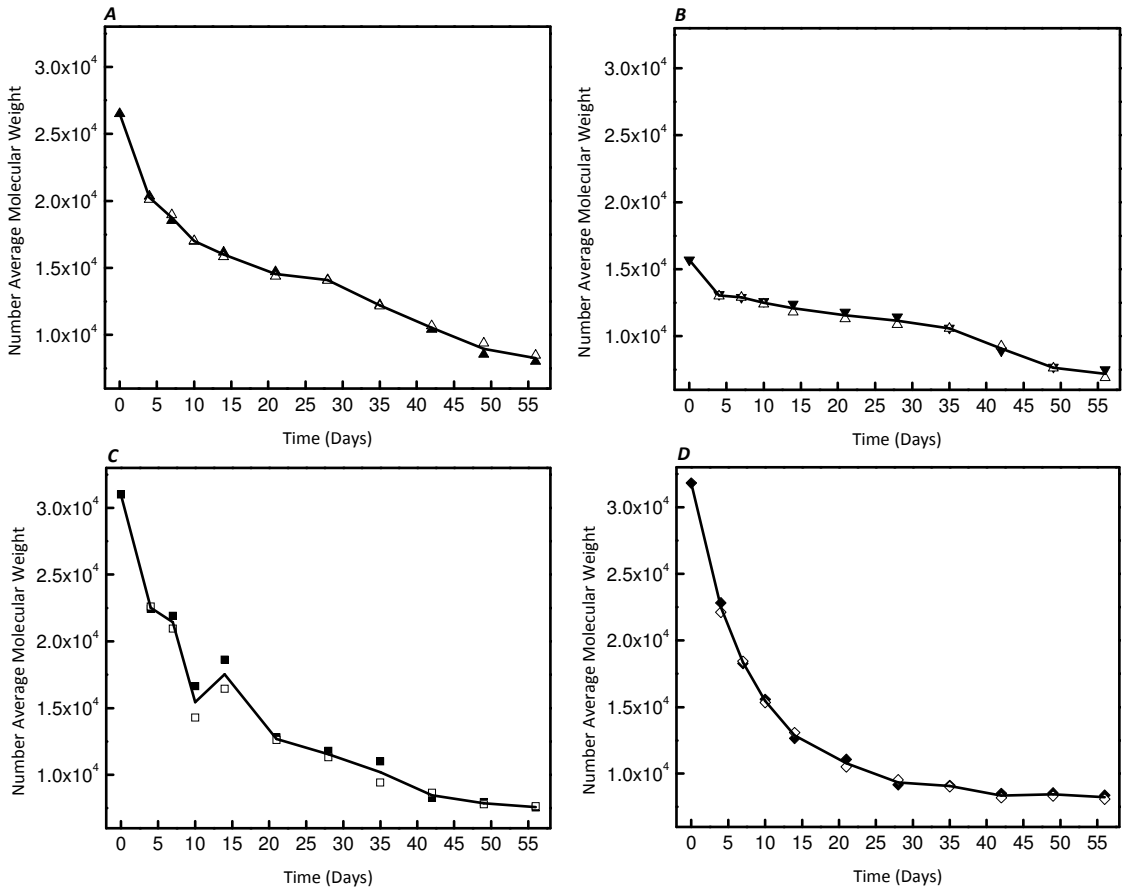


Figure S5. The spread of number average molecular weight (M_n) decrease of **poly LG(26k)** (A), **poly LG(16k)** (B), **R-SAP** (C) and **R-ROP** (D) as a function of degradation time. The solid symbols and open symbols represent independent experiment results. The line is plotted by the average of M_n from each experiment result.

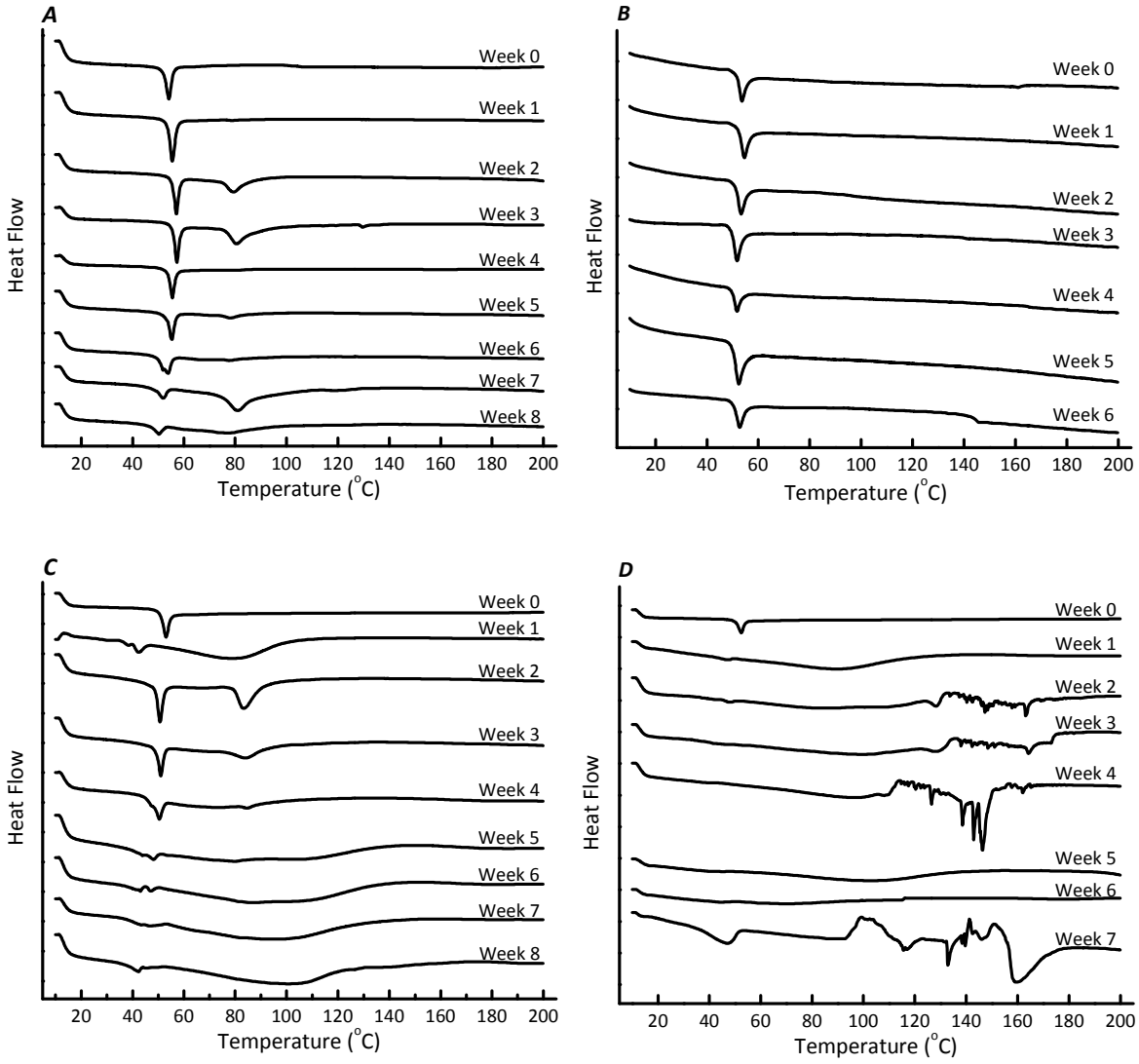


Figure S6. DSC thermograms of **poly LG(26k)** (A), **poly LG(16k)** (B), **R-SAP** (C) and **R-ROP** (D) as a function of degradation time. Note: weeks 5,6,7 for (D) are omitted in the figure in the main article because the sample size was sufficiently small after 4 weeks of degradation to make the data traces unreliable. The thermograms for **poly LG(26k)** and **R-ROP** (weeks 1-4) are repeated here to facilitate comparison.

Table S1. Glass transition data of PLGAs.[a]

	T_g (^oC)								
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Poly LG(26k)	47.0	47.4	47.2	47.2	46.9	44.6	43.0	41.4	41.2
Poly LG(16k)	45.6	46.1	44.0	41.0	40.7	44.3	43.3	-[b]	-[b]
R-SAP	45.7	46.3	46.0	44.5	43.1	42.2	40.2	40.0	33.6
R-ROP	45.9	40.5	42.8	-[c]	-[c]	-[c]	-[c]	-[c]	-[c]

[a]The T_gs were calculated from the 2nd heating cycle of the freeze-dried PLGAs after hydrolysis experiment on each week. [b]The T_gs of poly LG(16k) on week 7 and 8 were not available because the sample size is too small to give reliable DSC thermographs. [c]The T_gs of R-ROP after week 2 could not be determined from the thermographs.

References:

(1) Stayshich, R. M.; Meyer, T. Y. *J. Am. Chem. Soc.* **2010**, *132*, 10920-10934.
(2) Jain, R. A. *Biomaterials* **2000**, *21*, 2475-2490.
(3) Kasperczyk, J. *Macromol. Symp.* **2001**, *175*, 19-31.
(4) Hausberger, A. G.; DeLuca, P. P. *J. Pharm. Biomed. Anal.* **1995**, *13*, 747-60.
(5) Dong, C. M.; Qiu, K. Y.; Gu, Z. W.; Feng, X. D. *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 4179-4184.
(6) Gilding, D. K.; Reed, A. M. *Polymer* **1979**, *20*, 1459-64.