The University of Texas at Austin
McKetta Department
of Chemical Engineering

Stochastic and Deterministic Analysis of Reactivity Ratios in the Partially Reversible Copolymerization of Lactide and Glycolide

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Thank you!

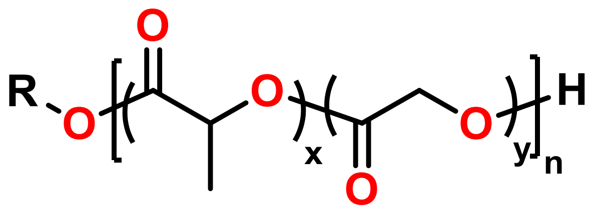
Dr. Feng Zhang

Mark Costello

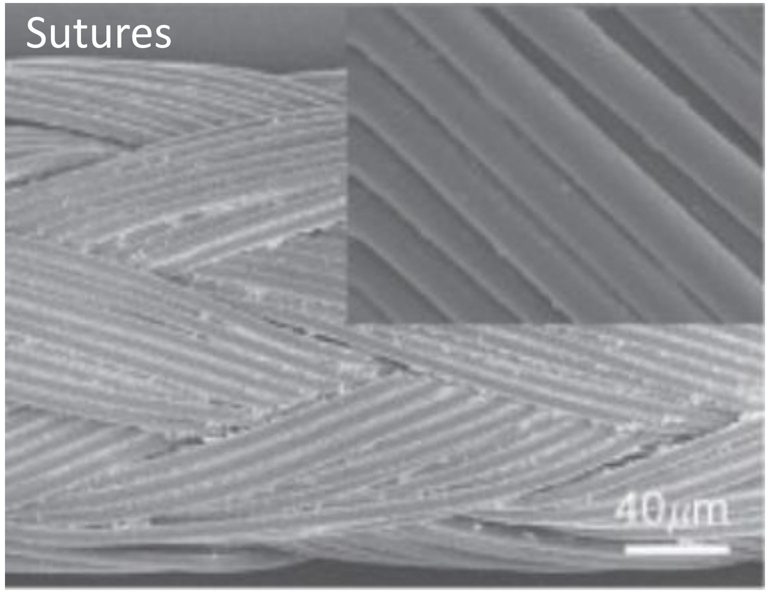
Dr. Beibei Chen

FDA U.S. FOOD & DRUG ADMINISTRATION

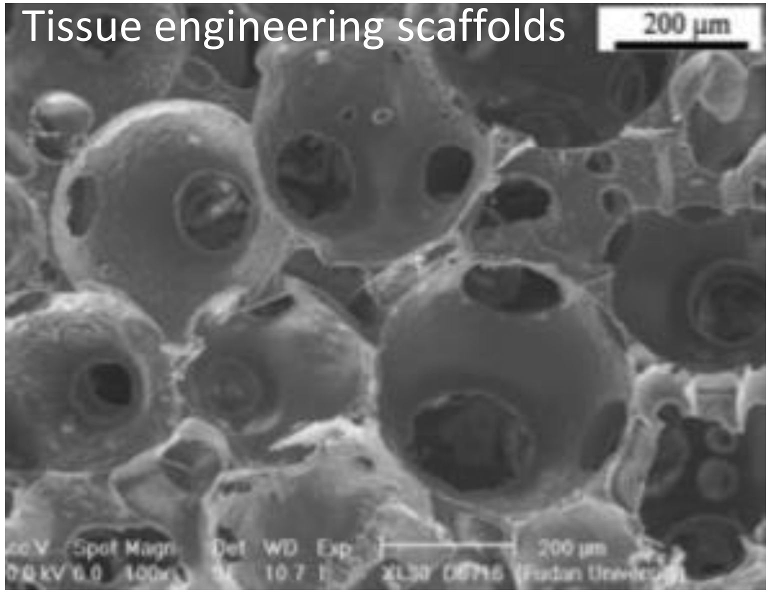
Poly(lactide-co-glycolide) (PLGA) is a biodegradable polymer that is used in pharmaceutical applications



PLGA



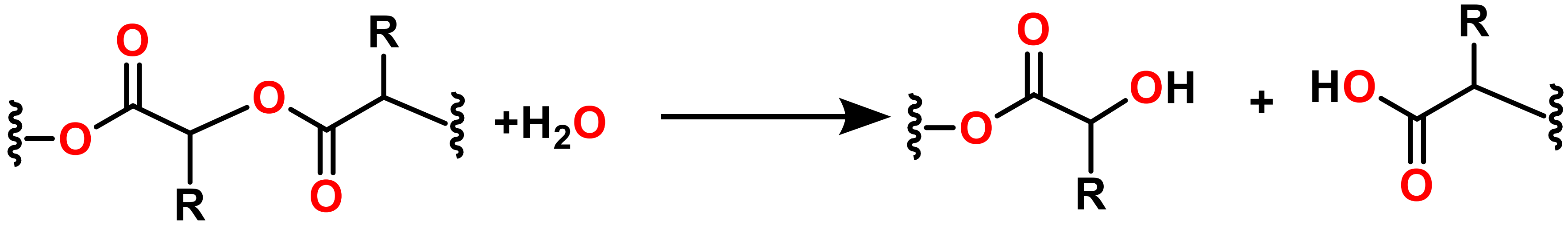
Antimicrobial and Biodegradable PLGA Medical Sutures with Natural Grapefruit Seed Extracts. *Mater. Lett.* **2013**, *95*, 40–43. https://doi.org/10.1016/j.matlet.2012.12.090.



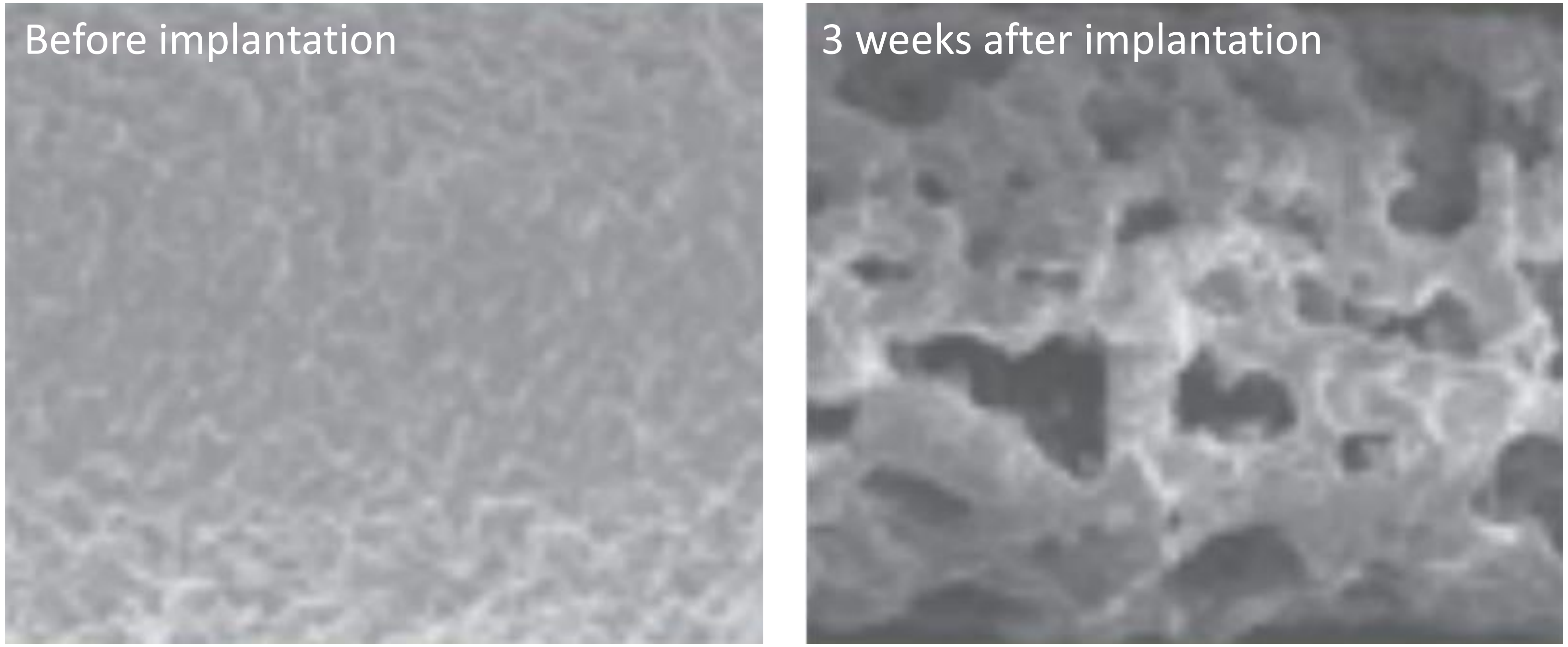
Junchuan Zhang; Hong Zhang; Linbo Wu; Jiandong Ding. Fabrication of Three Dimensional Polymeric Scaffolds with Spherical Pores. *J. Mater. Sci.* **2006**, *41* (6), 1725–1731. https://doi.org/10.1007/s10853-006-2873-7.

OZURDEX® Mechanism of Action | For HCPs https://hcp.ozurdex.com/mechanism-of-action (accessed Mar 27, 2021).

PLGA biodegrades to non-toxic products



R= -H or -Me



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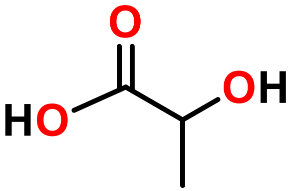
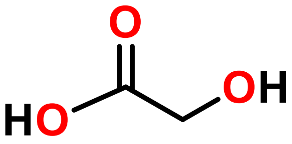


Lactic acid



Glycolic acid

Makadia, H. K.; Siegel, S. J. Poly Lactic-Co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. *Polymers* **2011**, *3* (3), 1377–1397. https://doi.org/10.3390/polym3031377.

Long-acting drug implants can improve patient outcomes

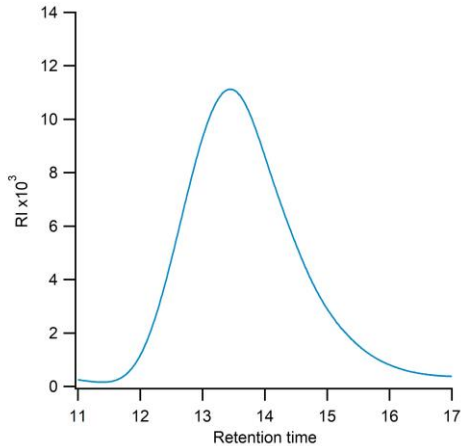
- Lower dosage frequency
- Better patient compliance
- Potentially lower drug toxicity

There are over 20 FDA-approved products which use PLGA as an excipient, but no generic version have been approved

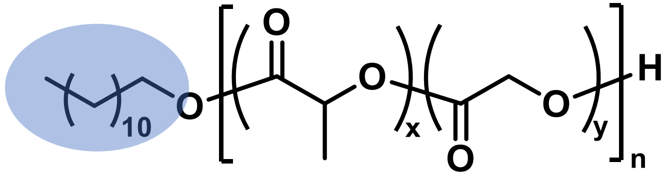
Wang, Y.; Qu, W.; Choi, S. H. FDA's Regulatory Science Program for Generic PLA/ PLGA-Based Drug Products. *Am. Pharm. Rev.* **2016**, *20*, 5–10.

Multiple characteristics affect PLGA degradation and drug release

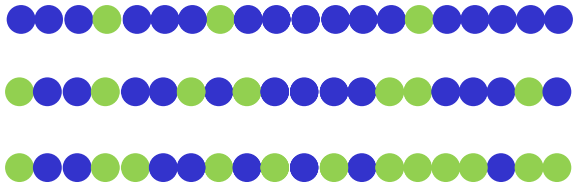
Molecular weight



End group



Composition (L:G ratio)



Sequencing

block

gradient

random

alternating

Understanding sequencing will expand PLGA's usefulness as an excipient

- Enable better design of new PLGA products
- ~~Improve the FDA's ability to regulate current PLGA products~~

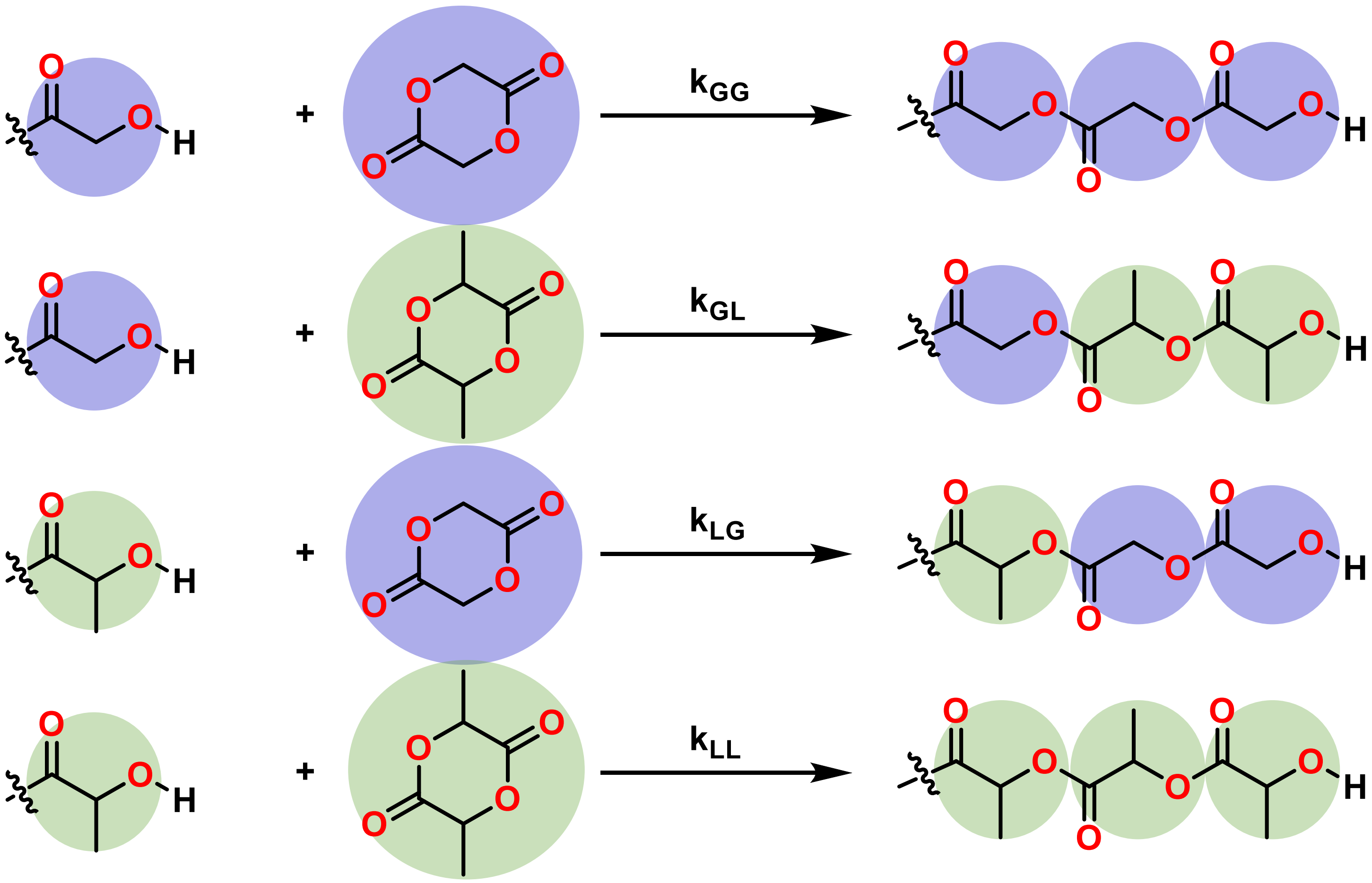
Not easily characterized or controlled

Li, J.; Rothstein, S. N.; Little, S. R.; Edenborn, H. M.; Meyer, T. Y. The Effect of Monomer Order on the Hydrolysis of Biodegradable Poly(Lactic- *Co* -Glycolic Acid) Repeating Sequence Copolymers. *J. Am. Chem. Soc.* **2012**, *134* (39), 16352–16359. https://doi.org/10.1021/ja306866w.

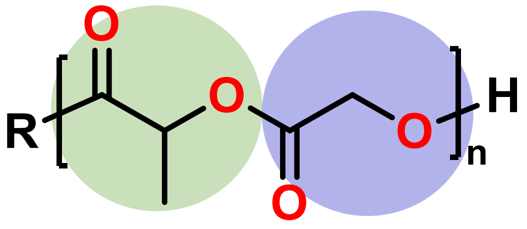
Copolymer sequence is usually understood in terms of reactivity ratios

$$r_G = \frac{k_{GG}}{k_{GL}}$$

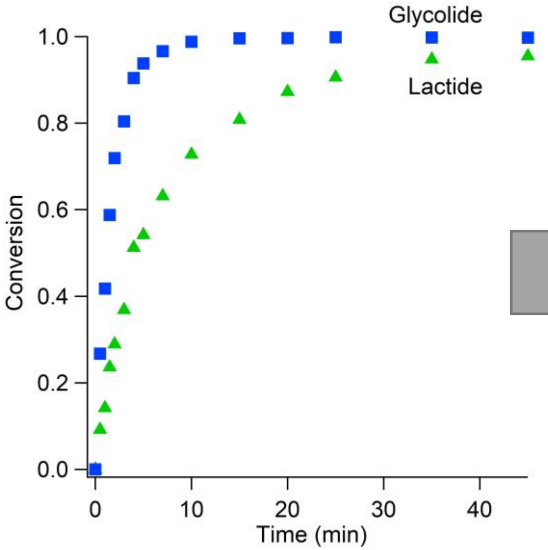
$$r_L = \frac{k_{LL}}{k_{LG}}$$



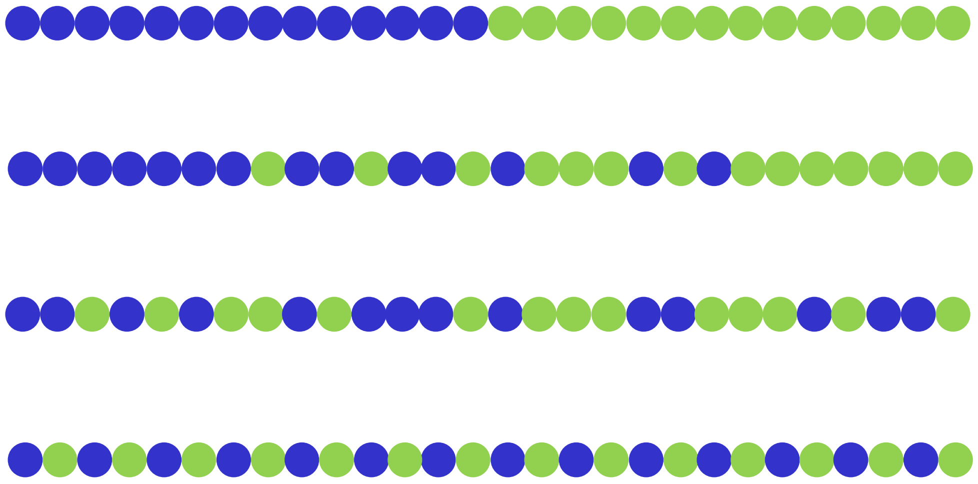
Copolymer sequence is currently understood in terms of reactivity ratios



Kinetic monomer consumption data

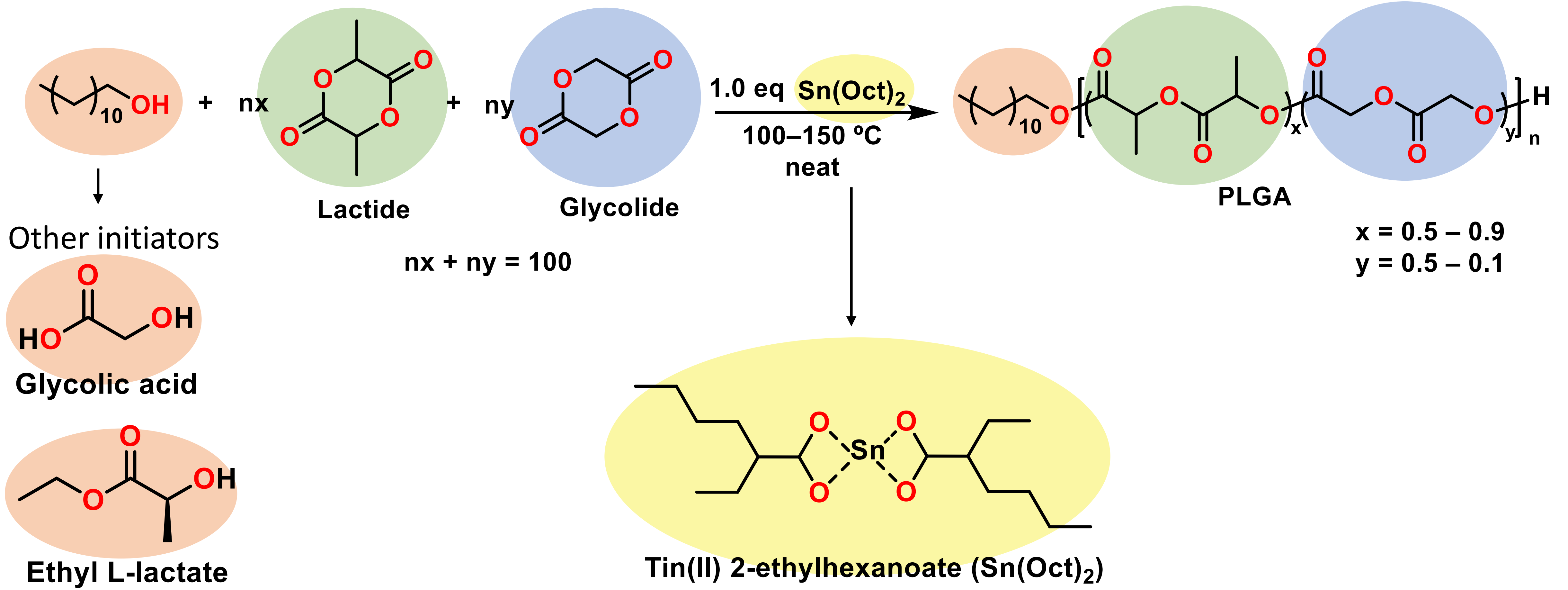


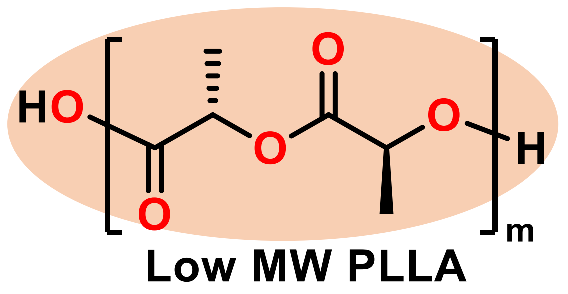
Reactivity ratios	Microstructure
$r_G, r_L >> 1$	Blocky
$r_G > 1, r_L < 1$	Gradient
$r_G, r_G \approx 1$	Random
$r_G, r_L << 1$	Alternating



Unfortunately, current models aren't sufficient for PLGA

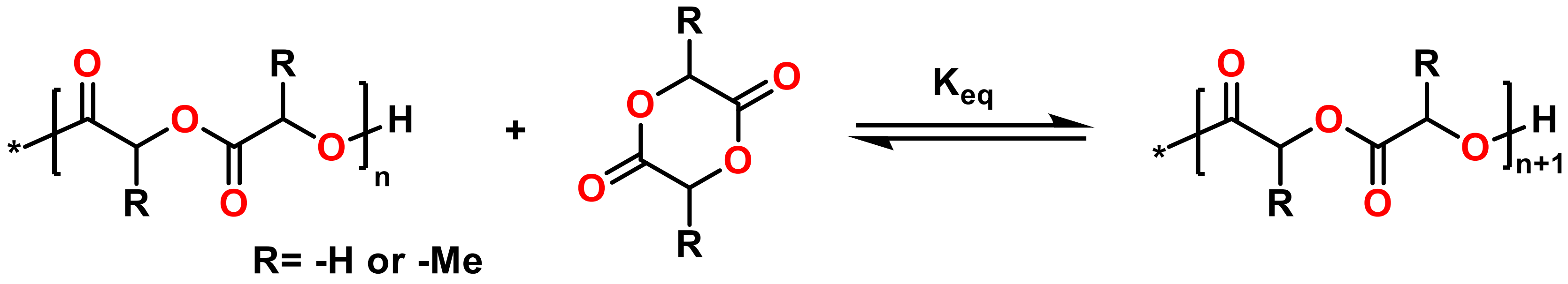
PLGA is synthesized from cyclic dimers

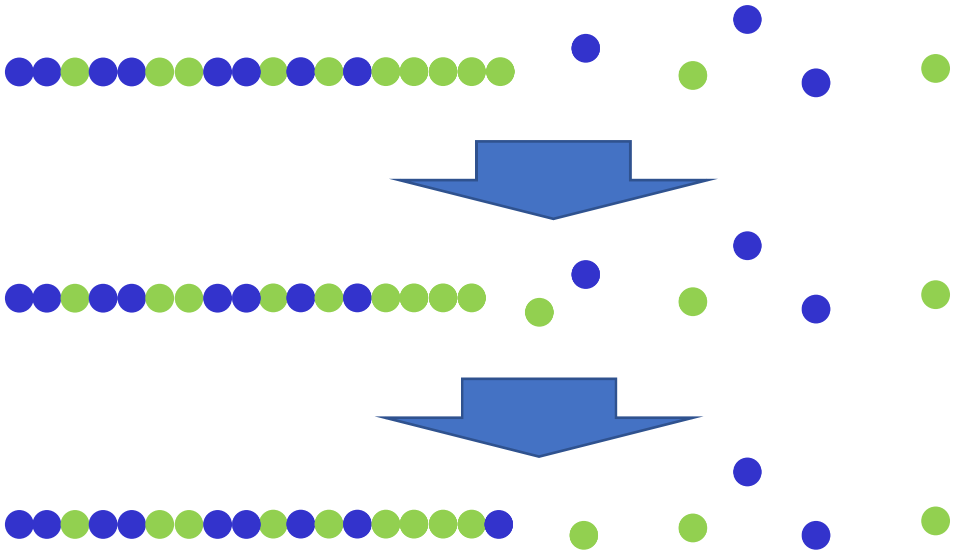




Also catalyzes reverse and transesterification reactions!

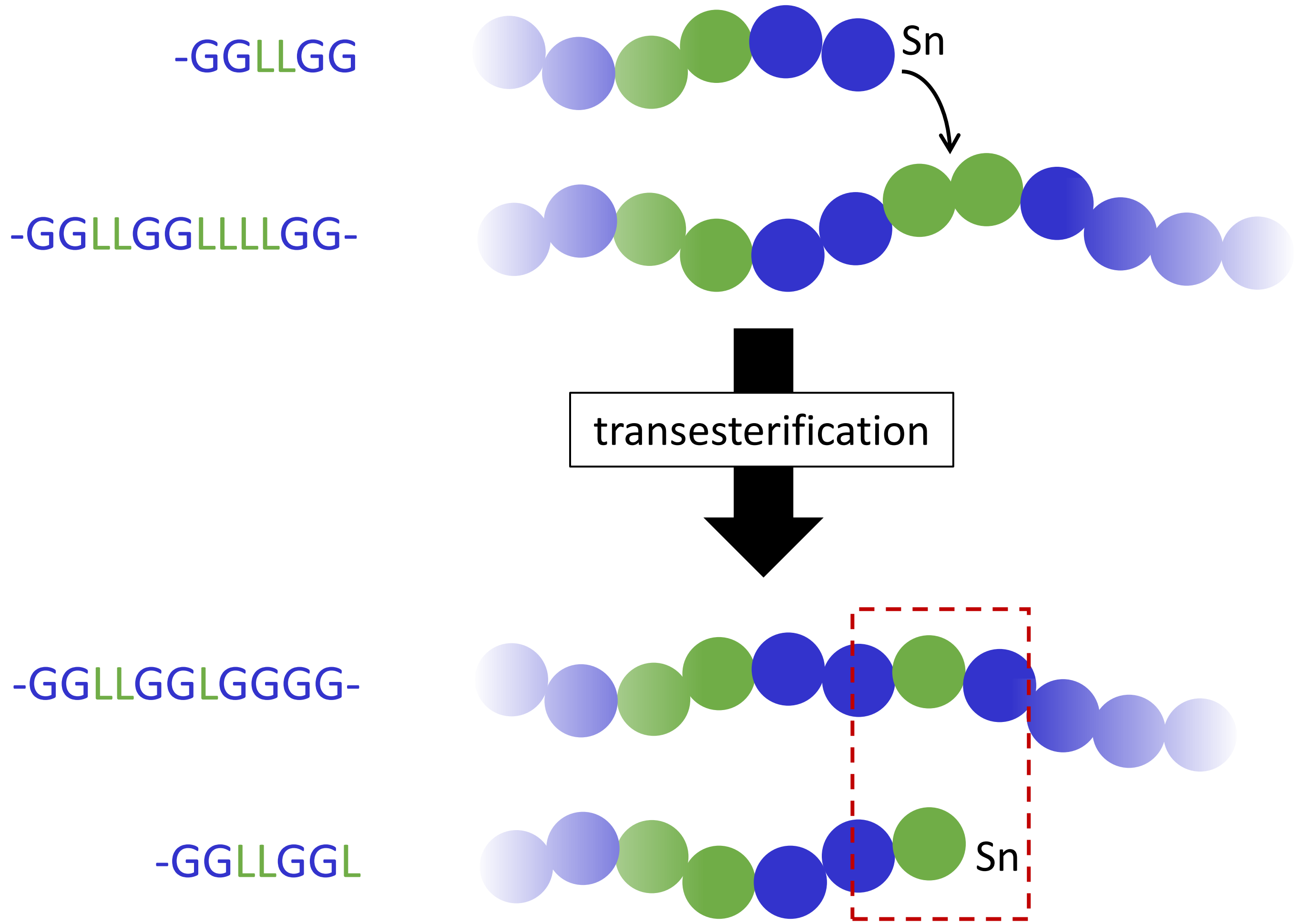
Sequence is altered throughout the polymerization as a result of equilibrium





These reactions are not accounted for in the equations used to calculate reactivity ratios, so any reactivity ratios we calculate with those models are inaccurate

Transesterification scrambles sequence throughout synthesis



Even if we calculate accurate reactivity ratios, the sequence is also affected by transesterification

- Calculate accurate reactivity ratios that account for reversibility
- Measure kinetics of transesterification
- Link both to actual sequence through modeling

Equations describing PLGA copolymerization are complicated

Initiation reactions

$$r_1 = k_G c_G(t) c_I(t)$$
$$r_2 = k_L c_L(t) c_I(t)$$

Forward polymerization reactions

$$r_3 = k_{GG} c_{P_G}(t) c_G(t)$$
$$r_4 = k_{GL} c_{P_G}(t) c_L(t)$$
$$r_5 = k_{LG} c_{P_L}(t) c_G(t)$$
$$r_6 = k_{LL} c_{P_L}(t) c_L(t)$$

Depolymerization reactions

$$r_7 = k_{G-G} c_{P_{GGG}}(t)$$
$$r_8 = k_{G-L} c_{P_{GLL}}(t)$$
$$r_9 = k_{L-G} c_{P_{LGG}}(t)$$
$$r_{10} = k_{L-L} c_{P_{LLL}}(t)$$

Transesterification

$$r_{11} = k_T c_{ends} c_{esters}$$

$$\frac{dc_I}{dt} = -r_1 - r_2$$

$$\frac{dc_G}{dt} = -r_1 - r_3 - r_5 + r_7 + r_9$$

$$\frac{dc_L}{dt} = -r_2 - r_4 - r_6 + r_8 + r_{10}$$

$$\frac{dc_{P_G}}{dt} = r_1 - r_4 + r_5 + r_8 - r_9$$

$$\frac{dc_{P_L}}{dt} = r_2 + r_4 - r_5 - r_8 + r_9$$

Because of depolymerization reactions, we need to know the concentration of every possible polymer sequence, which gives 2^n equations (n=max. degree of polymerization)
Too many equations to solve

Two ways we can circumvent mathematical challenges

- Instead of integrating 2^n deterministic equations, solve the problem stochastically

Divide the interval 0 to 1 according to the probability of each possible reaction (based on rate equations)

Generate a random number between 0 and 1 to select the next reaction that will occur

Generate another random number to choose which molecule will be reacted

Generate a third random number which will be used to update the time according to the Gillespie algorithm, $t_{n+1} = t_n - \frac{\log(a)}{r_{tot}}$

- Make some simplifying assumptions so that there are fewer equations to solve

- Preliminary data shows little glycolide reversibility and fast glycolide consumption → assume only lactide is reversible, and chain ends are always lactide by end of reaction

Initiation reactions

$$r_1 = k_G c_G(t) c_I(t)$$

$$r_2 = k_L c_L(t) c_I(t)$$

Forward polymerization reactions

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$$r_5 = k_{LG} c_{P_L}(t) c_G(t)$$

$$r_6 = k_{LL} c_{P_L}(t) c_L(t)$$

Depolymerization reactions

$$r_{10} = k_{L-L} c_{P_{LLL}}(t)$$

- Valid only for low glycolide composition

Gillespie, D. T. Exact Stochastic Simulation of Coupled Chemical Reactions. *J. Phys. Chem.* **1977**, *81* (25), 2340–2361. https://doi.org/10.1021/j100540a008.

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composition

Both models include a third reactivity ratio to represent lactide reversibility

$$r_G = \frac{k_{GG}}{k_{GL}}, \quad r_L = \frac{k_{LL}}{k_{LG}}, \quad r_R = \frac{k_{L-L}}{k_{LL}}$$

$$r_1 = k_G c_G(t) c_I(t)$$
$$r_2 = k_L c_L(t) c_I(t)$$

Forward polymerization reactions

$$r_3 = k_{GG} c_{P_G}(t) c_G(t)$$
$$r_4 = k_{GL} c_{P_G}(t) c_L(t)$$
$$r_5 = k_{LG} c_{P_L}(t) c_G(t)$$
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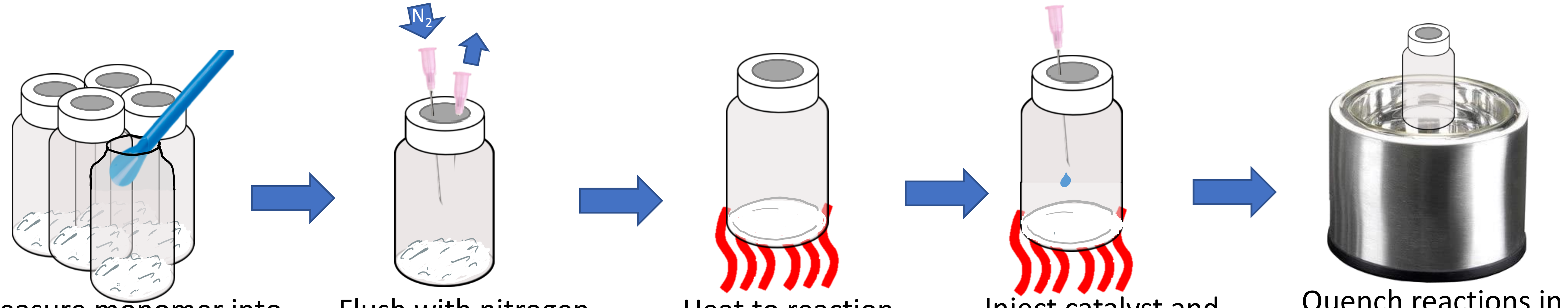
Depolymerization reactions

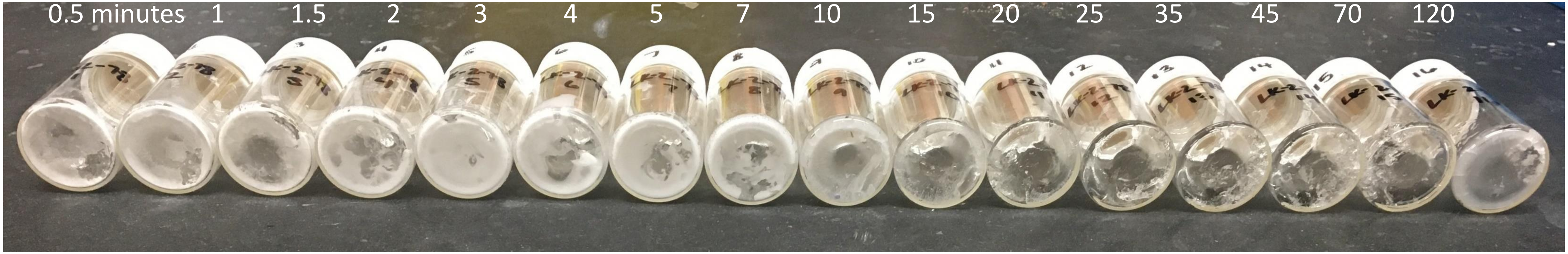
$$r_{10} = k_{L-L} c_{P_{LLL}}(t)$$

Gillespie, D. T. Exact Stochastic Simulation of Coupled Chemical Reactions. *J. Phys. Chem.* **1977**, *81* (25), 2340–2361. https://doi.org/10.1021/j100540a008.

Kinetic data for PLGA has been collected

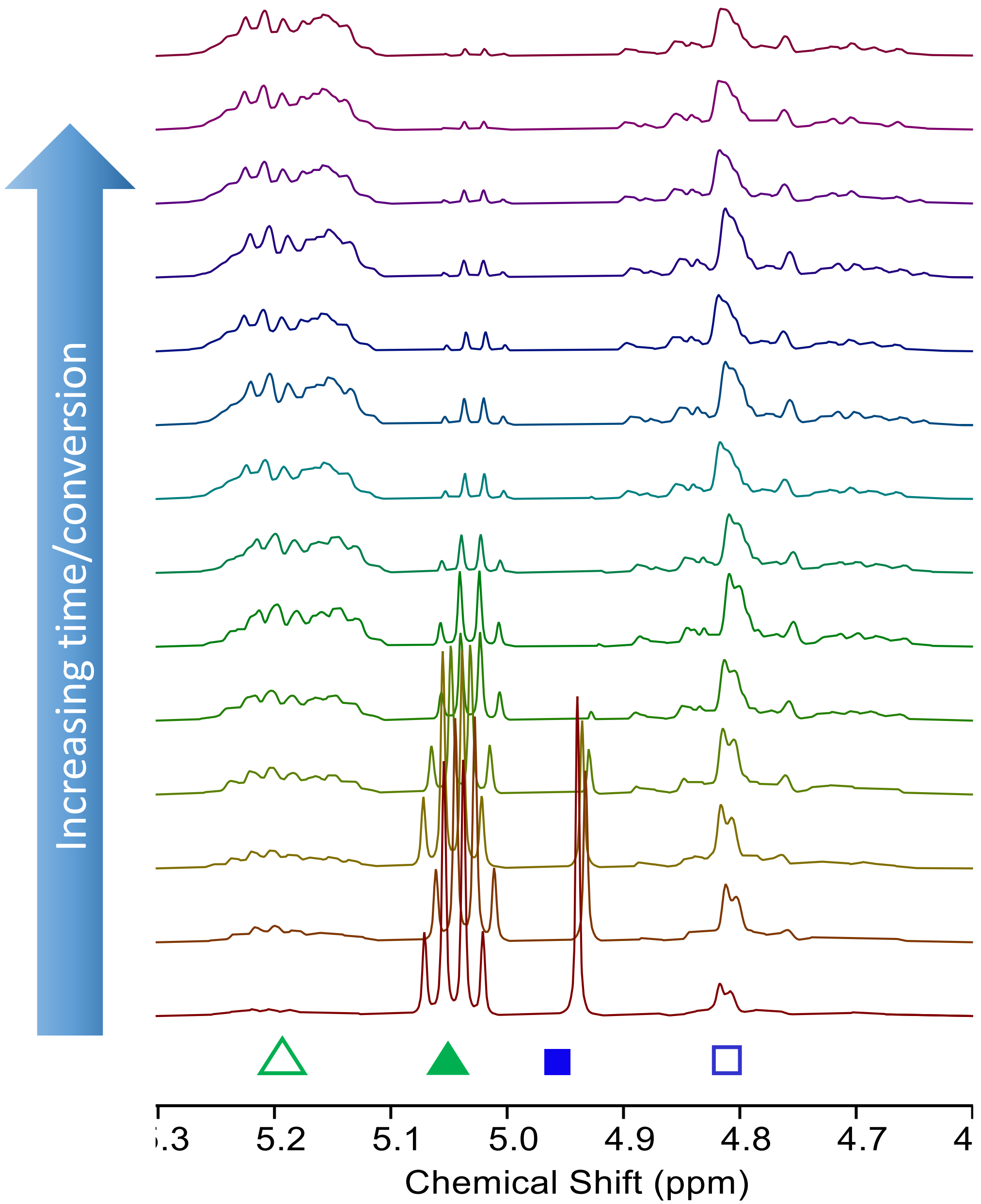


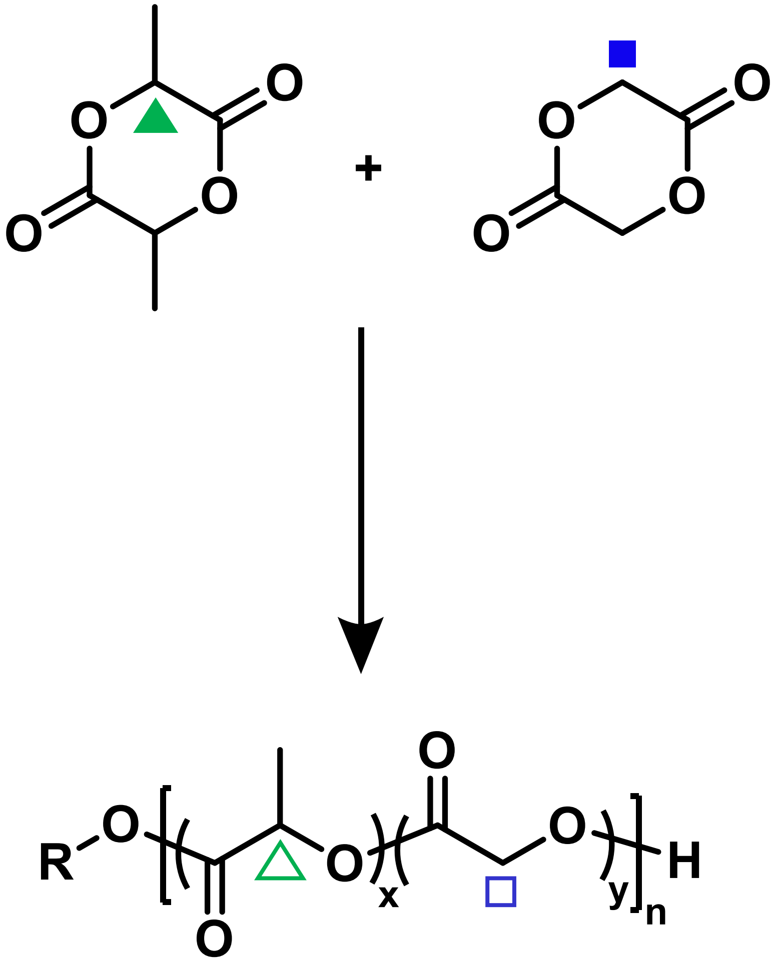
Measure monomer into 12-16 separate vials → Flush with nitrogen → Heat to reaction temperature → Inject catalyst and initiator → Quench reactions in liquid nitrogen



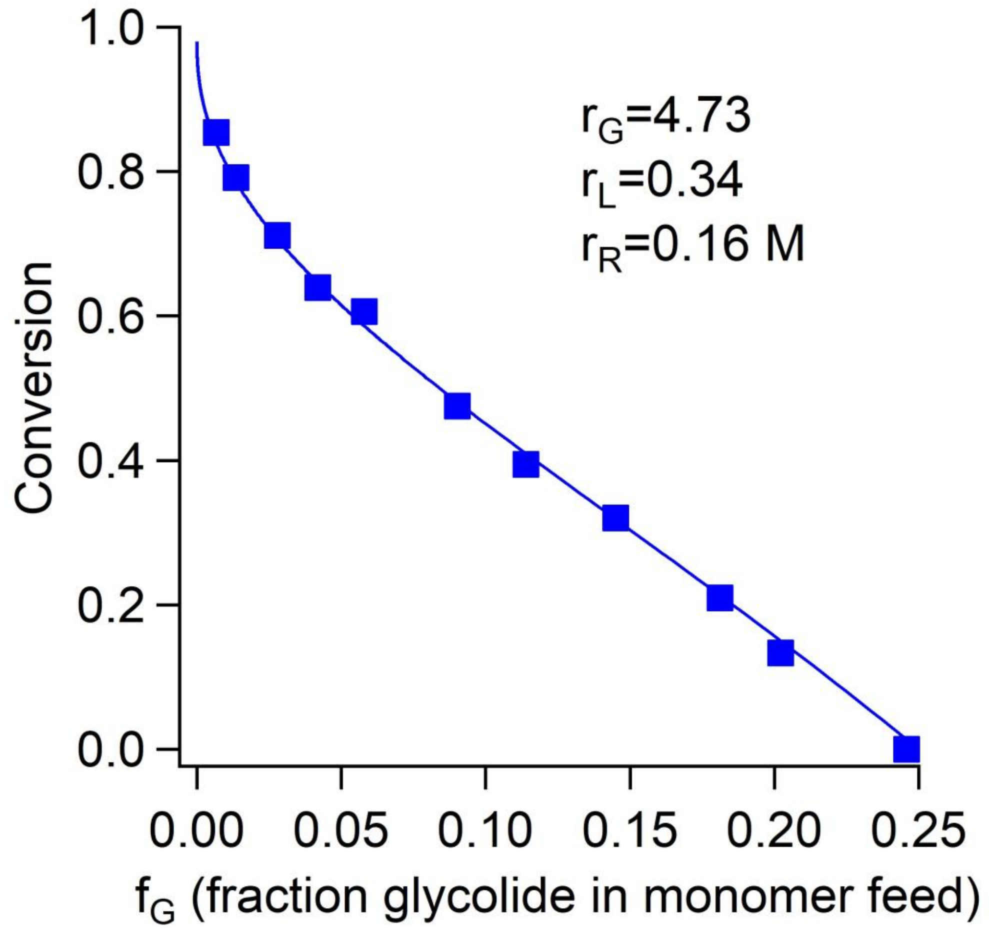
Increasing time/conversion

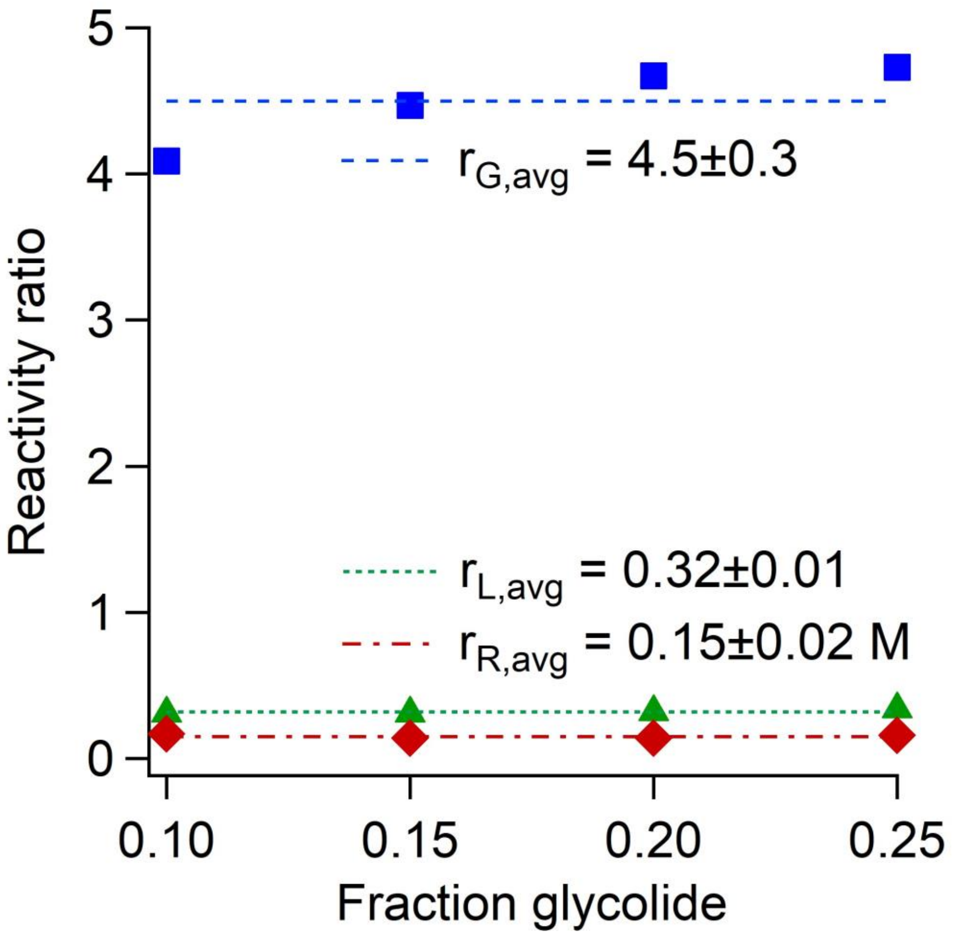
^{1}H NMR Spectroscopy was used to measure conversion



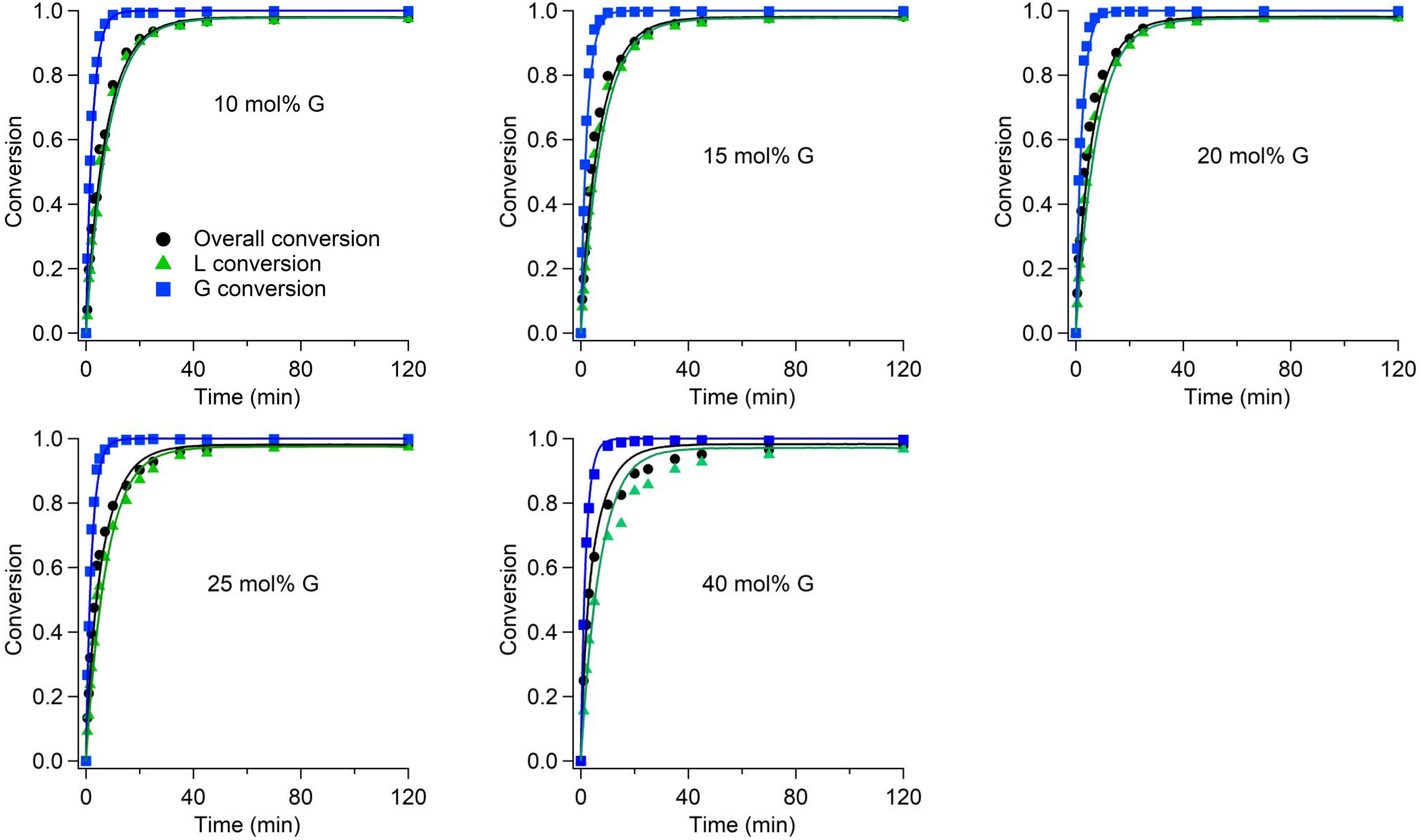


Experimental data with low glycolide content was fit to the simplified deterministic model



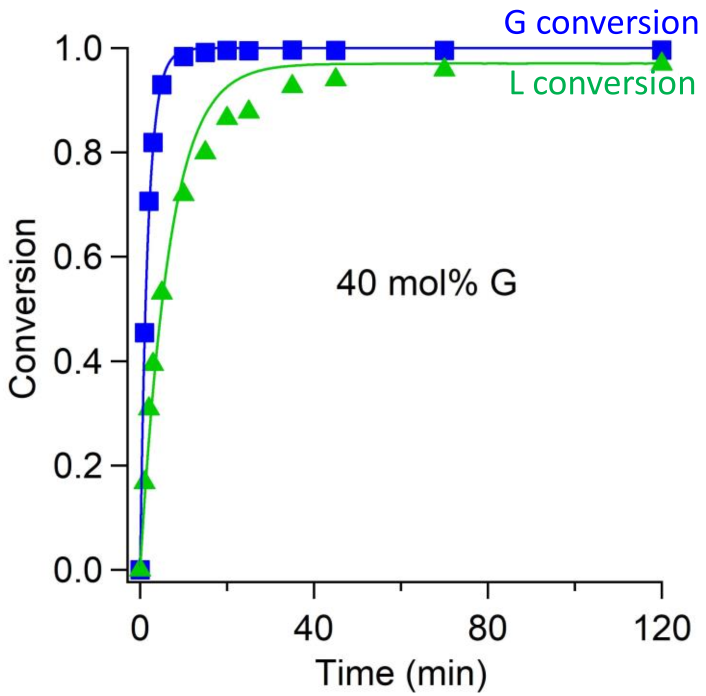


Stochastic modeling confirms accuracy of reactivity ratios

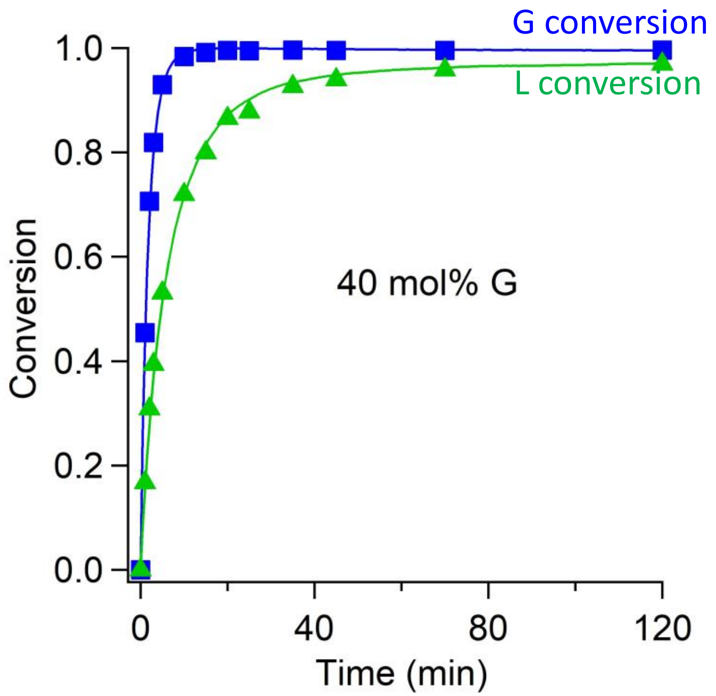


Reactivity ratio accuracy can be improved by fitting directly to the stochastic model

SD Model



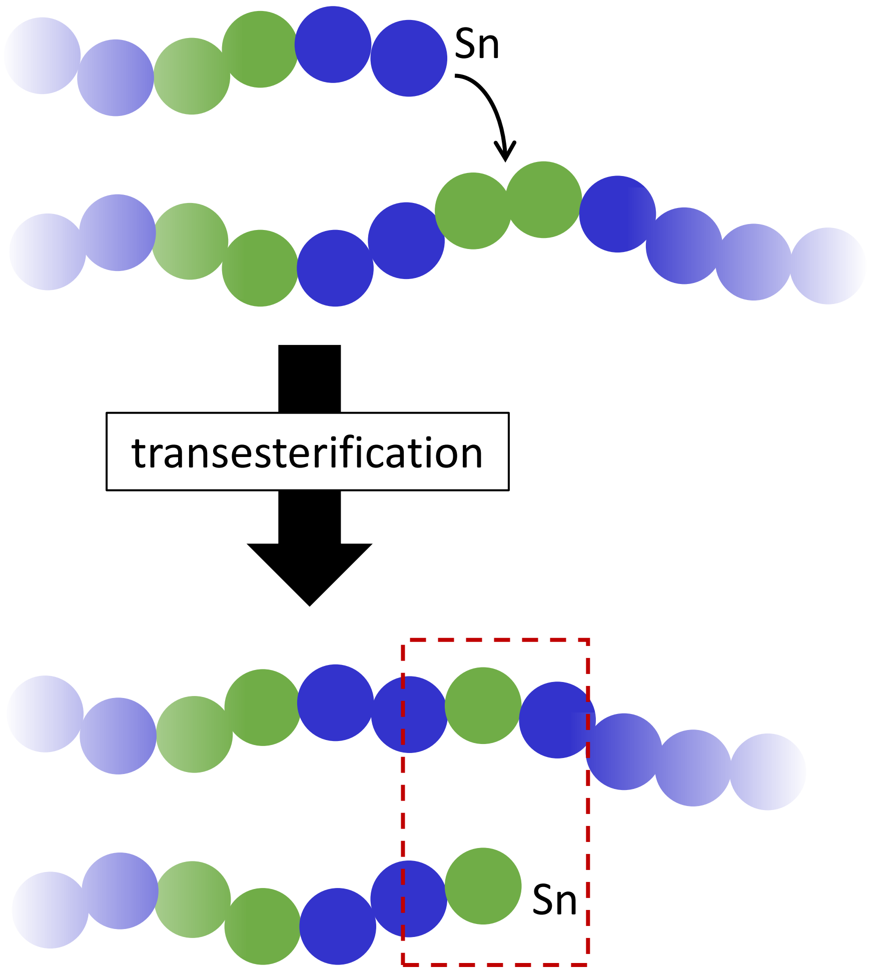
Stochastic model



$$SSR(k_{GG}, k_{LG}, k_{LG}, k_{LL}, k_{G-G}, k_{G-L}, k_{L-G}, k_{L-L}) = \sum_{i=1}^{i=n_p} (p_G(t_i)) - p_{G,i})^2 + \sum_{j=n_p+1}^{n_p+n'_p} (p_L(t_{j-n_p}) - p_{L,j-n_p})^2$$

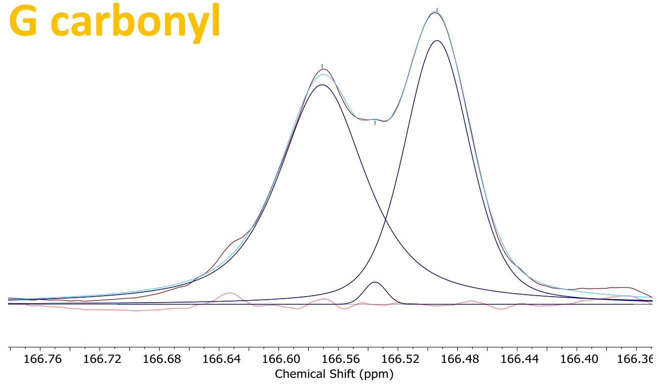
PLGA reactivity ratios alone don't solve all the problems

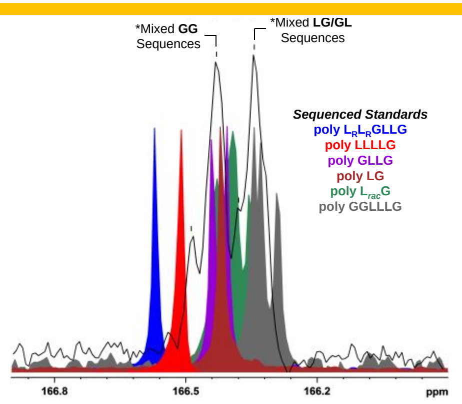
Transesterification has a major effect on PLGA sequencing but is not captured in reactivity ratios



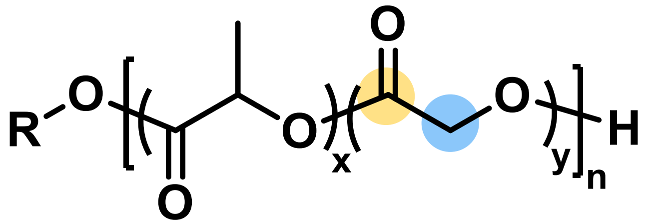
There is some disagreement about the best way to experimentally measure PLGA blockiness/sequence

G carbonyl

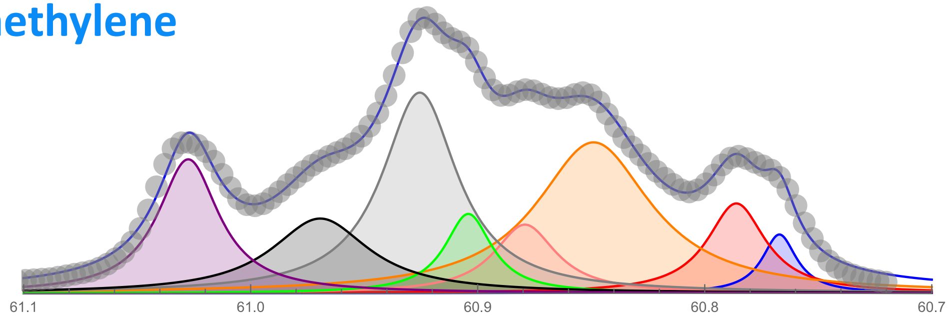




Washington et. al. *Biomaterials* **2017**, *117*, 66–76. https://doi.org/10.1016/j.biomaterials.2016.11.037.

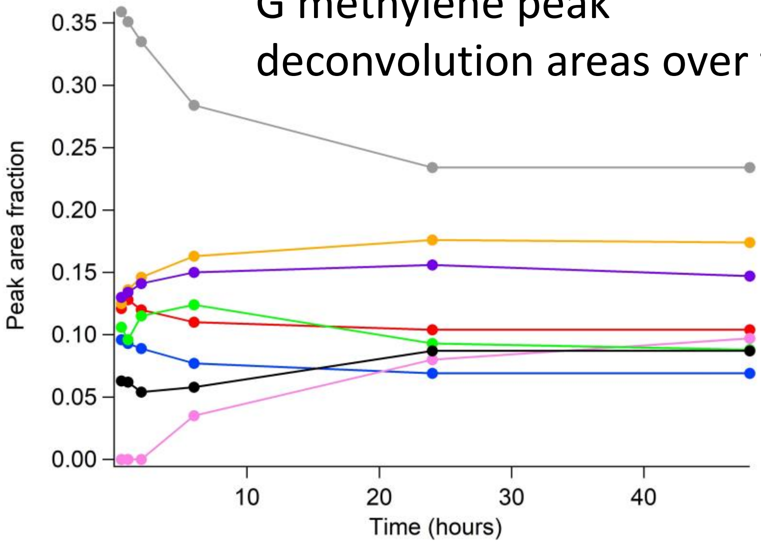


G methylene

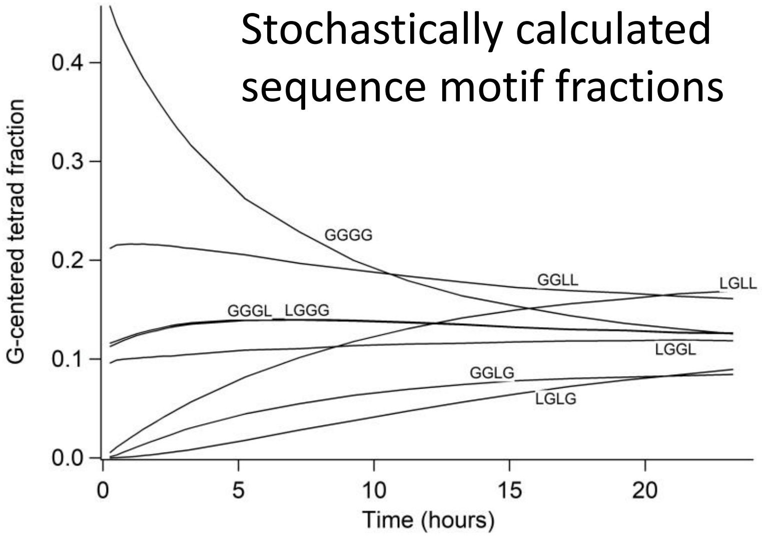


We can further leverage the stochastic model to begin to solve these additional issues

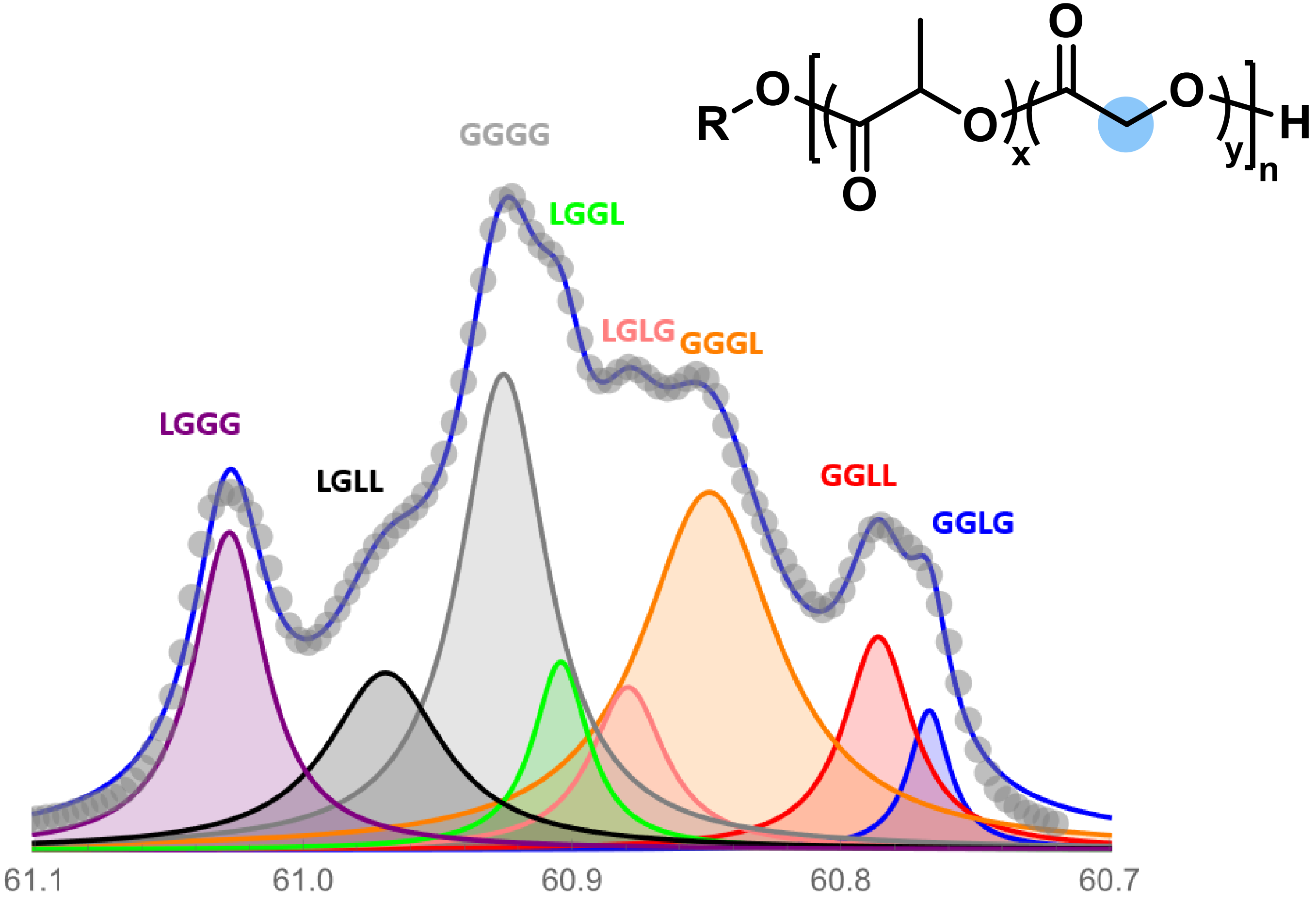
G methylene peak deconvolution areas over time



Stochastically calculated sequence motif fractions

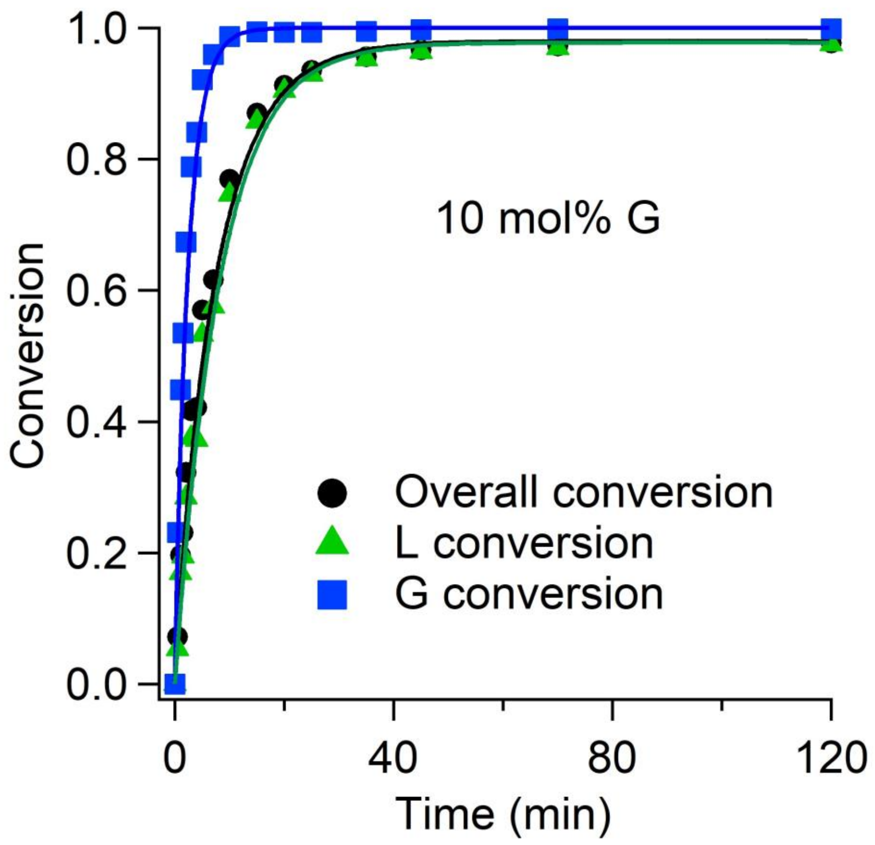


Proposed glycolyl methylene peak assignments



New fitting methods enable analysis of complex copolymerization kinetics and sequence

Reactivity ratio determination which accounts for reverse reactions



Complex 13C NMR peak assignment for improved experimental sequence measurement