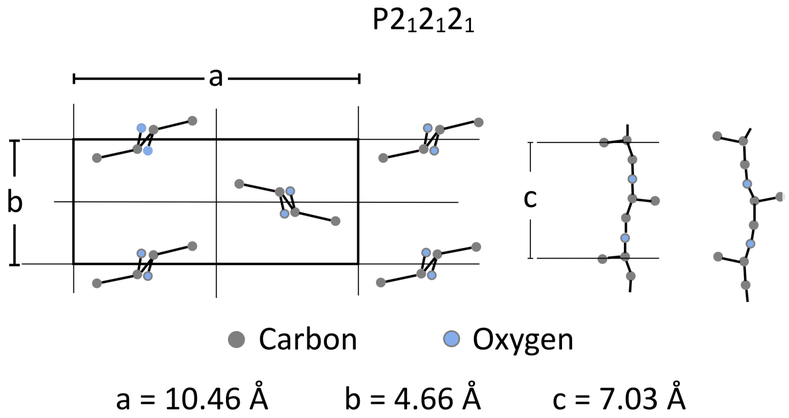

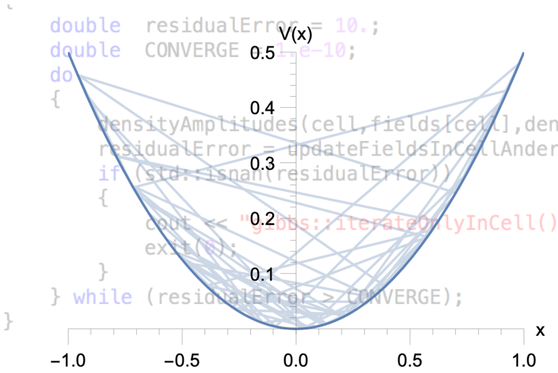

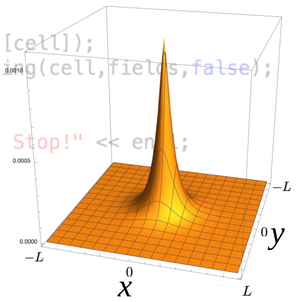

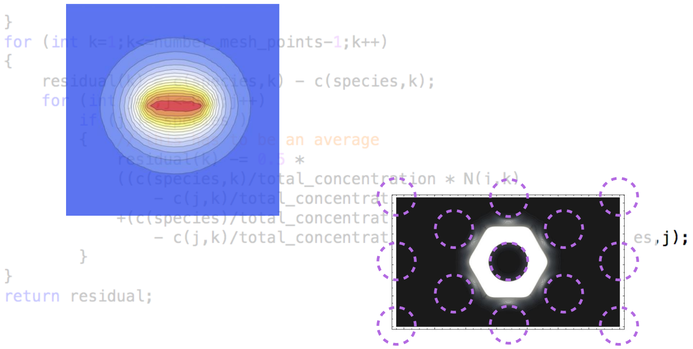

Understanding and Characterizing the Sequence Blockiness of Poly(lactide-co-glycolide)

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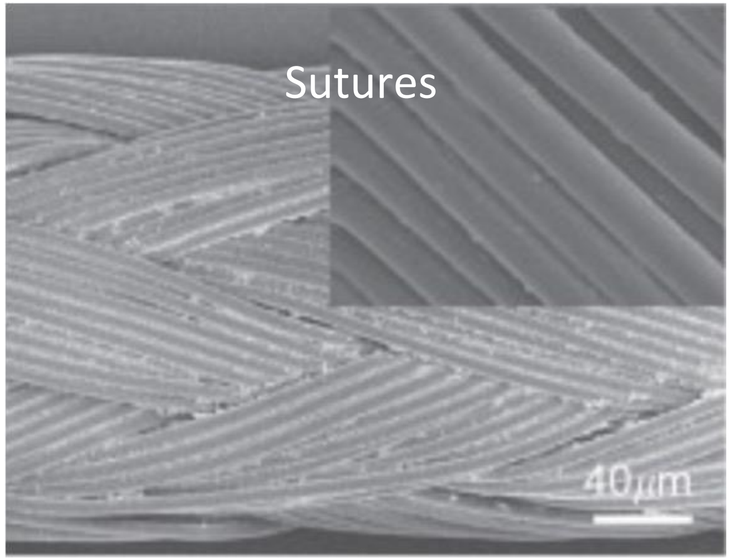
Acknowledgement

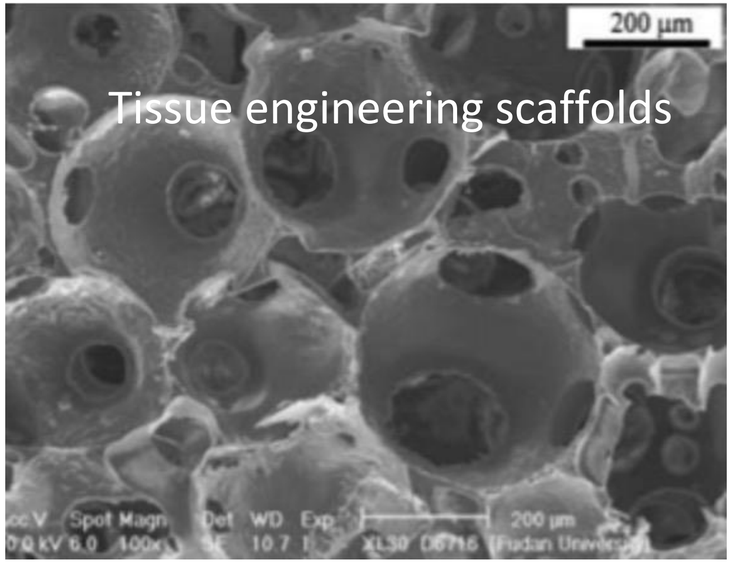
Louise Kuehster

This research was supported by the Broad Agency Announcement (BAA) contract number 75F40120C00198 from the U.S. Food and Drug Administration (FDA). The content is solely the responsibility of the authors and does not necessarily represent the official views or policies of the U.S. FDA.

Poly(lactide-co-glycolide) (PLGA) is an important biomedical material used in drug delivery

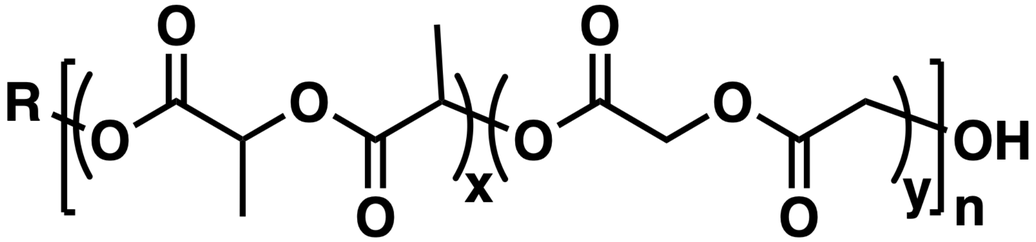


Antimicrobial and Biodegradable PLGA Medical Sutures with Natural Grapefruit Seed Extracts. Mater. Lett. 2013, 95, 40–43. https://doi.org/10.1016/j.matlet.2012.12.090.



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

Poly(lactide-co-glycolide) (PLGA) is used in many name-brand drug products

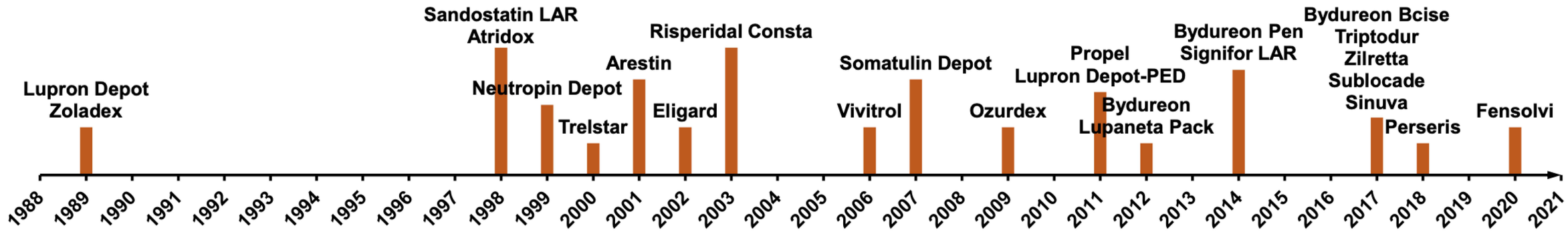
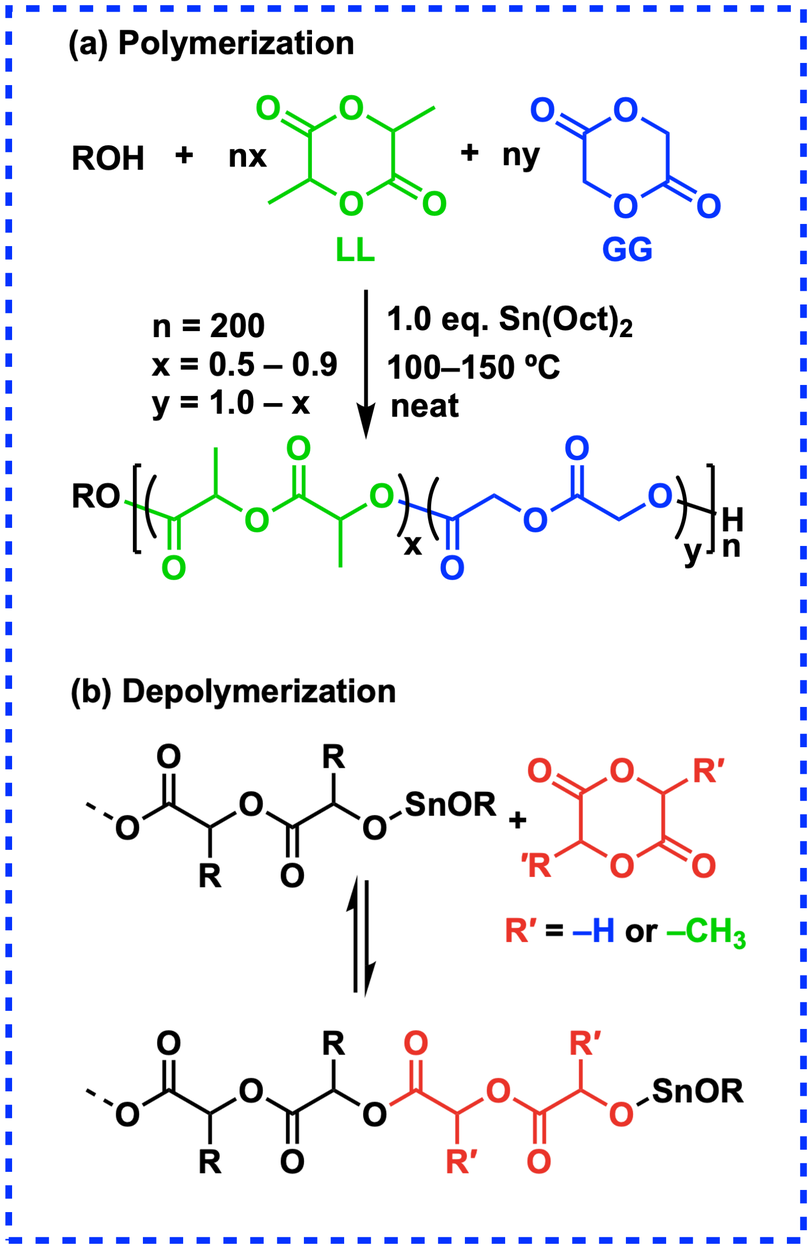


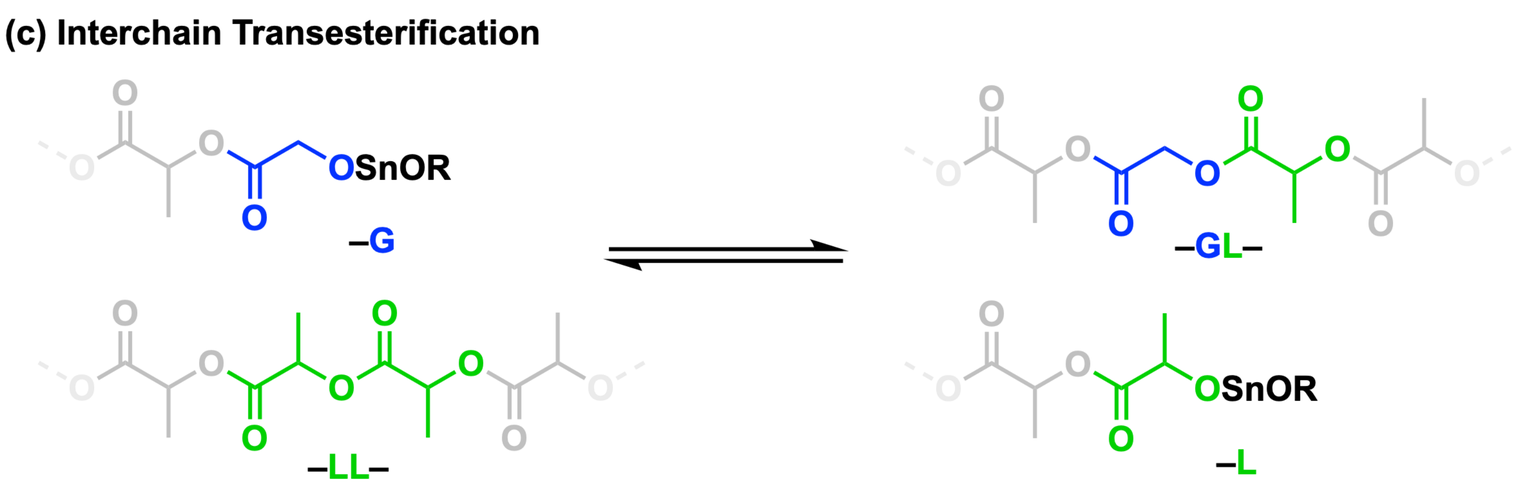
Figure credit Dr. Mark Costello

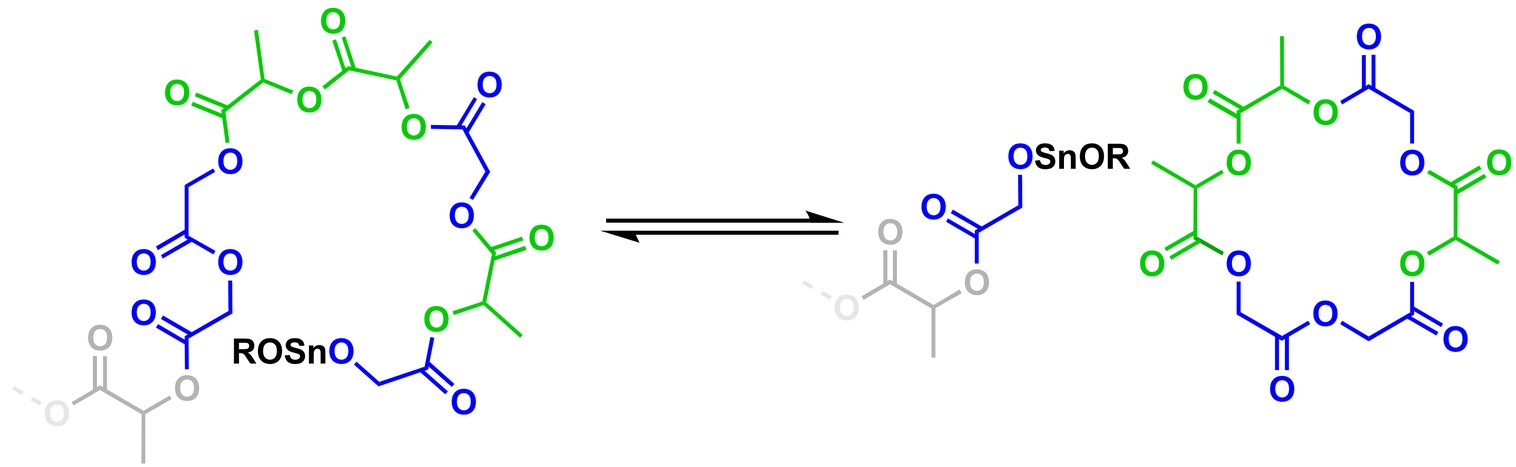
- 25 FDA–approved, PLGA–based injectable formulations
- Only one PLGA–based product is available despite many products existing past their window of patent protection and market exclusivity
- **PLGA is a complex material and we need new conceptual tools to establish sameness in terms of lactide and glycolide sequencing which impacts properties.**

Lim YW, Tan WS, Ho KL, Mariatulqabtiah AR, Abu Kasim NH, Abd Rahman N, et al. Challenges and Complications of Poly(lactic-co-glycolic acid)-Based Long-Acting Drug Product Development. Pharmaceutics. 2022;14(3). Wang Y, Qin B, Xia G, Choi SH. FDA's Poly (Lactic-Co-Glycolic Acid) Research Program and Regulatory Outcomes. AAPS J. 2021;23(4):92.

The *equilibrium* copolymerization of lactide and glycolide is complex. Standard models of copolymerization do not work







Reversibility alone renders the problem intractable using traditional deterministic mathematical modeling methods

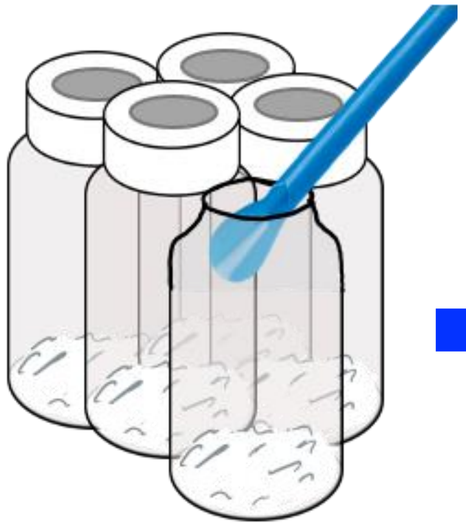
Challenge: A *reversible* copolymerization requires 2^n coupled linear ordinary differential equations for a minimally correct description of the system (w/o transesterification)

- We must know the concentration of each unique sequence in order to describe reversibility accurately
- *ca.* 10^{30} equations for n = 100 (w/o molecular weight dispersity)?!?!
- **We need new conceptual and computational tools to understand this system**

$$c_{P_{GG}} = c_{P_{GGGG}} + c_{P_{LLGG}} = c_{P_{GGGGGG}} + c_{P_{GGLLGG}} + c_{P_{LLGGGG}} + c_{P_{LLLLGG}} = \cdots$$

$$c_{P_{LL}} = c_{P_{GGLL}} + c_{P_{LLLL}} = c_{P_{LLGGLL}} + c_{P_{GGGGLL}} + c_{P_{GGLLLL}} + c_{P_{LLLLLL}} = \cdots$$

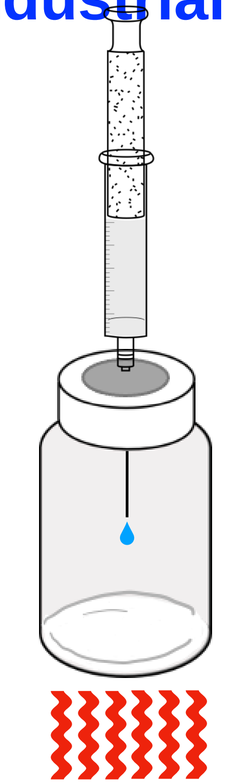
… but first, some experimental observations. Copolymerizations are done under conditions similar to those used industrially



measure monomer mixture into 12–16 vials

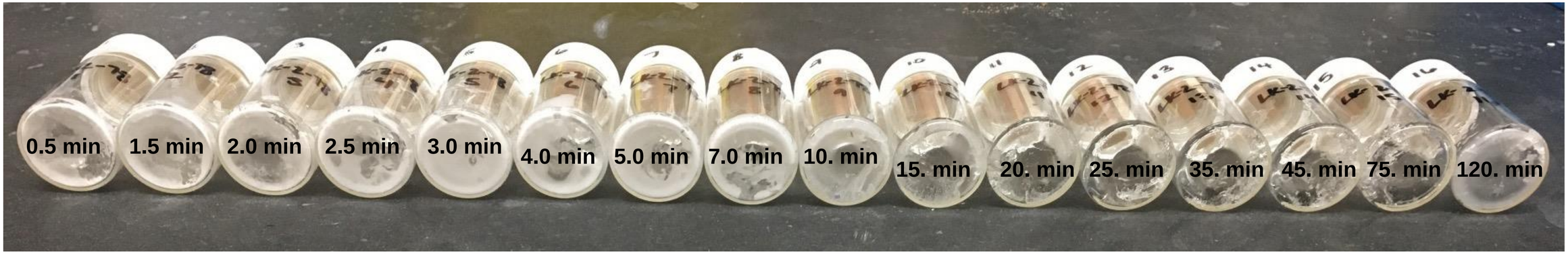
purge with nitrogen

heat to 120 °C

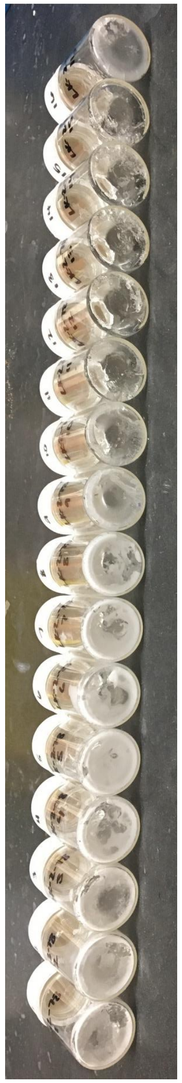


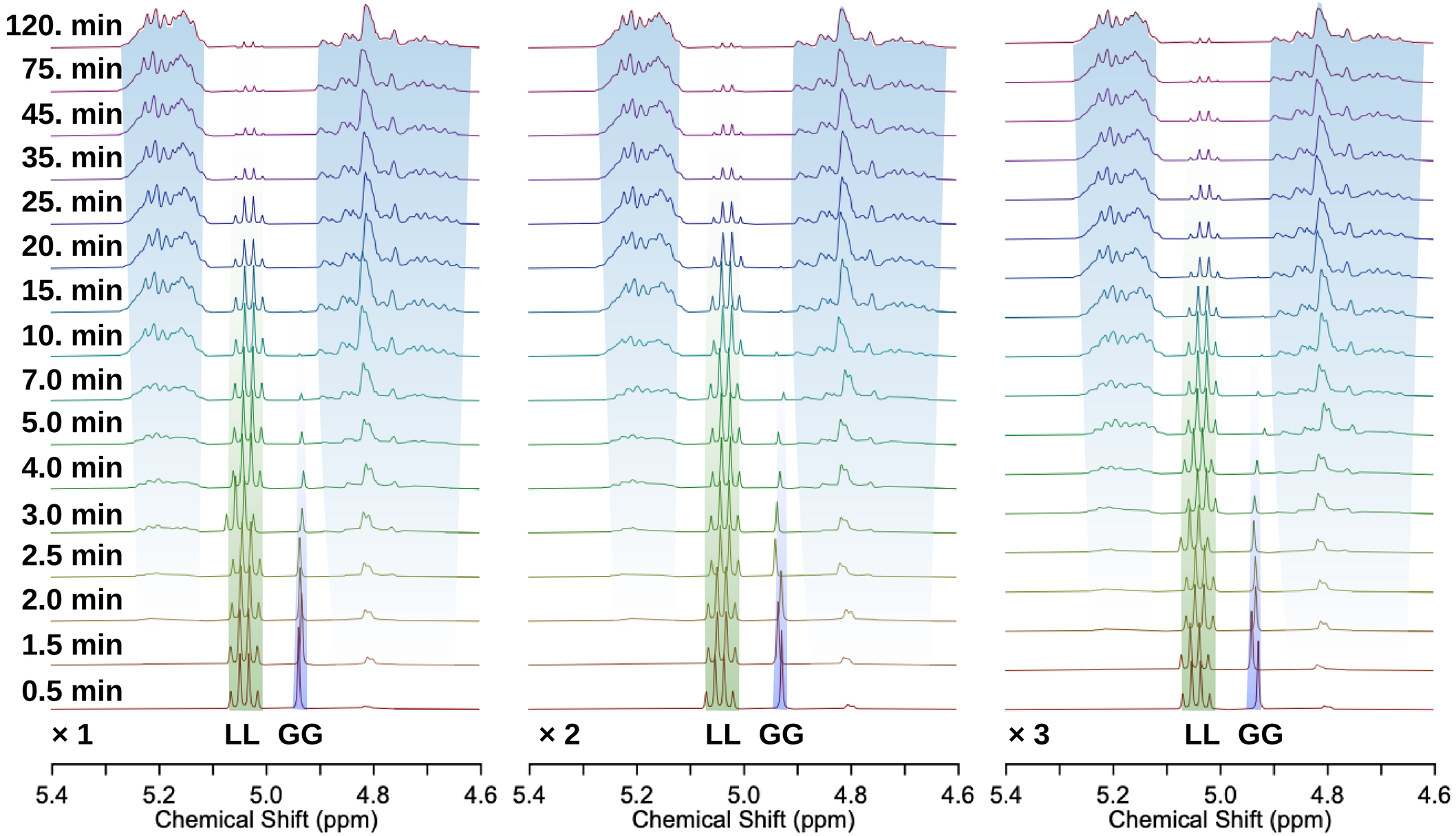
add catalyst $Sn(Oct)_2$

quench in LN2 after set time interval

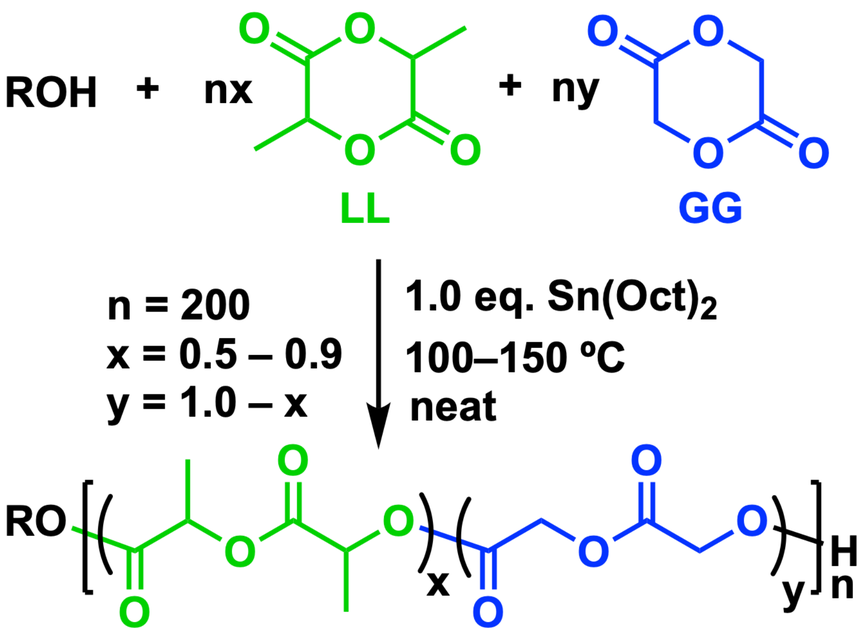


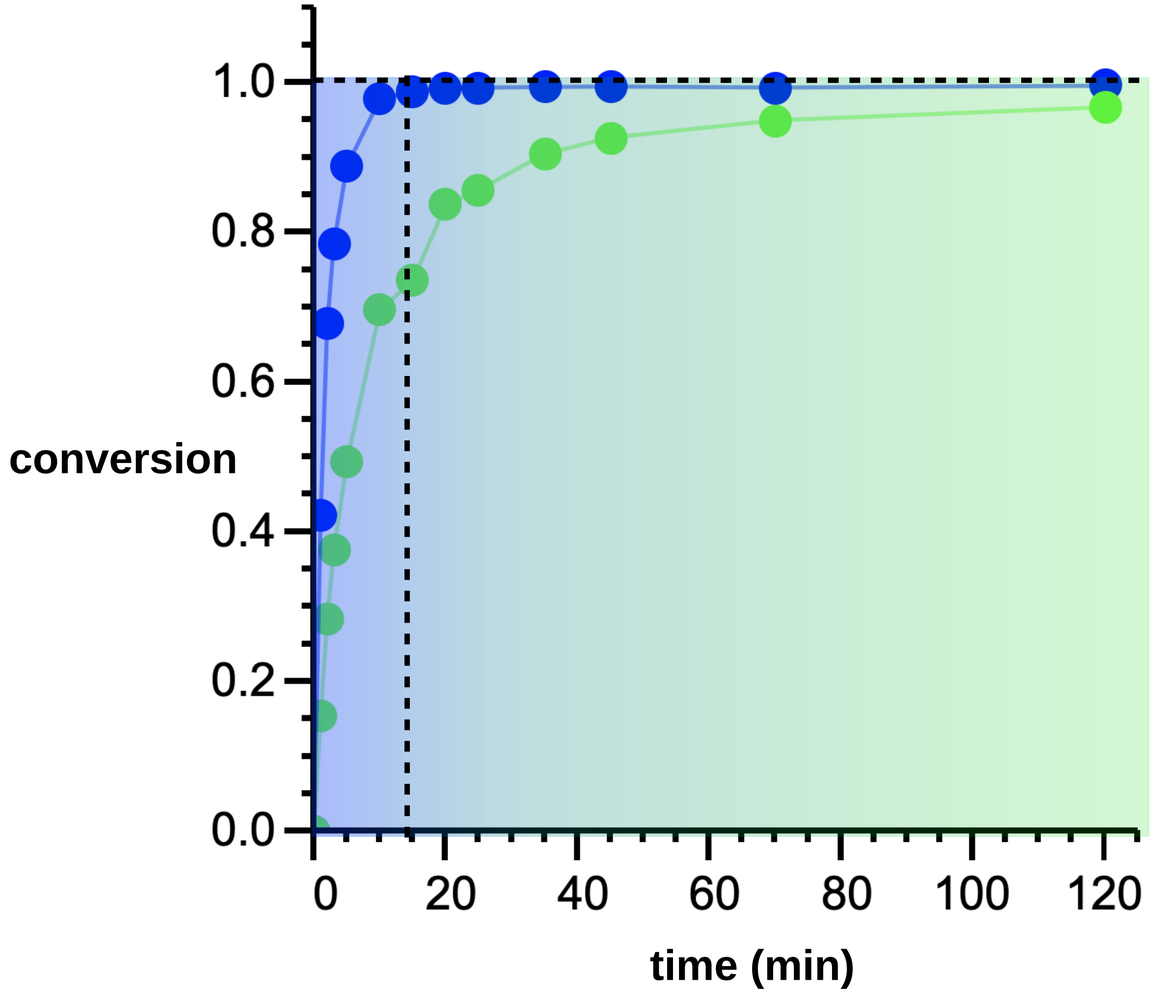
Measure composition using ^{1}H NMR spectroscopy (3× 75:25 L:G)

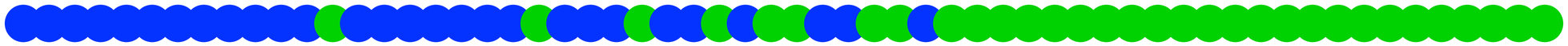




Glycolide was consumed early in the copolymerization. We expect a gradient copolymer with lactide enriched near the chain terminus







Given that glycolide reacts faster than lactide and does not exhibit reversibility, we make the following simplifications valid if $c_G << c_L$ ($[G]_0 << [L]_0$)

Initiation

$$\rho_1 = k_G c_G(t) c_I(t)$$
$$\rho_2 = k_L c_L(t) c_I(t)$$

Polymerization

$$\rho_3 = k_{GG} c_{P_G}(t) c_G(t)$$
$$\rho_4 = k_{GL} c_{P_G}(t) c_L(t)$$
$$\rho_5 = k_{LL} c_{P_L}(t) c_L(t)$$
$$\rho_6 = k_{LG} c_{P_L}(t) c_G(t)$$

Depolymerization

$$\rho_7 = k_{G-G} c_{P_{GG}}(t)$$
$$\rho_8 = k_{G-L} c_{P_{GL}}(t)$$
$$\rho_9 = k_{L-G} c_{P_{LG}}(t)$$
$$\rho_{10} = k_{L-L} c_{P_{LL}}(t)$$

Transesterification

$$\rho_{11} = k_T c_{ends} c_{esters}$$

$$\frac{dc_I}{dt} = -\rho_1 - \rho_2$$
$$\frac{dc_G}{dt} = -\rho_1 - \rho_3 - \rho_6 + \rho_7 + \rho_9$$
$$\frac{dc_L}{dt} = -\rho_2 - \rho_4 - \rho_5 + \rho_8 + \rho_{10}$$
$$\frac{dc_{P_G}}{dt} = \rho_1 - \rho_4 + \rho_6 + \rho_8 - \rho_9$$
$$\frac{dc_{P_L}}{dt} = \rho_2 + \rho_4 - \rho_6 - \rho_8 + \rho_9$$

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

Fitting a numerical model to data and estimating parameters is like any non-linear regression calculation… it just takes a little longer

SSR = sum of square residuals

The difference between your data and model (evaluated at the same points) gives you a number

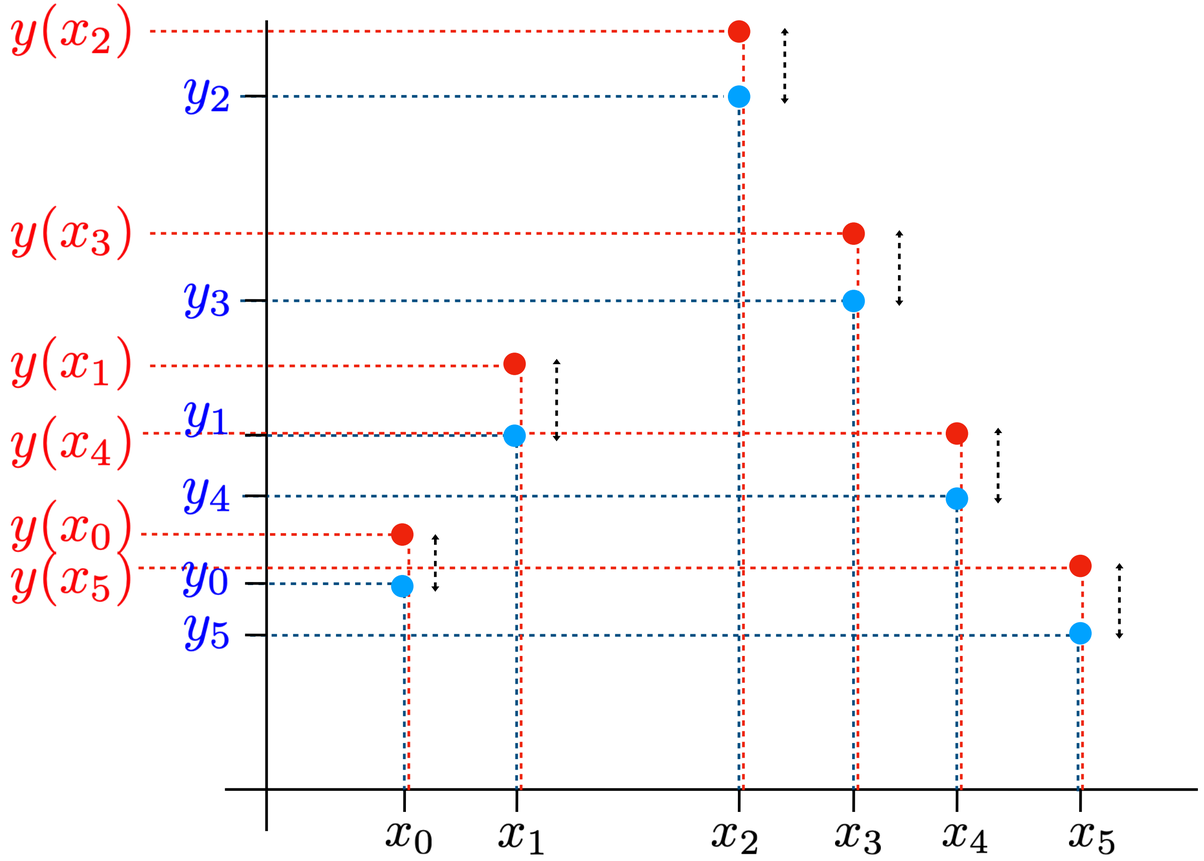
$$SSR(\mathbf{k}) = \sum_i^N (y_i - y(x_i))^2$$

a function of your model parameters – what you want to find

e.g., a linear model:

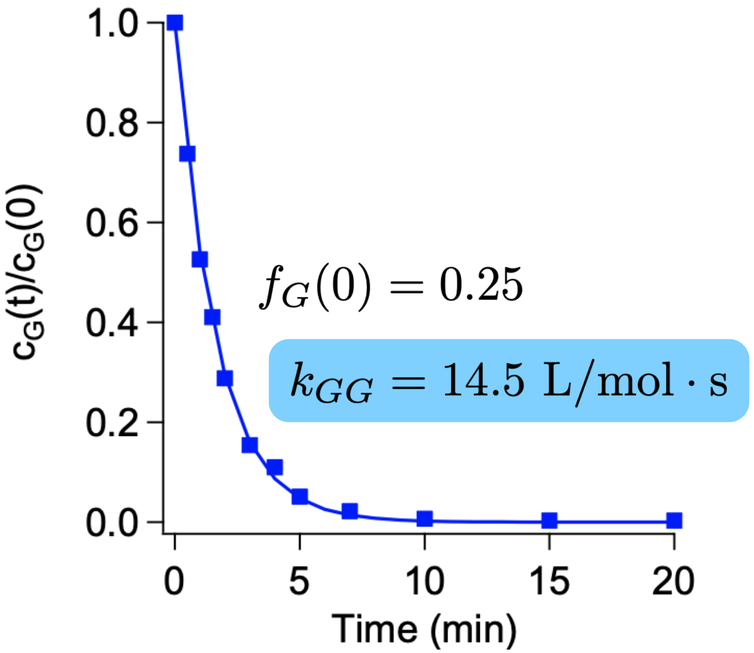
$$y(x) = mx + b$$

$$SSR(m,b) = \sum_i^N (y_i - mx_i - b)^2$$



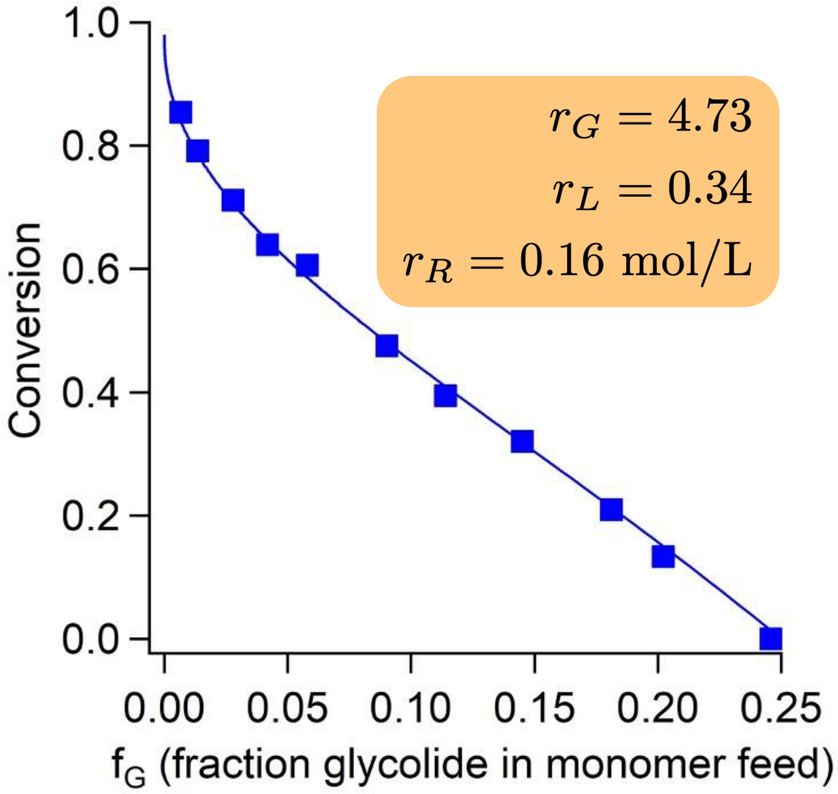
Experimental data with low glycolide concentration were fit to the simplified deterministic model – three steps yield all rate constants

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

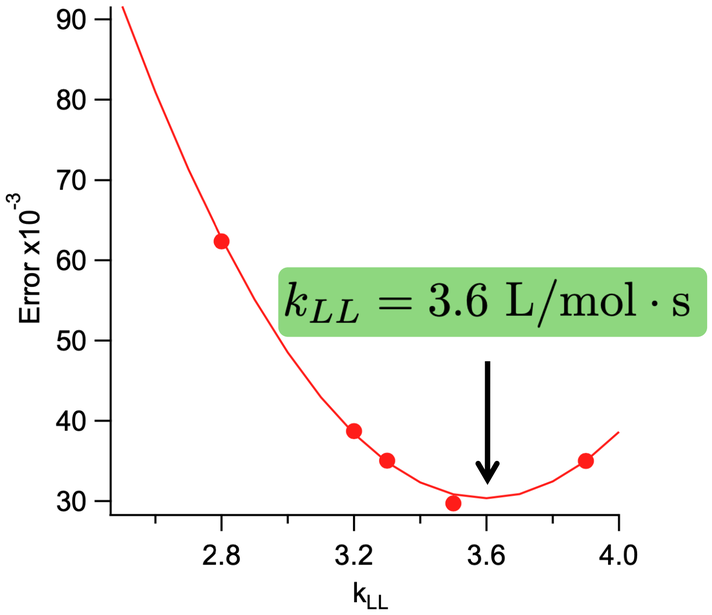


$$\frac{c_G(t)}{c_G(0)} = e^{-k_{GG}c_I(0)t}$$

*assuming non-terminal kinetics

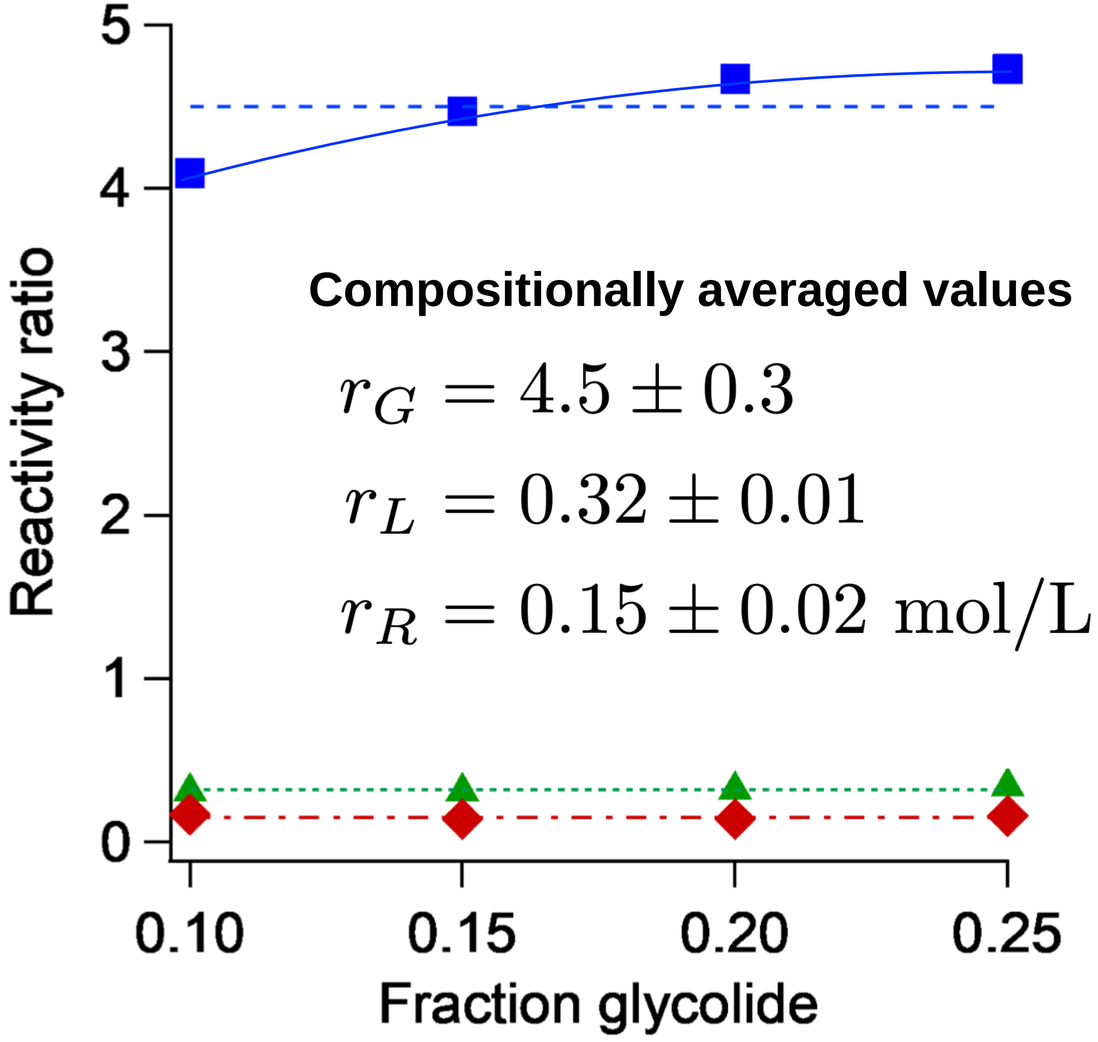


fit to numerical simplified deterministic model



quadratic fit of k_{LL} with k_{GG}, r_G, r_L, r_R held constant

The slight compositional dependence indicates that the simplified deterministic model is missing something important



- Assumption 1: Lactide-only on chain end
- Assumption 2: Glycolide irreversible
- Assumption 3: Effects of transesterification not included

Another numerical approach that has fewer assumptions is needed to help us figure this out

Computational model II: Stochastic model regression (SMR)

Challenge: A *reversible* copolymerization requires 2^n coupled linear ordinary differential equations for a correct description of the system (w/o transesterification)

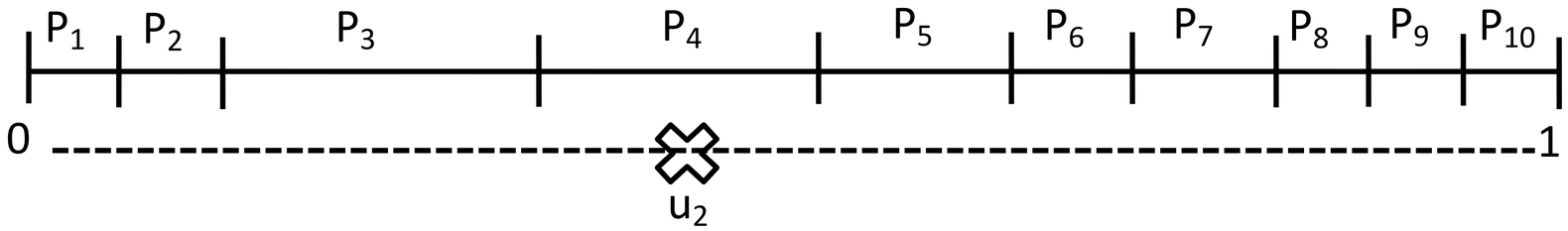
- We must know the concentration of each unique sequence in order to describe reversibility accurately
- *Ca.* 10^{30} equations for n = 100 (w/o dispersity)?!?!
- **We need new conceptual and computational tools to understand this system**

- A stochastic (probabilistic) kinetic calculation is perfectly suited for the PLGA problem
  - Gillespie algorithm:
  - The probability of reaction *i* ($\rho_{i\ [=]\ rate\ (M/s)}$) occurring:

$$P_i = \frac{\rho_i}{\sum_j \rho_j}$$

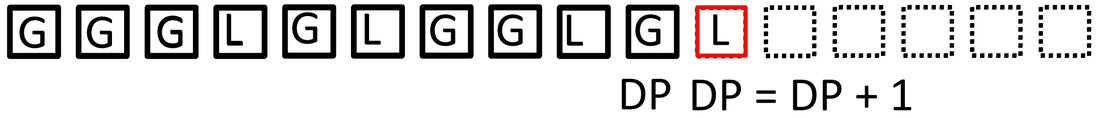
  - All integer arithmetic with a fixed number of possible polymer chains. Simulation is done on a small volume of the polymerization (*e.g.* 10^{-19} L with 8000 polymer chains)
  - We generate two random number at every within [0,1], s_1 and s_2. s_1 selects the reaction, s_2 sets the sojourn time τ

Computational model II: Stochastic model regression (SMR)



$$\rho_4 = k_{GL} c_{P_G}(t) c_L(t)$$

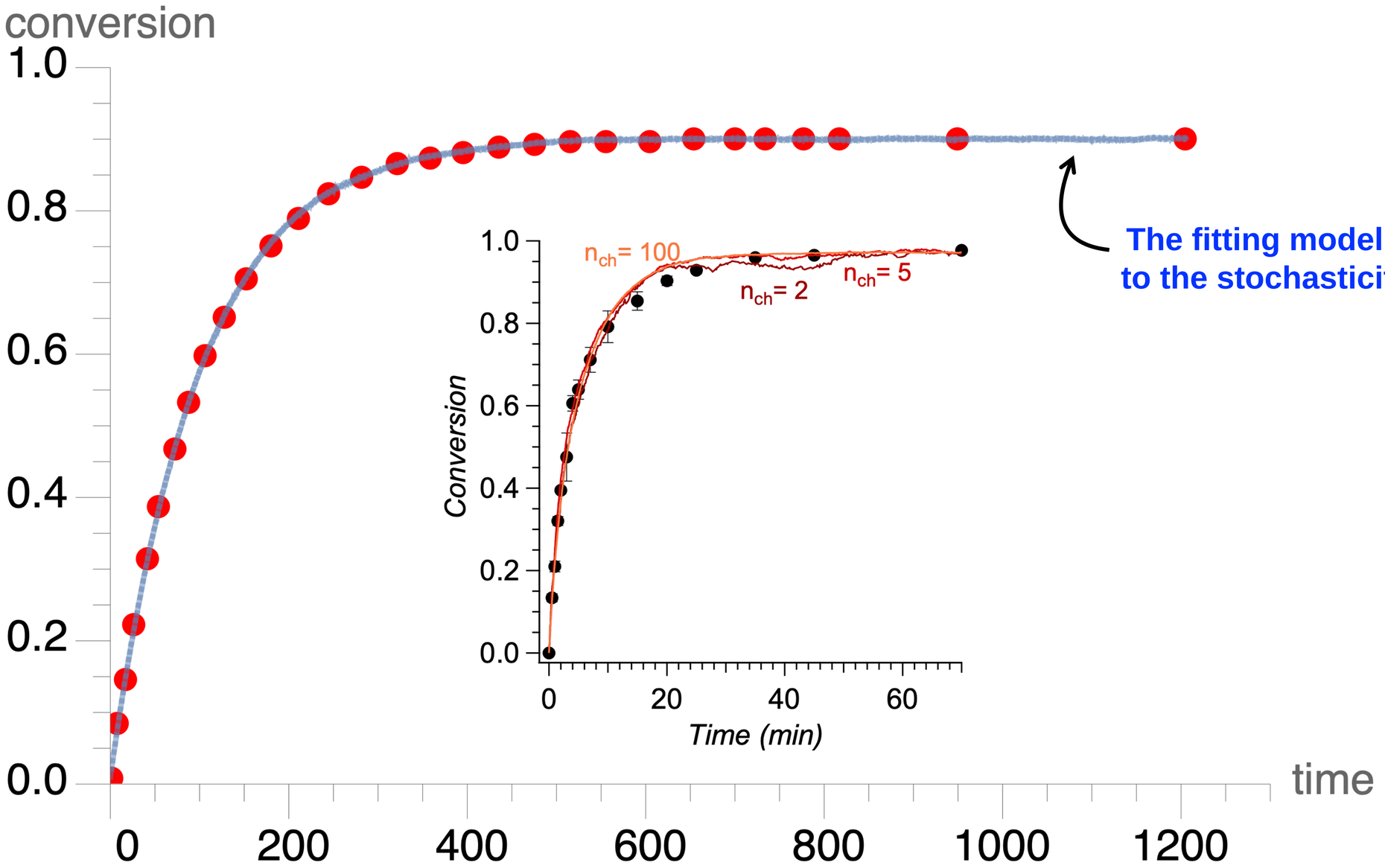
Generate s_3 [1,N_{chains}] until appropriate end-group (G) found



Update simulation time: $\tau = -\frac{\log s_2}{\sum_j r_j}$

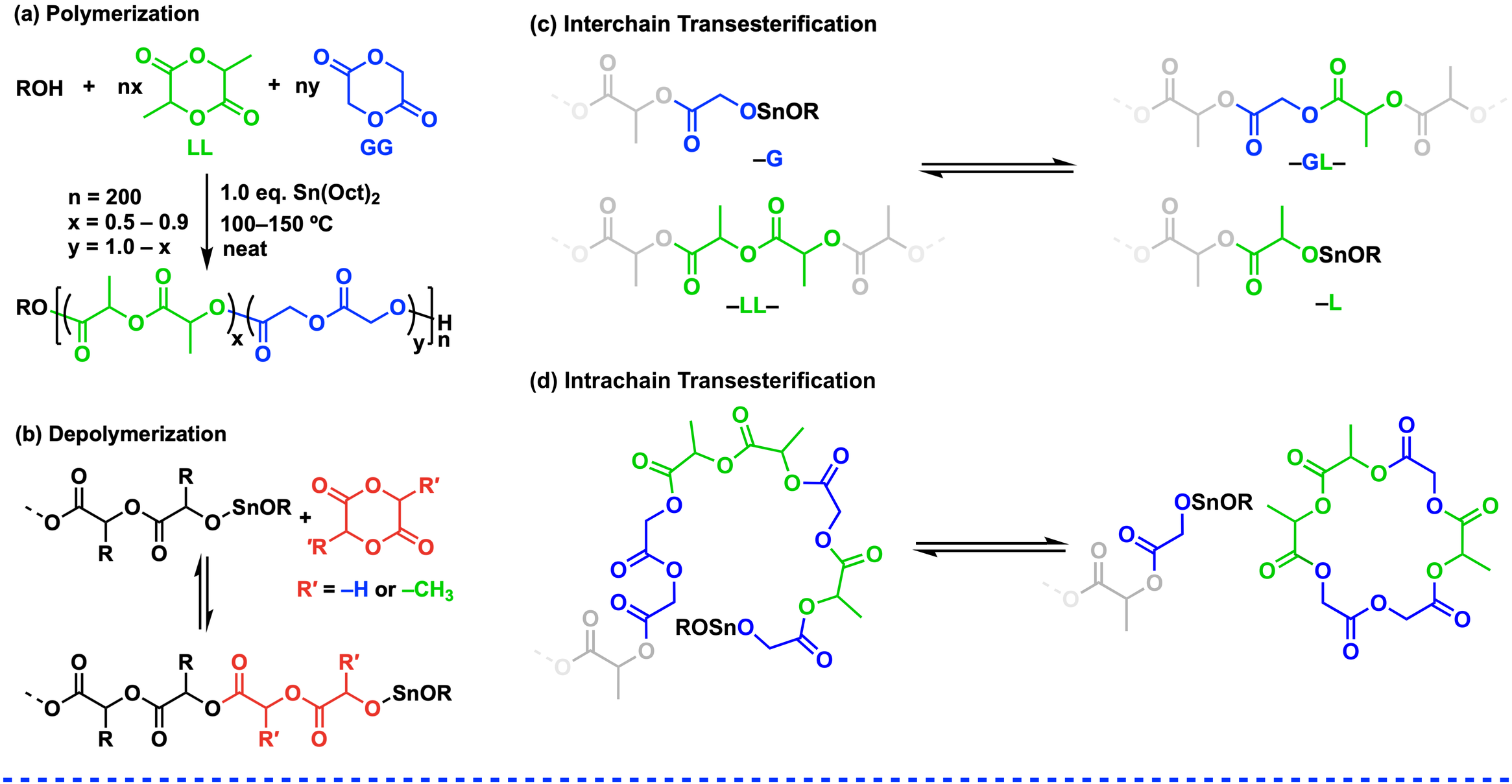
$$t = t + \tau$$

Being able to fit experimental data to a stochastic model allows one to learn quantitative information about inherently non-deterministic systems



The fitting model has noise due to the stochasticity of the model

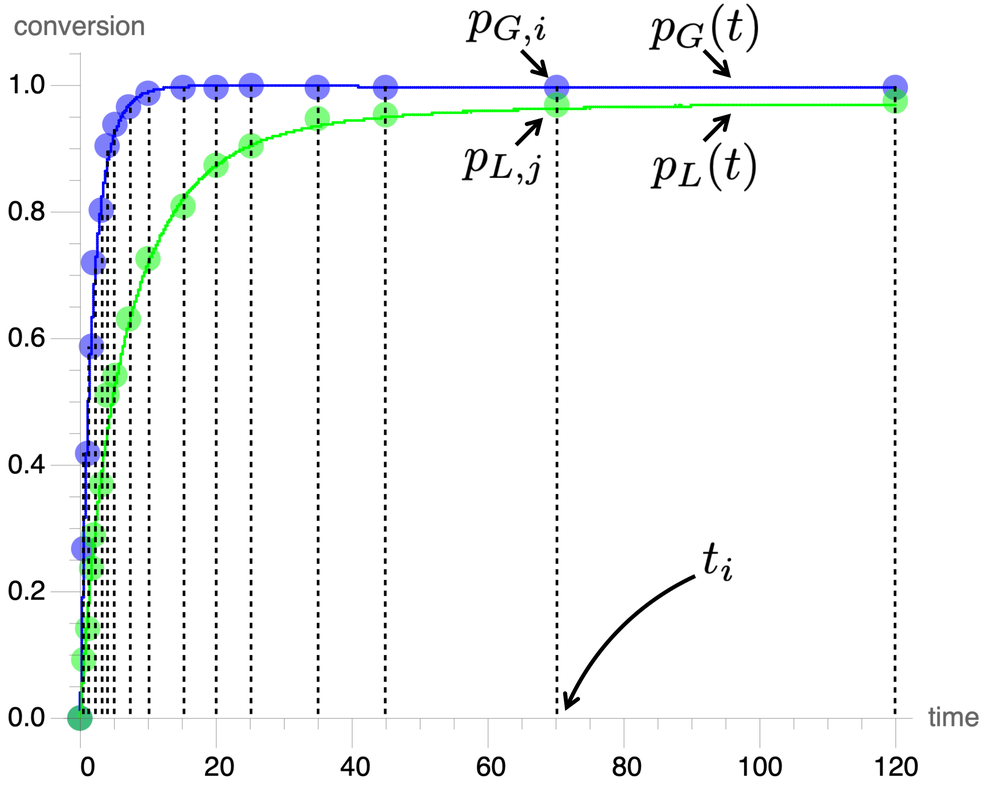
The new stochastic model allows us to incorporate the full complexity of the lactide/glycolide system. We will fit directly with the full model



Sum of squared residuals (SSR) is the function we seek to minimize. The lactide/glycolide system has nine parameters

$$SSR(k_{GG}, k_{GL}, k_{LL}, k_{LG}, k_{G-G}, k_{G-L}, k_{L-L}, k_{L-G}, k_T)$$

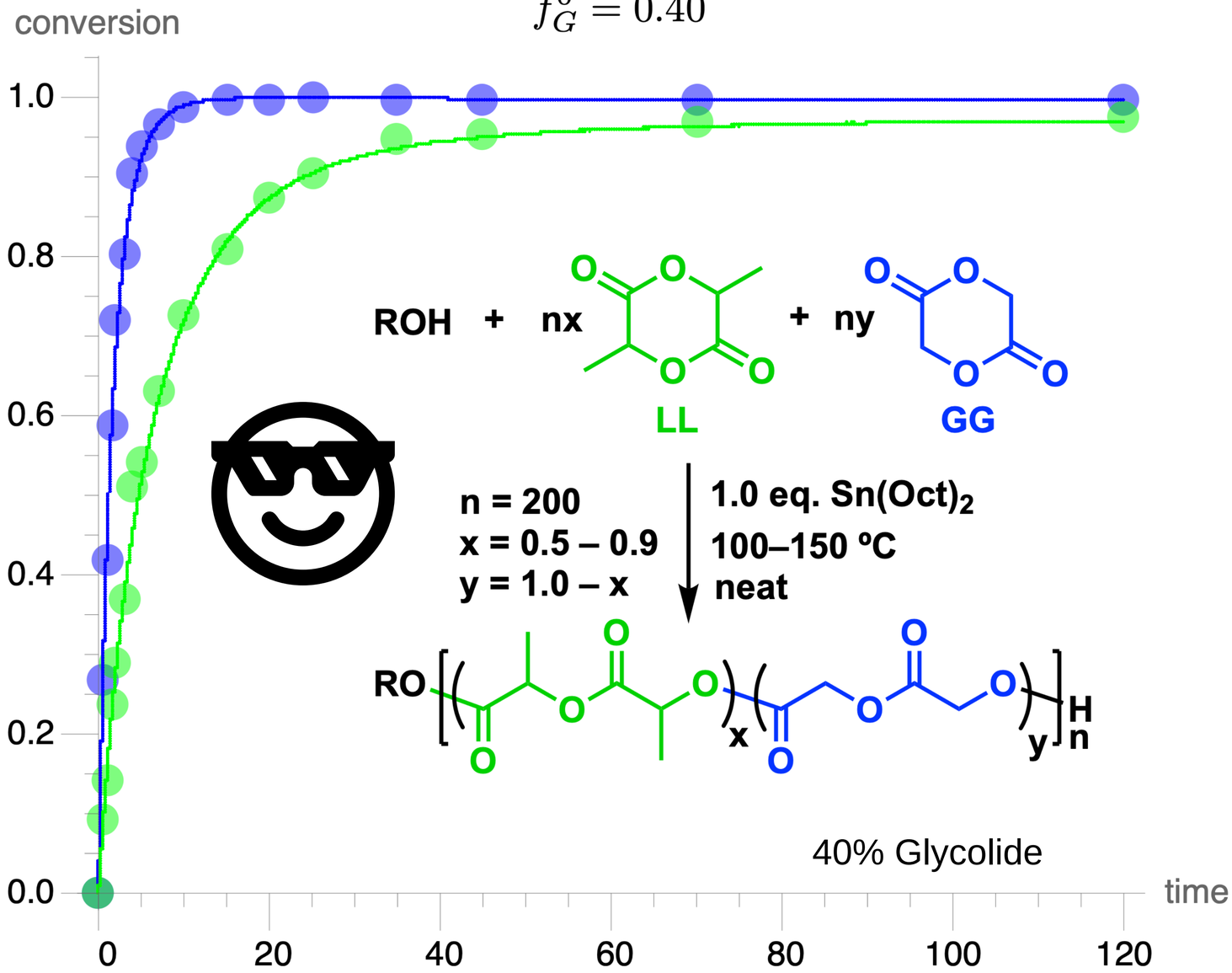
$$= \sum_{i=1}^{n_p} \left(p_G(t_i) - p_{G,i}\right)^2 + \sum_{j=n_p+1}^{n_p+n'_p} \left(p_L(t_{j-n_p}) - p_{L,j-n_p}\right)^2$$



- Match data and model (also data) using interpolation
- Whole system (L and G data) fit simultaneously
- Can change n_{chains} for higher speed, or higher precision
- Downhill simplex method used instead of derivative information
- Starting point important – we have a great starting point from our simplified deterministic fits
- Multiple starting points until SSR is below threshold

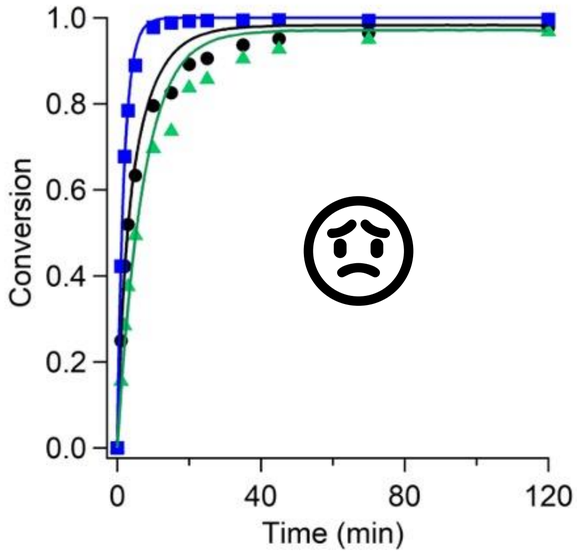
Fitting the fully parameterized stochastic model based on our earlier parameter estimates yields excellent fits to experimental data

$f_G^0 = 0.40$

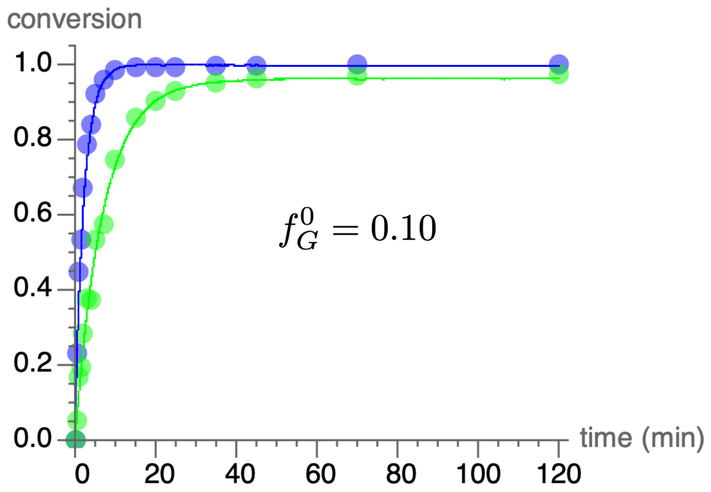


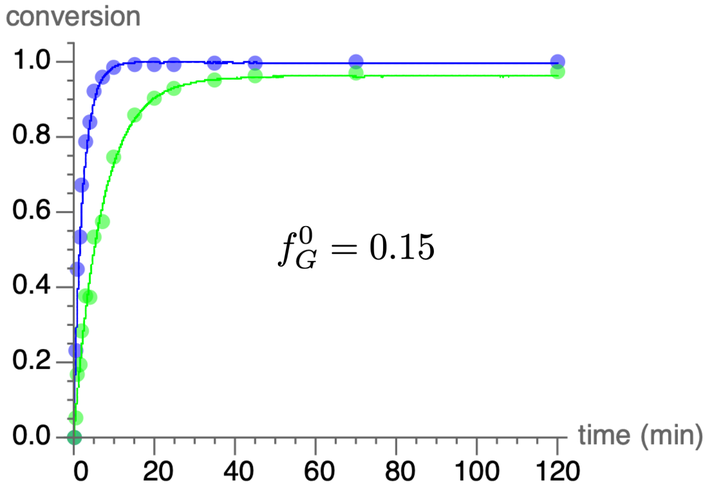
With simplified deterministic parameters

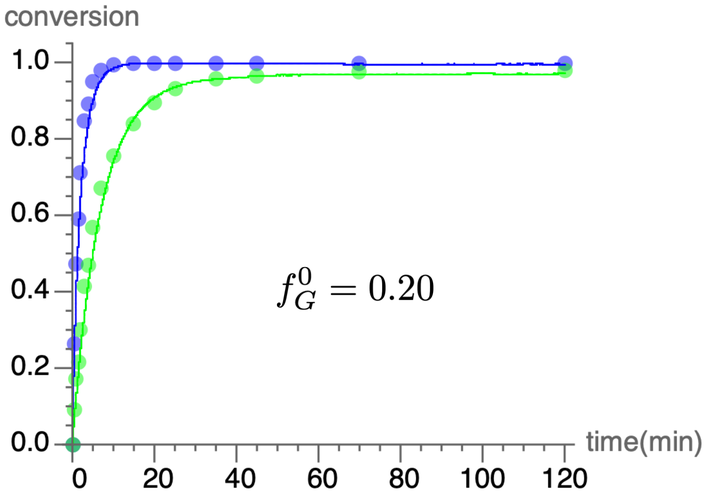
$f_G^0 = 0.40$

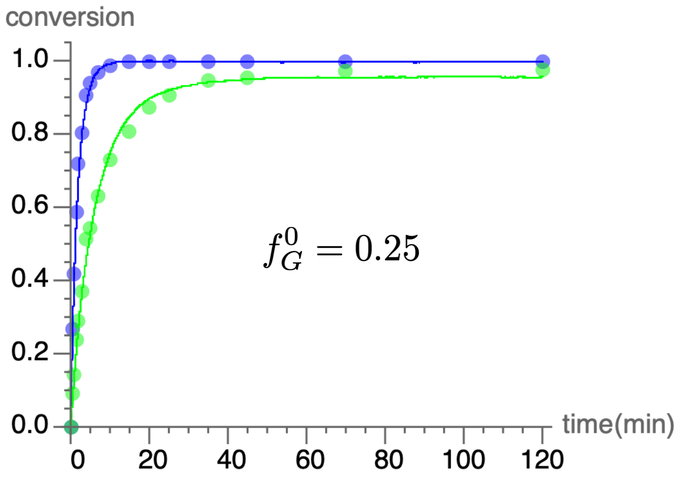


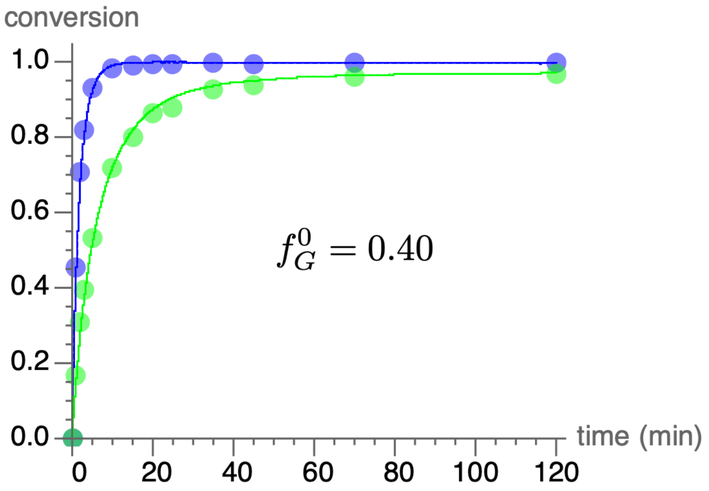
Fitting the fully parameterized stochastic model based on our earlier parameter estimates yields direct fits to experimental data



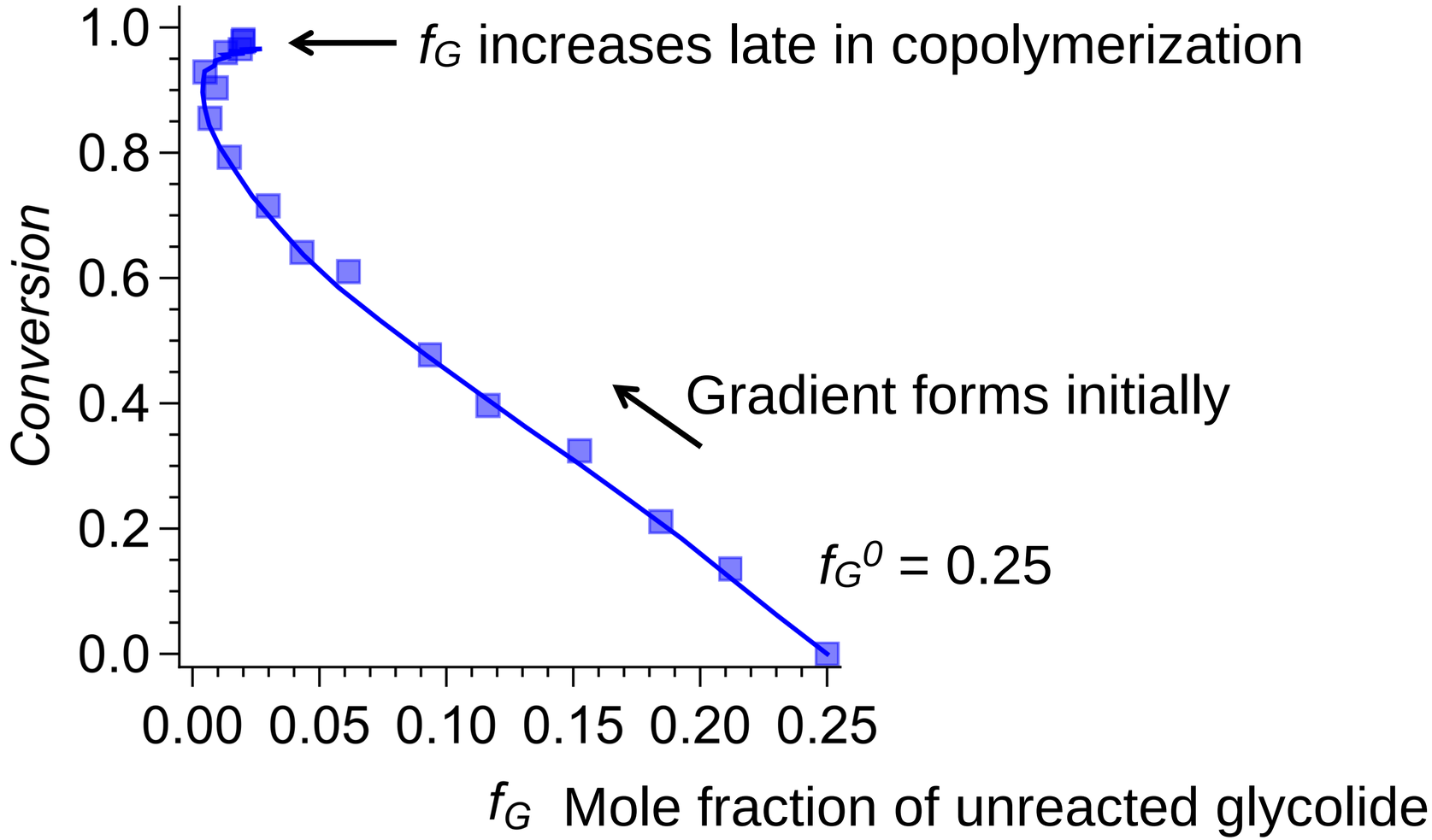








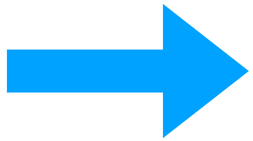
Fitting the fully parameterized stochastic model based on our earlier parameter estimates yields direct fits to experimental data



The parameterized stochastic model is now an oracle to answer any question we pose to the system

Simplified deterministic fit

$$r_G = 4.5 \pm 0.3$$
$$r_L = 0.32 \pm 0.01$$
$$r_R = 0.15 \pm 0.02 \text{ mol/L}$$

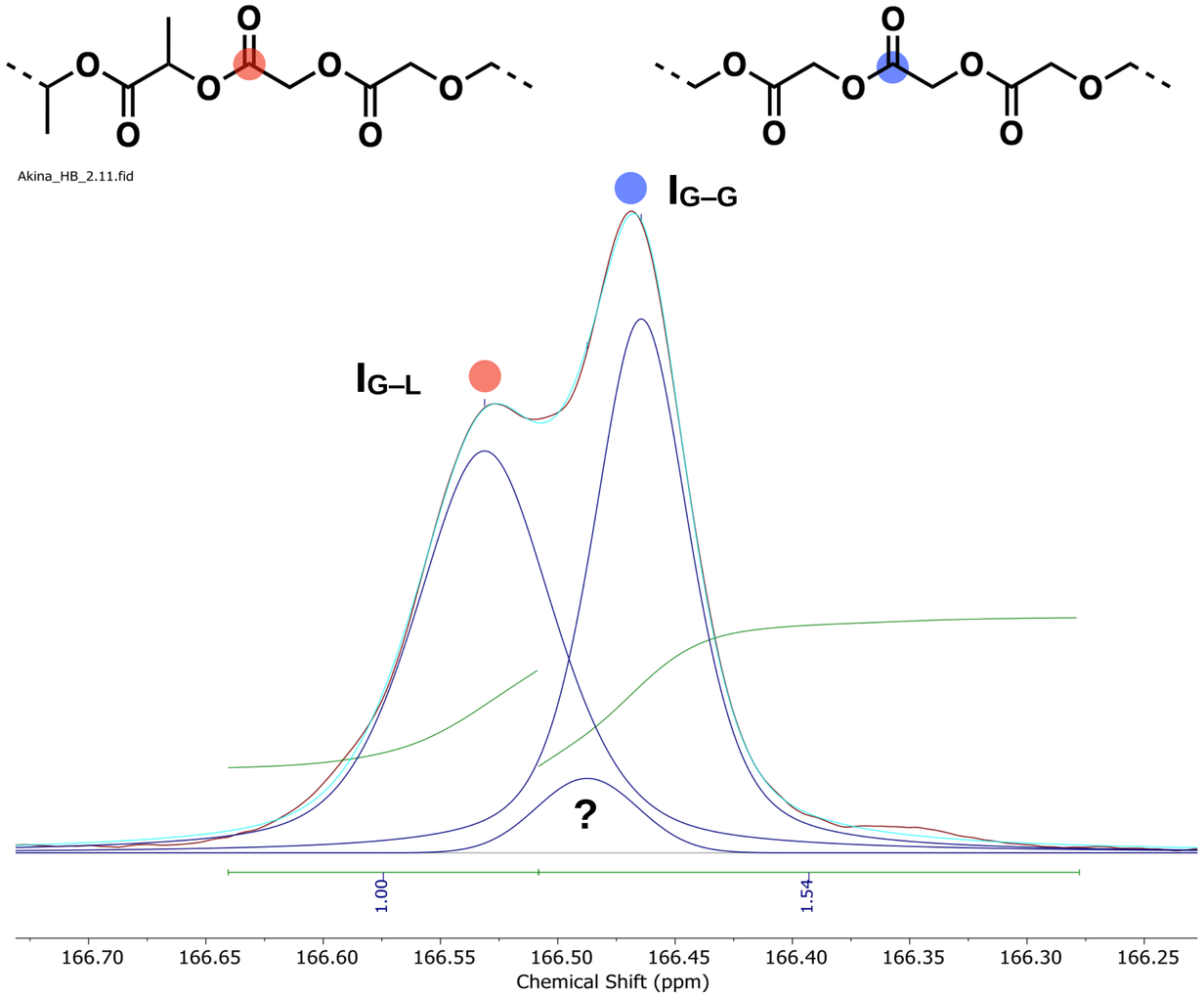


Full stochastic model fit – The PLGA oracle!

$$r_G = 4.426 \pm 0.886$$
$$r_L = 0.342 \pm 0.042$$
$$r_{G-G} = 0.041 \pm 0.014 \text{ mol/L}$$
$$r_{G-L} = 0.288 \pm 0.151 \text{ mol/L}$$
$$r_{L-L} = 0.286 \pm 0.057 \text{ mol/L}$$
$$r_{L-G} = 0.075 \pm 0.015 \text{ mol/L}$$
$$k_T = 0.010 \pm 0.009 \text{ L/mol} \cdot \text{s}$$

This is the machine we need to establish a test for blockiness

Let’s look at the “blockiness” definition and how it’s defined and currently measured using ^{13}C NMR spectroscopy

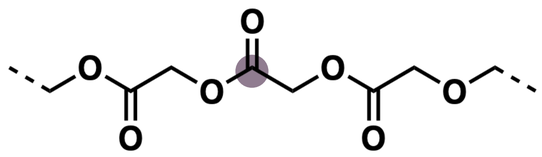


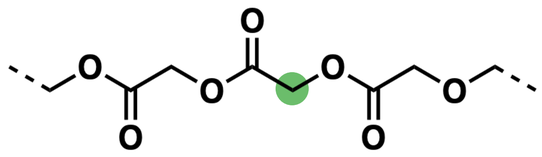
$$R_{cms} = \frac{I_{G-L}}{I_{G-G}}$$

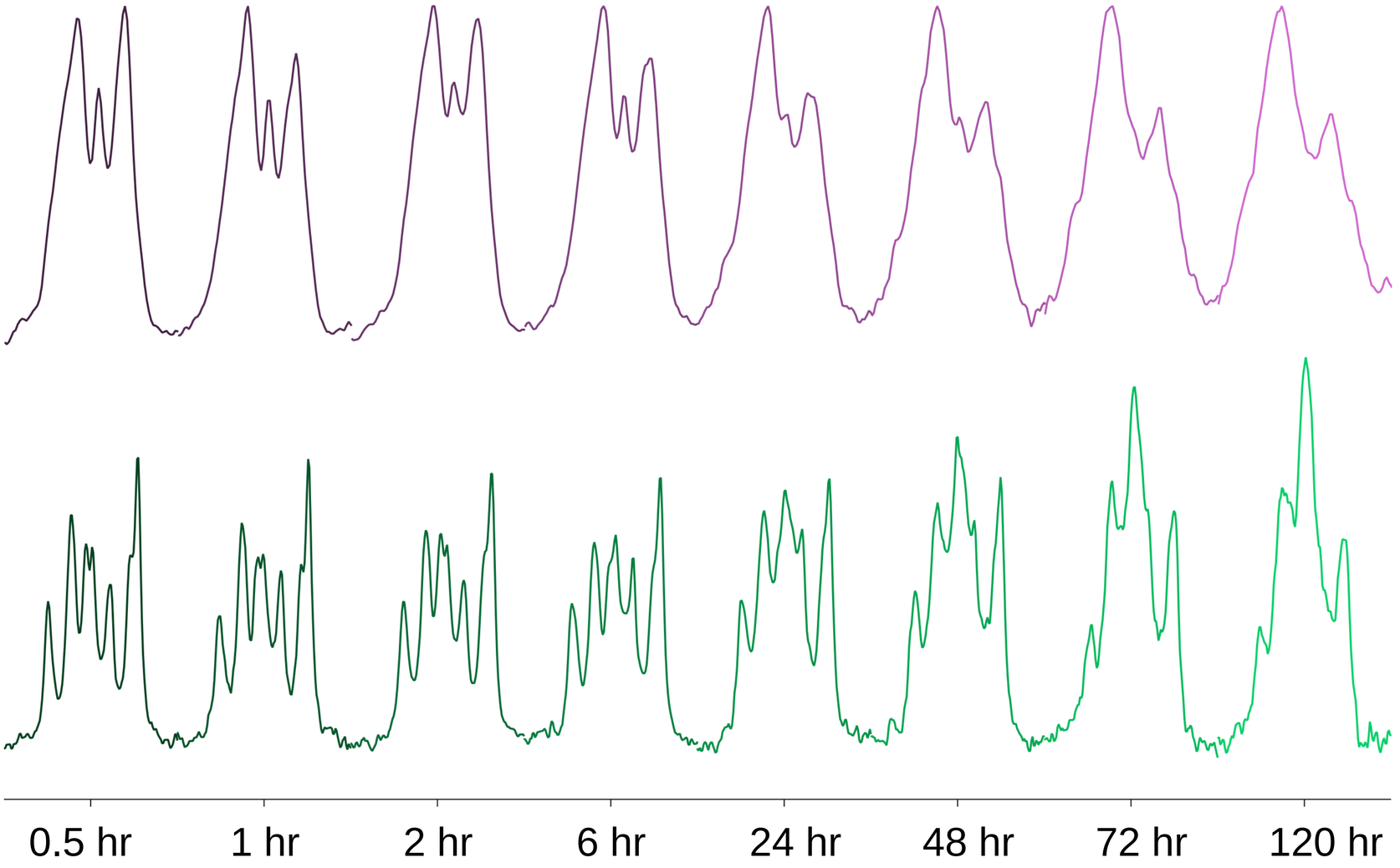
$$R_c = \frac{I_{G-G}}{I_{G-L}}$$

Sun, Jing, Jennifer Walker, Moritz Beck-Broichsitter, and Steven P. Schwendeman. “Characterization of Commercial PLGAs by NMR Spectroscopy.” *Drug Delivery and Translational Research*, August 20, 2021. https://doi.org/10.1007/s13346-021-01023-3.

^{13}C NMR spectroscopy data reveals changes in blockiness during co-polymerization



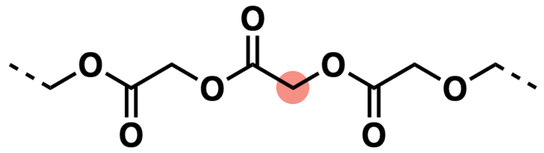




G-centered –CH_2– is less sensitive to stereochemistry

Dr. Michael Bingham

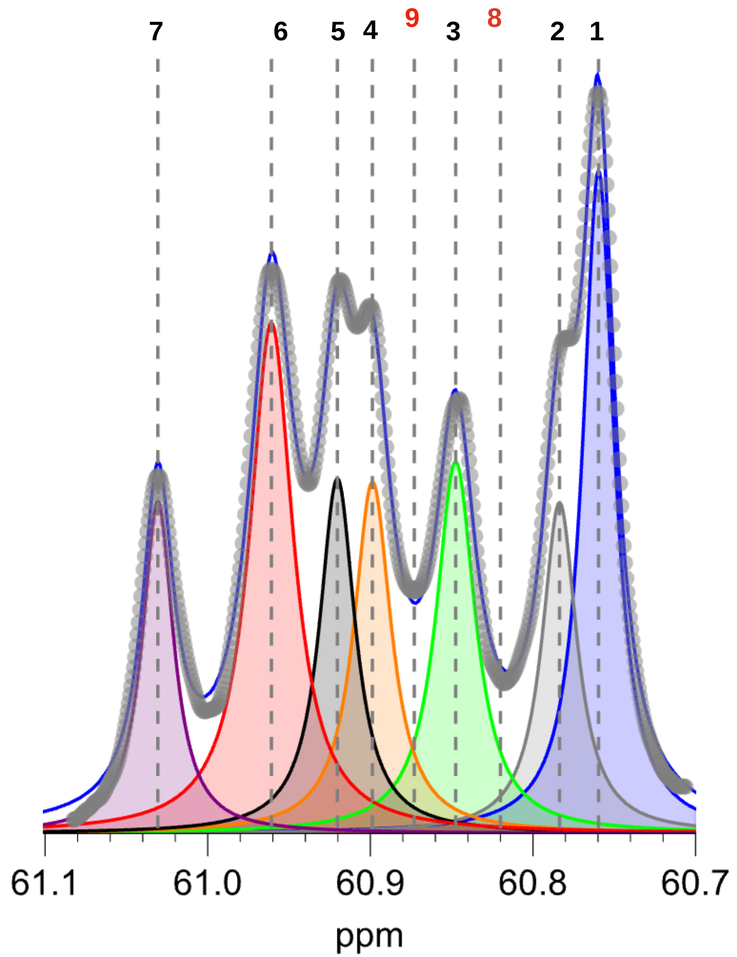
Analysis of ^{13}C NMR spectroscopy data can now be used to quantify sequence given the detail that the stochastic model enables



–CH₂– on glycolide repeat unit

Peaks that appear at later times in the copolymerization

Peaks present at start of copolymerization reaction

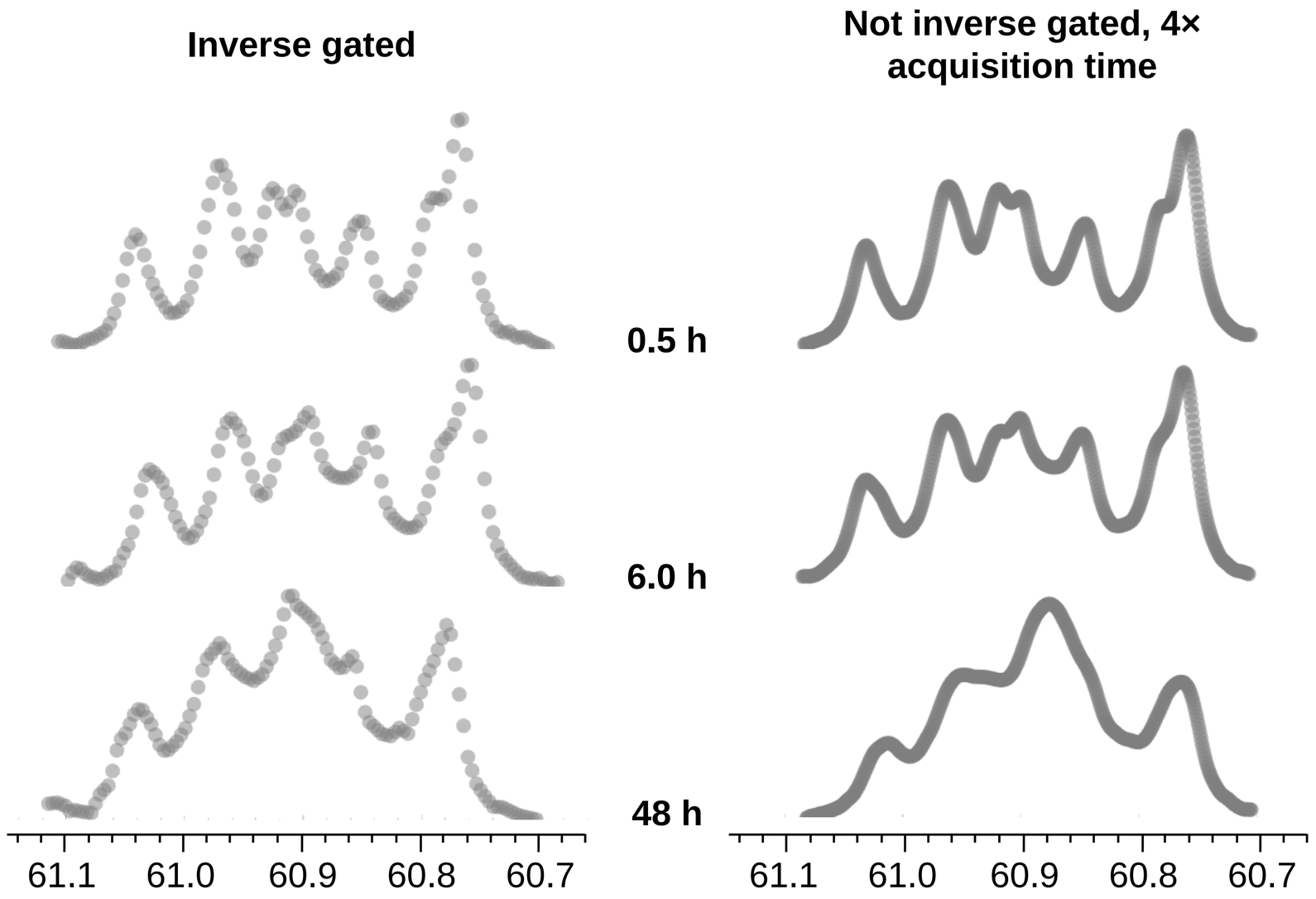


Deconvolutional fit function to each peak

$$P(x_{ppm}; A, \bar{x}_{ppm}, \sigma_x) = \frac{A\sigma_x}{2\pi\left((x_{ppm} - \bar{x}_{ppm})^2 + \frac{\sigma_x}{4}\right)}$$

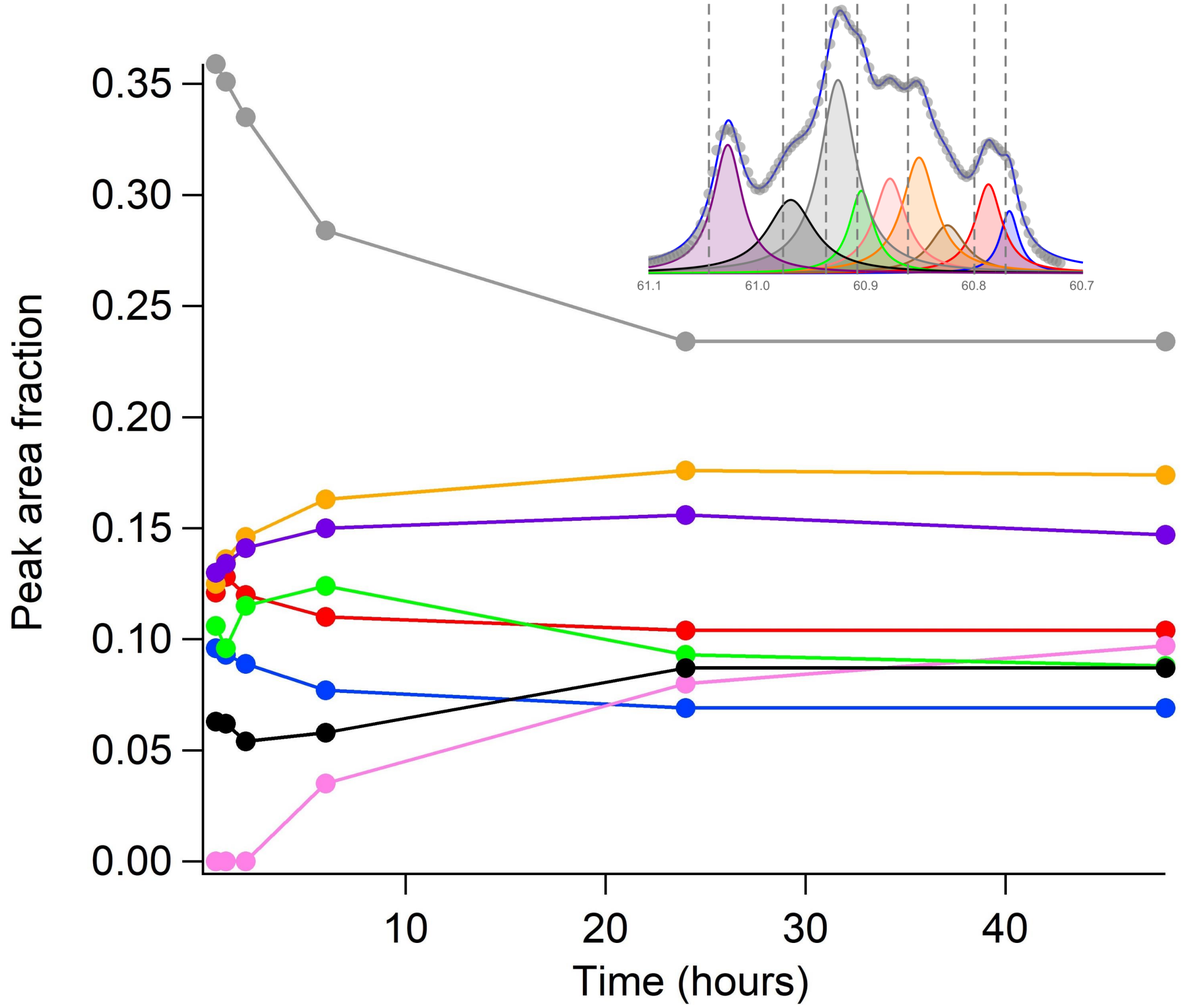
$$\int_{-\infty}^{\infty} P(x_{ppm}; A, \bar{x}_{ppm}, \sigma_x) dx_{ppm} = \frac{A}{\sigma_x}$$

Practical considerations for ^{13}C NMR spectroscopy measurements of glycolide-centered CH_2

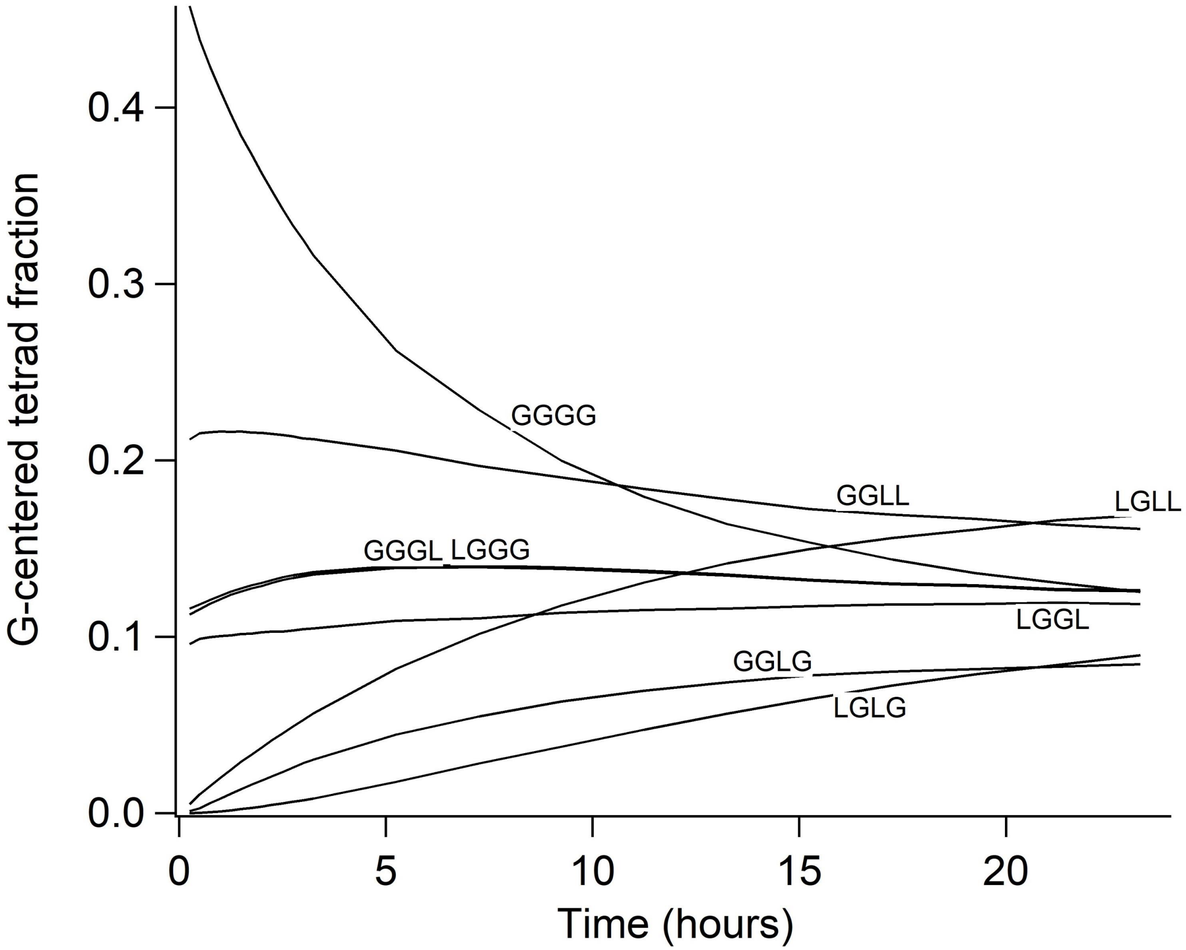


Compare ^{13}C NMR signals to stochastic prediction of tetrads

^{13}C NMR signal integrals - likely tetrads (XXXX)?

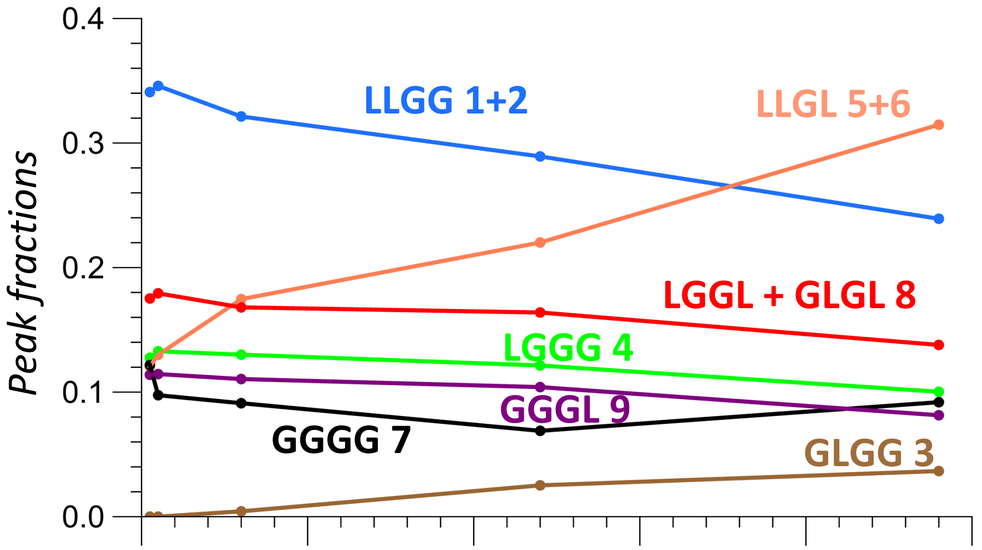


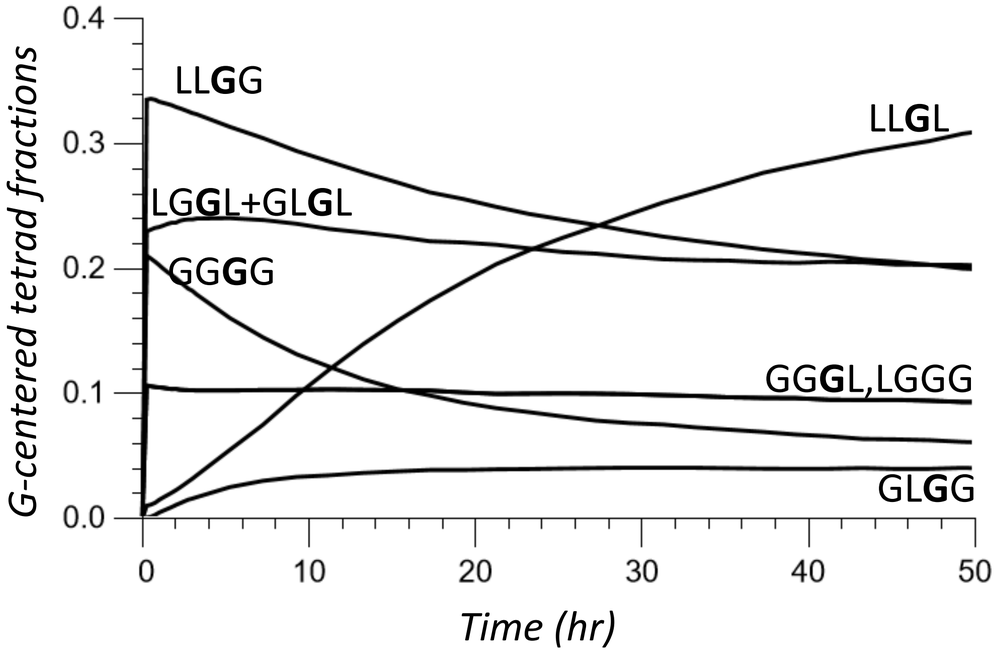
Stochastic prediction

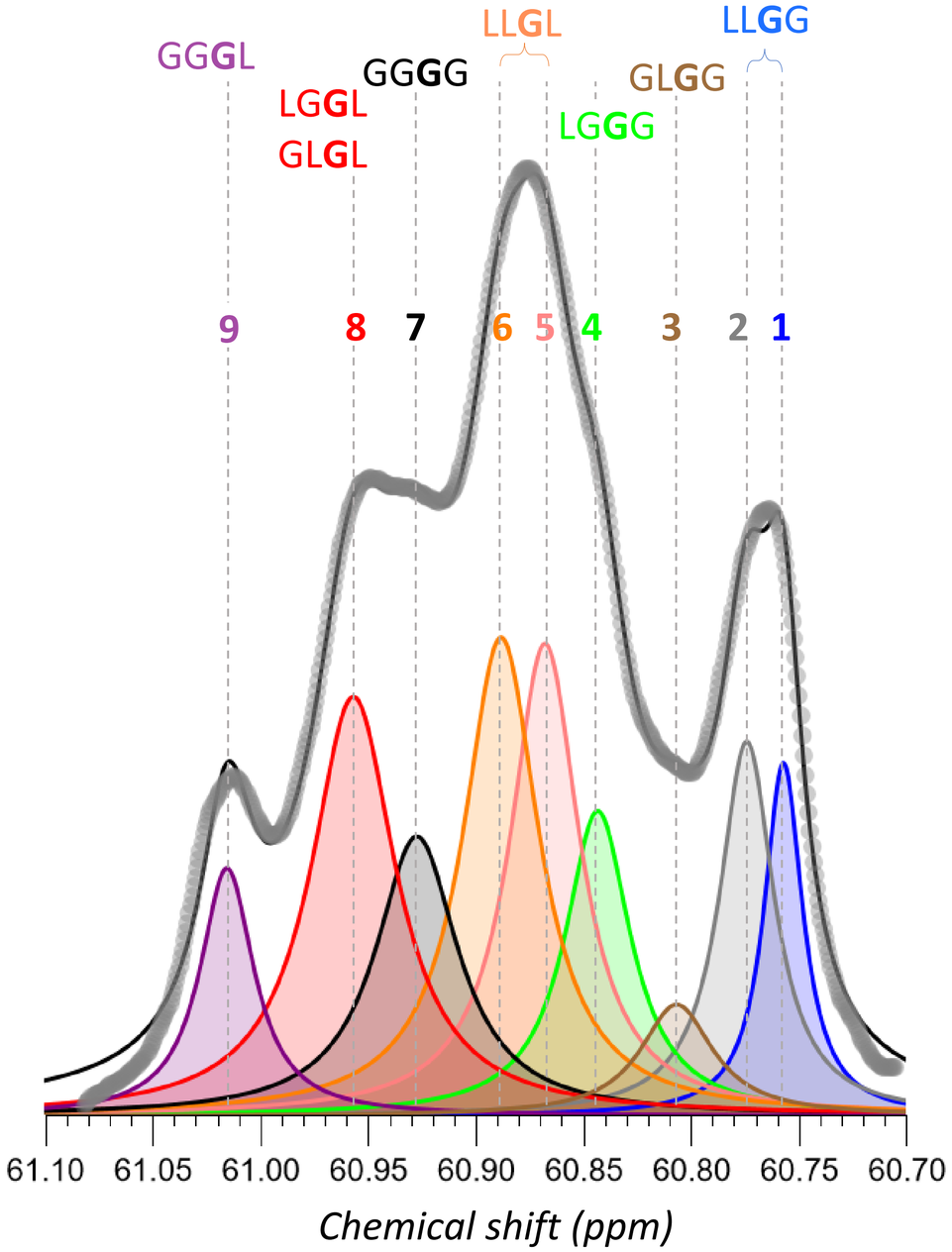


Comparing these trends allows us to make empirical assignments

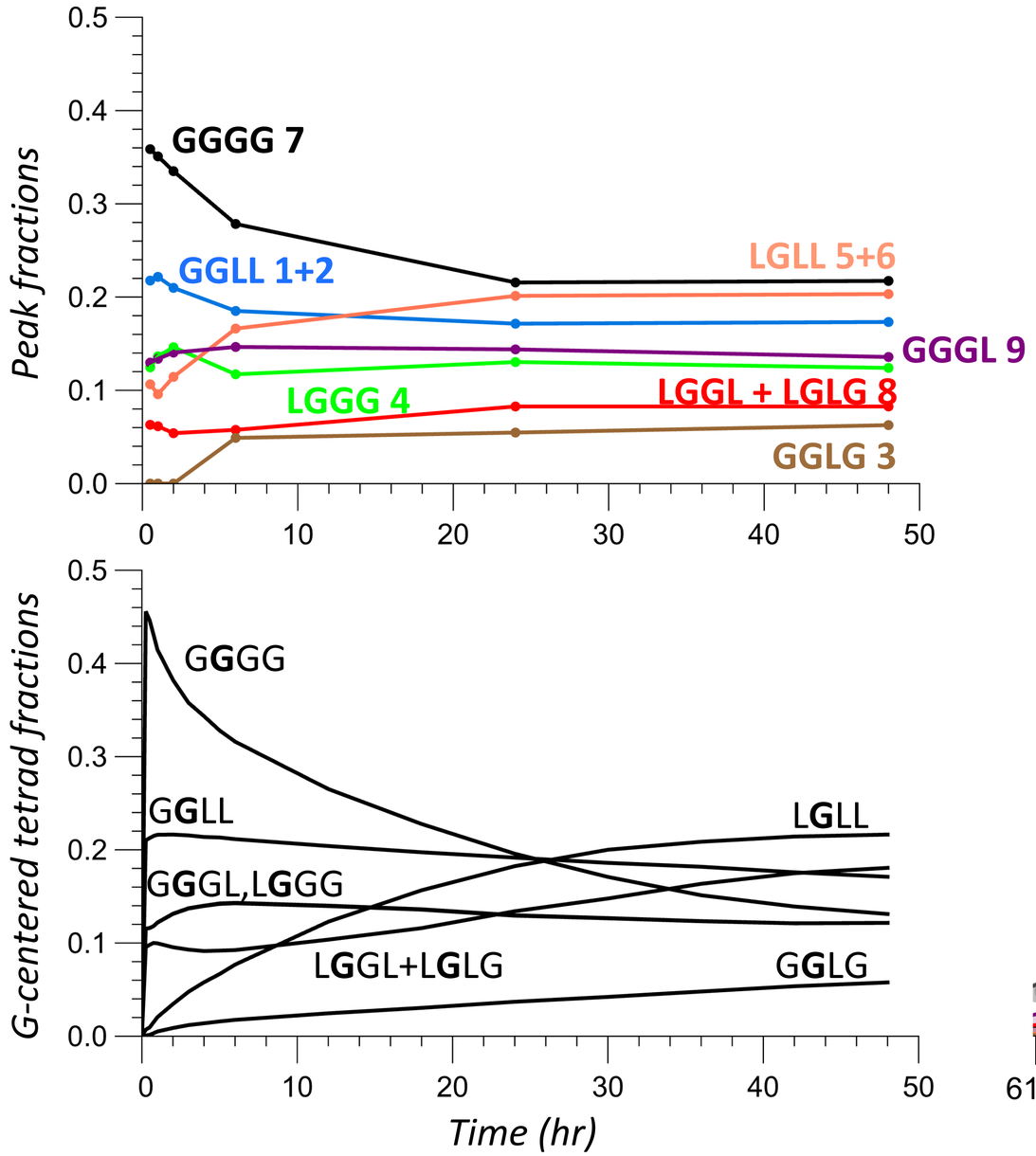
Proposed assignments agree with experimental data for 75:25 PLGA

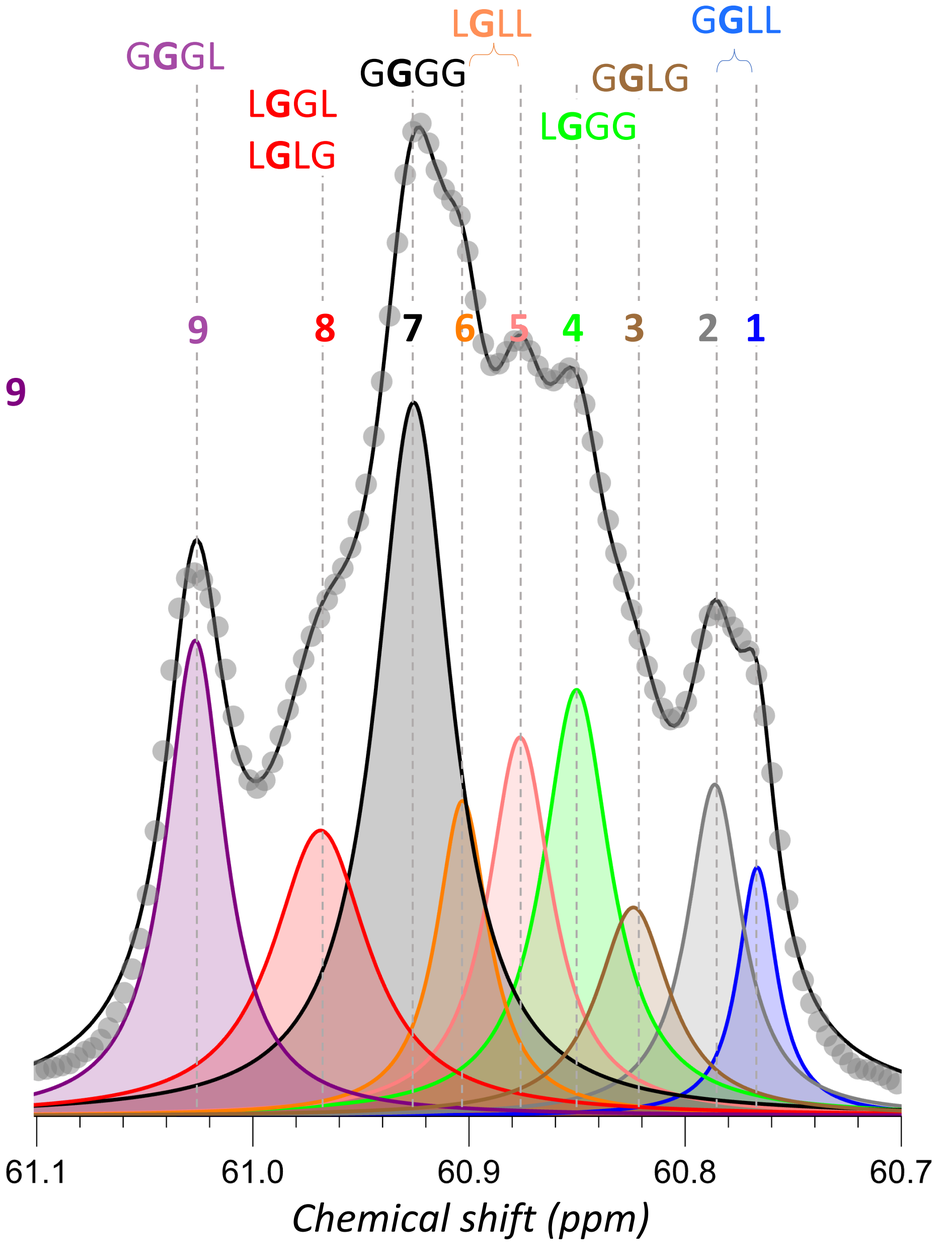






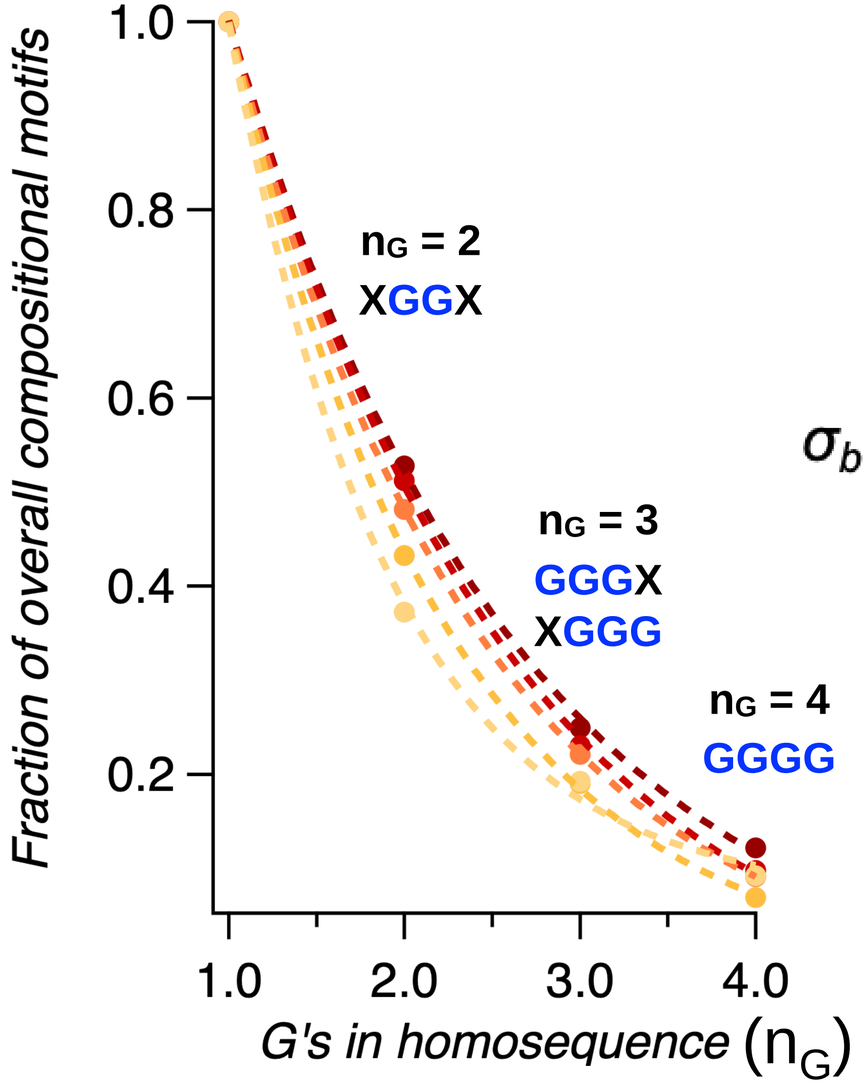
Peak assignments are consistent with 60:40 PLGA data too

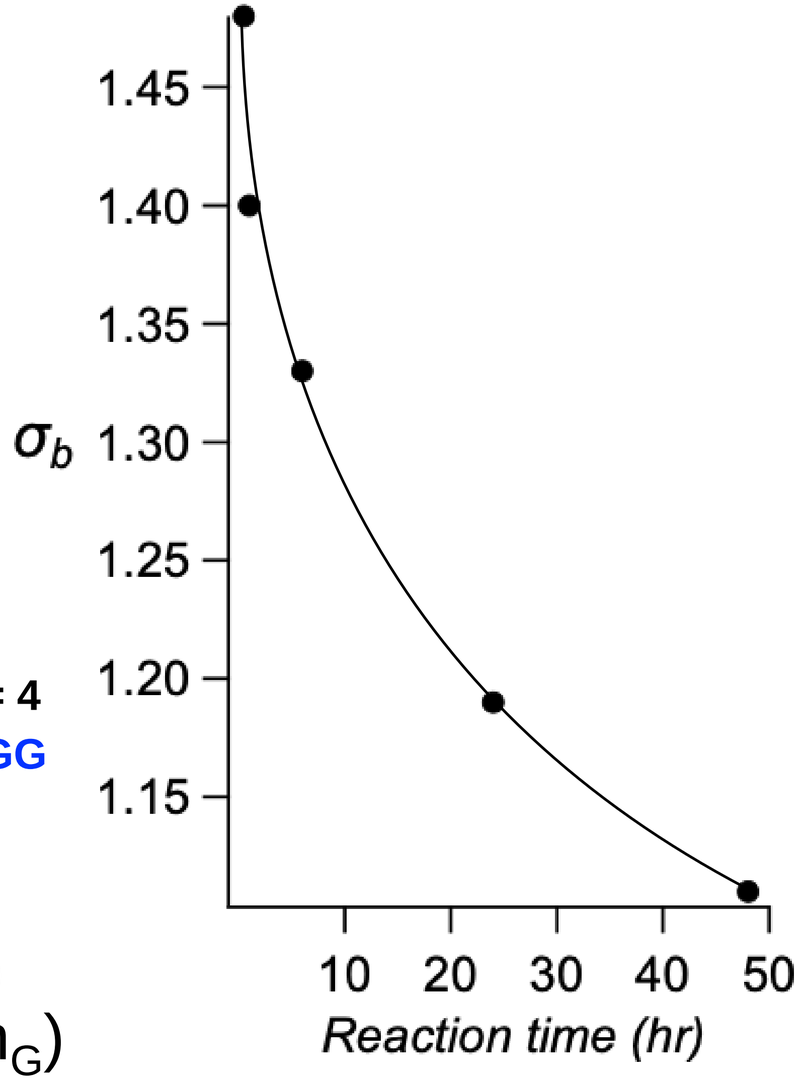


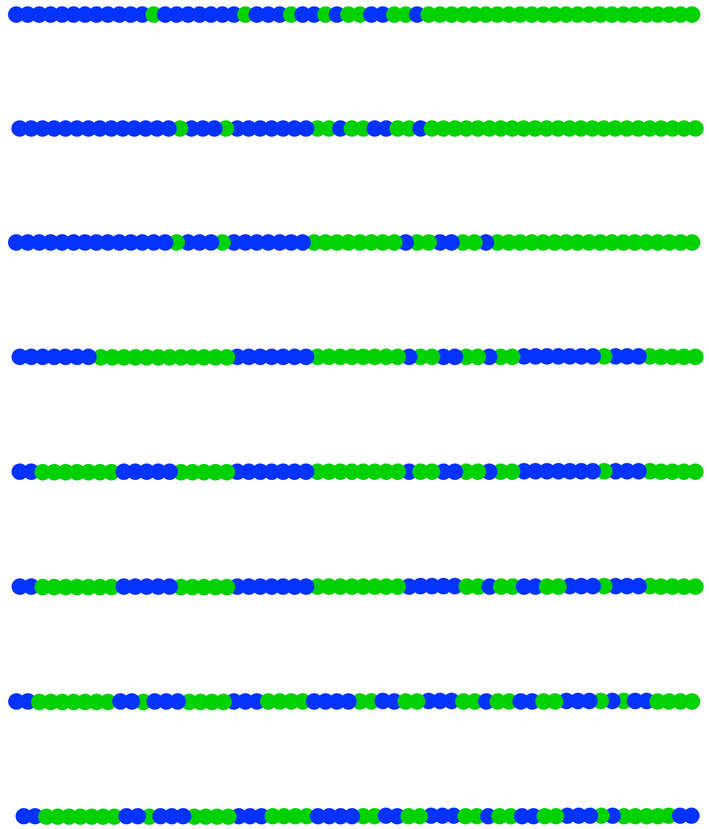


We now define the blockiness with the blockiness parameter σ_B from ^{13}C NMR spectroscopy data

$$f_{blockiness}(n_G) = e^{(1-n_G)/\sigma_b}$$







To summarize our technical innovations

- We developed a new method of data analysis by stochastic model regression to…
- train a complex copolymerization model to allow us to look under the hood of PLGA…
- and to establish a chemistry-informed description for the G/L repeat unit blockiness

The test establish sameness for blockiness for PLGA

- Collect high-quality ^{13}C NMR spectroscopy data focusing on 60.7–61.1 ppm
- Fit data to array of nine deconvolutional peak fit functions at fixed ppm
- Normalize total integral, sum integrals by block size ($n_G = 1$, $n_G = 2$, $n_G = 3$, *etc*.)
- Fit to $f(n_G, \sigma_b) = e^{(1-n_G)/\sigma_b}$ and report σ_B
- Contact me if help is needed: lynd@che.utexas.edu

Acknowledgement

This research was supported by the Broad Agency Announcement (BAA) contract number 75F40120C00198 from the U.S. Food and Drug Administration (FDA). The content is solely the responsibility of the authors and does not necessarily represent the official views or policies of the U.S. FDA.

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