Supplemental Information for "Increasing Molecular Mass in Enzymatic Lactone Polymerizations"

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Rate Parameter Error for Water Equilibrium Kinetics

Kinetic rate parameters, k_{W+} and k_{W-}, were fit to the water concentration measurements using Equation 2 in the main text. The values given in the main text were best fit parameters, with a coupled confidence interval due to equilibrium kinetics. The joint confidence interval for both parameters is shown below in Figure S1. The 95% confidence interval was calculated from the sum squared residuals of 20 individual data points fitting two parameters. A Monte Carlo simulation of the ordinary differential equation set was performed on 4000 pairs of randomly chosen kinetic parameters. The joint confidence interval error boundary was calculated in the same manner as reactivity ratios, described in detail elsewhere.[1]
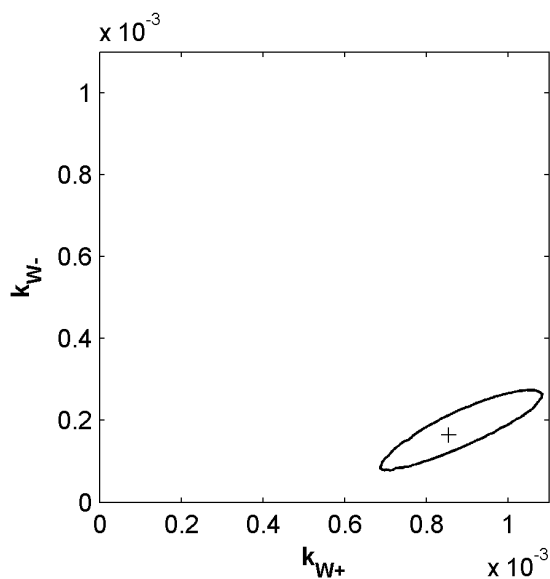

Figure S1. 95 % confidence interval for water equilibrium kinetic parameters. The cross point indicates the fitted parameters reported in the text.

Values for the kinetic parameters were varied over a range from 0 $s^{-1} < k < 1.5 \times 10^{-3}$ s^{-1} in random pairs. Using model and experimental results, a residual sum squared error was calculated for each pair. Error results were then interpolated using meshfit in MATLAB to determine the lowest sum squared error and confidence interval boundary. As expected, k_{W+} and k_{W-} are strongly correlated since both parameters were fitted from the same data set. However, the 95 % confidence interval has k_{W+} and k_{W-} at non-zero values over the entire envelope, so both parameters are required for an accurate prediction of water uptake kinetics by molecular sieves.

Enzymatic Ring Opening Polymerization Model

The enzymatic ring opening polymerization model of poly(ε-caporlactone) (PCL) was developed in MATLAB and solved with the ordinary differential equation solver *ode23tb*, a backward-forward step solver. A total of 6181 separate species were tracked. Free enzyme, water, ε-caprolactone (εCL), and water trapped within molecular sieves were non-PCL type chains tracked. Cyclic PCL chains were limited to chains with repeat units $i < 175$. Linear PCL chains and enzyme activated PCL chains were tracked for each chain length up to $i = 3000$. The two remaining tracked species were for linear and enzyme activated chains of $i > 3000$, which were considered unreactive. The chain concentration of these high molecular mass chains were minuscule (~10^{-9} mol/L) but tracked to balance the equation set. Extending the model to even higher chain lengths did not change the kinetic model results.

Initial conditions were the same for reactions with and without molecular sieves, described below in Table S1. Model data was reported at 500 s intervals for 18000 s. The total enzyme concentration should remain constant, and the deviation from the initial value was less than 0.0001%. Kinetic rate parameters for all reactions are given in Table S2.

Table S1. Initial species with non-zero concentrations at $t = 0$ s in the kinetic model.

Reactant	Initial Concentration
ε-caprolactone	3.0080 mol/L
Water	0.0213 mol/L
Enzyme	1.01×10^{-4} mol/L

Table S2. Kinetic rate parameters used to model in the enzymatic polymerization kinetic pathway, depicted in Scheme 1 in the main text.

Kinetic Parameters	Kinetic Rate Parameter (k_0)
k_1	150 L/mol/s
k_{2+}	36 000 L/mol/s
k_{2-}	4000 L/mol/s
k_{3+}	3000 L/mol/s
k_{3-}	3000 L/mol/s
k_{4+}	1500 1/s
k_{4-}	4000 L/mol/s
k_{W+}	8.1×10^{-4} s^{-1}
k_{W-}	1.5×10^{-4} s^{-1}

From model results, chain concentrations for each PCL length were modeled, along with enzyme activated chains and cyclic chains in solution. While the low molecular mass chains are tracked, the number-average molecular mass calculations only take into account chains greater than eight repeat units to be consistent with the cutoff used for GPC calculations. The predicted change in

the GPC trace at $t = 18000$ s is shown in Figure S2a for reactions with and without molecular sieves. Chain concentration for linear PCL chains at 18000 s is presented in Figure S2b.
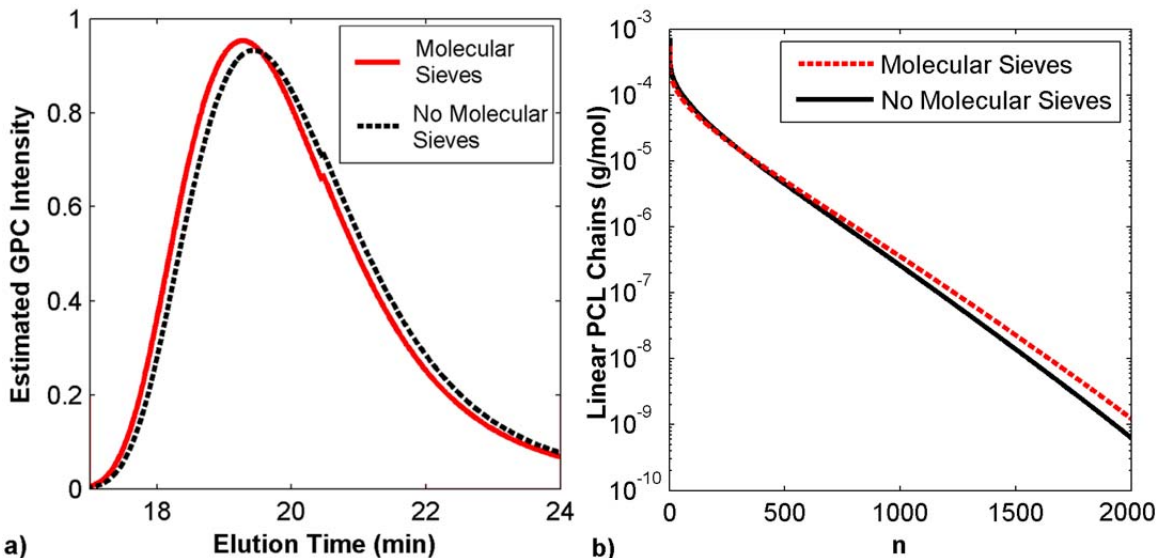

Figure S2. (a) Predicted GPC trace for the modeled chain distribution for reactions with and without molecular sieves as predicted by the kinetic model. (b) Modeled concentration of linear PCL chains at 18000 s.

M_n and the concentration of trapped water is shown in Table S1 for model results at $t = 18000$ s. If the water trapping reaction was not reversible, water would be continually removed from the reaction. The absolute concentration of linear PCL chains shows the greatest shift at higher molecular weights, with concentrations close to double in reactions with molecular sieves.

Table S1. Number average relative molecular mass and water trapped within molecular sieves at 18000 s for modeled reaction systems.

Modeled Reaction	M_n (g/mol) $t = 18000$ s	$[H_2O]_{MS}$ (mol/L) $t = 18000$ s
Without Molecular Sieves	13300	0
Molecular Sieves	14900	0.023
Molecular Sieves [$k_{W-} = 0$]	16400	0.041

Since water is the key component to higher molecular mass, the three end locations for the initial water concentration were tracked in the model. Water can remain in solution, be trapped in molecular sieves, or be incorporated into a linear PCL chain. Species containing water were summed for every time point, and the fraction of water in each state is shown in Figure S3. As

seen in Figure S3, the εCL polymerization would not be altered by the molecular sieves because the water fraction during polymerization ($t < 3000$ s) is nearly zero. Therefore, the molecular sieves fail to extract significant amounts of water prior to the completion of the ring opening step.
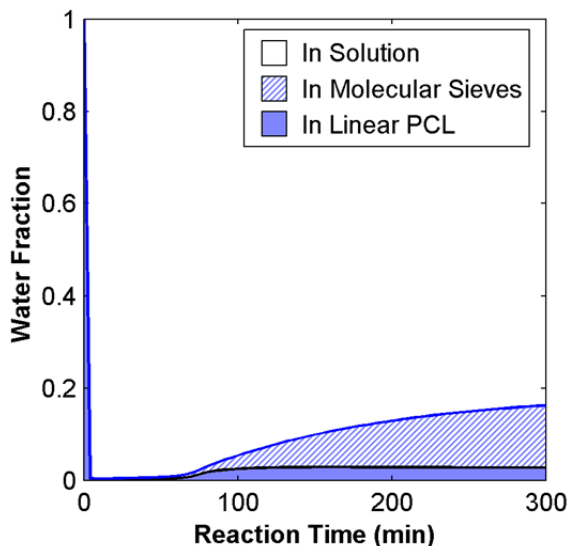

Figure S3. Water concentration fractions separated by location of water as a function of time for the reaction with molecular sieves. Water is present in solution, trapped in molecular sieves, or incorporated into PCL linear chains dependent on the reaction time.

Degradation Reactions

To see if the removal of trace water was applicable in all enzymatic processes, a degradation study was also performed. This reaction replaced dry toluene with water-equilibrated toluene and 1 mL of εCL with 1 g of enzymatically polymerized PCL. When 100 mg of Novozym 435 beads were added, the PCL in solution will degrade due to the excess water in the solution. Once enough time has passed, PCL will equilibrate at a new, lower M_n, molecular mass distribution. Once the reaction has reached a new equilibrium, 100 mg of molecular sieves were added to the solution, with aliquots taken at 5 h and 24 h. GPC results for the degradation reaction with molecular sieves are provided in Figure S4.
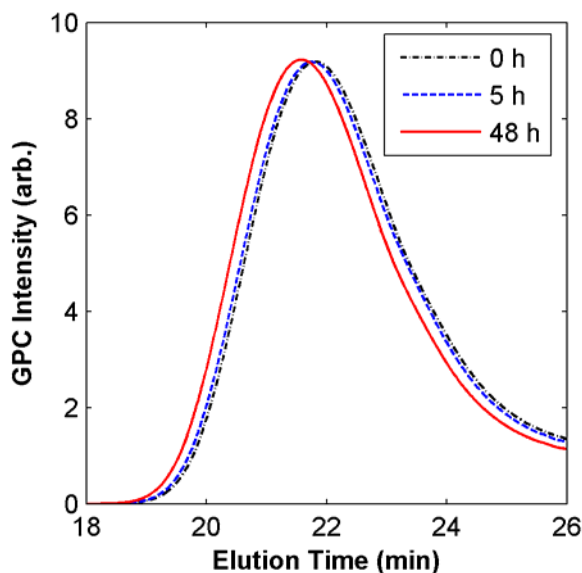
Figure S4. GPC for a degraded PCL after adding molecular sieves and 5 h or 24 h of reaction time. PCL was fully degraded and equilibrated at $t = 0$ h.

A shift in the molecular mass distribution to higher molecular masses was present, similar to the polymerization reaction. For the results shown in Figure S4, M_n increased by 11 %. In cases without molecular sieves, M_n remained constant or slightly decreased. With a higher total water concentration, the molecular sieve process may be unable to trap enough water to provide the same magnitude as seen during polymerization. If the number of chains were proportional to M_n, a complete recovery would require over half the initial water content to be trapped within molecular sieves. Since most of the initial water was consumed during degradation to create new linear PCL chains, a stronger water consumption reaction would be necessary to shift the equilibrium reaction.

References:

(1)   van Herk, A. M. *Journal of Chemical Education* **1995**, *72*, 138.