Supplementary Information

Activation of L-lactate oxidase by the formation of enzyme assemblies through liquid–liquid phase separation

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Supplementary Figure S1

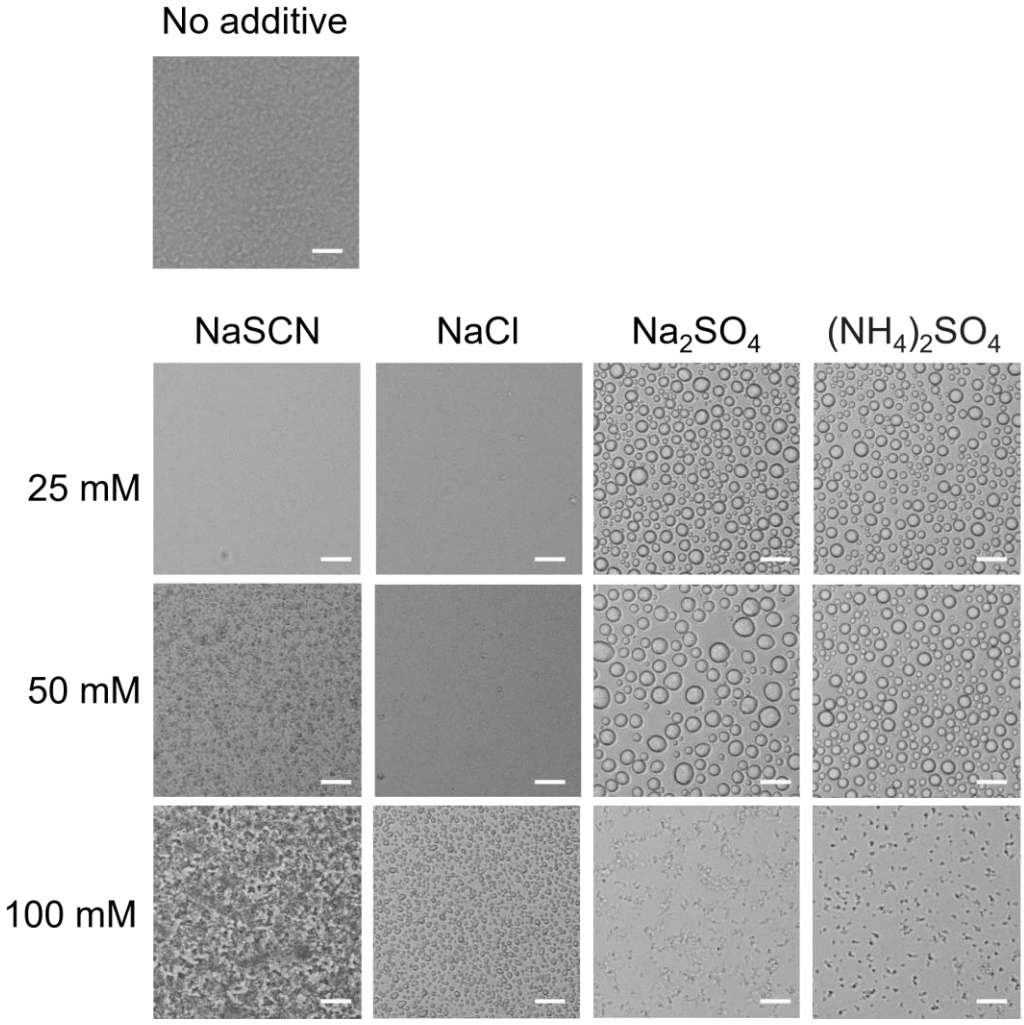

Fig. S1 Effect of the salt type and concentration on the formation of LOX-PLL assemblies. All solutions were prepared with 5 μM LOX, 1 mM PLL (concentrations refer to lysine monomer units), and salts (sodium thiocyanate: NaSCN, sodium chloride: NaCl, sodium sulfate: Na_2SO_4, ammonium sulfate: $((NH_4)_2SO_4)$ concentrations between 25–100 mM, and 20 mM Tris HCl (pH 8). Scale bar, 20 μm.

Supplementary Figure S2

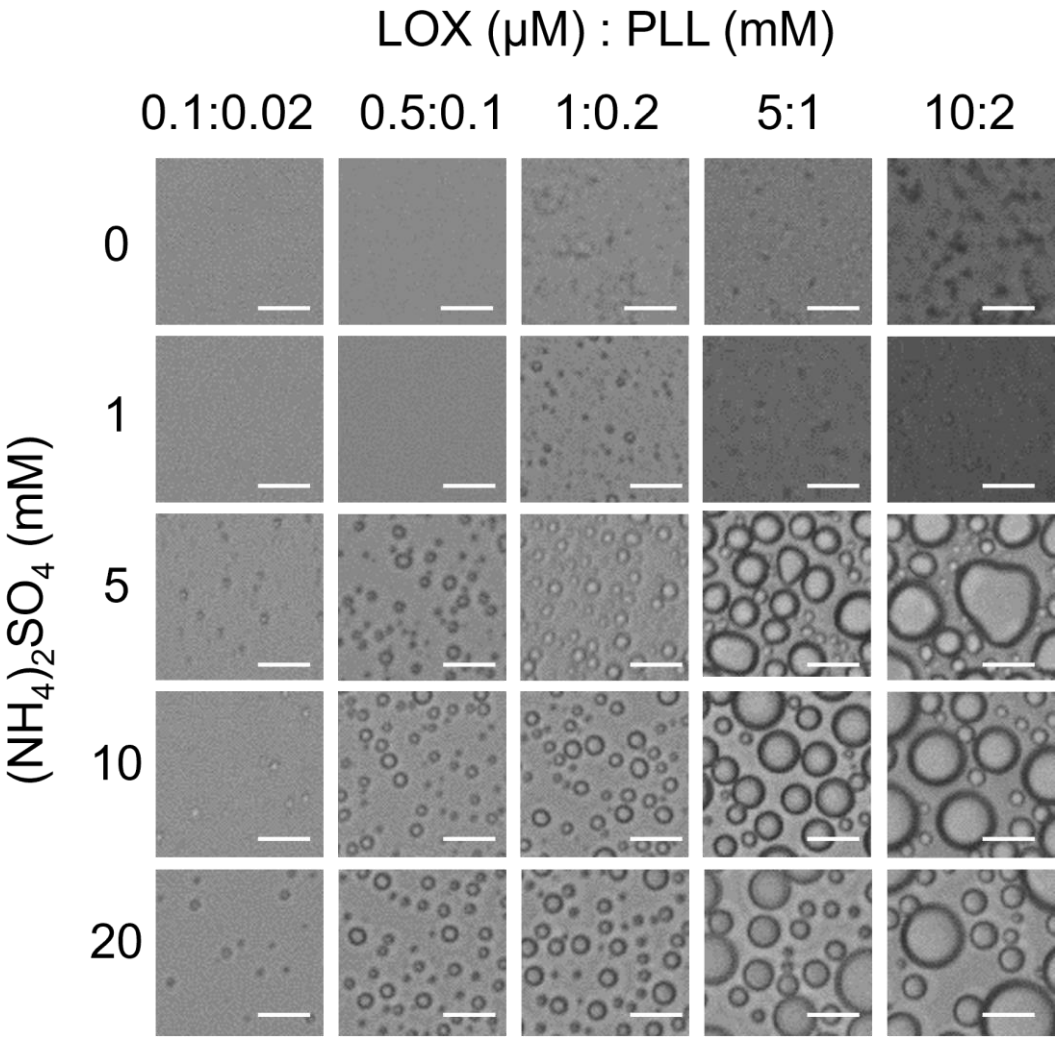

Fig. S2 Effect of ammonium sulfate on the formation of LOX-PLL assemblies. All solutions were prepared using 0.1–10 μM LOX, 0.02-2 mM PLL, 0–20 mM ammonium sulfate, and 20 mM Tris HCl (pH 8). Scale bar, 10 μm.

Supplementary Figure S3

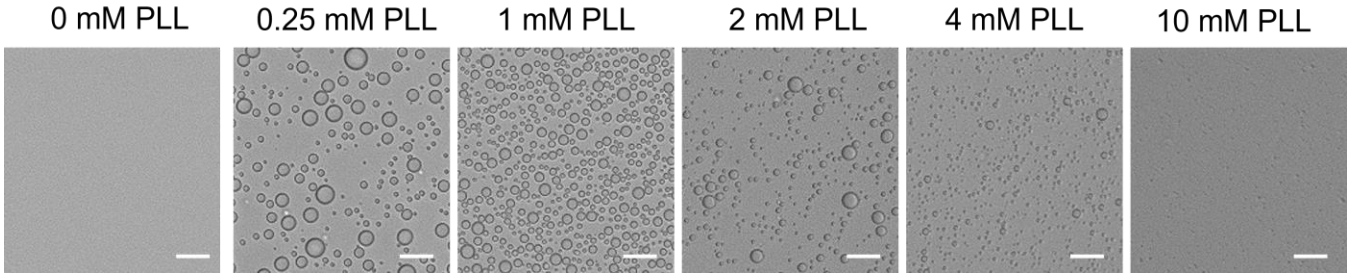

Fig. S3 Effect of PLL concentration on LOX-PLL droplet formation. All solutions were prepared with 5 μM LOX, 0–10 mM PLL, 10 mM $(NH_4)_2SO_4$, and 20 mM Tris-HCl (pH 8). Scale bar, 20 μm.

Supplementary Figure S4

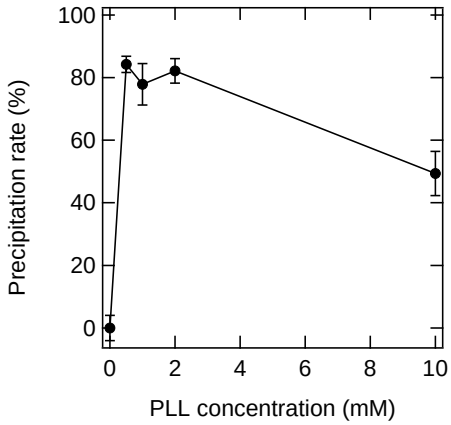

Fig. S4 Precipitation rate of LOX in the presence of PLL. All solutions were prepared with 5 μM LOX and PLL concentrations between 0 and 10 mM, 10 mM $(NH_4)_2SO_4$, and 20 mM Tris-HCl (pH 8).

Supplementary Figure S5

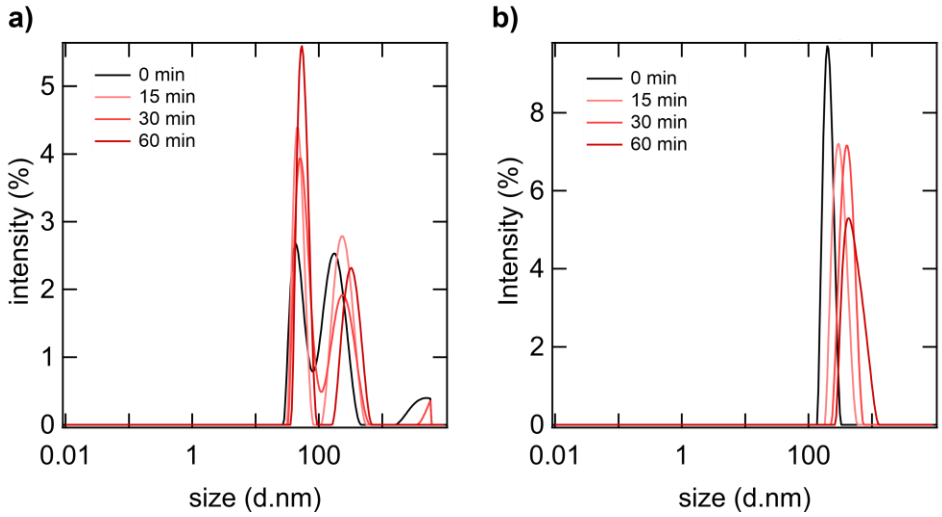

Fig. S5 Time-dependent changes in particle size of the LOX-PLL cluster **(a)** and droplet **(b).** All solutions contained 0.1 μM LOX, 0.02 mM PLL, 0 or 10 mM $(NH_4)_2SO_4$, and 20 mM Tris HCl (pH 8.0).

Supplementary Figure S6

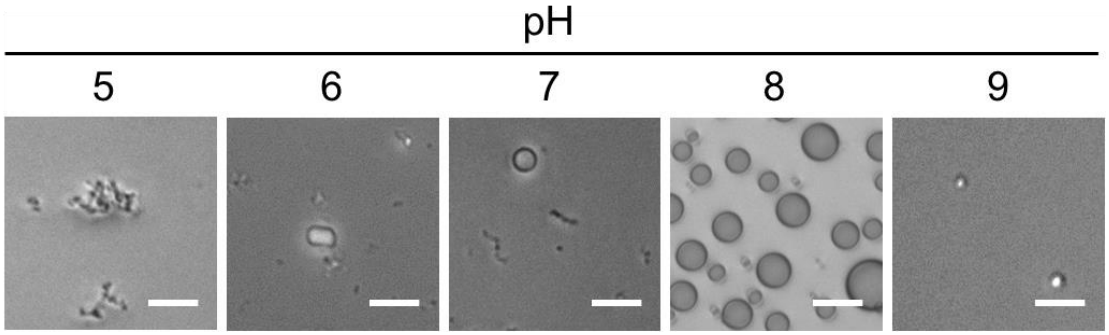

Fig. S6 Effect of PLL concentration on LOX-PLL droplet formation. All solutions were prepared with 5 μM LOX, 0–10 mM PLL, 10 mM $(NH_4)_2SO_4$, 20 mM MES, and 20 mM Tris-HCl (pH 5-9). Scale bar, 20 μm.

Supplementary Figure S7

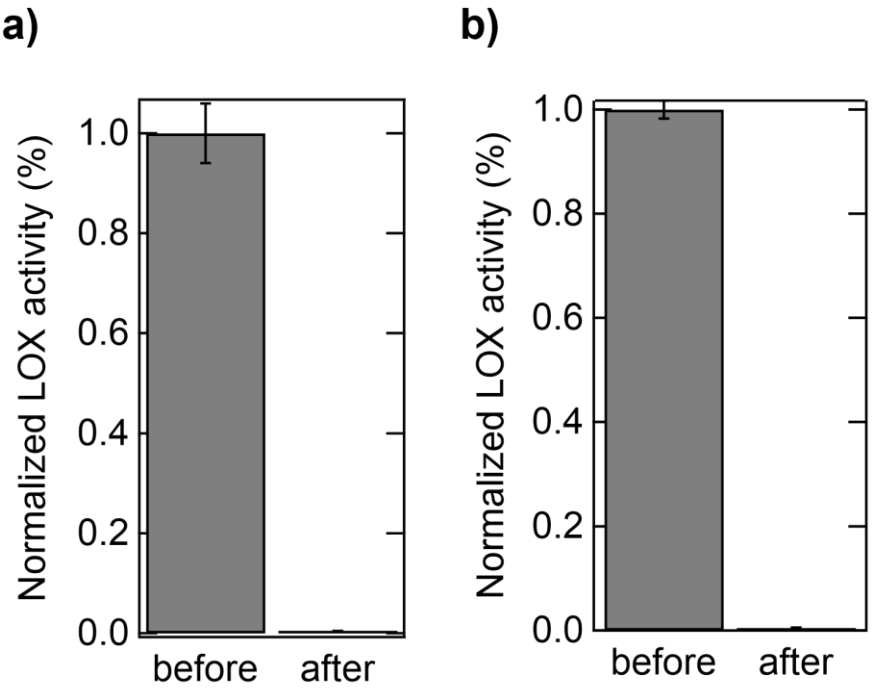

Fig. S7 Enzyme activity of LOX before and after centrifugation. **a**) Enzyme activities before and after the centrifugation of samples containing 0.1, 0.02 mM PLL, and 20 mM Tris HCl (pH 8) at 18,800 × g for 20 min. **b**) Enzyme activities before and after centrifugation of samples containing 0.1 μM LOX, 0.02 mM PLL, 10 mM $(NH_4)_2SO_4$, and 20 mM Tris HCl (pH 8) at 18,800 *g* for 20 min.

Supplementary Figure S8

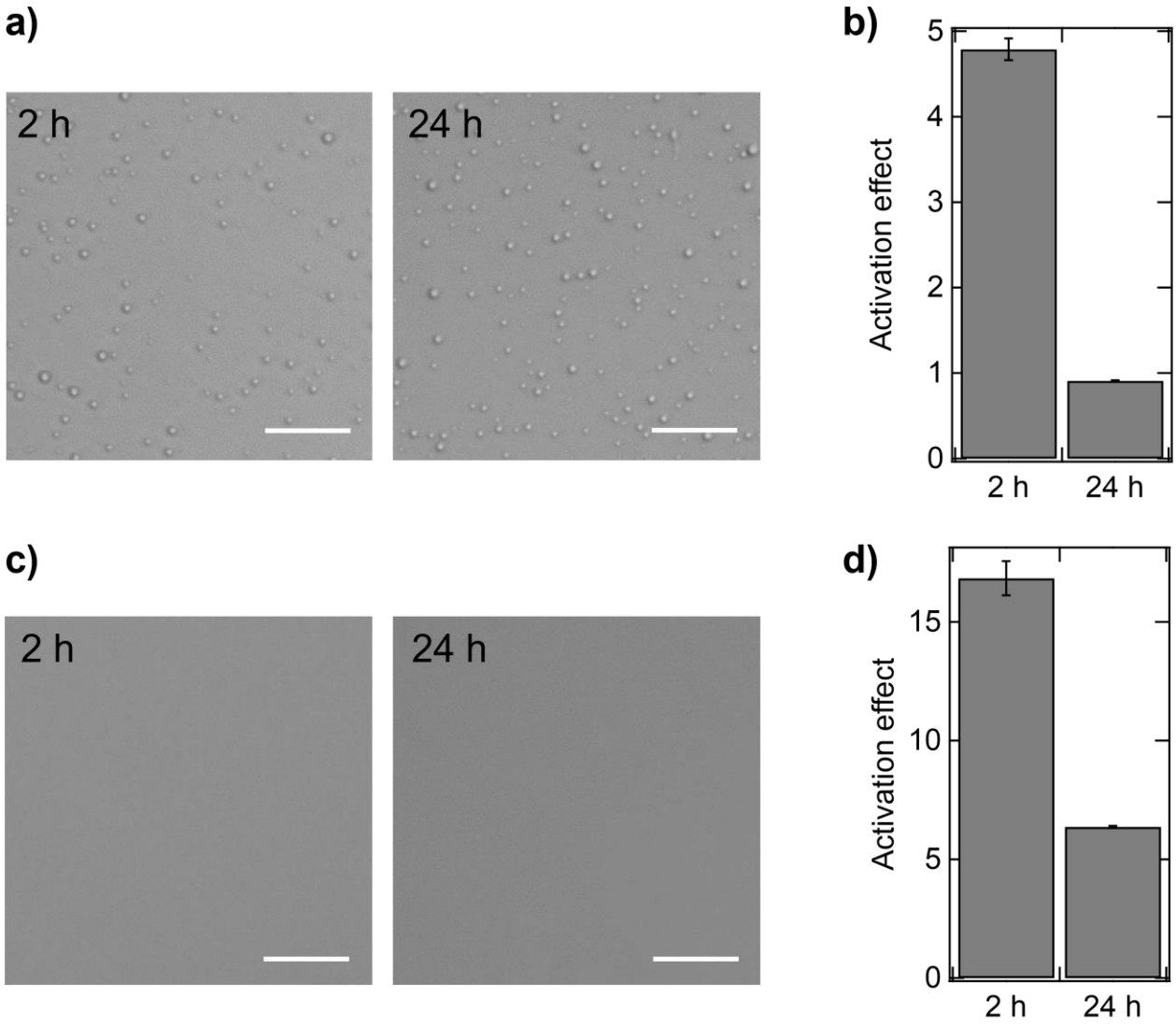

Fig. S8. Stability of droplets and $clusters_{lox}$. **a**) Bright-field field microscopic images of LOX-PLL droplets after 2 h and 24 h. The solution contained 0.1 μM LOX, 0.02 mM PLL, 10 mM $(NH_4)_2SO_4$, and 20 mM Tris HCl (pH 8). **b**) Activation effect of LOX in the droplets after 2 h and 24 h. The solution contained 0.1 μM LOX, 0.02 mM PLL, 10 mM $(NH_4)_2SO_4$, 0.1 mM DCIP, 1 mM L-lactic acid, and 20 mM Tris HCl (pH 8). **c**) Bright field microscopic images of $cluster_{lox}$ after 2 h and 24 h. The solution contained 0.1 μM LOX, 0.02 mM PLL, and 20 mM Tris HCl (pH 8). **d**) Activation effect of LOX in the $cluster_{lox}$ after 2 h and 24 h. The solution contained 0.1 μM LOX, 0.02 mM PLL, 0.1 mM DCIP, 1 mM L-lactic acid, and 20 mM Tris HCl (pH 8). Activation effect was defined as the initial reaction velocity in each condition divided by that in the absence of PLL and $(NH_4)_2SO_4$.

Supplementary Figure S9

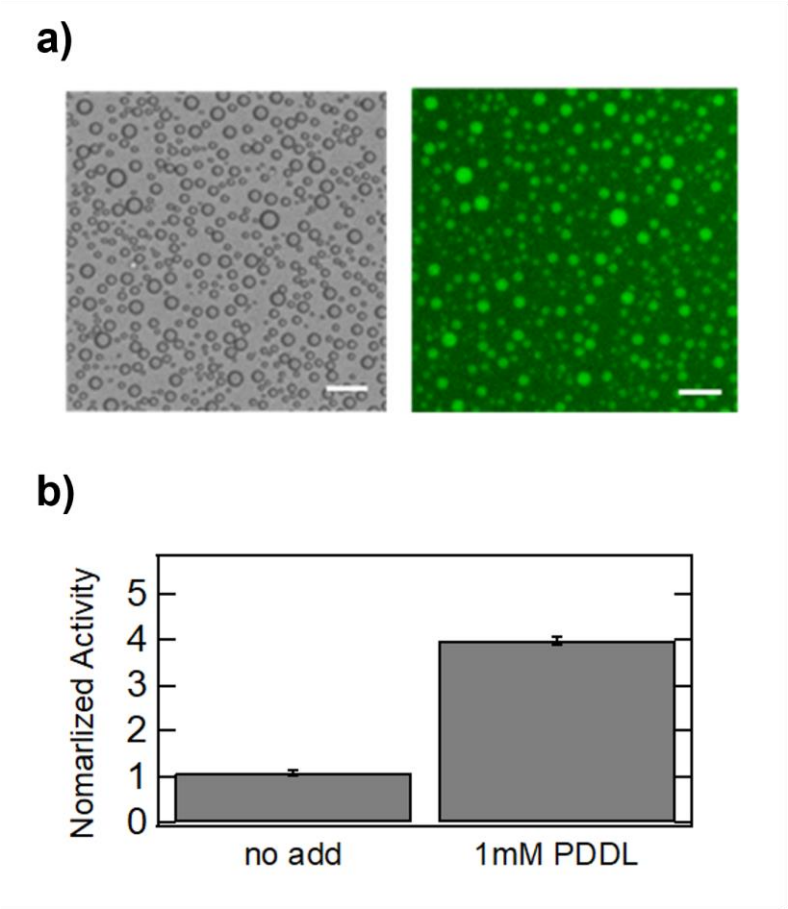

Fig. S9 Formation and activation of LOX-PDDL droplets. **a)** Blight field microscopic images of droplets (left) and fluorescent microscopic images of LOX (right). The solution contained 5 μM LOX, 1 mM PDLL, 6 mM $(NH_4)_2SO_4$, 20 mM Tris HCl, and 20 mM MES (pH 8). Scale bar, 20 μm. **b)** Normalized activity of LOX in the presence of 1 mM PDLL.

Supplementary Figure S10

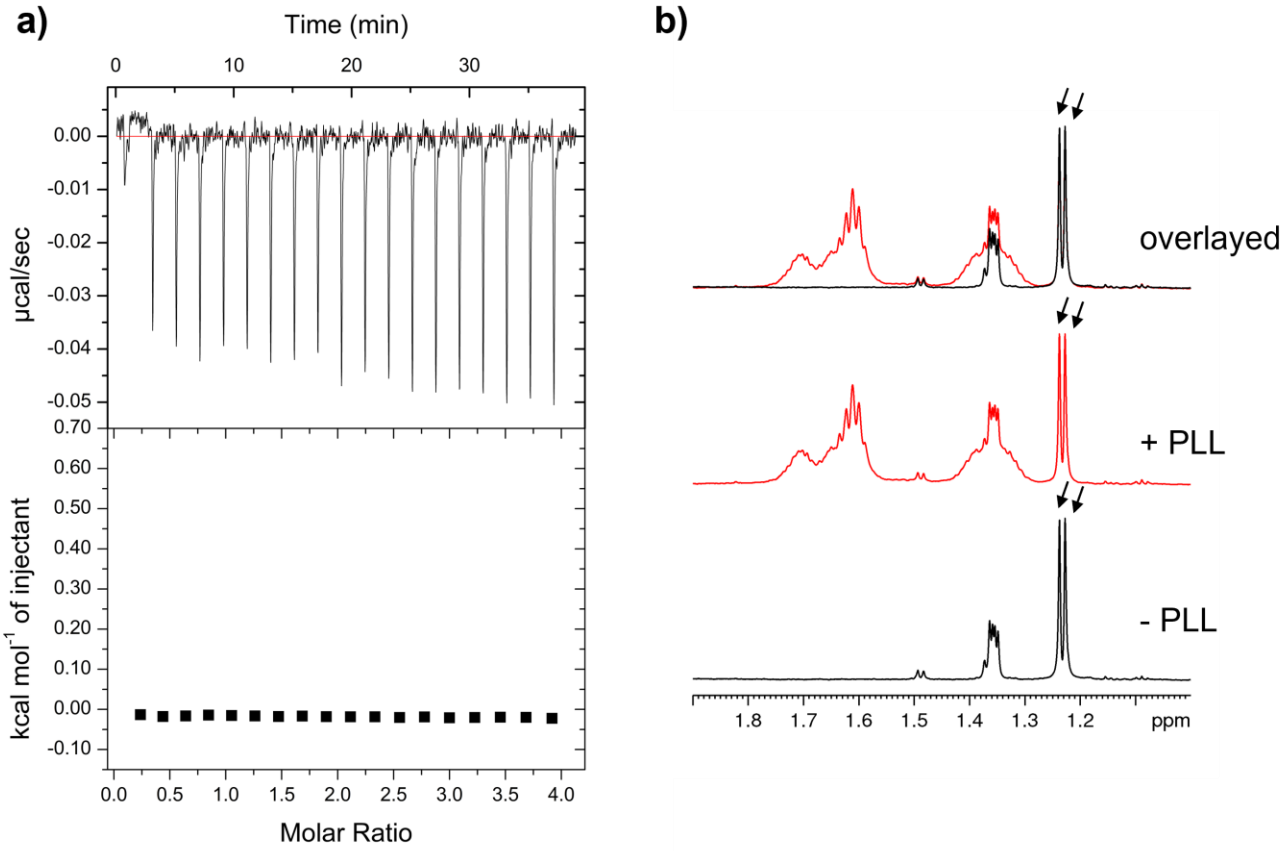

Fig. S10. Interaction of PLL with L-lactate. **a)** ITC analysis to quantify the interaction between PLL and L-lactate. **b)** 1H-NMR spectra of PLL and L-lactate in the presence and absence of the overlaid PLL.