SUPPORTING INFORMATION

Biocatalytic 3D Actuation in Liquid Crystal Elastomers via Enzyme Patterning

Albert Velasco Abadia[1], Katie M. Herbert[1], Timothy J. White[1,2], Daniel K. Schwartz[1], Joel L. Kaar[1]*

[1]Department of Chemical and Biological Engineering, University of Colorado, Boulder, CO 80309, USA

[2]Material Science and Engineering, University of Colorado, Boulder, CO 80309, USA

*Corresponding Author:
Joel L. Kaar
University of Colorado Boulder
Department of Chemical and Biological Engineering
Campus Box 596
Boulder, CO 80309
Tel: (303) 492-6031
Email: joel.kaar@colorado.edu

SUPPORTING FIGURES

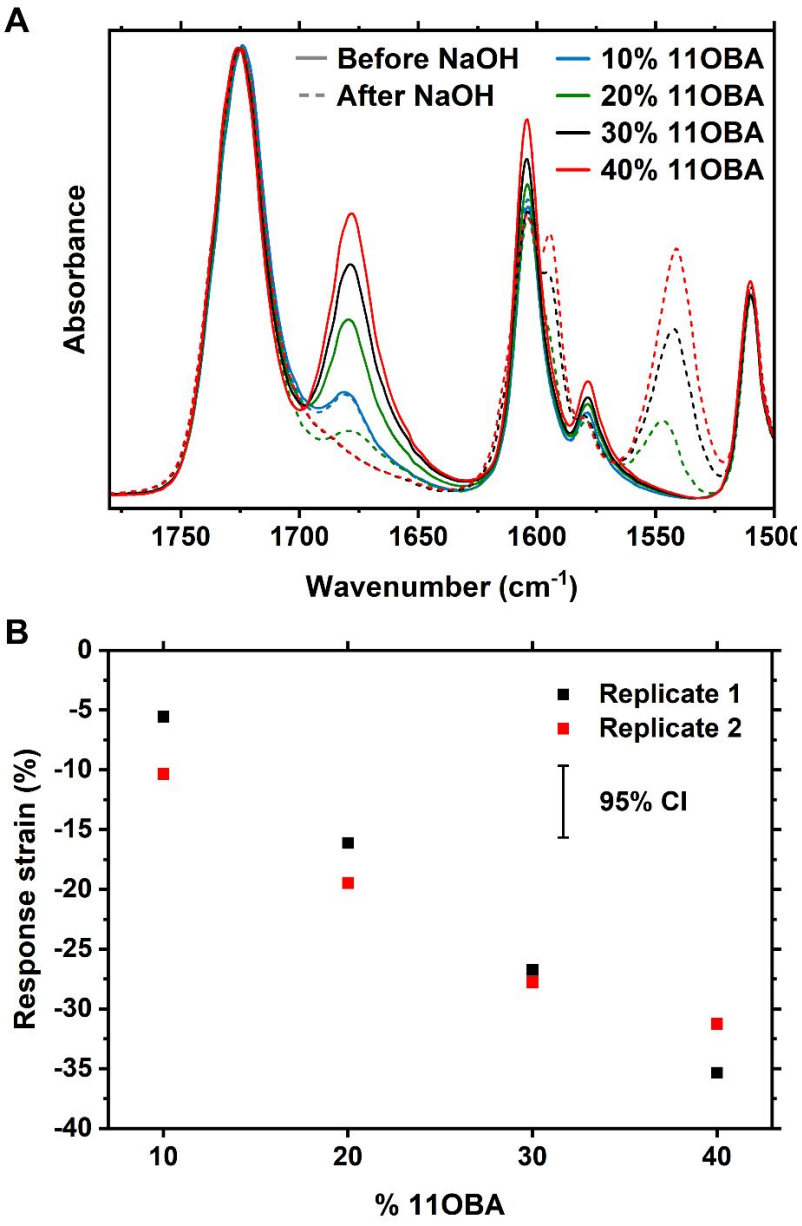

Figure S1. Characterization of LCE films with different relative fractions of 11OBA with respect to the monomer C6M. **A)** FTIR spectra of films before (solid line) and after (dashed line) exposure to 0.2M sodium hydroxide for 30 min. At 1678 cm^{-1} the C=O stretching corresponding to the carboxylic acid dimers was observed, which indicated the presence of hydrogen bonding. The emergence of the carboxylate antisymmetric stretching at 1541 cm^{-1} after base exposure indicated neutralization of carboxylic acids in 11OBA. **B)** Contractile strain of LCE films after exposure to 0.2M sodium hydroxide for 30 min. The error bar represents the 95% confidence interval of measurements.

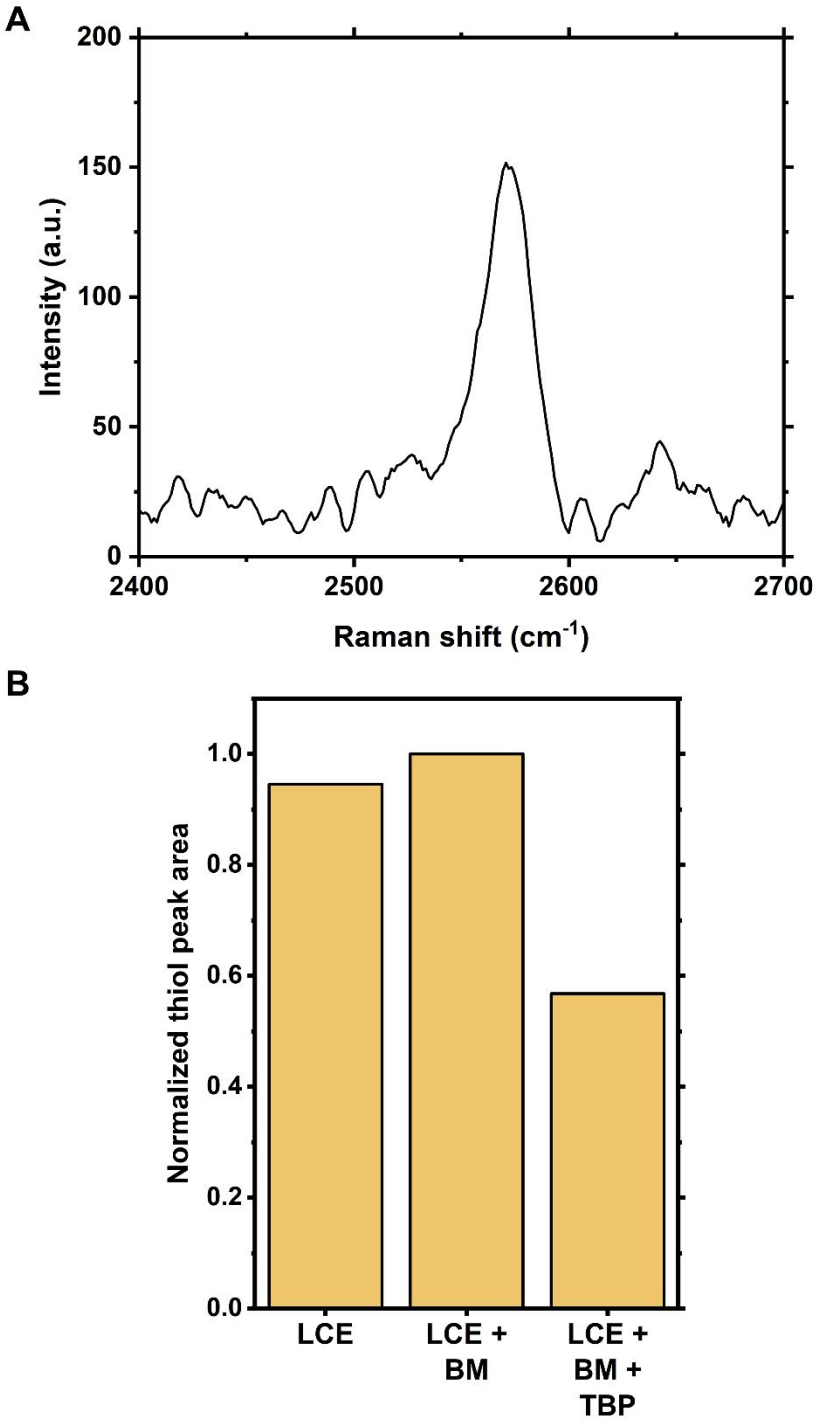

Figure S2. Characterization of the incorporation of the crosslinker (1,1-(methylenedi-4,1-phenylene)bismaleimide (BM) in LCEs by Raman spectroscopy. Bismaleimide incorporation was characterized by quantifying the decrease in the area of the peak associated with free thiols in the films. **A)** Representative Raman spectrum of LCE films showing the thiol S-H stretching peak at 2572 cm^{-1}. **B)** Evolution of the thiol peak after exposure of LCE films to 100 mM of the bismaleimide (in acetonitrile) with or without tributylphosphine (TBP, 1 mM).

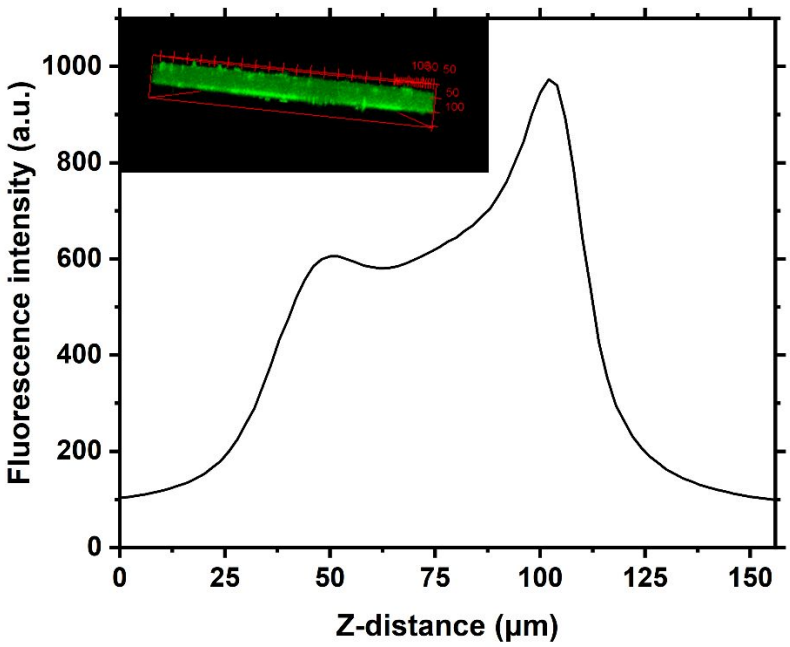

Figure S3. Z-stack imaging of an LCE_{Urease} film in which the urease was fluorescently tagged with carboxyfluorescein succinimidyl ester obtained by confocal microscopy. The plot shows the fluorescence intensity of the film throughout its thickness (z-distance represents the space in the z dimension of the film) while the inset shows the enzyme is uniformly immobilized throughout the thickness of LCEs.

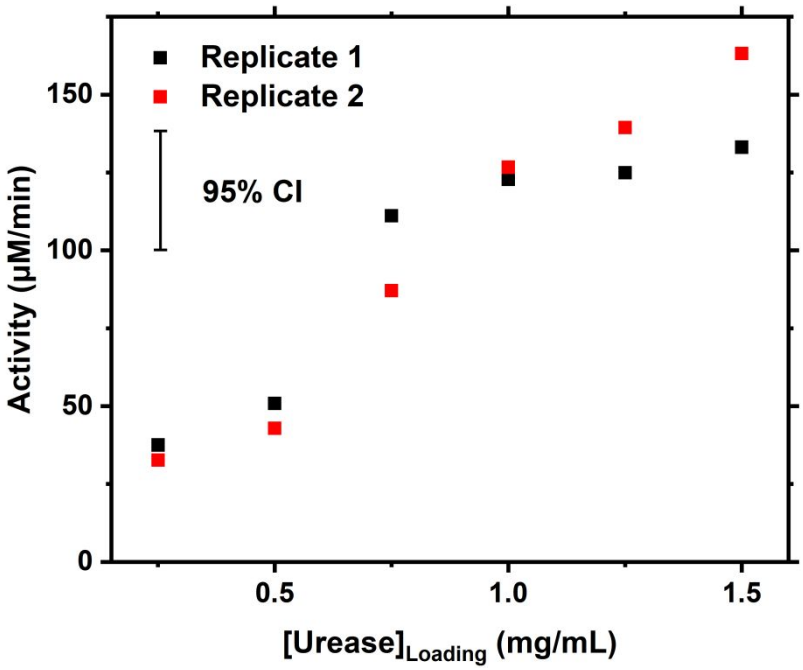

Figure S4. Activity of LCE_{Urease} films as a function of urease concentration in the solution used for the immobilization of the enzyme in the films. The error bar represents the 95% confidence interval of measurements.

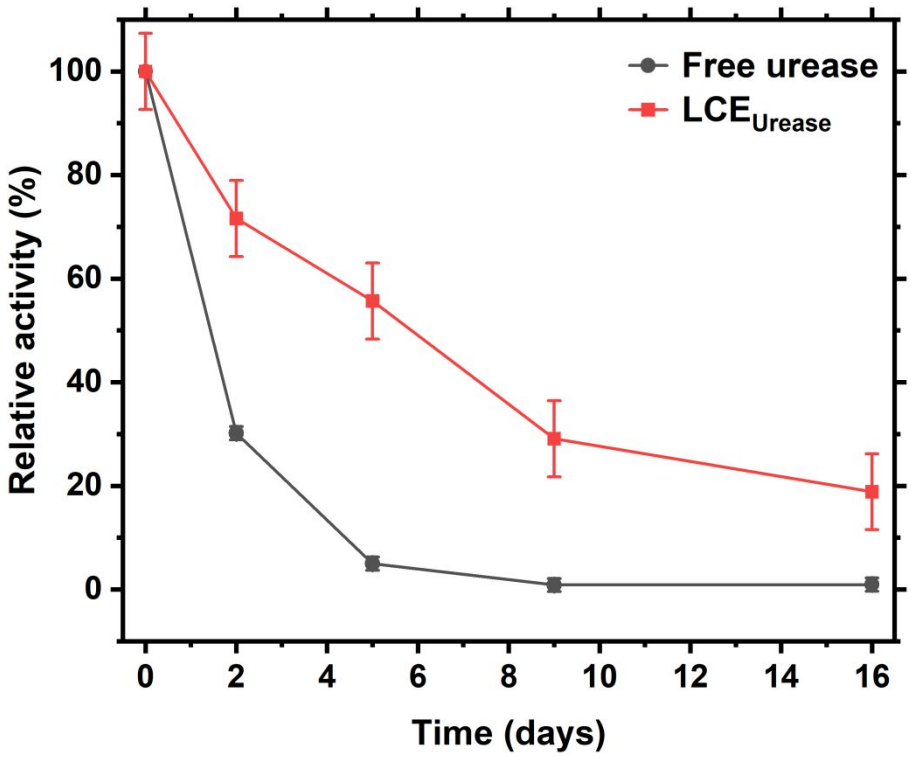

Figure S5. Stability of free urease and urease within LCE_{Urease} films when stored over several days at room temperature in buffer (HEPES 50 mM, pH 7.5). Stability was measured by assaying the relative inactivation of free urease and the LCE_{Urease} films over time (where the activity at each time point was normalized using the activity at day 0). The data points represent the mean of two (LCE_{Urease}) or three (free urease) independent replicates. The error bars, which may be too small to observe, represent the 95% confidence interval of measurements.

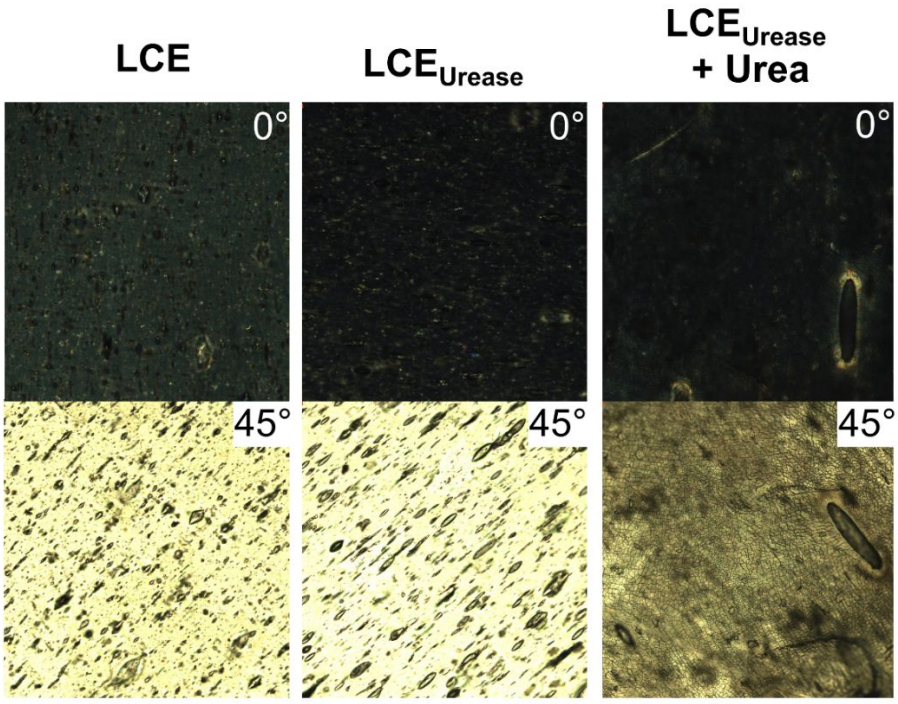

Figure S6. Polarized optical microscope images of LCE films, LCE$_{Urease}$ films, and LCE$_{Urease}$ films after exposure to urea. Images were captured with the films positioned in a 0° and a 45° angle with respect to the crosspolarizers. LCE and LCE$_{Urease}$ films displayed a large birefringence, suggesting a high degree of liquid crystal alignment, while the birefringence of the LCE$_{Urease}$ films decreased significantly after exposure to 50 mM urea in the presence of 500 mM sodium chloride for 24 h at room temperature.

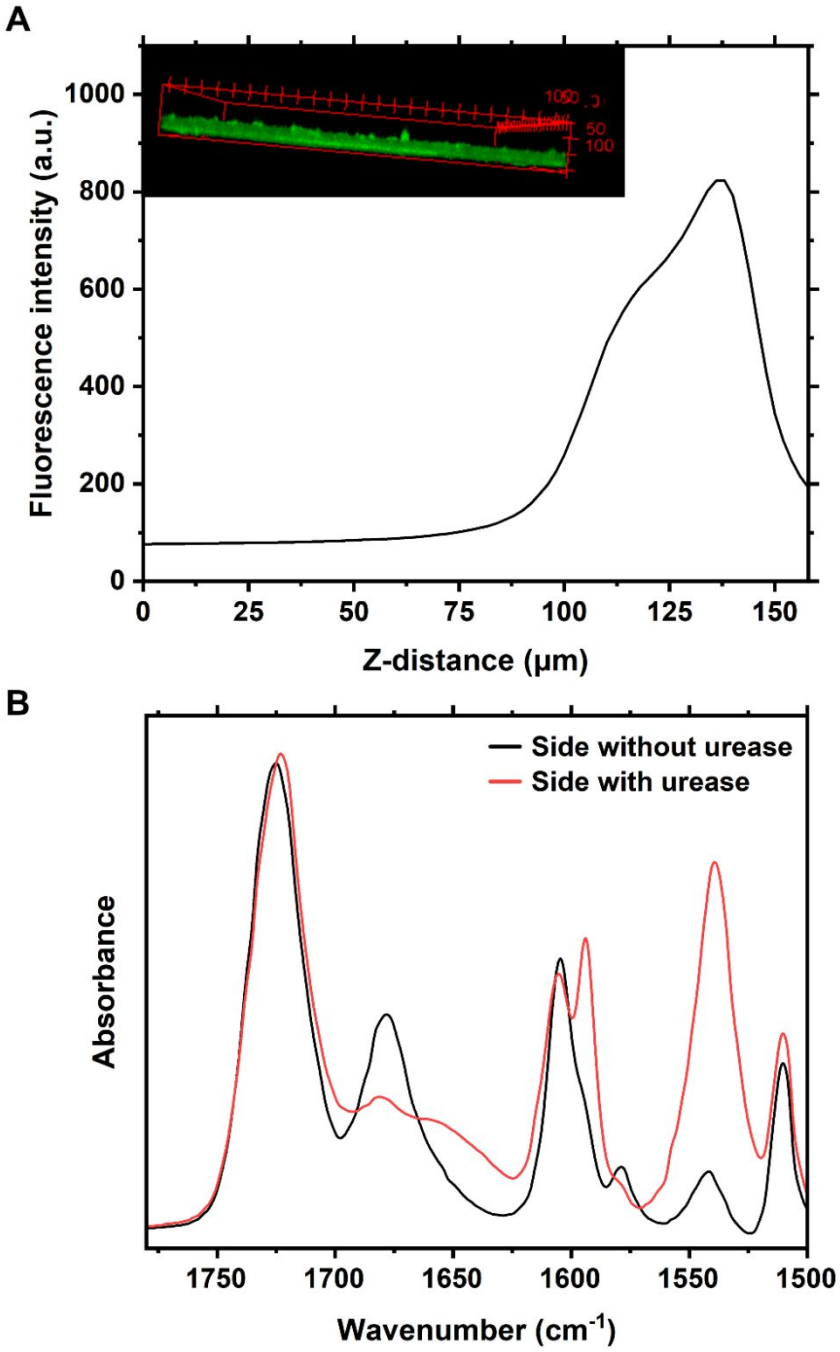

Figure S7. Characterization of LCE_{Urease} films in which urease was immobilized on only one side of the film. **A)** Confocal imaging of the LCE film with fluorescently tagged urease showing a plot of the fluorescence intensity of the film throughout its thickness. The inset confirms the fluorescently tagged urease is only immobilized on one side of the film. **B)** ATR-FTIR spectra of an LCE film with urease on one side before and after exposure to urea, showing a heterogeneous chemical composition. The neutralization of carboxylic acids (as evidenced by the decrease in the 1678 cm^{-1} carboxylic acid peak and the emergence of the 1541 cm^{-1} carboxylate peak) occurs predominantly on the side that contains urease.

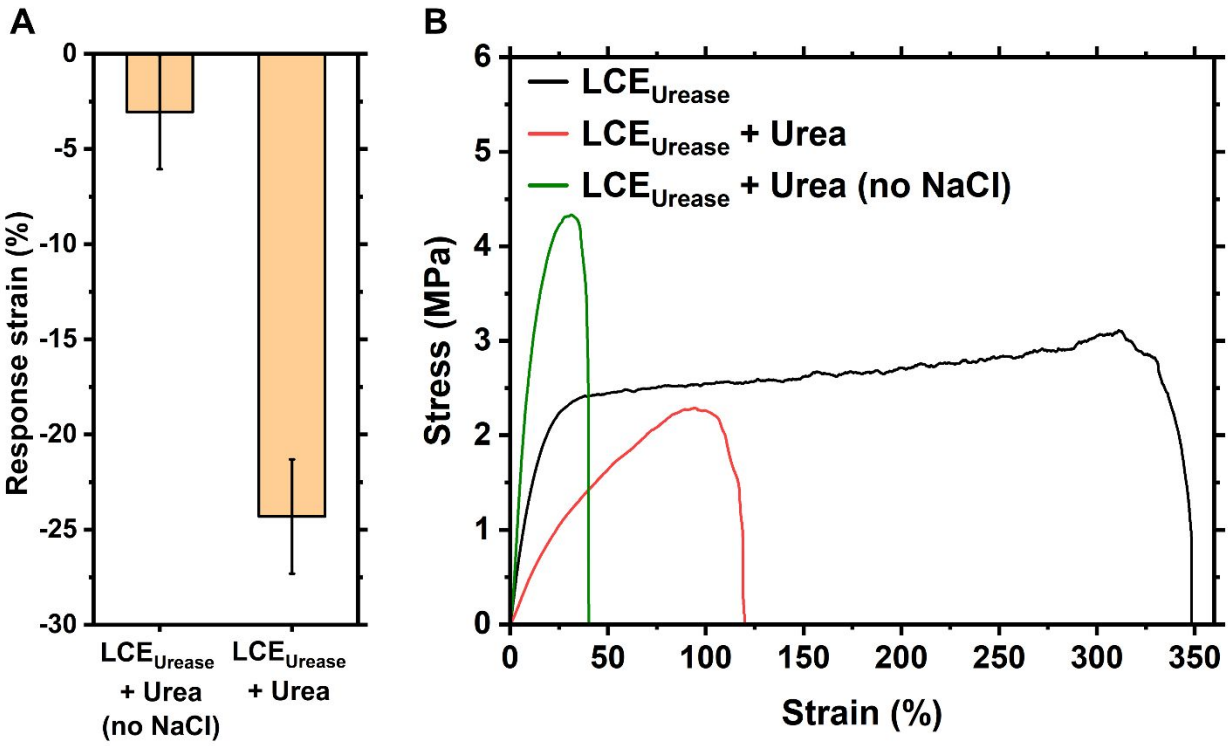

Figure S8. Effect of sodium chloride on the responsiveness and properties of LCE and LCE_{Urease} films. **A)** Response strain of LCE_{Urease} films after exposure to 50 mM urea with and without 500 mM sodium chloride for 24 h at room temperature. **B)** Stress-strain curves of LCE_{Urease} films after exposure to similar conditions. Each column represents the mean of two independent measurements. The error bars represent the 95% confidence interval of measurements.

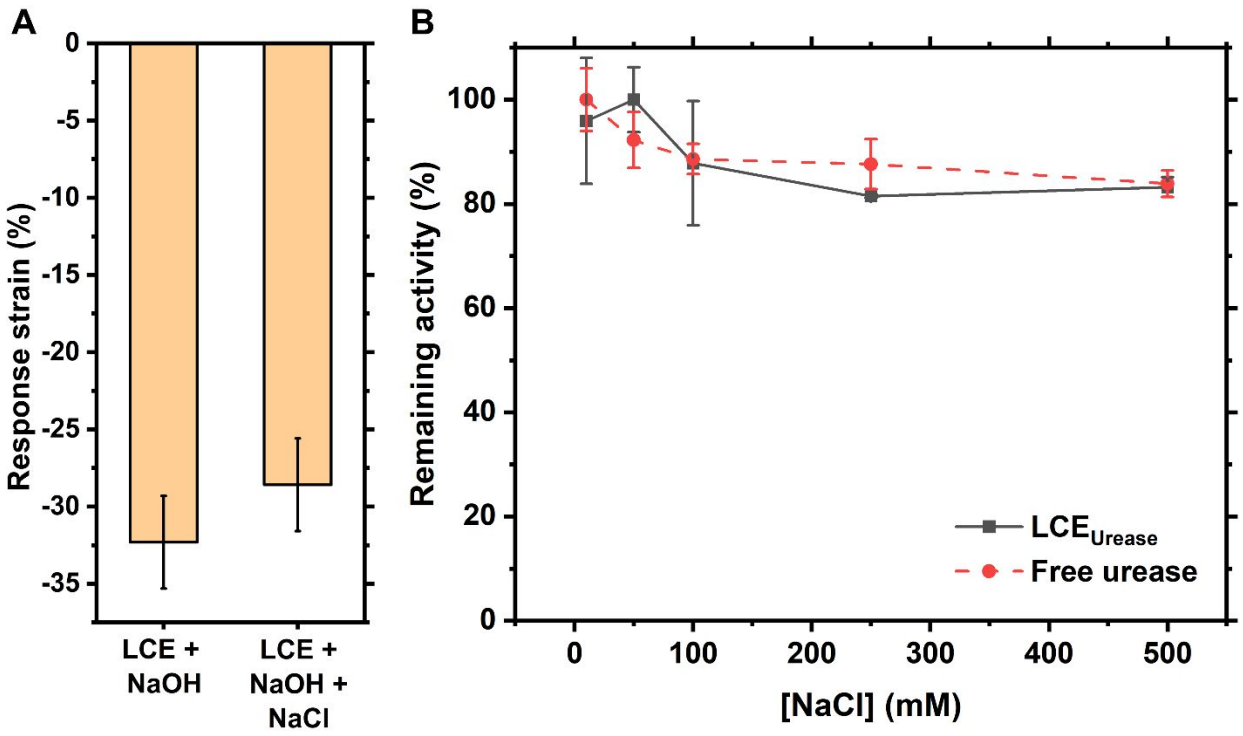

Figure S9. Effect of ionic strength on the response of LCE films as well as on the biocatalytic activity of LCE_{Urease} films. **A)** Response strain of LCEs after exposure to 10 mM sodium hydroxide with and without 500 mM sodium chloride for 30 min at room temperature. The response of LCE films to sodium chloride (500 mM) in the absence of sodium hydroxide was negligible. Each column represents the mean of two independent measurements. The error bars represent the 95% confidence interval of measurements. **B)** Relative activity of free urease and LCE_{Urease} films as a function of sodium chloride concentration. The activity was normalized using the highest activity in each data set. The data points represent the mean of two independent replicates. The error bars represent the 95% confidence interval of measurements.

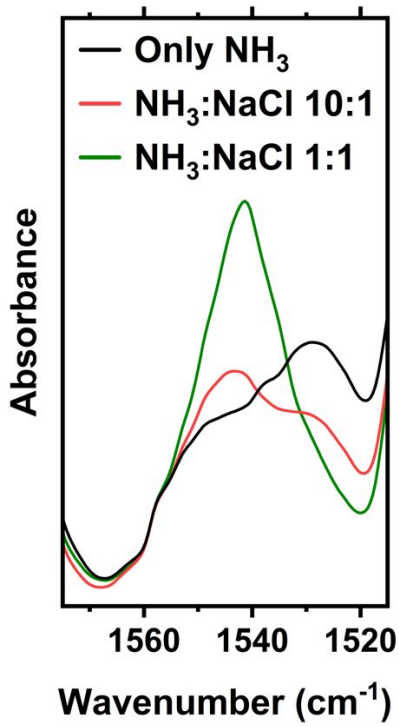

Figure S10. FTIR spectra of LCE films exposed to ammonia (black) as well as a 10:1 (red) and 1:1 (green) molar ratio of ammonia-to-sodium chloride for 24 h. For the ammonia only condition, the concentration of ammonia in the solution was 15 M. For the 10:1 ammonia-to-sodium chloride condition, the concentrations of ammonia and sodium chloride were 1 M and 0.1 M, respectively, For the 1:1 ammonia-to-sodium chloride condition, the concentrations of ammonia and sodium chloride were both 0.1 M. When reacted with just ammonia, the ammonium benzoate antisymmetric stretching peak at 1528 cm^{-1} was observed. At the 10:1 ammonia-to-sodium chloride condition, both the ammonium benzoate peak and the sodium benzoate peak (1541 cm^{-1}) were observed, suggesting that sodium was able to partially outcompete ammonium in the network structure. However, at the 1:1 ammonia-to-sodium chloride condition, the sodium ions appeared to fully outcompete the ammonium ions and only the sodium benzoate peak was observed. In all conditions, the 1678 cm^{-1} hydrogen-bonded peak disappeared, indicating full conversion of carboxylic acids to carboxylates.

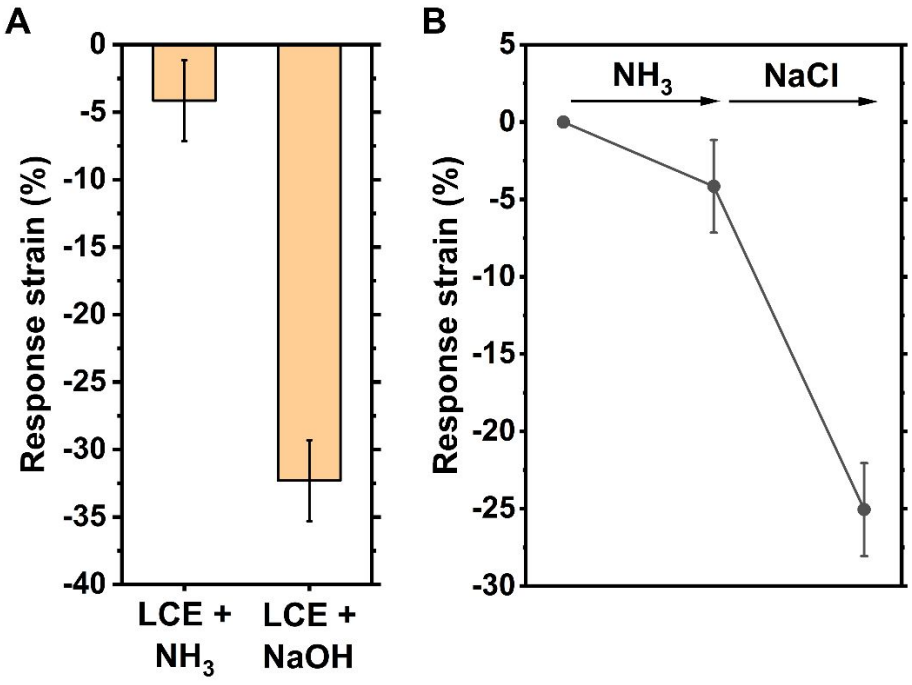

Figure S11. Effect of cation-related phenomena on the chemically induced actuation of the LCE films. **A)** Response strain of LCEs exposed to 15 M ammonia for 24 h or 0.2 M sodium hydroxide for 30 min at room temperature. The LCE films only showed a strong response when exposed to sodium hydroxide. **B)** Response strain of an LCE film initially exposed to 15 M ammonia for 24 h and subsequently exposed to 500 mM sodium chloride for 1 h. In between exposures, the film was rinsed thoroughly with DI water. The data points or columns represent the mean of two independent replicates. The error bars represent the 95% confidence interval of measurements.

SUPPORTING TABLES

Table S1. Dynamic mechanical analysis of LCE films with various conditions, including with and without urease as well as with and without urea, sodium hydroxide, and ammonia. From dynamic mechanical analysis, the Young's modulus, maximum stress during testing, and strain at break were determined. For determining the Young's modulus of the films, the modulus was calculated from the slope of elastic region of the stress-strain curve. Error estimates represent the standard deviation of at least three replicates.

Sample	Young's Modulus (MPa)	Maximum stress (MPa)	Strain at break (%)
LCE	6.7 ± 0.7	2.1 ± 0.5	279 ± 45
LCE + bismaleimide	5.1 ± 0.3	1.9 ± 0.2	283 ± 77
LCE_{Urease}	14.9 ± 0.5	3.6 ± 0.4	357 ± 14
LCE_{Urease} + urea	5.6 ± 1.6	1.9 ± 0.4	95 ± 20
LCE_{Urease} + urea (no salt)	40 ± 8	4.7 ± 0.6	32 ± 5
LCE + sodium hydroxide	2.0 ± 0.1	1.49 ± 0.04	180 ± 4
LCE + ammonia	40 ± 4	6.3 ± 0.4	55 ± 2