Supplementary information for:

Cell-sized mechanosensitive and biosensing compartment programmed with DNA

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Materials and Methods:

DNA constructions. The plasmids P70a-deGFP, P70a-S28, and P28a-deGFP have been described previously[1]. P28a-MscL and P28a-MscL-eGFP were obtained by first cloning MscL from *E. coli* K12 into a P28a backbone and then adding eGFP as a fusion protein. The pcDNA3-G-GECO construct was obtained from Takanari Inoue (Johns Hopkins University) and the calcium-sensing function was verified by monitoring G-GECO activity from external calcium flux through ionophore A23187 (Sigma Aldrich). G-GECO was then cloned into the pT7-CFE backbone for use in a mammalian cell-free expression system. Specifically, G-GECO was PCR amplified from of the pcDNA backbone and a 5' Kozak sequence and 3' CAAX sequence (in frame) added. The resulting PCR amplicon was cloned into the pT7-CFE empty vector between the plasmid IRES and poly-A tail sequence using BamHI and NotI. pT7-G-GECO DNA sequence was confirmed by sanger sequencing and its function was verified in bulk mammalian cell-free expression assays with and without calcium. Subsequently, restriction enzyme cloning from the pT7 construct into P70a-deGFP was carried out to obtain the P70a-G-GECO plasmid used in the present study. Due to lack of appropriate restriction cut sites, the gene for G-GECO was PCR amplified along with the 3' CAAX motif, and inserted by restriction enzyme cloning using PspXI and NcoI, in between the ribosome binding site and the T500 terminator sequences of the P70a-deGFP vector.

TXTL preparation and reactions. TXTL reactions are composed of the *E. coli* lysate, an amino acid mixture, and an energy buffer. Transcription and translation are performed by the endogenous molecular components provided by the *E. coli* cytoplasmic extract. A detailed description of the standard TXTL preparation has been reported previously in several articles[1-3]. The energy buffer is composed of: 50 mM HEPES pH 8, 1.5 mM ATP and GTP, 0.9 mM CTP

and UTP, 0.2 mg/ml tRNA, 0.26 mM coenzyme A, 0.33 mM NAD, 0.75 mM cAMP, 0.068 mM folinic acid, 1 mM spermidine, 30 mM 3-PGA, 2% PEG8000, either 10-15 mM maltose or 20-40 mM maltodextrin. A typical cell-free reaction is composed of 33% (volume) of *E. coli* crude extract. The other 66% of the reaction volume are composed of the energy mixture, the amino acids and plasmids. The amino acid concentration was adjusted between 1.5 mM and 3 mM of each of the 20 amino acids (always equimolar for the 20 amino acids). Mg-glutamate and K-glutamate concentrations were adjusted according to the plasmids used (typically 90 mM K-glutamate and 4 mM Mg-glutamate for P70a-deGFP). Cell-free TXTL reactions were carried out in a volume of 5 µl to 20 µl at 29-30°C.

Measurement of TXTL gene expression in bulk reactions. Quantitative measurements were carried out with the reporter protein deGFP (25.4 kDa, 1 mg/ml = 39.37 µM). deGFP is a variant of the reporter eGFP that is more translatable in cell-free systems. The excitation and emission spectra as well as fluorescence properties of deGFP and eGFP are identical, as previously reported[1]. The fluorescence of deGFP produced in batch mode reactions was measured on an H1m plate reader (Biotek Instruments, V-bottom 96-well plate, interval of three minutes, Ex/Em 488/525 nm). End-point measurements were carried out after 8-12 h of incubation. Pure recombinant eGFP (from either Cell Biolabs Inc. or purified in the lab) was used to make a linear calibration of intensity versus eGFP concentration for quantification on plate readers. Error bars are the standard deviations from at least three repeats. The measurements in batch more reactions for G-GECO were similar to deGFP. Measurements were recorded with a Synergy H1 plate reader (Biotek Instruments) in 96 V-bottom Nunc polypropylene plates. The temperature was set at 29°C for all conditions and kinetic measurements were carried out with an interval of one minute. The excitation and emission wavelengths were set at 488 nm and 525 nm respectively. The measurement run was stopped at a given time and restarted after externally adding calcium to the bulk reaction. This led to a delay of 1-2 minutes indicating the loss of at most two data points at the instant of calcium addition in the supplementary figures.

Liposome preparation. Liposomes were prepared using the water-in-oil emulsion transfer method. Lipids (Egg PC, Avanti Polar Lipids) were dissolved in mineral oil (Sigma-Aldrich) at 2 mg/ml. 2-8 µl reactions were added to 500 µl oil and vortexed to create an emulsion. This emulsion was added atop 10-20 µl of a feeding solution. The biphasic solution was centrifuged for 10 minutes at 1500 g to form liposomes. Single emulsion droplets were created by vortexing 6 µL of TXTL reaction in 30 µL of 2% Span 80 surfactant in mineral oil. Double emulsion templated vesicles were generated by a glass capillary device as described in previous works[4,5]. DOPC, cholesterol and Liss-Rhod PE (Avanti Polar Lipids) were mixed in a glass test tube and Argon gas was used to remove the solvent. The lipids were then placed in a dessicator for an hour following which they were resuspended in a 36:64 chloroform-hexane solution by volume. The final concentration of the lipids was kept constant at 6 mg/ml for all experiments when using this device. The inner solution was prepared by adding 1.5% polyvinyl alcohol (PVA) and 1 mM EGTA (final concentrations) to a standard TXTL cell-free reaction. The cell-free solution was then incubated at 29C for an hour before encapsulation. Outer solution composition was 20 mM K-HEPES, 80 mM KCl, 1.3% glycerol, 10% PVA, and 280 mM glucose (final osmolarity is 770 mOsm). The outer solution osmolarity was matched with the inner phase by adding appropriate concentration of glucose just before encapsulation. 10 mM $CaCl_2$ (final conc.) was added to this outer solution for resuspending the collected double emulsions. 50 µl of double emulsion collection was then added to 100 µl of the outer solution containing calcium for all experimental conditions except the hypo-osmotic shock with two plasmids, in which case 50 µl double emulsions was added to 100 µl of the hypo-osmotic solution. The hypo-osmotic solution composition was 20 mM K-HEPES, 80 mM KCl, 10% PVA, 400 mM glucose and 10 mM $CaCl_2$ (final osmolarity is 660mOsm). All osmolarities were measured on a Vapro-Osmometer (by

vapor pressure) with 10 µl of sample. Following resuspension in calcium-containing solutions, the double emulsions were incubated on a slide. Oil dewetting and lipid bilayer formation was observed within a short duration after double emulsion generation.

Measurement of TXTL gene expression in liposomes. The phospholipid vesicles were observed using an inverted microscope (Olympus, IX-81) equipped with Metamorph Advanced software. Liposomes expressing deGFP/eGFP were imaged with a GFP filter set (excitation 473 nm, emission 520 nm). To image the TRITC-dextran dye (Sigma-Aldrich) and BSA-TRITC dye (Sigma-Aldrich), a Texas Red filter set was used (excitation 556 nm, emission 617 nm). At least 40 lipid vesicles were monitored to generate the standard deviation shown in the error bars. The method used to quantify the deGFP protein expression has been described previously[1]. deGFP was expressed and quantified using the Biotek H1m plate reader. Then, deGFP protein at different, known concentrations was encapsulated in liposomes and standard curves of area vs intensity were created. By comparing the leading coefficients of these curves, we verify the scaling is linear. The CCD camera used was also verified to scale linearly with varying exposure times. The double emulsion templated vesicles were imaged with Olympus IX-81 spinning disk confocal microscope connected to an Andor iXOn 3 CCD camera, and operated with the Metamorph Advanced software. G-GECO and deGFP images were captured with a GFP filter set (excitation 488 nm, emission 525 nm) under constant exposure of 500 ms for all experimental conditions. A TRITC filter set (excitation 560 nm/emission 607 nm) was used to capture the fluorescent lipid images. The exposure was kept constant at 100 ms for lipid imaging. All images were background subtracted and their contrasts were matched for visual comparison. For the quantitative analysis of calcium detection under different concentrations, integrated density (ID) measurement was carried out in ImageJ. For each image, a box was drawn based on the size of a vesicle and the ID was calculated three times by shifting the box within the lumen of the vesicle. Next, using the same box, the background ID was calculated at three different positions in the image. The relative fluorescence intensity was then calculated by subtracting the average background ID from the average luminal ID and normalizing the result with the area of the box.

References

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Supplemental Figures:

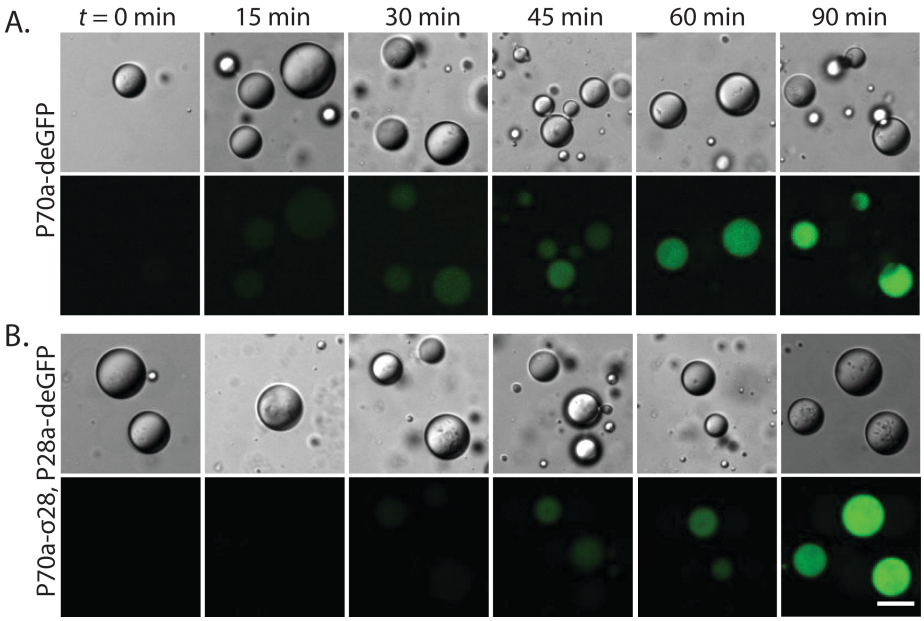

Figure S1. Encapsulation of TXTL reactions in single emulsions. P70a-deGFP, P70a-S28 and P28a-deGFP concentrations were the same as in Fig. 2A. Images represent a qualitative trend observed over 4-5 regions in the sample for three independent experiments. Scale bar: 50 μm.

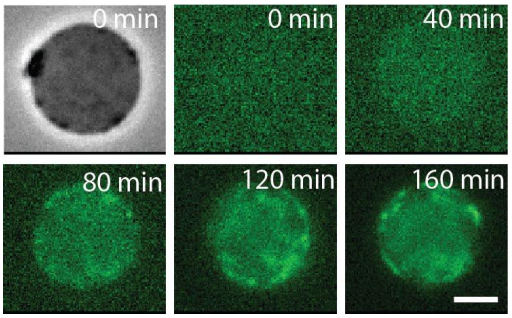

Figure S2. Synthesis of MscL-eGFP in liposomes (0.2 nM P70a-S28, 5 nM P28a-MscL-eGFP). Bright-field and fluorescence images shown at t = 0 min. Images taken at 40-minute interval with GFP channel. Scale bar: 5 μm.

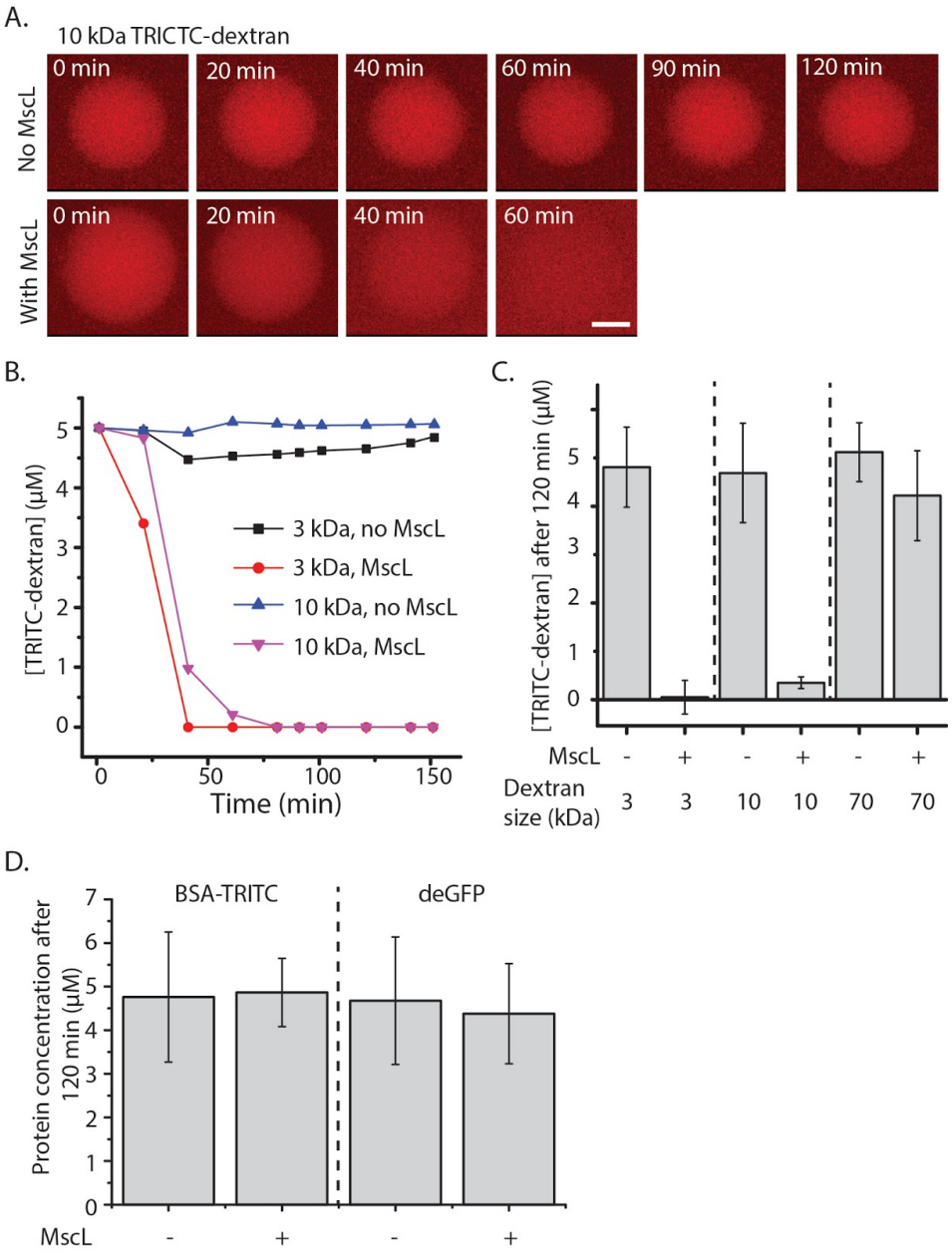

Figure S3. Leakage of TRITC-dextran of varying sizes in liposomes in the absence or presence of MscL. (A) Top: Fluorescence images of liposomes containing 10 kDa TRITC-dextran over a 2-hour period without P28a-MscL in the TXTL reaction. Bottom: Fluorescence images of liposomes containing 5 μM 10 kDa TRITC-dextran over a 60-minute period with P28a-MscL the TXTL reaction. Scale bar: 5 μm. (B) Liposome kinetics of 3 and 10 kDa TRITC-dextran concentration in the presence or absence of MscL. (C) Bar graph of average TRITC-dextran concentration at 0 and 120 minutes. 5 μM TRITC-dextran encapsulated in liposomes at t = 0. Sizes of dextran are 3, 10, and 70 kDa. - or + denote the absence or presence of MscL. Error bars shown are one standard deviation in each direction. Sample standard deviation is generated from the observation of 49, 51, 45, 43, 42, and 45 liposomes for 3-, 3+, 10-, 10+, 70-, and 70+, respectively (3- indicates 5 μM 3 kDa dextran and no MscL, 3+ indicates 5 μM 3 kDa

and 5 nM MscL plasmid, etc.) (D) Bar graph of average BSA-TRITC and deGFP concentration at 0 and 120 minutes. 5 μM BSA-TRITC or deGFP encapsulated in liposomes at t = 0. - or + denote the absence or presence of MscL. Error bars shown are one standard deviation in each direction. Sample standard deviation is generated from the observation of 41, 41, 42, and 45 liposomes for BSA-. BSA+, deGFP-, and deGFP+, respectively (BSA- indicates 5 μM BSA-TRITC and no MscL, BSA+ indicates 5 μM BSA-TRITC and 5 nM MscL plasmid, etc.)

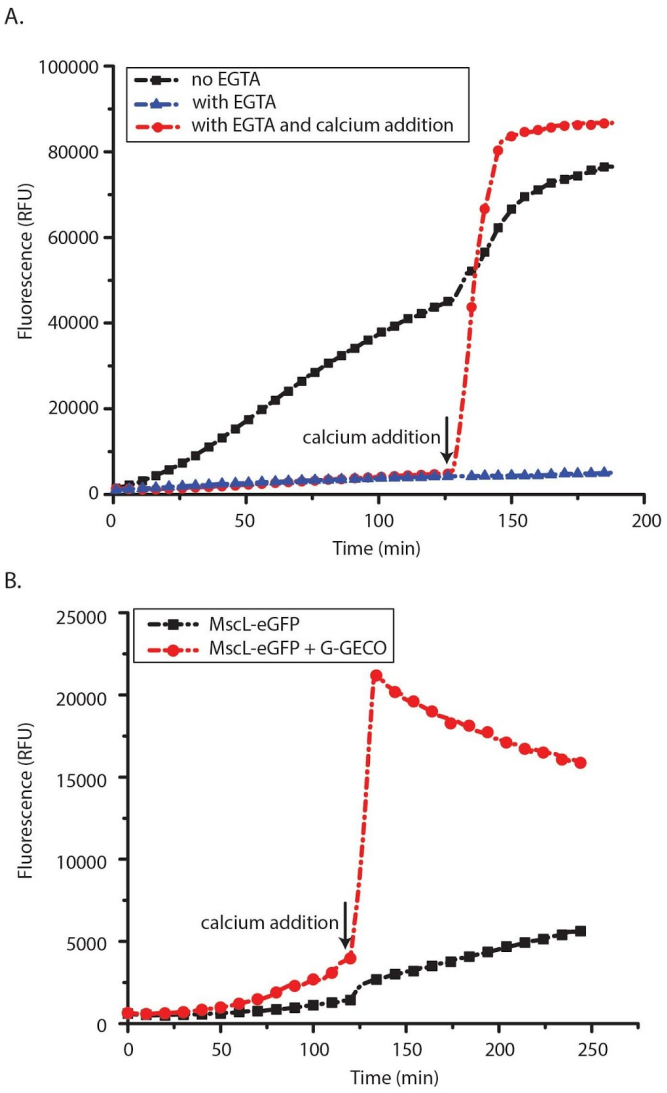

Figure S4. Expression kinetics of G-GECO in TXTL with and without calcium using P70a-G-GECO. (A) Calcium sensing by G-GECO in presence of 1 mM EGTA. 2 mM calcium chloride (final concentration) was added to bulk reaction at t = 125 min. Concentration of P70a-G-GECO plasmid was fixed at 1 nM for all cases. (B) Two-plasmid expression in bulk reaction. P70a-G-GECO, P70a-S28 and P28a-MscL-eGFP were added at final concentrations of 0.2 nM, 0.2 nM and 1 nM respectively. 1 mM calcium chloride was added after 120 minutes. TXTL reactions for both conditions contained 1 mM EGTA.

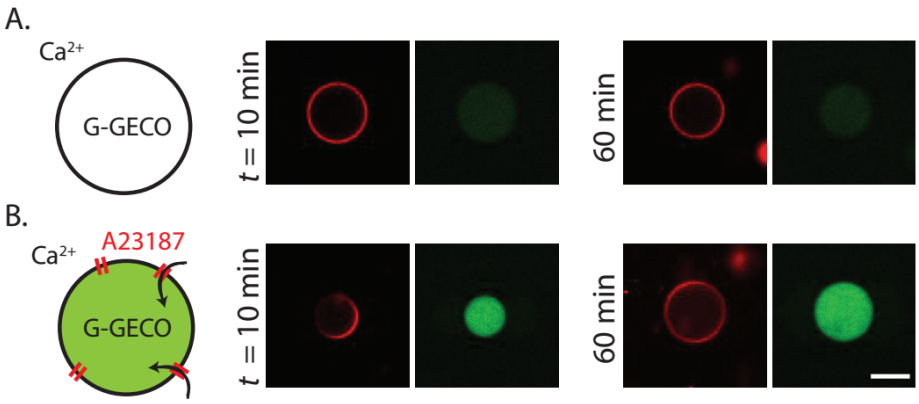

Figure S5. Calcium dependent increase in G-GECO fluorescence inside lipid vesicles. (A) Images showing the native fluorescence of cell-free expressed G-GECO after 3 hours of incubation. (B) Increase in fluorescence as observed upon addition of 1 µM A23187 (Calcium ionophore). The time points indicate incubation of vesicles after the addition of A23187 and the simultaneous control experiment without the ionophore. All cell-free reactions contained 1 mM EGTA. The calcium concentration in the outer solution was 10 mM. P70a-G-GECO plasmid was added at a concentration of 1.5 nM. Scale bar: 50 µm.

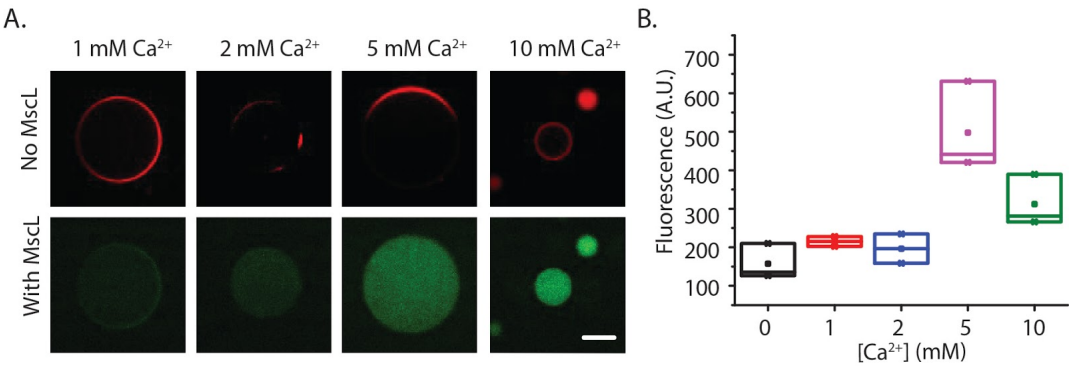

Figure S6. Osmotic downshock experiments of TXTL encapsulated vesicles (MscL and G-GECO) with different external calcium concentrations. (A) Fluorescence images of vesicles after 20 minutes of incubation following addition of hyposmotic media, for four different calcium concentrations. In contrast with the experimental condition in Figure 4, the vesicles were incubated for an hour after their generation, in isosmotic solution with no calcium, to allow for possible MscL incorporation into the lipid bilayer before the hyposmotic shock. (B) Box plot of the relative fluorescence intensities of vesicles from (A) at the same time point. The fluctuations in the relative intensities correspond to the values from six vesicles in each case. The plasmid concentrations for P70a-G-GECO, P70a-S28 and P28a-MscL were 1 nM, 0.4 nM, and 1.3 nM, respectively. EGTA was added at a concentration of 1 mM. Scale bar: 20 μm.