PPK Finale-lightweight

Status	Memoify
Tags	
AI summary	The research focuses on optimizing bulk reactions in PURE systems by comparing reactions with and without PEG 4K and including OptiPrep for liposome encapsulation. Detailed experimental setups, including component concentrations and volumes, are provided for various reaction conditions. Results indicate that the combined PPK+CP system yields the most intense GFP signal, with fluorescence measurements correlating with bulk reaction data.
Researchers	Ⓨ Yen-Yu Hsu
Keywords	

Overview

Data and images I want to add on top of Sarendra's experiments for DevNote.

Information and resources necessary for context; include links to other research notebooks as necessary.

Contents

Notebook

Bulk reactions:

Surendra has done excellent work optimizing the Energy module in bulk reactions. However, all of those reactions included PEG 4K, which is not typically used in standard PURE reactions. To provide a more direct comparison with our usual setup, and with conditions used by other

researchers, I plan to include data from reactions performed without PEG 4K, following the standard protocol we've used across all our experiments. In addition, it's important to include OptiPrep in the PURE reactions, as its use is essential for liposome encapsulation and therefore cannot be omitted in those experiments.

Repeat Sarendra's experiment and also test the reactions

1. With addition of optiprep
2. Without addition of PEG4K

@July 29, 2025

REACTION SETUP

3X Energy Mix:

Component	Stock concentration [mM]	Concentration of components in reaction [mM]	Concentration in Energy solution [mM]	Volume for the experimentFinal volume to add [µL]
HEPES	1000	50	150	16.5
Potassium glutamate	2500	100	300	13.2
NTP	100	2	6	6.6
tRNA [mg/mL]	50 (55.23)	3.5	10.5	20.9
TCEP	500	1	3	0.66
Folinic acid	5	0.02	0.06	1.32
Spermidine	200	2	6	3.3
Amino Acid solution	3.25	0.3	0.9	30.47
Water				17.05
Energy solution total		Final concentration [fold]		
		3		110

1. PURE with CP and 8 mM Mg2+ (w/PEG) (B1-3)

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
Energy solution-CP	3.00	×	1	×	11.67 (tRNA:2.2) 9.47

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
Sol B	3.33	x	1.00	x	10.51
deGFP DNA	120	ng/µL	3	nM	1.67
Mg-Acetate	200	mM	8	mM	1.40
Creatine phosphate	1000	mM	20	mM	0.70
PEG4K 40%	40	%	2	%	1.75
Water					7.30
Total volume [µL]					
35					

2. PURE with PolyP, PPK and 18 mM Mg2+ (w/PEG) (B4-6)

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
Energy solution-CP	3.00	×	1	×	11.67
Sol B	3.33	x	1.00	x	10.51
deGFP DNA	120	ng/µL	3	nM	1.67
Mg-Acetate	200	mM	18	mM	3.15
PEG4K 40%	40	%	2	%	1.75
PolyP	500	mM	30	mm	2.10
PPK2	57.5	uM	2	uM	1.22
Water					2.93
Total volume [µL]					
35					

3. PURE with CP, PolyP, PPK and 18 mM Mg2+ (w/PEG) (B7-9)

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
Energy solution-CP	3.00	×	1	×	11.67
Sol B	3.33	x	1.00	x	10.51

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
deGFP DNA	120	ng/µL	3	nM	1.67
Mg-Acetate	200	mM	18	mM	3.15
Creatine phosphate	1000	mM	20	mM	0.70
PEG4K 40%	40	%	2	%	1.75
PolyP	500	mM	30	mm	2.10
PPK2	57.5	uM	2	uM	1.22
Water					2.23
Total volume [µL]					
35					

4. PURE with CP and 8 mM Mg2+ (w/Optiprep) (B10-12)

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
Energy solution-CP	3.00	×	1	×	11.67
Sol B	3.33	x	1.00	x	10.51
deGFP DNA	120	ng/µL	3	nM	1.67
Mg-Acetate	200	mM	8	mM	1.40
Creatine phosphate	1000	mM	20	mM	0.70
Optiprep		%		%	1.16
Water					7.89
Total volume [µL]					
35					

5. PURE with PolyP, PPK and 18 mM Mg2+ (w/Optiprep) (B13-15)

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
Energy solution-CP	3.00	×	1	×	11.67

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
Sol B	3.33	x	1.00	x	10.51
deGFP DNA	120	ng/µL	3	nM	1.67
Mg-Acetate	200	mM	18	mM	3.15
Optiprep		%		%	1.16
PolyP	500	mM	30	mm	2.10
PPK2	57.5	uM	2	uM	1.22
Water					3.52
Total volume [µL]					
35					

6. PURE with CP, PolyP, PPK and 18 mM Mg2+ (w/Optiprep) (C1-3)

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
Energy solution-CP	3.00	×	1	×	11.67
Sol B	3.33	x	1.00	x	10.51
deGFP DNA	120	ng/µL	3	nM	1.67
Mg-Acetate	200	mM	18	mM	3.15
Creatine phosphate	1000	mM	20	mM	0.70
Optiprep		%		%	1.16
PolyP	500	mM	30	mm	2.10
PPK2	57.5	uM	2	uM	1.22
Water					2.82
Total volume [µL]					
35					

7. PURE with CP and 8 mM Mg2+ (C4-6)

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
Energy solution-CP	3.00	×	1	×	11.67
Sol B	3.33	x	1.00	x	10.51
deGFP DNA	120	ng/µL	3	nM	1.67
Mg-Acetate	200	mM	8	mM	1.40
Creatine phosphate	1000	mM	20	mM	0.70
Water					9.05
Total volume [µL]					
35					

8. PURE with PolyP, PPK and 18 mM Mg2+ (C7-9)

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
Energy solution-CP	3.00	×	1	×	11.67
Sol B	3.33	x	1.00	x	10.51
deGFP DNA	120	ng/µL	3	nM	1.67
Mg-Acetate	200	mM	18	mM	3.15
PolyP	500	mM	30	mm	2.10
PPK2	57.5	uM	2	uM	1.22
Water					4.68
Total volume [µL]					
35					

9. PURE with CP, PolyP, PPK and 18 mM Mg2+ (C10-12)

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
Energy solution-CP	3.00	×	1	×	11.67
Sol B	3.33	x	1.00	x	10.51

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
deGFP DNA	120	ng/µL	3	nM	1.67
Mg-Acetate	200	mM	18	mM	3.15
Creatine phosphate	1000	mM	20	mM	0.70
PolyP	500	mM	30	mm	2.10
PPK2	57.5	uM	2	uM	1.22
Water					3.98
Total volume [µL]					
35					

10. PURExpress Positive control (PC) reaction (w/PEG) (D1-3)

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
Sol A	2.50	×	1	×	14.00
Sol B	3.33	x	1.00	x	10.51
deGFP DNA	120	ng/µL	3	nM	1.67
PEG4K 40%	40	%	0	%	1.75
PolyP	500	mM	0	mm	0.00
PPK2	57.5	uM	0	uM	0.00
Water					7.07
Total volume [µL]					
35					

11. PURExpress Positive control (PC) reaction (w/optiprep) (D4-6)

Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
Sol A	2.50	×	1	×	14.00
Sol B	3.33	x	1.00	x	10.51
deGFP DNA	120	ng/µL	3	nM	1.67
Optiprep		%		%	1.16

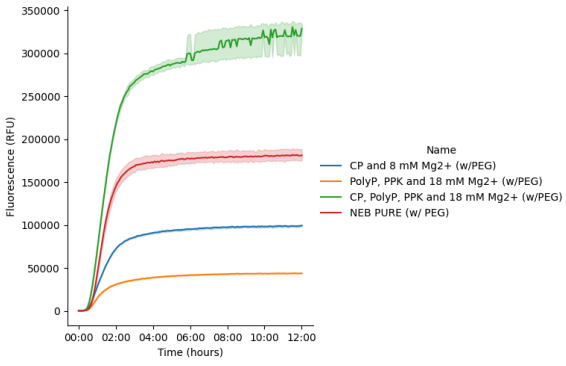
Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
Water					7.66
Total volume [µL]					
35					

12. PURExpress Positive control (PC) reaction (D7-9)

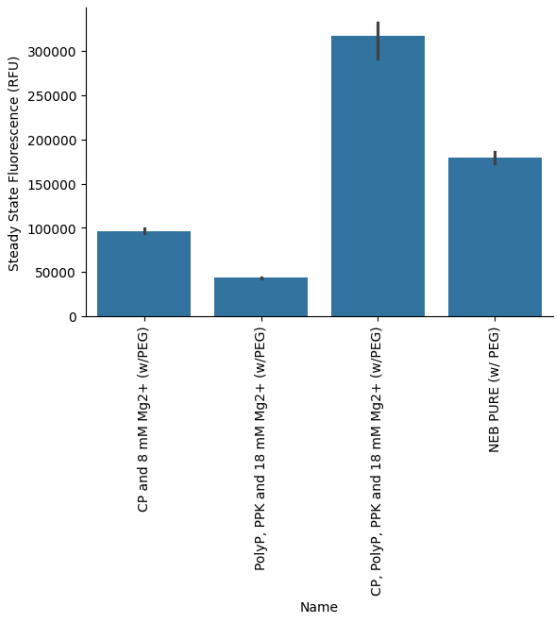
Component	Input concentration	Unit	Final concentration	Unit	Volume for one reaction [µL]
Sol A	2.50	×	1	×	14.00
Sol B	3.33	x	1.00	x	10.51
deGFP DNA	120	ng/µL	3	nM	1.67
Water					8.82
Total volume [µL]					
35					

RESULTS

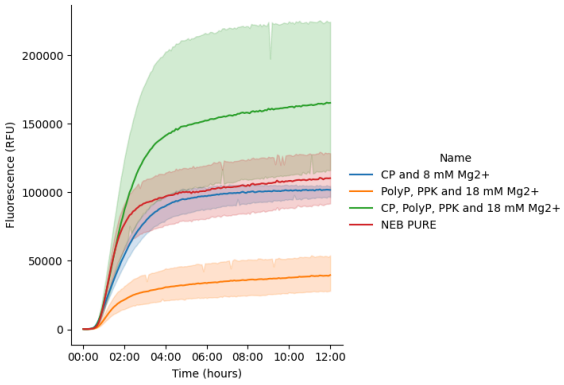
▼ PURE reactions with addition of PEG 4K.

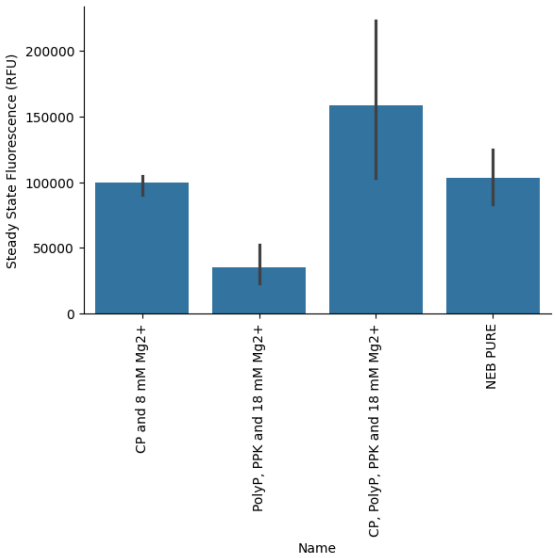


Reapting Sarenndra's experiments.

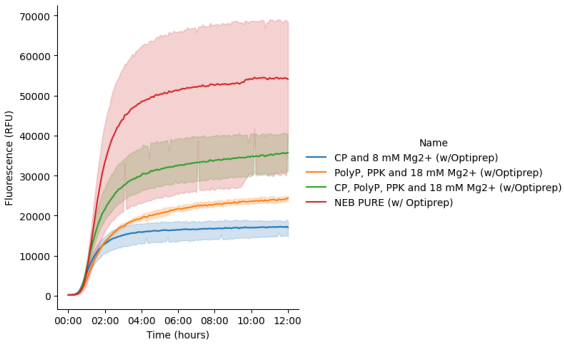


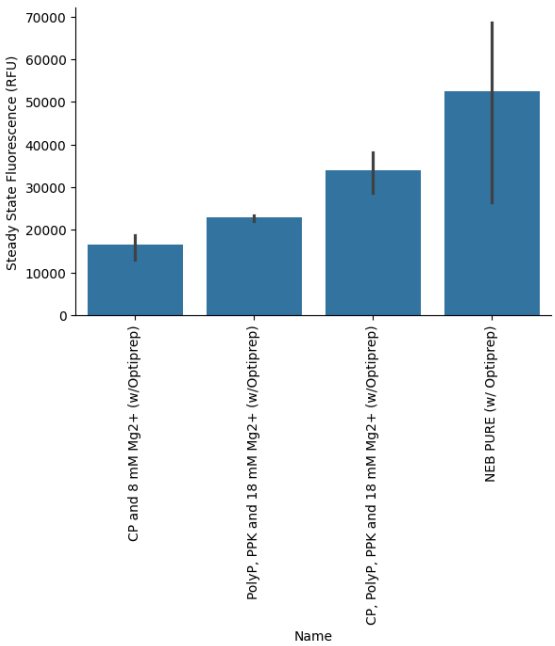
▼ PURE reactions

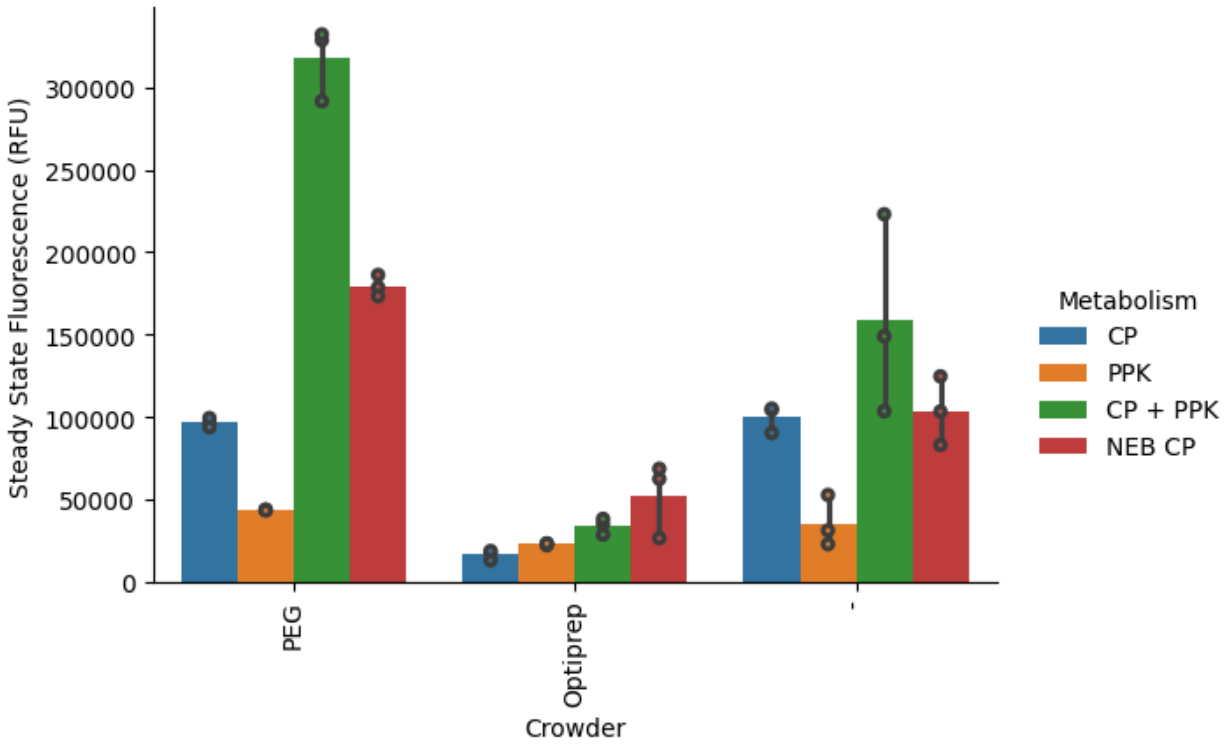




▼ PURE reactions with addition of Optiprep.







Combination graph of all samples.

Cell encapsulations:

Beside the bulk reactions, confocal images of liposomes encapsulating different PURE systems should also be included for the DevNote.

@August 11, 2025

PREPARATION OF ENERGY SOLUTION

3X Energy Mix:

Component	Stock concentration [mM]	Concentration of components in reaction [mM]	Concentration in Energy solution [mM]	Final volume to add [µL]
HEPES	1000	50	150	15
Potassium glutamate	2500	100	300	12
NTP	100	2	6	6
tRNA [mg/mL]	50 (55.23)	3.5	10.5	19
TCEP	500	1	3	0.6

Component	Stock concentration [mM]	Concentration of components in reaction [mM]	Concentration in Energy solution [mM]	Final volume to add [µL]
Folinic acid	5	0.02	0.06	1.2
Spermidine	200	2	6	3
Amino Acid solution	3.25	0.3	0.9	27.7
Water				15.5
Energy solution total		Final concentration [fold]		Final volume
		3		100

PREPARATION OF INNER SOLUTION

Name	CP PURE	PPK PURE	CP+PPK PURE	NEB PURE
3X Energy solution (µL)	16.68	16.68	16.68	0
Sol A (µL)	0	0	0	20
Sol B (µL)	15	15	15	15
pT7-deGFP (µL)	1.43	1.43	1.43	1.43
Mg-Acetate (µL)	2	4.5	4.5	0
Creatine phosphate (µL)	1	0	1	0
PolyP (µL)	0	3	3	0
PPK2 (µL)	0	1.74	1.74	0
Optiprep (µL)	0.84	0.84	0.84	0.84
Nucleus free water (µL)	13.05	6.81	5.81	12.73
Total volume [µL]	50	50	50	50

Note:

- [ ] **The concentrations of each component are listed below:**

  pOpen-pT7-deGFP: 120 ng/**µ**L

  Mg-Acetate: 200 mM

  Creatine phosphate: 1000 mM

  PolyP: 500 mM

  PPK: 57.5 µM

- [ ] **30 µL of the inner solution is used for bulk reaction detected using the platereader; whole 20 µL of the inner solution is used for making liposomes.**

PREPARATION OF LIPID-IN-OIL SOLUTION

Lipids

POPC: 16:0-18:1 PC (POPC)

Chol: cholesterol (plant)

Rhod-PE

Lipid-in-oil solution (~5mg/ ml): 70% POPC + 29.95% Chol + 0.05% Rhod

Lipids	Stock concentration (mg/mL)	MWt. (g/mol)	Target percentage (%)	Volume to add (µL)
POPC	25	760.08	70	162.2
Cholesterol	50	386.66	29.95	17.6
Rhod-PE	1	1301.72	0.05	5

1. Add 1 ml mineral oil in the 2mL small glass jar
2. Add lipids shown in the above table into the glass jar on top of the mineral oil
3. Vortex the lipid-in-oil mixture for 10 secs
4. Put the glass jar in the bead-loaded hot bath at ~55c for 3 hrs (keep the jar uncovered without lids in dark)
5. Place the jar (with lid) containing lipid-in-oil solution at RT for 10 mins before using

PREPARATION OF OUTER SOLUTION

	Stock concentration (mM)	Target concentration (mM)	Volume to add (µL)
Glucose	2000	800	600
Nucleus free water	x	x	900
Total volume [µL]			1500

FORMATION OF LIPOSOMES

☐ Set up a microfuge tube rack, with three 1.5 mL microfuge tubes per liposome encapsulation:

- ☐ For each reaction, label the two tubes:
  - ☐ **I** — Oil Emulsion

- ☐ **O** — Outer solution (800 mM Glucose)

☐ Add 20 µL of the inner solution prepared follwing the above table to tubes labelled **I**.

☐ Add 120 µL of the lipids-in-oil mixture on top of the PURE reactions in tubes labelled **I** and pipette vigorously until the emulsion becomes cloudy.

☐ Add 300 µL of Outer Solution to each of the tubes labelled **O**.

☐ Add 140 µL of the milky solution carefully on top of the outer solution in the tubes labelled **O.**

☐ Centrifuge at 9000 rcf / 4C / 10 min.

☐ Remove the top oil and resuspend the pellet in 100 µL of outer solution as the liposome solution.

☐ Transfer the liposome solution to one clean, sterile 1.5 mL Eppendorf tube.

MEASURE AND IMAGE LIPSOMES

Imaging using confocal microscopy (Operetta CLS):

While microscopy setups may vary, our performance data was collected using the following configuration.

☐ Add 30 µL of the outer solution (800 mM glucose) into 384-well glass bottom microplates.

☐ Add 20 µL of liposome solution on top of the outer solution in 384-well glass bottom microplates.

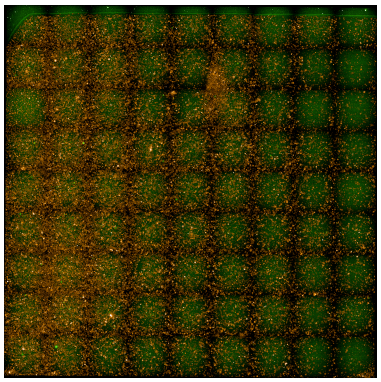
☐ Imaging conditions using Operetta:

- Temperature: 37 C degree
- Green fluorescence channel (1000 ms, exposure 95%) - excitation: 460 nm - 490 nm; emission: 500 nm - 550 nm.
- Red fluorescence channel (160 ms, exposure 95%) - excitation: 530 nm - 560 nm; emission: 570 nm - 650 nm.
- We capture a time lapse over 6 hrs with 10 min intervals.

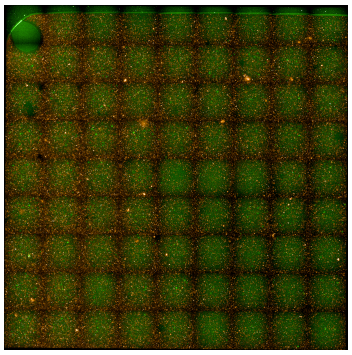
RESULTS

Whole well images at the endpoint:

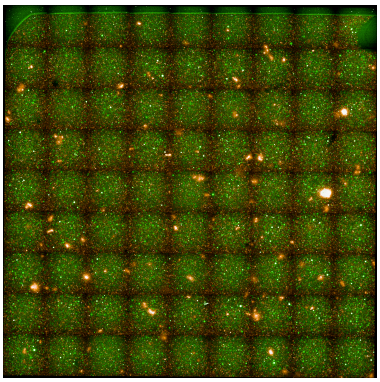
▼ Red + Green channels



Liposomes contain CP-PURE

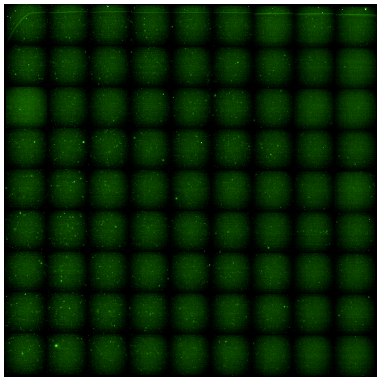


Liposomes contain PPK-PURE: 561 exp: 200; 488 exp: 1000

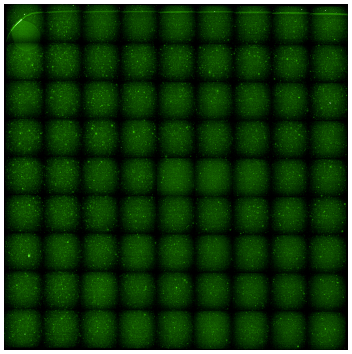


Liposomes contain CP+PPK-PURE: 561 exp: 200; 488 exp: 1000

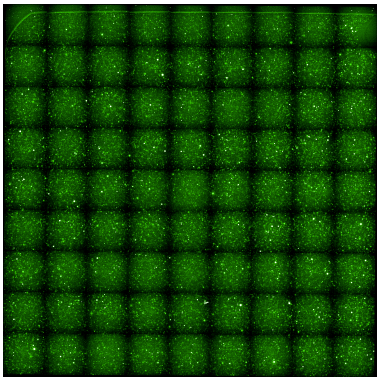
▼ Green channel



Liposomes contain CP-PURE

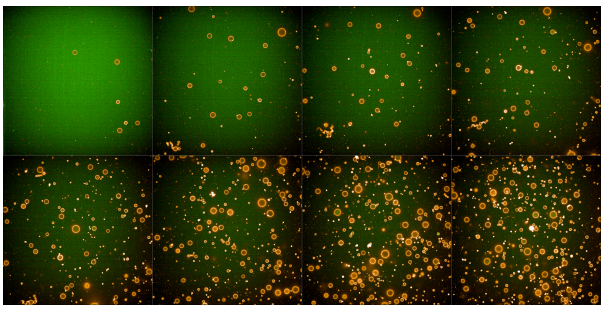


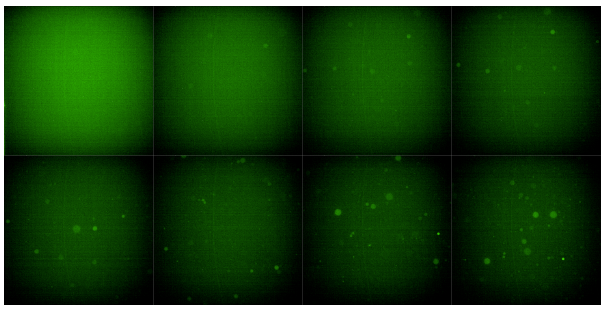
Liposomes contain PPK-PURE

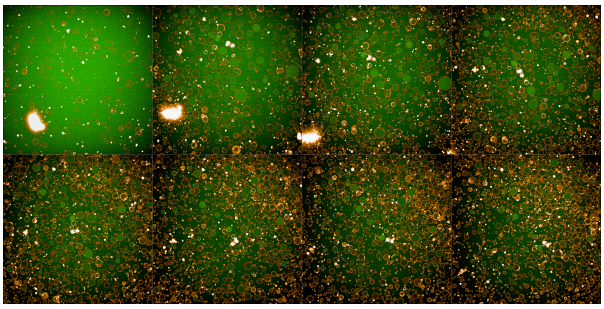


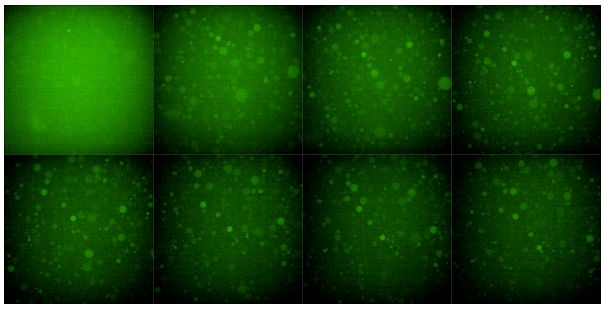
Liposomes contain CP+PPK-PUR

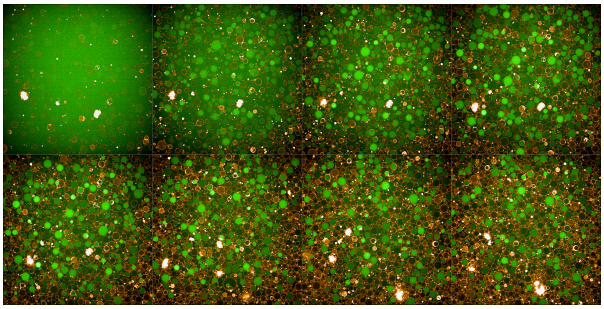
Focused view with timeseries images:

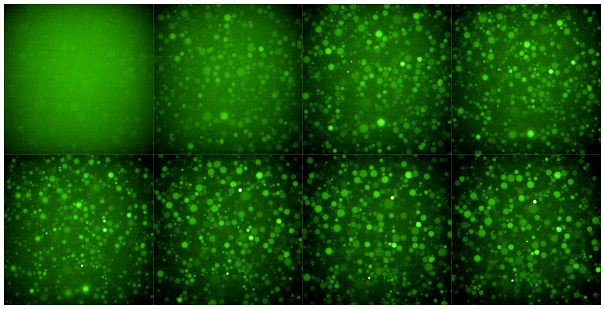






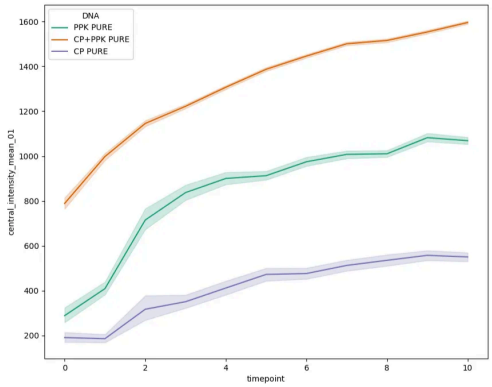




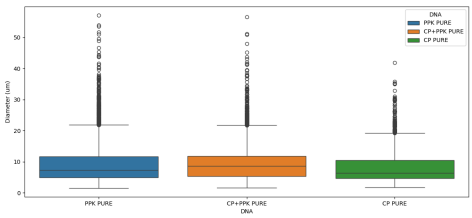


Data:

▼ PURE reactions with addition of Optiprep in cells - operetta.

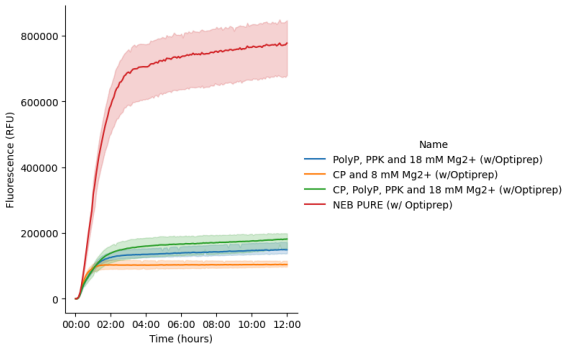


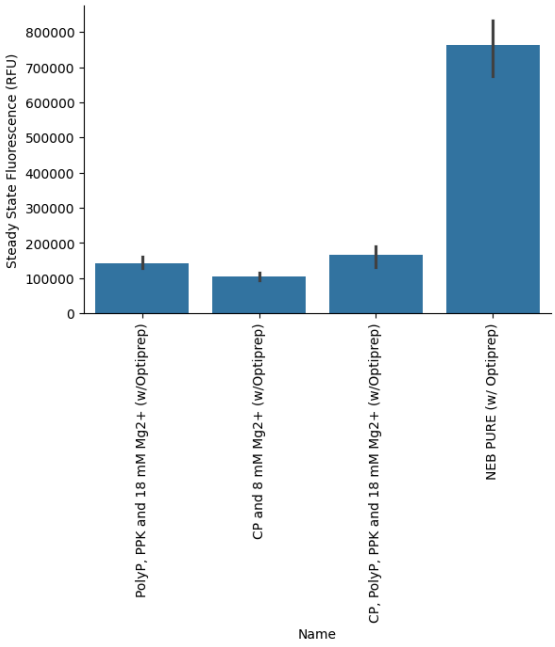
Expression of deGFP in liposomes overtime. The first timepoint was taken 40 mins after the reaction was prepared/ liposomes were made.



Size distribution

▼ PURE reactions with addition of Optiprep in bulk - platereader.





CONCLUSION

- All samples exhibit increasing green fluorescence over time; however, the combined PPK+CP system shows the most intense GFP signal.
- The relative intensities of green fluorescence align with the bulk reaction measurements obtained from the plate reader, following the order: CP+PPK PURE > PPK PURE > CP PURE.
- Liposomes containing NEB PURE continue to produce the highest GFP signals.