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Supplementary information

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Accelerated discovery of thermostable mRNA–lipid nanoparticle vaccines using data-efficient AI

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SUPPLEMENTARY INFORMATION

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SUPPLEMENTARY TABLE

Supplementary Table 1. Change in *in vitro* transfection efficiency of solid-state mRNA-LNPs after vacuum drying, normalized to corresponding soluble formulation.

LNP compositions	IL/HL/Chol/PEG	Clinically used	Fold change	Excipient(s)	Ref.
SM102/DSPC/Chol/DMG-PEG2000	50/10/38.5/1.5	Yes	-0.6	10% PVA+10% sucrose	1
SM102/DSPC/Chol/DMG-PEG2000	60/10/38.5/1.5	No	-0.2	10% PVA+10% sucrose	
SM102/DSPC/Chol/DMG-PEG2000	65/10/38.5/1.5	No	-0.5	10% PVA+10% sucrose	
SM102/DSPC/Chol/DMG-PEG2000	50/10/38.5/1.5	Yes	-0.62	5% PVA	2
SM102/DSPC/Chol/DMG-PEG2000	50/10/38.5/1.5	Yes	-0.82	5% PVA+2.5% sucrose	
SM102/DSPC/Chol/DMG-PEG2000	50/10/38.5/1.5	Yes	-0.75	5% PVA+10% sucrose	
SM102/DSPC/Chol/DMG-PEG2000	50/10/38.5/1.5	Yes	-0.81	5% PVA+15% sucrose	
SM102/DSPC/Chol/DMG-PEG2000	50/10/38.5/1.5	Yes	-0.95	5% PVA+10% trehalose	
SM102/DSPC/Chol/DMG-PEG2000	50/10/38.5/1.5	Yes	-0.9	5%PVA+10% glucose	
SM102/DSPC/Chol/DMG-PEG2000	50/10/38.5/1.5	Yes	-0.7	5% PVA +10% sucrose/ glucose	
SM102/DSPC/Chol/DMG-PEG2000	50/10/38.5/1.5	Yes	-0.8	5% PVA+10% sucrose/trehalose/glucose	
Lipid5/DOPE/Chol/C14-PEG2000	50/10/38.4/1.5	No	1.1	20% PVA/PVP	3
Lipid5/DOPE/Chol/C14-PEG2000	50/10/38.4/1.5	No	-0.3	20%PVP	
Lipid5/DOPE/Chol/C14-PEG2000	50/10/38.4/1.5	No	1.0	20% PVA/sucrose	
Lipid5/DOPE/Chol/C14-PEG2000	50/10/38.4/1.5	No	-0.9	20% PVP/sucrose	
ALC0315/DSPC/Chol/ALC-0159	46.3/9.4/42.7/1.6	Yes	1.08	6.15%PVA+16.73%PVP+ 4.67%sucrose+19.9%glucose+ 11.37%L-arginine HCl	This work
SM102/DSPC/Chol/DMG-PEG2000	50/10/38.4/1.5	Yes	1.03	10.07%PVA+17.45%PVP+ 22.22%sucrose+17.2%glucose +15.03%L-arginine HCl	

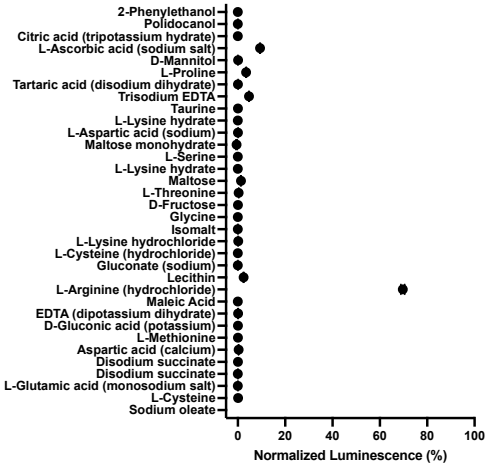
C14-PEG2000: 1-2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt)

Lipid 5: heptadecane-9-yl 8-((2-hydroxyethyl)(8-nonyloxy)-8-oxoctyl)amino)octanoate

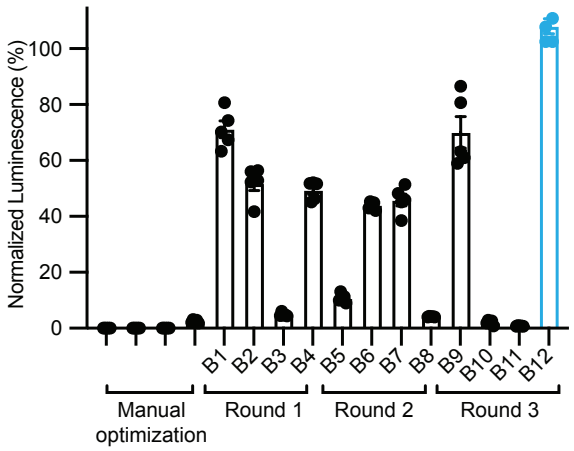
Supplementary Table 2. Gaussian Process hyperparameters used in optimization algorithm.

Hyperparameter	Initial Value	Optimization Range
l	$(1, \dots, 1) \in R^d$	$\left(\sqrt{10^{-3}}, \sqrt{10^{3}}\right)$
σ_f	1	$\left(\sqrt{10^{-3}}, \sqrt{10^{3}}\right)$
σ_n	10^{-2}	$(e^{-6}, 1)$

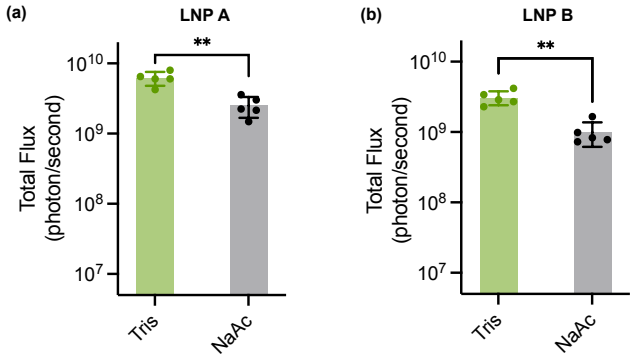
SUPPLEMENTARY FIGURES



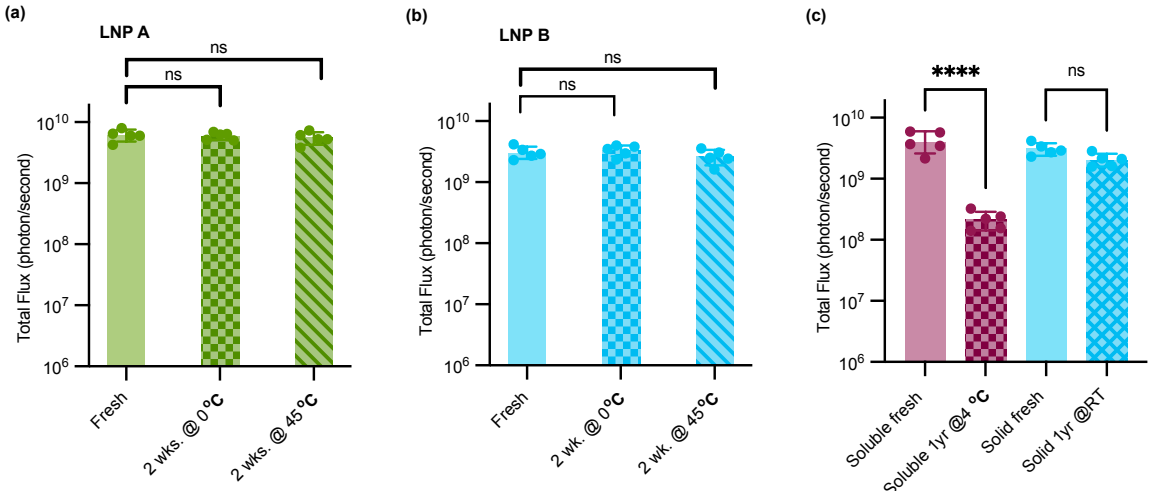
Supplementary Fig. 1 In vitro screening of amino acids for stabilizing mRNA-LNPs in solid states. Solid-state mRNA-LNPs (LNP A) were formulated with 10% (w/v) amino acids dissolved in 20 mM Tris buffer via vacuum drying, reconstituted in PBS, and treated with A549 cells. Luciferase expression was normalized against mRNA-LNPs with the same excipients maintained in solution.



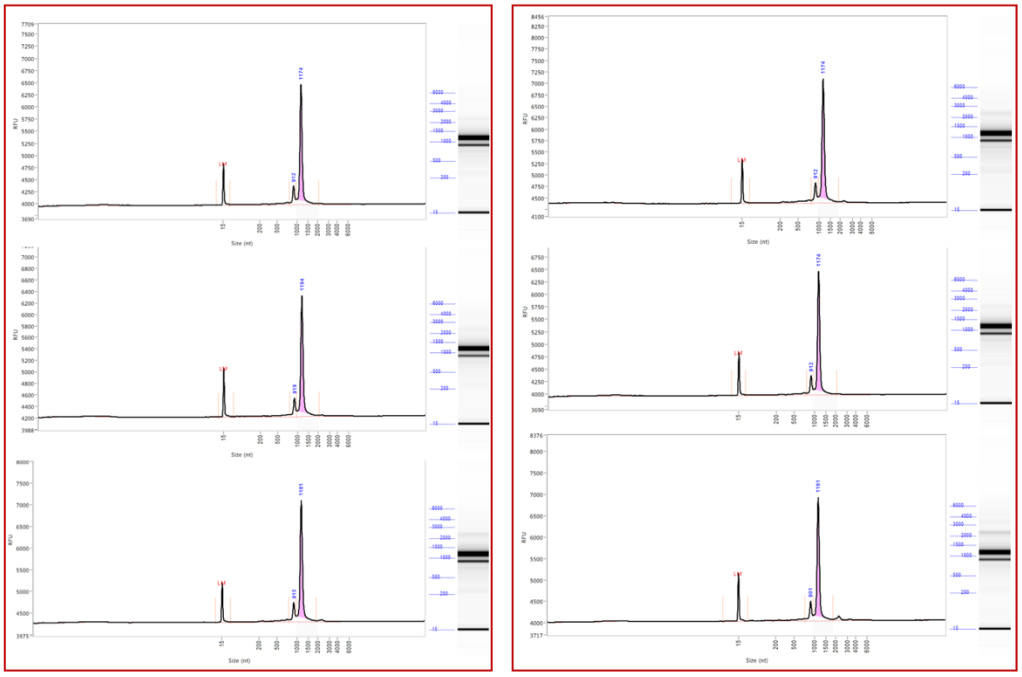
Supplementary Fig. 2 Optimization progress of formulating solid-state Fluc mRNA-LNPs. Plot showing improvement of *in vitro* transfection efficiency of solid-state Fluc mRNA-LNPs for LNP B. Optimized excipient formulation is highlighted in blue.



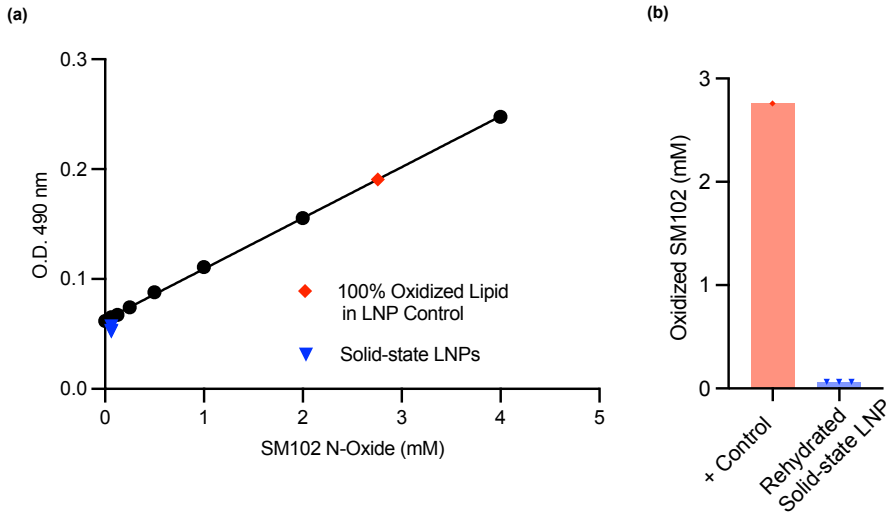
Supplement Fig. 3 Effect of buffer on *in vivo* protein expression of solid-state FLuc mRNA-LNP formulations. FLuc mRNA-LNPs formulated with the optimized excipient combinations were prepared in either Tris buffer (pH 7.5) or sodium acetate (NaAc, pH 5.5) for (a) LNP A and (b) LNP B lipid compositions.



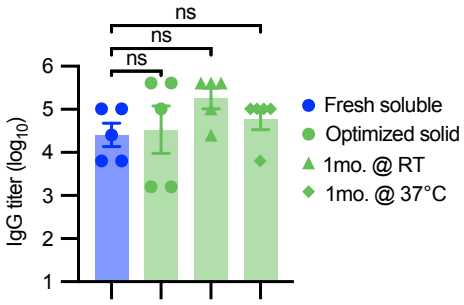
Supplement Fig. 4 *In vivo* validation of solid-state FLuc mRNA-LNPs stability across storage temperatures. Quantification of luminescence intensity at the injection site in mice at 6 h following intramuscular administration of reconstituted fresh or stored solid-state FLuc mRNA-LNPs (0.3 μg mRNA per mouse) for **(a)** LNP A and **(b)** LNP B. **(c)** Comparison of *in vivo* luminescence between soluble and solid-state FLuc mRNA-LNPs (0.3 μg mRNA per mouse, LNP B) after 1 year of storage. n = 5 biologically independent mice per group, one-way ANOVA with Tukey's test.



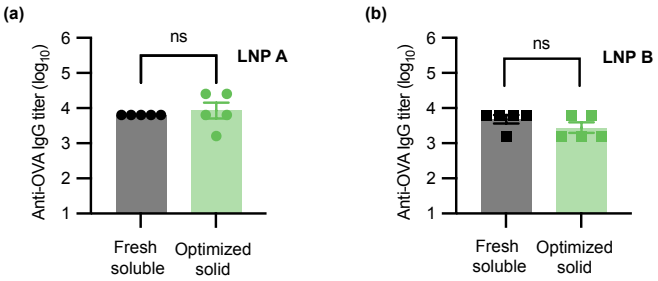
Supplementary Fig. 5 FEMTO Pulse traces of soluble mRNA-LNPs and reconstituted optimized solid-state mRNA-LNPs. Gel electrophoresis was performed on mRNA extracted from soluble LNPs (left panels) and reconstituted optimized solid-state LNPs (right panels).



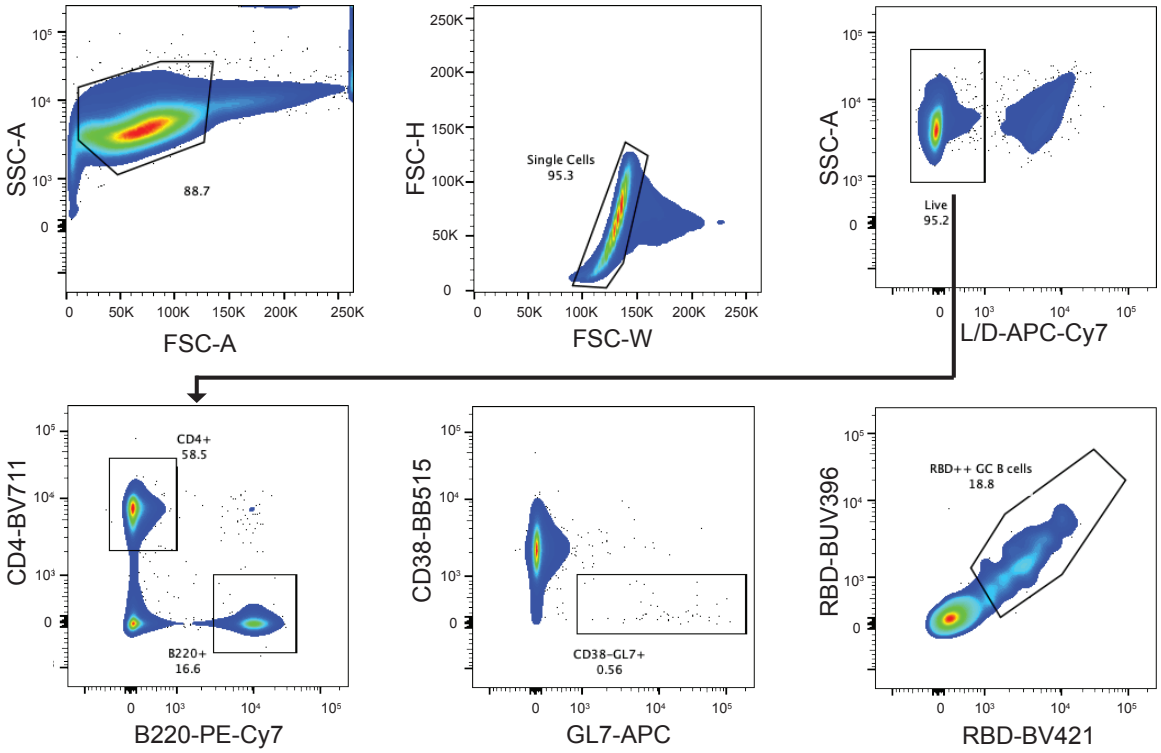
Supplementary Fig. 6 Quantification of oxidized lipid in solid-state mRNA-LNPs. (a) Standard curve generated using fully oxidized SM-102. (b) Concentration of oxidized SM-102 in 100% oxidized positive LNP control and reconstituted solid-state LNPs after vacuum drying.



Supplementary Fig. 7 Thermostability of solid-state RBD mRNA-LNPs. Serum anti-RBD IgG titers measured at week 5 in mice administered fresh soluble or reconstituted solid-state RBD mRNA-LNPs that were stored at room temperature or 37°C for one month (LNP A lipid composition; n = 5 biologically independent mice per group, one-way ANOVA with Tukey's test.)



Supplement Fig. 8 ***In vivo*** **validation of solid-state OVA mRNA-LNPs.** BL/6 mice were immunized with soluble or reconstituted solid-state OVA mRNA-LNPs via i.m. injections followed by a booster dose 2 weeks later. Plot shows serum anti-OVA IgG titers measured at week 3 for (a) LNP A and (b) LNP B lipid compositions (n = 5 biologically independent mice per group; 3 μg mRNA per mouse). Statistical significance was determined by two-tailed unpaired t test.



Supplementary Fig. 9 Lymph node flow cytometry gating strategy. Gating strategies for RBD-binding germinal center B cells derived from draining lymph nodes. Results are from one experiment. The gating strategy is reproduced in Fig. 4f.

References

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