

## Template-directed Synthesis of a Genetic Polymer in a Model Protocell

Sheref S. Mansy, Jason P. Schrum, Mathangi Krishnamurthy, Sylvia Tobé, Douglas A.

Treco and Jack W. Szostak\*

\*To whom correspondence should be addressed. Email:

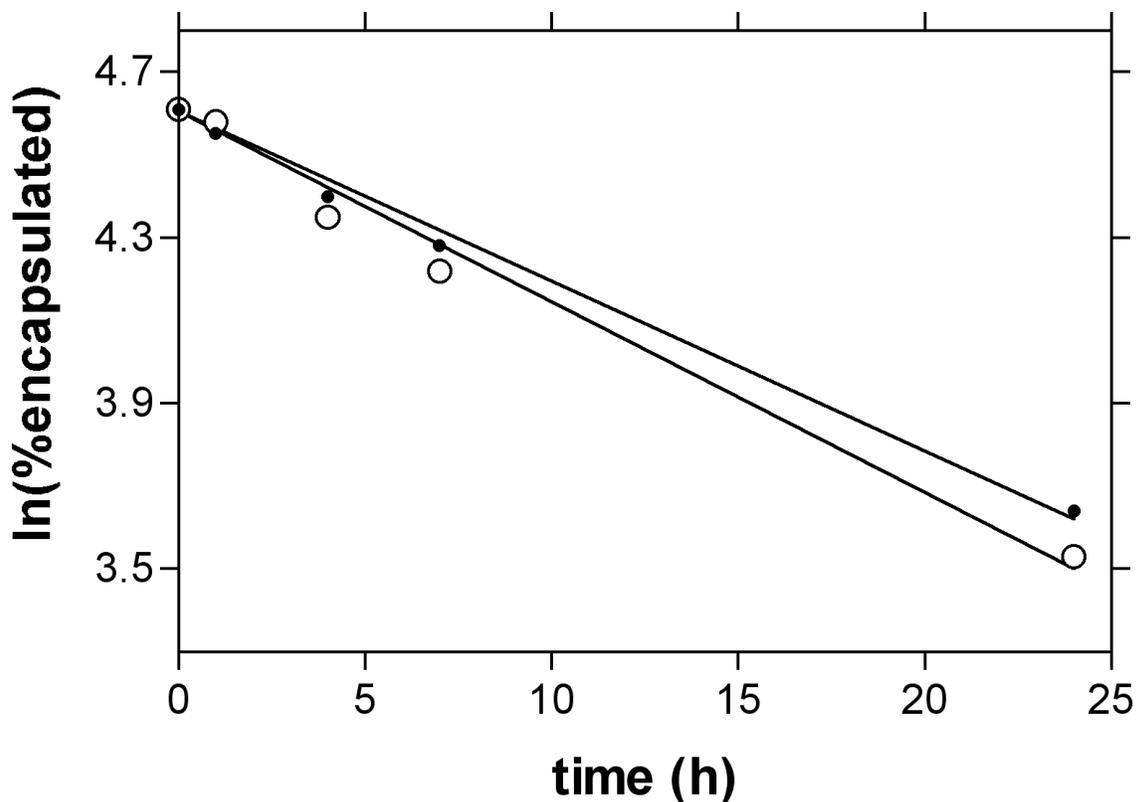
[szostak@molbio.mgh.harvard.edu](mailto:szostak@molbio.mgh.harvard.edu)

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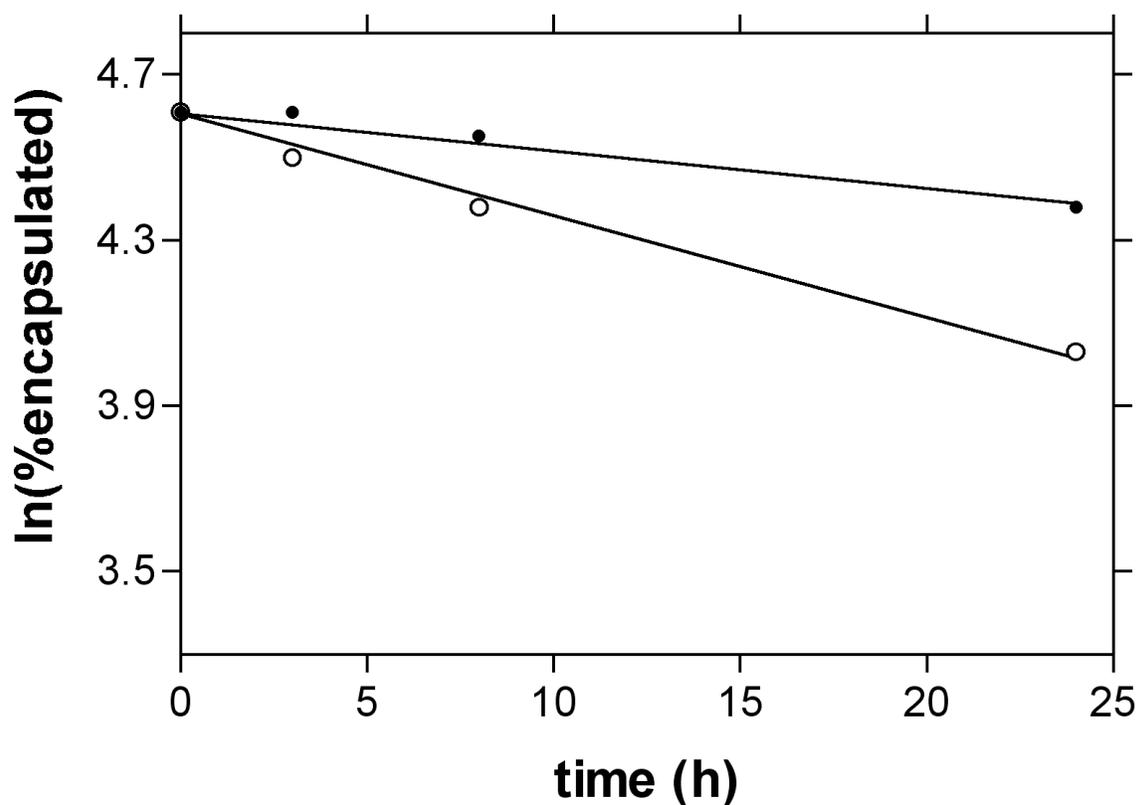
Supplementary Figures 1-7

Supplementary Table 1

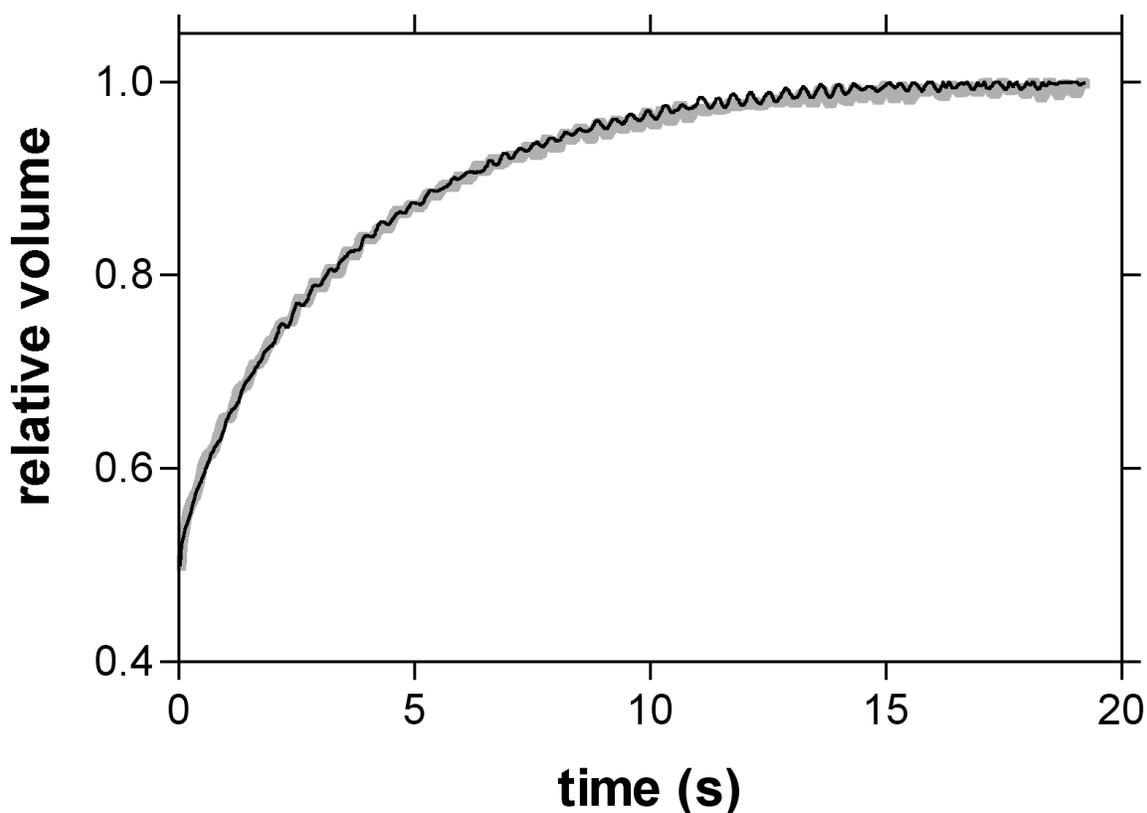
Supporting Online Text



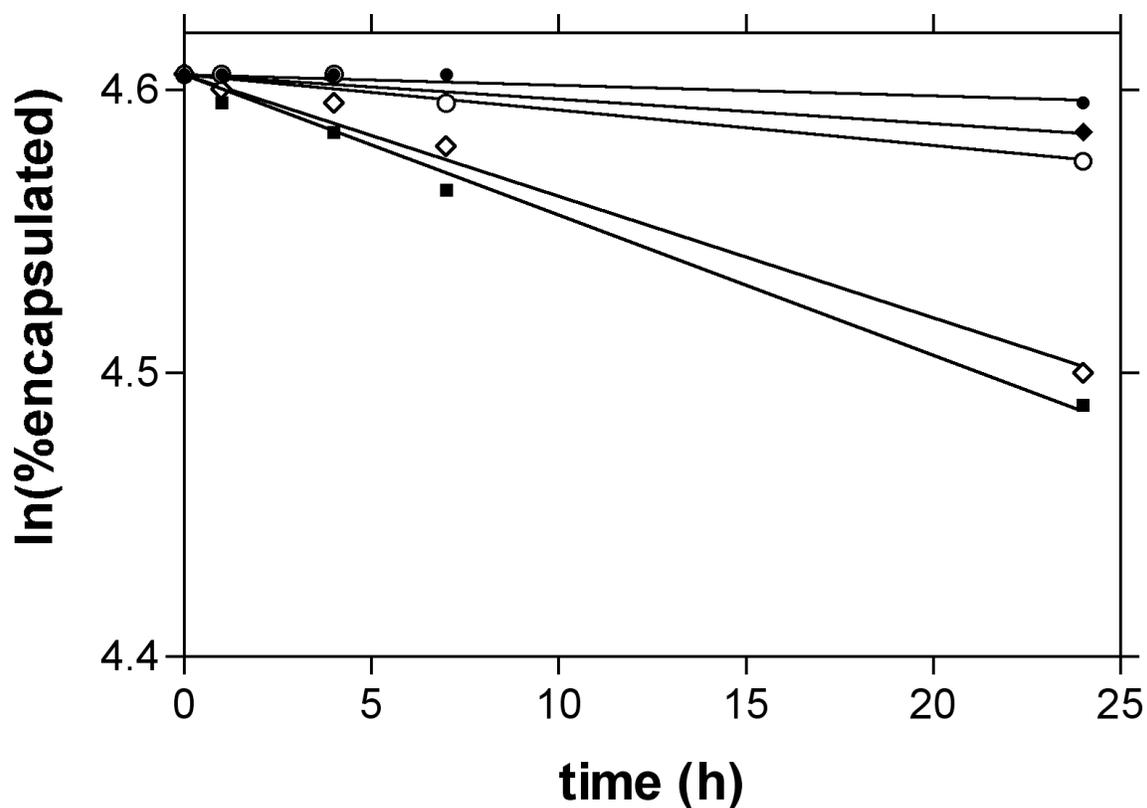
**Fig. S1.** Influence of  $\text{Mg}^{2+}$  on the permeability of uridine-5'-phosphorimidazole. Measurements were in the absence (●) or presence (○) of  $3 \text{ mM MgCl}_2$ . Nucleotide permeability was measured by quantifying leakage of the entrapped nucleotide at different time points by size-exclusion chromatography. The data shows a negligible influence of  $\text{Mg}^{2+}$  on uridine-5'-phosphorimidazole permeability. Solution conditions were  $0.2 \text{ M}$  sodium bicine,  $\text{pH } 8.5$ ,  $23 \text{ }^\circ\text{C}$ . Vesicles membranes were composed of 2:1 MA:GMM.



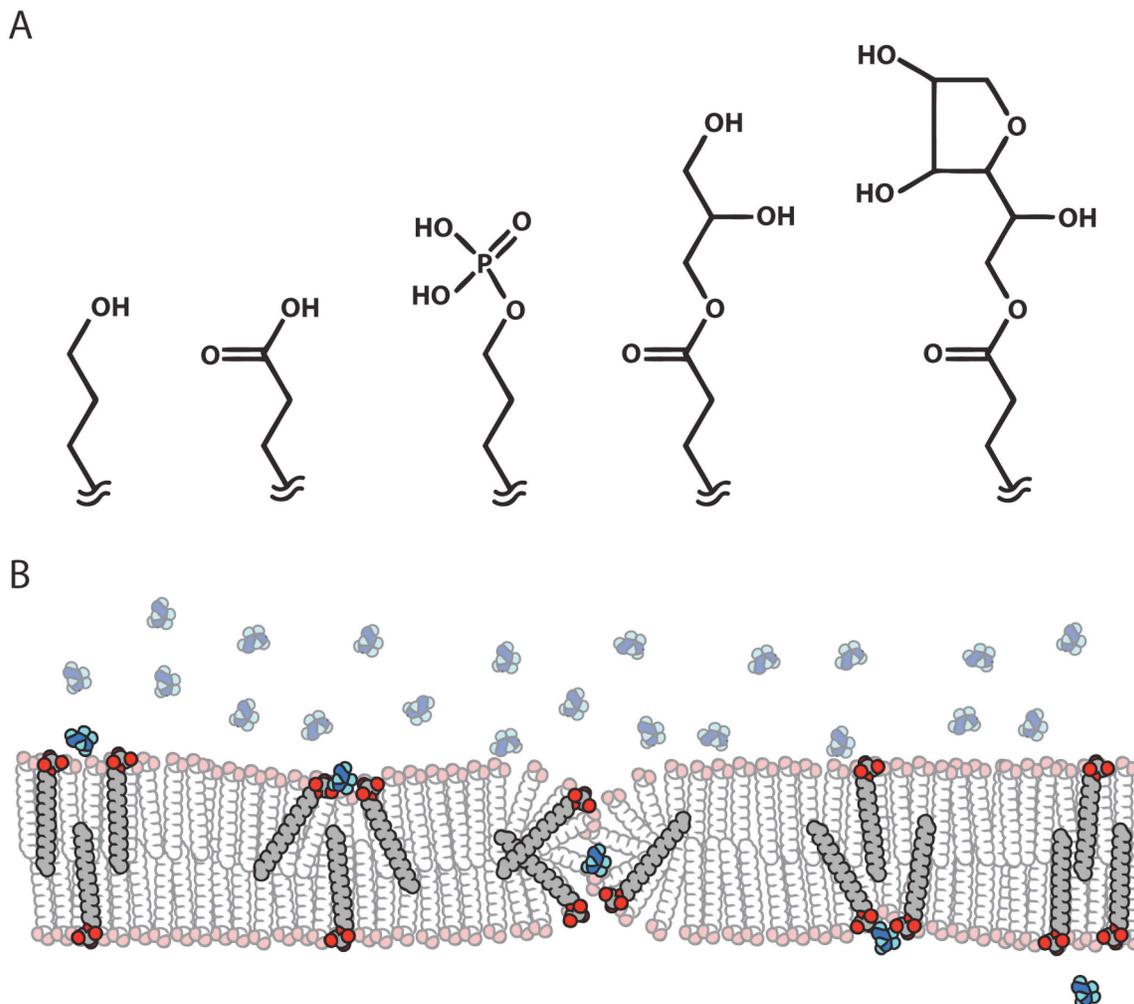
**Fig. S2.** Influence of 1-(2-hydroxyethyl)imidazole on the permeability of uridine-5'-phosphorimidazolide. Nucleotide measurements were either in the absence (●) or presence (○) of 100 mM 1-(2-hydroxyethyl)imidazole. Nucleotide permeability was measured by quantifying leakage of the entrapped nucleotide at different time points by size-exclusion chromatography. Solution conditions were 0.2 M sodium bicine, pH 8.5, 4 °C. Vesicles membranes were composed of 2:1 MA:GMM.



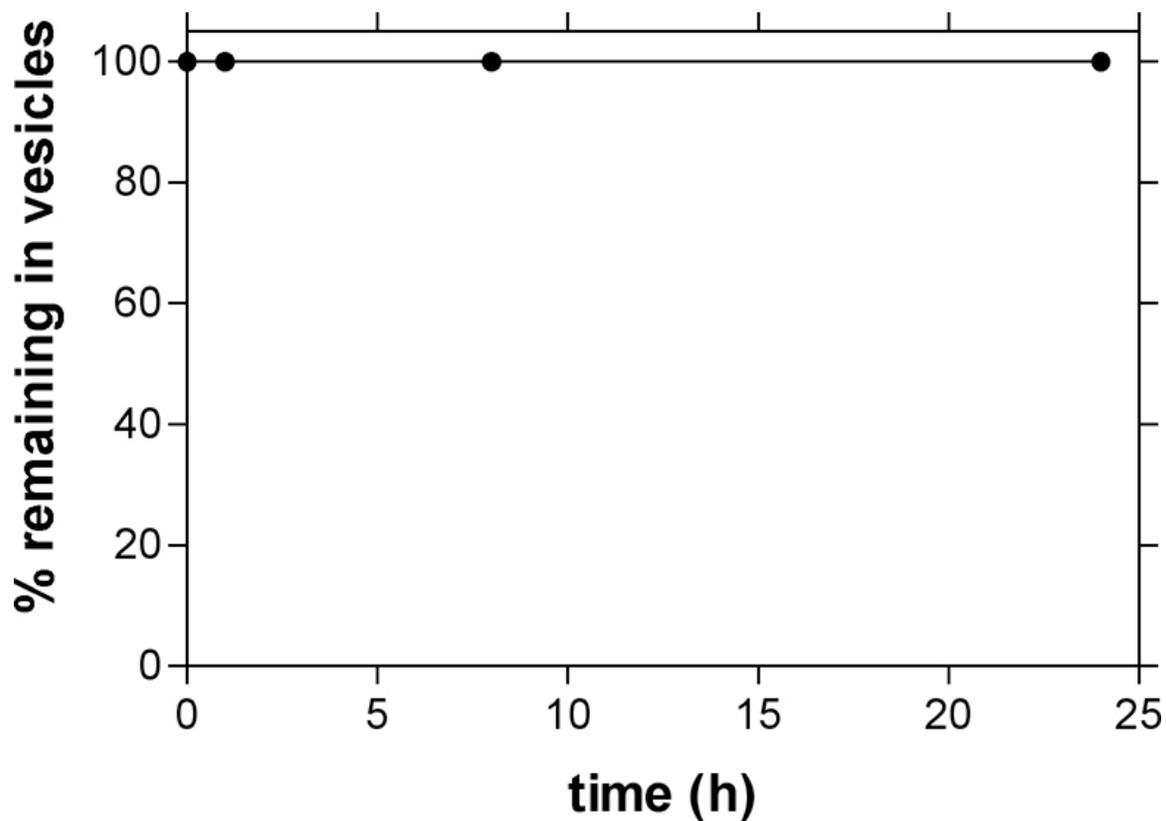
**Fig. S3.** Influence of 1-(2-hydroxyethyl)imidazole on ribose permeability. Ribose permeation was measured either in the absence (thin black line) or presence (thick grey line) of 100 mM 1-(2-hydroxyethyl)imidazole. Ribose permeability was measured by monitoring changes in 2:1 MA:GMM vesicle volume upon solute addition as described in the materials and methods. Solution conditions were 0.1 M POPSO, 3 mM EDTA, pH 8.2, 23 °C. 1-(2-hydroxyethyl)imidazole did not influence the permeability coefficient of ribose, which is consistent with a lack of significant interaction between the vesicle membrane and 1-(2-hydroxyethyl)imidazole.



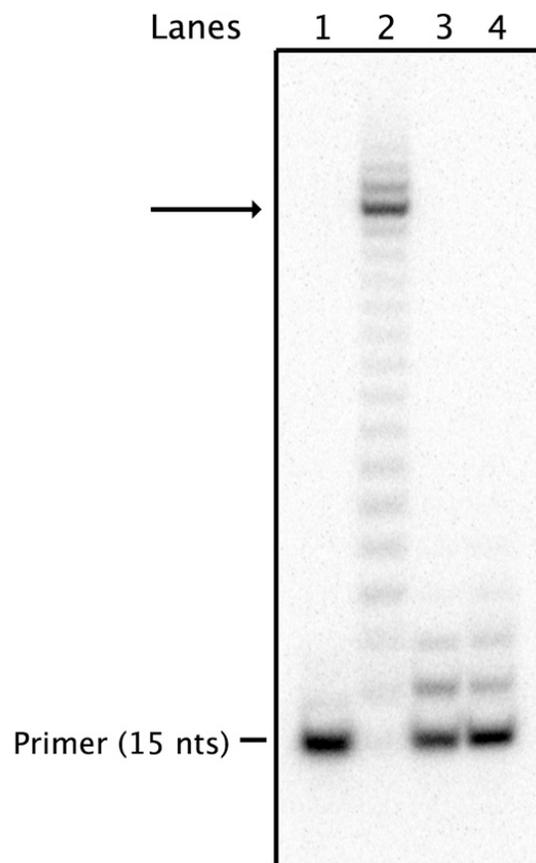
**Fig. S4.** Influence of membrane composition on UMP permeability. Nucleotide permeability was measured by quantifying leakage of the entrapped nucleotide at different times by size-exclusion chromatography. Solution conditions were 0.2 M sodium bicine, pH 8.5, 23 °C. Correlations between membrane composition and solute permeability are consistent for both ribose and UMP. ●, MA; ○, 2:1 MA:GMM; ◆, 2:1 capric acid:decanol; ■, 2:1 MA:farnesol; ◇, 4:1:1 capric acid:decanol:moncaprin.



**Fig. S5.** Mechanism of solute permeation. **(A)** Structures of single-chain lipid head groups. From left to right, alcohol, carboxylic acid, phosphate, glycerol monoester, and sorbitan. **(B)** Molecular model for mechanism of solute permeability. The hydrophilic portions of the solute interact with the hydrophilic head groups of the lipids; hydrophobic regions of the solute may also interact with the acyl chains of the lipids. The solute is carried across the membrane *via* a concerted flipping of the solute-lipid complex. Flipping, and thus permeation, are facilitated by decreased van der Waals interactions between the lipid molecules in the bilayer, and by stabilization of the highly curved intermediate structures.



**Fig. S6.** Retention of 5'-fluorescein-labeled dA<sub>10</sub>. Leakage of entrapped oligonucleotide was measured by size-exclusion chromatography of aliquots at different time points and fluorimetry. Solution conditions were 0.2 M sodium bicine, pH 8.5, 23 °C. Data were the same for all membrane compositions tested.



**Fig. S7.** Template dependent copying reactions. Lanes are as follows: (1) 3'-amino-terminated DNA primer; (2) template dC<sub>15</sub> copying reaction; (3) template dA<sub>15</sub> copying reaction; (4) reaction with no template. Solution conditions were 0.2 M sodium bicine, 100 mM 1-(2-hydroxyethyl)imidazole, pH 8.5, 4 °C. Template and 3'-amino-terminated radiolabeled DNA primer were reacted with 5 mM 2'-amino-2',3'-dideoxyguanosine-5'-phosphorimidazolide. Since 3'-amino nucleotides tend to cyclize, we used 2'-amino-nucleotides to avoid formation of the undesired cyclic nucleotide products. The arrow denotes full-length product.

**Table S1.** Solute permeability coefficients of model protocellular membranes. Sugar permeability coefficients are in  $10^{-8}$  cm/s and water permeability coefficients ( $P_w$ ) are in  $10^{-2}$  cm/s. Binary and ternary amphiphile mixtures are 2:1 and 4:1:1 molar ratios, respectively. <sup>a</sup>Measurements taken in the presence of 3 mM  $MgCl_2$ .

amphiphile	ribose	lyxose	arabinose	xylose	deoxyribose	$P_w$
myristoleate	24	4.6	3.2	3.0		2.0
myristoleate:monomyristolein	87	13	8.6	8.6	960	1.9
myristoleate:monomyristolein <sup>a</sup>	84	11	8.8	8.7		2.0
myristoleate:myristoleoyl alcohol	28	5.1	3.2	3.1	190	2.1
myristoleate:myristoleoyl phosphate	23					1.8
myristoleic acid:farnesol	410					2.7
myristoleic acid:lauric acid	12					2.0
myristoleate:capric acid	20					2.1
palmitoleate	12					1.1
palmitoleate:monopalmitolein	15					1.1
oleate	8.6	1.8	1.0	1.0	110	1.0
oleate:monoolein	13	3.1	1.6	1.5	110	0.91
oleate:sorbitan monooleate	35					1.2
linoleate	47					1.7
POPC	7.0	1.5	1.1	1.1		0.79
POPC <sup>a</sup>	7.1	1.8	1.3	1.1		0.78
capric acid:decanol	34	7.1	5.1	4.7		1.6
capric acid:decanol:monocaprin	360	91	76	70		2.0

## Supporting Online Text

### The effect of charge on nucleotide permeability

To test the specific role of charge in limiting nucleotide permeability, we measured the permeability of nucleotides in the presence and absence of 3 mM  $Mg^{2+}$ . The addition of  $Mg^{2+}$  results in the formation of  $Mg^{2+}$ :nucleotide complexes of reduced net charge. While we did not observe significant leakage of the  $Mg^{2+}$ :ATP complex (which retains a net negative charge of -2), we did observe slow permeation of AMP and ADP in the presence of 3 mM  $Mg^{2+}$  (Fig. 3A). At this concentration of  $Mg^{2+}$ , ADP crossed the membrane significantly more rapidly than AMP, most likely due to the much greater stability of the

$Mg^{2+}$ :ADP complex (which retains one net negative charge) compared to the net neutral  $Mg^{2+}$ :AMP complex. This level of  $Mg^{2+}$  had no influence on the permeation of neutral sugars, suggesting that the added  $Mg^{2+}$  did not affect the structure of the membrane *per se*, and that the enhanced nucleotide transport was indeed due to the formation of  $Mg^{2+}$ :nucleotide complexes of reduced net charge.

We observed very slow permeation of AMP, slightly faster permeation of 2'-deoxy-AMP due to the loss of one hydroxyl group, and similar permeation of 2'-amino, 2'-3' dideoxy-AMP; all three of these compounds bear two negative charges at pH 8.5. In contrast, all three phosphorimidazolides diffused across the membranes much more rapidly, as expected since these phosphoramidate monoesters are singly negatively charged.