

**Supplemental Materials for J. LeBarron and E. London “Highly hydrophilic segments attached to hydrophobic peptides translocate rapidly across membranes”**

**Supplemental Table 1.** Size of vesicles determined by dynamic light scattering. Vesicles containing 200  $\mu$ M POPC with 1 mol% peptide were diluted 14-fold before measuring dynamic light scattering. Values are averages/standard deviations of for independent samples. Avg = average; SD = standard deviation. A. ethanol vesicles; B. freeze-thaw vesicles.

**A:**

	Rh (nm)		Poly Disp (%)	
	<b>Avg</b>	<b>SD</b>	<b>Avg</b>	<b>SD</b>
blank	15.4	1.8	27.4	5.0
N <sub>0</sub> K <sub>2</sub>	20.8	2.3	41.2	10.9
N <sub>2</sub> K <sub>2</sub>	57.3	22.1	32.9	9.7
N <sub>6</sub> K <sub>2</sub>	63.3	6.5	22.5	9.8
Polio	22.3	5.4	45.0	9.9

**B:**

	Rh (nm)	Poly Disp (%)
blank	58	14
N <sub>2</sub> K <sub>2</sub>	59	9

**Supplemental Table 2.** Values for 345/325 and emission lambda max from experiments shown in Figure 3-6 before and after shifting pH. **A.** N<sub>0</sub>K<sub>2</sub>-flanked peptide; **B.** N<sub>2</sub>K<sub>2</sub>-flanked peptide; **C.** N<sub>6</sub>K<sub>2</sub>-flanked peptide; **D.** Polio-flanked peptide

**A:**

**N<sub>0</sub>K<sub>2</sub> -flanked peptide**

	pH change		0	12 sec	5 min	30 min	20 hr
EtOH vesicles	3.9 → 9.3	Ratio	0.83 ± 0.003	1.11 ± 0.014	1.11 ± 0.015	1.09 ± 0.038	1.08 ± 0.041
		<b>λ max</b>	<b>329</b>			<b>338</b>	<b>337</b>
100% POPC	9.1 → 3.7	Ratio	1.10 ± 0.003	0.86 ± 0.005	0.82 ± 0.006	0.82 ± 0.006	0.83 ± 0.031
		<b>λ max</b>	<b>338</b>			<b>328</b>	<b>328</b>
EtOH vesicles	3.9 → 9.3	Ratio	1.03 ± 0.005	1.09 ± 0.009	1.12 ± 0.005	1.12 ± 0.012	
		<b>λ max</b>	<b>334</b>			<b>339</b>	
60% POPC 40% chol	8.8 → 3.5	Ratio	1.13 ± 0.004	1.01 ± 0.003	0.99 ± 0.001	1.00 ± 0.001	
		<b>λ max</b>	<b>339</b>			<b>335</b>	
Freeze-thaw 100% POPC	4.2 → 9.9	Ratio	0.83 ± 0.001	1.01 ± 0.005	1.05 ± 0.007	1.04 ± 0.001	1.03 ± 0.014
		<b>λ max</b>	<b>328</b>			<b>337</b>	<b>334</b>
	9.2 → 3.7	Ratio	1.02 ± 0.004	0.75 ± 0.011	0.75 ± 0.006	0.75 ± 0.004	0.75 ± 0.003
		<b>λ max</b>	<b>335</b>			<b>326</b>	<b>324</b>
Freeze-thaw 60% POPC 40% chol	3.6 → 9.9	Ratio	0.99 ± 0.001	1.06 ± 0.005	1.10 ± 0.002	1.11 ± 0.042	1.26 ± 0.311
		<b>λ max</b>	<b>334</b>			<b>337</b>	<b>343</b>
	9.3 → 3.7	Ratio	1.08 ± 0.008	1.03 ± 0.041	1.00 ± 0.020	1.01 ± 0.007	0.99 ± 0.023
		<b>λ max</b>	<b>337</b>			<b>334</b>	<b>333</b>

**B:**

**N<sub>2</sub>K<sub>2</sub> -flanked peptide**

	pH change		0	12 sec	5 min	30 min	20 hr
EtOH vesicles	3.6 → 9.8	Ratio	0.78 ± 0.006	1.11 ± 0.003	1.06 ± 0.004	1.06 ± 0.004	1.03 ± 0.007
		<b>λ max</b>	<b>325</b>			<b>336</b>	<b>335</b>
100% POPC	9.9 → 3.9	Ratio	1.05 ± 0.004	0.76 ± 0.010	0.78 ± 0.007	0.79 ± 0.009	0.76 ± 0.005
		<b>λ max</b>	<b>335</b>			<b>325</b>	<b>324</b>
EtOH vesicles	3.9 → 9.6	Ratio	0.96 ± 0.003	1.08 ± 0.006	1.08 ± 0.004	1.07 ± 0.008	1.05 ± 0.020
		<b>λ max</b>	<b>332</b>			<b>337</b>	<b>337</b>
60% POPC 40% chol	10.1 → 3.5	Ratio	1.08 ± 0.006	0.94 ± 0.003	0.93 ± 0.004	0.94 ± 0.009	0.93 ± 0.030
		<b>λ max</b>	<b>337</b>			<b>331</b>	<b>331</b>

C:

**N<sub>6</sub>K<sub>2</sub> -flanked peptide**

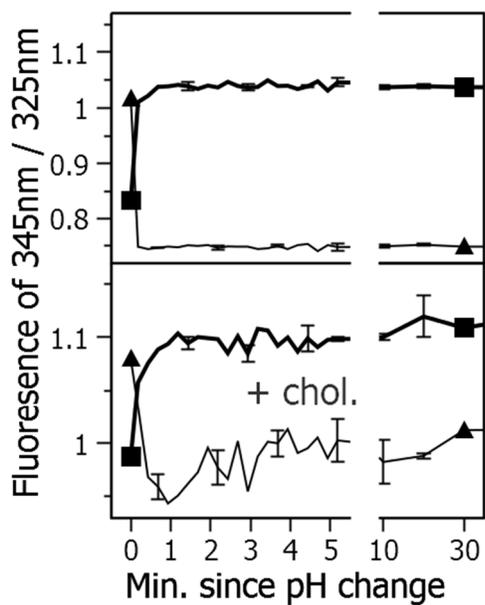
	pH change		0	12 sec	5 min	30 min	20 hr
EtOH vesicles 100% POPC	3.9 → 9.5	Ratio	0.80 ± 0.001	1.06 ± 0.012	1.07 ± 0.018	1.10 ± 0.002	
		<b>λ max</b>	<b>327</b>			<b>340</b>	
EtOH vesicles 60% POPC 40% chol	9.4 → 4.0	Ratio	1.05 ± 0.006	0.82 ± 0.018	0.80 ± 0.011	0.77 ± 0.005	
		<b>λ max</b>	<b>336</b>			<b>325</b>	
EtOH vesicles 60% POPC 40% chol	4.0 → 9.5	Ratio	1.12 ± 0.007	1.18 ± 0.019	1.23 ± 0.012	1.29 ± 0.011	
		<b>λ max</b>	<b>338</b>			<b>342</b>	
Freeze-thaw 100% POPC	9.5 → 3.9	Ratio	1.29 ± 0.008	1.21 ± 0.014	1.13 ± 0.028	1.14 ± 0.017	
		<b>λ max</b>	<b>342</b>			<b>338</b>	
Freeze-thaw 100% POPC	4.2 → 10.0	Ratio	0.76 ± 0.002	1.15 ± 0.026	1.18 ± 0.009	1.21 ± 0.014	1.20 ± 0.015
		<b>λ max</b>	<b>325</b>			<b>339</b>	<b>340</b>
Freeze-thaw 60% POPC 40% chol	8.7 → 3.7	Ratio	1.16 ± 0.009	0.87 ± 0.006	0.80 ± 0.012	0.78 ± 0.002	0.76 ± 0.011
		<b>λ max</b>	<b>340</b>			<b>325</b>	<b>324</b>
Freeze-thaw 60% POPC 40% chol	3.9 → 9.7	Ratio	1.07 ± 0.012	1.06 ± 0.052	1.12 ± 0.019	1.16 ± 0.004	1.28 ± 0.064
		<b>λ max</b>	<b>337</b>			<b>339</b>	<b>342</b>
Freeze-thaw 60% POPC 40% chol	10.0 → 3.5	Ratio	1.20 ± 0.008	1.19 ± 0.090	1.12 ± 0.025	1.10 ± 0.005	1.07 ± 0.022
		<b>λ max</b>	<b>341</b>			<b>338</b>	<b>338</b>

D:

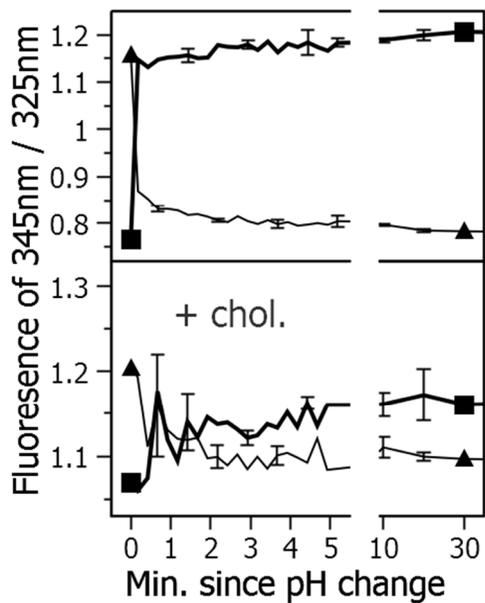
**Polio-flanked peptide**

	pH change		0	12 sec	5 min	30 min	20 hr
EtOH vesicles 100% POPC	3.6 → 9.9	Ratio	0.80 ± 0.001	1.05 ± 0.005	1.04 ± 0.004	1.05 ± 0.007	1.06 ± 0.003
		<b>λ max</b>	<b>328</b>			<b>336</b>	<b>337</b>
EtOH vesicles 60% POPC 40% chol	9.6 → 3.7	Ratio	1.05 ± 0.003	0.83 ± 0.003	0.82 ± 0.002	0.81 ± 0.003	0.80 ± 0.001
		<b>λ max</b>	<b>336</b>			<b>327</b>	<b>327</b>
EtOH vesicles 60% POPC 40% chol	3.5 → 9.9	Ratio	0.93 ± 0.012	1.04 ± 0.007	1.06 ± 0.005	1.06 ± 0.007	1.08 ± 0.011
		<b>λ max</b>	<b>332</b>			<b>337</b>	<b>336</b>
EtOH vesicles 60% POPC 40% chol	9.7 → 3.8	Ratio	1.09 ± 0.001	0.97 ± 0.009	0.97 ± 0.003	0.97 ± 0.003	0.95 ± 0.010
		<b>λ max</b>	<b>337</b>			<b>333</b>	<b>332</b>

**Supplemental Figure 1:** pH interconversion of N<sub>0</sub>K<sub>2</sub> flanked peptide between transmembrane inserted and the interfacial non-transmembrane inserted states using freeze-thaw vesicles. Graphs show ratio of Trp fluorescence emission intensities measured at 345 and 325 nm before and subsequent to pH shifts. All other conditions identical to Figure 3.

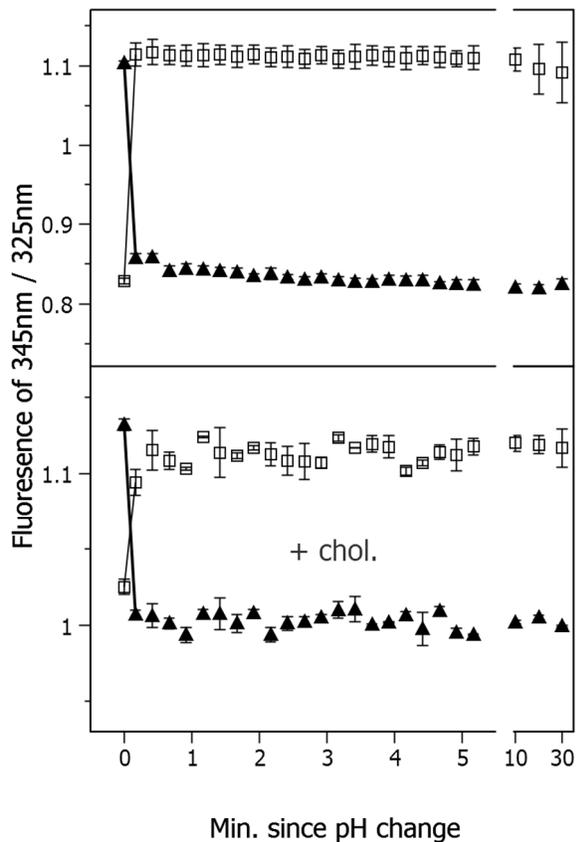


**Supplemental Figure 2:** pH interconversion of N<sub>6</sub>K<sub>2</sub> flanked peptide between transmembrane inserted and the interfacial non-transmembrane inserted states in freeze-thaw vesicles. All conditions identical to Supplemental Figure 1.

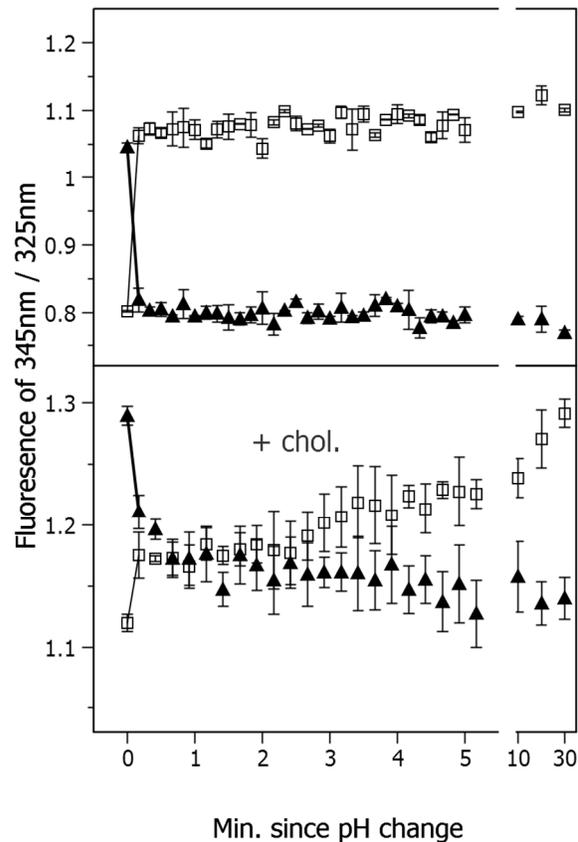


**Supplemental Figure 3: Full data for pH-induced interconversion of Asn flanked peptide.** Re-graph of data for ethanol dilution vesicles with all time points shown for Figures 3 and 5. The ratio of Trp fluorescence emission intensities at 345 and 325 nm before and subsequent to pH shifts is shown. Vesicles prepared at pH ~4 and shifted to pH ~10 represented by open squares. Filled triangles represent ethanol dilution vesicles prepared at pH ~10 and shifted to pH ~4. Other conditions identical to Figure 3. **A:** Vesicles containing 1 mol% N<sub>0</sub>K<sub>2</sub>-flanked peptide. **B:** Vesicles containing 1 mol% N<sub>6</sub>K<sub>2</sub>-flanked peptide.

**A:**



**B:**



**Supplemental Table 3. Comparison of effect of lipid composition on the wavelength of maximum fluorescence emission for peptides as a function of lipid composition and pH.** Peptides were incorporated during the formation of ethanol dilution vesicles at either pH ~4 or ~10 with lipid compositions shown. Fluorescence intensity and wavelength of maximum Trp emission ( $\lambda$  max) was recorded.  $\Delta \lambda$  max is the difference between  $\lambda$  max at acidic and basic pH. Values shown are averages from three samples.  $\lambda$  max values are reproducible to  $\pm 1$  nm. -It should be noted that DEuPC, a lipid with 22 carbon acyl chains, forms a bilayer too thick for the TM state form for the peptides of the type used in this study<sup>21, 22, 24, 26, 41, 43</sup> (and see Figure 1). The data shows that, at least for the N<sub>6</sub>K<sub>2</sub>-flanked and polio-flanked peptides, changing pH in DEuPC vesicles has a much smaller effect on  $\lambda$  max than in POPC vesicles, with or without cholesterol.

**N<sub>6</sub>K<sub>2</sub>- flanked peptide**

	pH	$\lambda$ Max.	$\Delta \lambda$ Max.
POPC	3.6	327	14
	9.8	341	
POPC 40 mol% chol	3.6	335	8
	9.6	343	
DEuPC	3.6	337	2
	9.8	339	
DEuPC 40 mol% chol	3.6	338	2
	9.9	340	

**Polio-flanked peptide**

POPC	3.5	328	9
	9.7	337	
POPC 40 mol% chol	3.6	330	7
	9.7	337	
DEuPC	3.5	336	3
	9.8	339	
DEuPC 40 mol% chol	3.9	336	2
	9.3	338	