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Redox Switching of an Artificial Transmembrane Signal Transduction System

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Supporting Information

General procedure for vesicle preparation

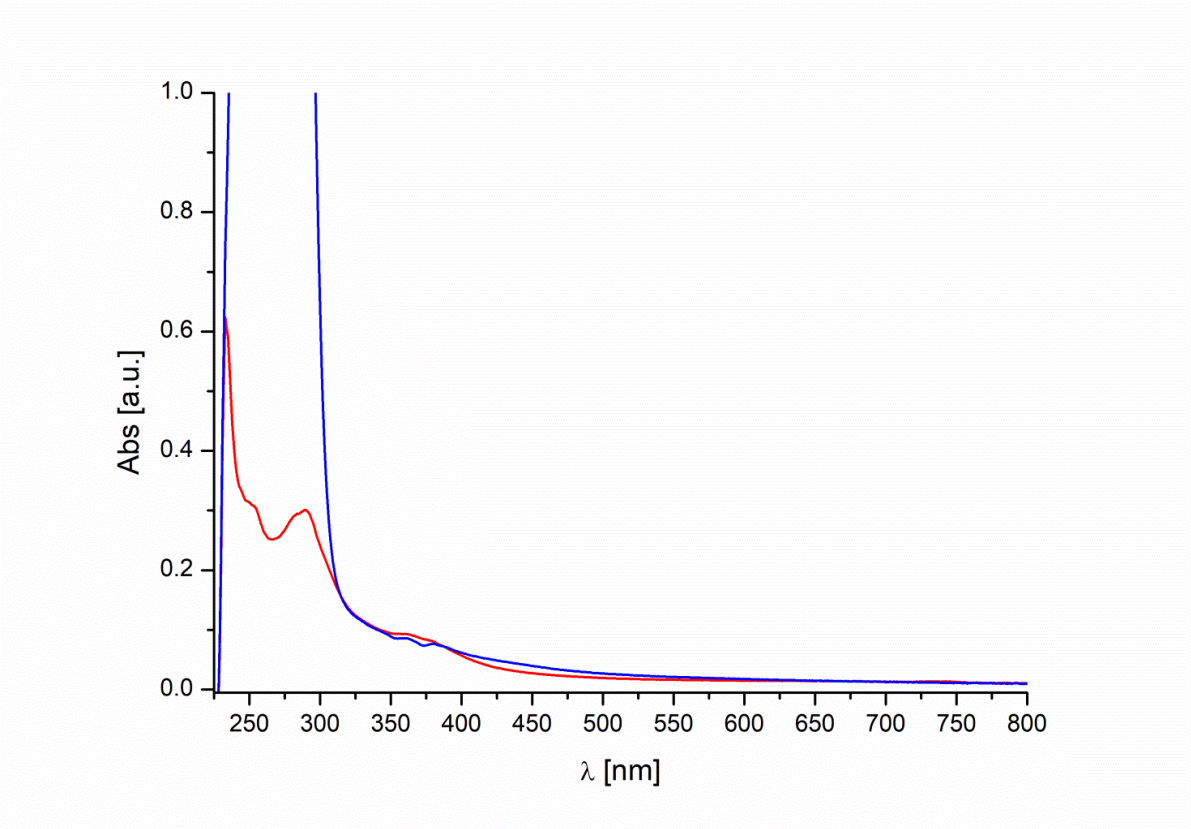
To an Eppendorf tip the following solutions were added: chloroform solution of the lipid 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) to obtain a final 1 mM concentration of lipids. The solvent was removed by flushing with nitrogen and the lipid film was dried under high vacuum for 2 hours. The lipid film was then rehydrated with PIPES (100 mM) at pH 6.7, NaCl (150 mM), $CdSO_4$ (250 μM) in water (HPLC grade) and a freshly prepared solution of the ester substrate **2** (250 μM). It was then sonicated for one minute. The suspension was subjected to five cycles of freeze-thaw using liquid nitrogen and a water bath at 40°C. The suspension was extruded nineteen times through a polycarbonate filter with 200 nm pores in an Avestin Lipofast apparatus,[1] and then the vesicles were separated by the bulk solution using GE Healthcare PD-10 desalting columns prepacked with Sephadex G-25 medium eluting with the buffered solution (PIPES (100 mM) at pH 6.7, NaCl (150 mM), $CdSO_4$ (250 μM) in water (HPLC grade)). The average size of the vesicles prepared with this method had been determined previously by Dynamic Light Scattering (DLS) measurements to be approximately 200 nm.[2]

The pH of the vesicles suspension was measured at end of each signaling experiment using a Mettler-Toledo “Seven Compact” pH meter equipped with an “In-lab Micro” electrode.

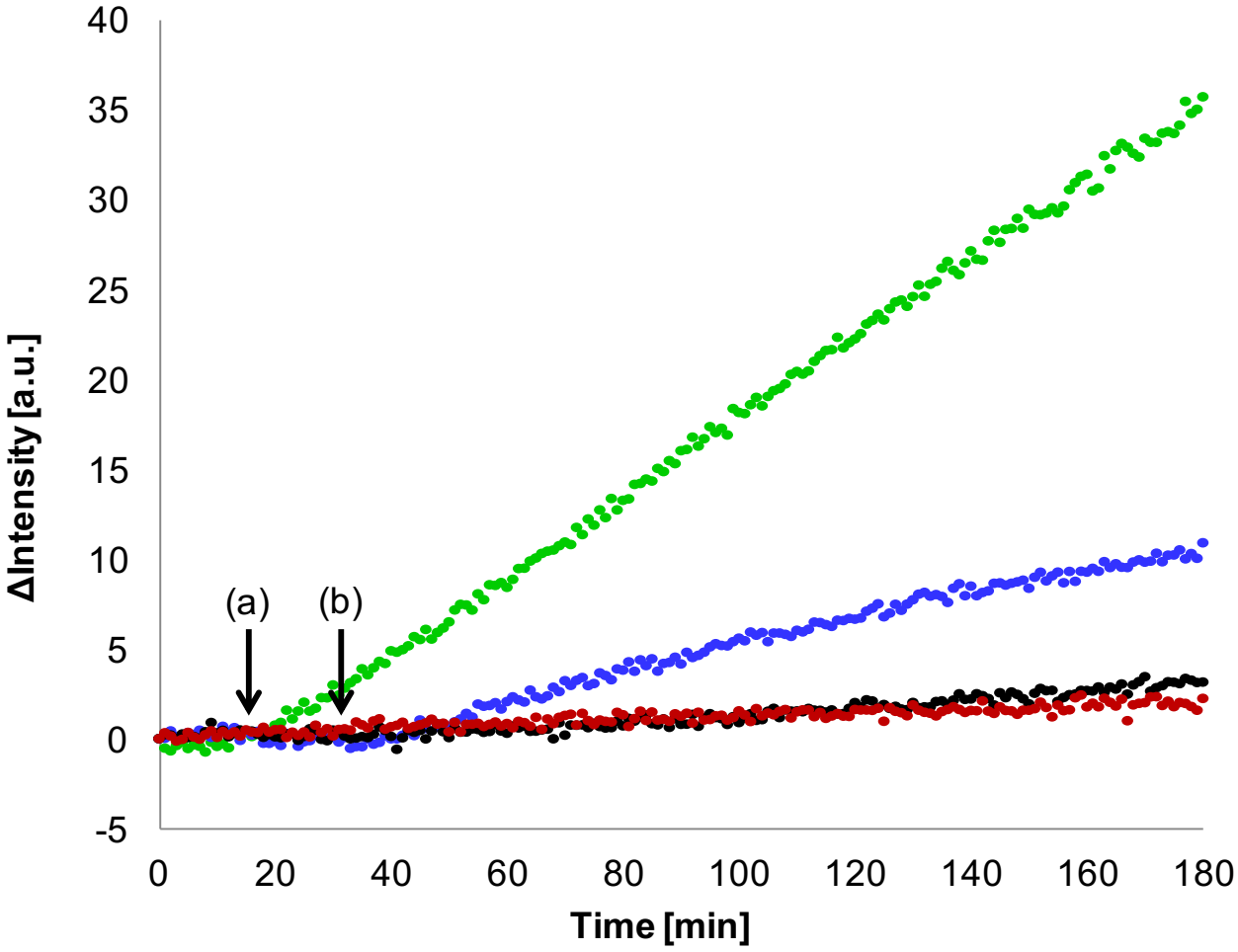
General procedure for fluorescence experiments

Fluorescence spectroscopic data were recorded using a Cary Eclipse fluorescence spectrophotometer (Agilent). Fluorescence excitation experiments were recorded using the following parameters: emission wavelength 510 nm, excitation range 380-480 nm, recorded at 1 minute intervals for 180 minutes. The excitation and emission slits were set at 5 nm for all the vesicle experiments.

Signal Transduction Experiments – Additional Data

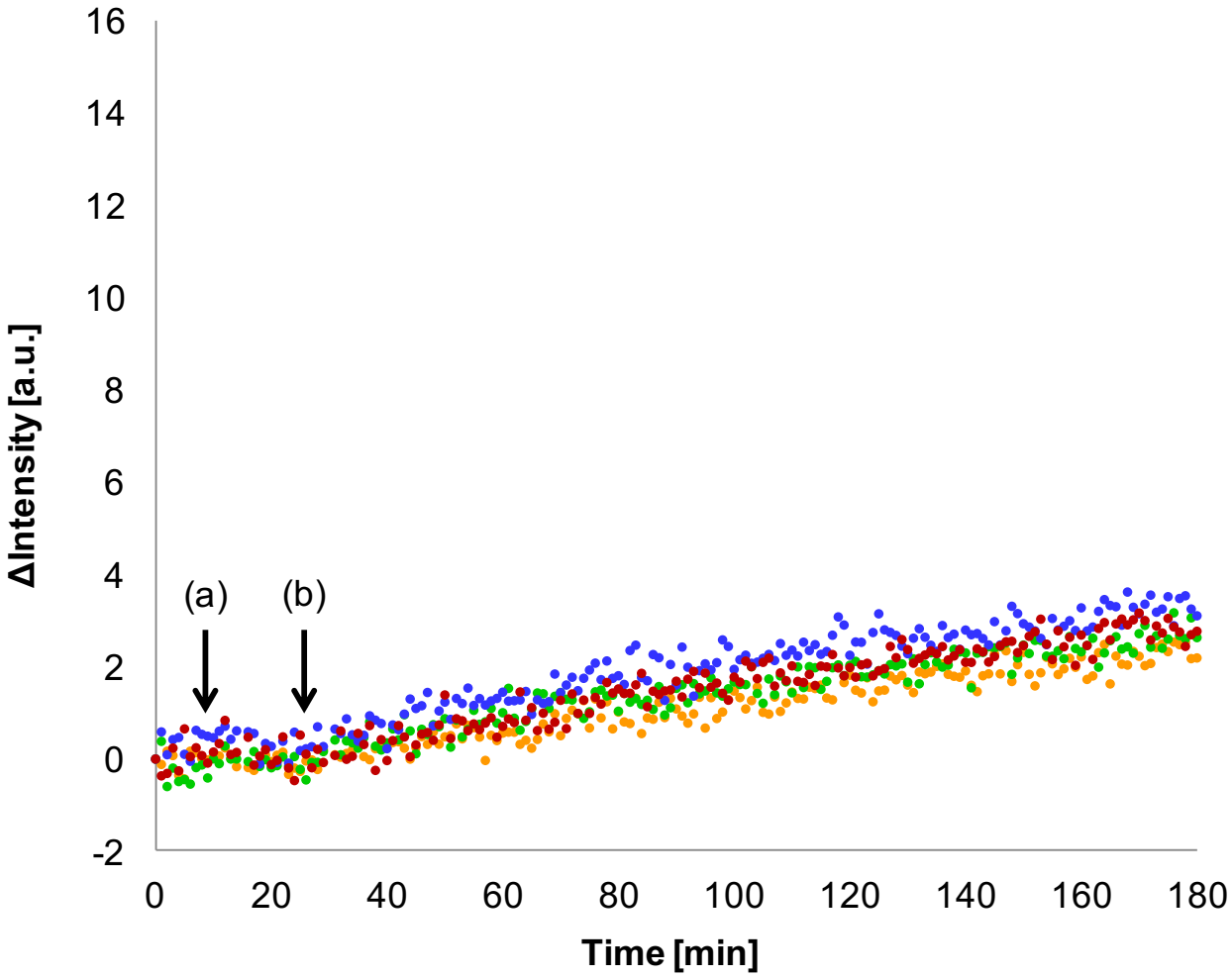


Supplementary Figure 1. UV-Vis spectra of the vesicles suspension upon the addition of **1**·Cu^{2+} (10 μM) in red followed by sodium ascorbate (0.1 mM) in blue. The experiment was conducted in 200 nm DOPC vesicles (1 mM final lipid concentration) that contained the ester substrate **2** (250 μM), in PIPES buffer (100 mM, pH 6.7) and NaCl (150 mM).

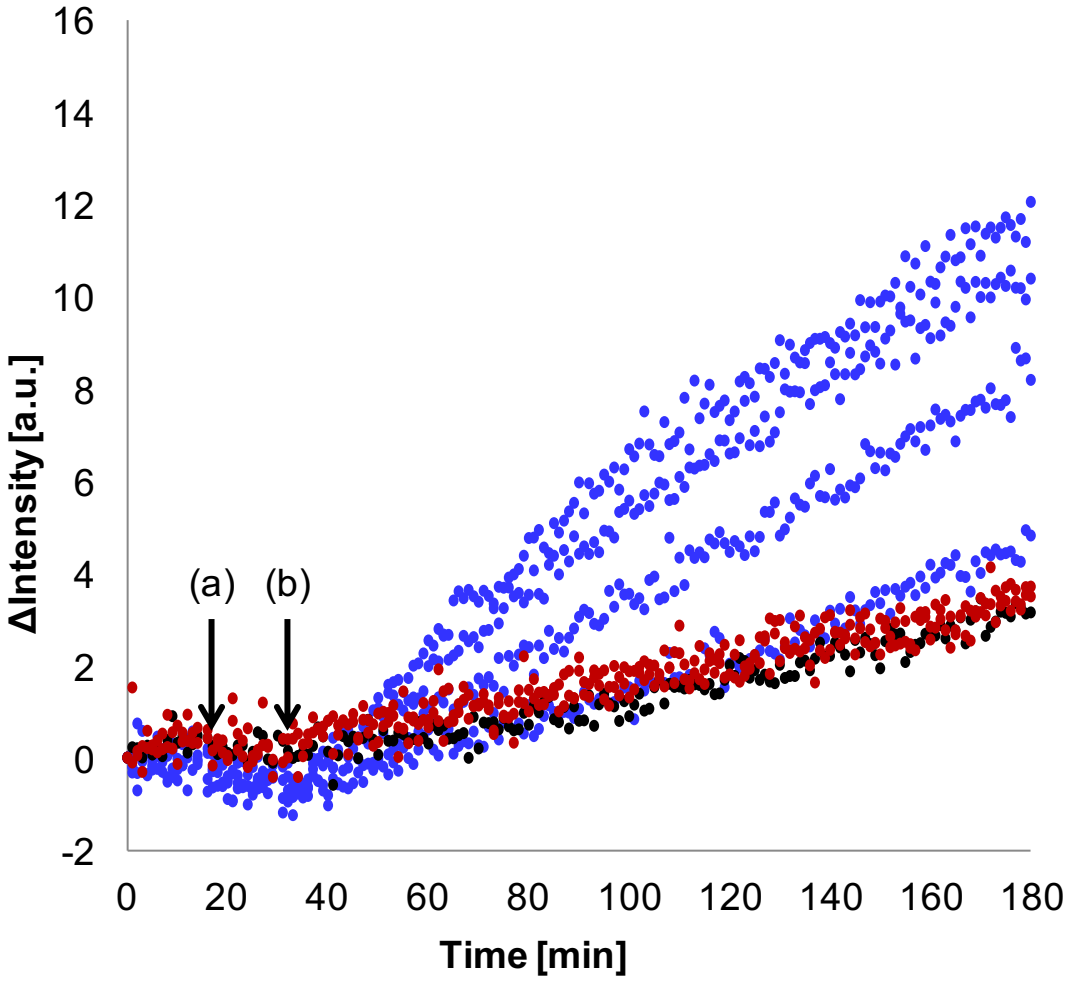


	1 (10 μM)	**Cu^{2+}** (10 μM)	**Sodium Ascorbate** (1 mM)	**Lipids**	
●	✓	✓	✓	✓	***ON***
●	✓	✓		✓	***OFF***
●	✓			✓	***ON***
●			✓	✓	***OFF***

Supplementary Figure 2. Control experiments for the signal transduction experiment. Time dependence of the fluorescence emission intensity at 510 nm (exciting at 415 nm) of a vesicles suspension following the external addition of (a) **1**·Cu^{2+} (10 μM) or Cu^{2+} (10 μM) and (b) sodium ascorbate (1 mM) as indicated in the table. All the experiments were conducted in 200 nm DOPC vesicles (1 mM final lipid concentration) that contained the ester substrate **2** (250 μM), in PIPES buffer (100 mM, pH 6.7), NaCl (150 mM) and $CdSO_4$ (250 μM).



Supplementary Figure 3. Control experiments for the signal transduction experiment. Time dependence of the fluorescence emission intensity at 510 nm (exciting at 415 nm) of a vesicles suspension following the external additions of (a) $CuCl_2$ (10 μM) and (b) sodium ascorbate (0.1 mM in orange, 0.5 mM in green, 1.0 mM in blue and 5.0 mM in red). All the experiments were conducted in 200 nm DOPC vesicles (1 mM final lipid concentration) that contained the ester substrate **2** (250 μM), in PIPES buffer (100 mM, pH 6.7), NaCl (150 mM) and $CdSO_4$ (250 μM).



Supplementary Figure 4. Comparison of EDTA (red) and sodium ascorbate (blue) input signals with a control experiment with no input signal (black). Time dependence of the fluorescence emission intensity at 510 nm (exciting at 415 nm) of a vesicle suspension following the external additions of **1**·Cu^{2+} (10 μM) at time point (a), and either increasing amounts of sodium ascorbate (0.1-5.0 mM, see main text) or EDTA (0.5-1.0 equivalents). All the experiments were conducted in 200 nm DOPC vesicles (1 mM final lipid concentration) that contained the ester substrate **2** (250 μM), in PIPES buffer (100 mM, pH 6.7), NaCl (150 mM) and $CdSO_4$ (250 μM).

Experiments in		pH
Figure 2	●	6.73
	●	6.73
	●	6.72
	●	6.70
	●	6.69
Figure 3	●	6.68
SFigure 2	●	6.73
	●	6.73
	●	6.74
	●	6.73
SFigure 3	●	6.74
	●	6.72
	●	6.73
	●	6.70
SFigure 4	●	6.73
	●	6.73
		6.72
		6.70
		6.69
	●	6.76
		6.75

Supplementary Table 1. pH of the vesicles suspension at the end of every signaling and control experiment reported in Figures 2 and 3, and in the Supplementary Figures 2, 3 and 4.

References

1) MacDonald, R. C.; MacDonald, R. I.; Menco, B. P.; Takeshita, K.; Subbarao, N. K.; Hu, L. R. *Biochim. Biophys. Acta* **1991**, 1061, 297-303.
2) Langton M. J., Williams N. H. and Hunter C. A. *J. Am. Chem. Soc.* **2017**, 139, 6461-6466.