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Supplementary information

Determining small-molecule permeation through lipid membranes

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SUPPLEMENTARY DATA

Determining small molecule permeation through lipid membranes

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Supplementary Tables

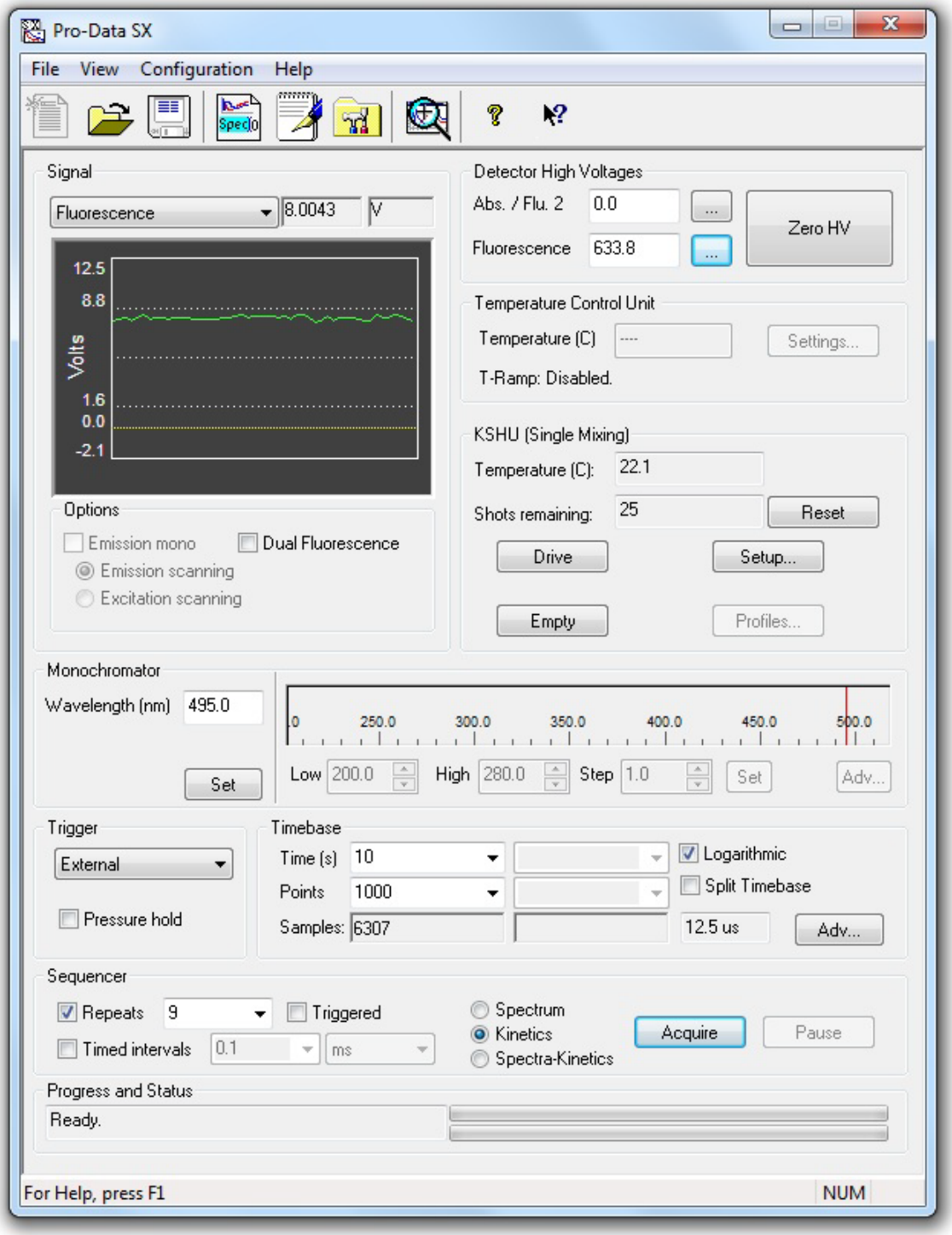
Osmolyte	Final concentration (mM)	M_W (g/mol)	pK_a (25°C)	Compound ID
KCl	52.5	74.55	N/A	
Sodium acetate	47.5	82.03	4.76	176
Sodium benzoate	45	144.1	4.19	243
Sodium butyrate	40	110.09	4.82	264
Sodium formate	50	68.01	3.75	284
Sodium L-lactate	50	112.06	3.86	612
Sodium propionate	40	96.06	4.88	1032
Sodium pyruvate	50	110	2.45	1060
Potassium sorbate	55	150.22	4.76	643460
Glycerol	120	92.09	14.4	753

Supplementary Table 1. Molecular weight and pK_a values of the used osmolytes. The pK_a values were taken from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), using the compound ID indicated in the last column. N/A, not applicable.

Amino acid	M_W (g/mol)	pI	pK_{a1}; pK_{a2}; pK_{a3} (25°C)	$LogP_{OW}$[1]	Molecular volume (Å^3)[1]	P_{POPC} *10^{-9} (cm/s)
Alanine	89.09	6.01	2.34; 9,60; /	-2.77	82.20	/
Arginine	174.2	10.76	2.17; 9.04; 12.48	-3.79	163.00	/
Asparagine	132.12	5.41	2.02; 8.80; /	-3.48	112.30	/
Aspartic acid	133.1	2.77	1.88; 9.60; 3.65	-3.61	103.70	/
Cysteine	121.15	5.05	1.96; 10.28; 8.18	-2.55	99.10	/
Glutamic acid	147.13	3.22	2.19; 9,67; 4.25	-3.11	127.50	/
Glutamine	146.15	5.65	2.17; 9.13; /	-3.51	120.50	/
Glycine	75.07	5.97	2.34; 9.60; /	-3.00	65.00	/
Histidine	155.16	7.59	1.82; 9.17; 6.00	-2.85	140.60	/
Isoleucine	131.17	6.02	2.36; 9.60; /	-1.8	131.70	7.52 ± 2.27
Leucine	131.17	5.98	2.36; 9.60; /	1.72	131.50	4.54 ± 1.56
Lysine	146.19	9.74	2.18; 8.95; 10.53	-3.77	144.30	/
Methionine	149.21	5.74	2.28; 9.21; /	-2.10	132.30	4.09 ± 0.22
Phenylalanine	165.19	5.48	1.83; 9.13; /	-1.44	155.80	64.8 ± 18.9
Proline	115.13	6.30	1.99; 10.60; /	-2.62	106.70	/
Serine	105.09	5.68	2.21; 9.15; /	-3.00	88.50	/
Threonine	119.12	5.60	2.09; 9.10; /	-2.83	105.30	/
Tryptophan	204.23	5.89	2.83; 9.39; /	-1.15	185.90	Excluded
Tyrosine	182.19	5.63	2.20; 9.11; 10.46	-2.11	162.70	Excluded
Valine	117.15	5.96	2.32; 9.62; /	-2.29	115.60	2.24 ± 0.32

Supplementary Table 2. Molecular weight, isoelectric point (pI), pK_a values, octanol-water partition coefficient ($LogP_{OW}$), molecular volume (Å^3) and permeability coefficients (cm/s) in POPC lipid vesicles of the proteinogenic amino acids. pK_{a1}, pK_{a2}, and pK_{a3} are pK_a values of the carboxyl group, the amino group, and the side chain, respectively.

Supplementary Figure

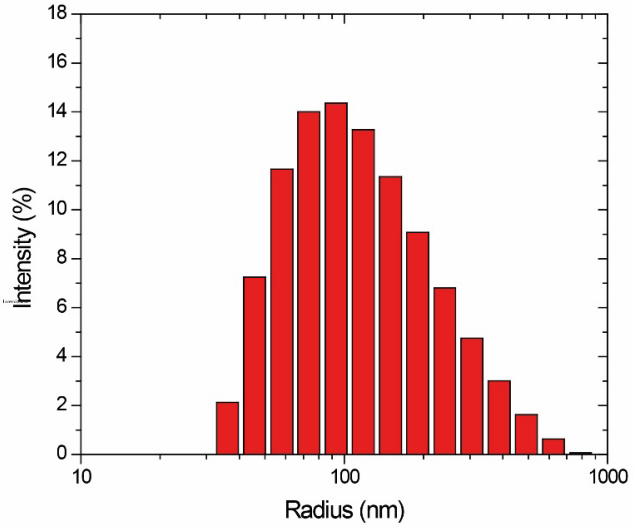


Supplementary Figure 1. Pro Data SX interface. Settings for the stopped flow kinetic measurements in calcein-loaded liposomes. The setup is indicated in step 33 of the protocol.

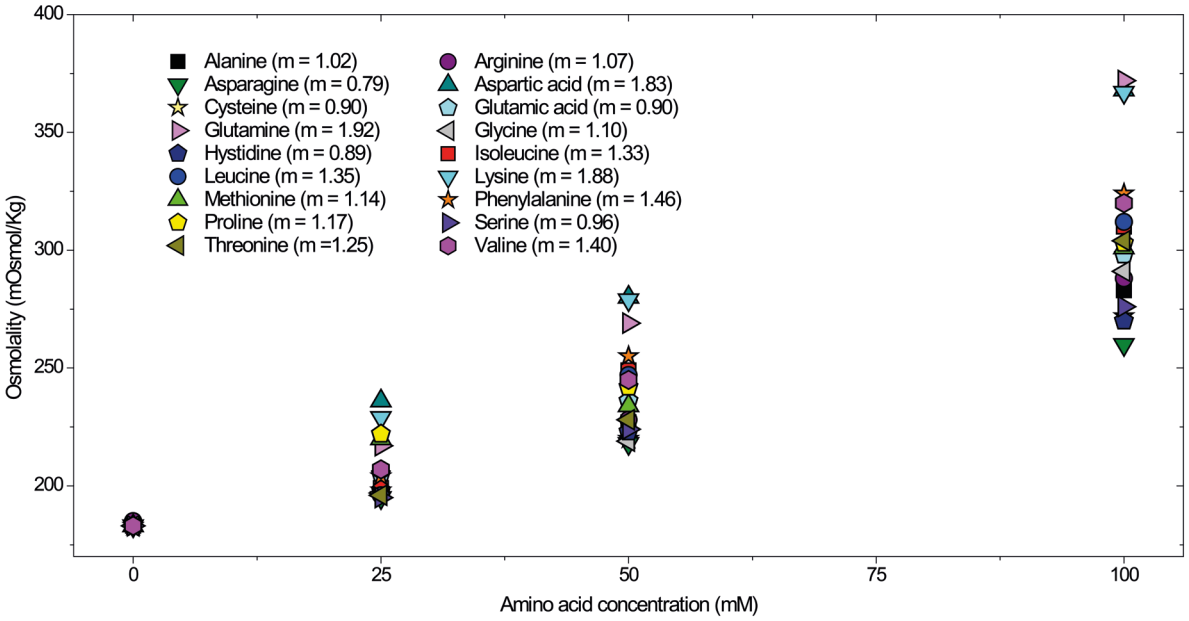
A

28.4034 0
36.02 2.12958
45.6792 7.24889
57.9284 11.6554
73.4625 14.0079
93.1621 14.3645
118.144 13.2753
149.826 11.3471
190.003 9.0763
240.954 6.80818
305.568 4.75086
387.509 3.01006
491.424 1.62914
623.203 0.631574
790.321 0.0652377
1002.25 0

B



Supplementary Figure 2. **Intensity weighted size distribution of DOPC vesicles. a,** Format of the csv file containing the size distribution of the vesicle solution to be fed to the fitting routine in MATLAB (step 49). The first and second columns correspond to radius (nm) and intensity (%), respectively. **b,** Size distribution of vesicles (LUVs) composed of DOPC from steps 43-44. The vesicles have been obtained by extrusion through polycarbonate filter with a pore diameter of 200 nm.



Supplementary Figure 3. Osmolality as a function of amino acid concentration. The slopes (m) of the plots are presented in the legend between brackets; the intercept q = 183.

BIBLIOGRAPHY

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