Supporting Information

Garcia-Celma et al. 10.1073/pnas.0902471106

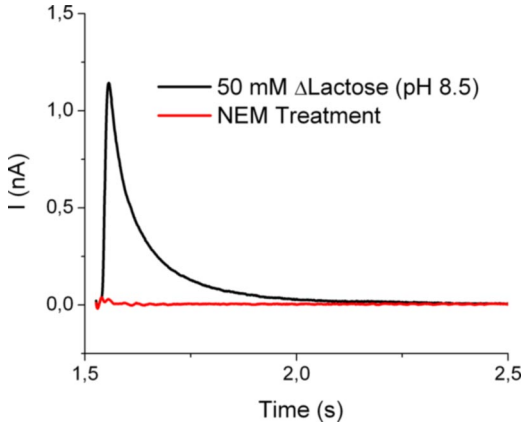

Fig. S1. Effect of the NEM treatment. The same solution exchange protocol as described for Fig. 1 was used. The nonactivating solution contained 50 mM glucose, whereas the activating solution contained 50 mM lactose. Both solutions were buffered at pH 8.5 with 100 mM potassium phosphate buffer plus 1 mM DTT. The trace in black corresponds to a 50 mM lactose concentration jump (50 mM ΔLactose) right before the addition of 2 mM *N*-ethylmaleimide (NEM). After 20 min, the transient current completely disappeared (red trace).

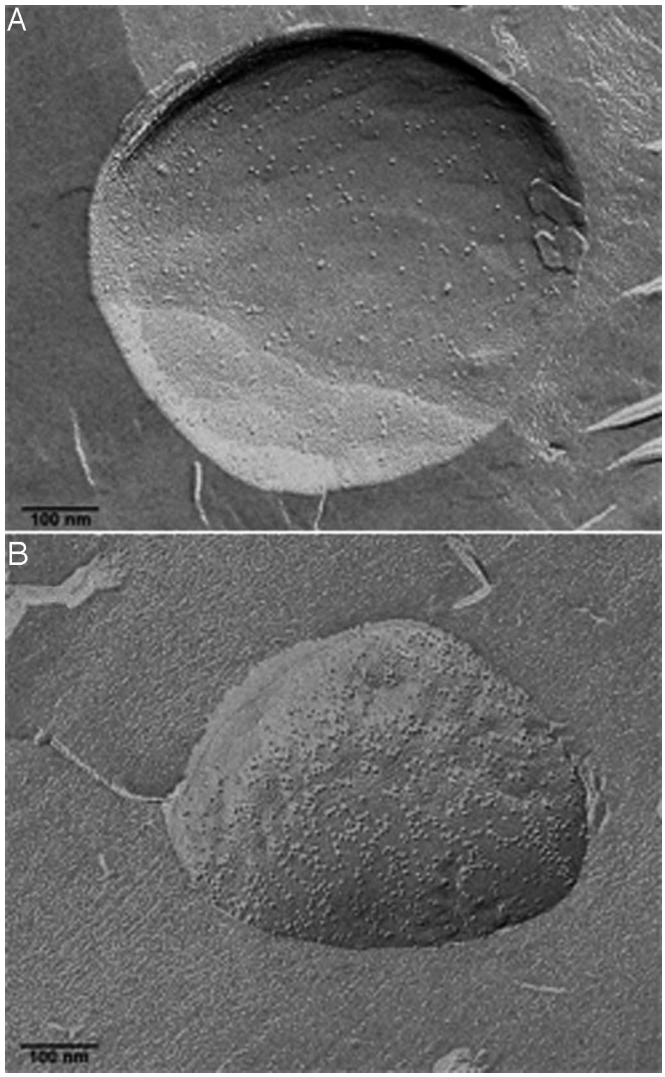

Fig. S2. Freeze–fracture electron microscope images of wild-type LacY proteoliposomes. When lipids and protein were mixed at lipid-to-protein ratio (LPR) = 10 (*A*), a protein particle density of ≈1,000 particles per μm^2 was observed. Reducing the LPR to 5 (*B*) increased in the protein particle density (≈4,500 particles per μm^2).

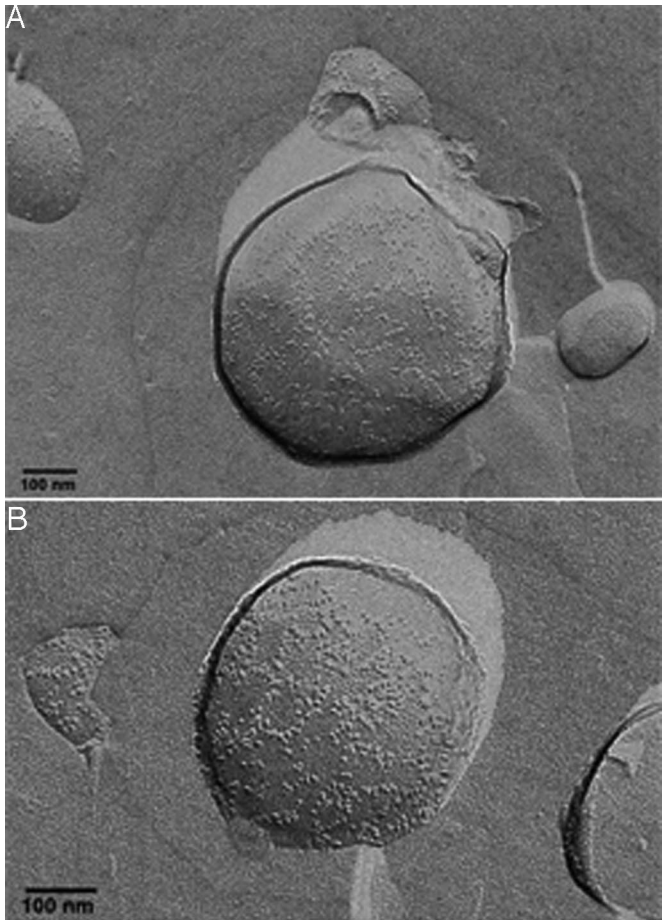

Fig. S3. Freeze–fracture images of E325 (*A* and Fig. S4*A*) and C154G (*B* and Fig. S4*B*) LacY. Both variants were reconstituted at LPR = 5 and present similar protein particle densities (≈3,500 particles per μm^2).

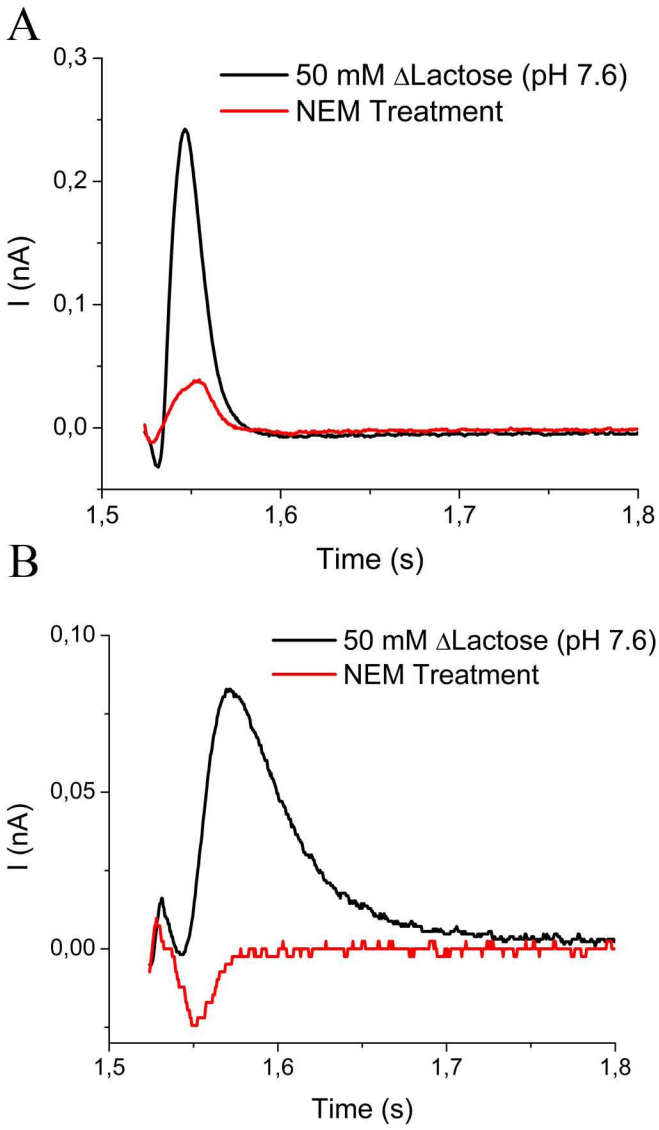

Fig. S4. Effect of the NEM treatment in the electrogenic activity of E325A (*A*) and C154G (*B*) LacY. The nonactivating solution contained 50 mM of glucose while the activating solution 50 mM of lactose in 100 mM of potassium phosphate buffer at pH 7.6 plus 1 mM DTT. The traces in black correspond to 50 mM lactose concentration jumps (50 mM ΔLactose) recorded with E325A LacY (*A*) or C154G Lac Y (*B*). Twenty minutes after the addition of 2 mM NEM the transient currents disappeared (red traces).

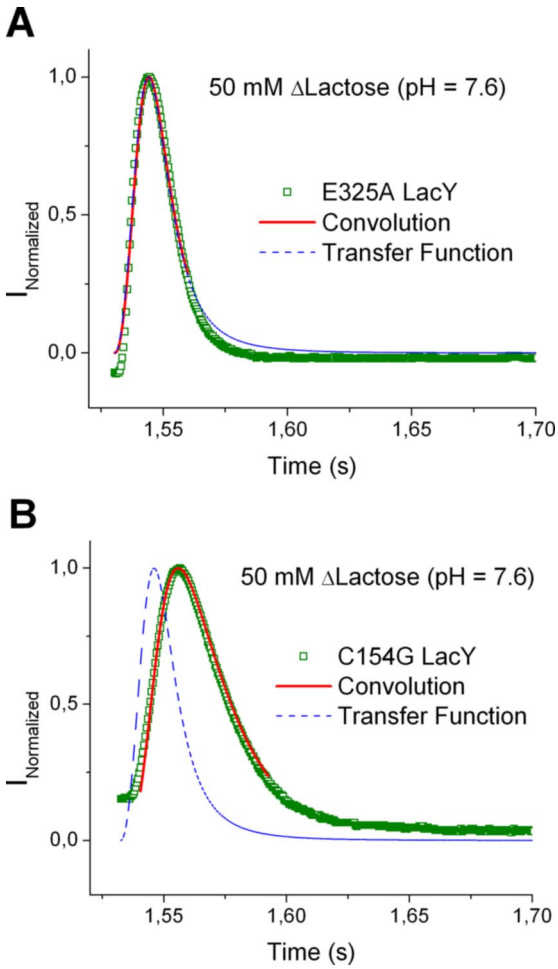

Fig. S5. Iterative least-square deconvolution of the normalized experimental transient currents generated by E325A and C154G LacY after application of 50 mM lactose concentration jumps at pH 7.6 (olive squares). The derivative of the substrate concentration rise at the surface of the solid-supported membrane (SSM), the transfer function of the system, was determined as previously described (1) and is shown in blue. Using an appropriate set of values for the k (rate constant) and t_0 (starting time of the transient), the transfer function is convoluted with a mono-exponential decaying functions $\exp[k \times (t - t_0)]$. In an iterative least-square algorithm, the result of the convolution is fitted to the measured current to obtain the parameters k and t_0. (*A*) The normalized experimental transient currents generated by E325A LacY (olive squares), and the result of the iterative least-square deconvolution algorithm when k is fixed to 1,000 s^{-1} (red trace), 200 s^{-1} (green trace), and 100 s^{-1} (black trace). The close identity between the transfer function and the experimental transient currents allows only a lower bound of $>200\ s^{-1}$ for the rate constant (red and green traces). (*B*) The normalized experimental transient currents generated by E325A LacY (olive squares), and the final result of the iterative least-square deconvolution algorithm (red trace). In this case, the transfer function is clearly distinguishable from the experimental transients, and the kinetics of the electrogenic event is resolved. For further details about the algorithm, see ref. 1.

1. Garcia-Celma JJ, et al. (2008) Rapid activation of the melibiose permease MelB immobilized on a solid-supported membrane. *Langmuir* 24:8119–8126.