

Supplementary Information for

Direct single-molecule detection of CoA-SH and ATP by the membrane proteins TMEM120A and TMEM120B

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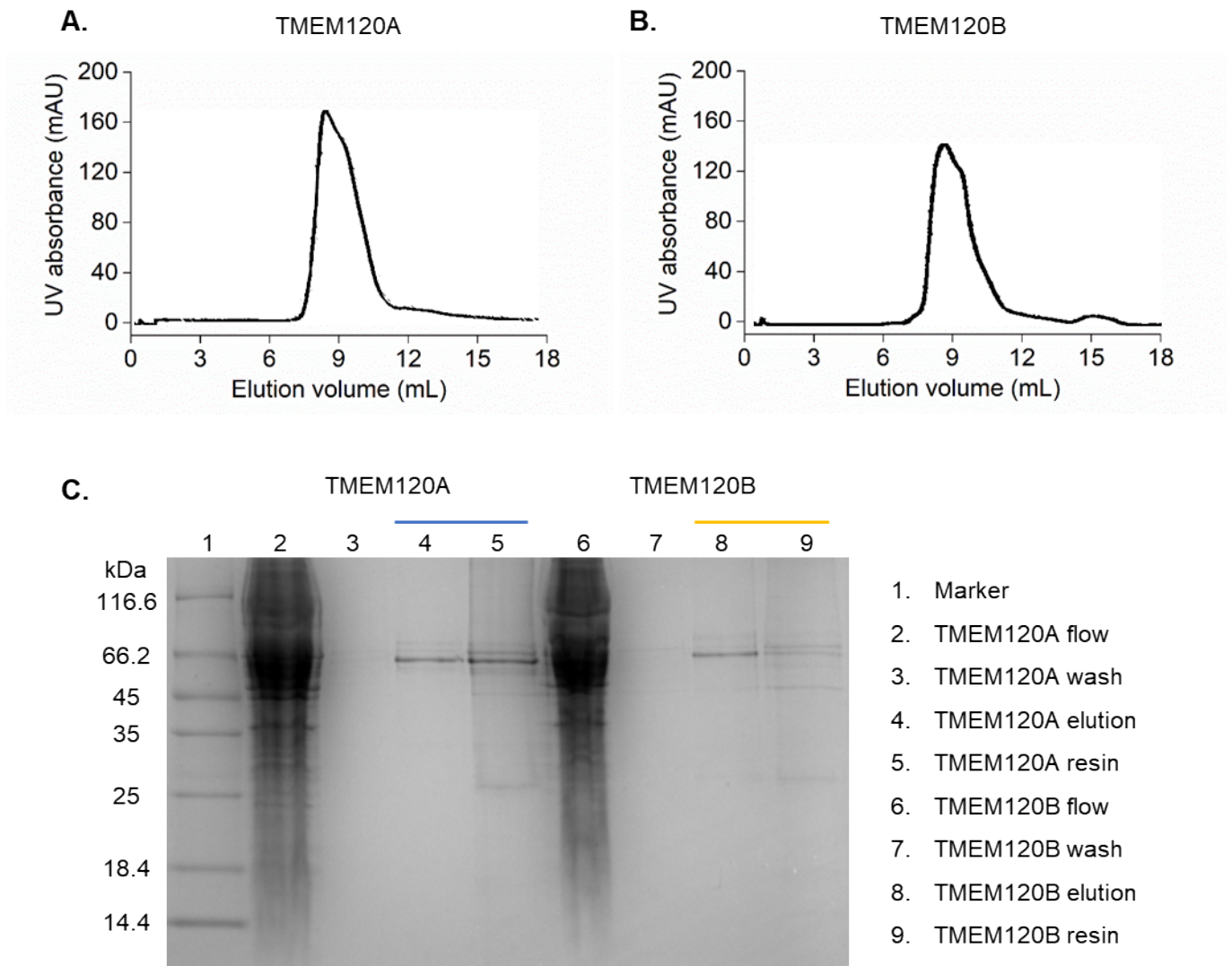


Figure 1. Representative chromatogram of gel filtration purification of TMEM120A (**A**) and TMEM120B (**B**). The fractions were analyzed by SDS-PAGE (**C**).

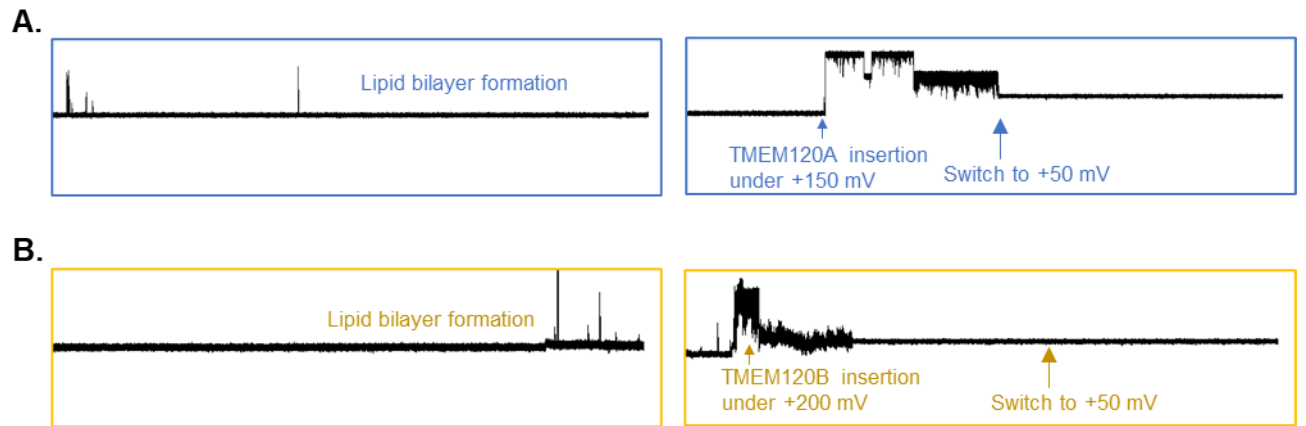


Figure 2. Current trace recording of TMEM120A/TMEM120B embedded into lipid bilayer membrane ((-*trans*) 100 mM NaCl - (-*cis*) 500 mM NaCl, 10 mM HEPES, pH 7.5.).

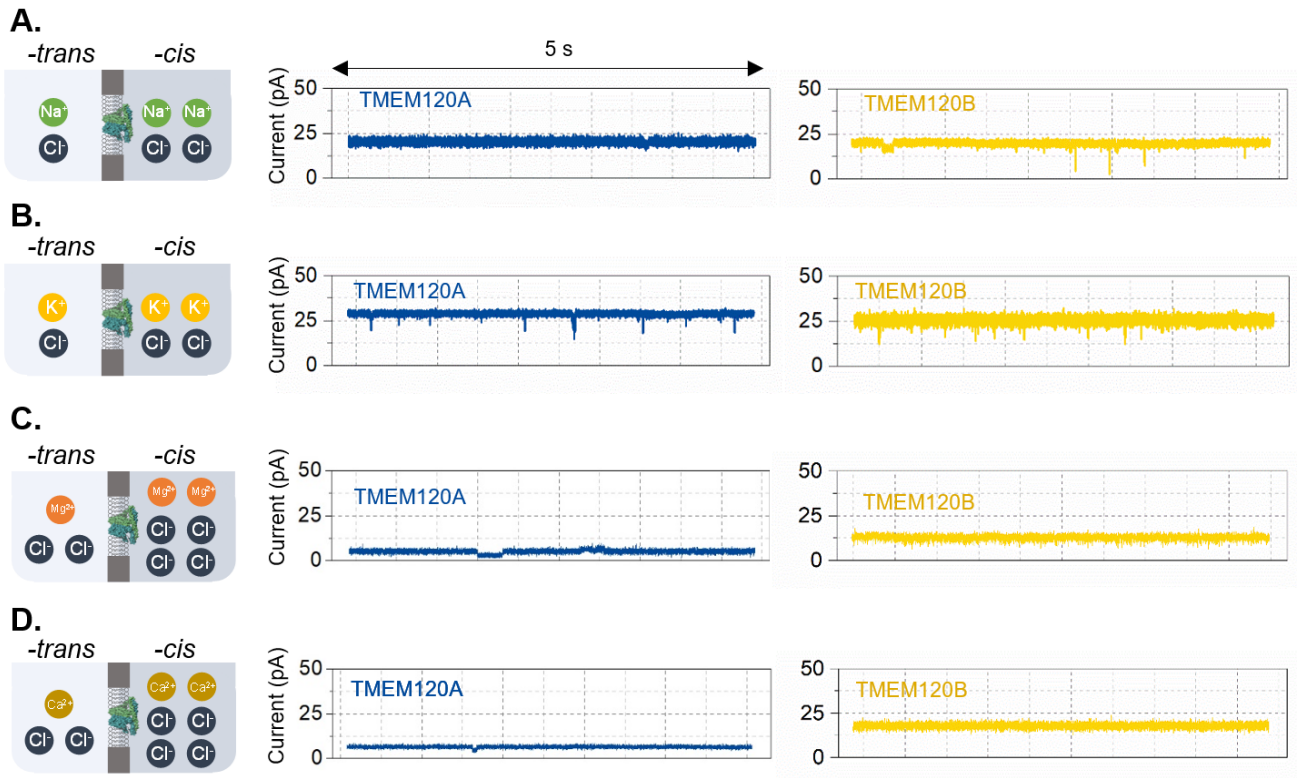


Figure 3. Current trace of TMEM120A and TMEM120B under different electrolytes conditions including 100 mM NaCl - 500 mM NaCl (**A**), 100 mM KCl - 500 mM KCl (**B**), 50 mM MgCl₂ - 250 mM MgCl₂ (**C**), 50 mM CaCl₂ - 250 mM CaCl₂ (**D**) (*-trans*) - (*-cis*), voltage: +20 mV).

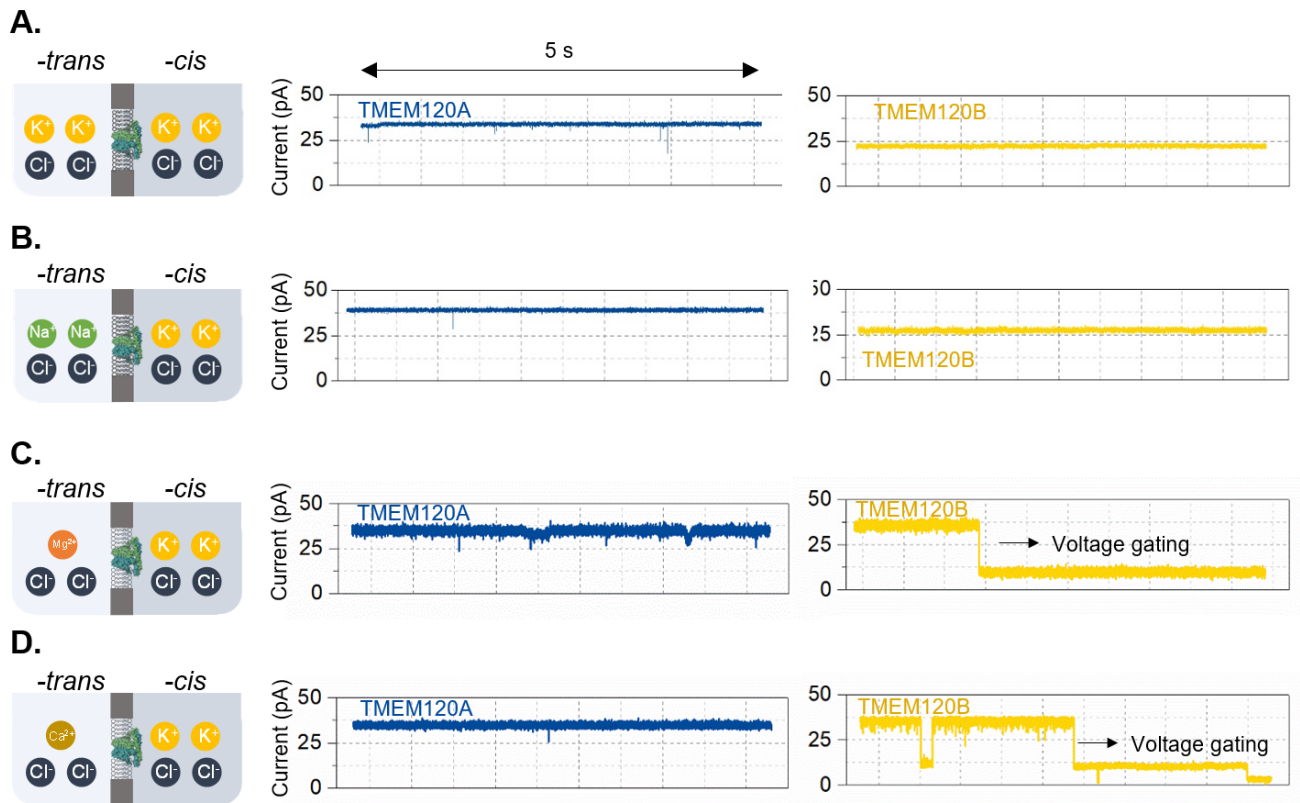


Figure 4. Current trace of TMEM120A and TMEM120B under different electrolytes conditions including 500 mM KCl - 500 mM KCl (**A**), 500 mM NaCl - 500 mM KCl (**B**), 250 mM MgCl₂ - 500 mM KCl (**C**) and 250 mM CaCl₂ - 500 mM KCl (**D**) (*-trans*) - (*-cis*), voltage: +20 mV).

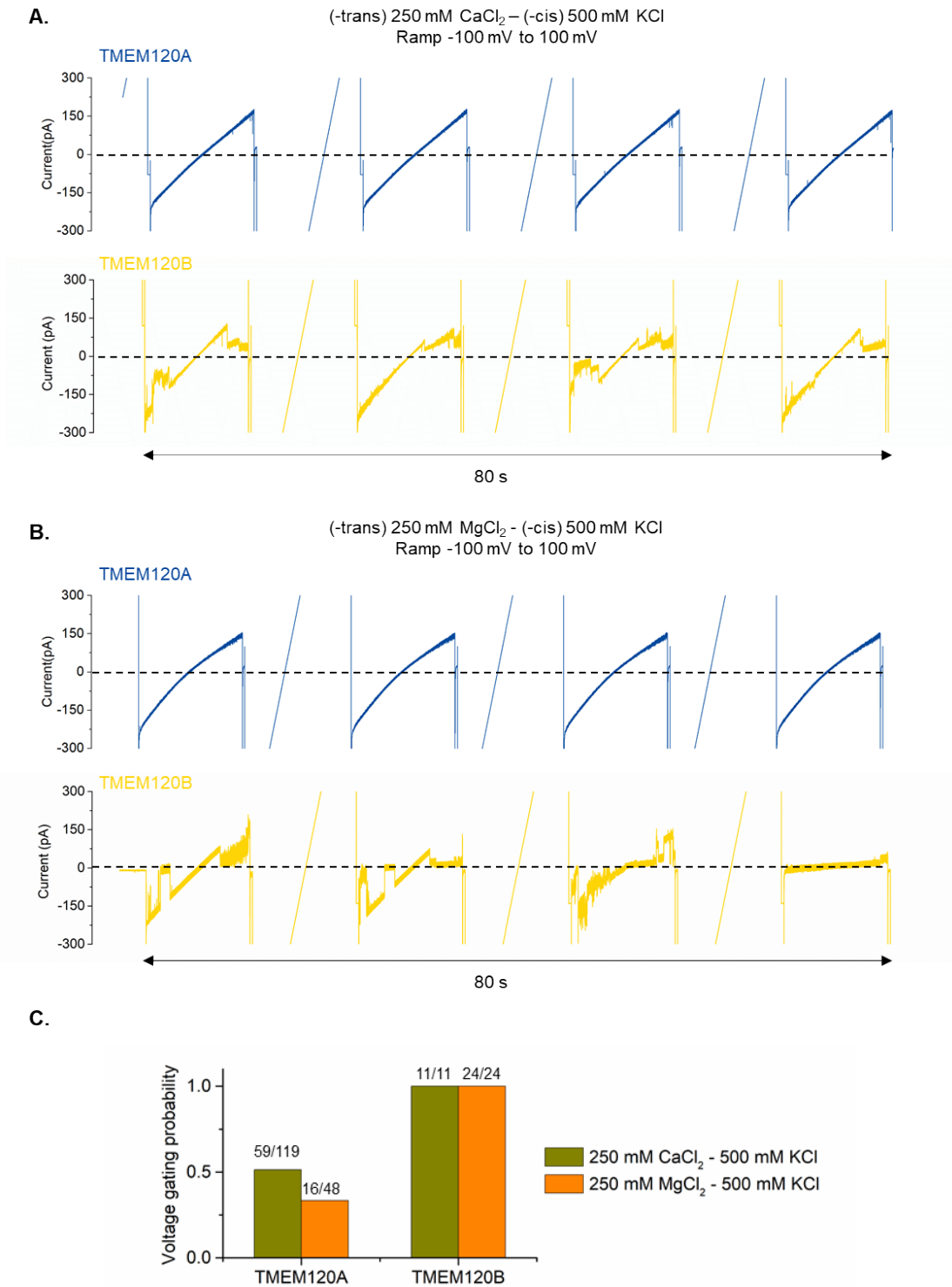


Figure 5. TMEM120B present higher voltage-gating probability than TMEM120A in the presence of Ca²⁺ and Mg²⁺. **A.** Ion condition: (-*trans*) 250 mM CaCl₂ – (-*cis*) 500 mM KCl, 10 mM HEPES, pH 7.5. **B.** Ion condition: (-*trans*) 250 mM MgCl₂ – (-*cis*) 500 mM KCl, 10 mM HEPES, pH 7.5. **C.** Voltage-gating probability of TMEM120A and TMEM120B under two different electrolyte conditions, with a voltage range from -100 mV to 100 mV.

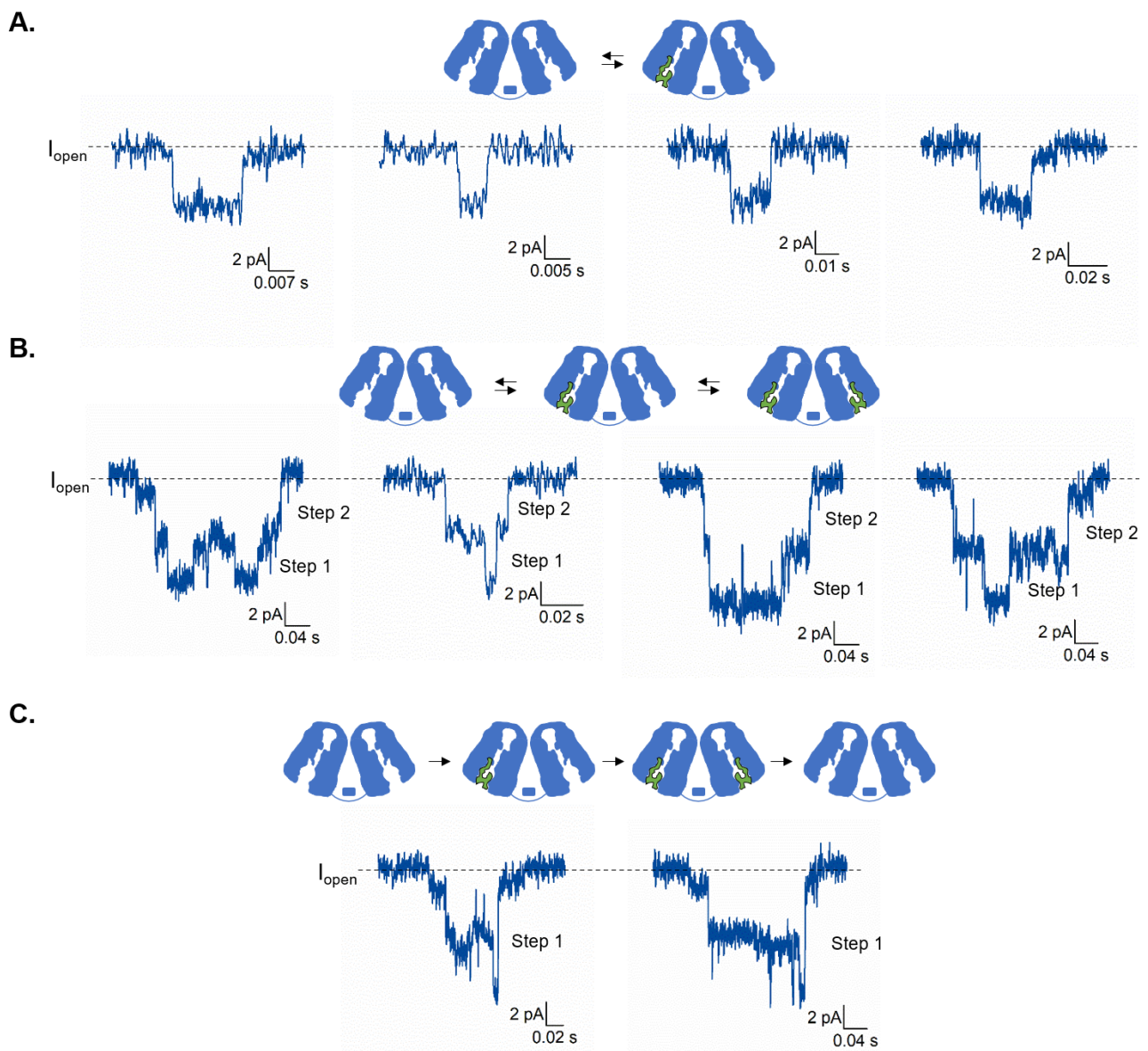


Figure 6. Represent signal and possible forms of interaction between COA-SH and TMEM120A. **A.** One-step block signal with relatively low blockage rate. **B.** Two-step block signal with relatively high blockage rate and two-step of dissociation. **C.** Two-step block signal with one step dissociation (+20 mV, (-*trans*) 100 mM NaCl - (-*cis*) 500 mM NaCl, 10 mM HEPES, pH 7.5.).

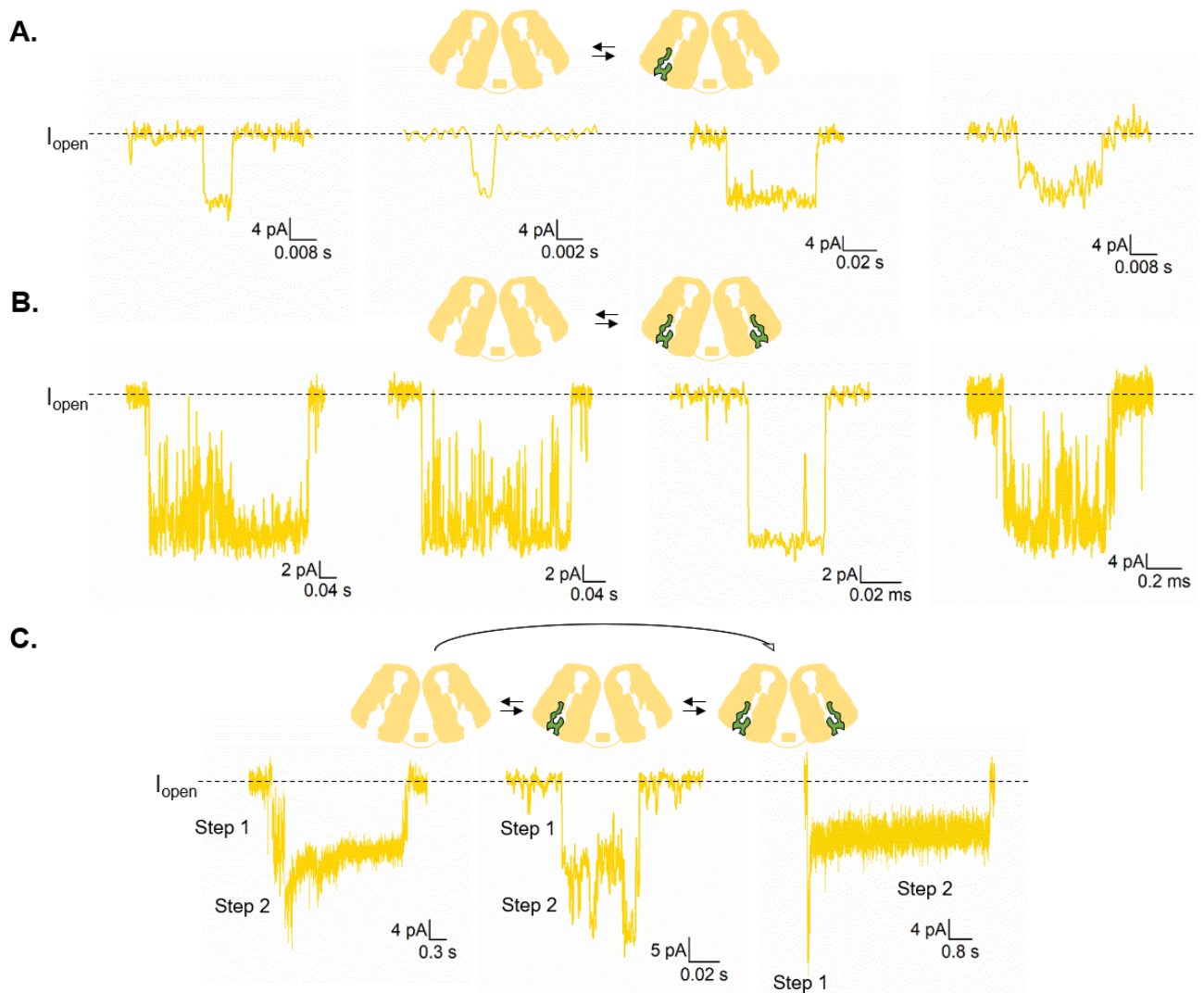


Figure 7. Represent signal and possible forms of interaction between COA-SH and TMEM120B. **A.** One-step block signal with relatively low blockage. **B.** One-step block signal with relatively high blockage rate. **C.** Block signal with two steps during block process (+20 mV, (-*trans*) 100 mM NaCl - (-*cis*) 500 mM NaCl, 10 mM HEPES, pH 7.5.).

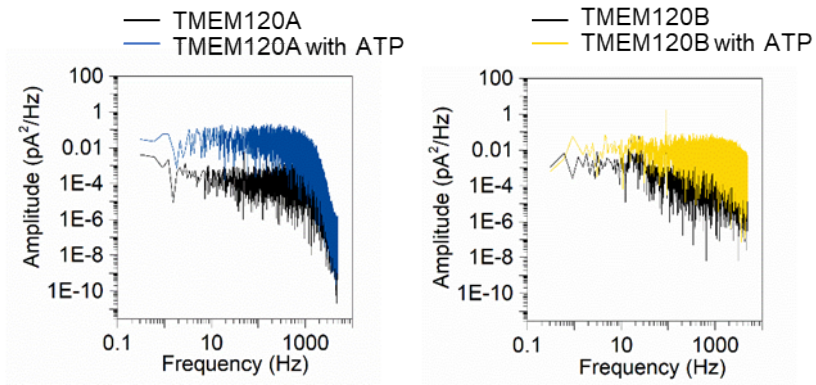


Figure 8. Power spectrum analysis of TMEM120A/TMEM120B control (black) and TMEM120A/TMEM120B with 1 mM ATP in the *-cis* side (blue/yellow). The electrolyte buffer is (*-trans*) 250 mM MgCl₂ - (*-cis*) 500 mM KCl. The voltage was +20 mV.

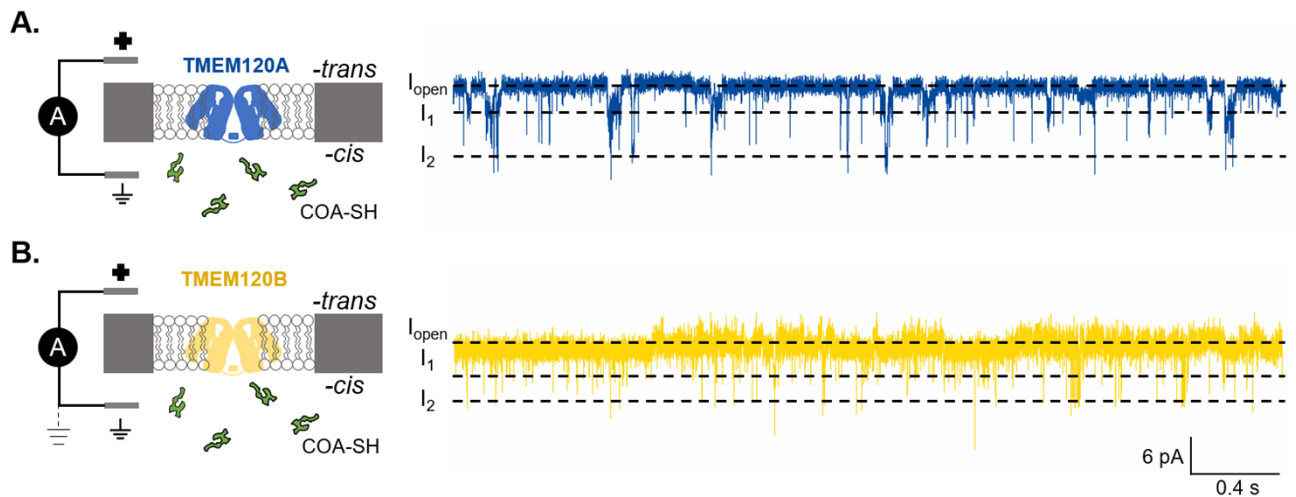


Figure 9. Current trace of COA-SH sensing by TMEM120A (blue) and TMEM120B(yellow) under the electrolyte condition of (-*trans*) 250 mM MgCl₂ - (-*cis*) 500 mM KCl. The voltage was +20 mV.