Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information for

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

Changjian Zhao[1, 2#], Mutian Chen[1, 2#], Xiaofeng Liu[3], Weidan Yuan[1], Kaiju Li[1, 2], Yu Wang[1, 2], Chen Chen[1, 2], Ming Zhang[1], Yuhan Dong[1, 2], Yuling Xiao[1], Dong Deng[3], Jia Geng[1, 2*].

1. Department of Laboratory Medicine, State Key Laboratory of Biotherapy, Med-X Center for Manufacturing, West China Hospital, Sichuan University and Collaborative Innovation Center, Chengdu, 610041, China.
2. Tianfu Jincheng Laboratory, City of Future Medicine, Chengdu 610500, China.
3. Division of Obstetrics, Key Laboratory of Birth Defects and Related Disease of Women and Children of MOE, State Key Laboratory of Biotherapy, West China Second Hospital, Sichuan University, Chengdu, 610041, Sichuan, China.

[#]These authors have contributed equally to this work.

**Correspondence to J.G. geng.jia@scu.edu.cn.*

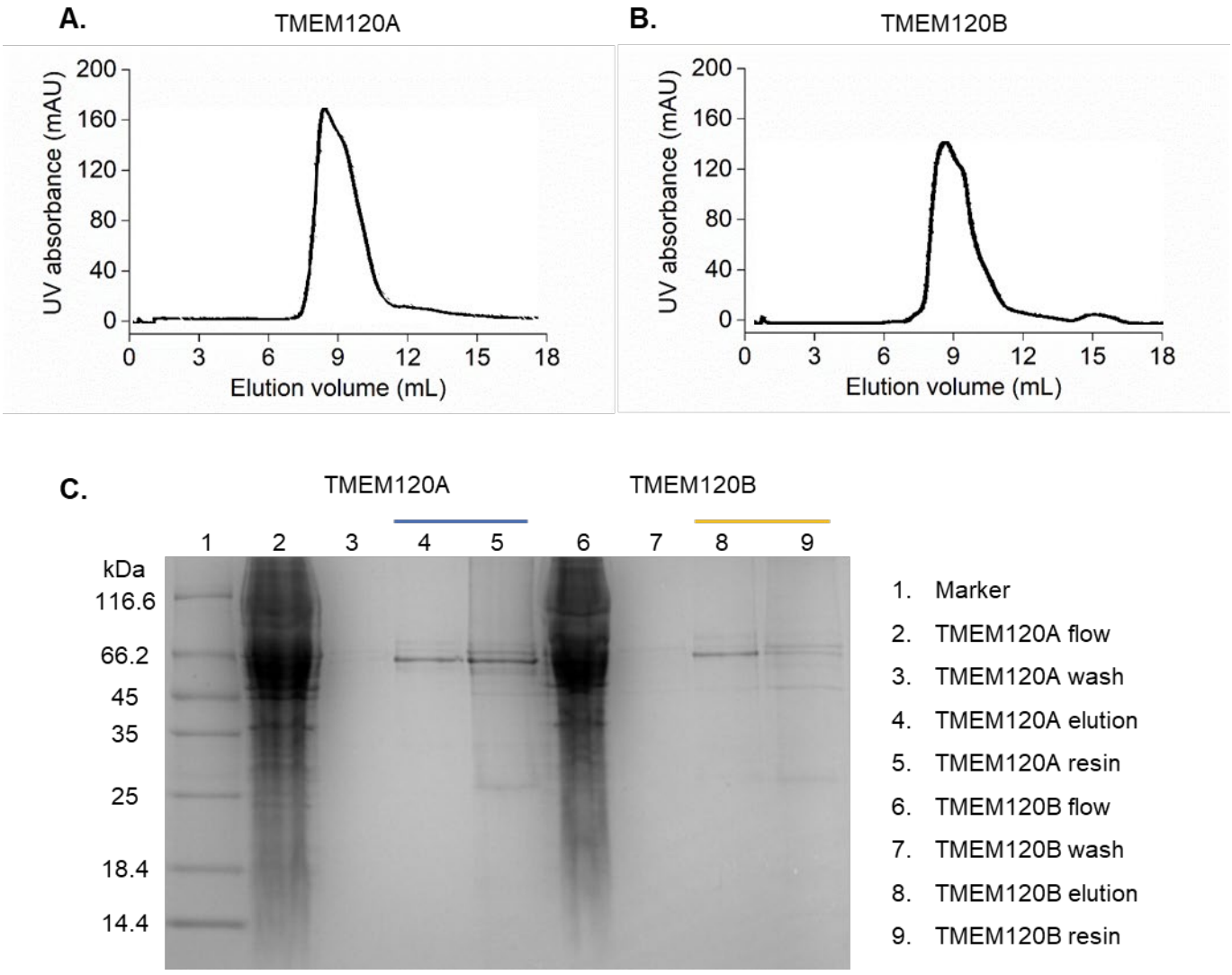

Figure 1. Representative chromatogram of gel filtration purification of TMEM120A (**A**) and TMEM120B (**B**). The fractions were analyzed by SDS-PAGE (**C**).

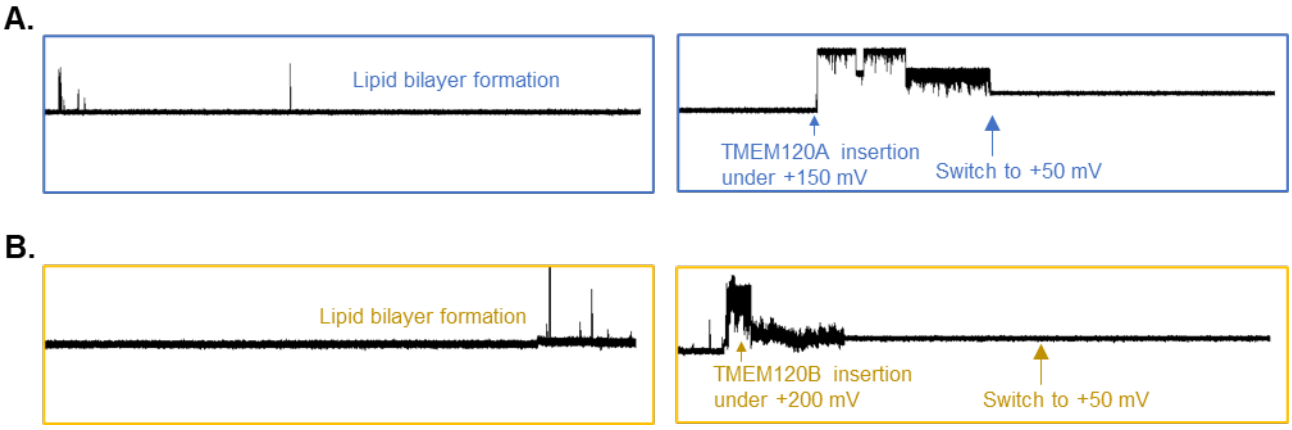

Figure 2. Current trace recording of TMEM120A/TMEM120B embedded into lipid bilayer membrane ((-*trans*) 100 mM NaCl - (-*cis*) 500 mM NaCl, 10 mM HEPES, pH 7.5.).

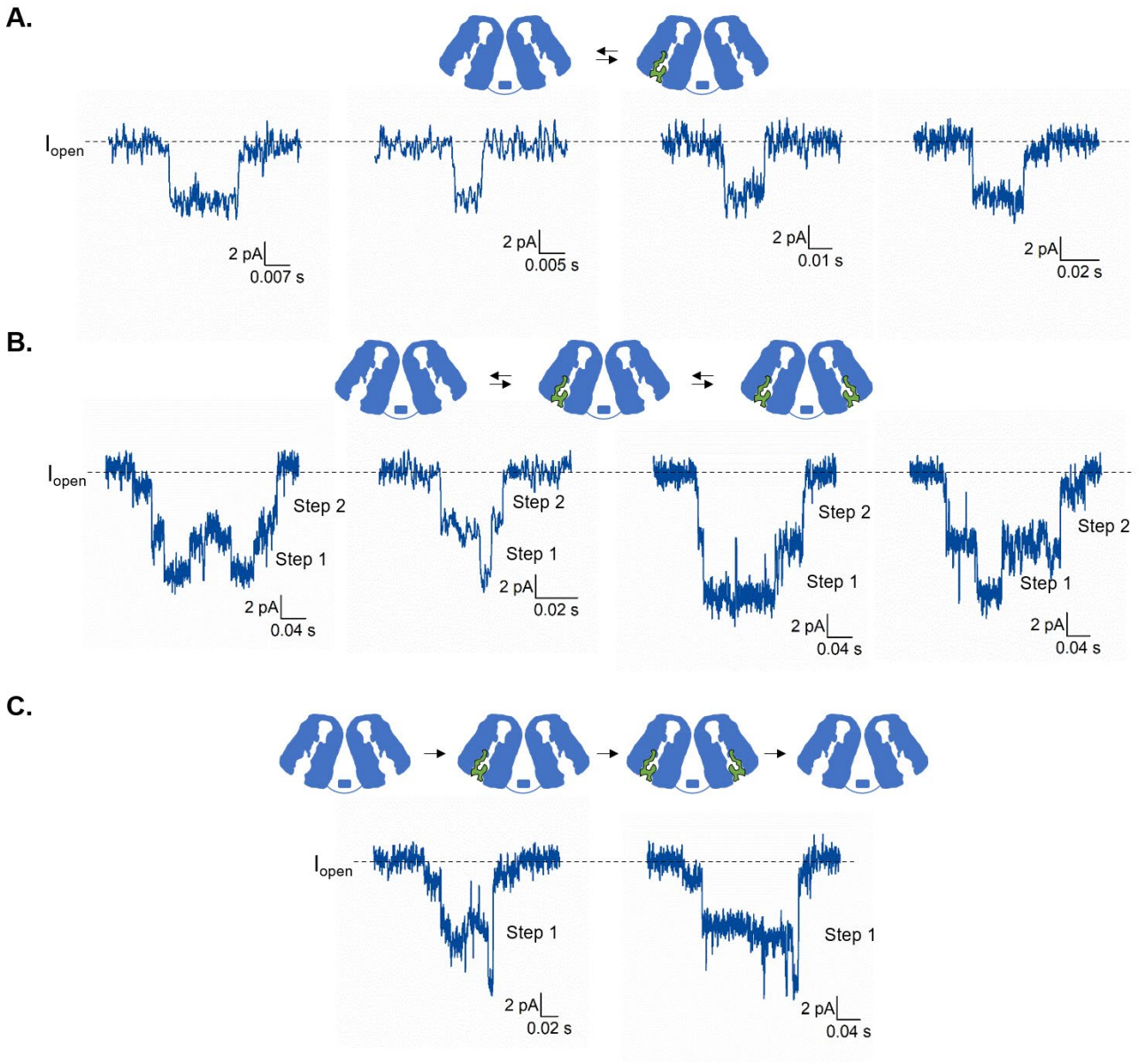

Figure 3. Represent signal and possible forms of interaction between COA-SH and TMEM120A. **A**. One-step block signal with relatively low blockage rate. **B**. Two-step block signal with relatively high blockage rate and two-step of dissociation. **C**. Two-step block signal with one step dissociation (+20 mV, (-*trans*) 100 mM NaCl - (-*cis*) 500 mM NaCl, 10 mM HEPES, pH 7.5.).

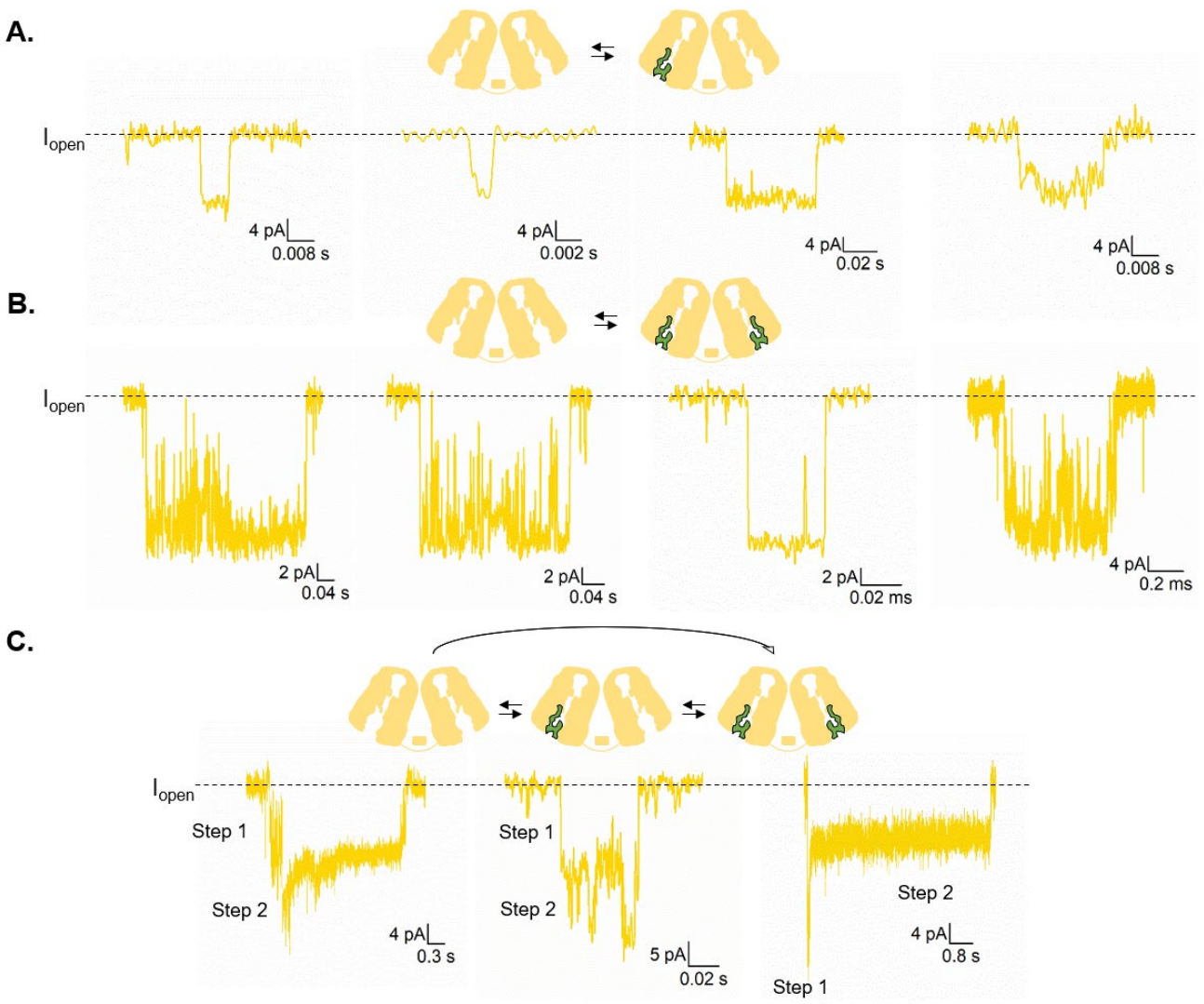

Figure 4. Represent signal and possible forms of interaction between COA-SH and TMEM120B. **A**. One-step block signal with relatively low blockage. **B**. One-step block signal with relatively high blockage rate. **C**. Block signal with two steps during block process (+20 mV, (-*trans*) 100 mM NaCl - (-*cis*) 500 mM NaCl, 10 mM HEPES, pH 7.5.).

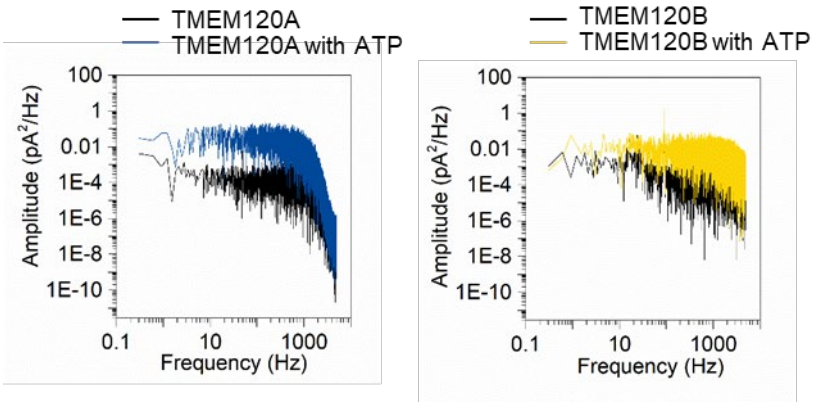

Figure 5. Power spectrum analysis of TMEM120A/TMEM120B control (black) and TMEM120A/TMEM120B with 1 mM ATP in the -*cis* side (blue/yellow). The electrolyte buffer is (-*trans*) 250 mM $MgCl_2$ - (-*cis*) 500 mM KCl. The voltage was +20 mV.

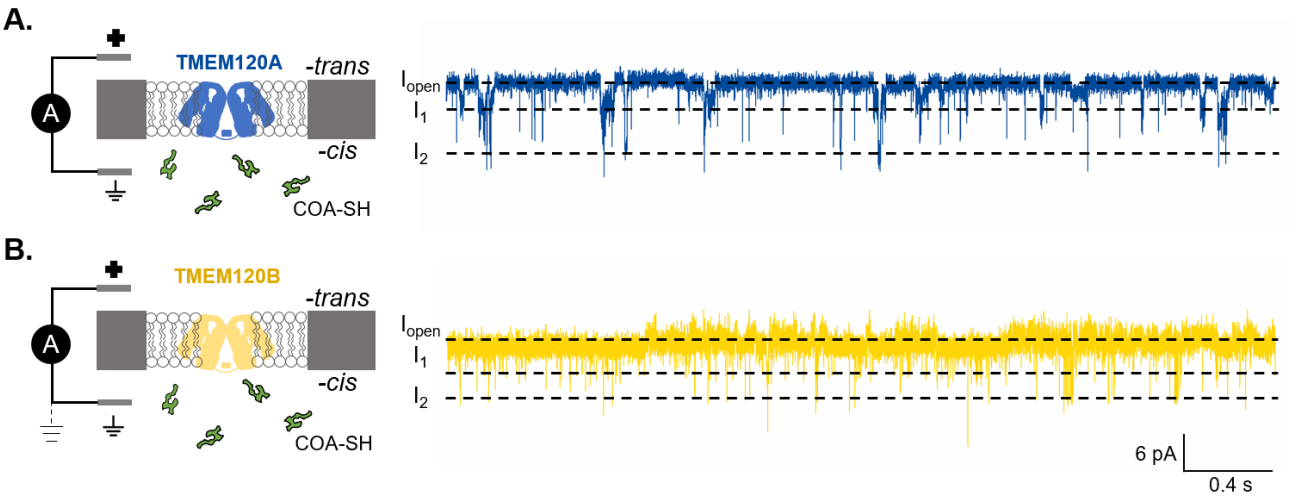

Figure 6. Current trace of COA-SH sensing by TMEM120A (blue) and TMEM120B(yellow) under the electrolyte condition of (-*trans*) 250 mM $MgCl_2$ - (-*cis*) 500 mM KCl. The voltage was +20 mV.

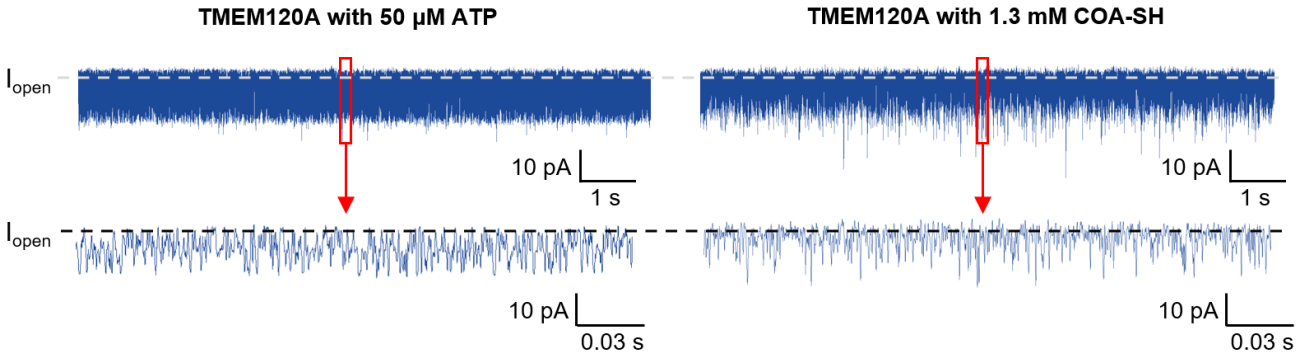

Figure 7. Using TMEM120A as an example, high concentrations of COA-SH or ATP can interfere with and block signal counts, thereby hindering quantitative detection. The electrolyte condition was -cis: 500 mM KCl, -trans: 250 mM $MgCl_2$, 10 mM Glycine-HCl, pH 4.5, with a sampling frequency of 10 kHz and a Bessel filter of 2.9 kHz, the voltage was +20 mV.