Supporting Information

Gao and Hwang 10.1073/pnas.1420676112

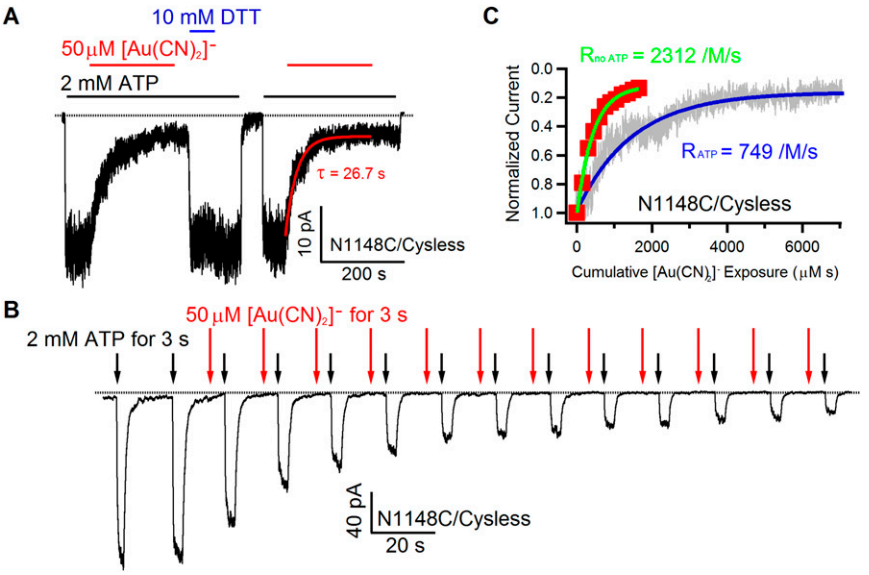

Fig. S1. Reactivity of N1148C (in TM12) to internal $[Au(CN)_2]^-$ in the presence (*A*) or absence (*B*) of ATP. (*A*) In the presence of ATP, 50 μM $[Au(CN)_2]^-$ inhibited N1148C–CFTR currents to a significant extent. Note the current recovered completely by DTT, but not by simple washout of $[Au(CN)_2]^-$, indicating that the ligand exchange reaction did occur between the cysteine and the probe. Fitting the current decay phase with a single exponential function (red line) yields a time constant of 26.7 s. (*B*) $[Au(CN)_2]^-$, when applied in the absence of ATP, diminished N1148C–CFTR currents even faster. (*C*) Comparison of the reaction rates for N1148C–CFTR with and without ATP. Red squares mark normalized current amplitudes extracted from *B*, and the gray trace represents the expanded current decay from *A*. Green and blue lines represent fits of the data with single exponential function. In this particular case, the reaction rate of N1148C–CFTR in the absence of ATP is ~3 times faster than that with ATP.

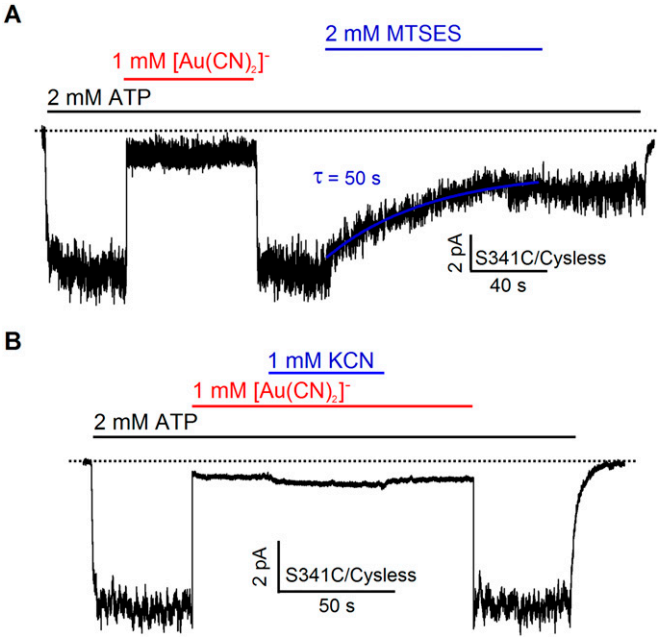

Fig. S2. $[Au(CN)_2]^-$ fails to react with S341C in the presence of ATP. (*A*) In an inside-out patch, the application of 1 mM $[Au(CN)_2]^-$ only resulted in a reversible inhibition, which was immediately relieved upon removal of $[Au(CN)_2]^-$. In the same patch, a later application of 2 mM MTSES caused irreversible current decay (blue line, single exponential fit of the current decay), indicating that this cysteine was either not reactive toward $[Au(CN)_2]^-$ or reactive but the response is subject to the continuous presence of $[Au(CN)_2]^-$. This latter scenario can be differentiated by adding CN^- to the $[Au(CN)_2]^-$–containing solution. As CN^- is a product of the reaction between cysteine side chain and $[Au(CN)_2]^-$, the addition of CN^- is expected to reverse the reaction and hence recover the current inhibited by $[Au(CN)_2]^-$ (1). However, CN^- did not restore the inhibited current (*B*), suggesting that the cysteine placed at position 341 is not susceptible to $[Au(CN)_2]^-$ ($n = 3$). Note a similar observation was made when 341C was probed with extracellularly applied $[Au(CN)_2]^-$ with or without CN^- (2).

1. Serrano JR, et al. (2006) CFTR: Ligand exchange between a permeant anion ([Au(CN)2]–) and an engineered cysteine (T338C) blocks the pore. *Biophys J* 91(5):1737–1748.
2. Alexander C, et al. (2009) Cystic fibrosis transmembrane conductance regulator: Using differential reactivity toward channel-permeant and channel-impermeant thiol-reactive probes to test a molecular model for the pore. *Biochemistry* 48(42):10078–10088.

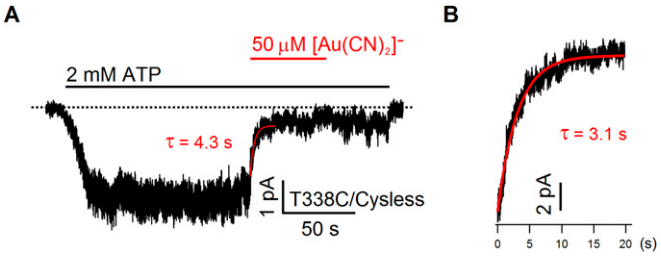

Fig. S3. Measurement of the reaction rate for T338C in response to cytoplasmic $[Au(CN)_2]^-$ at 30 mM internal $[Cl^-]$. (*A*) A real-time current trace of T338C–CFTR in an inside-out patch showing rapid current decay upon the addition of 50 μM $[Au(CN)_2]^-$. To maintain osmolarity and to avoid blockade of the pore by large organic anions, chloride in the perfusate was replaced by sucrose. The ranges of time constants estimated from curve fitting of the current decay with a single exponential function (red line) are 22.2–38.5 s at 154 mM $[Cl^-]$ and 1.4–6.4 s at 30 mM $[Cl^-]$. Unpaired Student's *t* test showed a significant difference between these two groups of data ($P < 0.001$). The calculated reaction rate at 30 mM $[Cl^-]$ (7,647 ± 1,563 /M/s, $n = 6$) is ~10-fold faster than that with 154 mM $[Cl^-]$ (752 ± 59 /M/s, $n = 7$), suggesting that intracellular $[Au(CN)_2]^-$ and chloride compete for the same binding site in the pore, probably at the presumed narrow region, as 338C resides right on the extracellular brink of this region. (*B*) Because the macroscopic current amplitude of T338C–CFTR is dramatically reduced due to the low $[Cl^-]$, to reassure our readers that the rates we measured were accurate, we summed up all of the current traces in six patches to build an ensemble current decay. Fitting this ensemble current decay with a single exponential function (red line) resulted in a current decay time constant of 3.1 s, equivalent to ~6,452 /M/s.

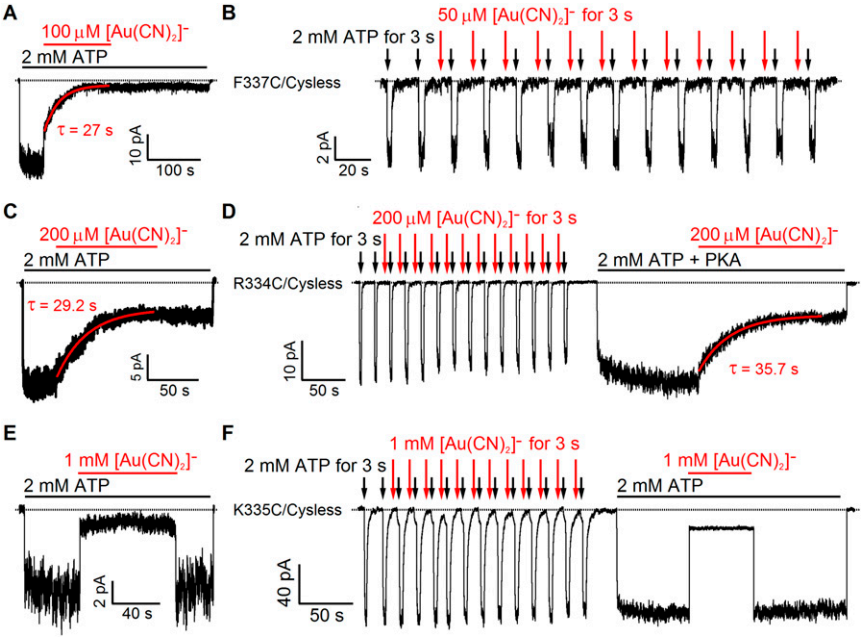

Fig. S4. Effects of internal $[Au(CN)_2]^-$ on F337C–CFTR, R334C–CFTR, and K335C–CFTR with and without ATP. (*A–D*) F337C and R334C were reactive to internal $[Au(CN)_2]^-$ only in the presence of ATP. However, internal $[Au(CN)_2]^-$ failed to react with K335C in the presence (*E*) or absence (*F*) of ATP.

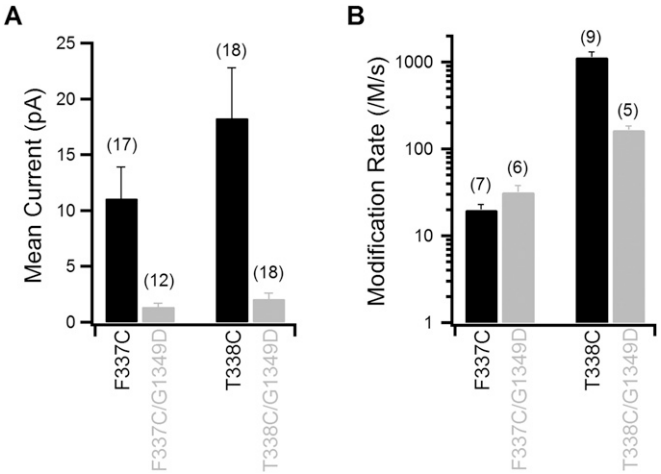

Fig. S5. Effects of the G1349D mutation on the mean current amplitude in inside-out patches and the reaction rates for cysteines introduced to positions 337 and 338. (*A*) Mean current amplitudes of F337C–CFTR and F337C/G1349D–CFTR in inside-out patches were 11.1 ± 2.8 pA and 1.4 ± 0.3 pA, respectively. This ~8-fold difference in macroscopic current amplitudes likely reflects an eightfold difference in P_o between F337C–CFTR and F337C/G1349D–CFTR (see *Results* for details). Similarly, introducing the G1349D mutation into T338C also caused a ~ninefold decrease of the P_o. Mean current amplitudes of T338C–CFTR and T338C/G1349D–CFTR were 18.3 ± 4.5 pA and 2.1 ± 0.5 pA, respectively. (*B*) Reaction rates of F337C and T338C by external $[Au(CN)_2]^-$ assessed in whole-cell experiments. Note a minor difference between F337C–CFTR (20 ± 3 /M/s) and F337C/G1349D–CFTR (32 ± 6 /M/s). However, the reaction rate for T338C (1,135 ± 166 /M/s) is ~10-fold higher than that of T338C/G1349D–CFTR (118 ± 18 /M/s), suggesting cysteine introduced at position 338 is preferentially modified in the open state. Number of data points is shown on the top of each bar.

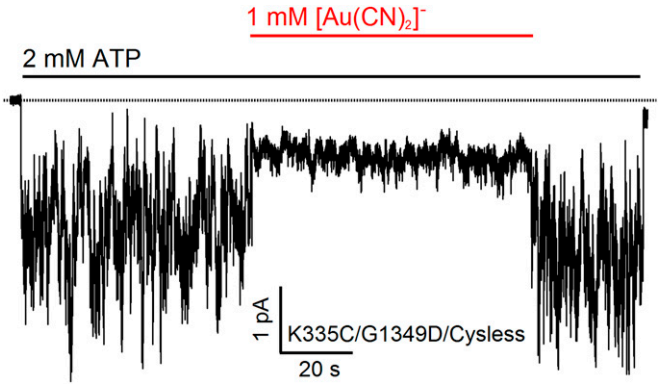

Fig. S6. A lack of reactivity of K335C/G1349D double mutant CFTR toward internal $[Au(CN)_2]^-$. When 1 mM $[Au(CN)_2]^-$ was applied internally to K335C/G1349D–CFTR, only a reversible blockade was observed ($n = 4$), in great contrast to a rapid reaction between this cysteine and external $[Au(CN)_2]^-$ in whole-cell experiments (cf., Fig. 3*D*), corroborating the idea that there is a physical barrier intracellular to this position in CFTR's closed state.

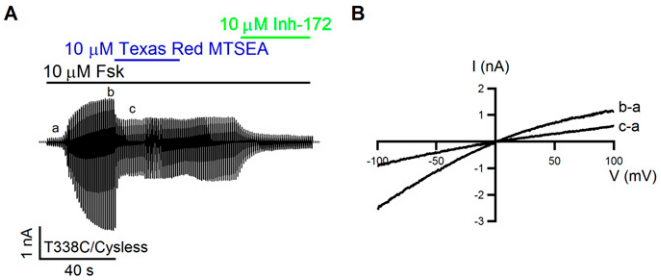

Fig. S7. Modification of T338C by external Texas red MTSEA. (*A*) In whole-cell experiments, 10 μM Texas red MTSEA irreversibly decreased the CFTR current by covalently modifying the cysteine introduced at position 338 ($n = 5$). A major fraction of the residual current following the application of Texas red MTSEA is sensitive to a specific CFTR inhibitor (Inh-172). (*B*) Current–voltage (I–V) relationship extracted from *A*. In this particular experiment, pipette $[Cl^-]$ was 125 mM, with aspartate and pyruvate replaced with CsCl to maintain the normal osmolarity. Ramp voltage pulses over –100 mV to 100 mV were applied every 5 s.