**Supplemental TEXT**

## T1. Differences in the reactivity of $[Au(CN)_2]^-$ and $[Ag(CN)_2]^-$

The reactivities of the two permeant pseudohalides, as judged by the production of a functional change either not reversed by washing or reversed by adding back $CN^-$, were not identical. As illustrated in **Fig. 4**, exposure of S341C CFTR to $[Ag(CN)_2]^-$ produced a component of irreversible inhibition whereas exposure to $[Au(CN)_2]^-$ did not. The nearly identical size, shape and hydration energy of these two permeant anions argues against any difference their ability to access engineered cysteines. Instead, these results may reflect the well-known differences in the coordination preferences of the Au(1) and Ag(1) metal centers. Au(1) strongly prefers a 2-coordinate complex, although an associative reaction mechanism would produce a transient intermediate, protein-S-$[Au(CN)_2]^{2-}$, leading to the final product protein-S-$[AuCN]^-$ (1). In contrast, Ag(1) can form stable 2- or 3- coordinate complexes so that either protein-S-$[Ag(CN)_2]^{2-}$ or protein-S-$[Ag(CN)]^-$ are possible products (2). In aqueous solution the di-cyano complex is more stable for Au(1) than for Ag(1) so that the energetics may favor the formation of the thiol complex with $[Ag(CN)_2]^-$ (3). One or both factors could account for the fact that $[Ag(CN)_2]^-$ was more likely than $[Au(CN)_2]^-$ to form a stable product with the thiolate moiety of substituted cysteines. The fact that $[Au(CN)_2]^-$ apparently forms a very stable product with some cysteines substituted into CFTR and not others suggests that factors other than the metal-thiolate interactions determine the stability of the complex. It may be, for example, that in some locations the $CN^-$ moiety experiences some local interactions with the protein that contribute to the formation of a stable complex.

## T2. Discussion of differences between the present study and previous studies

Cheung and Akabas (4,5) first examined the reactivity of cysteine-substituted CFTR constructs (wt background) toward MTS reagents and, although there is overlap with the results presented here, there are also significant differences. Cheung and Akabas (4,5) reported that I331C CFTR conductance was inhibited by exposure to $MTSEA^+$, but not $MTSES^-$, whereas we report here reactivity toward $MTSET^+$ and $MTSES^-$. On the other hand, the present results confirm their finding that L333C CFTR was reactive toward $MTSET^+$ and $MTSES^-$. We also recorded reactivity of both I331C and L333C CFTR toward $MTSEA^+$, but we have not used this compound for screening because it has been shown to cross biological membranes (6).

At positions 334 and 335, however, Cheung and Akabas (4,5) reported that cysteines reacted with all three MTS compounds, but detected functional effects that were uniformly inhibitory; whereas we have reported functional changes that were highly charge-dependent (7). Similarly, these investigators reported reactivity toward charged MTS reagents at residues 337, 341, 347, 351 and 352 that was not detected in our assays. Conversely, a cysteine at 338 was reported by Cheung and Akabas to be un-reactive (4), whereas we found non-selective reactivity at this locus (8,9). Cysteines at 343, 346, 349, and 350 were scored as un-reactive toward MTS reagents by Cheung et al. (4,5) as well as the present study.

Cysteines at 336, 340, 341, 342, 344 and 348, where we found selective reactivity toward channel-permeant reagents, were scored as un-reactive by Cheung et al. (4,5), but this difference is expected due to the differences in the thiol-directed probes utilized in the two studies. The remaining discrepancies between the two studies (at positions 336 and 338) have no obvious explanation at present, but could reflect, in part, the spontaneous reactions of engineered cysteines that can render them un-reactive toward exogenous reagents (9).

Chueng and Akabas reported reactivity of R352C/wt CFTR toward externally applied $MTSET^+$ and $MTSES^-$ (4, 5), but this reactivity was not seen by Smith et al. (7), nor in additional experiments conducted for the present study. Guinamard and Akabas (10) proposed that R352 could be a major

determinant of the anion to cation selectivity of CFTR based on NaCl dilution potentials measured in detached patch, single-channel recordings that compared R352Q and wt CFTR, but neither Smith et al. (7), St Aubin and Linsdell (11) nor Cui et al. (12) confirmed their findings.

Beck et al. (13) recently used MTS reagents as well as cadmium to probe cysteines substituted in the outer region of TM6. They confirmed the reactivity at positions 331 and 333 reported by Cheung and Akabas (4,5) and validated in the present work, and also described reactivity toward $Cd^{2+}$ at these sites. Their results with channel-*im*permeant reagents are broadly consistent with those reported here and earlier in Smith et al. (7), Liu et al. (8) and Serrano et al. (14), confirming reactivity toward $MTSEA^{+}$ at positions 334, 335 and 338. We also scored I336C CFTR as reactive toward MTS reagents, however, whereas Beck et al. (13) did not.

Fatehi and Linsdell (15) studied the reactivity of some of the same cysteine substituted constructs reported here and previously. They confirmed reactivity toward $MTSES^{+}$ and $MTSES^{-}$ of cysteines at 334, 335 and 338. However, they also presented evidence for reactivity of cysteines substituted at 337 and 341 toward $MTSET^{+}$ and $MTSES^{-}$ that was not detected in the present study, but had been reported by Cheung and Akabas (4,5). Because of the disparity between these results and our own, we re-sequenced our F337C CFTR and S341C CFTR constructs and repeated the tests for reactivity toward $MTSET^{+}$ and $MTSES^{-}$. We found no evidence for reactivity toward either reagent in experiments in which each oocyte served as its own control, so that even small changes in conductance would be readily detectable. In contrast, the results reported by Fatehi and Linsdell (15) were based on comparisons between patches of membrane from two different BHK cells so that the only parameter that could be compared was the shape of normalized I-V plots. In such a paradigm changes in the conductance, per se, are undetectable and it is impossible to determine the onset or the time course of any reaction. Furthermore, no attempt was made to reverse the reactions in real time in the same patch, as a confirmation that the expected reaction had actually occurred. Using the same approach, Fatehi and Linsdell (15) also assayed the reactivity of substituted cysteines toward the channel-permeant probe,

$[Au(CN)_2]^-$. Their results confirmed reactivity at 338 reported by Serrano et al. (14), but their assay procedure also indicated reactivity at positions 334, 335 and 341, but not at position 337. Our results confirm the finding of reactivity toward $[Au(CN)_2]^-$ at 334 and 335, but not at 341. We find S341C to be reactive toward $[Ag(CN)_2]^-$ but not $[Au(CN)_2]^-$. In contrast, we showed unambiguous cysteine reactivity toward $[Au(CN)_2]^-$ and $[Ag(CN)_2]^-$ at position 337. These differences may be attributable to the limitations inherent in the assay procedure employed by Fatehi and Linsdell (15) which relied solely on comparisons of the effect of KCN following various pre-treatments so that the reactions could not be followed in real time. Differing expression systems (BHK cells versus oocytes), however, may have also contributed.

**Supplemental References**


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**Supplemental Figures**

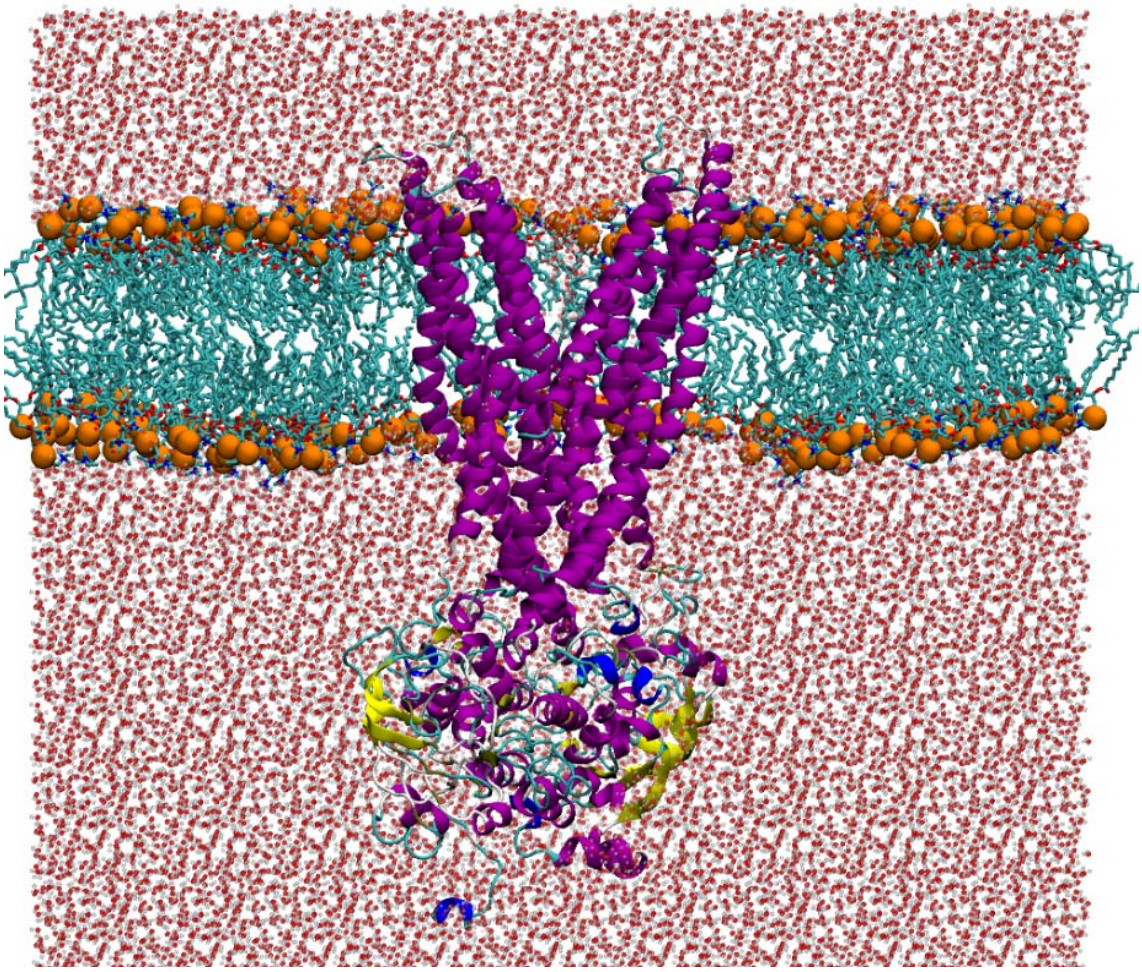

**Figure S1**. Snapshot of membrane protein simulation system. CFTR shown in cartoon representation, colored by secondary structure. DMPC bilayer shown in stick representation, colored by atom type. DMPC headgroup phosphorus atoms depicted as orange spheres. Water atoms shown as small spheres colored by atom type.

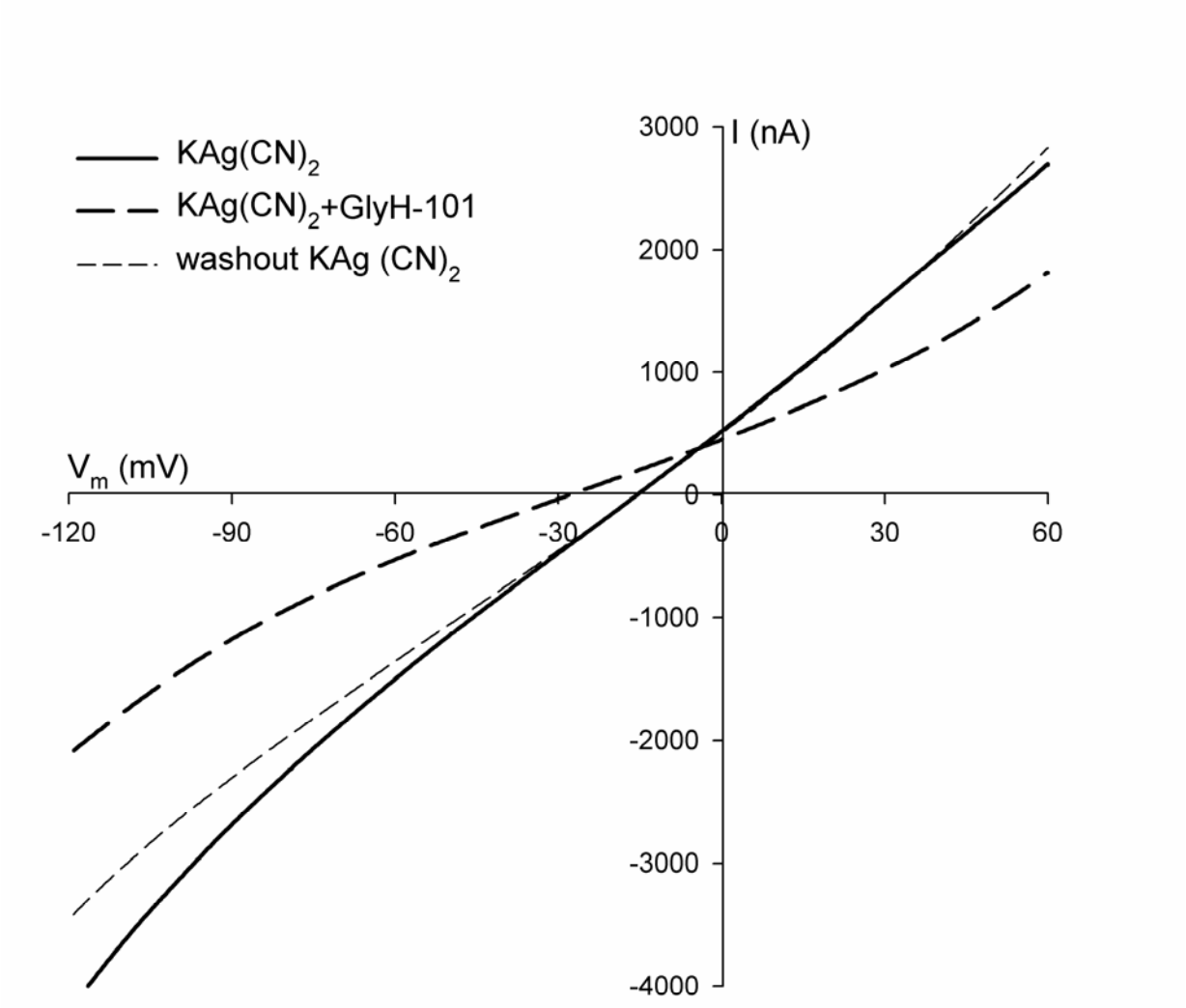


**Figure S2**. I-V curve from a representative oocyte expressing Cys-less CFTR recorded using two-electrode voltage clamp. The current was measured in a modified frog Ringer's solution rendered $Cl^-$ free (70 mM Na-gluconate, 30 mM $KAg(CN)_2$, 1mM Mg-aspartate, 1.8 mM Ba-acetate, 10 mM HEPES, pH 7.4). Inward current carried by $[Ag(CN)_2]^-$ entry into the cell was partially blocked reversibly by addition of 10 mM GlyH-101.

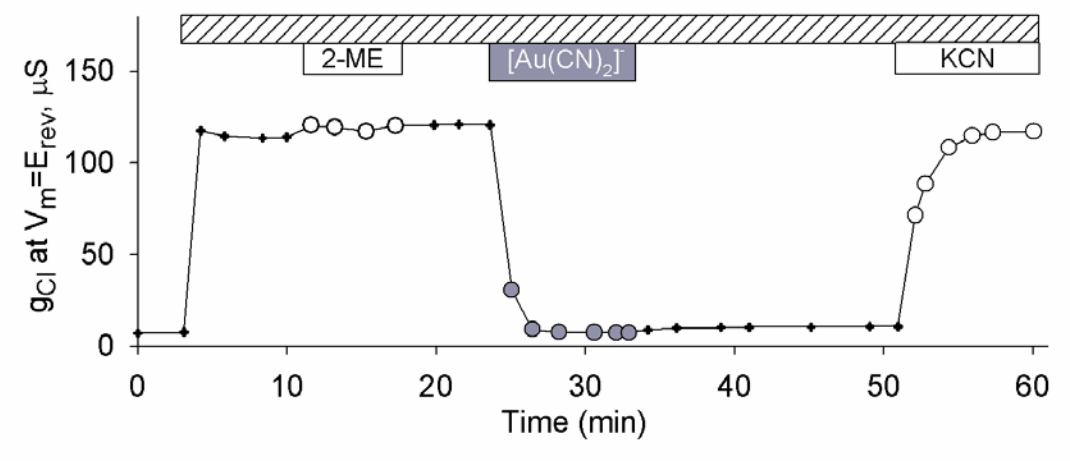


**Figure S3:** Reactivity of F337C/Cys-less CFTR toward $[Au(CN)_2]^-$ (1 mM) and reversal by KCN (1 mM).

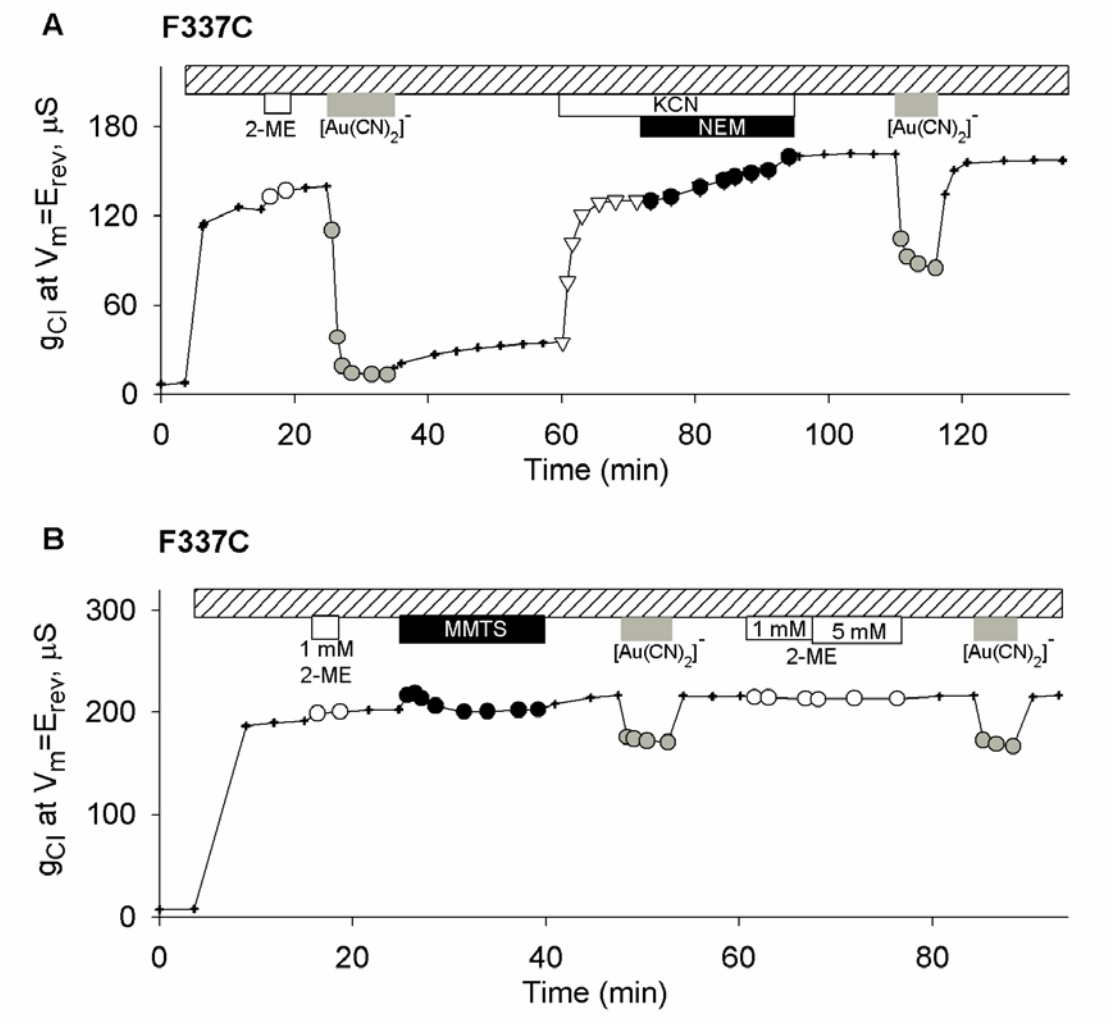


**Figure S4**. (**A**) Following block of F337C CFTR by $[Au(CN)_2]^-$ (1 mM) and reversal of the ligand exchange reaction by KCN (1mM), alkylating the thiol at 337 (NEM, 100 μM) blocked the reaction toward $[Au(CN)_2]^-$ ( 1mM). (**B**) Exposure of F337C CFTR to MMTS (200 μM) blocked the reaction toward $[Au(CN)_2]^-$ and the action of MMTS was not reversed by exposing the oocyte to 2-ME (1 mM, 5 mM).

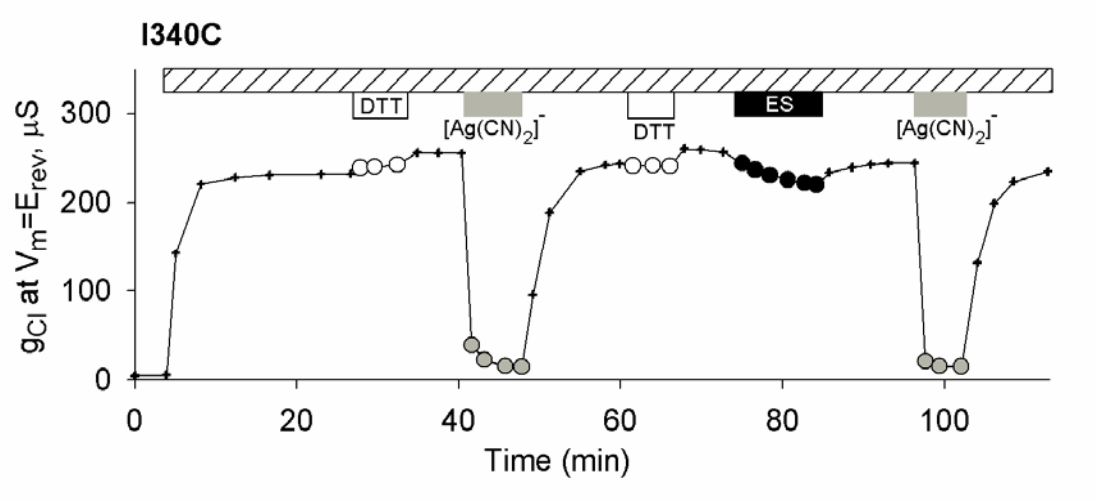


**Figure S5:** Reactivity of I340C CFTR toward $[Ag(CN)_2]^-$ (100 µM) but not $MTSES^-$ (1 mM). Stepwise reversal of $[Ag(CN)_2]^-$ block by KCN revealed the ligand substitution reaction and, like $MTSES^-$, neither IAM nor NEM blocked the reaction (not shown).

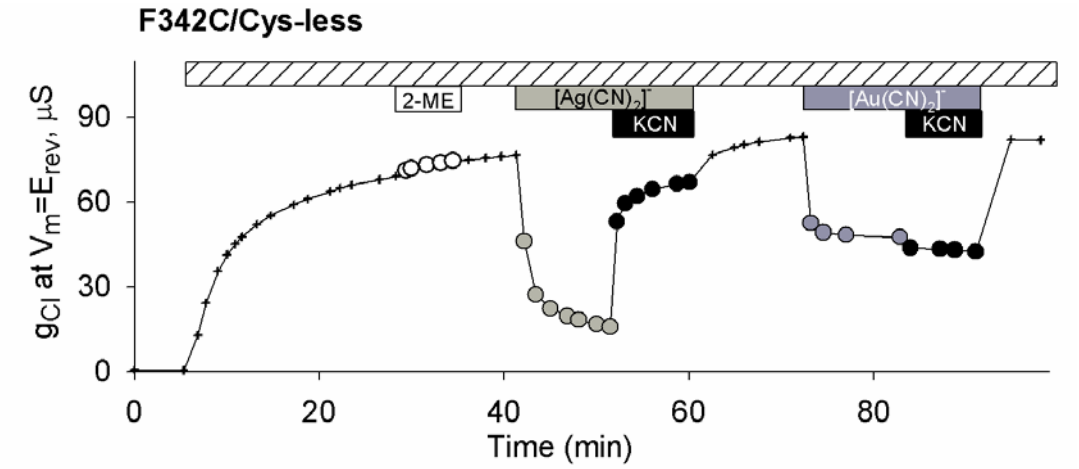


**Figure S6:** F342C/Cys-less CFTR is reactive toward $[Ag(CN)_2]^-$ (1 mM), but not $[Au(CN)_2]^-$ (1 mM). The KCN concentration is 500 µM.

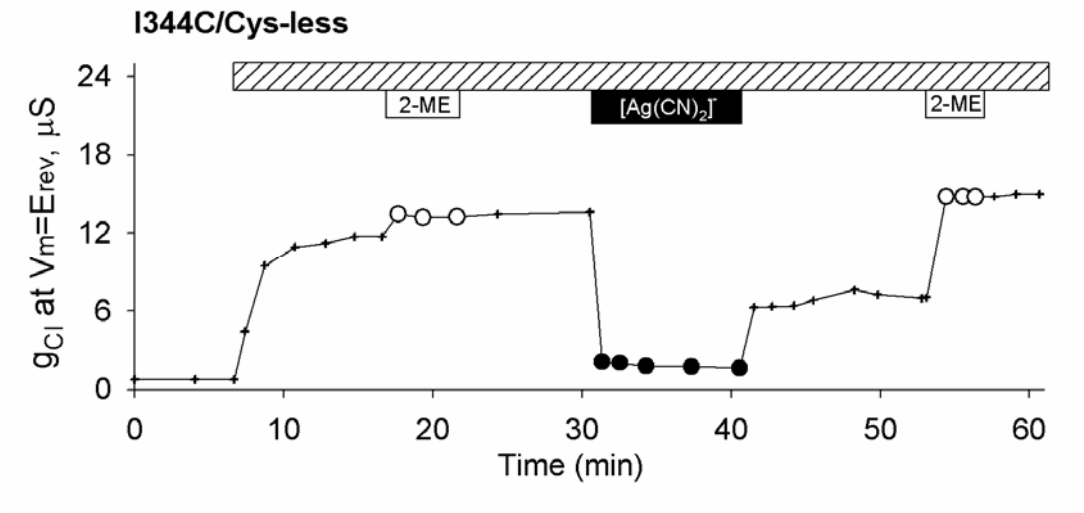


**Figure S7**: I344C/Cys-less CFTR is reactive towards $[Ag(CN)_2]^-$ (1 mM) and the reaction is reversed by 1 mM 2-ME. Note the partial reversibility following washout of $[Ag(CN)_2]^-$.

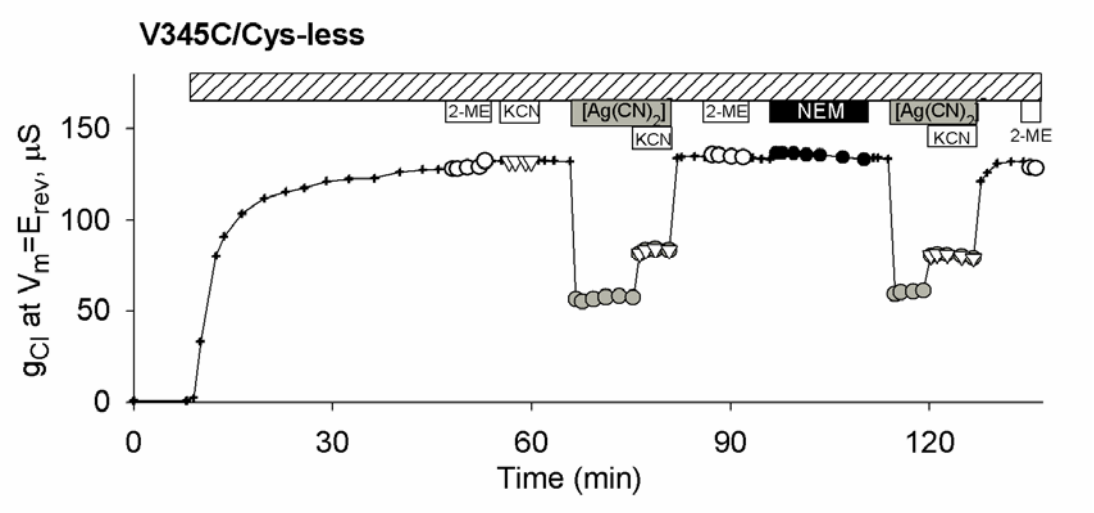


**Figure S8**: V345C/Cys-less CFTR is reactive toward $[Ag(CN)_2]^-$ (1 mM) but not NEM (100 µM). The concentrations of 2-ME and KCN were 1 mM and 100 µM, respectively.

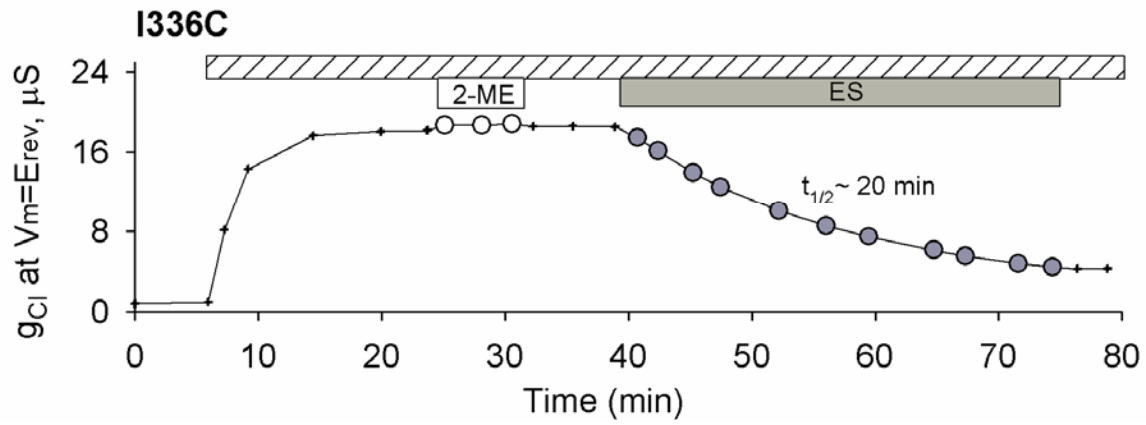


**Figure S9**: I336C CFTR is reactive toward $MTSES^-$, but the rate of reaction was at least ten times slower than a typical reaction seen in other mutants. The concentration of 2-ME and $MTSES^-$ are both 1 mM.