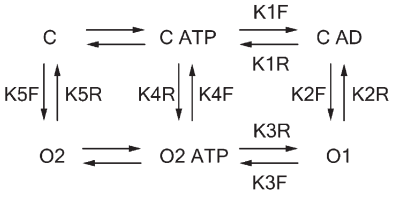
Jih et al., http://www.jgp.org/cgi/content/full/jgp.201210834/DC1

SUPPLEMENTAL DISCUSSION

Semiquantitative analysis of an equilibrium gating scheme for hydrolysis-deficient CFTR channels



(SCHEME S1)

As mentioned in Discussion, once ATP hydrolysis is abolished (e.g., in E1371S), the transitions between the remaining six states in Fig. 5 obey the principle of microscopic reversibility (Scheme S1), which means the product of the forward rates has to be equal to the product of the reverse rates in a closed loop (e.g., K1F · K2F · K3F · K4F = K1R · K2R · K3R · K4R). Thus, the stability of the NBD dimer in the open state would be represented by

$$\frac{K3F}{K3R} = \frac{K1R \bullet K2R \bullet K4R}{K1F \bullet K2F \bullet K4F}. \quad (S1)$$

Some of the values in Eq. S1 can be roughly estimated: As demonstrated in a previous study (Bompadre et al., 2007), G551D-CFTR, a disease-associated mutation, does not respond to ATP. Because the lack of a side chain at position 551 is critical for hydrogen bond formation between the negatively charged phosphate group of ATP and the backbone nitrogen in the signature sequence of an NBD dimer of ABC proteins (Dawson and Locher, 2006; Oldham et al., 2007; Ward et al., 2007), one can safely infer that this complete abolition of ATP responsiveness by the G551D mutation is not caused by a deficit in ATP binding but by an obliteration of NBD dimerization (i.e., C ATP→C AD transition). The fact that ATP does not elicit measurable response in the G551D mutant channel then indicates K5F/K5R ≅ K4F/K4R in Scheme S1. Thus, CFTR is not a classical ligand-gated channel but instead an NBD dimerization-gated channel.

Because the spontaneous openings occur rarely for WT-CFTR (K5R = 0.005 s^{-1}) and the closing rate of the spontaneous opening (K5F) is ∼2 s^{-1} (Bompadre et al., 2005a,b), K5R/K5F = 0.0025 and the value of K4R/K4F must be equally small. In the presence of saturating [ATP], the ATP-induced channel opening (C ATP→C AD→O1) is a much faster process that takes place within hundreds of milliseconds in WT-CFTR (presumably also in E1371S-CFTR). K1F (C ATP→C AD) and K2F (C AD→O1) are required to be faster than ∼1 s^{-1}, whereas the backward rate K1R (C AD→C ATP) should not be >>K2F for actualizing the ATP-induced opening rate within hundreds of milliseconds. On the other hand, channel closing in hydrolysis-deficient mutants after washing out ATP is extremely slow (e.g., a relaxation time constant >50 s for E1371S-CFTR) (Bompadre et al., 2005a,b). This suggests that either K2R or K1R/K2F is very small. To actualize a relaxation time constant of ∼50 s after washing out ATP, considering that K2F should be ∼1 s^{-1} or faster, the value of K2R · (K1R/K2F) could be roughly approximated as ∼0.02 s^{-1} or smaller.

Collectively,

$$\frac{K3F}{K3R} = \frac{K1R \bullet K2R \bullet K4R}{K1F \bullet K2F \bullet K4F} = \left(\frac{K1R \bullet K2R}{K2_F}\right) \bullet \left(\frac{1}{K1F}\right) \bullet \left(\frac{K4R}{K4F}\right) \leq \sim 0.0005.$$

That is, the NBD dimer of an open channel is exceedingly stable without ATP hydrolysis. We hence surmise that for WT-CFTR, ATP hydrolysis provides the energy to disrupt the minimum energy state to enact a speedy gating cycle.

REFERENCES

Bompadre, S.G., T. Ai, J.H. Cho, X. Wang, Y. Sohma, M. Li, and T.C. Hwang. 2005a. CFTR gating I: Characterization of the ATP-dependent gating of a phosphorylation-independent CFTR channel (ΔR-CFTR). *J. Gen. Physiol.* 125:361–375. http://dx.doi.org/10.1085/jgp.200409227

Bompadre, S.G., J.H. Cho, X. Wang, X. Zou, Y. Sohma, M. Li, and T.C. Hwang. 2005b. CFTR gating II: Effects of nucleotide binding on the stability of open states. *J. Gen. Physiol.* 125:377–394. http://dx.doi.org/10.1085/jgp.200409228

Bompadre, S.G., Y. Sohma, M. Li, and T.C. Hwang. 2007. G551D and G1349D, two CF-associated mutations in the signature sequences of CFTR, exhibit distinct gating defects. *J. Gen. Physiol.* 129:285–298. http://dx.doi.org/10.1085/jgp.200609667

Dawson, R.J., and K.P. Locher. 2006. Structure of a bacterial multidrug ABC transporter. *Nature.* 443:180–185. http://dx.doi.org/10.1038/nature05155

Oldham, M.L., D. Khare, F.A. Quiocho, A.L. Davidson, and J. Chen. 2007. Crystal structure of a catalytic intermediate of the maltose transporter. *Nature.* 450:515–521. http://dx.doi.org/10.1038/nature06264

Ward, A., C.L. Reyes, J. Yu, C.B. Roth, and G. Chang. 2007. Flexibility in the ABC transporter MsbA: Alternating access with a twist. *Proc. Natl. Acad. Sci. USA.* 104:19005–19010. http://dx.doi.org/10.1073/pnas.0709388104

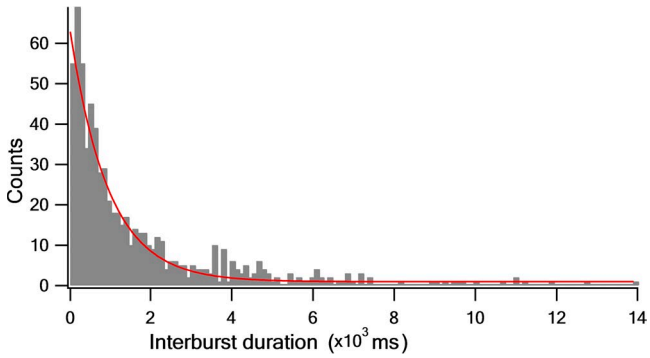


Figure S1. The interburst dwell-time histogram for R352C-CFTR. The histogram was fit with a single-exponential function and yielded a time constant of 944 ms.

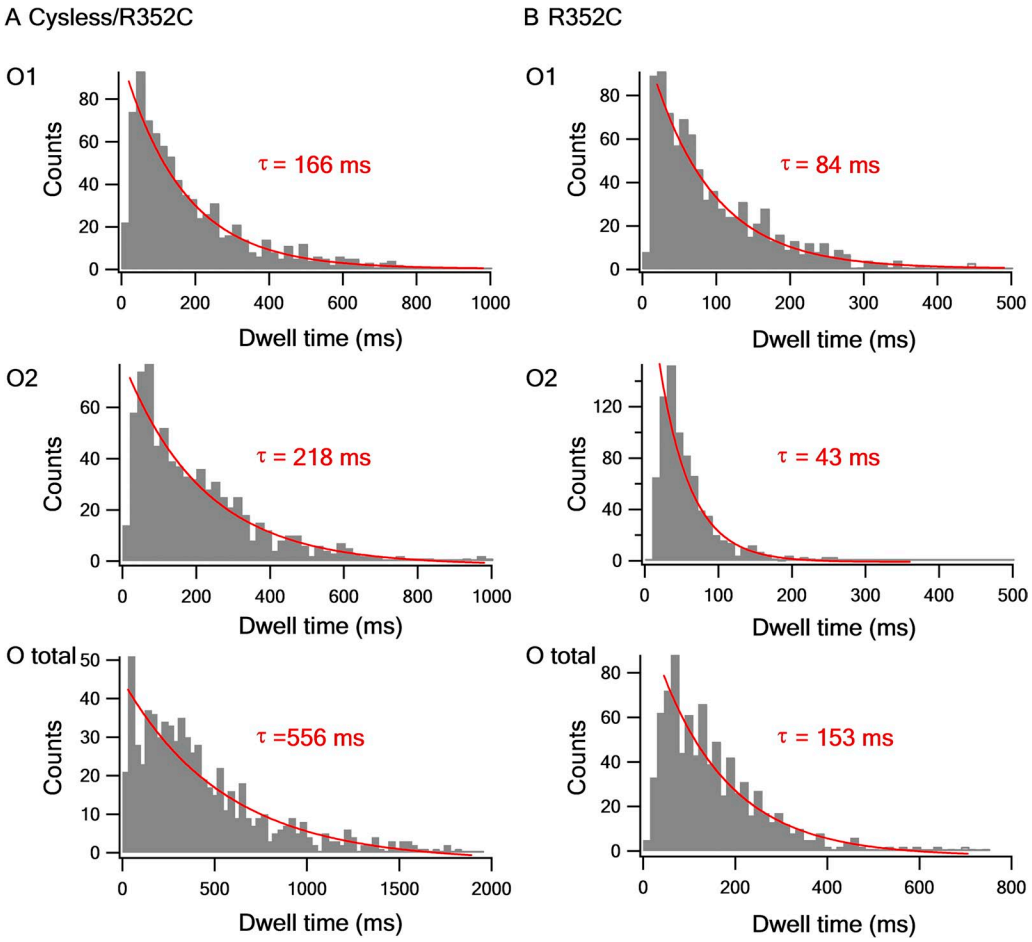


Figure S2. Dwell-time histogram for O1 and O2 states and opening burst in Cysless/R352C- and R352C-CFTR. (A and B) Dwell-time histograms of the O1 and O2 states and the opening burst (O total) for Cysless/R352C-CFTR (A) and R352C-CFTR (B). The histograms were fit with single-exponential functions, and the resulting time constant is marked next to each fitting curve.

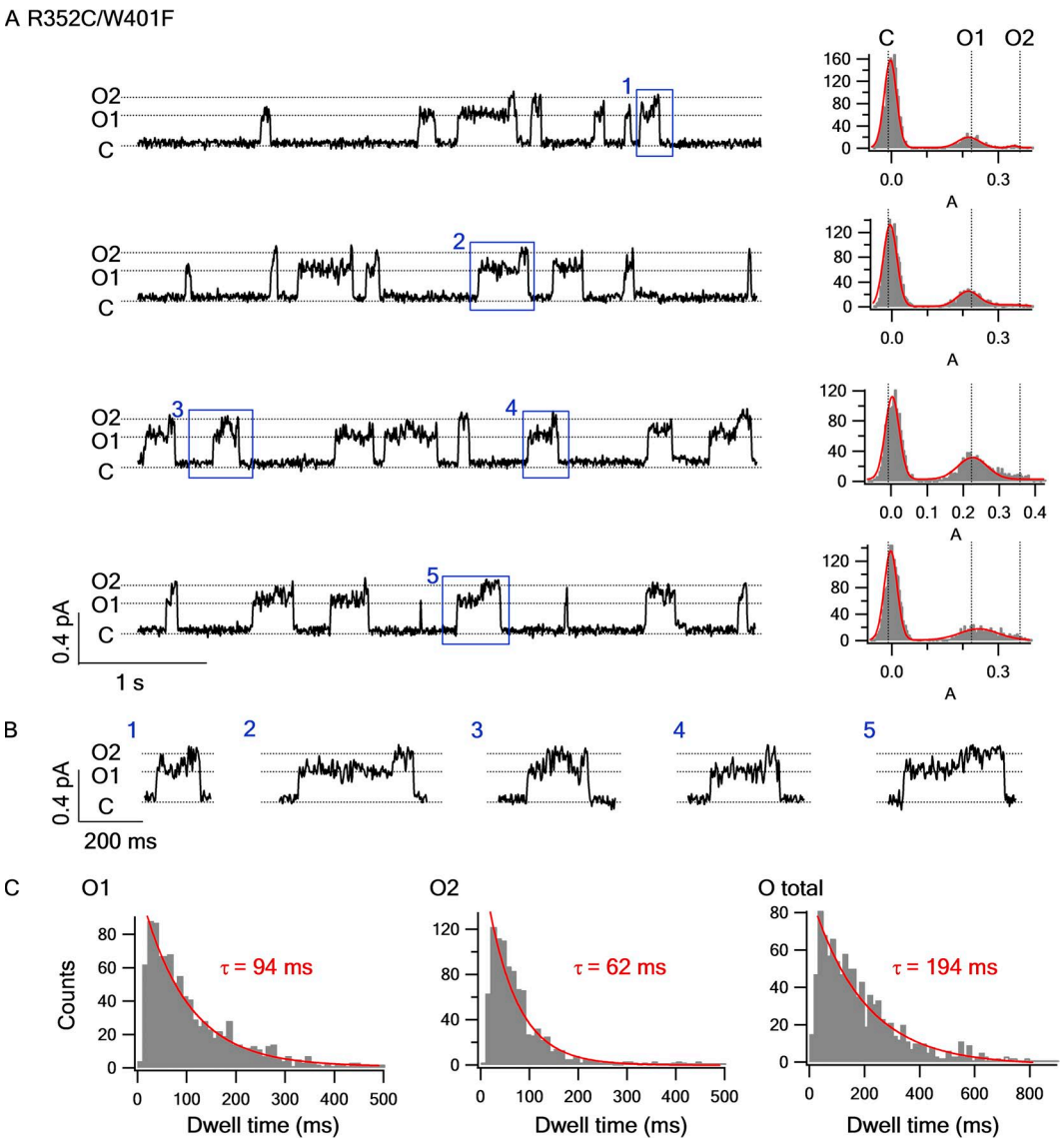


Figure S3. Gating kinetics of W401F/R352C-CFTR. (A) Representative traces and amplitude histograms for W401F/R352C-CFTR in the presence of 2.75 mM ATP. Consistent with the observation for R352C-CFTR, most of the opening bursts follow the C→O1→O2→C gating pattern. However, more reentry events were seen in W401F/R352C-CFTR (Table 1). (B) Sample opening events expanded from the traces in A. (C) Dwell-time histograms of the O1 and O2 states and the opening burst (O total). The histograms were fit with single-exponential functions, and the time constants are marked.