Locating a Plausible Binding Site for an Open Channel Blocker, GlyH-101, in the Pore of the Cystic Fibrosis Transmembrane Conductance Regulator

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SUPPLEMENTAL INFORMATION (Molecular Pharmacology)

Supplemental Figure S1 shows a steric clash between GlyH-101 in the predicted binding pose and an acetamide moiety covalently attached to a cysteine at position 338.

Supplemental Figure S2 illustrates the locations of charged residues in the extracellular vestibule of CFTR.

Supplemental Figure S3 illustrates reaction of S341C CFTR expressed in oocytes with NEM.

Supplemental Figure S4 shows the response of wt CFTR current to voltage jumps in the absence and presence of GlyH-101.

SUPPLEMENTAL FIGURES

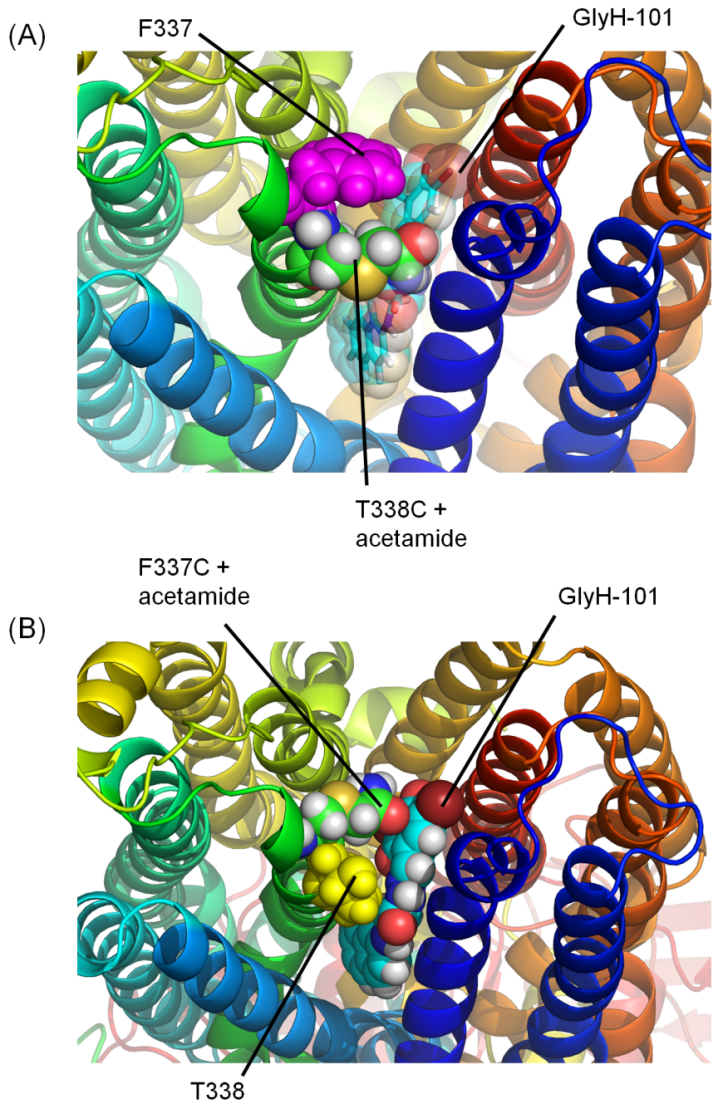

Figure S1. Top view of the CFTR channel from the extracellular side. An acetamide moiety added to T338C CFTR *in silico* is predicted to cause a steric clash with GlyH-101 whereas this is not the case with F337C CFTR. (A) GlyH-101 is shown in the predicted binding pose using a stick and semi-transparent van der Waals representations. F337 = magenta. TM6 = green. TM12 = red. An acetamide moiety was added to a cysteine at position 338. The alkylated side chain is shown in one of the conformations predicted by MacroModel (version 9.8, Schrödinger, LLC, New York, NY, 2010) in the absence of GlyH-101. For the alkylated T338C, carbon = green, oxygen = red, nitrogen = blue, sulfur = yellow, hydrogen = white. (B) GlyH-101 is shown in the predicted binding pose using a van der Waals respresentation. T338 = yellow. An acetamide moiety was added to a cysteine at position 337. The alkylated side chain is shown in one of the conformations predicted by MacroModel (version 9.8) in the absence of GlyH-101. For alkylated F337C CFTR, none of the predicted conformations of the alkylated cysteine resulted in a steric clash with GlyH-101. For the alkylated F337C, carbon = green, oxygen = red, nitrogen = blue, sulfur = yellow, hydrogen = white.

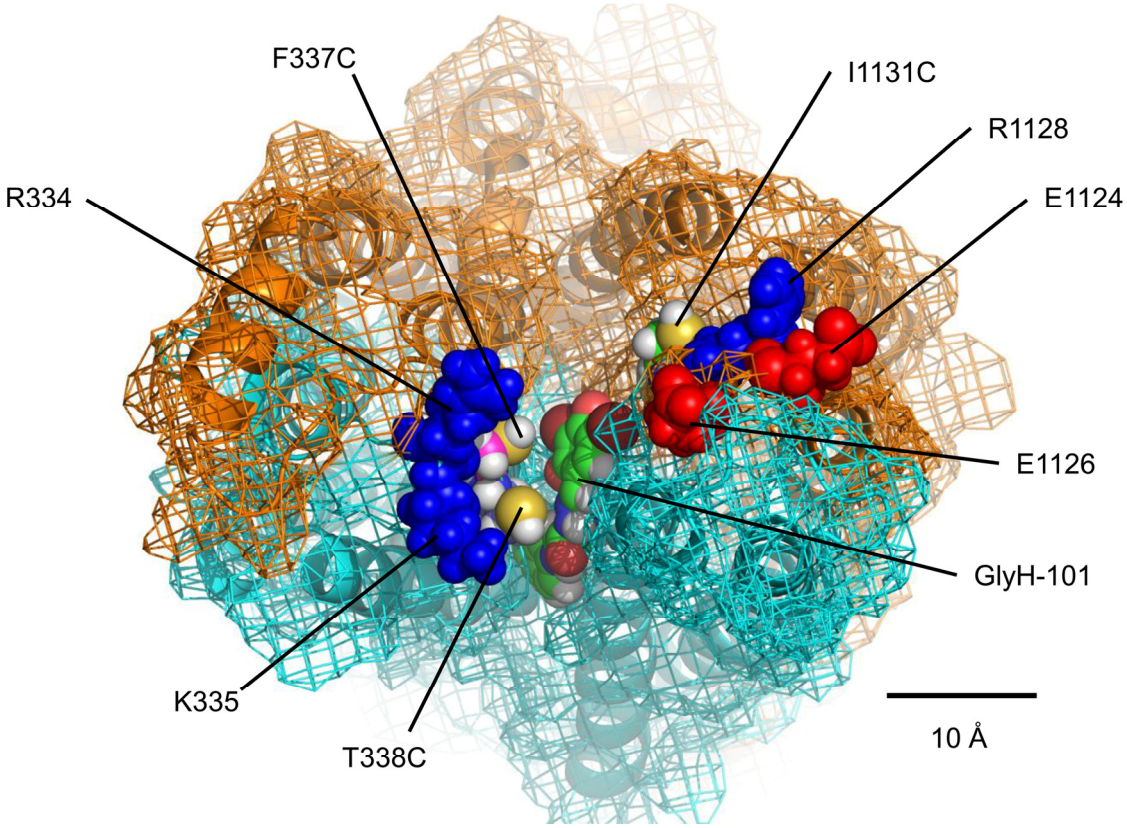

Figure S2. Top view of the CFTR channel from the extracellular side. F337, T338 and I1131 in the predicted binding pose of GlyH-101 (shown in **Figure 2**) were mutated to cysteines *in silico* (Maestro, Schrödinger Inc.). The carbon atoms of the cysteine at 337 are colored pink. Those for 338 and 1131 are blue and green respectively. Charged residues within the Debye length (9.2 Å in the Frog Ringer's solution) of the engineered cysteines are shown in blue (positive) and red (negative). The CFTR protein is represented in cartoon and its molecular surface is indicated by the mesh. Transmembrane domain 1 shown in cyan. Transmembrane domain 2 shown in orange.

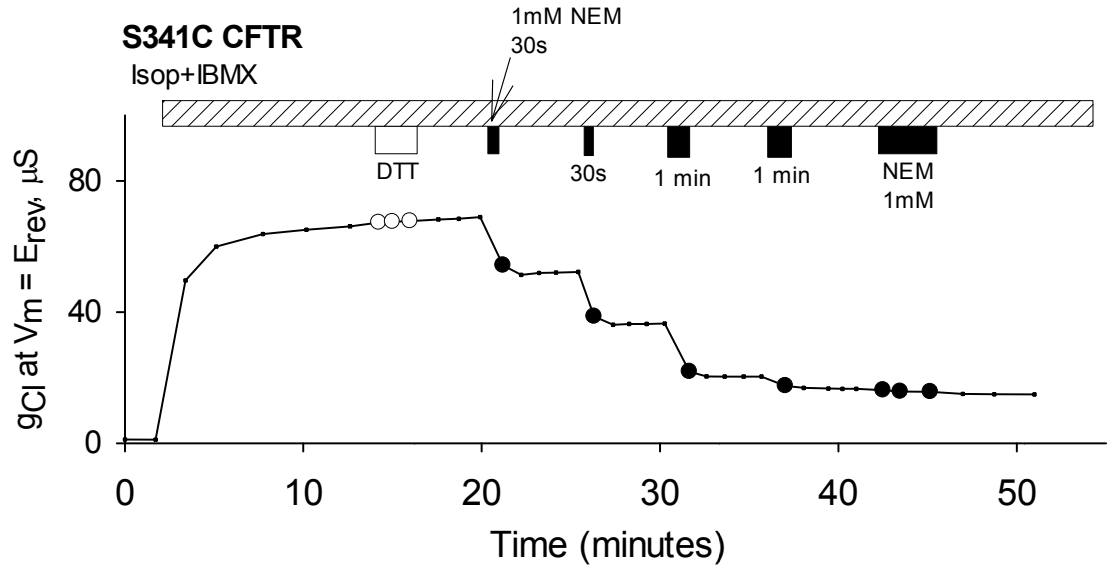

Figure S3. Covalent modification of S341C CFTR with NEM. Exposure of an oocyte to 30 s and 1 min pulses of 1 mM NEM resulted in irreversible reductions of conductance. The covalent labeling of S341C CFTR was almost complete after a cumulative exposure time of 2 min.

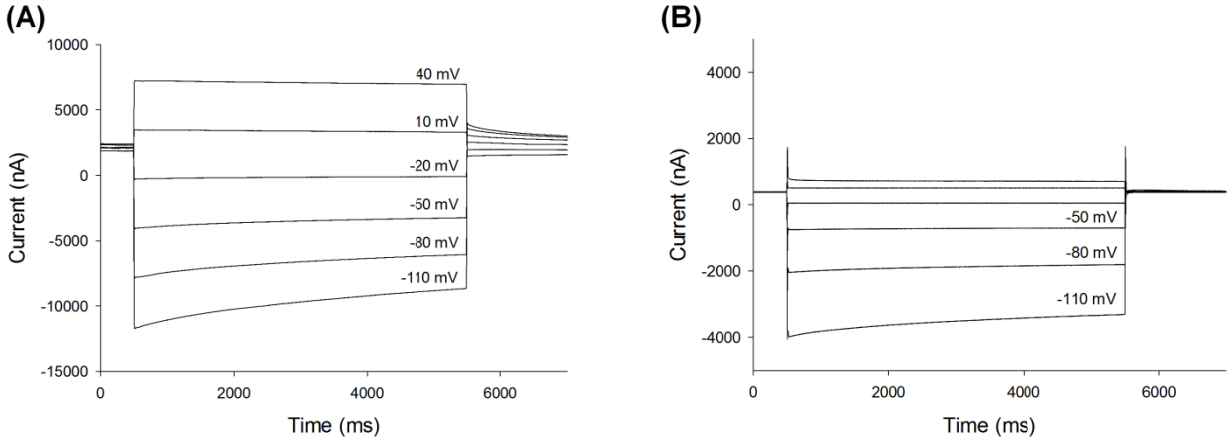

Figure S4. The wt CFTR channels were activated with a stimulating cocktail containing 10 µM isoproterenol and 1 mM IBMX. Six current traces were recorded in the absence (A) and presence (B) of 10 µM GlyH-101 and are shown superimposed. At the beginning of each trace, the voltage was held at 0 mV for 0.5 s. It was then stepped to either -110, -80, -50, -20, +10 or +40 mV for 5 s. After this step, the voltage was again held at 0 mV for 5 s.