SUPPLEMENTARY INFORMATION

Single-molecule detection of nitrogen mustards by covalent reaction within a protein nanopore

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Additional Figures

Figure S1 Single-channel recording of the reaction between melphalan and (T117C-D8RL3)$_1$(WT)$_6$. Single-channel current trace of (T117C-D8RL3)$_1$(WT)$_6$ with melphalan (50 μM) in the *trans* chamber in 20 mM CAPS, pH 10.5, 2 M NaCl, at ±100mV. The reaction point is marked by an arrow and was at 9 min in this case. The mean time to reaction was 7.0 ± 2.0 min (n = 3). Switches in the applied potential were made both before and after the reaction point (*).

Figure S2 Single-channel recording of the reaction between quinacrine mustard and (T117C-D8RL3)$_1$(WT)$_6$. a) Single-channel current trace of (T117C-D8RL3)$_1$(WT)$_6$ with quinacrine mustard (50 μM) in the *trans* chamber in 20 mM CAPS, pH 10.5, 2 M NaCl, at ±100mV. The reaction point is marked by an arrow and was at 10 min in this case. The mean time to reaction was 8.0 ± 2.0 min (n = 3). Switches in potential were made both before and after the reaction point (*). b) Characteristic current trace at +100 mV of the (T117C-D8RL3)$_1$(WT)$_6$ pore after reaction with quinacrine mustard. c) Characteristic current trace at -100 mV of the (T117C-D8RL3)$_1$(WT)$_6$ pore after reaction with quinacrine mustard.

Figure S3 Binding events of βCD-S-M to (M113F)$_7$. βCD-S-M is the product of the reaction of mechlorethamine with βCD-SH (or possibly the hemimustard derivative). a) Single-channel recording showing the binding of βCD-S-M to (M113F)$_7$. Level 1 represents the open pore current level. Level 2 represents the current level when βCD-SH is bound. Level 3 represents the current level when βCD-S-M is bound. b)

Expanded view of the current trace showing βCD-S-M binding to $(M113F)_7$ (*). c) Dissociation of βCD-S-M from $(M113F)_7$ (*).

Figure S4 Single-channel recording of the reaction between *N*-benzyl-*N,N*-di(2-chloroethyl)amine and $(T117C\text{-}D8RL3)_1(WT)_6$ and subsequent chain elongation arising from alternating reactions with glutathione (100 μM, *cis*) and *N*-benzyl-*N,N*-di(2-chloroethyl)amine (50 μM, *trans*). The reaction points are marked by arrows and numbered in order. M and G indicate mustard and glutathione reactions respectively. Switches in potential were made during the recording to show that the reaction steps could proceed at both positive and negative potentials (marked *).

Figure S1

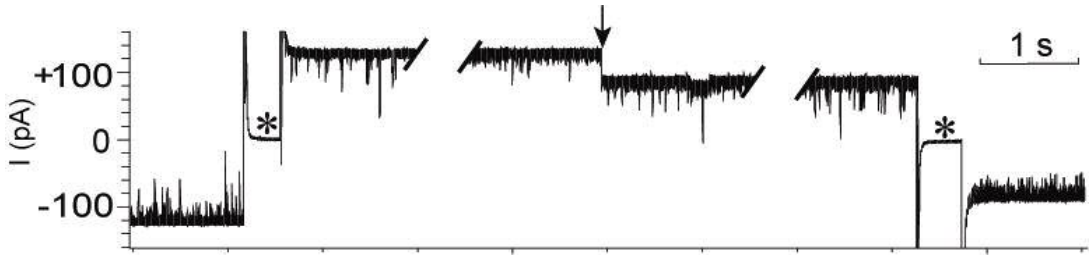

Figure S2

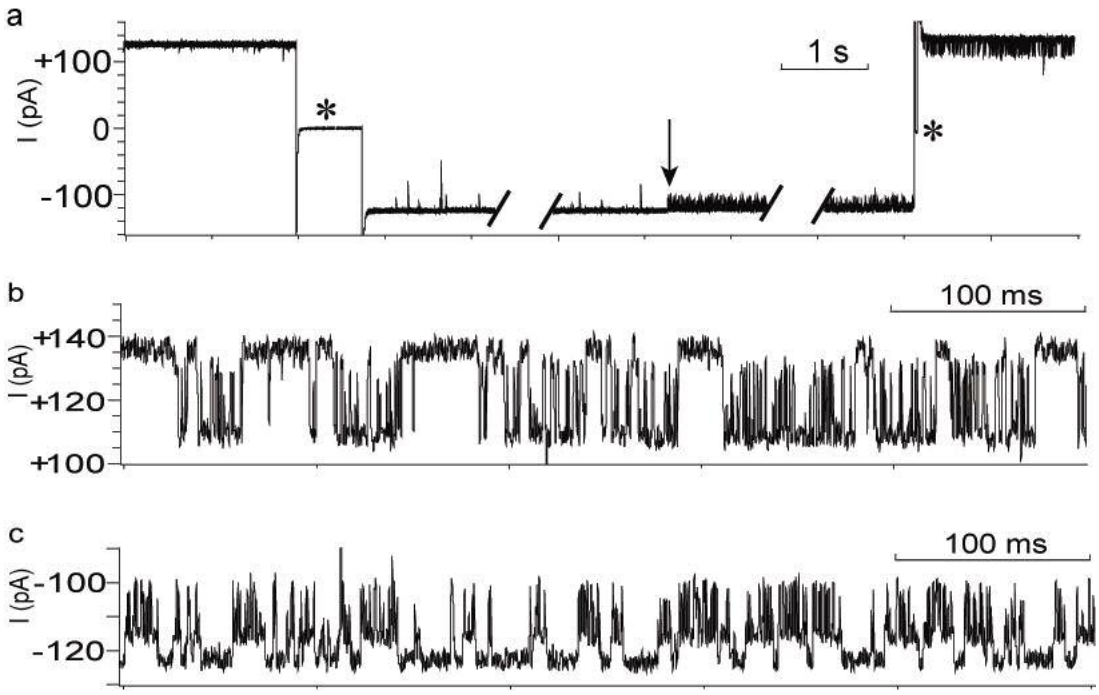

Figure S3

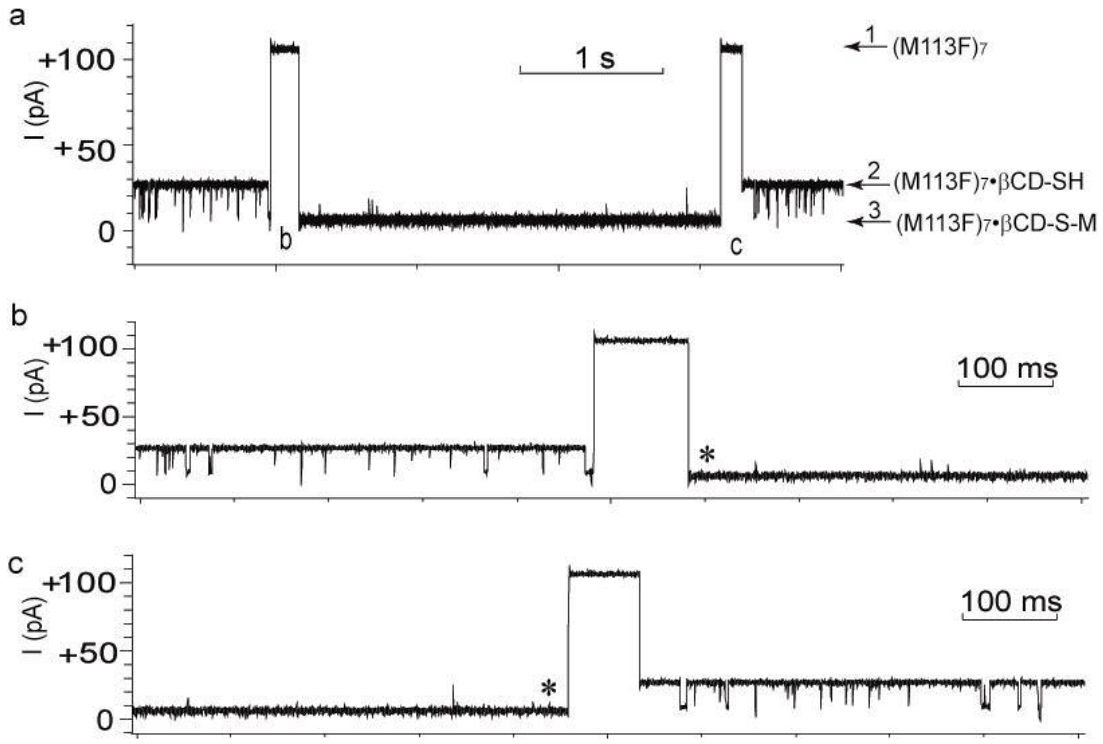

Figure S4