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| --- | --- | --- | --- | --- | --- | --- |
| **Simulation** | **Force Field** | **Voltage (mV)** | **Solution (mM)** | **Mutation** | **Restraint (kcal/mol)\*** | **Time scale (μs)** |
| **1** | AMBER | +200 | 150 mM KCl | W362F, S367T,V377T | 0 | 10.0 |
| **2** | AMBER\* | +200 | 150 mM KCl | W362F, S367T,V377T | 1 | 10.0 |
| **3** | AMBER | +200 | 150 mM KCl | W362F, S367T,V377T,I398N | 0 | 10.0 |
| **4** | CHARMM36m | +200 | 150 mM KCl | W362F, S367T,V377T | 0 | 10.0 |
| **5** | CHARMM36m | +200 | 150 mM KCl | W362F, S367T,V377T,I398N | 0 | 10.0 |
| **6** | CHARMM36m-NBFIX | +200 | 150 mM KCl | W362F, S367T,V377T | 0 | 10.0 |
| **7** | AMBER | +200 | 150 mM NaCl | W362F, S367T,V377T | 0 | 10.0 |
| **8** | CHARMM36m | +200 | 150 mM NaCl | W362F, S367T,V377T | 0 | 10.0 |

\*Harmonic restraints were applied to Cβ atoms of I398 to keep the isoleucine gate in the open state