Table S1. Statistics related to obtaining the "best rotamer" in mutant modeling

Mutant	Number of Available Rotamers	Clash Score[a]
K68N	5	**-1 (0.36)**[b], -1 (0.08), 0 (0.03), 48 (0.00), 53 (0.38)
N123A	1	**0 (1.00)**
A111N	9	**-1 (0.33)**, 0 (0.10), 4 (0.12), 4 (0.00), 7 (0.08), 8 (0.04), 30 (0.00), 39 (0.12), 48 (0.15)

[a]: A simple clash score is used to evaluate the rotamers when browsing through rotamer libraries. The "best rotamer" (shown in bold) is the one that totalizes the lowest score according to the following formula (Guex, N. and Peitsch, M.C. (1997) SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis*, **18**, 2714-2723.):

Clash Score = (4 × number of clashes with backbone N, CA, and C atoms) + (3 × number of clashes with backbone O atoms) + (2 × number of clashes with sidechains atoms) − number of H bonds − (4 × number of SS bonds)

[b]: Probability to be found in this conformation is shown in parenthesis.

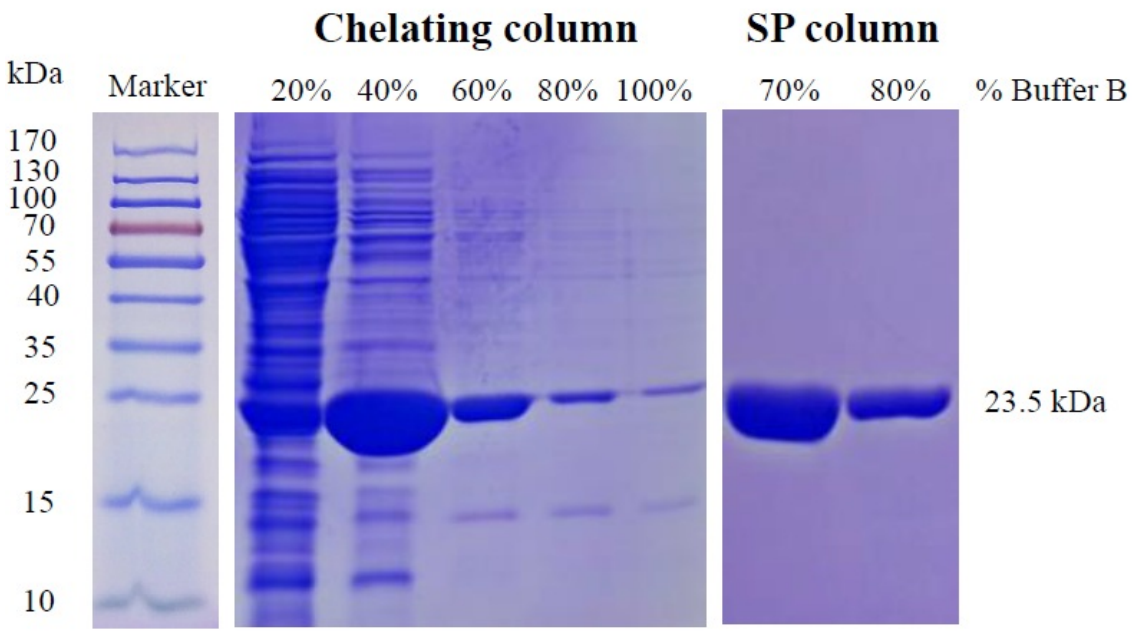

Figure S1. 12% SDS-PAGE analysis of *E. coli* MUG-K68N protein. The cell pellet from a 500-ml culture grown to late exponential phase was suspended in 7 ml of sonication buffer and sonicated at output 5 for 3 x 1 min with 1 min rest on ice between intervals using a Q125 sonicator (Qsonica). The lysate was clarified by centrifuging at 12,000 rpm at 4°C for 20 min. The supernatant was transferred into a fresh tube and loaded into a 1 ml HiTrap chelating column. The bound protein in the column was washed with chelating buffer A [20 mM sodium phosphate (pH 7.4) and 500 mM NaCl] and eluted with a linear gradient of 0-100% chelating buffer B [20 mM sodium phosphate (pH 7.4), 500 mM NaCl and 500 mM imidazole]. The partially purified MUG-K68N protein was loaded onto HiTrap SP column equilibrated with HiTrap SP column buffer A [20 mM HEPES (pH 8.0), 1 mM EDTA and 0.1 mM DTT] and eluted with HiTrap SP column buffer B [20 mM HEPES (pH 8.0), 1 mM EDTA, 0.1 mM DTT and 1 M NaCl]. The purity of the protein is at least 95% after passing the SP column.

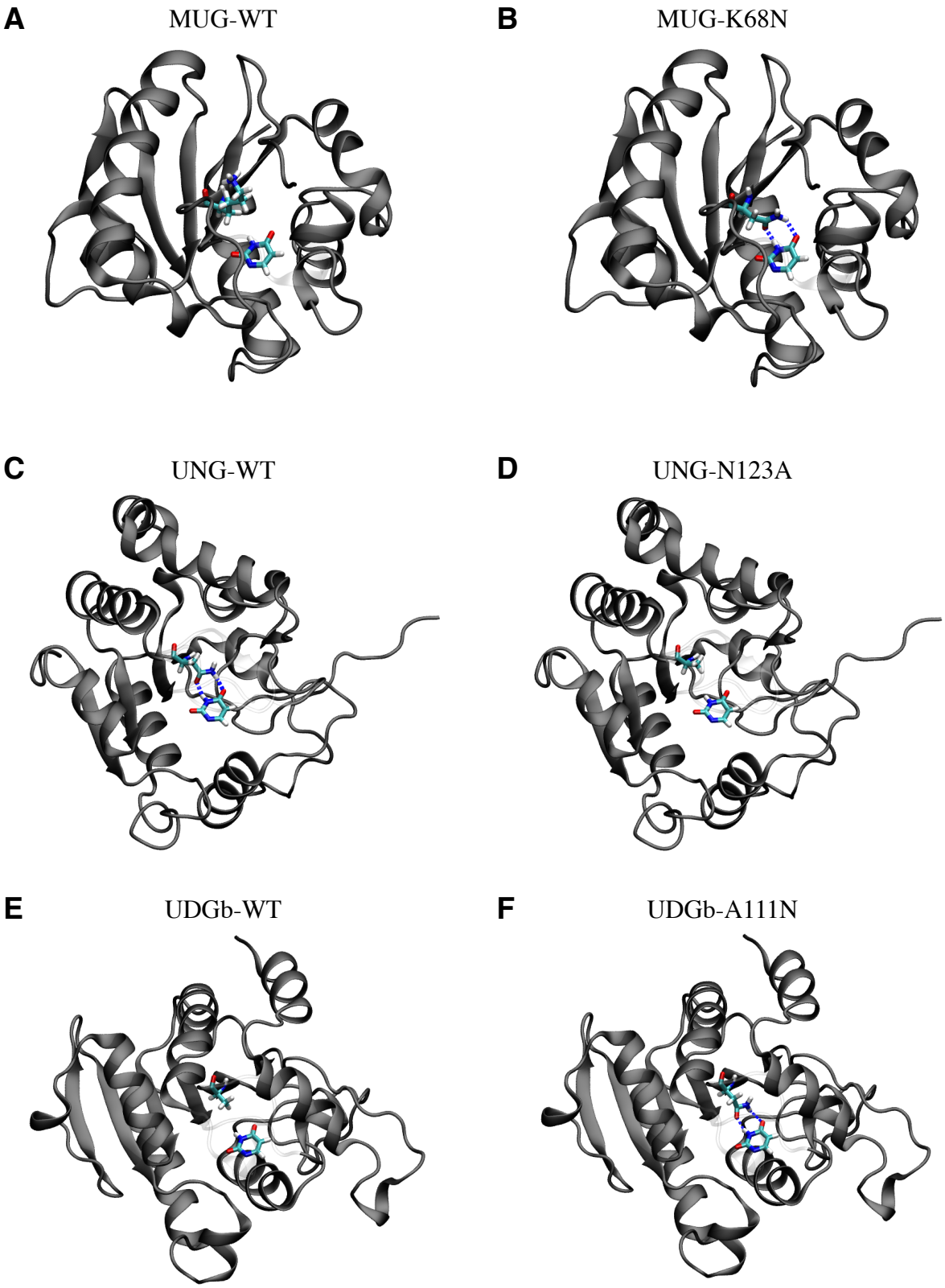

Figure S2. Zoom-out view of MUG, UNG and UNGb structures as shown in Figure 6. The segments that were removed for clarity in Figure 6 are shown in light gray. See legend for Figure 6 for other details.