

Supplementary files for: **Outline of an experimental design aimed to detect protein A mirror image in solution**

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Table S1. pKa constants for the initial native-like and mirror-image structures of Q10H protein.

| Residue | Native-like | Mirror-image |
|--------------|-------------|--------------|
| NEND | 8.2 | 7.9 |
| ASP3 | 3.6 | 2.9 |
| LYS5 | 10.8 | 10.9 |
| LYS8 | 10.7 | 12.4 |
| GLU9 | 2.6 | 3.6 |
| HIS10 | 5.8 | 7.3 |
| TYR15 | 9.5 | 9.5 |
| GLU16 | 4.2 | 3.7 |
| HIS19 | 7.1 | 7.0 |
| GLU25 | 3.6 | 3.4 |
| GLU26 | 3.8 | 3.8 |
| LYS36 | 11.1 | 10.9 |
| ASP37 | 3.5 | 3.7 |
| ASP38 | 3.1 | 3.3 |
| GLU48 | 3.4 | 3.2 |
| LYS50 | 11.8 | 10.9 |
| LYS51 | 11.6 | 11.3 |
| ASP54 | 2.6 | 3.1 |
| LYS59 | 9.9 | 9.9 |
| CEND | 3.9 | 3.8 |

Table S2. Low energies ionization states of native-like and mirror-image structures of Q10H protein.

| protein | Ionization state ^a | E _{pot} ^b | RMSD ^d |
|--------------|-------------------------------|-------------------------------|-------------------|
| native | 22 | 88.6 | 24.3 |
| | 20 | 100.1 | 25.6 |
| | 21 | 104.7 | 29.3 |
| Mirror-image | 22 | 183.8 | 24.5 |
| | 20 | 184.0 | 24.5 |
| | 02 | 188.7 | 24.1 |
| | 00 | 189.0 | 23.1 |

^a Ionization state of histidine residues H10 and H19, where 0,1,2 are integers designating the H⁺, the HID and the HIE forms, respectively. Thus, for example, the ionization state 20 means that H10 and H19 are in the HIE and H⁺ forms, respectively.

^b Average potential energy along the pH-constant 25ns MD trajectory, kcal/mole ;

^c RMSD energy-fluctuation, at 300K, along the pH-constant 25ns MD trajectory, kcal/mole.

Table S3. Average pKa constant and fractions of the His10 forms on the native-like and mirror-image conformations along the 25ns constant-pH MD simulation at pH 7.0.

| Protein | $\langle E_{\text{pot}} \rangle^{\text{d}}$ RMSD ^e | $\langle \text{pK} \rangle^{\text{g}}$ | His10 ^f | | | $\langle \text{pK} \rangle^{\text{g}}$ | His19 ^f | | |
|-----------------------------|--|--|-----------------------------|------------------|------------------|--|-----------------------------|------------------|------------------|
| | | | H ⁺ ^h | HID ⁱ | HIE ^j | | H ⁺ ^h | HID ⁱ | HIE ^j |
| Mirror-initial ^a | n/a | 7.6 | 0.76 | 0.08 | 0.16 | 7.1 | 0.58 | 0.14 | 0.37 |
| Mirror-image ^b | 183.9 (24.2) | 7.30 (0.21) | 0.65 (0.08) | 0.07 (0.02) | 0.28 (0.07) | 7.00 (0.21) | 0.49 (0.03) | 0.10 (0.01) | 0.41 (0.03) |
| Native-like ^c | 88.6 (24.3) | 6.20 (0.27) | 0.15 (0.06) | 0.09 (0.02) | 0.76 (0.08) | 7.03 (0.19) | 0.51 (0.06) | 0.08 (0.01) | 0.41 (0.06) |

^a Initial mirror-image structure;

^b MD optimized mirror-image structure;

^c MD optimized native-like structure;

^d Average potential energy, kcal/mole;;

^e RMSD due to energy fluctuations (in parenthesis), kcal/mole;;

^f Histidine residue number;

^g Average pK and, in parenthesis, its RMSD due to fluctuations;

^h Average fraction of ionization states and, in parenthesis, its RMSD due to fluctuations;

ⁱ Average fraction of HID tautomer and, in parenthesis, its RMSD due to fluctuations;

^j Average fraction of HIE tautomer and, in parenthesis, its RMSD due to fluctuations;

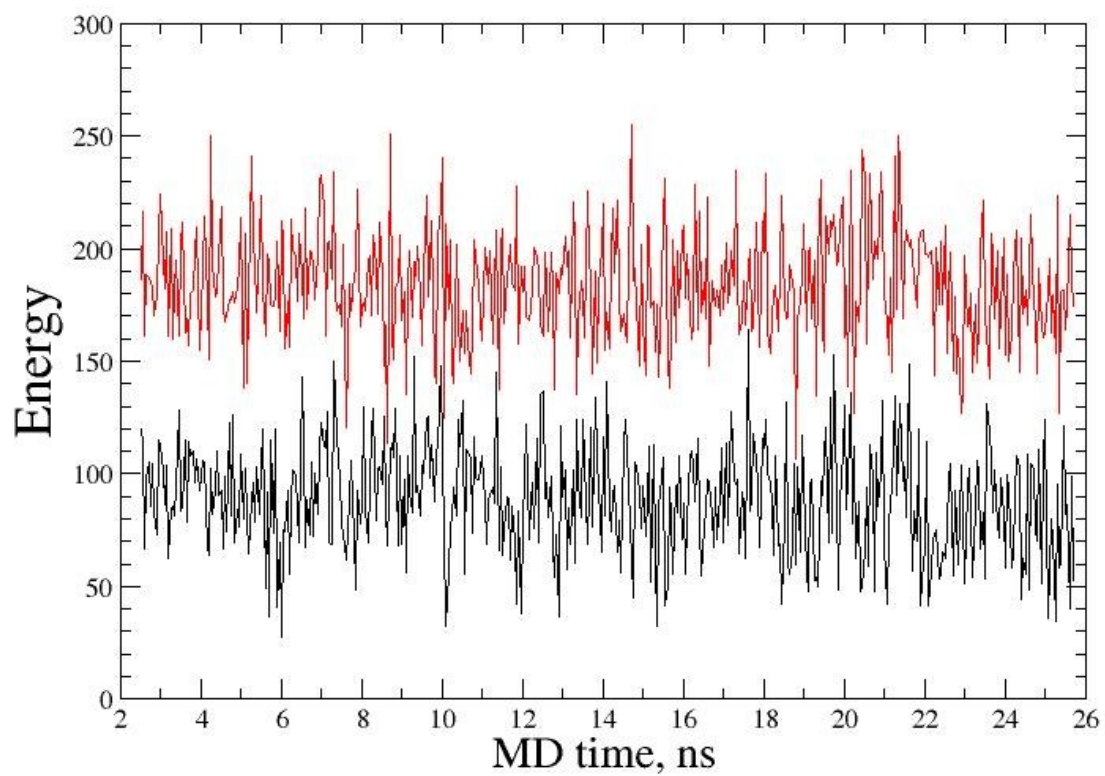


Figure S1. Potential energies for *native-like* and *mirror-image* structures of mutant Q10H of protein A (PDB ID 1BDD) along MD trajectories of 25ns; black – native-like; red – mirror-image.

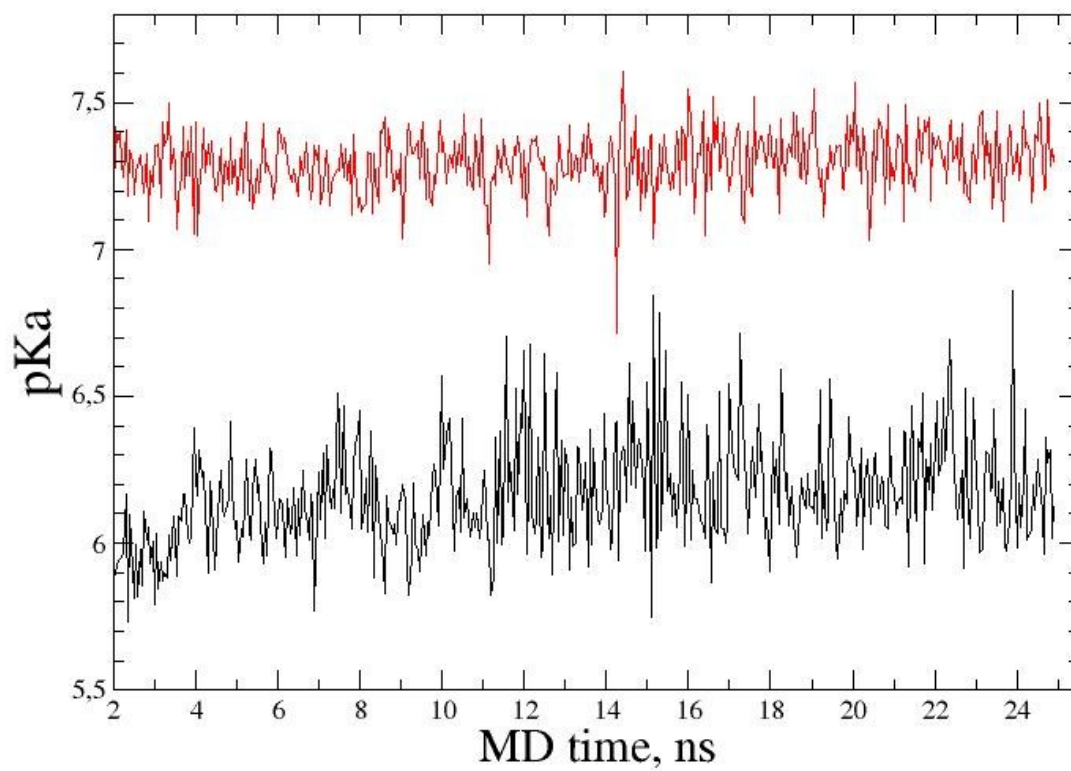


Figure S2. Ionization constant pKa of *native-like* and *mirror-image* structures of mutant Q10H of protein A (PDB ID 1BDD) along MD trajectories of 25ns; black – native; red – mirror-image.

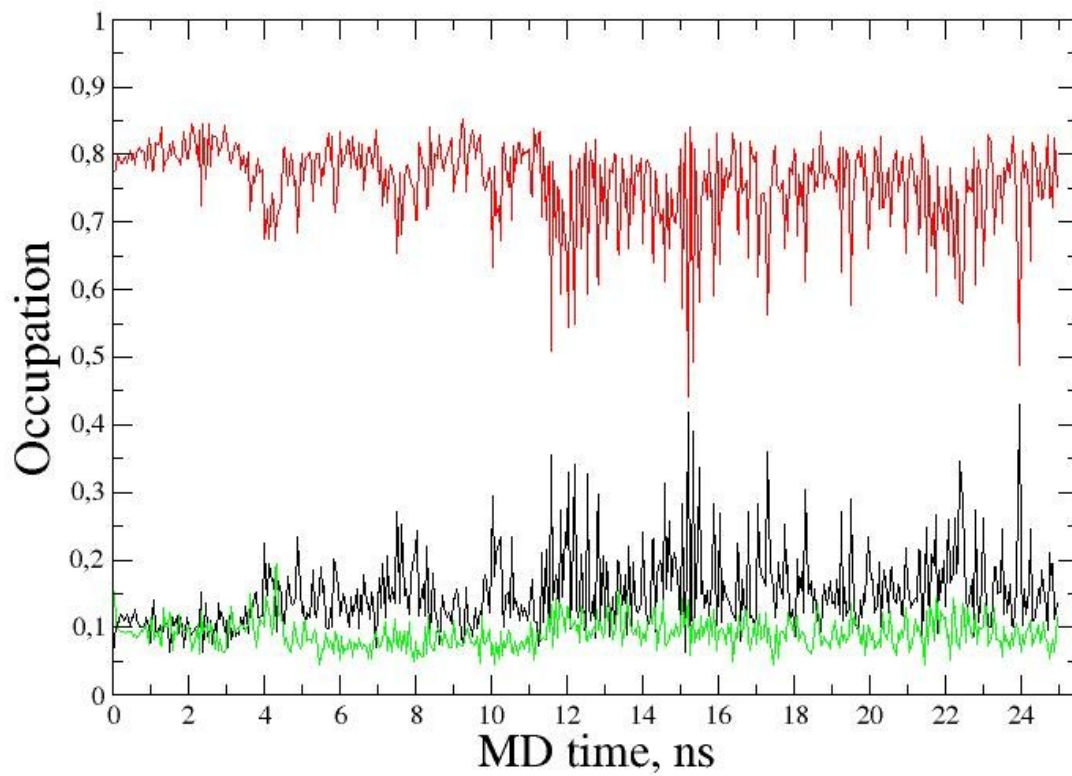


Figure S3a. Occupation of ionization states of histidine His10 for *native-like* structure of mutant Q10H; black – ionized state; red – HIE tautomer; green – HID tautomer.

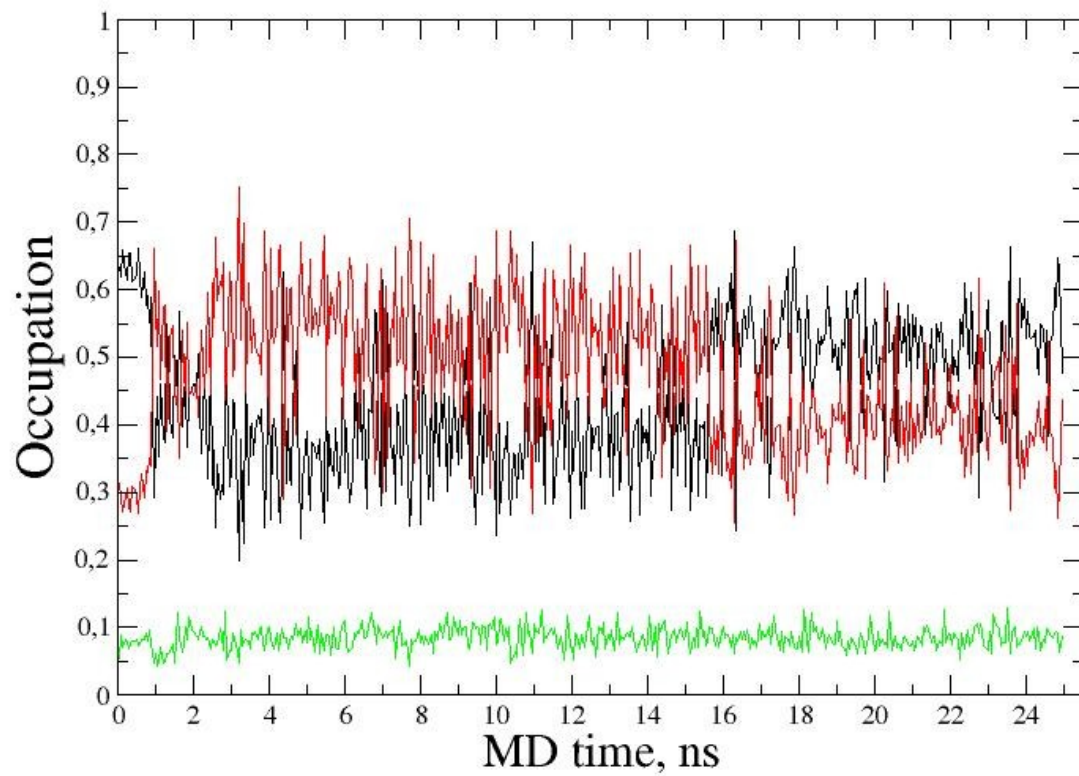


Figure S3b. Occupation of ionization states of histidine His10 for *mirror-image* structure of mutant Q10H; black – ionized state; red – HIE tautomer; green – HID tautomer.