Supplementary Information

Dynamical Characterization and Multiple Unbinding Paths of Two PreQ1 Ligands in One Pocket

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Supplemental Figures

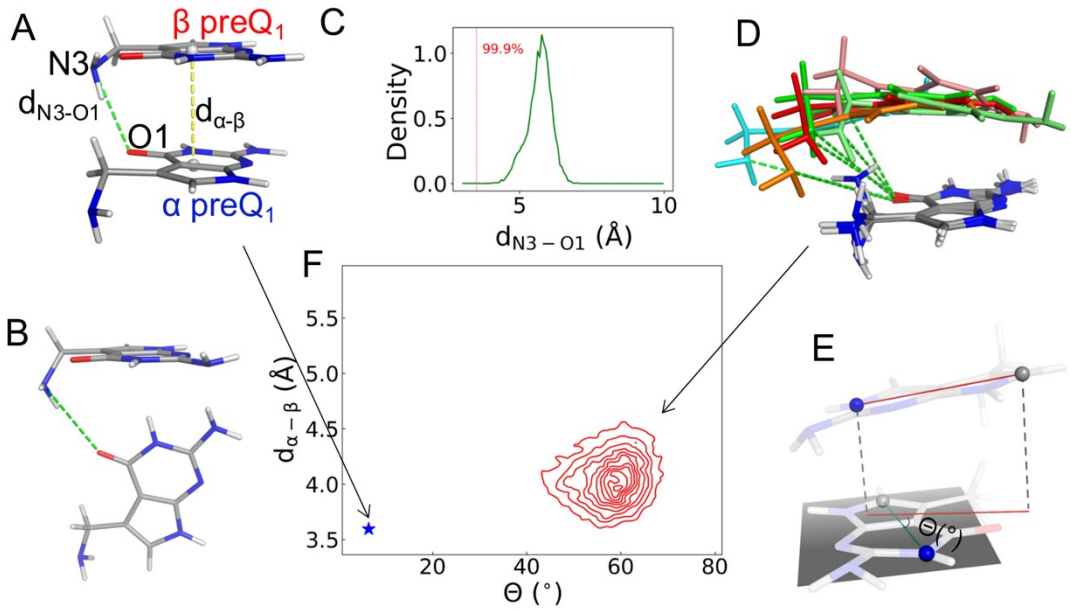

Fig. S1. Various conformations of two PreQ$_1$ in cMD simulations. (A) α and β sites preQ$_1$ ligands in the crystallographic structure, the distance ($d_{\alpha\text{-}\beta}$) between two purine centers is shown in a yellow dashed line. The hydrogen bond between N3 and O1 is shown in a green dashed line. (B) α and β sites preQ$_1$ in the optimized structure. (C) The Probability density of $d_{N3\text{-}O1}$ in cMD simulations. A dashed vertical line is drawn at 3.5Å, which separates the formed hydrogen bond distances from the rest. (D) Six representative structures from MD simulations are superimposed and aligned by α site preQ$_1$. (E) Illustration of an angle (Θ). A rectangle is drawn around the purine atoms of α site preQ$_1$, with a minimum of 1.0 Å separation. A line that passes through atoms C7 and N1 of β site preQ$_1$ and its projection on the plane are shown in red lines. The line that passes through atoms C7 and N1 of α site preQ$_1$ is shown in the green line. The intersection angle of two lines in the plane is labeled as Θ. (F) Density map in the space of two collective variables: $d_{\alpha\text{-}\beta}$ and Θ. Two collective variables of 7REX are indicated by a blue star.

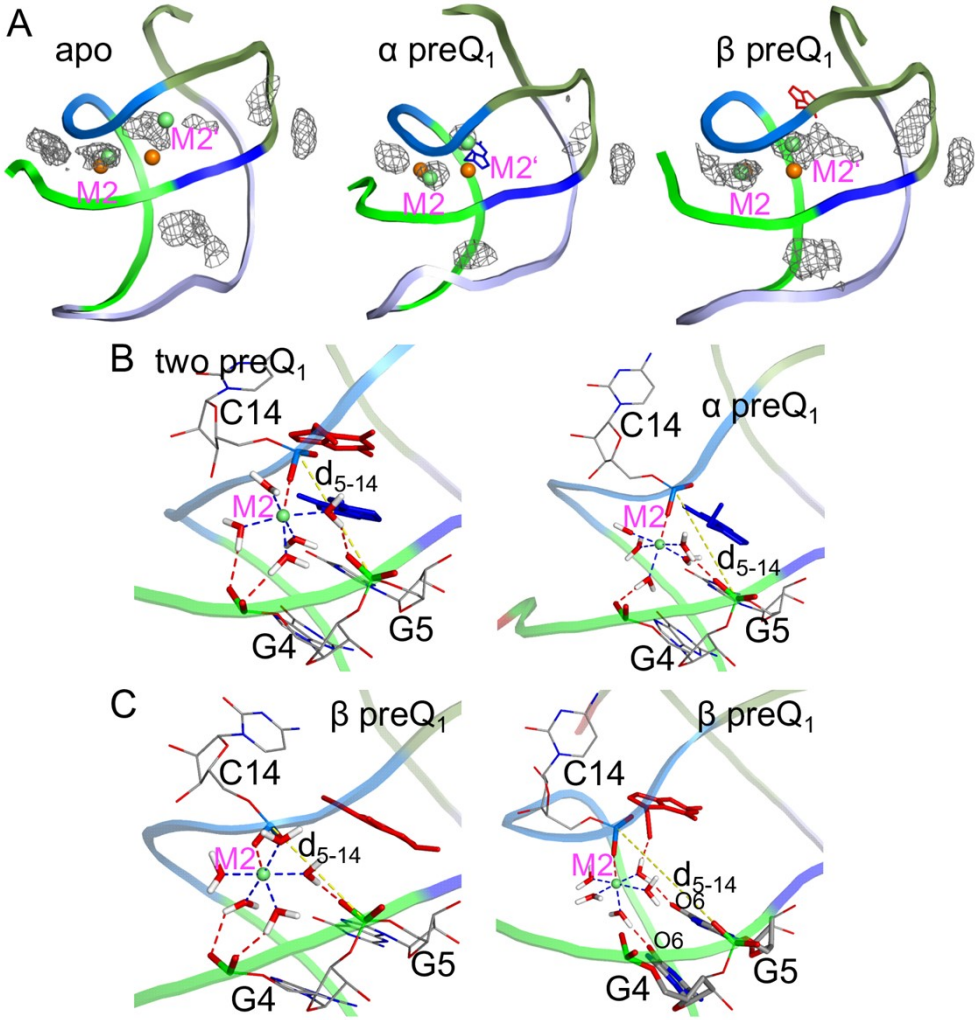

Fig. S2. Distributions of Mg^{2+} ions and effects of M2. (A) Density contours of Mg^{2+} ions in apo form, α $preQ_1$, and β $preQ_1$ bound complex, shown as grey mesh. Mg^{2+} ions located in the major groove from a representative structure of MD simulation are shown as green spheres and labeled as as M2 and M2'. (B) The coordination of the Mg^{2+} ion at the M2 site. The hydrogen bonds between the water molecules and the phosphate groups of G3, G4, and A14 are shown in red dashed lines. The $d_{5\text{-}14}$ is shown in yellow dashed line. (C) The indirect interaction between M2 and the nucleobases of G4 and G5.

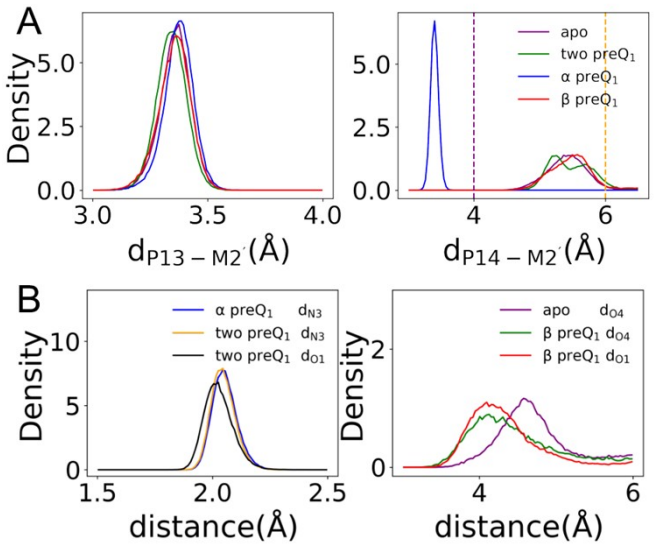

Fig. S3. Effects of M2' on the ligand binding. (A) Probability densities of $d_{P13\text{-}M3}$ and $d_{P14\text{-}M3}$ in cMD simulations. Two vertical dashed lines are drawn at 3.5 and 6.0Å colored in purple and yellow, respectively, which separates the direct interaction, indirect interaction, and none of interaction in the right panel. (B) Probability densities of distances in cMD simulations.

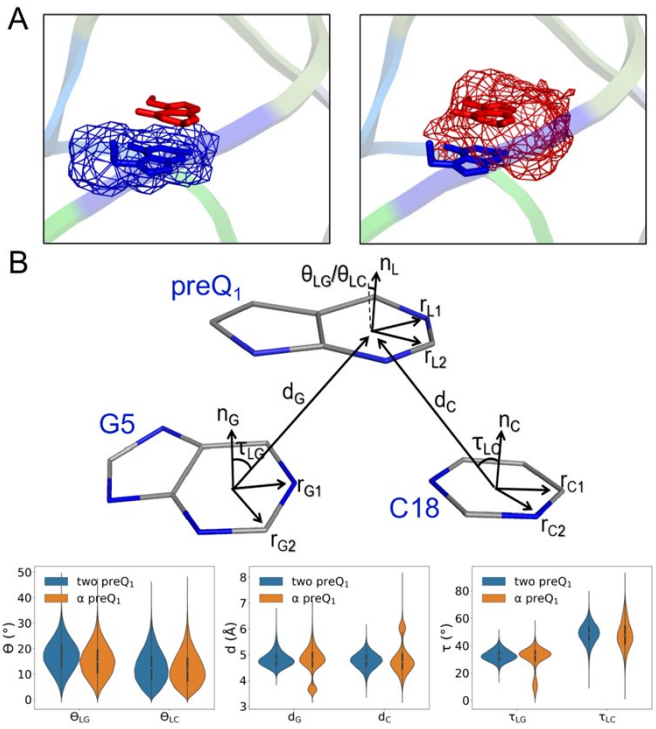

Fig. S4. The fluctuation of α and β sites preQ$_1$ ligands and the vdW interaction. (A) Density contours of α and β sites preQ$_1$ ligands are shown as wireframes in blue and red colors, respectively, in the two preQ$_1$ bound complex. (B) Top: Illustration of three parameters (*d*, θ, and τ) to evaluate the base stacking. The distance *d* is between two pyrimidine ring centers of the ligand and the nucleobase of G5/C18. The angle θ is between two normal vectors of the pyrimidine rings of the ligand and nucleobase of G5/C18. The angle τ is between two vectors, one vector is from the center of the pyrimidine ring of nucleobase of G5/C18 to the center of the pyrimidine ring of the ligand, and the other is the normal vector of the pyrimidine ring of nucleobase of G5/C18. Bottom: the densities of θ, *d*, and τ in a violin format.

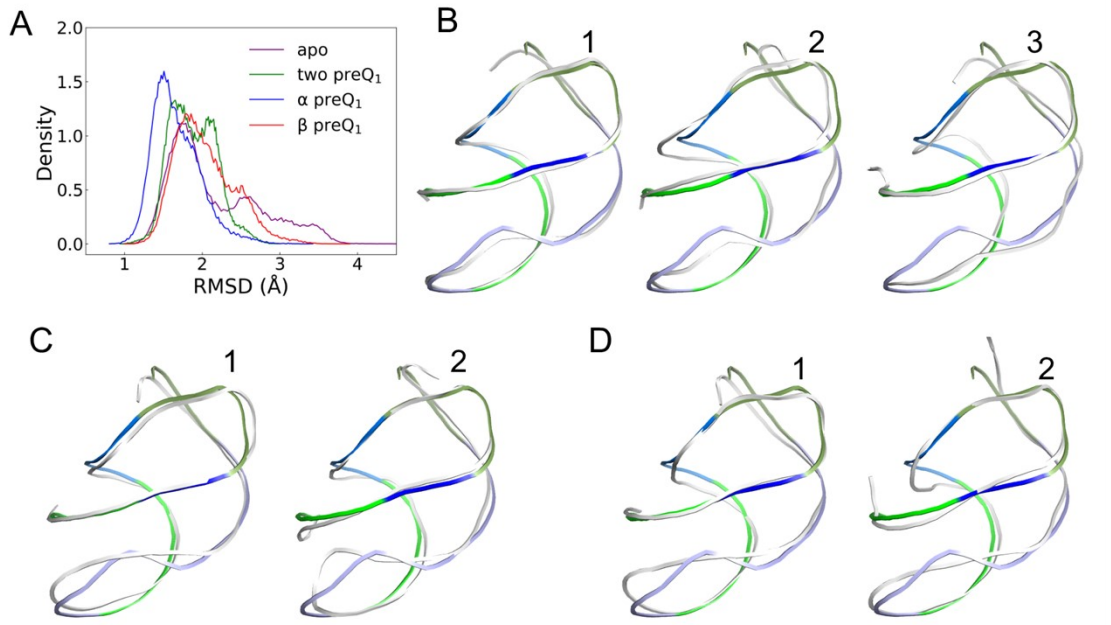

Fig. S5. The dynamical features. (A) The densities of RMSDs calculated with atoms P, O3', O5', C3', C4', and C5' relative to the starting structure. Structures from β $preQ_1$ (B) , two $preQ_1$ (C) , and α $preQ_1$ (D) bound complexes are displayed in superposition to 7REX.

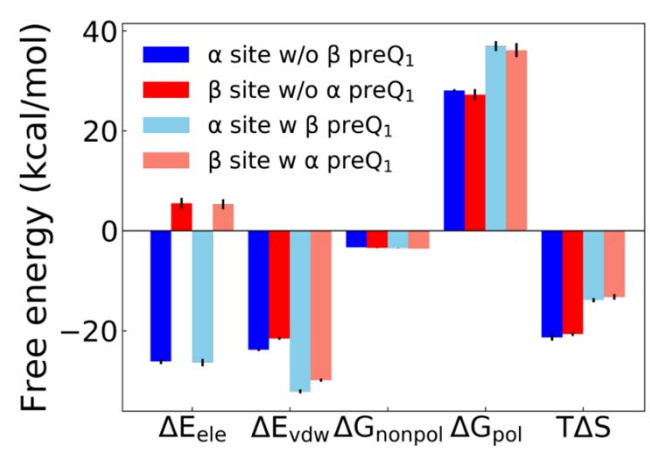

Fig. S6. The free energy terms. The free energy calculated by MM-PBSA method in two $preQ_1$ bound complex.

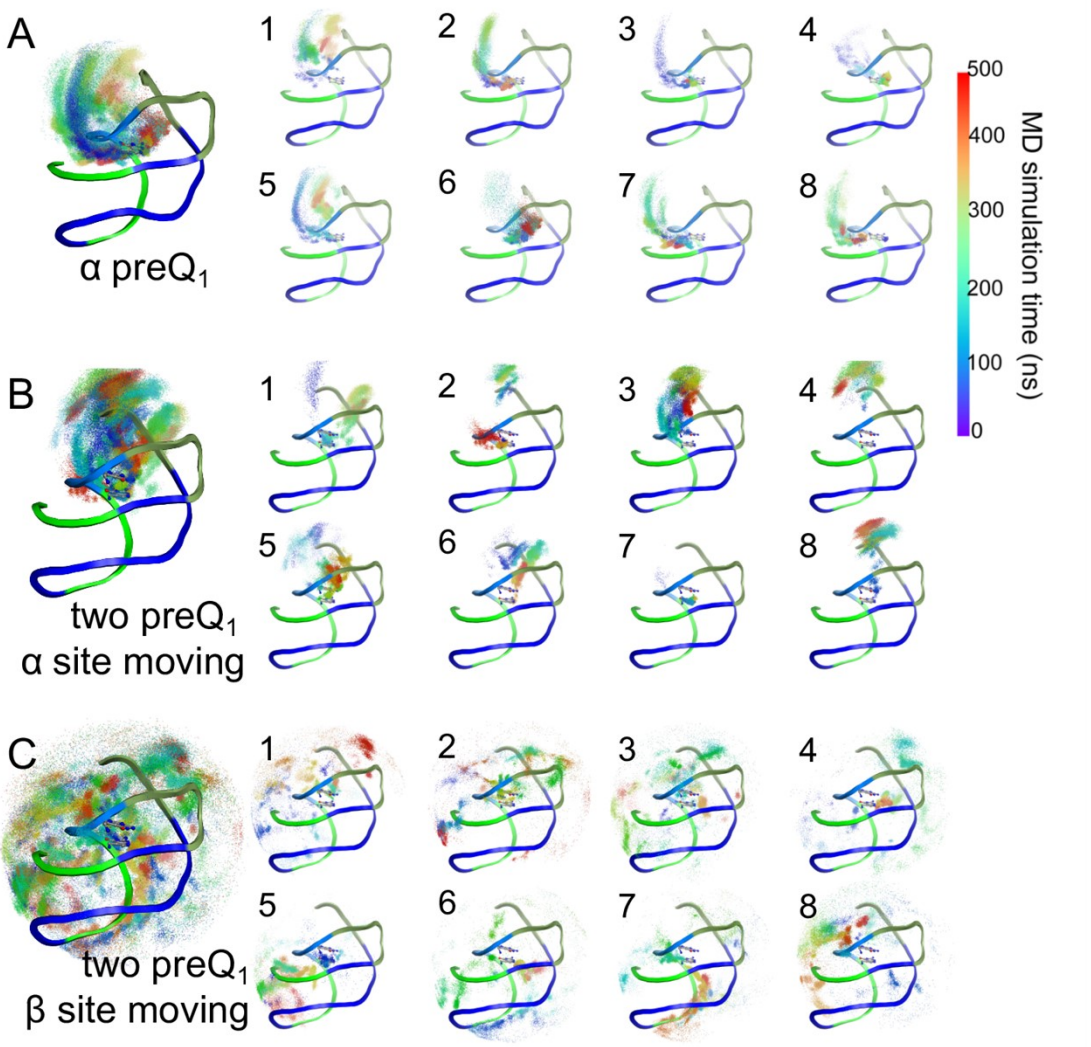

Fig. S7 The distribution of ligands in metadynamics simulations. The trajectories of ligand centers shown as dots colored according to the MD simulation time. The left larger panels are superimposition for eight trajectories, which are shown on the right eight small panels. The aptamer and bound ligands are shown in cartoon and stick representations and colored according to their secondary structure and the elements of atoms, respectively.

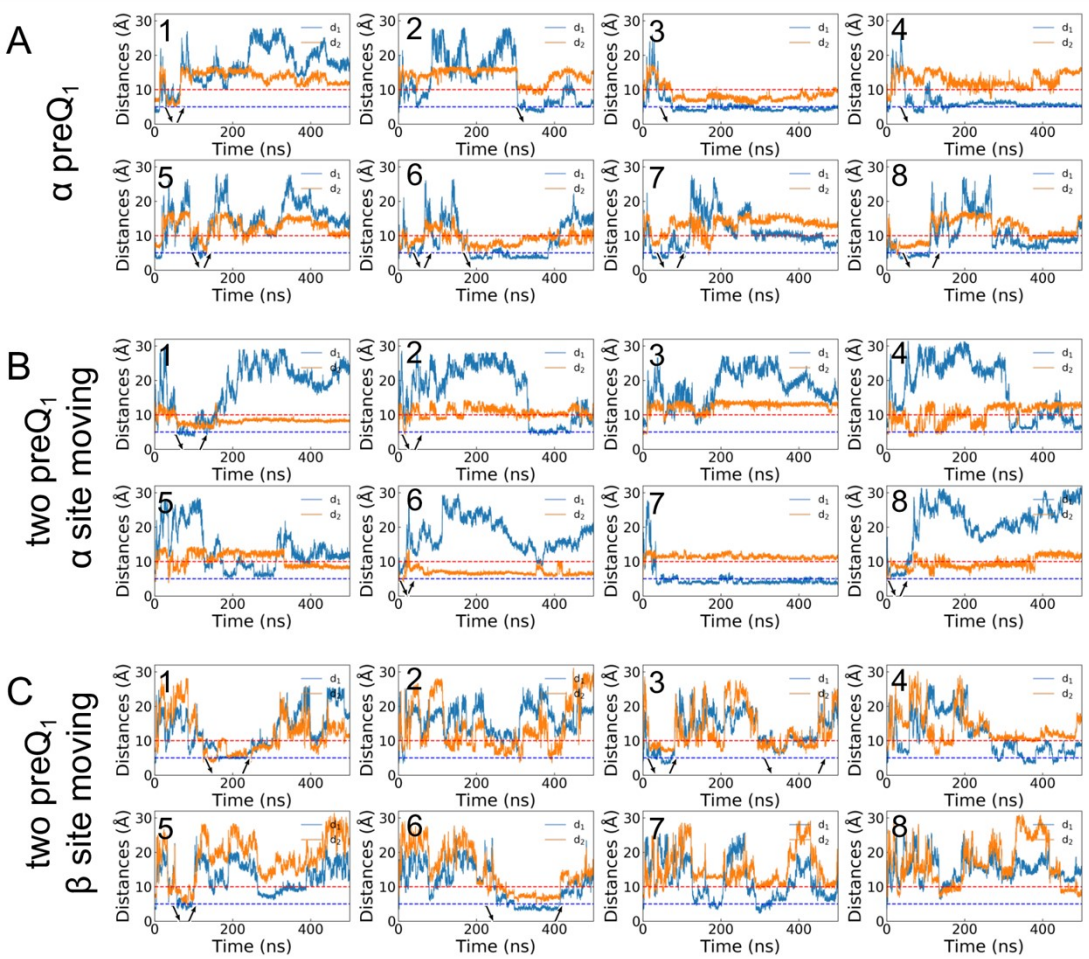

Fig. S8. Time traces of d_1 and d_2 in the metadynamics simulations.

Movie S1. A position interchange between β site $preQ_1$ and α site $preQ_1$ in one metadynamics simulation of β site $preQ_1$ moving in two $preQ_1$ complex. The unbinding β site $preQ_1$ moves to the position of α site $preQ_1$ and interacts with G5/C18 by base stacking, then α site $preQ_1$ rebinds to the position of β site $preQ_1$.

Movie S2. Ligand $preQ_1$ prefers to exit through the path1 in the metadynamics simulation of in α $preQ_1$ bound complex.

Movie S3. β site $preQ_1$ prefers to exit through the path2 in the metadynamics simulation of two $preQ_1$ complex.

Movie S4. α site $PreQ_1$ prefers to exit through the path2 in the metadynamics simulation of two $preQ_1$ complex.