

SUPPORTING INFORMATION

Complex formation of 5,10,15,20-tetrakis(*N*-methylpyridinium-4-yl)-21*H*,23*H*-porphyrin with G-quadruplex DNA

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SI-1. Determination of association constant (K_a) and number of binding sites (n) (Scatchard plots)

Firstly, 1:1 complex formation between TmPyP4 and (d(TTAGGG))₄ is considered as,



. The association constant (K_a) between them is defined as follows,

$$K_a = \frac{D_b}{C_f D_f}$$

, where C_f , D_f , and D_b are the concentrations of free TmPyP4, free (d(TTAGGG))₄, and the complex, respectively. The fraction of saturation of (d(TTAGGG))₄, p is defined as

$$p = \frac{D_b}{D_t}$$

, where $D_t (= D_f + D_b)$ is total concentration of (d(TTAGGG))₄. Using p , C_f , and D_t , the K_a value is written as follows,

$$K_a = \frac{D_b / D_t}{C_f (D_t - D_b) / D_t} = \frac{p}{C_f (1 - p)}$$

Secondly, when (d(TTAGGG))₄ has n independent binding sites with identical association constant, the fraction of saturation of (d(TTAGGG))₄, p is defined as follows:

$$p = \frac{r}{n}$$

, where r is an average number of TmPyP4 binding to (d(TTAGGG))₄. Then,

$$K_a = \frac{r / n}{C_f (1 - r / n)}$$

And rearranging the above equation gives

$$\frac{r}{C_f} = K_a (n - r)$$

Thus, a plot of r / C_f vs r has slope = $-K_a$, y-intercept = $n K_a$, and x-intercept = n .

We recorded the Soret absorbance at 440 nm (A_x) at various stoichiometric ratios (x) of (d(TTAGGG))₄ to 3 μ M of TmPyP4 solution. Then, the concentration of free TmPyP4, C_f is calculated as follows,

$$C_f = \frac{A_x - A_\infty}{A_0 - A_\infty} C_t$$

, where C_t , A_0 , and A_∞ are total concentration of TmPyP4 (3 μ M), the observed 440 nm-absorption, and the 440 nm-absorption when all TmPyP4 molecules bind to (d(TTAGGG))₄, respectively. The A_∞ value was determined by a curve-fitting of the 440 nm-absorption to an exponential curve. The molar ratio between the complex (= TmPyP4 bound (d(TTAGGG))₄) and total (d(TTAGGG))₄, r , is calculated as follows,

$$r = \frac{C_b}{D_t} = \frac{C_t - C_f}{x C_t}$$

Then, we obtained the Scatchard plots, as illustrated in Figure 3, and determined the association constant (K_a) and the number of binding site (n) .

SI-2. Determination of stabilization energy(ΔG from K_a)

Gibbs free energy change of complex formation is defined as follows,

$$\Delta G = -RT \ln K_a$$

, where K_a , R ($= 8.31 \text{ JK}^{-1}\text{mol}^{-1}$) and T are the association constant of complex formation, the gas constant and absolute temperature, respectively. (Moore, W. J. (1972) Physical chemistry, Prentice-Hall Inc., NJ.)

In our experiment, the association constant K_a was determined using Scatchard analysis described in SI-1 ($K_a = 6.2 \times 10^6 \text{ M}^{-1}$). Thus, $\Delta G = -38.7 \text{ kJmol}^{-1}$ at 25°C was obtained.

SI-3. Ring current effect of TmPyP4 porphyrin on the G4 – G6 imino proton shifts

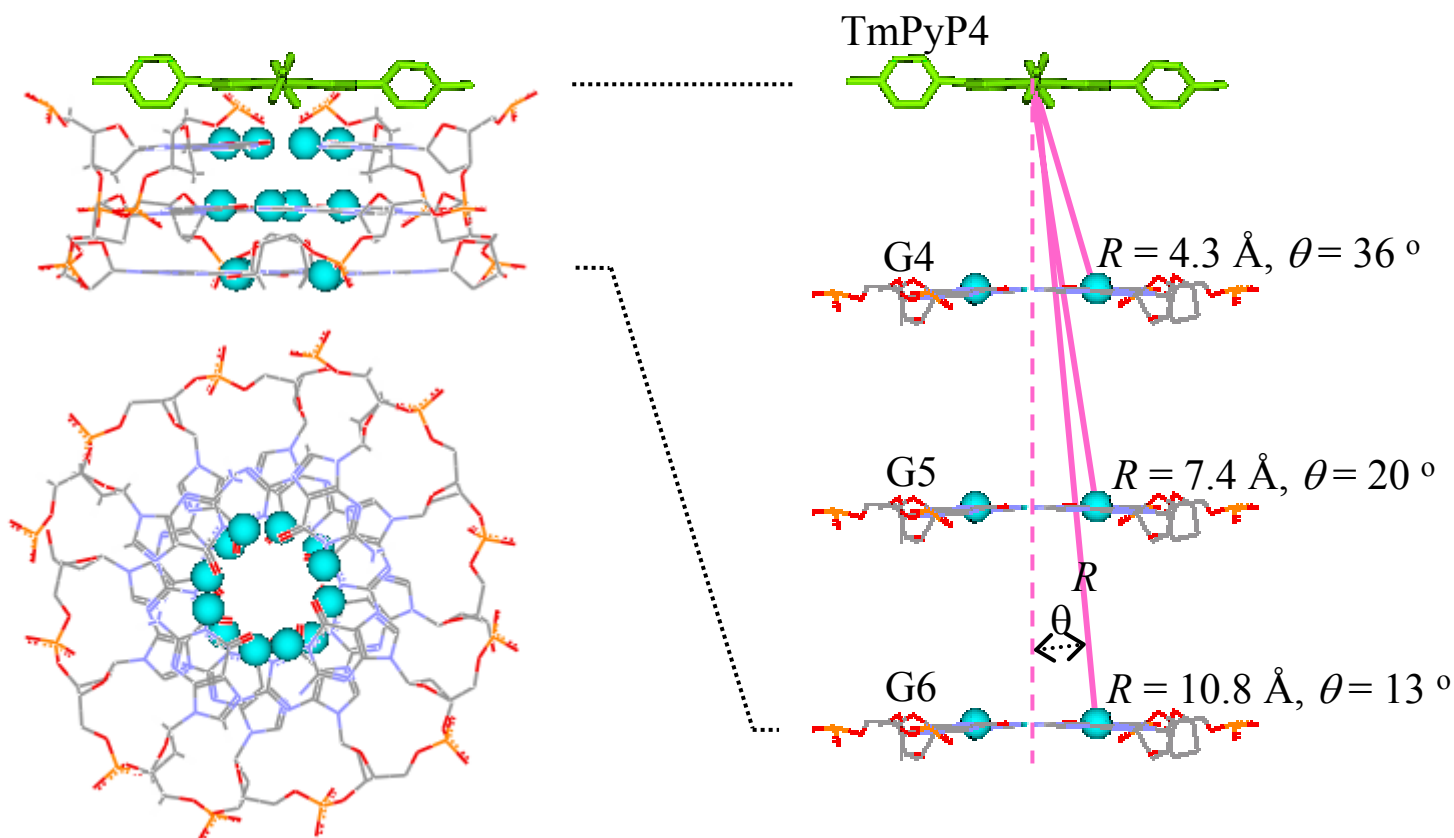
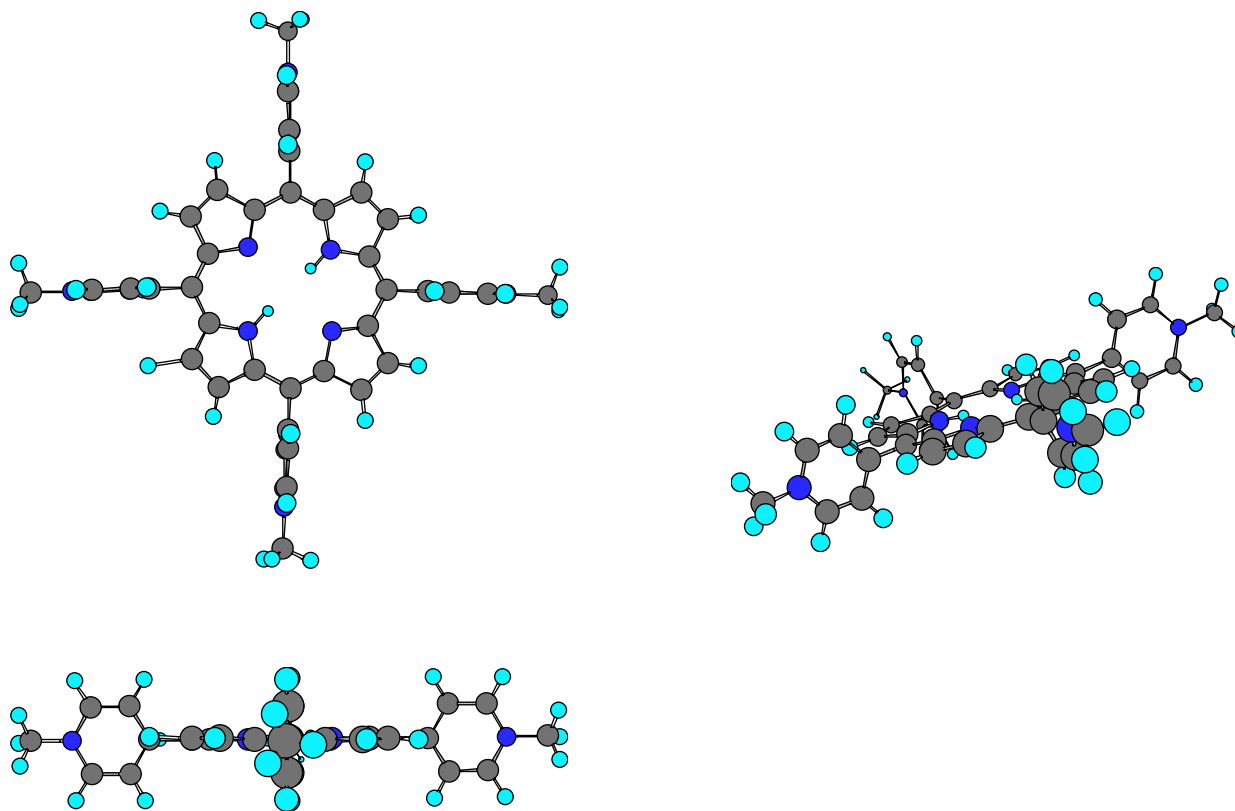


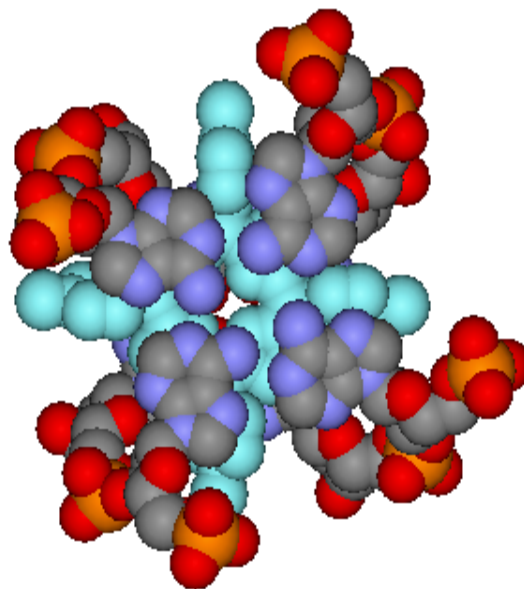
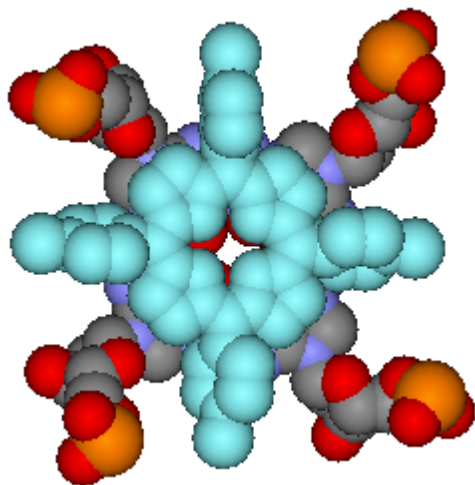
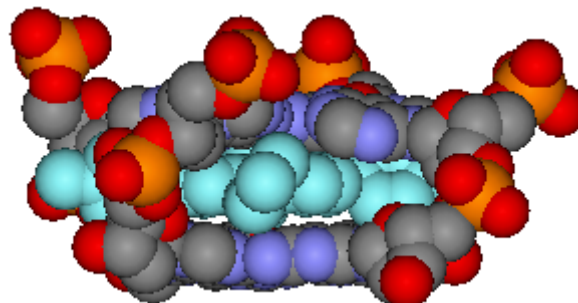
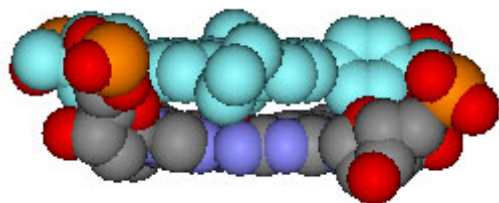
Figure Schematic drawing of imino proton (cyan) of G4 – G6-quartets (CPK color) and TmPyP4 (Green) (Left), and configuration of imino protons and TmPyP4 (Right).

Table Chemical shift induced by the ring current effect of TmPyP4 porphyrin

Proton	$\Delta\delta$ (ppm)	Free (ppm)	Calc. (ppm)
G4 Imino	-3.36	11.24	7.88
G5 Imino	-1.10	10.84	9.74
G6 Imino	-0.40	10.46	10.06



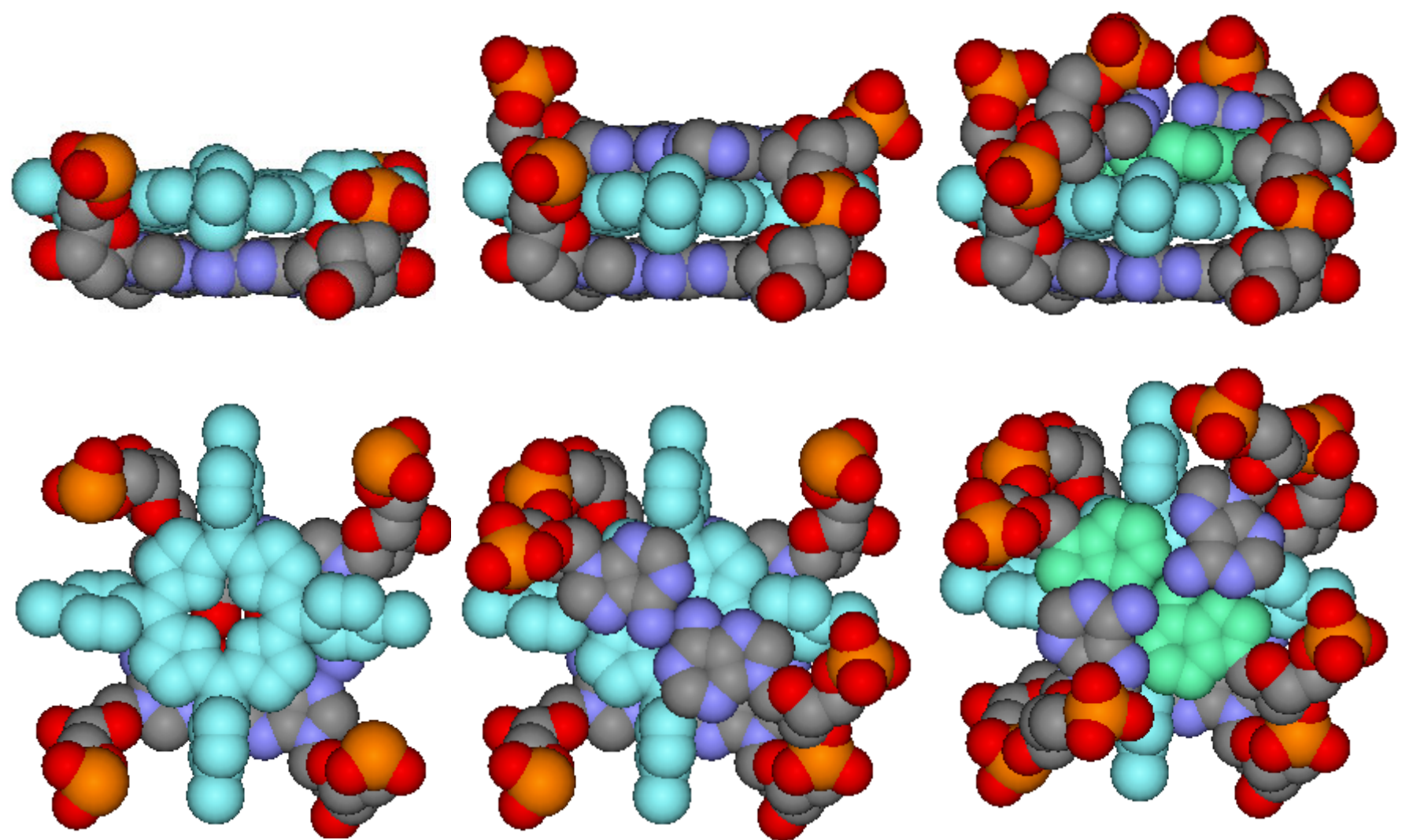
SI-4. An energy optimized-structure of TmPyP4 determined by PM3 calculation



TmPyP4 on the G4-quartet

A3-quartet on the TmPyP4-G4

SI-5. One of plausible model of TmPyP4 intercalation into the A3pG4 step proposed from molecular mechanics calculation. The energy optimized-structure of TmPyP4 (SI-4) was placed into the middle of the A3pG4 step which was taken from the NMR structure of (d(TTAGGGA))₄ (PDB: 1NP9) (46). One of the optimum structures, obtained from the molecular mechanics calculation using MM2 force field on the Chem3D (44), was illustrated. TmPyP4 on the G4-quartet (left) and four A3 bases stack on TmPyP4 (right). A3pG4 portion of (d(TTAGGG))₄ is illustrated in CPK color and TmPyP4 is shown in blue.



TmPyP4 on the G4-quartet

Two A3 bases on the TmPyP4 A3-G4 quadruplex DNA and TmPyP4

SI-6. Another plausible model of TmPyP4 intercalation into the A3pG4 step proposed from molecular mechanics calculation. The structure was obtained from a similar calculation as used in SI-5, with the displacement of two A3 adenine bases facing to each other by ~ 0.3 nm above the A3-quartet plane. TmPyP4 stacking on the G4-quartet (left), two A3 bases stacking on TmPyP4 (center), and the other two A3 bases partially covering the two A3 ones, shown in green, stacking on TmPyP4 (right). A3pG4 portion of (d(TTAGGG))₄ is illustrated in CPK color, exception being a pair of A3 adenine bases and TmPyP4 illustrated in green and blue, respectively.