

Supplementary Text S1.

K_D was calculated for binding of HMGB1a to CP- and OX-DNA in the TGGA, AGGC and CGGA sequence context from published gel-shift data(1,2). The equation for calculating K_D from θ (fraction of bound DNA), [DNA_{total}] and [Protein_{total}] was derived as follows:

$$\theta = \frac{[DNA_{bound}]}{[DNA_{total}]}$$

$$[DNA_{free}] = [DNA_{total}] - [DNA_{bound}]$$

$$[DNA_{free}] = [DNA_{total}] * (1 - \theta)$$

$$[Protein_{free}] = [Protein_{total}] - [Protein_{bound}]$$

$$[Protein_{free}] = [Protein_{total}] - [DNA_{bound}]$$

$$[Protein_{free}] = [Protein_{total}] - \theta * [DNA_{total}]$$

$$K_D = \frac{[Protein_{free}][DNA_{free}]}{[Complex]}$$

$$K_D = \frac{([Protein_{total}] - \theta * [DNA_{total}]) * ([DNA_{total}] * (1 - \theta))}{[DNA_{bound}]}$$

$$K_D = \frac{([Protein_{total}] - \theta * [DNA_{total}]) * ([DNA_{total}] * (1 - \theta))}{\theta * [DNA_{total}]}$$

$$K_D = \frac{([Protein_{total}] - \theta * [DNA_{total}]) * (1 - \theta)}{\theta}$$

The K_D 's were then used to calculate ratio of binding affinities between CP- and OX-DNA in different sequence contexts.

References

1. Malina, J., Novakova, O., Vojtiskova, M., Natile, G. and Brabec, V. (2007) Conformation of DNA GG intrastrand cross-link of antitumor oxaliplatin and its enantiomeric analog. *Biophysical Journal*, **93**, 3950-3962.
2. Wei, M., Cohen, S.M., Silverman, A.P. and Lippard, S.J. (2001) Effects of spectator ligands on the specific recognition of intrastrand platinum-DNA cross-links by high mobility group box and TATA-binding proteins. *J Biol Chem*, **276**, 38774-38780.

Supplementary Text S2

Pt Charge Derivation Protocol: The description of the derivation of partial charges of the Pt-GG adducts is described in detail in Sharma et al(1). Briefly, in order to determine the atomic partial charges of CP-GG and OX-GG adducts, the 9-methyl-guanine derivatives cis- [Pt(NH₃)₂(9-Me-Guo)₂]²⁺ (CP-meG2) and [Pt(trans-RR-1,2-diaminocyclohexane)(9-Me-Guo)₂]²⁺ (OX-meG2) were used. These derivatives were built from our NMR solution structures using Insight II. The atomic partial charges were determined using the Mulliken method implemented within Gaussian03 based on the NMR structure modified to the 9-methyl-guanine derivative. The density functional method B3LYP implemented within Gaussian03 was utilized; the LanL2DZ basis set was used for the platinum atom and 6-31Gd basis set was used for the rest of the atoms. Besides the atomic partial charges, other force field parameters of the Pt-GG adducts were referenced from AMBER parm99 force field parameters or from previous work by Yao et al(2). and Scheeff et al(3).

MD Simulations – Free Pt-DNA, DNA

i) Preparation of the System: The starting structures described in the Methods section were first modified by removal of hydrogen atoms in Insight II. The structures were then prepared by LEaP module of AMBER v8.0 as follows: First hydrogen atoms were added back according to the nucleotide templates in AMBER force field library. Secondly the structures were neutralized with Na⁺ ions. Thirdly the neutralized system was fully solvated in an octahedral water box using TIP3 model water molecules. The distance between the wall of the periodic box and the closest atom in the solute was set to be 12.5 Å. The Duan et al. force field(4,5) with the ff03 parameter set was used, as well as our self-defined force field parameters for Pt-GG adducts.

ii) Simulation Protocol: The whole system underwent 120 ps minimization and relaxation before a 10 ns production MD carried out by the SANDER module of AMBER v8.0. The non-bonded cutoff was set to be 9.0 Å and the non-bonded list was updated every 10 steps. The MD simulations were always carried out in NPT condition (constant pressure, using isotropic position scaling, at 1 atm (1 atm = 6.9 kPa), pressure relaxation time constant 0.2 ps in relaxation MD and 2 ps in production MD; constant temperature, using weak-coupling algorithm, at 300 K, heat bath coupling time constant 0.2 ps in relaxation MD and 1 ps in production MD) with a 1 fs time step. The SHAKE algorithm was applied to all bonds involving hydrogen atoms and in turn the regular force evaluation omitted for those bonds. The translational center-of-mass motion was removed every 1 ps. Every time when the system was heated, zero velocity information was inherited from the previous stage and a Maxwell distribution of velocities was re-established. In minimization and relaxation, a harmonic potential was applied to Pt-DNA adduct or DNA to restrain its motion. The restraint was gradually weakened and became zero at the final stage of relaxation. The following 10 ns production MD was carried out unrestrained.

The details of system minimization and relaxation were as follows. First, the whole system, including Pt-DNA adducts or undamaged DNA, water molecules and counter-ions, was minimized for 2000 steps using the steepest descent method in constant volume condition with a harmonic potential of 500 kcal/mol Å² (1 cal = 4.184 J) applied to Pt-DNA or DNA to fix its conformation. Second, the system was heated from 0 K to 300 K and kept in NVT (constant volume and constant temperature) condition with the harmonic restraints unchanged in a 20 ps relaxation MD. Third, the system was further relaxed in another short 20 ps MD in NPT condition with the same harmonic constraints. Fourth, the system was further minimized for 2000 steps of steepest descent in constant volume condition three times, with a weakening harmonic potential of 500, 50 and 5 kcal/mol Å² respectively. Fifth, the system was heated from 10 K to 300 K and kept in NVT condition with the harmonic restraints of 5 kcal/mol Å² in a 20 ps

relaxation MD. Sixthly, four rounds of 20 ps relaxation MD were carried out in NPT condition with a further weakening harmonic potential of 5, 1, 0.1 and 0 kcal/mol Å² respectively. Seventhly, the system was heated up from 10 K to 300 K in NVT condition with zero restraints in a 20 ps relaxation MD. Finally, the system was heated for the very last time from 100 K to 300 K at the beginning of 10 ns production MD.

MD Simulations – HMGB1a-Pt-DNA

i) Preparation of the System: The starting structures described in the Methods section were prepared in an manner identical to that of free Pt-DNA, except that LEaP v.9.0 was used and the crystallographic waters were retained while building the system.

ii) Simulation Protocol: The whole system underwent 140 ps minimization and relaxation before a 50 ns production MD carried out by the PMEMD module of AMBER v9.0. The MD simulation parameters were identical to the free Pt-DNA simulations.

The details of system minimization and relaxation were as follows. First, the whole system, including Pt-DNA adduct, protein, water molecules and counter-ions, was minimized for 5000 steps using the steepest descent method in constant volume condition. Second, the system was heated from 0 K to 300 K and in NVT (constant volume and constant temperature) conditions in a 20 ps relaxation MD. Third, the system was further relaxed in 100 ps MD in NPT. Finally, the system was heated from 200 K to 300 K in NVT condition for a 20 ps run before beginning the 50 ns production MD.

References

1. Sharma, S., Gong, P., Temple, B., Bhattacharyya, D., Dokholyan, N.V. and Chaney, S.G. (2007) Molecular dynamic simulations of cisplatin- and oxaliplatin-d(GG) intrastand cross-links reveal differences in their conformational dynamics. *J Mol Biol*, **373**, 1123-1140.
2. Yao, S., Plastaras, J.P. and Marzilli, L.G. (1994) A molecular mechanics AMBER-type force field for modeling platinum complexes of guanine derivatives. *Inorganic Chemistry*, **33**, 6061-6077.
3. Scheeff, E.D., Briggs, J.M. and Howell, S.B. (1999) Molecular modeling of the intrastrand guanine-guanine DNA adducts produced by cisplatin and oxaliplatin. *Molecular Pharmacology*, **56**, 633-643.
4. Duan, Y., Wu, C., Chowdhury, S., Lee, M.C., Xiong, G., Zhang, W., Yang, R., Cieplak, P., Luo, R., Lee, T. et al. (2003) A point-charge force field for molecular mechanics simulations of proteins based on condensed-phase quantum mechanical calculations. *J Comput Chem*, **24**, 1999-2012.
5. Cornell, W.D., Cieplak, P., Bayly, C.I., Gould, I.R., Merz, K.M., Ferguson, D.M., Spellmeyer, D.C., Fox, T., Caldwell, J.W. and Kollman, P.A. (1995) A Second Generation Force Field for the Simulation of Proteins, Nucleic Acids, and Organic Molecules. *Journal of the American Chemical Society*, **117**, 5179-5197.

Supplementary Figure S1. Average RMSD values for the MD simulations over time. The RMSD values for each of the 5 simulations compared to the corresponding starting structure for the undamaged DNA, CP-DNA and OX-DNA are shown for the full 10 ns of each simulation. RMSD at time t represents the average of RMSD in a 250 ps bin centered at t (running average). The five simulation trajectories starting from undamaged DNA, CP-DNA and OX-DNA structures with different initial MD velocities are represented in black, red, blue, green and violet.

Supplementary Figure S2. Conformational differences in overall distribution of helical parameters in the TGGA sequence context. The conformational differences in the central four base pairs between CP-, OX- and undamaged DNA in the TGGA sequence context are represented as a KS heat map of 3 possible comparisons; OX-DNA vs undamaged DNA, CP-DNA vs undamaged DNA and CP- vs OX-DNA. The KS ratio decreases in the order of Red > Blue > White in the heat map.

Supplementary Figure S3. Conformational differences between structures forming the G7-O6 hydrogen bond and structures forming no hydrogen bond. The conformational differences in the central four base pairs between structures forming G7-O6 hydrogen bond and structures forming no hydrogen bond to the drug are plotted for CP- and OX-DNA in the TGGA sequence context. We represent the differences as the KS ratio (described in Methods), which is color-coded. The KS ratio decreases in the order of Red > Blue > White.

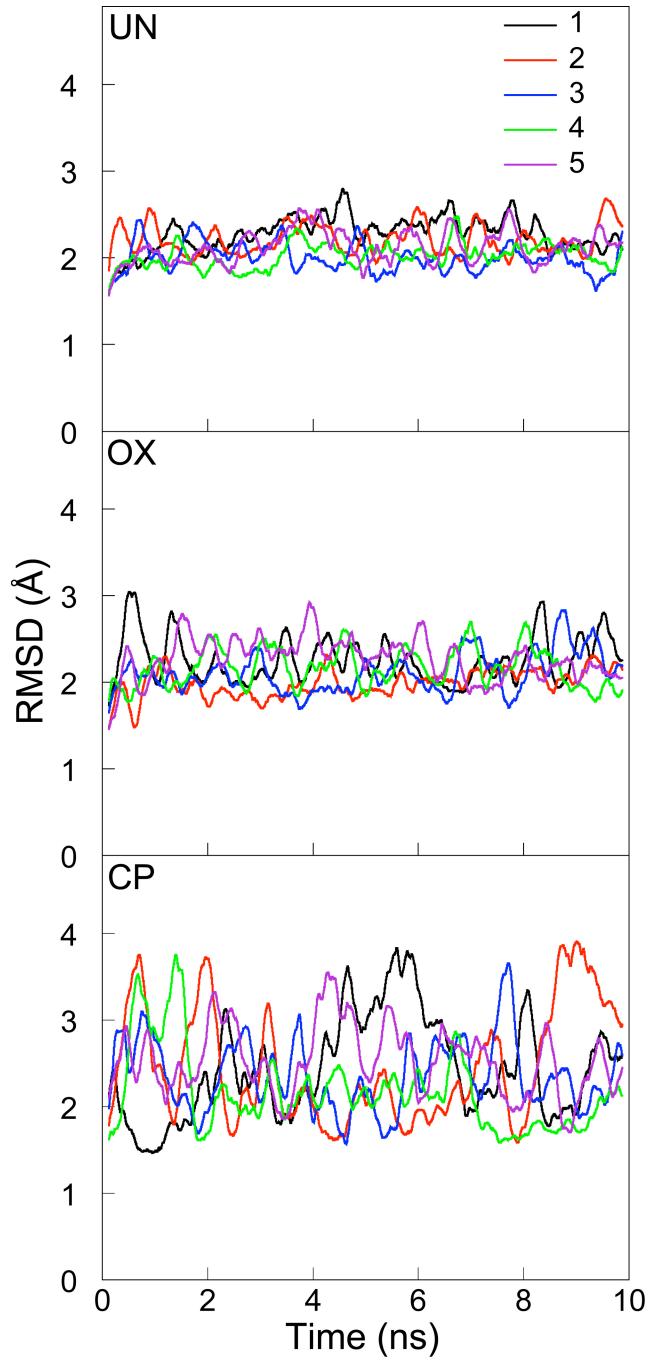
Supplementary Figure S4. Comparison of G7-A8 base-pair step parameters between free and protein bound Pt-DNA in the TGGA sequence context. Twist, slide, roll and shift parameters of the G7-A8 base-pair step are plotted for structures with CP-A8-N7 hydrogen bond, OX-T17-O4 hydrogen bond, CP- HMGB1a-Ser41-A8-N3 and OX-HMGB1a-Ser41-A8-N3 hydrogen bond. The distributions of free Pt-DNA parameters are plotted in black and those for HMGB1a bound Pt-DNA are plotted in red. Distributions of CP-DNA are represented as a solid curve, while those of OX-DNA are represented as a dashed curve. The frequency distribution for a particular hydrogen bonded species was obtained from structures that formed that particular hydrogen bond. However, the normalization was performed over the total number of structures (30,000 structures for free Pt-DNA and 45,000 for HMGB1a bound Pt-DNA) to show the relative abundance of different hydrogen bonded species. To enable comparison of free and bound Pt-DNA (which differ in sampling by an order of magnitude) in the same graph, we employ two Y-axes, which are color-coded.

Supplementary Figure S5. Bend Angle distributions of hydrogen-bonded species in the TGGA and AGGC sequence contexts. The bend angle distributions of different hydrogen-bonded species in CP-TGGA, OX-TGGA, CP-AGGC and OX-AGGC are plotted. The distribution for structures with no hydrogen bond (green), hydrogen bond to the adjacent base (blue), hydrogen bond to G7-O6 (red) and

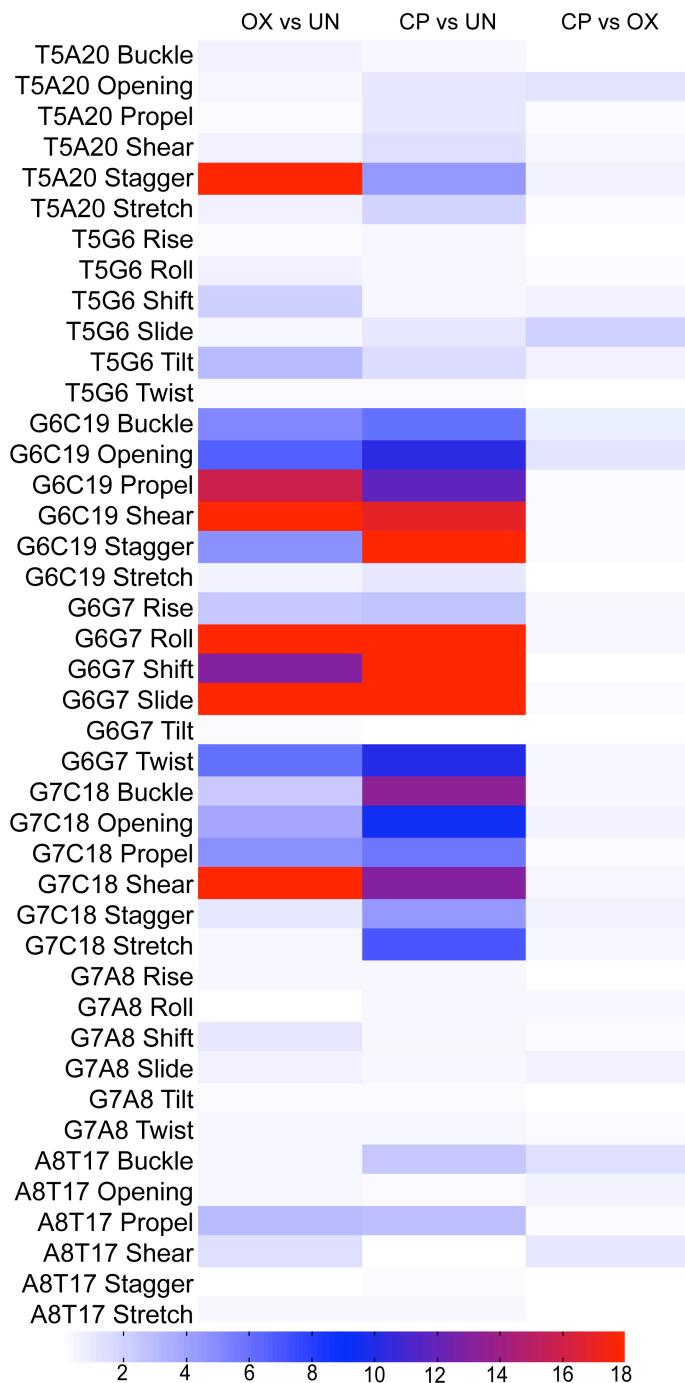
structures with hydrogen bonds to both G7-O6 and A5-N7 (black) are plotted. The frequency distribution for a particular hydrogen bonded species was obtained from structures that formed that particular hydrogen bond. However, the normalization was performed over the total number of structures (30,000 structures for Pt-DNA in the TGGA sequence context and 60,000 structures for Pt-DNA in the AGGC sequence context) to show the relative abundance of different hydrogen bonded species. The bend angle of DNA in the crystal structure of HMGB1a bound to CP-DNA is shown as a vertical dashed line.

Supplementary Figure S6. G6-G7 roll parameter of different hydrogen bonded species in CP- and OX-DNA adducts in the TGGA and AGGC sequence contexts. The distribution of G6-G7 roll of different hydrogen-bonded species in CP-TGGA, OX-TGGA, CP-AGGC and OX-AGGC are plotted. The distribution for structures with no hydrogen bond (green), hydrogen bond to the adjacent base (blue), hydrogen bond to G7-O6 (red) and structures with hydrogen bonds to both G7-O6 and A5-N7 (black) are plotted. The frequency distribution was obtained as described in Supplementary Figure S5. The left panel shows the distribution for the whole range of roll values, while the right panel is zoomed into the distributions at roll > 40°.

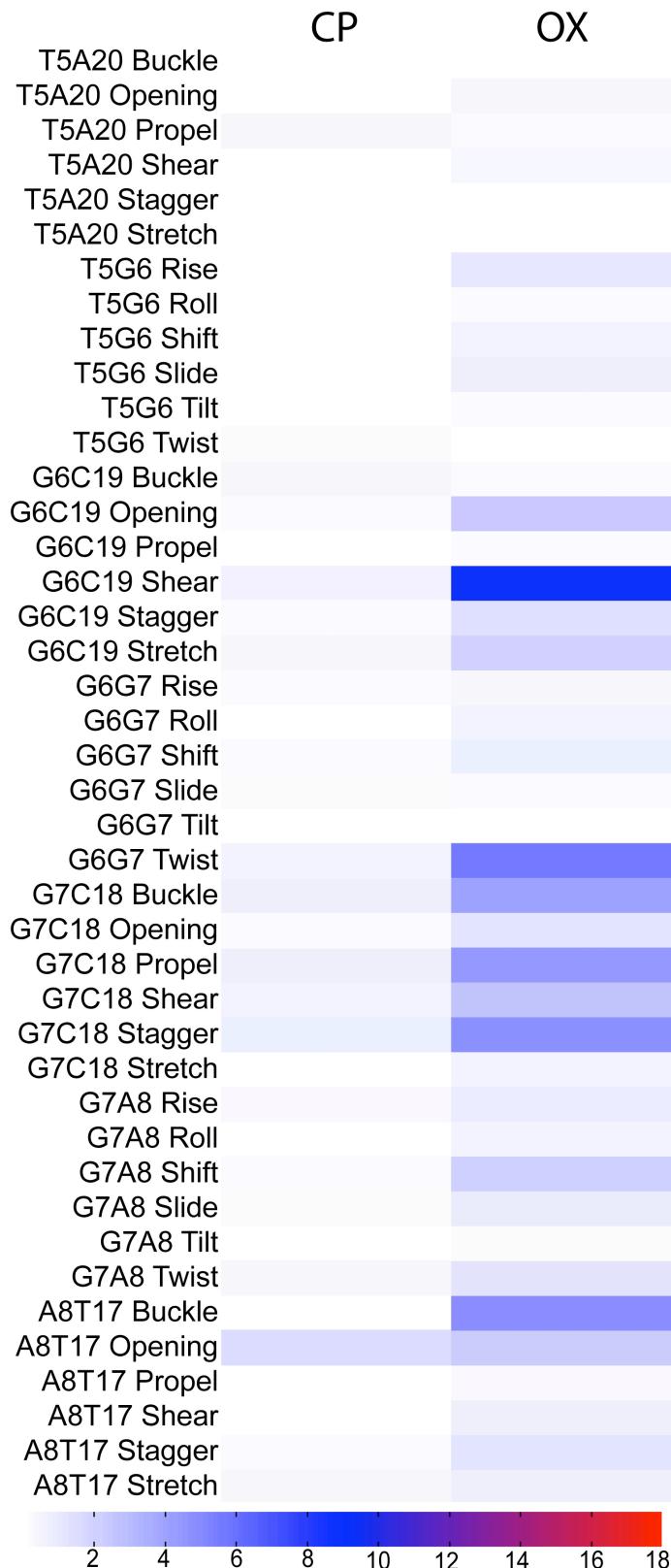
Supplementary Figure S1



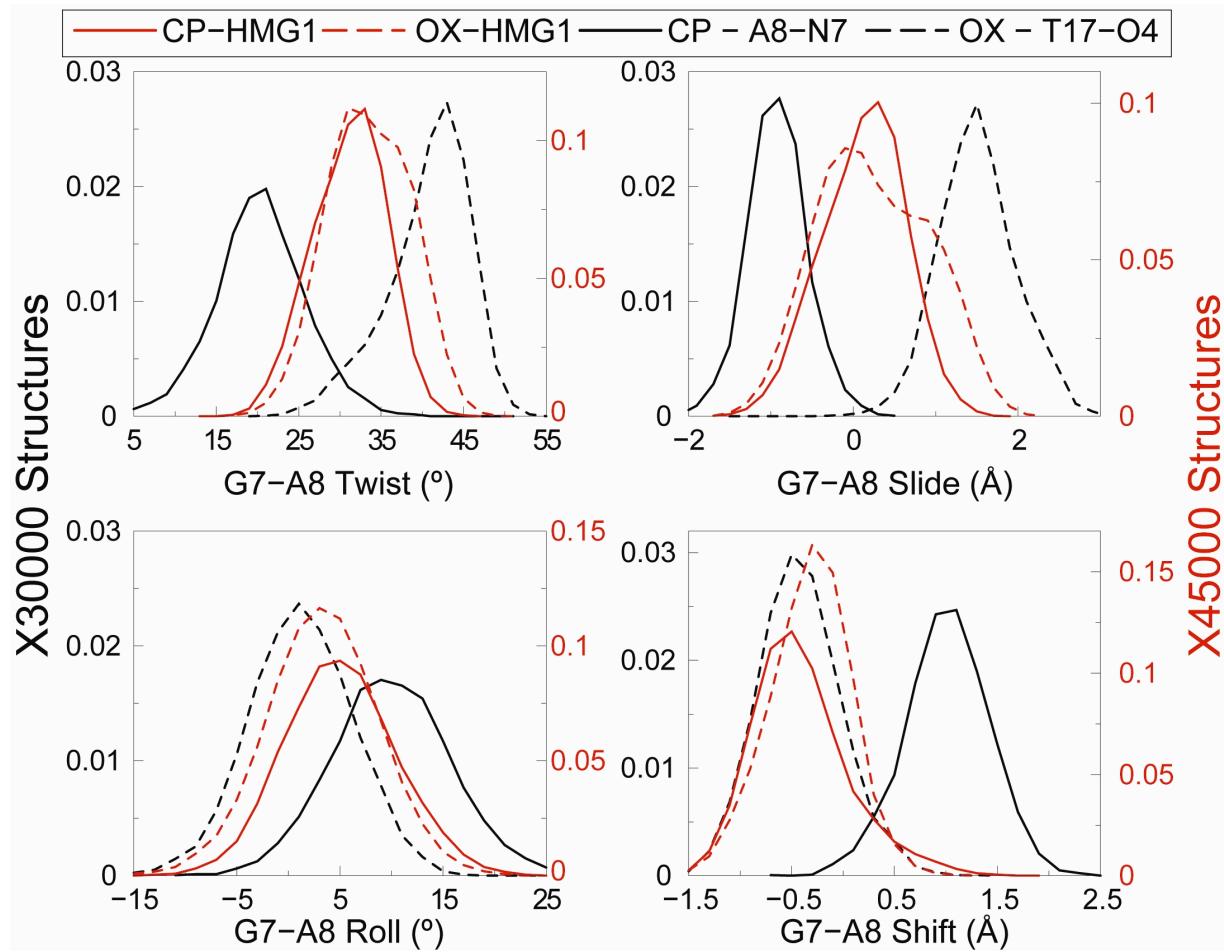
Supplementary Figure S2



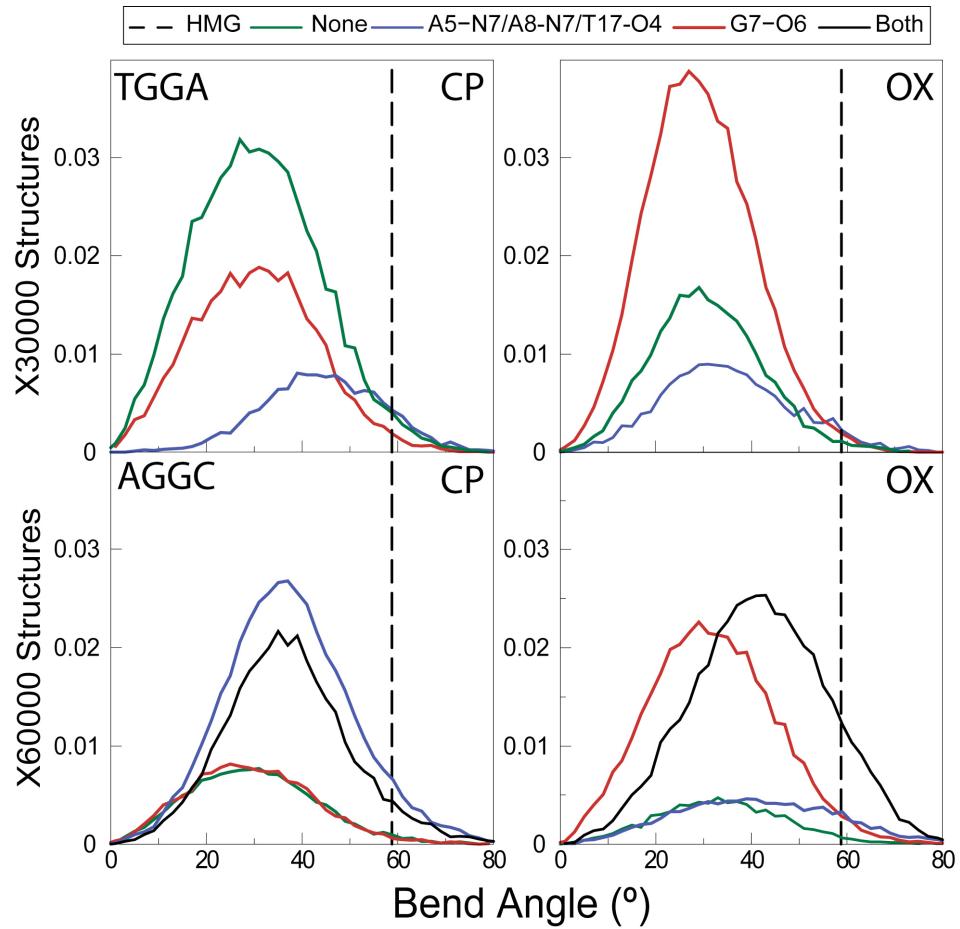
Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5



Supplementary Figure S6

