

Supplementary Information

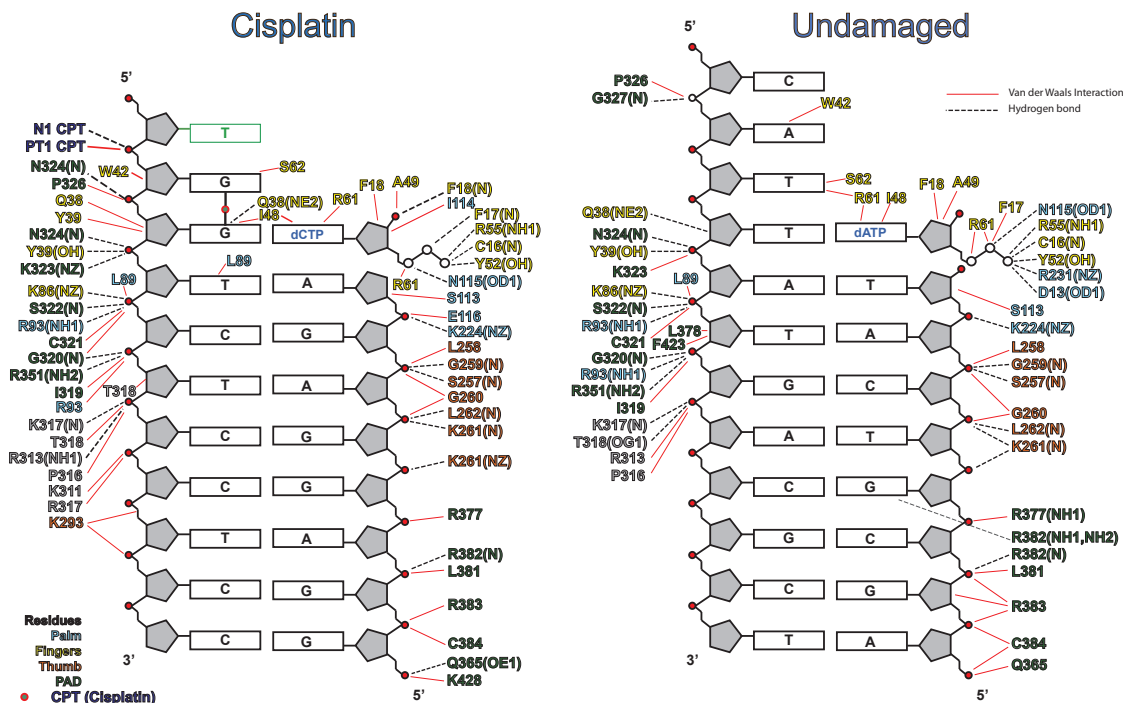
Structural basis for cisplatin DNA damage tolerance by human polymerase η during cancer chemotherapy

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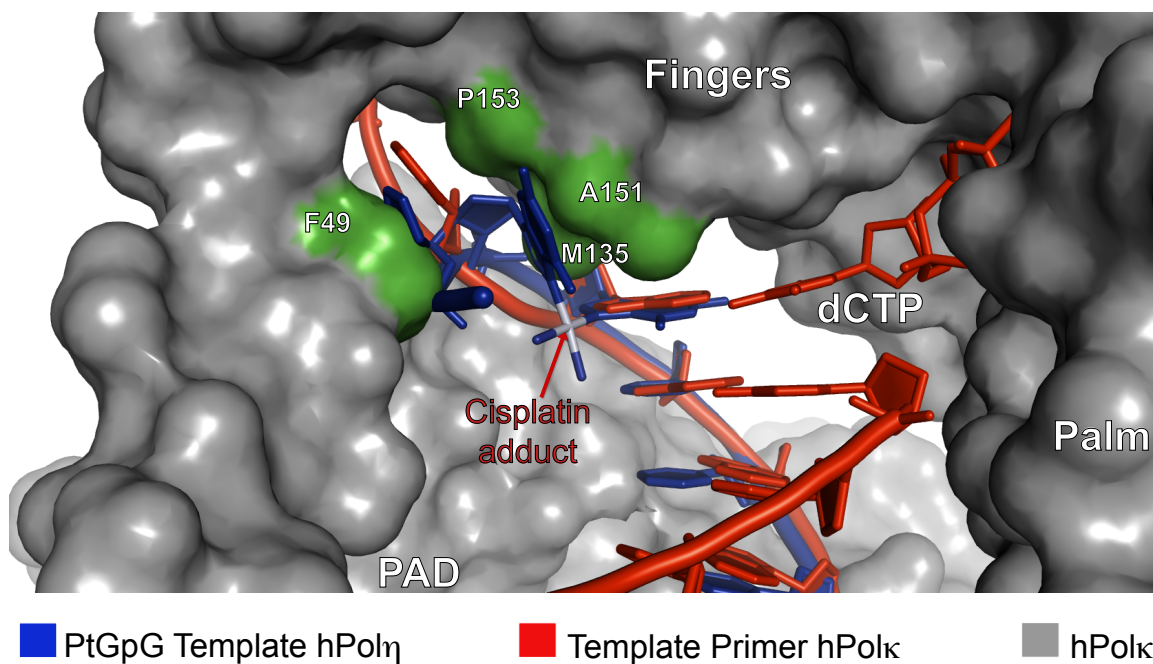
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Supplementary Figure 1. A schematic comparison of hydrogen bonds and van der Waals interactions between human Polη and DNA in the ternary complexes bound to PtGpG and undamaged DNA (PDB Id. 3MR2, Ref. 1). Hydrogen bonds are indicated by broken black lines (determined by interaction distance $< 3.2\text{\AA}$) and van der Waals interactions are indicated by continuous red lines (determined by interaction distance $< 4.2\text{\AA}$). The residues are colored to match the domain they belong to: cyan for the palm domain, yellow for the fingers domain, orange for the thumb domain, and green for the PAD. Cisplatin (CPT) is indicated as a red outlined disc.

hPolk



Supplementary Figure 2. Comparison between human Pol η and Pol κ (based on superimposition of their template strands). Molecular surface of human Pol κ (PDB Id. 2OH2, Ref. 2) when superimposed via the PtGpG template strand of human Pol η (blue) and template strand of human Pol κ (red). PtGpG sterically overlaps, M135, P153 and A151 of the fingers domain and other residues (F49) of N-clasp domain of hPol κ (green).

DNA; Lanes 5-8, DNA synthesis by wild type, R61A, Q38A, and R61AQ38A mutant hPol η , respectively, on cisplatin-damaged DNA. A schematic of the DNA substrate is shown on top. DNA lengths are shown on the left, and the position of the GG cisplatin lesion is given on the right. Reactions containing wild type or mutant Pol η (0.5 nM) and DNA substrate (10 nM) were carried out at 37 C $^{\circ}$ for 10 min. 25 μ M each of dATP, dCTP, dGTP and dTTP were included in the reaction.

Supplementary Table 1. Catalytic efficiency of wild type and mutant hPol η for dCTP incorporation opposite the 3'G of an undamaged GG sequence or one containing a cisplatin intra-strand cross-link.

hPolη	Template residue*	k_{cat} (min⁻¹)	K_{m} (μM)	Efficiency ($k_{\text{cat}}/K_{\text{m}}$)	Efficiency relative to WT
WT	undamaged G	19.1 \pm 0.1	0.9 \pm 0.04	21.2	-
	3'G CisPt	19.1 \pm 0.4	0.8 \pm 0.08	24.0	-
Q38A	undamaged G	12.4 \pm 0.2	1.2 \pm 0.1	10.3	0.49
	3'G CisPt	12.7 \pm 0.2	1.6 \pm 0.1	7.9	0.33
R61A	undamaged G	13.8 \pm 0.1	1.4 \pm 0.06	9.9	0.47
	3'G CisPt	11.6 \pm 0.2	0.6 \pm 0.06	19.3	0.80
Q38A R61A	undamaged G	11.0 \pm 0.4	5.1 \pm 0.6	2.2	0.10
	3'G CisPt	8.8 \pm 0.3	3.5 \pm 0.4	2.5	0.10

* The sequence of the entire 78 nt oligonucleotide template is given in the Methods section. The undamaged and cisplatin containing template DNAs are identical in sequence, and differ only in the absence or presence of the cisplatin lesion, respectively.

Reactions containing wild type or mutant Pol η (0.05 nM) and DNA substrate (10 nM) were carried out at 37 C^o for 5 min. The dCTP was included at concentrations ranging from 0.05 – 50 μM .

References

1. Biertumpfel, C., Zhao, Y., Kondo, Y., Ramon-Maiques, S., Gregory, M., Lee, J.Y., Masutani, C., Lehmann, A.R., Hanaoka, F., and Yang, W. (2010). Structure and mechanism of human DNA polymerase ϵ . *Nature* 465, 1044-1048.
2. Lone, S., Townson, S.A., Uljon, S.N., Johnson, R.E., Brahma, A., Nair, D.T., Prakash, S., Prakash, L., and Aggarwal, A.K. (2007). Human DNA polymerase κ encircles DNA: implications for mismatch extension and lesion bypass. *Mol Cell* 25, 601-614.