# Copper *bis*-dipyridoquinoxaline is a potent DNA intercalator that induces superoxide-mediated cleavage *via* the minor groove

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(S1) Characterisation of Cu-DPQ

(S2) X-Ray Crystallography

(S3) DNA binding experiments

(S4) DNA damage studies on SC pUC19

(S5) DNA damage studies on linear DNA

## (S1) Characterisation of [Cu(DPQ)<sub>2</sub>(NO<sub>3</sub>)](NO<sub>3</sub>)



Figure. S1 ESI-MS spectra of Cu-DPQ complex.



Figure. S2 ATR-FTIR spectra of DPQ ligand [Cu(DPQ)<sub>2</sub>(NO<sub>3</sub>)](NO<sub>3</sub>).



Figure. S3 UV-Vis stability study of Cu-Phen, Cu-DPQ-Phen and Cu-DPQ (5 mM DMF).

#### (S2) X-ray crystallography of [Cu(DPQ)<sub>2</sub>(NO<sub>3</sub>)]NO<sub>3</sub>·2H<sub>2</sub>O



**Figure S4.** Structure of  $[Cu(DPQ)_2(NO_3)](NO_3)\cdot 2H_2O$  showing the two independent cations and the labelling scheme for the non-carbon atoms. Hydrogen atoms omitted for clarity, 50% probability ellipsoids.

The data were collected at 150(2)K on a Bruker-Nonius Apex II CCD diffractometer using  $MoK_{\alpha}$  radiation ( $\lambda = 0.71073$ Å) and were corrected for Lorentz-polarisation effects. Data were processed as a 2-component twin (by rotation about reciprocal axis [-0.002 0.001 1.000]) and corrected for absorption (TWINABS). The structure was solved by dual space methods (SHELXT) and refined on F<sup>2</sup> in SHELXLE using all the reflections (SHELXL with HKLF 5 data). All the non-hydrogen atoms were refined using anisotropic atomic displacement parameters and hydrogen atoms bonded to carbon were inserted at calculated positions using a riding model. Hydrogen atoms of solvate water were not located or included in the refinement since partial-occupancy and disorder meant no single H-bonding network could be identified (Fig 3). One of the two uncoordinated nitrate anions was disordered and modelled with 60:40 occupancy of two overlapping sites. Only one of the solvate water molecules (O1) was full occupancy, the rest were refined with 50% occupancy over six independent sites. Parameters for data collection and refinement are summarised in Table 1. [Cu(DPQ)<sub>2</sub>(NO<sub>3</sub>)](NO<sub>3</sub>)·2H<sub>2</sub>O is a solvomorph of previously reported structure [Cu(DPQ)<sub>2</sub>(NO<sub>3</sub>)](NO<sub>3</sub>).<sup>1</sup>

## Table S1 Crystal data for [Cu(DPQ)<sub>2</sub>(NO<sub>3</sub>)](NO<sub>3</sub>)·2H<sub>2</sub>O

$C_{28}H_{16}CuN_9O_3\cdot NO_3\cdot 2(H_2O)$	F(000) = 1404	
$M_r = 688.08$	$D_{\rm x} = 1.631 {\rm ~Mg~m^{-3}}$	
Monoclinic, <i>P</i> 2 <sub>1</sub>	Mo K $\alpha$ radiation, $\lambda = 0.71073$ Å	
<i>a</i> = 7.3882 (12) Å	Cell parameters from 4719 reflections	
<i>b</i> = 29.205 (5) Å	$\theta = 2.6 - 22.3^{\circ}$	
c = 13.363 (2)  Å	$\mu = 0.85 \text{ mm}^{-1}$	
$\beta = 103.686 \ (3)^{\circ}$	T = 150  K	
V = 2801.4 (8) Å <sup>3</sup>	Lath, pale green	
Z = 4	$0.64 \times 0.12 \times 0.02 \text{ mm}$	

### Data collection

Bruker APEX 2 CCD diffractometer	5048 independent reflections
Graphite monochromator	4386 reflections with $I > 2\sigma(I)$
ω rotation with narrow frames scans	$\theta_{max} = 25.0^{\circ},  \theta_{min} = 2.1^{\circ}$
Absorption correction: multi-scan TWINABS-2012/1 (Bruker,2012) was used for absorption correction. Final HKLF 4 output contains 39211 reflections, Rint = 0.0845 (12298 with I > 3sig(I), Rint = 0.0444)	$h = -8 \rightarrow 8$
$T_{\min} = 0.579, \ T_{\max} = 0.745$	$k = 0 \rightarrow 34$
5048 measured reflections	$l = 0 \rightarrow 15$

#### Refinement

Refinement on $F^2$	Secondary atom site location: difference Fourier map
Least-squares matrix: full	Hydrogen site location: inferred from neighbouring sites
$R[F^2 > 2\sigma(F^2)] = 0.057$	H-atom parameters constrained
$wR(F^2) = 0.138$	$w = 1/[\sigma^2(F_o^2) + (0.0874P)^2 + 0.8348P]$ where $P = (F_o^2 + 2F_c^2)/3$
S = 1.07	$(\Delta/\sigma)_{\rm max} < 0.001$
5048 reflections	$\Delta \rangle_{\rm max} = 0.64 \ {\rm e} \ {\rm \AA}^{-3}$
911 parameters	$\Delta \rangle_{\rm min} = -0.68 \ {\rm e} \ {\rm \AA}^{-3}$
333 restraints	Absolute structure: No quotients, so Flack parameter determined by classical intensity fit
Primary atom site location: dual	Absolute structure parameter: 0.08 (2)

Cell refinement: *SAINT* v8.34A (Bruker, 2013); data reduction: *SAINT* v8.34A (Bruker, 2013); program(s) used to solve structure: SHELXT<sup>2</sup>; program(s) used to refine structure: *SHELXL2018*/3<sup>3</sup> and shelXle<sup>4</sup>; molecular graphics: *Mercury*<sup>5</sup>; software used to prepare material for publication: Olex2<sup>6</sup>, *publCIF*.<sup>7</sup>

Cu1—N1	2.041 (11)	Cu2—N9	2.035 (11)
Cu1—N4	2.006 (10)	Cu2—N12	2.052 (10)
Cu1—N5	2.089 (9)	Cu2—N13	2.089 (11)
Cu1—N8	1.949 (11)	Cu2—N16	1.979 (10)
Cu1—O21	2.144 (9)	Cu2—O31	2.092 (8)

Table S2. Selected Geometric parameters (Å, °) for [Cu(DPQ)<sub>2</sub>(NO<sub>3</sub>)](NO<sub>3</sub>)·2H<sub>2</sub>O

Table S3. Potential hydrogen bond distances (Å) for [Cu(DPQ)<sub>2</sub>(NO<sub>3</sub>)](NO<sub>3</sub>)·2H<sub>2</sub>O

01 - 02	2.75 (0.02)
01 - 021_\$1	2.92 (0.01)
02 - 06	2.74 (0.04)
02 - 041	3.04 (0.03)
02 - 042	2.98 (0.03)
03 - 04	2.57 (0.03)
03 - 06_\$2	2.77 (0.03)
03 - 07	3.09 (0.03)
03 - 042	2.87 (0.03)
04 - 051	2.90 (0.04)
04 - 053_\$2	3.00 (0.03)
05 - 043_\$3	2.77 (0.03)
05 - 053	2.71 (0.03)
06 - 051	2.77 (0.03)
06 - 051'	2.94 (0.04)
06 - 07	2.63 (0.04)
07 - 032	3.04 (0.03)
07 - 051	3.17 (0.03)

Symmetry equivalents: \$1 -1-x, -1/2+y, 1-z, \$2 1+x, y, z, \$3 -1+x, y, z



**Figure S5.** Unit cell plot for  $[Cu(DPQ)_2(NO_3)](NO_3)\cdot 2H_2O$  showing H-bonding (dashed green lines) and face stacking of the DPQ ligands.

## (S3) DNA binding experiments



**Figure S6.** Topoisomerase-I mediated DNA relaxation assay in the presence of A. Cu-Phen and B. Cu-DPQ-Phen.

## (S4) DNA damage studies on SC pUC19



**Figure S7.** DNA cleavage reactions of Cu-DPQ-DPQ in the presence of 1 mM Na-*L*-Asc on pUC19 (lanes 2-5). Pre-treatment of plasmid with 100  $\mu$ M EDTA (lanes 6-9) or 100  $\mu$ M neocuprione (lanes (10-13) prior to complex exposure.

#### (S5) DNA damage studies on linear DNA

#### Sequence generated by PCR:

Gctgcaaaacgtctgcgacctgagcaacaacatgaatggtcttcggtttccgtgtttcgtaa agtctggaaacgcggaagtcagcgccctgcaccattatgtt**ccgg**atctgcatcgcaggatg ctgctggctaccctgtggaacacctacatctgtattaacgaagcgctggcattgaccctgag tgatttttctctggtcccgccgcatccatacgccagttgtttaccctcacaacgttccagt aa**ccgg**gcatgttcatcatcagtaacccgtatcgtgagcatccattcggtatcatcggaa cattacccccatgaacagaaatcccccttacacggaggcatcagtgaccaaacaggaaaaaa ccgcccttaacatggcccgctttatcagaagccagacattaacgcttctggagaaactcaac gagctggacgggatgaacaggcagacatctgtgaatcgcttcacgaccacgctgatgagct ttaccgcagctgcctgcgtttcggtgatgacggtgaaaaccccgtcagggcgg cggagacggtcacagctgttcgtgaggatg<u>ccgg</u>gagcagacaagccgtcagggcgg tcagcggtgttggcgggtgcgagcagacagacagtgagcatagcggagg gtatactggcttaactatgcggcatcagagcagattgtactgagagtgcaccatatgcggtg tgaaataccgcacagatgcgtaaggagaaaataccgcatcaggcgctttccgc

Forward: 5' -gctgcaaaacgtctgcgacc-3', Reverse: 5' -cgcatcaggcgctcttccgc-3'

Treatment with Hpall and Mspl results in 5 bands of varying length:

- 1. Start  $\rightarrow$  first internal CCGG 104 bases
- 2. 1st CCGG  $\rightarrow$  2nd CCGG 147 bases
- 3. 2nd CCGG  $\rightarrow$  3rd CCGG 307 bases
- 4. 3rd CCGG  $\rightarrow$  4th CCGG 34 bases
- 5. 4th CCGG  $\rightarrow$  end 206 bases

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