Supplementary Material (ESI) for Chemical Communications

X-ray diffraction analyses of the natural isoquinoline alkaloids Berberine and Sanguinarine in complex with double helix DNA d(CGTACG)

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Supplementary Information

Crystallization and Data Collection Details

The deoxyhexanucleotide d(CGTACG) was purchased from Jena Bioscience (Jena Germany). Berberine and sanguinarine were supplied by Sigma-Aldrich Co. Ltd, USA.

A search for crystallization conditions were performed using a home-made crystallization screen based on the Nucleic Acid Mini Screen (Hampton Research) at 296 K and the sitting drop vapor diffusion method. Drops were formed mixing 1µl of a solution 2 mM of DNA duplex, 1µl of a solution 2 mM of the drug and 2µl of the screen conditions. Final optimized crystallization condition contain 10% MPD, 40 mM Na Cacodylate pH=6.5, 12 mM spermine tetra-HCl, 80 mM sodium chloride and 20 mM magnesium chloride for both complexes. Drops were equilibrated against a 30% MPD reservoir. Yellow (berberine complex) and orange (sanguinarine complex) crystals grew in about one and a half month.

Data collection on crystals of the DNA-drugs complexes were performed at 100 K, using as cryoprotectant the mother liquor solution with an increased concentration of MPD (30%). Data for the berberine complex were collected on an Oxford Diffraction instrument equipped with a sealed tube Enhance Ultra (Cu) and a Onyx CCD detector to a maximum resolution of 2.3 Å. Data for the sanguinarine complex were collected using synchrotron radiation at the ID29 Beamline, ESRF Grenoble, using a wavelength of 0.9763 Å and a ADSC Q315R detector to a maximum resolution of 2.3 Å. Data were integrated and scaled using the program XDS [1].

Data of the two complexes are hemihedrally twinned. The possibility of twinning of the data was suggested by the analysis of the intensity distribution. The Phenix.xtriage module of the PHENIX program [2] was used to analyze data for twinning. The multivariate Z scores from the L-tests of PHENIX were well above the expected value of 3.5 for both data sets. A high Z-score value indicates that the data differ significantly from what is expected for untwinned data. The results of the twinning tests and twinning law are shown in Table 2.

Furthermore data were submitted to the Merohedral crystal twinning server at UCLA [3] in the real space group $P3_221$ that indicated a twinning fraction $\alpha = 0.108$ for the berberine complex and $\alpha = 0.216$ for the sanguinarine complex.

Structure Determination and Refinement

The structure of the berberine complex was solved by the Molecular Replacement technique using the program Molrep [4] and the coordinates of the hexamer duplex d(CGTACG) complexed with an intercalating anthraquinone derivative (PDB code 1XCS) as a starting model, without all the heteroatoms. The structure of the Sanguinarine complex was solved by the same technique using the coordinate of the "two molecules" unit of the Berberine complex.

A spheric density was present in the Fo-Fc electron density maps that could be interpreted as a metal ion. Magnesium and sodium cations were present in the crystallization conditions, nevertheless after the introduction of these two metal ions positive electron density in the Fo-Fc map was still present at that position. The residual electron density in the Fo-Fc map was then interpreted, taking into account also the distances with neighbouring atoms, as a calcium ion, that was present in the MPD used for the crystallization as impurity.

The two models were refined with the program Refmac5 [5] from the CCP4 program suite [6], using the automatic twin refinement. The Rfactor and R_{free} decrease substantially in both cases at the stage in which this option was introduced in the refinement. Manual rebuilding of the model was performed using the program Coot [7]. No density was present for the 5'-Cs residues during refinement.

Data processing and refinement statistics are summarized in table.1.

Protein coordinates have been deposited with the Protein Data Bank **(**Protein Data Bank accession number 3NP6, 3NX5).

References

1) W. J. Kabsch, *Appl. Cryst.* **26**, 795-800 (1993).

2) *Adams et al., Acta Cryst **D58**, 1948-1954 (2002).*

3) T.O. Yeates, Methods Enzymol. **276**, 344-358 (1997).

4) A. A. Vagin and A. Teplyakov, *J. Appl. Cryst.*, **30** 1022-1025 (1997).

*5) G.N. Murshudov, A. A. Vagin and E. J. Dodson, Acta Cryst. **D53**, 240-255 (1997).*

6) Collaborative Computational Project, Number 4. Acta Crystallogr. ***D50**, 760-763 (1994).*

7) P. Emsley, B. Lohkamp, W. Scott, K. Cowtan, Acta Cryst. *D**66**, 486-501 (2010).*

Table S1. Twinning and intensity statistics summary.

	$d(CGTACG)_2$-Berberine	$d(CGTACG)_2$-Sanguinarine
Twinning tests		
For acentric data		
$\langle I^2\rangle/\langle I\rangle^2$	2.016	1.929
$\langle F\rangle^2/\langle F^2\rangle$	0.818	0.838
$\langle E^2-1\rangle$	0.712	0.672
Multivariate Z score L-test	5.292	9.549
Possible twin operator	*-h,-k,l*	
Estimated twin fraction		
Britton analisys	0.094	0.208
H-test	0.137	0.247

The expected values for twinning tests are $\langle I^2\rangle/\langle I\rangle^2$ untwinned = 2.000, perfect twin =1.500; $\langle F\rangle^2/\langle F^2\rangle$. untwinned = 0.785, perfect twin = 0.885; $\langle E^2-1\rangle$ untwinned = 0.736;perfect twin = 0.541

Table S2. Summary of Data Collection and Atomic Model Refinement Statistics. Values in parentheses are for the highest resolution shell

	Berberine/$(CGTACG)_2$	**Sanguinarine/$(CGTACG)_2$**
Data Collection		
Wavelength (Å)	1.542	0.976
Space group	$P3_221$	$P3_221$
Cell dimension (Å)	a= 29.99, c=119.07	a=30.37, c= 118.26
Limiting resolution (Å)	39.4-2.3 (2.3-2.4)	39.69-2.3 (2.43-2.3)
Unique reflections	3159	3006
R_{sym} (%)	7.6 (55.8)	5.2 (11.2)
Multiplicity	32.9 (11.6)	4.9 (2.85)
Completeness overall (%)	100 (99.8)	96.3 (85.1)
<I/σ(I)>	29.04 (4.8)	20.99 (6.5)
Refinement		
Resolution range (Å)	30.0-2.3	30.0-2.3
Unique reflections, working/free	3014/139	2719/289
Rfactor (%)	23.87	25.52
Rfree(%)	30.14	28.33
r.m.s.d. bonds(Å)	0.007	0.010
r.m.s.d. angles (°)	1.842	2.403

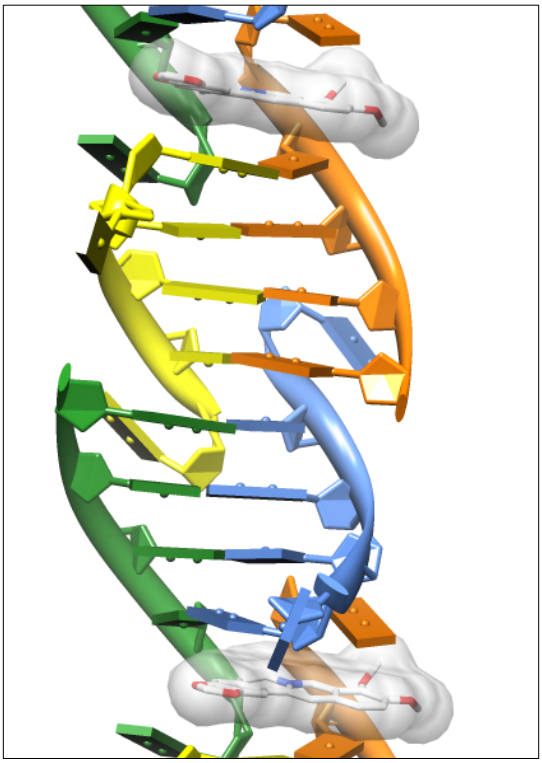

Figure S1: Berberine/CGTACG complex: DNA helices and stacked Berberine molecules. Symmetry related molecules are represented in the same colour

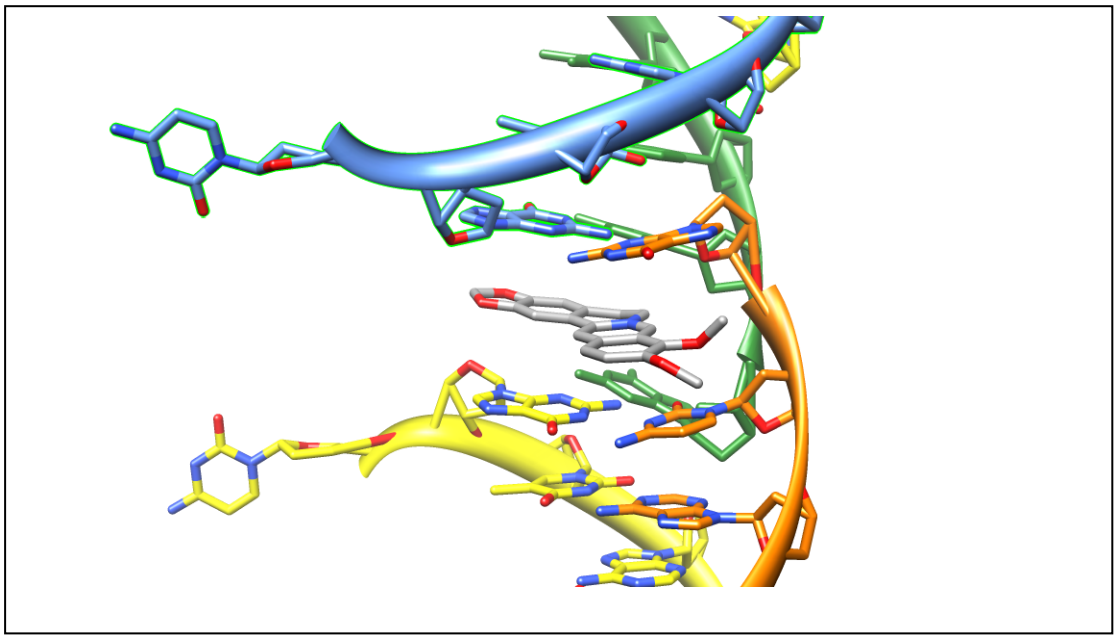

Figure S2: Berberine molecule intercalated at the interface of two “two-molecules” DNA units

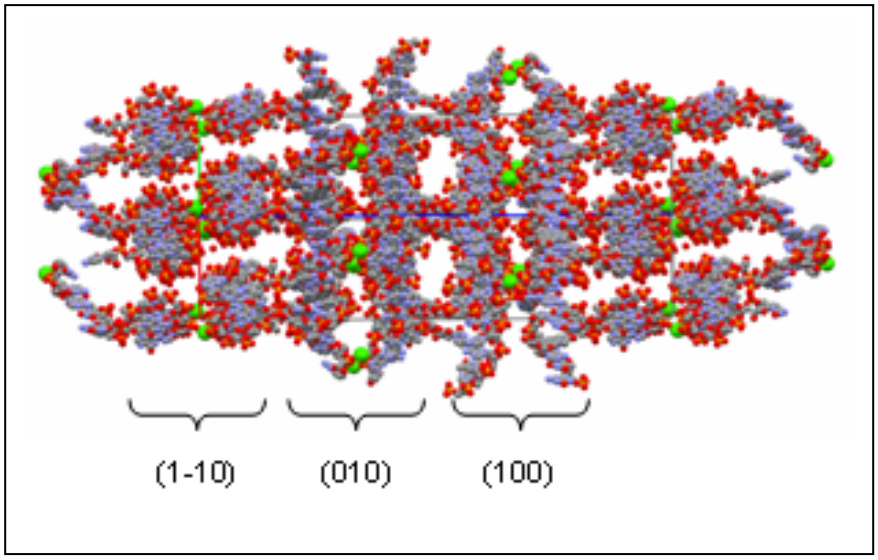

Figure S3: Crystal packing of the Sanguinarine/CGTACG complex.

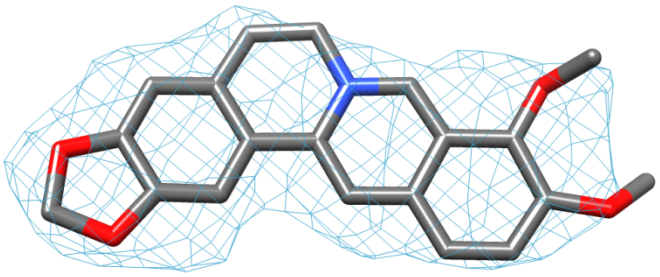

Figure S4: Skeleton and OMIT electron density map for Berberine contoured at 1.5σ level.