

## **Supporting Information**

### **Depicting the DNA Binding and Cytotoxicity Studies Against Human Colorectal Cancer of Aqua-bis(1-formyl-2-naphtholato- $k^2O,O'$ )copper(II): A Biophysical and Molecular Docking Perspective**

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#### ***Crystallography***

Single crystal X-ray data of complex **1** was collected on a Bruker SMART APEX CCD diffractometer using monochromatic Mo-K $\alpha$  radiation ( $\lambda = 0.71073$  Å) at 100(2) K. The linear absorption coefficients and the anomalous dispersion corrections were mentioned in the International Tables for X-ray crystallography. Using Olex2 [1], the structure was solved with the olex2.solve [1] structure solution program using Charge Flipping and refined with the olex2.refine [2] refinement package using Gauss-Newton minimization. All hydrogen atoms were located in difference Fourier maps of the structures and refined isotropically. All non-H atoms were refined anisotropically. The crystallographic data for complex **1** were summarized in Table S1.

#### ***Cytotoxicity Studies***

##### ***Cell line and Chemicals***

Cell line, human colorectal carcinoma (HCT116) and human embryonic kidney cells (HEK293) was achieved from ATCC, USA. Dulbecco's modified Eagle's medium (DMEM), penicillin–streptomycin–neomycin (PSN), antibiotic, fetal bovine serum (FBS), trypsin and

ethylenediaminetetraacetic acid (EDTA) were purchased from Gibco BRL (Grand Island, NY, USA). Tissue culture plastic ware was purchased from NUNC (Roskilde, Denmark). 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and other dyes were purchased from Thermo Fisher Scientific (USA).

### ***Cell Culture***

Human colorectal carcinoma (HCT116) and human embryonic kidney cells (HEK293) was cultured in DMEM with 10% fetal bovine serum (FBS) and 1% antibiotics (PSN) at 37 °C in a humidified atmosphere under 5% CO<sub>2</sub>. After 75–80% confluence, the cells were harvested with 0.025% trypsin and 0.52 mM EDTA in phosphate buffered saline (PBS), and seeded at a required density to allow them to re-equilibrate a day before the start of the experiment [3].

### ***MTT Assay***

The MTT assay was carried out to estimate the cell viability [4]. The cells were plated in 96-well plates and treated with different concentrations (0–20 µM) of complex **1** for 24h. After 24h, MTT solution was added and incubated for four hours. After the incubation period of MTT, formazan was solubilized with acidic isopropanol and the absorbance of the solution was measured at 595 nm using an ELISA reader.

### ***Quantification of Apoptosis and Necrosis Using Flow Cytometry***

Determination of Apoptosis and necrosis were analyzed by flow cytometry using an Annexin-V FITC/PI apoptosis/necrosis detection kit (Calbiochem, CA, USA). Treated cells ( $1 \times 10^6$ ) were washed and stained with Annexin-V-FITC and PI in accordance with the manufacturer's instructions. The percentages of viable, apoptotic (early and late) along with necrotic cells were estimated by flow cytometry (BD LSRFortessa, San Jose, CA, USA) [4,5].

### ***Determination of Mitochondrial ROS (mtROS)***

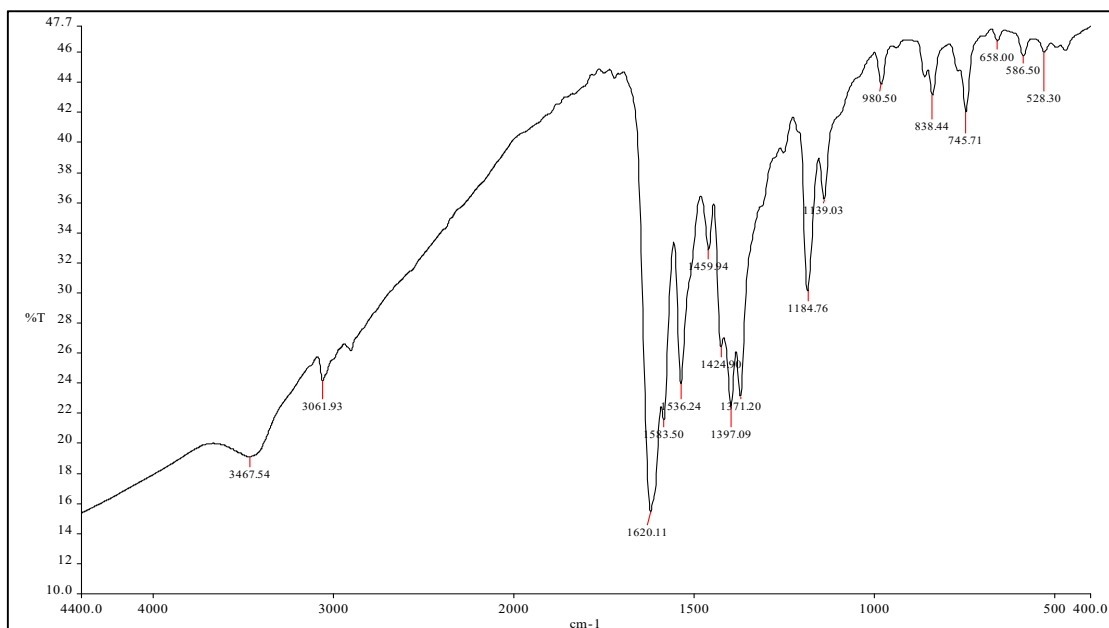
Mitochondria are the primary source of the amplification of reactive oxygen species (ROS) [6] production in mammalian cells, and it plays a major part in the stimulation of apoptosis in a variety of cells. To evaluate the intercellular mtROS, we incubated the complex **1** treated cells with 10  $\mu$ M H2DCFH-DA (2', 7'-dichlorofluorescein diacetate) at 37 °C for 25 min in the presence of Mitotracker-red, before analysis by confocal microscopy (FV 10i, Olympus, Japan) [7].

### ***DNA Fragmentation Assay***

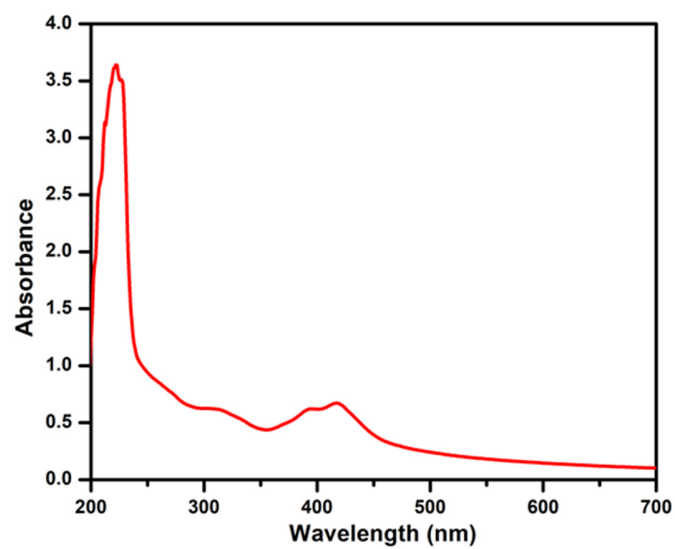
Cells were treated with complex **1** for 24 h and the resultant fragmented DNA was measured using commercially available kits according to the manufacturer protocol [8].

### ***Statistical Analysis***

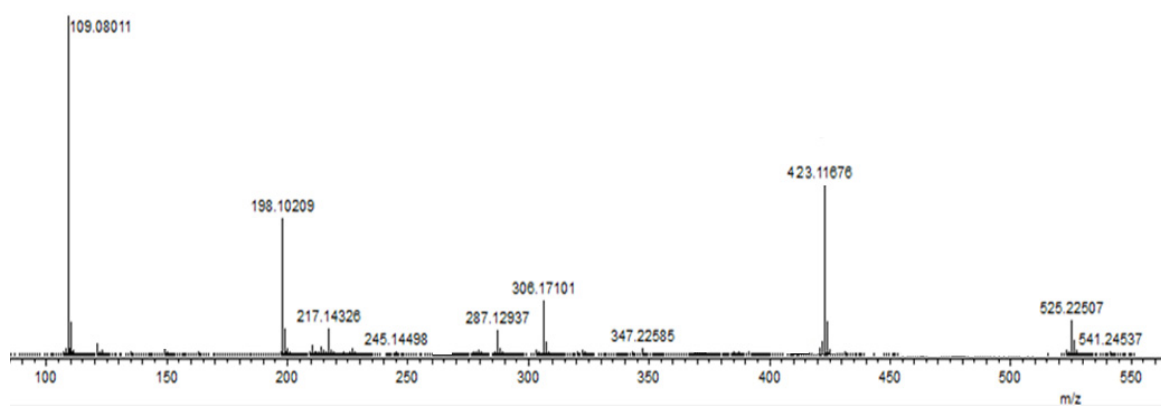
Results were represented as mean  $\pm$  SEM of multiple data points. Statistical significance in deference was calculated by the analysis of variance (ANOVA) using OriginPro (version 8.0) software.



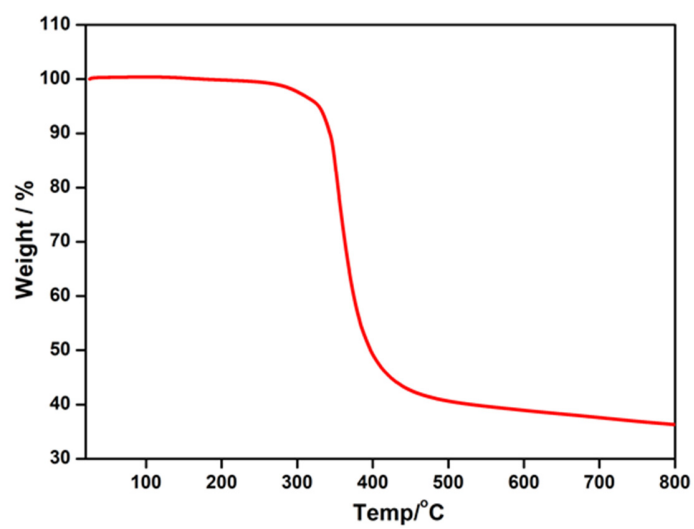
**Figure S1: IR spectrum of complex 1**



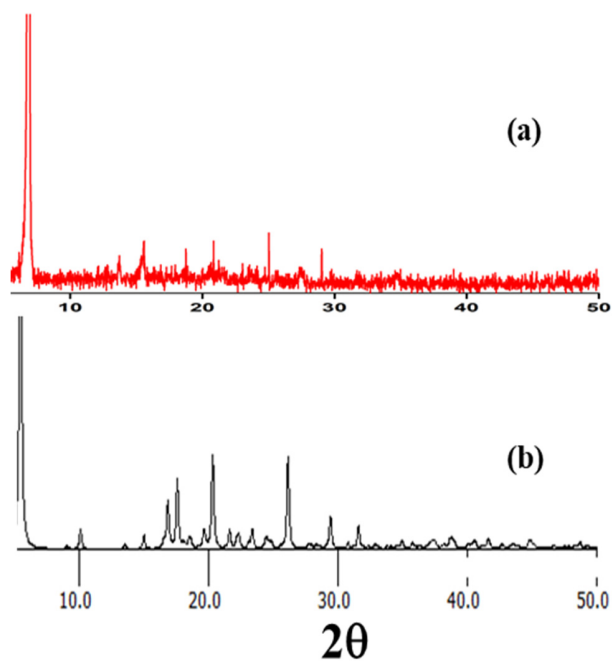
**Figure S2.** UV-Vis spectrum of complex **1**



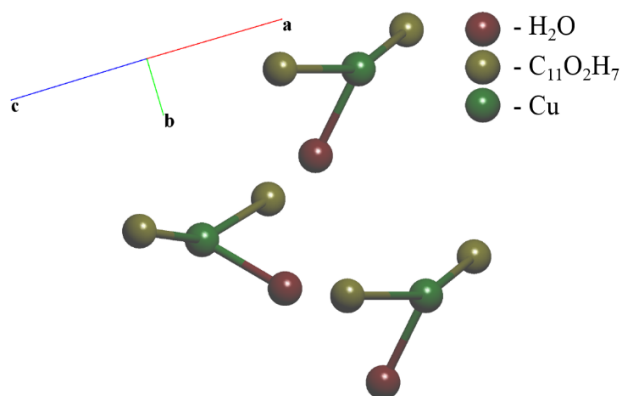
**Figure S3.** ToF-MS spectrum of complex **1**



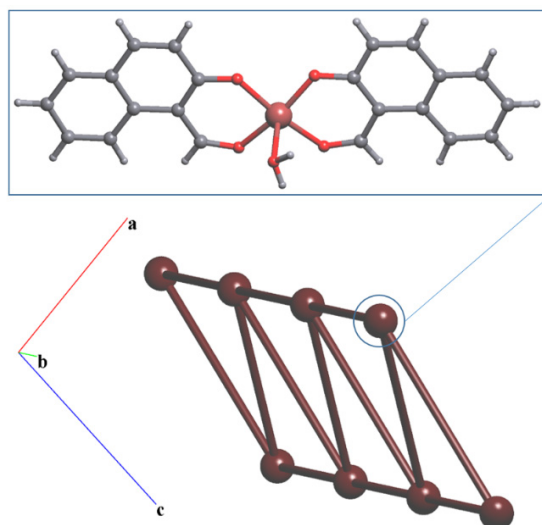
**Figure S4.** TGA pattern of complex **1**



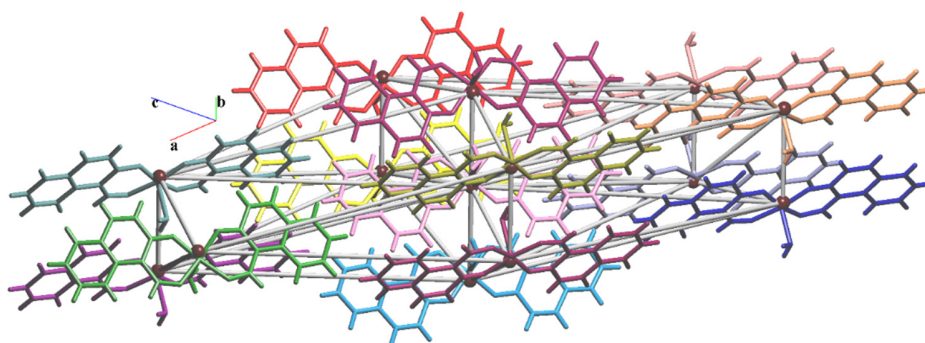
**Figure S5.** PXRD patterns (a) as-synthesized and (b) simulated of complex **1**



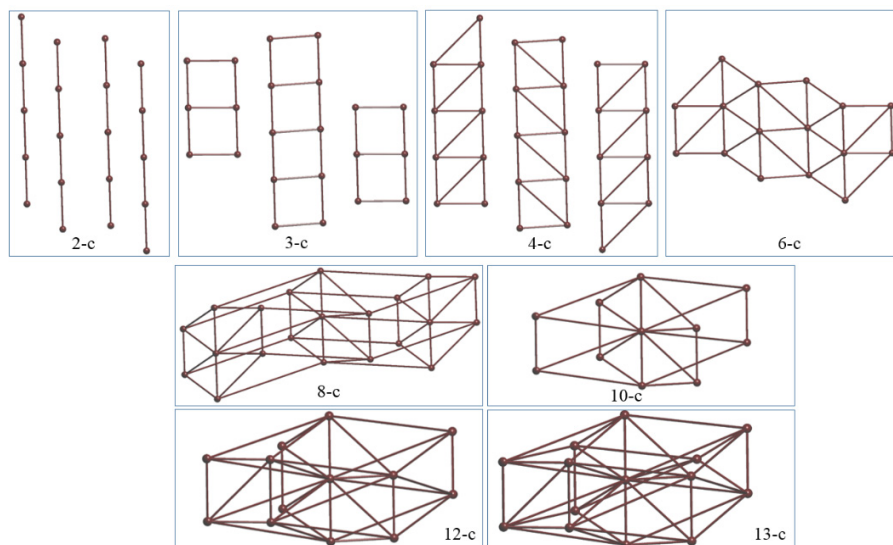
**Figure S6.** Standard representation of molecular complexes in the crystal.



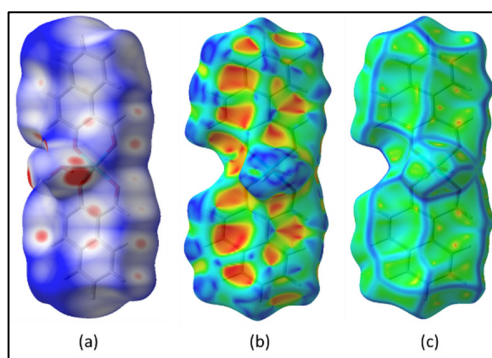
**Figure S7.** The 4-nodal net of **(3,6)(1,2)** topological type obtained within standard representation of H-bonded molecular MOFs.



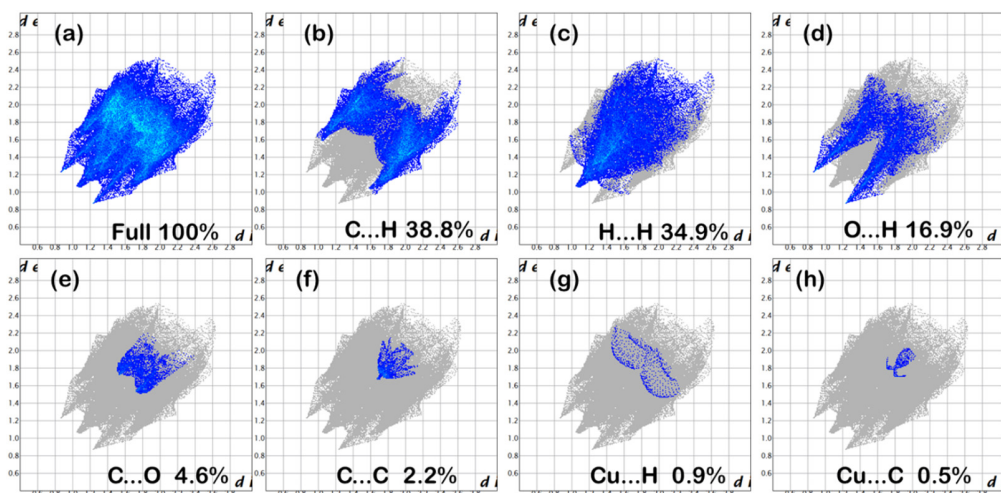
**Figure S8.** The combined atomic and 13-c simplified nets with 13T5 topology.



**Figure S9.** Order of the subnets that describe the packing of the structure on different levels of  $\Omega_i$ .



**Figure S10.** Representation of Hirshfeld surface mapped over (a)  $d_{\text{norm}}$ , (b) Shape-index, and (c) curvedness of the complex **1**



**Figure S11.** Representation of 2D fingerprint plot of complex **1**

**Table S1.** Details of the receptors used in this docking study

Name of receptor	PDB ID	size of grid			center of grid		
		x	y	z	x	y	z
DNA	1BNA	20	26	42	14.78	20.976	8.807

**Table S2.** Important bond lengths and bond angles for complex **1**

#### Bond Length (Å)

Atom	Atom	Length/Å	Atom	Atom	Length/Å
Cu1	O1	1.904(5)	C7	C8	1.386(10)
Cu1	O2	1.955(5)	C8	C9	1.413(10)
Cu1	O3	1.902(5)	C9	C10	1.460(9)
Cu1	O4	1.938(5)	C10	C11	1.424(10)
Cu1	O5	2.255(5)	C12	C13	1.439(10)
O1	C1	1.294(8)	C12	C21	1.426(10)
O2	C11	1.256(9)	C13	C14	1.339(10)
O3	C12	1.290(8)	C14	C15	1.432(10)
O4	C22	1.250(9)	C15	C16	1.406(10)
C1	C2	1.432(10)	C15	C20	1.422(10)
C1	C10	1.419(10)	C16	C17	1.393(11)
C2	C3	1.343(10)	C17	C18	1.383(11)
C3	C4	1.437(10)	C18	C19	1.374(10)



C4	C5	1.396(10)	C19	C20	1.425(10)
C4	C9	1.423(10)	C20	C21	1.456(10)
C5	C6	1.361(11)	C21	C22	1.427(10)
C6	C7	1.419(11)			

### Bond Angles (°)

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
O2	Cu1	O1	90.9(2)	C8	C9	C4	116.5(6)
O3	Cu1	O1	83.8(2)	C10	C9	C4	119.1(6)
O3	Cu1	O2	167.4(2)	C10	C9	C8	124.4(6)
O4	Cu1	O1	171.7(2)	C9	C10	C1	119.6(6)
O4	Cu1	O2	92.30(19)	C11	C10	C1	120.4(6)
O4	Cu1	O3	91.44(19)	C11	C10	C9	120.0(6)
O5	Cu1	O1	98.0(2)	C10	C11	O2	128.5(6)
O5	Cu1	O2	88.8(2)	C13	C12	O3	116.3(6)
O5	Cu1	O3	103.3(2)	C21	C12	O3	124.8(6)
O5	Cu1	O4	89.8(2)	C21	C12	C13	118.9(6)
C1	O1	Cu1	130.1(5)	C14	C13	C12	121.0(7)
C11	O2	Cu1	126.0(4)	C15	C14	C13	122.5(7)
C12	O3	Cu1	128.2(5)	C16	C15	C14	120.6(7)
C22	O4	Cu1	125.9(4)	C20	C15	C14	118.8(6)
C2	C1	O1	117.2(6)	C20	C15	C16	120.6(7)
C10	C1	O1	123.9(6)	C17	C16	C15	120.8(7)
C10	C1	C2	118.9(6)	C18	C17	C16	118.8(6)
C3	C2	C1	121.5(7)	C19	C18	C17	121.9(7)
C4	C3	C2	122.0(7)	C20	C19	C18	121.1(7)
C5	C4	C3	120.9(7)	C19	C20	C15	116.8(6)
C9	C4	C3	118.7(6)	C21	C20	C15	119.1(6)
C9	C4	C5	120.4(7)	C21	C20	C19	124.0(6)
C6	C5	C4	122.3(7)	C20	C21	C12	119.6(6)
C7	C6	C5	118.9(7)	C22	C21	C12	119.8(6)
C8	C7	C6	119.5(7)	C22	C21	C20	120.6(6)
C9	C8	C7	122.5(7)	C21	C22	O4	128.0(6)

**Table S3.** H-bonding details of complex **1**

S. No	Donor --- H...Acceptor	D - H	H...A	D...A	D-H...A
<b>1</b>	O(5) --H(5A) ..O(1)	0.85	2.50	3.068(7)	125
<b>2</b>	O(5) --H(5A) ..O(3)	0.85	2.20	3.036(7)	167'

3	O(5) --H(5B) ..O(1)	0.85	2.20	2.838(8)	132
4	C(19) --H(19) ..O(2)	0.93	2.60	3.468(8)	156

**Table S4.** Multilevel analysis of molecular complex packing as monomer.

Nº	Node degrees	$\Omega_i$ , %	Dimensionality of net	Topological type
1	2-c	14.15	1D [010]	2C1
2	3-c	13.11	1D [010]	(4,4)(0,2)
3	4-c	12.45	1D [010]	(3,6)(1,2)
4	6-c	11.70	2D (100)	<b>hxl</b>
5	8-c	4.18	3D	<b>hex</b>
6	10-c	3.53	3D	10T1393
7	12-c	3.21	3D	12T25
8	13-c	0.90	3D	13T5

## References

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# CHECK CIF REPORT

## checkCIF/PLATON report

Structure factors have been supplied for datablock(s) AC2N\_0m\_a  
THIS REPORT IS FOR GUIDANCE ONLY. IF USED AS PART OF A REVIEW PROCEDURE FOR PUBLICATION, IT SHOULD NOT REPLACE THE EXPERTISE OF AN EXPERIENCED CRYSTALLOGRAPHIC REFEREE.

No syntax errors found. CIF dictionary Interpreting this report

### Datablock: AC2N\_0m\_a

Bond precision: C-C = 0.0098 Å Wavelength=0.71073

Cell: a=16.1069(14) b=5.4478(5) c=19.531(2)

alpha=90 beta=95.936(3) gamma=90

Temperature: 100 K

Calculated Reported

Volume 1704.6(3) 1704.6(3)

Space group P 21/c P 1 21/c 1

Hall group -P 2ybc -P 2ybc

Moiety formula C22 H16 Cu O5 C22 H16 Cu O5

Sum formula C22 H16 Cu O5 C22 H16 Cu O5

Mr 423.90 423.91

Dx, g cm<sup>-3</sup> 1.652 1.652

Z 4 4

Mu (mm<sup>-1</sup>) 1.315 1.315

F000 868.0 869.8

F000' 869.66

h, k, lmax 19, 6, 23 19, 6, 22

Nref 3029 3017

Tmin, Tmax 0.619, 0.789

Tmin' 0.585

Correction method= Not given

Data completeness= 0.996 Theta(max)= 25.050

R(reflections)= 0.0731( 2465)

wR2(reflections)=

0.2262( 3017)

S = 1.110 Npar= 254

The following ALERTS were generated. Each ALERT has the format

**test-name ALERT alert-type alert-level.**

Click on the hyperlinks for more details of the test.

#### Alert level A

DIFF003\_ALERT\_1\_A \_diffrn\_measurement\_device\_type is missing

Diffractionmeter make and type. Replaces \_diffrn\_measurement\_type.

PLAT058\_ALERT\_1\_A Maximum Transmission Factor Missing ..... ?

PLAT059\_ALERT\_1\_A Minimum Transmission Factor Missing ..... ?

PLAT183\_ALERT\_1\_A Missing \_cell\_measurement\_reflns\_used Value .... Please Do !

PLAT184\_ALERT\_1\_A Missing \_cell\_measurement\_theta\_min Value ..... Please Do !

PLAT185\_ALERT\_1\_A Missing \_cell\_measurement\_theta\_max Value ..... Please Do !

#### Alert level B

PLAT107\_ALERT\_2\_B Twinning Matrix Invalid in Centrosymmetric SPGR ? Check

PLAT930\_ALERT\_2\_B FCF-based Twin Law ( 1 0-2) Est.d BASF 0.28 Check

#### Alert level C

ABSTY02\_ALERT\_1\_C An \_exptl\_absorpt\_correction\_type has been given without a literature citation. This should be contained in the

\_expt1\_absorpt\_process\_details field.

Absorption correction given as multi-scan

DIFMX02\_ALERT\_1\_C The maximum difference density is > 0.1\*ZMAX\*0.75

The relevant atom site should be identified.

PLAT094\_ALERT\_2\_C Ratio of Maximum / Minimum Residual Density .... 2.74 Report

PLAT097\_ALERT\_2\_C Large Reported Max. (Positive) Residual Density 2.45 eA-3

PLAT341\_ALERT\_3\_C Low Bond Precision on C-C Bonds ..... 0.00979 Ang.

PLAT906\_ALERT\_3\_C Large K Value in the Analysis of Variance ..... 3.536 Check

PLAT911\_ALERT\_3\_C Missing FCF Refl Between Thmin & STh/L= 0.596 9 Report

#### Alert level G

PLAT068\_ALERT\_1\_G Reported F000 Differs from Calcd (or Missing)... Please Check

PLAT073\_ALERT\_1\_G H-atoms ref, but \_hydrogen\_treatment Reported as constr Check

PLAT083\_ALERT\_2\_G SHELXL Second Parameter in WGHT Unusually Large 22.54 Why ?

PLAT769\_ALERT\_4\_G CIF Embedded explicitly supplied scattering data Please Note

PLAT794\_ALERT\_5\_G Tentative Bond Valency for Cu1 (II) . 2.27 Info

PLAT870\_ALERT\_4\_G ALERTS Related to Twinning Effects Suppressed .. ! Info

PLAT909\_ALERT\_3\_G Percentage of I>2sig(I) Data at Theta(Max) Still 67% Note

PLAT910\_ALERT\_3\_G Missing # of FCF Reflection(s) Below Theta(Min). 3 Note

PLAT913\_ALERT\_3\_G Missing # of Very Strong Reflections in FCF .... 1 Note

PLAT931\_ALERT\_5\_G CIFcalcFCF Twin Law ( 1 0-2) Est.d BASF 0.28 Check

PLAT933\_ALERT\_2\_G Number of OMIT Records in Embedded .res File ... 2 Note

PLAT941\_ALERT\_3\_G Average HKL Measurement Multiplicity ..... 4.4 Low

PLAT960\_ALERT\_3\_G Number of Intensities with I < - 2\*sig(I) ... 15 Check

PLAT982\_ALERT\_1\_G The Cu-f' = 0.3413 Deviates from IT-value = 0.3201 Check

PLAT983\_ALERT\_1\_G The Cu-f" = 1.2895 Deviates from IT-Value = 1.2651 Check

6 **ALERT level A** = Most likely a serious problem - resolve or explain

2 **ALERT level B** = A potentially serious problem, consider carefully

7 **ALERT level C** = Check. Ensure it is not caused by an omission or oversight

15 **ALERT level G** = General information/check it is not something unexpected

12 ALERT type 1 CIF construction/syntax error, inconsistent or missing data

6 ALERT type 2 Indicator that the structure model may be wrong or deficient

8 ALERT type 3 Indicator that the structure quality may be low

2 ALERT type 4 Improvement, methodology, query or suggestion

2 ALERT type 5 Informative message, check

It is advisable to attempt to resolve as many as possible of the alerts in all categories. Often the minor alerts point to easily fixed oversights, errors and omissions in your CIF or refinement strategy, so attention to these fine details can be worthwhile. In order to resolve some of the more serious problems it may be necessary to carry out additional measurements or structure refinements. However, the purpose of your study may justify the reported deviations and the more serious of these should normally be commented upon in the discussion or experimental section of a paper or in the "special\_details" fields of the CIF. checkCIF was carefully designed to identify outliers and unusual parameters, but every test has its limitations and alerts that are not important in a particular case may appear. Conversely, the absence of alerts does not guarantee there are no aspects of the results needing attention. It is up to the individual to critically assess their own results and, if necessary, seek expert advice.

#### Publication of your CIF in IUCr journals

A basic structural check has been run on your CIF. These basic checks will be run on all CIFs submitted for publication in IUCr journals (*Acta Crystallographica*, *Journal of Applied Crystallography*, *Journal of Synchrotron Radiation*); however, if you intend to submit to *Acta Crystallographica Section C* or *E* or *IUCrData*, you should make sure that full publication checks are run on the final version of your CIF prior to submission.

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Please refer to the *Notes for Authors* of the relevant journal for any special instructions relating to CIF

submission.

### Validation response form

Please find below a validation response form (VRF) that can be filled in and pasted into your CIF.

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# start Validation Reply Form
_vrf_DIFF003_AC2N_0m_a
;
PROBLEM: _diffrn_measurement_device_type is missing
RESPONSE: ...
;
_vrf_ABSTY02_AC2N_0m_a
;
PROBLEM: An _exptl_absorpt_correction_type has been given without
RESPONSE: ...
;
_vrf_DIFMX02_AC2N_0m_a
;
PROBLEM: The maximum difference density is > 0.1*ZMAX*0.75
RESPONSE: ...
;
_vrf_PLAT058_AC2N_0m_a
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PROBLEM: Maximum Transmission Factor Missing ..... ?
RESPONSE: ...
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PROBLEM: Missing _cell_measurement_theta_max Value ..... Please Do !
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;
_vrf_PLAT094_AC2N_0m_a
;
PROBLEM: Ratio of Maximum / Minimum Residual Density .... 2.74 Report
RESPONSE: ...
;
_vrf_PLAT097_AC2N_0m_a
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PROBLEM: Large Reported Max. (Positive) Residual Density 2.45 eA-3
RESPONSE: ...
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_vrf_PLAT341_AC2N_0m_a
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PROBLEM: Low Bond Precision on C-C Bonds ..... 0.00979 Ang.
RESPONSE: ...
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PROBLEM: Large K Value in the Analysis of Variance ..... 3.536 Check
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RESPONSE: ...  
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_vrf_PLAT911_AC2N_0m_a  
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PROBLEM: Missing FCF Refl Between Thmin & STh/L= 0.596 9 Report  
RESPONSE: ...  
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# end Validation Reply Form  
PLATON version of 13/07/2021; check.def file version of 13/07/2021  
Datablock AC2N_0m_a - ellipsoid plot
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