Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Accompanying the manuscript

Protein Engineering with Artificial Chemical Nucleases

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S-1: Preparation of the metal complex

Materials: Chemicals and reagents of analytical grade were purchased from Sigma-Aldrich Ireland and used without further purification.

Preparation of $[Cu(Phen)_2(H_2O)](NO_3)_2$ (**Cu-Phen**) was prepared according to the method reported by Prisecaru *et al*.[1] Briefly, copper(II) nitrate hemi(pentahidrate) (0.51 g, 2.1 mmol) and 1,10-phenanthroline monohydrate (0.85 g, 4.2 mmol) was refluxed in ethanol for 2 hours at 90 ^{o}C. On cooling to room temperature a green solid was filtrated, washed with a minimum amount of cold ethanol and then air-dried. Yield: 0.90 g (77 %). Blue crystals, suitable for X-ray analysis (**S-2**), were obtained upon slow evaporation over 14 days.

S-2: X-ray crystallography

Description.

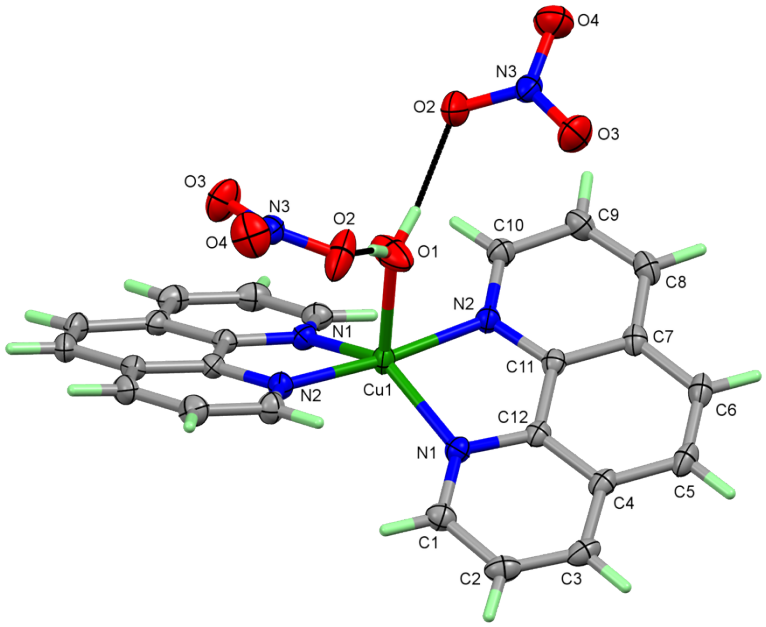

Figure S1. Perspective view of $[Cu(phen)_2(H_2O)](NO_3)_2$ drawn with 50% ADPs for the non-hydrogen atoms. A 2-fold axis runs through Cu1 and O1.

The structure of the discrete $[Cu(phen)_2(H_2O)](NO_3)_2$ complex is shown in Fig S1. A 2-fold rotation axis runs through Cu1 and O1 so that the asymmetric unit comprises one phen ligand and one nitrate anion, along with a half-occupancy copper ion and water molecule. The copper ion is 5-coordinate, with geometry intermediate between square pyramidal and trigonal bipyramidal (τ = 0.53 (Addison et al., 1984)). The coordinated water molecule is hydrogen bonded to the nitrate anion (O1--- O2 2.7150 (18) Å).

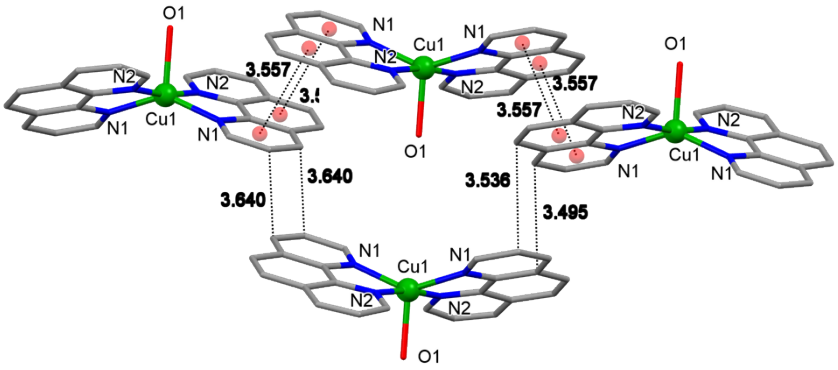

Figure S2. π-stacking interactions

The complexes are packed into columns running parallel to the b axis by □-stacking interactions (Figures S2 & S3).

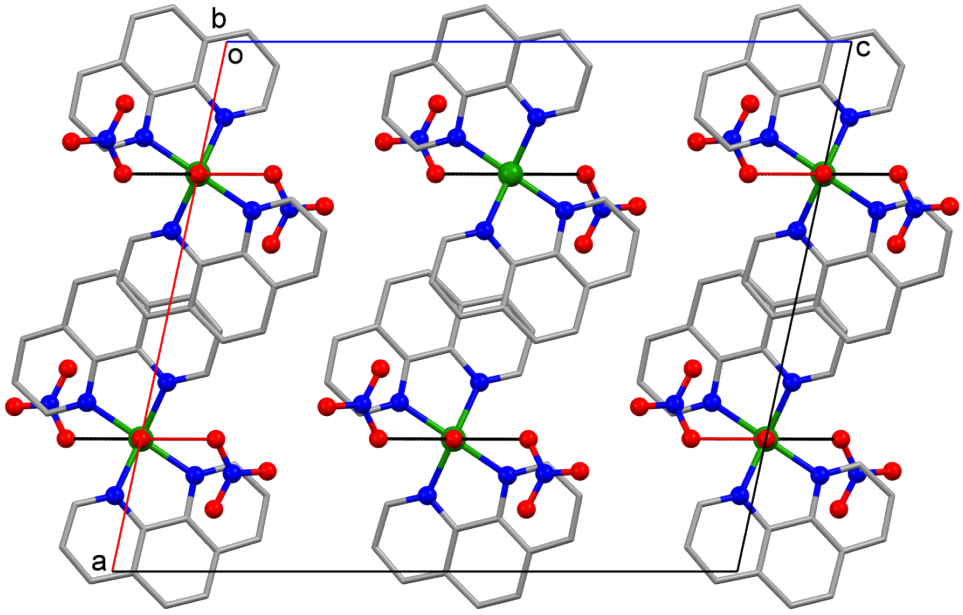

Figure S3. Packing diagram viewed down the b axis.

The complex was found in the CSD (version 5.36, Feb 2015; Allen, 2002) a number of times; the reported cells are shown in Table S1. In the present case, the *I*2/a space group was chosen over *C*2/c to reduce the value of the *β* angle but the reduced call is the same as the earlier reports, except that by Szpakolski et al. (2010) which appears to be a genuinely different polymorph. However, in each case the basic structure and packing are very similar.

Table S1. Comparison of reported unit cells for $[Cu(phen)_2(H_2O)](NO_3)_2$.

a (Å)	*b* (Å)	*C* (Å)	α (º)	β (º)	γ (º)	Vol (Å^3)	Space group	*R*1 (%) *T* (K)	Ref
16.6086(15)	7.1589(6)	19.141(3)	90	102.179(1)	90	2224.63	*I*2/a	2.78 (150)	This work
19.375(5)	7.469(5)	15.775(5)	90	100.782(5)	90	2242.53	*C*2/c	2.67 (130)	Szpakolski et al., 2010
22.58(2)	7.23(1)	16.59(2)	90	123.6(1)	90	2255.86	*C*2/c	11.8 (293)	Nakai et al., 1975
22.642(5)	7.2810(15)	16.614(3)	90	123.76(3)	90	2277.07	*C*2/c	5.26 (RT)	Zhou at al., 2011
22.640(5)	7.254(2)	16.604(3)	90	123.76(2)	90	2267.06	*C*c	4.24 (293)	Catalan et al., 1995
7.0836(3)	11.7898(3)	14.2951(4)	78.079(2)	79.862(3)	73.782(3)	1112.68	*P*-1	5.76 (150)	Boutebdja et al., 2014

Experimental

Data were collected at 150(2) K with Mo *K*α radiation using a Bruker-Nonius APEX-II CCD diffractometer. Crystal data, data collection and structure refinement details for $[Cu(phen)_2(H_2O)](NO_3)_2$ are summarized is the tables below. All hydrogen atoms bonded to carbon were added in calculated positions with C−H = 0.95 Å and $U_{iso}(H) = 1.2U_{eq}(C)$. The one independent hydrogen atom for the hydrogen bonded water molecule was located from the difference map and its coordinates refined under geometric constraints with $U_{iso}(H) = 1.5U_{eq}(O)$.

Computer programs: Data collection: Bruker *APEX2*; cell refinement: Bruker *SAINT*; data reduction: Bruker *SAINT*; program(s) used to solve structure: SHELXT (Sheldrick 2015a); program(s) used to refine structure: *SHELXL2014*/6 (Sheldrick, 2015b); molecular graphics: Bruker *SHELXTL*, *Mercury* (Maccrae *et al.*, 2006); software used to prepare material for publication: *publCIF* (Westrip, 2010).

CCDC 1403896 contains the supplementary crystallographic data for this structure. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

Table S2. Crystal data.

$C_{24}H_{18}CuN_4O{\cdot}2(NO_3)$	$F(000) = 1156$
$M_r = 565.98$	$D_x = 1.690$ Mg m^{-3}
Monoclinic, *I*2/*a*	Mo *K*α radiation, λ = 0.71073 Å
a = 16.6086 (15) Å	Cell parameters from 6719 reflections
b = 7.1589 (6) Å	θ = 2.2–29.4°
c = 19.141 (3) Å	μ = 1.05 mm^{-1}
β = 102.179 (1)°	*T* = 150 K
V = 2224.7 (4) Å^3	Lozenge, green
Z = 4	0.28 × 0.25 × 0.09 mm

Data collection

Bruker APEX 2 CCD diffractometer	2884 reflections with $I > 2\sigma(I)$
Radiation source: fine-focus sealed tube	$R_{int} = 0.018$
ω rotation with narrow frames scans	$\theta_{max} = 29.5°$, $\theta_{min} = 2.2°$

Absorption correction: multi-scan *SADABS* v2012/1, (Krause et al., 2015)	h = -22→22
T_{min} = 0.656, T_{max} = 0.746	k = -9→9
12031 measured reflections	l = -26→26
3092 independent reflections	

Refinement

Refinement on F^2	Primary atom site location: dual
Least-squares matrix: full	Secondary atom site location: difference Fourier map
$R[F^2 > 2\sigma(F^2)]$ = 0.028	Hydrogen site location: mixed
$wR(F^2)$ = 0.080	H atoms treated by a mixture of independent and constrained refinement
S = 1.10	$w = 1/[\sigma^2(F_o^2) + (0.0413P)^2 + 2.0021P]$ where $P = (F_o^2 + 2F_c^2)/3$
3092 reflections	$(\Delta/\sigma)_{max} < 0.001$
176 parameters	$\Delta\rangle_{max}$ = 0.48 e Å^{-3}
0 restraints	$\Delta\rangle_{min}$ = -0.20 e Å^{-3}

Special details

Geometry. All esds (except the esd in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell esds are taken into account individually in the estimation of esds in distances, angles and torsion angles; correlations between esds in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell esds is used for estimating esds involving l.s. planes.

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å^2)

	x	y	z	U_{iso}*/U_{eq}
Cu1	0.7500	0.60178 (3)	0.5000	0.02030 (8)
O1	0.7500	0.3012 (3)	0.5000	0.0457 (5)
H1A	0.7446 (18)	0.238 (4)	0.4653 (13)	0.069*
N1	0.64316 (7)	0.69821 (16)	0.52232 (6)	0.0200 (2)
N2	0.68110 (7)	0.58253 (16)	0.40086 (6)	0.0201 (2)
C1	0.62633 (9)	0.7642 (2)	0.58286 (7)	0.0236 (3)
H1	0.6701	0.7807	0.6232	0.028*
C2	0.54584 (9)	0.8100 (2)	0.58866 (8)	0.0271 (3)

H2	0.5358	0.8553	0.6327	0.033*
C3	0.48173 (9)	0.7895 (2)	0.53122 (8)	0.0262 (3)
H3	0.4271	0.8187	0.5352	0.031*
C4	0.49782 (8)	0.72449 (19)	0.46593 (8)	0.0220 (3)
C5	0.43526 (8)	0.6982 (2)	0.40247 (9)	0.0266 (3)
H5	0.3798	0.7294	0.4029	0.032*
C6	0.45418 (9)	0.6295 (2)	0.34193 (8)	0.0261 (3)
H6	0.4115	0.6098	0.3010	0.031*
C7	0.53772 (8)	0.58617 (18)	0.33867 (7)	0.0214 (3)
C8	0.56131 (9)	0.5153 (2)	0.27728 (7)	0.0256 (3)
H8	0.5209	0.4895	0.2353	0.031*
C9	0.64301 (9)	0.4839 (2)	0.27865 (8)	0.0264 (3)
H9	0.6598	0.4387	0.2373	0.032*
C10	0.70167 (9)	0.5191 (2)	0.34149 (7)	0.0240 (3)
H10	0.7581	0.4970	0.3418	0.029*
C11	0.60022 (8)	0.61580 (17)	0.39950 (7)	0.0186 (2)
C12	0.57981 (8)	0.68133 (18)	0.46438 (7)	0.0189 (2)
N3	0.68465 (7)	0.03411 (17)	0.34185 (7)	0.0246 (2)
O2	0.74951 (8)	0.0990 (2)	0.37991 (9)	0.0494 (4)
O3	0.61735 (8)	0.0568 (2)	0.35893 (8)	0.0415 (3)
O4	0.68915 (9)	-0.0521 (2)	0.28652 (7)	0.0435 (3)

Atomic displacement parameters (Å^2)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
Cu1	0.01397 (12)	0.02876 (14)	0.01747 (12)	0.000	0.00173 (8)	0.000
O1	0.0738 (14)	0.0238 (8)	0.0314 (9)	0.000	-0.0072 (9)	0.000
N1	0.0189 (5)	0.0207 (5)	0.0206 (5)	0.0000 (4)	0.0048 (4)	0.0008 (4)
N2	0.0173 (5)	0.0233 (5)	0.0193 (5)	0.0006 (4)	0.0028 (4)	-0.0002 (4)
C1	0.0260 (6)	0.0229 (6)	0.0227 (6)	-0.0010 (5)	0.0070 (5)	-0.0006 (5)
C2	0.0324 (7)	0.0235 (7)	0.0294 (7)	0.0019 (5)	0.0152 (6)	0.0000 (5)
C3	0.0237 (6)	0.0220 (6)	0.0365 (8)	0.0037 (5)	0.0141 (6)	0.0032 (5)
C4	0.0186 (6)	0.0185 (6)	0.0295 (7)	0.0010 (4)	0.0066 (5)	0.0044 (5)
C5	0.0150 (6)	0.0260 (7)	0.0377 (8)	0.0013 (5)	0.0034 (5)	0.0067 (6)
C6	0.0177 (6)	0.0266 (7)	0.0306 (7)	-0.0013 (5)	-0.0025 (5)	0.0063 (5)
C7	0.0192 (6)	0.0205 (6)	0.0228 (6)	-0.0022 (4)	0.0009 (5)	0.0044 (5)
C8	0.0266 (7)	0.0277 (7)	0.0199 (6)	-0.0051 (5)	-0.0012 (5)	0.0015 (5)
C9	0.0296 (7)	0.0285 (7)	0.0209 (6)	-0.0030 (6)	0.0047 (5)	-0.0027 (5)

C10	0.0215 (6)	0.0280 (7)	0.0228 (6)	0.0012 (5)	0.0050 (5)	-0.0016 (5)
C11	0.0170 (6)	0.0178 (6)	0.0203 (6)	-0.0005 (4)	0.0022 (4)	0.0026 (4)
C12	0.0176 (6)	0.0167 (6)	0.0225 (6)	-0.0001 (4)	0.0043 (5)	0.0024 (4)
N3	0.0206 (5)	0.0217 (5)	0.0310 (6)	0.0020 (4)	0.0046 (5)	0.0010 (5)
O2	0.0221 (6)	0.0468 (8)	0.0733 (10)	0.0008 (5)	-0.0037 (6)	-0.0247 (7)
O3	0.0245 (6)	0.0536 (8)	0.0495 (8)	-0.0003 (5)	0.0147 (5)	-0.0070 (6)
O4	0.0475 (8)	0.0505 (8)	0.0332 (6)	0.0092 (6)	0.0098 (6)	-0.0082 (6)

Geometric parameters (Å, º)

Cu1—N2^{i}	2.0033 (12)	C4—C12	1.4027 (18)
Cu1—N2	2.0033 (12)	C4—C5	1.435 (2)
Cu1—N1	2.0317 (11)	C5—C6	1.355 (2)
Cu1—N1^{i}	2.0317 (11)	C6—C7	1.436 (2)
Cu1—O1	2.1520 (18)	C7—C11	1.4030 (18)
N1—C1	1.3344 (17)	C7—C8	1.409 (2)
N1—C12	1.3636 (17)	C8—C9	1.370 (2)
N2—C10	1.3334 (17)	C9—C10	1.4018 (19)
N2—C11	1.3591 (17)	C11—C12	1.4334 (18)
C1—C2	1.403 (2)	N3—O3	1.2395 (17)
C2—C3	1.367 (2)	N3—O4	1.2416 (18)
C3—C4	1.411 (2)	N3—O2	1.2552 (17)
N2^{i}—Cu1—N2	172.11 (7)	C12—C4—C5	119.09 (13)
N2^{i}—Cu1—N1	100.01 (5)	C3—C4—C5	123.68 (13)
N2—Cu1—N1	82.70 (5)	C6—C5—C4	120.96 (13)
N2^{i}—Cu1—N1^{i}	82.70 (5)	C5—C6—C7	121.06 (13)
N2—Cu1—N1^{i}	100.01 (5)	C11—C7—C8	117.34 (12)
N1—Cu1—N1^{i}	140.27 (7)	C11—C7—C6	118.93 (13)
N2^{i}—Cu1—O1	86.06 (3)	C8—C7—C6	123.73 (13)
N2—Cu1—O1	86.06 (3)	C9—C8—C7	119.44 (13)
N1—Cu1—O1	109.86 (3)	C8—C9—C10	119.55 (13)
N1^{i}—Cu1—O1	109.86 (3)	N2—C10—C9	122.40 (13)
C1—N1—C12	118.07 (11)	N2—C11—C7	122.99 (12)
C1—N1—Cu1	130.93 (10)	N2—C11—C12	117.02 (11)
C12—N1—Cu1	110.92 (8)	C7—C11—C12	119.98 (12)
C10—N2—C11	118.25 (12)	N1—C12—C4	123.31 (12)
C10—N2—Cu1	129.26 (10)	N1—C12—C11	116.79 (11)

C11—N2—Cu1	111.77 (9)	C4—C12—C11	119.90 (12)
N1—C1—C2	121.93 (13)	O3—N3—O4	120.67 (14)
C3—C2—C1	120.30 (13)	O3—N3—O2	120.64 (14)
C2—C3—C4	119.12 (13)	O4—N3—O2	118.69 (14)
C12—C4—C3	117.23 (13)		

Symmetry code: (i) -*x*+3/2, *y*, -*z*+1.

Hydrogen-bond geometry (Å, º)

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
O1—H1*A*···O2	0.79 (3)	1.93 (3)	2.7150 (18)	171 (3)

Document origin: *publCIF* [Westrip, S. P. (2010). *J. Apply. Cryst.*, **43**, 920-925].

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S-3: Chemical nuclease activity

Reactions were carried out according to the literature procedure previously reported by this group.[2] Briefly, in a total volume of 20 μL using 80 mM HEPES buffer (Fisher) at pH 7.2 with 25 mM NaCl, an aliquot of the stock complex (prepared in DMF) was mixed with 1μg of F8 DNA and1 μL of 20 mM Na-L-ascorbate. Samples were incubated at 37 ^{o}C for 45 min before being quenched with 100 μM EDTA, 100 μM neocuproine and 6x loading dye (Fermentas), containing 10 mMTris-HCl (pH 7.6), 0.03% bromophenol blue, 0.03% xylene cyanol, 60% glycerol and 60 mM EDTA, then loaded onto agarose gel (2%) containing 2.0 μL of GelRed^{TM} (10,000X). Electrophoresis was completed at 80 V for 1.5 h using a wide mini-sub cell (BioRad) in 1XTAE buffer (Millipore).

S-4: Digestion of F8 DNA and generation of recombinant scFV libraries

F8 DNA was digested using DNaseI (NEB). The reaction µL mixture containing 5 µL DNase I reaction buffer (10x), 5 ug F8 DNA, 20 µL H_2O was heated to 37°C before the addition of 1 µL DNase I and the reaction was carried out for 20 minutes at 37°C. Digested DNA was ethanol precipitated, re-suspended in H_2O and analysed by 2% gel electrophoresis.

Digested DNA was self annealed using primerless PCR, carried out for 45 cycles. This mixture contain 10 µL Red Taq buffer, 10 µL digested DNA, 1 µL Red Taq and 29 µL H_2O. The resulting PCR products were diluted 1 in 25 in fresh PCR reaction mix containing 35 µL H_2O 10 µL Red Taq reaction mixture, 2 µL re-annealed DNA, 1 µL red Taq and the scFv amplified using 1 µL of 10 µM CSC-F and 1 µL of 10 µM CSC-B primers. Initially 15 cycles were used to amplify from the re-annealed DNase I and **CuPhen** digested DNA. Due to low levels of 750 bp fragment recovery from the AMN digested DNA samples this was subsequently upped to 30 cycles to improve the DNA yield.

Table S3. Direct binding ELISA results against PSA.

Plate 1	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0758	0.052	0.0596	0.0545	0.0564	0.0592	0.0532	0.0668	0.0549	0.0677	0.0839	0.0998
B	0.0581	0.054	0.0676	0.0533	0.0521	0.0515	0.0564	0.0554	0.0523	0.0532	0.0697	2.6999
C	0.0583	0.063	0.0533	0.0549	0.0512	0.0531	0.0529	0.0687	0.0537	0.0688	0.0642	0.1385
D	0.055	0.0561	0.0886	1.8551	0.1417	0.1132	0.0518	0.0537	0.0514	0.0884	1.1085	0.6259
E	0.059	0.0668	0.0558	0.0596	0.0567	0.0531	0.348	0.081	0.0564	0.0553	0.0553	0.0536
F	0.0595	0.058	0.0516	0.0581	0.0603	0.0664	0.0578	0.3516	0.133	0.0764	0.0544	1.8962
G	0.0685	0.06	0.0521	0.0585	0.1348	0.122	0.0575	2.0665	1.2971	0.0805	0.0625	0.0637
H	0.0766	0.1203	2.2928	0.0831	0.0623	0.0614	0.0612	1.7718	0.2058	0.0777	0.0684	1.597

Plate 2	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0603	0.056	0.8575	0.0534	0.0623	0.0554	0.0621	0.0541	1.2773	0.0612	0.0538	1.9705
B	0.0471	1.0034	0.1229	0.0508	0.0495	0.0535	0.0573	0.0533	0.0547	0.0476	0.0564	0.0492
C	0.0562	0.0589	0.0567	0.0516	0.0524	0.1407	0.0719	0.0546	0.0567	0.0406	0.0508	0.0532
D	0.0628	0.053	0.0571	0.8486	0.0692	0.0639	0.1075	0.0586	0.0729	0.0506	0.0549	0.0666
E	0.0628	0.0797	0.0594	0.0515	0.0867	0.1846	0.0527	0.053	0.057	0.4078	0.0697	0.099
F	0.0532	0.0403	0.0803	0.0544	0.0498	0.0508	1.6401	2.1542	0.7624	0.0507	0.0515	0.0606
G	0.0567	0.056	0.0568	0.0586	0.105	0.0603	0.0628	0.0671	0.0595	0.061	0.0732	0.1443
H	0.0641	0.0606	0.0504	0.0856	0.0557	1.2489	0.0903	0.0567	0.0562	0.0541	0.055	0.0657

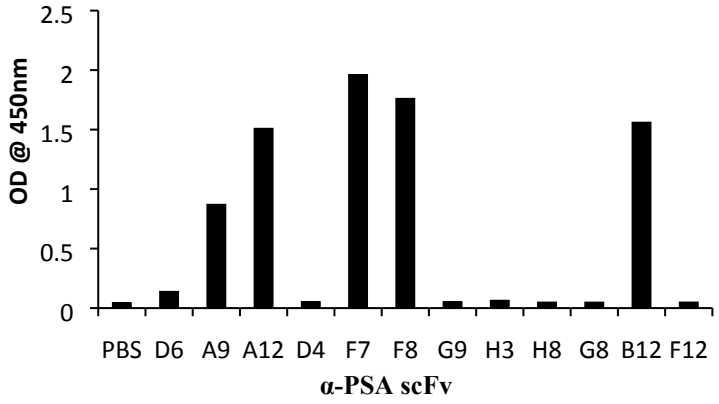

Figure S3. ELISA of α-PSA scFv clones against immobilised PSA. Detection was carried out using a HRP labelled anti-HA antibody. The assay was developed using 100 µl of TMB solution, stopped with 50 µl of 10% HCL and the absorbance read at 450nm.

Table S4: Properties of 10 sample sequenced clones from the DNase I library.

Clone no.	Unique sequences	Type of change	No. of AA changes
DNaseI 1	Yes	AA change	2 within
DNaseI 2	Yes	AA change	1 within (>20 at N-terminus)
DNaseI 3	Yes		2 within (>20 at N-terminus)
DNaseI 4	Yes		>12 at N-terminus, missing C-terminus
DNaseI 5	Yes	AA change	3 within (>20 at N-terminus)
DNaseI 6	No		
DNaseI 8	Yes	AA change	2 within
DNaseI 9	Yes	AA change	2 within
DNaseI 11	Yes	AA deletion	1 within

*Sfi*I (NEB) restriction digests were carried out to prepare Comb3XSS, DNaseI, and **CuPhen** scFv DNA samples for ligation. DNA and vector samples were ligated using T4 DNA ligase (NEB) and transformed into freshly prepared electrocompetent *E. coli* XL-1 blue cells. The resulting libraries were of sufficient number with empty vector backgrounds <10% (Figure S3).

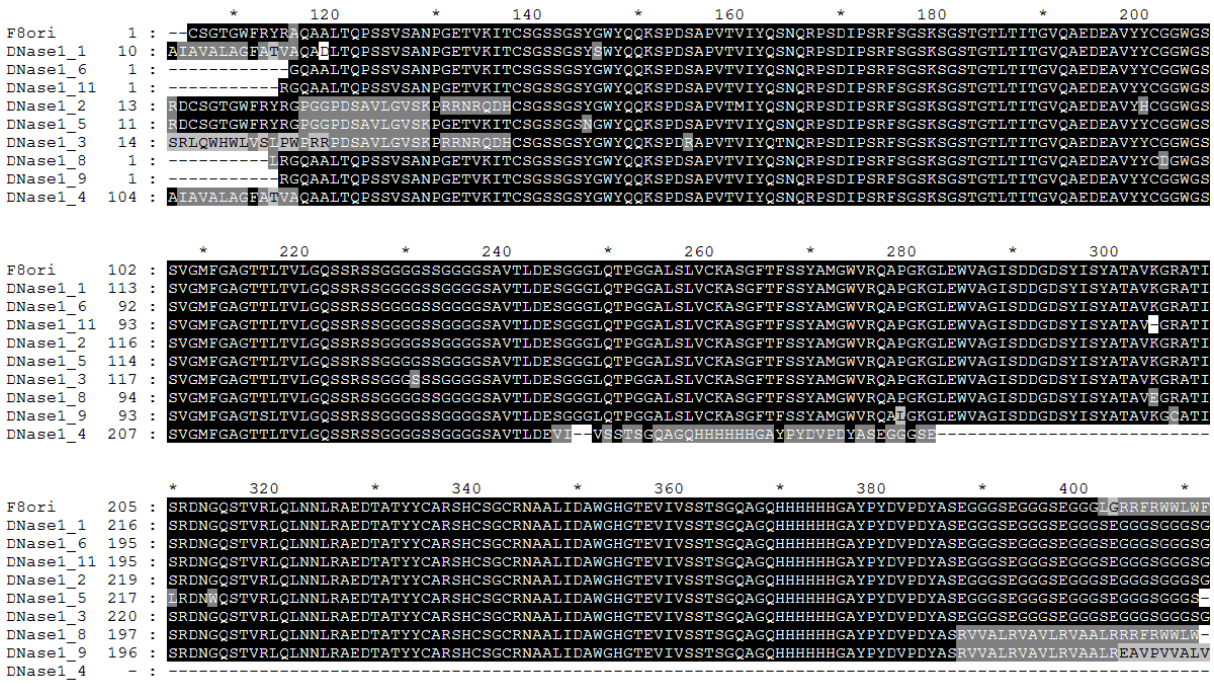

Figure S4: Protein alignment of sequencing results for 9 DNaseI digested library sample clones.

Ligation reactions	
DNase1 transformations)	1.4 µg pComb + 1 µg DNase1 scFv (Performed 4x 1 µl
Cu-Bis-Phen	0.7 µg pComb + 0.35 µg BIS scFv (Performed 5 x 1 µl transformations)
pComb	1.4 µg pComb (Performed 1 x 1 µl transformation)
Output Libraries	
DNaseI	1.56 x 10^7 (Background 0.96%)
Cu-Bis-Phen	5.26 x 10^7 (Background 0.18%)
pComb	3.75 x 10^4

Figure S4. Library ligation reactions and output library results from successful transformations.

Table S5: Properties of 12 sequenced sample clones from the **CuPhen** library.

Clone no.	Unique sequences	Type of change	No. of AA changes
CuPhen 1	No same as F8		
CuPhen 2	Yes	Deletions and insertions	
CuPhen 3	No same as F8		
CuPhen 4	Short read		
CuPhen 5	No same as F8		
CuPhen 6	No same as F8		
CuPhen 7	Yes	Deletions and insertions (7,9 and 10 similar)	
CuPhen 8	No same as F8		
CuPhen 9	Yes	Deletions and insertions (7,9 and 10 similar)	
CuPhen 10	Yes	Deletions and insertions (7,9 and 10 similar)	
CuPhen 11	No same as F8		
CuPhen 12	Yes	Deletions and insertions	

Table S6: ELISA screening of DNase I clones from panning output round 3. Positive wells are highlighted in yellow.

DNaseI Plate A	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0534	0.0747	0.6348	0.0507	0.0516	0.0521	0.0519	0.055	0.8066	0.0545	0.5516	0.0492
B	0.0553	0.0663	0.0615	0.058	0.737	0.0531	0.0558	0.053	0.0559	0.0549	0.0567	0.0528
C	0.0602	0.0604	0.0638	0.0657	0.0914	0.0511	0.0555	0.0514	1.1282	0.057	0.7323	1.0938
D	0.0531	0.0603	1.1161	0.0602	0.0689	0.0499	0.0625	0.8628	0.0534	0.0553	0.6635	0.0521
E	0.0588	0.0544	1.0496	0.0522	1.0737	0.8877	0.0513	0.0479	0.0547	0.0547	0.0545	0.0553
F	0.0514	0.0555	0.0551	0.0508	0.1403	0.4113	0.6984	0.0498	0.0513	0.0531	0.0522	0.0476
G	1.0503	0.0514	0.5512	0.05	0.0416	0.0506	0.8513	0.0487	0.0513	0.0514	0.9505	0.053
H	0.0654	0.0544	0.0526	0.0509	0.7204	0.0449	0.6877	0.0487	0.0484	0.9704	1.0164	0.8998

DNaseI Plate B	1	2	3	4	5	6	7	8	9	10	11	12
A	0.066	0.1423	0.9454	0.0615	1.1526	0.5986	0.8156	0.0884	0.0545	0.1104	0.0539	0.5155
B	1.1126	0.0878	0.0682	0.9516	0.0649	1.0523	0.8475	0.0592	0.0633	0.0652	1.1393	0.057
C	0.4024	0.3196	0.6571	0.105	0.1322	0.0638	0.6394	0.0588	0.0617	0.9984	0.0614	0.0616
D	0.0638	0.8815	1.0204	0.0793	0.0647	0.0649	0.8363	0.0696	0.0619	0.0693	1.1619	0.9608
E	0.1038	0.0659	0.0667	0.0705	0.0645	0.0637	1.0514	0.0673	0.0615	1.0576	0.5568	0.0709
F	0.8039	0.7265	0.8344	0.0632	0.063	1.166	0.0622	1.0608	0.0789	0.9987	0.0602	0.0644
G	1.0943	0.0665	1.0434	0.6949	1.094	0.0765	0.0739	0.0668	1.1392	0.0686	0.0538	0.9246
H	0.086	0.0726	1.0101	0.9771	0.0617	0.9314	0.0731	1.1274	0.0661	1.1719	0.0583	0.1099

Table S7: ELISA screening of **CuPhen** clones from panning output round 2. Positive wells are highlighted in yellow**.**

Bis plate 1	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0551	0.0496	0.5397	0.5808	0.5734	0.0502	0.0547	0.5559	0.0608	0.5339	0.0484	0.0515
B	0.0498	0.5095	0.5404	0.0484	0.6314	0.5256	0.0507	0.5047	0.6625	0.0638	0.0495	0.566
C	0.6991	0.0481	0.6641	0.666	0.7476	0.0613	0.6671	0.0613	0.3312	0.7308	0.0782	0.6649
D	0.5836	0.6348	0.0481	0.0507	0.0566	0.0539	0.5622	0.554	0.5605	0.5929	0.0494	0.0648
E	0.0533	0.6429	0.0464	0.0471	0.5231	0.7431	0.5308	0.6106	0.0475	0.6044	0.3914	0.5903
F	0.7712	0.5941	0.6421	0.6302	0.0475	0.6818	0.7343	0.6316	0.6553	0.184	0.5669	0.0529
G	0.0539	0.6216	0.0468	0.6744	0.5661	0.6927	0.0493	0.0563	0.0522	0.7285	0.5998	0.4201
H	0.643	0.05	0.0452	0.0452	0.0447	0.0507	0.0455	0.0454	0.0502	0.0453	0.0464	0.0574

Bis Plate 2	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0583	0.0474	0.0479	0.0522	0.0489	0.0516	0.0514	0.0572	0.047	0.0565	0.0471	0.053
B	0.0589	0.0558	1.0778	0.0474	0.0438	0.0505	0.0521	0.0469	1.2495	0.0498	0.8952	0.0466
C	1.1048	0.0504	0.0481	0.0489	0.0467	0.0484	0.0494	0.0482	0.0484	0.0712	0.0462	0.048
D	0.0524	0.0471	0.0549	0.0488	0.048	0.0746	0.0476	0.0526	0.0473	0.0463	0.0733	0.0467
E	0.0525	0.067	0.0533	0.0478	0.0484	0.0497	0.0467	0.0473	0.0445	0.0477	0.0514	0.0556
F	0.0458	0.0576	0.0561	0.0521	0.0484	0.0516	0.0472	0.0551	0.049	0.0481	0.0492	0.0531
G	0.0521	0.0478	0.2502	0.064	0.0409	0.0472	0.0457	0.0573	0.0504	0.0494	0.0588	0.0477
H	0.0675	0.2227	0.0607	0.0514	0.2879	0.0533	0.0496	0.0586	0.053	0.0512	0.0572	0.0639

Table S8: Comparison of diversity within sequences of DNase I and **CuPhen** phage display output clones.

No of full length reads	**No. of different sequences at DNA level**	**% overall sequences with bp changes**	**No. of unique sequences with changes at protein level**	**% sequences with changes at protein level**
DNaseI				
31	21	77.42%	18	58%
BIS				
43	21	48.84%	13	30.23%

S-5: Digestion analysis of DNA sequences containing varying G-C content using Agilent Bioanalyzer 2100

In a total volume of 20 µL, ~600 ng dsDNA (*ca*. 200 ng of 120 bp DNA 36% G-C + 200 ng of 160 bp DNA 50% G-C + 200 ng of 140 bp DNA 64% G-C) was incubated for 5 min at room temperature with 5 µM **CuPhen** (dissolved in DMF) and 25 mM NaCl in 80 mM HEPES buffer. The reaction was started by the addition of 100 µM Na-*L*-ascorbate to the DNA mixture with the solution being incubated at 37° C thereafter. Reactions were stopped at fixed intervals from 5-60 min with all experiments being conducted in duplicate.

The three sequences of differential G-C content and length were generated from pUC19 by PCR using the following primer sets:

120bp; 36% : Forward 5' gatcttttctacggggtctg 3', Reverse 5' gatttaaaacttcattttta 3'

140bp; 64%: forward 5' tcgcgcgtttcggtgatgacg 3' Reverse 5' ccagccccgacacccgccaac 3'

160bp; 50%: forward 5' ttatcgccactggcagcagc 3', Reverse 5'ctctttttccgaaggtaactggc 3'

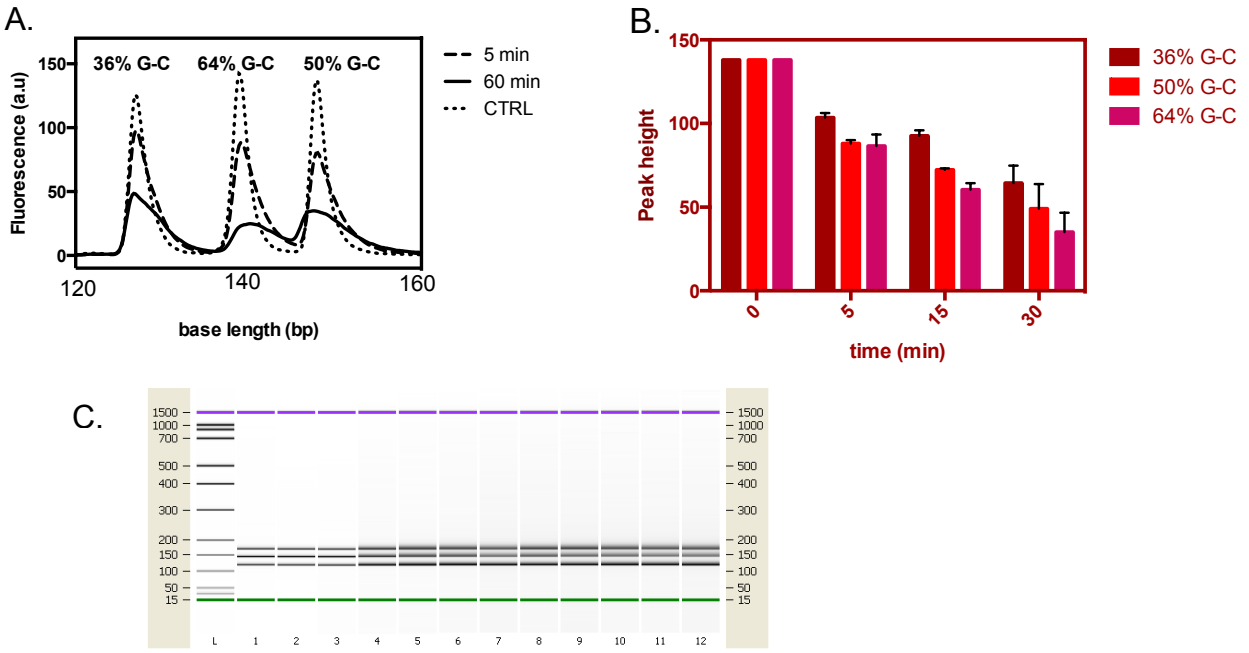

Fig. S.5 Electrograms of 600 ng dsDNA sequences containing varying amounts of G-C content exposed to metal complex (5 µM) between 0 – 60 min on the Bioanalyzer 2100 with DNA 1000 microfluidic chips. **A.** Reduction in DNA fluorescence upon exposure to 5 µM **Cu-Phen**, **B.** DNA degradation (from peak area analysis of duplicates experiments, error bars ± S.D.) of DNA sequences of varying G-C content exposed to Cu-Phen complex between 0-30 min, **C.** Typical electropherogram generated by the Bioanalyzer 2100, L = ladder, lane 1-3 = DNA control (120 bp DNA 36% G-C, 200 ng of 160 bp DNA 50% G-C and 200 ng of 140 bp DNA 64% G-C) lanes 4-12 = DNA + Cu-Phen exposed between 5-60 min.

References

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