Supporting Information

Efficient plasmid DNA cleavage by copper(II) complexes of 1,4,7-triazacyclononane ligands featuring xylyl-linked guanidinium groups

Linda Tjioe,[a] Anja Meininger,[a1] Tanmaya Joshi,[a] Leone Spiccia[a] and Bim Graham[b*]*

[a]School of Chemistry, Monash University, Clayton, Vic 3800, Australia

[b]Department of Medicinal Chemistry and Drug Action, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Vic 3052, Australia.

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[1] Current address: School of Chemistry, Ruprecht-Karls-Universität Heidelberg.

Table S01. Crystallography collection and refinement data

Compound	**C1**	**C3**
empirical formula	$C_{18}H_{34}Cl_3CuN_7O$	$C_{17}H_{35}Cl_3CuN_6O_2$
M/g mol^{-1}	534.41	525.40
cryst syst	Monoclinic	Monoclinic
space group	$P2_1/c$	$P2_1/c$
a/Å	17.9182(5)	11.8641(5)
b/Å	9.5518(3)	7.3579(4)
c/Å	14.7300(4)	28.275(1)
β/deg	107.315(1)	101.193(2)
V/Å^3	2406.8(1)	2421.3(2)
Z	4	4
T/K	123(2)	123(2)
λ/Å	0.71073	0.71073
D_{calcd}/g cm^{-3}	1.475	1.441
M(Mo Kα)/mm^{-1}	1.265	1.258
no. data measured	17289	22848
unique data (R_{int})	5519 (0.0500)	7051 (0.0243)
observed data [I > 2(σ)I]	4525	6065
final R1, wR2 (obsd data)	0.0489[a], 0.0815[b]	0.0267[a], 0.0660[b]
final R1, wR2 (all data)	0.0646, 0.0878	0.0341, 0.0692
ρ_{min}, ρ_{max}/*e* Å^{-3}	-0.550, 0.454	-0.371, 0.524

[a] $R = \Sigma(|F_o| - |F_c|)/ \Sigma |F_o|$. [b] $R' = [\Sigma w(|F_o| - |F_c|)^2/ \Sigma F_o^2]^{1/2}$, where $w = [\sigma^2(F_o)]^{-1}$.

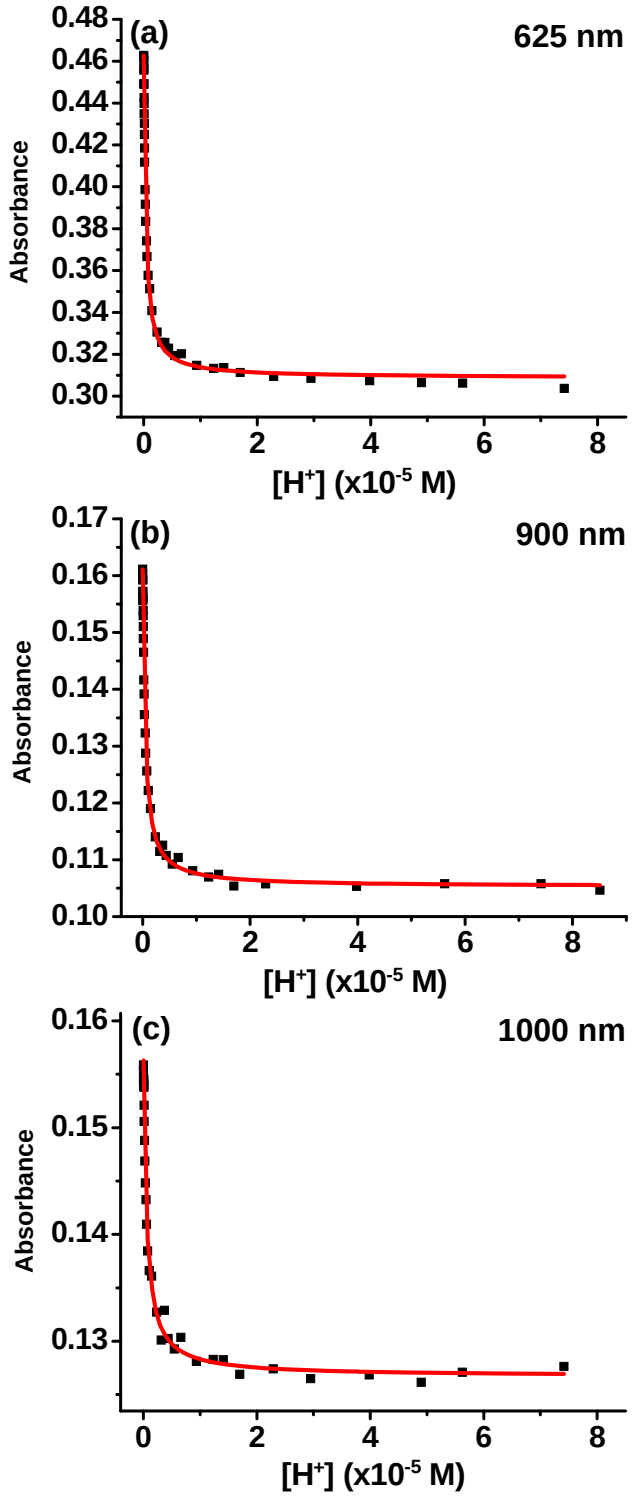


Figure S01. Absorbance *vs*. [H^+] graphs at selected wavelengths obtained from spectrophotometric pH titration of **C1** (7.5 mM) with 10 M HCl. (a) – (c) 625, 900 and 1000 nm, respectively.

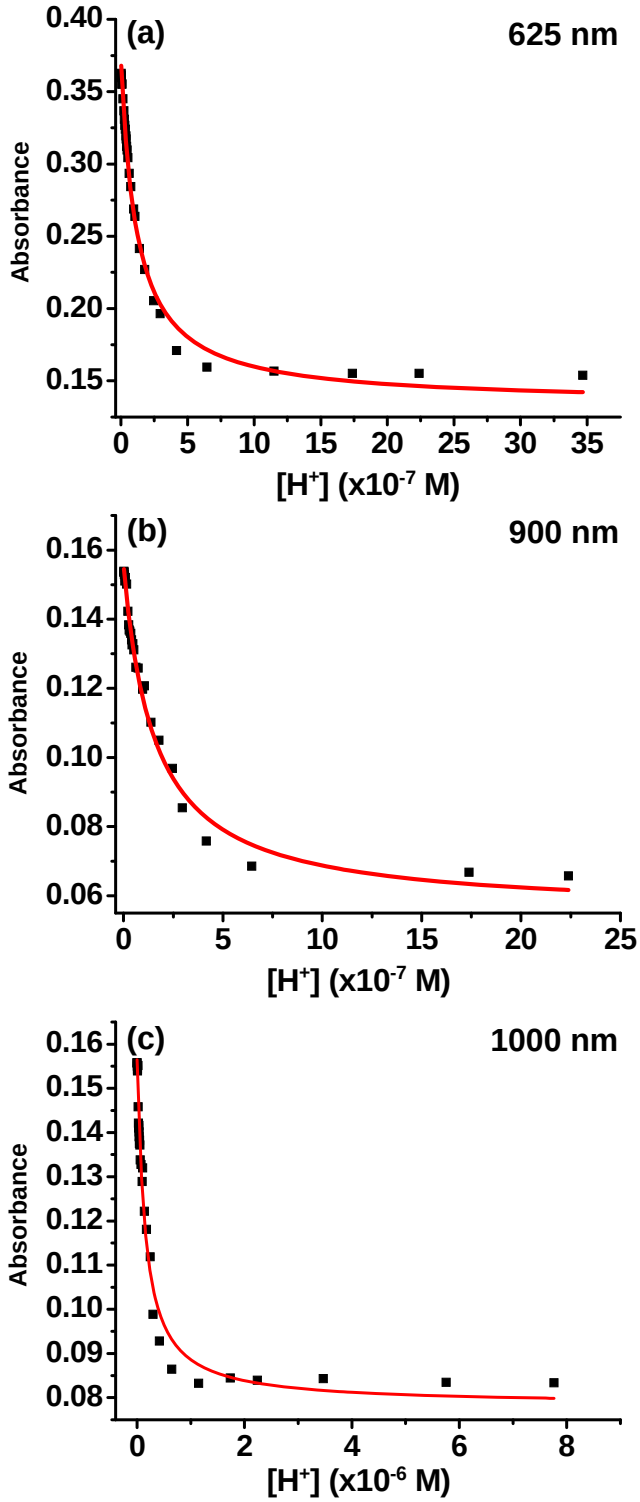


Figure S02. Absorbance *vs*. [H^+] graphs at selected wavelengths obtained from spectrophotometric pH titration of **C2** (7.5 mM) with 10 M HCl. (a) – (c) 625, 900 and 1000 nm, respectively.

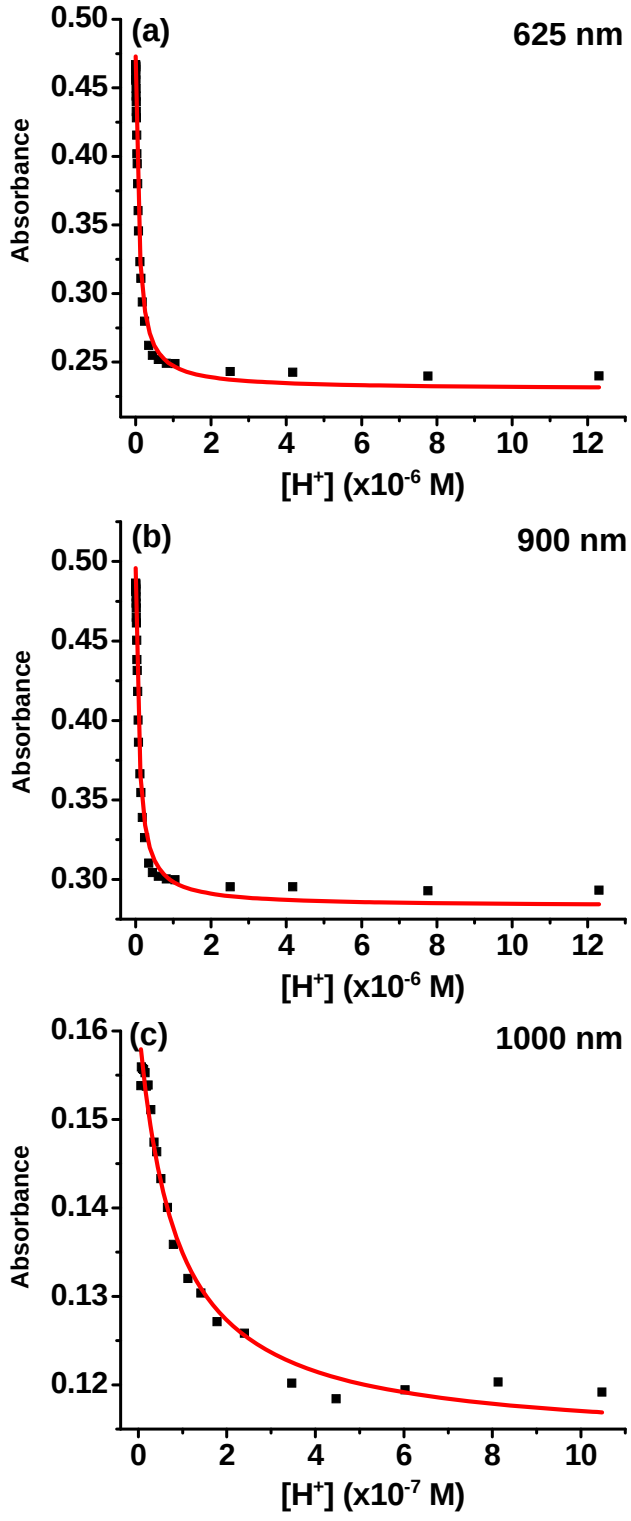


Figure S03. Absorbance *vs*. [H^+] graphs at selected wavelengths obtained from spectrophotometric pH titration of **C3** (7.5 mM) with 10 M HCl. (a) – (c) 625, 900 and 1000 nm, respectively.

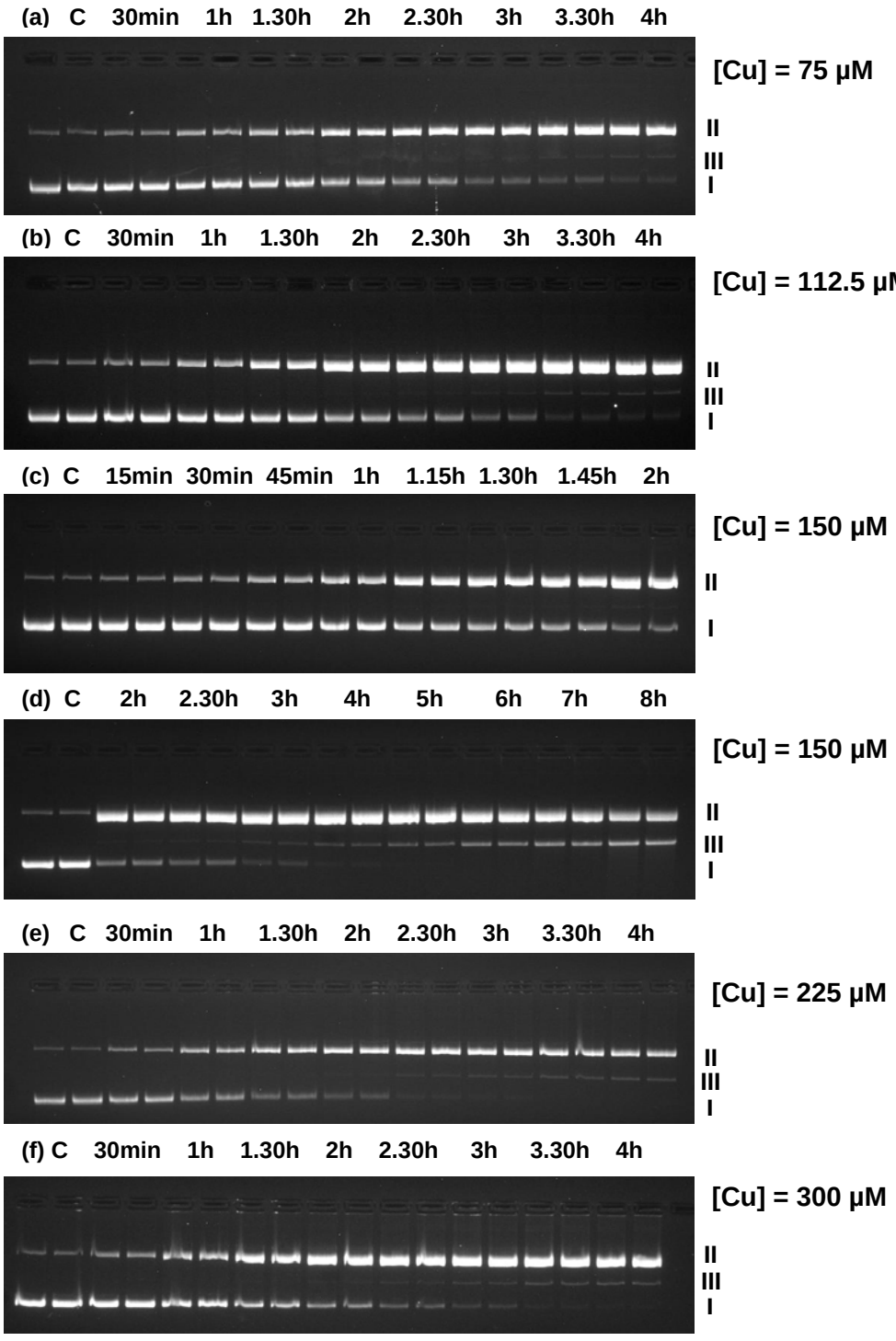


Figure S04. Agarose gels (1%) showing cleavage of pBR 322 plasmid DNA (38 µM bp) incubated at different concentrations of **C1** in 40 mM HEPES buffer, pH 7.0 at 37 ºC for various time intervals. Lane 1 & 2: DNA control, lane 3 & 4: 30 min, lane 5 & 6: 1 h, lane 7 & 8: 1.30 h, lane 9 & 10: 2 h, lane 11 & 12: 2.30 h, lane 13 & 14: 3 h, lane 15 & 16: 3.30 h, lane 17 & 18: 4 h. (a) – (f) 75, 112.5, 150, 225, 300 µM respectively.

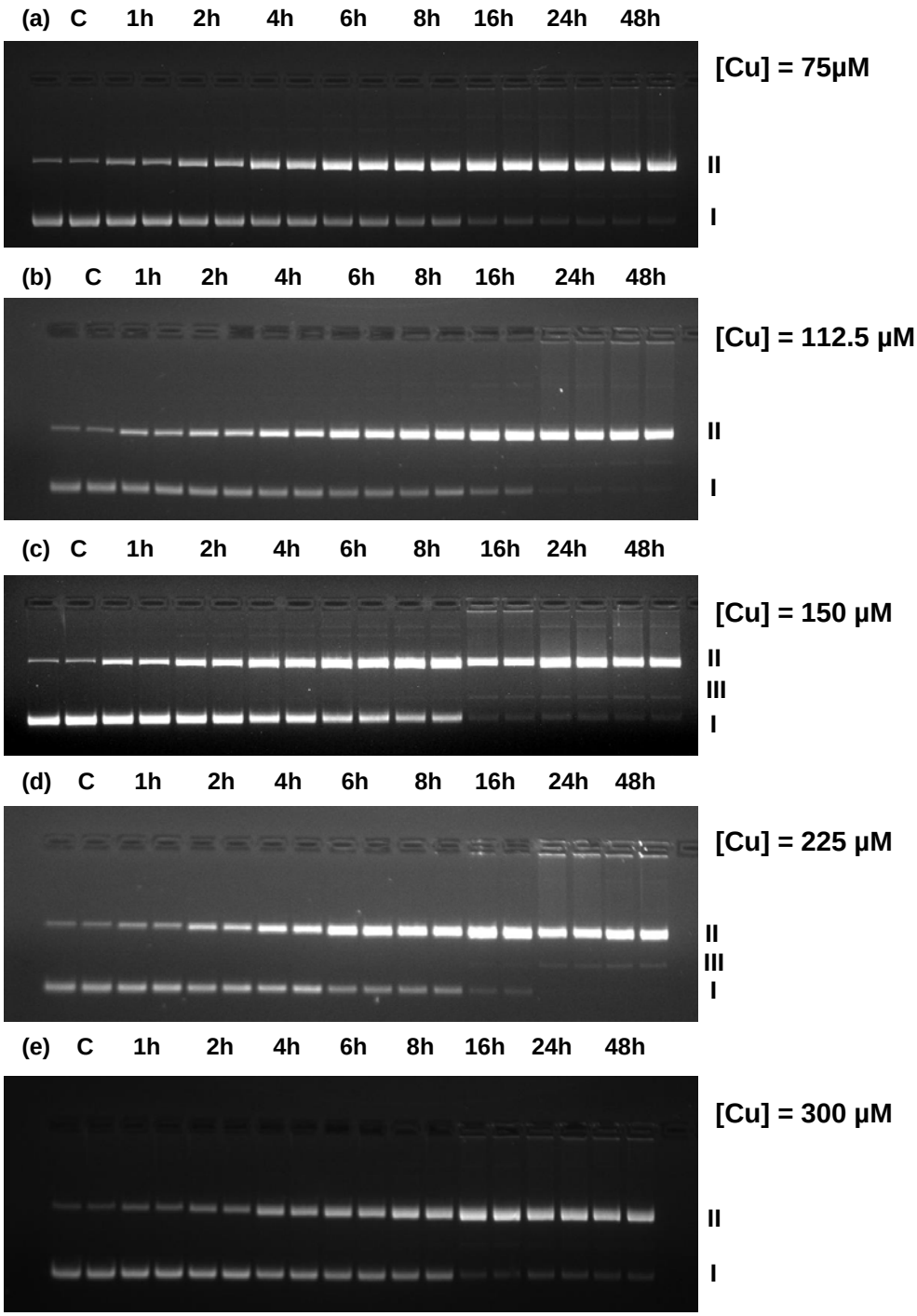


Figure S05. Agarose gels (1%) showing cleavage of pBR 322 plasmid DNA (38 μM bp) incubated at different concentrations of **C2** in 40 mM HEPES buffer, pH 7.0 at 37 ºC for various time intervals. Lane 1 & 2: DNA control, lane 3 & 4: 1 h, lane 5 & 6: 2 h, lane 7 & 8: 4 h, lane 9 & 10: 6 h, lane 11 & 12: 8 h, lane 13 & 14: 16 h, lane 15 & 16: 24 h, lane 17 & 18: 48 h. (a) – (e) 75, 112.5, 150, 225, 300 μM respectively.

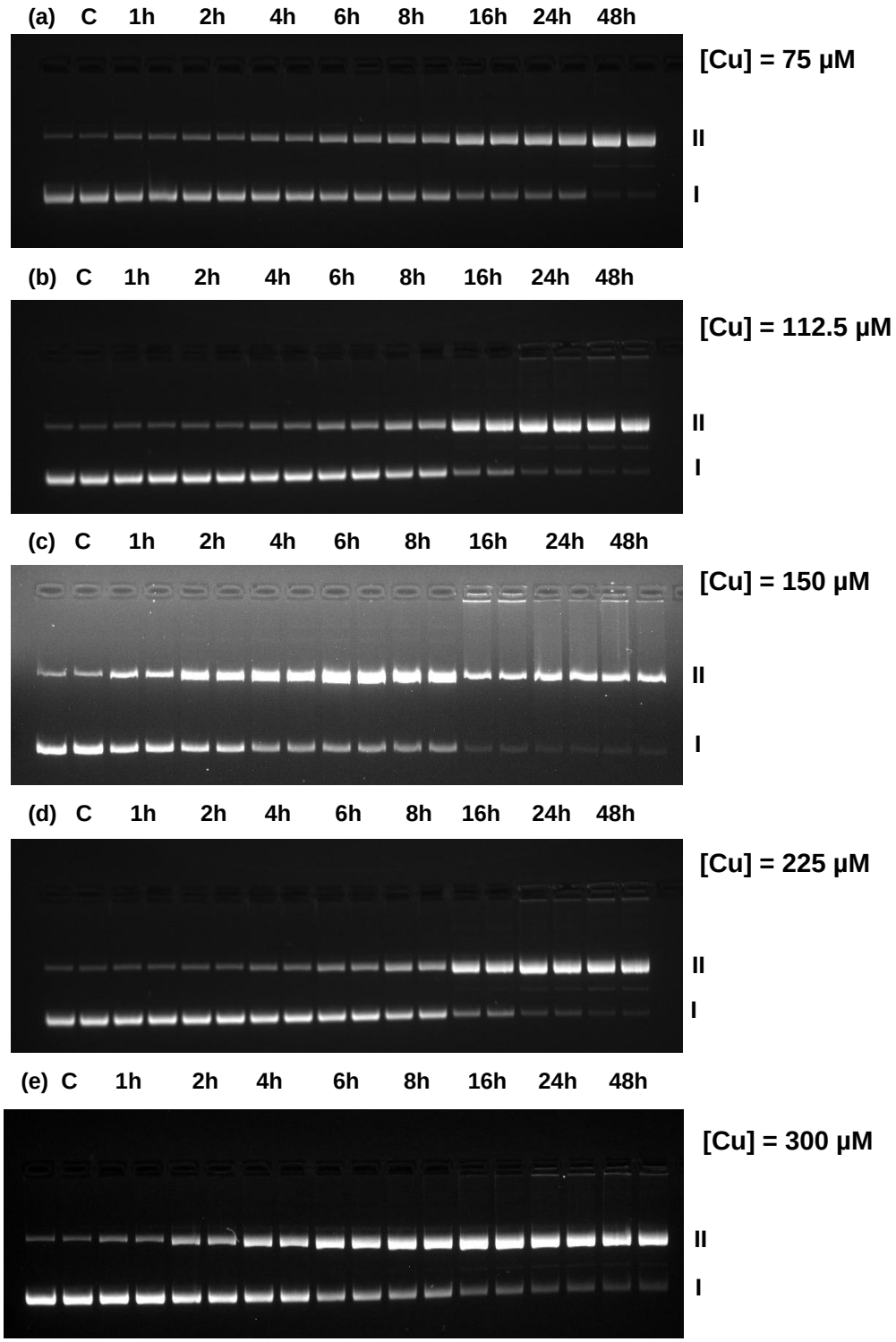


Figure S06. Agarose gels (1%) showing cleavage of pBR 322 plasmid DNA (38 µM bp) incubated at different concentrations of **C3** in 40 mM HEPES buffer, pH 7.0 at 37 ºC for various time intervals. Lane 1 & 2: DNA control, lane 3 & 4: 1 h, lane 5 & 6: 2 h, lane 7 & 8: 4 h, lane 9 & 10: 6 h, lane 11 & 12: 8 h, lane 13 & 14: 16 h, lane 15 & 16: 24 h, lane 17 & 18: 48 h. (a) – (e) 75, 112.5, 150, 225, 300 µM respectively.

Table S02. Apparent initial first-order rate constants of DNA cleavage reactions at various concentrations of **C1, C2**, and **C3.** The experiments were performed at 37 ºC in 40 mM HEPES buffer, pH 7.0.

C1		**C2**	
Concentration (μM)	k_{obs} ($\times 10^{-5}$ s^{-1})	Concentration (μM)	k_{obs} ($\times 10^{-5}$ s^{-1})
75	3.09 ± 0.43	75	5.24 ± 0.12
112.5	4.35 ± 0.32	112.5	6.41 ± 0.14
150	27.1 ± 0.28	150	8.15 ± 1.02
225	12.5 ± 0.39	225	5.65 ± 0.41
300	10.3 ± 0.48	300	2.51 ± 0.25

C3	
Concentration (μM)	k_{obs} ($\times 10^{-5}$ s^{-1})
75	2.69 ± 0.45
112.5	3.85 ± 0.41
150	6.70 ± 0.30
225	5.18 ± 0.31
300	2.55 ± 0.43

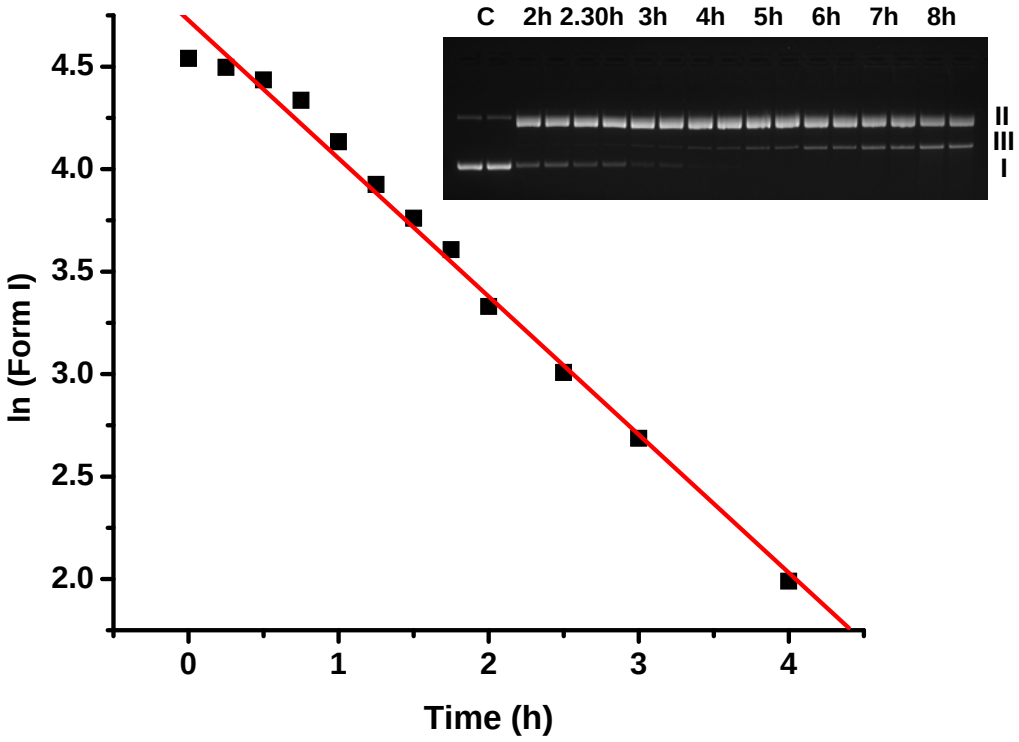


Figure S07. Kinetic profile of pBR 322 plasmid DNA (38 μM bp) cleavage promoted by **C1** (150 μM). The experiments were performed at 37 ºC in 40 mM HEPES buffer, pH 7.0.

Table S03. DNA cleavage promoted by **C1** under aerobic conditions.

		% DNA		
Time (h)	ln(Form I)	Form I	Form II	Form III
0	4.54	93.7	6.27	0
0.25	4.50	89.7	10.3	0
0.5	4.44	84.4	15.6	0
0.75	4.34	76.4	23.6	0
1	4.13	62.4	37.6	0
1.25	3.92	50.6	49.4	0
1.5	3.76	43.0	57.0	0
1.75	3.61	36.9	63.1	0
2	3.33	27.9	72.1	0
2.30	3.01	20.2	79.2	0.6
3	2.69	14.7	84.0	1.3
4	1.99	7.3	89.7	2.9
5	-	0	94.8	5.2
6	-	0	89.4	10.6
7	-	0	84.0	16.0
8	-	0	74.8	25.2

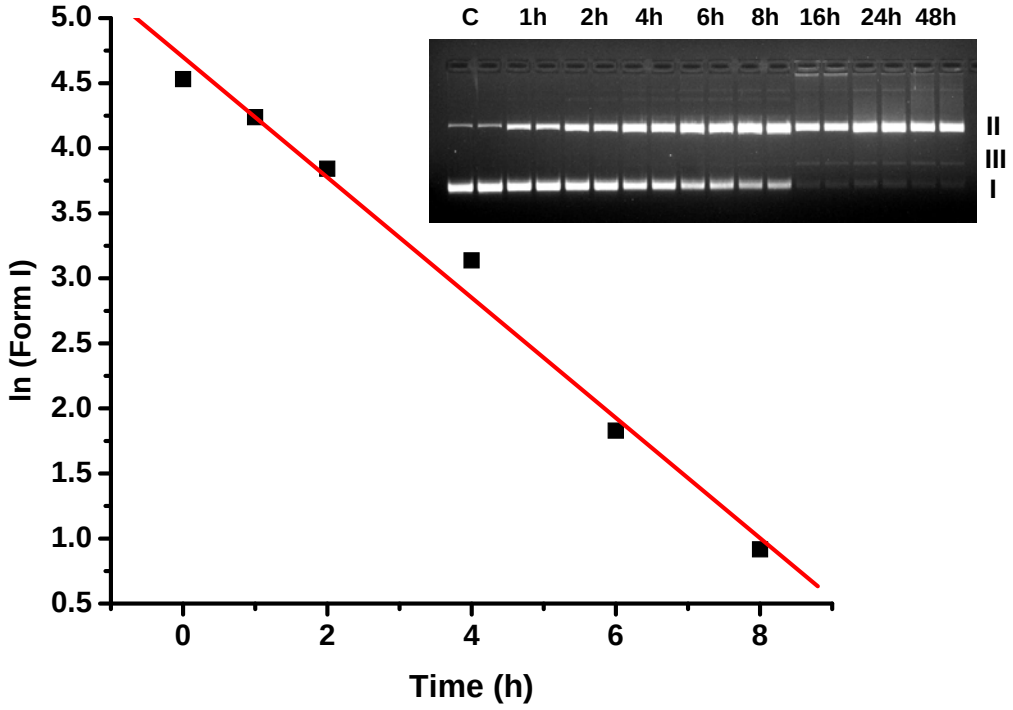


Figure S08. Kinetic profile of pBR 322 plasmid DNA (38 µM bp) cleavage promoted by **C2** (150 µM). The experiments were performed at 37 ºC in 40 mM HEPES buffer, pH 7.0.

Table S04. DNA cleavage promoted by **C2** under aerobic conditions.

		% DNA	
Time (h)	ln(Form I)	Form I	Form II
0	4.53	92.9	7.1
1	4.24	69.3	30.7
2	3.84	46.6	53.4
4	3.14	23.0	77.0
6	1.83	6.23	93.8
8	0.92	2.50	97.5
16	-	0	100
24	-	0	100
48	-	0	100

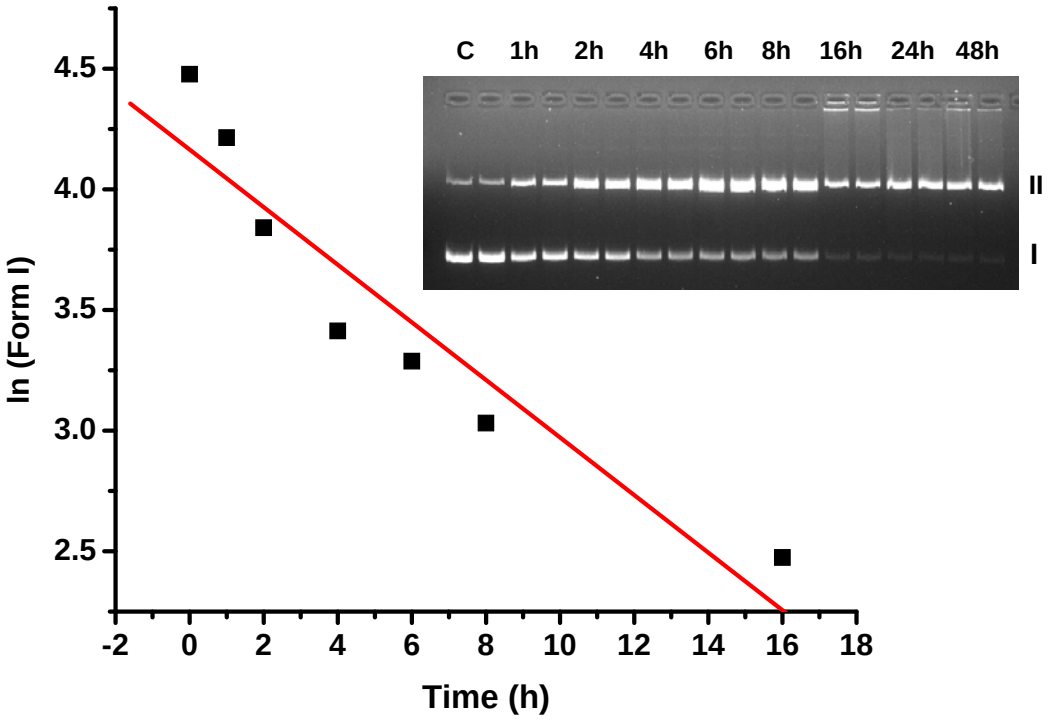


Figure S09. Kinetic profile of pBR 322 plasmid DNA (38 μM bp) cleavage promoted by **C3** (150 μM). The experiments were performed at 37 ºC in 40 mM HEPES buffer, pH 7.0.

Table S05. DNA cleavage promoted by **C3** under aerobic conditions.

		% DNA	
Time (h)	ln(Form I)	Form I	Form II
0	4.48	88.0	12.0
1	4.21	67.6	32.4
2	3.84	46.6	53.4
4	3.41	30.4	69.6
6	3.29	26.8	73.2
8	3.03	20.7	79.3
16	2.47	11.9	88.1
24	-	0	100
48	-	0	100

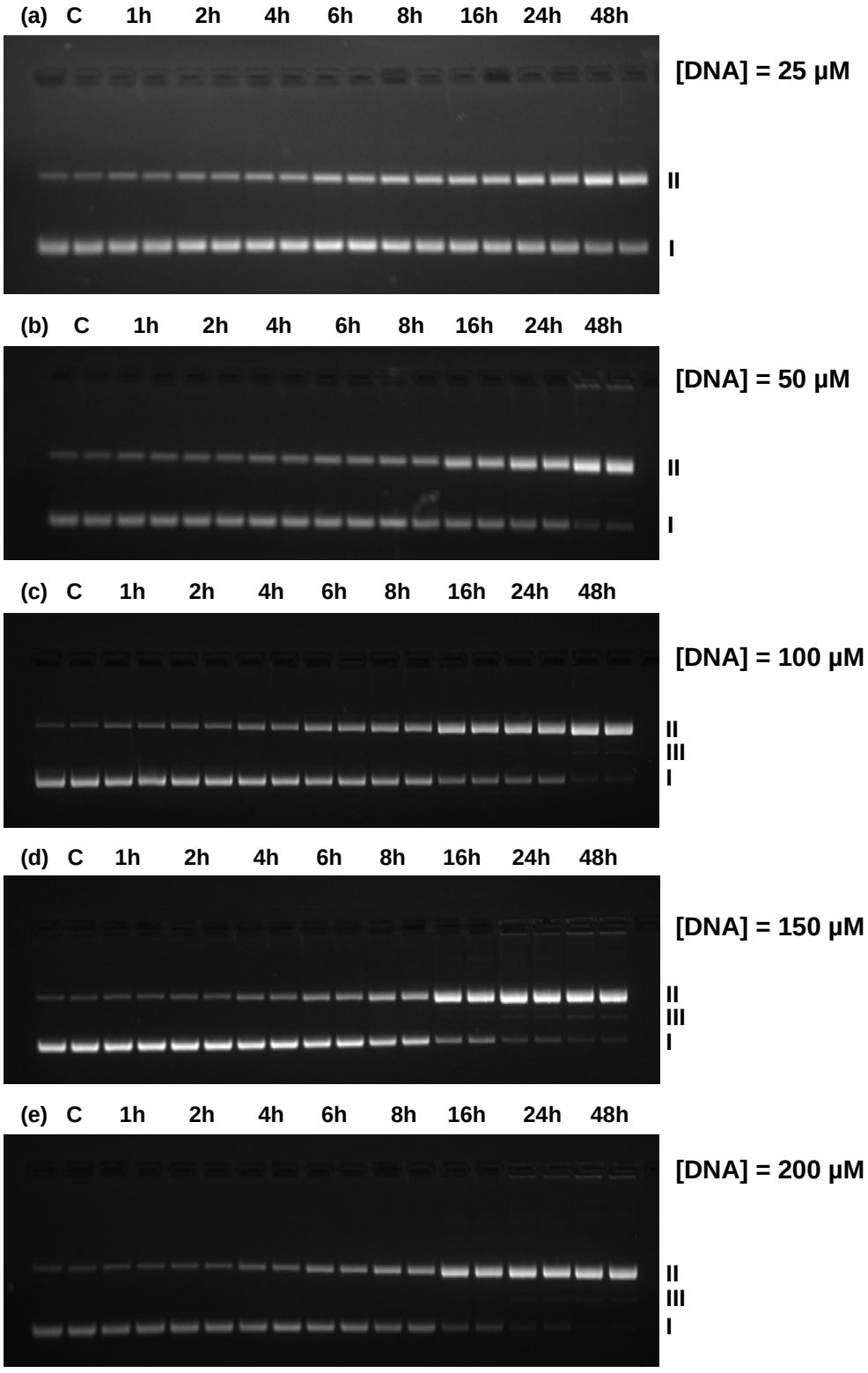
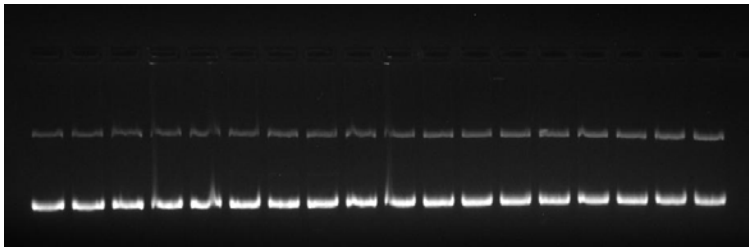


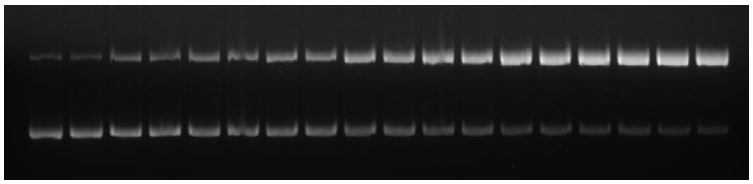
Figure S10. Agarose gels (1%) showing cleavage of pBR 322 plasmid DNA using 50 µM complex **C1** with different concentrations of the plasmid pBR 322 DNA in 40 mM HEPES buffer, pH 7.0 at 37 ºC for various time intervals. Lane 1 & 2: DNA control, lane 3 & 4: 1 h, lane 5 & 6: 2 h, lane 7 & 8: 4 h, lane 9 & 10: 6 h, lane 11 & 12: 8 h, lane 13 & 14: 16 h, lane 15 & 16: 24 h, lane 17 & 18: 48 h. (a) – (e) 25, 50, 100, 150, 200 µM respectively.

(a) C 15m 30m 45m 1h 1.15h 1.30h 1.45h 2h



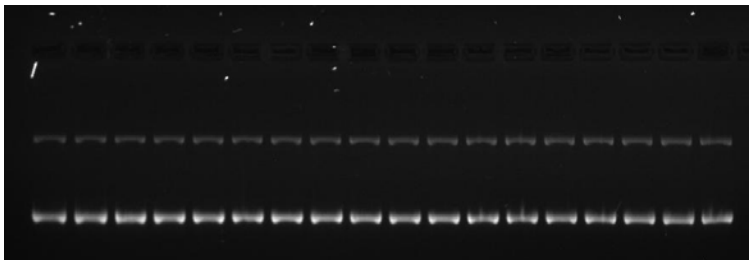
[NaCl] = 20 mM

(b) C 2h 2.30h 3h 4h 5h 6h 7h 8h



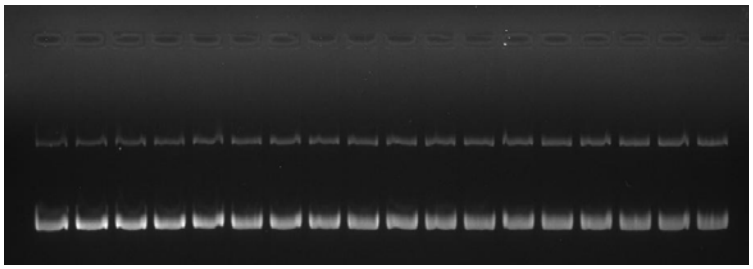
[NaCl] = 20 mM

(c) C 15m 30m 45m 1h 1.15h 1.30h 1.45h 2h



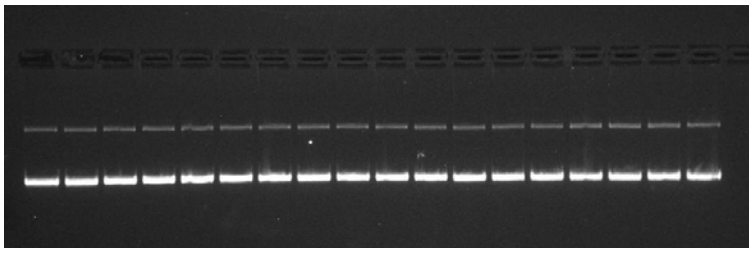
[NaCl] = 100 mM

(d) C 2h 2.30h 3h 4h 5h 6h 7h 8h



[NaCl] = 100 mM

(e) C 15m 30m 45m 1h 1.15h 1.30h 1.45h 2h



[NaCl] = 250 mM

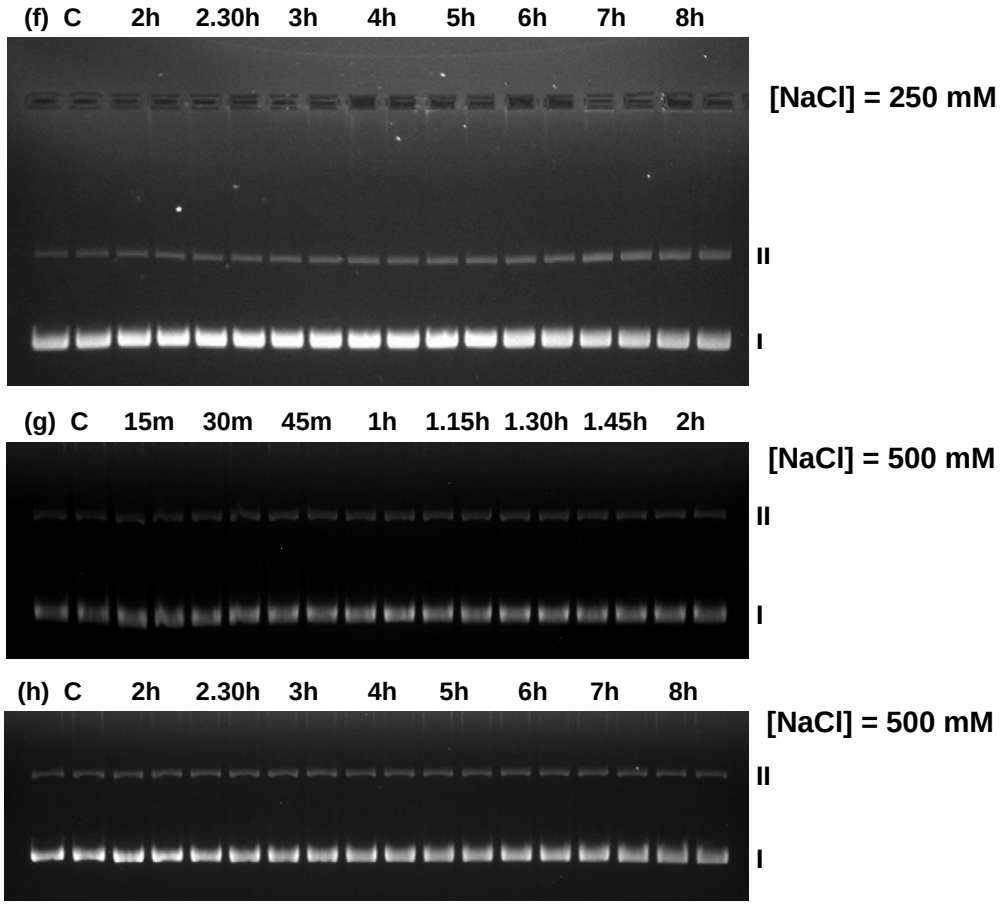


Figure S11. Agarose gels (1%) showing cleavage of pBR 322 plasmid DNA (38 μM bp) incubated using 150 μM complex **C1** with different concentrations of NaCl in 40 mM HEPES buffer, pH 7.0 at 37 ºC for various time intervals. First gel of each concentration of NaCl: Lane 1 & 2: DNA control, lane 3 & 4: 15 mins, lane 5 & 6: 30 mins, lane 7 & 8: 45 mins, lane 9 & 10: 1 h, lane 11 & 12: 1.15 h, lane 13 & 14: 1.30 h, lane 15 & 16: 1.45 h, lane 17 & 18: 2 h. Second gel of each concentration of NaCl: Lane 1 & 2: DNA control, lane 3 & 4: 2 h, lane 5 & 6: 2.30 h, lane 7 & 8: 3 h, lane 9 & 10: 4 h, lane 11 & 12: 5 h, lane 13 & 14: 6 h, lane 15 & 16: 7 h, lane 17 & 18: 8 h. (a) – (h) 20, 100, 250, 500 mM respectively.

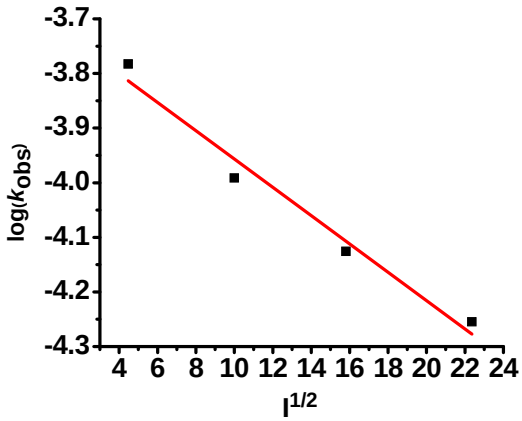


Figure S12. A plot of log (k_{obs}) vs. $[I]^{1/2}$.

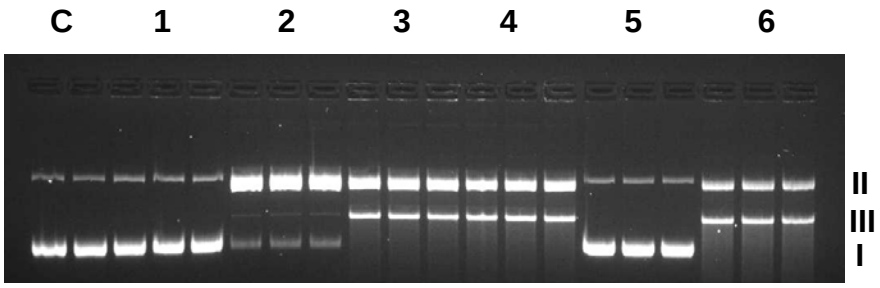


Figure S13. Agarose gel (1%) showing cleavage of pBR 322 plasmid DNA (38 μM bp) incubated with **C1** (150 μM) in presence of standard radical scavengers at 37 ºC in 40 mM HEPES buffer, pH 7.0 for 6 h. Lane C: DNA control, lane 1: DNA + **C1** + KI (10 mM), lane 2: DNA + **C1** + NaN_3 (10 mM), lane 3: DNA + **C1**, lane 4: DNA + **C1** + DMSO (10 mM), lane 5: DNA + Guanidine (150 μM), lane 6: DNA + **C1** + ^{t}BuOH (10 mM).

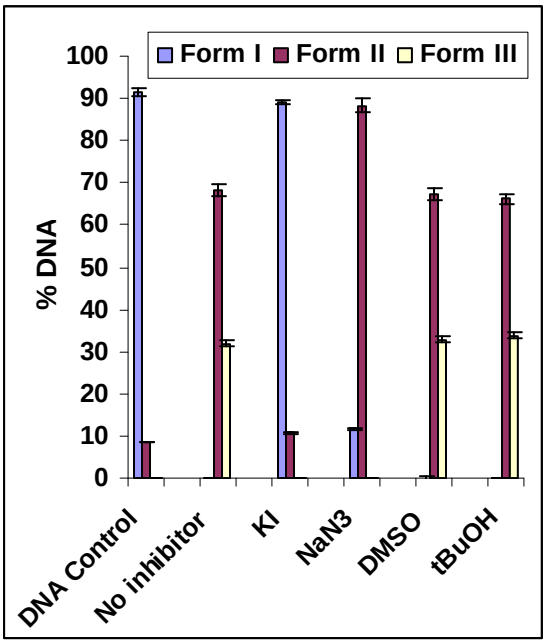


Figure S14. Bar graph showing cleavage of pBR 322 plasmid DNA by **C1** in the presence of standard radical scavengers for hydroxyl radical (10 mM KI, DMSO or ^{t}BuOH), and singlet oxygen (10 mM NaN_3), incubated for 6 h at 37 °C in HEPES buffer (pH 7.0).

Table S06. DNA cleavage promoted by **C1** in the presence of standard radical scavengers.

Scavengers	% DNA		
	Form I	Form II	Form III
DNA control	91.5	8.5	0
KI	89.2	10.8	0
NaN_3	11.7	88.3	0
DMSO	0	67.2	32.8
^{t}BuOH	0	66.2	33.8
Guanidine	92.4	7.6	0
No scavenger	0	68.1	31.9

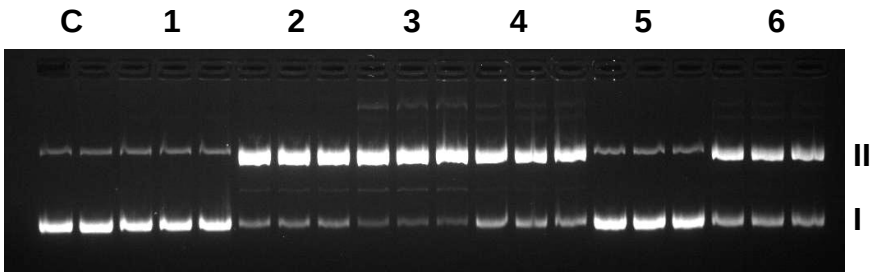


Figure S15. Agarose gel (1%) showing cleavage of pBR 322 plasmid DNA (38 μM bp) incubated with **C2** (150 μM) in presence of standard radical scavengers at 37 ºC in 40 mM HEPES buffer, pH 7.0 for 6 h. Lane C: DNA control, lane 1: DNA + **C2** + KI (10 mM), lane 2: DNA + **C2** + NaN_3 (10 mM), lane 3: DNA + **C2**, lane 4: DNA + **C2** + DMSO (10 mM), lane 5: DNA + Guanidine (150 μM), lane 6: DNA + **C2** ^{t}BuOH (10 mM).

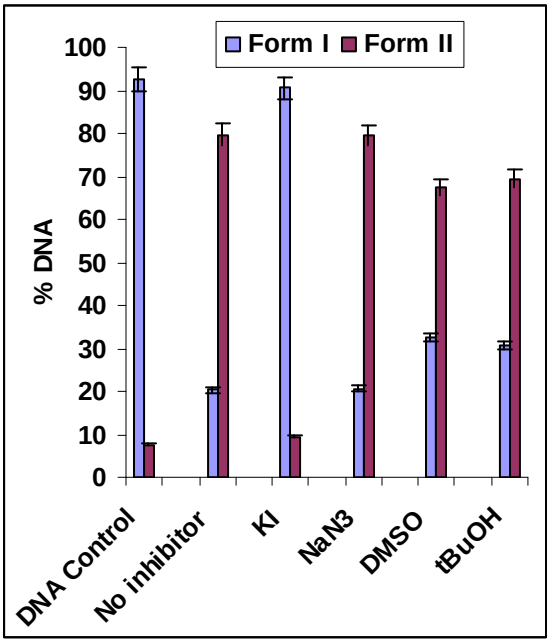


Figure S16. Bar graph showing cleavage of pBR 322 plasmid DNA by **C2** in the presence of standard radical scavengers for hydroxyl radical (10 mM KI, DMSO or ^{t}BuOH), and singlet oxygen (10 mM NaN_3), incubated for 6 h at 37 °C in HEPES buffer (pH 7.0).

Table S07. DNA cleavage promoted by **C2** in the presence of standard radical scavengers.

Scavengers	% DNA	
	Form I	Form II
DNA control	92.5	7.5
KI	90.5	9.5
NaN_3	20.6	79.4
DMSO	32.6	67.4
^{t}BuOH	30.6	69.4
Guanidine	89.7	10.3
No scavenger	20.2	79.8

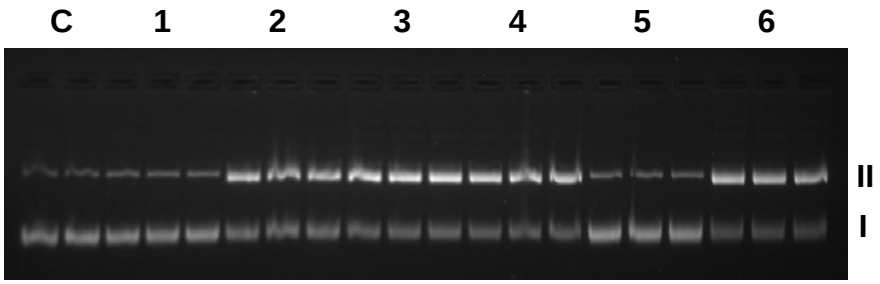


Figure S17. Agarose gel (1%) showing cleavage of pBR 322 plasmid DNA (38 μM bp) incubated with **C3** (150 μM) in presence of standard radical scavengers at 37 ºC in 40 mM HEPES buffer, pH 7.0 for 6 h. Lane C: DNA control, lane 1: DNA + **C3** + KI (10 mM), lane 2: DNA + **C3** + NaN_3 (10 mM), lane 3: DNA + **C3**, lane 4: DNA + **C3** + DMSO (10 mM), lane 5: DNA + Guanidine (150 μM), lane 6: DNA + **C3** + ^{t}BuOH (10 mM).

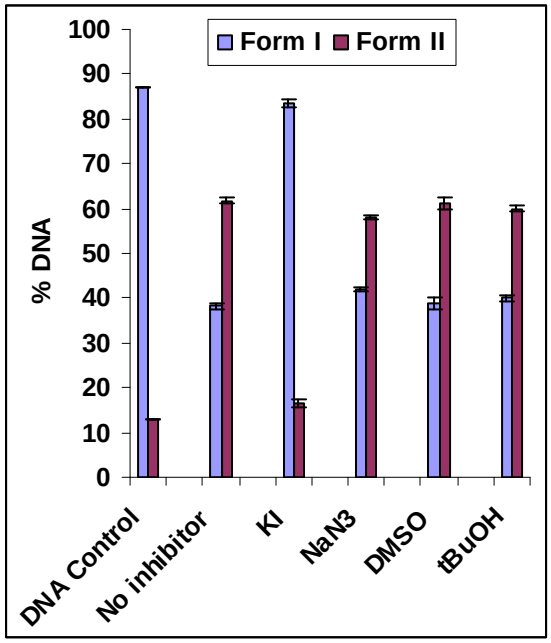


Figure S18. Bar graph showing cleavage of pBR 322 plasmid DNA by **C3** in the presence of standard radical scavengers for hydroxyl radical (10 mM KI, DMSO or ^{t}BuOH), and singlet oxygen (10 mM NaN_3), incubated for 6 h at 37 °C in HEPES buffer (pH 7.0).

Table S08. DNA cleavage promoted by **C3** in the presence of standard radical scavengers.

Scavengers	% DNA	
	Form I	Form II
DNA control	87.2	12.8
KI	83.6	16.4
NaN_3	42.0	58.0
DMSO	38.8	61.2
^{t}BuOH	40.0	60.0
Guanidine	84.6	15.4
No scavenger	38.4	61.6

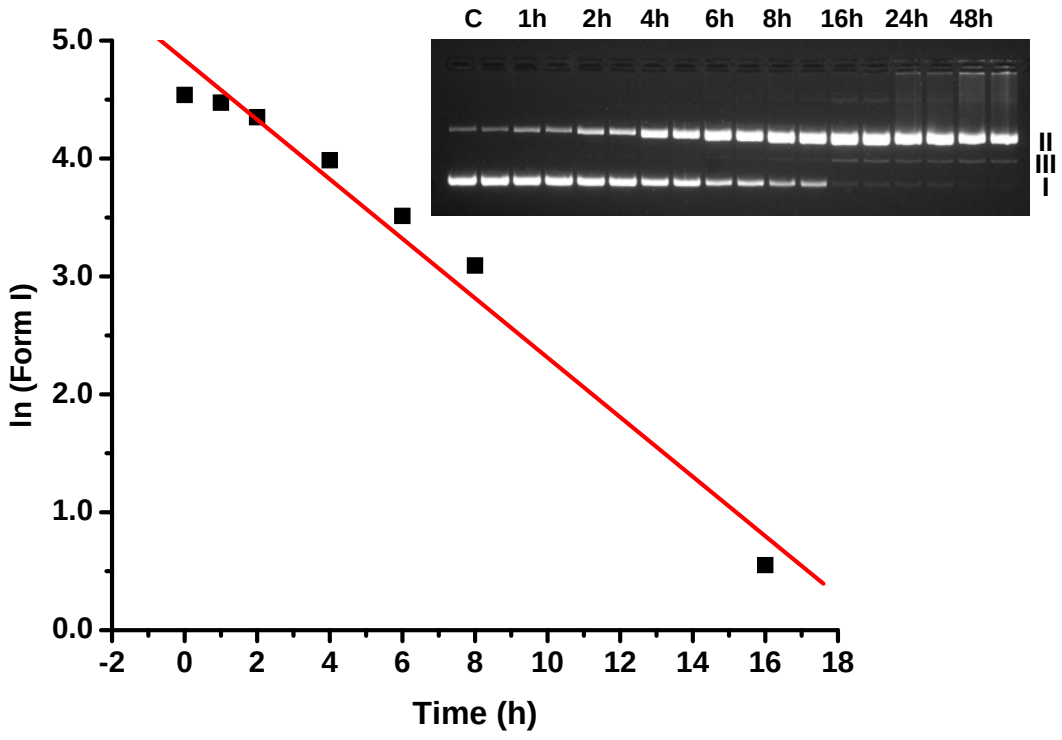


Figure S19. Kinetic profile of pBR 322 plasmid DNA (38 μM bp) cleavage promoted by **C1** (150 μM) under anaerobic conditions. The experiments were performed at 37 ºC in 40 mM HEPES buffer, pH 7.0.

Table S09. DNA cleavage promoted by **C1** under anaerobic conditions.

		% DNA	
Time (h)	ln(Form I)	Form I	Form II
0	4.54	93.6	6.4
1	4.47	87.7	12.3
2	4.35	77.5	22.5
4	3.99	53.8	46.2
6	3.51	33.6	66.5
8	3.09	22.0	78.0
16	0.55	1.7	98.3
24	-	0	100
48	-	0	100

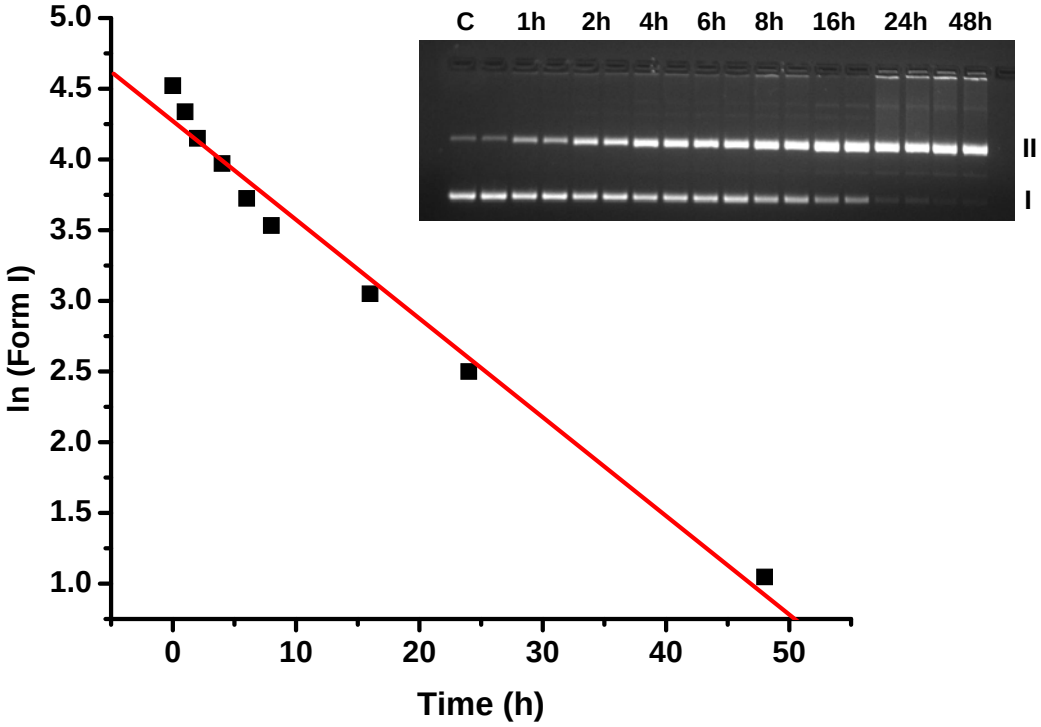


Figure S20. Kinetic profile of pBR 322 plasmid DNA (38 μM bp) cleavage promoted by **C2** (150 μM) under anaerobic conditions. The experiments were performed at 37 ºC in 40 mM HEPES buffer, pH 7.0.

Table S10. DNA cleavage promoted by **C2** under anaerobic conditions.

		% DNA	
Time (h)	ln(Form I)	Form I	Form II
0	4.52	91.9	8.1
1	4.37	76.5	23.5
2	4.15	63.4	36.6
4	3.97	53.1	46.9
6	3.72	41.5	58.5
8	3.53	34.2	65.8
16	3.05	21.5	78.5
24	2.50	12.0	88.0
48	1.05	2.9	97.1

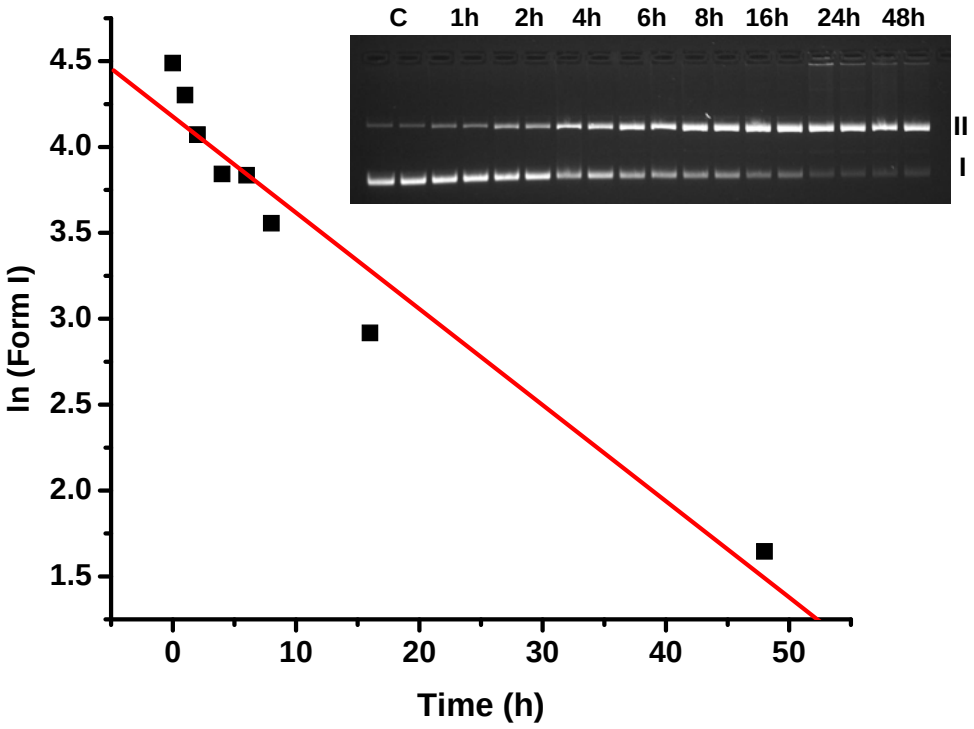


Figure S21. Kinetic profile of pBR 322 plasmid DNA (38 μM bp) cleavage promoted by **C3** (150 μM) under anaerobic conditions. The experiments were performed at 37 ºC in 40 mM HEPES buffer, pH 7.0.

Table S11. DNA cleavage promoted by **C3** under anaerobic conditions.

		% DNA	
Time (h)	ln(Form I)	Form I	Form II
0	4.49	89.0	11.0
1	4.30	73.8	26.2
2	4.07	58.6	41.4
4	3.84	46.7	53.3
6	3.84	46.3	53.7
8	3.56	35.0	65.0
16	2.92	18.5	81.5
24	1.94	6.9	93.1
48	1.65	5.2	94.8

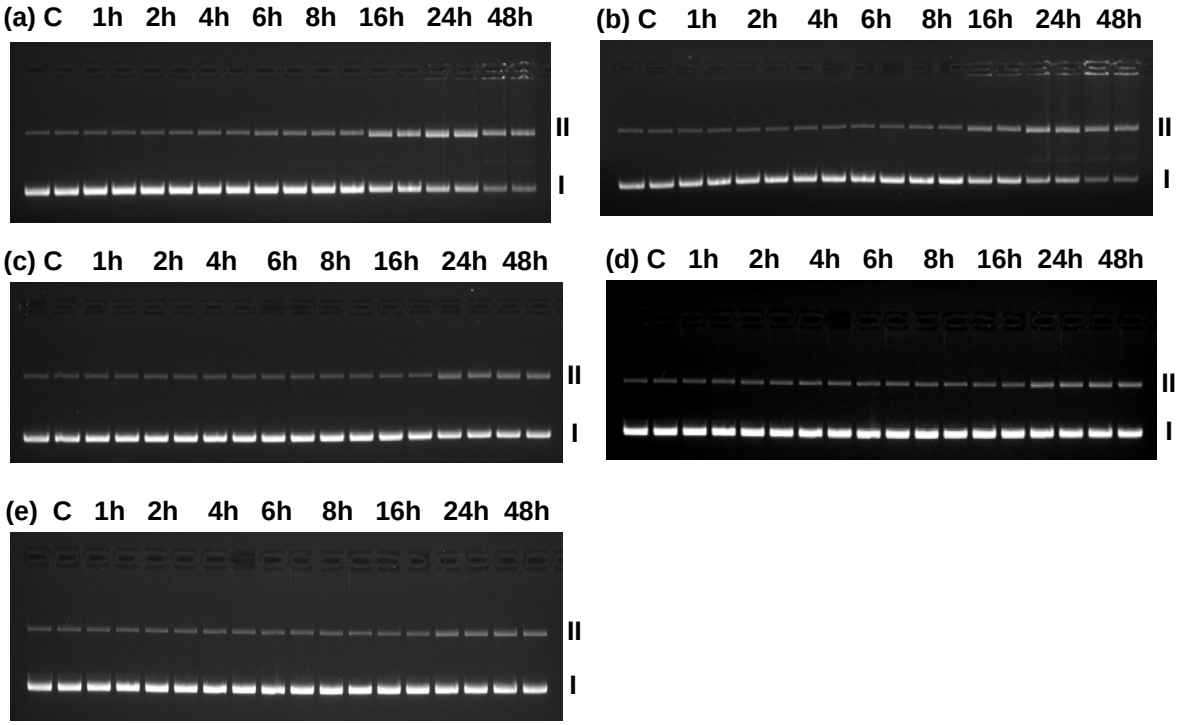


Figure S22. Agarose gels of pBR 322 plasmid DNA treated with 150 μM of non-metalated ligand, $\mathbf{L^1}$ (a), $\mathbf{L^2}$ (b) and $\mathbf{L^3}$ (c) in 40 mM HEPES buffer (pH 7.0), (d) Agarose gel of pBR 322 plasmid DNA treated with 150 μM $CuCl_2$ in 40 mM HEPES buffer (pH 7.0), (e) Agarose gel of pBR 322 plasmid DNA treated with HEPES buffer only (40 mM, pH 7.0) at 37 ºC. Lane 1 & 2: DNA control, lane 3 & 4: 1 h, lane 5 & 6: 2 h, lane 7 & 8: 4 h, lane 9 & 10: 6 h, lane 11 & 12: 8 h, lane 13 & 14: 16 h, lane 15 & 16: 24 h, lane 17 & 18: 48 h.

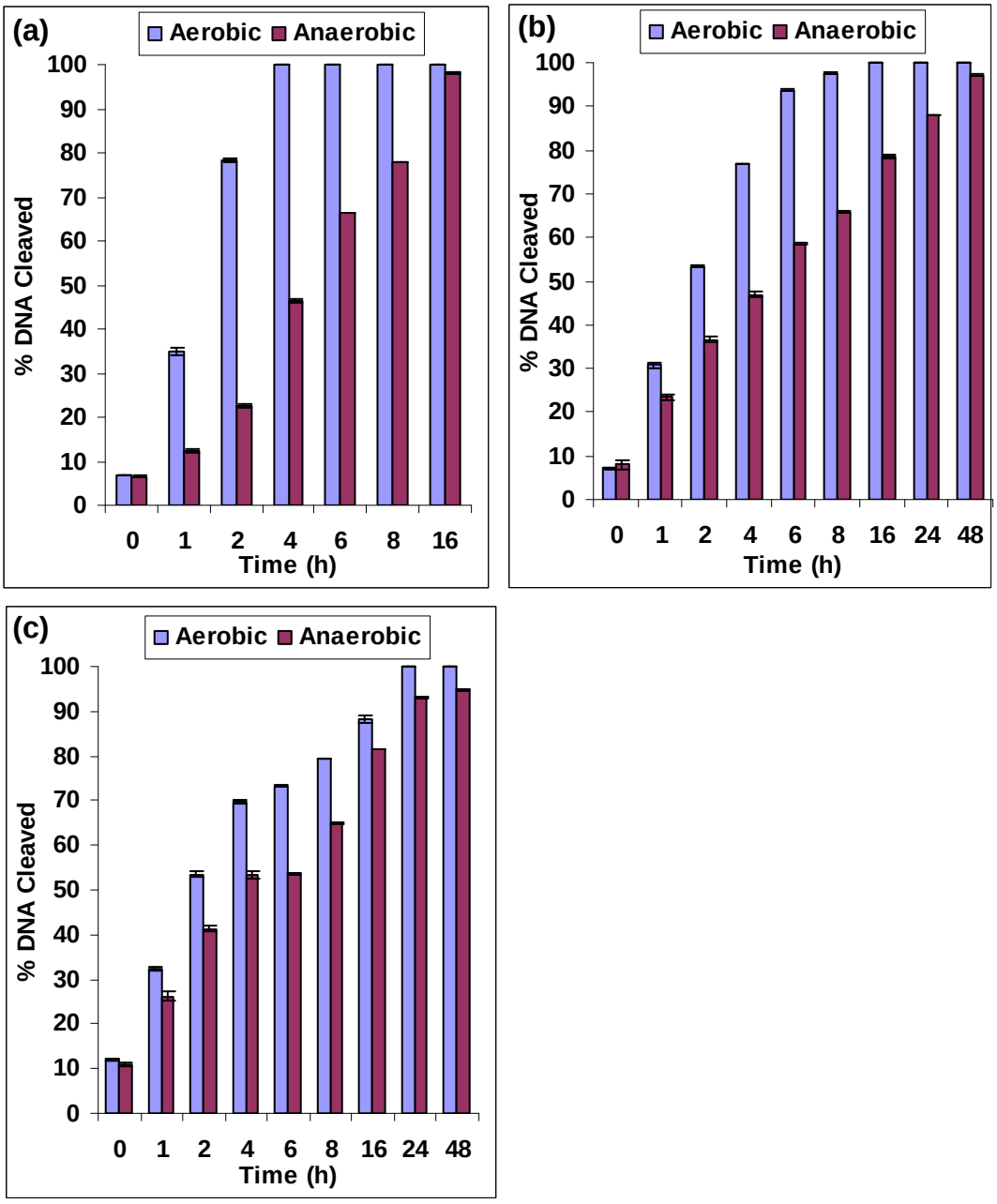


Figure S23. Extent of aerobic and anaerobic cleavage of pBR322 plasmid DNA by **C1** (a), **C2** (b) and **C3** (c) ([**C1**], [**C2**] and [**C3**] = 150 μM in 40 mM HEPES (pH 7.0) at 37 ºC over defined time intervals).