

**Supporting Information for**  
**Metal Ion Interactions in the Active Site of Human**  
**Topoisomerase II $\alpha$**

**Joseph E. Deweese, F. Peter, Guengerich, Alex B. Burgin, and Neil Osheroff**

CONTENTS

FIGURE S1: DynaFit script and results files for fitting of pre-equilibrium  $k$  vs.  $Mg^{2+}$  ion concentration using variable values for the metal ion dissociation, DNA cleavage, and ligation steps and a fixed value for the metal ion binding.

FIGURE S2: DynaFit script and results files for fitting of pre-equilibrium  $k$  vs.  $Mg^{2+}$  ion concentration using variable values for the DNA cleavage and ligation steps and fixed values for the metal ion binding and dissociation.

FIGURE S1. DynaFit script and results files for fitting of pre-equilibrium  $k$  vs.  $Mg^{2+}$  ion concentration using variable values for the metal ion dissociation, DNA cleavage, and ligation steps and a fixed value for the metal ion binding. See Fig. 3 of main text for plot. *A*, the reaction scheme with variable values for the metal ion dissociation ( $k_{-1}$ ), DNA cleavage ( $k_2$ ), and ligation ( $k_{-2}$ ) steps using a fixed value for the metal ion binding. *B*, same as in *A* with different starting values for  $k_{-1}$ ,  $k_2$ , and  $k_{-2}$ .

## A

### SCRIPT

```
;DYNAFIT script file ./test/
;Basic mechanism for TOPO
; all units in nM, sec
;Trial 8

[task]

data = velocities
task = fit

[mechanism]

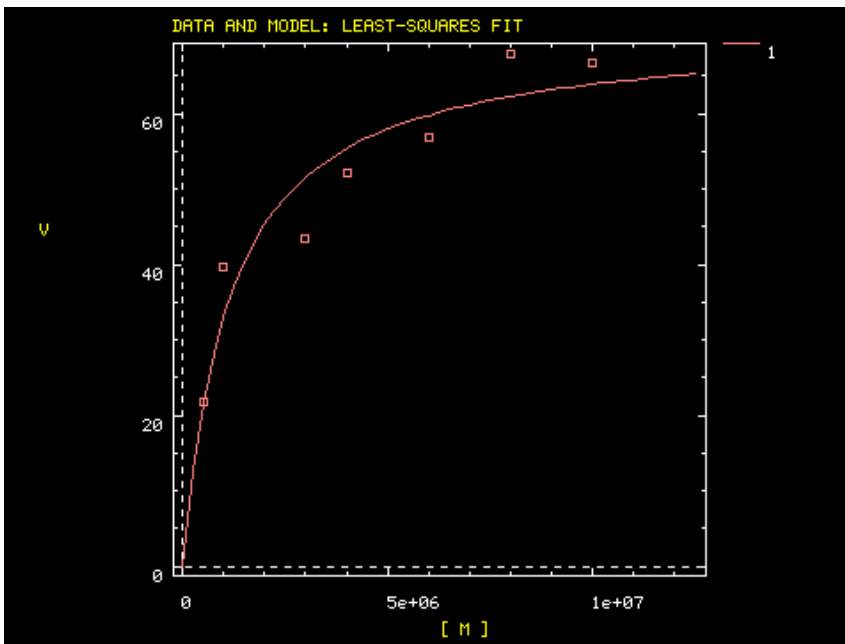
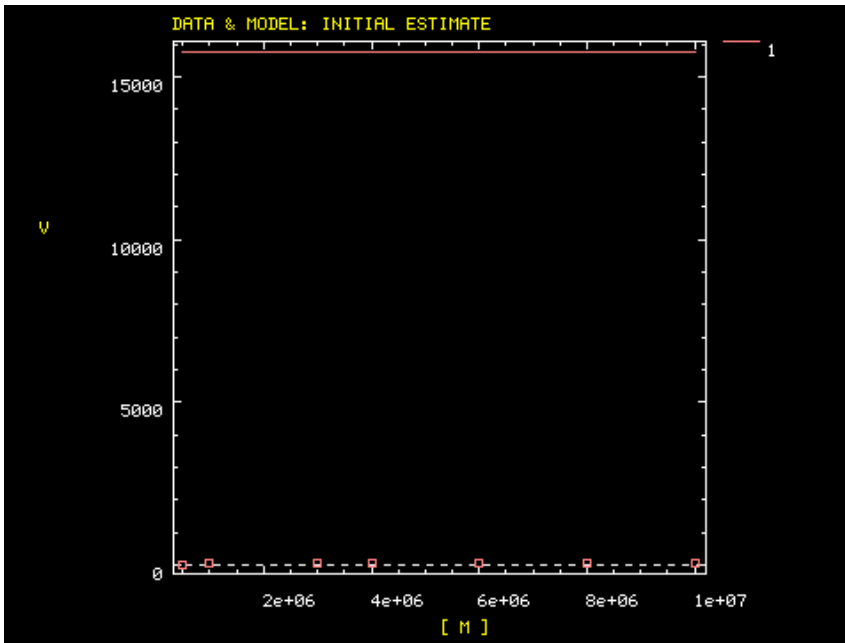
ED + M <==> EDM : k1 k-1
EDM <==> EPM : k2 k-2

[constants]
k1 = 1, k-1 = 200?
k2 = 200?, k-2 = 40?

[sweep]
[velocity]
variable M
file ./scripts/TopoIIrun.txt
[concentrations]
ED = 100

[responses]
EPM = 1
[progress]
delay = 0.001
[output]
directory ./projects/data/output/x
[end]
```

### RESULTS



SCRIPT FILE

-----  
TASK

Fit of initial velocities

DATA

file :scripts:TopoIIrun.txt

OUTPUT

LEAST-SQUARES FIT

mean square 23.2506  
standard deviation 4.82189  
log(determinant) -5.82  
log(condition number) -6.72  
Marquardt parameter 1.64e+04  
execution time (min) 0.05611  
datapoints 7  
parameters 3  
iterations 20  
subiterations 39  
function evaluations 63  
error status 0

PARAMETERS & STANDARD ERRORS

Set	Parameter	Initial	Fitted	Error	%Error
	k-1	200	1.161e+06	310000	27
	k2	200	8.056	290	3600
	k-2	40	2418	36000	1500

COVARIANCE MATRIX

Set	Parameter	Covariances		
	k-1	a	a	
	k2	b	76	b
	k-2	c	76	100

EIGENVECTORS AND EIGENVALUES

		Eigenvectors		
Eigenvalues		0.00	0.37	2.63
log(C)		7.03	0.85	0.00
Set	Parameter	1	2	3
	k-1	1	0	84
	k2	2	-70	37
	k-2	3	-70	-37

CONDITION INDICES

Set	Parameter	Index	7	1	0
	k-1	0	72	27	
	k2	50	13	36	
	k-2	49	14	36	

PLOTS

Best-fit plot: :projects:data:output:x:tab:fit\_01.tab

## B

### SCRIPT

```
;DYNAFIT script file ./test/
;Basic mechanism for TOPO
; all units in nM, sec
;Trial 10

[task]

data = velocities
task = fit

[mechanism]

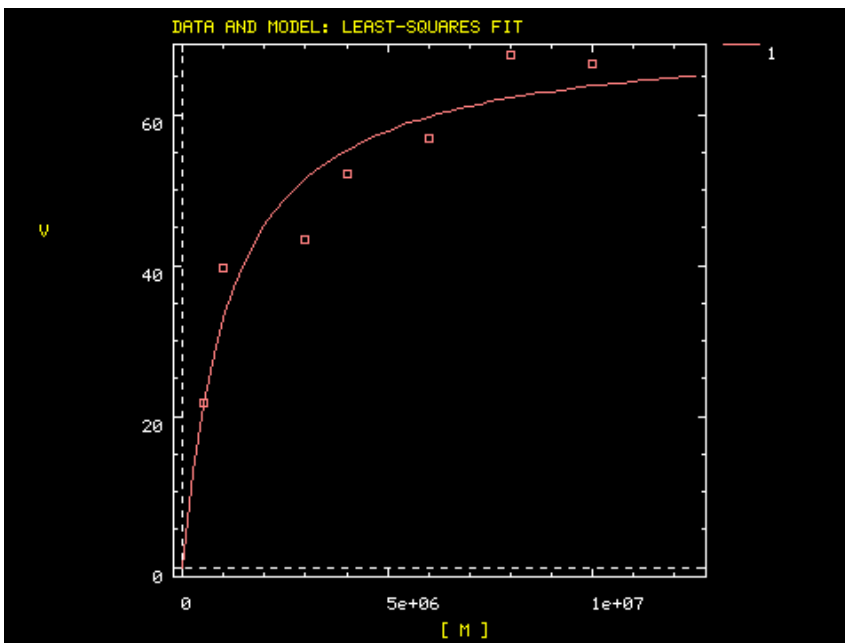
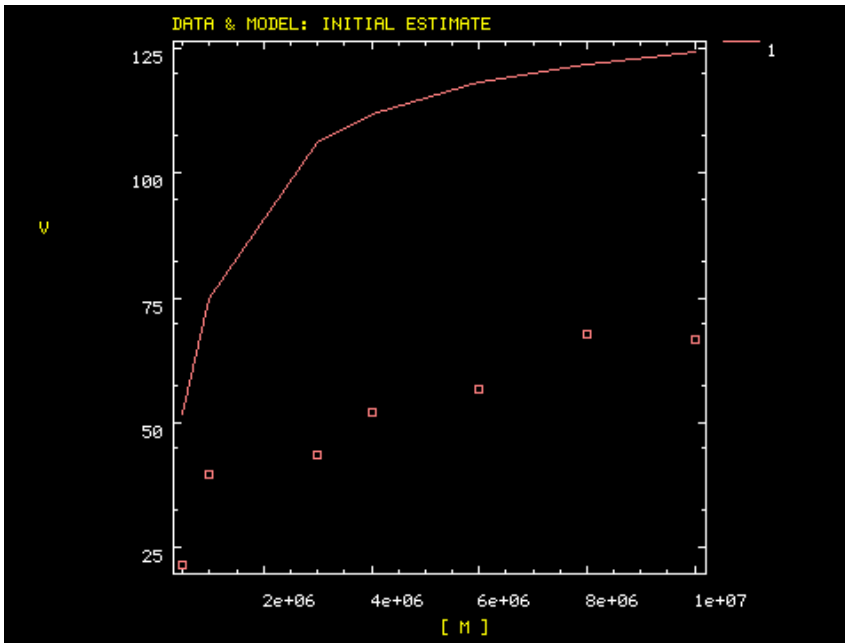
ED + M <==> EDM : k1 k-1
EDM <==> EPM : k2 k-2

[constants]
k1 = 0.01, k-1 = 10000?
k2 = 10?, k-2 = 2000?

[sweep]
[velocity]
    variable M
    file ./scripts/TopoIIrun.txt
[concentrations]
    ED = 100

[responses]
    EPM = 1
[progress]
    delay = 0.001
[output]
    directory ./projects/data/output/x
[end]
```

### RESULTS



SCRIPT FILE

-----  
TASK

Fit of initial velocities

DATA

file :scripts:TopoIIrun.txt

OUTPUT

#### LEAST-SQUARES FIT

```
mean square          23.2609
standard deviation    4.82295
log(determinant)     -2.78
log(condition number) -8.92
execution time (min)  0.01333
datapoints           7
parameters           3
iterations           10
function evaluations  11
error status         1
```

#### PARAMETERS & STANDARD ERRORS

Set	Parameter	Initial	Fitted	Error	%Error
	k-1	10000	13860	470000	3400
	k2	10	7.218	3100	43000
	k-2	2000	2311	430000	19000

#### COVARIANCE MATRIX

Set	Parameter	Covariances		
	k-1	a	a	
	k2	b	100	b
	k-2	c	100	100

#### EIGENVECTORS AND EIGENVALUES

		Eigenvectors			
Eigenvalues		0.00	0.21	2.79	
log(C)		8.90	1.11	0.00	
Set	Parameter	1	2	3	
	k-1	1	-1	83	55
	k2	2	-71	37	-58
	k-2	3	-70	-40	58

#### CONDITION INDICES

		Index	9	1	0
Set	Parameter				
	k-1	0	69	30	
	k2	50	14	34	
	k-2	49	16	34	

#### PLOTS

Best-fit plot: :projects:data:output:x:tab:fit\_01.tab

**FIGURE S2.** DynaFit script and results files for fitting of pre-equilibrium  $k$  vs.  $Mg^{2+}$  ion concentration using variable values for the DNA cleavage and ligation steps and fixed values for the metal ion binding and dissociation. See Fig. 3 of main text for plot. A, the

reaction scheme with variable values for the cleavage ( $k_2$ ) and ligation ( $k_{-2}$ ) steps using fixed values for the metal ion binding ( $k_1$ ) and dissociation ( $k_{-1}$ ). *B*, same as *A* using different starting values for  $k_2$  and  $k_{-2}$ .

## A

### SCRIPT

```
;DYNAFIT script file ./test/
;Basic mechanism for TOPO
; all units in nM, sec
;Trial 12

[task]

data = velocities
task = fit

[mechanism]

ED + M <==> EDM : k1 k-1
EDM <==> EPM : k2 k-2

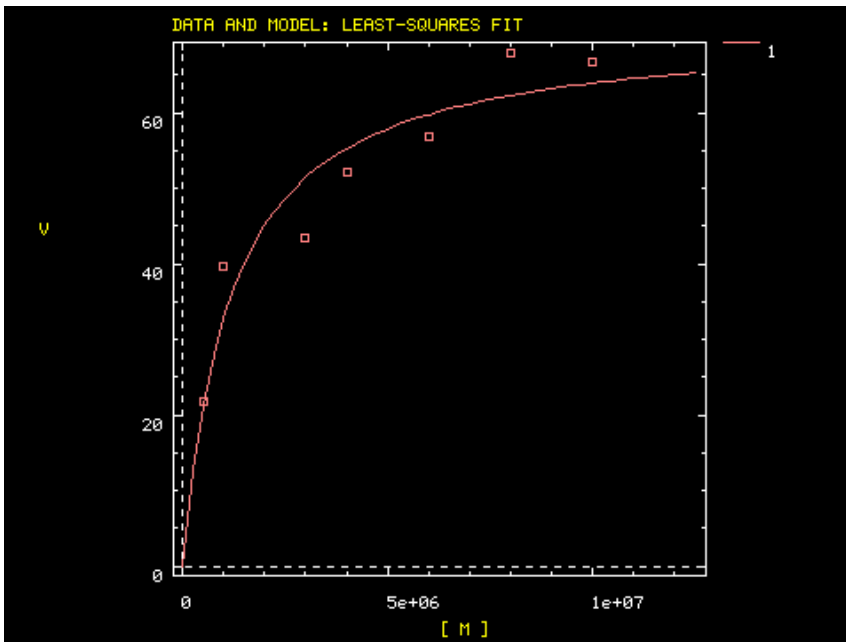
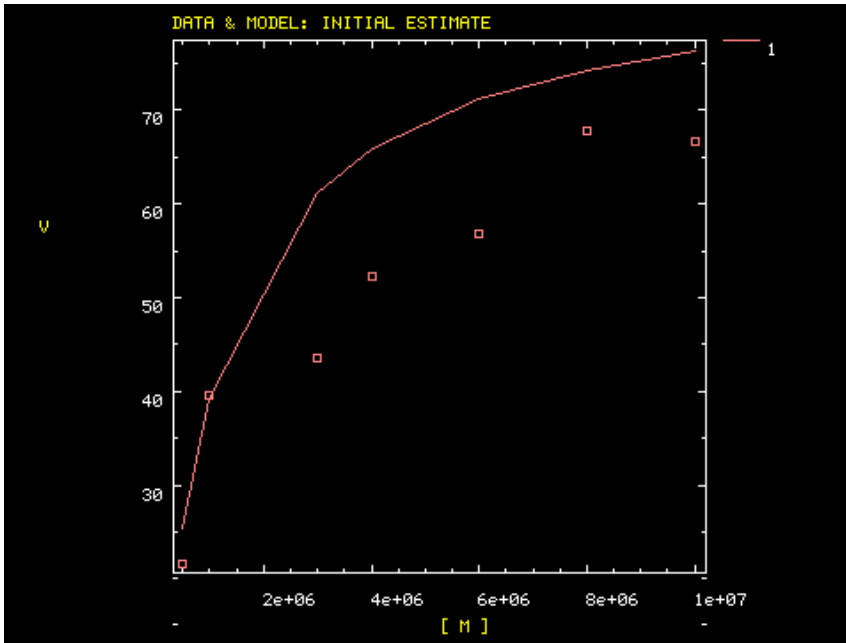
[constants]
k1 = 0.1, k-1 = 120000
k2 = 7?, k-2 = 2100?

[sweep]
[velocity]
    variable M
    file ./scripts/TopoIIrun.txt
[concentrations]
    ED = 100

[responses]
    EPM = 1
[progress]
    delay = 0.001
[output]
    directory ./projects/data/output/x
[end]
```

### RESULTS





SCRIPT FILE

-----  
TASK

Fit of initial velocities

DATA

file :scripts:TopoIIrun.txt

OUTPUT

LEAST-SQUARES FIT

```
mean square          23.2593
standard deviation   4.82279
log(determinant)    -1.23
log(condition number) -1.98
Marquardt parameter  32
execution time (min) 0.03778
datapoints          7
parameters          2
iterations           23
subiterations        19
function evaluations 45
error status         0
```

PARAMETERS & STANDARD ERRORS

Set	Parameter	Initial	Fitted	Error	%Error
	k2	7	6.472	1	16
	k-2	2100	2198	170	7.9

COVARIANCE MATRIX

Set	Parameter	Covariances	
	k2	a	a
	k-2	b	98

EIGENVECTORS AND EIGENVALUES

	Eigenvalues	Eigenvectors	
	0.02	1.98	
	1.98	0.00	
Set	Parameter	1	2
	k2	1	-70 -70
	k-2	2	-70 70

CONDITION INDICES

Set	Parameter	Index	2	0
	k2	49	49	
	k-2	49	49	

PLOTS

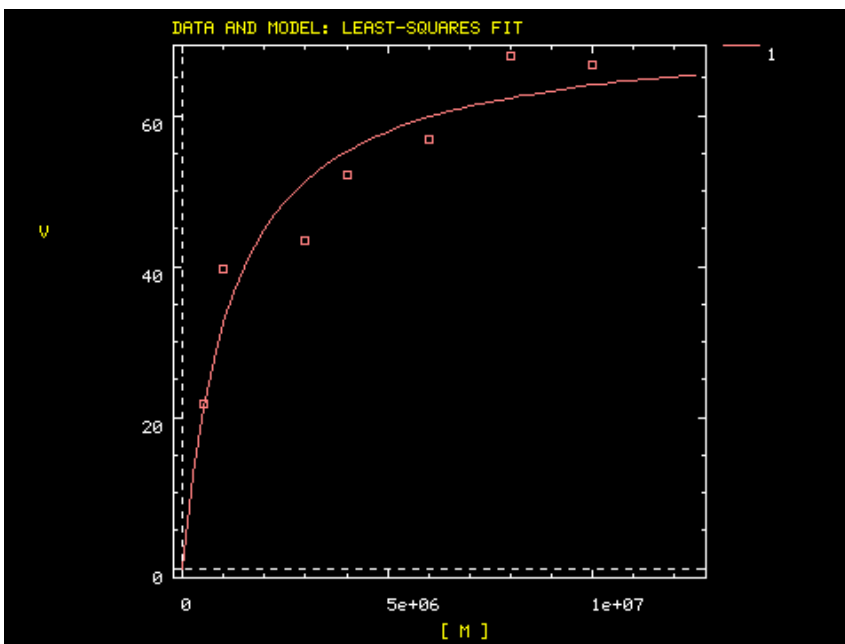
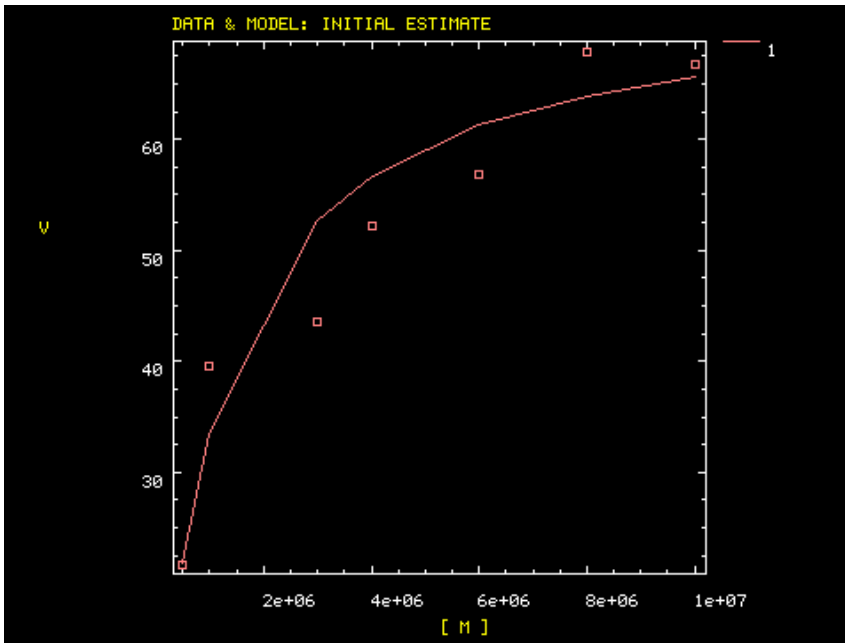
Best-fit plot: :projects:data:output:x:tab:fit\_01.tab

**B**

## SCRIPT

```
;DYNAFIT script file ./test/  
;Basic mechanism for TOPO  
; all units in nM, sec  
;Trial 16  
  
[task]  
  
data = velocities  
task = fit  
  
[mechanism]  
  
ED + M <==> EDM : k1 k-1  
EDM <==> EPM : k2 k-2  
  
[constants]  
k1 = 0.1, k-1 = 120000  
k2 = 2?, k-2 = 1000?  
  
[sweep]  
[velocity]  
variable M  
file ./scripts/TopoIIrun.txt  
[concentrations]  
ED = 100  
  
[responses]  
EPM = 1  
[progress]  
delay = 0.001  
[output]  
directory ./projects/data/output/x  
[end]
```

## RESULTS



SCRIPT FILE

-----  
TASK

Fit of initial velocities

DATA

file :scripts:TopoIIrun.txt

OUTPUT

LEAST-SQUARES FIT

```
mean square          23.2976
standard deviation   4.82676
log(determinant)     -2.89
log(condition number) -3.88
Marquardt parameter  1.31e+05
execution time (min) 0.03777
datapoints           7
parameters            2
iterations            11
subiterations        40
function evaluations  55
error status          0
```

PARAMETERS & STANDARD ERRORS

Set	Parameter	Initial	Fitted	Error	%Error
	k2	2	1.969	3.1	160
	k-2	1000	1009	1500	150

COVARIANCE MATRIX

Set	Parameter	Covariances
	k2	a a
	k-2	b 100

EIGENVECTORS AND EIGENVALUES

	Eigenvalues	Eigenvectors	
	0.00	2.00	
	3.88	0.00	
Set	Parameter	1	2
	k2	1	-70
	k-2	2	-70

CONDITION INDICES

Set	Parameter	Index	4	0
	k2	50	50	
	k-2	50	50	

PLOTS

Best-fit plot: :projects:data:output:x:tab:fit\_01.tab