Supplementary Information for

Recombinant butelase-mediated cyclization of the p53-binding domain of the oncoprotein MdmX stabilized protein conformation as a promising model for structural investigation

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Author Contributions: NP carried out the molecular cloning, protein preparation, ligation reaction; XC and NP performed CD, DSC and ITC assays; XC and HL conducted the NMR analysis; ZK, ZY and J Y performed protein purification; XC and MG did structure calculation; YH, MG and ZS designed the experiments and wrote the paper. Z.S. Y.H. and S. L. conceived of the project.

Materials and Methods

Mass spectrometric assay of the molecular weights of eMdmX and cMdmX

Protein sample from SEC purification was desalted with 1 mL Sep-Pak C18 cartridge. The elution from the cartridge was freeze-dried. The protein powder was dissolved in 50% acetonitrile and mixed with a mass spectrometric solution, which contained 50% acetonitrile and 2% formic acid, in a ratio of 1:1.

The mixture was subjected to ESI-MS analysis using a Thermo Q Exactive mass spectrometer (South-Central University for Nationalities, Wuhan, China).

RESULTS

Table S1. Data collection and refinement statistics of N-Mdm2 in complex with nutlin-3a.

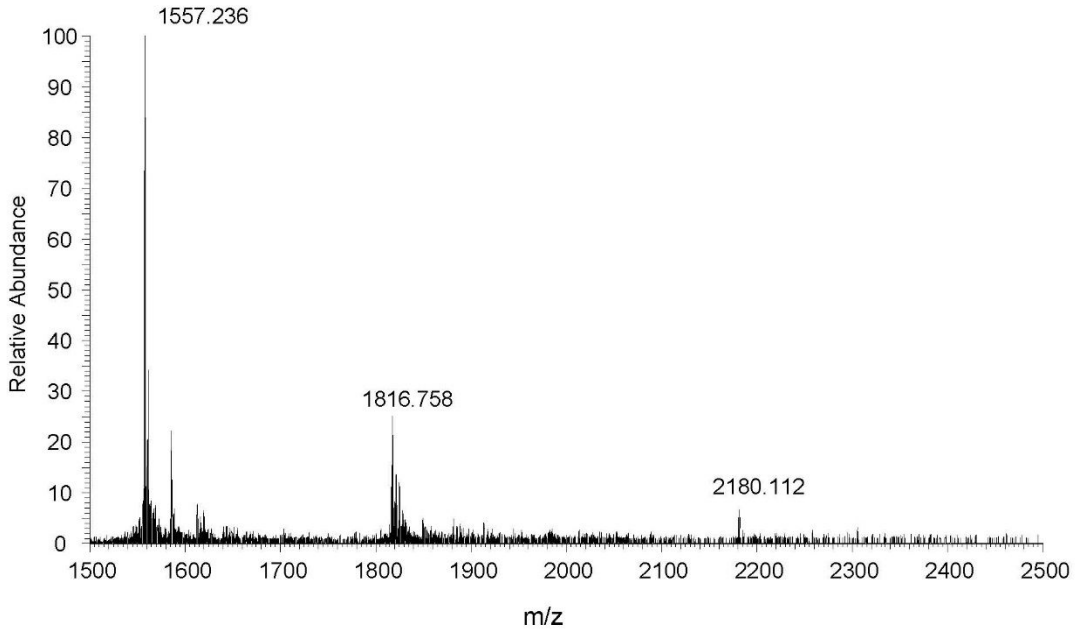
Data collection	
Space group	$P2_12_12_1$
Cell dimensions (Å)	42.5, 43.0, 54.5
a, b, c, α, β, γ	90.00°, 90.00°, 90.00°
Resolution (Å)	33.78 – 1.35
R_{merge}	0.05
$\langle I/\sigma(I)\rangle$	2.01 (at 1.35Å)
Completeness (%)	100
Redundancy	12.1
Refinement	
Resolution (Å)	33.78 – 1.35
No. reflections	22471
R_{work} / R_{free}	0.226/0.254
Number of atoms	
Protein	1460
B-factors	
Protein	20.2
R.m.s. deviations	
Bond lengths (Å)	0.0240
Bond angles (°)	2.314

Table S2. Comparison of kinetic parameters of rBTase with native butelase 1

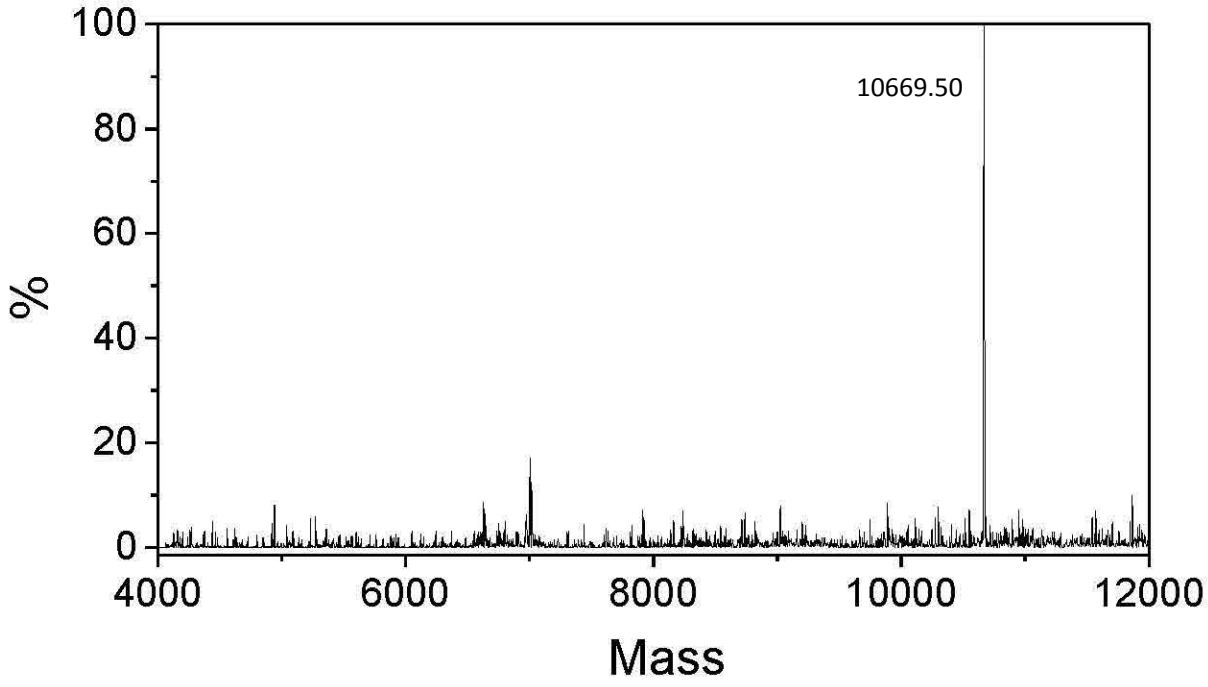
Substrate	k_{cat} (s^{-1})	K_m (μM)	k_{cat} / K_m (M^{-1} s^{-1})	Data source
kB1-NHV	2.15 ± 0.08	223 ± 12	9,641	This work
kB1-NHV	2.28 ± 0.05	213 ± 10	10,700	Nguyen et al, 2014

Data are means of triplicate experiments ± SD.

a


1557.236
1816.758
2180.112
Relative Abundance
m/z
100
90
80
70
60
50
40
30
20
10
0
1500
1600
1700
1800
1900
2000
2100
2200
2300
2400
2500

b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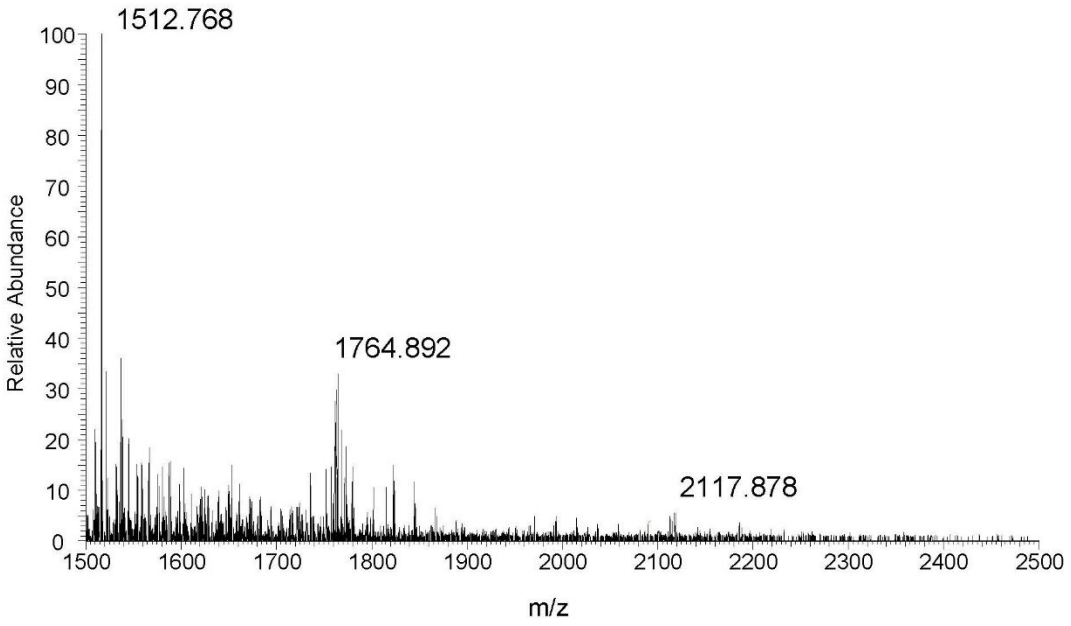
%
Mass
100
80
60
40
20
0
4000
6000
8000
10000
12000

c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1764.892
2117.878
Relative Abundance
m/z
100
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80
70
60
50
40
30
20
10
0
1500
1600
1700
1800
1900
2000
2100
2200
2300
2400
2500

d

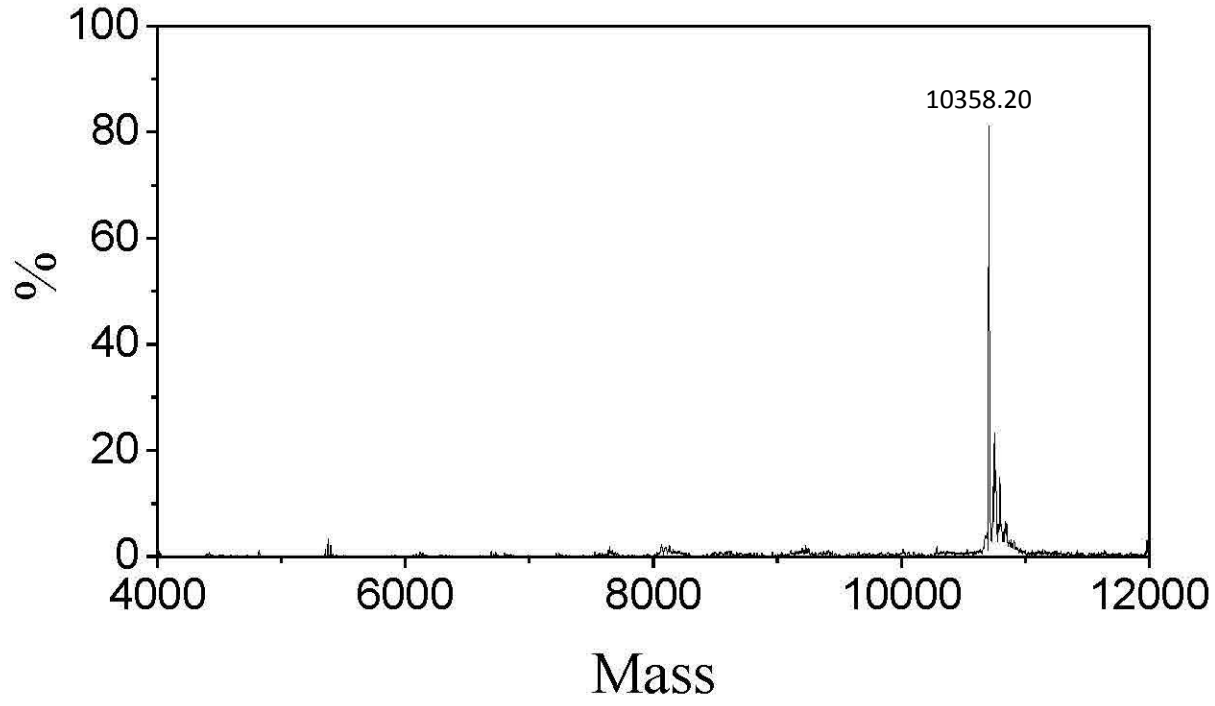
10358.20
%
Mass
100
80
60
40
20
0
4000
6000
8000
10000
12000

Figure S1. Mass spectra of eMdmX and cMdmX. The experimental molecular weights of eMdmX and cMdmX are 10669.45 Dalton and 10358.20 Dalton, respectively, matching their theoretical values. **a**. The mass spectrometric profile of eMdmX; **b**. The deconvolution of the mass spectrometric data of eMdmX. **c**. The mass spectrometric profile of cMdmX; **d**. The deconvolution of the mass spectrometric data of cMdmX.

Table S3. Summary on thermodynamics parameters of eMdmX and cMdmX during heat denaturation

	eMdmX	**cMdmX**
T_m (°C)	44	47
ΔC_p (kcal/mol.K)	2.3	2.8
ΔH_{cal} (kcal/mol)	81.5	86.9
ΔH_v (kcal/mol)	88.3	91.2

Table S4. Summary on the thermodynamics parameters of eMdmX and cMdmX titrated with nutlin-3a

	eMdmX	**cMdmX**
n	0.9± 0.2	1.1 ± 0.2
K_d (μM)	19.3 ± 3.5	8.6 ± 2.6
ΔH (kcal/mol)	-3.3 ± 1.9	-3.8 ± 1.8
-TΔS (kcal/mol)	-2.8 ± 1.2	-3.3 ± 1.7

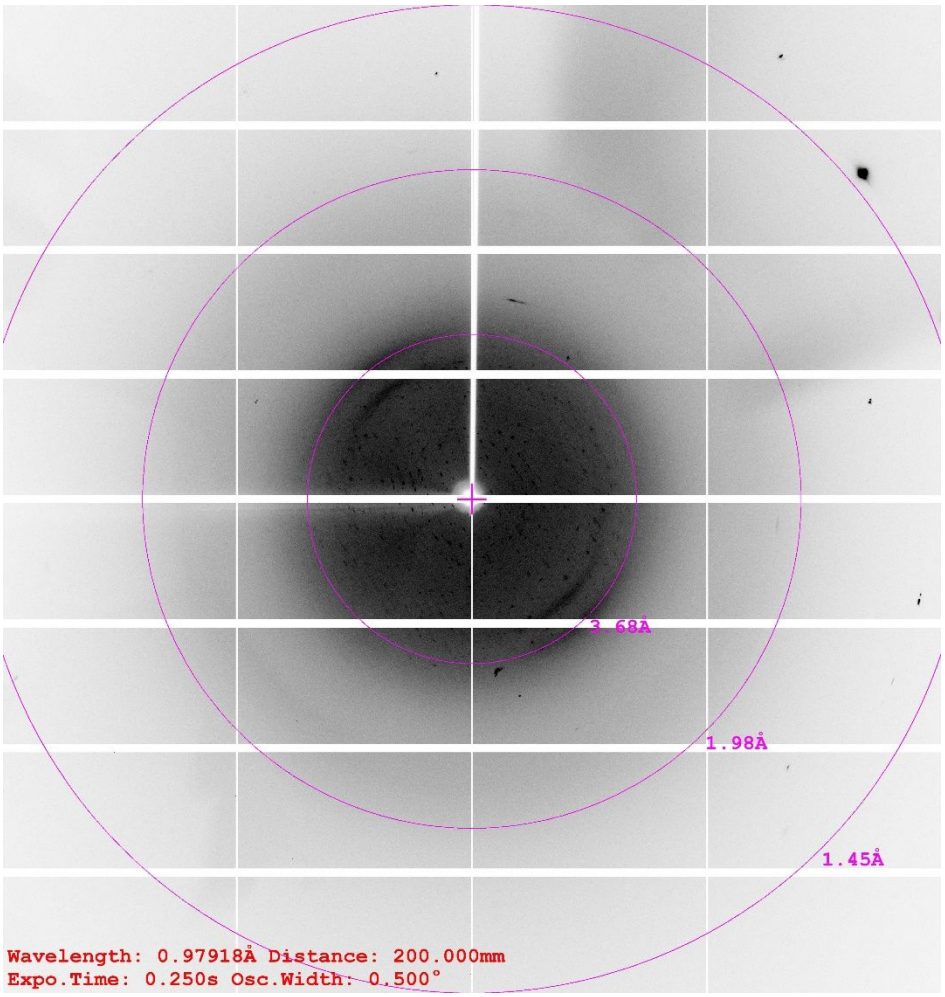

Figure S2. An X-ray diffraction map of the crystal of cMdmX/nutlin-3 complex.

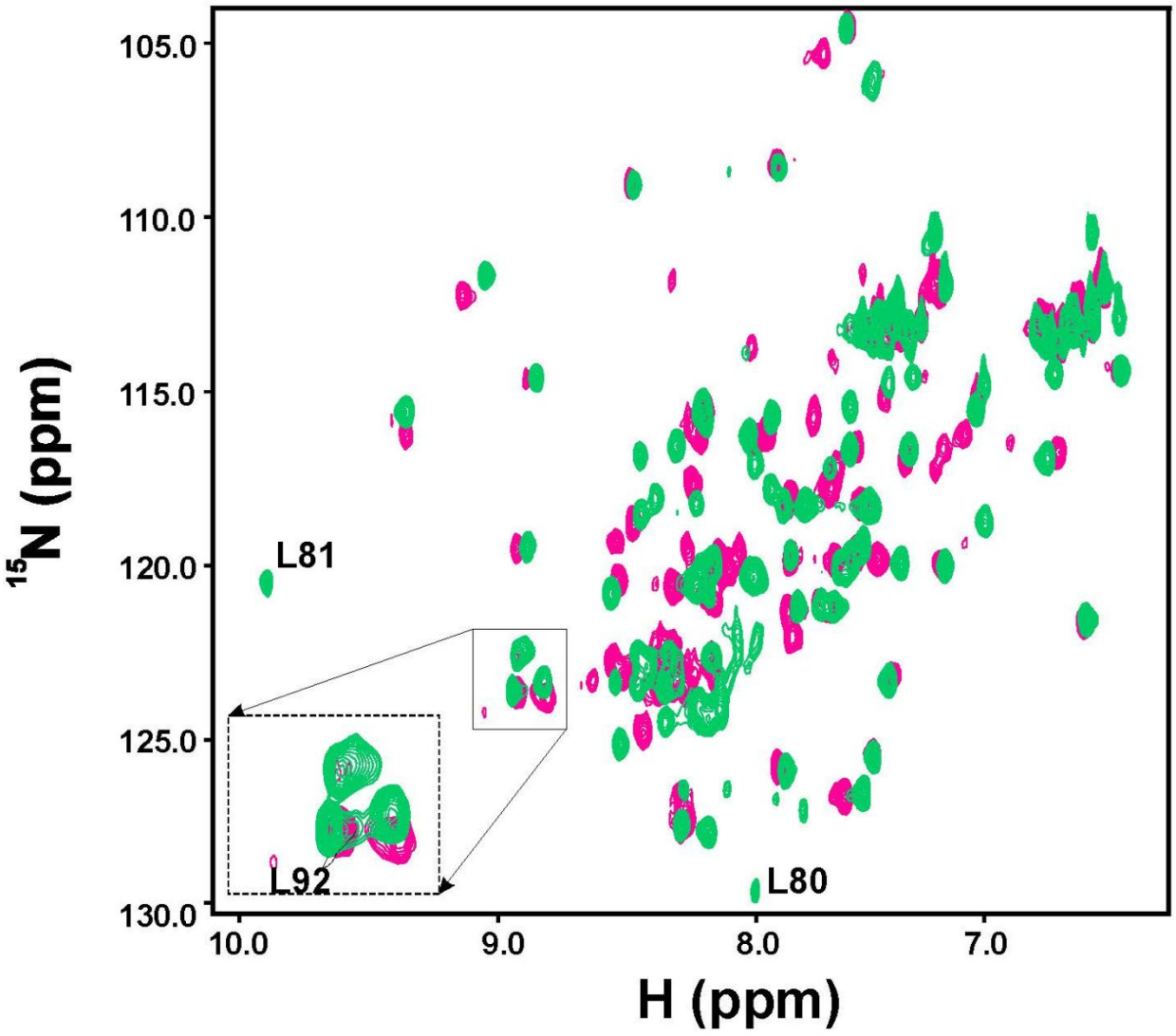

Figure S3. Effect of cyclization on ^{15}N-^{1}H HSQC NMR spectrum of N-eMdmX. ^{15}N-^{1}H HSQC spectrum of cMdmX in complex with nutlin-3a (*green*) was superimposed on that of eMdmX in complex with nutlin-3a (*red*). Spectral region around L92 peak was enlarged and deepen for clear view.