Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

DNA Nanoribbon as a potent Inhibitor of Metallo-β-Lactamases †

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Materials and Instruments

General chemicals were purchased from Sigma-Aldrich and were used without further purification. All DNA oligodeoxynucleotides were purchased from Invitrogen (Shanghai, China) and were used without further purification. All water was purified by a Milli-Q water purification system. UV-vis absorbance spectra were recorded on a UV 8453 spectrometer (Agilent, U.S.A.). Fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrometer (Shimadzu, Japan). Isothermal titration calorimetry (ITC) experiments were performed on a Malvern MicroCal ITC 200 instrument. CD spectra were acquired from a J-1500 CD spectrometer (JASCO Company, Hachioji, Japan). Atomic Force Microscopy images were collected on a MultiMode 8 AFM with NanoScope V Controller (Bruker, Inc.). Thermal Denaturation Studies were processed on a StepOnePlus Real-time PCR Systems(Applied Biosystems,U.S.A.) The DNA origami sample was cooled in a PCR machine (ABI 9700).

Over-expression and purification of Mβ Ls

NDM-1 (B1): NDM-1 was overexpressed and purified as previously described.[1] E. coli BL21(DE3) cells were first transformed with the over-expression plasmid pET26b-NDM-1 and the cells were plated on LB-agar plates containing 25μ g/mL kanamycin. The crude protein NDM-1 was further purified by run through a G75 column. Protein purity was ascertained by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and protein concentration was determined using Beer’ s law and an extinction coefficient of 27,960 $M^{-1}cm^{-1}$ at 280 nm.

VIM-2 (B1): VIM-2 was overexpressed and purified as previously described.[2] E. coli BL21(DE3) cells were first transformed with the over-expression plasmid pET24a-VIM-2 and the cells were plated on LB-agar plates containing 25μ g/mL kanamycin.The crude protein VIM-2 was run through a G75 column and eluted with 30 mM Tris, pH 7.6, containing 200 mM NaCl. Protein purity was ascertained by SDS PAGE and protein concentration was determined using Beer’s law and an extinction coefficient of 28,500 M^{-1} cm^{-1} at 280 nm.

ImiS (B2): ImiS was overexpressed and purified as previously described.[3] E. coli BL21(DE3) cells were first transformed with the over-expression plasmid pET-26b-ImiS and the cells were plated on LB-agar plates containing 25μ g/mL kanamycin. The crude protein ImiS was run through a G75 column and eluted with 30 mM Tris, pH 7.0, containing 200 mM NaCl. Protein purity was ascertained by SDS PAGE and protein concentration was determined using Beer’s law and an extinction coefficient of 37,250 $M^{-1}cm^{-1}$ at 280 nm.

L1 (B3): L1 was overexpressed and purified as previously described.[4] E. coli BL21(DE3) cells were first transformed with the over-expression plasmid pET26b(+)-L1 and the cells were plated on LB-agar plates containing 25μ g/mL kanamycin. The crude protein L1 was run through a G75 column and eluted with 30 mM Tris, pH 8.5, containing 200 mM NaCl. Protein purity was ascertained by SDS PAGE and protein concentration was determined using Beer’s law and an extinction coefficient of 54,614 $M^{-1}cm^{-1}$ at 280 nm.

Preparation of DNA Nanoribbon

Thermal Annealing : all the staple strands and the scaffold strand(in the molar ratio of 1:1) were mixed to a volume of 100μ L in 1×TA/Mg^{2+} buffer consisting of 40 mM Tris (pH 7.4), 20 mM acetic acid, and 12.5 mM magnesium acetate. Successful folding for the DNR1 was observed at the following conditions: subjecting the mixture to a thermal-annealing ramp that cooled from 90 ℃ to 20 ℃ over the course of 2 h. Successful folding for the DNR2 was observed at the following conditions: thermal annealing by rapid heating to 90 ℃ followed by slow cooling to 4 ℃ over 24 h. The sample was purified using Microcon centrifugal filter devices (100 kDa MWCO, 3000 g speed, 10 min, 3 times) followed by washing with the 1×TA/Mg^{2+} buffer to remove single stranded DNA after assembly. The concentration of the DNRs after purification (C_{after}) could be calculated from the absorbance of the DNR before and after the purification, A_{before} and A_{after} by the following equation:

$$C_{after} = \frac{A_{after}}{A_{before}} C_{before}$$

We use the concentration of *on*e copy unit to represent the concentration of DNRs in this report. The concentration of copy units before purification (C_{before}) was assumed equal to the scafford DNA concentration quantified by measuring OD260 with the extinction coefficient ε 260 calculated with IDT' s OligoAnalyzer.

Enzymatic Kinetic Study

The inhibition studies were conducted at 25℃ using imipenem(40μ M) as substrate of ImiS and cefazolin V (40μ M) as substrate of VIM-2, NDM-1 and L1. The inhibitor concentrations were varied from 1 to 12.5 nM in 1×TA/Mg^{2+}. The enzyme and inhibitor were pre-incubated for 10 min before adding cefazolin/imipenem monitored at 262/300 nm to determine the initial velocity. The kinetic experiment was done in triplicate and the average values were recorded. The inhibitor concentrations causing 50% decrease of enzyme activity (IC_{50}) was calculated based on the kinetic data.

Isothermal Titration Calorimetry

Isothermal titration calorimetry (ITC) experiments were performed on a Malvern MicroCal iTC 200 instrument at 25 ℃. During all experiments the reference cell was filled with degassed 1×TA/Mg^{2+}. Before starting the measurement, the system was equilibrated at the desired 25 ℃. 200μ L of NDM-1 solution (final concentration of 80 nM) in the absence and presence of DNR1 was delivered to the sample cell. After the preliminary equilibration period when the instrument reached the final temperature of 25 ℃, an additional 60 s delay period was allowed to generate the baseline used in the subsequent data analyses. After a 60 s delay, each reaction was automatically initiated by adding 38 μ L of cefazolin (1 mM) into the enzyme-containing sample cell. The stirring rate of the syringe was set to 750 rpm. Heat flow was recorded as a function of time. Data were collected every 1 s until the signal reached the baseline and continued to be recorded for appropriate time to generate the final baseline. Baseline data points before the injection and after the completion of reaction were removed prior to any calculations. The thermal power P (microcalories/second) is directly proportional to the rate of the reaction. According to this relationship the rates of substrate hydrolysis are calculated.

AFM characterization

Samples were prepared by deposition of 2μ L onto freshly cleaved mica, and left to adsorb to the surface for 2 min. They were scanned in tapping mode on a MultiMode 8 AFM with NanoScope V Controller (Bruker, Inc.).

Gel Electrophoresis

For the gel electrophoresis experiment, DNR1 (2 μM) was treated with NDM-1(50 μM) in 1× TA/Mg^{2+}buffer, and the samples were analyzed by 12% (w ⁄v) polyacrylamide gels(29:1 monoacrylamide:bisacrylamide) for 150 minutes at 4 °C at a constant 80 V in 1×TA/Mg^{2+}buffer.The gel was soaked in a SYBR green solution to illuminate the DNA nanoribbons after completion of the electrophoresis and then it was stained in Coomassie Blue to identify the existence of the enzyme.

Competitive Binding Experiment

In competitive binding experiment, a 2 mL solution of 62.5 nM DNR1 and 0.5×SYBR® Gold nucleic acid gel stain was titrated by NDM-1 over a range of NDM-1 concentrations from 0—40μ M. For all fluorescence measurements, the entrance and exit slits were both maintained at 5 nm. The sample was excited at 495 nm and its emission observed at 537 nm. All experiments were conducted at room temperature(25℃) in a buffer containing 1×TA/Mg^{2+}(pH 7.4) .

Circular Dichroic

The CD spectra of DNR1 and NDM-1 were recorded on a Jasco J-810 spectropolarimeter at 25.0 ℃. Each sample solution was scanned in the range of 200—300 nm. The CD spectrum, which represented the average of three scans, was generated from which the buffer background had been subtracted.

Thermal Denaturation Studies

Thermal denaturation experiments were performed on a StepOnePlus Real-time PCR Systems (Applied Biosystems,U.S.A.). In DNA melting experiment,the fluorescence intensity of SYBR/DNR1([DNR1] = 0.1 μM)was monitored at varying intervals of temperature from 20 to 95℃at 537 nm. The same experiment was repeated for DNR1-NDM1 complex ([DNR1] = 0.1 μM, [NDM-1] = 33 μM) to determine the change in melting temperature (Tm) of DNR1-NDM1 complex as compared to DNR1 alone. The fluorescence intensity recorded in each case was plotted as function of temperature. The melting temperature (Tm) was detected as the midpoint of the melting transition as determined by the maxima of the first derivative plots.

Design of DNA Nanoribbon and Sequence

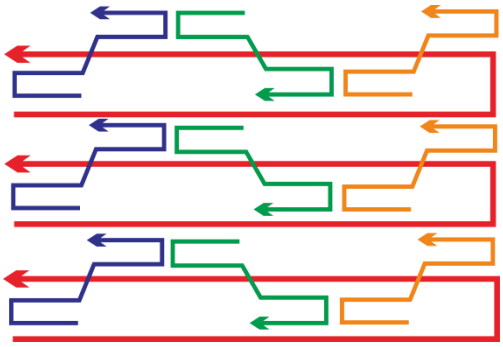

Fig. S1 Schematic illustration of the folding pathway of DNR1.

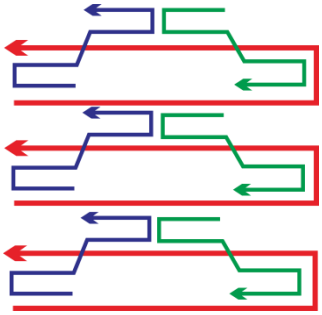

Fig. S2 Schematic illustration of the folding pathway of DNR2.

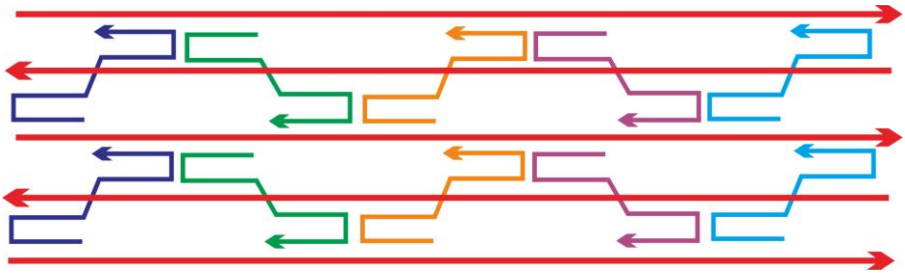

Fig. S3 Schematic illustration of the folding pathway of DNR3.

Table S1 Oligonucleotide sequences used in this work.

DNR1-scafford	5'-CAG GGC TGG GCA TAG AAG TCA GGG CAG AGA CGA GTT GAG AAT ACG AGT TGA GAA TAC GAG TTG AGA ATC CGA CCA TTG TGC GCT ATC TTC ATC TTA-3'
DNR1-staple 1	5'-CAG CCC TGT AAG ATG AAG ATA GCG TCT ATG CC-3'
DNR1-staple 2	5'-CCC TGA CTC ACA ATG GTC GGA TTC CGT CTC TG-3'
DNR1-staple 3	5'-TCT CAA CTT CAA CTC GTA TTC TCA ACT CGT AT-3'
DNR2-scafford	5'-CAGGGCTG GGCATAGA AGTCAGGG CAGAGACG GAATCCGA CCATTGTG CGCTATCT TCATCTTA-3'
DNR2-staple 1	5'-CAG CCC TGT AAG ATG AAG ATA GCG TCT ATG CC-3'
DNR2-staple 2	5'-CCC TGA CTC ACA ATG GTC GGA TTC CGT CTC TG-3'
DNR3-scafford1	CAGGGCTG GACATAGA AGTCAGAT CAGAGACG ACTTGAGA ATAGGATT CGAGTATA TGCGTACC TTGTCCGA CCATTGTG

DNR3-scafford2	CCAATCAA TCCGTCCT GTCAGACC TGCGAATG TGCCCATC CGATAATC GGTCATGG TGAAGCTG CCAGTCCC AAGAGTTA
DNR3-staple 1	CAGCCCTG TAACTCTT GGGACTGG TCTATGTC
DNR3-staple 2	ATCTGACT CAGCTTCA CCATGACC CGTCTCTG
DNR3-staple 3	TCTCAAGT GATTATCG GATGGGCA AATCCTAT
DNR3-staple 4	TATACTCG CATTCGCA GGTCTGAC GGTACGCA
DNR3-staple 5	TCGGACAA AGGACGGA TTGATTGG CACAATGG
DNR4-scafford	CTCCTAAC TGTTCTAA GAGTCGAG CTGAACCG TGGACGAG GGACCAGA CCCCGGAA GTAATGAC AAGCATGA ATGGTACT AAGTGAGC GAGTGCTG
DNR4-staple 1	GTTAGGAG CAGCACTC GCTCACTT TTAGAACA
DNR4-staple 2	CTCGACTC AGTACCAT TCATGCTT CGGTTCAG
DNR4-staple 3	CTCGTCCA GTCATTAC TTCCGGGG TCTGGTCC
tetrahedron-1	5'-GAG CGT TAG CCA CAC ACA CAG TC-3'
tetrahedron-2	5'-TTA GGC GAG TGT GGC AGA GGT GT-3'
tetrahedron-3	5'-CGC CTA AAC AAG TGG AGA CTG TG-3'
tetrahedron-4	5'-AAC GCT CAC CAC TTG AAC ACC TC-3'
random ssDNA	5'-CCTCTAATTCCAGGAG-3'
dsDNA-1	5'-CTCCTGGAATTAGAGG-3'
dsDNA-2	5'-CCTCTAATTCCAGGAG-3'

Supplementary Results

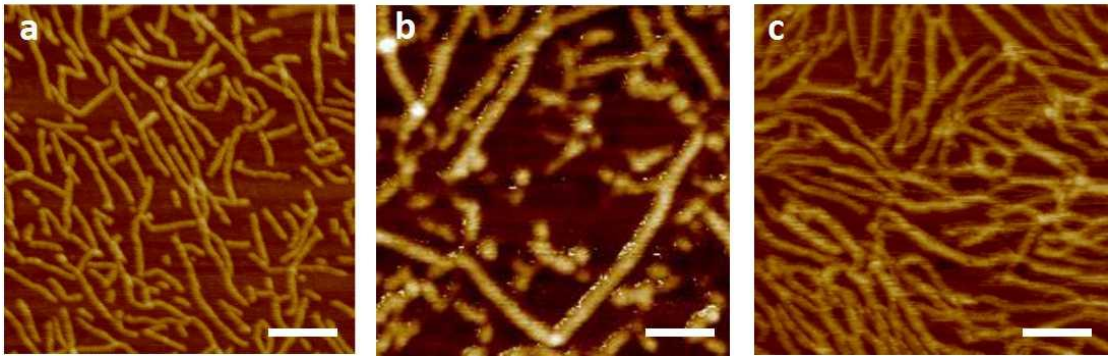

Fig. S4 Atomic force microscopy images of DNR2, DNR3 and DNR4. Scale bars, 100 nm.

Table S2 IC_{50} of different DNA nanostructures against metallo-β-lactamase (unit: nM)

	NDM-1[a]	VIM-2[a]	ImiS[b]	L1[a]
DNR1	3.32	-	11.76	5.66
DNR2	-	-	-	-
DNR3	-	-	-	-
DNR4	40	-	-	-
tetrahedron	-	-	-	-
random ssDNA	-	-	-	-
dsDNA	-	-	-	-
DNR1-scafford	-		-	-
DNR1-staple 1	-		-	-
DNR1-staple 2	-		-	-
DNR1-staple 3	-		-	-
mixture Ⅰ[c]	-		-	-
DNR4-scafford	-			
DNR4- staple 1	-			
DNR4- staple 2	-			
DNR4- staple 3	-			
mixture Ⅱ[d]	-			

[a] Substrate used: cefazolin. [b] Substrate used: imipenem. [c] The mixture of all the DNA sequences added for assembling DNR1. [d] The mixture of all the DNA sequences added for assembling DNR4.

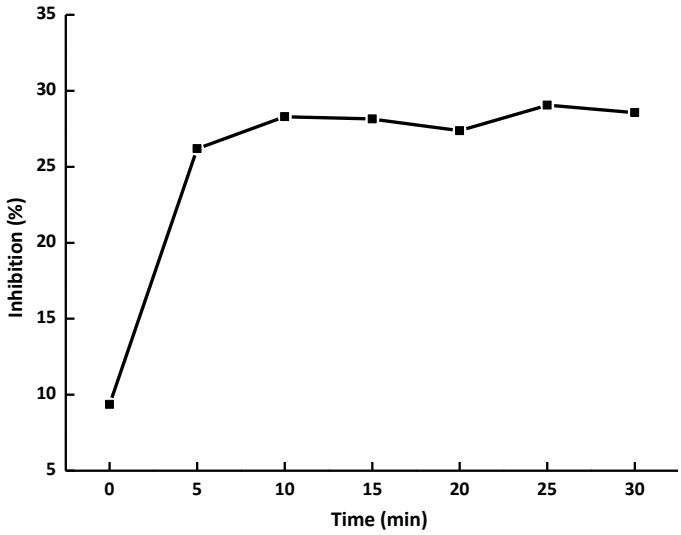

Fig. S5 Inhibition (%) of cefazolin catalyzed by NDM-1 incubation with DNR1 for 0-30 min.

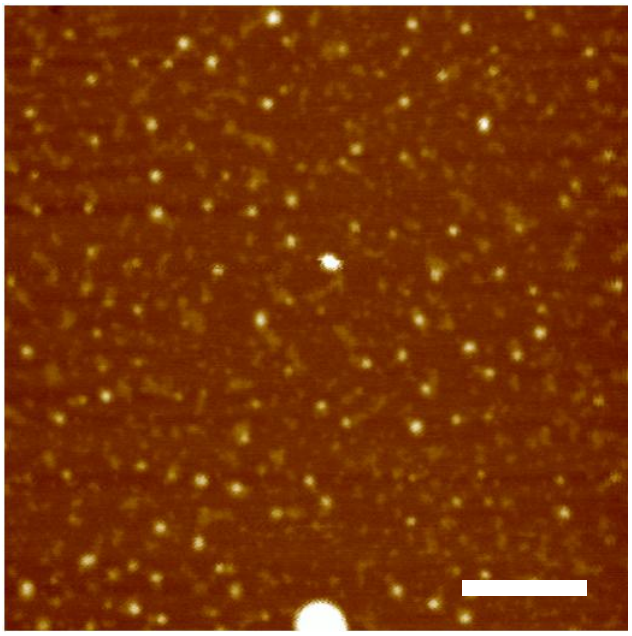

Fig. S6 Atomic force microscopy images of NDM-1. Scale bars, 200 nm.

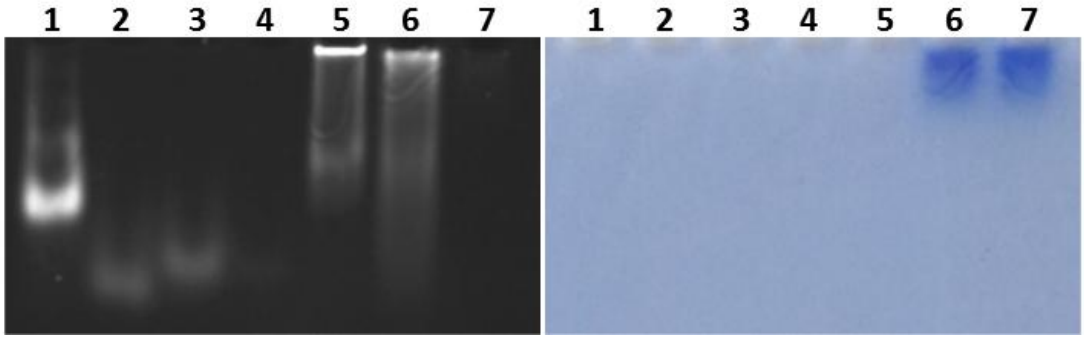

Fig. S7 Electrophoretic Mobility Shift Assay. On the left, the 12% polyacrylamide gel was stained by SYBR® Gold nucleic acid gel stain. On the right, the gel was stained by Coomassie Brilliant Blue R250. About 50 μM NDM-1 and 2 μM DNR1 were used to make the complex. (Lane 1: scafford, Lane 2: staple1, Lane 3: staple2, Lane 4: staple3, Lane 5: DNR1, Lane 6: DNR1/NDM-1 complex, Lane 7: NDM-1)

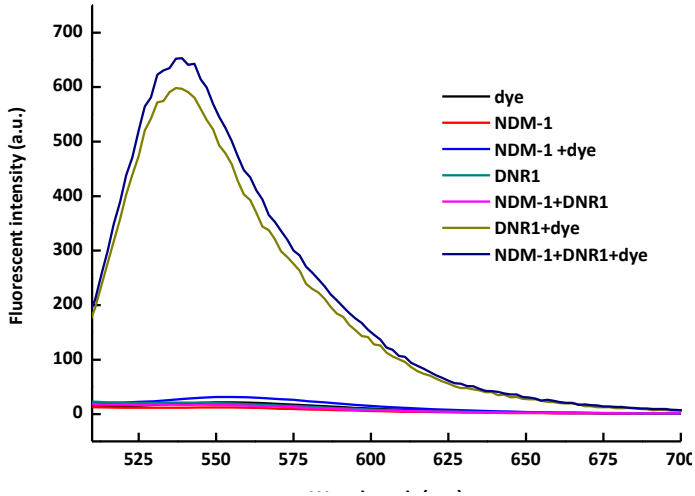

Fig. S8 The fluorescence Emission Spectrum of SYBR dye-DNR1 complex in the absence and presence of NDM-1, DNR1, NDM-1, dye, NDM-1/dye, NDM-1/DNR1. Ex=495 nm, Em=537 nm.

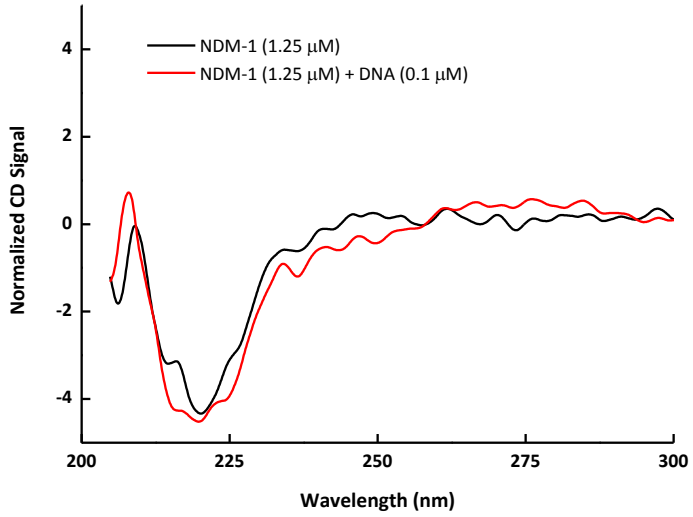

Fig. S9 CD spectrum of NDM-1 in the absence and presence of DNR1. Black line free NDM-1 (1.25 μM), red line NDM-1 (1.25 μM) with DNR1 (0.1 μM).

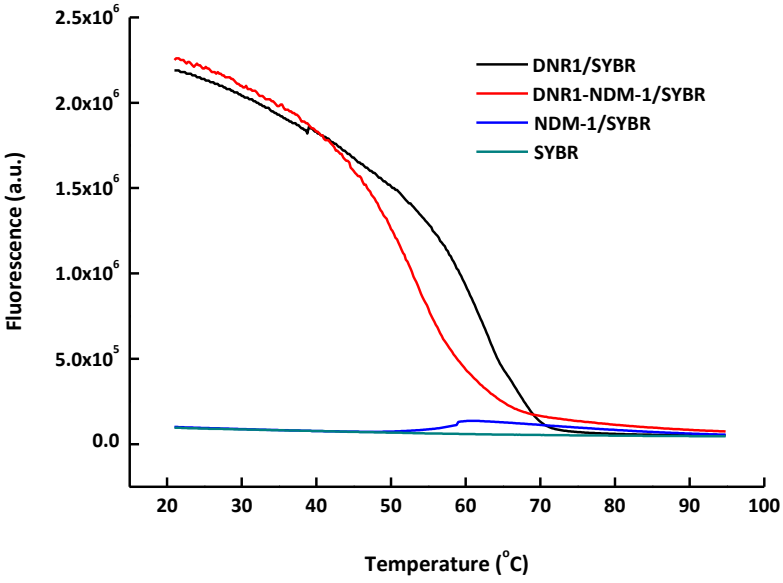

Fig. S10 The melting curves of SYBR(green), NDM-1/SYBR (blue), DNR1/SYBR (black),DNR1-NDM-1/SYBR (red).[DNR1] = 0.1 μM, [NDM-1] = 33 μM. Ex=470 nm, Em=570 nm.

References

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