Electronic Supplementary Information

Biomolecualr Logic Gate for Analysis of the New Delhi Metallo-β-Lactamase (NDM)-Coding Gene with Concurrent Determination of Its Drug Resistance-Encoding Fragments

Experimental Section

Oligonucleotides

DNA probes tagged with thiol group, FITC, and biotin employed in this study were synthesized by MWG biotech (Ebersberg, Germany). All of these modified oligonucleotides were purified using high performance liquid chromatography (HPLC), examined with a matrix-assisted laser desorption-ionization time-of-flight (MALDI–TOF) system, and subsequently quantified using optical-density (OD) measurement. Synthetic DNA targets were synthesized and purified using polyacrylamide gel electrophoresis (PAGE) by Protech (Taipei, Taiwan). Primers for asymmetric polymerase chain reaction were synthesized and purified using reverse phase cartridge (RPC) by MDBio (Taipei, Taiwan). Sequences of all oligonucleotides are elaborated in Table S1.

Materials

3-Mercaptopropionic acid (MPA, ≧99%), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$, ≧99%), 3,3′,5,5′-tetramethylbenzidine (TMB, ≧98%), D-(+)-Glucose (≧99.5%), hydrogen peroxide solution (H_2O_2, 34.5-36.5%), dimethyl Sulfoxide (DMSO, ≥99.7%), albumin from bovine serum (BSA, ≧98%), casein from bovine milk, citric acid monohydrate (A.C.S. grade), glycerol (A.C.S. grade) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium chloride (NaCl, ≧99%), potassium phosphate monobasic and dibasic (A.C.S. grade), and acetic acid (glacial, A.C.S. grade) were obtained from J. T. Baker (Phillipsburg, NJ). HRP-conjugated FITC-antibody (α-FITC-HRP) and avidin-conjugated glucose oxidase (Av-GOx) were acquired from NOVUS Biologicals (Littleton, CO) and Rockland (Gilbertsville, PA), respectively. Dithiothreitol (DTT) and ethylenediaminetetraacetic acid (EDTA, 99.5%) were purchased from PlusOne. 40% Agarose B low eletroendosmosis (EEO), dNTP (dATP, dCTP, dGTP, dTTP, 10 mM of each in 0.6 mM Tris-HCl, pH 7.5), and Phusion High-Fidelity DNA

Polymerase (2U/μL) was acquired from Bio Basic Inc. (Markham, Canada), GeneDirex (Taoyuan, Taiwan), and Thermo Scientific (Waltham, MA), respectively. 100 bp DNA ladder, 6X DNA loading dye, SYBR Gold (10,000X concentrated in DMSO), and SYBR Green I (10,000X concentrated in DMSO) were obtained from Invitrogen (Carlsbad, CA).

Reagents for the genosensor

Buffers used in current study includes: (i) immobilization buffer (IB): 10 mM phosphate, 1 M NaCl, 1 mM $MgCl_2$, pH 7.5; (ii) hybridization buffer (HB), 2.5 % BSA in IB; (iii) washing buffer (WB), 10 mM phosphate, 170 mM NaCl, 1 mM $MgCl_2$, pH 7.6; (iv) binding buffer (BB), 0.5% casein in WB. The substrate solution for glucose oxidase (GOx) and HRP cascade reaction was composed of 250 mM D-(+)-glucose and 0.1 mg/mL TMB in phosphate/citrate buffer, pH 5.0.

Asymmetric polymerase chain reaction (aPCR)

The forward and reverse primers (FP and RP in Table S1) for asymmetric amplification of a fragment in NDM gene were designed by the Tm Calculator of Applied Biosystems. To increase the efficiency and specificity, the two primers were designed to have a 2-3 °C-difference in melting temperatures and added in unequal concentrations[1, 2]{Sanchez, 2004 #22;Pierce, 2005 #23}. The aPCR product was generated from a 100 μL PCR mixture composed of 1X Phusion GC buffer / 0.2 μM dNTP / 1 μM FP / 0.2 μM RP / 21 ng NDM gene / 2 units of Phusion High-Fidelity DNA polymerase. Reactions were performed with 60 cycles of aPCR in MiniOpticon™ Thermal Cycler (Bio-Rad, Hercules, CA). The amplification cycles include 1 cycle of 30 sec at 98 °C followed by 60 cycles of 98 °C for 10 sec, 63.7 °C for 20 sec, and 72 °C for 30 sec, and a final extension was executed at 72 °C for 10 min.

Agarose gel electrophoresis

Products of the aPCR were verified by running gel electrophoresis to determine the sizes and purity of amplified fragments. The agarose gel was composed of 2.5% (w/v) agarose B (low EEO) in Tris-Acetic acid-EDTA (TAE) buffer consisted of 40 mM Tris base, 20 mM acetic acid, and 2 mM EDTA. Before being loaded to the gel, all samples, including DNA ladders, were mixed with 6X DNA

loading dye. Gels were run in the TAE buffer on the Wide Mini Horizontal Electrophoresis System (Major Science, Saratoga, CA) at 80 V for 50 min. After electrophoresis, gels were immersed and stained in 1X SYBR Gold solution (from stock diluted in running buffer) for 30 min. Stained gels were then photographed by MS Compact 0.4 Megapixel CCD Image System (Model UVCI-01-312, Major Science) in which samples were excited by 312 nm UV and detected at the range of 537~563 nm.

Detection of NDM-encoding gene

0.5 µM capture DNA were mixed with 200 µM DTT in IB and incubated for 15 min at ambient temperature (25 °C). Aliquots (6 µL) of this DNA/DTT solution were casted over each working electrode (2.5 mm^2 in area) in the 16-sensor Au array (Genefluidics, Irwindale, CA), followed by overnight incubation at 28 °C in humidified surroundings. DTT was used to effectively reduce the disulfide bond formed in dimer of the thiolated capture probe and offer anti-fouling ability (against physical adsorption of enzyme/protein[3, 4]. After being washed with cold de-ionized H_2O and dried with nitrogen, the modified Au electrodes were subsequently treated with 4 µL of 1 mM 3-mercaptopropionic acid (MPA) in WB for 1 h at room temperature to avoid nonspecific binding of DNA to the gold surface. The washing and drying processes were iterated to give the sensors ready for functionalization. Before challenging the system, targeted DNAs were mixed with reporter probes (F and B in table S1) and incubated for 15 min. Then 4 µL of the DNA hybrid was dropped onto the working electrode to hybridize with the assembled DNA probe at 28 °C for 30 min. After being rinsed with ice-cold WB and dried with nitrogen, 2 µL of the solution containing 10 µg/mL α-FITC-HRP and 50 µg/mL Av-GOx in BB were added and incubated for 30 min at 28 °C. Subsequently, the sensors were washed and dried. To ensure reliable output signals throughout the investigations, the sensory chip was equipped with a tailor-designed adaptor system to facilitate measurements. In the measurement, 35 µL of the substrate solution was casted to cover the three electrodes of the sensors which stood at room temperature for 3 min to expedite enzymatic reaction. A typical cyclic voltammogram (CV) was displayed in Fig. S11. From the CV, an applied potential at -0.15 V (vs. the pseudo-Au reference electrode) was determined to provide sufficient overpotential driving TMB(ox) to TMB(red).

Upon the determination of the desired operating potential, chronoamperometry, instead of cyclic voltammetry, was performed to collect the current of the system, as a result of the inherent capability of chronoamperometry to reliably reflect the amplitude of faradaic current. Those electrochemical experiments were conducted using a CHI 1021C Multi-Potentiostat (CH Instruments, Austin, TX).

Melting curve analysis

Before melting curve analysis, thermodynamic parameters of the hybridization between the biotin-tagged probe (B) with wild-type target (MT) or mutant (N) were computed by using DINAMelt web server[5]. The salt concentration was set as 1 M NaCl, and the oligonucleotide concentration for B, MT and N was 0.5, 0.1, and 0.1 μM, respectively. The outputted graphics are illustrated in Figure S9 and S10.

0.5 μM B was mixed with 0.1 μM MT or N in IB containing 2X SYBR Green I. Samples were incubated at 25 °C for 15 min on MiniOpticon Real-Time PCR System (Bio-Rad), followed by a melting program which started from 25 °C and increased to 90 °C at 0.5 °C increments. Between each increment, the temperature was held for 5 seconds, followed by a fluorescence detection.

Table S1. Oligonucleotides used in this study

Designation	**Sequence and modification (5'→3')**
M	AAA GTT GGG CGC GGT TGC TG – $(CH_2)_3$ – SH
E	TCA CCA CCG CCA GCG CGA CCG – $(CH_2)_3$ – SH
F	FITC$(CH_2)_6$ – TTTTT CTT GTC CTG ATG CGC
B	Biotin – TTTTT GGC ATC ACC GAG ATT GCC GAG
MT	GCG CAT CAG GAC AAG TTT TTT TTC AGC AAC CGC GCC CAA CTT TTT TTT TTT CTC GGC AAT CTC GGT GAT GCC
ET	CGG TCG CGC TGG CGG TGG TGA CTC ACG C GCG CAT CAG GAC AAG TT TTT TT CTC GGC AAT CTC GGT GAT GCC
Q	GCG CAT GAC GAC AAG TTT TTT TTC AGC AAC CGC GCC CAA CTT TTT TTT TTT CTC GGC AAT CTC GGT GAT GCC
N	GCG CAT CAG GAC AAG TTT TTT TTC AGC AAC CGC GCC CAA CTT TTT TTT TTT CTC GGC GCC CTC GGT GAT GCC

QN	GCG CAT GAC GAC AAG TTT TTT TTC AGC AAC CGC GCC CAA CTT TTT TTT TTT CTC GGC GCC CTC GGT GAT GCC
FP	GGA TCA AGC AGG AGA TCA ACC
RP	CGC GGC GTA GTG CTC AGT G

Selection of sequences

We searched NDM-1 in protein data bank (PDB, http://www.rcsb.org/) to acquire the reported crystal structures of NDM-1. Among the 21 structure records, nine crystal structures are complex with seven beta-lactam antibiotics (Table S2). We compiled R scripts (elaborated below) to identify the potential active sites surrounding the beta-lactam antibiotics (also named as ligand below). Briefly, for every PDB record, center of the ligand was first determined by taking mean of coordination (in X-, Y-, and Z-axis) of each atom involved in the molecule. Distances between either two atoms were evaluated, and the largest one was deemed as the size of the ligand. We defined a spherical space, centered at ligand's center and a radius in 1.5-fold ligand molecular size. Amino acid residues (in NDM) whose alpha-carbon are inclusive in this defined spherical space were selected. Identical procedures were applied to the nine PDB records. An exemplified result obtained from PDB 3Q6X in complex with ZZ7 is detailed below (Table S3). Amino acid residues (27 a.a.) which appeared in every NDM structures were summarized in Table S3 (highlighted in red). Then serial amino acid fragments ($\geq$ 4 a.a. residues) among the generated 27 a.a. was found to be a.a. 73-76, 121-125, and 218-224. Nucleotide sequences characteristic to these amino acid fragments were also identified to GTCGCTTCCAAC (217-228), GCGCATCAGGACAAG (652-672), and CTCGGCAATCTCGGTGATGCC (361-375). The conservation of nucleotide sequence was evaluated by aligning all available NDM genes. Subsequently we BLAST the three nucleotide sequences against all complete genomes and plasmids in microbial nucleotide BLAST[6] to evaluate sequence specificity. The nucleotide sequence GTCGCTTCCAAC (217-228) was not specific to NDM because it was recognized in many microbial genomes, thereby was excluded from a candidate gene representative of active site. The rest, GCGCATCAGGACAAG and CTCGGCAATCTCGGTGATGCC, were utilized as the characteristic sequences for the active site. To obtain a NDM-specific gene for capture probe used in recognition purpose, we retrieved the nucleotide sequence residing between the position 376 and 651 for probe design using unique probe selector (UPS)[7]. Such the 276 bp fragment was uploaded to result in a unique 20 bp capture probe sequence based on its pangenomic level and the non-redundant NCBI nucleotide database. The top-ranked probe sequence CAGCAACCGCGCCCAACTTT was selected to be the capture probe. A detailed schematic flowchart for identifying nucleotide sequences specific for NDM and characteristic of its catalytic sites can be seen in Figure S1.

Table S2 Crystal structures in PDB complexed with beta-lactam antibiotics

PDB ID	Ligand ID	Ligand name	Structure title
3Q6X	ZZ7	(2R,4S)-2-[(R)-{[(2R)-2-AMINO-2-PHENYLACETYL]AMINO}(CARBOXY)METHYL]-5,5-DIMETHYL-1,3-THIAZOLIDINE-4-CARBOXYLIC ACID	Crystal structure of NDM-1 in complex with hydrolyzed ampicillin
4EY2	0RM	(2R,4S)-2-{(R)-CARBOXY[(2,6-DIMETHOXYBENZOYL)AMINO]METHYL}-5,5-DIMETHYL-1,3-THIAZOLIDINE-4-CARBOXYLIC ACID	Crystal structure of NDM-1 bound to hydrolyzed methicillin
4EYB	0WO	(2R,4S)-2-[(R)-CARBOXY{[(5-METHYL-3-PHENYL-1,2-OXAZOL-4-YL)CARBONYL]AMINO}METHYL]-5,5-DIMETHYL-1,3-THIAZOLIDINE-4-CARBOXYLIC ACID	Crystal structure of NDM-1 bound to hydrolyzed oxacillin
4EYF	PNK	(2R,4S)-2-{(R)-CARBOXY[(PHENYLACETYL)AMINO]METHYL}-5,5-DIMETHYL-1,3-THIAZOLIDINE-4-CARBOXYLIC ACID	Crystal structure of NDM-1 bound to hydrolyzed benzylpenicillin
4EYL	0RV	(2S)-2-[(1S,2R)-1-CARBOXY-2-HYDROXYPROPYL]-4-{[(3S,5S)-5-(DIMETHYLCARBAMOYL)PYRROLIDIN-3-YL]SULFANYL}-3-METHYL-2H-PYRROLE-5-CARBOXYLIC ACID	Crystal structure of NDM-1 bound to hydrolyzed meropenem
4H0D	ZZ7	(2R,4S)-2-[(R)-{[(2R)-2-AMINO-2-PHENYLACETYL]AMINO}(CARBOXY)METHYL]-5,5-DIMETHYL-1,3-THIAZOLIDINE-4-CARBOXYLIC ACID	New Delhi Metallo-beta-Lactamase-1 Complexed with Mn from Klebsiella pneumoniae
4HKY	FPM	(5R,6S)-6-(1-HYDROXYETHYL)-7-OXO-3-[(2R)-OXOLAN-2-YL]-4-THIA-1-AZABICYCLO[3.2.0]HEPT-2-ENE-2-CARBOXYLIC ACID	New Delhi Metallo-beta-Lactamase-1, Complexed with Cd and Faropenem
4HKY	SFR	(2R)-2-[(1S,2R)-1-CARBOXY-2-HYDROXYPROPYL]-5-[(2R)-TETRAHYDROFURAN-2-YL]-2,3-DIHYDRO-1,3-THIAZOLE-4-CARBOXYLIC ACID	New Delhi Metallo-beta-Lactamase-1, Complexed with Cd and Faropenem
4HL1	ZZ7	(2R,4S)-2-[(R)-{[(2R)-2-AMINO-2-PHENYLACETYL]AMINO}(CARBOXY)METHYL]-5,5-DIMETHYL-1,3-THIAZOLIDINE-4-CARBOXYLIC ACID	Crystal Structure of New Delhi Metallo-beta-Lactamase-1, Complexed with Cd and Ampicillin
4HL2	ZZ7	(2R,4S)-2-[(R)-{[(2R)-2-AMINO-2-PHENYLACETYL]AMINO}(CARBOXY)METHYL]-5,5-DIMETHYL-1,3-THIAZOLIDINE-4-CARBOXYLIC ACID	New Delhi Metallo-beta-Lactamase-1 1.05 A structure Complexed with

Hydrolyzed Ampicillin

Table S3 Seventy amino acid residues selected from PBD 3Q6X in complex with ZZ7 using our complied script

PDB.ID	Ligand.ID	chain	x	y	z	resSeq	resName
3Q6X	ZZ7	A	15.672	-21.653	29.154	33	PRO
3Q6X	ZZ7	A	15.185	-19.252	32.061	34	THR
3Q6X	ZZ7	A	14.825	-16.469	29.471	35	ILE
3Q6X	ZZ7	A	13.66	-17.319	25.977	36	GLY
3Q6X	ZZ7	A	10.988	-19.472	24.382	37	GLN
3Q6X	ZZ7	A	18.536	-20.471	18.686	62	THR
3Q6X	ZZ7	A	21.606	-20.61	20.927	63	SER
3Q6X	ZZ7	A	21.892	-20.604	24.702	64	TYR
3Q6X	ZZ7	A	24.15	-18.783	27.139	65	LEU
3Q6X	ZZ7	A	24.452	-20.789	30.351	66	ASP
3Q6X	ZZ7	A	24.45	-20.787	30.355	66	ASP
3Q6X	ZZ7	A	24.564	-18.245	33.208	67	MET
3Q6X	ZZ7	A	25.877	-19.934	36.362	68	PRO
3Q6X	ZZ7	A	20.616	-18.505	36.663	70	PHE
3Q6X	ZZ7	A	20.24	-20.999	33.771	71	GLY
3Q6X	ZZ7	A	20.03	-20.698	29.995	72	ALA
3Q6X	ZZ7	A	19.313	-17.491	28.091	73	VAL
3Q6X	ZZ7	A	18.069	-18.213	24.549	74	ALA
3Q6X	ZZ7	A	18.915	-15.881	21.637	75	SER
3Q6X	ZZ7	A	17.432	-16.127	18.146	76	ASN
3Q6X	ZZ7	A	18.623	-15.172	14.667	77	GLY
3Q6X	ZZ7	A	22.972	-12.564	13.809	90	ASP
3Q6X	ZZ7	A	25.633	-14.821	15.266	91	THR
3Q6X	ZZ7	A	25.122	-17.116	18.253	92	ALA
3Q6X	ZZ7	A	26.412	-16.076	21.69	93	TRP
3Q6X	ZZ7	A	29.789	-17.746	21.042	94	THR
3Q6X	ZZ7	A	24.516	-8.651	12.675	118	VAL
3Q6X	ZZ7	A	22	-6.411	14.38	119	THR
3Q6X	ZZ7	A	24.029	-4.587	17.08	120	HIS
3Q6X	ZZ7	A	27.252	-4.752	19.117	121	ALA
3Q6X	ZZ7	A	26.241	-7.026	22.02	122	HIS
3Q6X	ZZ7	A	27.272	-10.624	22.717	123	GLN
3Q6X	ZZ7	A	23.981	-12.296	21.771	124	ASP

3Q6X	ZZ7	A	24.137	-10.793	18.255	125	LYS
3Q6X	ZZ7	A	27.908	-10.5	17.581	126	MET
3Q6X	ZZ7	A	29.611	-12.91	19.957	127	GLY
3Q6X	ZZ7	A	30.429	-15.393	17.178	128	GLY
3Q6X	ZZ7	A	28.029	-1.222	15.589	145	SER
3Q6X	ZZ7	A	31.179	0.254	19.225	148	LEU
3Q6X	ZZ7	A	32.344	-3.387	18.861	149	ALA
3Q6X	ZZ7	A	34.261	-1.062	23.276	151	GLN
3Q6X	ZZ7	A	31.881	-4.011	23.85	152	GLU
3Q6X	ZZ7	A	34.462	-6.833	23.739	153	GLY
3Q6X	ZZ7	A	33.365	-7.703	20.16	154	MET
3Q6X	ZZ7	A	15.942	2.05	21.24	188	GLY
3Q6X	ZZ7	A	17.423	-1.044	22.863	189	HIS
3Q6X	ZZ7	A	20.762	0.452	21.818	190	THR
3Q6X	ZZ7	A	21.712	3.385	19.602	191	SER
3Q6X	ZZ7	A	22.992	0.996	16.899	192	ASP
3Q6X	ZZ7	A	20.136	-1.516	16.746	193	ASN
3Q6X	ZZ7	A	13.301	-6.964	15.234	206	GLY
3Q6X	ZZ7	A	16.267	-8.401	17.152	207	GLY
3Q6X	ZZ7	A	16.479	-7.201	20.766	208	CYS
3Q6X	ZZ7	A	14.501	-4.032	20.062	209	LEU
3Q6X	ZZ7	A	11.2	-5.957	19.992	210	ILE
3Q6X	ZZ7	A	9.834	-8.053	22.86	211	LYS
3Q6X	ZZ7	A	6.351	-9.564	27.796	215	ALA
3Q6X	ZZ7	A	9.866	-6.118	30.524	217	SER
3Q6X	ZZ7	A	11.872	-4.483	27.74	218	LEU
3Q6X	ZZ7	A	14.948	-6.415	28.947	219	GLY
3Q6X	ZZ7	A	18.328	-4.759	29.439	220	ASN
3Q6X	ZZ7	A	17.837	-0.988	29.265	221	LEU
3Q6X	ZZ7	A	21.42	-0.255	30.392	222	GLY
3Q6X	ZZ7	A	22.473	0.948	26.901	223	ASP
3Q6X	ZZ7	A	18.944	2.034	25.837	224	ALA
3Q6X	ZZ7	A	17.616	5.348	24.578	225	ASP
3Q6X	ZZ7	A	12.855	-11.355	17.781	248	MET
3Q6X	ZZ7	A	15.857	-12.246	19.894	249	SER
3Q6X	ZZ7	A	14.321	-13.756	23.026	250	HIS

3Q6X	ZZ7	A	10.813	-15.044	22.269	251	SER

R Scripts used to identify the potential active sites surrounding the beta-lactam antibiotics

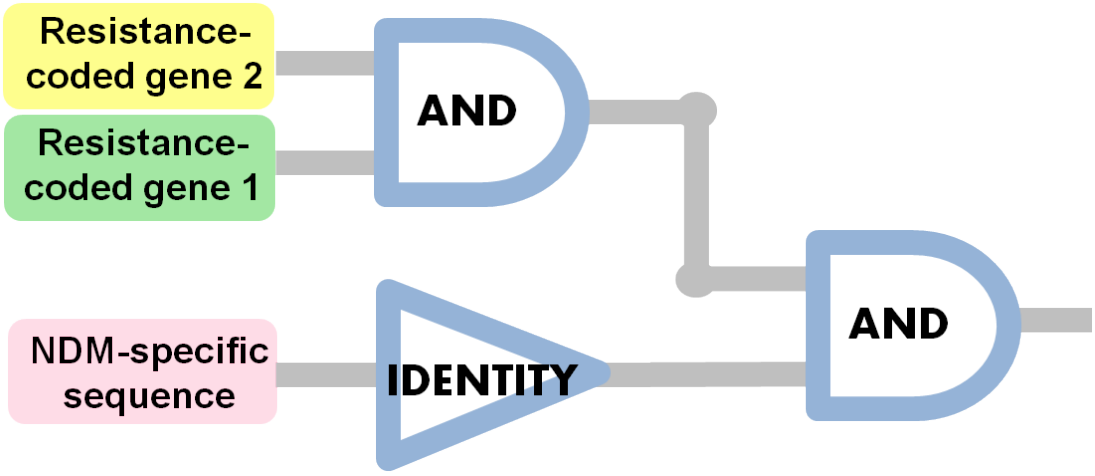
```
PDB.ligand.list <- read.table("PDB/PDB.ligand.2.txt",sep="\t",header=T)
#########
##PDB.ligand.2.txt
##PDB ID Ligand ID
##3Q6X   ZZ7
##4EY2   0RM
##4EYB   0WO
##4EYF   PNK
##4EYL   0RV
##4H0D   ZZ7
##4HKY   FPM
##4HKY   SFR
##4HL1   ZZ7
##4HL2   ZZ7
#########
CA.near.ligand.all <- NA
for (i in 1:dim(PDB.ligand.list)[1]){
file.1 <- paste("PDB/",PDB.ligand.list[i,"PDB.ID"],".pdb",sep="")
## Please download the nine pdb structures from http://www.rcsb.org/
## For example, 3Q6X.pdb can be downloaded via
http://www.rcsb.org/pdb/download/downloadFile.do?fileFormat=pdb&compressio
n=NO&structureId=3Q6X

      PDB.file <- readLines(file.1)
      ########################################
      # HETATM: the atomic coordinate records for atoms within "non-standard"
groups. These records are used for atoms presented in HET groups, e.g. ZZ7
      ########################################

      HETATM <- PDB.file[grep("HETATM",PDB.file)]
      ligand <- HETATM[grep(as.character(PDB.ligand.list[i,"Ligand.ID"]),HETATM)]

      chain <- sapply(strsplit(ligand,character(0)),"[[",22)
      coor.x <- sapply(strsplit(ligand,character(0)),"[",c(31:38))
```

```
        coor.y <- sapply(strsplit(ligand,character(0)),"[",c(39:46))
        coor.z <- sapply(strsplit(ligand,character(0)),"[",c(47:54))

        x <- as.numeric(apply(coor.x,2,paste,collapse=""))
        y <- as.numeric(apply(coor.y,2,paste,collapse=""))
        z <- as.numeric(apply(coor.z,2,paste,collapse=""))

        ligand.coor <-
data.frame(PDB.ID=PDB.ligand.list[i,"PDB.ID"],Ligand.ID=PDB.ligand.list[i,"Ligand.ID"],
chain,x,y,z)

        ########################################
        # ATOM: the atomic coordinates for standand residues
        ########################################

        ATOM <- PDB.file[grep("ATOM",PDB.file)]
        CA <- ATOM[grep(" CA ",ATOM)]

        resname.atom <- sapply(strsplit(CA,character(0)),"[",c(18:20))
        chain.atom <- sapply(strsplit(CA,character(0)),"[[",22)
        resSeq.atom <- sapply(strsplit(CA,character(0)),"[",c(23:26))
        coor.x.atom <- sapply(strsplit(CA,character(0)),"[",c(31:38))
        coor.y.atom <- sapply(strsplit(CA,character(0)),"[",c(39:46))
        coor.z.atom <- sapply(strsplit(CA,character(0)),"[",c(47:54))

        resname.atom <- as.character(apply(resname.atom,2,paste,collapse=""))
        resSeq.atom <- as.numeric(apply(resSeq.atom,2,paste,collapse=""))
        x.atom <- as.numeric(apply(coor.x.atom,2,paste,collapse=""))
        y.atom <- as.numeric(apply(coor.y.atom,2,paste,collapse=""))
        z.atom <- as.numeric(apply(coor.z.atom,2,paste,collapse=""))

        atom.coor <-
data.frame(PDB.ID=PDB.ligand.list[i,"PDB.ID"],Ligand.ID=PDB.ligand.list[i,"Ligand.ID"],
chain=chain.atom,x=x.atom,y=y.atom,z=z.atom,resSeq=resSeq.atom,
resName=resname.atom)

        ### Processed by chain
        chain.num <- names(table(ligand.coor$chain))
```

```
	CA.near.ligand.tmp <- NA
	for (m in 1:length(chain.num)){
		ligand.coor.subset <- subset(ligand.coor,chain == chain.num[m])

		############ To determine the pair-wise distances between each two
atoms of ligand
		diameter.fold <- 1.5		# A user-defined fold
		distance.matrix <- NA
		for (j in 1:dim(ligand.coor.subset)[1]){
			distance <- sqrt((ligand.coor.subset$x[j] - ligand.coor.subset$x)^2 +
(ligand.coor.subset$y[j] - ligand.coor.subset$y)^2 + (ligand.coor.subset$z[j] -
ligand.coor.subset$z)^2)
			distance.matrix <- rbind(distance.matrix,distance)
		}
		distance.matrix <- distance.matrix[-1,]

		## The maximum distance among the ligand atoms
		diameter <- max(distance.matrix)

		## The center coordinate of ligand structure
		ligand.mid.coor <- apply(ligand.coor.subset[,c("x","y","z")],2,mean)

		## To determin the distance between the ligand center and each alpha
carbon
		connect.chain <- subset(atom.coor,chain == chain.num[m])
		distance <- sqrt((ligand.mid.coor["x"] - connect.chain$x)^2 +
(ligand.mid.coor["y"] - connect.chain$y)^2 + (ligand.mid.coor["z"] -
connect.chain$z)^2)
		## To identify which amino acid residue reside in active sites
		CA.near.ligand <- connect.chain[which(distance <= (diameter *
diameter.fold)),]
		CA.near.ligand.tmp <- rbind(CA.near.ligand.tmp,CA.near.ligand)
	}

	CA.near.ligand.all <- rbind(CA.near.ligand.all,CA.near.ligand.tmp[-1,])

}
```

```
CA.near.ligand.all <- CA.near.ligand.all[-1,]

write.table(CA.near.ligand.all,
file="PDB/CA.near.ligand.all.txt",sep="\t",row.names=F,col.names=T,quote=F)

ligand.resSeq <- table(CA.near.ligand.all[,c("Ligand.ID","resSeq")])

bind2ligand <- apply(ligand.resSeq,2,function(x)sum(x != 0))
bind2ligand.with.ligandnum <- rbind (bind2ligand,ligand.resSeq)

bind2all.ligand<- which(bind2ligand.with.ligandnum["bind2ligand",] == 7)
colnames(bind2ligand.with.ligandnum[,which(bind2ligand.with.ligandnum["bind2lig
and",] == 7)])
#[1] "65"   "67"   "73"   "74"   "75"   "76"   "93"   "121" "122" "123" "124" "125"
"189" "190" "207" "208" "209" "211" "218" "219" "220" "221" "222" "223" "224"
"249" "250"
```

(A)



(B)

NDM-specific sequence	Resistance-coded gene 1	Resistance-coded gene 2	Output
1	1	1	1
1	1	0	0
1	0	1	0
1	0	0	0
0	1	1	0
0	1	0	0
0	0	1	0
0	0	0	0

Figure S1. Equivalent circuit (A) and truth table (B) computed for NDM analysis. In line with the scheme 1 and Boolean circuit shown, the facile processing of this biomolecular logic gate system relies on integration of three hybridization activities, two affinity recognition events, and concatenated biocatalytic reactions that lead to a resulting output, displayed as the electrochemical transformation of oxidized TMB (TMB(ox)) to reduced TMB (TMB(red)). Hybridization of the immobilized capture probes (**M**) and the targeted segment (**I**) forms the backbone of the **Identity** gate. Concurrently, an **AND** gate leveraging two hybridization events of the targeted segments (**II** and **III**), against FITC-conjugated reporter (**F**) and biotin-conjugated

reporter (**B**) probes, respectively, operates in parallel with the **Identity** gate. Subsequently, both glucose oxidase–labeled avidin (Av-GOx) and hydrogen peroxidase–labeled anti-FITC (α-FITC-HRP), bound to corresponding antigens, enable an enzymatic cascade reaction to occur. That is, once the substrates (glucose, TMB) are present, GOx catalyzes the oxidation of glucose to produce H_2O_2, which is further reduced by HRP along with the resulting TMB(ox) to configure the **AND** gate utility. Via such the processing, the biomolecular logic gate is able to recognize 'NDM gene' *AND* (not *OR*) in connection to its two drug resistance-encoding fragments (coding gene of biocatalytic activity of metallo-β-lactamase). From an ultimate point of view, this is a triple-input design of Boolean circuit.

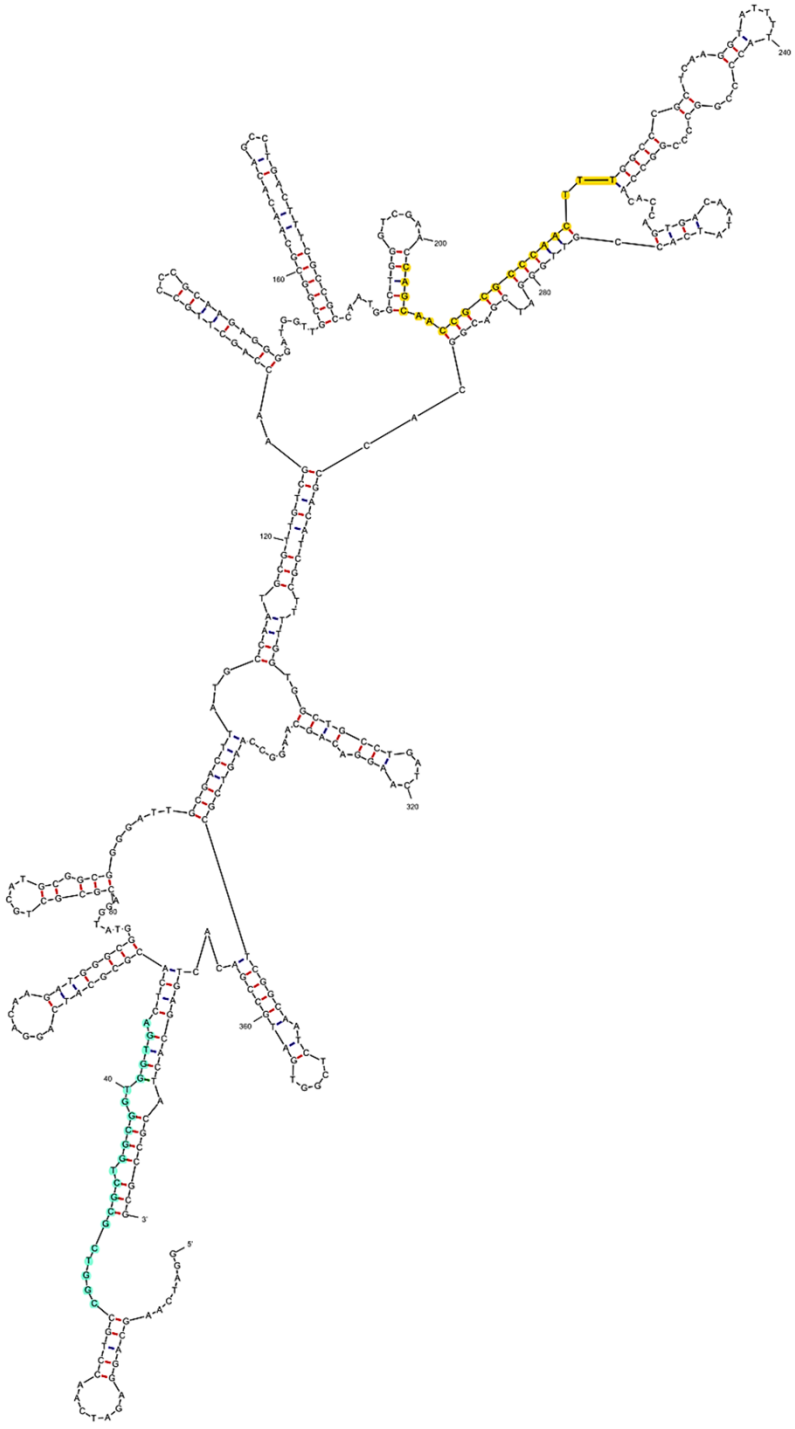


Figure S2A. The envisaged secondary structure of the expected aPCR product predicted using mfold web server at 28 ˚C in the presence of 1 M NaCl and 1 mM $MgCl_2$. The sequence highlighted with yellow is the site hybridized with FITC-tagged probe and the cyan one with biotin-labeled probe. mfold web server: http://mfold.rna.albany.edu/?q=mfold/DNA-Folding-Form

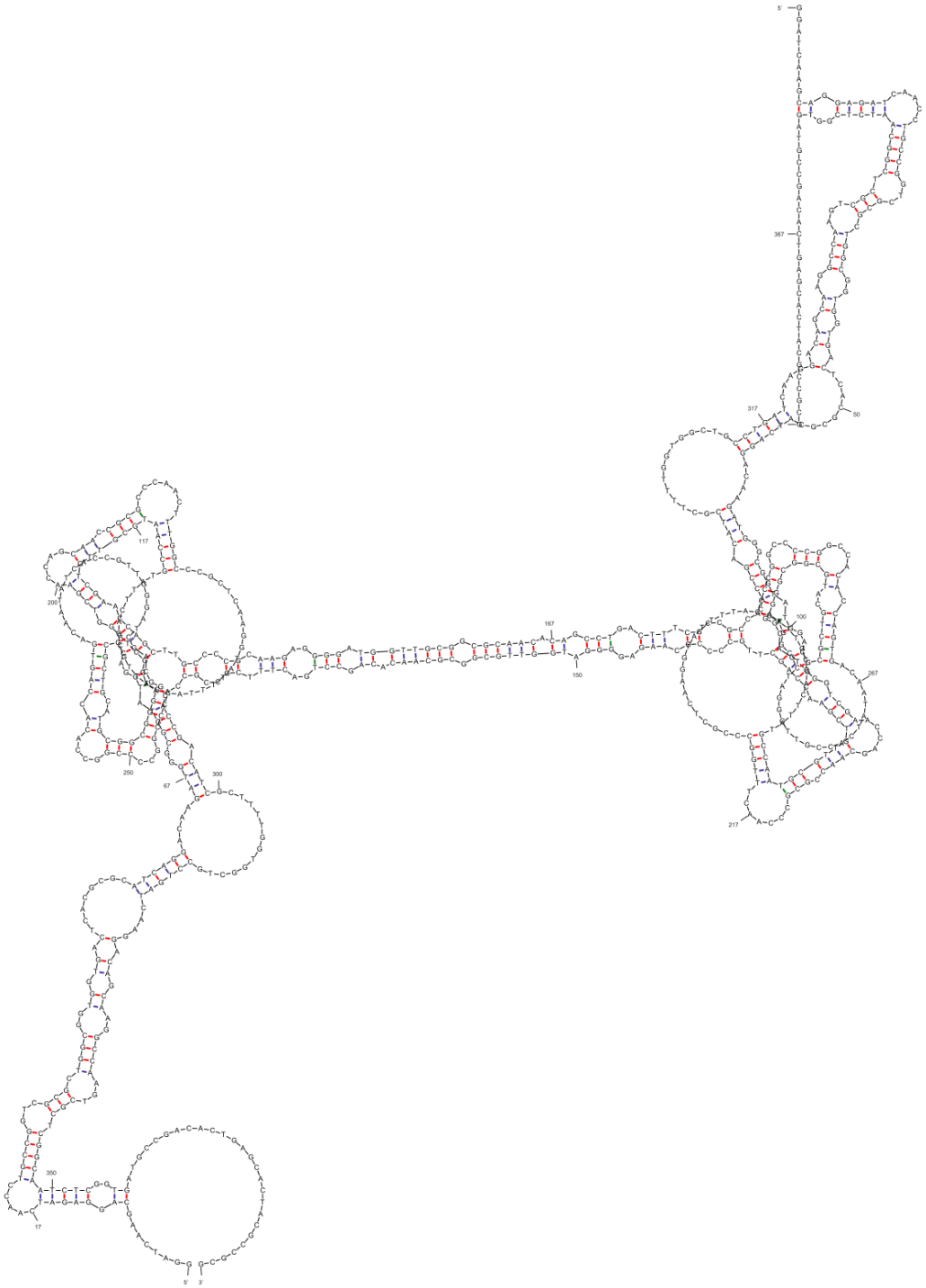


Figure S2B. The possible dimer structure formed by the expected aPCR product predicted using DINAMelt web server at 28 ˚C in the presence of 1 M NaCl.

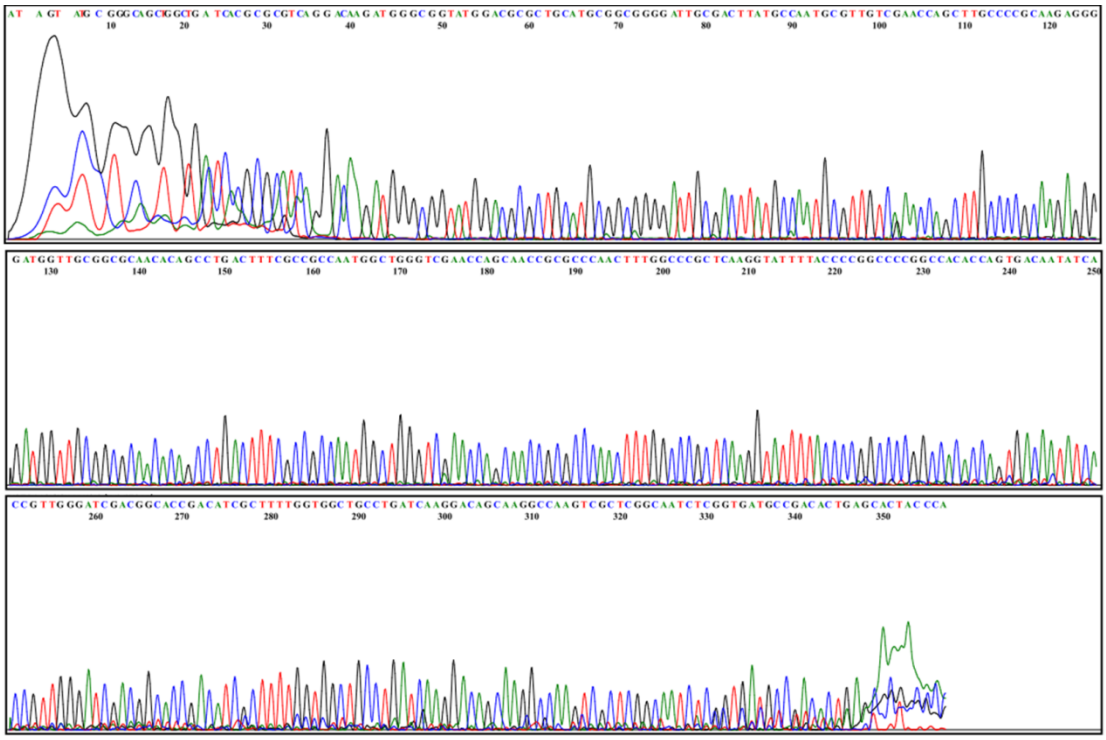


Figure S3. The sequencing result of the band **a** in Figure 1B with the forward primer (FP) used in aPCR.

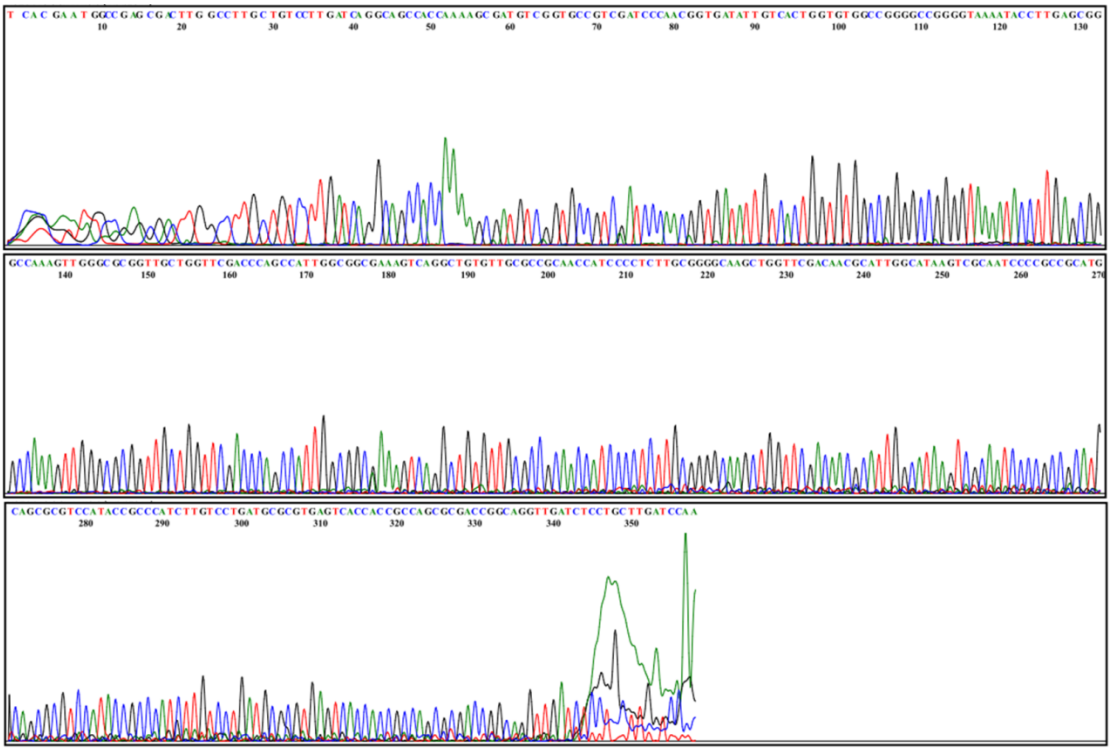


Figure S4. The sequencing result of the band **a** in Figure 1B with the reverse primer (RP) used in aPCR.

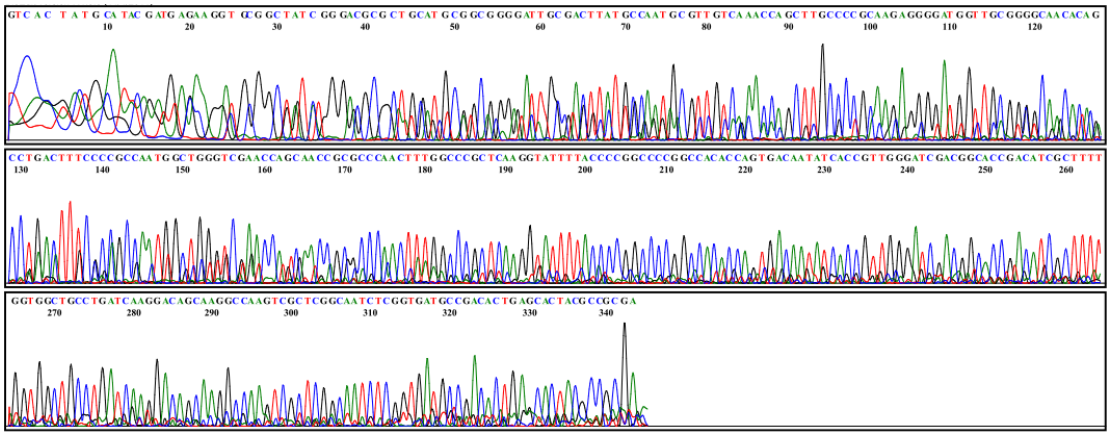


Figure S5. The sequencing result of the band **b** in Figure 1B with the forward primer (FP) used in aPCR.

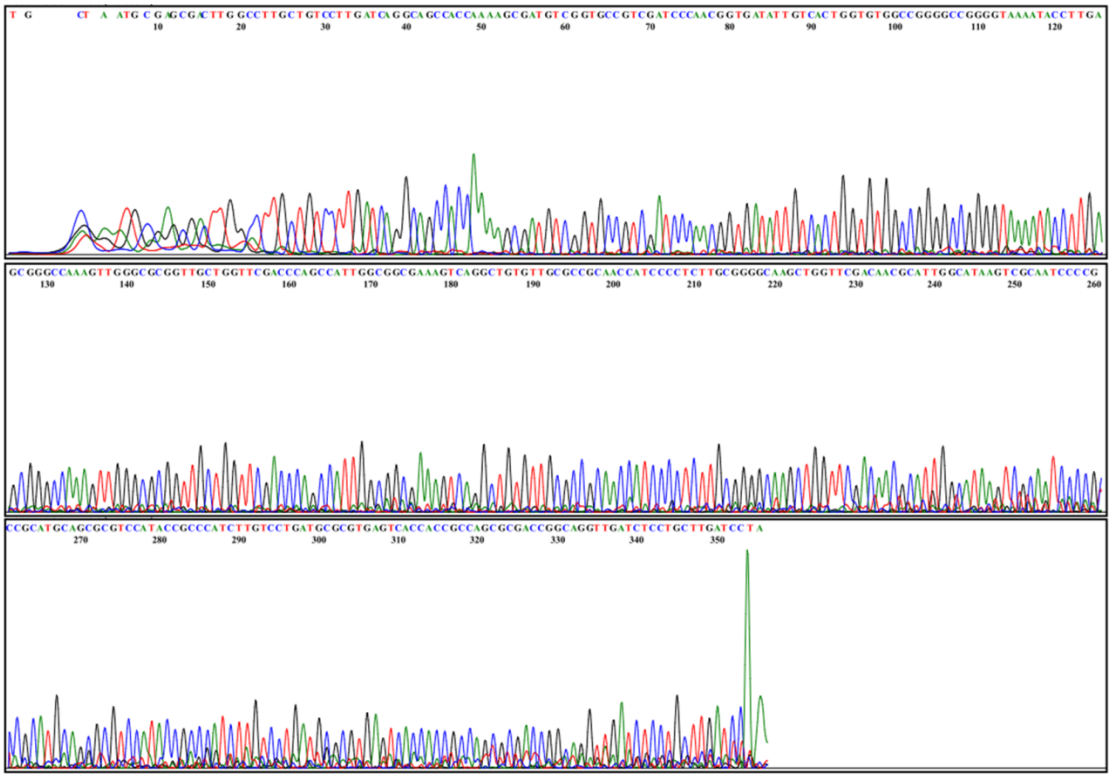


Figure S6. The sequencing result of the band **b** in Figure 1B with the reverse primer (RP) used in aPCR.

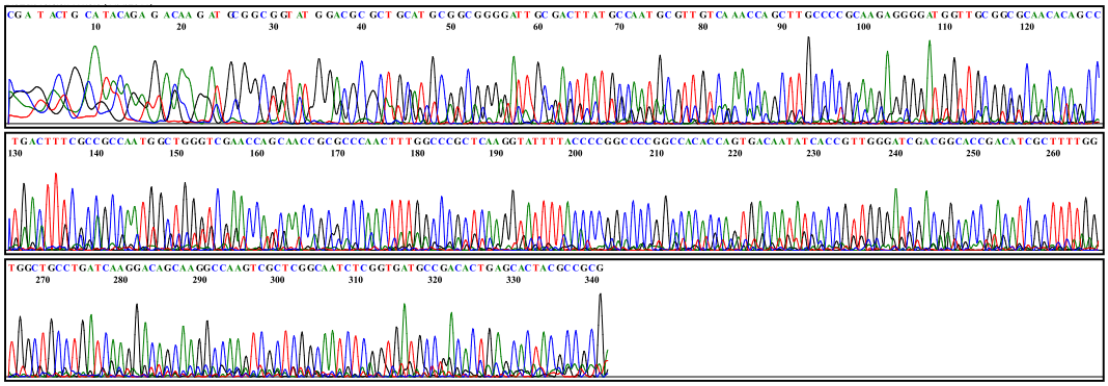


Figure S7. The sequencing result of the band **c** in Figure 1B with the forward primer (FP) used in aPCR.

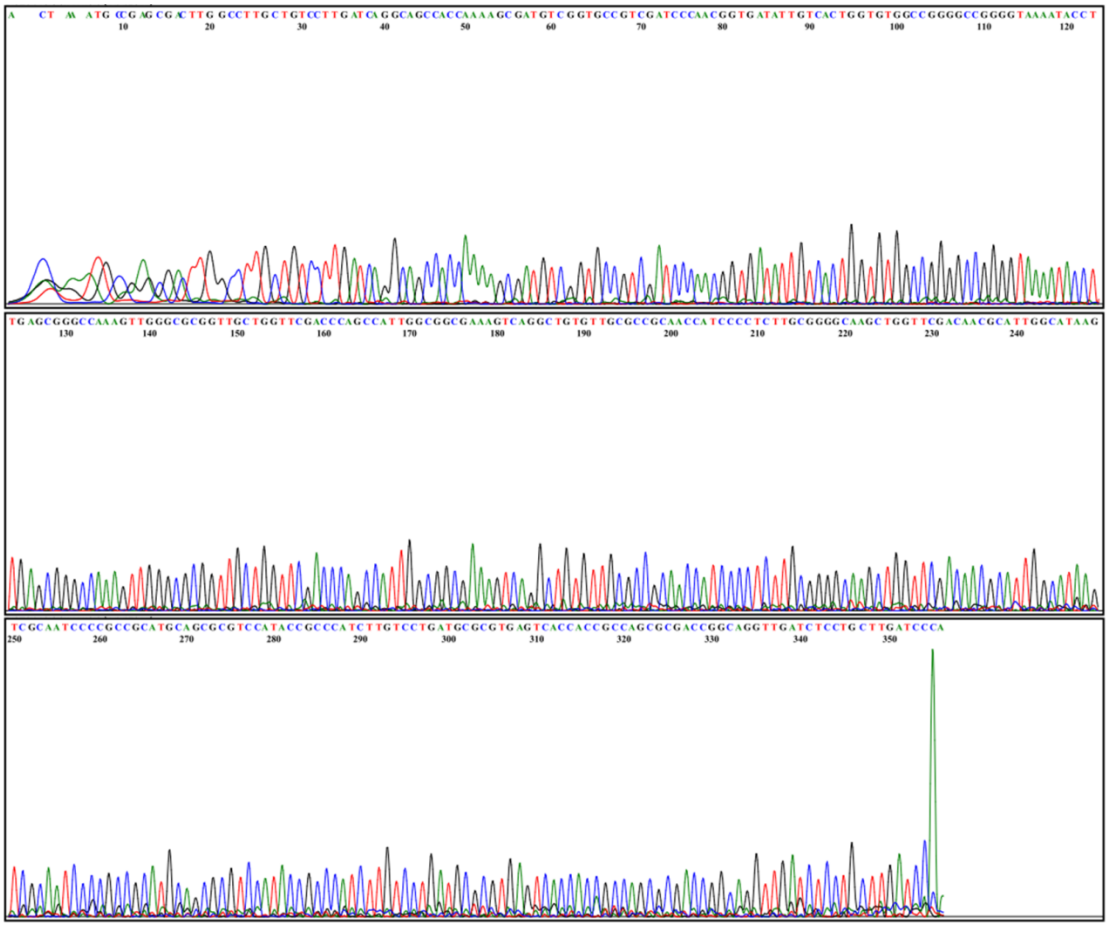


Figure S8. The sequencing result of the band **c** in Figure 1B with the reverse primer (RP) used in aPCR.

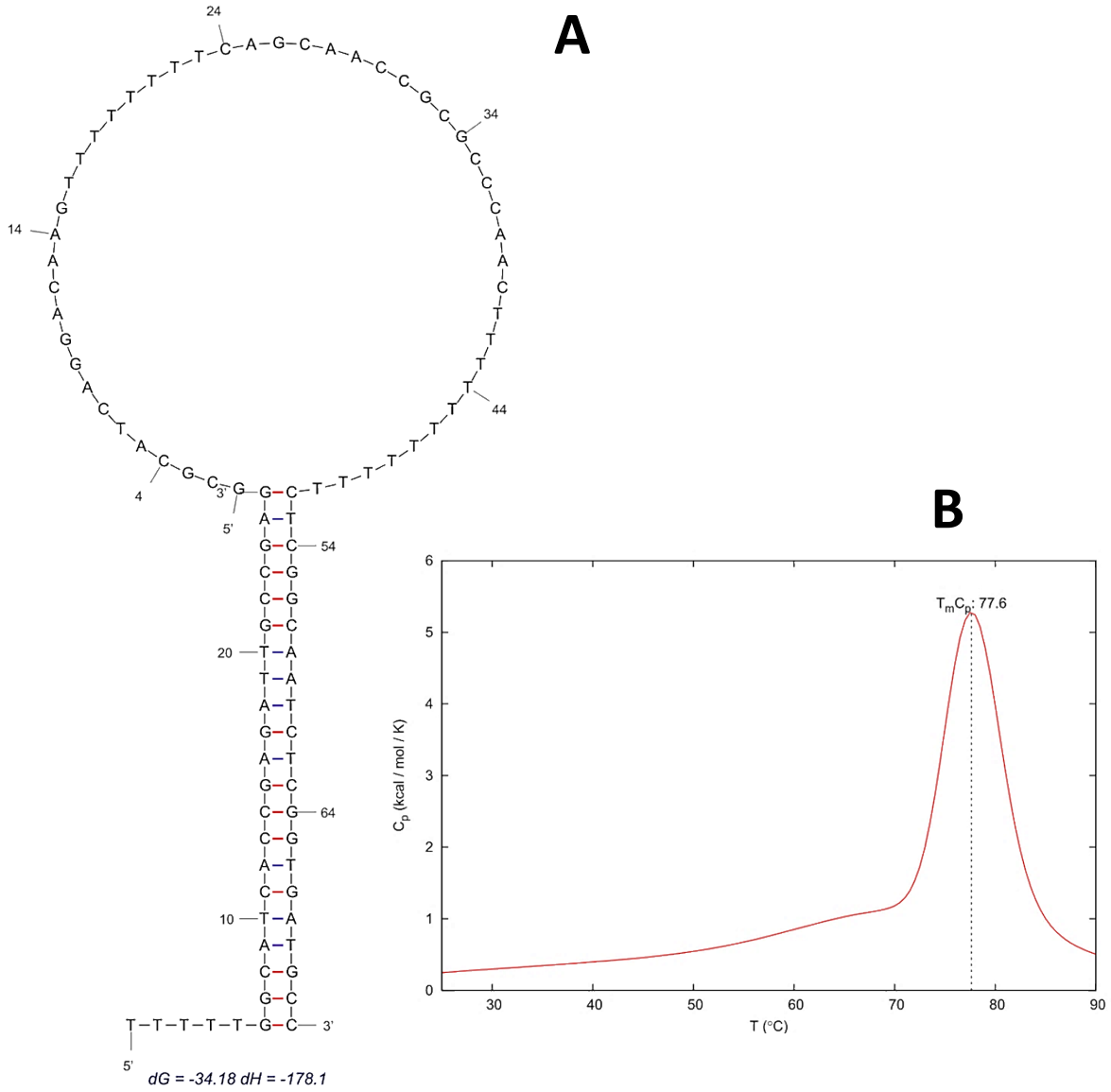


Figure S9. (A) The hybridized complex composed of Biotin-
type target (MT) and its predicted Gibb's free energy indica
(B) The heat capacity plot of the B-MT hybridization ens
temperature. The value of simulated melting temperature (
shown on the peak as 77.6 ° C which is consistent with the

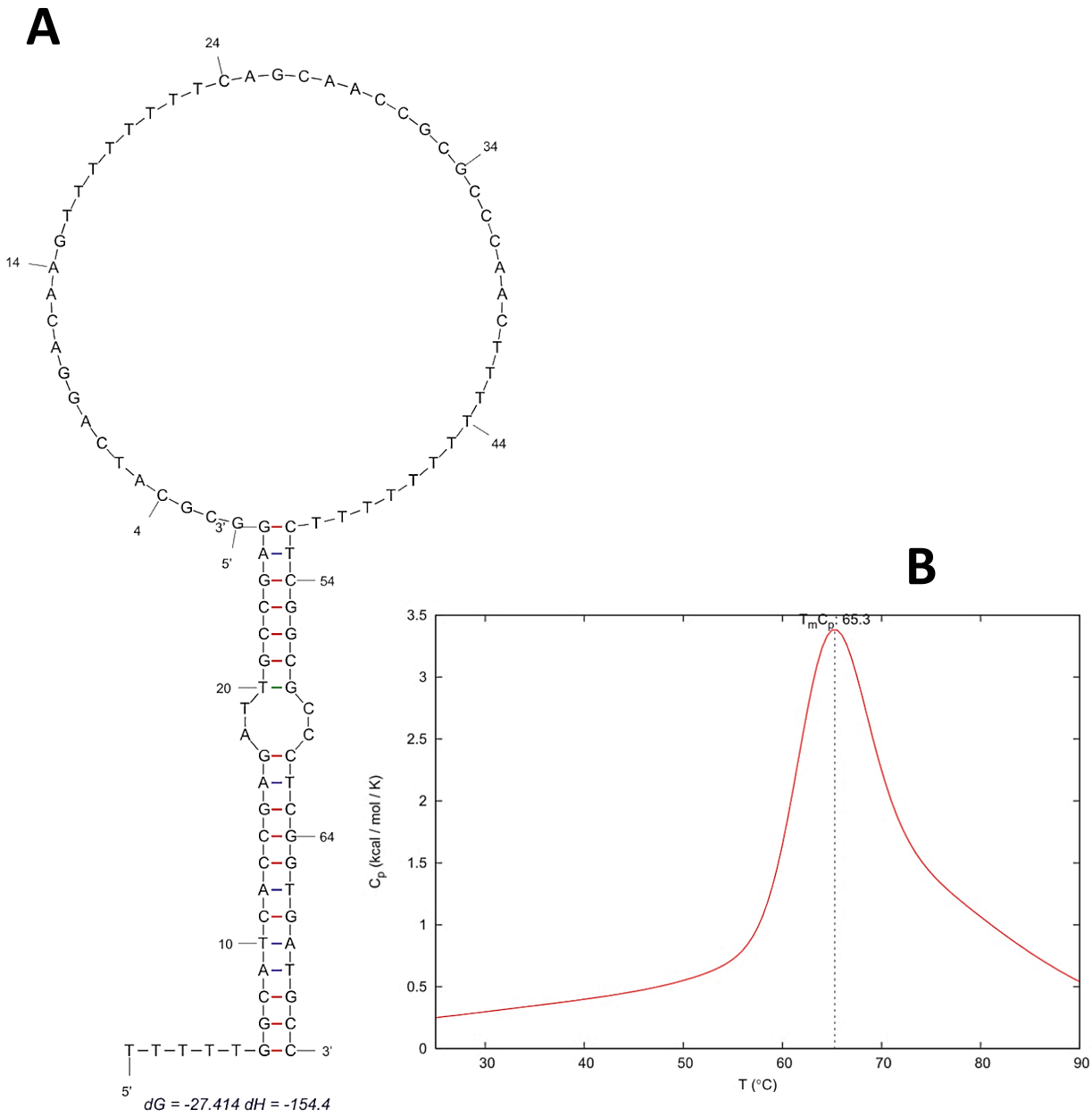


Figure S10. (A) The hybridized complex composed of bio
N220A mutant (N) and its predicted Gibb's free ene
structure. (B) The heat capacity plot of the B-N hybridizati
of temperature The value of simulated melting temperatu
is shown on the peak as 77.6 ° C which is consistent with

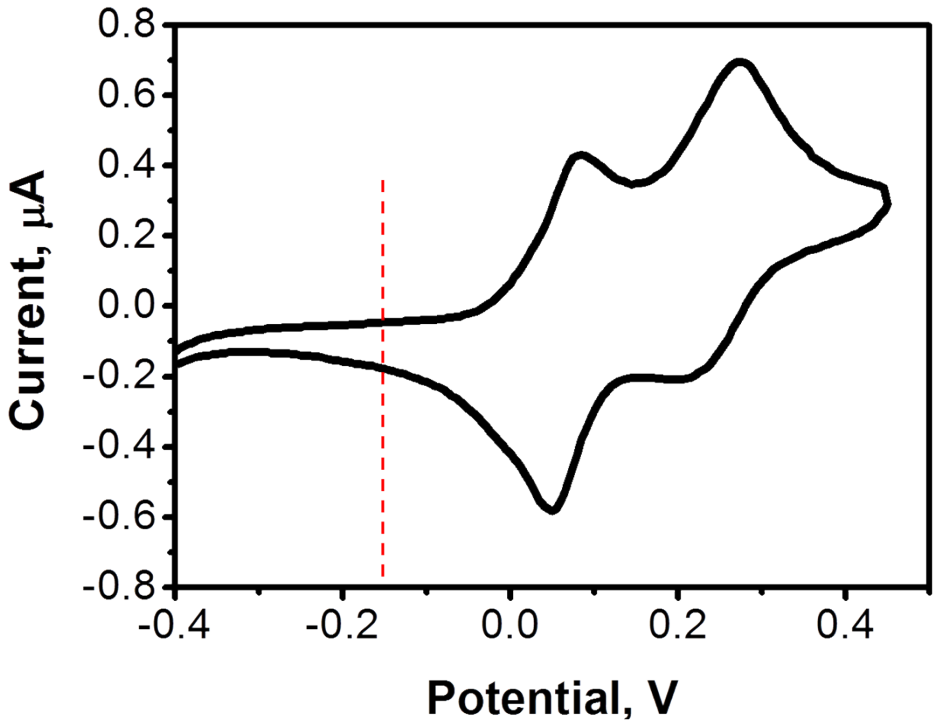


Figure S11. A typical cyclic voltammogram (CV) of the system in the present of the substrate solution (250 mM D-(+)-glucose and 0.1 mg/mL TMB in phosphate/citrate buffer, pH 5.0).

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