



Literature Report XII

J|A|C|S
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

pubs.acs.org/JACS

Article

Visualizing the Dynamic Metalation State of New Delhi Metallo- β -lactamase-1 in Bacteria Using a Reversible Fluorescent Probe

Radhika Mehta, Dann D. Rivera, David J. Reilley, Dominique Tan, Pei W. Thomas, Abigail Hinojosa, Alesha C. Stewart, Zishuo Cheng, Caitlyn A. Thomas, Michael W. Crowder, Anastassia N. Alexandrova, Walter Fast,* and Emily L. Que*

Reporter: Wenchao Jiang

Date: 2021-06-03

CV of Corresponding Authors



Emily L. Que

The University of Texas at Austin

College of Natural Sciences, Department of Chemistry, Assistant Professor

Inorganic platforms for medical imaging

Chemical tools for studying cellular metal ion homeostasis



Walter L. Fast

The University of Texas at Austin

College of Pharmacy, Division Head and Professor of Chemical Biology and Medicinal Chemistry

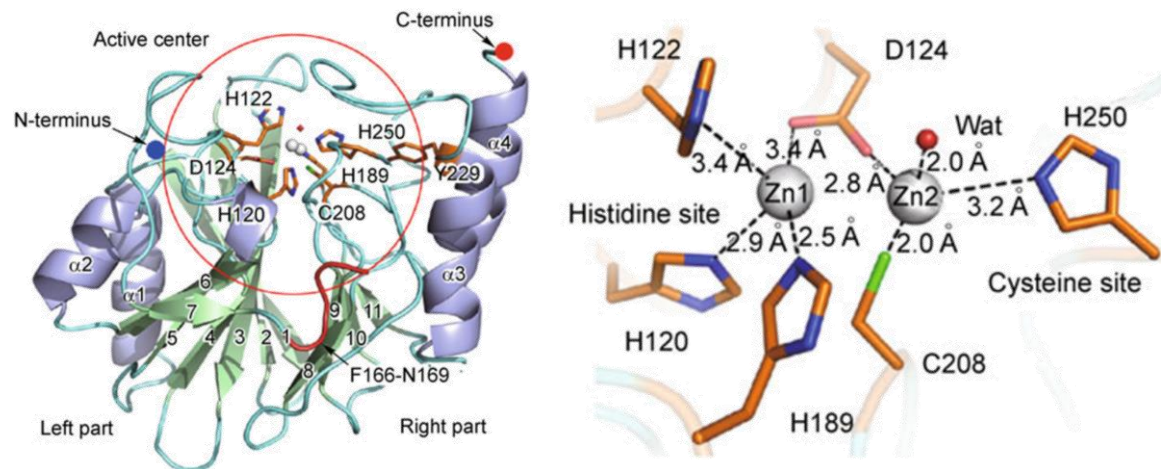
Countering Antibiotic Resistance Mechanisms

Blocking Interbacterial Signaling Pathways

Regulation of Nitric Oxide Production Through Methylated Arginines

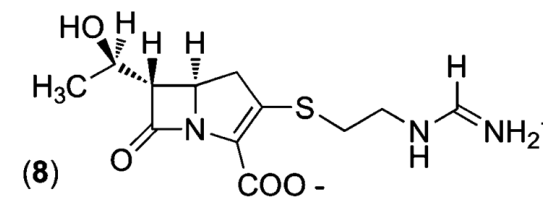
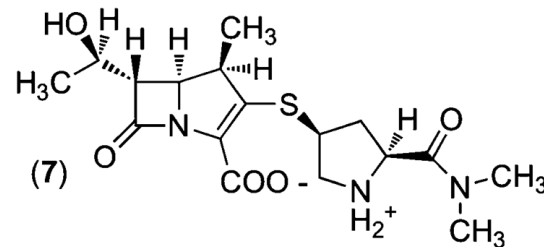
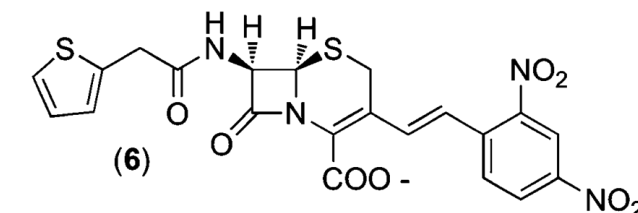
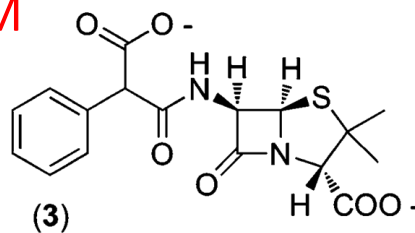
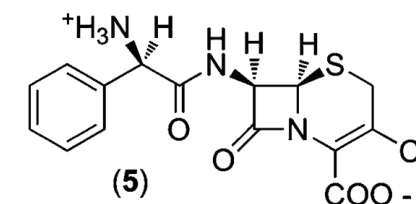
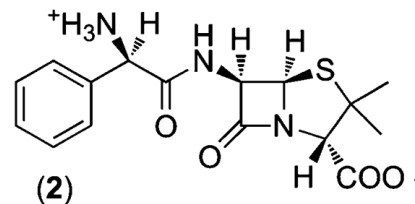
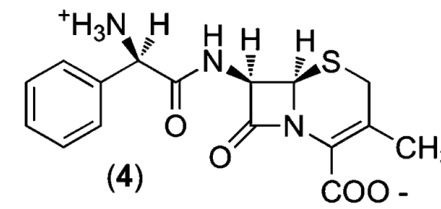
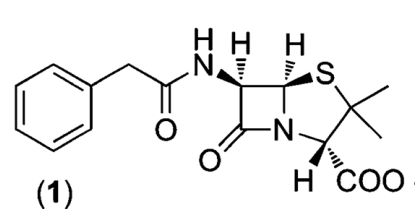
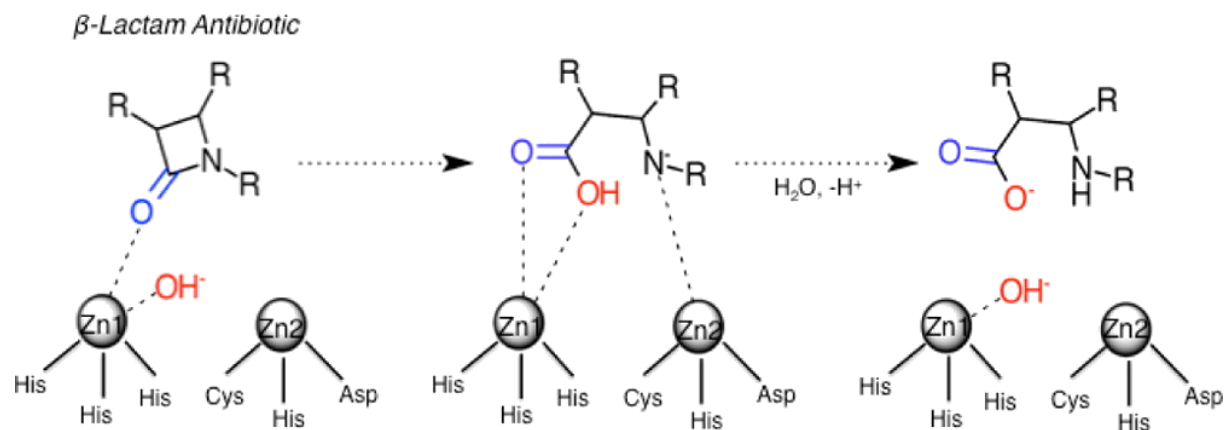
Developing Novel Covalent Enzyme Inhibitors

New Delhi Metallo- β -lactamase-1 and β -lactams

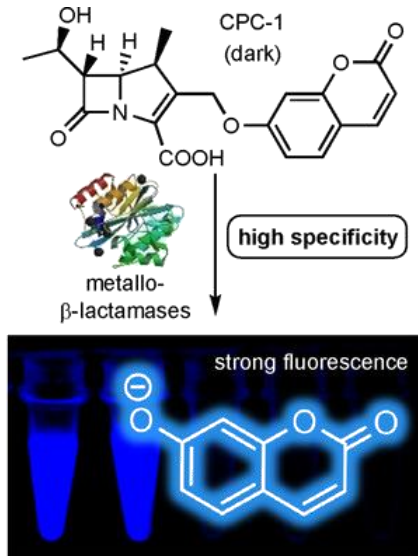


NDM-1 $K_d, Zn1 \approx 1 \text{ nM}$; $K_d, Zn2 \approx 1 \mu\text{M}$

NDM-15 $K_d, Zn2 = 120 \text{ nM}$

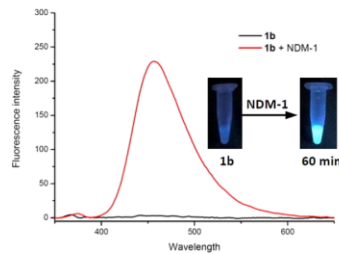
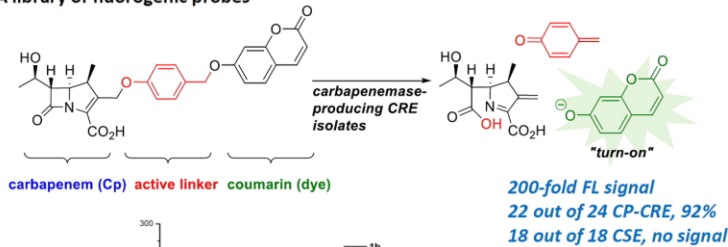


Existing NDM-1 targeted fluorophores

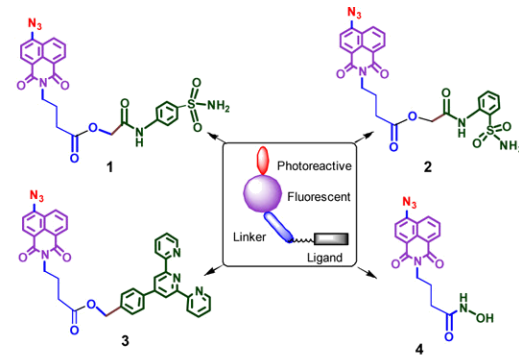


ChemBioChem 2019, 20, 511

A library of fluorogenic probes



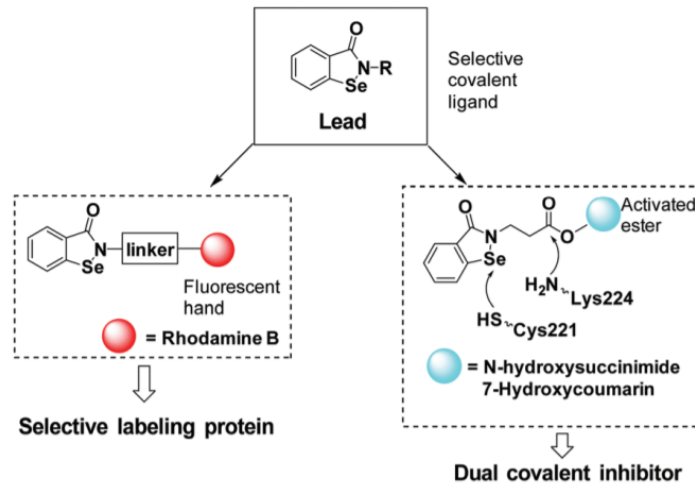
Bioorg. Chem. 2020, 94, 103405



A

- (A) The photoaffinity probes 1-4.
 (B) SDS-PAGE analysis of NDM-5-capture by various sulfonamides 1-4 as visualized by UV and Coomassie Blue
 (C & D) Fluorescence Microscopy pictures of *E. coli* cells expressed with NDM-5 and NDM-7 respectively

ACS Omega 2019, 4, 6, 10891–10898

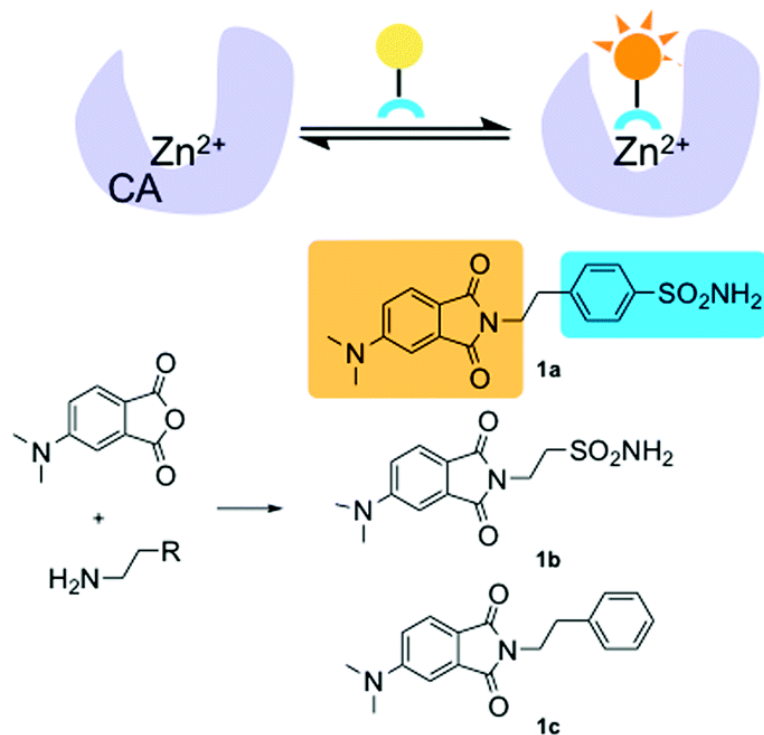


Chem. Commun., 2018,54, 4802

Irreversible "switch on" probes

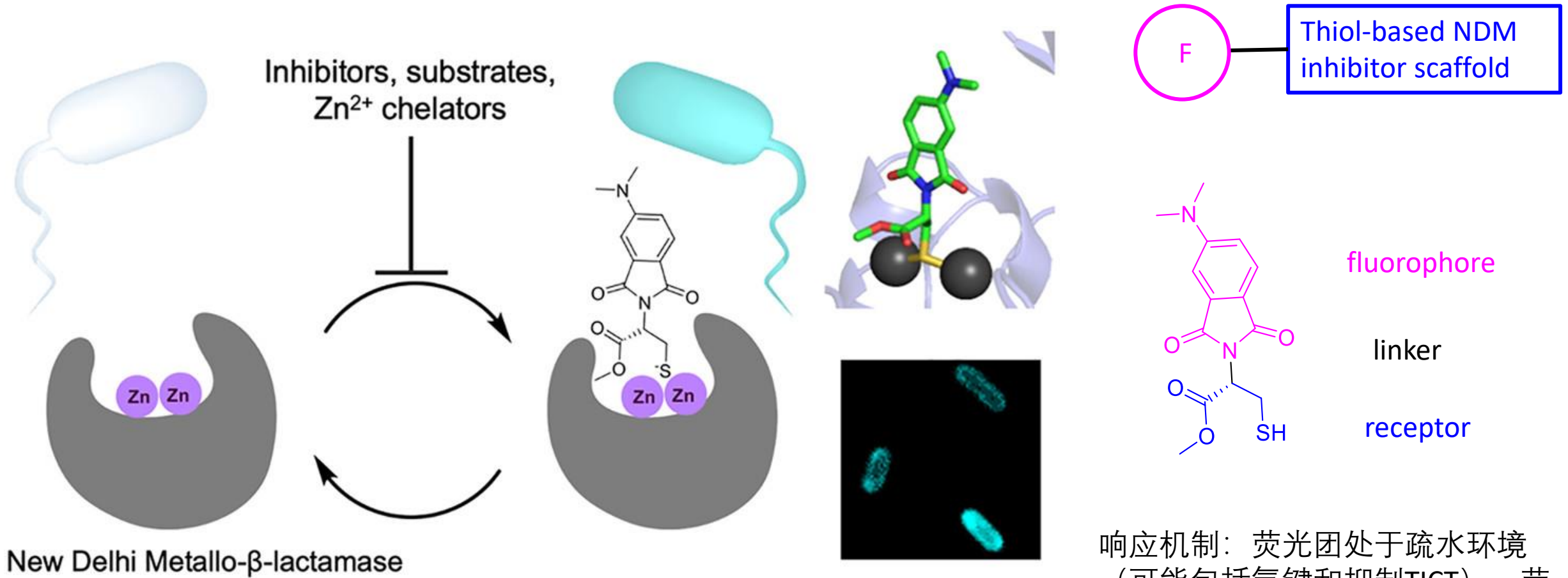
Unable to report on metalation and lack the ability to monitor dynamic reversible changes

Detecting zinc-bound carbonic anhydrase

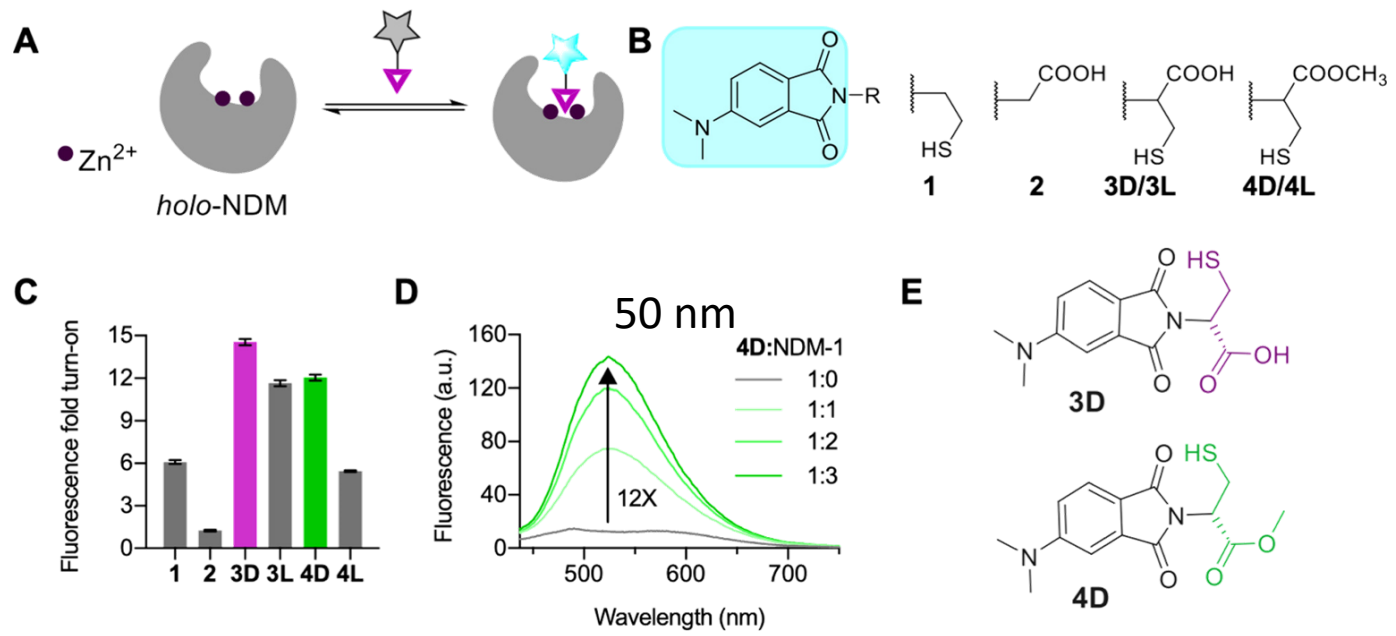


		Toluene	CHCl ₃	DMSO	MeOH	HEPES buffer
1a	λ_{abs} (nm)	388 (388) [†]	398	400 (401) [†]	397	420 (402) [†]
	ϵ (M ⁻¹ cm ⁻¹)	11800	9100	4800	5000	4800
	λ_{em} (nm)	470	485	512	550	575
	Φ	0.9	0.81	0.13	0.014	0.004
1b	λ_{abs} (nm)	391	398	398	397	417
	ϵ (M ⁻¹ cm ⁻¹)	5400	4600	5000	4800	5100
	λ_{em} (nm)	477	496	516	560	580
	Φ	0.97	0.88	0.14	0.018	0.003
1c	λ_{abs} (nm)	386	395	399	397	420
	ϵ (M ⁻¹ cm ⁻¹)	8000	8000	7200	6500	7200
	λ_{em} (nm)	465	485	512	548	590
	Φ	0.9	1	0.23	.03	-

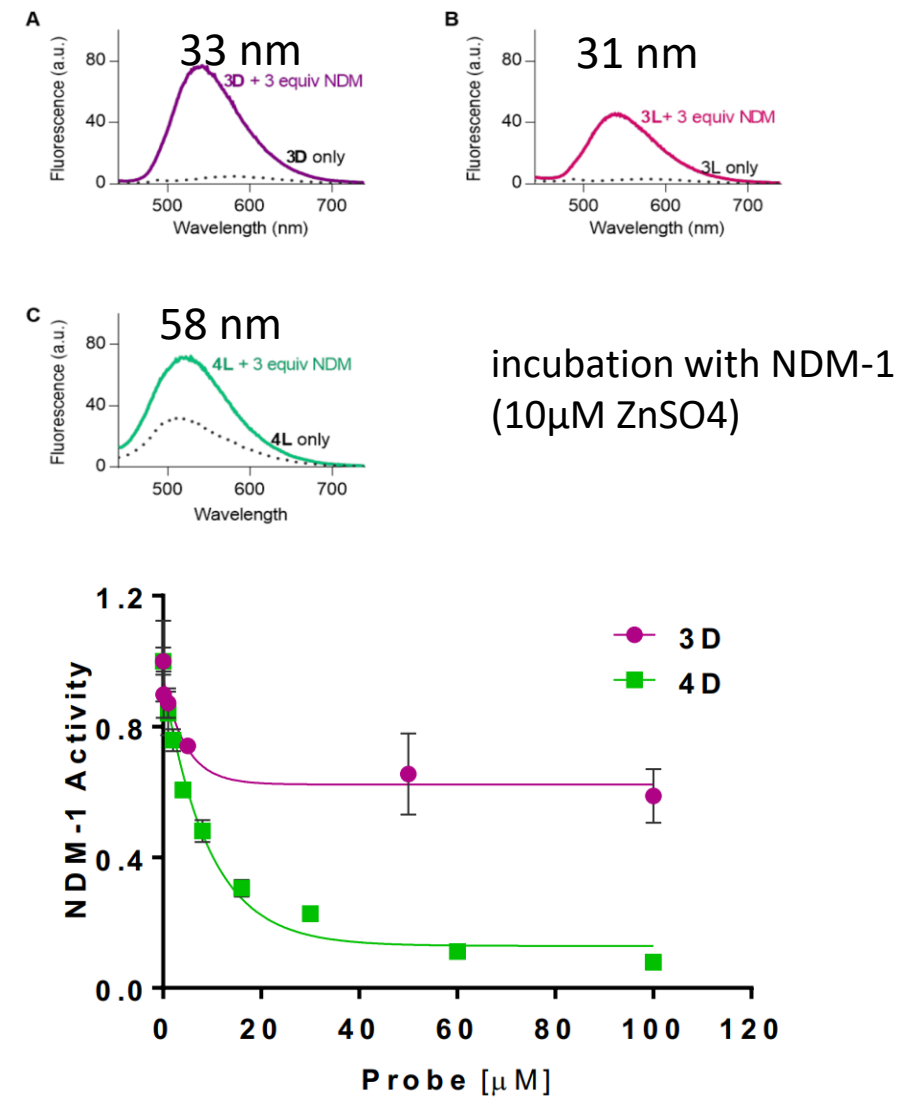
Reversible fluorescent detector for NDM metalation



Properties of the Probes

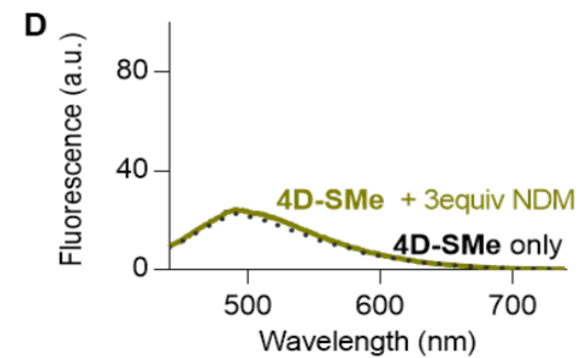
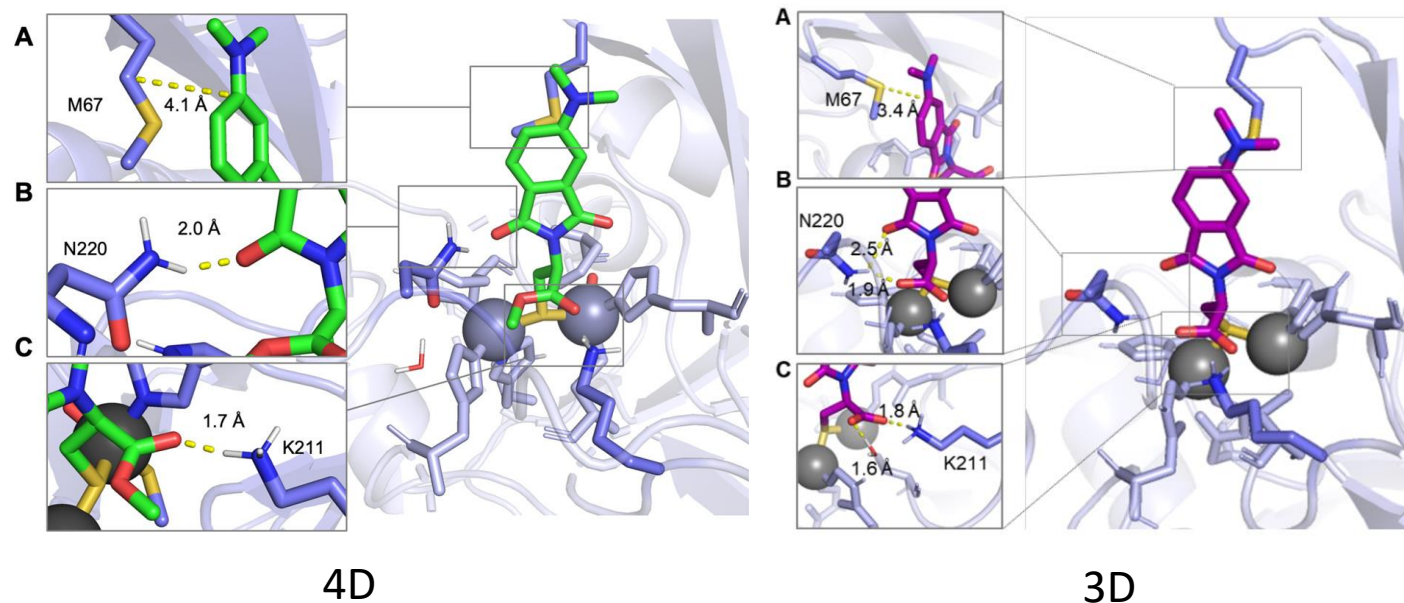


	1	2	3D	3L	4D	4L
Φ (MeOH)	0.008	0.021	0.028	0.027	0.007	0.012
ϵ ($M^{-1}cm^{-1}$)	4211	7100	3658	4652	5395	5035

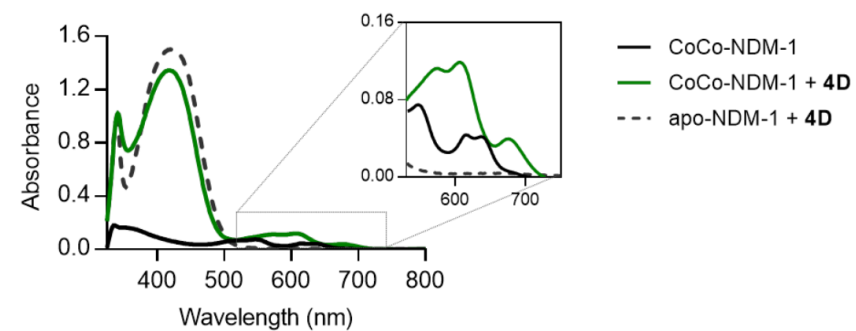


λ_{ex} = 417–420 nm and λ_{em} = 575–580 nm in HEPES buffer

Probes 3D and 4D

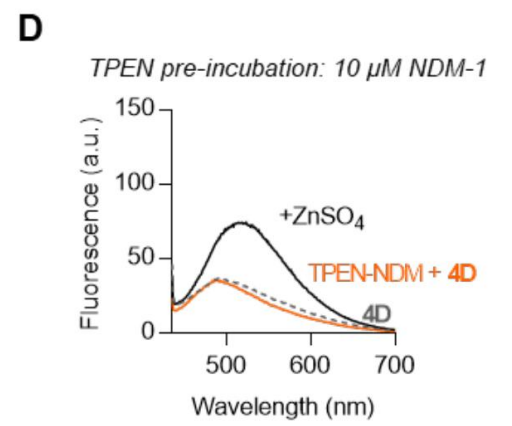
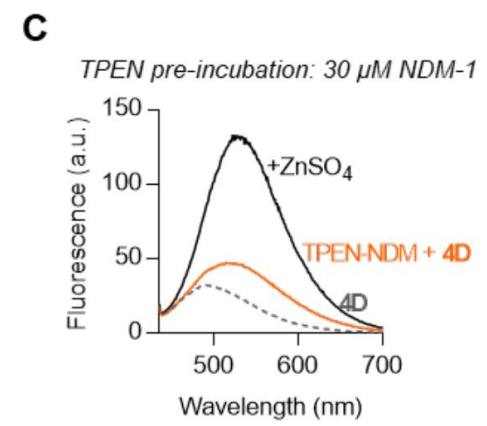
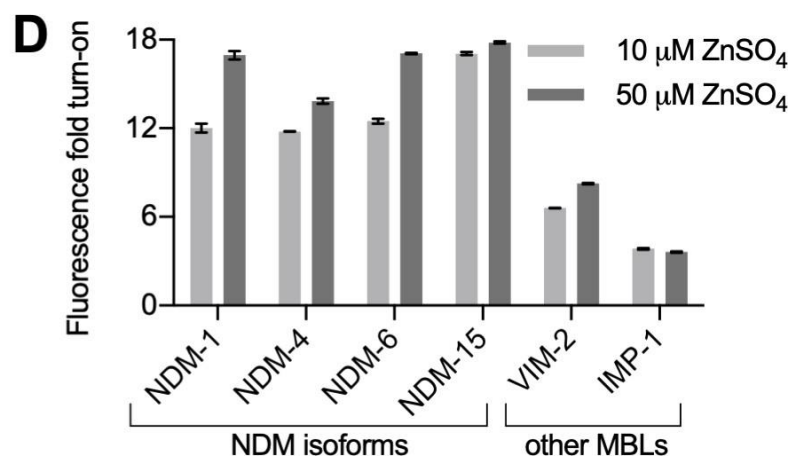
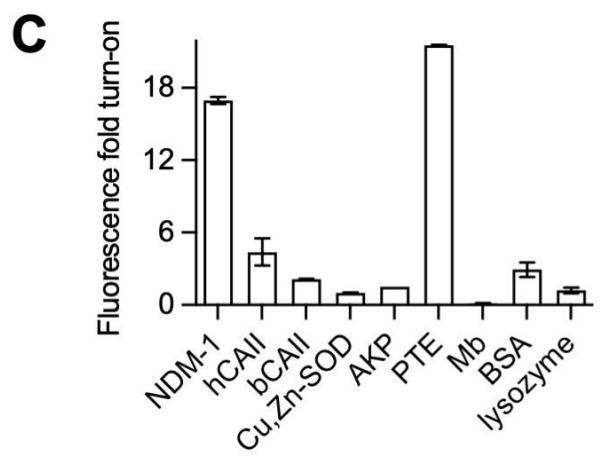
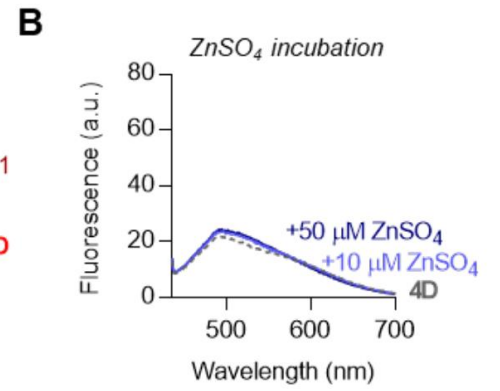
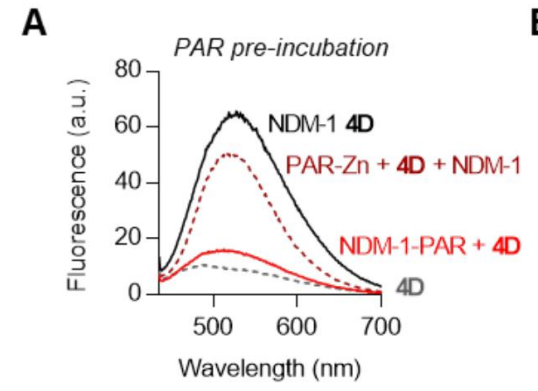
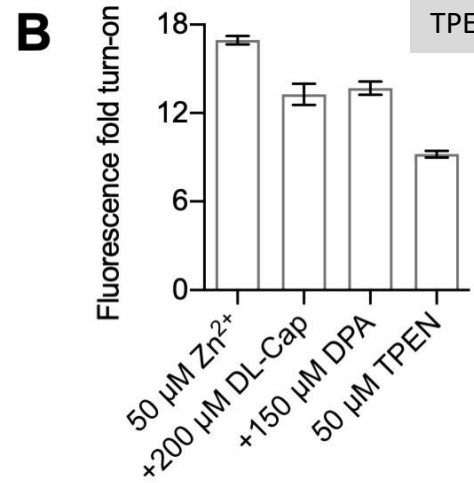
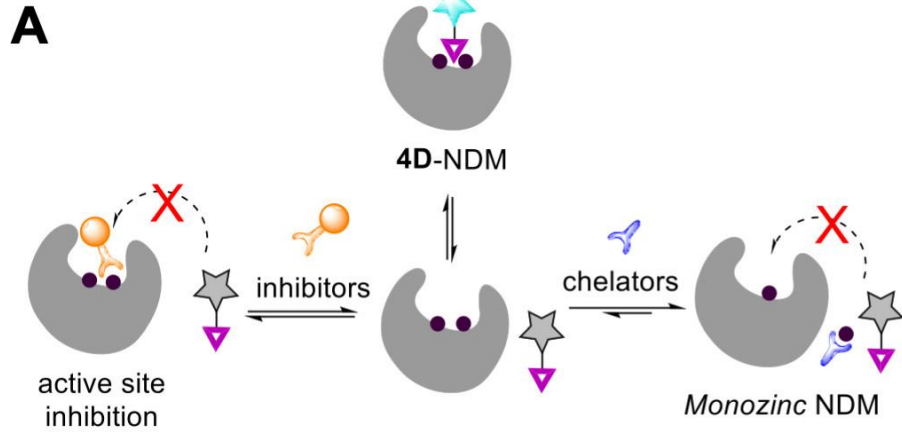


probe	minimum values		distances (Å)			
	binding penalties (kcal/mol)	metal angle variance (deg)	(thiol) S-Zn1	(thiol) S-Zn2	(carboxyl) O-NH (K211)	(carbonyl) O-NH (N220)
3D	10.5	7.96	2.3	2.4	1.8	2.5
4D	4.2	7.52	2.3	2.4	1.7	2.0

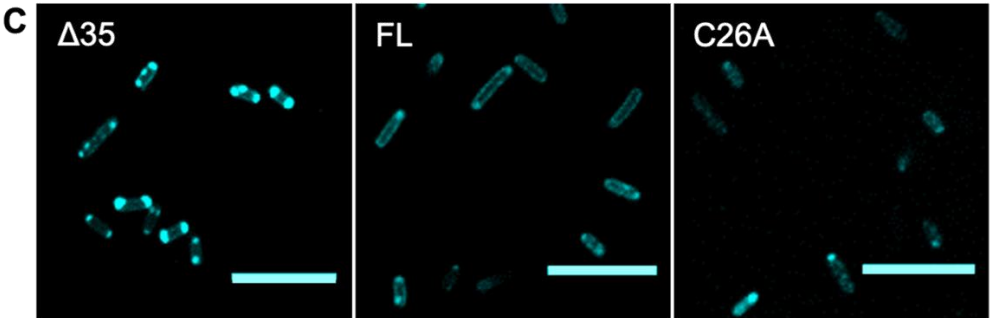
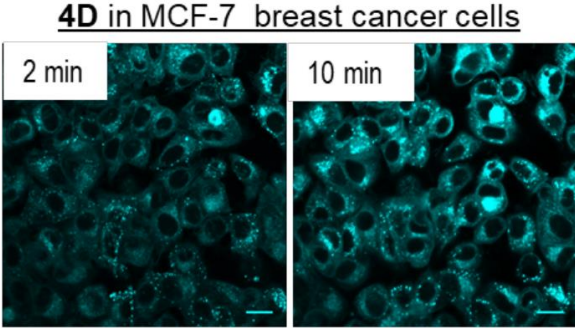
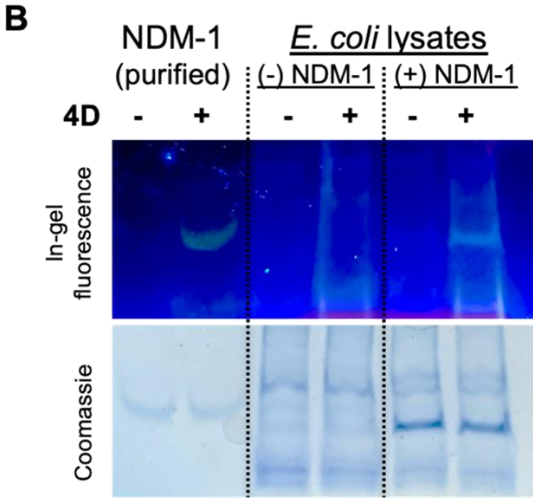
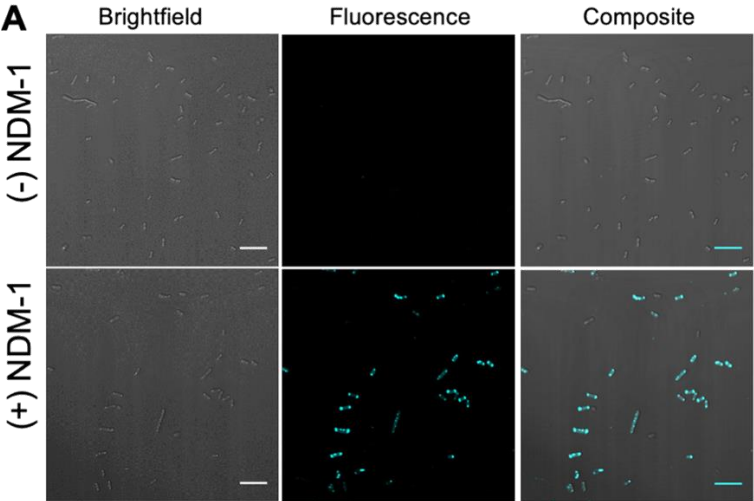


Reversibility and Selectivity of Probe 4D

Cap: 竞争抑制剂
 DPA: 能广泛作用于二价金属的弱螯合剂同时能与NDM-1结合
 TPEN: 膜透性强锌螯合剂



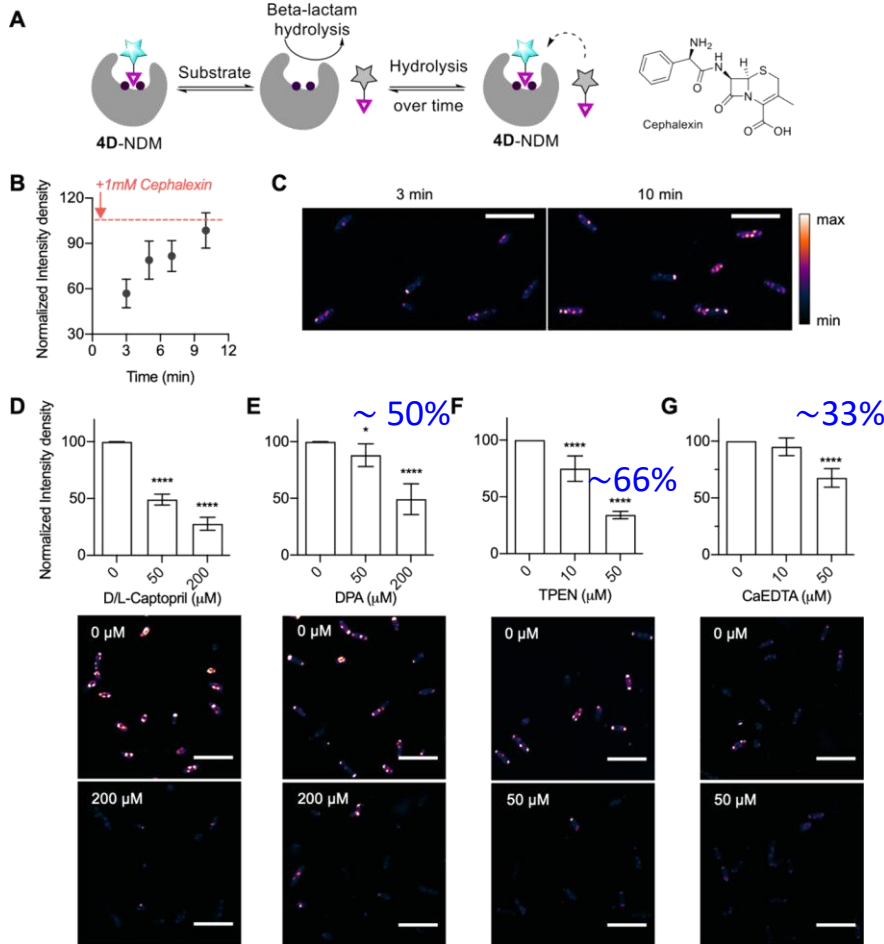
Application of 4D in Cells and Cell Lysates



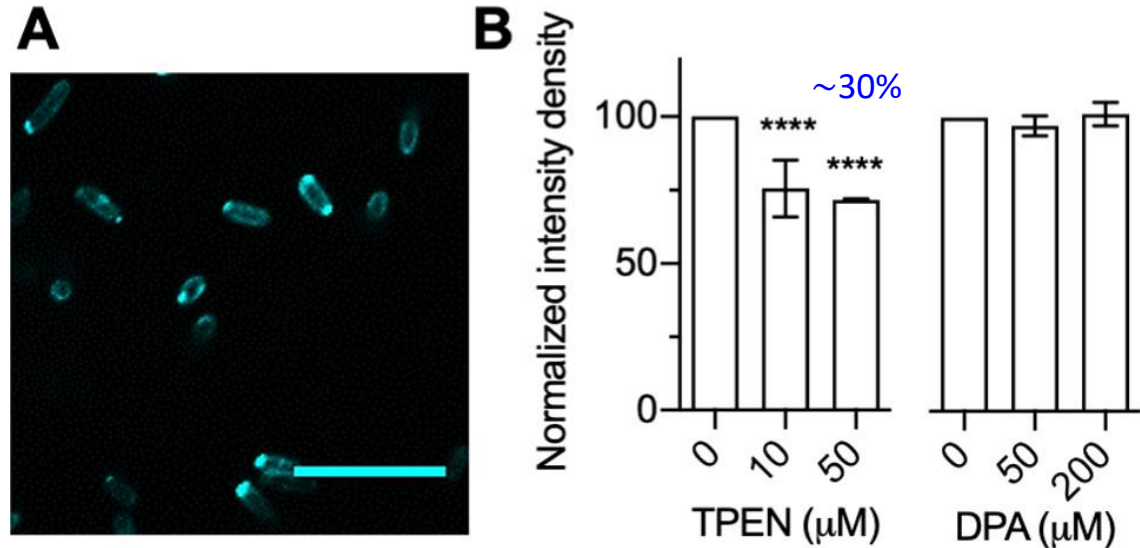
FL: 定位于外膜的内叶，将NDM催化域置于质周空间内
 C26A: 相比FL突变了一个氨基酸导致在质周空间积累
 $\Delta 35$: 在周质空间，具有很好的水溶性

The Dynamic Metalation State of NDM

Metalation State of NDM-1 in Bacteria

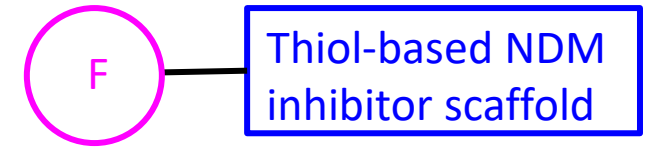


Metalation Status of NDM-15

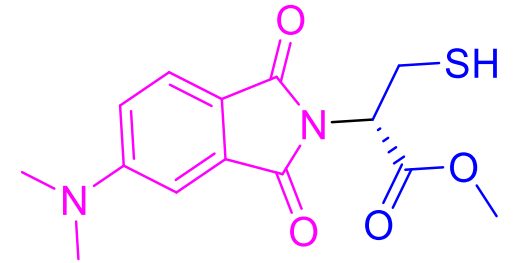


DPA: 一种弱的二价金属螯合剂
 TPEN: 膜透性强锌螯合剂, 相当于内源性锌螯合剂
 CaEDTA: 胞外锌螯合剂

Summary



Report a novel probe, 4D, to monitor the dynamic metalation state of NDM within *E. coli*



- Reversible: displaced by competition with active-site ligands or by demetalation of NDM monitoring of dynamic alterations to the active-site metal content
- Specific for NDM in *E. coli* by native gel electrophoresis with cell lysates
- Image dizinc NDM expressed in the *E. coli* periplasm and can report on dynamic changes : substrate , inhibitor , demetalation by cell-permeable or cell-impermeable zinc chelators
- NDM-15 is more resistant to demetalation by zinc chelators

Screening new inhibitors for NDM and ascertaining the efficacy of target engagement in vivo

Higher-specificity probes for more complex biological samples