



High Resolution Crystal Structure of CTX-M-15 in Complex with the New β -lactamase Inhibitor NXL104

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Introduction (1)

Extended-spectrum β -lactamases (ESBLs) represent important resistance factors to β -lactam antibiotics in Gram-negative bacteria, as they confer resistance to penicillins and expanded-spectrum cephalosporins

The CTX-M-type enzymes, which naturally exhibit ESBL properties, appeared later than the TEM- or SHV-derived ESBL variants, and now number at least 90 variants

They are the most prevalent ESBLs in many settings and have a worldwide diffusion

CTX-M-15 belongs to the CTX-M-1 group (5 AA substitutions) and shows a broad geographical diffusion (clonal spread, see also presentation 1257, Paterson)

Thus far, 29 CTX-M X-ray structures have been determined (TOHO-1 and mutants, CTX-M-9, CTX-M-14, CTX-M-16 and CTX-M-27), some of which in complex with inhibitors

Rossolini *et al.*, Clin. Infect. Dis. (2008) 46(S1):33

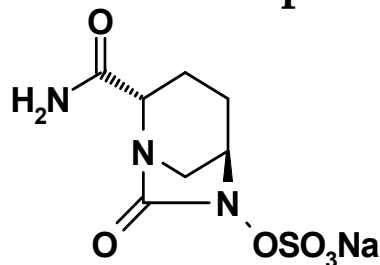
Ibuka *et al.*, Biochemistry (2003) 42:10634

Chen *et al.*, J. Mol. Biol. (2005) 348:349



Introduction (2)

NXL104 is a new β -lactamase inhibitor (trans-7-oxo-6-(sulfooxy)-1,6-diazabicyclo[3.2.1]octan-2-carboxamide, a representative of a novel class of bridged bicyclico[3.2.1]diazabicyclo-octanones) that inactivates active serine- β -lactamases by forming a stable covalent complex



It shows excellent inhibition properties of clinically-relevant enzymes belonging to Ambler classes A and C, including:

- TEM-, SHV-, CTX-M-type ESBLs (and also other minor variants)
- Class A carbapenemases (most notably KPC-2)
- Resident AmpC-type β -lactamases (*E. coli*, *P. aeruginosa*)
- Plasmid-mediated class C β -lactamases (e.g. CMY-type enzymes)

Currently in clinical development (phase II) and to be associated with third- and fifth-generation cephalosporins (e. g. NXL104-ceftazidime [Novexel S.A.] or NXL104-ceftaroline [Forest Laboratories Inc.]



Methods and Scope

CTX-M-15 was produced using a T7-based *E. coli* expression system and purified to homogeneity using ion-exchange and size-exclusion chromatography.

The interaction between CTX-M-15 and NXL104 was investigated by kinetic methods

Crystallization was performed at a protein concentration of 8 mg/ml using the sitting drop method and crystallization conditions manually refined (complexes were obtained by co-crystallization of CTX-M-15 with 3 mg/ml NXL104) (pH, 8.0).

Diffraction data were collected at the BM24 beamline at the ESRF (Grenoble, France). Data were scaled and initial model obtained by molecular replacement using the structure of TOHO-1 (PDB code, 1IYS) as the search model. The final model was obtained after cycles of manual building and structure refinement.

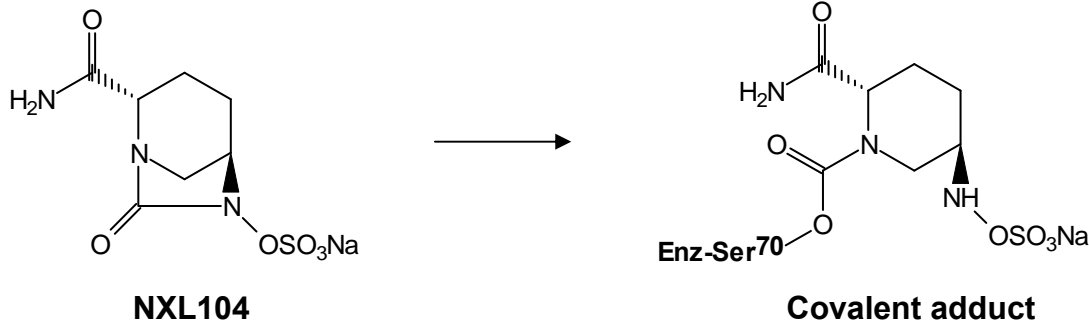
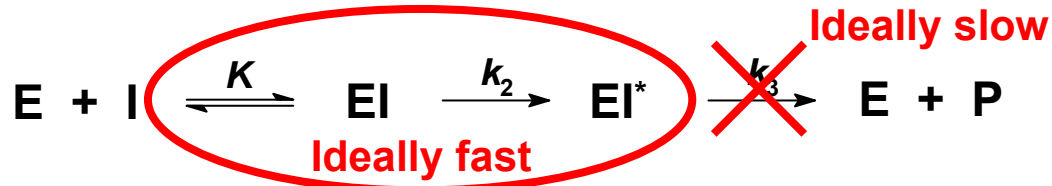
Obtain kinetic and structural information on the interaction between β -lactamases and NXL104



Results (1) - Enzymology

The kinetic parameters of inactivation were investigated using the reporter substrate method

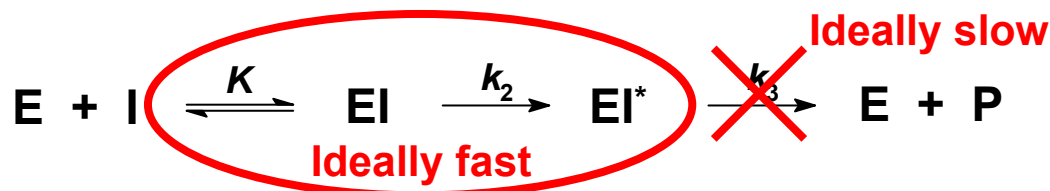
Clavulanic acid (CLAV) and tazobactam (TZB) were used as comparators



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Inhibitor	IC ₅₀ (nM)	k ₂ (s ⁻¹)	K (μM)	k ₂ /K (M ⁻¹ .s ⁻¹)	t _{1/2} ^{acylation} (s)	k ₃ (s ⁻¹)	t _{1/2} ^{deacyl} (min)
CLAV	12	0.08	0.53	1.6 x 10 ⁵	8	5.4 x 10 ⁻⁴	20
TZB	6	0.18	0.22	8.2 x 10 ⁵	4	4.9 x 10 ⁻⁴	25
NXL104	5	0.25	0.59	4.3 x 10 ⁵	3	≤ 5.9 x 10 ⁻⁷	≥ 19,000

NXL104 shows:

- “acylation” efficiencies similar to that of CLAV and TAZ
- 1,000-fold slower “deacylation”



Results (2) – Diffraction data collection

Data collection statistics		
	Native CTX-M-15	CTX-M-15:NXL-104 complex
X-ray source	ESRF BM14U	ESRF BM14U
Wavelength (Å)	0.910	0.918
Data collection temp. (K)	100	100
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
Cell dimensions (Å)	a=44.698, b=45.730, c=117.086	a=44.483, b=45.686, c=117.721
Subunits/asu	1	1
Matthews coefficient (Å ³ Da ⁻¹)	2.08	2.08
Solvent cont. (%)	41.01	41.01
Resolution limits (Å)	29.27 – 1.10 (1.16-1.10)	23.54-1.10 (1.16-1.10)
Reflections measured	767395 (109593)	596968 (85566)
Unique reflections	97955 (14022)	98124 (14148)
Completeness (%)	99.8 (99.2)	99.9 (100.0)
R _{merge} (%)	7.5 (43.8)	5.1 (14.5)
Multiplicity	7.8 (7.8)	6.1 (6.0)
I/σ(I)	14.9 (4.3)	20.3 (11.2)

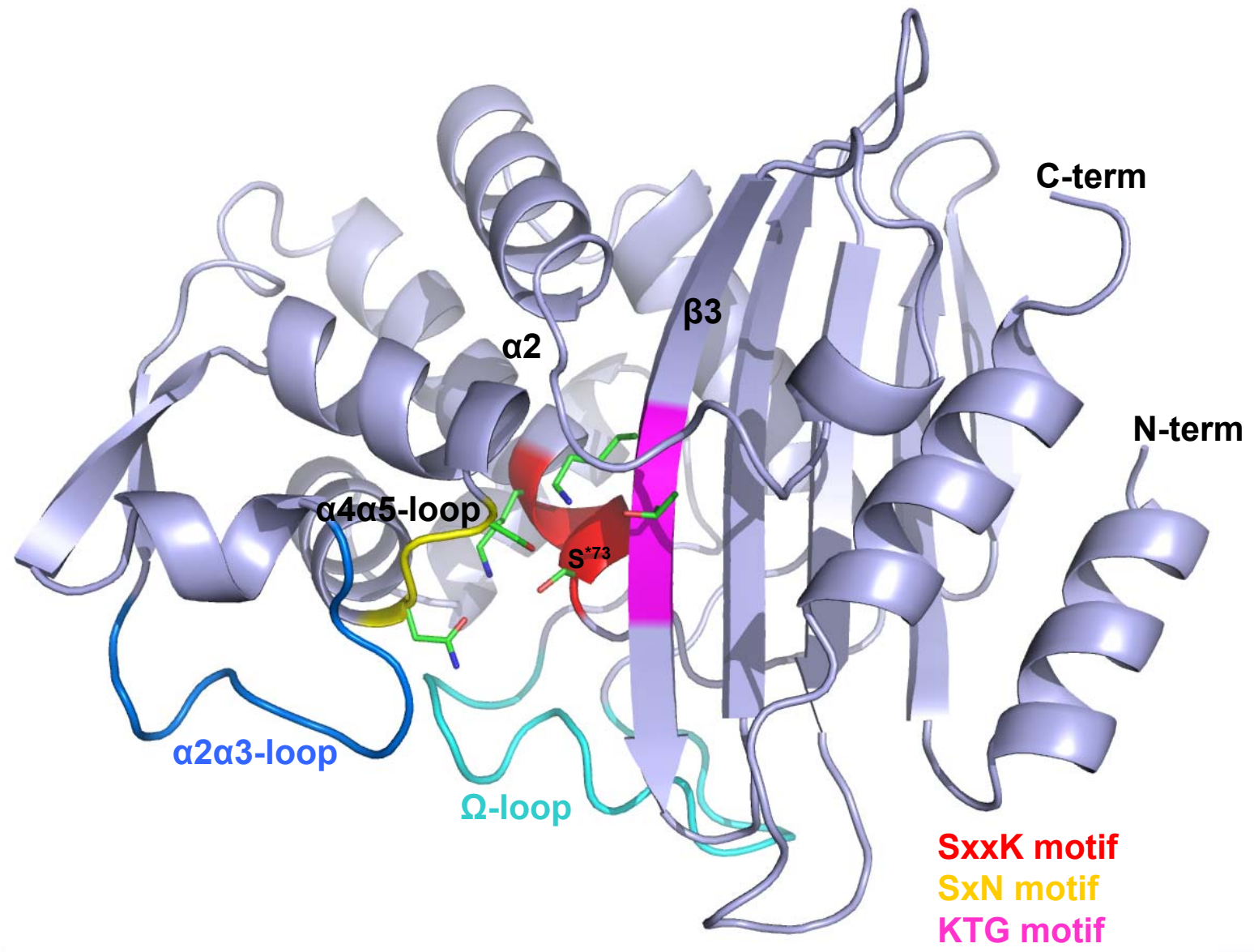


Results (3) – Refinement statistics

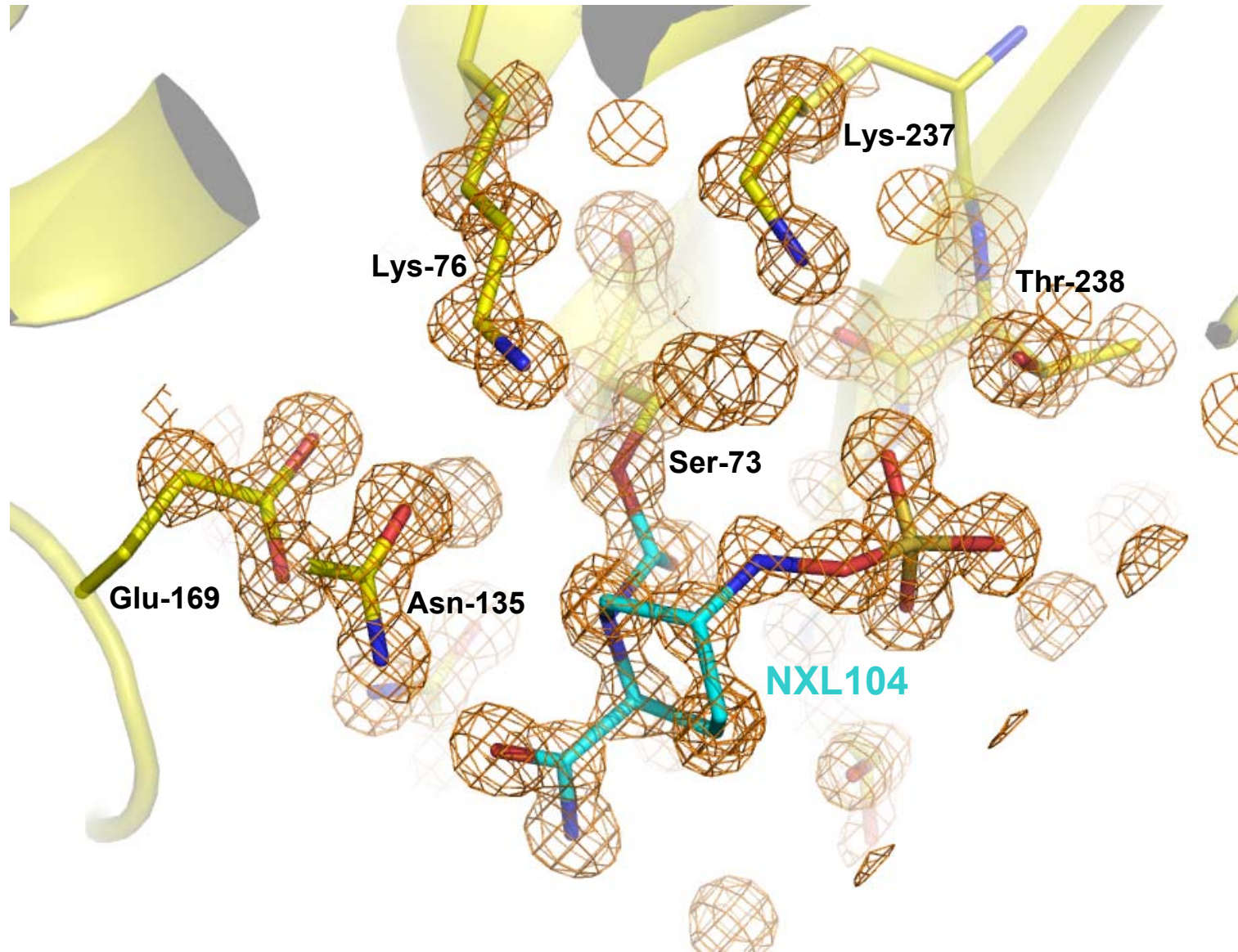
	Native CTX-M-15	CTX-M-15:NXL-104 complex
Resolution range (Å)	29.27-1.10 (1.13-1.10)	22.85-1.10 (1.13-1.10)
Number of reflections	93067 (6736)	93135 (6786)
Refinement type	Anisotropic + calc hydrogens	Anisotropic + calc hydrogens
R _{cryst} (%)	14.56 (17.90)	13.86 (11.30)
R _{free} set size	5.0	5.0
R _{free} (%)	15.65 (19.20)	14.85 (13.62)
Protein atoms	1941	1954
Ligand atoms	-	17 (NXL 104)
Water molecules	299	296
Ethylene glycol	4	10
Chloride ions	2	1
Sulphate ions	4	3
Average B factors (Å ²)	6.14	5.34
r.m.s.d. bond lengths (Å)	0.005	0.005
r.m.s.d. bond angles (°)	1.128	1.150
r.m.s.d. planes (Å)	0.004	0.004
r.m.s.d. chiral centers (Å ³)	0.063	0.064



Results (4) – The native structure



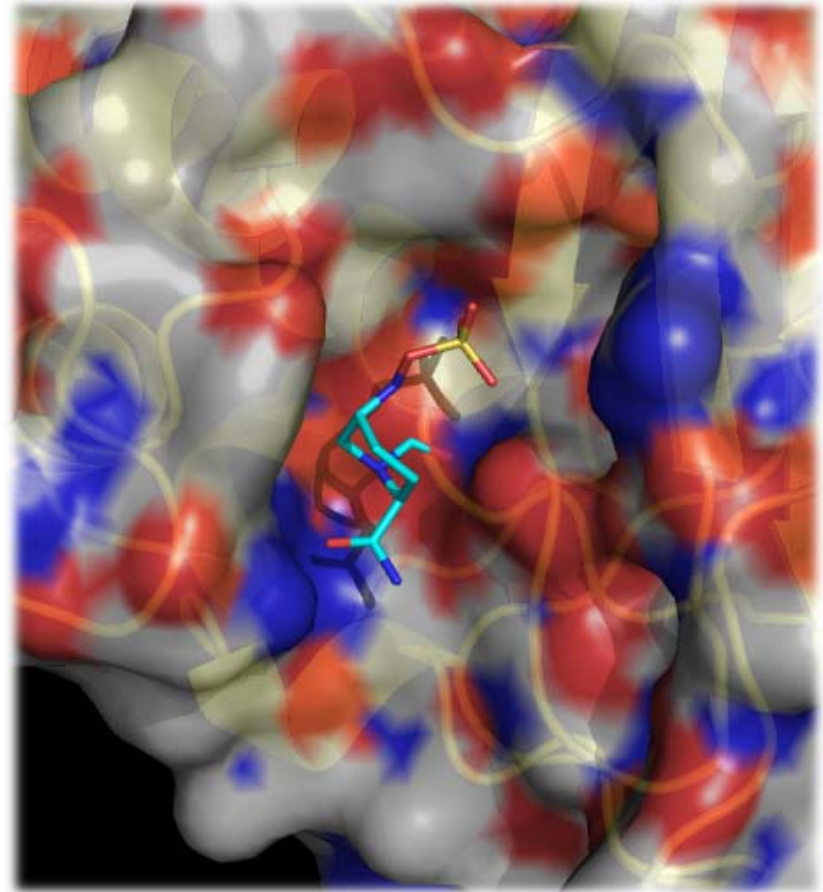
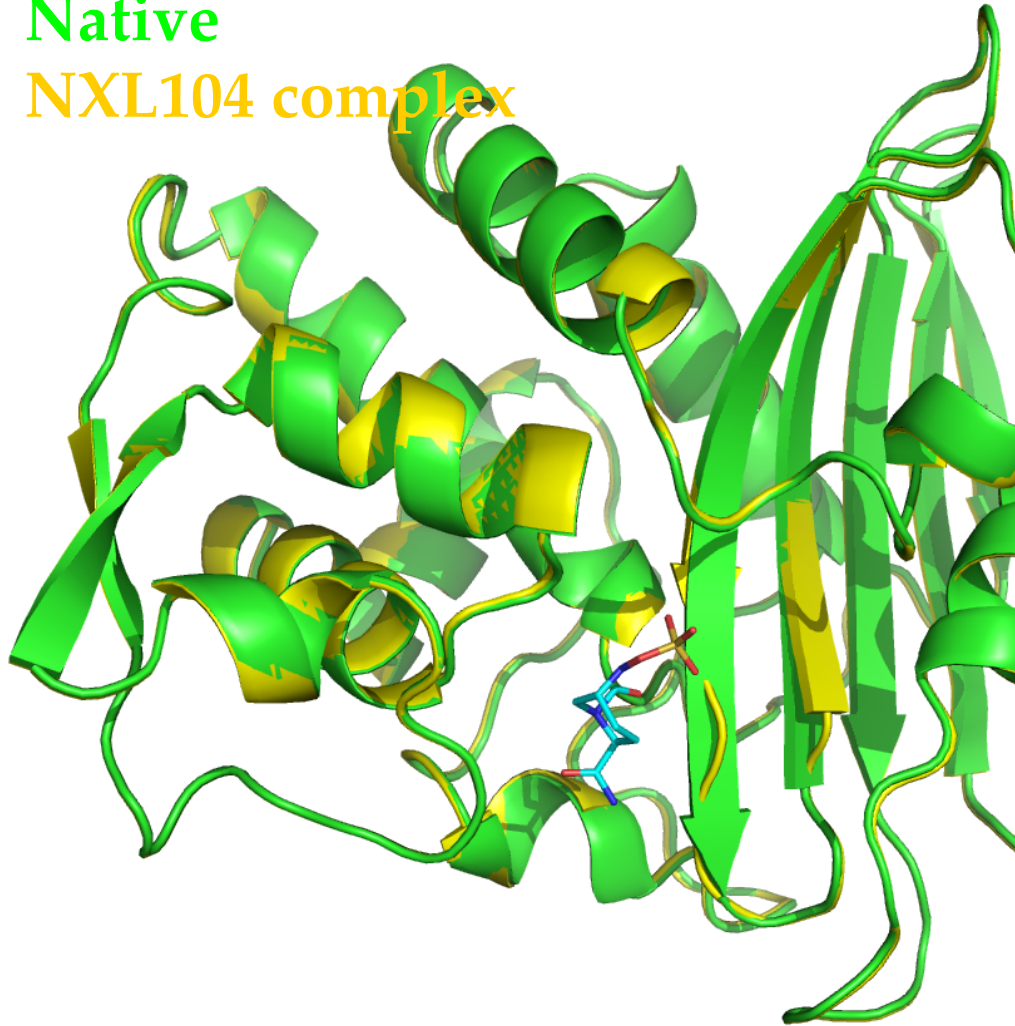
Results (5) – Complex with NXL104



Results (6) – Native and complex structures

Native

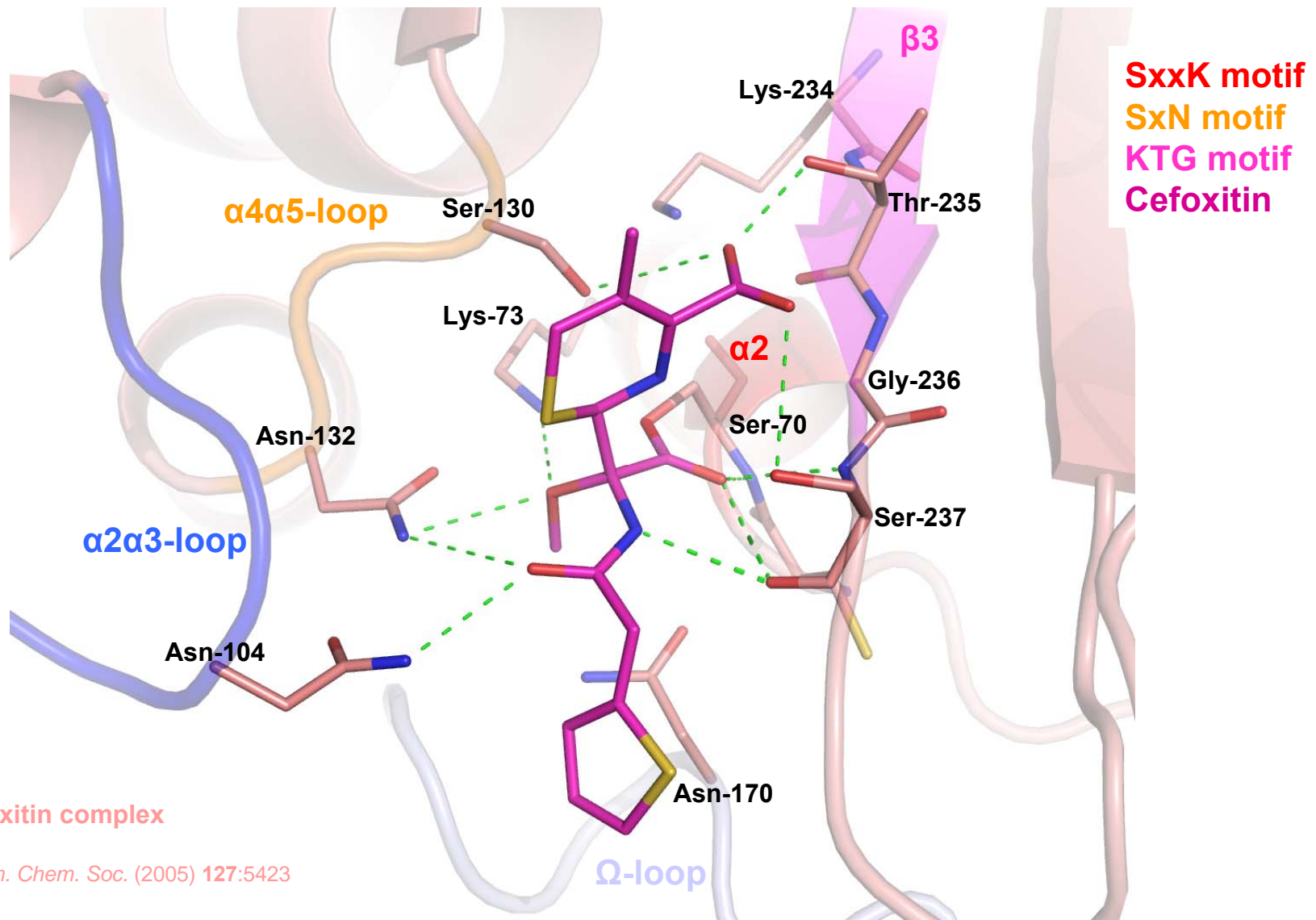
NXL104 complex



Rmsd, 0.118 Å (241 C α atoms)

The binding of NXL104 minimally affects the enzyme tertiary structure

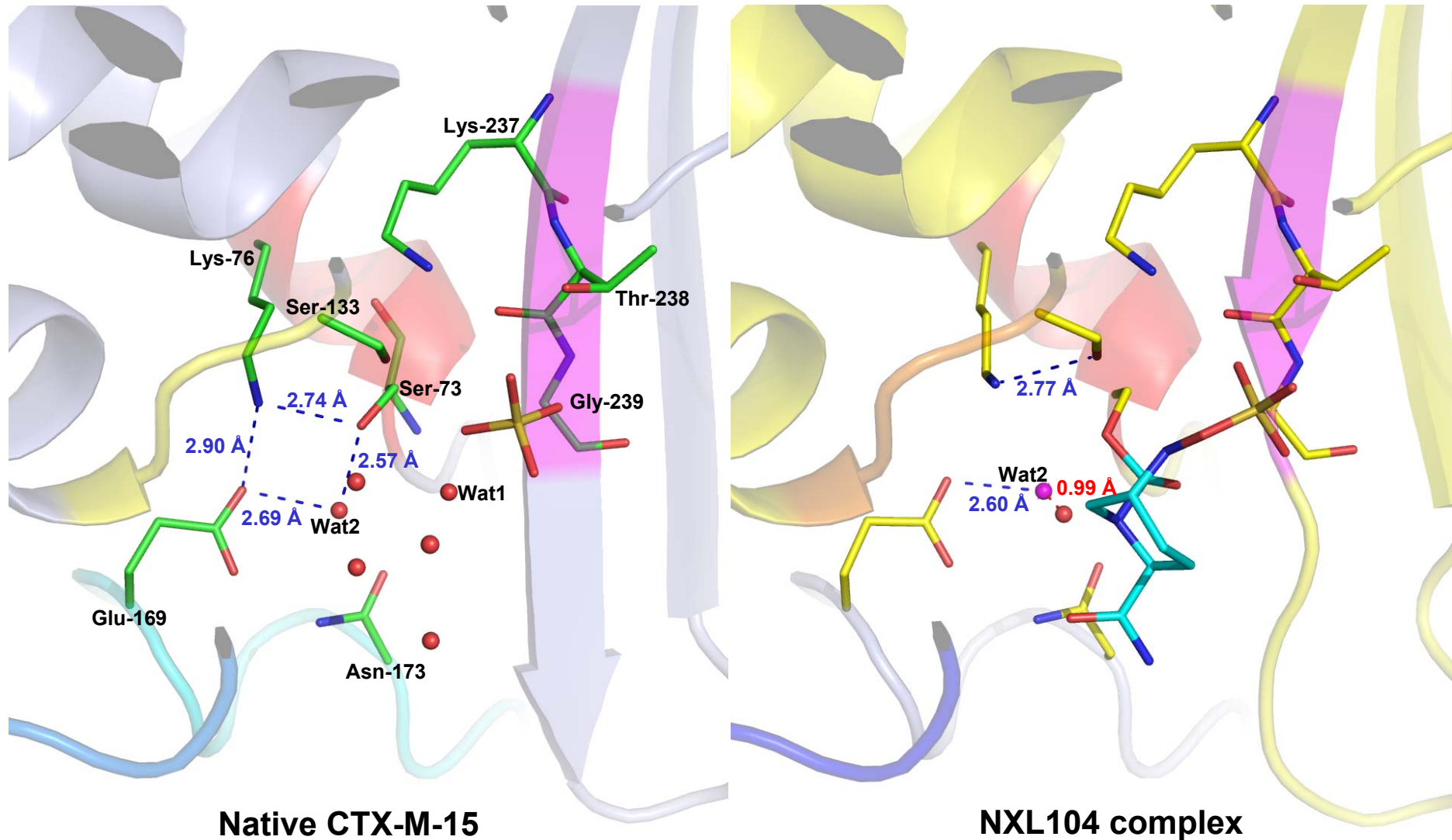
Results (7) – Interaction with NXL104



NXL104 interacts with many conserved residues of BLs



Results (8) – Comparison of the active sites



Active site Lys73 adopts a different conformation in the complex



Conclusions

- NXL104 is a potent inactivator of β -lactamases, that forms extremely stable covalent adducts
- A high resolution structure of CTX-M-15 was obtained, in the native form and in complex with NXL104
- Unlike clavulanate or tazobactam, NXL104 does not undergo chemical rearrangements in the active site
- Bound NXL104 is stabilized by polar interactions with conserved residues of β -lactamases
- In the complex, the putative deacylation water molecule is displaced by ~ 1 Å



Further information

Poster #	Poster title	Session #
NXL104 inactivation kinetics		
C1-1374	The Nature of Inhibition of TEM-1 β -Lactamase by the Non- β -Lactam Inhibitor NXL104	156
Ceftazidime/NXL104		
A1-005	Efficacy of Ceftazidime (CAZ)/NXL104 Combination in Murine Septicaemia caused by CTX-M-producing Enterobacteriaceae species	2
A1-006	Pharmacokinetics (PK) and Efficacy of Ceftazidime (CAZ) / NXL104 combination in a Murine Pneumonia Model Caused by an AmpC-Producing Klebsiella pneumoniae	2
A1-007	Effect of Age and Gender on the Pharmacokinetics (PK) and Safety of NXL104 in Healthy Subjects (Protocol NXL104/1004)	2
E-186	In Vitro Antibacterial Activity of Ceftazidime in Combination with the BLI NXL104	13
E-188	Anti-Anaerobic Activity of NXL104 in Combination with β -lactams and Metronidazole	13
E-192	In vitro activity of NXL104/ceftazidime against β -lactamase producing anaerobic bacteria	13
E-194	Activity of Ceftazidime/NXL104 and Select Comparators Against Geographically Diverse Clinical Isolates of Pseudomonas aeruginosa	13
B-1339	Efficacy of NXL104 in Combination with Ceftazidime in Murine Infection Models	154
Ceftaroline/NXL104		
A1-002	Optimal Administration of Ceftaroline (CPT) Plus NXL104 (NXL) for Cell Kill and Resistance Suppression for Enterobacter cloacae	2
B-1339a	In vivo antibacterial efficacy of ceftaroline (CPT) combined with the β -lactamase inhibitor NXL104 in a murine septicemia model	154
F1-1492	Spectrum and Activity of Ceftaroline Combined with NXL-104 Tested against a Challenge Collection of Pathogens with Well Characterized Resistances	164
F1-1493	Bactericidal Activity of Ceftaroline Combined with NXL-104 against Critical Targeted Organisms Possessing Various Resistance Mechanisms	164
F1-1494	Activity of Chequerboard Combinations of Ceftaroline and NXL104 vs β -Lactamase Producers	164

