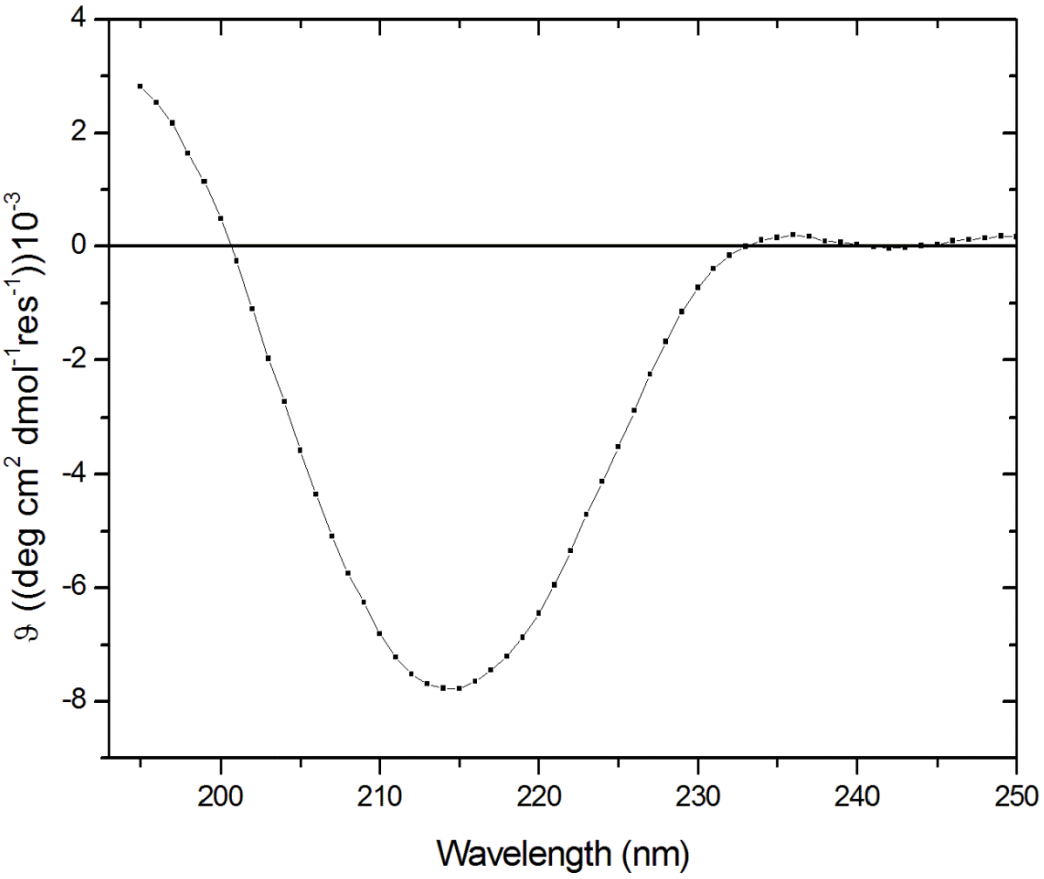
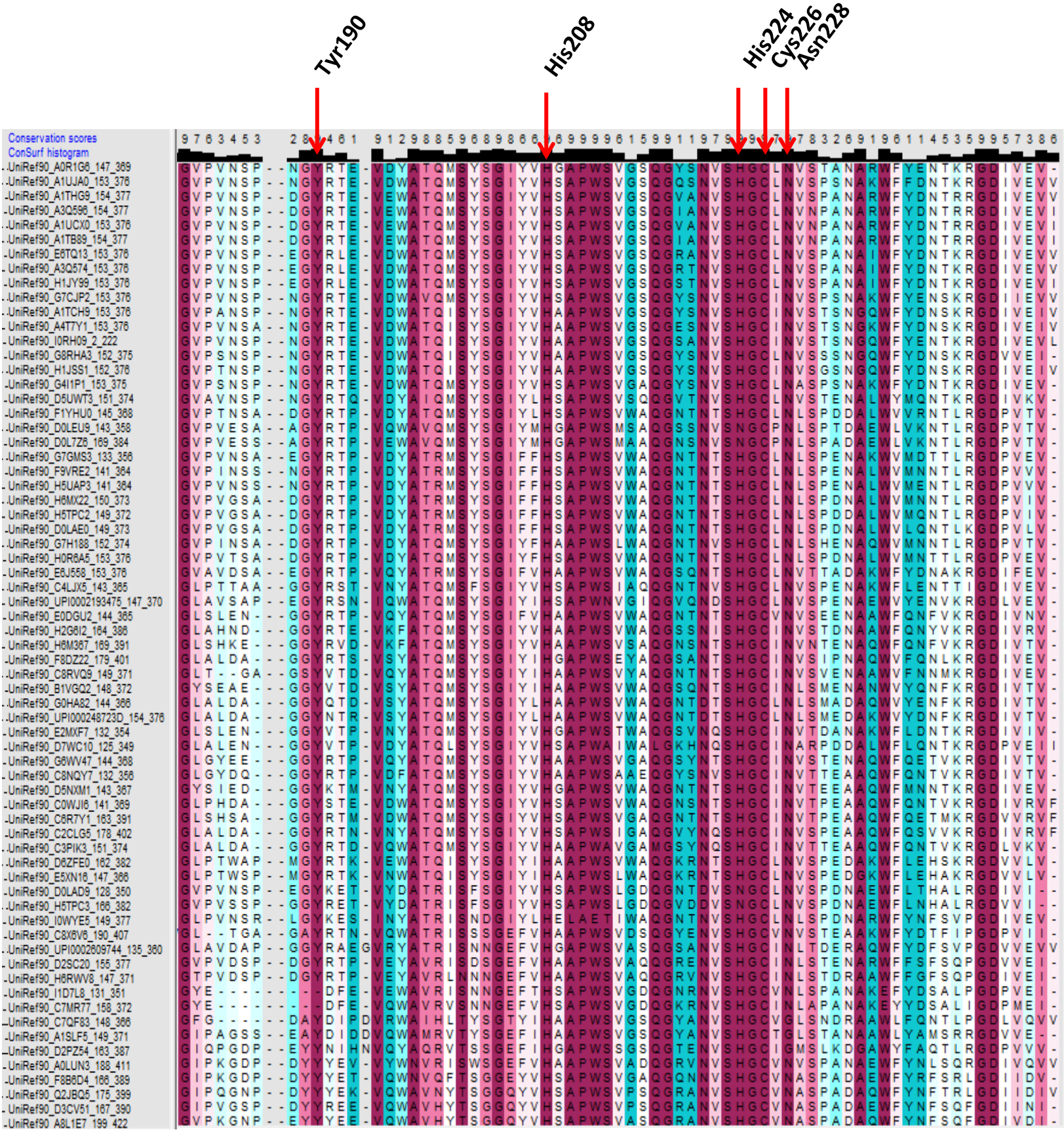
Supplementary Material

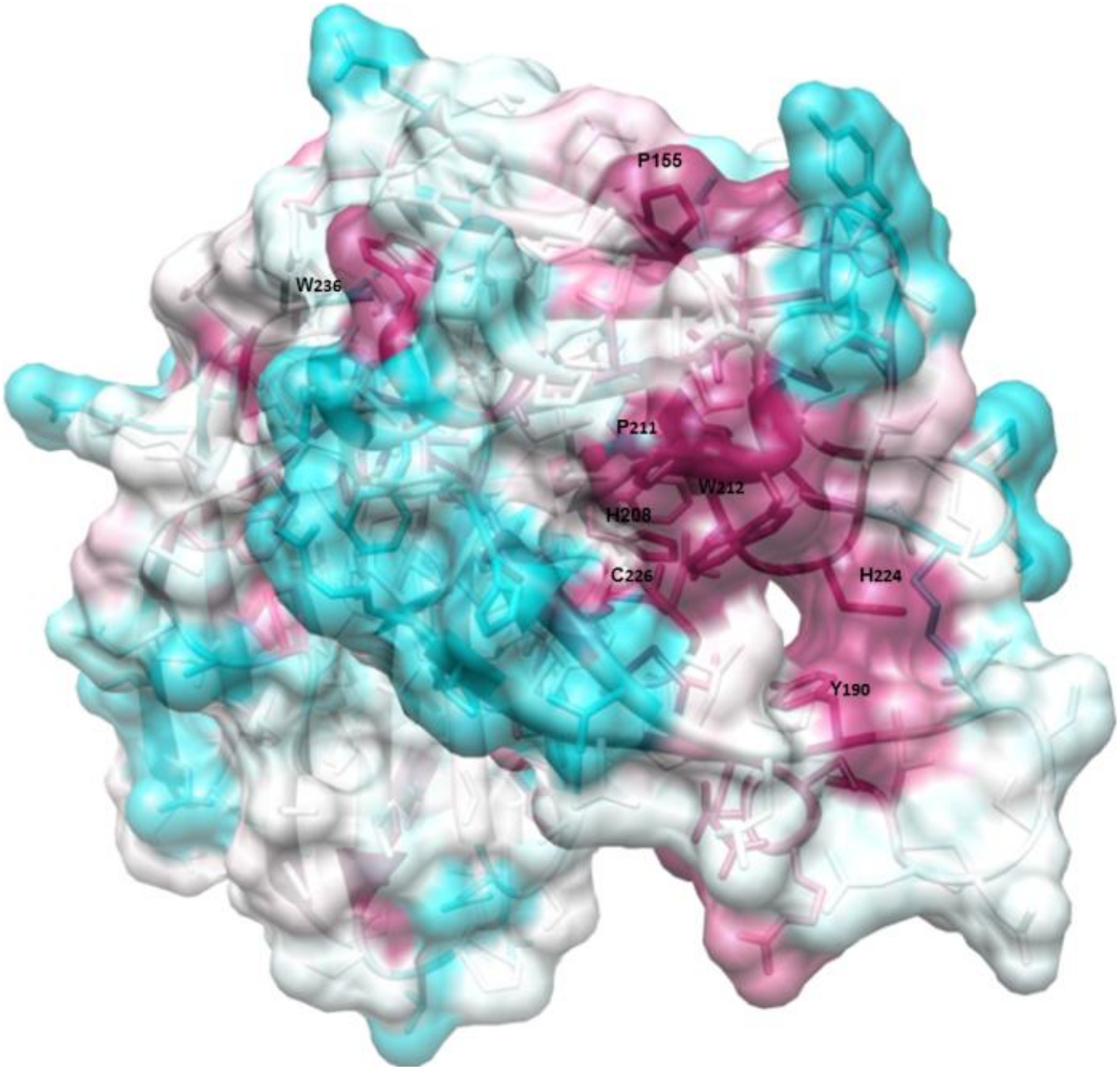
Supplementary Figure S1. CD spectrum of Ldt_{Mt1} (0.2 mg/mL) in 10 mM sodium phosphate buffer, pH 7.0.



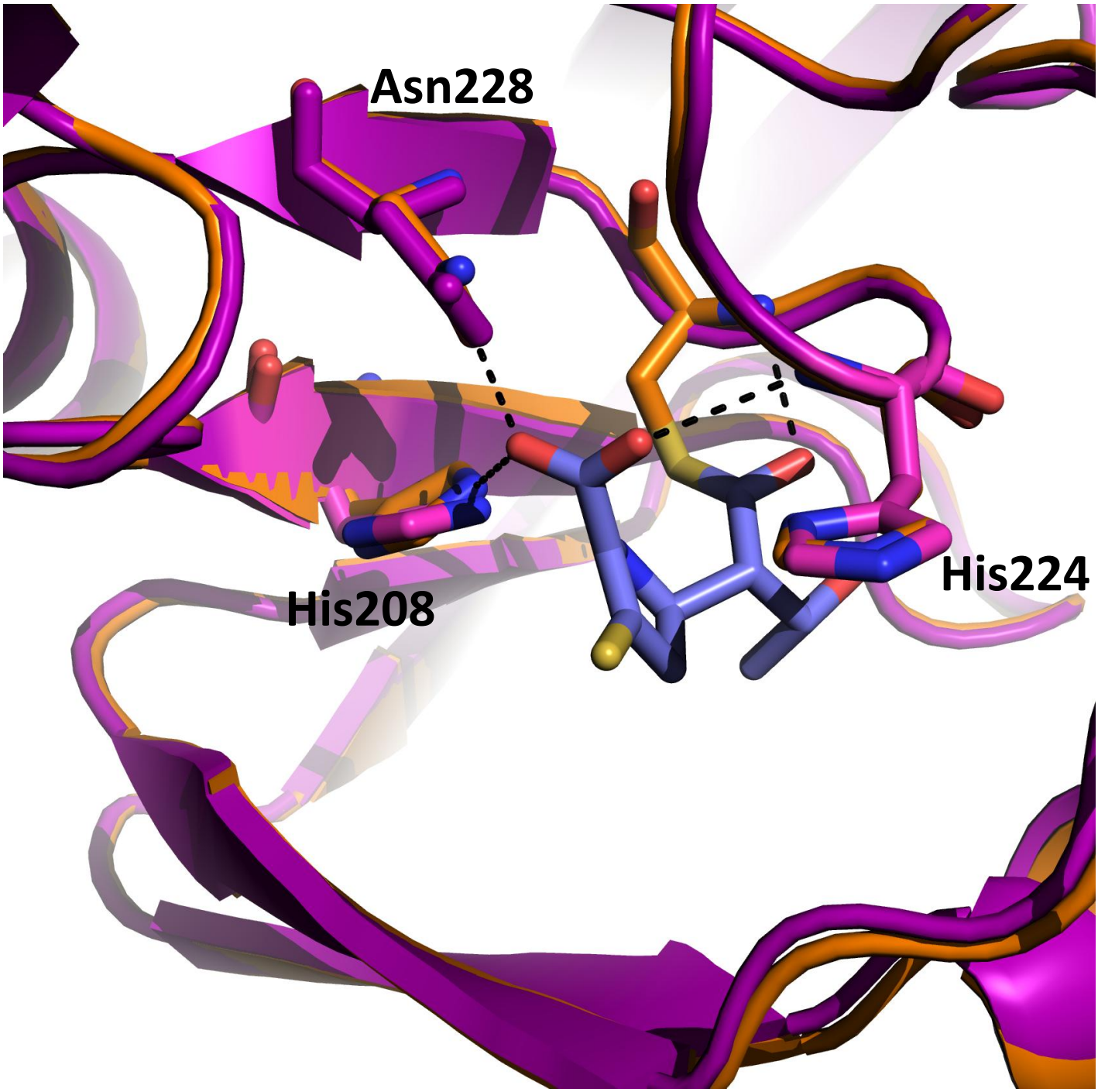
Supplementary Figure S2. Consurf sequence alignment showing the conserved residues on the surface of Ldt_{Mt1}. Residue coloring, reflecting the degree of residue conservation over the entire domain family, ranges from magenta (highly conserved) to cyan (variable). Key residues involved in hydrogen bonding with imipenem are highlighted.



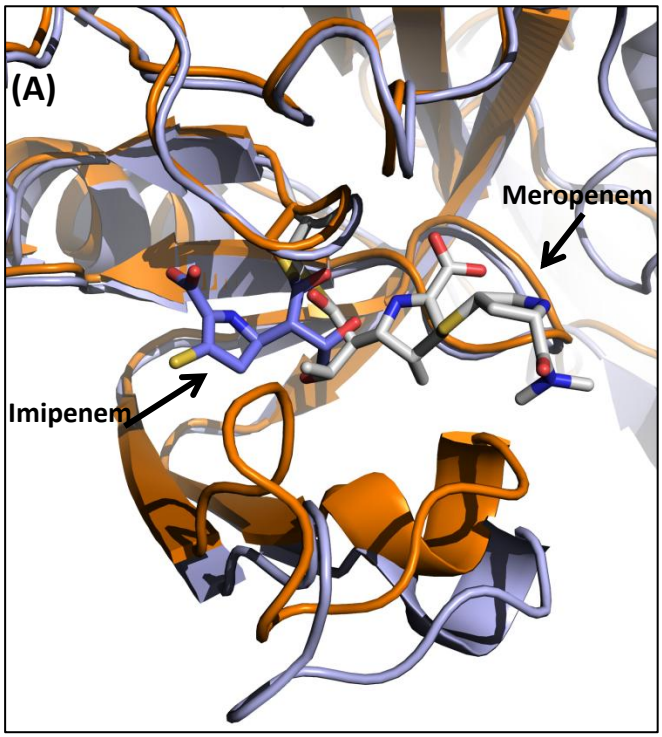
Supplementary Figure S3. Degree of residue conservation mapped on Ldt_{Mt1} surface. Residue coloring, reflecting the degree of residue conservation over the entire domain family, ranges from magenta (highly conserved) to cyan (variable). Conserved residues are labeled.

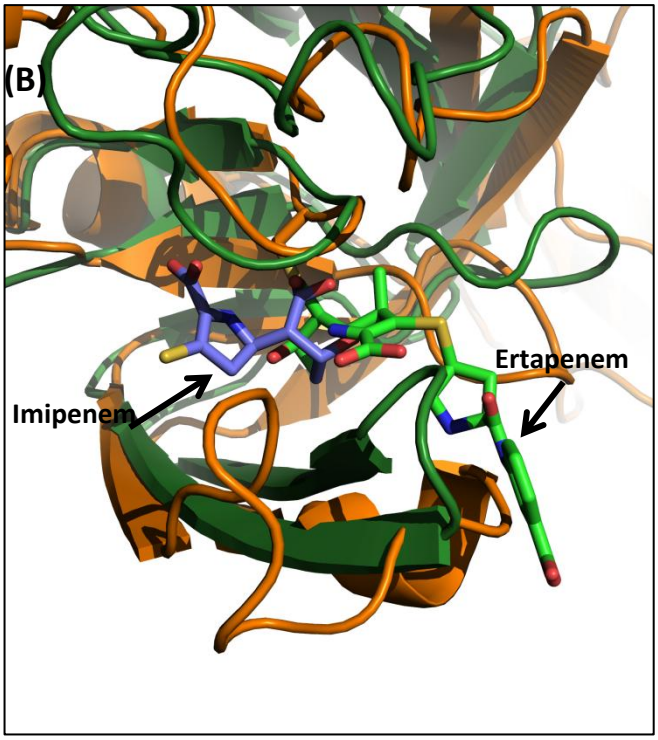


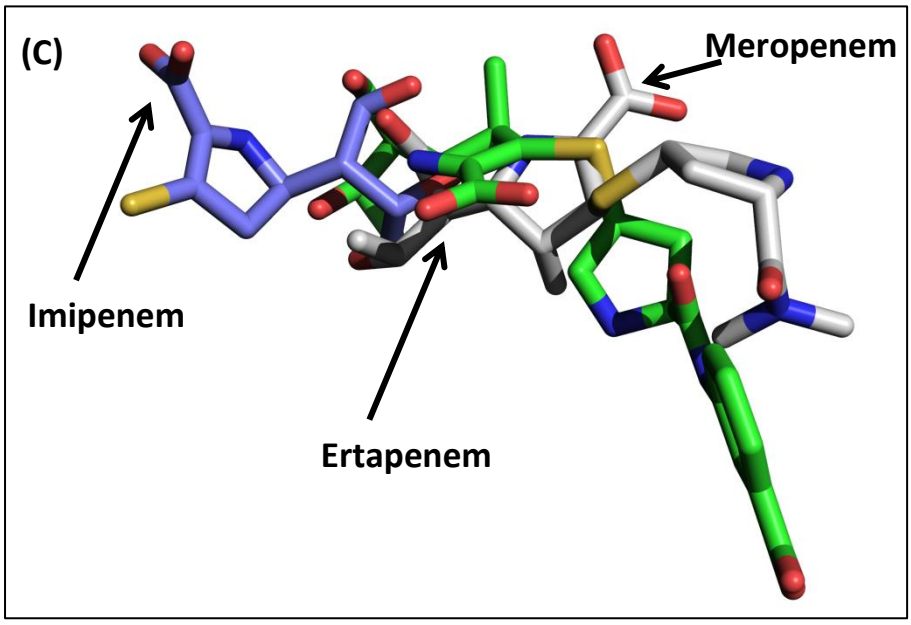
Supplementary Figure S4. Cartoon representation of the superposition of catalytic domains of Ldt_{Mt1} in its apo form (magenta) and in complex with imipenem (orange). The imipenem molecule is drawn in stick representation.



Supplementary Figure S5. Comparison of β-lactam binding modes to LD-transpeptidases. Cartoon representation of the superposition of catalytic domains of the Ldt_{Mt1} in complex with imipenem (orange) and (A) Ldt_{Mt2} in complex with meropenem (light blue, PDB code 4gsu) and (B) LD-transpeptidase from *E. faecium* in complex with ertapenem (PDB code 3zgp). (C) Superposition of the three binding modes. Arrows point at the carboxyl group of β-lactams, which points in different directions in the three complexes.







Supplementary Table S1. Structurally homologue IG domains, as calculated by the DALI server.

PDB	Z score	Rmsd (Å)	No. of aligned residues	Sequence identity (%)	Description
13so3-B	6.5	2.8	76	8	SUPPRESSOR OF TUMORIGENICITY 14 PROTEIN
1cf8-L	6.3	2.9	76	11	CATALYTIC ANTIBODY 19A4 (LIGHT CHAIN)
3hi1-L	6.3	2.8	76	9	GLYCOPROTEIN 120
1u6a-L	6.2	2.8	76	9	F105 LIGHT CHAIN
3ls5-L	6.2	2.8	76	7	LIGHT CHAIN OF ANTIBODY FAB FRAGMENT
3ls4-L	6.2	2.8	75	7	LIGHT CHAIN OF ANTIBODY FAB FRAGMENT
1hq4-C	6.2	2.9	76	11	ANTIBODY HA5-19A4 FAB LIGHT CHAIN
1iai-M	6.2	2.9	76	5	IDIOTYPIC FAB 730.1.4 (IGG1) OF VIRUS
1ors-A	6.1	2.9	75	9	33H1 FAB LIGHT CHAIN
1iqd-A	6.0	2.9	76	7	HUMAN MONOCLONAL BO2C11 FAB LIGHT CHAIN
1rhh-C	6.0	2.9	76	8	FAB X5, LIGHT CHAIN
2brr-L	6.0	2.9	75	8	MN20B9.34 ANTI-P1.4 ANTIBODY, FAB HEAVY CHAIN
1ken-L	6.0	2.9	76	9	HEMAGGLUTININ HA1
1ken-U	6.0	2.9	76	9	MOLECULE: HEMAGGLUTININ HA1