Impact of allosteric inhibitors on MRSA pyruvate kinase conformational dynamics

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A comparative molecular dynamics study of the methicillin-resistant *Staphylococcus aureus* (MRSA) pyruvate kinase (PK) is presented, with and without allosteric inhibitors bound. PK is an attractive target to develop novel antibiotics since it has been identified as a crucial 'hub protein' in MRSA interactome [1], implying high sensitivity to mutations and thus low probability to develop further resistance. Moreover, it has been shown to be critical for bacterial survival. Some potent and selective compounds, able to inhibit both MRSA PK enzymatic activity and bacterial growth in vitro, are already available [2, 3, 4]. However, the allosteric mechanisms governing PK inhibition in bacterial pathogens are poorly understood. Comparing all-atom MD simulations of 3 μs length of the 250 kDa *apo* and *holo* tetramers of MRSA PK, we show that binding of inhibitors at the level of the so-called small interface (between C domains) makes the lid covering the active site (B-domain) at a distance about 45 Å away from the small interface less mobile. We considered as a positive control an anti-PK natural compound (cis-3,4-dihyrohyrohamacanthin B) for which crystallographic data are available (PDB ID 3T07) and as putative inhibitors some similar new compounds with a pyrazine core. The reduced dynamic movement of the lid domain (in terms of extent or frequency) could be a mechanistic explanation for the activity of these inhibitors because this region needs both to open, to allow reagents and products to diffuse in and out, and to close, to hold back the catalytically active potassium and magnesium ions.

[1] A Cherkasov et al., J. Proteome Res., 2011, 10(3):1139-1150.

[2] R Zoraghi et al., J. Biol. Chem., 2011, 286(52):44716-25.

[3] CG Veale et al., J. Nat. Prod., 2015, 78(3):355-62.

[4] C Labrière et al., Eur. J. Med. Chem., 2017, 125:1-13.