Deamination of lysine or β-lysine (Fig. 1) are enzymatic reactions that have a high biotechnological interest as part of the metabolic pathways for the production of adipic acid, that is the industrially most important di-carboxylic acid. However, enzymes able to deaminate these substrates have not been identified so far. We selected 3-methylaspartate-ammonia lyase (MAL, EC 4.3.1.2) as an enzyme that potentially could act on or could be engineered to act on the target substrates.

Here, we aimed at studying MAL, characterizing the binding and inhibition properties of the target substrates and other five additional chemicals with a similar structure to both the natural and the target substrates were also tested (Fig. 2, left panel).

1. The substrates spatially fit in the pocket
Docking results showed that all the tested substrates spatially fit in the catalytic pocket (Fig. 2). However, the binding affinities obtained for the tested ligands are lower than for the natural substrate, suggesting that the ligands are weak or very transient binders.

2. β-lysine is a competitive inhibitor
β-lysine and β-glutamic acid are competitive and mixed inhibitors of MAL, respectively, indicating that they bind close to or in the catalytic pocket (Fig. 3). Interestingly, lysine does not inhibit MAL. These results suggest that the amino group positioned in the β-carbon (as in β-lysine and β-glutamic acid) is needed for the binding of substrate in MAL.

3. Aspartate shows a poor binding in MAL compared to 3-methylaspartate
Of the 7 tested compounds, MAL was active only towards aspartate, showing a decrease in the apparent affinity constant of 141-fold compared to 3-methylaspartate (Fig. 4).

These results suggest that the presence of the methyl group (in 3-methylaspartate) and the interactions that it forms in the catalytic pocket are essential for the proper binding of the substrate.

CONCLUSIONS
The binding of the substrate in MAL is highly structured. Of the seven tested substrates, only β-lysine and β-glutamic acid provide a chemical structure suitable to form the hydrogen bonds needed for the proper positioning of the substrate in MAL binding site (showing a non-polar group in the β-carbon, correctly oriented in the hydrophobic pocket and an amino group in the β-carbon). This study provides the groundwork necessary for future studies on engineering MAL substrate specificity.