In order to design mutant aminoacyl-tRNA synthetases to facilitate the incorporation of unnatural amino acids into proteins in vivo, it is useful to understand the structural mechanism by which they facilitate the incorporation of some amino acids but not others. The mechanism by which Leucyl-tRNA synthetase performs hydrolytic proofreading against certain substrates, natural and non-natural, but not against others is not clear. Previous work has identified Threonine 252 to be a conserved and critical residue for substrate specificity, and that substitution of larger amino acids at position 252 allows more incorporation of substrates that would normally be proofread against by the wildtype synthetase, and suggested that bound waters may help to limit the size of the editing cavity. By removing the crystal waters, adding explicit solvent, and performing molecular dynamics, we found that the amino acid side-chain binding pocket enlarged and that the synthetase could no longer discriminate between isoleucine and methionine. We suggest that the hydrogen bond network between Arg249 and the phosphate of the substrate guides the formation of the hydrophobic side-chain binding pocket. Starting from the crystal structure (including the crystal waters) and performing energy minimization and combinatorial side-chain replacement of protein residues and the ligand side-chain, we predict relative binding energies with trends in good agreement to previous aminoacylation experiments. Thus, we have high confidence in our predictions for different substrates that have not been studied as thoroughly yet. In vivo incorporation assays are being performed in the lab to further investigate the T252V mutant in particular to see if T252’s hydrophilic nature rather than its size is necessary for proofreading against certain substrates.