

Electronic Supplementary Information (ESI) for:

**Identification of productive inhibitor binding orientation in fatty acid
amide hydrolase (FAAH) by QM/MM mechanistic modelling**

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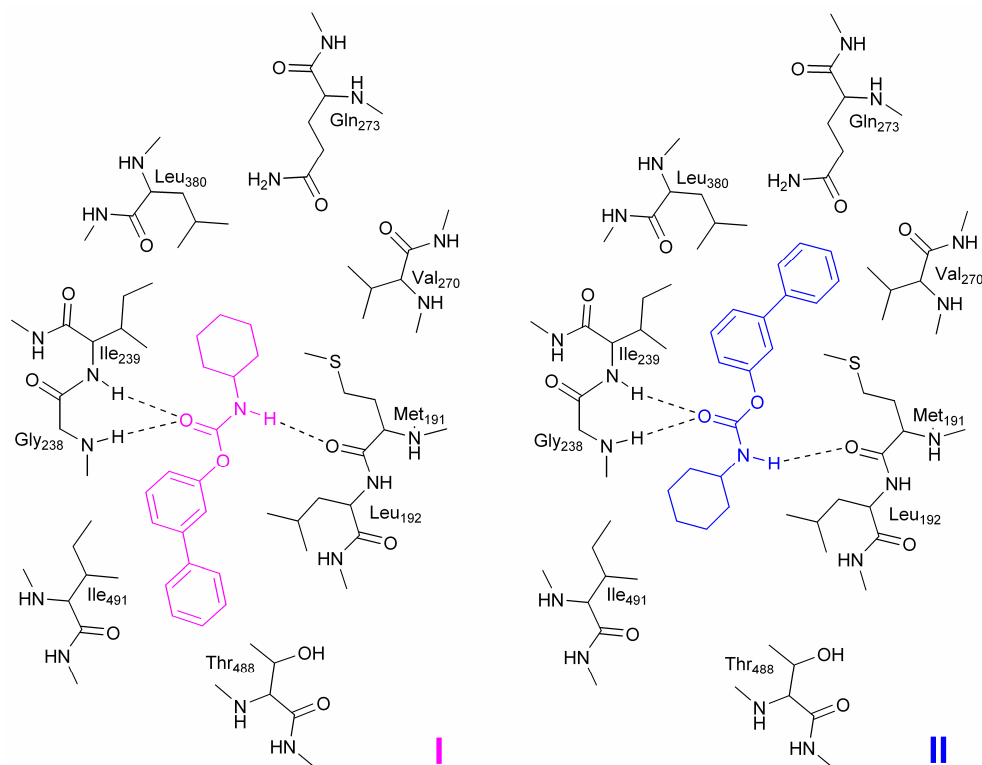
Molecular Dynamics (MD) simulations

The simulations were focused on the active site of FAAH-URB524 complexes built as described in the main text. The obtained complexes were equilibrated by MD simulation applying the following procedure. All protein residues with at least one atom within 25 Å of the centre (O1 of Ser241) were selected while all the others were deleted. The system was solvated by superimposing a 60 Å edge pre-equilibrated box of 8000 TIP3P water molecules and deleting any added water molecules of which the oxygen was within 2.6 Å of another non-hydrogen atom and farther than 25 Å from the centre. 10 ps of Langevin dynamics¹ at a temperature of 300 K was performed to equilibrate the water molecules, keeping all the other atoms fixed. Then the system was prepared for stochastic boundary MD simulation: (I) throughout, a 4 Å buffer zone was defined as all atoms further than 21 Å away from the center of the sphere, in which the non-solvent heavy atoms were harmonically restrained to their crystallographic coordinates with force constants based on model average *B*-factors.^{2,3} These restraints were scaled (linearly and in four steps) from zero at 21 Å from the center of the system, to a maximum at 25 Å. (II) Langevin dynamics were applied for the buffer region using frictional coefficients of 250 ps⁻¹ for non-hydrogen protein atoms and 62 ps⁻¹ on water oxygen atoms. (III) A deformable boundary potential was also applied for the water oxygens. (IV) The CHARMM22 force field⁴ (including

CHARMM22 protein parameters)⁵ was used to generate a trajectory using a time step of 1.0 ps for the integration, and a cutoff distance for non bonded interactions of 12 Å. The simulation was divided into two phases: (i) a heating phase of 30 ps needed to increase the temperature from 0 to 300 K; (ii) an equilibration phase of 100 ps at 300 K. The equilibrated structures were then employed for QM/MM calculations (see scheme S1 for a representation of the conformers). Each MD simulation was extended up to 370 ps and a second snapshot was taken for further QM/MM calculations (see below).

FAAH-URB524 starting structures

A two-dimensional diagram describing the main interactions between FAAH and the URB524 inhibitor in each orientation is shown in scheme S1. In binding orientation **I**, the *m*-biphenyl group is located in the ACP channel, in close to proximity to Ile491, and Thr488, whereas the cyclohexyl ring points toward Ile238. In binding orientation **II**, the *O*-aryl moiety, laying in the CA channel, interacts favorably with four lipophilic residues, namely Ile238, Leu380, Val270 and Met191 whereas the meta position of the distal ring faces the polar sidechain of Gln273.



Scheme S1: Two-dimensional representation of URB524 docked in orientations **I** (pink) and **II** (blue). Hydrogen bonds between the inhibitor and the enzyme are highlighted with dark dotted lines.

Reaction coordinates definition

The distance-dependent reaction coordinate restraints were applied using the RESD command of CHARMM. The value for each reaction coordinate was defined by the subtraction of one interatomic distance from another for three atoms (e.g. for a proton transfer the distance between the accepting oxygen and the moving hydrogen was subtracted from the distance between that hydrogen and the donating oxygen). When a third distance was included in one reaction coordinate an additional restraint was applied, which was only active when a given distance fell below or exceeded a given limit during a geometry optimization. In the case of R_x , the stepwise increase of the reaction coordinate was freely distributed among the three distances during energy minimization, with an additional constraint that the distance $d[C,O1]$ could only decrease to ensure the overall progress of the reaction. A similar approach has been successfully applied to FAAH,³ and also to a class A β -lactamase by Hermann et al.⁶

Breakage of the tetrahedral intermediate (TI)

Breakdown of the TI was investigated employing other reaction coordinates. In particular, step 2 and 3 of the reaction path were also combined together to have a less supervised description of the process. The following reaction coordinates were applied: $[d(O2, H1) - d(OAr, H1) + d(C, OAr)]$ describing proton transfer from Ser217 to the aromatic oxygen of the biphenyl fragment together with leaving group expulsion; $[d(N, H2) - d(O2, H2)]$ describing proton transfer from Lys142 to Ser217. The resulting three-dimensional PM3-CHARMM22 PES indicated that a stepwise process was favoured over a concerted one. However, as these surfaces resulted rough, we preferred to separate the breakage of the TI in two consecutive steps as described in the manuscript.

PM3-CHARMM22 energy profiles

Figure S1 shows the calculated PM3-CHARMM22 energy profiles for the carbamylation reaction of Ser241 by URB524 in orientation **I** (pink) and **II** (blue). The energies indicate that the reaction occurs much more easily in orientation **II** (barrier of 40 kcal mol⁻¹) than in **I** (barrier of 57 kcal mol⁻¹) consistently with the B3LYP/6-31+G(d)//PM3-CHARMM22 profiles reported in the manuscript.

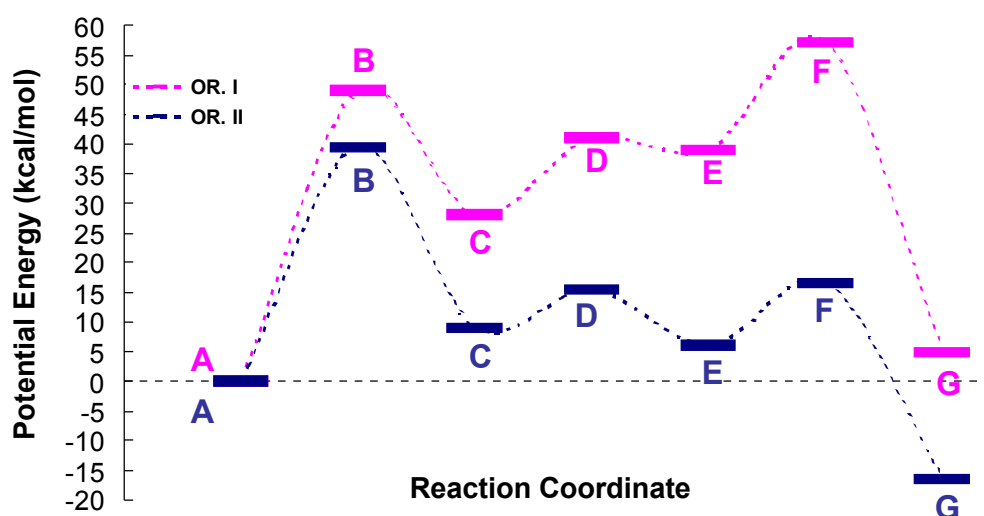


Fig. S1. PM3-CHARMM22 energy profile for carbamylation of Ser241 for orientations **I** (magenta) and **II** (blue).

Effect of the starting structure on calculated energy profiles

A second starting structure (from increasing the time of MD simulations up to 370 ps) was taken for each orientation of the inhibitor (**I** and **II**), and was used to model the carbamylation reaction using the same procedure described in the main text. Figure S2 shows the calculated PM3-CHARMM22 energy profiles for these structures. In this case also, the reaction takes place more readily in orientation **II** (barrier of 40 kcal mol⁻¹) than in **I** (barrier of 57 kcal mol⁻¹), similar to the profiles shown in Fig S1 and in the main text.

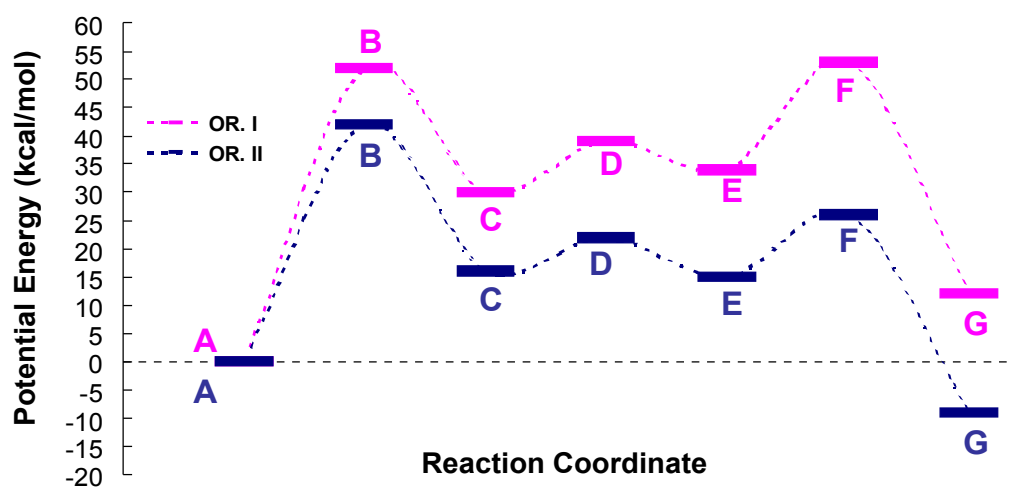


Fig. S2. PM3-CHARMM22 energy profiles for orientations I (magenta) and II (blue) for structures taken after 370 ps of MD simulation.

References

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