Insights into ligand-protein binding from local mechanical response

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1. Selection of spring constant and pulling velocity for alanine dipeptide

In order to select the suitable simulation parameters, different spring constants $k$ were tested (see Figure S1, A). It was found that stiffer $k$ and slow pulling velocities resulted in the reversible work values. Spring constant of 2000 kcal/(Å$^2$·mol) was chosen and Figure S1, B indicates it was sufficiently hard enough to steer the system to target point.

**Figure S1.** (a) Various trial targeted MD simulations performed with different spring constant $k$ (in kcal/(Å$^2$·mol)) (b) Line in the black displays the behavior of the system along with centre of the harmonic oscillator (red line) with spring $k = 2000$ kcal/(Å$^2$·mol). Red line represents the center of the moving harmonic constraining potential.
2. Representative sketch of the residues of CDK5 involved in the binding process

Figure S2. Residues involved in the (R)-roscovitine/CDK5 interaction. Carbon atoms of the residues of the protein are highlighted with green sticks. Carbon atoms of the ligand are highlighted with yellow sticks. The hinge region is shown with green cartoons while the glycine rich loop is evidenced in orange. All the rest of the protein is represented with white cartoons.
3. Selection of the pulling length in undocking of (R)-rosocovitine/CDK5

Test undocking experiments were performed so to decide the final pulling length for the production steered-MD runs. Coordination number, which is a measure of the number of contacts, was chosen to detect the complete detachment of the ligand from the protein. Detachment of the ligand in case of highest work and lowest work test trajectories on two test runs are shown in Figure S3 in terms of coordination number of the ligand with the protein. At 20 Å the protein-ligand interactions are lost and this justify the selection of the pulling length to be limited at 22 Å.

**Figure S3.** Dashed line: high work test trajectory. Continuous line: low work test trajectory. Coordination of the ligand with the C-alpha of the protein during in the entire trajectory is shown as function of the pulling coordinate.
4. Effect of steering on the structure of the CDK5

After the selection of the pulling velocity for the production steered-MD simulations, it was necessary to check whether the adopted pulling regime disrupted the protein structure. Therefore, we checked the RMSD of C-alpha of the binding pocket. It was found that there was no erratic change in the conformation in the binding pocket along with the rest part of the protein in both low work and high work test trajectories (see Figure S4) thus confirming that the work values obtained were not due to a major unfolding of the structure.

![Figure S4](image)

**Figure S4.** Dashed line: C-alpha RMSD of the binding pocket for the high work trajectory, continuous line: C-alpha RMSD of the binding pocket for the low work trajectory.
5. Cartesian-MDS representation of drug unbinding trajectories

We analyzed the unbinding trajectories obtained for (R)-roscovitine/CDK5 complex obtained via steered-MD along the distance between the ligand and the binding pocket of the target. The trajectories were analyzed by collecting the RMSD of the heavy atoms of the ligand respect to its crystal position as a function of the distance from the binding pocket. These coordinates are then processed via MDS in an identical way as done for alanine dipeptide thus producing a Cartesian-MDS representation. As expected, the RMSD metrics lumps together high and low work trajectories. This is particularly evident for distances lower than 10 Å.

![Cartesian MDS representation of the ligand along the distance between the ligand and the binding pocket. The color of the spheres evidences the work exerted in that specific portion of the space. Please compare with Figure 7 of the main text.](image)

**Figure S5.** Cartesian MDS representation of the ligand along the distance between the ligand and the binding pocket. The color of the spheres evidences the work exerted in that specific portion of the space. Please compare with Figure 7 of the main text.
6. Enhanced picture for LMR representation

Figure S6. Since the absolute color coding used in Figure 7 and 8 does not enhance sufficiently the heterogeneity of LMR within a distance bin, we here report the same snapshots as in Figure 8 but we adopt a “relative” color coding, where the blue conformation of the ligand represent the minimum LMR while the red represents the highest LMR at a given distance. It is evident that, while passing from B to C, the configurations with the lower LMR are located closer to the glycine-rich loop while the higher LMR values correspond to conformations that retain a contact with hinge region.

7. Steering experiment on (R)-roscovitine/CDK5 system with constrained residues

In the MDS representation of 50 work trajectories, one outlier was observed at 9 Å (see MDS point denoted with C in Figure 7 and 9). In order to determine the reason of such anomalous behavior, careful conformational analysis was carried out by visual inspection. It was found that outlier was corresponding to peculiar structural motion of the protein (hinge region in the CDK5). Here we show that if this particular motion of the protein is suppressed, the position of the outlier in the MDS representation changes and this demonstrates the one-to-one relationship between observed structural features and the MDS representation.

Steering experiment is carried out from the starting geometry of the trajectory producing the MDS anomalous behavior (see MDS points denoted with C in Figures 7 and 9 and denoted with a purple circle in Figure S6, A) using an identical set up as discussed under (R)-roscovitine/CDK5 section. Those particular residues that were supposed to be responsible for the specific motion in the protein (see Figure 9C in the manuscript) were kept constrained by means of RMSD harmonic restraint during the simulation. In Figure S6, B the MDS representation for all the 50 simulations is reproduced up to 9.5 Å and shows that the RMSD restrained trajectory changes position in the MDS representation (see purple colored MDS points in Figure S6, B that substitutes the one in Figure S6, A). This effect clearly evidences that the position of this particular trajectory in the MDS in Figure S6, A observed at 9 Å is evidently due to this backbone rearrangement. Following this, another steering experiment is
carried out as a control so to demonstrate that the RMSD restraint does not cause
dramatic shift of other MDS points while affecting the outlier only. For doing this, a
starting geometry of one the mainstream trajectories (see MDS points colored in
green in Figure S6, A) was chosen where particular structural feature demonstrated by
outlier was absent. A comparison of MDS representations in Figures S6, A and S6, B
for the green trajectories, shows that the MDS positions deviate minimally. This
discarded the possibility that the introduction of the restraint was producing a rigid
shift on all the representations.

Figure S7. MDS representation of the mechanical response plotted against the
steering coordinate obtained from the CDK5/(R)-roscovitine system. The MDS point
colored in purple at 9 Å corresponds to the trajectory showing the anomalous
behavior. The green points belong to one of the mainstream trajectory where
anomalous structural motion corresponding to the outlier is absent. A: unconstrained
runs. B: two runs where the backbone atoms displaying the motion were kept
constrained. The outlier shifts to the centre when the RMSD restraint is applied. The
same constraint applied to a trajectory (in green) where this anomalous behavior is not
observed does not produce any remarkable shift in the MDS representation.