Metadynamics Simulations Reveal Binding of PAR2 to its Trypsin Activated N-terminus

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The proteinase-activated receptors (PAR) belong to the class A of G protein-coupled receptor (GPCR) and show an unusual mechanism of receptor activation. In contrast to most GPCR which are activated by diffusible ligands like small molecules, lipids, peptides or proteins, the PARs are activated by proteolytic modification. Here, serine proteases like trypsin or thrombin cleave a part the N-terminus of the receptors exposing a new terminal sequence, subsequently activating the receptor. Despite being associated with diseases like arthritis [1], asthma [2] and cancer [3], the exact pathophysiological role of PAR is not fully understood.

Up to date, four crystal structures of PARs have been resolved, three of which in complex with a small molecule antagonist [4,5] and one in complex with a PAR2-antibody [5], with two of the small molecules binding to allosteric sites of the receptor. What is not yet clear, however, is the binding of the receptor to the proteinase exposed signaling sequence of the N-terminus or other molecules with agonistic properties.

We describe an approach to deduce the stages N-terminus binding to the PAR2 applying metadynamics simulations [6,7]. With this enhanced sampling method, the N-terminus continuously explores new conformations and interactions with the receptor along a given direction. In our case from the core of the receptor to the extracellular side. A bias is introduced to shift the ligand from low energy conformation to low energy conformation. The introduced bias to shift the ligand away from a certain binding mode allows the calculation of absolute binding energies, therefore enabling the deduction of potential stages of N-terminus binding.

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