# An Ensemble-Based SMD Workflow that Predicts the Residence Time of $A_{2A}$ Receptor Ligands

Potterton, A.G.[1], Heifetz, A.[1, 2], Coveney, P.V.[3, 4], Townsend-Nicholson, A.[1]

[1] Department of Structural and Molecular Biology, Division of Biosciences, Darwin Building, University College London, Gower Street, London, WC1E 6BT, UK

[2] Evotec (U.K.) Ltd., 114 Innovation Drive, Milton Park, Abingdon, OX14 4RZ, UK

[3] Centre for Computational Science, Department of Chemistry, University College London, Gower Street, London, WC1H 0AJ, UK

[4] Computational Science Laboratory, Institute for Informatics, Faculty of Science, University of Amsterdam, Amsterdam, 1098XH, The Netherlands

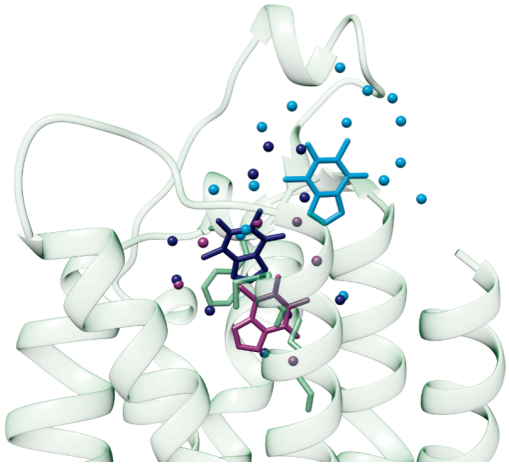

***Figure 1*** *Interactions of the dissociating ligand, Theophylline, with water (shown as spheres) and the receptor residues (shown in green stick representation). The ligand and waters are coloured purple, dark blue and light blue to indicate timepoints of 0, 5 and 10 ns, respectively, in the simulation.*

## 1. Prediction of drug target residence time using ensemble-based MD

Drug-target residence time, the lifetime of the ligand-receptor complex, is said to be better than binding affinity at predicting *in vivo* efficacy. Computational prediction of drug-target residence time, using standard molecular dynamics (MD), is challenging as experimental dissociation times are approximately $10^7$ longer than the simulation times that are currently feasible. We therefore applied steered MD (SMD) to forcibly speed up ligand dissociation. To ensure the interactions of the dissociating ligand with the receptor residues and water, ***Figure 1***, are reproducible, ensemble analysis was performed. We applied this method to 17 ligands of a prototypical GPCR (G protein-coupled receptor), all of which had associated published experimental kinetic binding data. Our results reveal that the computationally-calculated change in ligand-water interaction energy correlates strongly with experimentally-determined residence time ($R^2 = 0.79$). Further, the residues that interact with the dissociating ligand in these simulations are known experimentally to affect binding affinity and residence time. These experimental data indicate that our ensemble-based SMD protocol[1] is a novel, rapid and reproducible method for the rationalisation and determination of drug-target relative residence time.

## 2. Implementation into industry

After conducting the proof of concept study with the $A_{2A}$ adenosine receptor, the ensemble-based SMD protocol was carried out on ligands of the $M_3$ muscarinic receptor at Evotec. Like the $A_{2A}$ adenosine receptor, the $M_3$ muscarinic receptor is a class A GPCR, although it presents a more challenging target as there are fewer structural and kinetic ligand binding data available and the receptor has a much deeper binding pocket. The protocol was modified to increase simulation length by 50%, from 10 to 15 nanoseconds, to permit the ligand to dissociate as for the $A_{2A}$ adenosine receptor simulations. This workflow is currently being used as part of the lead optimisation phase of a drug discovery programme for a client, predicting and rationalising the effect of ligand substitutions on relative residence time.

[1] A. Potterton *et al.*, "Ensemble-Based Steered Molecular Dynamics Predicts Relative Residence Time of A2A Receptor Binders," *J. Chem. Theory Comput.*, Mar. 2019.