HTVS protocol to identify non-covalent inhibitors of CRM1

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The protein function depends on its subcellular localization, as it determines the access to binding partners and enzymes that catalyze post-translational modifications. The best-studied export protein is the Chromosome Region Maintenance 1 (CRM1, also known as XPO1 or exportin 1), which is a transversal protein across all eukaryotic cells. Inhibition of CRM1 has long been idealized for the treatment of cancer and several viruses and it consists of binding a compound to the NES-binding groove to prevent the association of CRM1 with its cargo. However, all known inhibitors of CRM1 establish a covalent bond with Cys528, leading to high toxicities and impairing its *in vivo* application.

Until recently, all known inhibitors bound covalently to the NES-binding groove. However, in a recent paper, Lei *et al.* presented the first inhibitor that was able to bind non-covalently to the NES-binding groove - the non-covalent CRM1 inhibitor 1 (NCI-1) [1]. Unfortunately, and despite the name suggesting otherwise, this inhibitor also binds covalently to Cys528 in the *wild-type* form. Nevertheless, NCI-1 ability to bind to CRM1 non-covalently serves as a proof-of-concept that such inhibitors are viable and can be developed.

With the intent of discovering non-covalent inhibitors of CRM1, a high-throughput virtual screen (HTVS) protocol was developed and implemented using a database provided by our collaborator, Prof. Romano Silvestri (Head of Medicinal Chemistry, Sapienza Univ., Italy). This HTVS was done using both the NES-binding groove from the crystallographic structure available in the PDB (ID: 6TVO) and two new conformations sampled from MD simulations, which we expect to be better descriptors of the apo structure. The top rank compounds were selected and are now being tested experimentally by our collaborator, Professor Wolfgang Link (University of Madrid, Spain).

1. Lei Y, An Q, Shen X-F, Sui M, Li C, Jia D, et al. Structure-Guided Design of the First Noncovalent Small-Molecule Inhibitor of CRM1. J Med Chem. 2021;64: 6596–6607.