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Type	Oral communication
Session	Protein structure and function
Title	Computational evaluation of the synergic effect of a small molecule on Lumacaftor F508del-CFTR rescue ability
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Motivation

Cystic Fibrosis (CF) is the most common lethal monogenic disorder in Caucasians. It is mainly caused by the mutation of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR), an ion chloride channel located in the plasma membrane of the epithelial cells. CFTR is a membrane protein composed of five domains: two intracellular nucleotide-binding domains (NBD1 and 2), two membrane-spanning domains (MSD1 and 2) in turn composed of twelve Transmembrane helices (TM1 to 12) connected by four Intracellular and six Extracellular Loop (ICL1 to 4, and ECL1 to 6), and one regulatory domain (R) [1]. The protein is so composed: MSD1–NBD1–R–MSD2–NBD2. The deletion of phenylalanine 508 (F508del) in the NBD1 domain is the most common CFTR mutation, disrupting the interaction between the NBD1 domain and the ICL4 loop. Nowadays, CF therapies for F508del-CFTR have significantly enhanced the mean survival age of patients [2]. However, the development of alternative strategies is required because the burden of CF care remains very high.
Drug repositioning is a strategy able to find a new target for already approved drugs, reducing development cost and time. In previous studies, we found a druggable pocket (DP1) for the marketed CF drug Lumacaftor in complex with F508del-CFTR [3], located in the interface region between the NBD1 domain and ICL4 loop. We have also identified druggable DP1 sub-regions [4], that could be targeted by repositioned small drugs to produce a synergic effect with Lumacaftor in its F508del-CFTR rescue ability. Here, we present our progress in targeting these DP1 sub-regions.

Methods

The results from a previous drug repositioning experiment on DP1 were re-evaluated focusing on small molecules. The docking study was performed on 846 AIFA drugs over 18 apo F508del-CFTR conformations previously obtained [3,4]. The resulting best pose of each drug with a molecular weight < 500 Da was selected. The remaining poses were filtered based on their proximity with the Lumacaftor inside the DP1, selecting the ones > 1.5 Å and < 5.0 Å. Then, the selected drugs were evaluated for their safety on CF patients, finding nicotinamide (NAM) as a hit.
Two different sets of 50 ns three MD replicas experiments (Amber18 package) were set up to evaluate: (1) which drug (Lumacaftor or NAM) firstly binds into the DP1; (2) if the drug is able to induce a fitting of the binding pocket; (3) if the first drug favors the binding of the second one.
The starting complexes were: (1) the docking pose of NAM inside the DP1 sub-region of the apo F508del-CFTR; (2) the docking pose of NAM inside the DP1 sub-region of the F508del-CFTR-VX809 complex.
The trajectory analyses were performed with the AmberTools package, to evaluate the stability of the complexes and the binding poses of the two drugs inside DP1.

Results

Starting from a previous drug repositioning experiment [4], we carried out a further drug repositioning analysis to preliminarily evaluate a potential hit able to bind into the DP1 sub-regions in combination with Lumacaftor. From the analysis of the docking repositioning, 1462 docking poses, obtained from the filter steps described in methods, were sorted from the lowest molecular weight, and the CF safety evaluation resulted in finding nicotinamide (NAM) as a hit. NAM is a natural compound, an amide of vitamin B3, and it is already used as a food supplement.
Computationally, the combined MD simulation between two drugs in the same pocket is a stimulating topic to deal with. The challenge has been to ascertain which drug firstly binds into the DP1 and its ability to induce a fitting of the binding pocket that possibly favors the binding of the second drug.
Results of the first set of MD carried out by evaluating the docking pose of NAM inside the sub-region of DP1 in the apo F508del-CFTR, show that NAM does not find a suitable DP1 sub-pocket to accommodate,

and it remains unstable.
So, we hypothesized a DP1 sub-region fitting for the binding of NAM led by Lumacaftor. To test this hypothesis, the second set of MD, combining the docking pose of NAM inside the DP1 sub-region of F508del-CFTR-VX809 [4], was carried out. The results of the first two replicas confirmed the above hypothesis, highlighting an H-bond between NAM and R1070 of ICL4. This interaction could recover the disrupted interaction between the NBD1 domain and ICL4 loop, thus supporting the Lumacaftor rescue ability in a synergic way.
Up to now, the third MD replica and the Surface Plasmon Resonance analyses to validate NAM binding and its possible combination with VX809 inside the apo F508del-CFTR are ongoing.

Info

References:
[1] Rogan M.P., Stoltz D.A., Hornick D.B.; Chest. 2011, 139, 1480-1490.
[2] Elborn J.S.; Lancet 2016, 388, 2519-2531.
[3] D'Ursi P., Uggeri M., Urbinati C., Millo E., Paiardi G., Milanesi L., Ford R.C., Clews J., Meng X., Bergese P., Ridolfi A., Pedemonte N., Fossa P., Orro A., Rusnati M.; Sensors and Actuators B-Chemical 2019, 301, 127131.
[4] Orro A., Uggeri M., Rusnati M., Urbinati C., Pedemonte N., Pesce E., Moscatelli M., Padoan R., Cichero E., Fossa P., D'Ursi P.; European Journal of Medicinal Chemistry 2021, 213, 113186.

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Availability	-

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