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Development of novel methodologies for the identification of CFTR-targeted drugs: a multidisciplinary approach using Real Time Surface Plasmon Resonance interaction assay supported by bioinformatics strategies on HPC infrastructures

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Background. Cystic fibrosis (CF) is due to dysfunctions of CFTR whose most common mutation, F508Δ, localized in NBD1 domain, causes an abnormal conformation of F508Δ-CFTR that is withhold in the endoplasmic reticulum. The small amount of receptor that reaches the plasma membrane exhibits low activity (gating defect).

Hypothesis & objectives. Current CF therapies are aimed at symptoms alleviation, calling for new drugs to rescue F508Δ-CFTR trafficking (correctors) or gating (potentiators), to the identification of which this study has sought to make an original contribution.

Methods. An integrated approach between computational Methods. and surface plasmon resonance (SPR) was here applied to better understand the molecular mechanisms of the known VX809 corrector and to characterize new compounds

Results. Molecular dynamics unmasked two binding pockets (BPs) in NBD1. BP1 is smaller in wild type (WT) than in F508Δ-CFTR, due to two loops (drug site 1 and 2) making contact and closing the BPs. In F508Δ-NBD1, all the ligands tested bind BP1, VX809 and FCG in drug site 1, EN371B, EN277I and EN371A in drug site 2. VX809 binding to F508Δ-NBD1 is stable and samples the same space of the WT, indicating a conformational recovery. FCG association is unstable, indicating a different binding site. EN371A is inactive, moving away from the BP. SPR validated molecular modeling: all the compounds except EN371A bind F508Δ-NBD1 with affinities higher than that of keratin 8 (K8). VX809 and FCG are stronger binders than EN2771 and EN371B. When the compounds were assayed for their capacity to affect the F508Δ-NBD1/K8 interaction, EN277I was ineffective while EN371B prevented the interaction, indicating a competition with K8 for the same BP. VX809 or FCG originated instead an additional signal, indicating their binding to the receptor in a site distinct from that of K8.

Spin-off for research & clinical purposes. SPR analysis validates and complements in silico predictions sustaining the integration of bioinformatics and SPR as effective in speeding up the discovery of new CFTR-targeted drugs.

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