Molecular modelling, molecular docking and molecular dynamics simulation techniques applied to the design of a novel APC/C inhibitor

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The Anaphase-promoting complex/cyclosome (APC/C) is an E3 cullin-RING ubiquitin ligase, composed of 19 subunits and 1 co-activator, either cell division cycle 20 (CDC20) or fizzy and cell division cycle 20 related 1 (FZR1, best known as CDH1). This huge complex is involved in cell cycle regulation by targeting proteins for degradation by the 26S proteasome. It regulates many cellular processes, including cell division, senescence, differentiation and apoptosis. In particular, the main function of APC/C, in association with CDC20, is to trigger the transition from metaphase to anaphase by mediating the degradation of specific proteins, such as cyclin B1 and securin [1]. APC/C-CDC20 is also the main target of the spindle assembly checkpoint (SAC), a surveillance mechanism monitoring and ensuring correct mitosis execution [2]. APC/C malfunctioning has been causally linked to genomic and chromosomal instability and is considered a major driver of tumorigenesis.
Recently, high resolution cryo-EM structures of human APC/C have been published, allowing to understand the tertiary and quaternary structure organization of this complex [3][4]. The "baseball glove" convex structure of APC links functional and regulatory aspects of this complex (Figure 1). The catalytic core catalyses the transfer of ubiquitin to an E2 complex active site upon binding to the E2-ubiquitin conjugate, marking proteins for degradation. The tetratricopeptide repeat (TPR) lobe, instead, accounts for co-activators binding. Co-activators are essential components of APC/C because they regulate the timing of the cell cycle, allowing the specific binding of different substrates to the complex [1]. The binding of APC/C with its co-activators is mainly mediated by interactions between a C-terminal IR tail, which is present in both co-activators, and the binding pocket of the CDC27 subunit (also known as APC3).
Here, to study this complex in different phases of the cell cycle, we selected two cryo-EM structures of APC/C, APC/C-CDC20 [4] and APC/C-CDH1 bound to the inhibitor Emi1 [3], from the PDB database. Since many regions in the structures were missing or not solved at atomic resolution, we entirely modelled them using the web-server I-Tasser [5], while several loops have been included using the *de novo* procedure implemented in the Modeller software [6].
We then focused on the evidence that APC/C in complex with the proto-oncogene CDC20, can be efficiently inhibited by the TAME molecule (Tosyl-L-Arginine Methyl Ester). This compound structurally mimics the IR tail of CDC20 and inhibits APC/C by entering the binding pocket of CDC27, displacing the co-activator, and thereby forming a higher affinity interaction with CDC27 [7]. The displacement of the co-activator leads to the inhibition of APC/C, resulting in mitotic arrest and cell death via mitotic catastrophe.
Following APC/C modelling, we performed some preliminary docking studies using the AutoDock Vina program [8] with the aim of identifying the pattern of interactions of CDC27 with the IR tail of CDC20 or between CDC27 and TAME. The comparison of the obtained results highlights differences in the pattern of residues involved in the interactions, and in the binding mode of the two ligands. To identify the energy contribution of the residues involved, we set up classical molecular dynamics simulation of the APC/C-CDC20 complex, in order to evaluate the IR tail binding affinity through the MM/GBSA method [9]. These results will help to rationally modify the TAME moieties to increase the affinity for CDC27, improving its ability to inhibit the APC/C and induce apoptosis in cancer cells.

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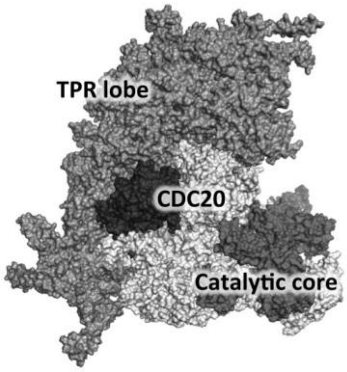

Figure 1. Complete APC/C structure, main functional elements are shown by labels.