Poster Presentation

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X-ray Crystallography: essential tool for protein characterization and ligand optimization

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Human 17β-hydroxysteroid dehydrogenase type 14 (h17β-HSD14) is the latest identified 17β-HSD member of the Short-chain Dehydrogenase-Reductase super family (SDR). In vitro, h17β-HSD14 catalyzes the NAD+ dependent oxidation at position 17 of estradiol (E2) and 5-androstene-3β,17β-diol (5-diol) to estrone and dehydroepiandrosterone, respectively. This protein has not yet been thoroughly investigated and its physiological role remains unknown. The enzyme is composed by 270 AA and its weight is 28.6 kDa. Two variants of h17β-HSD14 are known. The first one was isolated from the retina and contains a serine at position 205 (S205). An allelic variant differs only by a threonine at this position, and was identified from melanotic melanoma cells (T205). The in vitro turnover of both variants for E2 and 5-diol is similar.[1]
Northern blot experiments revealed that h17β-HSD14 is predominantly expressed in brain, placenta and liver. As it is expressed in the brain, it may become a potential target for the treatment of neuronal diseases, where estrogens were demonstrated to have neuroprotective effects. Potent and selective inhibitors are also useful tools to study the role of enzymes in vivo.
Recently, we characterized the apo (S205), holo (S205 and T205) and ternary complex crystal structures of h17β-HSD14 with estrone as well as with nonsteroidal inhibitors (T205).[1, 2] Crystal structures of the protein-ligand complex were necessary for the understanding of the inhibitors SAR and their further optimization. In fact, they revealed how small changes at the inhibitor's substituents can cause variations of their binding modes.
In order to further characterize the enzyme, we performed site-directed mutagenesis studies, where we analyze the biochemical consequence of these mutations on enzymatic turnover using a fluorescence-based assay.
In parallel, with the goal to discover new inhibitor scaffolds, we initiated a fragment-based lead discovery (FBLD) campaign by screening a 96 fragment library assembled considering a slightly extended "Rule of 3" as guideline. We believe that crystallographic fragment screening is a promising approach which may lead to the identification of more hits than other biophysical screening methods, especially for those ligands that show weak binding affinity,[3] while providing essential structural information about binding modes, prerequisite for their further optimization.
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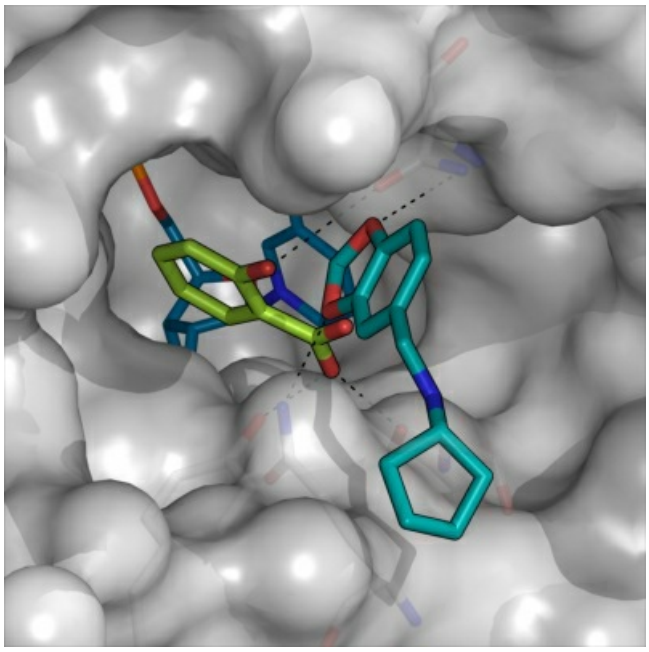

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