Transthyretin amyloid as a model target for the modulation of protein-protein interactions: combining the reach of structure- and ligand-based virtual screening methods

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1 Introduction

Most proteins exert their biological functions as components of protein complexes. In recent years, the understanding of the crucial role of protein-protein interactions, both in physiological and pathological processes, led to the realization that the modulation of specific protein interactions is of great pharmaceutical interest. In the case of amyloid diseases – including Alzheimer's disease, Familial Amyloid Polyneuropathy (FAP), type 2 diabetes, and several others –, the formation of protein aggregates and fibrils are at the source of cytotoxicity. Amyloid fibrils and aggregates are a product of deviant interactions and assembly of conformational intermediates found along the unfolding pathway of certain proteins.

Transthyretin (TTR) is a homotetrameric protein present in the blood plasma and cerebral spinal fluid, and is implicated in the deposition of amyloid fibrils in the peripheral nerves and heart tissue, in disorders such as FAP, Familial Amyloid Cardiomiopathy and Senile Systemic Amyloidosis. Although its biological function is not fully understood, TTR is known to bind and transport the hormone thyroxine. The formation of amyloid aggregates and fibrils of TTR involves an initial step whereby the native tetramer dissociates to monomers with altered tertiary structure. This step is critical for amyloid formation and can be modulated by the binding of thyroxine-like compounds to the two equivalent thyroxine-binding pockets of tetrameric TTR. Over the last decade, several small molecules have been screened for their ability to stabilize the tetrameric form of the protein and thereby prevent amyloid formation, but the undesirable side-effects associated with the discovered binders, or their inability to strongly bind to TTR in the plasma, are still a major obstacle.

The availability of a large amount of structural data of complexes of TTR with a number of ligands renders this protein an appealing target for the design of small organic molecules to interfere with amyloid formation. In this work, we attempt to answer important questions: Are there any systematic structural differences across multiple complexes and between the two thyroxine-binding sites of TTR? Does the available information account for the negative cooperativity displayed by most binders? Which docking algorithms and scoring functions better handle the characteristics of these pockets, and therefore, identify drug candidates? We present the framework of extensive molecular modeling studies applied to TTR: We explored ligand-based methods – from 2D fingerprints to 3D shape similarity – and docking and scoring algorithms, identifying the most appropriate receptor structures for Virtual Screening (VS). Moreover, we extracted common pharmacophoric features from known active compounds and

devised receptor- and ligand-based pharmacophore models. The VS performance of the different methods is evaluated through ROC enrichment plots, on a benchmark set comprising of 22 experimental actives and 738 decoys selected through a protocol similar to the one proposed by Huang *et al.* (2006).

The combination of multiple structure- and ligand-based virtual screening methods brings consensus to the elucidation of novel TTR amyloid inhibitors.

References

Huang, N., Soichet, B.K. and Irwin, J.J. (2003). Benchmarking sets for molecular docking. *Journal of Medicinal Chemistry*, **49**, 6789-6801.