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# Selection of Transthyretin Amyloid Inhibitors

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## Abstract
Amyloidosis is a group of clinical disorders caused by the aggregation of specific proteins into abnormal extracellular deposits. Today, 31 different proteins have been linked to amyloid diseases including transthyretin-related amyloidosis (ATTR). ATTR occurs through the aggregation of either wild-type plasma protein transthyretin (TTR) or a mutated form. TTR is a homotetramer that under normal circumstances functions as a carrier of thyroxine and retinol binding protein. The aggregation cascade requires dissociation of the tetramer into monomers, and preventing this dissociation represents a potential mode of intervention. Interestingly, small molecules, referred as kinetic stabilizers, can bind to TTR's thyroxine-binding site (TBS) and such molecules are currently being used as a therapeutic approach to impair tetramer dissociation. The efficacy of TTR stabilization is directly correlated to the binding affinity of the ligand to TBS. However, the binding of the ligand to TTR *in vivo* can be affected by other plasma components resulting in poor efficacy. Thus, the selectivity of ligands is an important parameter. We have designed an assay where the ability to stabilize TTR can be directly evaluated in plasma and we have investigated the stabilizing effect of nine potential TTR binders (*Paper I*). The results, surprisingly, revealed that the binding affinity of molecules has a poor correlation to its selectivity. However, the nature of protein-ligand complex formation can also be described by enthalpic (ΔH) and entropic (ΔS) energy contributions. ΔH represents the change in chemical bonds and frequently requires a higher order of orientation compared to the ΔS component, which mainly represents the hydrophobic effect via the exclusion of water. We hypothesized that ligands possessing high ΔH in binding to their co-partner would also be more specific in a complex environment such as plasma. By applying a thermodynamic analysis using isothermal titration calorimetry, we found that the selectivity in plasma correlates well with the ΔH contribution and might, therefore, be a better predictor for selectivity.

Luteolin was found to be a highly selective stabilizer of TTR and was investigated further (*Paper II*). The ligand displayed a significant rescuing effect in both cell culture and animal models. However, luteolin undergoes rapid enzymatic degradation in the liver and this impairs its use as a potential therapeutic drug. To attempt to circumvent this issue, we modified the most exposed hydroxyl group thus rendering the molecule inert towards glucuronidation (*Paper III*). The substitutions resulted in higher stability in the face of hepatic degradation molecules, but they also affected the selectivity in a negative manner. The screening for new TTR stabilizers resulted in the discovery of tetrabromobisphenol A, which displayed a very high selectivity (*Paper IV*). This study also included a comparison with the drug Vyndaqel™ which currently is in clinically use, and showed how the dosage could be altered to acquire a better level of saturation and possibly also a better clinical effect. Taken together we present new molecules with the ability to stabilize TTR, and these can serve as scaffolds for the design of new drugs. We present a method to measure the efficacy of a TTR-stabilizing drugs in a complex matrix and as well as a way to adjust the dosage of existing drugs. We also show that the selectivity of a drug is affected by the relative proportion of ΔH and ΔS, and this is of interest for drug design in general.

## Keywords
TTR, transthyretin, transthyretin-related amyloidosis (ATTR), TTR-stabilizing drugs, selectivity

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