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**Crystal Structure of Human Transthyretin in Complex with Iodo-Diflunisal, a Potent Amyloid Inhibitor.** Gales, L,<sup>a</sup> Macedo-Ribeiro, S,<sup>b</sup> Arsequell, G,<sup>c</sup> Valencia, G,<sup>c</sup> Saraiva, MJ,<sup>a</sup> Damas, AM.<sup>a</sup> <sup>a</sup> Instituto de Biologia Molecular e Celular & Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal. <sup>b</sup> Centro de Neurociências de Coimbra, Coimbra, Portugal. <sup>c</sup> Instituto de Investigaciones Químicas y Ambientales (CSIC) de Barcelona, Barcelona, Espana. E-mail: lgales@ibmc.up.pt

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Transthyretin (TTR) is a plasma protein, with an extensive  $\beta$ -sheet conformation, implicated in the transport of thyroxine and vitamin A. The misfolding of this protein, due to a point mutation or change in the environment, and subsequent aggregation into amyloid fibrils is related to Familial Amyloidotic Polineuropathy diseases. TTR is a homotetramer, with  $\beta$  sandwich monomers composed of two four-stranded  $\beta$  sheets. The four monomers assemble, around the central channel of the protein, where two thyroxine molecules can accommodate simultaneously. Several small molecules, that bind in the hormone binding cavity, are being tested as inhibitors TTR amyloid fibril formation, since they stabilize the protein native tetrameric fold. One of the most potent inhibitors was proved to be diflunisal [1]. This encouraged us to look for diflunisal structure-related compounds with even higher TTR affinity and determine their protein-drug three-dimensional structure. Here we report the 1.7 Å crystal structure of TTR in complex with one of the most promising ligands: iodo-diflunisal. Iodo-diflunisal binds very deep in the hormone binding channel. The fluorine-substituted phenyl ring is positioned deeply in the interior of the binding cavity. The two fluorine atoms are involved in extensive hydrophobic interactions with Ala 108, Leu 110, Ser 117, Thr 118 and Thr 119 of two adjacent TTR monomers that may help to stabilize the native tetrameric structure of the protein. The iodine-substituted phenyl ring is positioned at the entry of the hormone binding channel. The iodine atom establishes hydrophobic interactions with the side chains of Thr 106, Ala 108, Thr 119 and Val 121 and the carboxylate substituent forms a weak hydrogen interaction with Ne of Lys 15 of one of the monomers (distance of 3.5 Å). Furthermore, the ligand binding induces the rotation of the side chains of Ser 117 and Thr 119 leading to formation of new intersubunit hydrogen bonds. The results clearly show why this compound stabilizes the native structure of TTR.

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**Crystal structure of the quorum sensing protein TraM and its interaction with the transcriptional regulator TraR.** Alessandro Vannini, Cinzia Volpari and Stefania Di Marco, IRBM, Italy. E-mail: stefania\_dimarco@merck.com

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Quorum sensing is a term that reflects the ability of bacteria to control the expression of specific operons in a cell density-dependent manner and is based on the production, release and "sensing" of small signal molecules called autoinducers, which accumulate in the environment as a function of cell density. Obeying to quorum sensing is the soil bacterium *A. tumefaciens*, responsible for substantial loss of perennial crops worldwide [1]. Pathogenesis involves transfer of the Tumor-inducing (Ti) plasmid from the bacterium to the host cell nucleus and the subsequent transcription of opines and phytohormones, which cause large tumors to form on stems. The conjugal transfer of the Ti plasmid is strictly controlled by a LuxRI-type quorum sensing circuit composed of the activator TraR (homologous to LuxR), a *cis*-acting DNA inverted repeat called *trabox*, and the autoinducer, N-(3-oxo-octanoyl)-L-homoserine lactone. Recently, the crystal structure of TraR bound to its autoinducer and to DNA has been reported [2]. Additional regulatory proteins modulate the activity of LuxR-type proteins, such as defective LuxR homologues that form inactive heterodimers and other regulators that form inhibitory complexes [3]. Among these, the Ti plasmid encoded protein TraM from *A. tumefaciens*, modulates TraR-dependent transcriptional activation by direct protein-protein interaction. TraM acts as an anti-activator of TraR by binding to its C-terminal domain and therefore prevents TraR from binding DNA [3]. This inhibition is required for the normal operation of the quorum-sensing pathway and, as a consequence, null mutations in TraM result in constitutive conjugation even at low population density. TraM acts either to prevent TraR from initiating transcription of the *tra* regulon under non-inducing condition, or to shut down it efficiently when the opine signal is no longer available during infection. Thus, TraM plays a key role in determining the threshold level of the bacterial population required for initiating the Ti plasmid conjugal transfer [3]. We have determined the crystal structure of the recombinant TraM protein. The structure reveals two molecules per asymmetric unit, arranged as a dimer. In order to understand the molecular basis of TraR-TraM interaction, we have reconstituted and characterized, *in vitro*, the complex. Dimeric TraM binds tightly dimeric TraR with an equimolar ratio, forming a stable oligomeric complex of ~150 KDa. A model of the TraR/TraM complex is proposed.

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