agents to prevent bacterial infections. In addition, carbohydrate arrays can be used as a screen for such agents, as demonstrated in initial work by Disney and Seeberger in which they examine the relative inhibitory ability of three mannose-derived compounds.

The work shown herein by Disney and Seeberger is uncomplicated in its presentation. By using just five monosaccharides, standard array techniques, and fluorescently labeled bacteria, they elegantly demonstrate the potential of sugar microarrays for fast assessment of bacterial contamination. This simple study opens the way for the molecular dissection of very complex carbohydrate-bacterial interactions and for the use of such information to create both rapid diagnostic techniques and new therapeutic agents.

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# Polychlorinated Biphenyls Inhibit Amyloid Fibril Formation

**In this issue of *Chemistry & Biology*, Purkey et al. [1] compare the binding of PCBs and hydroxylated PCBs (polychlorinated biphenyls) with the human serum protein transthyretin. Hydroxylated PCBs appear to bind with higher selectivity to transthyretin relative to other serum proteins and in so doing inhibit amyloid fibril formation.**

The report by Purkey et al. [1], in this issue marks a comeuppance for PCBs—specifically, hydroxylated PCBs. PCBs were first used commercially in the U.S. in 1929 and soon became practically indispensible in fluids applications where thermal resistance and low conductivity are essential, e.g., heat transfer, hydraulics, and capacitors. By the 1960s there was mounting evidence for PCB toxicity. As the extraordinary environmental persistence of PCBs became apparent, U.S. production was finally banned in 1977. Arguably, PCBs have become the molecular poster child for environmental accumulation. Since PCBs will remain in the environment for many decades, significant efforts have been made to identify the most harmful PCBs and their mechanisms of toxicity.

PCBs were manufactured and used as mixtures of several dozen of the 209 possible congeners. Unfortunately, these mixtures of PCBs exert pleiotropic physiological effects that are difficult to tease apart. The toxicology of PCBs is further complicated by their metabolism into hydroxylated PCBs with a vague resemblance to estrogens and thyroxin (Figure 1). A number of PCBs and hydroxylated PCBs have been previously shown to bind to transthyretin in vitro, but there has been little direct information about the binding of these compounds to transthyretin in serum where various proteins compete for lipophilic ligands [2–4].

Transthyretin is a homotetrameric protein that serves two functions in humans. The transthyretin tetramer transports up to two molecules of thyroxin in a constricted tunnel formed at the tetramer interface. A second function of the transthyretin tetramer is the association with retinol binding protein (RBP), which is believed to protect RBP and its retinol ligand from glomerular filtration in the kidneys. Unfortunately, the transthyretin tetramer is poised to malfunction. The dissociation of monomeric subunits from the transthyretin tetramer is the rate-determining step toward misfolding and misassembly into amyloidogenic plaques.

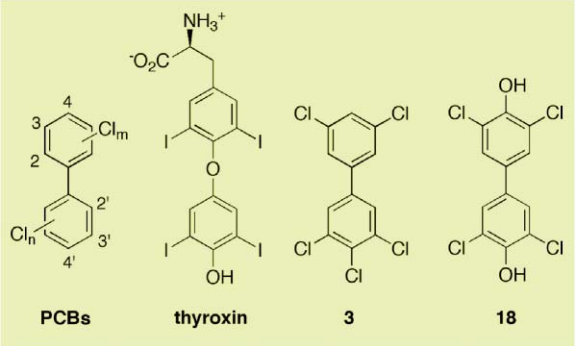


Figure 1. Structures of Ligands for the Transthyretin Tetramer

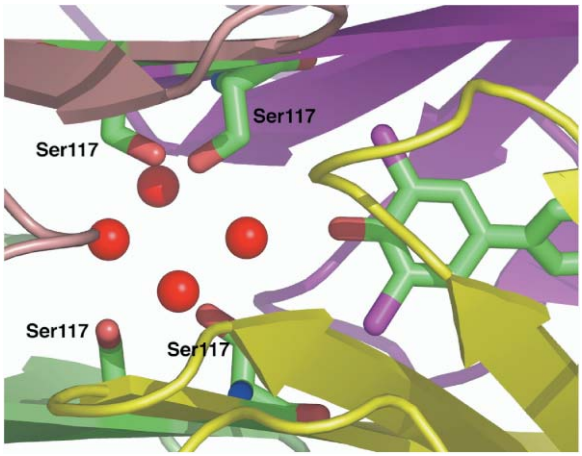


Figure 2. A Depiction of How the Four Ser117 Side Chains of the Transthyretin Tetramer and the Hydroxyl of PCB **18** Converge on Bound Water Molecules

The transthyretin subunits are colored in green, yellow, pink, and purple. Bound water molecules are shown as red spheres.

These plaques are believed to cause senile systemic amyloidosis, familial amyloid cardiomyopathy, and the familial amyloid polyneuropathies—collectively referred to as ATTR. Senile systemic amyloidosis is associated with wild-type transthyretin and slow onset, whereas the familial amyloidoses are associated with genetic mutations that can lead to symptoms in patients aged in their 30s or 40s. In the worst cases, familiar cardiomyopathy presents as congestive heart failure. Neuropathic symptoms include diminished sensation and or pain, particularly in the extremities. Unfortunately, the only treatment available for ATTR is to transplant the liver, which is the source of transthyretin.

Surprisingly, the thyroxin binding function of the transthyretin tetramer does not appear to be necessary for either thyroxin transport or RBP binding, so efforts have been directed toward the identification of ligands that bind to and stabilize the tetrameric native state of transthyretin, thereby inhibiting monomer dissociation [5, 6]. Thus, PCBs that bind to the transthyretin tetramer and inhibit disassembly could serve as leads for the development of inhibitors of transthyretin amyloidogenesis.

Purkey and coworkers carried out two signficant assays on 22 PCBs—8 PCBs reported to bind tightly ($K_D$ < 50 nM) to transthryetin and 14 4-hydroxy-PCBs. An antibody capture assay was used to assess binding stoichiometry in human serum where proteins like albumin and thyroxin binding protein can compete for binding. PCBs that bound to the transthyretin tetramer with stoichiometries closest to the theoretical upper limit of 2:1 PCB/tetramer were deemed most selective for transthyretin over other proteins. All 14 of the hydroxylated PCBs exhibited binding stoichiometries between 0.7 and 1.9 ligand/transthyretin, only 2 of the 8 PCBs (3,3′,5,5′-tetrachlorobiphenyl and 3,3′,4,5,5′-pentachlorobiphenyl **3**) appeared to bind appreciably to transthyretin. While the binding stoichiometries that were measured are actually lower limits, the data suggest that the hydroxylated PCBs bind selectively to the transthyretin tetramer, whereas un-hydroxylated PCBs do not.

The hydroxylated PCBs were assayed for the ability to inhibit acid-induced amyloid fibril formation. All of the hydroxylated PCBs inhibited fibril formation when added to 3.6 μM transthyretin at equimolar concentration; three of them appeared even more effective than the analgesic flufenamic acid, a promising inhibitor of transthyretin amyloid fibril formation [7]. The results are consistent with the idea that ligand binding can lead to kinetic stabilization of the tetramer.

In order to triangulate structural data with the serum binding data and the fibril inhibition data, cocrystal structures were obtained with four of the hydroxylated PCBs bound pairwise in the transthyretin tetramers. The best fibril inhibitor, 4,4′-dihydroxy-3,3′5,5′-tetrachlorobiphenyl **18** adopts a nonplanar conformation that places each of the four chlorine substituents in one of the four halogen binding pockets of the transthyretin tetramer.

The most deeply bound of the two phenolic groups engages two of the four subunits in a hydrogen bonding network (Figure 2). The network connects the phenolic hydroxyl, with bound waters and the Ser117 sidechains on opposing transthyretin subunits. In contrast, in the crystal structure of the transthyretin•thyroxin$_2$ complex 1IE4.pdb, the phenolic hydroxyl group of thyroxin is not observed to engage in such a network. Instead, the thyroxin hydroxyl forms a hydrogen bond to water which is in turn hydrogen bonded to Thr119.

The results reported by Purkey et al. in this issue challenge the traditional notion that PCBs have no beneficial physiological effects. While the simple hydroxylated PCBs studied in this work are not yet ready for the clinic, they do provide useful information for the design of improved inhibitors of transthyretin amyloid fibril formation.

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