

## De Novo Enzymes Development for PFAS Compounds Degradation

**Dayal Saran** (dsaran@allonnia.com) and Kent Sorenson (ksorenson@allonnia.com) (Allonnia, Boston, MA)

Galit Meshulam-Simon (galit@aetherbio.com) (Aether, Menlo Park, CA)

**Background/Objectives.** Per- and polyfluoroalkyl substances (PFAS) are a family of persistent, toxic compounds commonly found in consumer products like cookware, food packaging, and in firefighting foams. PFAS manufacturing and processing facilities, airports, and military installations that use firefighting foams are some of the main sources of PFAS found in air, soil, and water, including sources of drinking water. Studies indicate that PFAS compounds can cause reproductive and developmental, liver and kidney, and other immunological diseases.

The EPA is leading the national effort to reduce PFAS risks to the public through implementation of its PFAS Action Plan and through active engagement and partnership with, states, industry groups and local communities. Current solutions for PFAS remediation are focused on sequestration techniques via adsorption media and then transfer to a landfill or incineration facility. However, such solutions are insufficient in that they are transporting PFAS compounds from one place to another, rather than remediating, PFAS contaminations.

Currently there are various technologies under development that are focused on complete destruction (defluorination) of PFAS compounds. Some of those technologies such as plasma based destruction, sonolysis, chemical oxidation, reductive destruction are shown to be promising but tend to be energy intensive, expensive or impractical at desired scale.

**Approach/Activities.** Biological remediation could represent a simple, environmentally safe and cost-effective technology to treat PFAS-contaminated sources. Although, to date, there are no examples of successful PFAS bioremediation, there are a several reports published from academic labs showing some degradation and defluorination of PFAS from bacterial and fungal microbes isolated from the contaminated sites.

Fluoroacetate dehalogenase (FacD), a promising biodegradation enzyme candidate, has been reported as the only non-metallic enzyme to catalyze the cleavage of the strong C–F bond using 2-fluoropropionic acid as a substrate. Here, we designed multiple libraries of FacD mutants using machine learning and in silico enzyme modeling methods that can potentially degrade pentafluoropropionic acid, PFOA and many other per-fluorinated compounds.

**Results/Lessons Learned.** In this presentation we will describe the approach we used to screen a total of around 10000 single, double and multiple mutants of FacD in a HTP format to identify most optimal enzymes capable of degrading perfluorinated compounds. We saw more than 50% degradation of PFOA, PFOS and other perfluorinated compounds under in vitro conditions. This work will be followed by testing the best hits for optimal activity under environmental conditions.