Modeling the Ability of Cyclodextrins to Bind Short-Chain PFAS

Grant Proposal

Lydia Metcalf

Massachusetts Academy of Math and Science at Worcester Polytechnic Institute

Worcester, Massachusetts

Modeling the Ability of Cyclodextrins to Bind Short-Chain PFAS

Abstract

This project aims to use cyclodextrin compounds to adsorb per- and polyfluoroalkyl substances (PFAS) from water. PFAS are a growing problem in the modern environment. PFAS have a negatively charged head and a hydrophobic fluorinated tail. Current remediation strategies include adsorption with activated carbon and biochar. These work acceptably for PFAS with longer tails (long-chain or legacy PFAS), but legacy PFAS are being phased out for PFAS with shorter tails (short-chain PFAS). Current removal solutions rely on hydrophobic interactions and do not work for short-chain PFAS, which are less hydrophobic due to the shorter length of the hydrophobic part (the tail). In this project, we used computational chemistry to assess cyclodextrins as a remediation strategy for short-chain PFAS. Cyclodextrins are hollow truncated cone-shaped compounds that have a hydrophobic interior surface and hydroxyl groups along the edge which are capable of forming hydrogen bonds with another compound. These hydrogen bonds formed with the head of PFAS can compensate for the weaker hydrophobic interactions between short-chain PFAS tails and the interior surface. When docking the natural cyclodextrins to PFAS in AutoDock Vina, it was found that beta-cyclodextrins and gamma-cyclodextrins were significantly more effective than alpha-cyclodextrins at the a = 0.01 significance level. Additionally, several modified cyclodextrins were assessed with regards to their ability to bind PFAS. The results of this project can be used to create a more effective way to adsorb PFAS and a solution for the millions of people whose water is contaminated with these toxic chemicals.

Keywords: PFAS (per- and polyfluorinated alkyl substances), cyclodextrins, Sugammadex, water remediation, water contamination, computational chemistry, docking

Modeling the Ability of Alpha- and Gamma- Cyclodextrins to Bind Short-Chain PFAS

Per- and polyfluoroalkyl substances, or PFAS, are some of the most pressing contaminants affecting natural water sources in the present day. PFAS can cause harm to the environment and may have impacts on human health as well. PFAS is a common contaminate in well-water and is known to have endocrine-disrupting and cancer-causing effects (Fenton et al., 2021). These substances are widespread and do not break down significantly once released into the environment, which leads to their eventual bioaccumulation and persistence in the environment (Dickman & Aga, 2022). PFAS consists of a polar head group and a long, fluorinated tail. This structure causes them to be both hydrophobic and lipophobic. Legacy or long-chain PFAS, such as PFOS and PFOA, have longer hydrophobic tails, as opposed to short-chain or emerging PFAS with shorter chain lengths. Several promising solutions for their cleanup have been proposed utilizing the chemical structure of the PFAS molecule, such as physical adsorption by various substances (Dickman & Aga, 2022). However, due to discoveries of the toxicity of longer-chain legacy PFAS, the chemical industry has shifted away from the use of legacy PFAS to that of modern short-chain PFAS. These were initially thought to be less toxic than legacy PFAS; however, it has been suggested that short-chain PFAS may have some of the same problems as their longer-chain counterparts (Li et al., 2020). In addition, short-chain PFAS are more hydrophilic than legacy PFAS due to the shorter length of the hydrophobic tail part, which lowers the efficacy of hydrophobic interactions with adsorbents commonly utilized for legacy PFAS cleanup (Li et al., 2020). Therefore, it is clear that short-chain PFAS should be given a high level of priority when deciding areas for further research surrounding PFAS remediation.

Currently, one of the most promising remediation methods for PFAS of either chain length is the usage of various adsorbents. Granular activated carbon (GAC) and super-fine powder activated carbon (SPAC) are widely used adsorbents for long-chain PFAS. Still, their adsorption capability is often affected by natural organic materials existing in the environment. Also, they cannot effectively adsorb short-chain PFAS. In one study, SPAC exhibited an adsorption rate four orders of magnitude lower for PFBS, a short-chain PFAS, than for PFOS, a long-chain PFAS, and it was noted that long-chain PFAS may outcompete shorter-chain PFAS for adsorption sites, further decreasing the adsorption ability for short-chain PFAS (Murray et al., 2019). In addition, this material can be more costly than

some methods. The indicated adsorption mechanisms were primarily hydrophobic and electrostatic interactions (Dickman & Aga, 2022).

Due to these problems, biochars and modified biochars have been proposed as a more effective solution to the problem of PFAS contamination. Biochar is a carbon-based adsorbent derived from pyrolyzed natural biomass and has been shown to have better results than the leading solutions of GAC in some studies (Militao et al., 2023). As such, it is a cost-effective method for the removal of longer-chain PFAS.

One study using biochar-impregnated alginate beads found that beads with a certain biochar content had up to 99% removal efficiency for PFOS, a type of legacy PFAS, at a starting PFOS concentration of 100 μg L^{-1}. For PFBS, one of the newer short-chain PFAS, using similar beads in which the biochar had been modified with diammonium phosphate prior to its addition yielded a removal efficiency of 39.3%. This experimental efficiency was comparable to commercial GAC. Therefore, the biochars used in this study proved more effective towards legacy PFAS but do show some promise toward future applications for short-chain PFAS (Militao et al., 2023).

Another study investigated the effects of doping reed straw-derived biochar with iron and copper to modify the adsorption capacity for PFBA and PFPeA, two short-chain PFAS. Both types of doped biochar had higher adsorption capacities for both PFAS, showing some promise to this method (Liu et al., 2024). However, better short-chain solutions are still necessary.

One new approach to this problem is the use of cyclodextrins. Cyclodextrins consist of six (alpha-cyclodextrin), seven (beta-cyclodextrin), or eight (gamma-cyclodextrin) glucose units arranged in a ring structure. This results in a unique truncated cone shape, where the interior of the cone is hydrophobic and can encapsulate smaller molecules and the exterior of the cone is hydrophilic, with the hydroxyl groups along the smaller edge able to hydrogen bond with the negatively charged oxygen atoms in the PFAS head. (Esteso & Romero, 2024). Cyclodextrins, specifically the beta-cyclodextrin variety, have been used to adsorb AmPr-FHxSA with moderate efficiency, suggesting it could be a novel solution to the PFAS issue.

One study tested beta-cyclodextrins with an attached positively-charged functional group to bind various short-chain PFAS. It was found that PFAS thread through the ring cavity of the cyclodextrin. The tail will have

hydrophobic interactions with the hydrophobic cavity, and the head will have hydrogen bonding interactions with the hydroxyl groups on the smaller edge of the cyclodextrin. Although hydrophobic interactions are weaker in short-chain PFAS than in legacy PFAS, the electrostatic interactions from the attached functional group were comparable to adding another hydrogen bond. Therefore, cyclodextrins are a promising candidate for short-chain PFAS (Weiss-Errico & O'Shea, 2019).

This project proposes to study the interactions between various types of short-chain PFAS and alpha- and gamma-cyclodextrins. To the best of the author's knowledge, these types of cyclodextrin have not been previously studied with regard to PFAS. Therefore, molecular modeling techniques will be employed to examine whether these could be viable candidates for the adsorption of PFAS.

Section II: Specific Aims

This proposal's objective is to find a viable solution to short-chain PFAS contamination in water sources by modeling the intermolecular attractions between cyclodextrins and PFAS with PyMol and Autodock Vina and further analyzing the resulting interactions to determine the feasibility of each cyclodextrin-PFAS interaction.

Our long-term goal is to determine the viability of alpha-cyclodextrin, beta-cyclodextrin, and gamma-cyclodextrin and modifications thereof for adsorption of various short-chain PFAS compounds. The central hypothesis of this proposal is that alpha-cyclodextrins will have a different docking score for a sample of PFAS compounds than the average docking score for the same sample of PFAS compounds with beta-cyclodextrins. The rationale is that cyclodextrins are capable of both hydrophobic interactions by way of the interior cavity and hydrogen bonds along the large and small edges. The specific type of cyclodextrins was utilized because alpha-cyclodextrins have only six glucose subunits, rather than the seven glucose subunits that comprise beta-cyclodextrins, which would allow the comparatively small PFAS to bind into the cavity without being interfered with by larger molecules such as natural organic matter (NOM). The work we propose here will further our understanding of possibilities for the cleanup of PFAS in the environment, provide a theoretical basis which further research can expand upon by performing a physical experiment to confirm our results, and potentially have significant positive impacts for the many people whose well water is contaminated with PFAS in the U.S.

Modeling the Ability of Cyclodextrins to Bind Short-Chain PFAS

Specific Aim 1: The first specific aim of this project is to model the binding of perfluorobutanoic acid (PFBA), a type of short-chain PFAS, to beta-cyclodextrins, which have seven glucose subunits in their ring structure and some literature evidence for adsorption of PFAS.

Specific Aim 2: The second specific aim of this project is to model the binding of PFBA, the compound tested in the first specific aim, to alpha- and gamma- cyclodextrins, which have six and eight glucose subunits, respectively, and remain untested for PFAS.

Specific Aim 3: The third specific aim of this project is to create a modified cyclodextrin with positive residues and test its effectiveness in docking PFBA against the cyclodextrin identified in the second specific aim as being the most effective (alpha, beta, or gamma).

The expected outcome of this work is that the project will identify the most likely basic cyclodextrin candidate for the adsorption and removal of PFAS. The candidate selected will then serve as the starting point for further research, including the modification of the cyclodextrin to better adsorb PFAS. The results from this work will provide a foundation for the long-term goal of creating a cyclodextrin with attached cationic residues and testing its ability to dock anionic short-chain PFAS.

Section III: Project Goals and Methodology

Relevance/Significance: Across the U.S., an estimated 71 to 95 million people (20% of the U.S. population) are currently drinking water from PFAS-contaminated groundwater sources, according to an October 2024 USGS study in *Science* (Tokranov et al., 2024). PFAS have been shown to be linked with an increased risk of certain cancers and to have endocrine disrupting effects on humans (Dickman & Aga, 2022). Therefore, PFAS is a major problem in the field of water contamination. Existing solutions for PFAS are costly or have drawbacks, one major drawback being that many existing removal methods only consider long-chain or legacy PFAS. These PFAS have been mainly phased out in favor of the newer short-chain PFAS. The chemical structure of short-chain PFAS has a shorter hydrophobic tail, which makes them less able to be adsorbed by conventional methods.

Innovation: This project aims to tackle the problem of short-chain PFAS cleanup, which is ignored in much of the existing literature. The technique of molecular docking will be borrowed from the field of pharmacology and drug discovery to simulate the interaction of short-chain PFAS molecules with cyclodextrins, a potential adsorbent.

Modeling the Ability of Cyclodextrins to Bind Short-Chain PFAS

Molecular docking usually involves the simulated binding of a target drug molecule (the ligand) into a large macromolecule protein (the receptor). However, it can also be used with non-biological molecules. To the author's knowledge, cyclodextrins and PFAS have not previously been modeled in this manner, providing a basis for the novelty of this project's approach.

Methodology: The first step of the methodology is to download the appropriate files of the 3D structure of each cyclodextrin receptor and each PFAS ligand. The files will come from various sources, including PubChem and the Japan Protein Data Bank. If the files are not already in the appropriate format for preparation for docking, which is .pdb extension, they will be converted to .pdb format using the free software OpenBabel (O'Boyle et al., 2011). The cyclodextrins are determined to be the three base types of cyclodextrin. These are alpha- with six glucose subunits comprising its ring structure, beta- with seven glucose subunits, and gamma- with eight glucose subunits. The PFAS of interest were found using the EPA's CompTox database, specifically their list of prioritized PFAS. For the first trial, PFBA (perfluorobutanoic acid) was chosen as a model PFAS due to its simple structure and relative prominence. Next, the .pdb files of the ligand and receptor will be converted to .pdbqt format using AutoDockTools (the reason for this step is to include required information about partial charges and AutoDock-specific atom types, such as whether an atom is capable of hydrogen bonding or not). AutoDockTools also generates a grid box (the search space where the ligand is to bind) in this step. Finally, the docking simulation is run with AutoDock Vina, which outputs a docking score for the nine most favorable poses (Eberhardt et al., 2021; Trott & Olson, 2010).

Specific Aim #1:

The first specific aim of this project is to model the binding of perfluorobutanoic acid (PFBA), a type of short-chain PFAS, to beta-cyclodextrins, which have seven glucose subunits in their ring structure, with some evidence suggesting potential interactions with PFAS (Abaie et al., 2024). The objective is to find a control for the rest of the experiment by docking PFAS to beta-cyclodextrin, which has previously been studied with respect to PFAS (though short-chain PFAS are underexplored in previous work). Our approach (methodology) starts by finding the openly available files for the 3D structure of beta-cyclodextrin and PFBA. The .pdb file for beta-cyclodextrin was acquired through the Japan Protein Data Bank and checked for any glaring errors by opening it in PyMOL. For

PFBA, the 3D structure was downloaded in .xml format through PubChem and converted to .pdb format using OpenBabel software. Next, AutoDockTools was used to convert both files to .pdbqt and generate the grid box, or search space. Finally, AutoDock Vina was run, and the docking scores and poses were recorded. Our rationale for this approach is based on studies in the field of drug discovery which use a similar method of docking, substituting cyclodextrins for proteins.

Justification and Feasibility. Molecular docking and other simulations are very helpful to determine the feasibility of a cyclodextrin candidate without a physical experiment. similar approach has been previously studied with the complex of Sugammadex, a modified gamma-cyclodextrin, and rocuronium (Anderson et al., 2024). This paper explored the docking of a certain drug, rocuronium, into the cavity of the cone-shaped Sugammadex, the drug used to recapture it from the body. The paper was able to identify the best-scoring poses and identify new poses that might exist in solution that had not yet been shown experimentally (Anderson et al., 2024).

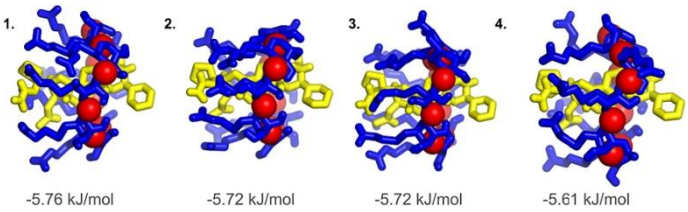

Figure 1: The four generated best-scoring poses for the complexation of rocuronium into Sugammadex. Here, the cyclodextrin is depicted in blue and red and rocuronium in yellow. This is an example of docking with cyclodextrin as receptor instead of a protein. Figure from (Anderson et al., 2024).

Summary of Preliminary Data. When the docking simulation was run, beta-cyclodextrin showed promising results in binding PFAS. Although PFOA and PFOS, the two long-chain PFAS, showed the best binding (as expected), promising scores were also obtained for short-chain PFAS, as shown in the graph.

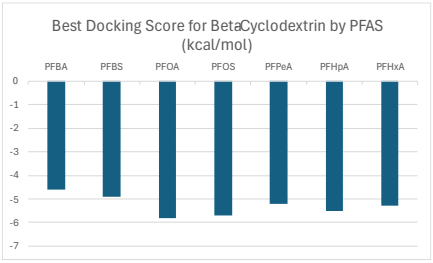

Figure 2: The highest recorded docking score for each PFAS tested when docked to beta-cyclodextrin.

Expected Outcomes. The overall outcome of this aim is to generate docking scores for top docking poses for the host-guest complex of beta-cyclodextrin and PFBA. This knowledge will be used for a positive control for the other two types of cyclodextrin, as there is some literature evidence for its use in this application.

Potential Pitfalls and Alternative Strategies. We expect some unreliability in the results, as molecular docking programs do not output actual binding energy values but a “docking score” based on a necessarily

imperfect scoring function. Therefore, an alternative strategy is to confirm and contextualize the results with molecular modeling software.

Specific Aim #2:

The second specific aim of this project is to model the binding of PFBA, the compound tested in the first specific aim, to alpha- and gamma- cyclodextrins, which have six and eight glucose subunits, respectively, and remain untested for PFAS. The objective is to find which cyclodextrin is most likely to bind PFAS by docking PFBA to alpha- and gamma- cyclodextrins. Our approach (methodology) starts by finding the openly available files for the 3D structure of alpha- and beta-cyclodextrins through the Japan Protein Data Bank. Next, AutoDockTools was used to convert both files to .pdbqt and generate the grid box, or search space. (The previous files from the first aim were used for PFBA). Finally, AutoDock Vina was run, and the docking scores and poses were recorded. Our rationale for this approach is based on studies in the field of drug discovery, which use a similar method of docking, substituting cyclodextrins for proteins.

Justification and Feasibility. See the justification for the first specific aim.

Summary of Preliminary Data. For nearly all PFAS tested, including two common long-chain PFAS and five common short-chain PFAS, beta-cyclodextrin had the highest docking score, closely followed by gamma-cyclodextrin (Figure 3). Alpha-cyclodextrin was not as effective. The average maximum docking score for each cyclodextrin is shown in the graph. In a two-sample t-test, beta-cyclodextrin was shown to have a significantly different average than alpha-cyclodextrin but was not significantly different than gamma-cyclodextrin.

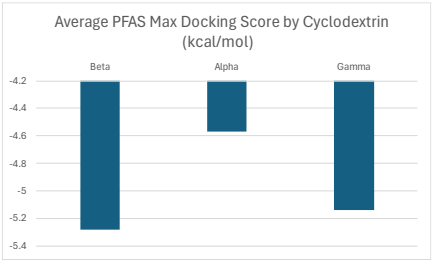

Figure 3: The average of the highest recorded docking scores for each PFAS, by cyclodextrin type.

Expected Outcomes. The overall outcome of this aim is to generate docking scores for top docking poses for the host-guest complex of alpha- and gamma-cyclodextrins and PFBA. This knowledge will be used to find the best cyclodextrin candidate for hosting PFBA.

Potential Pitfalls and Alternative Strategies. We expect some unreliability in the results, as molecular docking programs do not output actual binding energy values but a “docking score” based on a necessarily

imperfect scoring function. Therefore, an alternative strategy is to confirm and contextualize the results with molecular modeling software.

Specific Aim #3

The third specific aim of this project is to create a modified cyclodextrin with positive residues and test its effectiveness in docking PFBA against the cyclodextrin identified in the second specific aim as being the most effective (alpha, beta, or gamma). The objective is to create an ideal cyclodextrin compound for removal of short-chain PFAS. The methodology is to base our structure off Sugammadex, a gamma-cyclodextrin with the terminal carboxyl groups replaced with anionic residues and chemical moieties that increase binding to the cationic drug rocuronium (Anderson et al., 2024). It is expected that the negatively charged PFAS head group and the positively charged cyclodextrin moieties will have interactions in much the same way.

Justification and Feasibility. Sugammadex is a cyclodextrin-based drug used to remove cationic drugs from the body and works effectively due to the electrostatic interactions between the anionic groups and the positively charged parts of the drug (Nag et al., 2013). The molecule created in this aim is to have cationic residues where Sugammadex has anionic, to bind and remove anionic PFAS in a similar way.

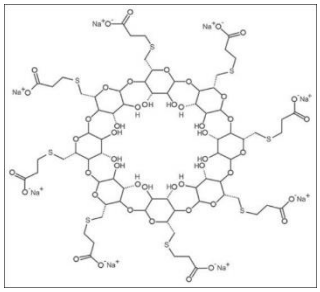

Figure 4: The chemical structure of Sugammadex (Nag et al., 2013)

Summary of Preliminary Data. N/A

Expected Outcomes. To create an effective remediation strategy for short-chain PFAS based on preexisting solutions and techniques borrowed from adjacent fields.

Potential Pitfalls and Alternative Strategies. The solution proposed may be expensive to manufacture and it may be difficult to create. To remedy these errors, outside assistance from experts in the field will be required.

Section III: Resources/Equipment

N/A – Using open-source software and/or working under an educational license.

Section V: Ethical Considerations

This research should not have ethical considerations during the experiment phase, but real-world application of the results should make sure access to the remediation strategy is equitable to households of different wealth statuses.

Section VI: Timeline

The preliminary data will be acquired by the December STEM fair on December 9th, 2024. Data collection will continue through the month of December and the month of January, finishing by the end of January 2025. The project presentation is expected to take place at the February STEM fair on February 20th, 2025.

Section VIII: References

Abaie, E., Kumar, M., Kumar, N., Sun, Y., Guelfo, J., Shen, Y., & Reible, D. (2024). Application of β-Cyclodextrin Adsorbents in the Removal of Mixed Per- and Polyfluoroalkyl Substances. *Toxics (Basel)*, *12*(4), 264. https://doi.org/10.3390/toxics12040264

Anderson, A., García-Fandiño, R., Piñeiro, Á., & O'Connor, M. S. (2024). Unraveling the molecular dynamics of sugammadex-rocuronium complexation: A blueprint for cyclodextrin drug design. *Carbohydrate Polymers*, *334*, 122018. https://doi.org/https://doi.org/10.1016/j.carbpol.2024.122018

Dickman, R. A., & Aga, D. S. (2022). A review of recent studies on toxicity, sequestration, and degradation of per- and polyfluoroalkyl substances (PFAS). *Journal of Hazardous Materials*, *436*, 129120. https://doi.org/https://doi.org/10.1016/j.jhazmat.2022.129120

Eberhardt, J., Santos-Martins, D., Tillack, A. F., & Forli, S. (2021). AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. *Journal of Chemical Information and Modeling*, *61*(8), 3891-3898. https://doi.org/10.1021/acs.jcim.1c00203

Esteso, M. A., & Romero, C. M. (2024). Cyclodextrins: Properties and Applications. *Int J Mol Sci*, *25*(8). https://doi.org/10.3390/ijms25084547

Fenton, S. E., Ducatman, A., Boobis, A., DeWitt, J. C., Lau, C., Ng, C., Smith, J. S., & Roberts, S. M. (2021). Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research. *Environ Toxicol Chem*, *40*(3), 606-630. https://doi.org/10.1002/etc.4890

Li, F., Duan, J., Tian, S., Ji, H., Zhu, Y., Wei, Z., & Zhao, D. (2020). Short-chain per- and polyfluoroalkyl substances in aquatic systems: Occurrence, impacts and treatment. *Chemical Engineering Journal*, *380*, 122506. https://doi.org/https://doi.org/10.1016/j.cej.2019.122506

Liu, N., Li, Y., Zhang, M., Che, N., Song, X., Liu, Y., & Li, C. (2024). Efficient adsorption of short-chain perfluoroalkyl substances by pristine and Fe/Cu-loaded reed straw biochars. *Science of The Total Environment*, *946*, 174223. https://doi.org/https://doi.org/10.1016/j.scitotenv.2024.174223

Militao, I. M., Roddick, F., Fan, L., Zepeda, L. C., Parthasarathy, R., & Bergamasco, R. (2023). PFAS removal from water by adsorption with alginate-encapsulated plant albumin and rice straw-derived biochar. *Journal of Water Process Engineering*, *53*, 103616. https://doi.org/https://doi.org/10.1016/j.jwpe.2023.103616

Murray, C. C., Vatankhah, H., McDonough, C. A., Nickerson, A., Hedtke, T. T., Cath, T. Y., Higgins, C. P., & Bellona, C. L. (2019). Removal of per- and polyfluoroalkyl substances using super-fine powder activated carbon and ceramic membrane filtration. *Journal of Hazardous Materials*, *366*, 160-168. https://doi.org/https://doi.org/10.1016/j.jhazmat.2018.11.050

Nag, K., Singh, D. R., Shetti, A. N., Kumar, H., Sivashanmugam, T., & Parthasarathy, S. (2013). Sugammadex: A revolutionary drug in neuromuscular pharmacology. *Anesth Essays Res*, *7*(3), 302-306. https://doi.org/10.4103/0259-1162.123211

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. *Journal of Cheminformatics*, *3*(1), 33. https://doi.org/10.1186/1758-2946-3-33

Tokranov, A. K., Ransom, K. M., Bexfield, L. M., Lindsey, B. D., Watson, E., Dupuy, D. I., Stackelberg, P. E., Fram, M. S., Voss, S. A., Kingsbury, J. A., Jurgens, B. C., Smalling, K. L., & Bradley, P. M. (2024). Predictions of groundwater PFAS occurrence at drinking water supply depths in the United States. *Science*, *386*(6723), 748-755. https://doi.org/doi:10.1126/science.ado6638

Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, *31*(2), 455-461. https://doi.org/https://doi.org/10.1002/jcc.21334

Weiss-Errico, M. J., & O'Shea, K. E. (2019). Enhanced host–guest complexation of short chain perfluoroalkyl substances with positively charged β-cyclodextrin derivatives. *Journal of inclusion phenomena and macrocyclic chemistry*, *95*(1-2), 111-117. https://doi.org/10.1007/s10847-019-00930-w