Reticular Synthesis of Metal-Peptide Frameworks for Enhanced Enantioselective Separation of Chiral Drugs

In pharmaceutics, over half of all drugs exhibit chirality or “handedness.” Of those, the majority are marketed as a racemic 50:50 mix of their non-superimposable mirrored stereoisomers, also known as enantiomers[1]. Typically, one (eutomer) enantiomer utilizes a different metabolic pathway to make it pharmacologically more active than its (distomer) counterpart[1]. In some cases, one enantiomer configuration is toxic to the human body. Separation of chiral molecules is performed either during stereoselective synthesis or through challenging separation techniques, thus, the process of delivering an enantiopure drug to the market is an essential, yet timely and expensive feat. A simpler mechanism for enantioseparation during the early phases of a drug’s discovery could reduce lengthy purification steps and save valuable time and resources to make newer drugs more viable in the market[2].

I started conducting research in the Materials Discovery Lab at OSU in the summer of 2021 under the guidance of Dr. Kyriakos Stylianou who provided me the freedom to study a project that I am passionate about. My fixation with organic chemistry and chemical engineering inspired me to seek research in improving how we deliver vital organic chemicals. After discussion with Dr. Stylianou and review of the literature on utilizing porous materials for chiral separation, today, I am fortunate to enjoy the complete autonomy to study the design, synthesis, and pore tunability of metal-organic frameworks (MOFs) for enantioselective chiral drug separation.

MOFs are an organic-inorganic subclass of coordination polymers arrayed through a network of positively charged metal ions bound to organic ligands or “linkers”. These metal ions form nodes that coordinate with the organic ligands in a repeating sequence to create a hollow framework with large internal surface areas and pore aperture. Engineering MOFs with customizable ligands allows for tunability of these pore channels to selectively separate molecules. Reticular synthesis for instance is performed by spatially separating characteristic organic-inorganic building units with pillar ligands of increasing size. This process creates a family of isoreticular MOFs whose pore apertures can be tuned to separate drugs based on size selectivity of the molecules entering through the pore channels.

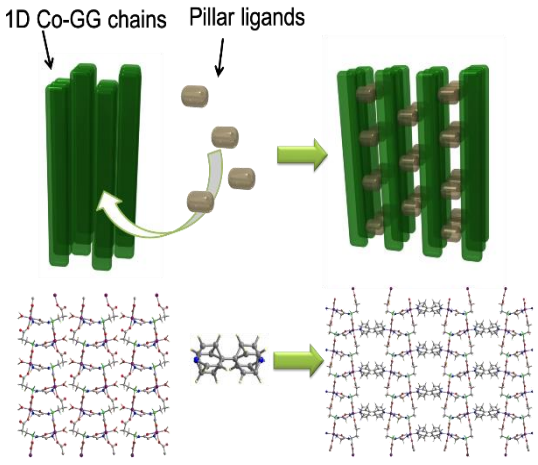

Figure 1. The use of pillar ligands in the synthesis induces the separation of the 1D chains affording porous and chiral peptide-based MOFs.

I chose a metal-dipeptide framework as the basis for my MOFs. Peptides offer structural versatility and plentiful coordinate sites for metal binding[3]. Further tunability in pore characteristics comes from the chirality of the coordinate dipeptide framework and its amino acid constituents. These isoreticular homochiral MOFs show evidence in dictating efficiency of adsorption through enantiomeric excess depending on the size of the molecules[3]. Specifically, cobalt glycyl-L(S)-glutamate (Co-L-GG) forms 1-dimensional rigid ladder-shaped structures (**Figure 1**), ideal for

reticular synthesis; however, it lacks accessible pores[3]. Incorporation of 4,4'-bipyridine (bipy) ligand readily replaces coordinated water molecules of Co-L-GG to create the pre-existing Co-L-GGbipy MOF. I experimented with different bipyridyl analogue ligands with different permutations of solvents and concentrations while holding the Co-L-GG framework constant. Through systematic effort, I synthesized Co-L-GGbipy as well as five novel Co-L-GGbipy(R) MOFs with varying sized ligands. Due to a comparable structural topology among isoreticular MOFs, I performed Powder X-Ray Diffraction (PXRD) to verify for similar crystallinity (**Figure 2**). The largest intensity diffraction peaks centered around 5 degrees are the predominant lattice planes in all my MOFs, confirming their structures. From my previous experience with solid-state synthesis and X-ray characterization of perovskites, I knew diffraction plane scattering at a smaller 2θ incidence angle occurred in materials with larger unit cells. I reasoned that by increasing the size of the pillar ligands and, therefore, the unit cells, I could expect a diffraction at a smaller 2θ angle. PXRD verified my hypothesis as the intensity peak of the MOF with the longest ligand, bipyBP, had the smallest diffraction angle at 3.62 degrees and the MOF using bipy with the smallest length had the largest diffraction angle at 6.06 degrees (**Figure 2**).

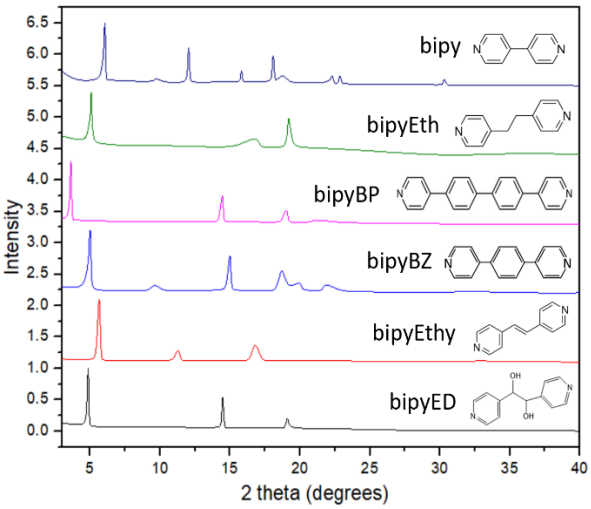

Figure 2. Powder X-Ray Diffraction data for all synthesized Co-L-GGbipy(R) MOFs. Analogue of ligand extensions used for each are shown.

A challenge in gauging MOF performance is finding and operating under conditions that these MOFs will not collapse under. Water by-products must be carefully considered for reactions involving Co-L-GGbipy(R) to prevent hydrolysis of peptide bonds. I performed Thermogravimetric Analysis to record their thermal stability up to 300°C, and all synthesized MOFs showed solvent stability through PXRD when I immersed them overnight in liquid methanol, dimethylformamide and tetrahydrofuran.

For my chiral drug, I chose racemic Penicillamine (Pen) to analyze the pore size impact for enantioselective adsorption. D-Pen is an intended antiarthritic and is used to treat Wilson's Disease whereas L-Pen is toxic and inhibits the production of vitamin B6. I measured 1,000 ppm solutions of both enantiomers and ran each through a chiral gas chromatography (GC) to obtain an enantiopure reference. Since Pen would be soluble and my MOFs are stable under a methanol environment, I chose methanol as the solvent for GC. Glycyl-L(S)-glutamate exhibits chiral recognition sites in the pore channels of the framework and so I predict that these sites will preferentially bind to the L(S)-enantiomer of racemic drugs. Single-Crystal XRD on Co-L-GGbipy and Co-L-GGbipyED showed that separation will be possible within accessible pore apertures of 115Å^3 and 179Å^3, respectively. Once I optimize my conditions, I will mix equal amounts of the D and L enantiomers into two vials with methanol and immerse both MOFs into each racemic DL-Pen solution at room temperature for 48 hours. Once filtered, I will compare the solution chiral GC data with the reference to determine the preferential enantioselective adsorption and enantiomeric excess (**Figure 3)**.

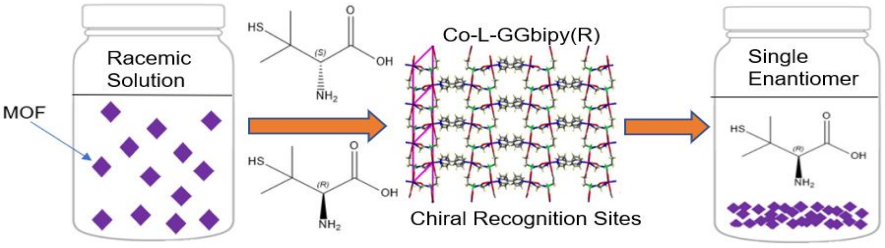

Figure 3. Schematic showing separation of DL-Penicillamine (DL-Pen). Pore diffusion occurs in homochiral Co-L-GGbipy(R) and leaves a single stereoisomer to be collected for chiral gas chromatography.

My MOFs did not come without design failures. Several of the pillar ligands were incompatible without specific synthesis conditions. I noticed from synthesis of Co-L-GGbipyBP, that the use of benzyl alcohol solvent accrued collectible powders in the bottom of the vials that were set aside at room temperature for not being sufficiently soluble. Motivated by this, I reasoned I could instead elucidate the reaction pathway that my MOF undergoes. I resynthesized Co-L-GGbipy using benzyl alcohol as the solvent at room temperature along with trials heated at 85°C in the oven for 2, 4, 6, and 8 hours. PXRD revealed crystal evidence of the Co-L-GG framework in the previous failed syntheses and the timed trials showed a steady recession of the Co-L-GG framework into the recognized MOF structure as length of heating increased.

These results were galvanizing. By understanding the reaction mechanisms, I can improve my synthesis conditions to increase the yield and purity of these and newer isoreticular MOFs. With more robust and diverse ligands to utilize, we can find more applications regarding the separation capabilities of different drugs. For instance, my Co-L-GGbipyBZ and Co-L-GGbipyBP MOFs feature pillar ligands with additional aromatic phenyl structures within the pores which, during diffusion, may interact and bind with other aromatic chiral molecules or drugs such as Ibuprofen; further research could use my MOFs to investigate separation efficiency between aromatic and aliphatic chiral drugs. With my experience in synthesis, future projects will involve testing the impact of pore size using my isoreticular homochiral MOFs for enantioselective catalysis in promoting the synthesis of enantiopure drugs; thus, further reducing drug purification steps and making them more accessible to the public.

Research and chemical synthesis were intimidating at first, but I have since gained confidence and fascination in the development of purposeful materials to help find better alternatives for drug delivery. My goal is to finalize my project, draft my manuscript and publish my work on MOFs for drug separation to the American Chemistry Society (ACS) Chemistry of Materials journal. I plan on participating in the ACS Conference in the fall so I can communicate my project with the scientific community and seek collaborations with other materials researchers on new areas utilizing functional isoreticular MOFs.

References

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[3] Stylianou, K. C.; Gómez, L.; Imaz, I.; Verdugo-Escamilla, C.; Ribas, X.; Maspoch, D. Engineering Homochiral Metal-Organic Frameworks by Spatially Separating 1D Chiral Metal-Peptide Ladders: Tuning the Pore Size for Enantioselective Adsorption. *Chemistry - A European Journal* **2015**, *21* (28), 9964–9969.