Investigating molecular blood-brain-barrier permeability

Lab Final Project
PHYSCI 2 at Harvard College
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Introduction

The blood-brain-barrier (BBB) is a selective semipermeable membrane that separates circulating blood from the brain and extracellular fluid in the central nervous system, and is responsible for regulation of CNS homeostasis and protection of the brain microenvironment from toxins, pathogens, and other threats (Daneman & Prat, 2015; Obermeier et al., 2013). At every level of the neurovascular tree, endothelial cells of the BBB line the capillaries and are surrounded by pericytes, astrocytes, microglia, extracellular matrix components, and peripheral immune cells, which together form the neurovascular unit (Iadecola, 2017). The physical properties of the neurovascular unit – most importantly, the continuous tight junctions which connect the non-fenestrated endothelial cells – restrict the paracelllular and transcellular movememnt of molecules from the bloodstream into the brain (Figure 1) (Abbott et al., 2010; Langen et al., 2019). Other key features of the BBB which govern passage into the brain include specific molecular transporters which facilitate both the influx of nutrients and the efflux of toxins; catalytic enzymes such as intracellular monoamine oxidase and cytochrome P450 which degrade potential toxins; and extravascular structures such as the endothelial glycocalyx and astrocytic endfeet which modulate BBB function (Abbott et al., 2006; Daneman & Prat, 2015; Langen et al., 2019).

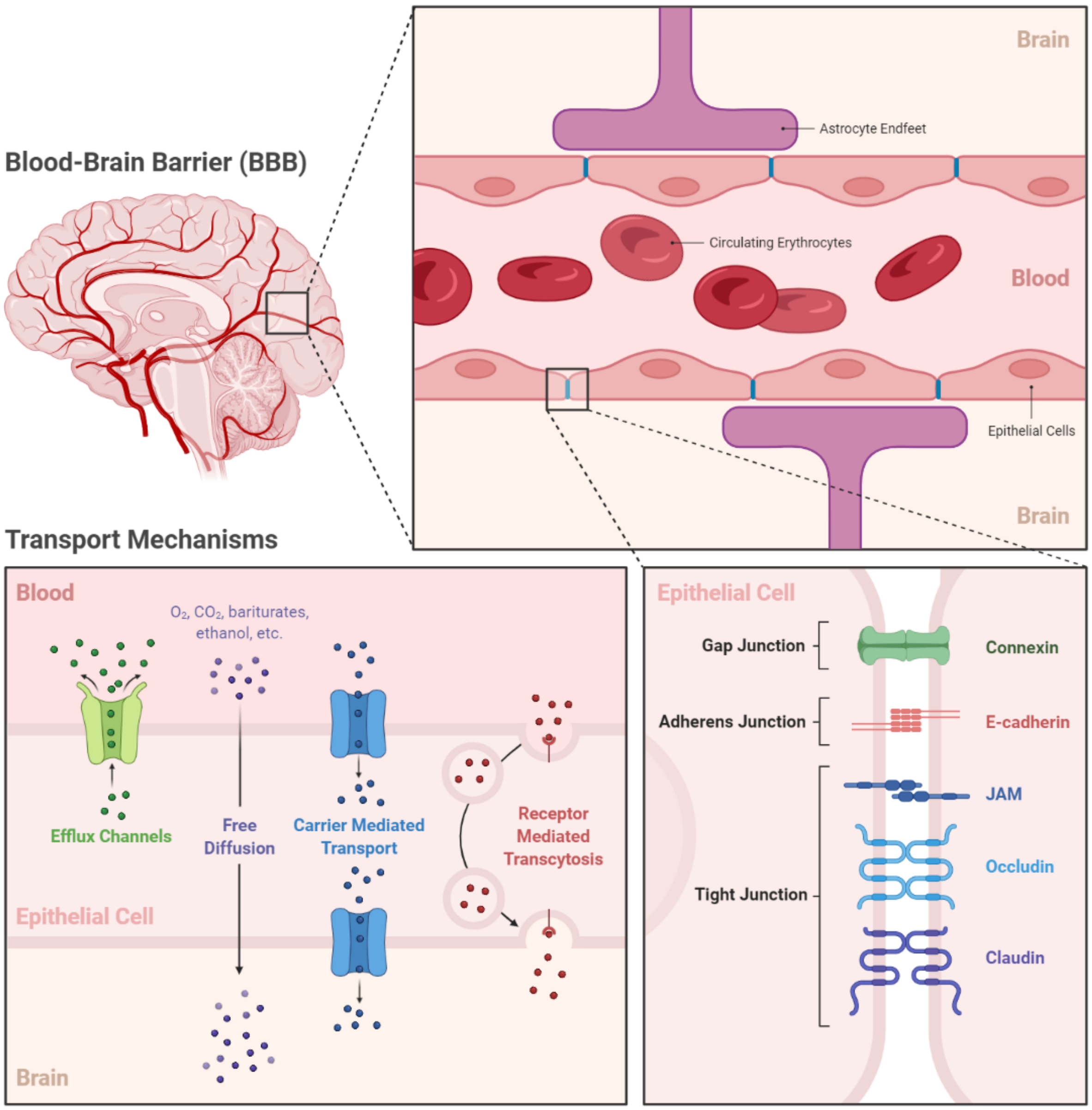


Figure 1: Transport regulatory functions of the blood-brain barrier. Figure from ayushnoori/graph-bbb on GitHub and created using Biorender.com.

In addition to regulating the passage of nutrients into the brain and protecting the brain microenvironment from invaders or pathogens, the BBB also prevents more than 98% of small molecule drugs and macromolecular therapeutics from reaching the brain. This poses a major obstacle in the treatment of neurological disorders which often remain refractory to treatment due to the inability of drugs to cross the BBB (Wu et al., 2023). The BBB is therefore a major target of drug delivery research, and understanding the permeability of molecules to the BBB is of great interest to facilitate the development of new neurotherapeutics. Our own previous work has attempted to predict BBB permeability (for example, see ayushnoori/graph-bbb on GitHub), but this has been limited by lack of molecular diversity and interpretability. In this project, we aim to investigate the relationship between molecular structure and BBB permeability using a structurally diverse dataset of 1058 compounds with known BBB permeabilities.

Research Question

Here, we investigate the relationships between BBB permeability and molecular properties such as molecular weight, number of hydrogen bond donors and acceptors, number of rotatable bonds, and number of rings.

Methodology and Results

We use techniques learned during the Fall 2023 semester in PHYSCI 2 Lab at Harvard College to investigate the relationships between molecular properties of interest and BBB permeability. We leverage a new diverse molecular database of BBB permeability with chemical descriptors, recently published in *Nature Scientific Data* in 2021:

Meng, F., Xi, Y., Huang, J. & Ayers, P. W. A curated diverse molecular database of blood-brain barrier permeability with chemical descriptors. *Sci Data* **8**, 289 (2021).

Please also see theochem/B3DB and Issue #174 of mims-harvard/TDC on GitHub. After retrieving and pre-processing our data, we calculate several molecular features of 1058 compounds as well as numerical logBB values for each compound, where logBB is the logarithm of the brain-plasma concentration ratio:

$$\log BB = \log \frac{C_{brain}}{C_{blood}}$$

Then, we apply curve fitting methods learned in Lab 4 and Lab 5 to fit various biologically-informed models to the data. We visualize our data and results and, based on visual inspection, generate hypotheses for relationships between molecular features and logBB. Finally, we use χ^2_{red}-testing to select from multiple competing models of the data and compare the goodness-of-fit of each.

Our experimental design, methodology, and results are described in detail below. First, we load relevant libraries.

```
In [ ]: # standard imports
import os
import numpy as np
import pandas as pd
import matplotlib.pyplot as plt

# molecular manipulation
from rdkit import Chem, DataStructs
from rdkit.Chem import AllChem
from rdkit.Chem import Descriptors

# clustering
from sklearn.cluster import AgglomerativeClustering
```

```
# path manipulation
from pathlib import Path

# import project configuration
import project_config
from lab_functions import *
```

Next, we read the Meng *et al*. dataset into a `pandas` data frame (Meng *et al.*, 2021). This dataset, known as the Blood-Brain Barrier Database (B3DB) was compiled from more than 50 published resoures and contains BBB permeability data for 1058 compounds. Each row in the date frame corresponds to a unique compound and each column corresponds to a chemical descriptor or logBB value.

```
In [ ]: # read in data
        # define this as the directory of the cloned project
        project_config.PROJECT_DIR = Path("/Users/jaredhn/codespace/ps2-lab")
        data = pd.read_csv(project_config.PROJECT_DIR / 'B3DB_regression.tsv', sep='\t
        data.head()
```

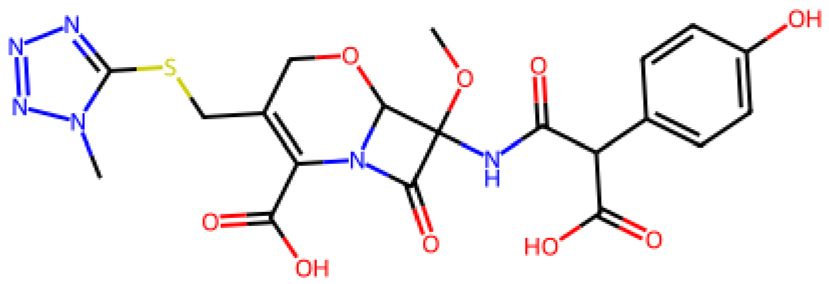
Out[ ]:

	NO.	compound_name	IUPAC_name	
0	1	moxalactam	7-[[2-carboxy-2-(4-hydroxyphenyl)acetyl]amino]...	CN1C(=NN=N1)SCC2=C(N3C(C(C3=O)(N
1	2	schembl614298	(2s,3s,4s,5r)-6-[[(4r,4ar,7s,7ar,12bs)-7-hydro...	CN1CC[C@]23[C@@H]4[C@H]1CC5=C2C(
2	3	morphine-6-glucuronide	(2s,3s,4s,5r)-6-[[(4r,4ar,7s,7ar,12bs)-9-hydro...	CN1CC[C@]23[C@@H]4[C@H]1CC5=C2C(
3	4	2-[4-(5-bromo-3-methylpyridin-2-yl)butylamino]...	2-[4-(5-bromo-3-methylpyridin-2-yl)butylamino]...	CC1=NC=C(C=C1)CC2CNC(NC2=O)NCCCC(
4	5	NaN	NaN	c1(c2c3n(c4c(C(N(C)C3)=O)c(Cl)

We use the RDKit library to calculate various molecular features from the SMILES structures. Simplied molecular-input line-entry system, or SMILES, is a specification for describing the structure of chemical species using short ASCII strings. RDKit is an open-source cheminformatics toolkit that supports many common tasks in cheminformatics, including molecular property calculation.

```
In [ ]: # get molecule from SMILES
        data['mol'] = data['SMILES'].apply(Chem.MolFromSmiles)

        # visualize first molecule
        print("Compound Name: ", data['compound_name'].iloc[0])
        data['mol'].iloc[0]
```

```
Compound Name:  moxalactam
```

Out[ ]: 

Next, we use the RDKit library to calculate molecular features from SMILES strings. We calculate the following molecular features for each compound:

- Average molecular weight, which reflects the distribution of isotopes of the molecule's atoms.
- Exact molecular weight, which gives the molecular weight of the most common isotopes of each atom in the molecule.
- Average molecular weight excluding hydrogens.
- Number of hydrogen bond acceptors.
- Number of hydrogen bond donors.
- Number of heavy atoms.
- Number of aromatic rings.
- Number of total rings.
- Number of rotatable bonds.

In [ ]:
```
# to calculate all 210 descriptors
# descriptors = data['mol'].apply(lambda x: pd.Series(Chem.Descriptors.CalcMol

# get average molecular weight (MolWt)
data['mol_wt'] = data['mol'].apply(lambda x: Descriptors.MolWt(x))

# get exact molecular weight (ExactMolWt)
data['exact_mol_wt'] = data['mol'].apply(lambda x: Descriptors.ExactMolWt(x))

# get average molecular weight ignoring hydrogens (HeavyAtomMolWt)
data['heavy_atom_mol_wt'] = data['mol'].apply(lambda x: Descriptors.HeavyAtomM

# get average number of hydrogen bond acceptors (NumHAcceptors)
data['num_h_acceptors'] = data['mol'].apply(lambda x: Descriptors.NumHAccepto

# get average number of hydrogen bond donors (NumHDonors)
data['num_h_donors'] = data['mol'].apply(lambda x: Descriptors.NumHDonors(x))

# get number of heavy atoms (HeavyAtomCount)
data['heavy_atom_count'] = data['mol'].apply(lambda x: Descriptors.HeavyAtomCo

# get number of aromatic rings (NumAromaticRings)
data['num_aromatic_rings'] = data['mol'].apply(lambda x: Descriptors.NumAromat

# get number of rings (NumRings)
data['num_rings'] = data['mol'].apply(lambda x: Descriptors.RingCount(x))
```

```
# get number of rotatable bonds (NumRotatableBonds)
data['num_rotatable_bonds'] = data['mol'].apply(lambda x: Descriptors.NumRotat

# show first 5 rows of data
data.head()
```

Out[ ]:

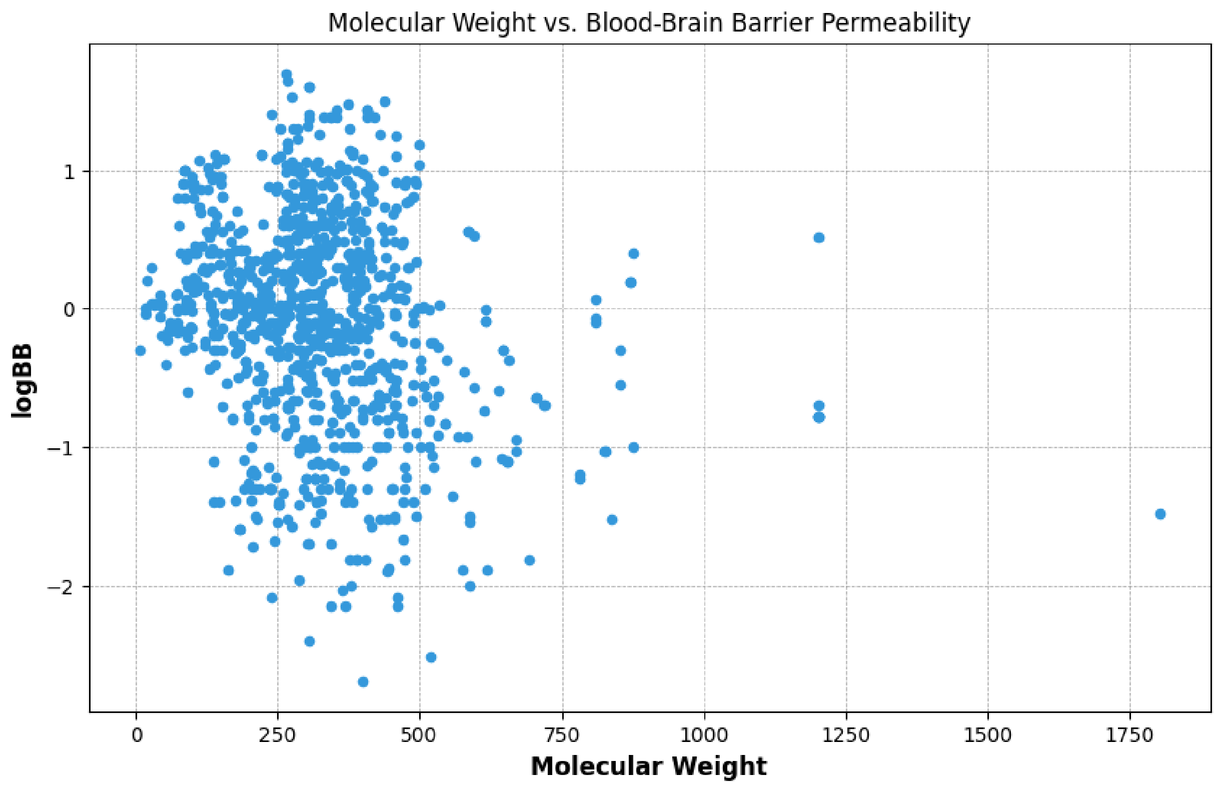
	NO.	compound_name	IUPAC_name	
0	1	moxalactam	7-[[2-carboxy-2-(4-hydroxyphenyl)acetyl]amino]...	CN1C(=NN=N1)SCC2=C(N3C(C(C3=O)(N
1	2	schembl614298	(2s,3s,4s,5r)-6-[[(4r,4ar,7s,7ar,12bs)-7-hydro...	CN1CC[C@]23[C@@H]4[C@H]1CC5=C2C(
2	3	morphine-6-glucuronide	(2s,3s,4s,5r)-6-[[(4r,4ar,7s,7ar,12bs)-9-hydro...	CN1CC[C@]23[C@@H]4[C@H]1CC5=C2C(
3	4	2-[4-(5-bromo-3-methylpyridin-2-yl)butylamino]...	2-[4-(5-bromo-3-methylpyridin-2-yl)butylamino]...	CC1=NC=C(C=C1)CC2CNC(NC2=O)NCCCC(
4	5	NaN	NaN	c1(c2c3n(c4c(C(N(C)C3)=O)c(Cl)

We visualize the relationship between molecular weight and logBB. Note that the logBB is the logarithm of the ratio of the concentration of the compound in the brain to the concentration in the plasma.

$$\log BB = \log \frac{C_{brain}}{C_{blood}}$$

If the logBB is positive, then a compound is BBB-permeable and vice versa.

In [ ]:
```
# create function to plot data
def make_scatter(data, x, y, xlabel, ylabel, title = None, color = '#3498db'):

    # create title
    if title is None:
        title = xlabel + ' vs. ' + ylabel

    # set figure dimensions
    plt.figure(figsize=(10, 6))

    # plot data points
    plt.scatter(data[x], data[y], s=20, color=color)

    # set title and axis labels
    plt.title(title)
    plt.xlabel(xlabel, fontweight='bold', size=12)
    plt.ylabel(ylabel, fontweight='bold', size=12)

    # add a gray dashed grid in the background
    plt.grid(axis = "both", color='gray', linestyle='--', linewidth=0.5, alpha
    plt.gca().set_axisbelow(True)
    # return plot
    return plt

# plot molecular weight vs. logBB
plt = make_scatter(data, 'mol_wt', 'logBB', 'Molecular Weight', 'logBB', title
plt.show()
```



Since we seek to perform χ^2_{red}-testing, we require uncertainties for our measurements. The most important uncertainty to account for is not in the molecular weight; rather, there is biological uncertainty in logBB, or the measured brain-plasma concentration ratio. However, the B3DB database does not provide uncertainties. Therefore, we must compute a proxy uncertainty measurement.

Since we are not provided with uncertainties on logBB, we cluster molecules by molecular similarity (*e.g.*, Morgan fingerprint), motivated by the underlying biological assumption that compounds with highly similar molecular structures and properties would also have highly similar brain-plasma concentration ratios. Thus, for the purposes of uncertainty estimation, we treat molecules within the same cluster as equivalent observations, and take the standard error of logBB values within each cluster as the uncertainty on logBB for that cluster.

First, we use the RDKit library to calculate Morgan fingerprints for each compound. Morgan fingerprints, also known as extended-connectivity fingerprint ECFP4, are a type of circular fingerprint, which encode the local chemical environment of a molecule by iteratively applying a hashing function to a molecule's substructures. Here, we use a radius of 2, which means that the hashing function is applied to all substructures within 2 bonds of each atom in the molecule, and a bit length of 1024.

In [ ]:
```
# compute fingerprints
data['fingerprints'] = data['mol'].apply(lambda x: Chem.AllChem.GetMorganFinge

# convert to numpy arrays
np_fingerprints = []
for fp in data['fingerprints']:
    array = np.zeros((0, ), dtype=np.int8)
    DataStructs.ConvertToNumpyArray(fp, array)
    np_fingerprints.append(array)
```

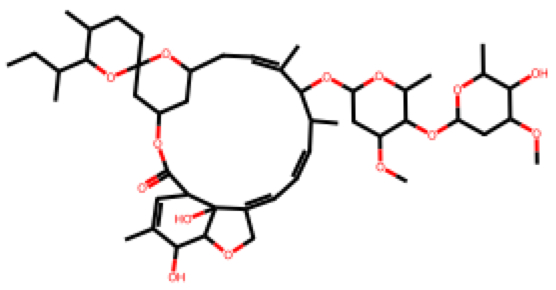
Next, we perform clustering on the Morgan fingerprints to group molecules by molecular similarity. We use agglomerative clustering, whcih recursively merges pairs of clusters based on the linkage distance between the clusters; in this case, we use the Ward linkage criterion, which minimizes the variance of the clusters being merged.

In [ ]:
```
# perform hierarchical clustering
num_clusters = 50  # You can adjust the number of clusters
clustering = AgglomerativeClustering(n_clusters = num_clusters, metric = 'eucl
cluster_labels = clustering.fit_predict(np_fingerprints)
```

We assign cluster identity to each compound and visualize molecules from the same cluster to confirm the structural similarity between the molecules.

In [ ]:
```
# assign cluster identity
data['cluster'] = cluster_labels

# within each cluster, compute SEM of logBB
cluster_summary = data.groupby('cluster')['logBB'].agg(['mean', 'sem'])

# assign mean and SEM to all molecules by cluster
data = data.merge(cluster_summary, left_on = 'cluster', right_index = True)

# we visualize molecules of cluster 40
Chem.Draw.MolToImage(data[data['cluster'] == 40]['mol'][30])
```

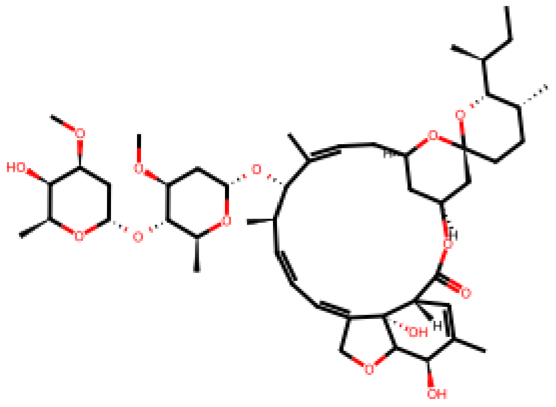
Out[ ]:



Plot a second molecule from cluster 40 to demonstrate that the clustering is reasonable.

In [ ]:
```
# plot second molecule
Chem.Draw.MolToImage(data[data['cluster'] == 40]['mol'][178])
```

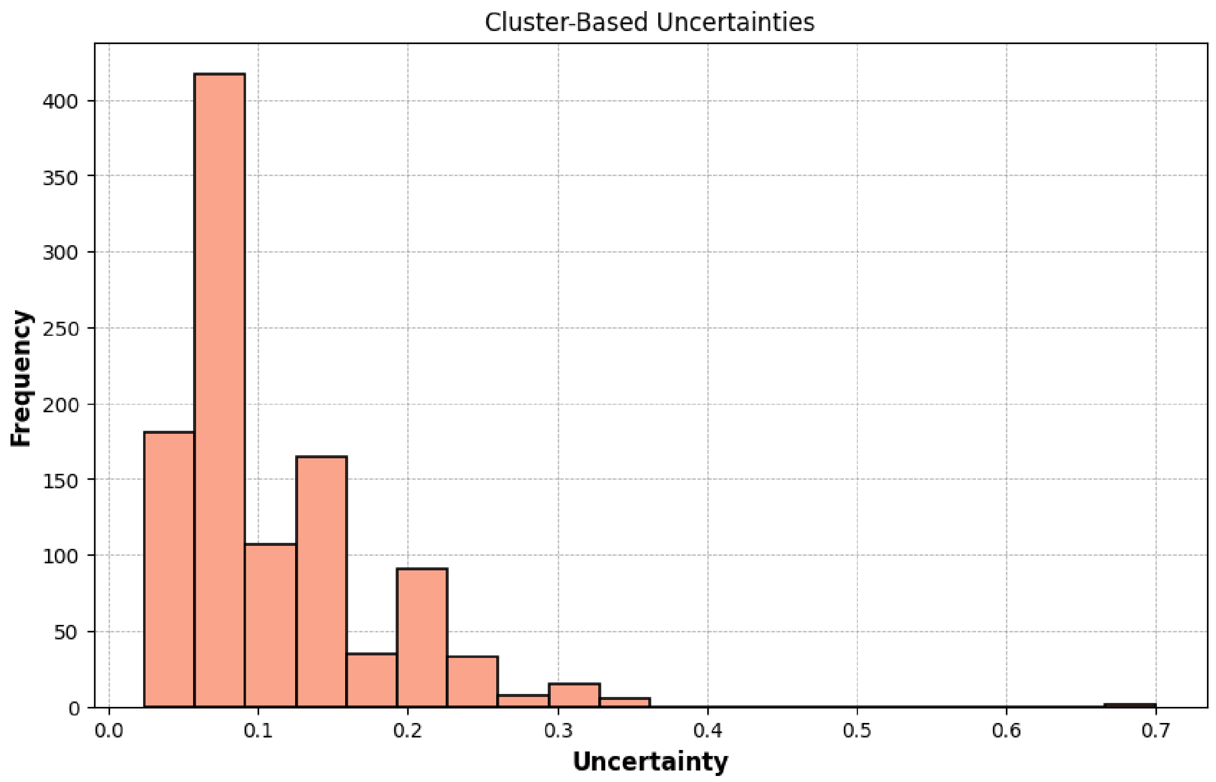
Out[ ]:



Finally, we plot a histogram of the uncertainties.

In [ ]:
```
# set figure dimensions
plt.figure(figsize=(10, 6))

# plot data points
plt.hist(data['sem'], bins = 20, color = '#F9A48B', edgecolor = 'black', linew

# set title and axis labels
plt.title('Cluster-Based Uncertainties')
plt.xlabel('Uncertainty', fontweight='bold', size=12)
plt.ylabel('Frequency', fontweight='bold', size=12)
```

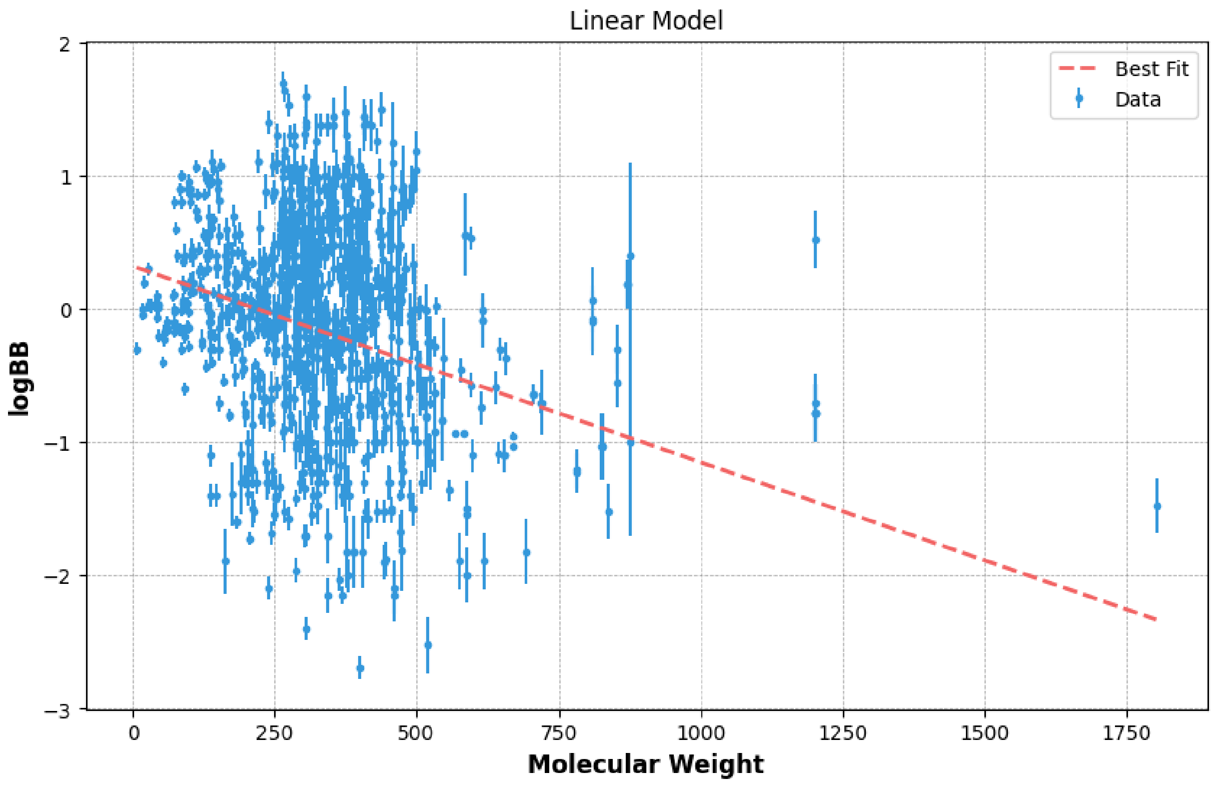
```
# add a gray dashed grid in the background
plt.grid(axis = "both", color='gray', linestyle='--', linewidth=0.5, alpha=0.7
plt.gca().set_axisbelow(True)
```

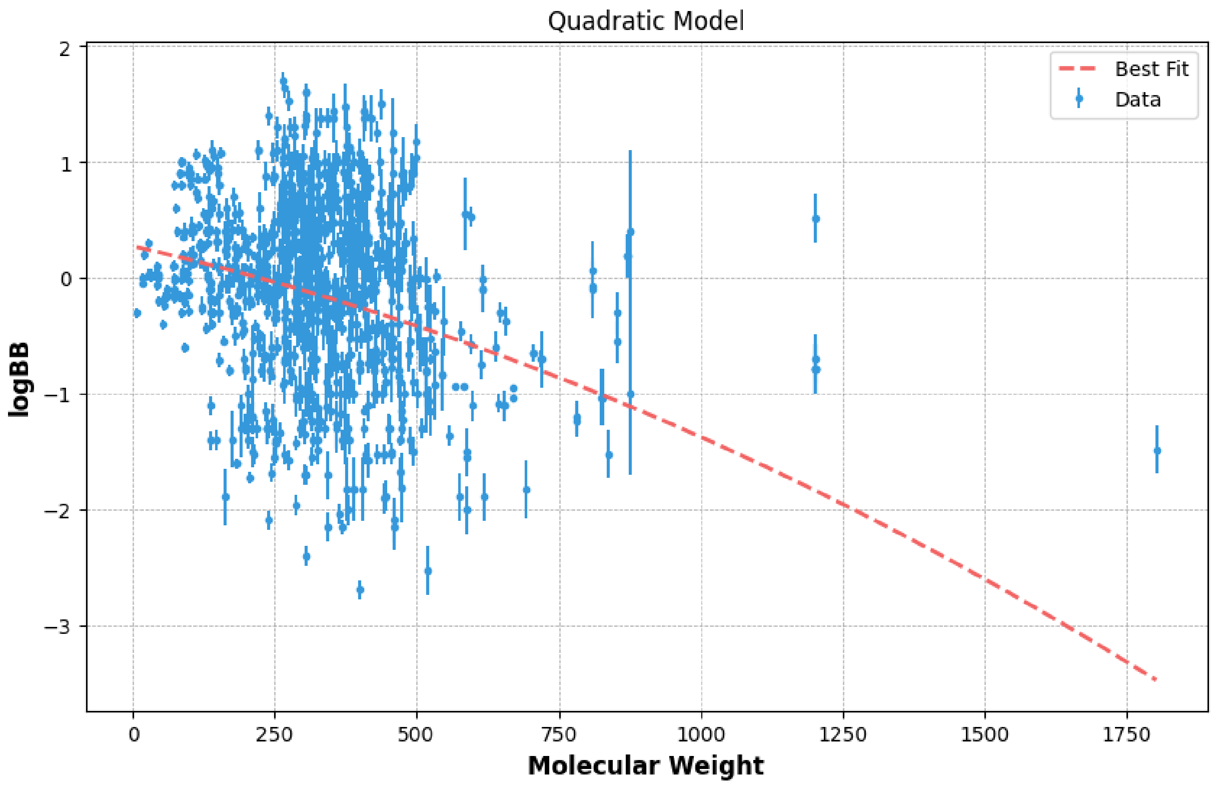


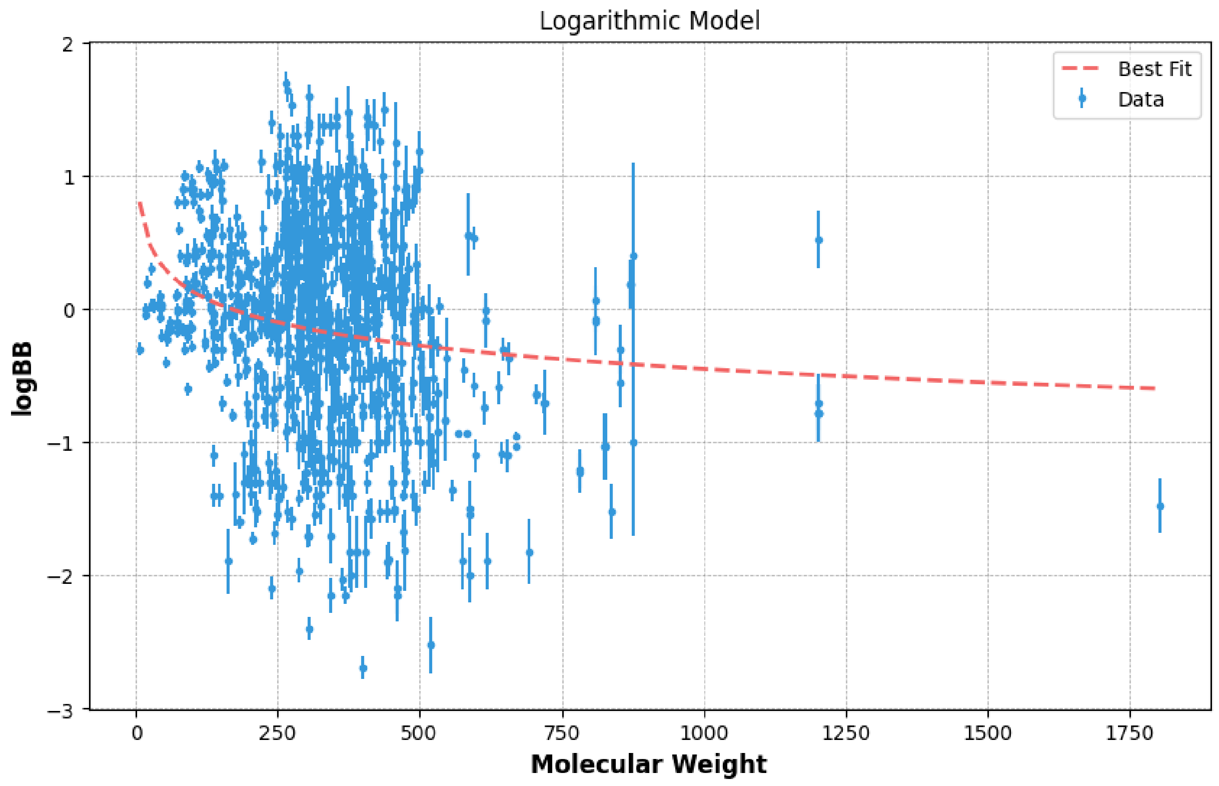
Next, we fit a linear model to the data.

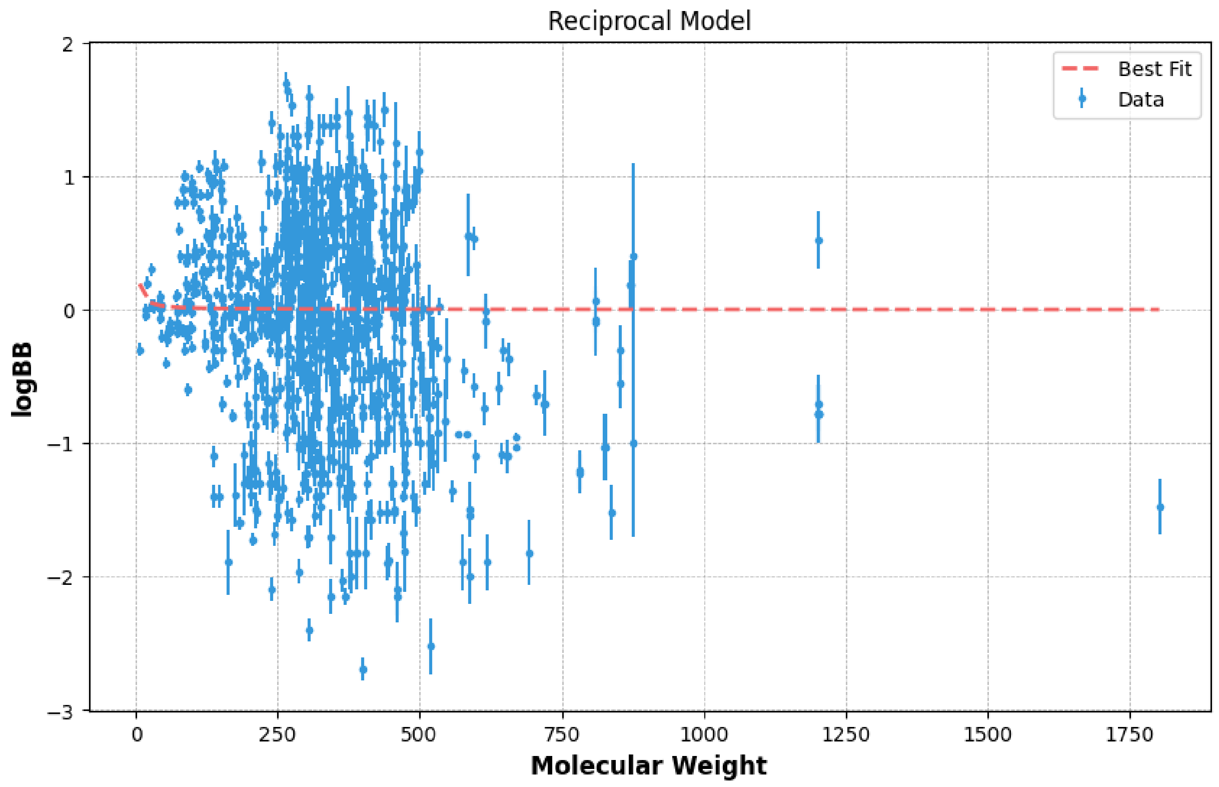
In [ ]:
```
# subset columns
x_value = data['mol_wt'].to_numpy()
y_value = data['logBB'].to_numpy()
y_error = data['sem'].to_numpy()

# define linear model
def linear_model(x, slope, y_int):
  y = x*slope + y_int
  return y

# define quadratic model
def quadratic_model(x, a, b, y_int):
  y = a * x**2 + b*x + y_int
  return y

# define logarithmic model
def logarithmic_model(x, a, y_int):
  y = a * np.log(x + 0.000001) + y_int
  return y

# define
def reciprocal_model(x, a):
  y = a / (x + 0.000001)
  return y

# fit linear and quadratic models
```

```
fitparams, fiterrs = mycurvefit(linear_model, x_value, y_value, y_error, 'Mole
fitparams, fiterrs = mycurvefit(quadratic_model, x_value, y_value, y_error, 'M
fitparams, fiterrs = mycurvefit(logarithmic_model, x_value, y_value, y_error, 
fitparams, fiterrs = mycurvefit(reciprocal_model, x_value, y_value, y_error, '
Best Fit Parameters:

         Independent Variable: Molecular Weight
         Dependent Variable: logBB
         Model: Linear Model
         P1 =  -0.00147 +/- 2e-05
         P2 =  0.32189 +/- 0.00468

 Fit Metrics:

         Degrees of freedom (N-d):  1056
         Reduced Chi Squared = 66.752

Best Fit Parameters:

         Independent Variable: Molecular Weight
         Dependent Variable: logBB
         Model: Quadratic Model
         P1 =  -0.0 +/- 0.0
         P2 =  -0.00111 +/- 4e-05
         P3 =  0.27563 +/- 0.00658

 Fit Metrics:

         Degrees of freedom (N-d):  1055
         Reduced Chi Squared = 66.721

Best Fit Parameters:

         Independent Variable: Molecular Weight
         Dependent Variable: logBB
         Model: Logarithmic Model
         P1 =  -0.25187 +/- 0.00338
         P2 =  1.29096 +/- 0.01826

 Fit Metrics:

         Degrees of freedom (N-d):  1056
         Reduced Chi Squared = 69.863

Best Fit Parameters:

         Independent Variable: Molecular Weight
         Dependent Variable: logBB
         Model: Reciprocal Model
         P1 =  1.33221 +/- 0.20458

 Fit Metrics:

         Degrees of freedom (N-d):  1057
         Reduced Chi Squared = 75.629
```


Linear Model
Best Fit
Data
logBB
Molecular Weight
2
1
0
−1
−2
−3
0
250
500
750
1000
1250
1500
1750


Quadratic Model
Best Fit
Data
logBB
Molecular Weight
2
1
0
−1
−2
−3
0
250
500
750
1000
1250
1500
1750

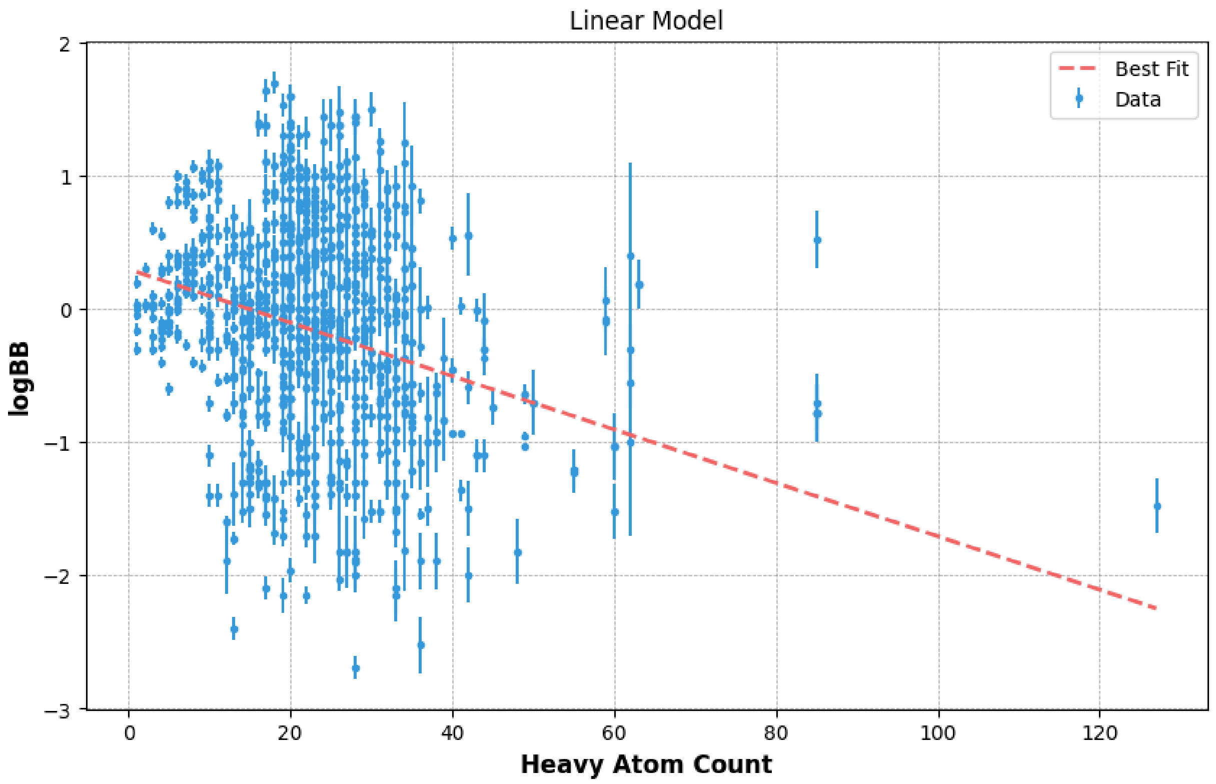


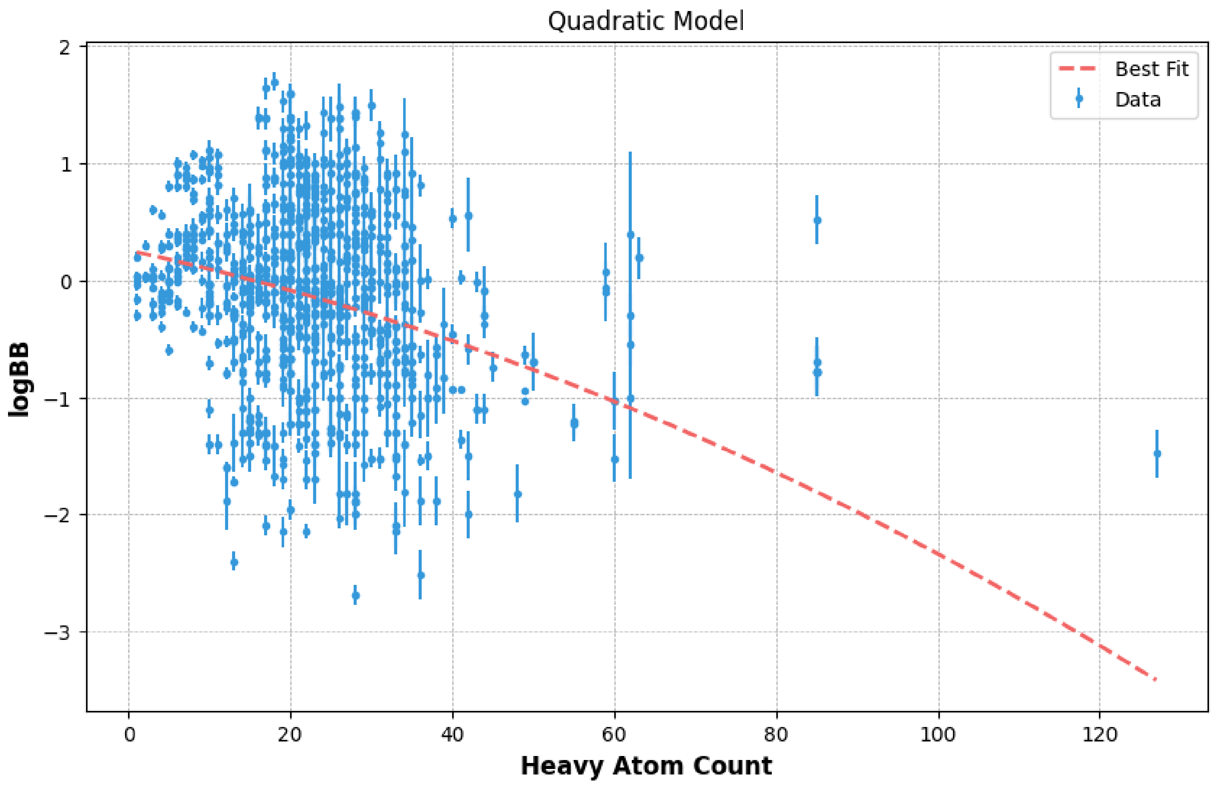


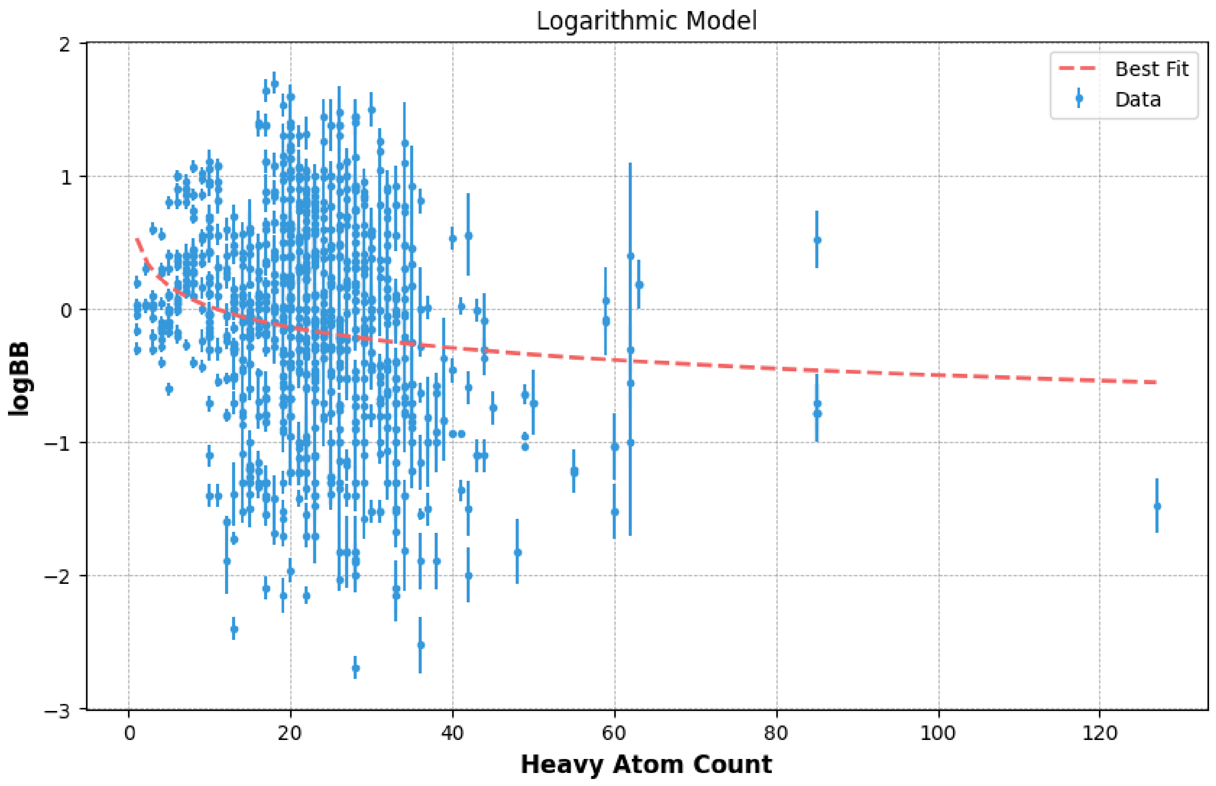
We repeat the linear and quadratic fits for other variables of interest.

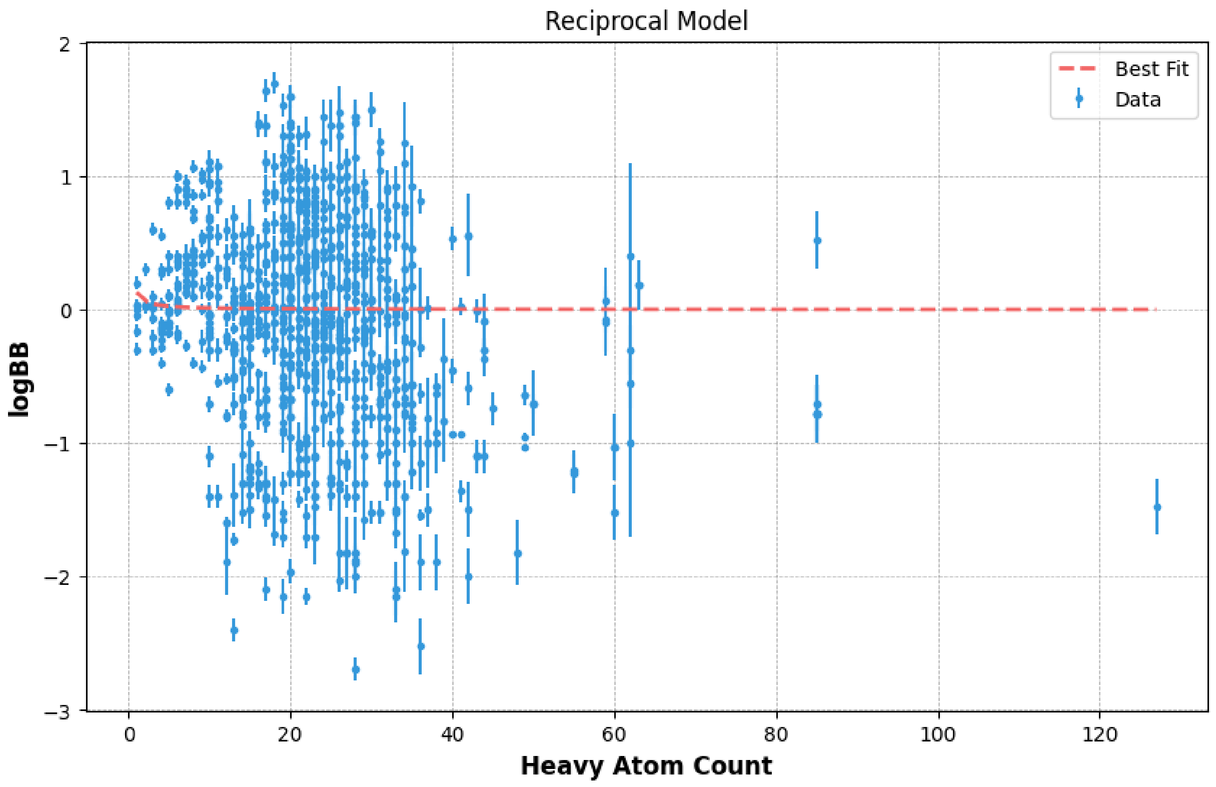
In [ ]:
```
# fit models to logBB vs. heavy atom count
x_value = data['heavy_atom_count'].to_numpy()
tparams, fiterrs = mycurvefit(linear_model, x_value, y_value, y_error, 'Heavy
fiarams, fiterrs = mycurvefit(quadratic_model, x_value, y_value, y_error, 'Hea
fitparams, fiterrs = mycurvefit(logarithmic_model, x_value, y_value, y_error, 
fitparams, fiterrs = mycurvefit(reciprocal_model, x_value, y_value, y_error, '
```

```
Best Fit Parameters:

         Independent Variable: Heavy Atom Count
         Dependent Variable: logBB
         Model: Linear Model
         P1 =  -0.02004 +/- 0.00021
         P2 =  0.29819 +/- 0.00448

 Fit Metrics:

         Degrees of freedom (N-d):  1056
         Reduced Chi Squared = 66.854

Best Fit Parameters:

         Independent Variable: Heavy Atom Count
         Dependent Variable: logBB
         Model: Quadratic Model
         P1 =  -0.00011 +/- 1e-05
         P2 =  -0.01487 +/- 0.00053
         P3 =  0.25436 +/- 0.00608

 Fit Metrics:

         Degrees of freedom (N-d):  1055
         Reduced Chi Squared = 66.81

Best Fit Parameters:

         Independent Variable: Heavy Atom Count
         Dependent Variable: logBB
         Model: Logarithmic Model
         P1 =  -0.22338 +/- 0.00306
         P2 =  0.53252 +/- 0.00844

 Fit Metrics:

         Degrees of freedom (N-d):  1056
         Reduced Chi Squared = 70.088

Best Fit Parameters:

         Independent Variable: Heavy Atom Count
         Dependent Variable: logBB
         Model: Reciprocal Model
         P1 =  0.13013 +/- 0.01338

 Fit Metrics:

         Degrees of freedom (N-d):  1057
         Reduced Chi Squared = 75.579
```


Linear Model
Best Fit
Data
logBB
Heavy Atom Count
2
1
0
−1
−2
−3
0
20
40
60
80
100
120


Quadratic Model
Best Fit
Data
logBB
Heavy Atom Count
2
1
0
−1
−2
−3
0
20
40
60
80
100
120

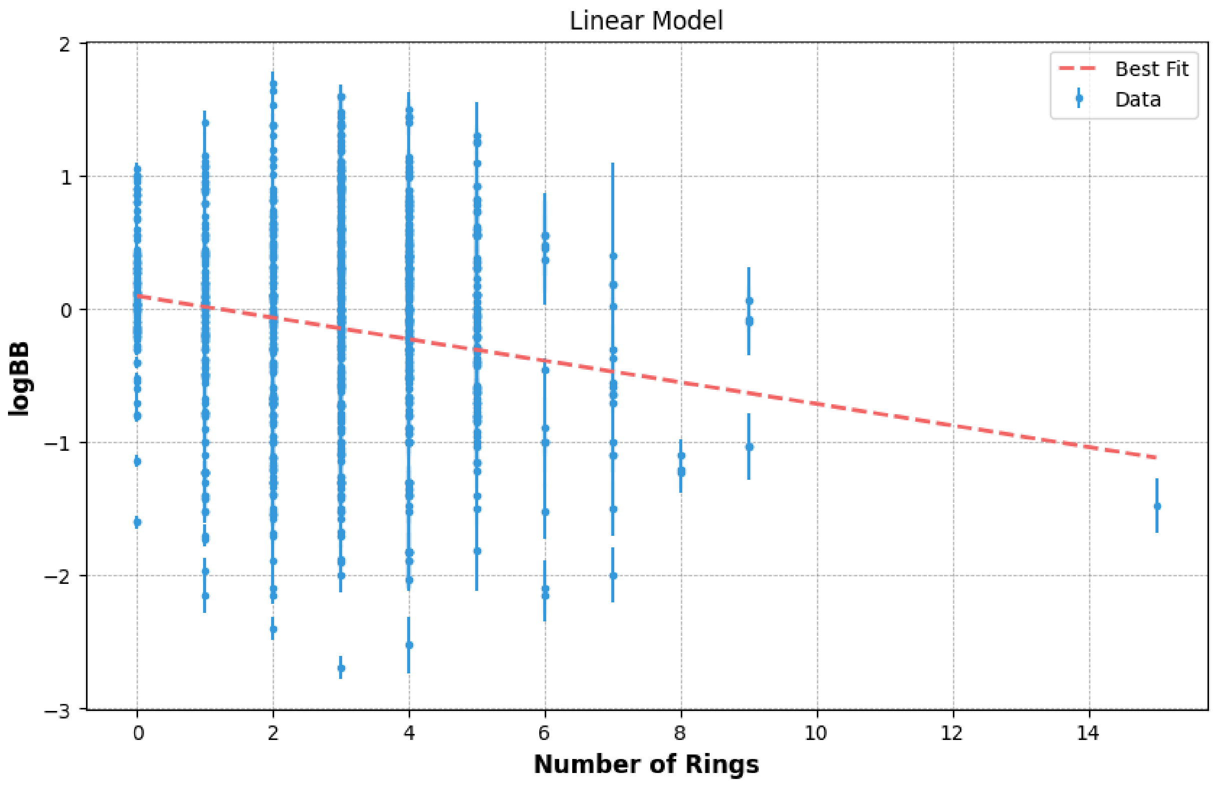


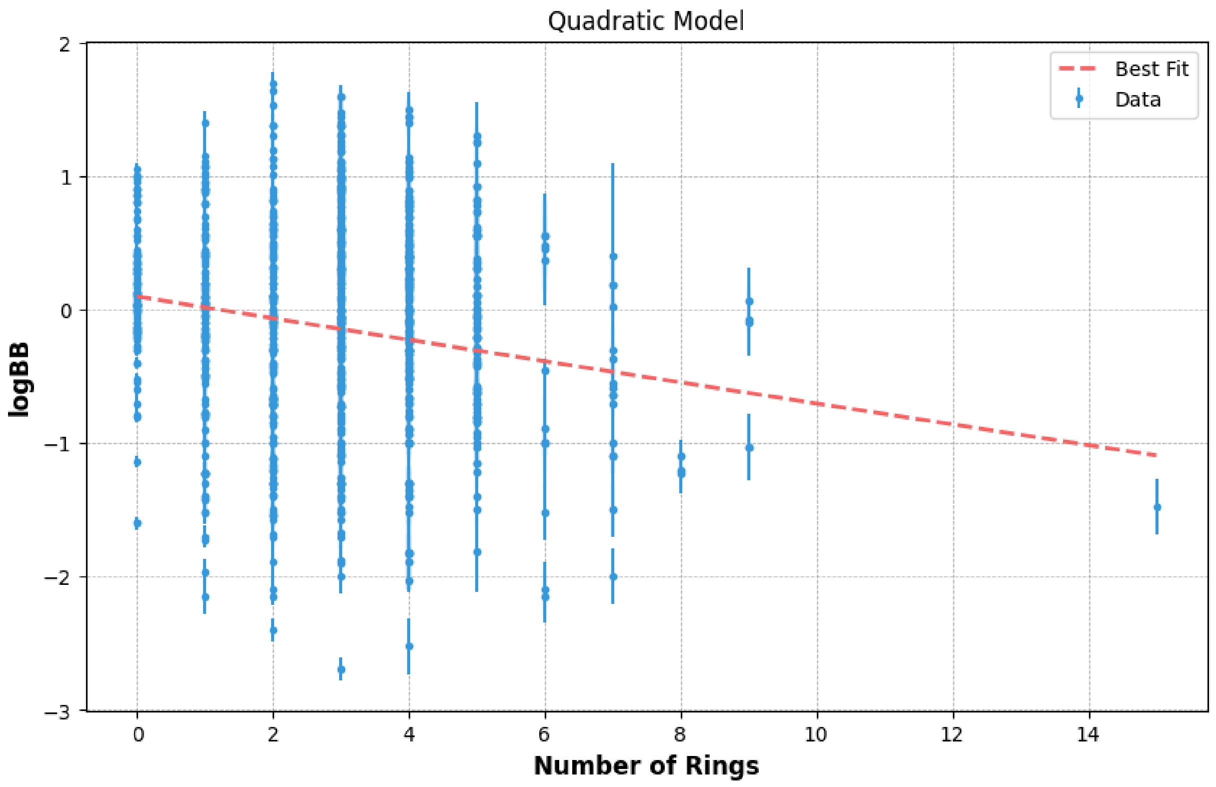


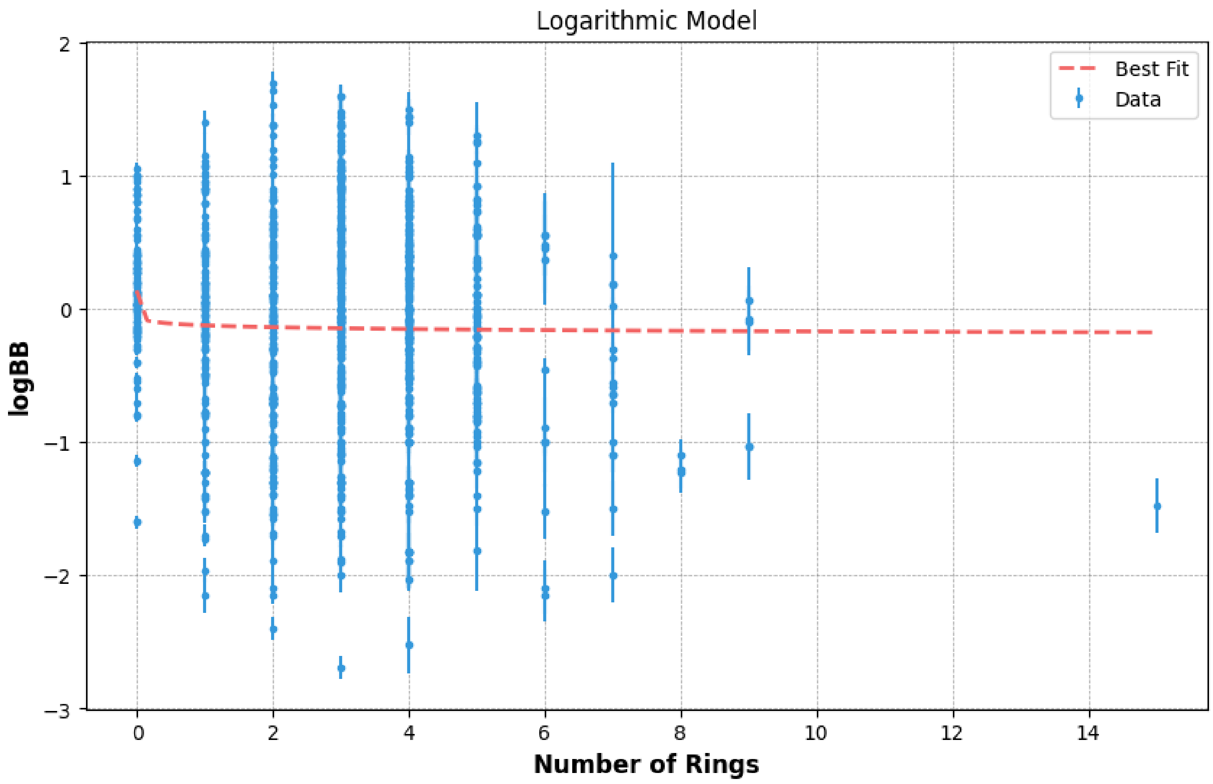
We repeat this process for number of rings.

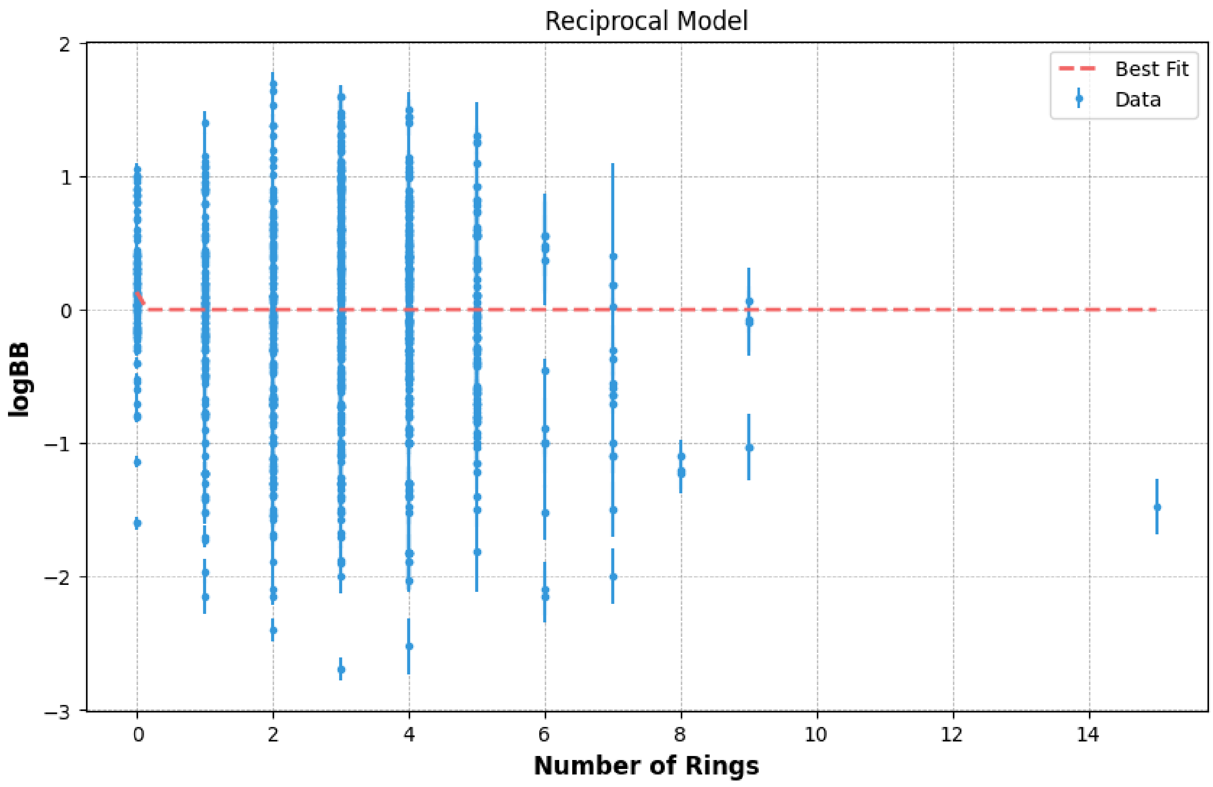
In [ ]:
```
# fit models to logBB vs. number of rings
x_value = data['num_rings'].to_numpy()
fitparams, fiterrs = mycurvefit(linear_model, x_value, y_value, y_error, 'Numb
fitparams, fiterrs = mycurvefit(quadratic_model, x_value, y_value, y_error, 'N
fitparams, fiterrs = mycurvefit(logarithmic_model, x_value, y_value, y_error,
fitparams, fiterrs = mycurvefit(reciprocal_model, x_value, y_value, y_error, '
```

```
Best Fit Parameters:

         Independent Variable: Number of Rings
         Dependent Variable: logBB
         Model: Linear Model
         P1 =  -0.08095 +/- 0.00137
         P2 =  0.09804 +/- 0.00355

 Fit Metrics:

         Degrees of freedom (N-d):  1056
         Reduced Chi Squared = 71.826

Best Fit Parameters:

         Independent Variable: Number of Rings
         Dependent Variable: logBB
         Model: Quadratic Model
         P1 =  0.00016 +/- 0.00066
         P2 =  -0.08169 +/- 0.00345
         P3 =  0.09845 +/- 0.00395

 Fit Metrics:

         Degrees of freedom (N-d):  1055
         Reduced Chi Squared = 71.894

Best Fit Parameters:

         Independent Variable: Number of Rings
         Dependent Variable: logBB
         Model: Logarithmic Model
         P1 =  -0.01904 +/- 0.00035
         P2 =  -0.12306 +/- 0.00262

 Fit Metrics:

         Degrees of freedom (N-d):  1056
         Reduced Chi Squared = 72.355

Best Fit Parameters:

         Independent Variable: Number of Rings
         Dependent Variable: logBB
         Model: Reciprocal Model
         P1 =  0.0 +/- 0.0

 Fit Metrics:

         Degrees of freedom (N-d):  1057
         Reduced Chi Squared = 74.79
```


Linear Model
Best Fit
Data
logBB
Number of Rings
2
1
0
−1
−2
−3
0
2
4
6
8
10
12
14


Quadratic Model
Best Fit
Data
logBB
Number of Rings
2
1
0
−1
−2
−3
0
2
4
6
8
10
12
14

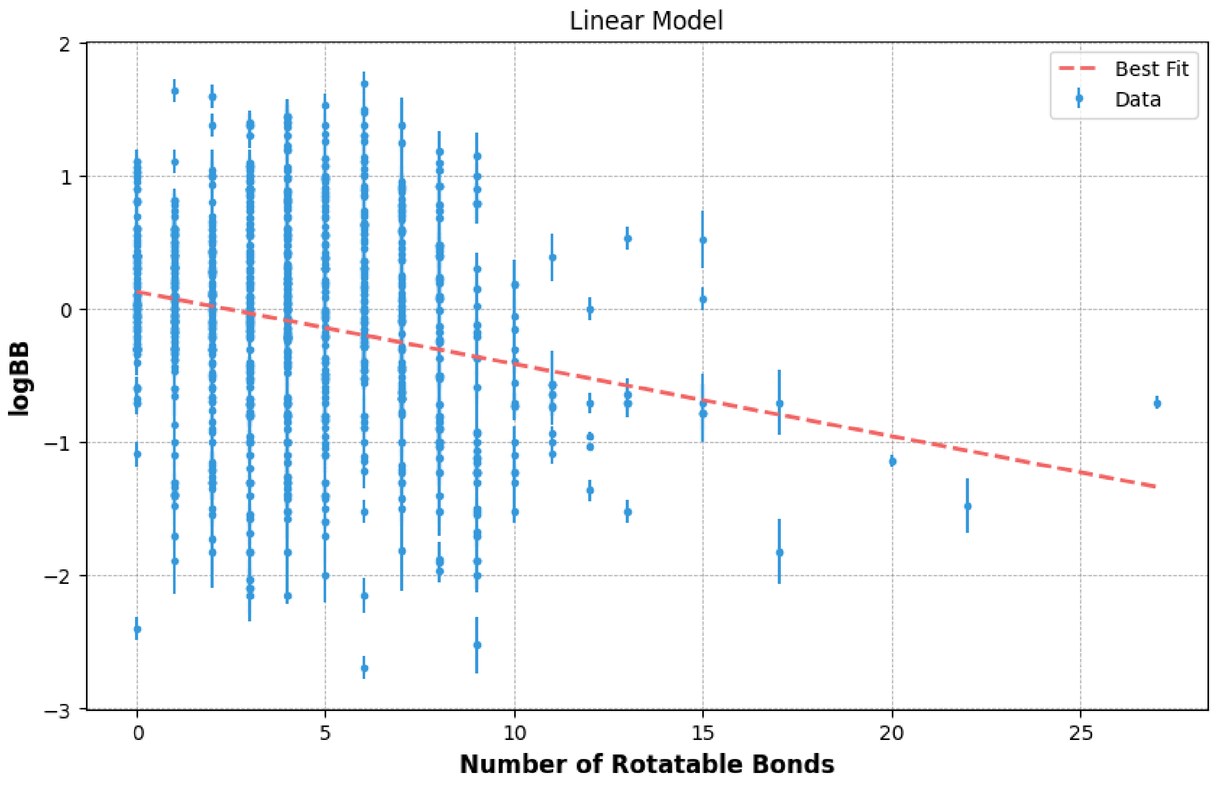


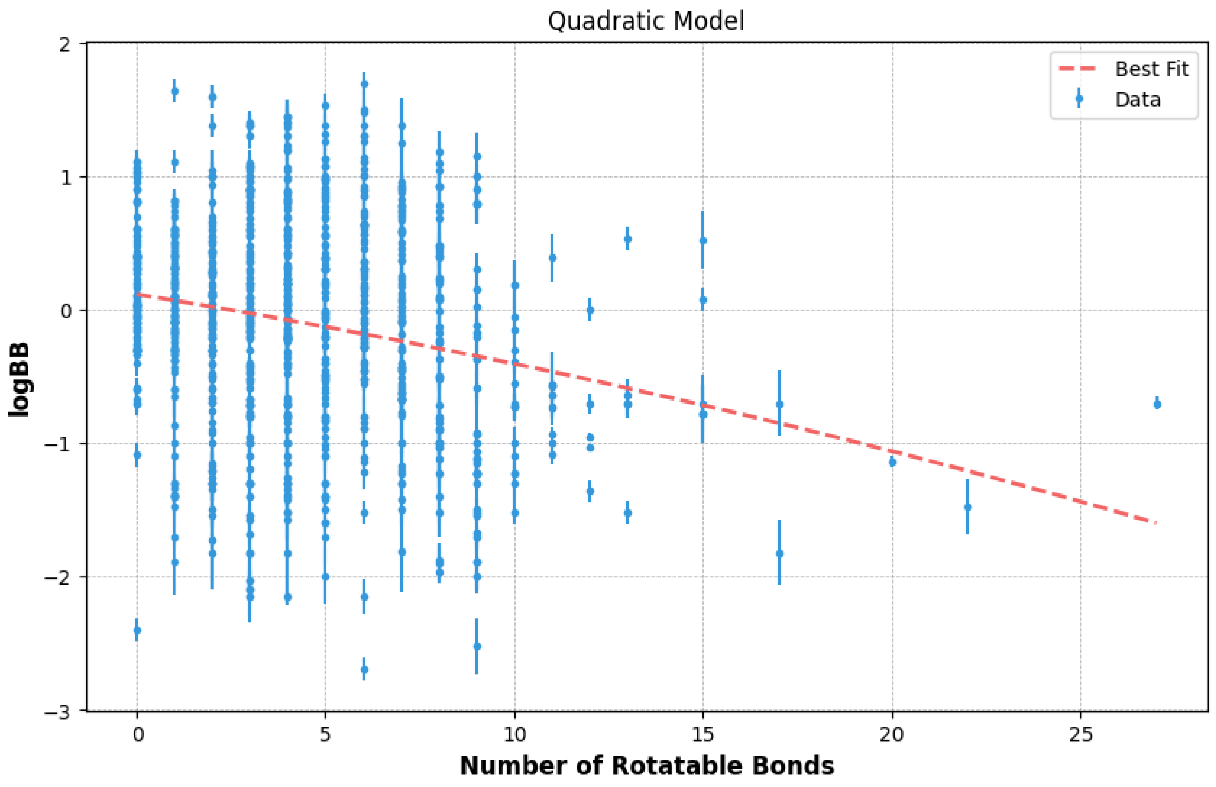


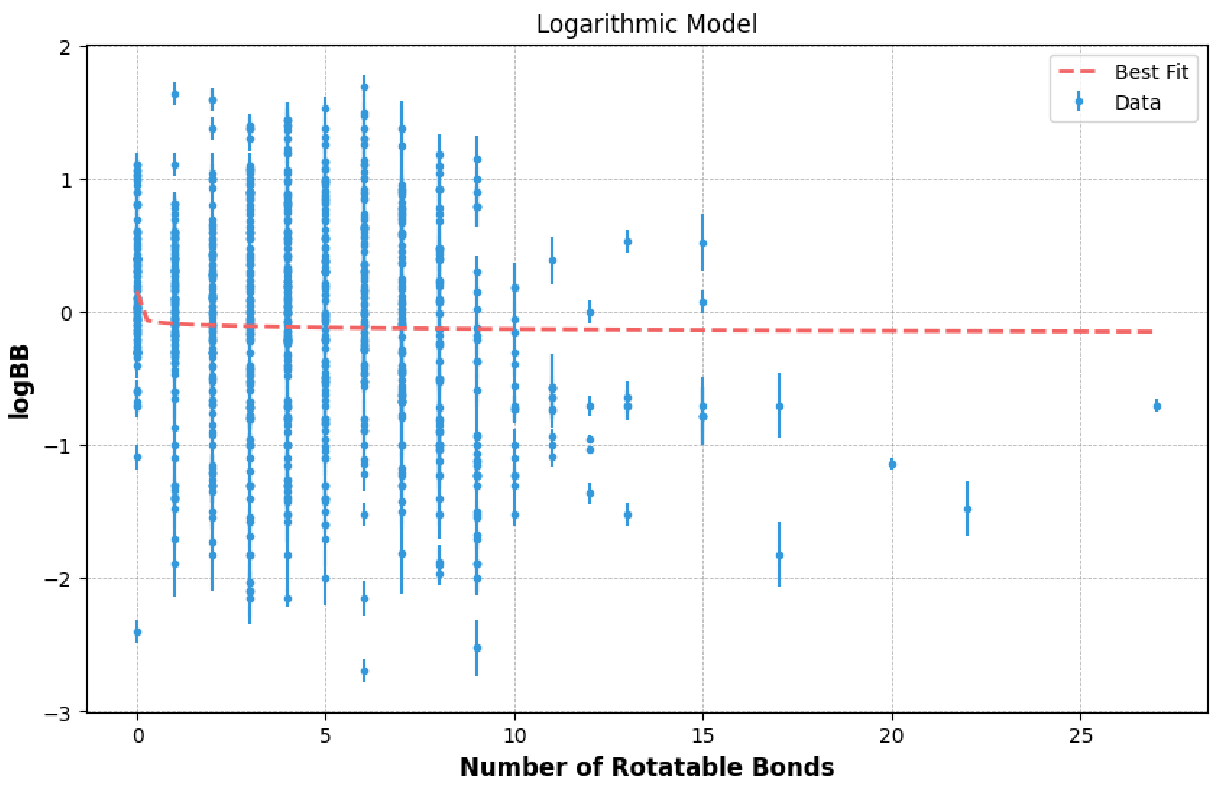
We repeat this process for number of rotatable bonds.

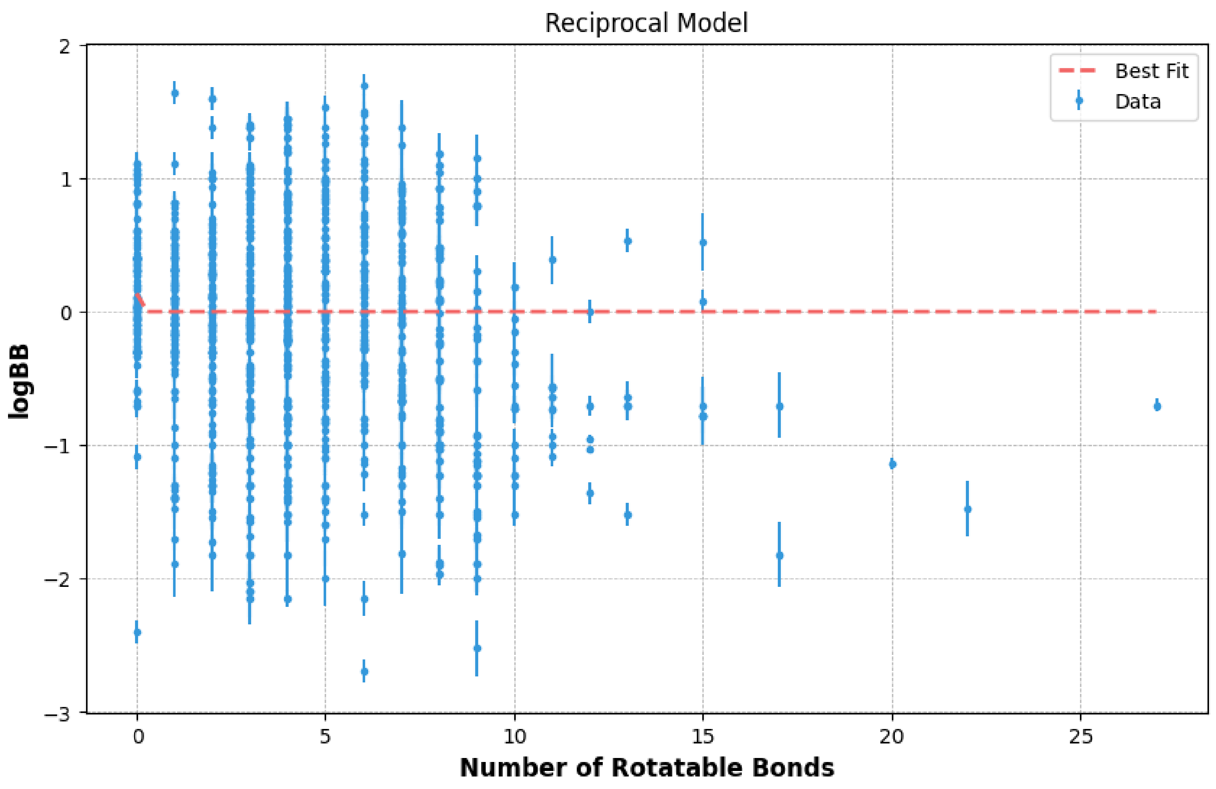
```
In [ ]: # fit models to logBB vs. number of rotatable bonds
        x_value = data['num_rotatable_bonds'].to_numpy()
        fitparams, fiterrs = mycurvefit(linear_model, x_value, y_value, y_error, 'Numb
        fitparams, fiterrs = mycurvefit(quadratic_model, x_value, y_value, y_error, 'N
        fitparams, fiterrs = mycurvefit(logarithmic_model, x_value, y_value, y_error,
        fitparams, fiterrs = mycurvefit(reciprocal_model, x_value, y_value, y_error, '
```

```
Best Fit Parameters:

         Independent Variable: Number of Rotatable Bonds
         Dependent Variable: logBB
         Model: Linear Model
         P1 =  -0.0542 +/- 0.0007
         P2 =  0.12962 +/- 0.00338

 Fit Metrics:

         Degrees of freedom (N-d):  1056
         Reduced Chi Squared = 69.456

Best Fit Parameters:

         Independent Variable: Number of Rotatable Bonds
         Dependent Variable: logBB
         Model: Quadratic Model
         P1 =  -0.00067 +/- 9e-05
         P2 =  -0.0453 +/- 0.00137
         P3 =  0.11424 +/- 0.00395

 Fit Metrics:

         Degrees of freedom (N-d):  1055
         Reduced Chi Squared = 69.468

Best Fit Parameters:

         Independent Variable: Number of Rotatable Bonds
         Dependent Variable: logBB
         Model: Logarithmic Model
         P1 =  -0.01787 +/- 0.0004
         P2 =  -0.08749 +/- 0.00242

 Fit Metrics:

         Degrees of freedom (N-d):  1056
         Reduced Chi Squared = 73.229

Best Fit Parameters:

         Independent Variable: Number of Rotatable Bonds
         Dependent Variable: logBB
         Model: Reciprocal Model
         P1 =  0.0 +/- 0.0

 Fit Metrics:

         Degrees of freedom (N-d):  1057
         Reduced Chi Squared = 75.07
```


Linear Model
Best Fit
Data
logBB
Number of Rotatable Bonds
2
1
0
−1
−2
−3
0
5
10
15
20
25


Quadratic Model
Best Fit
Data
logBB
Number of Rotatable Bonds
2
1
0
−1
−2
−3
0
5
10
15
20
25

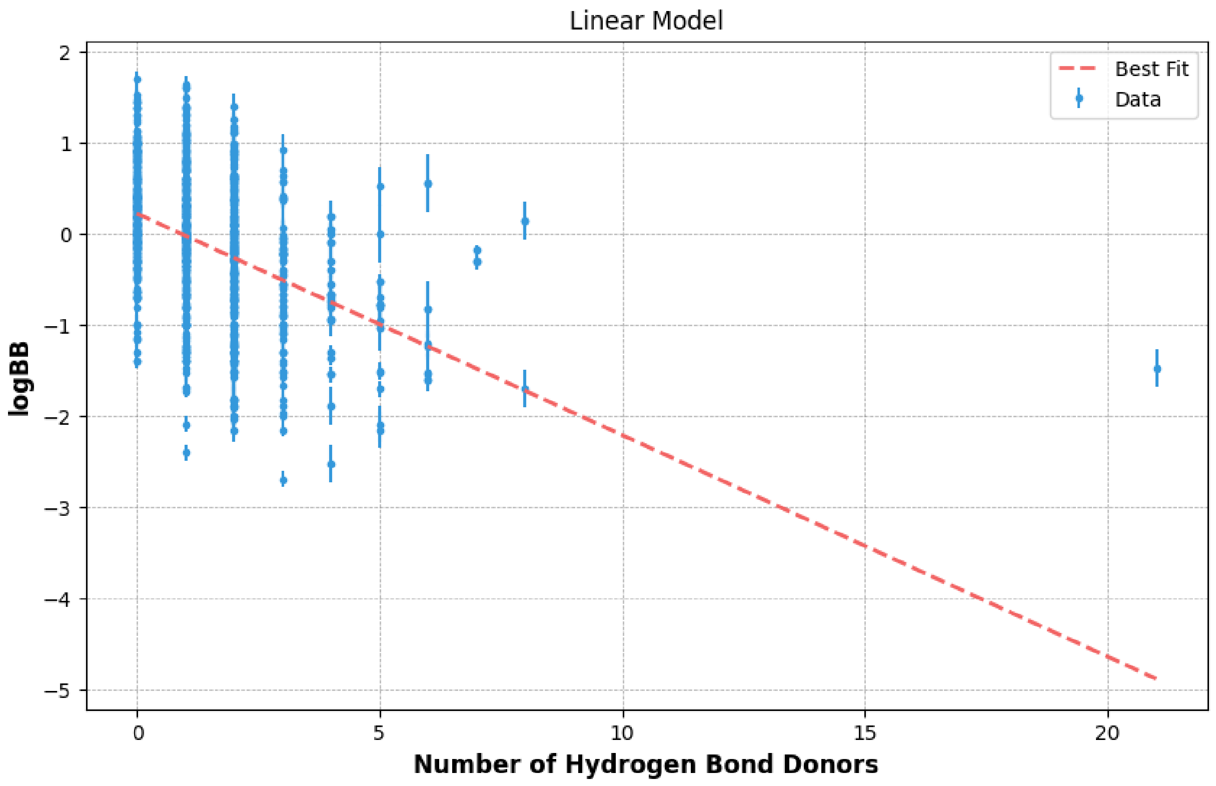


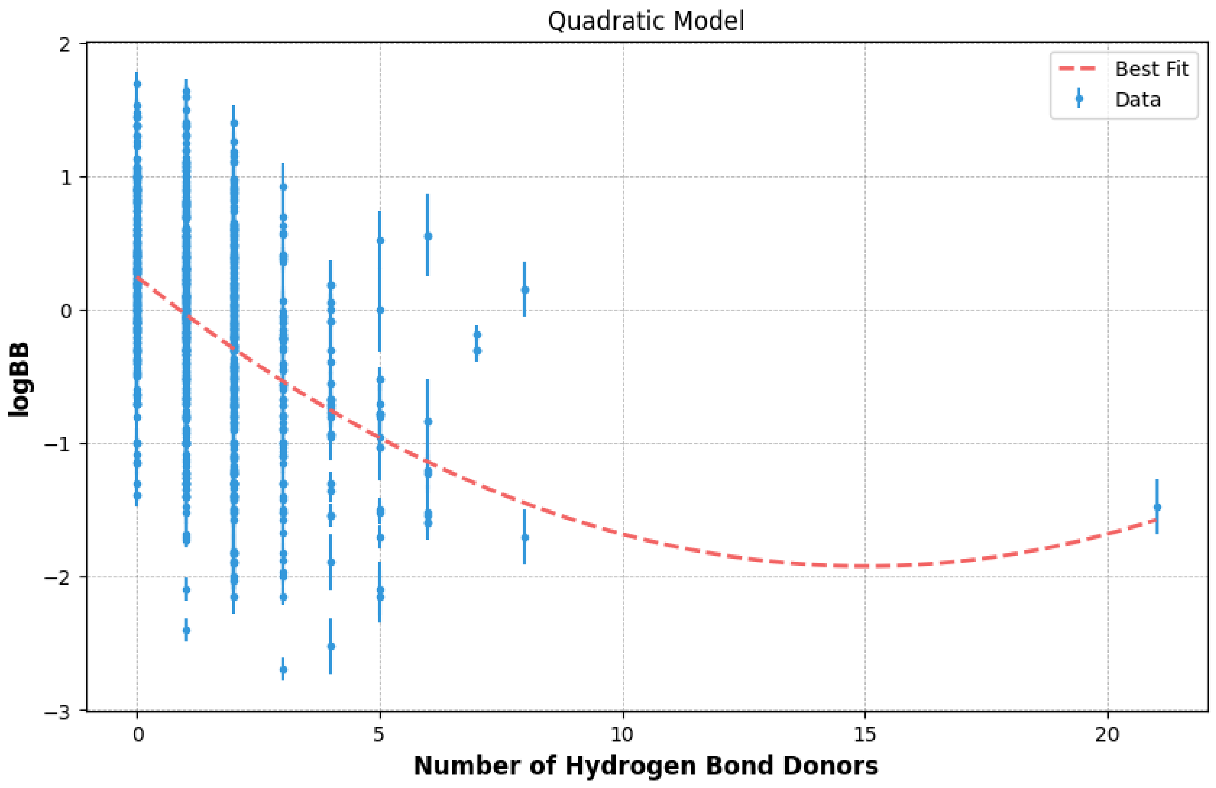


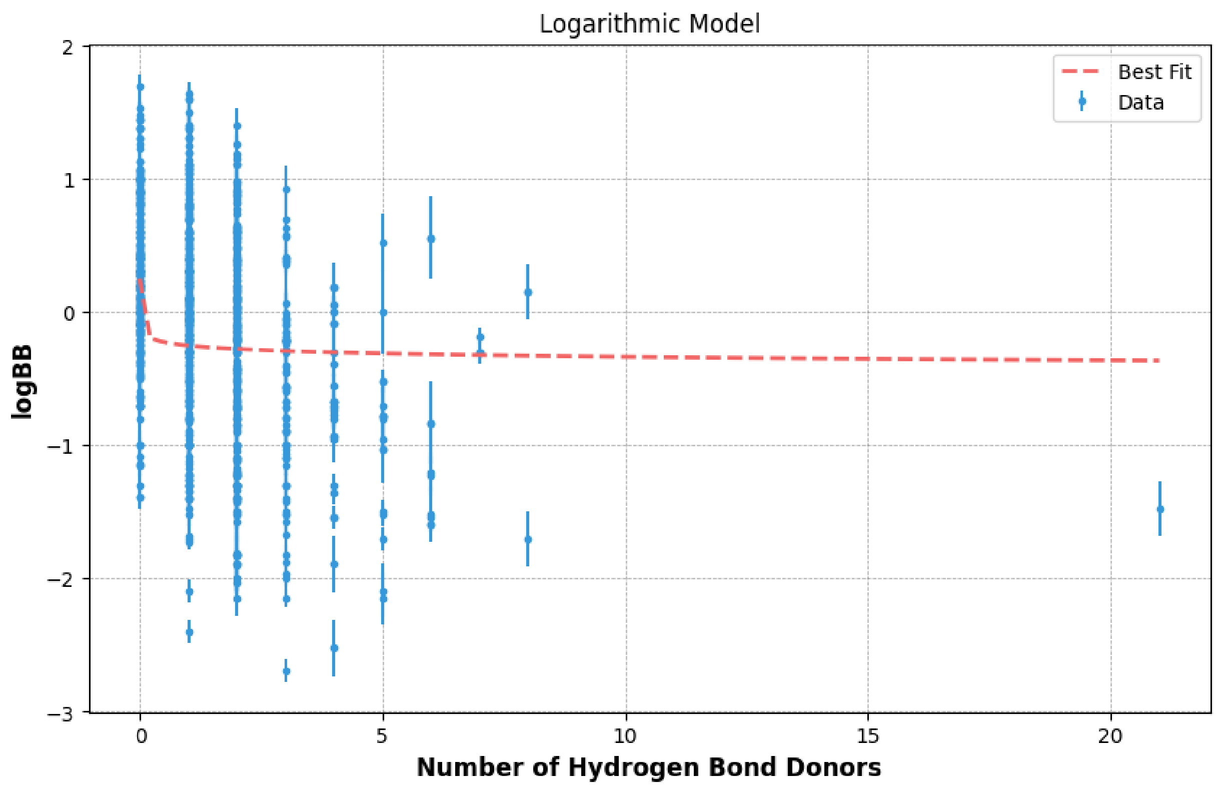
Finally, we repeat this process for the number of hydrogen bonds donors and acceptors.

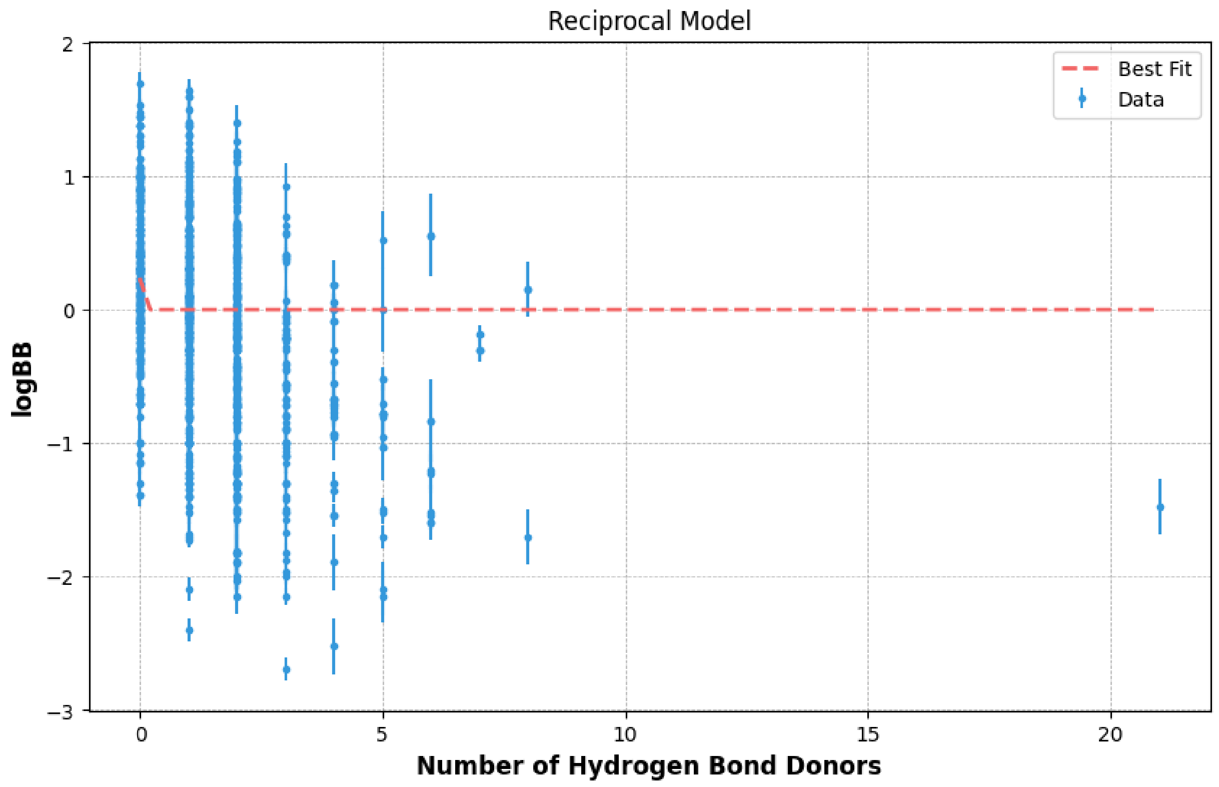
```
In [ ]: # fit models to logBB vs. number of hydrogen bond donors
x_value = data['num_h_donors'].to_numpy()
fitparams, fiterrs = mycurvefit(linear_model, x_value, y_value, y_error, 'Numb
fitparams, fiterrs = mycurvefit(quadratic_model, x_value, y_value, y_error, 'N
fitparams, fiterrs = mycurvefit(logarithmic_model, x_value, y_value, y_error,
fitparams, fiterrs = mycurvefit(reciprocal_model, x_value, y_value, y_error, '
```

```
Best Fit Parameters:

         Independent Variable: Number of Hydrogen Bond Donors
         Dependent Variable: logBB
         Model: Linear Model
         P1 =  -0.24314 +/- 0.00173
         P2 =  0.22366 +/- 0.00308

 Fit Metrics:

         Degrees of freedom (N-d):  1056
         Reduced Chi Squared = 56.382

Best Fit Parameters:

         Independent Variable: Number of Hydrogen Bond Donors
         Dependent Variable: logBB
         Model: Quadratic Model
         P1 =  0.00964 +/- 0.00049
         P2 =  -0.28923 +/- 0.00291
         P3 =  0.2466 +/- 0.0033

 Fit Metrics:

         Degrees of freedom (N-d):  1055
         Reduced Chi Squared = 56.069

Best Fit Parameters:

         Independent Variable: Number of Hydrogen Bond Donors
         Dependent Variable: logBB
         Model: Logarithmic Model
         P1 =  -0.03654 +/- 0.00033
         P2 =  -0.25183 +/- 0.00292

 Fit Metrics:

         Degrees of freedom (N-d):  1056
         Reduced Chi Squared = 63.655

Best Fit Parameters:

         Independent Variable: Number of Hydrogen Bond Donors
         Dependent Variable: logBB
         Model: Reciprocal Model
         P1 =  0.0 +/- 0.0

 Fit Metrics:

         Degrees of freedom (N-d):  1057
         Reduced Chi Squared = 71.683
```


Linear Model
Best Fit
Data
logBB
Number of Hydrogen Bond Donors


Quadratic Model
Best Fit
Data
logBB
Number of Hydrogen Bond Donors

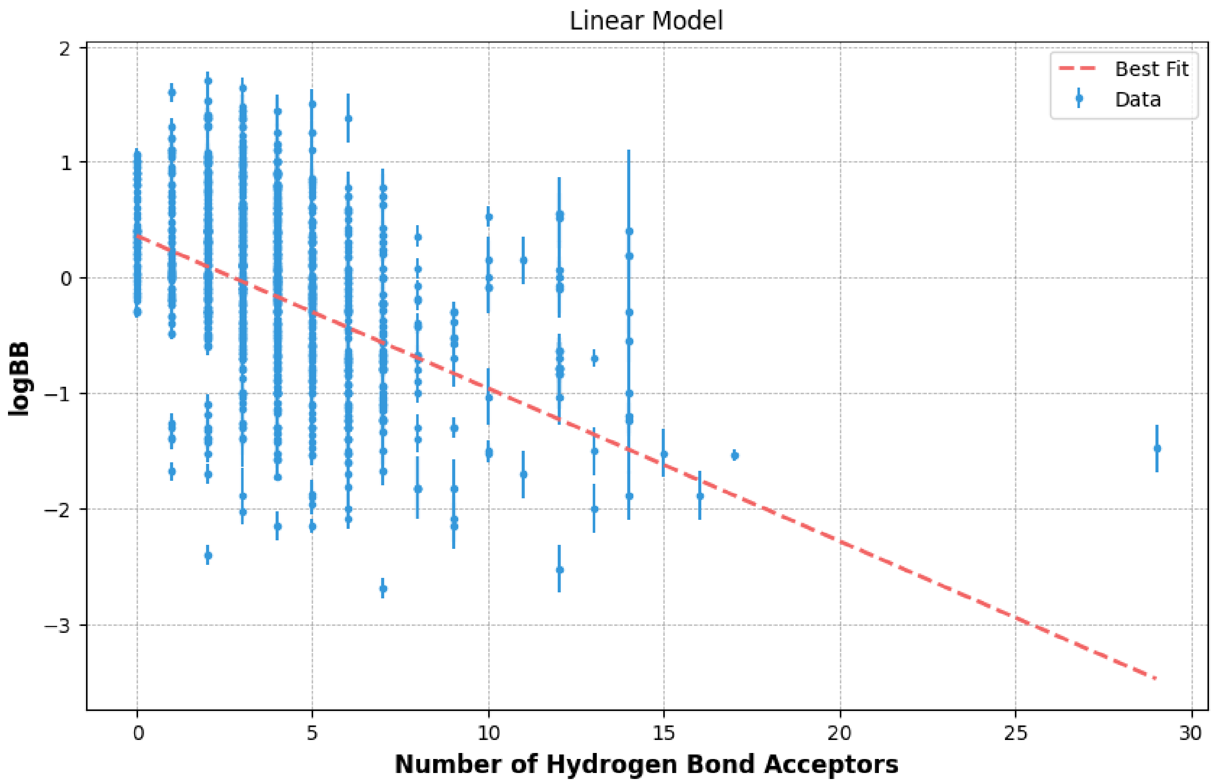


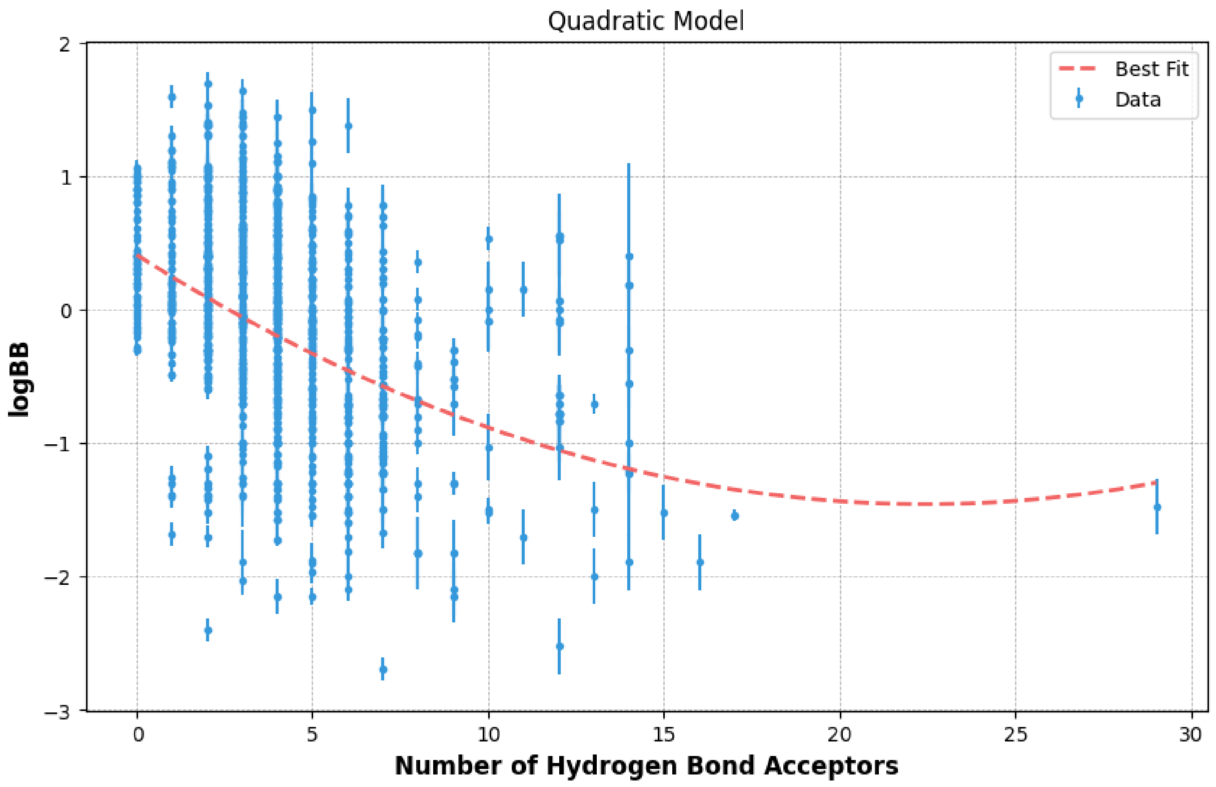


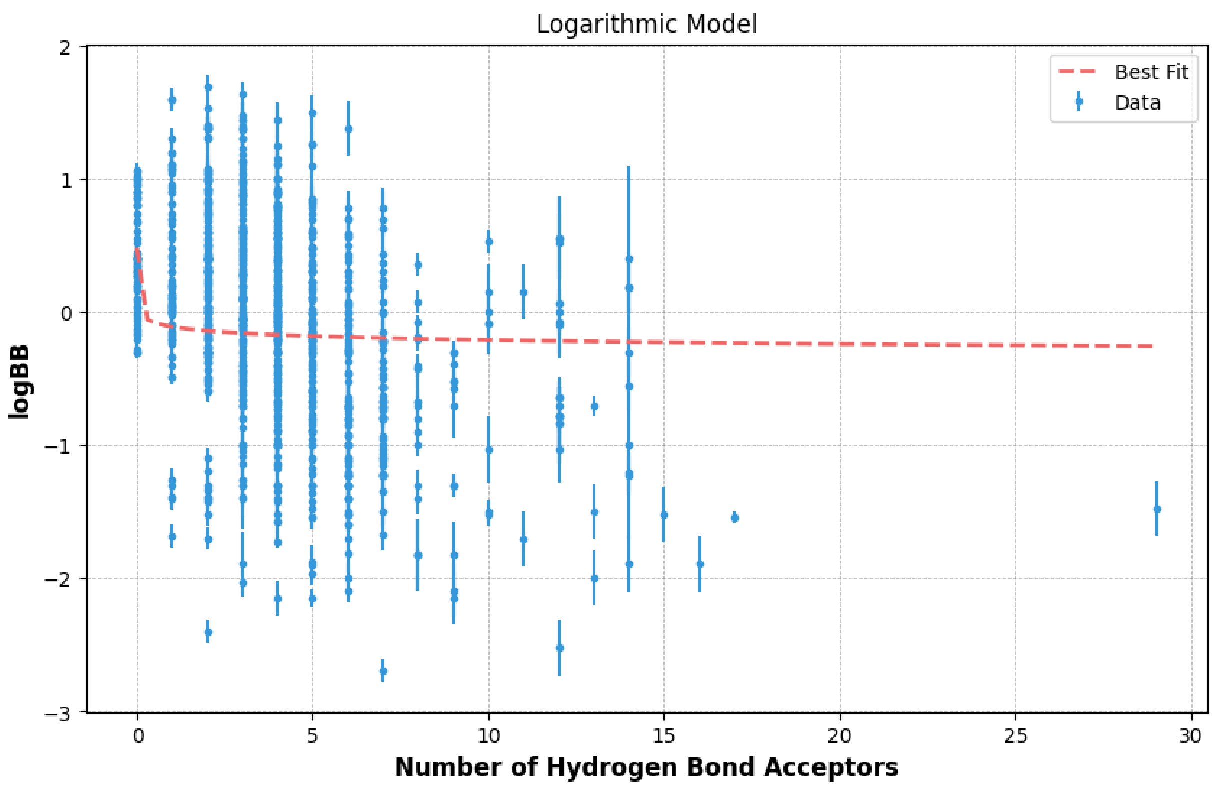
Again, for number of hydrogen bond acceptors.

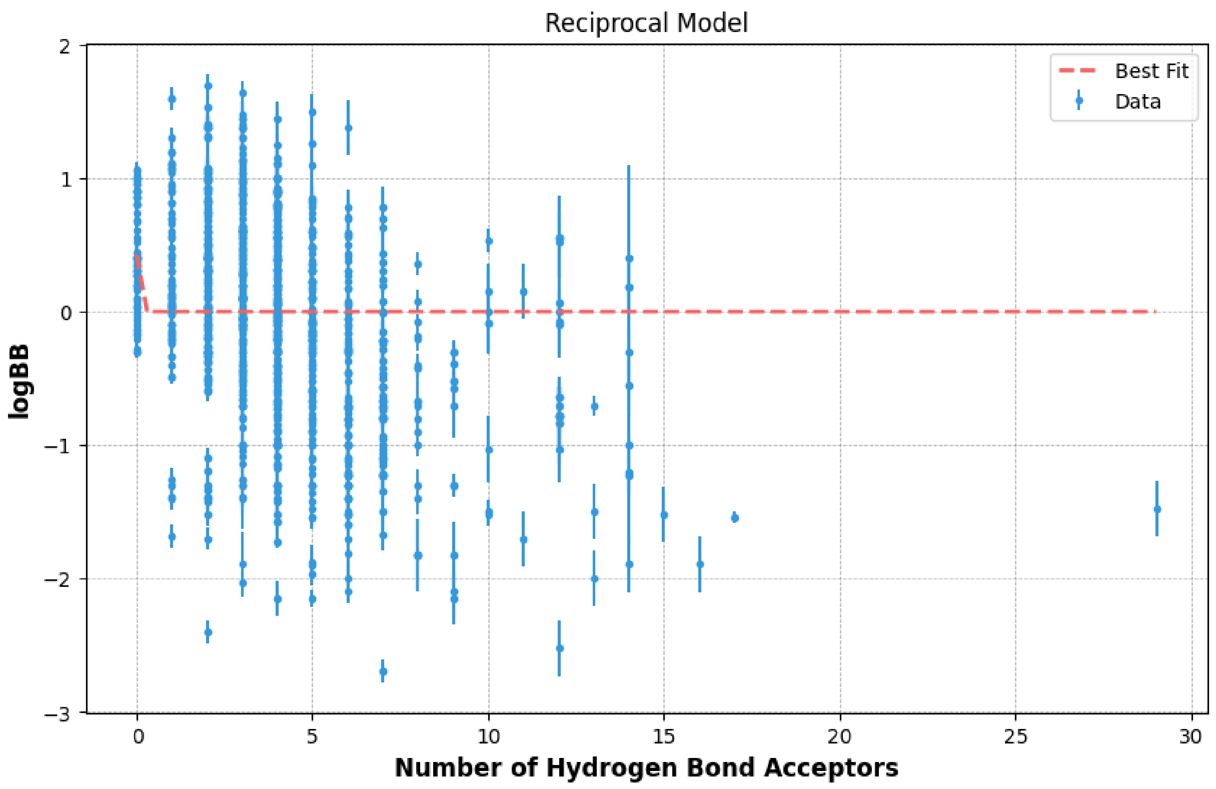
```
In [ ]: # fit models to logBB vs. number of hydrogen bond acceptors
        x_value = data['num_h_acceptors'].to_numpy()
        fitparams, fiterrs = mycurvefit(linear_model, x_value, y_value, y_error, 'Numb
        fitparams, fiterrs = mycurvefit(quadratic_model, x_value, y_value, y_error, 'N
        fitparams, fiterrs = mycurvefit(logarithmic_model, x_value, y_value, y_error,
        fitparams, fiterrs = mycurvefit(reciprocal_model, x_value, y_value, y_error, '
```

```
Best Fit Parameters:

         Independent Variable: Number of Hydrogen Bond Acceptors
         Dependent Variable: logBB
         Model: Linear Model
         P1 =  -0.13224 +/- 0.00093
         P2 =  0.3613 +/- 0.00378

 Fit Metrics:

         Degrees of freedom (N-d):  1056
         Reduced Chi Squared = 56.173

Best Fit Parameters:

         Independent Variable: Number of Hydrogen Bond Acceptors
         Dependent Variable: logBB
         Model: Quadratic Model
         P1 =  0.00371 +/- 0.00018
         P2 =  -0.16636 +/- 0.00187
         P3 =  0.40912 +/- 0.00441

 Fit Metrics:

         Degrees of freedom (N-d):  1055
         Reduced Chi Squared = 55.808

Best Fit Parameters:

         Independent Variable: Number of Hydrogen Bond Acceptors
         Dependent Variable: logBB
         Model: Logarithmic Model
         P1 =  -0.04297 +/- 0.00043
         P2 =  -0.11071 +/- 0.00239

 Fit Metrics:

         Degrees of freedom (N-d):  1056
         Reduced Chi Squared = 65.611

Best Fit Parameters:

         Independent Variable: Number of Hydrogen Bond Acceptors
         Dependent Variable: logBB
         Model: Reciprocal Model
         P1 =  0.0 +/- 0.0

 Fit Metrics:

         Degrees of freedom (N-d):  1057
         Reduced Chi Squared = 70.725
```


Linear Model
logBB
Number of Hydrogen Bond Acceptors
Best Fit
Data
2
1
0
−1
−2
−3
0
5
10
15
20
25
30


Quadratic Model
logBB
Number of Hydrogen Bond Acceptors
Best Fit
Data
2
1
0
−1
−2
−3
0
5
10
15
20
25
30





Discussion and Conclusion

Here, we fit several models to the data to investigate the relationships between BBB permeability and molecular properties such as molecular weight, number of heavy atoms, number of rings, number of rotatable bonds, and number of hydrogen bond donors and acceptors.

Molecular Weight

When investigating the relationship between molecular weight and BBB-permeability, we found little difference between the linear and quadratic models, with χ^2_{red} values of 66.752 and 66.721. Other models, such as logarithmic and reciprocal functions, have χ^2_{red} values of 69.863 and 75.629, respectively. Since all χ^2_{red} values are $\gg 1$, the linear, quadratic, logarithmic, and reciprocal models all do not explain the data.

However, the linear model is given by the equation $y = -0.00147x + 0.32189$, where y is logBB and x is the molecular weight. This indicates that there is a negative correlation between molecular weight and BBB permeability, which is consistent with the literature and with our intuition: as the size of a molecule increases, it is less likely to cross the BBB. The uncertainties on our measurements are small: the measured slope is $-0.00147 \pm 2 \times 10^{-5}$ and the measured intercept is 0.32189 ± 0.00468. We found similar results for other variables of interest.

Number of Heavy Atoms

When investigating the relationship between number of heavy atoms and BBB-permeability, we found little correlation with our given models. For the linear model, with a function of $y = -0.02004x + 0.29819$, the χ^2_{red} value is 66.854. For the quadratic model, the χ^2_{red} value is 75.579. For the logarithmic model, the χ^2_{red} value is 70.088. For the reciprocal model, the χ^2_{red} value is 75.579. While the smallest χ^2_{red} value out of these models (linear has the smallest, with a value of 66.854) is $\gg 1$, the negative slope of the linear function makes sense: since the number of heavy atoms is correlated with molecular weight and molecular size, we expect molecules with increased numbers of heavy atoms to have decreased BBB-permeability.

Number of Rings

When investigating the relationship between number of rings and BBB-permeability, we found little correlation with our given models. For the linear model, with a function of $y = -0.08095x + 0.00137$, the χ^2_{red} value is 71.826. For the quadratic model, the χ^2_{red} value is 75.07. For the logarithmic model, the χ^2_{red} value is 72.355. For the reciprocal model, the χ^2_{red} value is 74.79. While the smallest χ^2_{red} value out of these models (linear has the smallest) is $\gg 1$, the negative slope of the linear function makes sense. The presence of more rings is correlated with greater molecular size and weight; therefore, our results are consistent with the literature and intuition: as ring count and therefore molecular size increases, a molecule would be less likely to cross the BBB.

Number of Rotatable Bonds

When investigating the relationship between number of rotatable bonds and BBB-permeability, we found little minimum correlation with our given models. For the linear model, with a function of $y = -0.0542x + 0.0007$, the χ^2_{red} value is 69.456. For quadratic model, the χ^2_{red} value is 75.07. For the logarithmic model, the χ^2_{red} value is 73.229. For the reciprocal model, the χ^2_{red} value is 75.07. On initial thought, this is counterintuitive, since we expect the number of rotatable bonds of a molecule to correlate with increased flexibility and number of possible conformations, and therefore perhaps with increased capacity to bind to a BBB transporter and traverse the BBB. However, we reason that larger molecules often possess more rotatable bonds; therefore, increased number of rotatable bonds is also correlated with increased molecular size. Based on previous intuition and the literature, the negative slope in the fitted linear function thus makes sense.

Number of Hydrogen Bond Donors and Acceptors

When investigating the relationship between number of hydrogen bond donors and BBB-permeability, we found little correlation with our models. The linear model, with a function of $y = -0.24314x + 0.22366$, has a χ^2_{red} value of 56.382. The quadratic model has a χ^2_{red} value of 56.069, the logarithmic model has a χ^2_{red} value of 63.655, and the reciprocal model has a χ^2_{red} value of 71.683. All models have χ^2_{red} values $\gg 1$, indicating poor fit. However, as the linear and quadratic models fit best, we intepret the negative slope to mean as the number of hydrogen bond donors increases, BBB-permeability decreases. This makes sense because molecules with hydrogen bonds are polar, so they cannot naturally pass through the blood-brain barrier.

When investigating the relationship between number of hydrogen bond acceptors and BBB-permeability, we also found little correlation with our models. The linear model, with a function of $y = -0.133224x + 0.3613$, has a χ^2_{red} value of 56.173. The quadratic model has a χ^2_{red} value of 55.808, the logarithmic model has a χ^2_{red} value of 65.611, and the reciprocal model has a χ^2_{red} value of 70.725. Again, all models have χ^2_{red} values $\gg 1$, indicating poor fit. The linear and quadratic model fit best, so we can intepret the negative slope to mean as the number of hydrogen bond acceptors increases, BBB-permeability decreses. Similarly to hydrogen bond donor count, this makes sense because molecules with hydrogen bonds are polar, so they are excluded from passage through the blood-brain barrier.

Thus, we conclude that large macromolecules are excluded from the brain; however, our simplistic linear, quadratic, logarithmic, and reciprocal models are insufficient to explain the relationship between molecular properties – such as molecular weight, number of rotatable bonds, number of heavy atoms, number of rings, number of rotatable bonds, and number of hydrogen bond donors and acceptors – and BBB-permeability.

References

1. Abbott, N. J., Patabendige, A. A. K., Dolman, D. E. M., Yusof, S. R. & Begley, D. J. Structure and function of the blood–brain barrier. *Neurobiology of Disease* **37**, 13–25 (2010).

2. Abbott, N. J., Rönnbäck, L. & Hansson, E. Astrocyte–endothelial interactions at the blood–brain barrier. *Nat Rev Neurosci* **7**, 41–53 (2006).

3. Daneman, R. & Prat, A. The Blood–Brain Barrier. *Cold Spring Harb Perspect Biol* **7**, a020412 (2015).

4. Iadecola, C. The neurovascular unit coming of age: a journey through neurovascular coupling in health and disease. *Neuron* **96**, 17–42 (2017).

5. Langen, U. H., Ayloo, S. & Gu, C. Development and Cell Biology of the Blood-Brain Barrier. *Annual Review of Cell and Developmental Biology* **35**, 591–613 (2019).

6. Meng, F., Xi, Y., Huang, J. & Ayers, P. W. A curated diverse molecular database of blood-brain barrier permeability with chemical descriptors. *Sci Data* **8**, 289 (2021).

7. Obermeier, B., Daneman, R. & Ransohoff, R. M. Development, maintenance and disruption of the blood-brain barrier. *Nat Med* **19**, 1584–1596 (2013).

8. Wu, D. et al. The blood–brain barrier: structure, regulation, and drug delivery. *Sig Transduct Target Ther* **8**, 1–27 (2023).