# August 22, 2012

The following is code to calculate zdiff's. It's been tested; the details are in "june 2012 lab meeting log".

**from** **sundries** **import** CIDict

**from** **Bio.PDB** **import** PDBParser

**import** **warnings**

**from** **Bio** **import** AlignIO

**def** z(residue):

*'''Returns the z coordinate of a residue object's Calpha.'''*

**return** residue.child\_dict['CA'].get\_coord()[2]

**class** **Gap**(object):

*'''Represents a gap in a Position object.'''*

**pass**

**class** **Position**(object):

**def** \_\_init\_\_(self, pairs):

self.residues = CIDict(pairs)

**def** zdiff(self, template\_id, unknown\_id):

template\_res = self.residues[template\_id]

unknown\_res = self.residues[unknown\_id]

**if** type(template\_res) **is** Gap **or** type(unknown\_res) **is** Gap:

**return** None

**else**:

**return** z(template\_res) - z(unknown\_res)

**def** resi(self, stru\_id):

**return** self.residues[stru\_id].get\_id()[1]

**class** **NotFoundError**(Exception):

**pass**

**class** **Zdiff**(object):

**def** \_\_init\_\_(self, \*stru\_seq\_pairlist):

pos\_inputs = list()

**for** structure, sequence **in** stru\_seq\_pairlist:

residues = structure.get\_residues()

pairs\_for\_pos = list()

**for** letter **in** sequence:

**if** letter == '-':

seq\_unit = Gap()

**else**:

seq\_unit = residues.next()

pairs\_for\_pos.append((structure.get\_id(), seq\_unit))

pos\_inputs.append(pairs\_for\_pos)

self.positions = [Position(id\_res\_pairlist) \

**for** id\_res\_pairlist **in** zip(\*pos\_inputs)]

**def** get(self, template\_id, unknown\_id, resi, start=0):

**for** pos **in** self.positions[start:]:

**if** pos.resi(unknown\_id) == resi:

**return** pos.zdiff(template\_id, unknown\_id)

**raise** NotFoundError('resi {} of {} not found' \

.format(resi, unknown\_id))

**def** report(self, template\_id, unknown\_id):

output = (pos.zdiff(template\_id, unknown\_id) \

**for** pos **in** self.positions)

**return** filter(**lambda** x: x **is** **not** None, output)

**with** warnings.catch\_warnings():

warnings.simplefilter('ignore')

scry\_stru = PDBParser().get\_structure('1A0S', 'aligned\_1A0S.pdb')

maltoporin\_stru = PDBParser().get\_structure('1AF6', 'aligned\_1AF6.pdb')

alignment = AlignIO.read('Swiss-PDB structural alignment.aln', 'clustal')

**for** seq\_record **in** alignment:

**if** seq\_record.id == 'aligned\_1A0S':

scry\_seq = seq\_record

**if** seq\_record.id == 'aligned\_1AF6':

maltoporin\_seq = seq\_record

output = Zdiff((scry\_stru, scry\_seq), (maltoporin\_stru, maltoporin\_seq))

**with** open('NEW 1a0s as template.txt', 'w') **as** f:

f.writelines(str(zdiff) + '**\n**' \

**for** zdiff **in** output.report('1A0S', '1AF6'))

**with** open('NEW 1af6 as template.txt', 'w') **as** f:

f.writelines(str(zdiff) + '**\n**' \

**for** zdiff **in** output.report('1af6', '1a0s'))

My current goal is to turn this code into a module that can be accessed with easy-to-remember commands, and check that this module can reproduce the old results.

Copying some of the data from "june 2012 lab meeting" so I can try to replcate it. From "june 2012 lab meeting/zdiff calculator validation", I copied two structures, an alignment, and a list of zdiffs. The list of zdiffs does not have resi's, so I am pretending that I know that it is in order by resi. These files are:  
aligned\_1A0S.pdb  
aligned\_1AF6.pdb  
OLD 1a0s as template.txt  
Swiss-PDB structural alignment.aln

They all have their original names, except for "OLD 1a0s as template.txt", which used to be "NEW 1a0s as template.txt".

Remade the zdiff's with new code. The zdiff's at the top and bottom came out correct, and there was the right number of them, so I figure it probably works.

The code I used was this:

**from** sundries **import** CIDict

**from** Bio.PDB **import** PDBParser

**import** warnings

**from** Bio **import** AlignIO

# The guts that it runs on

**def** z(residue):

'''Returns the z coordinate of a residue object's Calpha.'''

**return** residue.child\_dict['CA'].get\_coord()[2]

**class** Gap(object):

'''Represents a gap in a Position object.'''

**pass**

**class** Position(object):

**def** \_\_init\_\_(self, pairs):

self.residues = CIDict(pairs)

**def** zdiff(self, template\_id, unknown\_id):

template\_res = self.residues[template\_id]

unknown\_res = self.residues[unknown\_id]

**if** type(template\_res) **is** Gap **or** type(unknown\_res) **is** Gap:

**return** None

**else**:

**return** z(template\_res) - z(unknown\_res)

**def** resi(self, stru\_id):

'''Return resi of position in specified structure'''

# Get the residue of the specified structure that is in this

# position in the alignment.

target = self.residues[stru\_id]

# It might actually be a gap; return None of it is

**if** type(target) **is** Gap:

**return** None

# Otherwise, return the id of the residue. It's a Biopython

# Residue object, so this is done with its get\_id() method.

**else**:

**return** self.residues[stru\_id].get\_id()[1]

**class** NotFoundError(Exception):

**pass**

**class** Zdiff(object):

**def** \_\_init\_\_(self, \*stru\_seq\_pairlist):

pos\_inputs = list()

**for** structure, sequence **in** stru\_seq\_pairlist:

residues = structure.get\_residues()

pairs\_for\_pos = list()

**for** letter **in** sequence:

**if** letter == '-':

seq\_unit = Gap()

**else**:

seq\_unit = residues.next()

pairs\_for\_pos.append((structure.get\_id(), seq\_unit))

pos\_inputs.append(pairs\_for\_pos)

self.positions = [Position(id\_res\_pairlist) \

**for** id\_res\_pairlist **in** zip(\*pos\_inputs)]

**def** get(self, template\_id, unknown\_id, resi, start=0):

**for** pos **in** self.positions[start:]:

**if** pos.resi(unknown\_id) == resi:

**return** pos.zdiff(template\_id, unknown\_id)

**raise** NotFoundError('resi {} of {} not found' \

.format(resi, unknown\_id))

**def** report(self, template\_id, unknown\_id):

output = (pos.zdiff(template\_id, unknown\_id) \

**for** pos **in** self.positions)

**return** filter(lambda x: x **is** **not** None, output)

**def** resi\_report(self, template\_id, unknown\_id):

output = ((pos.resi(template\_id),

pos.zdiff(template\_id, unknown\_id)) \

**for** pos **in** self.positions)

**return** filter(lambda x: x[1] **is** **not** None, output)

# The API for making zdiff files

**def** calc(template\_name, target\_name, template\_structure\_filename,

target\_structure\_filename, alignment\_filename,

write\_to, comment, format\_='clustal'):

# Open relevant files

with open(template\_structure\_filename, 'r') as template\_structure\_file,\

open(target\_structure\_filename, 'r') as target\_structure\_file,\

open(alignment\_filename, 'r') as alignment\_file:

# Load structures with Biopython's PDB file parser

# Daniel's aligned structures are missing some inessential

# information, and as a consequence the parser gives thousands

# of warnings. Gotta ignore these.

with warnings.catch\_warnings():

warnings.simplefilter('ignore')

template\_structure = PDBParser().\

get\_structure(template\_name,

template\_structure\_file)

target\_structure = PDBParser().\

get\_structure(target\_name,

target\_structure\_file)

# Open alignment using Biopython's parser

alignment = AlignIO.read(alignment\_filename, format\_)

# Find the template and target sequences in the alignment

**for** seq\_record **in** alignment:

**if** seq\_record.id == template\_name:

templ\_seq = seq\_record

**if** seq\_record.id == target\_name:

targ\_seq = seq\_record

# Calculate zdiff

results = Zdiff((template\_structure, templ\_seq),

(target\_structure, targ\_seq))

# Write results to a file

with open(write\_to, 'w') as o:

# Write some coments so I know which zdiff file this is

o.write('# Template: ')

o.write(template\_name + ' (' + template\_structure\_filename + ')\n')

o.write('# Target: ')

o.write(target\_name + ' (' + target\_structure\_filename + ')\n')

o.write('# Alignment: ' + alignment\_filename + '\n')

o.write('# ' + comment + '\n')

# Write the actual data

# Weird quirk of the zdiff objects - before giving a report, it

# requires the id's of the structures. I don't know if I wrote

# it to support more than two, or if I was planning to, or what.

**for** resi, zdiff **in** results.resi\_report(template\_name, target\_name):

o.write(str(resi) + ', ' + str(zdiff) + '\n')

I ran the "calc" function as so:

calc('aligned\_1A0S', 'aligned\_1AF6', 'aligned\_1A0S.pdb', 'aligned\_1AF6.pdb', 'Swiss-PDB structural alignment.aln', 'new.zdiff', 'reproduction of structural alignment to test code')

I copied the folder "june 2012 lab metting/pdbs from hhomp" to "zdiff module/pdbs from hhomp". This has the pdb structures of the proteins of known structure that are nearby other proteins of known structure in the HHOMP clustermap.

# September 4, 2012

Daniel's derivation of Ezβ involves aligning the sequences of PDB structures to whatever HHOMP clusters they had the closest match with in HHOMP's search.

Some of these HHOMP clusters have proteins of known structure in them. The proteins of known structure are not in the alignments, but they are mentioned in the cluster description. The alignment with OMPLA in it has the sequence from 1QD6 in the alignment, though, with "pdb" in the title. But the others don't have sequences with "pdb" in their titles, and in one case I searched the sequences for one matching a pdb file but couldn't find it.

Sometimes these structures are the very same that are in Daniel's dataset. Sometimes they are not, though. I will find the zdiff between the predicted structures of these proteins using proteins from Daniel's dataset, and the real structures.

I found five clusters that I can use for this purpose.

cluster73: 18.1.1 and 18.1.2  
Daniel's dataset: Sucrose porin (1A0S), mapped to cluster73  
HHOMP's dataset: Maltoporin (2MPR) in 18.1.1

cluster99: 16.1.1 and 16.1.2  
Daniel's: OmpC (2J1N), mapped to cluster99  
HHOMP's: PhoE (1PHO) and OmpF (2OMF), both in 16.1.1  
I've heard OmpC is pretty similar to these two, the seq identity might be really high

cluster71: 14.1.1, 14.1.5, and 14.1.7  
Daniel's: TodX (3BS0), mapped to cluster71  
HHOMP: FadL (1T16) in 14.1.5

cluster?:  
Daniel's: HasR (3CSL) mapped to cluster 22.4.6  
HHOMP's: BBtuB (1NQE) in 22.4.5  
These are basically on opposite sides of the 22-stranded cluster, but the HHOMP guys put them both in 22.4. I hope that means they're similar.  
However, no small contains both of these. Only cluster124, 139, 149, 152, and 153, and all those clusters are huge, much bigger than the clusters the sequences in Daniel's datgaset were mapped to.  
I'm leaving these ones out.

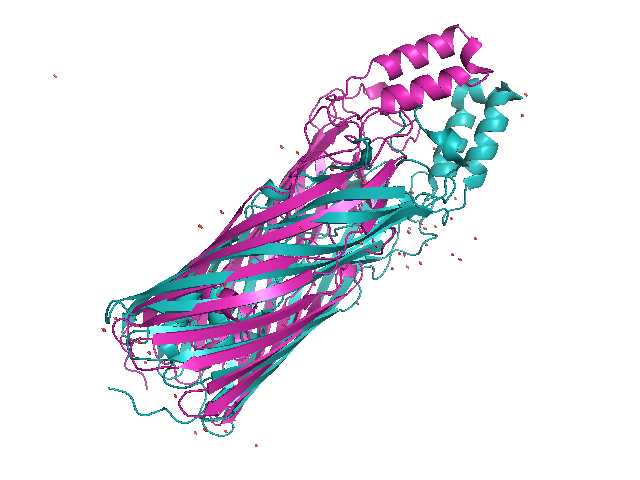
cluster18: 22.1.3, 22.1.4, 22.1.5, 22.1.6, 22.1.7  
Daniel's: FauA (3EFM), mapped to cluster18  
HHOMP'S: FhuA (1BY5) in 22.1.4  
It looks like a lot, but this all looks like one big blob on the clustermap. Still, I guess if they were more closely related, it would be concentrated to a point, and the sequences find their closest relatives.

First, I need to make aligned structures (of the structures in HHOMP, so that the z coordinate of each atom represents its distance from the plane running through the center of the membrane. I will do this by structural alignment to the homolog with which I will calculate zdiff.

I created a folder called "zdiff module/comparison structures". In this folder I made subfolders "cluster73", "cluster99", "cluster71" and "cluster18". IN each subfolder I put one of Daniel's aligned structures that was mapped to this cluster (file taken from "pymol/structures", which in turn was sent to me by Daniel a long long time ago, before I kept good records), as well as the structure (or structure**s**, in the case of cluster99) that HHOMP lists as belonging to the cluster (downloaded from www.pdb.org).

I opened Daniel's aligned structure of 3EFM and 1BY5 in PyMOL, and aligned 1BY5 to 3EFM using the "cealign" command. According to the PyMOL wiki, CEAlign is a structure-based alignment that does not use sequence information. I saved the result as "zdiff module/comparison structures/cluster18/1BY5 aligned to daniel's 3EFM.pdb".

I opened Daniel's aligned structure of 3BS0 and the PDB's 1T16. This has two chains, chain A and B, each of which is a β barrel. I deleted chain B using PyMOL's "remove" command. I aligned 1T16 to aligned\_3BS0 using cealign. I saved it as "1T16 aligned to daniel's 3BS0", following the same naming scheme. I also loaded Daniel's aligned structure of 1T16 and compared them. One of them is magenta and one of them is cyan in the picture below. It is oriented along the right axis. However, the difference in rotation is important. There has to be a strand-by-strand matchup with 3BS0; it has to have the same rotation about that axis as 3BS0.



Same procedure with 2MPR onto Daniel's 1A0S, had to delete all but chain A in 2MPR as with 1T16.

Did the same with 1POR and 2OMF, onto Daniel's 2J1N.

# September 9, 2012

I created multiple sequence aglinments with ClustalW, each containing the sequence of a PDB structure that is in an HHOMP cluster, the HHOMP cluster that contains it (according to the annotations of the clusters, since these sequences do not always appear in the MSA of the clusters given by HHOMP), and a structure from Daniel's dataset that was mapped to that cluster by HHOMP's search function. The alignments are in “zdiff module/gonnet aligned”. They were created using the following Python function:

***def zdiff\_align(matrix, output\_dir):***

***walk = list(os.walk('comparison structures'))***

***# Get the names of the clusters:***

***# [0][1] is the list of foldernames in the rootmost directory***

***clusters = walk[0][1]***

***for path, folder\_list, file\_list in walk[1:]:***

***# Establish what cluster we're talking about***

***for name in clusters:***

***if name in path:***

***cluster = name***

***for filename in file\_list:***

***# Align each pair of an HHOMP structure, and the structure in***

***# our dataset that was matched to the same cluster, with***

***# all the sequences of the cluster***

***match = re.match("(....) aligned to daniel's (....)\.pdb",***

***filename)***

***if match is None:***

***continue***

***# Retrieve from the regex match the pdbid of the protein in***

***# HHOMP:***

***hhomp\_pdbid = match.group(1)***

***# Retrieve from the regex match the pdbid of the protein in***

***# our dataset that was mapped to this cluster:***

***our\_pdbid = match.group(2)***

***# Retrieve the path names of the PDB files representing both***

***hhomp\_path = path + '/' + match.group(0)***

***our\_path = path + '/aligned\_{0}.pdb'.format(our\_pdbid)***

***# Make the alignment***

***# Create the output directory if it does not already exist***

***try:***

***# This will not successfully make the directory if***

***# nested directories would have to be created***

***os.mkdir(output\_dir)***

***except OSError:***

***# This is the error you'd get if the directory already***

***# exists***

***# So, our work is done, don't worry about it:***

***pass***

***output\_path = output\_dir + '/{}, {} as target, {} as template'\***

***.format(cluster, hhomp\_pdbid,***

***our\_pdbid)***

***alignments.align(output\_path, cluster, matrix,***

***hhomp\_path, our\_path)***

I ran this function with these arguments:

***zdiff\_align('gonnet', 'gonnet\_aligned')***

This function uses the “alignments” module that I wrote. The current code of this module can be found by looking at today's commit on my github repository, at “github.com/sinisterdexter/beta-barrel-oligomerization”.