

BCH222 - Ramachandran-Plot Data

Reading

Lovell et al. (2003) Proteins: Struct Funct Genomics **50**, 437 on validation around the C α (Section on C β deviation is optional.)

Why is the C α geometry important for validation?

Where is the γ -turn region located on the ϕ, ψ plot? _____

What residue type occurs most in the "left leg" below the β region? _____

Why don't cis prolines occur in the smaller, central peak of the Pro distribution?

What are two criteria that can help positively validate an individual outlier residue?

Gly->Ala mutational studies show that the general-case amino-acids in allowed but not favored ϕ, ψ regions are destabilized by approximately _____ kcal/mol?

When residues in secondary structure are omitted from the ϕ, ψ distribution, what changes?
_____ What does not change?

Graphics etc. assignment

To work in a spreadsheet file of Ramachandran data and in two different types of kinemages showing aspects of that data, and to evaluate specific outliers.

1. [rama4400-bch222-2011_short.csv.zip](#) (5.1MB)

Download the csv (comma-separated values) file, unzip it, and read it into Excel (or another spreadsheet program, if you prefer). How many total residues of data are there (last row number -1)? _____ Select all data (from upper-left column label to last lower-right entry), choose Data/Sort, say you have a header row, and sort on the "restype" column. Scroll down to find the Pro rows. How many are there? _____ Now select just the Pro data, and sort on the "omega" column. Scroll to the middle values near zero to find the cis prolines. How many are there between -30° and $+30^\circ$? _____ So, what is the percentage of cis prolines in this data? _____ For later, note that Pro 128 of 2fp2 has an aberrant omega angle of -52° . Quit without saving.

Go to <http://molprobity.biochem.duke.edu>, and fetch the 2fp2 file. Add hydrogens, analyze contacts & geometry, and download the multi-kin. Download the 2fp2 2Fo-Fc map from the electron density server at <http://eds.bmc.uu.se/eds/> (look for the Maps item on the left panel, calculate the map, and download it with a right-click). Open the multi-kin in KiNG. Use Edit/find point to center on Pro 128, zoom in, and turn on mainchain & sidechains. Is it buried or exposed? _____ Measure the omega angle for the peptide between Pro 127 and 128: _____° What two types of outliers are close? _____, _____ Is Pro 127 at a chain terminus or a disordered section? _____ Turn on H-bonds; how many are there to the peptides around 127-128? _____ Use Tools/Structural biology/Electron density maps to read in the map. Are the backbone O atoms in strong density? _____ Do the Pro rings show clearly? _____ Find residue B 128; is it a Rama outlier? _____ Is its electron density good? _____ Overall, do you think it's established that this omega angle is unusual? _____

2. [prepro-noGP_Blt30_wh.kin.gz](#) (636KB)

Open the prePro Ramachandran kinemage in KiNG, and go to the "smaller" view to see the plot edges. Note that the prePro distribution is a quite distorted version of the general Rama plot, with a sharp, rounded edge bitten out from the lower-right of the β region. Center near that edge and zoom in; is the highest concentration of data points near the center or near the ends of that curved edge? _____ Go back to the "smaller" view. There are two outlier datapoints very far from any contours, Trp 151 from file _____ and Ala 81 from file _____. Unfortunately there are no EDS maps for either of them. The Trp is probably correct and the Ala probably not, but one cannot be sure without the electron density. Locate the outlier point well to the left of the α -helical region; it is _____ from file 1hfu. Turn on Tools/Show XYZ coordinates; its ϕ, ψ is _____, _____.

For the outlier residue you just identified, repeat the process you did for the 2fp2 Pro, to make and download a multi-kin and a 2Fo-Fc map and view the outlier in KiNG. Is it buried or exposed? _____ Turn on H-bonds; how many are there to the peptides around 205-206? _____ Read in the 2Fo-Fc map. Are the backbone O atoms in strong density? _____ Do the sidechain shapes show clearly? _____ There is a bad clash between the Pro C δ and the previous the C β which can be lessened but not entirely removed by idealizing the Pro rotamer. Zoom out a bit; what interesting site can you find close by? _____

Overall, do you think it's established that this conformation is genuinely strained? _____

3. [rama4400_short_aaSort.kin.gz](#) (636KB)

Open the high-dimensional phi,psi kinemage in KiNG. Turn off Gly to see the expected ϕ, ψ distribution (note that this data does not have pre-Pro omitted). Turn sideways to see that each

amino-acid type is at a different level in Z. Look at view2 to see a closeup of the α -helical "comma". View4 shows psi vs tau (the bond angle at C α); although the scale is compressed, you can see that tau varies systematically with psi. Choose view3 to see the distribution vs omega; the red points in the center near omega=0 are the _____.

Choose view6 to see the ϕ, ψ distribution near omega=0. To add the general-case contours, append the kin file [general-noGPIVpreP_justContours_atZ90.kin](#). Turn the Pro button on & off to see which of these cis peptides are Pro (705 are Pro, 136 non-Pro). Turn on Tools/Show XYZ coordinates and pick points near the left edge of where the red Pro points start; that's at around phi = _____°

Go back to view1, and turn on each amino-acid type, one at a time.

Which type occupies all 4 corners of the ϕ, ψ plot? _____

Which two have the greatest concentration in the α peak? _____

Which two have almost no points in the L α region? _____, _____

Which two have the most points in the L α region? _____, _____

Which one has 4 distinct clusters in the β /extended region? _____