Prepare Project

- File > Projects Browser
- Click New Folder
- Browse to Appropriate Folder
- In File Dialog enter "nvjworkshop"
- Click Save
- Click New
- Enter "ubiquitin"
- Use IUPAC mode - Yes
- Link to another directory - No
- Directory doesn't exist, Create - Yes
- Should now have a green "ubiquitin" in Available Projects
- Outside of NvJ, browse to nvjworkshop->ubiquitin folder. Inspect contents
- Open nvjworkshop->ubiquitin->datasets folder
- Open another OS folder and browse to nvjdata->ubiquitin
- Move all the .nv and .par files to the project datasets folder
- Move the ubiq.seq and ubiq.pdb file to the project structures folder
- In the Projects Browser,(with Ubiquitin Project Selected) Click the Load button
- This will open all the datasets
- Close the Project Browser **Save**

Display and peak pick HNCO

- Open Datasets Table (Datasets > Datasets Table)
- Select the HNCO Experiment,Click Draw, Click Create
- Close Datasets Table **Save2**
- Adjust Contour Level of HNCO experiment
- Set on center plane, then show all planes and check level
- Set Level to 1.5, Click disk icon on SpecAttributes File Panel **Save3**
- Peak pick spectrum
- Goto PeakPick Tab of Spectrum Attributes Panel
- Click Pick, should get 79 peaks **Save4**
- Zoom into 8.4-7.2, 177-182.5 **Save5**
- Right click on spectrum, change cursor to peak delete

Delete weak peaks (67, 68, 70 , 71) **Save6**

- Display Peak Table (Peaks > Show Peak Table)
- Sort by Intensity and delete remaining weak peak (55) by clicking delete icon and top
- Compress and Degap List (Peaks > Edit > Compress and Degap, Yes)
- Close Peak Table **Save7**
- Lower contour level to 0.5
- Note peak at 10.1, 179.5 ppm
- Change cursor to PeakAdd, and click on above peak
- Return cursor to Crosshair (3 button) **Save8**

- Inspect Peaks
  - Return Level to 1.5
  - Display Peak Inspector (should be set to display hnco)
  - Goto SpecAttributes Peak Tab and set Show Mode to Expand
  - Goto first Peak in Inspector
  - Adjust spectrum display view bounds to give nice view
  - Change ShowMode to ExpandFixed
  - In Peak Table of Spectrum Attributes panel set Planes to 3 **Save9**
  - Step through all peaks and ensure that no nearby peaks missed, and any noise peaks deleted.
  - Return to full view of HNCO and click Heart Icon (favorites). Save as HNCO, then close window.
- Display, peak pick, save parameters, save favorite for other backbone experiments
  - HNCA Level 0.155, 203 Peaks, **Save10**
  - HNCACB Level 2.5, 319 Peaks, Pos/Neg Contours, **Save11**
  - HNCACO, Level 0.25, 152 Peaks, **Save12**
  - HNCOCA, Level 0.3, 114 Peaks, **Save13**
  - HNCOCACB, Level 0.08, 71 Peaks, **Save14** Load Protein Sequence
- Choose Molecules>Read/Write Topology>Sequence File
- Navigate to Projects Structures folder and open "ubiq.seq" **Save15**
- Verify Sequence is read in
  - Assign>Atoms menu item to display Atom Table
    Enter *.ca and hit return in entry next to Filter
    Scroll through and check sequence
    Close Atom Table

- Assign>Sequence menu tiem to display Sequence Browser
  Verify sequence and close browser
- Molecule>Analysis>Viewer to see Molecular Graphics
  Select Atoms... Backbone
  Display Add.. Lines
  Select Atoms... CA
  Display Add... Labels
  Close Viewer

Quit NMRViewJ and Restart as test to make sure you really are saving data

- Quit
- Restart
- Open Project Browser
- Select ubiquitin project
- Click Open
- Check Atoms in Atoms>Assign Table
- Open Peak Inspector and Check various lists

Setup RunAbout

- Open RunAbout from Analysis Window
- Set Reference list
  Select hnco list in top row, middle chooser (with triangle)
  Select hnco list in top row, left side chooser (combobox)
  "i" circle should be white, 75 peaks shown to right of "i" circle
- Open backbone lists
  Select hnca list in top row (backbone section), middle chooser (with triangle)
  Select hnco list in top row (backbone section), left side chooser (combobox)
  "i" circle should be white, 203 peaks shown to right of "i" circle
  Repeat for hncacb, hncaco, hnco, hncoca and hncocacb experiments in successive rows **Save16**
- Check Patterns
  Click the circle "i" button for the various lists and check the patterns
    Should be i.hn for all HN dimensions
    Should be i.n for all N dimensions

Should be pattern representative of experiment for C dimensions

HNCA i-1,i.ca

HNCACB i,i-1.cb{$intensity < 0},ca{$intensity > 0.0}

Make sure your CBs are negative and CA positive, or reverse intensities in pattern

HNCACO i-1,i.c

HNCO i-1.c

HNCOCA i-1.ca

HNCOCACB i-1.cb

- Set Tolerances

Either use (or modify) defaults in Tolerances section, and click Set or, click Auto to set tolerances based on median linewidths

For tutorial, click Auto

Check tolerances by clicking circle "i" icons. **Save17**

Peak Edit Mode

- Click Mode button at top of RunAbout window and select "Edit Peaks"
- Change RunAbout Tabbed window to the **Helm** Tab
- Display Spectrum Attributes View Panel, and click in each RunAbout Window
- Study View panel as you click mouse in different spectra.
- Understand the arrangement.
- Step through reference peaks by clicking up/down arrows next to peak number in Helm
- Experiment with the Controls in the View tab
- Turn off and on rows by clicking the check boxes in the Active Column (click Refresh each time)
- Turn off a full check box and set carbon shift range in MinY and MaxY columns
- Turn on and off the N Wings
- Return to cb,ca,c on and Full, and N wings on
- Look for
- Peak alignment
- Each window has vertical line at center, are peaks that you think are from same amide on that line
- Peak picking threshold
- Are there peaks with intensity on center lines, that don't

have peak boxes (perhaps you picked at too high a level)
- Are there more peaks than you might expect (perhaps you picked at too low of a level)
- Adjust alignment manually
- Goto peak 0 (for example)
- Make sure cursor is in crosshair mode (Use the Cursor menu at top of RunAbout)
- Position the black vertical crosshair in top row, 2nd column, a little too the left of peak (we're unaligning peaks)
- Change to the RunAbout Actions tab
- Click the Alignment - Manual button and answer Yes, Yes, Yes
- Now, move the crosshair on top of the peak and repeat above step to restore alignment.
- Adjust Alignment Automatically
- Click the Alignment - Automatic button in the Actions tab
- Watch for messages in the NMRViewJ console indicating the alignment changes. When five lists have been shown it's done. **Save18** (note, dataset reference is stored in .par files for each dataset, not STAR file)

Filter and Trim Peak Lists

- Go to the Actions Tab in RunAbout
- Select the HNCO list as the Filter Peak List
- Click the Filter Button
- Look at output in the NvJ console showing how many peaks deleted in each list **Save19**
- Check the trim number. Should be about 10% above number of residues (84)
- Click Trim
- Look at output in the NvJ console showing how many peaks deleted in each list **Save20**
- Click Compress/Degap to ensure that peaks marked for deletion are gone **Save21**

Cluster Peaks

- Click the Cluster Peaks button

- Should have result in console like: 4 cluster(s) with 1 peaks 1 cluster(s) with 2 peaks 5 cluster(s) with 9 peaks 7 cluster(s) with 10 peaks 31 cluster(s) with 11 peaks 13 cluster(s) with 12 peaks 8 cluster(s) with 13 peaks 5 cluster(s) with 14 peaks 1 cluster(s) with 18 peaks
- Switch to Cluster Edit Mode
- Change to the Helm Tab
- Inspect the Spectra to see that the layout is the same as in Peak Edit Mode
- Note that the peaks are now labeled with a cluster number.
- Use the LabelType menu at top of RunAbout to change the labels (return to Cluster label mode) **Save22**
- Find the Clusters area (contains alternating black and blue numbers with "Clusters / All " at top.
- There's one number for each cluster.
- Each cluster number corresponds to the peak number of the HNCO peak that is in each cluster
- Change the combobox at the top of Cluster area (All,Correct,Lonely,Missing, Extra).
- Try different values and then show different clusters (click on a cluster number to see that cluster) or click the up/down arrows in the Edit Cluster section to step through clusters.
- Observe
  - The pattern of peaks in the spectra
  - The list of peaks in the Peaks in Cluster section
  - The number of peaks in the NPeaks entry in the Current Cluster section (and its color)
  - Asterisks next to peaks in the "Peaks in Cluster"
  - Yellow triangles in spectrum display (likely spots for missing peaks, new and probably not very reliable feataure)
  - Orange triangles in spectrum display (likely spots for extra (artifactual peaks)
- Lonely Clusters
- View them with the Lonely Setting in the Clusters section
- Go to the Actions Tab and click Purge Lonely
- Where'd they go **Save23**
- Clusters with Missing Peaks
- View them with the Missing setting in the Clusters section
- Yellow triangles show next most intense peaks (that weren't

picked). At least useful in showing which spectra have missing peaks, and sometimes point to the correct spot.

Cluster 2, Lower contour levels and see that there is intensity at the yellow triangle, but the missing peak is probably because the beta of the two residues in cluster overlap each other.

Clusters 26 and 49, Triangle probably correctly points to missing intra residue peak in hncaco

Goto cluster 26, Click in hncaco H-C window, hit the "z" key.

Zoom window appears.

Place mouse over peak that wasn't pciked (at about 175.7 ppm), rest mouse pointer over peak, click the "a" key (note key descriptions at bottom of spectrum)

Click z key again to unzoom **Save24**

- Clusters with Extra Peaks
- View them with the Extra setting in the Clusters section
- Check out cluster 0
- Weak peak in hncoca (marked with asterisk in "Peaks in Cluster" area and orange triangle in spectra (only visible as weak peak in H,N view
- Zoom into hncoca H,N view, select the weak peak, click the "d" key
- Check out cluster 6
- Find the weak peak
- Click the "Trim" button
- Note that when you click Trim (or remove peak manually, the cluster no longer has extra peaks and disappears from "Extra" list)
- Click on Trim button in a few clusters, note that you jump to the next cluster with extra peaks
- Get tired of doing them one by one, Go to Actions panel and click "Trim All"
- Go back to Helm, refresh "Extra" setting, note there are no more clusters with "Extra" peaks. **Save25**
- Remember that weak peak we added
- Goto cluster 70

- Lower contour levels, Is it real, how do we know?
- Zoom into the hncoca, hnca, hncocacb and hncacb spectra and add peaks till there are 8 in cluster **Save26**

The Cluster Inspector

- Basic features
- Set Clusters display mode to All
- Goto cluster 0
- Click the blue "i" button (i for Inspector)
- Check out the Yellow boxes
- Look at the amino acids lists on the left and right sides. Which ones are highlighted in blue.
- Think about the CA and CB shifts for the i-1 and i peaks and compare with the highlighted amino acids.
- Check out clusters 1, 2 and 3 and the highlighted amino acids
- Peak Type Assignment
- Goto cluster 11
- Look carefully at what's in the yellow boxes. Change the PPM / PeakNum setting at top of inspector
- Compare the red/blue bars in inspector with the colors of peaks in spectra
- Look at the position of the labels on peaks in spectra. Are they all the same.
- Compare the peak label positions with the peak number positions in inspector
- Look at a column of boxes in Inspector, what are the shifts in the three rows at bottom?
- Note the weak negative peak in hncacb box. Goto spectrum, zoom in, find peak, delete it **Save27**
- Note that peak 47 (at 42.6 ppm) appears as a i-1 and i CB peak. Click on its i-1 box and drag it over to the i position.

  You've changed the type of this peak, check out the bottom three rows of shifts.
- There's one missing peak. The i-1.CA of the HNCACB experiment. We can add it by zooming into the hncacb experiment and adding a peak next to the existing CA.
- If we look at the inspector, we see that the new peak, 273,

as an i.CA. Drag it over to the i-1 position.

- Now we have cluster 11 complete. **Save28**
- Check out cluster 32. It has an artifactual peak in the hncacb. We can delete it directly in the Inspector by hovering the mouse over it and clicking the Delete key. **Save29**
- Fix up 33,39, 40, 41, 44, 4?,54, 57, **Save29**

Linking Clusters

- Change the mode to Edit Links
- Go to Cluster 0
- Examine the spectra display.
- Use the Spectrum Attributes panel
- Click in each window and understand what is being displayed
- Click the various entries in the left list box in Matching Clusters and study the change in the left column of spectra.
- Repeat with right list box and right column of spectra
- Compare the top left list entry "9 2.65 3 0 RAV" with the next one "20 0.93 1 62 AV"
- Click between the two and study the change in the left spectra column. How do the peaks in the left most column compare with the peaks in the columns to the right.
- You're at cluster 0. Look at the first row in left list box.
- Go to cluster 9. Look at the entries in the right list box and the right hand spectra.
- Confirm Links
- In Cluster 0 highlight left listbox row 0 (with cluster 9) and click the Check icon.
- Note the listbox changes to yellow and the in row 0 changed to R_V
- Click the left pointing blue arrow
- Where are you now? Why?
- Look at the Sequence display. Note the green and magenta highlights.
- Look at the Clusters area. Note the change in the order of cluster numbers. See the Fragment
- Continue confirming clusters and clicking the left pointing blue arrow.

- End up at cluster 53. What's different about the row of information in listbox.
- Look at sequence display. What residue are we at?
- Go back to cluster 0 and work in the right hand direction.
- Get to cluster 68. Why is the third column of the first right hand list box row a "2" not three (look at sequence).
- Confirm cluster and continue
- Continue to cluster 40. Give up! **Save31**

- Check out the Graphs Tab
- Click Draw
- Use crosshairs to zoom in as in normal spectrum

Goto Cluster 1

Confirm left hand cluster (to cluster 16)
Study sequence coloring and Match Scores section
Goto Cluster 16 and confirm it
Study sequence coloring and Match Scores section
Continue till you've confirmed the cluster 4-51 link
Check out sequence display and Match scores

- Extending matches automatically

At cluster 4, click the Extend button (below Clusters area).
Wow **Save32**
Click on ILE 61 in Sequence display. Note that you jump to the corresponding cluster (12)
Why did the auto extension stop here?
Look at the first row of left box (43 1.80 2 12 RAV)
Go to the Parameters tab and check out the "Extend Min Matches" and "Extend Score" settings
Return to the Helm, and check out the left two spectra in top row.
Look at the Labels on the peaks, Compare them with the labels on the peaks in middle and bottom row.
Goto cluster 43 and click the blue "i" icon to get the Cluster Inspector
Delete the weak CB peak in the HNCACB and drag the strong negative peak from i-1 to i.CB
Look at Matching Clusters score
Go back to cluster 12 and click Extend
Yippee

- Freezing Clusters
    - Click on a cluster in the longest fragment
    - Note the sequence display
    - Click the Freeze button
    - Repeat for the other fragment
    - Open Atom table (Assign > Atoms)
    - Enter a filter of *.ca,cb,h,n and scroll to bottom of table and note that the C terminus residues are assigned
- Goto cluster 2 and Click extend **Save33**
- Manually confirm the C terminus glycine of new fragment and click extend again. **Save34**
- Goto N terminus of Fragment (click on F4 in sequence box)
- Look at score information and hncacb-hncocacb spectra
    - Zoom in on hncacb (at top left)
    - Add (hover mouse over peak intensity and click "a") a new peak to the hncacb list.
    - Zoom back out
    - Check out the scores and manually confirm 26-45
    - Manually confirm to N terminus
    - Freeze Fragment **Save35**
- Repeat process for remaining clusters
- From cluster 5, extend check (the 57 to 54 connection is interesting) Add peak to 54, you might need to adjust the position of a peak in 57) **Save36**
- Hmmm, 54-57 isn't viable? Confirm 54-64.
- Now things look OK, confirm 57-54 **Save37**
- Only thing left is 61-62. 62 is a little funky. Use Cluster Inspector and delete CB peak (gly doesn't have CB)
- Now we can confirm 61-62
- Freeze that fragment to add in the C-terminal gly **Save39**
- Click on each fragment and freeze again just to be sure everything is saved to an atom assignment **Save40**

Study Assignments

- Assign > Atoms Panel
- Filter *.ca,cb,c,n,h
- Sort by PPM
- What atoms are unassigned

- Reference>BMRB_Avg
- Sort by DPPM
- What Atoms have large deviations
- Experiment with Reference>Delta Mode Setting
- Experiment with different Reference Set Values

- LACS Plot
- Assign>Atoms Panel, File>Analyze Shifts > LACS PLot
- * Click on CA, CB,C in LACS Plot
- Look at intersection of two lines (for CA and CB) modes

- Compare with Existing Assignments
- Atoms Panel, at top, change "Set" to "1"
- Atoms Panel, File> Read Chemical Shifts > Read PPM From STAR3 to...
- Browse to bmrb17439.str (in nvjwworkshop/nvjwdata/ubiquitin folder) and open it
- PPM Set 0, OK (this referes to the first set (set 0) to be found in STAR file)
- Toggle between Set 0 and Set 1
- From Reference Menu (Atoms Window), Choose Reference Set 1
- Now sort by DPPM. This is a comparison of our assignments with those in the bmr17439 file.

- Do various plots in Sequence Display (CSI, SPP, PSSI)

Sidechain Assignments with HCCH Tocsy

- Peak pick Tocsy
- From Datasets Table, Draw hcchtoc18 at contour level 0.12
- Draw all planes **Save41**
- Goto PeakPick Tab of Spectrum Attributes Panel
- Adjust Noise Slider to 10.5
- Click Pick (with list name box empty)
- Message appears "Slice rms not measured...", Click Yes
- Should have 1693 peaks **Save42**
- Goto center plane, then navigate up and down planes.
- Verify that peaks near, but not on water stripe picked

- Remove wiggly peaks
- Open Peak Inspector and select the hcchtocsy experiment
- Select Peaks->Edit>Filter menu item
- Highlight wiggle removal checkbox (bottom of window)

- Select H1 dimension, and Click proceed
- Repeat for H2 and C dimensions
- Number of peaks at top right should be 1191
- Close Filter window **Save43**
- Save tocsy window as favorite and then close
- RunAbout Tocsy Mode
- Open up RunAbout
- Select the hcchtoc18 list in menu to the right of hcchtocsy in Sidechain section of Parameters
- Click the red "i" to bring up pattern window.
- Set patterns, tolerance and relation as i.h* 0.012 i.h* 0.024 D3 c.h* 0.518
- Click Apply and close Pattern window (circle "i" should now be white) **Save44**
- Goto Actions Tab and click "Cluster Peaks" in the HCCH Peaks section (not the H,N section)
- Change mode to HCCH Clusters and switch to Helm **Save45 Save46**
- Browse through a few clusters and figure out what you're seeing
- What are the lines (compare with numbers in the list box in helm)
- What's the green line
- Why is it sometimes orange
- Enter 500 in HCCH Cluster and hit return.
- Should jump to 379 (last cluster)
- Step through clusters
- Delete 0 (note that clusters renumbered)
- Add diagonal to 1 (Click "Add Diag." button)
- Delete 2, Delete new 2, Delete 5, 5 again
- Continue deleting noise and adding diagonals through about peak 37 **Save47**
- Go to Actions panel and click "Auto Add Diagonal" **Save48**
- Step through all clusters, deleting artifactual ones and adding remaining diagonals
- I ended up with 263 clusters **Save49**
- An 3D 15N NOE HSQC experiment is useful
- Display the noesqc experiment at level 0.2 and draw all planes
- Peak pick whole spectrum with noise slider at 10.5

- I got 3100 peaks **Save50**
- Open Peak>Edit>Couple
- Set Dim 1 max to 24 Hz and click the couple button
- Now 2754 peaks **Save51**
- Do Wiggle Removal (Peak>Edit>Filter) on all three dimensions
- Now 1443 peaks **Save52**
- In RunAbout Parameter pane set n15Noe peak (Sidechain section) to be noesqc
- Display Pattern dialog (click "i") and set Pattern,Tolerance, Relation to: i.hn 0.04 D3 j.h* 0.06 i.n 0.3
- Click Apply and Close Pattern dialog. Go back to Helm, Reset Mode to HCCH Assignment
- You should now see four strip windows **Save53**
- Refresh display by either typing a return key in the Residue box, or going to the next residue and back. You should end up on Residue 1
- Open the spectrum attributes panel and look at what is displayed in windows 1,2 and 4
- In The HCCH Peaks area try changing the "All", "Check Peak", and "Check Int" setting. Observe the change in the number of peaks.
- Turn on the "Check Peak" setting
- The left most box (1) is showing protons (presumably HA) that have peaks at the CA shift of this residue, and which also have peaks at the CB shift (because we have the "Check Peak" box on. The box should contain peaks 1000,233, 253, and 168.
- Click on each of the rows in the list and note how the leftmost spectrum changes. Note the vertical green line at the shift of the peak your clicking on, and the horizontal green line that intersects it at the same shift (showing diagonal peak).
- As you click on the different peaks note the changing entries in the protons list box. Which protons seem reasonable for a methionine.
- Use information from NOESY spectrum
- With a NOE Res setting (near top of Helm) of "1", the NOE spectrum is shown for the region of the next residue.

What NOEs are you likely to see in this strip.

- Turn on crosshairs (Cursor menu at top of RunAbout).
- Slide Crosshairs through the NOESY spectrum and note how the peaks in the first window align with the NOE peaks. Which of the choices of HA in list "1" give peaks that line up with the NOESY.
- Assign the HA atom
- Click on peak 168 in the protons list (which has the same shift)
- Note that the carbons list now has entries. Click on the top one (best scoring) and observe the third spectrum.
- Also note the color changes in the Atom Assignments section and any numbers (0.73) in the right hand column. The 0.73 is the probability that the proton shift of 4.146 and carbon shift of 54.8 is consistent with the ha and ca of a Met residue. The green indicates that those atoms have a significant probability of being the assignment.
- Click on the ha (should be green button). Note that the chemical shift of the peak is entered.
- Assign the other atoms
- Click on peak 187 in list, and then top scoring carbon peak. Looks, from probability score most like the hg*
- Click on the hg2 button, note message about using Carbon plane. Say no. Note assignment for hg2 entered in, but not the carbon shift
- Make sure cursor is in selector mode. In the third spectrum click on one of the peaks along the green vertical line (one of the Met1 protons).
- Now click the hg2 button again. Note, no message, and the carbon is assigned.
- hg3 is probably on top of hg2, so we can assign it the same value. Note the message you get about reassigning the carbon. Just say no.
- Assign the hb2 and hb3 atoms (at 2.025 and 1.899). Because the CB shift is assigned from the backbone experiments we won't be prompted to assign it. **Save54**
- Assign Residue 3 **Save55**

- Assign Residue 4, Note, no good HA proton in Check Peak mode, try Check Int. **Save56**
- Assign 5
- Ignore **Save57 Save58**
- Assigning 5 doesn't seem right. Peaks 53,63,75 seem like Val, but aren't showing up in list 1
- Expand HCCHdiag tolerance (only peaks that are diagonal show up in list 1)
- Change from 0.01 to 0.02 in Parameters Pane
- Assign through Res 15 **Save60**
- Cluster 18 missing a peak
- Continue through residue 31 **Save61**
- Cluster 37 missing peak (Lys HE) Goto Cluster editor and add **Save62**
- Assign through 36 **Save63**
- Finish going through assignments **Save64 Save65 Save66 Save67**