Protocol for Guanidinium Denaturation Studies

I. Make Stock Solutions

A. Make ~ 7.5 M Gua·HCl titration
   1. Dissolve 358 g Gua·HCl in ~30 mL water
   2. Volume will expand
   3. Add small amounts of water until solid is entirely dissolved
   4. Bring volume up to 500 mL
   5. Measure concentration of solution by refractive index measurements (Pace CN, *Meth. Enzymol.* **1986**, *131*, 266-280)

B. Make ~ 500 μM peptide solution
   1. Purge ~ 100 mL of water with Argon
   2. Pipet 10 mL of water into Falchion Tube
   3. Add 5.7 mg TCEP and dissolve (TCEP reduces thiols)
   4. Add 25 mg L16C and dissolve
   5. Aliquot into 10 1 mL eppendorf tubes
   6. Store 9 eppendorf tubes in freezer
   7. Determine concentration using last eppendorf tube
      a. Send 200 μL away for amino acid analysis (Core facilities)
      b. Determine thiol concentration using Ellman's test
         1. Turn on UV spectrometer
         2. Make four solutions of 1.5 μL, 1.0 μL, 0.5 μL, 0 μL peptide solution in 3.0 mL 0.1 M sodium phosphate buffer pH 8
         3. Add 100 uL DTNB reagent solution to each vial
         4. Mix and allow to stand for 15 minutes
         5. Take UV spectrum of each solution (500-350nm)
         6. Plot line of A(410 nm) vs μL peptide solution added
         7. [thiol] in stock = (3000 x slope) / 13650

II. Go to spectrometer to warm up lamp (Room 2609)

A. Sign in!
B. Turn on CD spectrometer
   1. Flip large switch on right and smaller one on left

III. Make Solutions for Titration

A. Make peptide solution (5 μM peptide, 5 mM K^+-Phosphate)
   1. Thaw one of the peptide solutions stored in the refrigerator

2. Purge ~ 100 mL water and 500 mM K^+-Phosphate (pH 8.0) with Argon
3. Mix the following solutions
   14.7 mL water
   150 μL 500 mM K^+-Phosphate (pH 8.0)
   150 μL peptide solution
4. pH to desired pH using 1.0 M HCl or 1.0 M NaOH

B. Make Gua·HCl/peptide solution (Gua·HCl ~ 7M, 5 μM peptide, 5 mM K^+-Phosphate)
1. Purge Gua·HCl stock solution with argon
2. Mix the following solutions
   14.7 mL Gua·HCl stock solution
   150 μL 500 mM K^+-Phosphate (pH 8.0)
   150 μL peptide solution
3. pH to desired pH using 1.0 M HCl or 1.0 M NaOH

C. Purge both solutions with argon for ~ 5 minutes after mixing to remove any gas that might lead to bubble formation in the titrator

IV. Go to Spectrometer with

A. Peptide and Gua·HCl/Peptide Solutions (≥ 15 mL each)
B. Empty 40 mL Falchion Tube (for Waste)
C. Water (40 mL)
D. Quartz cuvette (1 cm pathlength) with square top
E. Styrofoam block to stabilize Falchion Tubes
F. Pipetman (1000 mL) with tips
G. Box of Kimwipes
H. 3.5” Disk

V. At the spectrometer

A. Spark Lamp by pressing red button (read lamp time and record)
B. Turn on computer by flipping middle switch
C. Prepare titrator with Gua·HCl/peptide solution
1. Place both intake (right) and export (left) tubes into H_2O
2. Flip switch on titrator down
3. Allow syringes to fill & dispense 5 times
4. Center switch
5. Place tubes in Gua·HCl/peptide solution
6. Repeat steps 2-4

D. Prepare cuvette
1. Place 2.0 mL peptide solution in cuvette
2. Place stir bar in cuvette
3. Place titrator cap on top
4. Tap out any bubbles
5. Place cuvette in CD at position 0

6. Put top on CD instrument

E. Prepare computer
   1. Log in
   2. Start CD-202 program
   3. Load Gua·HCl titration configuration
      a. From FILE menu, choose READ CONFIGURATION FILE
      b. Choose File “GuaTit.CFG” in D:/btfarrer/BABYtitrations/
   4. Check to verify conditions
      a. Choose “Configure Experiment”
      b. Type description of experiment
      c. Type file name
         (To turn counter to 1, choose “Experiment Configuration” and “set experiment counter” and reset to 1)
      d. Wavelength = 222 nm
      e. Bandwidth = 1 nm
      f. Make sure auto slit closure is selected
      g. Temperature = 25 C
      h. Choose Titration under “Type”
         1. Under “Experiment Configurations” choose “Titration Configuration”
         2. Initial titrant in cell = 0 M
            Titrant concentration in syringe = 7.63 M
            Sample in cell = 5×10^{-6} M
            Sample in syringe = 5×10^{-6} M
            Target concentration = 6.6 M
            Concentration step = 0.2 M
            Stir Time (min) = 2.0
            Averaging time = 1.0 sec
            Sample Volume = constant
            Initial cell volume = 2.0 mL
         3. Hit review for list of concentrations, time, and volume
            Are they reasonable
         4. Hit okay
      i. Exit and save configuration

F. Start Stirring
   1. Select Control Panel
   2. Select Stir Control
   3. Set Speed = 50; Turn “ON”; Unclick “Stir During Measurements”

G. Run Experiment
   1. Check to make sure wavelength is set to 222 nm
   2. Hit the “Run Experiment” Key

H. After Experiment is Done, Save Data to Disk

I. Do reverse titration
   1. Prepare Titrator with peptide solution (Section IV-C above)
      (Note: Do not remove solution from cuvette)
   2. Set up computer for Reverse titration

a. See IV-E-3 to load titration configuration “Revers.CFG”
b. See IV-E-4 to check conditions (Same as above except:)
1. Initial titrant in cell = 6.6 M
Titrant concentration in syringe = 0 M

	Domain1	Domain2
Target concentration =	1.0 M	0.5 M
Concentration step =	0.2 M	0.1 M
Equilibration Time =	2 min.	2 min.

3. Start Stirring (See IV-F)
4. Run Experiment
5. After Experiment is complete, save data

J. Shutdown Computer
K. Sign out
L. Turn off all switches below CD before leaving

VI. Work up data as described in Creighton TE, Protein Structure: A Practical Approach, 2nd Ed., 1997, Chapter 12, p 299-322