Biophysical *Journal*
Computational Tool

Biophysical Society

PyFolding: Open-Source Graphing, Simulation, and Analysis of the Biophysical Properties of Proteins

Alan R. Lowe,[1,2,3,*] Albert Perez-Riba,[4] Laura S. Itzhaki,[4] and Ewan R. G. Main[5,*]
[1]London Centre for Nanotechnology, University College London, London, United Kingdom; [2]Department of Structural and Molecular Biology, University College London, London, United Kingdom; [3]Department of Biological Sciences, Birkbeck College, University of London, London, United Kingdom; [4]Department of Pharmacology, University of Cambridge, Cambridge, United Kingdom; and [5]School of Biological and Chemical Sciences, Queen Mary University of London, London, United Kingdom

ABSTRACT For many years, curve-fitting software has been heavily utilized to fit simple models to various types of biophysical data. Although such software packages are easy to use for simple functions, they are often expensive and present substantial impediments to applying more complex models or for the analysis of large data sets. One field that is reliant on such data analysis is the thermodynamics and kinetics of protein folding. Over the past decade, increasingly sophisticated analytical models have been generated, but without simple tools to enable routine analysis. Consequently, users have needed to generate their own tools or otherwise find willing collaborators. Here we present *PyFolding*, a free, open-source, and extensible Python framework for graphing, analysis, and simulation of the biophysical properties of proteins. To demonstrate the utility of *PyFolding*, we have used it to analyze and model experimental protein folding and thermodynamic data. Examples include: 1) multiphase kinetic folding fitted to linked equations, 2) global fitting of multiple data sets, and 3) analysis of repeat protein thermodynamics with Ising model variants. Moreover, we demonstrate how *PyFolding* is easily extensible to novel functionality beyond applications in protein folding via the addition of new models. Example scripts to perform these and other operations are supplied with the software, and we encourage users to contribute notebooks and models to create a community resource. Finally, we show that *PyFolding* can be used in conjunction with Jupyter notebooks as an easy way to share methods and analysis for publication and among research teams.

INTRODUCTION

The past decade has seen a shift in the analysis of experimental protein folding and thermodynamic stability data from the fitting of individual data sets using simple models to increasingly complex models using global optimization over multiple large data sets [examples include (1–21)]. This shift in focus has required moving from user-friendly, but expensive software packages to bespoke solutions developed in computing environments such as MATLAB and Mathematica or by using in-house solutions [examples include (3,6,12,21,22)]. However, as these methods of analysis have become more essential, simple curve-fitting software no longer provides sufficient flexibility to implement the models. Thus, there is an increasing need for substantially more computational expertise than previously required. In this respect, the protein folding field contrasts with other fields, for example x-ray crystallography, where free or inexpensive and user-friendly interfaces and analysis packages have been developed (23).

Here, we present *PyFolding*, a free, open-source, and extensible framework for graphing, analysis, and simulation. At present, it is customized for the analysis and modeling of protein folding kinetics and thermodynamic stability. To demonstrate these and other functions, we present a number of examples as Jupyter notebooks. The software, coupled with the supplied models/Jupyter (iPython) notebooks, can be used by researchers with less programming expertise to access more complex models/analyses and share their work with others. Moreover, *PyFolding* also enables researchers to automate the time-consuming process of combinatorial calculations, fitting data to multiple models or multiple models to specific data. This enables novice users to simply replace the filenames of the data sets with their own and execute the same calculations for their systems. For more advanced users, new models and functionality can be added with ease by utilizing the template models. The Jupyter notebooks provided also show

Submitted September 21, 2017, and accepted for publication November 27, 2017.

*Correspondence: a.lowe@ucl.ac.uk or e.main@qmul.ac.uk

Editor: Daniel Raleigh.
https://doi.org/10.1016/j.bpj.2017.11.3779

how *PyFolding* provides an easy way to share analysis for publication and among research teams.

MATERIALS AND METHODS

PyFolding was developed using Python 2.7 and additional libraries NumPy, SciPy, and Matplotlib. Analyses were performed on either an i5 Macbook Pro with 8 Gb RAM running macOS Sierra, a Dell Precision T3600 Workstation running Ubuntu 16.04LTS with 64 Gb RAM and an NVIDIA GTX1080 GPU, or a virtual PC running Windows 10 (64 bit) in VirtualBox on an i7 Macbook Air. Example data for the associated notebooks were taken from existing publications or extracted from original publications using *engauge digitizer* (https://github.com/markummitchell/engauge-digitizer). The *PyFolding* software, notebooks, and example data are distributed through *github* at https://github.com/quantumjot/PyFolding.

RESULTS AND DISCUSSION

PyFolding is implemented in Python and is distributed as a lightweight, open-source library through *github* and can be downloaded with instructions for installation from the authors' site (https://github.com/quantumjot/PyFolding). *PyFolding* has several dependencies, requiring Numpy, SciPy, and Matplotlib. These are now conveniently packaged in several Python frameworks, enabling easy installation of *PyFolding* even for those who have never used Python before (described in the "SETUP.md" file of *PyFolding* and as a series of instructional videos to demonstrate the installation and use of *PyFolding*; https://github.com/quantumjot/PyFolding/wiki). As part of *PyFolding*, we have provided many commonly used folding models as standard, such as two- and three-state equilibrium folding and various equivalent kinetic variations (Supporting Material, *Jupyter notebooks 1–4 and 8*). Functions and models themselves are open source and are thus available for inspection or modification by both reviewers and authors. Moreover, due to the open-source nature, users can introduce new functionality by adding new models into the library, building upon the template classes provided. We encourage users to contribute notebooks and models to create a community resource.

Fitting and evaluation of typical folding models within *PyFolding*

PyFolding uses a hierarchical representation of data internally. Proteins exist as objects that can have metadata as well as multiple sets of kinetic and thermodynamic data associated with them. Input data such as chevron plots or equilibrium denaturation curves can be supplied as comma separated value files (CSV). Once loaded, each data set is represented in *PyFolding* as an object, associating the data with numerous common calculations. Models are represented as functions that can be associated with the data objects you wish to fit. As such, data sets can have multiple models and vice versa enabling automated fitting and evaluation (Supporting Material, *Jupyter notebooks 1–3*). Parameter estimation for simple (non-Ising) models is performed using the Levenberg-Marquardt nonlinear least-mean-squares optimization algorithm to optimize the appropriate objective function [as implemented in SciPy (24)]. The output variables (with SE) and fit of the model to the data set (with R^2 coefficient of determination and 95% confidence levels) can be viewed within *PyFolding* and/or the fit function and parameters written out as a CSV file for plotting in your software of choice (Supporting Material, *Jupyter notebooks 1–3*). Importantly, by representing proteins as objects, containing both kinetic and equilibrium data sets, *PyFolding* enables users to perform and automate higher-level calculations such as Φ-value analysis (25,26), which can be tedious and time-consuming to perform otherwise (Supporting Material, *Jupyter notebook 3*). Moreover, users can define their own calculations so that more complex data analysis can be performed. For example, multiple kinetic phases of a chevron plot (fast and slow rate constants of folding) can be fitted to two linked equations describing the slow and fast phases of a three-state folding regime (Fig. 1; Supporting Material, *Jupyter notebook 4*). We believe that this type of fitting is extremely difficult to achieve with the commercial curve-fitting software commonly employed for analyzing these data, owing to the complexity of parameter sharing among different models and data sets.

More complex fitting, evaluation, and simulations using the Ising model

Ising models are statistical, thermodynamic, nearest-neighbor models that were initially developed for ferromagnetism (27,28). Subsequently, they have been used with great success in both biological and nonbiological systems to describe order-disorder transitions (12). Within the field of protein folding and design, they have been used in a number of instances to model phenomena such as helix-to-coil transitions, β-hairpin formation, prediction of protein folding rates/thermodynamics, and with regards to the postulation of downhill folding (6,12,20,29–34). Most recently, two types of one-dimensional (1-D) variants have been used to probe the equilibrium and kinetic un/folding of repeat proteins (3,12,17,21,22,35,36). The most commonly used, and mathematically less complex, has been the 1-D homopolymer model (also called a homozipper). Here, each arrayed element of a protein is treated as an identical, equivalent, independently folding unit, with interactions between units via their interfaces. Analytical partition functions describing the statistical properties of this system can be written. By globally fitting this model to, for example, chemical denaturation curves for a series of proteins that differ only by their number of identical units, the intrinsic energy of a repeated unit and the interaction energy between the folded units can be delineated.

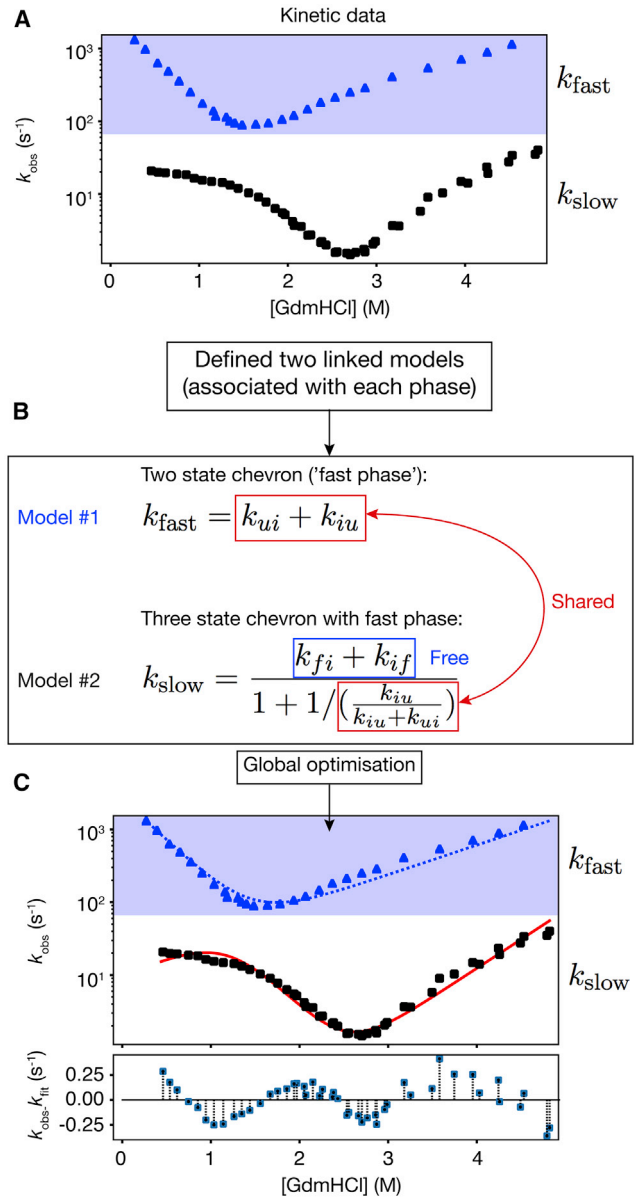


FIGURE 1 Work flow example of the fitting linked equations in *PyFolding*. (*A*) Unfolding and folding kinetics (*chevron plots*) showing the distinct fast and slow phases for the three-state folding thermophilic AR protein (tANK) identified in the archaeon *Thermoplasma* (2) are loaded into *PyFolding* as chevron objects. (*B*) Two linked models (functions) are associated with the chevron data. These describe the fast (*model 1*) and slow phases (*model 2*) of the chevrons. Certain rate constants and their associated *m*-values are shared between the two models. The other parameters are "free" and associated and fitted only in the slow-phase model. (*C*) Global optimization within *PyFolding* enables simultaneous fitting of the two models with shared parameters to the two respective phases. The resultant fits for the fast (*blue dotted line*) and slow phases (*red solid line*) are shown overlaid on the observed data. The residuals show the difference between the slow-phase observations and fit. These calculations can be found in Supporting Material, *Jupyter notebook 4*. GdmHCl, guanidinium chloride. To see this figure in color, go online.

However, this simplified model cannot describe the majority of naturally occurring proteins where subunits differ in their stabilities, and varying topologies and/or noncanonical interfaces exist. In these cases, a more sophisticated and mathematically more complex heteropolymer Ising model must be used. Here, the partition functions required to fit the data are dependent on the topology of interacting units and thus are unique for each analysis.

At present, there is no freely available software that can globally fit multiple folding data sets to a heteropolymer Ising model, and only a few that can adequately implement a homopolymer Ising model. Therefore, most research groups have had to develop bespoke solutions to enable analysis of their data (3,21,22,35,36). Significantly, in *PyFolding* we have implemented methods to enable users to easily fit data sets of proteins with different topologies to both the homozipper and heteropolymer Ising models. To achieve this goal, *PyFolding* presents a flexible framework for defining any nondegenerate 1-D protein topology using a series of primitive protein folding "domains/modules" (Fig. 2). Users define their proteins' 1-D topology from these domains (Supporting Material, *Jupyter notebooks 5–6*). *PyFolding* will then automatically calculate the correct partition function for the defined topology, using the matrix formulation of the model [as previously described (12)], and globally fit the equations to the data as required (Supporting Material, *Jupyter notebooks 5–6*). The same framework also enables users to simulate the effect of changing the topology, a feature that is of great interest to those engaged in rational protein design (Supporting Material, *Jupyter notebook 7*).

To determine a globally optimal set of parameters that minimizes the difference between the experimental data sets and the simulated unfolding curves, *PyFolding* uses the stochastic differential evolution optimization algorithm (37) implemented in SciPy (24). In practice, experimental data sets may not adequately constrain parameters during optimization of the objective function, despite yielding an adequate curve fit to the data. It is therefore essential to carefully assess the output of the model to verify the validity of any topologies and the resultant parameters. A description of how *PyFolding* provides the error estimates and determines how constrained parameters are is given in the error analysis section below. As with the simpler models, *PyFolding* can be used to visualize the global minimum output variables (with SEs) and the fit of the model to the data set (with R^2 coefficient of determination) (Supporting Material, *Jupyter notebooks 5–6*). The output can also be exported as a CSV file for plotting in your software of choice. In addition, *PyFolding* outputs a graphical representation of the topology used to fit the data and a graph of the denaturant dependence of each subunit used (Fig. 2). Thus, *PyFolding* enables nonexperts to create and analyze protein folding data sets with either a homopolymer or heteropolymer Ising model for any reasonable 1-D protein

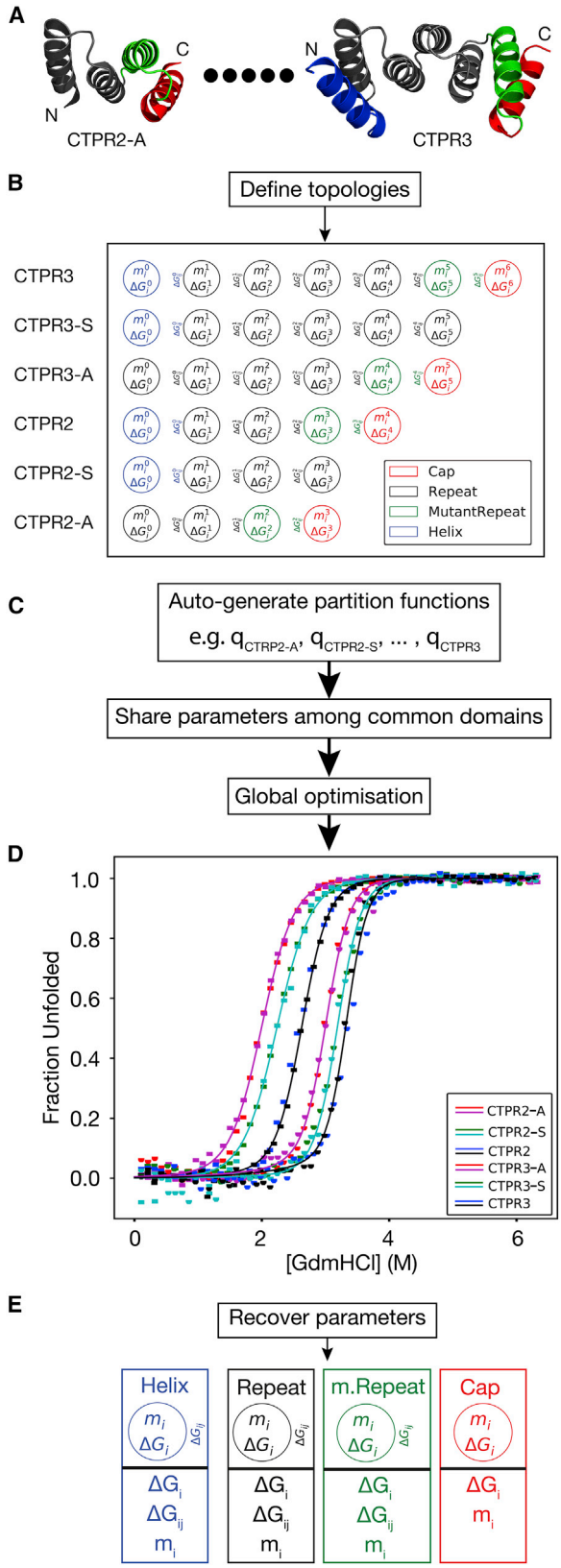


FIGURE 2 Work flow example of global optimization of a heteropolymer Ising model in *PyFolding*. (*A*) Guanidinium chloride (GdmHCl)-induced equilibrium denaturations of a series of single-helix deletion CTPRn proteins are loaded into *PyFolding* as EquilibriumDenaturation objects. In the figure, we schematically represent these as individual protein structures corresponding to the smallest in the series (*CTPR2-A*) up to (*dots*) the largest (*CTPR3*) (3). The figures were made with Pymol and individual helices are colored by the user-defined topology used by the Ising model: helix (*blue*), repeat (*black*), a mutant repeat (*green*), or a cap (*red*). (*B*) Using *PyFolding*'s built-in primitive protein folding "domains/modules," one can define topologies for each protein in the series. Each primitive is a container for several thermodynamic parameters to describe the intrinsic and interfacial stability terms. (*C*) Using the topologies defined in (*B*), *PyFolding* will automatically generate the appropriate partition functions (q) for each protein in the series using a matrix formulation, and share parameters between other proteins in the series. (*D*) A final global fitting step finds the optimal set of parameters to describe the series. (*E*) The optimal parameters (and their estimated errors/confidence intervals) for each domain primitive are recovered and output for the user. These calculations can be found in Supporting Material, *Jupyter notebook 6*. To see this figure in color, go online.

topology. Moreover, once the 1-D topology of your protein has been defined, *PyFolding* can also be used to simulate and thereby predict folding behavior of both the whole protein and the subunits that it is composed of (Supporting Material, *Jupyter notebook 7*). In principle, this type of approach could be extended to higher dimensional topologies, thus providing a framework to enable rational protein design.

Error analysis

We calculate various metrics to assess the quality of the output from *PyFolding*. All independent nonconstant variables are reported with a SE of each parameter, i:

$$\mathrm{SE}(i) = \mathrm{cov}(i,i) \times \sqrt{\frac{\sum \left(y_{\mathrm{fit}} - y_{\mathrm{obs}}\right)^2}{d}}, \qquad (1)$$

where cov is the covariance matrix (where $\mathrm{cov}(i,i)$ represents the variance of parameter i), y_{fit} are the y-values of the fit at the observed x-values, y_{obs} are the observed y-values of the data, and d represents the degrees of freedom (the number of data points minus the number of free variables). From these values, we can also calculate the confidence interval (nominally at 95%), where the confidence interval for parameter i is

$$\mathrm{CI}(i) = P_i \pm t(95\%, d) \times \mathrm{SE}(i)\,, \qquad (2)$$

where P_i is the value of parameter i and $t(95\%, d)$ is the t-distribution at 95% with d degrees of freedom. Finally, we report the coefficient of determination (R^2) as a statistical measure of the error between the data and the fitted model:

$$R^2 = 1 - \frac{\sum \left(y_{\mathrm{fit}} - y_{\mathrm{obs}}\right)^2}{\sum \left(y_{\mathrm{fit}} - \overline{y_{\mathrm{obs}}}\right)^2}, \qquad (3)$$

where $\overline{y_{\mathrm{obs}}}$ represents the mean of the observed data.

In all models other than the heteropolymer Ising model, we utilize a gradient optimizer such as the Levenberg-Marquardt algorithm that yields a covariance matrix of the fitted parameters. However, since we must utilize a different optimization method (the differential evolution optimizer) for the global fitting of heteropolymer Ising models, we calculate the errors in a slightly different way. The optimizer does not yield a covariance matrix as default, so we calculate a numerical approximation based on the Jacobian matrix (here, a matrix of numerical approximations of all the partial differentials of all variables) as follows:

$$\mathrm{cov} = \left(\mathrm{J}^{\mathrm{T}}\,\mathrm{J}\right)^{-1} \times \mathrm{MSE}, \tag{4}$$

where J is the Jacobian matrix, and MSE is the mean squared error of the fit.

In *PyFolding*, we have provided estimates of the standard error and confidence intervals for each parameter (calculated as described above) using this numerical approximation of the covariance matrix. In general, estimating errors for the parameters or the uniqueness of the solution in heteropolymer models is a complex problem, owing to the method of optimization used. Interestingly, Aksel and Barrick (12) used Bootstrap analysis to evaluate parameter confidence intervals. However, many of the published studies either do not describe how error margins were determined or simply list the error between the data and curve fit. Here, when confronted with ill-posed data sets or poorly chosen topologies, which can produce an adequate curve fit to the data (as measured by R^2), *PyFolding*'s numerical error approximation becomes unstable, leading to large errors. Thus, in evaluating the determinant of the Jacobian as well as the estimated errors, it is possible to assess the quality of the model and the validity of the solution: large errors show that the model parameters are not properly constrained. In such cases, *PyFolding* raises the appropriate warnings to enable the user to quickly interpret the results and adjust the topologies and members of a data set appropriately.

CONCLUSIONS

Here we have shown that *PyFolding*, in conjunction with Jupyter notebooks, enables researchers with minimal programming expertise the ability to fit both typical and complex models to their thermodynamic and kinetic protein folding data. The software is free and can be used to both analyze and simulate data with models and analyses that expensive commercial user-friendly options cannot. In particular, we have incorporated the ability to fit and simulate equilibrium unfolding experiments with user defined protein topologies, using a matrix formulation of the 1-D heteropolymer Ising model. This aspect of *PyFolding* will be of particular interest to groups working on protein folds composed of repetitive motifs such as Ankyrin repeats and tetratricopeptide repeats, given that these proteins are increasingly being used as novel antibody therapeutics (38–41) and biomaterials (42–47). Further, as analysis can be performed in Jupyter notebooks, it enables novice researchers to easily use the software and for groups to share data and methods. We have provided a number of example notebooks and accompanying video tutorials as a resource accompanying this manuscript, enabling other users to recreate our data analysis and modify parameters. Finally, due to *PyFolding*'s extensible framework, it is straightforward to extend, thus enabling fitting and modeling of other systems or phenomena such as protein-protein and other protein-binding interactions. Such extensions can be rapidly and seamlessly deployed as a community resource, thus broadening the functionality of the software.

SUPPORTING MATERIAL

Supporting Materials and Methods are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(17)35041-5.

AUTHOR CONTRIBUTIONS

A.R.L. wrote the software. A.R.L. and E.R.G.M. developed the models. A.R.L., E.R.G.M., L.S.I., and A.P.-R. tested the software and performed data analysis and simulations. A.R.L. and E.R.G.M. created the supplemental Jupyter Notebooks. E.R.G.M. created the online tutorials. E.R.G.M. and A.R.L. wrote the manuscript, and all authors edited and approved the manuscript.

ACKNOWLEDGMENTS

We would like to thank Dr. Jonathan Phillips for insightful discussion, helpful comments and suggestions.

L.S.I. acknowledges the support of a Senior Fellowship from the UK Medical Research Foundation. A.P.-R. was supported by a Biotechnology and Biological Sciences Research Council Doctoral Training Programme scholarship and an Oliver Gatty Studentship. E.R.G.M. and L.S.I. laboratories acknowledge support from a Leverhulme Trust project grant.

REFERENCES

1. Main, E. R., K. F. Fulton, and S. E. Jackson. 1999. Folding pathway of FKBP12 and characterisation of the transition state. *J. Mol. Biol.* 291:429–444.
2. Löw, C., U. Weininger, …, J. Balbach. 2008. Structural insights into an equilibrium folding intermediate of an archaeal ankyrin repeat protein. *Proc. Natl. Acad. Sci. USA.* 105:3779–3784.
3. Millership, C., J. J. Phillips, and E. R. G. Main. 2016. Ising model reprogramming of a repeat protein's equilibrium unfolding pathway. *J. Mol. Biol.* 428:1804–1817.
4. Jackson, S. E., and A. R. Fersht. 1991. Folding of chymotrypsin inhibitor 2. 1. Evidence for a two-state transition. *Biochemistry.* 30:10428–10435.
5. Schätzle, M., and T. Kiefhaber. 2006. Shape of the free energy barriers for protein folding probed by multiple perturbation analysis. *J. Mol. Biol.* 357:655–664.

6. Naganathan, A. N., and V. Muñoz. 2014. Thermodynamics of downhill folding: multi-probe analysis of PDD, a protein that folds over a marginal free energy barrier. *J. Phys. Chem. B.* 118:8982–8994.
7. Ferreiro, D. U., and P. G. Wolynes. 2008. The capillarity picture and the kinetics of one-dimensional protein folding. *Proc. Natl. Acad. Sci. USA.* 105:9853–9854.
8. Barrick, D., D. U. Ferreiro, and E. A. Komives. 2008. Folding landscapes of ankyrin repeat proteins: experiments meet theory. *Curr. Opin. Struct. Biol.* 18:27–34.
9. DeVries, I., D. U. Ferreiro, …, E. A. Komives. 2011. Folding kinetics of the cooperatively folded subdomain of the IκBα ankyrin repeat domain. *J. Mol. Biol.* 408:163–176.
10. Maxwell, K. L., D. Wildes, …, K. W. Plaxco. 2005. Protein folding: defining a "standard" set of experimental conditions and a preliminary kinetic data set of two-state proteins. *Protein Sci.* 14:602–616.
11. Wensley, B. G., S. Batey, …, J. Clarke. 2010. Experimental evidence for a frustrated energy landscape in a three-helix-bundle protein family. *Nature.* 463:685–688.
12. Aksel, T., and D. Barrick. 2009. Analysis of repeat-protein folding using nearest-neighbor statistical mechanical models. *Methods Enzymol.* 455:95–125.
13. Mallam, A. L., and S. E. Jackson. 2007. A comparison of the folding of two knotted proteins: YbeA and YibK. *J. Mol. Biol.* 366:650–665.
14. Scott, K. A., L. G. Randles, and J. Clarke. 2004. The folding of spectrin domains II: phi-value analysis of R16. *J. Mol. Biol.* 344:207–221.
15. Hutton, R. D., J. Wilkinson, …, L. S. Itzhaki. 2015. Mapping the topography of a protein energy landscape. *J. Am. Chem. Soc.* 137:14610–14625.
16. Tsytlonok, M., P. O. Craig, …, L. S. Itzhaki. 2013. Complex energy landscape of a giant repeat protein. *Structure.* 21:1954–1965.
17. Javadi, Y., and E. R. Main. 2009. Exploring the folding energy landscape of a series of designed consensus tetratricopeptide repeat proteins. *Proc. Natl. Acad. Sci. USA.* 106:17383–17388.
18. Lowe, A. R., and L. S. Itzhaki. 2007. Biophysical characterisation of the small ankyrin repeat protein myotrophin. *J. Mol. Biol.* 365:1245–1255.
19. Xu, M., O. Beresneva, …, H. Roder. 2012. Microsecond folding dynamics of apomyoglobin at acidic pH. *J. Phys. Chem. B.* 116:7014–7025.
20. Garcia-Mira, M. M., M. Sadqi, …, V. Muñoz. 2002. Experimental identification of downhill protein folding. *Science.* 298:2191–2195.
21. Aksel, T., A. Majumdar, and D. Barrick. 2011. The contribution of entropy, enthalpy, and hydrophobic desolvation to cooperativity in repeat-protein folding. *Structure.* 19:349–360.
22. Kajander, T., A. L. Cortajarena, …, L. Regan. 2005. A new folding paradigm for repeat proteins. *J. Am. Chem. Soc.* 127:10188–10190.
23. Winn, M. D., C. C. Ballard, …, K. S. Wilson. 2011. Overview of the CCP4 suite and current developments. *Acta Crystallogr. D Biol. Crystallogr.* 67:235–242.
24. Jones, E., T. Oliphant, …, P. Peterson. 2001. SciPy: Open source scientific tools for Python. http://www.scipy.org.
25. Serrano, L., A. Matouschek, and A. R. Fersht. 1992. The folding of an enzyme. III. Structure of the transition state for unfolding of barnase analysed by a protein engineering procedure. *J. Mol. Biol.* 224:805–818.
26. Fersht, A. R., A. Matouschek, and L. Serrano. 1992. The folding of an enzyme. I. Theory of protein engineering analysis of stability and pathway of protein folding. *J. Mol. Biol.* 224:771–782.
27. Brush, S. G. 1967. History of the Lenz-Ising model. *Rev. Mod. Phys.* 39:883–893.
28. Niss, M. 2005. History of the Lenz-Ising model 1920–1950: from ferromagnetic to cooperative phenomena. *Arch. Hist. Exact Sci.* 59:267–318.
29. Zimm, B. H., and J. K. Bragg. 1959. Theory of the phase transition between helix and random coil in polypeptide chains. *J. Chem. Phys.* 31:526–535.
30. Muñoz, V., P. A. Thompson, …, W. A. Eaton. 1997. Folding dynamics and mechanism of beta-hairpin formation. *Nature.* 390:196–199.
31. Muñoz, V., and W. A. Eaton. 1999. A simple model for calculating the kinetics of protein folding from three-dimensional structures. *Proc. Natl. Acad. Sci. USA.* 96:11311–11316.
32. Kubelka, J., E. R. Henry, …, W. A. Eaton. 2008. Chemical, physical, and theoretical kinetics of an ultrafast folding protein. *Proc. Natl. Acad. Sci. USA.* 105:18655–18662.
33. Kubelka, G. S., and J. Kubelka. 2014. Site-specific thermodynamic stability and unfolding of a de novo designed protein structural motif mapped by 13C isotopically edited IR spectroscopy. *J. Am. Chem. Soc.* 136:6037–6048.
34. Lai, J. K., G. S. Kubelka, and J. Kubelka. 2015. Sequence, structure, and cooperativity in folding of elementary protein structural motifs. *Proc. Natl. Acad. Sci. USA.* 112:9890–9895.
35. Wetzel, S. K., G. Settanni, …, A. Plückthun. 2008. Folding and unfolding mechanism of highly stable full-consensus ankyrin repeat proteins. *J. Mol. Biol.* 376:241–257.
36. Aksel, T., and D. Barrick. 2014. Direct observation of parallel folding pathways revealed using a symmetric repeat protein system. *Biophys. J.* 107:220–232.
37. Storn, R., and K. Price. 1997. Differential evolution - A simple and efficient heuristic for global optimization over continuous spaces. *J. Glob. Optim.* 11:341–359.
38. Rasool, M., A. Malik, …, M. A. Kamal. 2017. DARPins bioengineering and its theranostic approaches: emerging trends in protein engineering. *Curr. Pharm. Des.* 23:1610–1615.
39. Jost, C., and A. Plückthun. 2014. Engineered proteins with desired specificity: DARPins, other alternative scaffolds and bispecific IgGs. *Curr. Opin. Struct. Biol.* 27:102–112.
40. Ernst, P., and A. Plückthun. 2017. Advances in the design and engineering of peptide-binding repeat proteins. *Biol. Chem.* 398:23–29.
41. Cortajarena, A. L., F. Yi, and L. Regan. 2008. Designed TPR modules as novel anticancer agents. *ACS Chem. Biol.* 3:161–166.
42. Sawyer, N., E. B. Speltz, and L. Regan. 2013. NextGen protein design. *Biochem. Soc. Trans.* 41:1131–1136.
43. Main, E. R., J. J. Phillips, and C. Millership. 2013. Repeat protein engineering: creating functional nanostructures/biomaterials from modular building blocks. *Biochem. Soc. Trans.* 41:1152–1158.
44. Grove, T. Z., L. Regan, and A. L. Cortajarena. 2013. Nanostructured functional films from engineered repeat proteins. *J. R. Soc. Interface.* 10:20130051.
45. Phillips, J. J., C. Millership, and E. R. G. Main. 2012. Fibrous nanostructures from the self-assembly of designed repeat protein modules. *Angew. Chem. Int.Engl.* 51:13132–13135.
46. Grove, T. Z., and L. Regan. 2012. New materials from proteins and peptides. *Curr. Opin. Struct. Biol.* 22:451–456.
47. Grove, T. Z., J. Forster, …, L. Regan. 2012. A modular approach to the design of protein-based smart gels. *Biopolymers.* 97:508–517.

Biophysical Journal, Volume *114*

Supplemental Information

PyFolding: Open-Source Graphing, Simulation, and Analysis of the Biophysical Properties of Proteins

Alan R. Lowe, Albert Perez-Riba, Laura S. Itzhaki, and Ewan R.G. Main

Contents

1 Generic Data Importer Function Notebook

[Authors] ARL & ERGM

This Notebook aims to show an example of importing generic data into PyFolding and fitting it with a user defined model. Once you have tested it here you can add it to your models.py from the PyFolding installation and setup PyFolding again to incorporate it into your local copy or simply copy it into whichever notebook you are using.

So as always lets load up PyFolding:

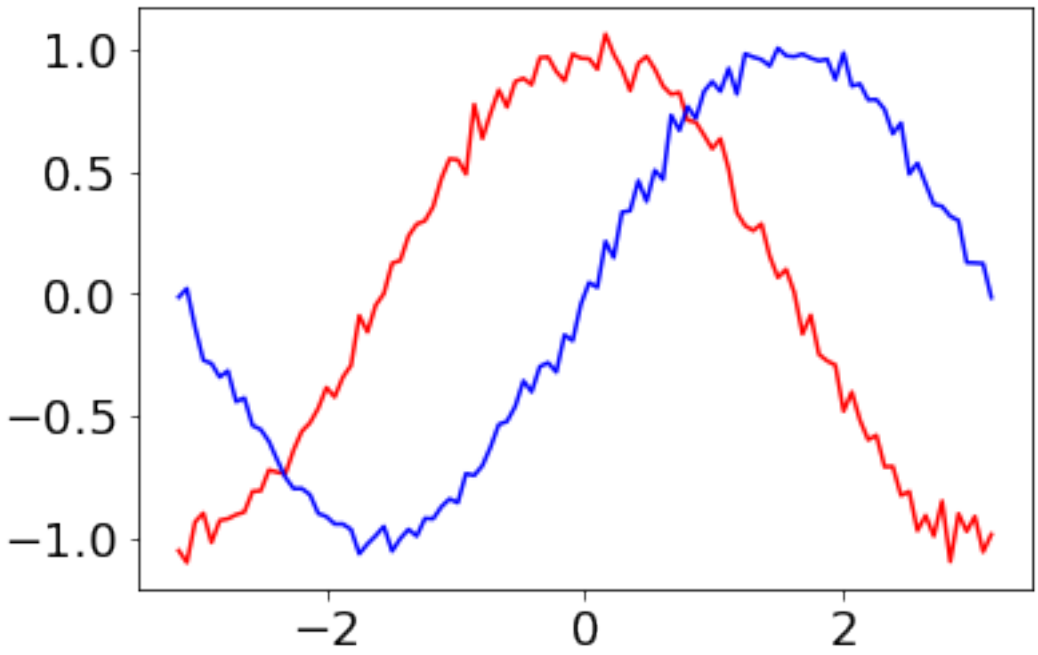
In [1]:
```
#First off let's load pyfolding & pyplot into this ipython notebook
# (pyplot allows us to plot more complex figures of our results):

%matplotlib inline
import pyfolding
from pyfolding import models

# let's use some other libraries also
import matplotlib.pyplot as plt
import numpy as np
```

```
<IPython.core.display.Javascript object>


PyFolding: Jupyter autoscrolling has been disabled
```

NOTE: This section just autogenerates a CSV file of data to be imported using the generic importer

---

In [2]:
```
from pyfolding import utils
from collections import OrderedDict
x = np.linspace(-np.pi, np.pi, 100)
y_cos = np.cos(x) + np.random.randn(len(x))*0.05
y_sin = np.sin(x) + np.random.randn(len(x))*0.05

plt.figure()
plt.plot(x, y_cos,'r-', x, y_sin,'b-')
plt.show()

data = OrderedDict([('x',x),('cos',y_cos),('sin',y_sin)])

utils.write_CSV('../examples/waves.csv', data)
```




```
Writing .csv file (../examples/waves.csv)...
```

1.1 Loading any data set

Here we will load a data set which contains sine and cosine functions over the range $(-\pi, \pi]$ to demonstrate how PyFolding can deal with arbitrary datasets. We will then plot and fit a simple mode to recover the amplitude and phase of the waves.

```
In [3]: # loading the data - The kinetics of each protein is in one .csv
        # as per PyFolding SI Notebooks 1 and 2

        pth = "../examples/"
        wave_data = pyfolding.read_generic_data(pth,"waves.csv")

In [4]: print wave_data

<pyfolding.core.GenericData object at 0x113fc3150>
```

We can use this generic object as we would any other

For example, let's extract the datasets found within, and plot x versus y (y defaults to the first label, e.g. 'cos' in this case)

```
In [5]: print wave_data.labels

['x', 'cos', 'sin']
```

```
In [6]: print wave_data.x

[-3.14159265 -3.07812614 -3.01465962 -2.9511931  -2.88772658 -2.82426006
 -2.76079354 -2.69732703 -2.63386051 -2.57039399 -2.50692747 -2.44346095
 -2.37999443 -2.31652792 -2.2530614  -2.18959488 -2.12612836 -2.06266184
 -1.99919533 -1.93572881 -1.87226229 -1.80879577 -1.74532925 -1.68186273
 -1.61839622 -1.5549297  -1.49146318 -1.42799666 -1.36453014 -1.30106362
 -1.23759711 -1.17413059 -1.11066407 -1.04719755 -0.98373103 -0.92026451
 -0.856798   -0.79333148 -0.72986496 -0.66639844 -0.60293192 -0.53946541
 -0.47599889 -0.41253237 -0.34906585 -0.28559933 -0.22213281 -0.1586663
 -0.09519978 -0.03173326  0.03173326  0.09519978  0.1586663   0.22213281
  0.28559933  0.34906585  0.41253237  0.47599889  0.53946541  0.60293192
  0.66639844  0.72986496  0.79333148  0.856798    0.92026451  0.98373103
  1.04719755  1.11066407  1.17413059  1.23759711  1.30106362  1.36453014
  1.42799666  1.49146318  1.5549297   1.61839622  1.68186273  1.74532925
  1.80879577  1.87226229  1.93572881  1.99919533  2.06266184  2.12612836
  2.18959488  2.2530614   2.31652792  2.37999443  2.44346095  2.50692747
  2.57039399  2.63386051  2.69732703  2.76079354  2.82426006  2.88772658
  2.9511931   3.01465962  3.07812614  3.14159265]
```

```
In [7]: print wave_data.y

[ -1.05184198e+00  -1.10086036e+00  -9.34577866e-01  -8.98684831e-01
  -1.01876844e+00  -9.28422902e-01  -9.21834189e-01  -9.05072298e-01
  -8.94156059e-01  -8.09748407e-01  -8.07323495e-01  -7.21774418e-01
  -7.31338883e-01  -7.37619719e-01  -6.40085902e-01  -5.63761858e-01
  -5.28692200e-01  -4.69408225e-01  -3.84358928e-01  -4.23246500e-01
  -3.42038709e-01  -2.93540838e-01  -8.99022064e-02  -1.56712701e-01
  -4.70741508e-02   2.21520170e-04   1.24822637e-01   1.35374655e-01
   2.36447385e-01   2.84709078e-01   2.98650228e-01   3.56154884e-01
   4.71149537e-01   5.51995373e-01   5.48471932e-01   4.89630950e-01
   7.75252581e-01   6.35591971e-01   7.39330183e-01   8.34019948e-01
   7.64425698e-01   8.68623007e-01   8.81952824e-01   8.55746330e-01
   9.66528664e-01   9.68849809e-01   9.09940500e-01   8.71076422e-01
   9.80475110e-01   9.64203100e-01   9.62147890e-01   9.19320898e-01
   1.06287708e+00   9.79996528e-01   9.19656691e-01   8.31644631e-01
   9.43496470e-01   9.71987785e-01   9.19820064e-01   8.50062902e-01
```

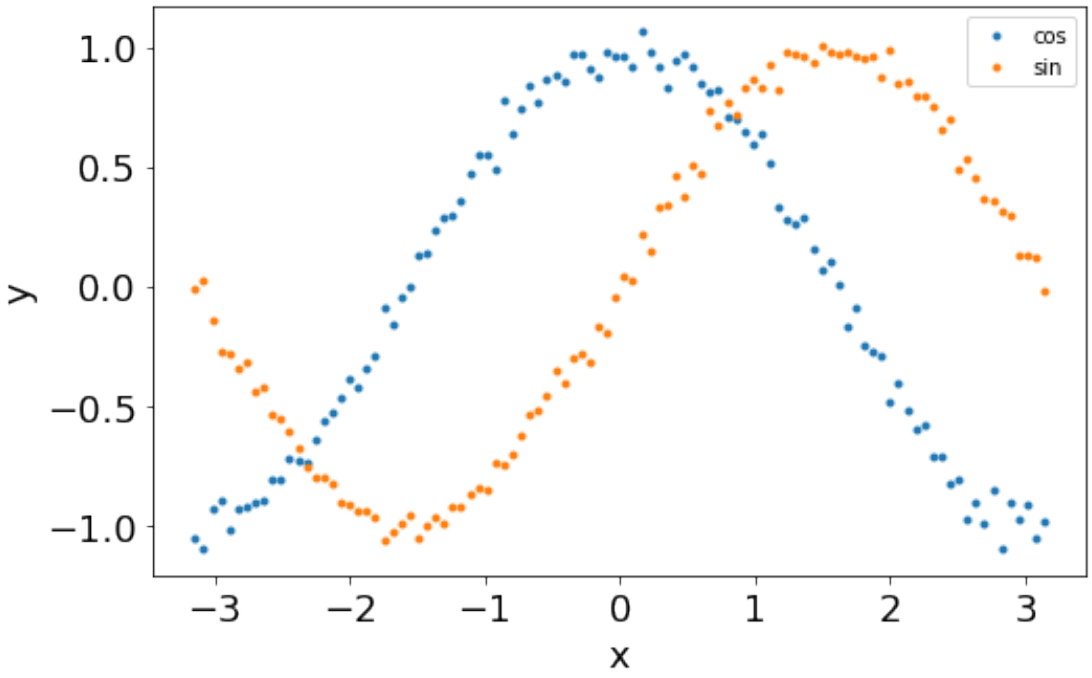
```
 8.16184298e-01   8.24049638e-01   7.10046130e-01   7.02083818e-01
 6.49232729e-01   5.93266790e-01   6.34566975e-01   5.10333001e-01
 3.31677692e-01   2.78595731e-01   2.58089568e-01   2.85880648e-01
 1.53620903e-01   6.60839402e-02   9.97124703e-02   4.06463880e-03
-1.65095090e-01  -8.79775962e-02  -2.48359633e-01  -2.75328948e-01
-2.92200664e-01  -4.81186925e-01  -4.02666378e-01  -5.18212035e-01
-5.98537310e-01  -5.79979894e-01  -7.08734247e-01  -7.07753630e-01
-8.26412974e-01  -8.10114098e-01  -9.70090371e-01  -9.08403141e-01
-9.92313207e-01  -8.49087297e-01  -1.09725395e+00  -9.01188317e-01
-9.73493283e-01  -9.12334689e-01  -1.05720339e+00  -9.85473702e-01]
```

1.2 Plotting data and fitting to a new model

Note that we can call plot and fit this to an arbitrary function (i.e. it doesn't have to be anything to do with protein folding) using all of the same tools

---

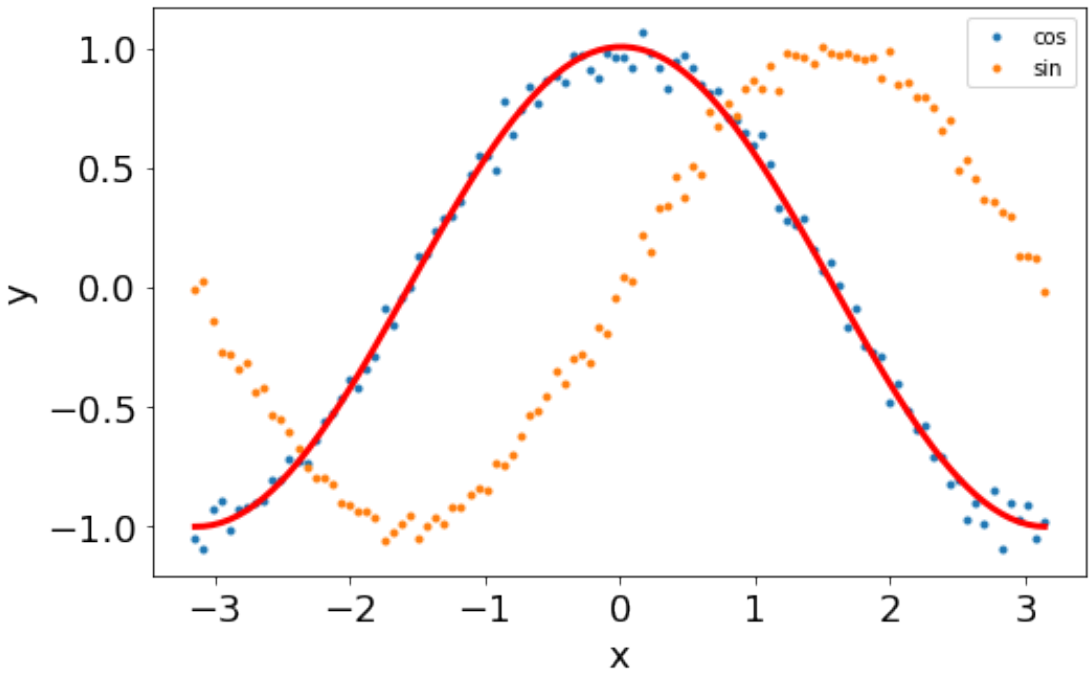
```
In [8]: wave_data.plot()
```



1.2.1 Sinusoidal model with phase and amplitude

Lets define a sinusoidal model with phase and amplitude. We can use this to extract the amplitude and phase of the waves...

```
In [9]: class Wave(pyfolding.FitModel):
            """ Sine wave with amplitude and phase
            """
            def __init__(self):
                pyfolding.FitModel.__init__(self)
                fit_args = self.fit_func_args
                self.params = tuple( [(fit_args[i],i) for i in xrange(len(fit_args))] )
                self.default_params = np.array([1., 0.])



            def fit_func(self, x, amplitude, phase):
                return amplitude*np.sin(x+phase)

In [10]: wave_data.fit_func = Wave
         wave_data.fit()

================================================================================
Fitting results
================================================================================
ID: waves
Model: Wave
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) amplitude    1.00419 ± 0.00045            95% CI[   1.00408,    1.00431]
(f) phase        1.55891 ± 0.00045            95% CI[   1.55879,    1.55902]
--------------------------------------------------------------------------------
R^2: 0.99389
================================================================================



In [11]: wave_data.plot()
```




```
In [12]: print np.pi/2
```

```
1.57079632679
```

End of Notebook

---

2 Generating New Models/ Equations Notebook

[Author] ERGM & ARL

This Notebook aims to show an example of importing generic data into PyFolding and fitting it with a user defined model. Once you have tested it here you can add it to your models.py from the PyFolding installation and setup PyFolding again to incorporate it into your local copy or simply copy it into whichever notebook you are using.

So as always lets load up PyFolding:

```
In [1]: # Load up pyfolding, etc.
        %matplotlib inline
        import pyfolding
        from pyfolding import models

        # let's use some other libraries also
        import matplotlib.pyplot as plt
        import numpy as np

        # Command imports pyfolding models
        from pyfolding.models import *
```

```
<IPython.core.display.Javascript object>
```

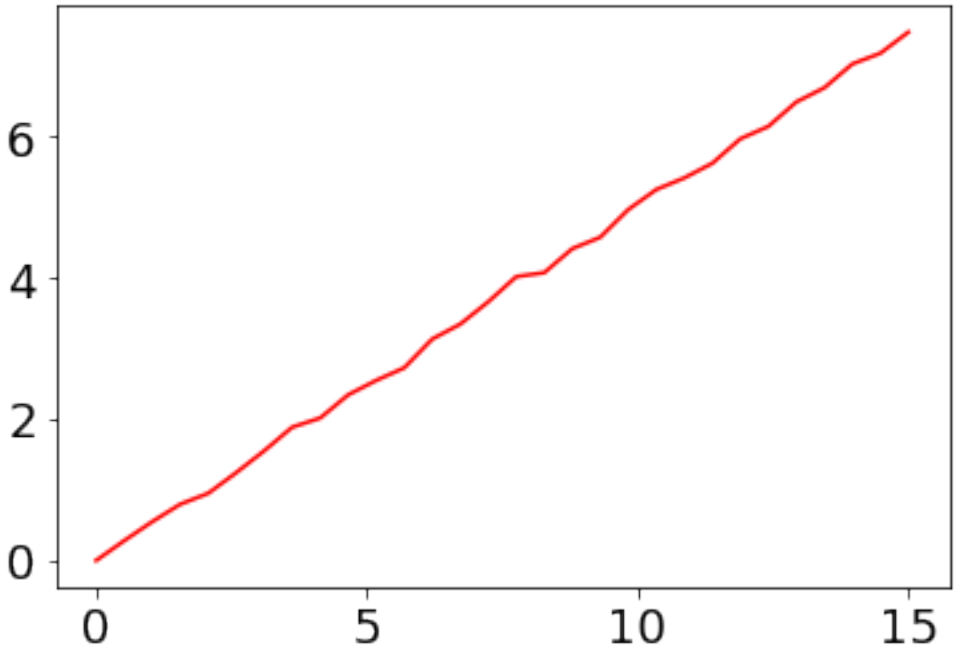
```
PyFolding: Jupyter autoscrolling has been disabled
```

NOTE: The cell below just autogenerates a CSV file of data to be imported using the generic importer

---

```
In [2]: # Lets create some data

        from pyfolding import utils
        from collections import OrderedDict
        x = np.linspace(0, 15, num=30)
        y_straight_line = 0.5*(x) + np.random.randn(len(x))*0.05

        plt.figure()
        plt.plot(x, y_straight_line,'r-')
```

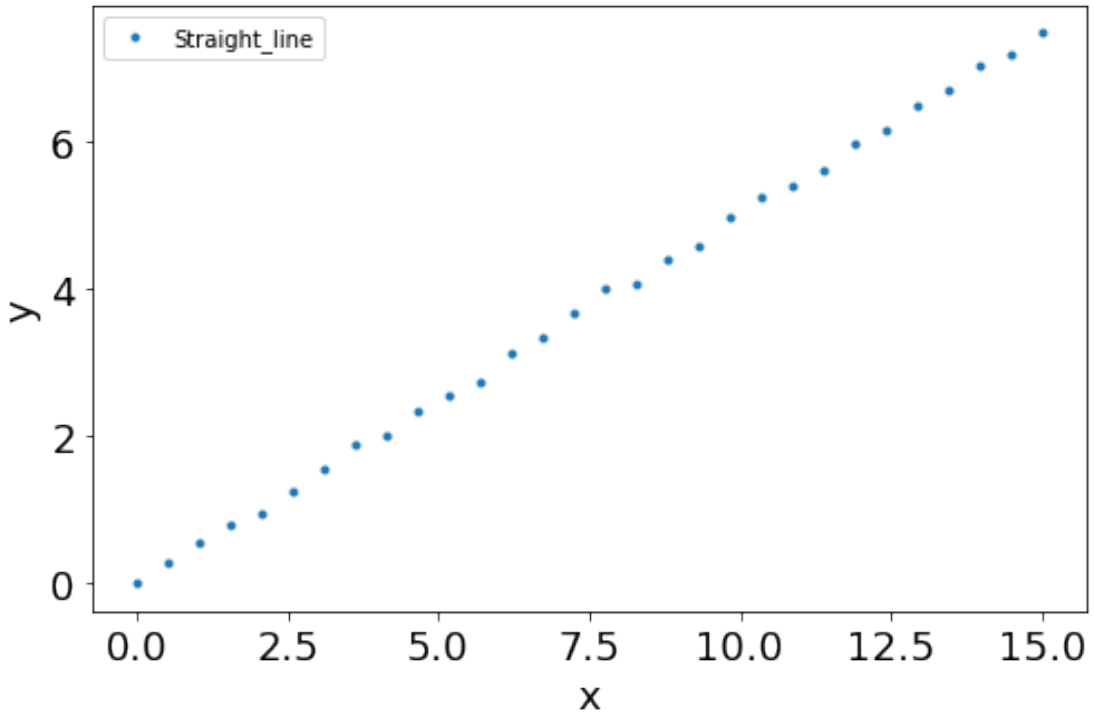
```
plt.show()

data = OrderedDict([('x',x),('Straight_line',y_straight_line)])

utils.write_CSV('../examples/test_data.csv', data)
```




```
Writing .csv file (../examples/test_data.csv)...
```

2.1 Loading the Generic Data into PyFolding

```
In [3]: # Lets load this generic data

        pth = "../examples/"
        test_data = pyfolding.read_generic_data(pth,"test_data.csv")

        # Lets plot it to confirm it is functioning correctly in pyFolding
        test_data.plot()
```




```
In [4]: #If we want to set our temperature

        pyfolding.set_temperature(25.0)
```

```
Set temperature to 25.00°C
(NOTE: Careful, this sets the temperature for all subsequent calculations)
```

2.2 Format for model definition

In the PyFolding there is a python file that defines all the models that we have inputted into PyFolding called models.py. These have the following format (I have used TwoStateEquilibrium as an example)

```
    class TwoStateEquilibrium(core.FitModel):

"""Two state equilibrium denaturation curve - No sloping baseline.

Folding Scheme:
    N <-> D
```

```
Params:
    F = Fraction unfolded
    m = m-value
    x = denaturant concentration (M)
    d50 = denaturant midpoint (M)
    R = Universal Gas Constant (kcal.mol-1.K-1)
    T = Temperature (Kelvin)

Reference:
    Clarke and Fersht. Engineered disulfide bonds as probes of
    the folding pathway of barnase: Increasing the stability
    of proteins against the rate of denaturation.
    Biochemistry (1993) vol. 32 (16) pp. 4322-4329"""

def __init__(self):
    core.FitModel.__init__(self)
    fit_args = self.fit_func_args
    self.params = tuple( [(fit_args[i],i) for i in xrange(len(fit_args))] )
    self.default_params = np.array([1.5, 5.])
    self.verified = True


def fit_func(self, x, m, d50):
    F = ( np.exp((m*(x-d50))/core.temperature.RT)) / (1.+np.exp((m*(x-d50))/core.temperature.RT)
    return F

@property
def equation(self):
    return r'F = \frac{\exp( m(x-d_{50})) / RT} { 1+\exp(m(x-d_{50}))/RT}'
```

2.3 New Model Formatting

The parts that you need to use the make a new equation/model are:

---

class TwoStateEquilibrium(core.FitModel):

```
def __init__(self):
    core.FitModel.__init__(self)
    fit_args = self.fit_func_args
    self.params = tuple( [(fit_args[i],i) for i in xrange(len(fit_args))] )
    self.default_params = np.array([1.5, 5.])
    self.verified = True
```

```
def fit_func(self, x, m, d50):
    F = ( np.exp((m*(x-d50))/core.temperature.RT)) / (1.+np.exp((m*(x-d50))/core.temperature.RT)
    return F
```

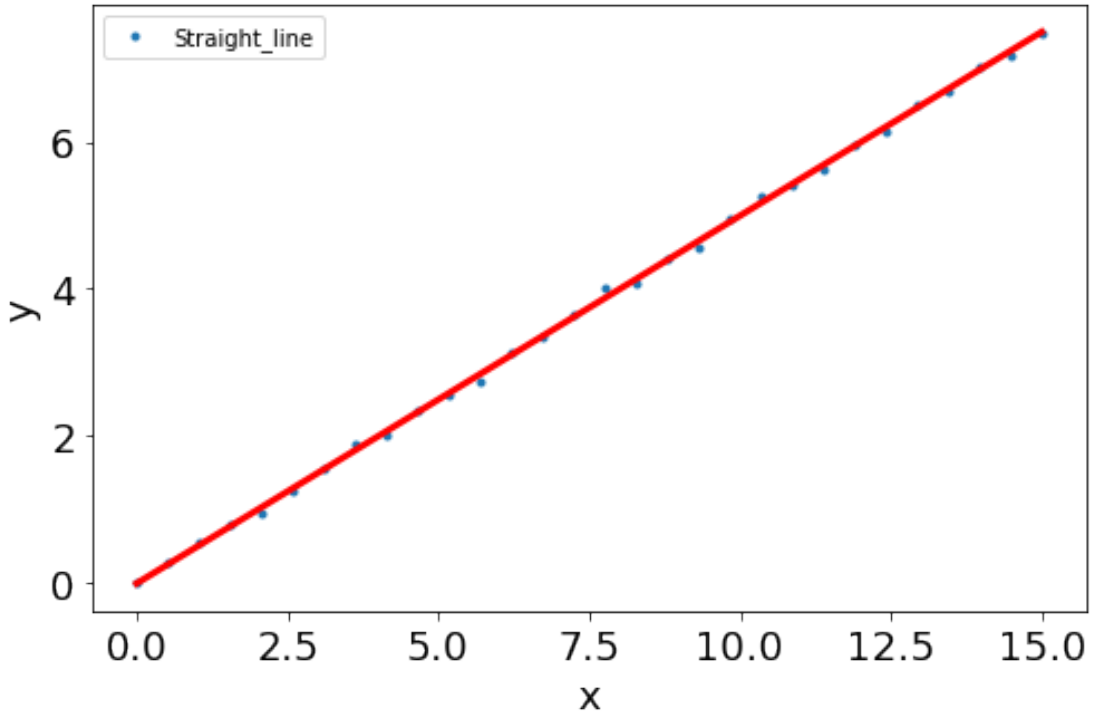
So lets use these and create our own equation and run it in this notebook (no need to modify the folder models.py) to fit the straight line data we generated above:

```
In [5]: # lets give our equation a name
        class straightline(core.FitModel):

        # We don't need to touch any of this next part except "self.default_params"
        # "self.default_params" are the initial parameters the model will use so lets change
        # the values in the brackets:
        # "np.array([1., 1.])".

            def __init__(self):
                core.FitModel.__init__(self)
                fit_args = self.fit_func_args
                self.params = tuple( [(fit_args[i],i) for i in xrange(len(fit_args))] )
                self.default_params = np.array([1., 1.])

        # This is our actual equation we will be using,
        # so lets input a straight line i.e. y = mx+c
            def fit_func(self, x, gradient, intercept):
                y = (gradient*x)+intercept
                return y

In [6]: # Lets fit our earlier generated data and see if the model works!

        test_data.fit_func = straightline
        test_data.fit()
```

```
================================================================================
Fitting results
================================================================================
ID: test_data
Model: straightline
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) gradient     0.50094 ± 0.00014             95% CI[   0.50090,    0.50097]
(f) intercept   -0.01329 ± 0.00120             95% CI[  -0.01359,   -0.01299]
--------------------------------------------------------------------------------
```

```
R^2: 0.99938
================================================================================
```

```
In [10]: # Now lets plot the data with the fit
         test_data.plot()
```



NoteBook End

---

3 SI Notebook 1 - Fitting equilibrium and kinetic folding data to simple models

[Author] ERGM

In this notebook we will show how equilbrium and kinetic folding data can be imported into a notebook and fitted to folding models. We also show how you can automate fitting data to a series of models and automate using different starting parameters/variables!

If you are less script/computer orientated, you can simply change the data paths/variables, etc for your proteins and re-run the jupyter notebook ("Kernal/Restart & Run all" from the menu above).

3.1 Data Format

Datasets should be in .csv files where:

1. The 1st row should contain the data titles
2. the 1st column should contain the x-values
3. The subsequent columns should contain the y-values.
4. You can have different data sets in different .csv files or all in one (as long as there is only one x-value column).
5. If you wish to perform global analyses on folding or equilibrium data, the datasets concerned must be in the same .csv
6. Except for global analyses using the Ising model, here each dataset must have its own .csv

---

Example .csv structure:

[Urea] (M)	Fraction Unfolded FKBP12
0	-0.00207
0.267	0.00307
...	...

First off lets load pyfolding & pyplot into this ipython notebook (pyplot allows us to plot more complex figures of our results):

```
In [1]: # use this command to tell Jupyter to plot figures inline with the text
        %matplotlib inline
```

```
        # import pyfolding, the pyfolding models and ising models
        import pyfolding
        from pyfolding import *

        # import the package for plotting, call it plt
        import matplotlib.pyplot as plt

        # import numpy as well
        import numpy as np
```

```
<IPython.core.display.Javascript object>


PyFolding: Jupyter autoscrolling has been disabled
```

Now, we need to load some data to analyse.

I will import the equilibrium denaturation & unfolding/folding kinetics of wild-type FKBP12 from:

Main E.R.G., Fulton K.F. & Jackson S.E. (1999) ‘‘The folding pathway of FKBP12 and characterisation of the transition state.’’ Journal of Molecular Biology, 291, 429-444.

3.1.1 Considerations

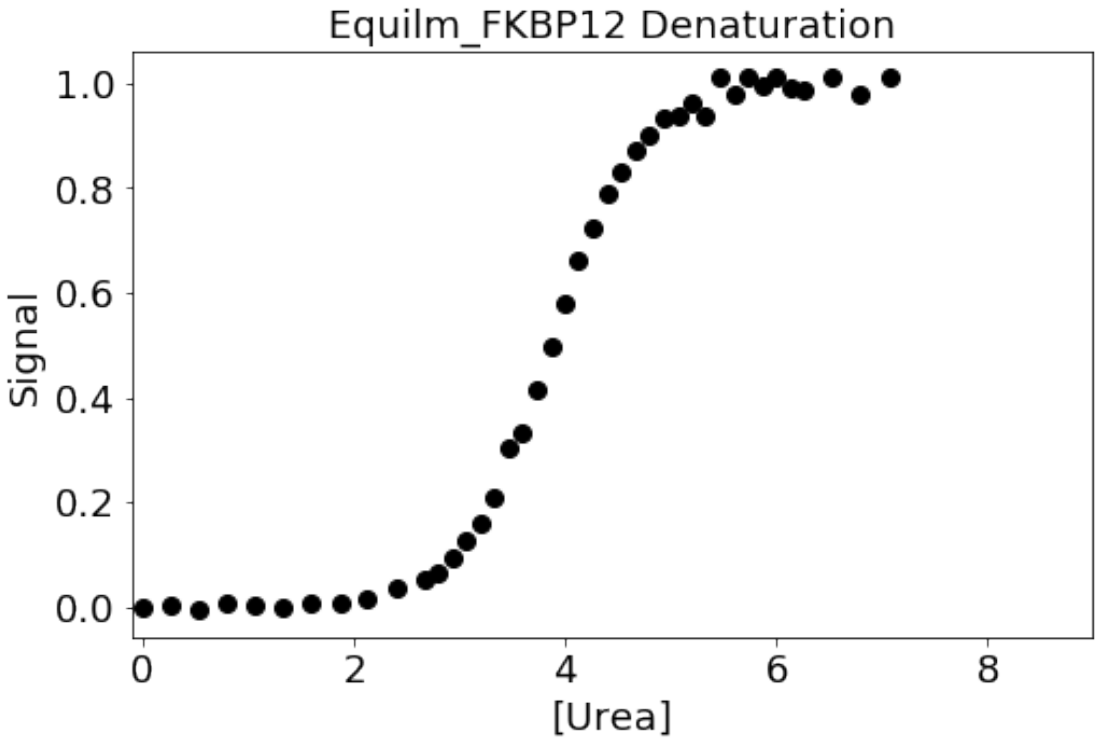
1. Kinetics data should be entered as rate constants (k) and NOT as the log of the rate constant.
2. There can be no “empty” x-axis cells in the .csv file for kinetics data.

In [2]:
```
        # start by loading a data set
        # arguments are "path", "filename"

        pth = "../examples/FKBP12/"
        Equilm_FKBP12 = pyfolding.read_equilibrium_data(pth,"Equilm_FKBP12.csv")
        Kinetics_FKBP12 = pyfolding.read_kinetic_data(pth,"Kinetics_FKBP12.csv")
```

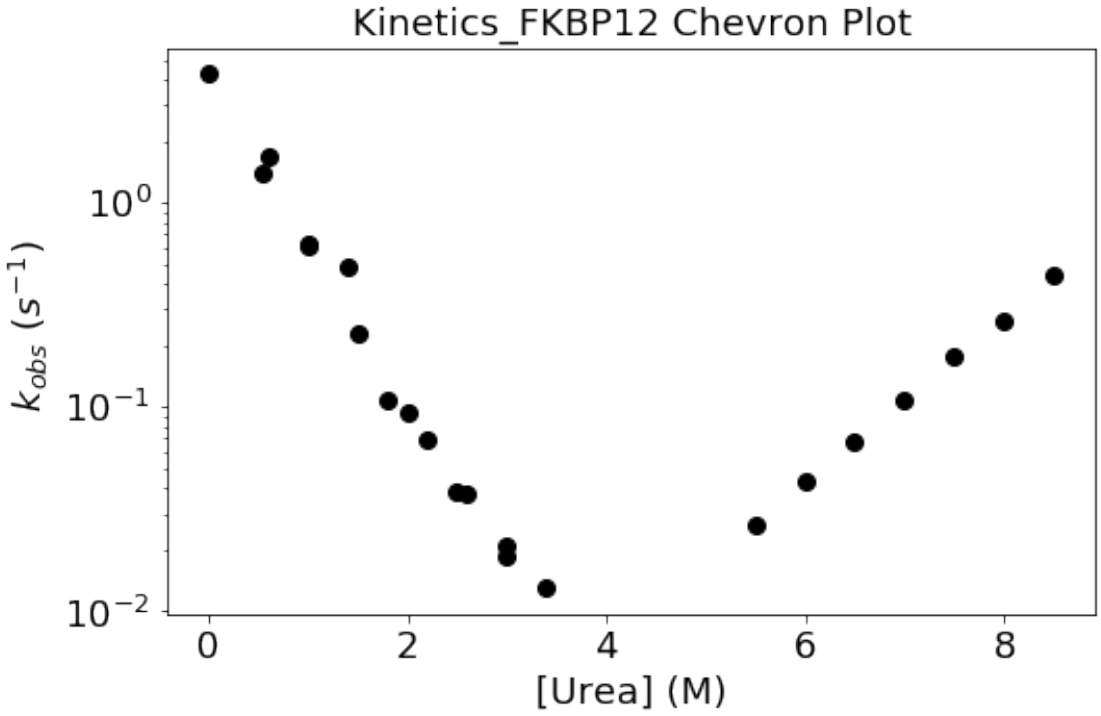
3.2 Plotting Data

OK, now we can try plotting the data to protein folding models.

In [3]:
```
        # now plot the equilm data
        Equilm_FKBP12.plot()
```



In [4]:
```
# now the kinetics
Kinetics_FKBP12.plot()
```



3.3 Fitting Data

OK, now we can try fitting the data to protein folding models. We will start by fitting the equilibrium data to a two state folding model, without sloping baslines (as the data has been processed as Fraction Unfolded).

3.3.1 List the models in pyfolding:

```
In [5]: # Command imports pyfolding models
        from pyfolding.models import *

        # command lists models
        list_models()

        # After the model name:
        #'Verified: True' - model rigorously tested and it functions as expected.
        #'Verified:False' - model tested, but not rigourously.
```

```
Out[5]: [('ChevronPolynomialFit', 'Verified: True'),
         ('HeteropolymerIsingEquilibrium', 'Verified: False'),
         ('HomozipperIsingEquilibrium', 'Verified: True'),
         ('ParallelTwoStateChevron', 'Verified: False'),
         ('ParallelTwoStateUnfoldingChevron', 'Verified: False'),
         ('TemplateModel', 'Verified: False'),
         ('ThreeStateChevron', 'Verified: True'),
         ('ThreeStateDimericIEquilibrium', 'Verified: True'),
         ('ThreeStateEquilibrium', 'Verified: True'),
         ('ThreeStateFastPhaseChevron', 'Verified: True'),
         ('ThreeStateMonoIEquilibrium', 'Verified: True'),
         ('ThreeStateSequentialChevron', 'Verified: True'),
         ('TwoStateChevron', 'Verified: True'),
         ('TwoStateChevronMovingTransition', 'Verified: True'),
         ('TwoStateDimerEquilibrium', 'Verified: True'),
         ('TwoStateEquilibrium', 'Verified: True'),
         ('TwoStateEquilibriumSloping', 'Verified: True')]
```

3.3.2 Print a model and its parameters, etc:

```
In [6]: # print the info
        models.TwoStateEquilibrium().info()
```

$$F = \frac{\exp(m(x - d_{50}))/RT}{1 + \exp(m(x - d_{50}))/RT}$$

```
Two state equilibrium denaturation curve - No sloping baseline.

        Folding Scheme:
                N <-> D

        Params:
                F = Fraction unfolded
                m = m-value
                x = denaturant concentration (M)
                d50 = denaturant midpoint (M)
                R = Universal Gas Constant (kcal.mol-1.K-1)
                T = Temperature (Kelvin)

        Reference:
                Clarke and Fersht. Engineered disulfide bonds as probes of
                the folding pathway of barnase: Increasing the stability
                of proteins against the rate of denaturation.
                Biochemistry (1993) vol. 32 (16) pp. 4322-4329
```

In [7]:
```
# Printing the model variables

# The function has two parts:
# part1 states the model you want to find about: "******()"
# part2 prints the variables: ".fit_func_args"

TwoStateEquilibrium().fit_func_args
```

Out[7]:
```
['m', 'd50']
```

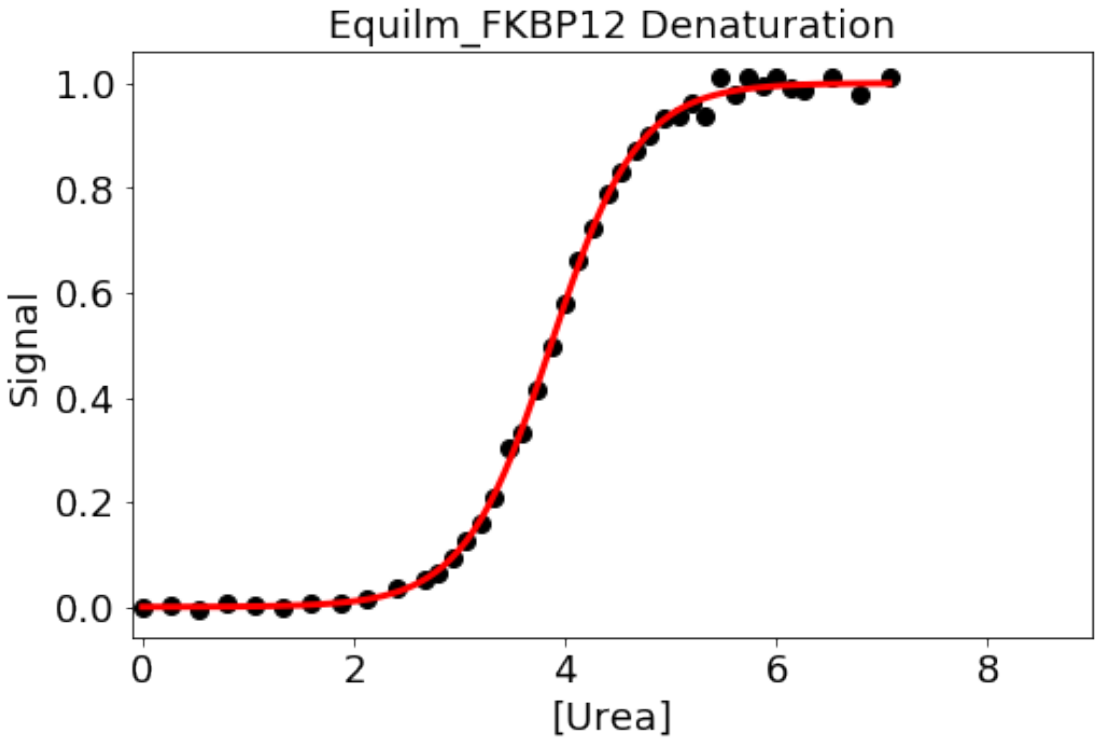
3.3.3 Fitting the Data.

Or we can skip straight to fitting the data.

In [8]:
```
# Set temperature to 25.00°C
# (NOTE: Careful, this sets the temperature for all subsequent calculations)
pyfolding.set_temperature(25.)

#1st select the fit function and associates it with the data
Equilm_FKBP12.fit_func = models.TwoStateEquilibrium
```

```
        #then fit it.
        #in the brackets you can define starting values for the variables -
        #input in the order printed above with the".fit_func_args'
        Equilm_FKBP12.fit(p0=[2,4])

        # We can print the resultant graph using either of these commands:
        # 1. "Equilm_FKBP12.plot()" or
        # 2. "pyfolding.plot_equilibrium(Equilm_FKBP12)"

        # lets do the first & save out the resultant graph

        Equilm_FKBP12.plot(save='/Users/ergm/Desktop/test.pdf')
```

```
Set temperature to 25.00°C
(NOTE: Careful, this sets the temperature for all subsequent calculations)
================================================================================
Fitting results
================================================================================
ID: Equilm_FKBP12
Model: TwoStateEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) m      1.43404 ± 0.00022            95% CI[   1.43399,    1.43410]
(f) d50    3.86730 ± 0.00007            95% CI[   3.86728,    3.86732]
--------------------------------------------------------------------------------
R^2: 0.99933
================================================================================
```




```
In [9]: # save out the fit
        Equilm_FKBP12.save_fit('/users/ergm/test.csv')
```

We can fit the kinetic data separately too, lets first print the equation we want to use:

```
In [10]: # printing the equation for viewing
         models.TwoStateChevron().info()

         # Lets also see what the input variables are also
         models.TwoStateChevron().fit_func_args
```

$$k_{obs} = k_f + k_u$$

where:

$$\begin{aligned} k_f &= k_f^{H_2O} \exp(-m_{kf} x) \\ k_u &= k_u^{H_2O} \exp(m_{ku} x) \end{aligned} \tag{1}$$

thus:

$$k_{obs} = k_f^{H_2O} \exp(-m_{kf} x) + k_u^{H_2O} \exp(m_{ku} x)$$

```
 Two state chevron plot.

        Folding Scheme:
                N <-> D

        Params:
                k obs = rate constant of unfolding or refolding at a particular denaturant conce
                kf = rate constant of refolding at a particular denaturant concentration
                mf = the gradient of refolding arm of the chevron
                ku = rate constant of unfolding at a a particular denaturant concentration
                mu = the gradient of unfolding arm of the chevron
                x = denaturant concentration (M)

        Reference:
                Jackson SE and Fersht AR.  Folding of chymotrypsin inhibitor 2.
                1. Evidence for a two-state transition.
                Biochemistry (1991) 30(43):10428-10435.
```

```
Out[10]: ['kf', 'mf', 'ku', 'mu']
```

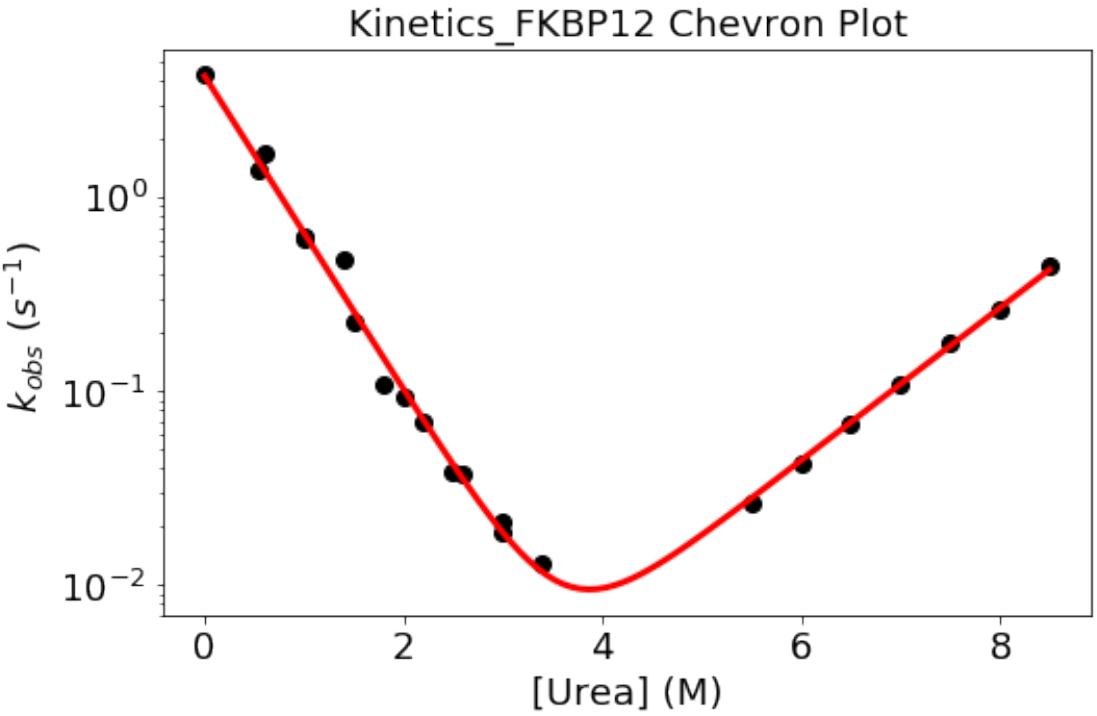
```
In [11]: # 1st select the fit function and associates it with the data
         Kinetics_FKBP12.fit_func = models.TwoStateChevron

         # 2nd fit the data with initial values (input these as per the list of input variables
         Kinetics_FKBP12.fit(p0=[4,2,0.0001,1])
```

```
================================================================================
Fitting results
================================================================================
ID: Kinetics_FKBP12
Model: TwoStateChevron
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) kf    4.21728 ± 0.05339            95% CI[   4.20408,    4.23048]
(f) mf    1.86223 ± 0.00703            95% CI[   1.86049,    1.86396]
(f) ku    0.00019 ± 0.00001            95% CI[   0.00019,    0.00019]
(f) mu    0.90557 ± 0.00799            95% CI[   0.90360,    0.90755]
--------------------------------------------------------------------------------
R^2: 0.99234
================================================================================
```

We can print the resultant graph: This is the same as when plotting the Equilm data and the commands are either: Kinetics_FKBP12.plot() or pyfolding.plot_chevron(Kinetics_FKBP12)

Lets use the first

```
In [12]: Kinetics_FKBP12.plot()
```

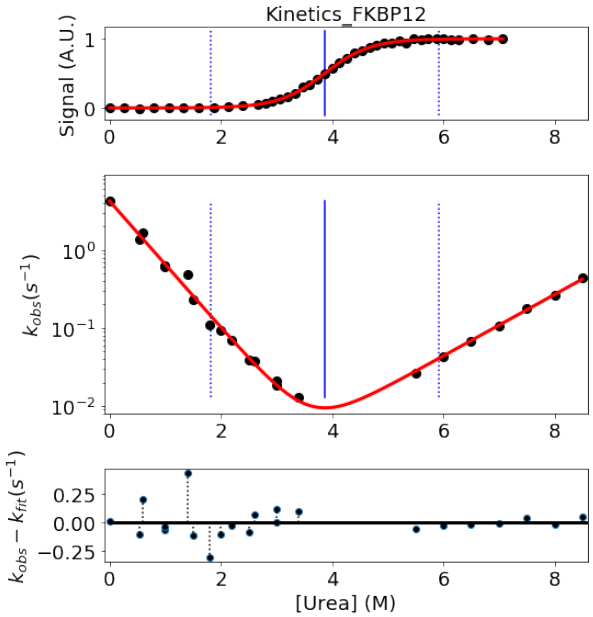


3.4 Plotting Data to a Fancier Graph

We can also plot a fancier graph that shows both Equilm and Kinetics together

---

```
In [13]: pyfolding.plot_figure(Equilm_FKBP12,
                               Kinetics_FKBP12,
                               display=True,
                               pth= '/users/ergm/desktop', save=True)

         # the "pth" and save=True part writes out a pdf of the figure below.
```



Data-set: Equilm_FKBP12

Equilibrium Model: TwoStateEquilibrium
m: 1.43404 ± 0.00022
d50: 3.86730 ± 0.00007
Folding midpoint: 3.87 M
R^2: 1.00

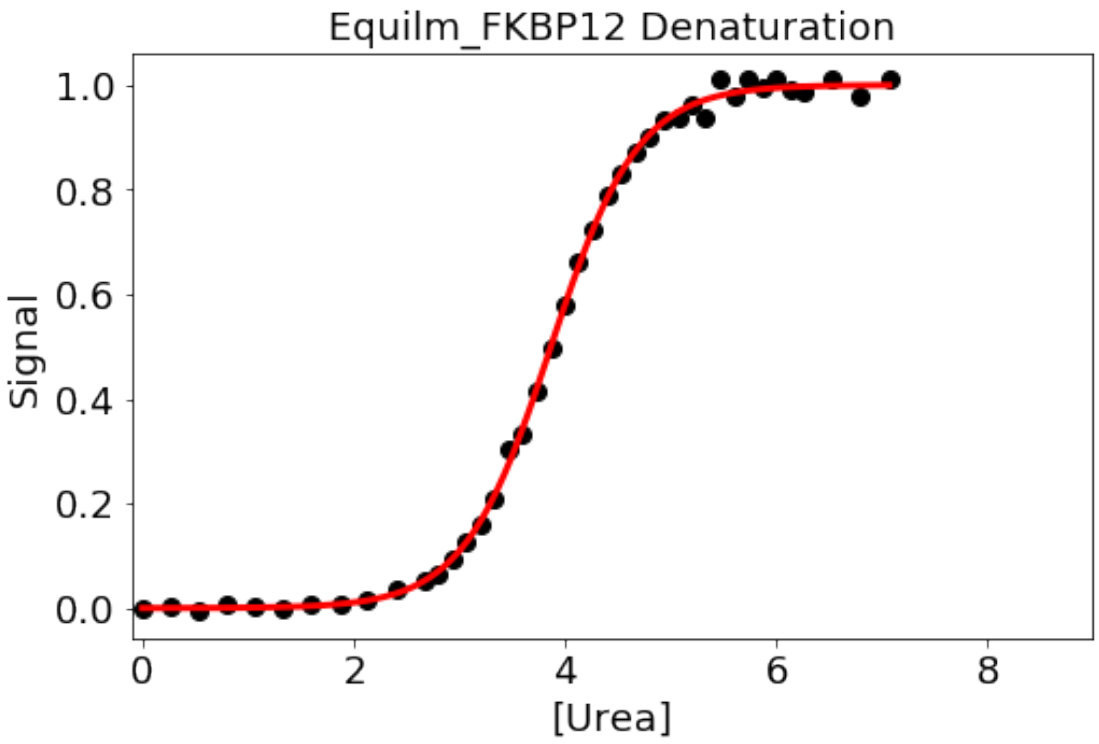
Kinetic Model: TwoStateChevron
Fit Standard Error: 0.03
kf: 4.22e+00 ± 5.34e-02
mf: 1.86e+00 ± 7.03e-03
ku: 1.92e-04 ± 1.08e-05
mu: 9.06e-01 ± 7.99e-03
R^2: 0.99

3.5 Fit to multiple models

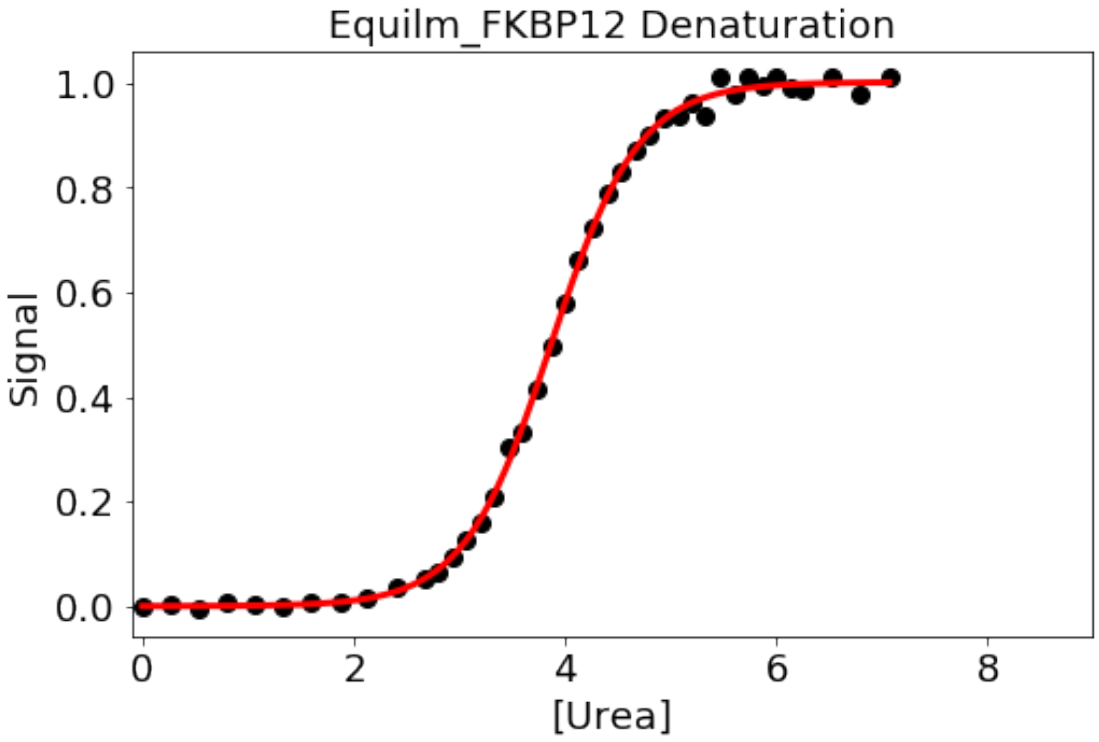
In [14]:
```
# make a list of models to be used to fit
models_to_fit = [models.TwoStateEquilibrium,
                 models.TwoStateEquilibriumSloping]

# and now lets fit them
for model in models_to_fit:
    Equilm_FKBP12.fit_func = model
    Equilm_FKBP12.fit()
    Equilm_FKBP12.plot()
```

```
================================================================================
Fitting results
================================================================================
ID: Equilm_FKBP12
Model: TwoStateEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) m      1.43404 ± 0.00022              95% CI[   1.43399,    1.43410]
(f) d50    3.86730 ± 0.00007              95% CI[   3.86728,    3.86732]
--------------------------------------------------------------------------------
```

```
R^2: 0.99933
================================================================================
```




```
================================================================================
Fitting results
================================================================================
ID: Equilm_FKBP12
Model: TwoStateEquilibriumSloping
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) alpha_f   -0.00000 ± 0.00008            95% CI[  -0.00002,    0.00002]
(f) beta_f    -0.00005 ± 0.00006            95% CI[  -0.00007,   -0.00004]
(f) alpha_u    0.99945 ± 0.00051            95% CI[   0.99932,    0.99958]
(f) beta_u     0.00035 ± 0.00008            95% CI[   0.00033,    0.00037]
(f) m          1.43029 ± 0.00058            95% CI[   1.43014,    1.43043]
(f) d50        3.86799 ± 0.00021            95% CI[   3.86793,    3.86804]
--------------------------------------------------------------------------------
R^2: 0.99933
================================================================================
```



As you can see, each of the models has found a good fit with the default initial variables.

We can also get PyFolding to iterate over a range of initial parameters used (incase, for example, fitting your data is very dependent on your starting parameters).

```
In [15]: #1st select the fit function and associates it with the data
         Equilm_FKBP12.fit_func = models.TwoStateEquilibrium

         # iterate over some different parameters
         for m in xrange(1,3):
             for d50 in xrange (2,4):
                 Equilm_FKBP12.fit(p0=[m,d50])
```

```
================================================================================
Fitting results
================================================================================
ID: Equilm_FKBP12
Model: TwoStateEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) m      1.43404 ± 0.00022            95% CI[   1.43399,    1.43410]
(f) d50    3.86730 ± 0.00007            95% CI[   3.86728,    3.86732]
--------------------------------------------------------------------------------
R^2: 0.99933
================================================================================


================================================================================
Fitting results
================================================================================
ID: Equilm_FKBP12
Model: TwoStateEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) m      1.43404 ± 0.00022            95% CI[   1.43399,    1.43410]
(f) d50    3.86730 ± 0.00007            95% CI[   3.86728,    3.86732]
--------------------------------------------------------------------------------
R^2: 0.99933
================================================================================


================================================================================
Fitting results
================================================================================
ID: Equilm_FKBP12
Model: TwoStateEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) m      1.43404 ± 0.00022            95% CI[   1.43399,    1.43410]
(f) d50    3.86730 ± 0.00007            95% CI[   3.86728,    3.86732]
--------------------------------------------------------------------------------
R^2: 0.99933
================================================================================


================================================================================
Fitting results
================================================================================
```

```
ID: Equilm_FKBP12
Model: TwoStateEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) m      1.43404 ± 0.00022            95% CI[   1.43399,    1.43410]
(f) d50    3.86730 ± 0.00007            95% CI[   3.86728,    3.86732]
--------------------------------------------------------------------------------
R^2: 0.99933
================================================================================
```

End of this Notebook.

---

4 SI Notebook 2 - Fittting more complicated models to some example data

[Author] ERGM

In this notebook we will fit more complicated models to some example data. Here the idea is to show that we can fit multiple models in series to folding data. The models are slightly more complex than two-state folding (Pyfolding SI Notebook 1.) These models can be used with in bought software, but with PyFolding we can automate and is Free!

If you are less script/computer orientated, you can simply change the data paths/variables, etc for your proteins and re-run the jupyter notebook ("Kernal/Restart & Run all" from the menu above).

In this notebook I will be using data from the paper below:

```
Mapping the Topography of a Protein Energy Landscape
Hutton, R. D., Wilkinson, J., Faccin, M., Sivertsson, E. M., Pelizzola, A.,
Lowe, A. R., Bruscolini, P. & Itzhaki, L. S. J Am Chem Soc (2015) 127, 46: 14610-25
```

[http://pubs.acs.org/doi/10.1021/jacs.5b07370]

4.1 Data Format

See PyFolding SI Notebook 1 for how to set up your .csv files for import

4.1.1 Special Considerations

1. If you wish to perform global analyses on folding data, the datasets of the same WT/mutant concerned must be in the same .csv
2. Kinetics data should be entered as rate constants (k) and NOT as the `log` of the rate constant.
3. There can be no "empty" cells in the x-axis variables in the .csv file for kinetics data.

Example .csv structure:

urea	k1	k2
8.59	1.72	
8.77	1.96	
9.00	2.41	
0.54	5.44	23.6
0.79	5.94	21.2
1.02	6.56	21.6
…	…	…

In [1]:
```
# As in previous notebook, lets load pyfolding & pyplot into this ipython notebook
#(pyplot allows us to plot more complex figures of our results):

%matplotlib inline
import pyfolding
from pyfolding import models

# let's use some other libraries also
import matplotlib.pyplot as plt
import numpy as np
```

```
<IPython.core.display.Javascript object>


PyFolding: Jupyter autoscrolling has been disabled
```

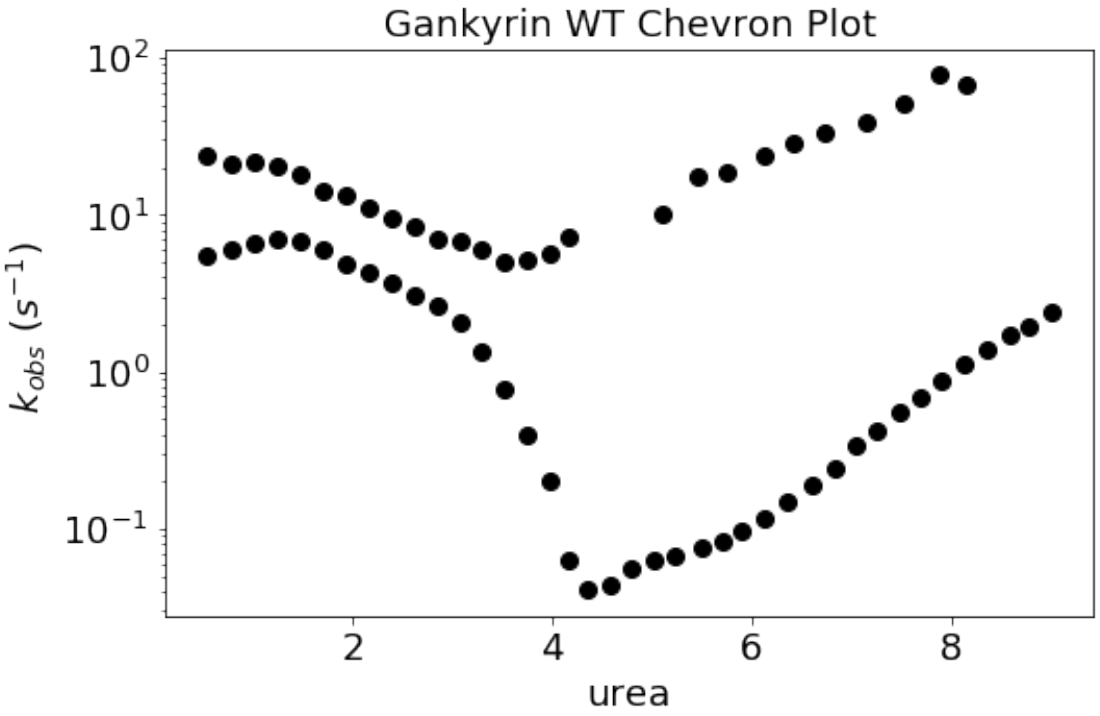
Now, we need to load some data to analyse.

In [2]:
```
# loading the data -
pth = "../examples/Gankyrin"
GankyrinChevron = pyfolding.read_kinetic_data(pth,"GankyrinWTChevron.csv")

# let's give this dataset a good name
GankyrinChevron.ID = 'Gankyrin WT'
```

4.2 Plotting Chevron phases

Let's plot the chevron phases:

In [3]:
```
# easy plotting of the entire dataset ...
GankyrinChevron.plot()
```



Note that the measurements were performed at 25ºC. This is the default for pyFolding. However lets set this to show you how.

```
In [4]: pyfolding.set_temperature(25.0)
```

```
Set temperature to 25.00°C
(NOTE: Careful, this sets the temperature for all subsequent calculations)
```

4.3 Fitting Data To Multiple Models

4.3.1 List Models

Lets first list the models that are already in PyFolding.

We can list the models in pyfolding:

```
In [5]: # Command imports pyfolding models
        from pyfolding.models import *

        # command lists models
        list_models()

        # After the model name:
        #'Verified: True' - the model rigourously tested and it functions as expected.
        #'Verified:False' - the model has not been rigourously tested.
```

```
Out[5]: [('ChevronPolynomialFit', 'Verified: True'),
         ('HeteropolymerIsingEquilibrium', 'Verified: False'),
         ('HomozipperIsingEquilibrium', 'Verified: True'),
         ('ParallelTwoStateChevron', 'Verified: False'),
         ('ParallelTwoStateUnfoldingChevron', 'Verified: False'),
         ('TemplateModel', 'Verified: False'),
         ('ThreeStateChevron', 'Verified: True'),
         ('ThreeStateDimericIEquilibrium', 'Verified: True'),
         ('ThreeStateEquilibrium', 'Verified: True'),
         ('ThreeStateFastPhaseChevron', 'Verified: True'),
         ('ThreeStateMonoIEquilibrium', 'Verified: True'),
         ('ThreeStateSequentialChevron', 'Verified: True'),
         ('TwoStateChevron', 'Verified: True'),
         ('TwoStateChevronMovingTransition', 'Verified: True'),
         ('TwoStateDimerEquilibrium', 'Verified: True'),
         ('TwoStateEquilibrium', 'Verified: True'),
         ('TwoStateEquilibriumSloping', 'Verified: True')]
```

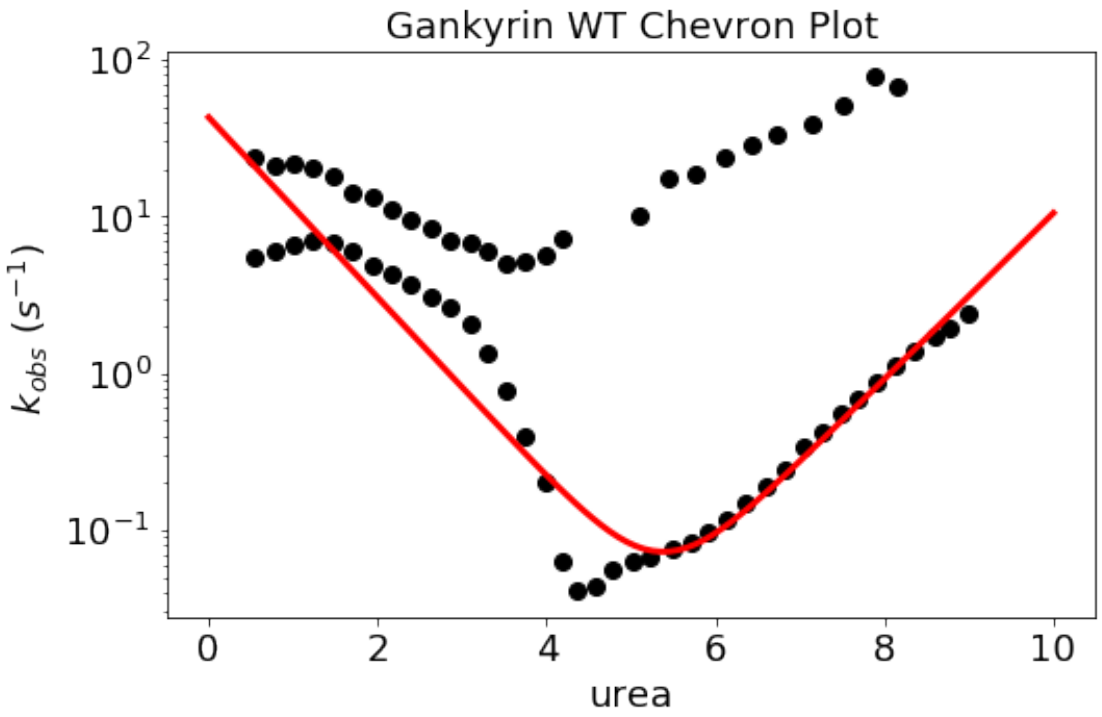
4.3.2 Fit to multiple models

Now lets fit to multiple models

```
In [9]: # make a list of models to be used to fit in the square brackets
        test_models = [models.TwoStateChevron,
                       models.ThreeStateChevron,
                       models.ThreeStateSequentialChevron,
                       models.ThreeStateFastPhaseChevron,
                       models.TwoStateChevronMovingTransition,
                       models.ChevronPolynomialFit]

        #now tell Pyfolding to fit your data to each model
        for model in test_models:
            GankyrinChevron.fit_func = model    # selects fit function & associates with data
```

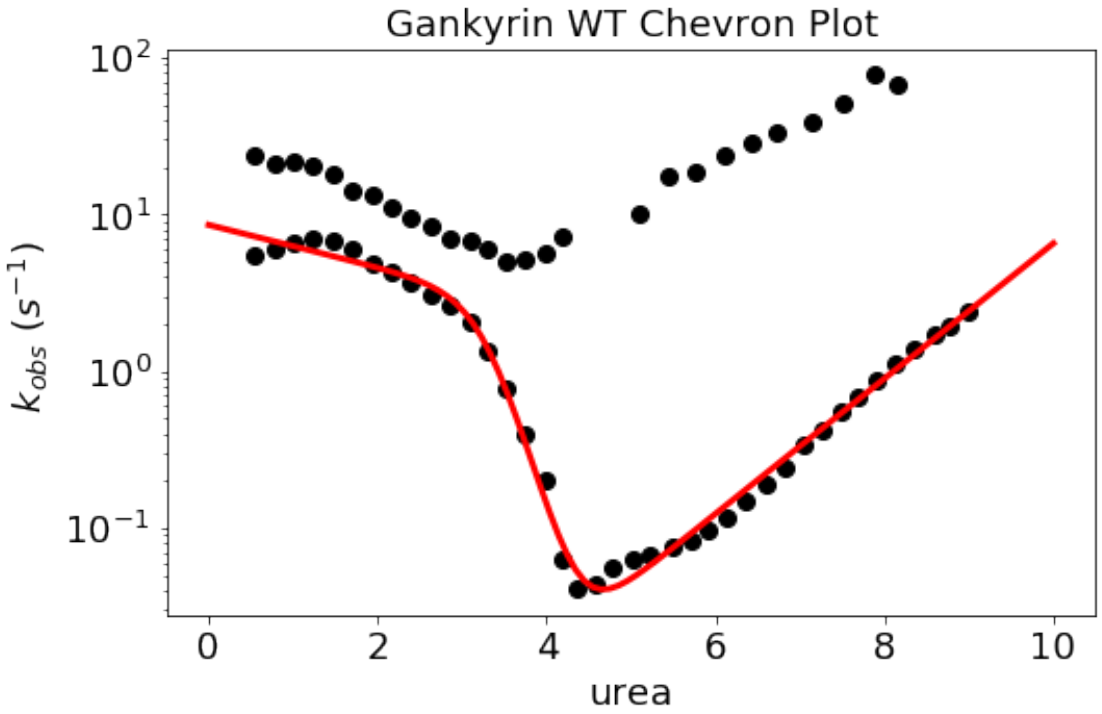
```
GankyrinChevron.fit()      # then fit it.
GankyrinChevron.plot()  # plots it. Add (components=True) to plot component k's.
```

```
================================================================================
Fitting results
================================================================================
ID: Gankyrin WT
Model: TwoStateChevron
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) kf   42.82617 ± 7.83661            95% CI[  40.86319,   44.78916]
(f) mf    1.32179 ± 0.06202            95% CI[   1.30625,    1.33733]
(f) ku    0.00005 ± 0.00004            95% CI[   0.00004,    0.00006]
(f) mu    1.21849 ± 0.09285            95% CI[   1.19524,    1.24175]
--------------------------------------------------------------------------------
R^2: 0.89052
================================================================================
```



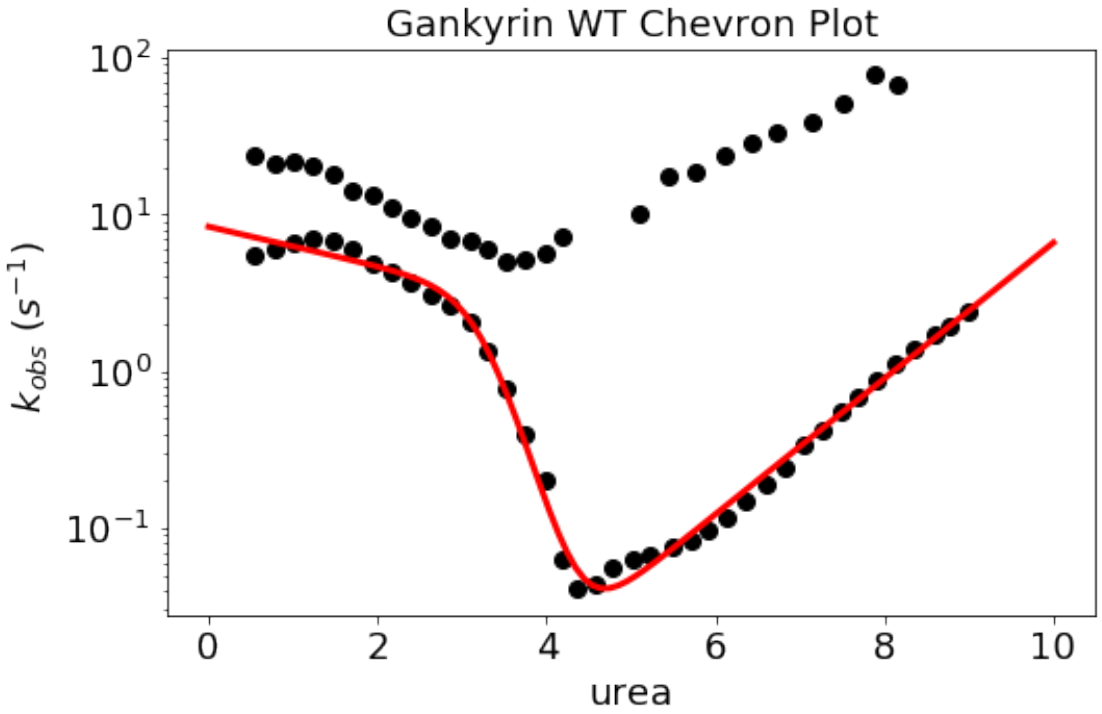

```
================================================================================
Fitting results
```

```
================================================================================
ID: Gankyrin WT
Model: ThreeStateChevron
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) kfi    0.00032 ± 0.00001            95% CI[   0.00032,    0.00032]
(f) mif   -0.99293 ± 0.00453            95% CI[  -0.99406,   -0.99179]
(f) kif 3088061.04961 ± 591967.18085         95% CI[2939906.24233, 3236215.85690]
(f) mi    -5.23401 ± 0.04790            95% CI[  -5.24600,   -5.22202]
(f) Kiu    0.00000 ± 0.00000            95% CI[   0.00000,    0.00000]
(f) mu    -1.30465 ± 0.01243            95% CI[  -1.30776,   -1.30154]
--------------------------------------------------------------------------------
R^2: 0.99254
================================================================================
```



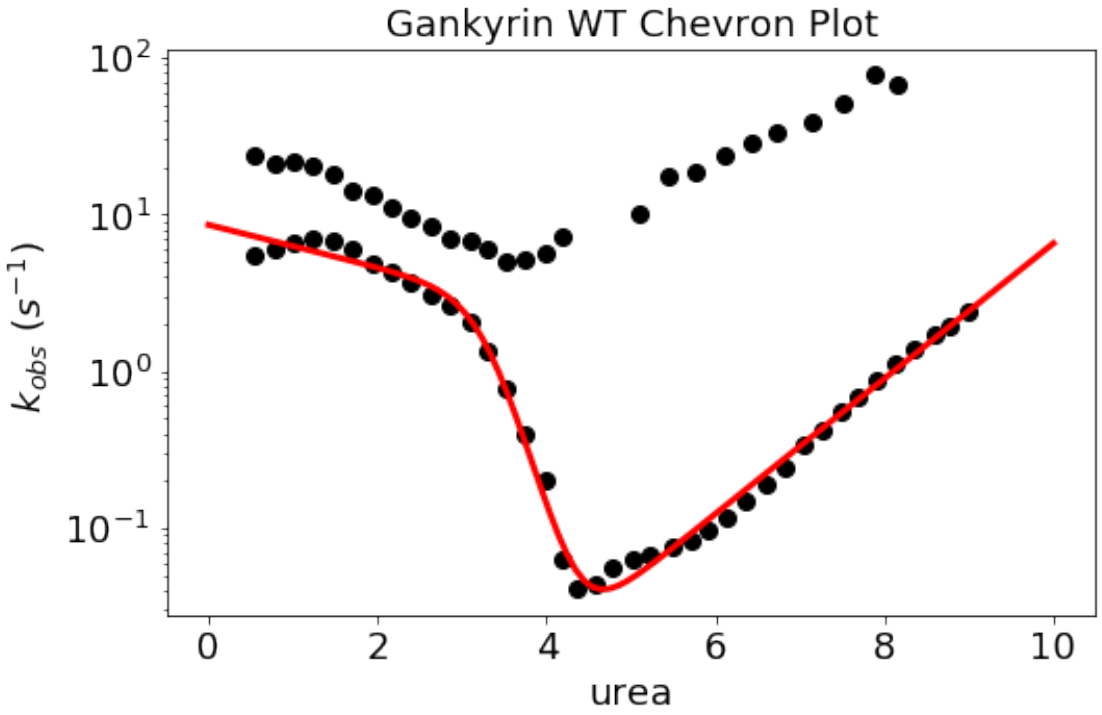

```
================================================================================
Fitting results
================================================================================
ID: Gankyrin WT
Model: ThreeStateSequentialChevron
```

```
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) kui    8.35101 ± inf                95% CI[      -inf,        inf]
(f) mui    0.28903 ± inf                95% CI[      -inf,        inf]
(c) kiu 10000.00000
(c) miu    0.00000
(f) kif 1976594059.17404 ± inf                95% CI[      -inf,        inf]
(f) mif    3.79062 ± inf                95% CI[      -inf,        inf]
(f) kfi    0.00031 ± inf                95% CI[      -inf,        inf]
(f) mfi    0.99698 ± inf                95% CI[      -inf,        inf]
--------------------------------------------------------------------------------
R^2: 0.99250
================================================================================
```



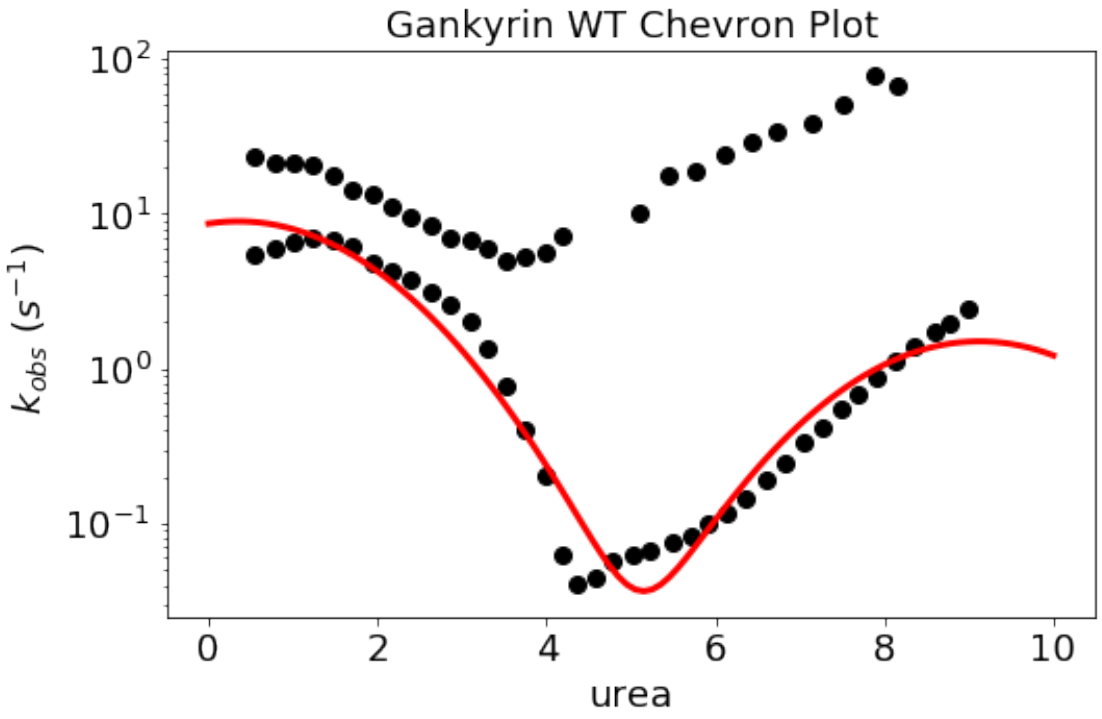

```
================================================================================
Fitting results
================================================================================
ID: Gankyrin WT
Model: ThreeStateFastPhaseChevron
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
```

```
Temperature: 25.00°C

(f) kui  726.43137 ± 328693556.72880          95% CI[-82183999.71540, 82185452.57815]
(f) mui    2.50450 ± 254385.90101           95% CI[-63602.74477, 63607.75378]
(f) kiu    0.00101 ± 456.74252            95% CI[-114.20037,  114.20239]
(f) miu    1.42452 ± 254385.89377           95% CI[-63603.82294, 63606.67198]
(f) kif 6166648.24920 ± 1373603.37261          95% CI[5823200.02911, 6510096.46929]
(f) mif    4.24069 ± 0.05691              95% CI[   4.22646,    4.25492]
(f) kfi    0.00032 ± 0.00001              95% CI[   0.00032,    0.00032]
(f) mfi    0.99294 ± 0.00484              95% CI[   0.99172,    0.99415]
--------------------------------------------------------------------------------
R^2: 0.99254
================================================================================
```



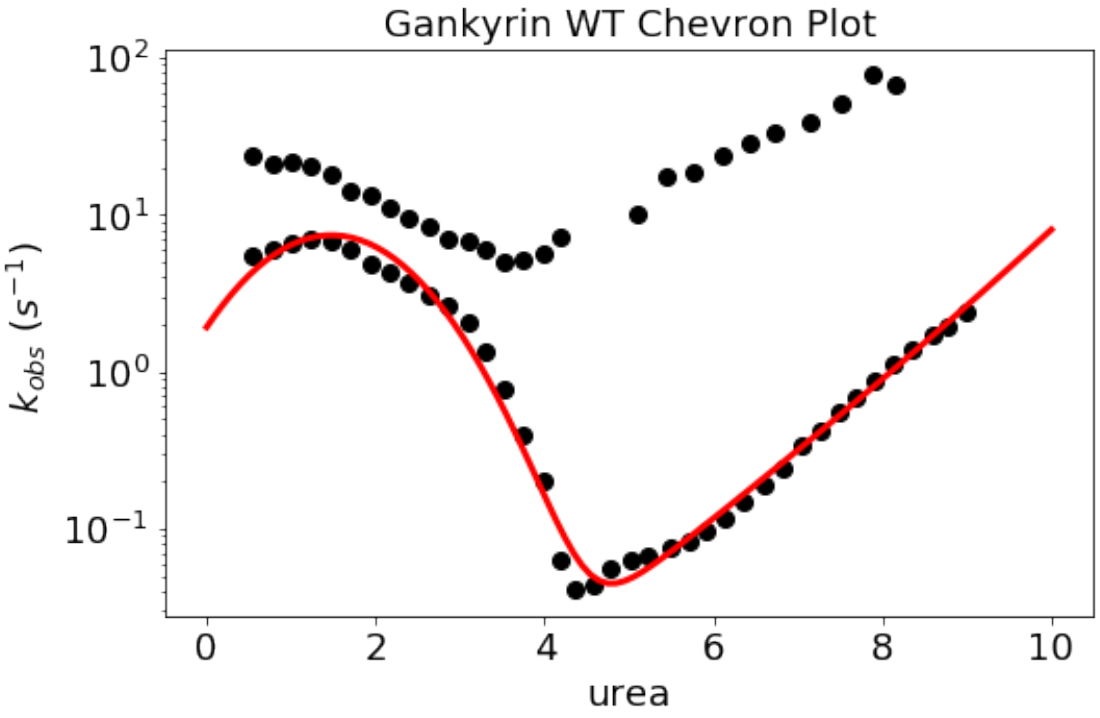

```
================================================================================
Fitting results
================================================================================
ID: Gankyrin WT
Model: TwoStateChevronMovingTransition
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C
```

```
(f) ku          0.00000 ± 0.00000              95% CI[   0.00000,    0.00000]
(f) mu          4.98449 ± 0.25547              95% CI[   4.92052,    5.04845]
(f) kf          8.64128 ± 1.19561              95% CI[   8.34192,    8.94064]
(f) mf          0.19051 ± 0.10469              95% CI[   0.16429,    0.21672]
(f) m_prime    -0.27314 ± 0.01801              95% CI[  -0.27764,   -0.26863]
--------------------------------------------------------------------------------
R^2: 0.94901
================================================================================
```




```
================================================================================
Fitting results
================================================================================
ID: Gankyrin WT
Model: ChevronPolynomialFit
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) ku          0.00062 ± 0.00020              95% CI[   0.00056,    0.00067]
(f) mu          0.76331 ± 0.09633              95% CI[   0.73920,    0.78742]
(f) mu_prime    0.01845 ± 0.00691              95% CI[   0.01672,    0.02018]
```

```
(f) kf          1.92293 ± 0.09362              95% CI[   1.89950,    1.94636]
(f) mf          1.82503 ± 0.04649              95% CI[   1.81339,    1.83666]
(f) mf_prime   -0.61791 ± 0.00971              95% CI[  -0.62034,   -0.61548]
--------------------------------------------------------------------------------
R^2: 0.98724
================================================================================
```



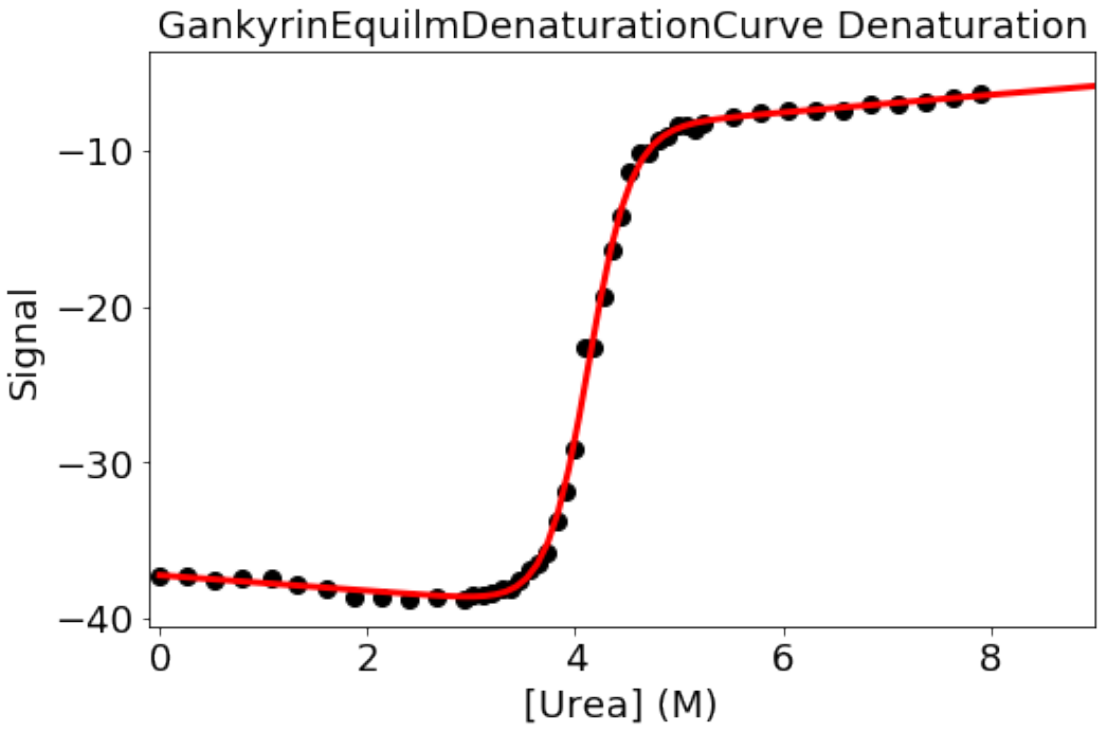
4.3.3 Fit to multiple models and output with prettier graphics

We can also fit to multiple models and output the "prettier" graphics (as in PyFolding SI Notebook 1)

```
In [7]: # First load in the Equilm denaturation (as per PyFolding SI Notebook 1)
        pth = "../examples/Gankyrin"
        GankyrinEquilm = pyfolding.read_equilibrium_data(pth,"GankyrinEquilmDenaturationCurve.cs

        # 1st select the fit function and associates it with the data
```

```
        GankyrinEquilm.fit_func = models.TwoStateEquilibriumSloping

        # then fit it.
        # the brackets enable you to define starting values for the variables see notebook 1
        GankyrinEquilm.fit(p0=[-37, 0.1, -15, 0.1, 3,4])

        # and plot to check all it OK
        GankyrinEquilm.plot()
```

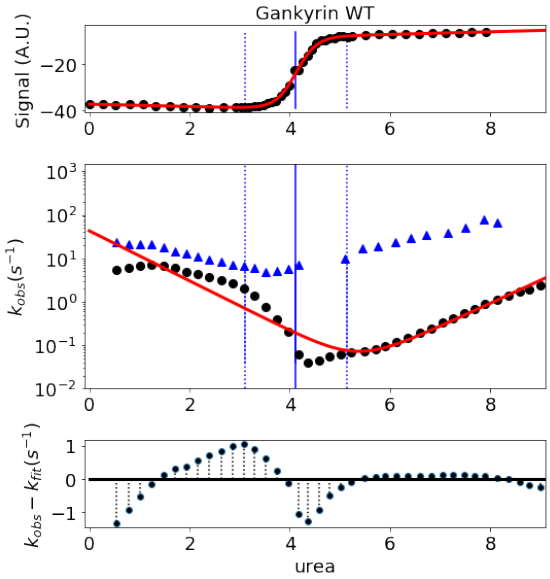
```
================================================================================
Fitting results
================================================================================
ID: GankyrinEquilmDenaturationCurve
Model: TwoStateEquilibriumSloping
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) alpha_f  -37.25158 ± 0.14271            95% CI[ -37.28741,  -37.21575]
(f) beta_f    -0.49562 ± 0.06439            95% CI[  -0.51179,   -0.47946]
(f) alpha_u   26.33776 ± 0.48279            95% CI[  26.21654,   26.45898]
(f) beta_u     1.06005 ± 0.09104            95% CI[   1.03719,    1.08291]
(f) m          2.90885 ± 0.05757            95% CI[   2.89439,    2.92330]
(f) d50        4.12424 ± 0.00486            95% CI[   4.12302,    4.12546]
--------------------------------------------------------------------------------
R^2: 0.99869
================================================================================
```



In [8]:
```
# again make a list of models to be used to fit in the square brackets
test_models = [models.TwoStateChevron,
               models.ThreeStateChevron,
               models.ThreeStateSequentialChevron,
               models.ThreeStateFastPhaseChevron,
               models.TwoStateChevronMovingTransition,
               models.ChevronPolynomialFit]

#now tell Pyfolding to fit your data to each model
for model in test_models:
    GankyrinChevron.fit_func = model # 1st select fit function & associate with data
    GankyrinChevron.fit() # then fit it.
    pyfolding.plot_figure(GankyrinEquilm, GankyrinChevron, display=True) # then plot
```

```
================================================================================
Fitting results
================================================================================
ID: Gankyrin WT
Model: TwoStateChevron
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) kf   42.82617 ± 7.83661            95% CI[  40.86319,   44.78916]
(f) mf    1.32179 ± 0.06202            95% CI[   1.30625,    1.33733]
```

```
(f) ku     0.00005 ± 0.00004              95% CI[    0.00004,     0.00006]
(f) mu     1.21849 ± 0.09285              95% CI[    1.19524,     1.24175]
--------------------------------------------------------------------------------
R^2: 0.89052
================================================================================
```

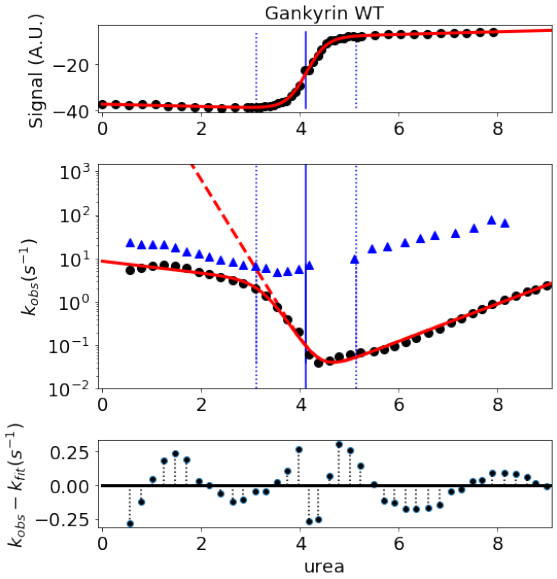


Data-set: GankyrinEquilmDenaturationCurve

Equilibrium Model: TwoStateEquilibriumSloping
alpha_f: -37.25158 ± 0.14271
beta_f: -0.49562 ± 0.06439
alpha_u: 26.33776 ± 0.48279
beta_u: 1.06005 ± 0.09104
m: 2.90885 ± 0.05757
d50: 4.12424 ± 0.00486
Folding midpoint: 4.12 M
R^2: 1.00

Kinetic Model: TwoStateChevron
Fit Standard Error: 0.09
kf: 4.28e+01 ± 7.84e+00
mf: 1.32e+00 ± 6.20e-02
ku: 5.37e-05 ± 3.76e-05
mu: 1.22e+00 ± 9.29e-02
R^2: 0.89

```
================================================================================
Fitting results
================================================================================
ID: Gankyrin WT
Model: ThreeStateChevron
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) kfi    0.00032 ± 0.00001              95% CI[    0.00032,     0.00032]
(f) mif   -0.99293 ± 0.00453              95% CI[   -0.99406,    -0.99179]
(f) kif 3088061.04961 ± 591967.18085          95% CI[2939906.24233, 3236215.85690]
(f) mi    -5.23401 ± 0.04790              95% CI[   -5.24600,    -5.22202]
(f) Kiu    0.00000 ± 0.00000              95% CI[    0.00000,     0.00000]
(f) mu    -1.30465 ± 0.01243              95% CI[   -1.30776,    -1.30154]
--------------------------------------------------------------------------------
R^2: 0.99254
================================================================================
```

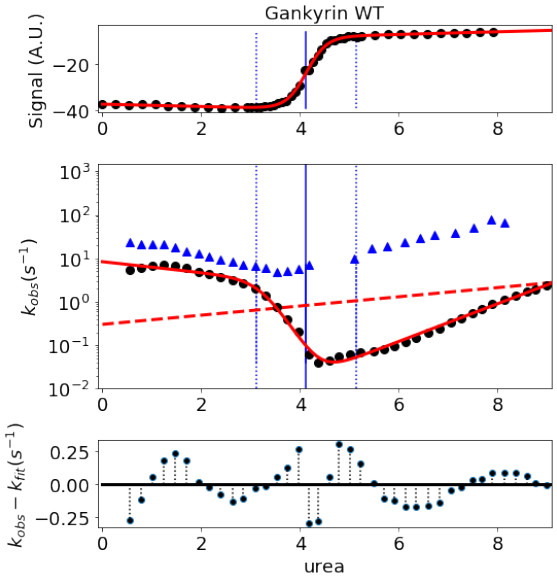


Data-set: GankyrinEquilmDenaturationCurve

Equilibrium Model: TwoStateEquilibriumSloping
alpha_f: -37.25158 ± 0.14271
beta_f: -0.49562 ± 0.06439
alpha_u: 26.33776 ± 0.48279
beta_u: 1.06005 ± 0.09104
m: 2.90885 ± 0.05757
d50: 4.12424 ± 0.00486
Folding midpoint: 4.12 M
R^2: 1.00

Kinetic Model: ThreeStateChevron
Fit Standard Error: 0.02
kfi: 3.19e-04 ± 1.02e-05
mif: -9.93e-01 ± 4.53e-03
kif: 3.09e+06 ± 5.92e+05
mi: -5.23e+00 ± 4.79e-02
Kiu: 2.77e-06 ± 5.12e-07
mu: -1.30e+00 ± 1.24e-02
R^2: 0.99

```
================================================================================
Fitting results
================================================================================
ID: Gankyrin WT
Model: ThreeStateSequentialChevron
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) kui    8.35101 ± inf                95% CI[      -inf,        inf]
(f) mui    0.28903 ± inf                95% CI[      -inf,        inf]
(c) kiu 10000.00000
(c) miu    0.00000
(f) kif 1976594059.17404 ± inf                95% CI[      -inf,        inf]
(f) mif    3.79062 ± inf                95% CI[      -inf,        inf]
(f) kfi    0.00031 ± inf                95% CI[      -inf,        inf]
(f) mfi    0.99698 ± inf                95% CI[      -inf,        inf]
--------------------------------------------------------------------------------
R^2: 0.99250
================================================================================
```

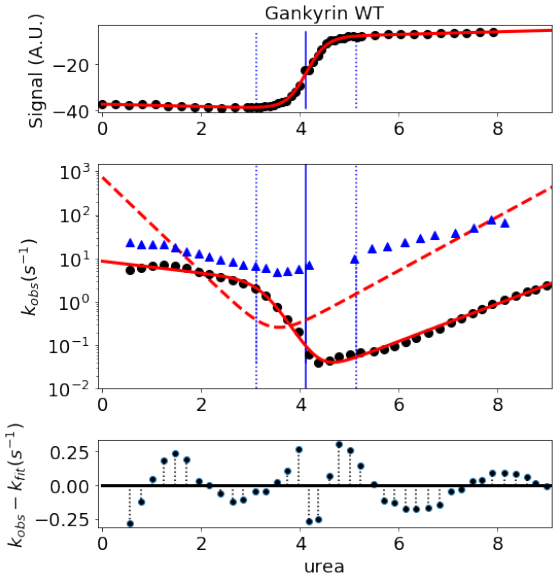


Data-set: GankyrinEquilmDenaturationCurve

Equilibrium Model: TwoStateEquilibriumSloping
alpha_f: -37.25158 ± 0.14271
beta_f: -0.49562 ± 0.06439
alpha_u: 26.33776 ± 0.48279
beta_u: 1.06005 ± 0.09104
m: 2.90885 ± 0.05757
d50: 4.12424 ± 0.00486
Folding midpoint: 4.12 M
R^2: 1.00

Kinetic Model: ThreeStateSequentialChevron
Fit Standard Error: 0.02
kui: 8.35e+00 ± inf
mui: 2.89e-01 ± inf
kiu: 1.00e+04 ± 0.00e+00
miu: 0.00e+00 ± 0.00e+00
kif: 1.98e+09 ± inf
mif: 3.79e+00 ± inf
kfi: 3.09e-04 ± inf
mfi: 9.97e-01 ± inf
R^2: 0.99

```
================================================================================
Fitting results
================================================================================
ID: Gankyrin WT
Model: ThreeStateFastPhaseChevron
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) kui  726.43137 ± 328693556.72880          95% CI[-82183999.71540, 82185452.57815]
(f) mui    2.50450 ± 254385.90101           95% CI[-63602.74477, 63607.75378]
(f) kiu    0.00101 ± 456.74252            95% CI[-114.20037,  114.20239]
(f) miu    1.42452 ± 254385.89377           95% CI[-63603.82294, 63606.67198]
(f) kif 6166648.24920 ± 1373603.37261          95% CI[5823200.02911, 6510096.46929]
(f) mif    4.24069 ± 0.05691              95% CI[   4.22646,    4.25492]
(f) kfi    0.00032 ± 0.00001              95% CI[   0.00032,    0.00032]
(f) mfi    0.99294 ± 0.00484              95% CI[   0.99172,    0.99415]
--------------------------------------------------------------------------------
R^2: 0.99254
================================================================================
```

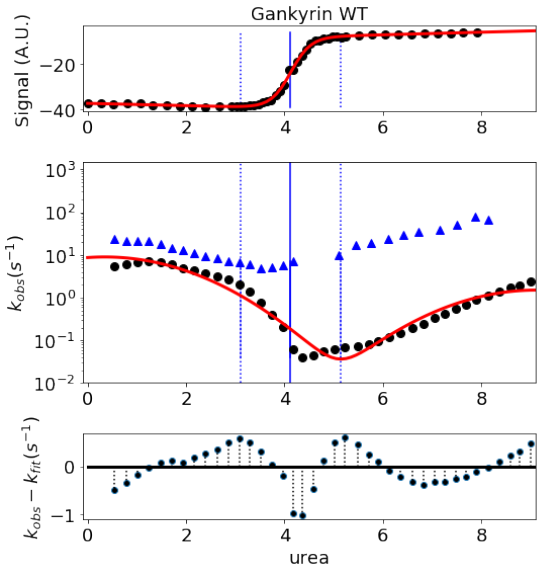


Data-set: GankyrinEquilmDenaturationCurve

Equilibrium Model: TwoStateEquilibriumSloping
alpha_f: -37.25158 ± 0.14271
beta_f: -0.49562 ± 0.06439
alpha_u: 26.33776 ± 0.48279
beta_u: 1.06005 ± 0.09104
m: 2.90885 ± 0.05757
d50: 4.12424 ± 0.00486
Folding midpoint: 4.12 M
R^2: 1.00

Kinetic Model: ThreeStateFastPhaseChevron
Fit Standard Error: 0.02
kui: 7.26e+02 ± 3.29e+08
mui: 2.50e+00 ± 2.54e+05
kiu: 1.01e-03 ± 4.57e+02
miu: 1.42e+00 ± 2.54e+05
kif: 6.17e+06 ± 1.37e+06
mif: 4.24e+00 ± 5.69e-02
kfi: 3.19e-04 ± 1.09e-05
mfi: 9.93e-01 ± 4.84e-03
R^2: 0.99

```
================================================================================
Fitting results
================================================================================
ID: Gankyrin WT
Model: TwoStateChevronMovingTransition
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) ku          0.00000 ± 0.00000            95% CI[   0.00000,    0.00000]
(f) mu          4.98449 ± 0.25547            95% CI[   4.92052,    5.04845]
(f) kf          8.64128 ± 1.19561            95% CI[   8.34192,    8.94064]
(f) mf          0.19051 ± 0.10469            95% CI[   0.16429,    0.21672]
(f) m_prime    -0.27314 ± 0.01801            95% CI[  -0.27764,   -0.26863]
--------------------------------------------------------------------------------
R^2: 0.94901
================================================================================
```

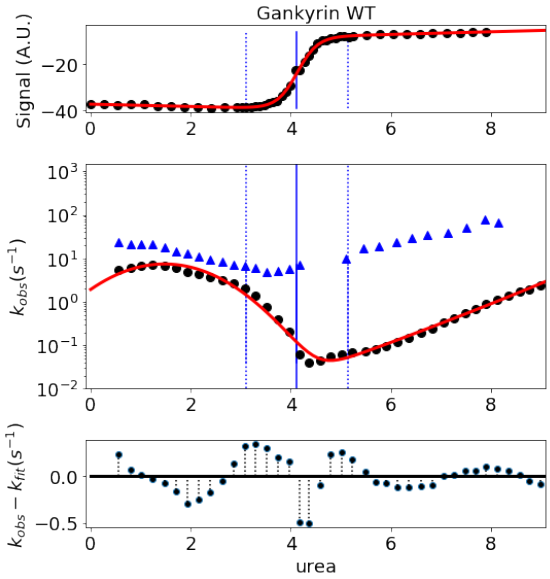


Data-set: GankyrinEquilmDenaturationCurve

Equilibrium Model: TwoStateEquilibriumSloping
alpha_f: -37.25158 ± 0.14271
beta_f: -0.49562 ± 0.06439
alpha_u: 26.33776 ± 0.48279
beta_u: 1.06005 ± 0.09104
m: 2.90885 ± 0.05757
d50: 4.12424 ± 0.00486
Folding midpoint: 4.12 M
R^2: 1.00

Kinetic Model: TwoStateChevronMovingTransition
Fit Standard Error: 0.06
ku: 1.99e-10 ± 1.80e-10
mu: 4.98e+00 ± 2.55e-01
kf: 8.64e+00 ± 1.20e+00
mf: 1.91e-01 ± 1.05e-01
m_prime: -2.73e-01 ± 1.80e-02
R^2: 0.95

```
================================================================================
Fitting results
================================================================================
ID: Gankyrin WT
Model: ChevronPolynomialFit
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) ku          0.00062 ± 0.00020            95% CI[   0.00056,    0.00067]
(f) mu          0.76331 ± 0.09633            95% CI[   0.73920,    0.78742]
(f) mu_prime    0.01845 ± 0.00691            95% CI[   0.01672,    0.02018]
(f) kf          1.92293 ± 0.09362            95% CI[   1.89950,    1.94636]
(f) mf          1.82503 ± 0.04649            95% CI[   1.81339,    1.83666]
(f) mf_prime   -0.61791 ± 0.00971            95% CI[  -0.62034,   -0.61548]
--------------------------------------------------------------------------------
R^2: 0.98724
================================================================================
```



Data-set: GankyrinEquilmDenaturationCurve

Equilibrium Model: TwoStateEquilibriumSloping
alpha_f: -37.25158 ± 0.14271
beta_f: -0.49562 ± 0.06439
alpha_u: 26.33776 ± 0.48279
beta_u: 1.06005 ± 0.09104
m: 2.90885 ± 0.05757
d50: 4.12424 ± 0.00486
Folding midpoint: 4.12 M
R^2: 1.00

Kinetic Model: ChevronPolynomialFit
Fit Standard Error: 0.03
ku: 6.15e-04 ± 2.01e-04
mu: 7.63e-01 ± 9.63e-02
mu_prime: 1.84e-02 ± 6.91e-03
kf: 1.92e+00 ± 9.36e-02
mf: 1.83e+00 ± 4.65e-02
mf_prime: -6.18e-01 ± 9.71e-03
R^2: 0.99

End of this Notebook.

---

5 SI Notebook 3 - Performing and automating higher-level calculations such as ΔGs & Phi-values

[Author] ERGM

In this notebook we will show how imported and fitted equilbrium and kinetic folding data (as per PyFolding SI Notebooks 1 and 2) can be associated with a particular protein (say WT or a mutant). This enables higher higher-level calculations such as ΔGs & Phi-value analysis to be achieved (also shown here).

If you are less script/computer orientated, you can simply change the data paths/variables, etc for your proteins and re-run the jupyter notebook ("Kernal/Restart & Run all" from the menu above).

5.1 Data Format

Please see PyFolding SI Notebooks 1 and 2 for the format your data has to be in to enable this type of analysis.

```
In [1]: #  First off lets load pyfolding & pyplot into this ipython notebook
        #(pyplot allows us to plot more complex figures of our results):

        %matplotlib inline

        # import pyfolding, the pyfolding models and ising models
        import pyfolding
        from pyfolding import *

        # import the package for plotting, call it plt
        import matplotlib.pyplot as plt

        # import numpy as well
        import numpy as np
```

```
<IPython.core.display.Javascript object>
```

```
PyFolding: Jupyter autoscrolling has been disabled
```

Now, we need to load some data to analyse. In this notebook I will be using data that I have digitized from the papers below:

```
Folding of chymotrypsin inhibitor 2. 1. Evidence for a two-state transition.
Jackson, S. E. & Fersht, A. R.
Biochemistry 30, 10428-10435 (1991).

Structure of the hydrophobic core in the transition state for folding of
chymotrypsin inhibitor 2: a critical test of the protein engineering
method of analysis.
Jackson, S. E., elMasry, N. & Fersht, A. R.
Biochemistry 32, 11270-11278 (1993).
```

[http://pubs.acs.org/doi/abs/10.1021/bi00107a010 & http://pubs.acs.org/doi/abs/10.1021/bi00093a002]

In [2]:
```
# start by loading a data set
# arguments are "path", "filename"
# We will do Equilm and Kinetics all together

pth = "../examples/CI2/"

#load CI2 WT equilm and kinetics
EquilmWT_CI2 = pyfolding.read_equilibrium_data(pth,"CI2_WT_Equilm.csv")
KineticsWT_CI2 = pyfolding.read_kinetic_data(pth,"CI2_WT_Kinetics.csv")

# load CI2 Mutant VA38
EquilmVA38_CI2 = pyfolding.read_equilibrium_data(pth,"CI2_VA38_Equilm.csv")
KineticsVA38_CI2 = pyfolding.read_kinetic_data(pth,"CI2_VA38_Kinetics.csv")


# load CI2 Mutant VA66
EquilmVA66_CI2 = pyfolding.read_equilibrium_data(pth,"CI2_VA66_Equilm.csv")
KineticsVA66_CI2 = pyfolding.read_kinetic_data(pth,"CI2_VA66_Kinetics.csv")


# load CI2 Mutant IV48
EquilmIV48_CI2 = pyfolding.read_equilibrium_data(pth,"CI2_IV48_Equilm.csv")
KineticsIV48_CI2 = pyfolding.read_kinetic_data(pth,"CI2_IV48_Kinetics.csv")
```

OK, to perform and automate higher-level calculations such as ΔGs & Phi-value analysis

5.2 Assign Data to a specific protein object

1st we need to assign data to a specific protein by making a "protein object"

In [3]:
```
#assign WT
WT = pyfolding.Protein(ID="CI2_WT")      # creating CI2_WT protein object
WT.equilibrium = EquilmWT_CI2            # assigning equilm data to the CI2_WT protein ob
WT.chevron = KineticsWT_CI2              # assigning kinetic data to the CI2_WT protein o

#assign VA38
VA38 = pyfolding.Protein(ID="CI2_VA38") # creating CI2_WT protein object
VA38.equilibrium = EquilmVA38_CI2        # assigning equilm data to the CI2_WT protein ob
VA38.chevron = KineticsVA38_CI2          # assigning kinetic data to the CI2_WT protein o

#assign VA66
VA66 = pyfolding.Protein(ID="CI2_VA66") # creating CI2_WT protein object
VA66.equilibrium = EquilmVA66_CI2        # assigning equilm data to the CI2_WT protein ob
VA66.chevron = KineticsVA66_CI2          # assigning kinetic data to the CI2_WT protein o

#assign IV48
IV48 = pyfolding.Protein(ID="CI2_IV48") # creating CI2_WT protein object
IV48.equilibrium = EquilmIV48_CI2        # assigning equilm data to the CI2_WT protein ob
IV48.chevron = KineticsIV48_CI2          # assigning kinetic data to the CI2_WT protein o

# lets put these in a python list for later on!
Proteins = [WT,
            VA38,
            VA66,
            IV48]
```

In [4]:
```
# make a python list of the data so we can fit data together and check everything
#loaded correctly

Equilm_curves = [EquilmWT_CI2,
                 EquilmVA38_CI2,
                 EquilmVA66_CI2,
                 EquilmIV48_CI2]

Kinetic_chevrons = [KineticsWT_CI2,
                    KineticsVA38_CI2,
                    KineticsVA66_CI2,
                    KineticsIV48_CI2]
```

5.3 Fit the Data

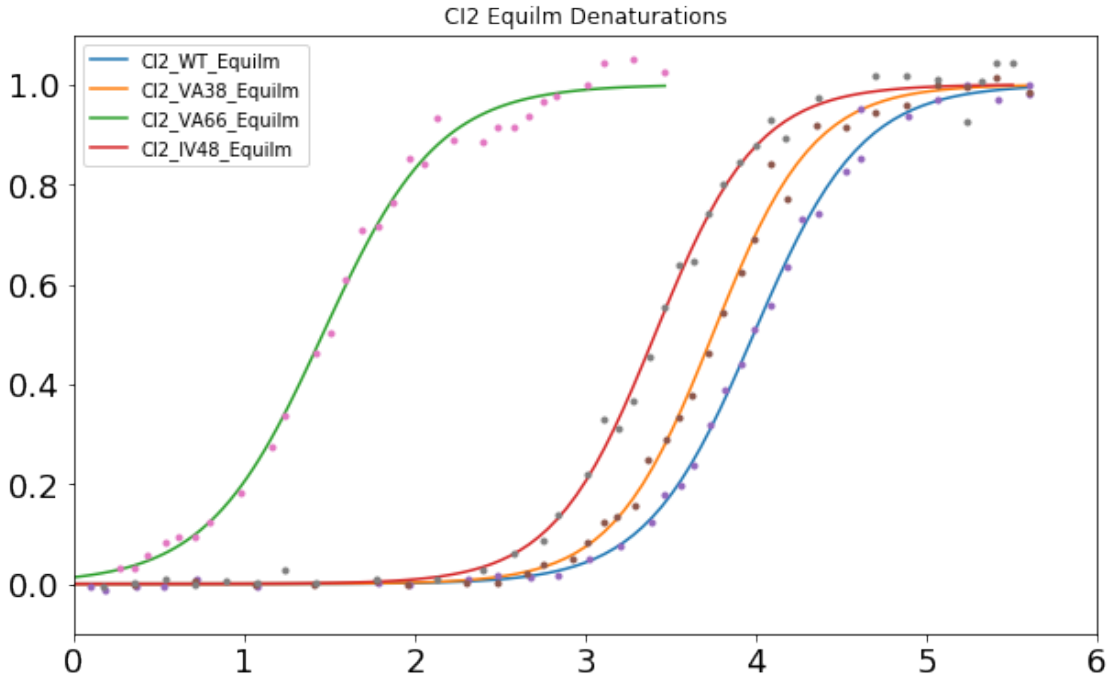
Now we need to fit the data. In a similar manner to previous notebooks, we can save time by getting PyFolding to automate the fitting all our data to the model we want:

```
In [5]: # Set temperature to 25.00°C
        # (NOTE: Careful, this sets the temperature for all subsequent calculations)
        pyfolding.set_temperature(25.)

        # fit all the data automatically

        # first lets fit the equilibrium denaturations (we will be using the lists we defined ab
        for c in Equilm_curves:
            c.fit_func = models.TwoStateEquilibrium
            c.fit(p0=[3, 2])
```

```
Set temperature to 25.00°C
(NOTE: Careful, this sets the temperature for all subsequent calculations)
================================================================================
Fitting results
================================================================================
ID: CI2_WT_Equilm
Model: TwoStateEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) m      1.86581 ± 0.00075            95% CI[   1.86563,    1.86600]
(f) d50    3.98689 ± 0.00013            95% CI[   3.98686,    3.98693]
--------------------------------------------------------------------------------
R^2: 0.99809
================================================================================


================================================================================
Fitting results
================================================================================
ID: CI2_VA38_Equilm
Model: TwoStateEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) m      2.00158 ± 0.00122            95% CI[   2.00127,    2.00188]
(f) d50    3.74752 ± 0.00019            95% CI[   3.74748,    3.74757]
--------------------------------------------------------------------------------
R^2: 0.99720
================================================================================


================================================================================
Fitting results
================================================================================
ID: CI2_VA66_Equilm
Model: TwoStateEquilibrium
```

```
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) m      1.74742 ± 0.00193            95% CI[   1.74693,    1.74790]
(f) d50    1.45999 ± 0.00042            95% CI[   1.45989,    1.46010]
--------------------------------------------------------------------------------
R^2: 0.99391
================================================================================


================================================================================
Fitting results
================================================================================
ID: CI2_IV48_Equilm
Model: TwoStateEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) m      1.96731 ± 0.00168            95% CI[   1.96689,    1.96773]
(f) d50    3.40891 ± 0.00026            95% CI[   3.40884,    3.40897]
--------------------------------------------------------------------------------
R^2: 0.99645
================================================================================
```

In [6]:
```
# Before we go further lets check everything looks good!

# the following commands plot all the Equilm curves & fits on one plot

plt.figure(figsize=(10,6))
for c in Equilm_curves:
    plt.plot(c.results.x_fit, c.results.y_fit, '-')
for c in Equilm_curves:
    plt.plot(c.x, c.y, '.')

# This is to make the plot look good!

plt.legend([c.ID for c in Equilm_curves], loc='best') # plots a legend & 'loc" command d
plt.title("CI2 Equilm Denaturations")                 # plots a title.
plt.ylim([-0.1, 1.1])                                 # y axis from 0 to 8
plt.xlim([0, 6])                                      # x axis from 0 to 5
plt.show()
```

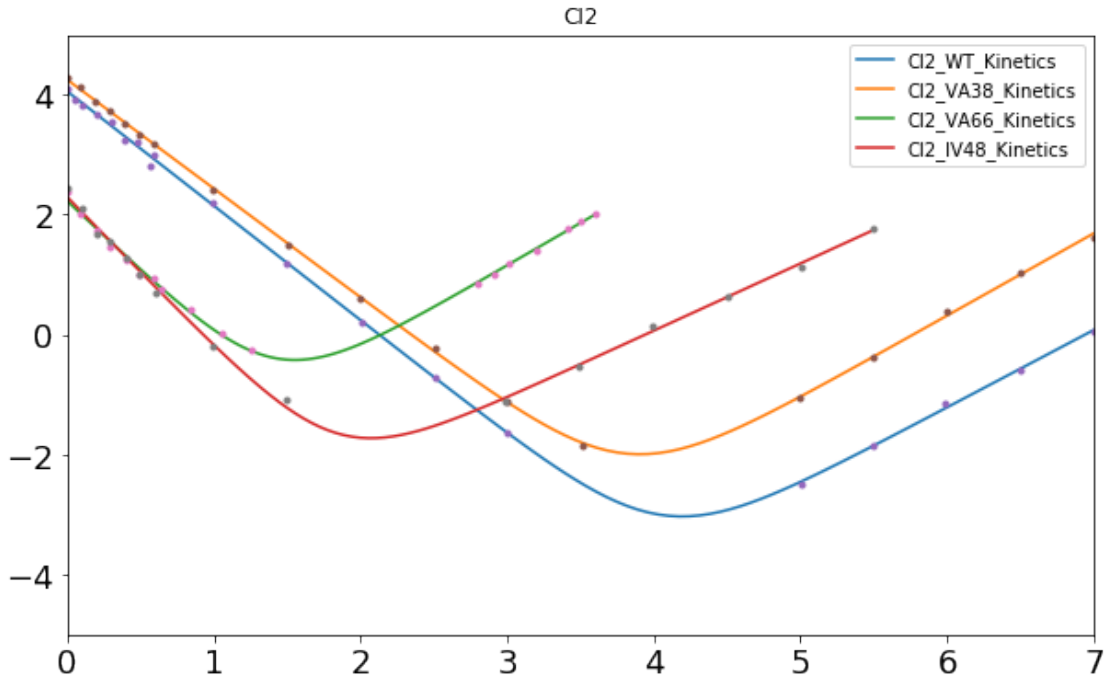



```
In [14]: # Now lets fit the kinetics
         for c in Kinetic_chevrons:
             c.fit_func = models.TwoStateChevron
             c.fit()
```

```
================================================================================
Fitting results
================================================================================
ID: CI2_WT_Kinetics
Model: TwoStateChevron
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) kf   57.74792 ± 0.08261            95% CI[  57.72761,   57.76823]
(f) mf    1.90608 ± 0.00111            95% CI[   1.90581,    1.90636]
(f) ku    0.00013 ± 0.00000            95% CI[   0.00013,    0.00013]
(f) mu    1.29050 ± 0.00248            95% CI[   1.28989,    1.29111]
--------------------------------------------------------------------------------
R^2: 0.99939
================================================================================


================================================================================
Fitting results
================================================================================
```

```
ID: CI2_VA38_Kinetics
Model: TwoStateChevron
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) kf   70.14623 ± 0.05693            95% CI[  70.13226,   70.16019]
(f) mf    1.81407 ± 0.00055            95% CI[   1.81393,    1.81420]
(f) ku    0.00037 ± 0.00000            95% CI[   0.00037,    0.00037]
(f) mu    1.37146 ± 0.00121            95% CI[   1.37116,    1.37175]
--------------------------------------------------------------------------------
R^2: 0.99960
================================================================================


================================================================================
Fitting results
================================================================================
ID: CI2_VA66_Kinetics
Model: TwoStateChevron
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) kf    9.26632 ± 0.02154            95% CI[   9.26103,    9.27160]
(f) mf    2.33134 ± 0.00486            95% CI[   2.33015,    2.33253]
(f) ku    0.04530 ± 0.00070            95% CI[   0.04512,    0.04547]
(f) mu    1.41589 ± 0.00482            95% CI[   1.41471,    1.41707]
--------------------------------------------------------------------------------
R^2: 0.99325
================================================================================


================================================================================
Fitting results
================================================================================
ID: CI2_IV48_Kinetics
Model: TwoStateChevron
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(f) kf    9.92141 ± 0.03494            95% CI[   9.91292,    9.92990]
(f) mf    2.50768 ± 0.00590            95% CI[   2.50624,    2.50911]
(f) ku    0.01212 ± 0.00017            95% CI[   0.01208,    0.01216]
(f) mu    1.11975 ± 0.00319            95% CI[   1.11897,    1.12052]
--------------------------------------------------------------------------------
R^2: 0.99578
================================================================================
```

In [8]:
```
# Again,before we go further lets check everything looks good!

# the following commands plot all the kinetics on one plot
plt.figure(figsize=(10,6))
for c in Kinetic_chevrons:
    plt.plot(c.results.x_fit, c.results.y_fit, '-')
for c in Kinetic_chevrons:
    plt.plot(c.x, c.y, '.')

# as before this is to make the plot look good!

plt.legend([c.ID for c in Kinetic_chevrons], loc='best')  # plots a legend & 'loc" comma
plt.title("CI2")                                          # plots a title.
plt.ylim([-5, 5])                                         # y axis from 0 to 8
plt.xlim([0, 7])                                          # x axis from 0 to 5
plt.show()
```



Now lets perform and automate some higher-level calculations such as Delta Gs & Phi-value analysis :

5.4 Calculating Free Energy of unfolding

In [9]:
```
# Lets start with the free energy of unfolding (ΔGD-N) [calculated from the equilm m-val
# we can get an individual values for each protein by calling up our protein objects we
# Lets try it for WT

print WT.deltaG
```

```
7.43880166046
```

In [10]:
```
# Now we can automate with the list we created at the start of this notebook {in cell (
# from the list in cell (3) the order should be: WT, VA38, VA66 & IV48

for c in Proteins:
    print "{0:s} --> delta G: {1:.2f}".format(c.ID, c.deltaG)
```

```
CI2_WT --> delta G: 7.44
CI2_VA38 --> delta G: 7.50
CI2_VA66 --> delta G: 2.55
CI2_IV48 --> delta G: 6.71
```

Protein	delta G
WT	7.53
VA38	7.54
VA66	2.57
IV48	6.80

The values from the paper are:

In [11]:
```
# We can also do calculations on any of the data we have.
#Lets calculate some DDG values for VA66

# Stability change in water between VA66 and WT (i.e. DDG from Equilibrium curves)
ddg = WT.deltaG - VA66.deltaG

# Free Energy change between Denatured and Transition State between VA66 and WT
#(i.e. DDG from folding rates in water)

ddts = constants.RT * np.log(WT.kf_H20 / VA66.kf_H20)
```

```
        print ddg, ddts
```

```
4.88758792146 0.354099720978
```

5.5 Calculating Phi Values

```
In [12]: # Lets finish by calculating some Phi Values
         # we can get an individual value by calling up our protein objects we defined
         # at the start of this notebook {in cell (3)}
         # Lets try it for the mutant VA38

         from pyfolding.phi import phi

         PhiValue = phi(WT,VA66)

         print PhiValue
```

```
0.0724487675042
```

```
In [13]: # Now we can automate with the list we created also at the start of this notebook {in c
         # from the list in cell (3) the order should be a Phi value for VA38, IV48 & IA48
         mut_proteins = Proteins[1:]

         for c in mut_proteins:
             c_phi_value = phi(WT, c)
             print "{0:s} --> Phi value: {1:.2f}".format(c.ID, c_phi_value)
```

```
CI2_VA38 --> Phi value: 0.45
CI2_VA66 --> Phi value: 0.07
CI2_IV48 --> Phi value: 0.46
```

5.5.1 As you can see this works. YAY!

However, the final Phi values are different from the original CI2 paper due to errors from digitizing the data. CI2_VA38 –> Phi value: 0.45 CI2_VA66 –> Phi value: 0.23 CI2_IV48 –> Phi value: 0.29

For example, VA38 - has a very small difference in stability from WT. Thus when digitising this causes a large error in the reported Phi values.

End of this Notebook.

---

6 SI Notebook 4 - Fitting multiple datasets with linked equations (share certain parameters between the models)

[Authors] ERGM, ARL

In this notebook, let's try to fit a complicated model to some example data. Here the idea is to fit two models to two datasets, but share some of the parameters between the two models. In real terms this is difficult to do in most bought fitting software, but we have the full power of a programming language at our disposal.

We can use the GlobalFit object to do exactly what we want. Let's start by getting some nice data digitised from this paper:

```
Structural insights into an equilibrium folding intermediate of an archaeal ankyrin repeat
protein. Low C, Weininger U, Neumann P, Klepsch M, Lilie H, Stubbs MT, and Balbach J
PNAS (2008) 105: 3779-3784
```

[http://www.pnas.org/cgi/doi/10.1073/pnas.0710657105]

Remember, if you are less script/computer orientated, you can simply change the data paths/variables, etc for your proteins and re-run the jupyter notebook ("Kernal/Restart & Run all" from the menu above).

6.1 Data Format

Please see PyFolding SI Notebooks 1 and 2 for the format your data has to be in to enable this type of analysis.

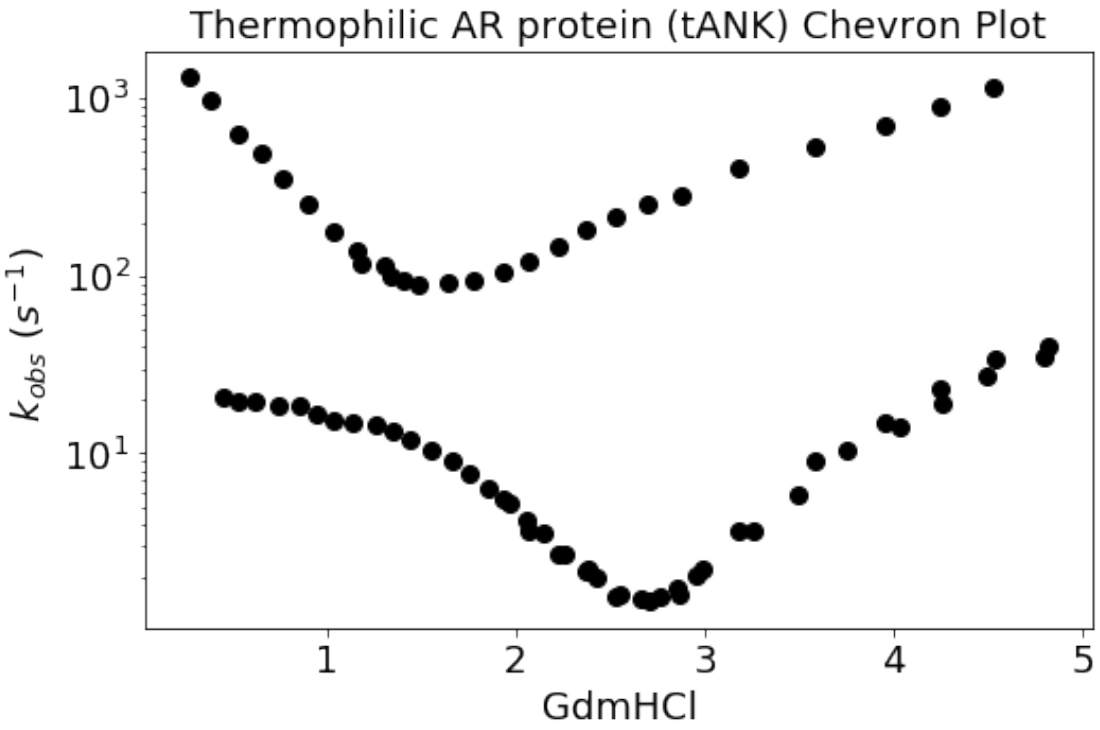
In [1]:
```
# First off let's load pyfolding & pyplot into this ipython notebook
# (pyplot allows us to plot more complex figures of our results):

%matplotlib inline
import pyfolding
from pyfolding import models

# let's use some other libraries also
import matplotlib.pyplot as plt
import numpy as np
```

```
<IPython.core.display.Javascript object>
```

```
PyFolding: Jupyter autoscrolling has been disabled
```

6.2 Load and Plot the Data

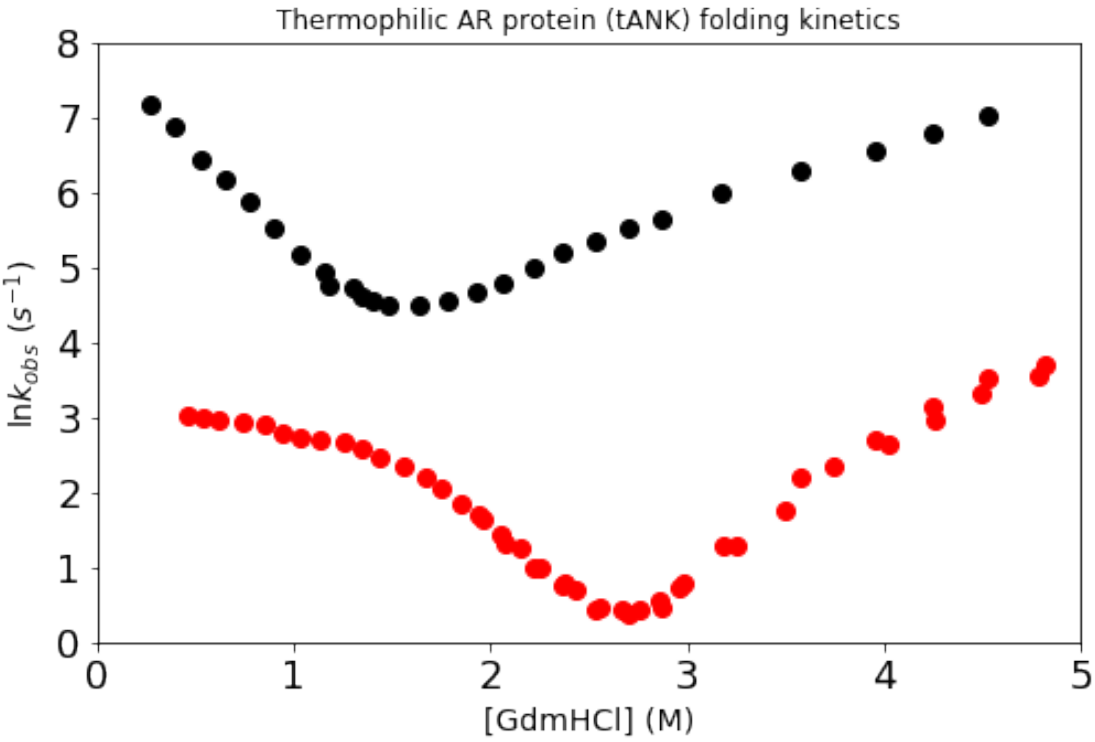
```
In [2]: # loading the data - The kinetics of each protein is in one .csv as per
        #PyFolding SI Notebooks 1 and 2

        pth = "../examples/tANK/"
        tANK_all = pyfolding.read_kinetic_data(pth,"ChevronAll.csv")

In [3]: # let's give this dataset a good name
        tANK_all.ID = 'Thermophilic AR protein (tANK)'

In [4]: # easy plotting of the entire dataset ...
        tANK_all.plot()
```



6.3 Custom Plotting

```
In [5]: # ...or, custom plotting

        k1_x, k1_y = tANK_all.chevron('k1') # defines the slower chevron rates
        k2_x, k2_y = tANK_all.chevron('k2') # defines the faster chevron rates

        plt.figure(figsize=(8,5))           # makes the figure 8cm by 5cm
        plt.plot(k1_x, k1_y, 'ro', markersize=8) # red filled circles, sized '8'
        plt.plot(k2_x, k2_y, 'ko', markersize=8) # black filled circles, sized '8'
```

```
plt.rc('xtick', labelsize=14)             # fontsize of the x tick labels
plt.rc('ytick', labelsize=14)             # fontsize of the y tick labels
plt.ylim([0, 8])                          # y axis from 0 to 8
plt.xlim([0, 5])                          # x axis from 0 to 5
plt.grid(False)                           # no grid on the graph
plt.xlabel('[GdmHCl] (M)', fontsize=14)   # x axis title with fontsize
plt.ylabel(r'$\ln k_{obs}$ $(s^{-1})$', fontsize=14)  # y axis title with fontsize
plt.title('Thermophilic AR protein (tANK) folding kinetics') # Plot title
plt.show()
```



6.4 Set temperature

Note that the measurements were performed at 15°C, so we need to adjust the temperature:

```
In [6]: pyfolding.set_temperature(15.0)

Set temperature to 15.00°C
(NOTE: Careful, this sets the temperature for all subsequent calculations)
```

6.5 List the Models used

In the paper they decided that their data showed that their protein was folding via a three state mechanism. So lets try fitting the digitized data to this model - three state chevron with fast phase. To do this, we need to share the parameters like this:

1. Fit a "two-state chevron" model to the fast phase, and share those params with:
2. A "three-state with fast phase" model for the slower phase

First of all lets print the information on each model:

```
In [7]: models.TwoStateChevron().info()
```

$$k_{obs} = k_f + k_u$$

where:

$$\begin{aligned} k_f &= k_f^{H_2O} \exp(-m_{kf} x) \\ k_u &= k_u^{H_2O} \exp(m_{ku} x) \end{aligned} \tag{2}$$

thus:

$$k_{obs} = k_f^{H_2O} \exp(-m_{kf} x) + k_u^{H_2O} \exp(m_{ku} x)$$

```
Two state chevron plot.

        Folding Scheme:
                N <-> D

        Params:
                k obs = rate constant of unfolding or refolding at a particular denaturant conce
                kf = rate constant of refolding at a particular denaturant concentration
                mf = the gradient of refolding arm of the chevron
                ku = rate constant of unfolding at a a particular denaturant concentration
                mu = the gradient of unfolding arm of the chevron
                x = denaturant concentration (M)

        Reference:
                Jackson SE and Fersht AR.  Folding of chymotrypsin inhibitor 2.
                1. Evidence for a two-state transition.
                Biochemistry (1991) 30(43):10428-10435.
```

```
In [8]: models.ThreeStateFastPhaseChevron().info()
```

$$k_{obs} = \frac{k_{fi} + k_{if}}{(1 + 1/K_{iu})}$$

where:

$$\begin{aligned} k_{fi} &= k_{fi}^{H_2O} \exp(m_{fi}x) \\ k_{if} &= k_{if}^{H_2O} \exp(-m_{if}x) \\ k_{iu} &= k_{iu}^{H_2O} \exp(m_{iu}x) \\ k_{ui} &= k_{ui}^{H_2O} \exp(-m_{ui}x) \\ K_{iu} &= \frac{k_{iu}}{k_{iu} + k_{ui}} \end{aligned} \tag{3}$$

```
Three state chevron with single intermediate.

        Folding Scheme: N <-> I <-> D

        Params:
                k obs = rate constant of unfolding or refolding at a particular denaturant conce
                kfi = microscopic rate constant for the conversion of folded to intermediate
                kif = microscopic rate constant for the conversion of intermediate to folded
                kiu = microscopic rate constant for the conversion of intermediate to unfolded
                kui = microscopic rate constant for the conversion of unfolded to intermediate
                Kiu = equilibrium constant for the rapid equilibration between intermediate & un
                mfi = m-value associated with the kinetic transition between folded & intermedia
                mif = m-value associated with the kinetic transition between intermediate & fold
                miu = m-value associated with the kinetic transition between intermediate & unfo
                mui = m-value associated with the kinetic transition between unfolded & intermed
                x = denaturant concentration (M)

        Reference:
                Parker et al. An integrated kinetic analysis of
                intermediates and transition states in protein folding reactions.
                Journal of molecular biology (1995) vol. 253 (5) pp. 771-86
```

6.6 Create a New model - called FastPhase

To fit the data to two equations at once where certain parameters are shared, we need to create a fast phase model, which is essentially a two state chevron, but where the parameters represent the folding and unfolding of the intermediate. We can do this *on-the-fly* by using the template model to create a new FastPhase** model:**

```
In [9]: class FastPhase(pyfolding.FitModel):
            """ Fast phase model
            """
            def __init__(self):
                pyfolding.FitModel.__init__(self)
                fit_args = self.fit_func_args
                self.params = tuple( [(fit_args[i],i) for i in xrange(len(fit_args))] )
                self.default_params = np.array([100., 1.3480, 5e-4, 1.])
                self.verified = True


            def fit_func(self, x, kui, mui, kiu, miu):
                k_obs = kui*np.exp(-mui*x) + kiu*np.exp(miu*x)
                return k_obs

            def error_func(self, y):
                return np.log(y)
```

6.7 Setting up a custom Global fit

```
In [10]: # We first need to differentiate the data into the two phases we will fit
         k1_x, k1_y = tANK_all.chevron('k1') # defines the slower chevron rates
         k2_x, k2_y = tANK_all.chevron('k2') # defines the faster chevron rates

         # start by setting up a GlobalFit object
         global_fit = pyfolding.GlobalFit()
         global_fit.fit_funcs = (models.ThreeStateFastPhaseChevron, FastPhase)
         global_fit.x = (k1_x, k2_x) # remember, k1 defines the slower chevron
         global_fit.y = (k1_y, k2_y) # & k2 defines are faster chevron
         global_fit.ID = ['slow_phase','fast_phase']
         global_fit.shared = ['kui', 'mui', 'kiu', 'miu']
         global_fit.initialise()
```

```
In [11]: # find out the order of params for p0
         print global_fit.params.keys()
```

```
['mui', 'miu', 'kui', 'kiu', 'kif_{slow_phase}', 'mif_{slow_phase}', 'kfi_{slow_phase}', 'mfi_{s
```

```
In [12]: # set some reasonable starting parameters for the fit
         p0 = np.array([1.7, 0.01, 1860., 2.7, 2652.0, 2.8, 14., 1.])
         bounds = ((-5.,-5.,0.,0.,0.,-5.,0.,-5), (5.,5.,1e4,1e4,1e4,5.,1e4,5.))

         # run the fit and get the results
         _,_ = global_fit.fit(p0=p0, bounds=bounds)
         results = global_fit.results
```

6.8 Display Results from custom Global fit

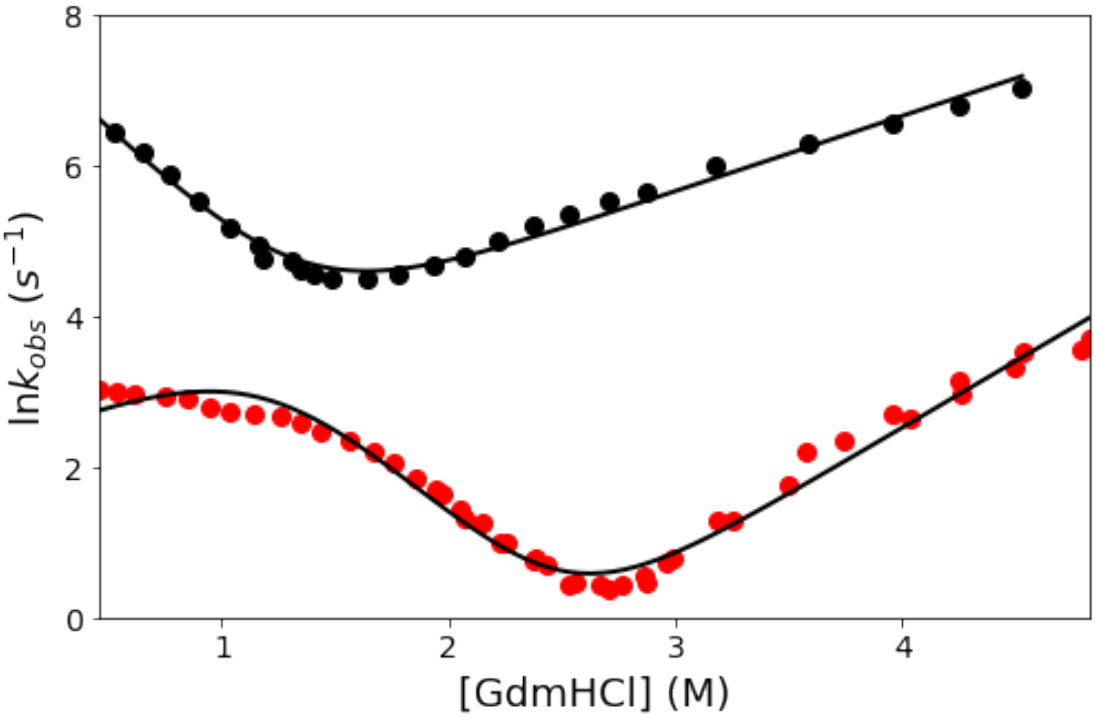
Display the results from the fitting. Note that in the results table, (f) means a free-variable, (s) is shared and (c) is constant.

```
In [13]: for r in results:
             r.display()
```

```
================================================================================
Fitting results
================================================================================
ID: slow_phase
Model: ThreeStateFastPhaseChevron
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 15.00°C

(s) kui 2652.18218 ± 54.43026           95% CI[2638.45729, 2665.90706]
(s) mui    2.82889 ± 0.02696            95% CI[   2.82210,    2.83569]
(s) kiu   14.30673 ± 0.37100            95% CI[  14.21318,   14.40028]
(s) miu    0.99906 ± 0.00828            95% CI[   0.99698,    1.00115]
(f) kif 1860.56417 ± 63.30515           95% CI[1844.60144, 1876.52691]
(f) mif    2.73831 ± 0.01748            95% CI[   2.73391,    2.74272]
(f) kfi    0.01057 ± 0.00038            95% CI[   0.01047,    0.01066]
(f) mfi    1.76794 ± 0.00936            95% CI[   1.76558,    1.77030]
--------------------------------------------------------------------------------
R^2: 0.97224
================================================================================


================================================================================
Fitting results
================================================================================
ID: fast_phase
Model: FastPhase
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 15.00°C

(s) kui 2652.18218 ± 52.85223           95% CI[2638.84949, 2665.51487]
(s) mui    2.82889 ± 0.02618            95% CI[   2.82229,    2.83550]
(s) kiu   14.30673 ± 0.36024            95% CI[  14.21585,   14.39760]
(s) miu    0.99906 ± 0.00804            95% CI[   0.99704,    1.00109]
--------------------------------------------------------------------------------
R^2: 0.98521
================================================================================
```

6.9 Plot the Figure of the custom Global fit

In [14]:
```
# now that we've fitted the data, plot a figure
plt.figure(figsize=(8,5))
plt.plot(k1_x, k1_y, 'ro', markersize=8)
plt.plot(k2_x, k2_y, 'ko', markersize=8)

# plot each of the chevrons...
for r in results:
    plt.plot(r.x_fit, r.y_fit, 'k-', linewidth=2)

plt.xlim([min(k1_x), max(k1_x)])
plt.rc('xtick', labelsize=18)     # fontsize of the x tick labels
plt.rc('ytick', labelsize=18)     # fontsize of the y tick labels
plt.grid(False)
plt.xlabel('[GdmHCl] (M)', fontsize=18)
plt.ylabel(r'$\ln k_{obs}$ $(s^{-1})$', fontsize=18)
plt.ylim([0, 8])
plt.show()
```



6.10 Plot custom Global fit with "prettier" graphics

We can also output this with the "prettier" graphics showing chevron & equilibrium curves together (as in PyFolding SI Notebook 1)

6.10.1 1st - we need to add the kinetic results to the protein object

---

```
In [15]: tANK_all.results = results[0]
         tANK_all.components = {'fast': np.exp(results[1].y_fit)}
         tANK_all.fit_func = models.ThreeStateFastPhaseChevron

Warning: overwriting fit result for <pyfolding.core.Chevron object at 0x114753a50>
```

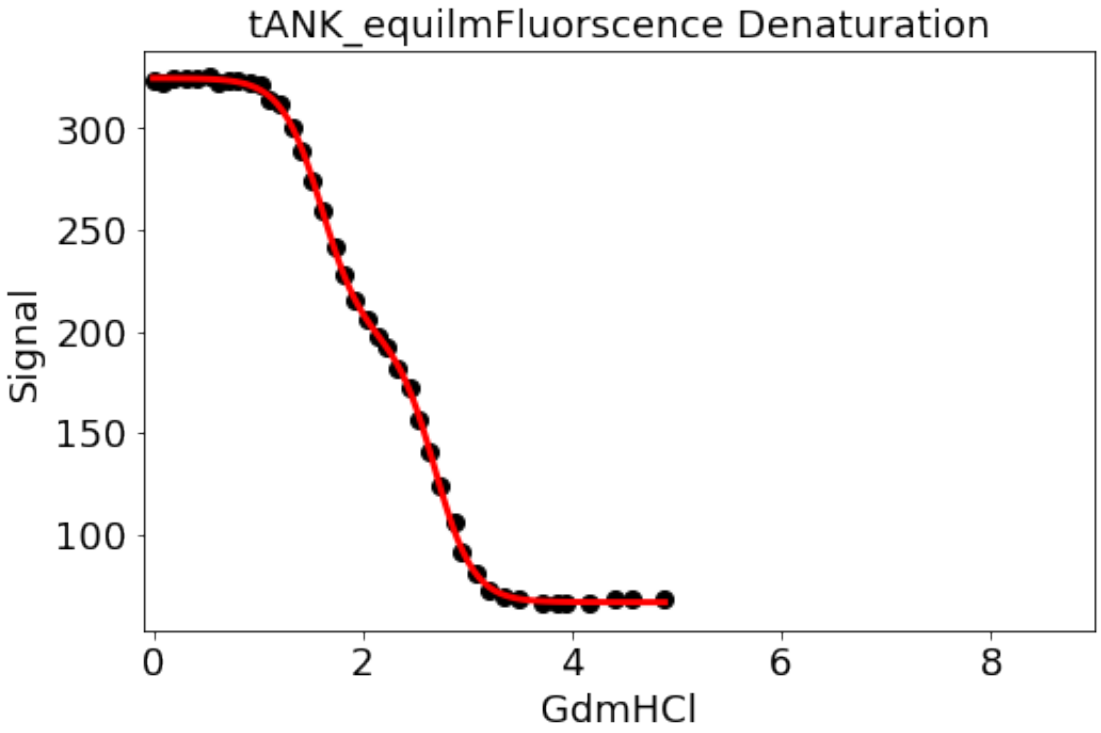
6.10.2 2nd - lets get the equilibrium data & fit to a three state model

---

```
In [16]: #1st load the data by adding the equilibrium
         tank_Equilm = pyfolding.read_equilibrium_data(pth, "tANK_equilmFluorscence.csv")

         #then select the fit function and associate it with the data
         tank_Equilm.fit_func = models.ThreeStateEquilibrium

         # Fit it with parameters (as the defaults ones are not close enough)
         tank_Equilm.fit(p0=[350,2,25,18.5,11.6,34,12.6])

         # Plot the figure to check
         tank_Equilm.plot()
```

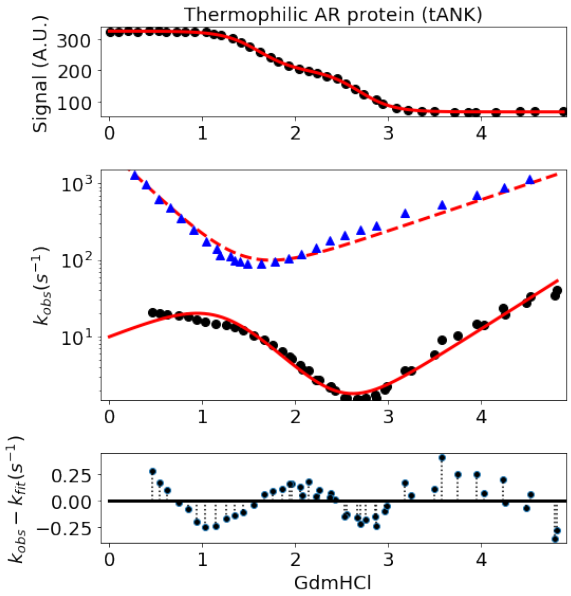
```
================================================================================
Fitting results
================================================================================
ID: tANK_equilmFluorscence
Model: ThreeStateEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 15.00°C

(f) Y_N   324.75027 ± 0.74190            95% CI[ 324.56451,  324.93603]
(f) Y_I   197.11446 ± 3.56063            95% CI[ 196.22293,  198.00599]
(f) Y_D    66.56156 ± 0.75910            95% CI[  66.37149,   66.75162]
(f) DGni    4.77711 ± 0.21538            95% CI[   4.72318,    4.83104]
(f) m_ni    2.96838 ± 0.14905            95% CI[   2.93106,    3.00570]
(f) DGid    8.33336 ± 0.44659            95% CI[   8.22154,    8.44518]
```

```
(f) m_id    3.09530 ± 0.15594             95% CI[   3.05626,    3.13435]
--------------------------------------------------------------------------------
R^2: 0.99983
================================================================================
```



6.10.3 3rd - lets plot both together & print out to pdf

In [17]: `pyfolding.plot_figure(tank_Equilm, tANK_all, display=True)`



Data-set: tANK_equilmFluorscence

Equilibrium Model: ThreeStateEquilibrium
Y_N: 324.75027 ± 0.74190
Y_I: 197.11446 ± 3.56063
Y_D: 66.56156 ± 0.75910
DGni: 4.77711 ± 0.21538
m_ni: 2.96838 ± 0.14905
DGid: 8.33336 ± 0.44659
m_id: 3.09530 ± 0.15594
R^2: 1.00

Kinetic Model: ThreeStateFastPhaseChevron
Fit Standard Error: 0.02
kui: 2.65e+03 ± 5.44e+01
mui: 2.83e+00 ± 2.70e-02
kiu: 1.43e+01 ± 3.71e-01
miu: 9.99e-01 ± 8.28e-03
kif: 1.86e+03 ± 6.33e+01
mif: 2.74e+00 ± 1.75e-02
kfi: 1.06e-02 ± 3.82e-04
mfi: 1.77e+00 ± 9.36e-03
R^2: 0.97

End of this Notebook.

---

7 SI Notebook 5 - Fitting to a HomoZipper/HomoPolymer Ising Model

[Author] ERGM

In this notebook we will show how equilbrium folding data can be imported into a notebook and fitted to Homopolymer Ising Model.

If you are less script/computer orientated, you can simply change the data paths and other parameters for your proteins and re-run the jupyter notebook ("Kernal/Restart & Run all" from the menu above).

7.1 Data Format

Please see PyFolding SI Notebooks 1 and 2 for the format your data has to be in to enable this type of analysis. Remember for Ising Model Analysis here each protein dataset (equilibrium denaturation curve) must have its own .csv

---

First off lets load pyfolding & pyplot into this ipython notebook (pyplot allows us to plot more complex figures of our results):

```
In [1]: # use this command to tell Jupyter to plot figures inline with the text
        %matplotlib inline

        # import pyfolding, the pyfolding models and ising models
        import pyfolding
        from pyfolding import *

        # import the package for plotting, call it plt
        import matplotlib.pyplot as plt

        # import numpy as well
        import numpy as np
```

```
<IPython.core.display.Javascript object>
```

```
PyFolding: Jupyter autoscrolling has been disabled
```

Now, we need to load some data to analyse. I will import the equilibrium denaturation of a series of CTPRa proteins from:

Phillips, J. J., Javadi, Y., Millership, C. & Main, E. R. Modulation of the multistate folding of designed TPR proteins through intrinsic and extrinsic factors. Protein Sci 21, 327-338 (2012).

I will load 7 denaturation curves that correspond to the following proteins:

Protein	Filename	No. of Helices (repeating unit)
CTPRa2	H2_25C.csv	5
CTPRa3	H3_25C.csv	7
CTPRa4	H4_25C.csv	9
CTPRa5	H5_25C.csv	11
CTPRa6	H6_25C.csv	13
CTPRa8	H8_25C.csv	17
CTPRa10	H10_25C.csv	21

7.2 Import Data, assign names and put into a list

We will load all of the data together, as follows:

In [2]:
```
# arguments are "path", "filename"
pth = "../examples/CTPRan"

#this is a set of commands to automate loading the data for each proteins
fn = ["H2_25C.csv","H3_25C.csv","H4_25C.csv",
      "H5_25C.csv","H6_25C.csv","H8_25C.csv","H10_25C.csv"]

#Here we are loading all the curves in a list called "proteins" and assigning them names
proteins = [pyfolding.read_equilibrium_data(pth,f) for f in fn]

# also store the number of helices for each protein
n_helices = [5, 7, 9, 11, 13, 17, 21]
```

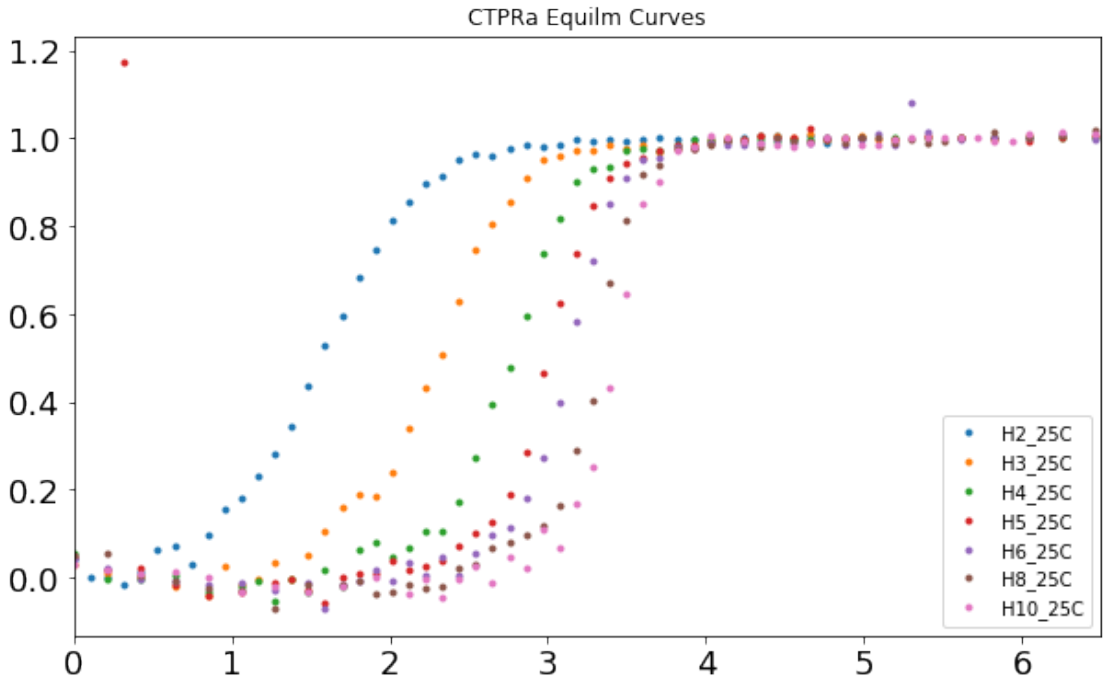
7.3 Set the Temperature

In [3]:
```
# Set temperature to 25.00°C
# (NOTE: Careful, this sets the temperature for all subsequent calculations)
pyfolding.set_temperature(25.)
```

```
Set temperature to 25.00°C
(NOTE: Careful, this sets the temperature for all subsequent calculations)
```

7.4 Plot the data

In [4]:
```
# the following commands plot all the data curves on one plot

plt.figure(figsize=(10,6))
for c in proteins:
    plt.plot(c.x, c.y, '.')

# the following commands plot all the data curves on one plot, where "loc" command deter
plt.legend([c.ID for c in proteins], loc='best')
plt.title("CTPRa Equilm Curves")
plt.xlim([0, 6.5])                                          # x axis from 0 to 6.5
plt.show()
```



7.5 Print information on the HomoZipper Ising Model

In [5]:
```
# printing the equation for viewing

models.HomozipperIsingEquilibrium().info()
```

the partition function (q) and thus fraction of folded protein (f) of n arrayed repeats are given by:

$$q = 1 + \frac{\kappa([\kappa\tau]^{n+1} - [n+1]\kappa\tau - n)}{(\kappa\tau + 1)^2}$$

$$f = \frac{1}{n}\sum_{i=0}^{n} i\frac{(n-i+1)\kappa^i\tau^{i-1}}{q} \tag{4}$$

where:

$$\kappa(x) = \exp\frac{-G_i}{RT} = \exp\frac{-G_{i,H_20} + m_i x}{RT}$$

$$\tau(x) = \exp\frac{-G_{i,i+1}}{RT}$$

```
Homopolymer Zipper Ising model

        Params:
                q = partition function
                f = fraction of folded protein
                Kappa = equilibrium constant of folding for a given repeating unit
                Tau = equilibrium constant of association between 2 repeating units
                n = number of repeating units
                x = denaturant concentration (M)
                Gi = intrinsic stability (folding energy) of a repeating unit i
                mi = denaturant sensitivity of the intrinsic stability of a repeating unit i
                Gi,i+1 = interface interaction energy between 2 repeating units
                R = Universal Gas Constant (kcal.mol-1.K-1)
                T = Temperature (Kelvin)

        Reference:
                Aksel and Barrick. Analysis of repeat-protein folding using
                nearest-neighbor statistical mechanical models.
                Methods in enzymology (2009) vol. 455 pp. 95-125
```

7.6 Fitting to a Homopolymer Ising model

We need to define a topology for each protein, this is essentially the domains of the protein in order (N->C)

Module	Abbreviation	Defined in Ising as
Module, type 1	r	RepeatDomain

Module	Abbreviation	Defined in Ising as
Module, type 2	Mr	MutantRepeatDomain
Module, type 3	h	HelixDomain
Module, type 4	l	LoopDomain
Module, type 5	Ml	MutantLoopDomain
Cap Module	c	CapDomain
Mut. Cap Module	Mc	MutantCapDomain
Decoupled Cap Domain	DC	DecoupleCapDomain

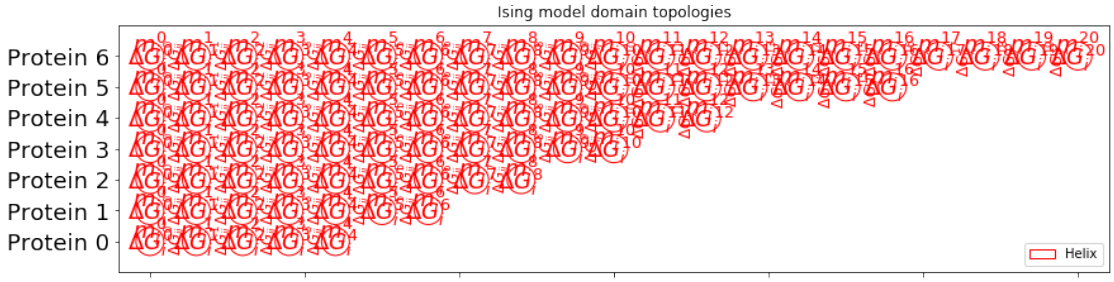
Protein topology can be defined via the following Ising motifs For Homopolymer fitting Helix, Repeat & loop motif functions are identical. We will need the differing types when using the Heteropolymer in the next Notebook. The nomenclature stems from our CTPR proteins analysis, as their repeated motifs can be defined in terms of either repeated TPRs, repeated helices and/or loops.

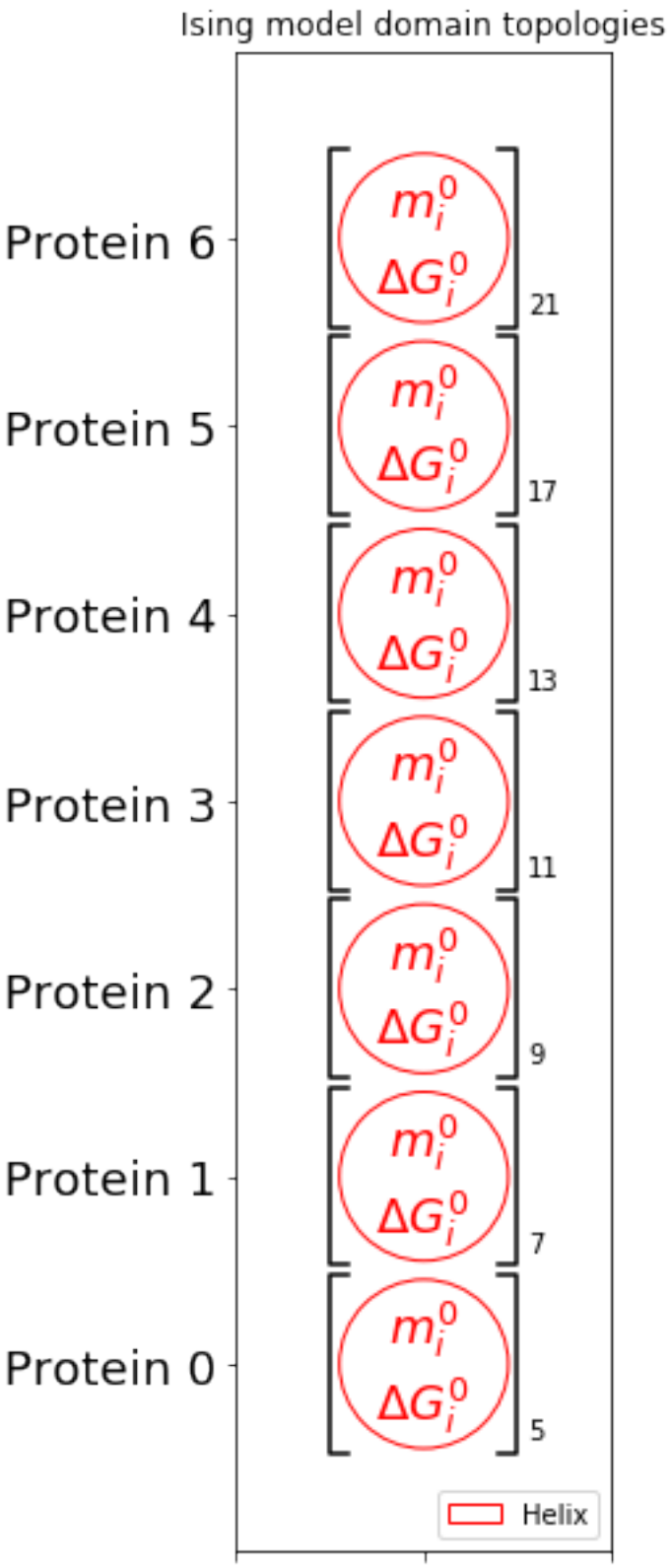
Each motif is defined as having:

1. an intrinisic stability term (Gi)
2. a denaturation dependence term (mi) - which is associated with Gi
3. an interface energy term (Gij) - defined as the interaction between folded units i and i-1, i.e. the interface of the i unit with the N-terminal unit i-1 to it.
4. The Ising model automatically takes into account the first N-terminal repeat does not have an interface term {ij} associated with it.

7.7 Set up Our Topology

```
In [6]: # set up the protein topologies automatically
        h = ising.HelixDomain

        # We have already defined the number of helices for each protein
        # in cell (3) above as "n_helices", so:
        topology = [[h]*n for n in n_helices]

        # plot them either in an expanded form
        ising.plot_domains(topology, collapse=False)

        # or in a collapsed form
        ising.plot_domains(topology, collapse=True)
```


Ising model domain topologies
Protein 6
Protein 5
Protein 4
Protein 3
Protein 2
Protein 1
Protein 0
Helix


Ising model domain topologies
Protein 6
Protein 5
Protein 4
Protein 3
Protein 2
Protein 1
Protein 0
m_i^0
ΔG_i^0
21
17
13
11
9
7
5
Helix

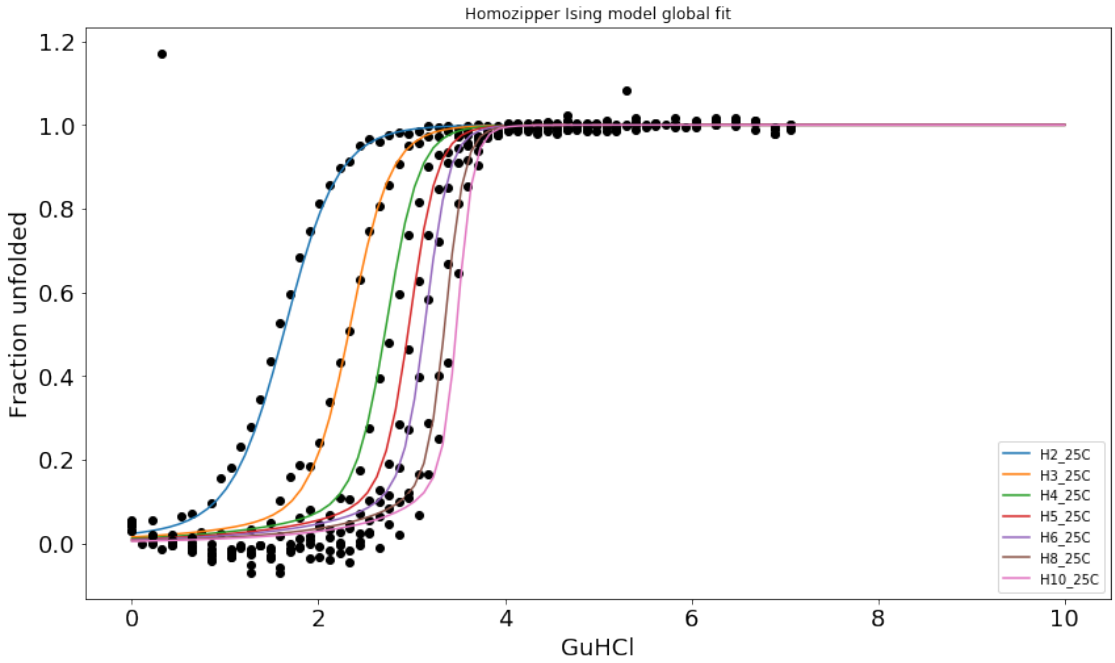
7.8 Automatic global fitting to the homozipper model

```
In [7]: # use a one-liner and save out the curve fits via: save='/Users/ergm/Desktop/test.csv'
        r_homo = ising.fit_homopolymer(proteins, n_helices, save='/Users/ergm/Desktop/test.csv')
```

```
================================================================================
Fitting results
================================================================================
ID: H2_25C
Model: HomozipperIsingEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(c) n               5.00000
(s) DG_intrinsic    3.52517 ± 0.00796            95% CI[   3.52315,    3.52719]
(s) m_intrinsic    -0.45188 ± 0.00090            95% CI[  -0.45211,   -0.45166]
(s) DG_interface   -5.29438 ± 0.01119            95% CI[  -5.29721,   -5.29154]
--------------------------------------------------------------------------------
R^2: 0.99659
================================================================================


================================================================================
Fitting results
================================================================================
ID: H3_25C
Model: HomozipperIsingEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(c) n               7.00000
(s) DG_intrinsic    3.52517 ± 0.00796            95% CI[   3.52315,    3.52719]
(s) m_intrinsic    -0.45188 ± 0.00090            95% CI[  -0.45211,   -0.45166]
(s) DG_interface   -5.29438 ± 0.01119            95% CI[  -5.29721,   -5.29154]
--------------------------------------------------------------------------------
R^2: 0.99637
================================================================================


================================================================================
Fitting results
================================================================================
ID: H4_25C
Model: HomozipperIsingEquilibrium
```

```
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(c) n               9.00000
(s) DG_intrinsic    3.52517 ± 0.00796            95% CI[   3.52315,    3.52719]
(s) m_intrinsic    -0.45188 ± 0.00090            95% CI[  -0.45211,   -0.45166]
(s) DG_interface   -5.29438 ± 0.01119            95% CI[  -5.29721,   -5.29154]
--------------------------------------------------------------------------------
R^2: 0.99366
================================================================================


================================================================================
Fitting results
================================================================================
ID: H5_25C
Model: HomozipperIsingEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(c) n              11.00000
(s) DG_intrinsic    3.52517 ± 0.00796            95% CI[   3.52315,    3.52719]
(s) m_intrinsic    -0.45188 ± 0.00090            95% CI[  -0.45211,   -0.45166]
(s) DG_interface   -5.29438 ± 0.01119            95% CI[  -5.29721,   -5.29154]
--------------------------------------------------------------------------------
R^2: 0.87746
================================================================================


================================================================================
Fitting results
================================================================================
ID: H6_25C
Model: HomozipperIsingEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(c) n              13.00000
(s) DG_intrinsic    3.52517 ± 0.00796            95% CI[   3.52315,    3.52719]
(s) m_intrinsic    -0.45188 ± 0.00090            95% CI[  -0.45211,   -0.45166]
(s) DG_interface   -5.29438 ± 0.01119            95% CI[  -5.29721,   -5.29154]
--------------------------------------------------------------------------------
R^2: 0.99559
================================================================================


================================================================================
Fitting results
```

```
================================================================================
ID: H8_25C
Model: HomozipperIsingEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(c) n              17.00000
(s) DG_intrinsic    3.52517 ± 0.00796            95% CI[   3.52315,    3.52719]
(s) m_intrinsic    -0.45188 ± 0.00090            95% CI[  -0.45211,   -0.45166]
(s) DG_interface   -5.29438 ± 0.01119            95% CI[  -5.29721,   -5.29154]
--------------------------------------------------------------------------------
R^2: 0.99526
================================================================================


================================================================================
Fitting results
================================================================================
ID: H10_25C
Model: HomozipperIsingEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(c) n              21.00000
(s) DG_intrinsic    3.52517 ± 0.00796            95% CI[   3.52315,    3.52719]
(s) m_intrinsic    -0.45188 ± 0.00090            95% CI[  -0.45211,   -0.45166]
(s) DG_interface   -5.29438 ± 0.01119            95% CI[  -5.29721,   -5.29154]
--------------------------------------------------------------------------------
R^2: 0.99435
================================================================================
```



End of this Notebook.

---

8 SI Notebook 6 - Fitting to a HeteroPolymer Ising Model

[Author] ERGM

In this notebook we will show how equilbrium folding data can be imported into a notebook and fitted to Heteropolymer Ising Model.

If you are less script/computer orientated, you can simply change the data paths and other parameters for your proteins and re-run the jupyter notebook ("Kernal/Restart & Run all" from the menu above).

8.1 Data Format

Please see PyFolding SI Notebooks 1 and 2 for the format your data has to be in to enable this type of analysis. Remember for Ising Model Analysis here each protein dataset (equilibrium denaturation curve) must have its own .csv

---

First off lets load pyfolding & pyplot into this ipython notebook (pyplot allows us to plot more complex figures of our results):

```
In [2]: # use this command to tell Jupyter to plot figures inline with the text
        %matplotlib inline

        # import pyfolding, the pyfolding models and ising models
        import pyfolding
        from pyfolding import models, ising

        # import the package for plotting, call it plt
        import matplotlib.pyplot as plt
```

```
<IPython.core.display.Javascript object>


PyFolding: Jupyter autoscrolling has been disabled
```

Now, we need to load some data to analyse. I will import the equilibrium denaturation curves of a series of CTPRn variants proteins taken from:

Millership C., Phillips J.J. & Main E.R.G. (2016) ''Ising model reprogramming of a repeat protein equilibrium unfolding pathway.'' Journal of Molecular Biology, 428 (9A), 1804-1817.

I will load 12 denaturation curves (two repeats of each expt) that correspond to the following proteins:

Protein	Filename	No. of Helices	Truncation
CTPR2ΔA	CTPR2APhos1_10C.csv	4	N-Terminal helix removed
CTPR2ΔA	CTPR2APhos2_10C.csv	4	N-Terminal helix removed
CTPR2ΔS	CTPR2SPhos1_10C.csv	4	C-Terminal helix removed
CTPR2ΔS	CTPR2SPhos2_10C.csv	4	C-Terminal helix removed
CTPR2	CTPR2Phos1_10C.csv	5	none
CTPR2	CTPR2Phos1_10C.csv	5	none
CTPR3ΔA	CTPR3APhos1_10C.csv	6	N-Terminal helix removed
CTPR3ΔA	CTPR3APhos2_10C.csv	6	N-Terminal helix removed
CTPR3ΔS	CTPR3SPhos1_10C.csv	6	C-Terminal helix removed
CTPR3ΔS	CTPR3SPhos2_10C.csv	6	C-Terminal helix removed
CTPR3	CTPR3Phos1_10C.csv	7	none
CTPR3	CTPR3Phos1_10C.csv	7	none

8.2 Import Data, assign names and put into a list

```
In [3]: # start by loading a data set
        # arguments are "path", "filename"
        pth = "../examples/CTPRn"
        CTPR2A1 = pyfolding.read_equilibrium_data(pth,"CTPR2APhos1_10C.csv")
        CTPR2A2 = pyfolding.read_equilibrium_data(pth,"CTPR2APhos2_10C.csv")
        CTPR2S1= pyfolding.read_equilibrium_data(pth,"CTPR2SPhos1_10C.csv")
        CTPR2S2 = pyfolding.read_equilibrium_data(pth,"CTPR2SPhos2_10C.csv")
        CTPR2_1= pyfolding.read_equilibrium_data(pth,"CTPR2Phos1_10C.csv")
        CTPR2_2 = pyfolding.read_equilibrium_data(pth,"CTPR2Phos2_10C.csv")
        CTPR3A1 = pyfolding.read_equilibrium_data(pth,"CTPR3APhos1_10C.csv")
        CTPR3A2 = pyfolding.read_equilibrium_data(pth,"CTPR3APhos2_10C.csv")
        CTPR3S1 = pyfolding.read_equilibrium_data(pth,"CTPR3SPhos1_10C.csv")
        CTPR3S2 = pyfolding.read_equilibrium_data(pth,"CTPR3SPhos2_10C.csv")
        CTPR3_1 = pyfolding.read_equilibrium_data(pth,"CTPR3Phos1_10C.csv")
        CTPR3_2 = pyfolding.read_equilibrium_data(pth,"CTPR3Phos2_10C.csv")

        # make a python list of the data
        curves = [CTPR2A1,CTPR2A2,
                  CTPR2S1,CTPR2S2,
                  CTPR2_1,CTPR2_2,
                  CTPR3A1,CTPR3A2,
                  CTPR3S1,CTPR3S2,
                  CTPR3_1, CTPR3_2]
```

8.3 Set Temperature

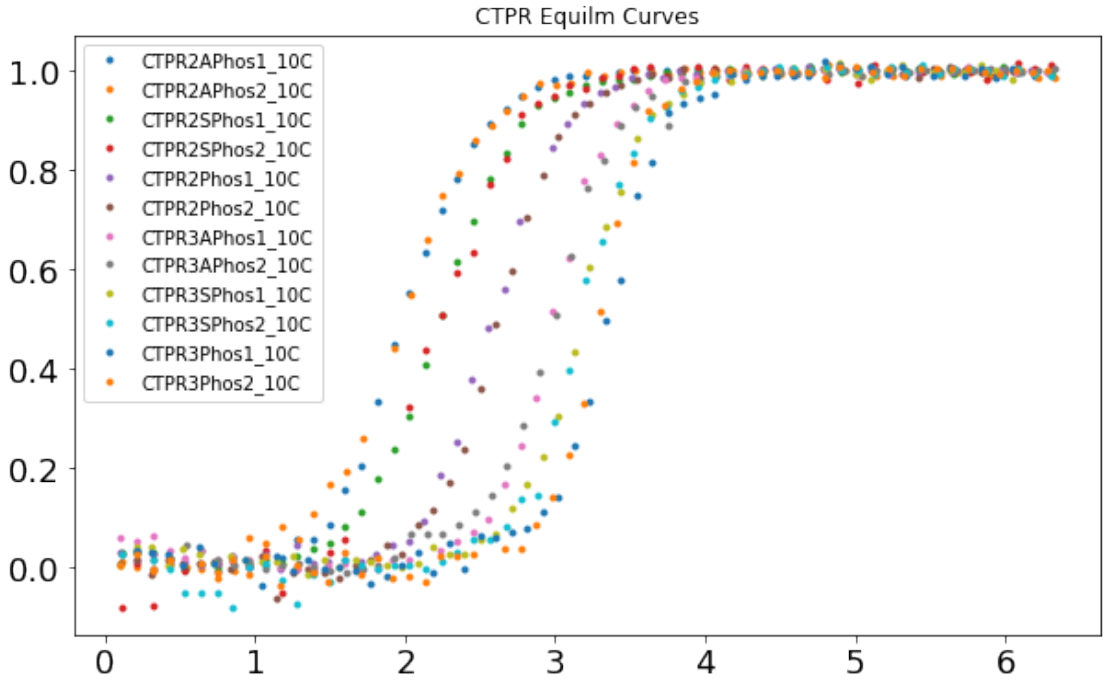
Here the expts were conducted at 10 degrees C, so lets set this up

```
In [4]: # set the temperature to 10 degrees C

        pyfolding.set_temperature(10.0)
```

```
Set temperature to 10.00°C
(NOTE: Careful, this sets the temperature for all subsequent calculations)
```

8.4 Plot the data

```
In [4]: # Lets check everything was loaded correctly by plotting all the data curves on one plot

        plt.figure(figsize=(10,6))
        for c in curves:
            plt.plot(c.x, c.y, '.')

        # the following commands plot all the data curves on one plot,
        # where "loc" command determines where the legend goes.
        plt.legend([c.ID for c in curves], loc='best')
        plt.title("CTPR Equilm Curves")
        plt.show()
```



8.5 Fitting to a Heteropolymer Ising model

8.6 Assign Topology

We need to define a topology for each protein, this is essentially the domains of the protein in order (N->C)

Module	Abbreviation	Defined in Ising as
Module, type 1	r	RepeatDomain
Module, type 2	Mr	MutantRepeatDomain
Module, type 3	h	HelixDomain
Module, type 4	l	LoopDomain
Module, type 5	Ml	MutantLoopDomain
Cap Module	c	CapDomain
Mut. Cap Module	Mc	MutantCapDomain
Decoupled Cap Domain	DC	DecoupleCapDomain

Protein topology can be defined via the following Ising motifs All modules functions are identical, except for the Decoupled Cap Domain and Cap domains. 1. The Decoupled Cap domain has been coded such that the there is no interface energy between itself and any domain N-terminal to it.
2. Cap domains are essentially identical to the other domains, except for clarity their interface energy term (G_ij) is not displayed when used in fitting. They should only be used at the C-terminus.

With regards to the other domains, you simply use them to define different modules present in your protein. The nomenclature stems from our various CTPR proteins analysis, as their repeated motifs can be defined in terms of either repeated TPRs, repeated helices and/or loops.

Each motif is defined as having:

1. an intrinisic stability term for each module (Gi)
2. a denaturation dependence term (m_i) - which is associated with Gi
3. an interface energy term (G_ij) - defined as the interaction between folded units i and i+1, i.e. the interface of the i unit with the unit C terminal to it (i+1).
4. The Ising model automatically takes into account the C-terminal repeat does not have an interface term {ij} associated with it.

Except:

1. the Cap Motifs/Domains (Cap + Mutant Cap) do not print their interface term {ij} for clarity.
2. Thus the Cap domain should only be used when you have a C-terminal deletion.
3. The Decoupled Cap Domain do not use an interface term (hence “decoupled”)

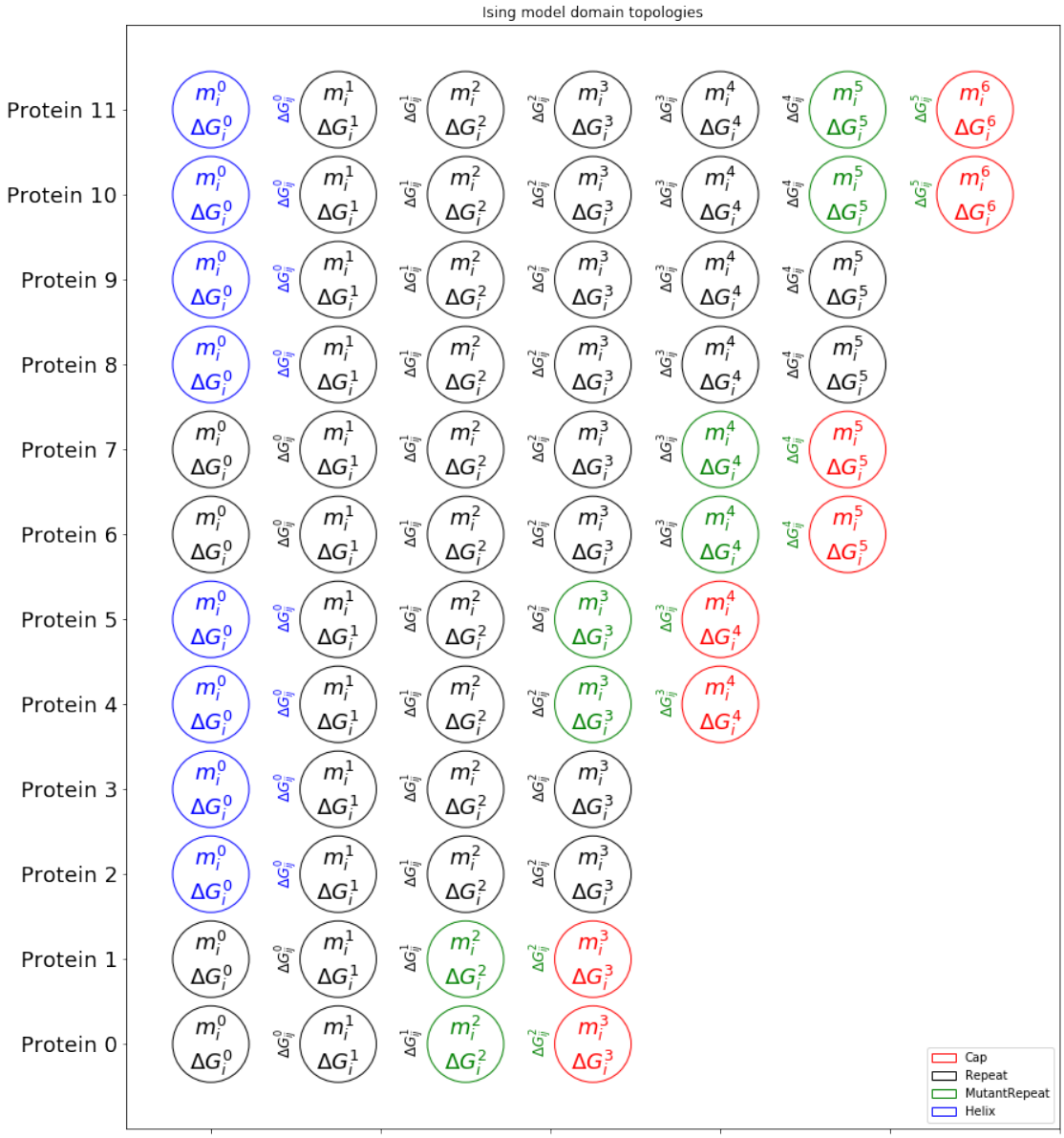
Lets list the motifs already present in the Ising model to check that the above is correct!

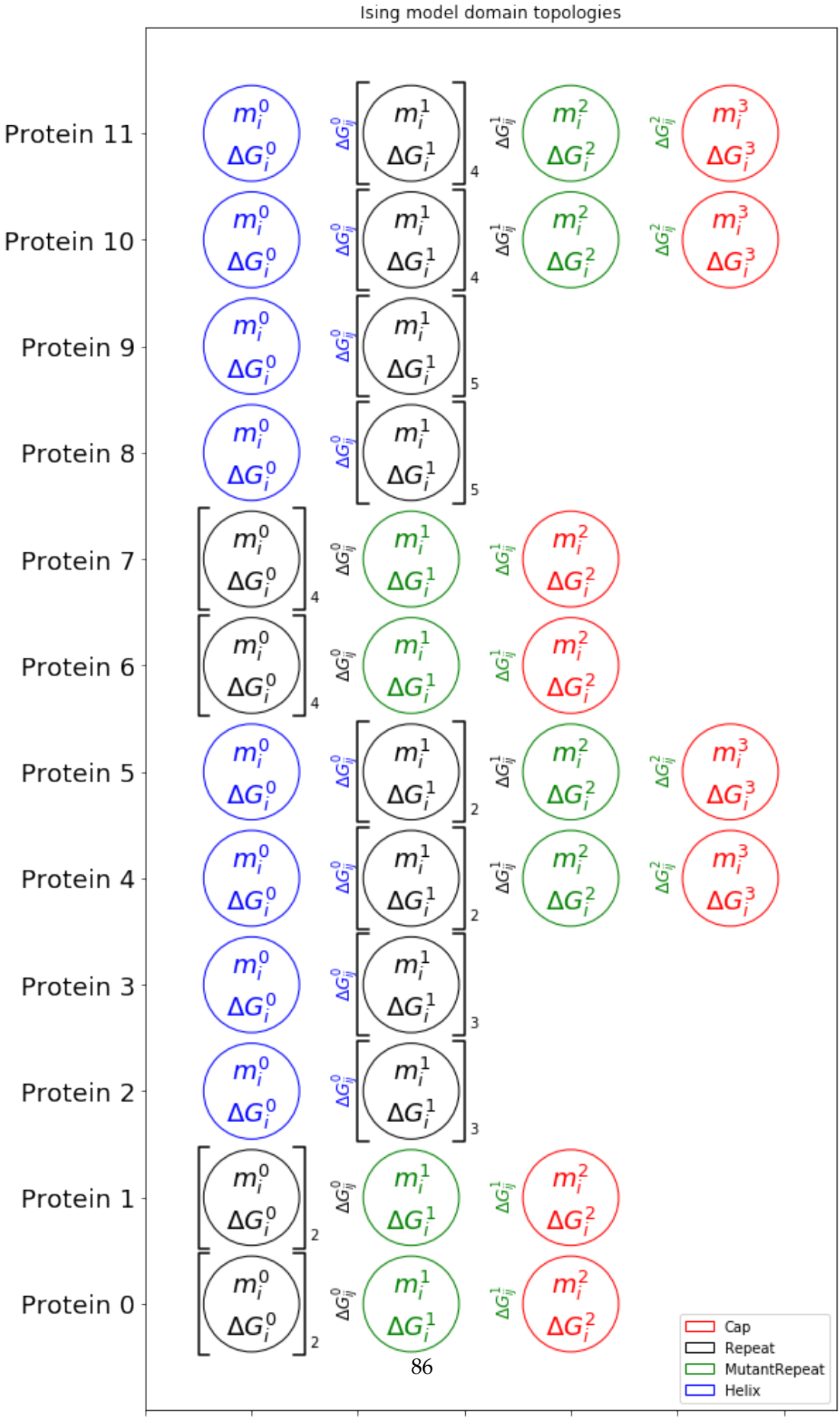
```
In [5]: # This command lists the motifs already present in the Ising model.
        ising.list_models()
```

```
Out[5]: ['CapDomain',
         'DecoupleCapDomain',
         'HelixDomain',
         'LoopDomain',
         'MutantCapDomain',
         'MutantLoopDomain',
         'MutantRepeatDomain',
         'RepeatDomain',
         'RepeatDomain_mij']
```

8.7 Plot our Chosen Topology

```
In [6]: #Define shorthand for Ising modules:
        r = ising.RepeatDomain
        h = ising.HelixDomain
        Mr = ising.MutantRepeatDomain
        c = ising.CapDomain
        Mc = ising.MutantCapDomain
        l = ising.LoopDomain
        Ml = ising.MutantLoopDomain

        #lets try topology A first, but need to add in double as two curves of each protein
        topology1 = [[r,r,Mr,c],[r,r,Mr,c],
                     [h,r,r,r], [h,r,r,r],
                     [h,r,r,Mr,c], [h,r,r,Mr,c],
                     [r,r,r,r,Mr,c], [r,r,r,r,Mr,c],
                     [h,r,r,r,r,r],[h,r,r,r,r,r],
                     [h,r,r,r,r,Mr,c], [h,r,r,r,r,Mr,c]]

        # plot them either in an expanded form
        ising.plot_domains(topology1, collapse=False)

        # or in a collapsed form
        ising.plot_domains(topology1, collapse=True)

        # NOTE: You can save the domain image by using the save keyword like this:
        # ising.plot_domains([topology], collapse=True, save="/Users/ergm/Desktop/domains.pdf")
```


Ising model domain topologies
Protein 11
Protein 10
Protein 9
Protein 8
Protein 7
Protein 6
Protein 5
Protein 4
Protein 3
Protein 2
Protein 1
Protein 0
Cap
Repeat
MutantRepeat
Helix


Ising model domain topologies
Protein 11
Protein 10
Protein 9
Protein 8
Protein 7
Protein 6
Protein 5
Protein 4
Protein 3
Protein 2
Protein 1
Protein 0
Cap
Repeat
MutantRepeat
Helix

8.8 Fit Data with our chosen Topology

In [8]:
```
# note: popsize is the population size for the optimiser (bigger means more likelihood o
# start with a popsize of 10, increase to 100-1000 for better fits:
# curves defines the data you will fit, topologies define what it fits to
# ising.fit_heteropolymer(curves, topologies, popsize=10)

# for example, let's just fit the CTPRn datasets to topology 1:

ising.fit_heteropolymer(curves[0:12], topology1[0:12], popsize=10, maxiter= 10000)

# NOTE: You can save the fits using the save keyword like this:
# ising.fit_heteropolymer(curves[0:12], topology1[0:12], popsize=10, maxiter= 10000
#                         , save="/Users/ergm/Desktop/FitResults")
```

```
Appending 12 curves to GlobalFitIsing...
 + added CTPR2APhos1_10C with topology ['Repeat', 'Repeat', 'MutantRepeat', 'Cap']
 + added CTPR2APhos2_10C with topology ['Repeat', 'Repeat', 'MutantRepeat', 'Cap']
 + added CTPR2SPhos1_10C with topology ['Helix', 'Repeat', 'Repeat', 'Repeat']
 + added CTPR2SPhos2_10C with topology ['Helix', 'Repeat', 'Repeat', 'Repeat']
 + added CTPR2Phos1_10C with topology ['Helix', 'Repeat', 'Repeat', 'MutantRepeat', 'Cap']
 + added CTPR2Phos2_10C with topology ['Helix', 'Repeat', 'Repeat', 'MutantRepeat', 'Cap']
 + added CTPR3APhos1_10C with topology ['Repeat', 'Repeat', 'Repeat', 'Repeat', 'MutantRepeat',
 + added CTPR3APhos2_10C with topology ['Repeat', 'Repeat', 'Repeat', 'Repeat', 'MutantRepeat',
 + added CTPR3SPhos1_10C with topology ['Helix', 'Repeat', 'Repeat', 'Repeat', 'Repeat', 'Repeat
 + added CTPR3SPhos2_10C with topology ['Helix', 'Repeat', 'Repeat', 'Repeat', 'Repeat', 'Repeat
 + added CTPR3Phos1_10C with topology ['Helix', 'Repeat', 'Repeat', 'Repeat', 'Repeat', 'MutantR
 + added CTPR3Phos2_10C with topology ['Helix', 'Repeat', 'Repeat', 'Repeat', 'Repeat', 'MutantR

Performing global optimisation of Ising model (12 curves, Population size: 10, Tolerance: 1.00E-
 - Fitting in progress (Iteration: 100, Convergence: 2.89931E-08, Timing: 2.32s per iteration)
 - Fitting in progress (Iteration: 200, Convergence: 2.27786E-08, Timing: 2.26s per iteration)
 - Fitting in progress (Iteration: 300, Convergence: 2.73710E-08, Timing: 2.44s per iteration)
 - Fitting in progress (Iteration: 400, Convergence: 3.07232E-08, Timing: 2.34s per iteration)
 - Fitting in progress (Iteration: 500, Convergence: 1.49071E-07, Timing: 9.31s per iteration)
 - Fitting in progress (Iteration: 600, Convergence: 2.56571E-07, Timing: 13.23s per iteration)
 - Fitting in progress (Iteration: 700, Convergence: 5.54906E-07, Timing: 13.57s per iteration)
 - Fitting in progress (Iteration: 800, Convergence: 4.74560E-07, Timing: 2.42s per iteration)
 - Fitting in progress (Iteration: 900, Convergence: 7.91965E-07, Timing: 2.15s per iteration)
 - Fitting in progress (Iteration: 1000, Convergence: 2.57162E-06, Timing: 2.03s per iteration)
 - Fitting in progress (Iteration: 1100, Convergence: 6.80320E-06, Timing: 2.03s per iteration)
 - Fitting in progress (Iteration: 1200, Convergence: 1.37043E-05, Timing: 2.03s per iteration)
 - Fitting in progress (Iteration: 1300, Convergence: 3.58656E-05, Timing: 2.03s per iteration)
 - Fitting in progress (Iteration: 1400, Convergence: 8.46884E-05, Timing: 2.31s per iteration)
 - Fitting in progress (Iteration: 1500, Convergence: 2.48855E-04, Timing: 2.25s per iteration)
```

```
 - Fitting in progress (Iteration: 1600, Convergence: 5.63081E-04, Timing: 2.04s per iteration)
 - Fitting in progress (Iteration: 1700, Convergence: 1.16502E-03, Timing: 2.26s per iteration)
 - Fitting in progress (Iteration: 1800, Convergence: 3.73319E-03, Timing: 2.27s per iteration)
 - Fitting in progress (Iteration: 1900, Convergence: 9.52760E-03, Timing: 2.37s per iteration)
 - Fitting in progress (Iteration: 2000, Convergence: 2.83025E-02, Timing: 2.42s per iteration)
 - Fitting in progress (Iteration: 2100, Convergence: 7.43200E-02, Timing: 2.40s per iteration)
 - Fitting in progress (Iteration: 2200, Convergence: 1.38931E-01, Timing: 2.42s per iteration)
 - Fitting in progress (Iteration: 2300, Convergence: 3.92334E-01, Timing: 2.18s per iteration)
 - Fitting in progress (Iteration: 2400, Convergence: 8.44591E-01, Timing: 2.24s per iteration)

Fitting results (NOTE: Careful with the errors here):

================================================================================
Fitting results
================================================================================
ID: CTPR2APhos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i           3.46641 ± 0.91527            95% CI[   3.23405,    3.69877]
(s) Repeat DG_ij         -6.15007 ± 0.61596            95% CI[  -6.30644,   -5.99369]
(s) Repeat m_i           -0.53090 ± 0.21582            95% CI[  -0.58569,   -0.47611]
(s) MutantRepeat DG_i     5.17597 ± 0.16560            95% CI[   5.13393,    5.21801]
(s) MutantRepeat DG_ij   -9.28958 ± 0.42047            95% CI[  -9.39632,   -9.18283]
(s) MutantRepeat m_i     -0.00790 ± 0.13650            95% CI[  -0.04255,    0.02676]
(s) Cap DG_i              4.89001 ± 0.41848            95% CI[   4.78377,    4.99625]
(s) Cap m_i              -1.25264 ± 0.93580            95% CI[  -1.49021,   -1.01507]
(s) Helix DG_i            6.87395 ± 1.29828            95% CI[   6.54435,    7.20354]
(s) Helix DG_ij          -9.94555 ± 1.30027            95% CI[ -10.27565,   -9.61545]
(s) Helix m_i            -0.63753 ± 2.80242            95% CI[  -1.34898,    0.07392]
--------------------------------------------------------------------------------
R^2: 0.99891
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR2APhos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i           3.46641 ± 0.91527            95% CI[   3.23405,    3.69877]
(s) Repeat DG_ij         -6.15007 ± 0.61596            95% CI[  -6.30644,   -5.99369]
(s) Repeat m_i           -0.53090 ± 0.21582            95% CI[  -0.58569,   -0.47611]
(s) MutantRepeat DG_i     5.17597 ± 0.16560            95% CI[   5.13393,    5.21801]
```

```
(s) MutantRepeat DG_ij   -9.28958 ± 0.42047            95% CI[  -9.39632,   -9.18283]
(s) MutantRepeat m_i     -0.00790 ± 0.13650            95% CI[  -0.04255,    0.02676]
(s) Cap DG_i              4.89001 ± 0.41848            95% CI[   4.78377,    4.99625]
(s) Cap m_i              -1.25264 ± 0.93580            95% CI[  -1.49021,   -1.01507]
(s) Helix DG_i            6.87395 ± 1.29828            95% CI[   6.54435,    7.20354]
(s) Helix DG_ij          -9.94555 ± 1.30027            95% CI[ -10.27565,   -9.61545]
(s) Helix m_i            -0.63753 ± 2.80242            95% CI[  -1.34898,    0.07392]
--------------------------------------------------------------------------------
R^2: 0.99894
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR2SPhos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i           3.46641 ± 0.91527            95% CI[   3.23405,    3.69877]
(s) Repeat DG_ij         -6.15007 ± 0.61596            95% CI[  -6.30644,   -5.99369]
(s) Repeat m_i           -0.53090 ± 0.21582            95% CI[  -0.58569,   -0.47611]
(s) MutantRepeat DG_i     5.17597 ± 0.16560            95% CI[   5.13393,    5.21801]
(s) MutantRepeat DG_ij   -9.28958 ± 0.42047            95% CI[  -9.39632,   -9.18283]
(s) MutantRepeat m_i     -0.00790 ± 0.13650            95% CI[  -0.04255,    0.02676]
(s) Cap DG_i              4.89001 ± 0.41848            95% CI[   4.78377,    4.99625]
(s) Cap m_i              -1.25264 ± 0.93580            95% CI[  -1.49021,   -1.01507]
(s) Helix DG_i            6.87395 ± 1.29828            95% CI[   6.54435,    7.20354]
(s) Helix DG_ij          -9.94555 ± 1.30027            95% CI[ -10.27565,   -9.61545]
(s) Helix m_i            -0.63753 ± 2.80242            95% CI[  -1.34898,    0.07392]
--------------------------------------------------------------------------------
R^2: 0.99946
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR2SPhos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i           3.46641 ± 0.91527            95% CI[   3.23405,    3.69877]
(s) Repeat DG_ij         -6.15007 ± 0.61596            95% CI[  -6.30644,   -5.99369]
(s) Repeat m_i           -0.53090 ± 0.21582            95% CI[  -0.58569,   -0.47611]
(s) MutantRepeat DG_i     5.17597 ± 0.16560            95% CI[   5.13393,    5.21801]
```

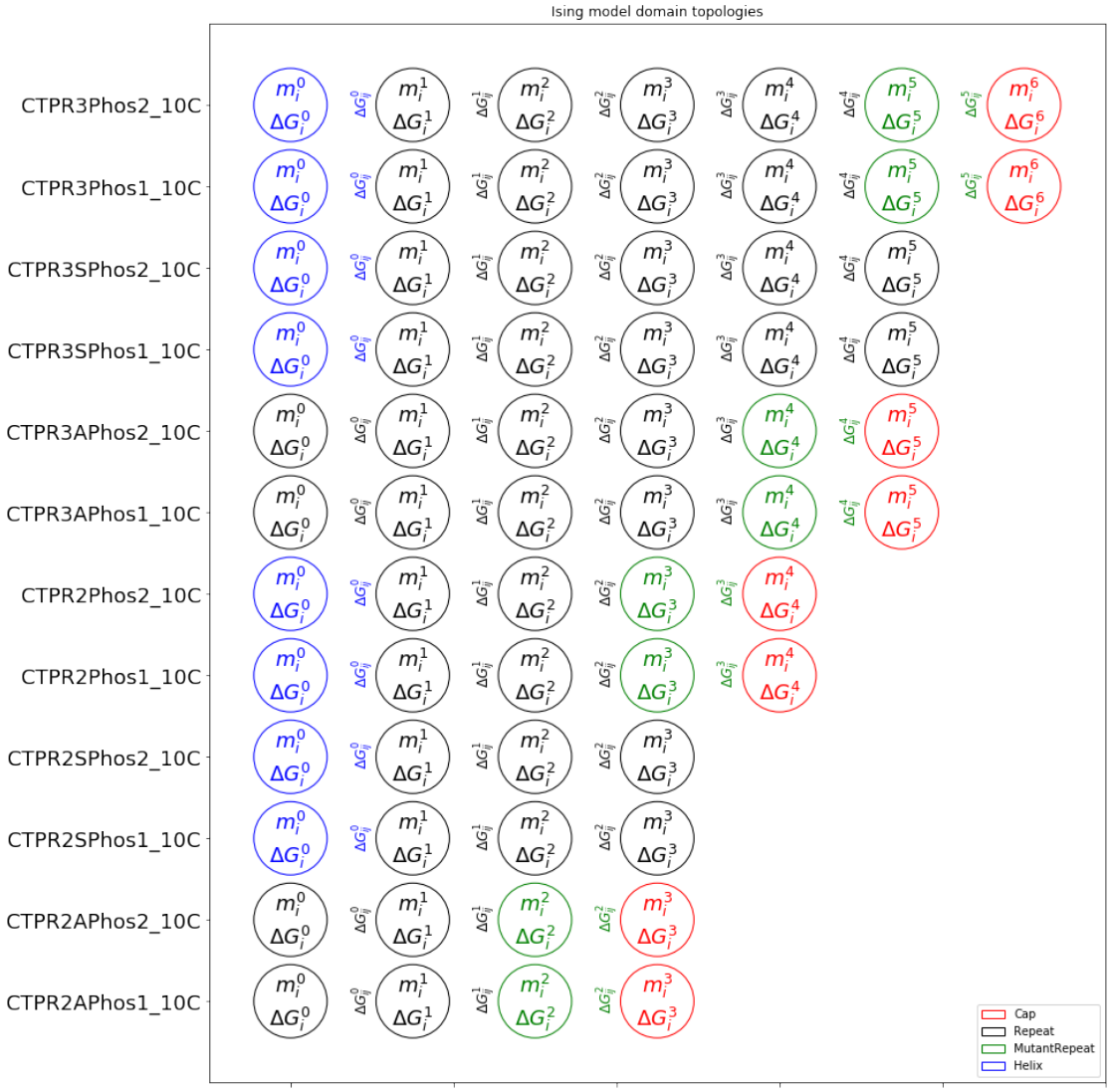
```
(s) MutantRepeat DG_ij   -9.28958 ± 0.42047            95% CI[  -9.39632,   -9.18283]
(s) MutantRepeat m_i     -0.00790 ± 0.13650            95% CI[  -0.04255,    0.02676]
(s) Cap DG_i              4.89001 ± 0.41848            95% CI[   4.78377,    4.99625]
(s) Cap m_i              -1.25264 ± 0.93580            95% CI[  -1.49021,   -1.01507]
(s) Helix DG_i            6.87395 ± 1.29828            95% CI[   6.54435,    7.20354]
(s) Helix DG_ij          -9.94555 ± 1.30027            95% CI[ -10.27565,   -9.61545]
(s) Helix m_i            -0.63753 ± 2.80242            95% CI[  -1.34898,    0.07392]
--------------------------------------------------------------------------------
R^2: 0.99492
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR2Phos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i           3.46641 ± 0.91527            95% CI[   3.23405,    3.69877]
(s) Repeat DG_ij         -6.15007 ± 0.61596            95% CI[  -6.30644,   -5.99369]
(s) Repeat m_i           -0.53090 ± 0.21582            95% CI[  -0.58569,   -0.47611]
(s) MutantRepeat DG_i     5.17597 ± 0.16560            95% CI[   5.13393,    5.21801]
(s) MutantRepeat DG_ij   -9.28958 ± 0.42047            95% CI[  -9.39632,   -9.18283]
(s) MutantRepeat m_i     -0.00790 ± 0.13650            95% CI[  -0.04255,    0.02676]
(s) Cap DG_i              4.89001 ± 0.41848            95% CI[   4.78377,    4.99625]
(s) Cap m_i              -1.25264 ± 0.93580            95% CI[  -1.49021,   -1.01507]
(s) Helix DG_i            6.87395 ± 1.29828            95% CI[   6.54435,    7.20354]
(s) Helix DG_ij          -9.94555 ± 1.30027            95% CI[ -10.27565,   -9.61545]
(s) Helix m_i            -0.63753 ± 2.80242            95% CI[  -1.34898,    0.07392]
--------------------------------------------------------------------------------
R^2: 0.99864
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR2Phos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i           3.46641 ± 0.91527            95% CI[   3.23405,    3.69877]
(s) Repeat DG_ij         -6.15007 ± 0.61596            95% CI[  -6.30644,   -5.99369]
(s) Repeat m_i           -0.53090 ± 0.21582            95% CI[  -0.58569,   -0.47611]
(s) MutantRepeat DG_i     5.17597 ± 0.16560            95% CI[   5.13393,    5.21801]
```

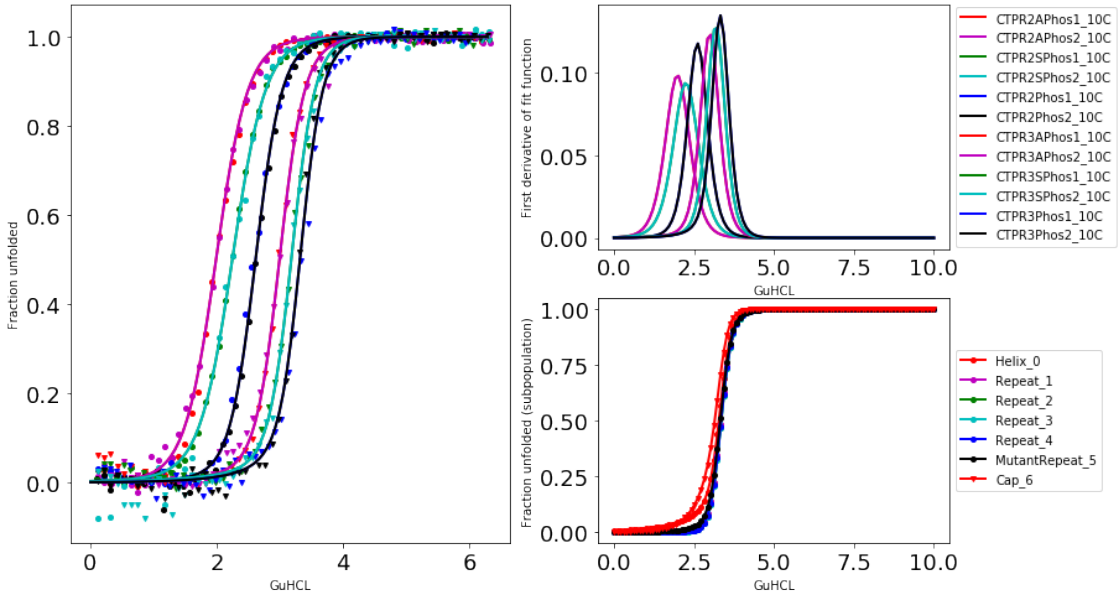
```
(s) MutantRepeat DG_ij   -9.28958 ± 0.42047            95% CI[  -9.39632,   -9.18283]
(s) MutantRepeat m_i     -0.00790 ± 0.13650            95% CI[  -0.04255,    0.02676]
(s) Cap DG_i              4.89001 ± 0.41848            95% CI[   4.78377,    4.99625]
(s) Cap m_i              -1.25264 ± 0.93580            95% CI[  -1.49021,   -1.01507]
(s) Helix DG_i            6.87395 ± 1.29828            95% CI[   6.54435,    7.20354]
(s) Helix DG_ij          -9.94555 ± 1.30027            95% CI[ -10.27565,   -9.61545]
(s) Helix m_i            -0.63753 ± 2.80242            95% CI[  -1.34898,    0.07392]
--------------------------------------------------------------------------------
R^2: 0.99897
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3APhos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i           3.46641 ± 0.91527            95% CI[   3.23405,    3.69877]
(s) Repeat DG_ij         -6.15007 ± 0.61596            95% CI[  -6.30644,   -5.99369]
(s) Repeat m_i           -0.53090 ± 0.21582            95% CI[  -0.58569,   -0.47611]
(s) MutantRepeat DG_i     5.17597 ± 0.16560            95% CI[   5.13393,    5.21801]
(s) MutantRepeat DG_ij   -9.28958 ± 0.42047            95% CI[  -9.39632,   -9.18283]
(s) MutantRepeat m_i     -0.00790 ± 0.13650            95% CI[  -0.04255,    0.02676]
(s) Cap DG_i              4.89001 ± 0.41848            95% CI[   4.78377,    4.99625]
(s) Cap m_i              -1.25264 ± 0.93580            95% CI[  -1.49021,   -1.01507]
(s) Helix DG_i            6.87395 ± 1.29828            95% CI[   6.54435,    7.20354]
(s) Helix DG_ij          -9.94555 ± 1.30027            95% CI[ -10.27565,   -9.61545]
(s) Helix m_i            -0.63753 ± 2.80242            95% CI[  -1.34898,    0.07392]
--------------------------------------------------------------------------------
R^2: 0.99848
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3APhos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i           3.46641 ± 0.91527            95% CI[   3.23405,    3.69877]
(s) Repeat DG_ij         -6.15007 ± 0.61596            95% CI[  -6.30644,   -5.99369]
(s) Repeat m_i           -0.53090 ± 0.21582            95% CI[  -0.58569,   -0.47611]
(s) MutantRepeat DG_i     5.17597 ± 0.16560            95% CI[   5.13393,    5.21801]
```

```
(s) MutantRepeat DG_ij   -9.28958 ± 0.42047            95% CI[  -9.39632,   -9.18283]
(s) MutantRepeat m_i     -0.00790 ± 0.13650            95% CI[  -0.04255,    0.02676]
(s) Cap DG_i              4.89001 ± 0.41848            95% CI[   4.78377,    4.99625]
(s) Cap m_i              -1.25264 ± 0.93580            95% CI[  -1.49021,   -1.01507]
(s) Helix DG_i            6.87395 ± 1.29828            95% CI[   6.54435,    7.20354]
(s) Helix DG_ij          -9.94555 ± 1.30027            95% CI[ -10.27565,   -9.61545]
(s) Helix m_i            -0.63753 ± 2.80242            95% CI[  -1.34898,    0.07392]
--------------------------------------------------------------------------------
R^2: 0.99813
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3SPhos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i           3.46641 ± 0.91527            95% CI[   3.23405,    3.69877]
(s) Repeat DG_ij         -6.15007 ± 0.61596            95% CI[  -6.30644,   -5.99369]
(s) Repeat m_i           -0.53090 ± 0.21582            95% CI[  -0.58569,   -0.47611]
(s) MutantRepeat DG_i     5.17597 ± 0.16560            95% CI[   5.13393,    5.21801]
(s) MutantRepeat DG_ij   -9.28958 ± 0.42047            95% CI[  -9.39632,   -9.18283]
(s) MutantRepeat m_i     -0.00790 ± 0.13650            95% CI[  -0.04255,    0.02676]
(s) Cap DG_i              4.89001 ± 0.41848            95% CI[   4.78377,    4.99625]
(s) Cap m_i              -1.25264 ± 0.93580            95% CI[  -1.49021,   -1.01507]
(s) Helix DG_i            6.87395 ± 1.29828            95% CI[   6.54435,    7.20354]
(s) Helix DG_ij          -9.94555 ± 1.30027            95% CI[ -10.27565,   -9.61545]
(s) Helix m_i            -0.63753 ± 2.80242            95% CI[  -1.34898,    0.07392]
--------------------------------------------------------------------------------
R^2: 0.99877
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3SPhos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i           3.46641 ± 0.91527            95% CI[   3.23405,    3.69877]
(s) Repeat DG_ij         -6.15007 ± 0.61596            95% CI[  -6.30644,   -5.99369]
(s) Repeat m_i           -0.53090 ± 0.21582            95% CI[  -0.58569,   -0.47611]
(s) MutantRepeat DG_i     5.17597 ± 0.16560            95% CI[   5.13393,    5.21801]
```

```
(s) MutantRepeat DG_ij   -9.28958 ± 0.42047            95% CI[  -9.39632,   -9.18283]
(s) MutantRepeat m_i     -0.00790 ± 0.13650            95% CI[  -0.04255,    0.02676]
(s) Cap DG_i              4.89001 ± 0.41848            95% CI[   4.78377,    4.99625]
(s) Cap m_i              -1.25264 ± 0.93580            95% CI[  -1.49021,   -1.01507]
(s) Helix DG_i            6.87395 ± 1.29828            95% CI[   6.54435,    7.20354]
(s) Helix DG_ij          -9.94555 ± 1.30027            95% CI[ -10.27565,   -9.61545]
(s) Helix m_i            -0.63753 ± 2.80242            95% CI[  -1.34898,    0.07392]
--------------------------------------------------------------------------------
R^2: 0.99731
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3Phos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i           3.46641 ± 0.91527            95% CI[   3.23405,    3.69877]
(s) Repeat DG_ij         -6.15007 ± 0.61596            95% CI[  -6.30644,   -5.99369]
(s) Repeat m_i           -0.53090 ± 0.21582            95% CI[  -0.58569,   -0.47611]
(s) MutantRepeat DG_i     5.17597 ± 0.16560            95% CI[   5.13393,    5.21801]
(s) MutantRepeat DG_ij   -9.28958 ± 0.42047            95% CI[  -9.39632,   -9.18283]
(s) MutantRepeat m_i     -0.00790 ± 0.13650            95% CI[  -0.04255,    0.02676]
(s) Cap DG_i              4.89001 ± 0.41848            95% CI[   4.78377,    4.99625]
(s) Cap m_i              -1.25264 ± 0.93580            95% CI[  -1.49021,   -1.01507]
(s) Helix DG_i            6.87395 ± 1.29828            95% CI[   6.54435,    7.20354]
(s) Helix DG_ij          -9.94555 ± 1.30027            95% CI[ -10.27565,   -9.61545]
(s) Helix m_i            -0.63753 ± 2.80242            95% CI[  -1.34898,    0.07392]
--------------------------------------------------------------------------------
R^2: 0.99722
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3Phos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i           3.46641 ± 0.91527            95% CI[   3.23405,    3.69877]
(s) Repeat DG_ij         -6.15007 ± 0.61596            95% CI[  -6.30644,   -5.99369]
(s) Repeat m_i           -0.53090 ± 0.21582            95% CI[  -0.58569,   -0.47611]
(s) MutantRepeat DG_i     5.17597 ± 0.16560            95% CI[   5.13393,    5.21801]
```

```
(s) MutantRepeat DG_ij    -9.28958 ± 0.42047          95% CI[  -9.39632,   -9.18283]
(s) MutantRepeat m_i      -0.00790 ± 0.13650          95% CI[  -0.04255,    0.02676]
(s) Cap DG_i               4.89001 ± 0.41848          95% CI[   4.78377,    4.99625]
(s) Cap m_i               -1.25264 ± 0.93580          95% CI[  -1.49021,   -1.01507]
(s) Helix DG_i             6.87395 ± 1.29828          95% CI[   6.54435,    7.20354]
(s) Helix DG_ij           -9.94555 ± 1.30027          95% CI[ -10.27565,   -9.61545]
(s) Helix m_i             -0.63753 ± 2.80242          95% CI[  -1.34898,    0.07392]
-------------------------------------------------------------------------------
R^2: 0.99765
===============================================================================
```





In [9]:
```
#Now do Topology 2
topology2 = [[r,r,r,c],[r,r,r,c],
             [h,r,r,r], [h,r,r,r],
             [h,r,r,r,c], [h,r,r,r,c],
             [r,r,r,r,r,c], [r,r,r,r,r,c],
             [h,r,r,r,r,r],[h,r,r,r,r,r],
             [h,r,r,r,r,r,c], [h,r,r,r,r,r,c]]

#Fit
ising.fit_heteropolymer(curves[0:12], topology2[0:12], popsize=10, maxiter= 10000)
```

```
Appending 12 curves to GlobalFitIsing...
 + added CTPR2APhos1_10C with topology ['Repeat', 'Repeat', 'Repeat', 'Cap']
 + added CTPR2APhos2_10C with topology ['Repeat', 'Repeat', 'Repeat', 'Cap']
 + added CTPR2SPhos1_10C with topology ['Helix', 'Repeat', 'Repeat', 'Repeat']
 + added CTPR2SPhos2_10C with topology ['Helix', 'Repeat', 'Repeat', 'Repeat']
 + added CTPR2Phos1_10C with topology ['Helix', 'Repeat', 'Repeat', 'Repeat', 'Cap']
 + added CTPR2Phos2_10C with topology ['Helix', 'Repeat', 'Repeat', 'Repeat', 'Cap']
 + added CTPR3APhos1_10C with topology ['Repeat', 'Repeat', 'Repeat', 'Repeat', 'Repeat', 'Cap']
 + added CTPR3APhos2_10C with topology ['Repeat', 'Repeat', 'Repeat', 'Repeat', 'Repeat', 'Cap']
 + added CTPR3SPhos1_10C with topology ['Helix', 'Repeat', 'Repeat', 'Repeat', 'Repeat', 'Repeat
 + added CTPR3SPhos2_10C with topology ['Helix', 'Repeat', 'Repeat', 'Repeat', 'Repeat', 'Repeat
 + added CTPR3Phos1_10C with topology ['Helix', 'Repeat', 'Repeat', 'Repeat', 'Repeat', 'Repeat'
 + added CTPR3Phos2_10C with topology ['Helix', 'Repeat', 'Repeat', 'Repeat', 'Repeat', 'Repeat'

Performing global optimisation of Ising model (12 curves, Population size: 10, Tolerance: 1.00E-
 - Fitting in progress (Iteration: 100, Convergence: 2.66817E-08, Timing: 1.68s per iteration)
```

```
 - Fitting in progress (Iteration: 200, Convergence: 1.06884E-07, Timing: 1.66s per iteration)
 - Fitting in progress (Iteration: 300, Convergence: 1.65748E-06, Timing: 1.70s per iteration)
 - Fitting in progress (Iteration: 400, Convergence: 6.98981E-05, Timing: 1.67s per iteration)
 - Fitting in progress (Iteration: 500, Convergence: 1.74531E-03, Timing: 1.60s per iteration)
 - Fitting in progress (Iteration: 600, Convergence: 6.79443E-02, Timing: 1.68s per iteration)
 - Fitting in progress (Iteration: 700, Convergence: 6.93502E-01, Timing: 1.79s per iteration)

Fitting results (NOTE: Careful with the errors here):

================================================================================
Fitting results
================================================================================
ID: CTPR2APhos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i     3.58860 ± 1.13612            95% CI[   3.30017,    3.87702]
(s) Repeat DG_ij   -6.23306 ± 1.48678            95% CI[  -6.61051,   -5.85561]
(s) Repeat m_i     -0.51206 ± 0.78378            95% CI[  -0.71104,   -0.31308]
(s) Cap DG_i        3.29234 ± 0.94705            95% CI[   3.05191,    3.53277]
(s) Cap m_i        -0.81869 ± 3.29559            95% CI[  -1.65535,    0.01796]
(s) Helix DG_i      6.72118 ± 1.47219            95% CI[   6.34744,    7.09493]
(s) Helix DG_ij    -9.99497 ± 1.47612            95% CI[ -10.36971,   -9.62022]
(s) Helix m_i      -0.70077 ± 4.45640            95% CI[  -1.83212,    0.43058]
--------------------------------------------------------------------------------
R^2: 0.99874
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR2APhos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i     3.58860 ± 1.13612            95% CI[   3.30017,    3.87702]
(s) Repeat DG_ij   -6.23306 ± 1.48678            95% CI[  -6.61051,   -5.85561]
(s) Repeat m_i     -0.51206 ± 0.78378            95% CI[  -0.71104,   -0.31308]
(s) Cap DG_i        3.29234 ± 0.94705            95% CI[   3.05191,    3.53277]
(s) Cap m_i        -0.81869 ± 3.29559            95% CI[  -1.65535,    0.01796]
(s) Helix DG_i      6.72118 ± 1.47219            95% CI[   6.34744,    7.09493]
(s) Helix DG_ij    -9.99497 ± 1.47612            95% CI[ -10.36971,   -9.62022]
(s) Helix m_i      -0.70077 ± 4.45640            95% CI[  -1.83212,    0.43058]
--------------------------------------------------------------------------------
R^2: 0.99901
```

```
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR2SPhos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i     3.58860 ± 1.13612            95% CI[   3.30017,    3.87702]
(s) Repeat DG_ij   -6.23306 ± 1.48678            95% CI[  -6.61051,   -5.85561]
(s) Repeat m_i     -0.51206 ± 0.78378            95% CI[  -0.71104,   -0.31308]
(s) Cap DG_i        3.29234 ± 0.94705            95% CI[   3.05191,    3.53277]
(s) Cap m_i        -0.81869 ± 3.29559            95% CI[  -1.65535,    0.01796]
(s) Helix DG_i      6.72118 ± 1.47219            95% CI[   6.34744,    7.09493]
(s) Helix DG_ij    -9.99497 ± 1.47612            95% CI[ -10.36971,   -9.62022]
(s) Helix m_i      -0.70077 ± 4.45640            95% CI[  -1.83212,    0.43058]
--------------------------------------------------------------------------------
R^2: 0.99952
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR2SPhos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i     3.58860 ± 1.13612            95% CI[   3.30017,    3.87702]
(s) Repeat DG_ij   -6.23306 ± 1.48678            95% CI[  -6.61051,   -5.85561]
(s) Repeat m_i     -0.51206 ± 0.78378            95% CI[  -0.71104,   -0.31308]
(s) Cap DG_i        3.29234 ± 0.94705            95% CI[   3.05191,    3.53277]
(s) Cap m_i        -0.81869 ± 3.29559            95% CI[  -1.65535,    0.01796]
(s) Helix DG_i      6.72118 ± 1.47219            95% CI[   6.34744,    7.09493]
(s) Helix DG_ij    -9.99497 ± 1.47612            95% CI[ -10.36971,   -9.62022]
(s) Helix m_i      -0.70077 ± 4.45640            95% CI[  -1.83212,    0.43058]
--------------------------------------------------------------------------------
R^2: 0.99491
================================================================================


================================================================================
Fitting results
================================================================================
```

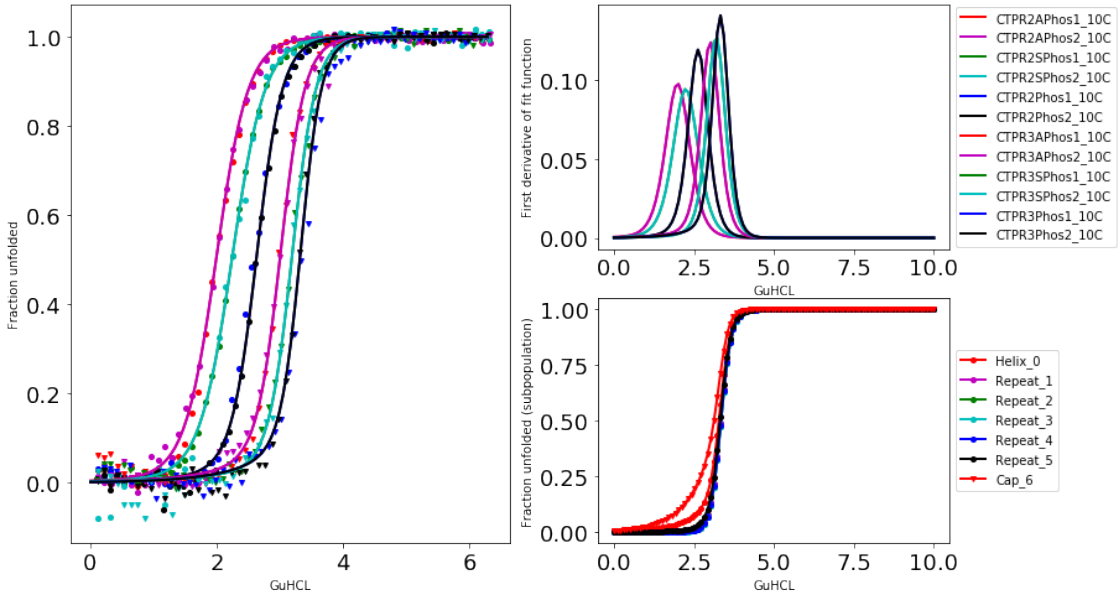
```
ID: CTPR2Phos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i     3.58860 ± 1.13612            95% CI[   3.30017,    3.87702]
(s) Repeat DG_ij   -6.23306 ± 1.48678            95% CI[  -6.61051,   -5.85561]
(s) Repeat m_i     -0.51206 ± 0.78378            95% CI[  -0.71104,   -0.31308]
(s) Cap DG_i        3.29234 ± 0.94705            95% CI[   3.05191,    3.53277]
(s) Cap m_i        -0.81869 ± 3.29559            95% CI[  -1.65535,    0.01796]
(s) Helix DG_i      6.72118 ± 1.47219            95% CI[   6.34744,    7.09493]
(s) Helix DG_ij    -9.99497 ± 1.47612            95% CI[ -10.36971,   -9.62022]
(s) Helix m_i      -0.70077 ± 4.45640            95% CI[  -1.83212,    0.43058]
--------------------------------------------------------------------------------
R^2: 0.99823
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR2Phos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i     3.58860 ± 1.13612            95% CI[   3.30017,    3.87702]
(s) Repeat DG_ij   -6.23306 ± 1.48678            95% CI[  -6.61051,   -5.85561]
(s) Repeat m_i     -0.51206 ± 0.78378            95% CI[  -0.71104,   -0.31308]
(s) Cap DG_i        3.29234 ± 0.94705            95% CI[   3.05191,    3.53277]
(s) Cap m_i        -0.81869 ± 3.29559            95% CI[  -1.65535,    0.01796]
(s) Helix DG_i      6.72118 ± 1.47219            95% CI[   6.34744,    7.09493]
(s) Helix DG_ij    -9.99497 ± 1.47612            95% CI[ -10.36971,   -9.62022]
(s) Helix m_i      -0.70077 ± 4.45640            95% CI[  -1.83212,    0.43058]
--------------------------------------------------------------------------------
R^2: 0.99888
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3APhos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i     3.58860 ± 1.13612            95% CI[   3.30017,    3.87702]
```

```
(s) Repeat DG_ij   -6.23306 ± 1.48678            95% CI[  -6.61051,   -5.85561]
(s) Repeat m_i     -0.51206 ± 0.78378            95% CI[  -0.71104,   -0.31308]
(s) Cap DG_i        3.29234 ± 0.94705            95% CI[   3.05191,    3.53277]
(s) Cap m_i        -0.81869 ± 3.29559            95% CI[  -1.65535,    0.01796]
(s) Helix DG_i      6.72118 ± 1.47219            95% CI[   6.34744,    7.09493]
(s) Helix DG_ij    -9.99497 ± 1.47612            95% CI[ -10.36971,   -9.62022]
(s) Helix m_i      -0.70077 ± 4.45640            95% CI[  -1.83212,    0.43058]
--------------------------------------------------------------------------------
R^2: 0.99838
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3APhos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i     3.58860 ± 1.13612            95% CI[   3.30017,    3.87702]
(s) Repeat DG_ij   -6.23306 ± 1.48678            95% CI[  -6.61051,   -5.85561]
(s) Repeat m_i     -0.51206 ± 0.78378            95% CI[  -0.71104,   -0.31308]
(s) Cap DG_i        3.29234 ± 0.94705            95% CI[   3.05191,    3.53277]
(s) Cap m_i        -0.81869 ± 3.29559            95% CI[  -1.65535,    0.01796]
(s) Helix DG_i      6.72118 ± 1.47219            95% CI[   6.34744,    7.09493]
(s) Helix DG_ij    -9.99497 ± 1.47612            95% CI[ -10.36971,   -9.62022]
(s) Helix m_i      -0.70077 ± 4.45640            95% CI[  -1.83212,    0.43058]
--------------------------------------------------------------------------------
R^2: 0.99824
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3SPhos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i     3.58860 ± 1.13612            95% CI[   3.30017,    3.87702]
(s) Repeat DG_ij   -6.23306 ± 1.48678            95% CI[  -6.61051,   -5.85561]
(s) Repeat m_i     -0.51206 ± 0.78378            95% CI[  -0.71104,   -0.31308]
(s) Cap DG_i        3.29234 ± 0.94705            95% CI[   3.05191,    3.53277]
(s) Cap m_i        -0.81869 ± 3.29559            95% CI[  -1.65535,    0.01796]
(s) Helix DG_i      6.72118 ± 1.47219            95% CI[   6.34744,    7.09493]
(s) Helix DG_ij    -9.99497 ± 1.47612            95% CI[ -10.36971,   -9.62022]
```

```
(s) Helix m_i      -0.70077 ± 4.45640            95% CI[  -1.83212,    0.43058]
--------------------------------------------------------------------------------
R^2: 0.99878
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3SPhos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i     3.58860 ± 1.13612            95% CI[   3.30017,    3.87702]
(s) Repeat DG_ij   -6.23306 ± 1.48678            95% CI[  -6.61051,   -5.85561]
(s) Repeat m_i     -0.51206 ± 0.78378            95% CI[  -0.71104,   -0.31308]
(s) Cap DG_i        3.29234 ± 0.94705            95% CI[   3.05191,    3.53277]
(s) Cap m_i        -0.81869 ± 3.29559            95% CI[  -1.65535,    0.01796]
(s) Helix DG_i      6.72118 ± 1.47219            95% CI[   6.34744,    7.09493]
(s) Helix DG_ij    -9.99497 ± 1.47612            95% CI[ -10.36971,   -9.62022]
(s) Helix m_i      -0.70077 ± 4.45640            95% CI[  -1.83212,    0.43058]
--------------------------------------------------------------------------------
R^2: 0.99734
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3Phos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i     3.58860 ± 1.13612            95% CI[   3.30017,    3.87702]
(s) Repeat DG_ij   -6.23306 ± 1.48678            95% CI[  -6.61051,   -5.85561]
(s) Repeat m_i     -0.51206 ± 0.78378            95% CI[  -0.71104,   -0.31308]
(s) Cap DG_i        3.29234 ± 0.94705            95% CI[   3.05191,    3.53277]
(s) Cap m_i        -0.81869 ± 3.29559            95% CI[  -1.65535,    0.01796]
(s) Helix DG_i      6.72118 ± 1.47219            95% CI[   6.34744,    7.09493]
(s) Helix DG_ij    -9.99497 ± 1.47612            95% CI[ -10.36971,   -9.62022]
(s) Helix m_i      -0.70077 ± 4.45640            95% CI[  -1.83212,    0.43058]
--------------------------------------------------------------------------------
R^2: 0.99653
================================================================================
```

```
================================================================================
Fitting results
================================================================================
ID: CTPR3Phos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Repeat DG_i     3.58860 ± 1.13612            95% CI[   3.30017,    3.87702]
(s) Repeat DG_ij   -6.23306 ± 1.48678            95% CI[  -6.61051,   -5.85561]
(s) Repeat m_i     -0.51206 ± 0.78378            95% CI[  -0.71104,   -0.31308]
(s) Cap DG_i        3.29234 ± 0.94705            95% CI[   3.05191,    3.53277]
(s) Cap m_i        -0.81869 ± 3.29559            95% CI[  -1.65535,    0.01796]
(s) Helix DG_i      6.72118 ± 1.47219            95% CI[   6.34744,    7.09493]
(s) Helix DG_ij    -9.99497 ± 1.47612            95% CI[ -10.36971,   -9.62022]
(s) Helix m_i      -0.70077 ± 4.45640            95% CI[  -1.83212,    0.43058]
--------------------------------------------------------------------------------
R^2: 0.99794
================================================================================
```


Ising model domain topologies
CTPR3Phos2_10C
CTPR3Phos1_10C
CTPR3SPhos2_10C
CTPR3SPhos1_10C
CTPR3APhos2_10C
CTPR3APhos1_10C
CTPR2Phos2_10C
CTPR2Phos1_10C
CTPR2SPhos2_10C
CTPR2SPhos1_10C
CTPR2APhos2_10C
CTPR2APhos1_10C
Cap
Repeat
Helix



In [10]:
```
#Now do Topology 3
topology3 = [[h,r,h,c], [h,r,h,c],
             [r,h,r,h], [r,h,r,h],
             [r,h,r,h,c], [r,h,r,h,c],
             [h,r,h,r,h,c], [h,r,h,r,h,c],
             [r,h,r,h,r,h], [r,h,r,h,r,h],
             [r,h,r,h,r,h,c], [r,h,r,h,r,h,c]]

#fit
ising.fit_heteropolymer(curves[0:12], topology3[0:12], popsize=10, maxiter= 10000)
```

```
Appending 12 curves to GlobalFitIsing...
 + added CTPR2APhos1_10C with topology ['Helix', 'Repeat', 'Helix', 'Cap']
 + added CTPR2APhos2_10C with topology ['Helix', 'Repeat', 'Helix', 'Cap']
 + added CTPR2SPhos1_10C with topology ['Repeat', 'Helix', 'Repeat', 'Helix']
 + added CTPR2SPhos2_10C with topology ['Repeat', 'Helix', 'Repeat', 'Helix']
 + added CTPR2Phos1_10C with topology ['Repeat', 'Helix', 'Repeat', 'Helix', 'Cap']
 + added CTPR2Phos2_10C with topology ['Repeat', 'Helix', 'Repeat', 'Helix', 'Cap']
 + added CTPR3APhos1_10C with topology ['Helix', 'Repeat', 'Helix', 'Repeat', 'Helix', 'Cap']
 + added CTPR3APhos2_10C with topology ['Helix', 'Repeat', 'Helix', 'Repeat', 'Helix', 'Cap']
 + added CTPR3SPhos1_10C with topology ['Repeat', 'Helix', 'Repeat', 'Helix', 'Repeat', 'Helix']
 + added CTPR3SPhos2_10C with topology ['Repeat', 'Helix', 'Repeat', 'Helix', 'Repeat', 'Helix']
 + added CTPR3Phos1_10C with topology ['Repeat', 'Helix', 'Repeat', 'Helix', 'Repeat', 'Helix',
 + added CTPR3Phos2_10C with topology ['Repeat', 'Helix', 'Repeat', 'Helix', 'Repeat', 'Helix',

Performing global optimisation of Ising model (12 curves, Population size: 10, Tolerance: 1.00E-
 - Fitting in progress (Iteration: 100, Convergence: 1.74023E-08, Timing: 1.66s per iteration)
```

```
 - Fitting in progress (Iteration: 200, Convergence: 5.09258E-08, Timing: 1.73s per iteration)
 - Fitting in progress (Iteration: 300, Convergence: 6.14388E-07, Timing: 1.91s per iteration)
 - Fitting in progress (Iteration: 400, Convergence: 8.90962E-06, Timing: 1.91s per iteration)
 - Fitting in progress (Iteration: 500, Convergence: 1.73486E-04, Timing: 1.88s per iteration)
 - Fitting in progress (Iteration: 600, Convergence: 1.09176E-03, Timing: 2.24s per iteration)
 - Fitting in progress (Iteration: 700, Convergence: 3.72576E-03, Timing: 2.30s per iteration)
 - Fitting in progress (Iteration: 800, Convergence: 2.86758E-02, Timing: 1.76s per iteration)
 - Fitting in progress (Iteration: 900, Convergence: 1.94056E-01, Timing: 1.87s per iteration)
 - Fitting in progress (Iteration: 1000, Convergence: 4.83044E-01, Timing: 1.87s per iteration)

Fitting results (NOTE: Careful with the errors here):

================================================================================
Fitting results
================================================================================
ID: CTPR2APhos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Helix DG_i      3.63211 ± 4.48305            95% CI[   2.49399,    4.77023]
(s) Helix DG_ij    -6.01364 ± 0.72695            95% CI[  -6.19819,   -5.82909]
(s) Helix m_i      -0.46349 ± 0.61955            95% CI[  -0.62077,   -0.30620]
(s) Repeat DG_i     6.99113 ± 2.24650            95% CI[   6.42081,    7.56145]
(s) Repeat DG_ij   -9.99998 ± 1.59313            95% CI[ -10.40444,   -9.59553]
(s) Repeat m_i     -0.60650 ± 5.86237            95% CI[  -2.09479,    0.88179]
(s) Cap DG_i        3.14314 ± 1.33059            95% CI[   2.80534,    3.48094]
(s) Cap m_i        -0.81343 ± 2.57567            95% CI[  -1.46732,   -0.15954]
--------------------------------------------------------------------------------
R^2: 0.99867
================================================================================



================================================================================
Fitting results
================================================================================
ID: CTPR2APhos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Helix DG_i      3.63211 ± 4.48305            95% CI[   2.49399,    4.77023]
(s) Helix DG_ij    -6.01364 ± 0.72695            95% CI[  -6.19819,   -5.82909]
(s) Helix m_i      -0.46349 ± 0.61955            95% CI[  -0.62077,   -0.30620]
(s) Repeat DG_i     6.99113 ± 2.24650            95% CI[   6.42081,    7.56145]
(s) Repeat DG_ij   -9.99998 ± 1.59313            95% CI[ -10.40444,   -9.59553]
(s) Repeat m_i     -0.60650 ± 5.86237            95% CI[  -2.09479,    0.88179]
(s) Cap DG_i        3.14314 ± 1.33059            95% CI[   2.80534,    3.48094]
```

```
(s) Cap m_i        -0.81343 ± 2.57567            95% CI[  -1.46732,   -0.15954]
--------------------------------------------------------------------------------
R^2: 0.99903
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR2SPhos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Helix DG_i      3.63211 ± 4.48305            95% CI[   2.49399,    4.77023]
(s) Helix DG_ij    -6.01364 ± 0.72695            95% CI[  -6.19819,   -5.82909]
(s) Helix m_i      -0.46349 ± 0.61955            95% CI[  -0.62077,   -0.30620]
(s) Repeat DG_i     6.99113 ± 2.24650            95% CI[   6.42081,    7.56145]
(s) Repeat DG_ij   -9.99998 ± 1.59313            95% CI[ -10.40444,   -9.59553]
(s) Repeat m_i     -0.60650 ± 5.86237            95% CI[  -2.09479,    0.88179]
(s) Cap DG_i        3.14314 ± 1.33059            95% CI[   2.80534,    3.48094]
(s) Cap m_i        -0.81343 ± 2.57567            95% CI[  -1.46732,   -0.15954]
--------------------------------------------------------------------------------
R^2: 0.99946
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR2SPhos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Helix DG_i      3.63211 ± 4.48305            95% CI[   2.49399,    4.77023]
(s) Helix DG_ij    -6.01364 ± 0.72695            95% CI[  -6.19819,   -5.82909]
(s) Helix m_i      -0.46349 ± 0.61955            95% CI[  -0.62077,   -0.30620]
(s) Repeat DG_i     6.99113 ± 2.24650            95% CI[   6.42081,    7.56145]
(s) Repeat DG_ij   -9.99998 ± 1.59313            95% CI[ -10.40444,   -9.59553]
(s) Repeat m_i     -0.60650 ± 5.86237            95% CI[  -2.09479,    0.88179]
(s) Cap DG_i        3.14314 ± 1.33059            95% CI[   2.80534,    3.48094]
(s) Cap m_i        -0.81343 ± 2.57567            95% CI[  -1.46732,   -0.15954]
--------------------------------------------------------------------------------
R^2: 0.99496
================================================================================
```

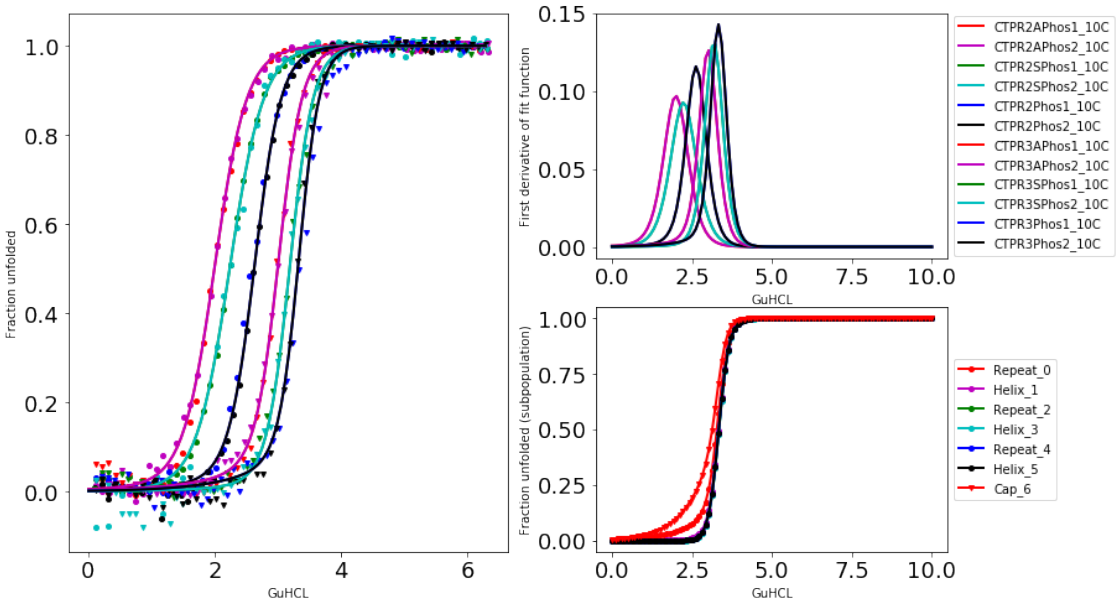
```
================================================================================
Fitting results
================================================================================
ID: CTPR2Phos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Helix DG_i      3.63211 ± 4.48305            95% CI[   2.49399,    4.77023]
(s) Helix DG_ij    -6.01364 ± 0.72695            95% CI[  -6.19819,   -5.82909]
(s) Helix m_i      -0.46349 ± 0.61955            95% CI[  -0.62077,   -0.30620]
(s) Repeat DG_i     6.99113 ± 2.24650            95% CI[   6.42081,    7.56145]
(s) Repeat DG_ij   -9.99998 ± 1.59313            95% CI[ -10.40444,   -9.59553]
(s) Repeat m_i     -0.60650 ± 5.86237            95% CI[  -2.09479,    0.88179]
(s) Cap DG_i        3.14314 ± 1.33059            95% CI[   2.80534,    3.48094]
(s) Cap m_i        -0.81343 ± 2.57567            95% CI[  -1.46732,   -0.15954]
--------------------------------------------------------------------------------
R^2: 0.99844
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR2Phos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Helix DG_i      3.63211 ± 4.48305            95% CI[   2.49399,    4.77023]
(s) Helix DG_ij    -6.01364 ± 0.72695            95% CI[  -6.19819,   -5.82909]
(s) Helix m_i      -0.46349 ± 0.61955            95% CI[  -0.62077,   -0.30620]
(s) Repeat DG_i     6.99113 ± 2.24650            95% CI[   6.42081,    7.56145]
(s) Repeat DG_ij   -9.99998 ± 1.59313            95% CI[ -10.40444,   -9.59553]
(s) Repeat m_i     -0.60650 ± 5.86237            95% CI[  -2.09479,    0.88179]
(s) Cap DG_i        3.14314 ± 1.33059            95% CI[   2.80534,    3.48094]
(s) Cap m_i        -0.81343 ± 2.57567            95% CI[  -1.46732,   -0.15954]
--------------------------------------------------------------------------------
R^2: 0.99881
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3APhos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
```

```
Temperature: 10.00°C

(s) Helix DG_i      3.63211 ± 4.48305            95% CI[   2.49399,    4.77023]
(s) Helix DG_ij    -6.01364 ± 0.72695            95% CI[  -6.19819,   -5.82909]
(s) Helix m_i      -0.46349 ± 0.61955            95% CI[  -0.62077,   -0.30620]
(s) Repeat DG_i     6.99113 ± 2.24650            95% CI[   6.42081,    7.56145]
(s) Repeat DG_ij   -9.99998 ± 1.59313            95% CI[ -10.40444,   -9.59553]
(s) Repeat m_i     -0.60650 ± 5.86237            95% CI[  -2.09479,    0.88179]
(s) Cap DG_i        3.14314 ± 1.33059            95% CI[   2.80534,    3.48094]
(s) Cap m_i        -0.81343 ± 2.57567            95% CI[  -1.46732,   -0.15954]
--------------------------------------------------------------------------------
R^2: 0.99844
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3APhos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Helix DG_i      3.63211 ± 4.48305            95% CI[   2.49399,    4.77023]
(s) Helix DG_ij    -6.01364 ± 0.72695            95% CI[  -6.19819,   -5.82909]
(s) Helix m_i      -0.46349 ± 0.61955            95% CI[  -0.62077,   -0.30620]
(s) Repeat DG_i     6.99113 ± 2.24650            95% CI[   6.42081,    7.56145]
(s) Repeat DG_ij   -9.99998 ± 1.59313            95% CI[ -10.40444,   -9.59553]
(s) Repeat m_i     -0.60650 ± 5.86237            95% CI[  -2.09479,    0.88179]
(s) Cap DG_i        3.14314 ± 1.33059            95% CI[   2.80534,    3.48094]
(s) Cap m_i        -0.81343 ± 2.57567            95% CI[  -1.46732,   -0.15954]
--------------------------------------------------------------------------------
R^2: 0.99816
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3SPhos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Helix DG_i      3.63211 ± 4.48305            95% CI[   2.49399,    4.77023]
(s) Helix DG_ij    -6.01364 ± 0.72695            95% CI[  -6.19819,   -5.82909]
(s) Helix m_i      -0.46349 ± 0.61955            95% CI[  -0.62077,   -0.30620]
(s) Repeat DG_i     6.99113 ± 2.24650            95% CI[   6.42081,    7.56145]
```

```
(s) Repeat DG_ij   -9.99998 ± 1.59313            95% CI[ -10.40444,   -9.59553]
(s) Repeat m_i     -0.60650 ± 5.86237            95% CI[  -2.09479,    0.88179]
(s) Cap DG_i        3.14314 ± 1.33059            95% CI[   2.80534,    3.48094]
(s) Cap m_i        -0.81343 ± 2.57567            95% CI[  -1.46732,   -0.15954]
--------------------------------------------------------------------------------
R^2: 0.99844
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3SPhos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Helix DG_i      3.63211 ± 4.48305            95% CI[   2.49399,    4.77023]
(s) Helix DG_ij    -6.01364 ± 0.72695            95% CI[  -6.19819,   -5.82909]
(s) Helix m_i      -0.46349 ± 0.61955            95% CI[  -0.62077,   -0.30620]
(s) Repeat DG_i     6.99113 ± 2.24650            95% CI[   6.42081,    7.56145]
(s) Repeat DG_ij   -9.99998 ± 1.59313            95% CI[ -10.40444,   -9.59553]
(s) Repeat m_i     -0.60650 ± 5.86237            95% CI[  -2.09479,    0.88179]
(s) Cap DG_i        3.14314 ± 1.33059            95% CI[   2.80534,    3.48094]
(s) Cap m_i        -0.81343 ± 2.57567            95% CI[  -1.46732,   -0.15954]
--------------------------------------------------------------------------------
R^2: 0.99747
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3Phos1_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Helix DG_i      3.63211 ± 4.48305            95% CI[   2.49399,    4.77023]
(s) Helix DG_ij    -6.01364 ± 0.72695            95% CI[  -6.19819,   -5.82909]
(s) Helix m_i      -0.46349 ± 0.61955            95% CI[  -0.62077,   -0.30620]
(s) Repeat DG_i     6.99113 ± 2.24650            95% CI[   6.42081,    7.56145]
(s) Repeat DG_ij   -9.99998 ± 1.59313            95% CI[ -10.40444,   -9.59553]
(s) Repeat m_i     -0.60650 ± 5.86237            95% CI[  -2.09479,    0.88179]
(s) Cap DG_i        3.14314 ± 1.33059            95% CI[   2.80534,    3.48094]
(s) Cap m_i        -0.81343 ± 2.57567            95% CI[  -1.46732,   -0.15954]
--------------------------------------------------------------------------------
R^2: 0.99655
```

```
================================================================================


================================================================================
Fitting results
================================================================================
ID: CTPR3Phos2_10C
Model: Heteropolymer Ising Model
Optimiser: scipy.optimize.differential_evolution
Temperature: 10.00°C

(s) Helix DG_i      3.63211 ± 4.48305            95% CI[   2.49399,    4.77023]
(s) Helix DG_ij    -6.01364 ± 0.72695            95% CI[  -6.19819,   -5.82909]
(s) Helix m_i      -0.46349 ± 0.61955            95% CI[  -0.62077,   -0.30620]
(s) Repeat DG_i     6.99113 ± 2.24650            95% CI[   6.42081,    7.56145]
(s) Repeat DG_ij   -9.99998 ± 1.59313            95% CI[ -10.40444,   -9.59553]
(s) Repeat m_i     -0.60650 ± 5.86237            95% CI[  -2.09479,    0.88179]
(s) Cap DG_i        3.14314 ± 1.33059            95% CI[   2.80534,    3.48094]
(s) Cap m_i        -0.81343 ± 2.57567            95% CI[  -1.46732,   -0.15954]
--------------------------------------------------------------------------------
R^2: 0.99795
================================================================================
```


Ising model domain topologies
CTPR3Phos2_10C
CTPR3Phos1_10C
CTPR3SPhos2_10C
CTPR3SPhos1_10C
CTPR3APhos2_10C
CTPR3APhos1_10C
CTPR2Phos2_10C
CTPR2Phos1_10C
CTPR2SPhos2_10C
CTPR2SPhos1_10C
CTPR2APhos2_10C
CTPR2APhos1_10C
Cap
Repeat
Helix



End of this Notebook.

---

9 SI Notebook 7 - Simulating Ising Models

[Author] ARL & ERGM

Here we are going to generate an Ising model representation of a protein and simulate the unfolding curve. There are several steps:

1. Initialise domains with their appropriate values (ΔG, m etc..)
2. Build a topology with these domains
3. Create the partition function
4. Simulate!

Let's start with loading PyFolding:

```
In [1]: %matplotlib inline
        import numpy as np
        import matplotlib.pyplot as plt

        import pyfolding
        from pyfolding import ising, models
```

```
<IPython.core.display.Javascript object>
```

```
PyFolding: Jupyter autoscrolling has been disabled
```

```
In [2]: # Lets see what modules we have by listing them
        ising.list_models()
```

```
Out[2]: ['CapDomain',
         'DecoupleCapDomain',
         'HelixDomain',
         'LoopDomain',
         'MutantCapDomain',
         'MutantLoopDomain',
         'MutantRepeatDomain',
         'RepeatDomain']
```

9.1 Initialise domains and give them their appropriate values

1st, we need some domains to build our protein. Let's use a cap domain and a repeat domain:

```
In [3]: # NOTE: we need to instantiate these domains, hence the () after their names

        cap = ising.CapDomain()
        repeat = ising.RepeatDomain()
```

2nd, we need to set the domain properties:

```
In [4]: cap.DG_i = -5.
        cap.m_i = -1.5

        repeat.DG_i = -1.
        repeat.m_i = -2.5
        repeat.DG_ij = -1.
```

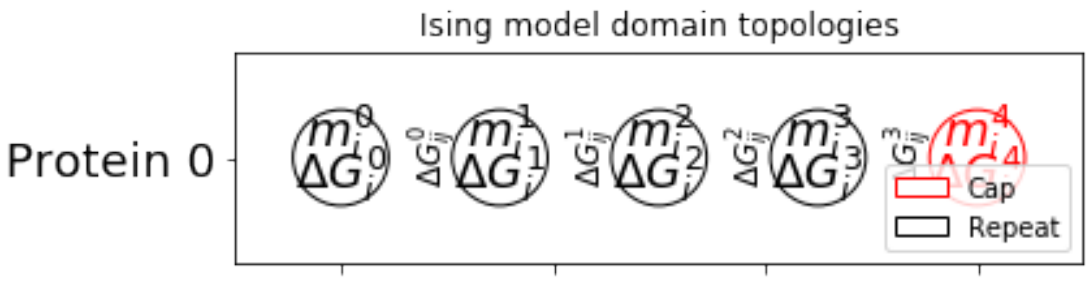
9.2 Build a topology

Having defined the properties of the domains, we need to design a protein topology to represent the protein we're simulating. Let's use a cap and three repeats to define our simulated protein:

```
In [5]: topology = [repeat, repeat, repeat, repeat, cap]
```

Let's plot our topology to make sure we've done everything correctly:

```
In [6]: # NOTE: You can now save the domain image by using the save keyword:
        # ising.plot_domains([topology], save="/Users/ubcg83a/Desktop/domains.pdf")

        ising.plot_domains([topology], collapse=False) #, save="/Users/ERGM/Desktop/domains.pdf"
```



9.3 Generate partition function

Having defined our protein topology, we can generate the partition function to go along with this:

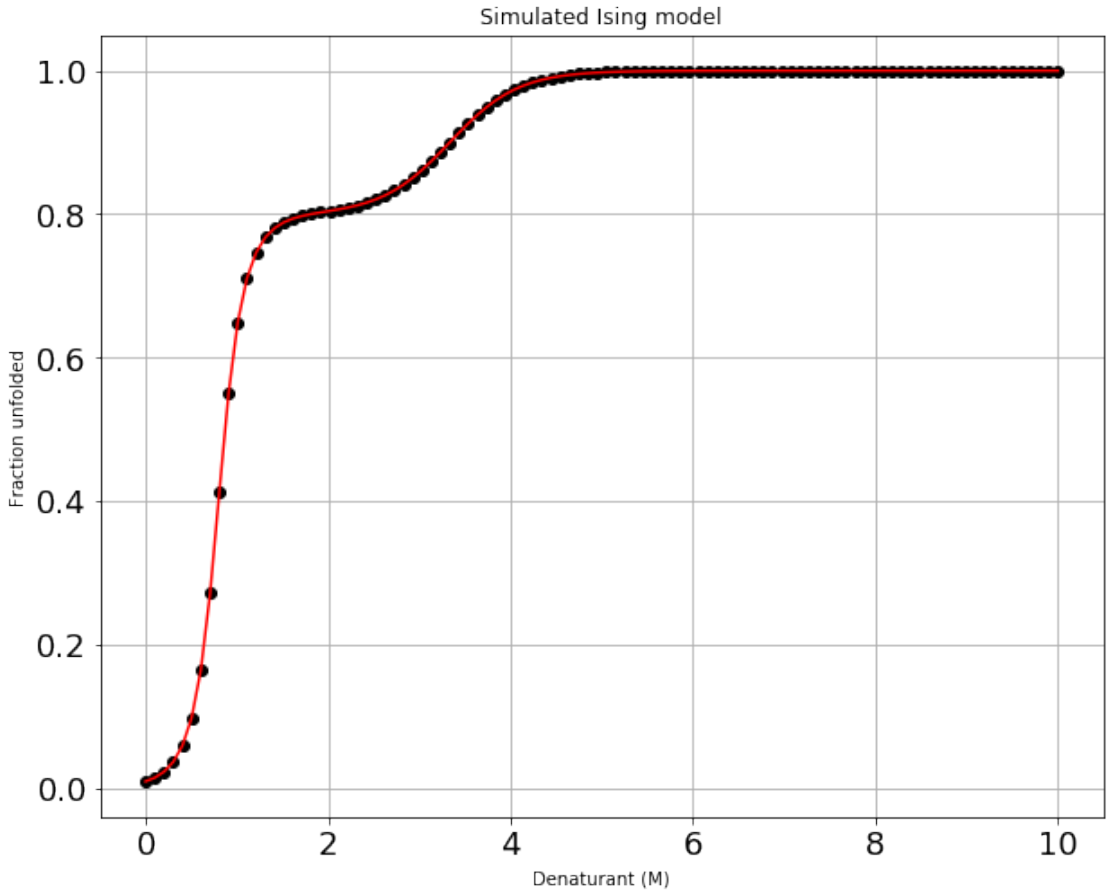
```
In [7]: partition = ising.IsingPartitionFunction( topology )
        pyfolding.set_temperature(10.)
```

```
Set temperature to 10.00°C
(NOTE: Careful, this sets the temperature for all subsequent calculations)
```

9.4 Simulate

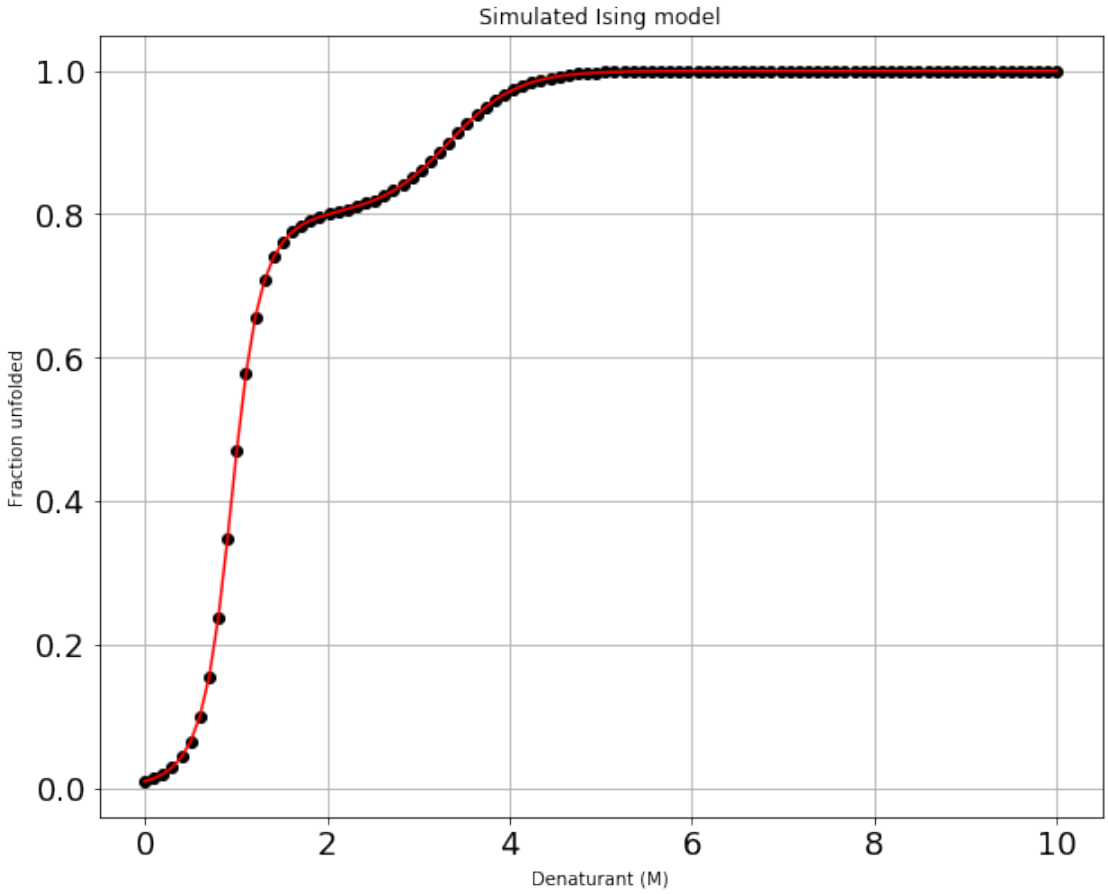
Now we can simulate our protein unfolding over a certain concentration range:

```
In [8]: x = np.linspace(0.,10.,100) # range of denaturants, 0-10M with 100 points (i.e. every 0.
        y = partition.theta(x)

        plt.figure(figsize=(10,8))
        plt.plot(x,y,'ko', x,y,'r-')
        plt.title("Simulated Ising model")
        plt.xlabel('Denaturant (M)')
        plt.ylabel('Fraction unfolded')
        plt.grid(True)
        plt.show()
```



Try changing a value of the model! Let's set m_i for the repeat to -2.1:

```
In [9]: repeat.m_i = -2.1
        y = partition.theta(x)

        plt.figure(figsize=(10,8))
        plt.plot(x,y,'ko', x,y,'r-')
        plt.title("Simulated Ising model")
        plt.xlabel('Denaturant (M)')
        plt.ylabel('Fraction unfolded')
        plt.grid(True)
        plt.show()
```



End of this Notebook.

---

10 SI Notebook 8 - Global fit Equilibrium Curves from a dimeric protein to dimeric equilibrium models

[Authors] ERGM & ARL

In this notebook we will show how equilbrium folding data from dimeric proteins can be imported into a notebook and fitted to dimeric Equilibrium denaturation models using global fits over multiple datasets.

If you are less script/computer orientated, you can simply change the data paths and other parameters for your proteins and re-run the jupyter notebook ("Kernal/Restart & Run all" from the menu above).

10.1 Data Format

Please see PyFolding SI Notebooks 1 and 2 for the format your data has to be in to enable this type of analysis. Remember for Ising Model Analysis here each protein dataset (equilibrium denaturation curve) must have its own .csv

---

First off lets load pyfolding & pyplot into this ipython notebook (pyplot allows us to plot more complex figures of our results):

```
In [1]: # use this command to tell Jupyter to plot figures inline with the text
        %matplotlib inline

        # import pyfolding, the pyfolding models and ising models
        import pyfolding
        from pyfolding import *

        # import the package for plotting, call it plt
        import matplotlib.pyplot as plt

        # import numpy as well
        import numpy as np
```

```
<IPython.core.display.Javascript object>
```

```
PyFolding: Jupyter autoscrolling has been disabled
```

Now, we need to load some data to analyse. I will import the equilibrium denaturations of dimeric tr-LcrH at a series of protein concentrations from:

Singh S.K., Boyle A.L. & Main E.R. (2013) ‘‘LcrH, a class II chaperone from the type three secretion system, has a highly flexible native structure.’’ J Biol Chem., 288, (6) 4048-55.

I will load 4 denaturation curves that correspond tr-LcrH at the following protein concentrations:

Protein	Filename	Total Protein Concentration
tr-LcrH	50uM_1.csv	50 uM
tr-LcrH	50uM_2.csv	50 uM
tr-LcrH	80uM_1.csv	80 uM
tr-LcrH	80uM_2.csv	80 uM

10.2 Import Data, assign names and put into a list

In [2]:
```
# we will load all of the data together, as follows:

# arguments are "path", "filename"
pth = "../examples/LcrH"

# this is a set of commands to automate loading the data
# for each denaturation
fn = ["50uM_1.csv","50uM_2.csv","80uM_1.csv","80uM_2.csv"]

# Here we are loading all the curves in a list called "proteins"
# and assigning them names
proteins = [pyfolding.read_equilibrium_data(pth,f) for f in fn]

# also store the total protein conentration for each denaturation
Pt = [50e-6, 50e-6, 80e-6, 80e-6]
```

10.3 Set Temperature

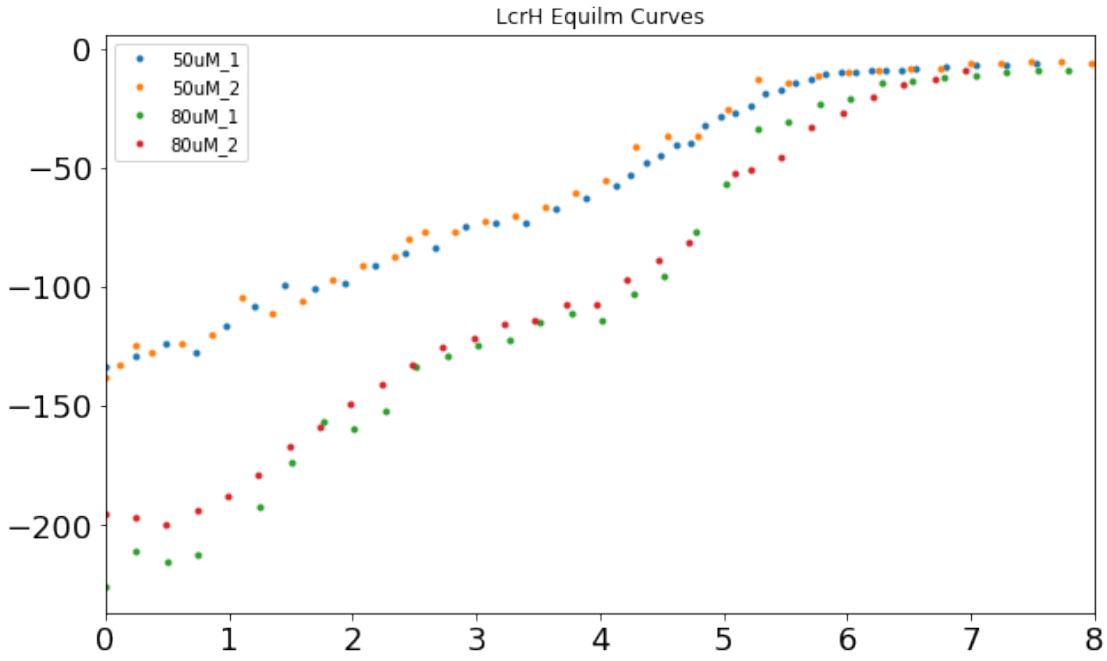
In [3]:
```
# Set temperature to 25.00°C
# (NOTE: Careful, this sets the temperature for all subsequent calculations)
pyfolding.set_temperature(25.)
```

```
Set temperature to 25.00°C
(NOTE: Careful, this sets the temperature for all subsequent calculations)
```

10.4 Plot Data

In [4]:
```
# the following commands plot all the data curves on one plot
```

```
plt.figure(figsize=(10,6))
for c in proteins:
    plt.plot(c.x, c.y, '.')

# the following commands plot all the data curves on one plot,
# where "loc" command determines where the legend goes.
plt.legend([c.ID for c in proteins], loc='best')
plt.title("LcrH Equilm Curves")
plt.xlim([0, 8])                # x axis from 0 to 8
plt.show()
```



In [5]:
```
# Command imports pyfolding models
from pyfolding.models import *

# command lists models
list_models()

# After the model name:
#'Verified: True' model has been rigourously and it functions as expected.
#'Verified:False' signifies that the model has not been rigourously tested.
```

Out[5]:
```
[('ChevronPolynomialFit', 'Verified: True'),
 ('HeteropolymerIsingEquilibrium', 'Verified: False'),
 ('HomozipperIsingEquilibrium', 'Verified: True'),
 ('ParallelTwoStateChevron', 'Verified: False'),
```

```
('ParallelTwoStateUnfoldingChevron', 'Verified: False'),
('TemplateModel', 'Verified: False'),
('ThreeStateChevron', 'Verified: True'),
('ThreeStateDimericIEquilibrium', 'Verified: True'),
('ThreeStateEquilibrium', 'Verified: True'),
('ThreeStateFastPhaseChevron', 'Verified: True'),
('ThreeStateMonoIEquilibrium', 'Verified: True'),
('ThreeStateSequentialChevron', 'Verified: True'),
('TwoStateChevron', 'Verified: True'),
('TwoStateChevronMovingTransition', 'Verified: True'),
('TwoStateDimerEquilibrium', 'Verified: True'),
('TwoStateEquilibrium', 'Verified: True'),
('TwoStateEquilibriumSloping', 'Verified: True')]
```

10.5 Fit Data to 3 state denaturation that unfolds via dimeric I

In [6]:
```
# We are going to fit this data to 3 state denaturation that unfolds via
# a dimeric intermediate (as per the J.B.C. paper)
# Lets print the equation & Info

models.ThreeStateDimericIEquilibrium().info()
```

$$Y_{rel} = Y_N F_N + Y_I F_I + Y_D F_D$$

expanded:

$$Y_{rel} = Y_N \cdot \frac{2PtF_D^2}{K_1K_2} + Y_I \frac{2PtF_D^2}{K_2} + Y_D * (F_D)$$

where:

$$F_D = \frac{-K_1K_2 + \sqrt{((K_1K_2)^2 + 8(1+K_1)(K_1K_2)Pt)}}{4Pt(1+K_1)} \tag{5}$$

$$K_1 = \exp \frac{-\Delta G^1_{H_20} + m_1 x}{RT}$$

$$K_2 = \exp \frac{-\Delta G^2_{H_20} + m_2 x}{RT}$$

```
Three State model for a dimer denaturation Equilibrium - Dimeric Intermediate.

    Folding Scheme:
            N2 <-> I2 <-> 2D

    Params:
            Y_rel = spectroscopic signal at a given concentration of urea
            Y_N = spectroscopic signal for native state
            Y_D = spectroscopic signal for denatured state
            Y_I = spectroscopic signal for intermediate state
            F_D = fraction denatured monomers
```

```
        F_N = fraction native dimers
        F_I = fraction intermediate dimers
        Pt = total protein concentration. This variable needs to be set per denaturation
        K1 = equilibrium contstant of unfolding native to intermediate state
        K2 = equilibrium contstant of unfolding intermediate to denatured state
        DG1 = stability of native state relative to intermediate state
        m1 = m-value of native to intermediate transition
        DG2 = stability of intermediate state relative to denatured state
        m2 = m-value of intermediate to denatured transition
        x = denaturant concentration (M)
        R = Universal Gas Constant (kcal.mol-1.K-1)
        T = Temperature (Kelvin)

    Reference:
        Mallam and Jackson. Folding studies on a knotted protein.
        Journal of Molecular Biology (2005) vol. 346 (5) pp. 1409-1421
```

10.6 Automatic global fitting to the Three State Dimeric Intermediate Equilibrium model

We could fit individual denaturations to this model. However, one aspect of the model is that it should be able to fit globally to a number of denaturations. We will try to fit this with the Three State Dimeric Intermediate Equilibrium model

---

```
In [7]: global_fit = core.GlobalFit()
        global_fit.fit_funcs = [models.ThreeStateDimericIEquilibrium
                                for i in xrange(len(proteins))]
        global_fit.constants = [(('Pt',c),) for c in Pt]
        global_fit.shared = ['DG1','DG2','m1','m2'] #
        global_fit.x = [p.x for p in proteins]
        global_fit.y = [p.y for p in proteins]
        global_fit.ID = [p.ID for p in proteins]

        global_fit.initialise()

In [8]: print global_fit.params.keys(), len(global_fit.params.keys())

['DG2', 'DG1', 'm1', 'm2', 'Y_N_{50uM_1}', 'Y_I_{50uM_1}', 'Y_D_{50uM_1}', 'Y_N_{50uM_2}', 'Y_I_


In [9]: # this commands gives our initial parameters for fitting
        p0 = [5.,1.7,1.,1.7,
              -200,-50,-10,
```

```
              -200,-50,-10,
              -200,-50,-10,
              -200,-100,-10]

        # this command gives fitting constraints
        b = bounds=((0.,0.,-3.,-3.,
                     -300,-300,-300,
                     -300,-300,-300,
                     -300,-300,-300,
                     -300,-300,-300),
                    (20.,20.,3.,3.,
                     0,0,0,
                     0,0,0,
                     0,0,0,
                     0,0,0))

        # this commands checks that our inputs for the initial parameters and
        # constraints (bounds) above
        # match the number of our variables
        print len(b[0]), len(b[1]), len(p0)

        #this commands tell pyfolding to fit our data
        out, covar = global_fit.fit( p0=p0, bounds=b )

        # print out the results of the fit
        for result in global_fit.results:
            result.display()
```

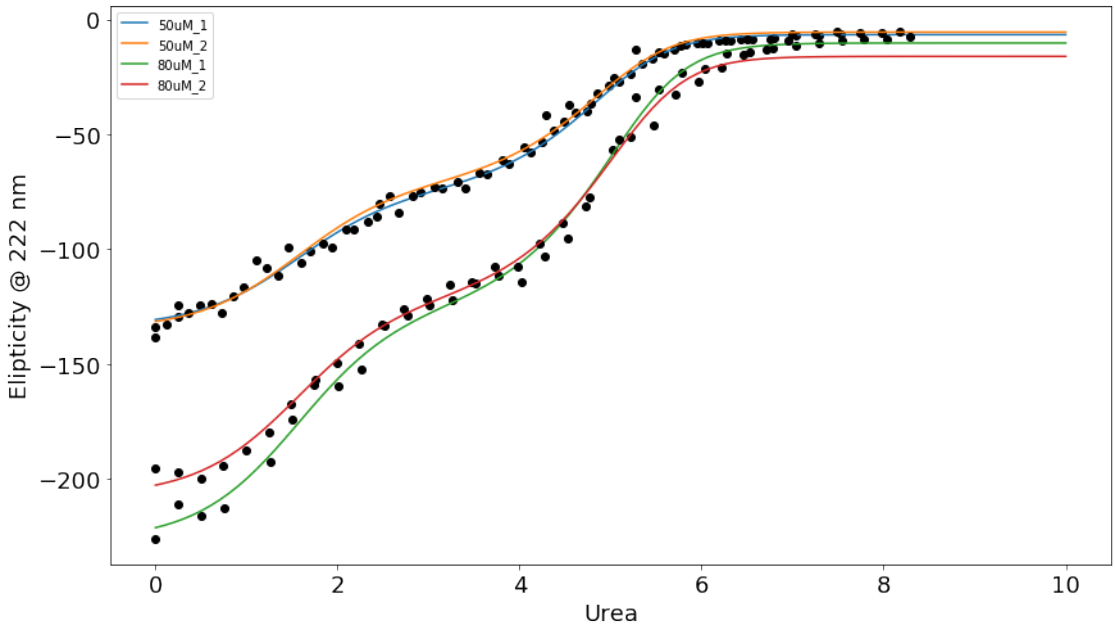
```
16 16 16
================================================================================
Fitting results
================================================================================
ID: 50uM_1
Model: ThreeStateDimericIEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(s) DG1    1.66962 ± 0.72748            95% CI[   1.48549,    1.85376]
(s) m1     1.07181 ± 0.44714            95% CI[   0.95863,    1.18498]
(s) DG2   13.91139 ± 2.41006            95% CI[  13.30137,   14.52140]
(s) m2     1.71687 ± 0.45270            95% CI[   1.60228,    1.83145]
(f) Y_N -133.91534 ± 10.10650           95% CI[-136.47341, -131.35727]
(f) Y_I  -75.49283 ± 11.70917           95% CI[ -78.45656,  -72.52911]
(f) Y_D   -6.57639 ± 3.97871            95% CI[  -7.58345,   -5.56934]
(c) Pt     0.00005
--------------------------------------------------------------------------------
R^2: 0.99661
================================================================================
```

```
================================================================================
Fitting results
================================================================================
ID: 50uM_2
Model: ThreeStateDimericIEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(s) DG1    1.66962 ± 0.72748            95% CI[   1.48549,    1.85376]
(s) m1     1.07181 ± 0.44714            95% CI[   0.95863,    1.18498]
(s) DG2   13.91139 ± 2.41006            95% CI[  13.30137,   14.52140]
(s) m2     1.71687 ± 0.45270            95% CI[   1.60228,    1.83145]
(f) Y_N -135.06375 ± 9.56663            95% CI[-137.48517, -132.64233]
(f) Y_I  -72.07005 ± 11.55312           95% CI[ -74.99427,  -69.14582]
(f) Y_D   -5.47739 ± 4.07509            95% CI[  -6.50884,   -4.44594]
(c) Pt     0.00005
--------------------------------------------------------------------------------
R^2: 0.99375
================================================================================


================================================================================
Fitting results
================================================================================
ID: 80uM_1
Model: ThreeStateDimericIEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(s) DG1    1.66962 ± 0.72748            95% CI[   1.48549,    1.85376]
(s) m1     1.07181 ± 0.44714            95% CI[   0.95863,    1.18498]
(s) DG2   13.91139 ± 2.41006            95% CI[  13.30137,   14.52140]
(s) m2     1.71687 ± 0.45270            95% CI[   1.60228,    1.83145]
(f) Y_N -226.85519 ± 13.79841           95% CI[-230.34773, -223.36265]
(f) Y_I -126.94931 ± 16.17772           95% CI[-131.04407, -122.85454]
(f) Y_D  -10.23378 ± 4.60544            95% CI[ -11.39946,   -9.06809]
(c) Pt     0.00008
--------------------------------------------------------------------------------
R^2: 0.99589
================================================================================


================================================================================
Fitting results
================================================================================
ID: 80uM_2
```

```
Model: ThreeStateDimericIEquilibrium
Optimiser: pyfolding.GlobalFit and scipy.optimize.curve_fit
Temperature: 25.00°C

(s) DG1    1.66962 ± 0.72748            95% CI[   1.48549,    1.85376]
(s) m1     1.07181 ± 0.44714            95% CI[   0.95863,    1.18498]
(s) DG2   13.91139 ± 2.41006            95% CI[  13.30137,   14.52140]
(s) m2     1.71687 ± 0.45270            95% CI[   1.60228,    1.83145]
(f) Y_N -207.47639 ± 12.61417           95% CI[-210.66918, -204.28360]
(f) Y_I -122.90586 ± 14.63107           95% CI[-126.60915, -119.20257]
(f) Y_D  -16.04299 ± 6.40061            95% CI[ -17.66305,  -14.42292]
(c) Pt     0.00008
--------------------------------------------------------------------------------
R^2: 0.99668
================================================================================
```

10.7 Plot the fitted data

```
In [10]: results = global_fit.results
         plt.figure(figsize=(14,8))
         for i, p in enumerate(proteins):
             plt.plot(p.x, p.y, 'ko')
             plt.plot(results[i].x_fit, results[i].y_fit, '-', label=p.ID)
         plt.legend()
         plt.xlabel(p.denaturant_label, fontsize=constants.FONT_SIZE)
         plt.ylabel('Elipticity @ 222 nm', fontsize=constants.FONT_SIZE)
         plt.show()
```

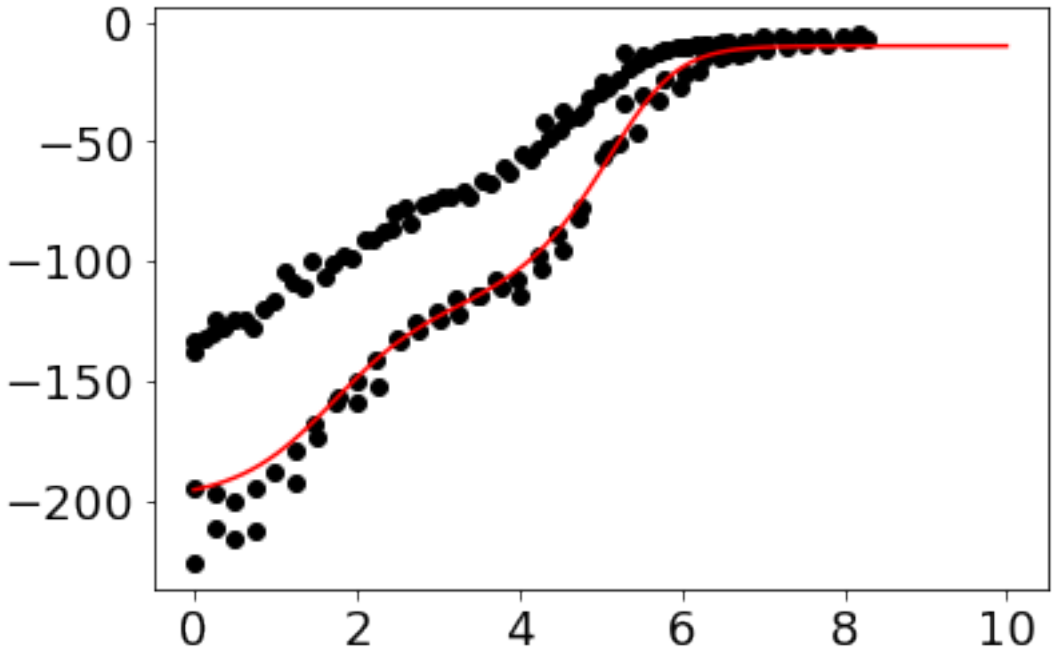


10.8 Simulate Data

This is also a good way to work out what initial parameters might work well

```
In [12]: #Lets first choose some values for simulation:
         DG1 = 1.7
         DG2 = 14.
         m1 = 1.
         m2 = 1.7
         Y_N = -200
         Y_I = -120
         Y_D = -10
         Pt = 80e-6

         # Then lets simulate what curve we would obtain with these values:
         x = np.linspace(0.,10.,100)
         m = models.ThreeStateDimericIEquilibrium()
         y = m(x, DG1, m1, DG2, m2, Y_N, Y_I, Y_D, Pt)

         #Then lets plot the simulation against the data we have:
         plt.figure()
         for i, p in enumerate(proteins):
             plt.plot(p.x, p.y, 'ko')
         plt.plot(x,y,'r-')
```

```
plt.show()
print y[0]
```




```
-195.679350161
```

End of this Notebook.
