ANALYSIS OF KINETIC MODELS OF GANKYRIN FOLDING

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1. PyFolding analysis

Fitting and testing of different kinetic models was performed using PyFolding.

1.1. **Downloading and installing PyFolding.** PyFolding is free, open source, Python based analysis tool for comparing different kinetic and thermodynamic models of protein folding. Input data in the form of chevron plots or equilbrium denaturation curves can be supplied as comma separated value (.CSV) files. PyFolding requires Numpy, Scipy and Matplotlib, and can be downloaded using:

```
git clone https://github.com/quantumjot/PyFolding.git
```

Fitting is performed using a Levenberg-Marquardt non-linear least squares optimisation, using the Scipy `optimize.curve_fit` algorithm[1].

1.2. **Error calculation.** Fit errors are calculated from the estimated covariance matrix of the parameters, following optimisation, and reported as the standard error of the mean of N samples:

$$X_{\text{Error}} = \frac{\sqrt{\text{Cov}[X, X]}}{\sqrt{N}} \tag{1}$$

1.3. **Models.** Currently, PyFolding supports the following models:

- Two-state equilibrium unfolding
- Three-state equilibrium unfolding
- Homozipper Ising model for equilibrium unfolding
- Two-state chevron
- Two-state with moving transition state chevron
- Three-state chevron with fast pre-equilibrium chevron
- Three-state chevron with fast phase chevron
- Three-state sequential barriers chevron
- Parallel two-state chevrons

[1] `http://docs.scipy.org/doc/scipy/reference/generated/scipy.optimize.curve_fit.html`

1.4. **Usage.** PyFolding can be used as such:

```
import folding
import models

# load the kinetic and equilibrium folding data
chevron = folding.read_kinetic_data("WT_chevron.csv")
equilibrium = folding.read_equilibrium_data("WT_equilibrium.csv")

# fit the equilibrium data to a two-state model
equilibrium.fit_func = models.TwoStateEquilibrium
equilibrium.fit()

# print the m-value and transition midpoint
print equilibrium.m_value
print equilibrium.midpoint

# print the stability
print equilibrium.deltaG

# use the midpoint (D_50) of the equilibrium curve as the kinetic midpoint
chevron.midpoint = equilibrium.midpoint

# now fit the chevron to a two-state model
chevron.fit_func = models.TwoStateChevron
chevon.fit()

# plot the output
folding.plot_figure(equilibrium, chevron)
```

2. Equilibrium models

2.1. **Two-state equilibrium unfolding.** Two-state equilibrium unfolding with sloping baselines:

$$\theta(x) = \frac{(\alpha_f + \beta_f \cdot x) + (\alpha_u + \beta_u \cdot x) \cdot (\exp(m \cdot (x - D_{50\%}))/RT)}{1. + \exp(m \cdot (x - D_{50\%}))/RT} \tag{2}$$

2.2. **Homozipper Ising model.** A simple homozipper Ising model for equilibrium unfolding:

$$\begin{aligned} \kappa(x) &= \exp(-(\Delta G_{intrinsic} - m_{intrinsic} \cdot x)/RT) \\ \tau(x) &= \exp(-(\Delta G_{interface} - m_{interface} \cdot x)/RT) \\ \theta &= \frac{\kappa}{n(\kappa\tau - 1)} \cdot \frac{n(\kappa\tau)^{n+2} - (n+2)(\kappa\tau)^{n+1} + (n+2)\kappa\tau - n}{(\kappa\tau - 1)^2 + \kappa((\kappa\tau)^{n+1} - (n+1)\kappa\tau + n)} \end{aligned} \tag{3}$$

3. Kinetic models

3.1. **Two-state chevron.** A simple two-state folding reaction with unfolded (U) and native (N) states:

$$\mathrm{U} \underset{k_u}{\overset{k_f}{\rightleftharpoons}} \mathrm{N} \tag{4}$$

$$\begin{aligned} k_f &= k_f^{(H_2O)} \cdot \exp(-m_f \cdot x) \\ k_u &= k_u^{(H_2O)} \cdot \exp(m_u \cdot x) \\ k_{obs} &= k_f + k_u \end{aligned} \tag{5}$$

3.2. **Two-state chevron with moving transition state.** A simple two-state folding reaction, but with a moving transition state:

$$\mathrm{U} \underset{k_u}{\overset{k_f}{\rightleftharpoons}} \mathrm{N} \tag{6}$$

$$\begin{aligned} k_f &= k_f^{(H_2O)} + \exp(-m_f \cdot x) + \exp(-m' \cdot x^2) \\ k_u &= k_u^{(H_2O)} + \exp(m_u \cdot x) + \exp(m' \cdot x^2) \\ k_{obs} &= k_f + k_u \end{aligned} \tag{7}$$

3.3. **Three-state chevron.** A simple three-state folding reaction with an on pathway intermediate state (I):

$$\mathrm{U} \underset{k_{iu}}{\overset{k_{ui}}{\rightleftharpoons}} \mathrm{I} \underset{k_{ni}}{\overset{k_{in}}{\rightleftharpoons}} \mathrm{N} \tag{8}$$

$$\begin{aligned} k_{ni} &= k_{ni}^{(H_2O)} \cdot \exp(-m_{in} \cdot x) \\ k_{in} &= k_{in}^{(H_2O)} \cdot \exp((m_i - m_{in}) \cdot x) \\ K_{iu} &= K_{iu}^{(H_2O)} \cdot \exp((m_u - m_i) \cdot x) \\ k_{obs} &= k_{ni} + k_{in}/(1 + 1/K_{iu}) \end{aligned} \tag{9}$$

Alternatively, the equilbrium constant for the intermediate, K_{iu}, can be expressed in terms of the microscopic rate constants for a fast phase:

$$\begin{aligned} k_{ui} &= k_{ui}^{(H_2O)} \cdot \exp(-m_{ui} \cdot x) \\ k_{iu} &= k_{iu}^{(H_2O)} \cdot \exp(m_{iu} \cdot x) \\ K_{iu} &= k_{iu}/(k_{iu} + k_{ui}) \end{aligned} \tag{10}$$

3.4. **Three-state chevron with metastable intermediate (I^*).**

$$\mathrm{U} \underset{k_{iu}}{\overset{k_{ui}}{\rightleftharpoons}} \mathrm{I^*} \underset{k_{ni}}{\overset{k_{in}}{\rightleftharpoons}} \mathrm{N} \tag{11}$$

$$\begin{aligned} \lambda_1 &= -(k_{ui} + k_{iu} + k_{in} + k_{ni}) \\ \lambda_2 &= k_{ui} \cdot (k_{in} + k_{ni}) + k_{iu} \cdot k_{ui} \\ k_{obs} &= \frac{1}{2} \cdot \left(-\lambda_2 \pm \sqrt{\lambda_2^2 - 4 \cdot \lambda_1} \right) \end{aligned} \tag{12}$$

with:

$$\begin{aligned} k_{obs}^{TS1} &= k_{ui} + (k_{ni}/k_{in}) \cdot k_{iu} \\ k_{obs}^{TS2} &= (k_{ui}/k_{iu}) \cdot k_{in} + k_{ni} \end{aligned} \tag{13}$$

3.5. **Parallel two-state chevrons.** Two parallel two-state chevrons (see section 3.1):

$$\begin{gathered} \mathrm{U} \underset{k_u^A}{\overset{k_f^A}{\rightleftharpoons}} \mathrm{N} \\ \mathrm{U} \underset{k_u^B}{\overset{k_f^B}{\rightleftharpoons}} \mathrm{N} \end{gathered} \tag{14}$$

$$\begin{aligned} k_f^A &= k_f^{A\,(H_2O)} \cdot \exp(-m_f^A \cdot x) \\ k_u^A &= k_u^{A\,(H_2O)} \cdot \exp(m_u^A \cdot x) \\ k_f^B &= k_f^{B\,(H_2O)} \cdot \exp(-m_f^B \cdot x) \end{aligned} \tag{15}$$

where, the unfolding of the second pathway is determined by the the parameters of the first pathway, in order to prevent hysteresis:

$$\begin{aligned} k_u^{B\,(H_2O)} &= (k_f^{B\,(H_2O)} \cdot k_u^{A\,(H_2O)})/k_f^{A\,(H_2O)} \\ m_u^B &= m_f^A + m_u^A - m_f^B \\ k_u^B &= k_u^{B\,(H_2O)} \cdot \exp(m_u^B \cdot x) \\ \lambda_A &= k_f^A + k_u^A \\ \lambda_B &= k_f^B + k_u^B \\ k_{obs} &= \lambda_A + \lambda_B \end{aligned} \tag{16}$$

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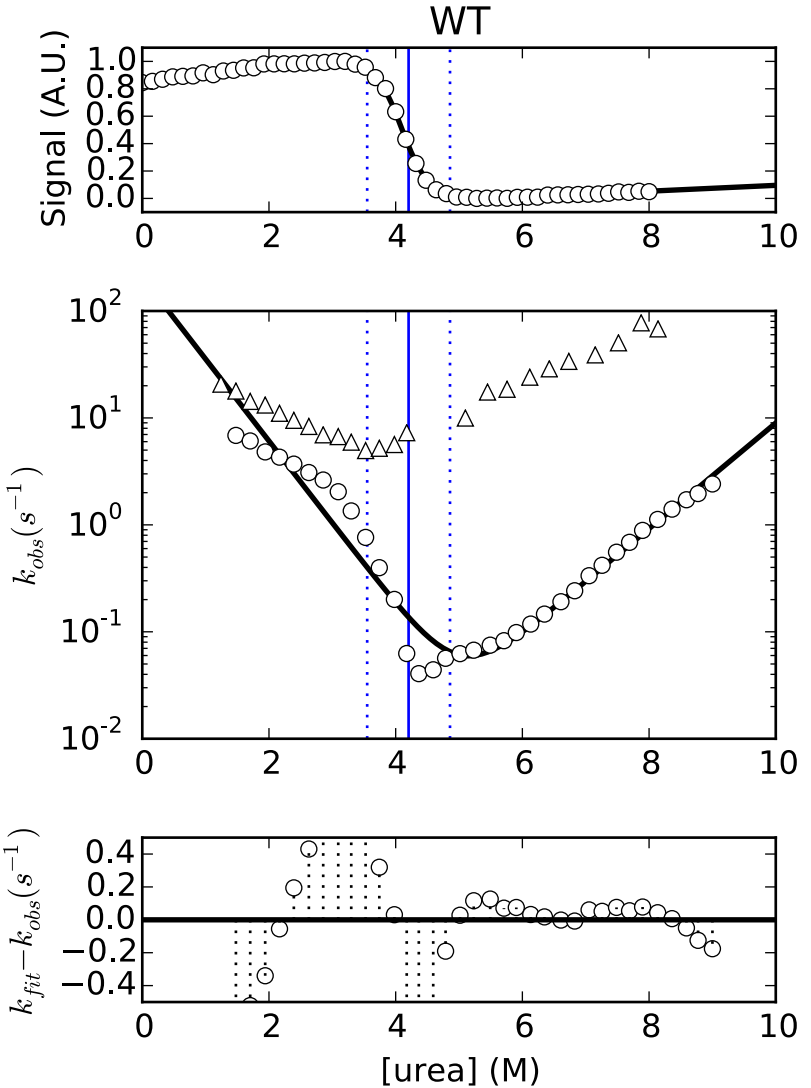
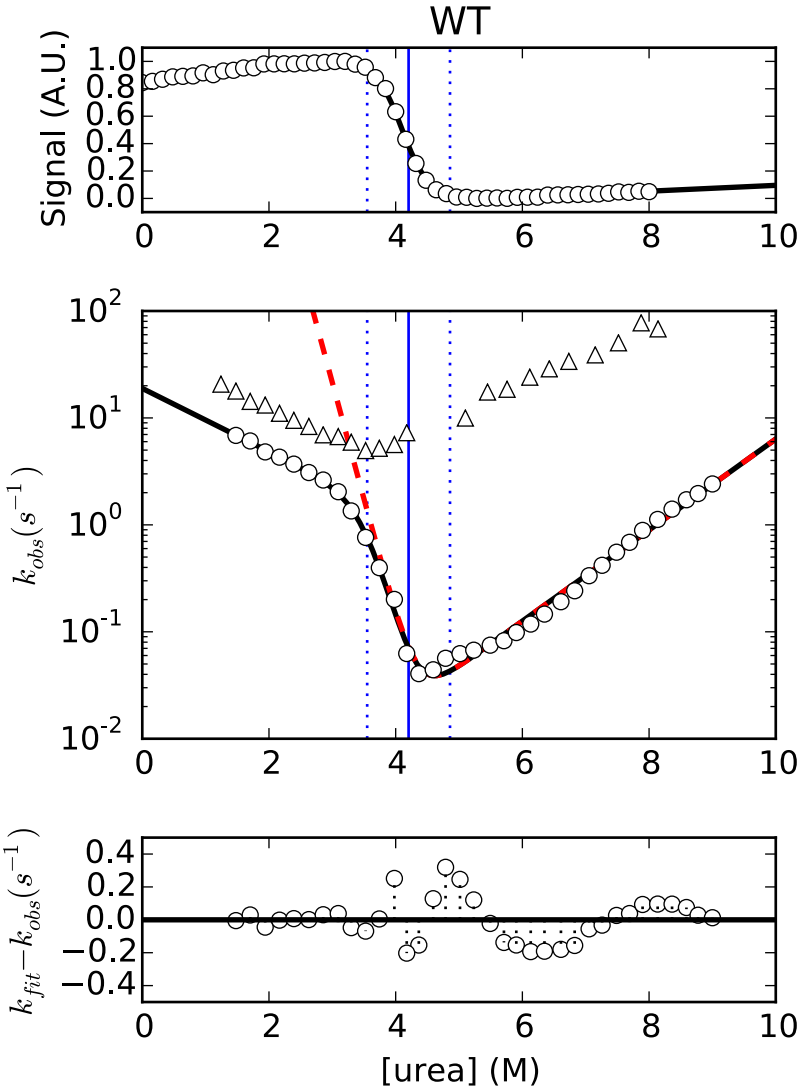
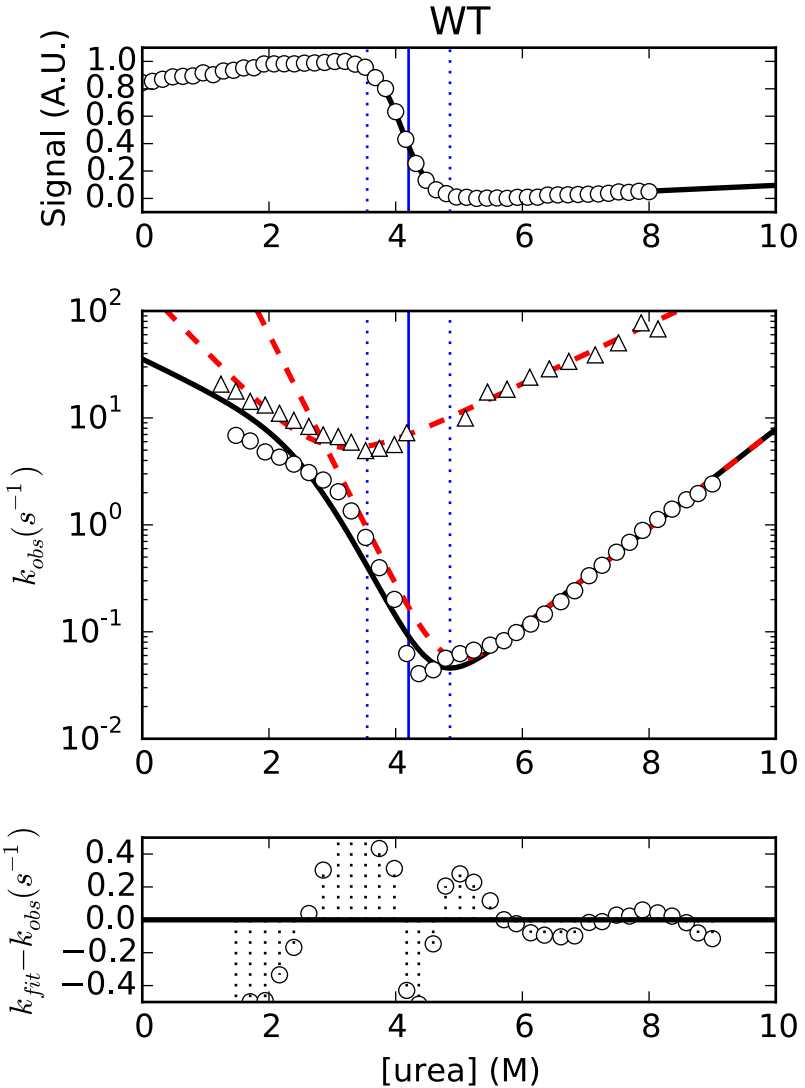


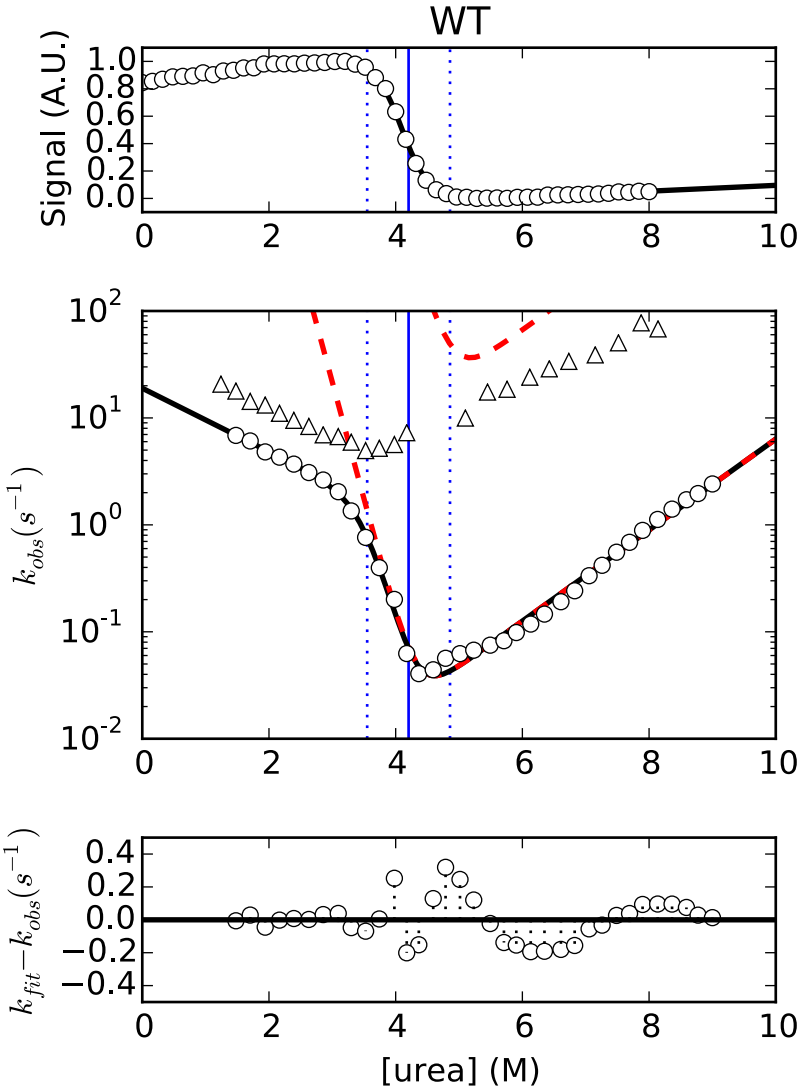
Data-set: WT
Model: TwoStateChevron
Folding midpoint 4.20 M
Fit Standard Error: 0.07
Fitting parameters:
kf: 2.07e+02 ± 1.30e+01
mf: 1.76e+00 ± 2.00e-02
ku: 1.03e-04 ± 1.26e-05
mu: 1.14e+00 ± 1.66e-02
R^2 : 0.94



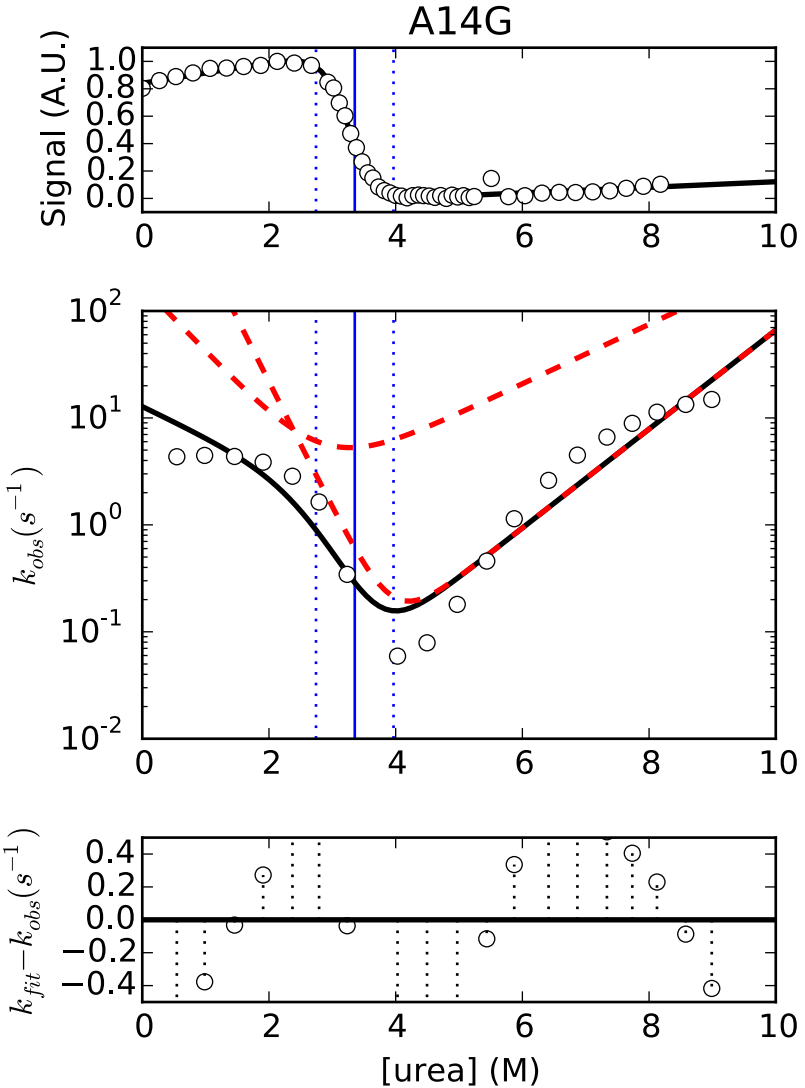
Data-set: WT
Model: ThreeStateChevron
Folding midpoint 4.20 M
Fit Standard Error: 0.02
Fitting parameters:
kfi: 3.52e-04 ± 1.01e-05
mif: -9.80e-01 ± 4.06e-03
kif: 7.51e+07 ± 2.19e+07
mi: -6.00e+00 ± 7.16e-02
Kiu: 2.52e-07 ± 6.83e-08
mu: -1.66e+00 ± 1.84e-02
R^2 : 0.99



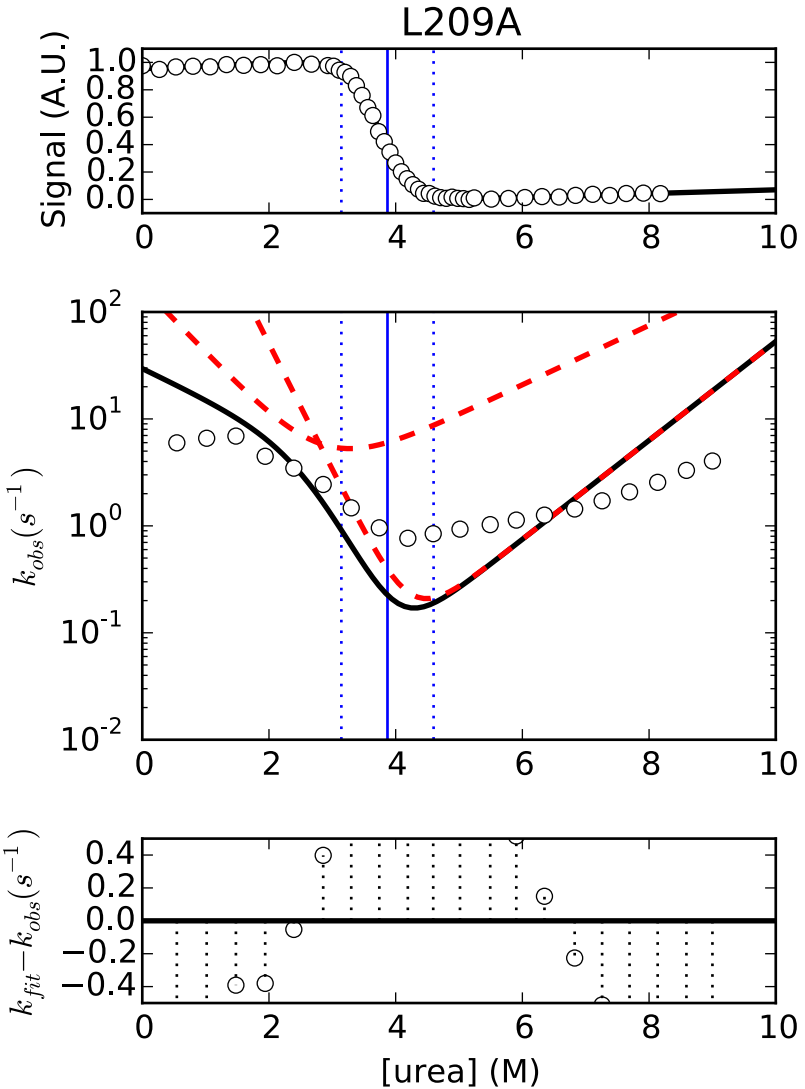
Data-set: WT
Model: ThreeStateFastPhaseChevron
Folding midpoint 4.20 M
Fit Standard Error: 0.05
Fitting parameters:
kui: 1.72e+02 ± inf
mui: 1.42e+00 ± inf
kiu: 4.45e-01 ± inf
miu: 6.41e-01 ± inf
kif: 1.38e+04 ± inf
mif: -2.71e+00 ± inf
kfi: 1.85e-04 ± inf
mfi: 1.07e+00 ± inf
R^2 : 0.97



Data-set: WT
Model: ThreeStateSequentialChevron
Folding midpoint 4.20 M
Fit Standard Error: 0.02
Fitting parameters:
kui: 4.11e+10 ± 1.08e+10
mui: 4.35e+00 ± 6.20e-02
kif: 1.90e+01 ± 7.44e-01
mif: 6.83e-01 ± 1.76e-02
kfi: 3.53e-04 ± 1.00e-05
mfi: 9.80e-01 ± 4.04e-03
R^2 : 0.99



Data-set: A14G
Model: ThreeStateFastPhaseChevron
Folding midpoint 3.35 M
Fit Standard Error: 0.12
Fitting parameters:
kui: 1.72e+02 ± inf
mui: 1.42e+00 ± inf
kiu: 4.45e-01 ± inf
miu: 6.41e-01 ± inf
kif: 4.96e+03 ± inf
mif: -2.71e+00 ± inf
kfi: 1.57e-03 ± inf
mfi: 1.07e+00 ± inf
R^2 : 0.90



Data-set: L209A
Model: ThreeStateFastPhaseChevron
Folding midpoint 3.87 M
Fit Standard Error: 0.21
Fitting parameters:
kui: 1.72e+02 ± inf
mui: 1.42e+00 ± inf
kiu: 4.45e-01 ± inf
miu: 6.41e-01 ± inf
kif: 1.15e+04 ± inf
mif: -2.71e+00 ± inf
kfi: 1.25e-03 ± inf
mfi: 1.07e+00 ± inf
R^2 : -0.79