# Supporting Information

**Bacteriorhodopsin folds through a poorly organized transition state**

Jonathan P. Schlebach, Nicholas B. Woodall, James U. Bowie, and Chiwook Park

## Appendices

Derivation of Eq. 2.

Proof that $\lambda_1 > k_h$ and $\lambda_2 < k_h$.

## Supplementary Figures

# Appendices

*Derivation of Eq. 2.* When a system is comprised of three species interconverting through unimolecular reactions, the relaxation of the system proceeds with two macroscopic rate constants ($\lambda_1$ and $\lambda_2$), which are solutions of the following equation:

$$\begin{vmatrix} \lambda-(k_{12}+k_{13}) & k_{21} & k_{31} \\ k_{12} & \lambda-(k_{21}+k_{23}) & k_{32} \\ k_{13} & k_{23} & \lambda-(k_{31}+k_{32}) \end{vmatrix} = 0 . \quad \text{(S1)}$$

In Eq. S1, $k_{ij}$ is the microscopic rate constant for the conversion from the i-th species to the j-th species. The kinetic mechanism of bR unfolding and refolding involves three species ($bR_F$, $bR_U$, and bO) with three kinetic constants ($k_f$, $k_u$, and $k_h$). By replacing $k_{12}$, $k_{21}$, and $k_{23}$ with $k_u$, $k_f$, and $k_h$, respectively, and set other kinetic constants to 0, Eq. S1 is applied to the kinetic mechanism of bR unfolding and folding.

$$\begin{vmatrix} \lambda-k_u & k_f & 0 \\ k_u & \lambda-(k_f+k_h) & 0 \\ 0 & k_h & \lambda \end{vmatrix} = 0 , \quad \text{(S2)}$$

Eq. S2 can be arranged into a quadratic equation:

$$\lambda^2-(k_f+k_u+k_h)\lambda+k_uk_h=0 \quad (2)$$

*Proof that $\lambda_1 > k_h$ and $\lambda_2 < k_h$.* A quadratic function $f(\lambda)$ can be defined using Eq. 2:

$$f(\lambda) = \lambda^2 - (k_f + k_u + k_h)\lambda + k_u k_h$$

When $\lambda = k_h$,

$$f(k_h) = {k_h}^2 - (k_f + k_u + k_h)k_h + k_u k_h = -k_f k_h \quad \text{(S3)}$$

Because $k_f$ and $k_h$ have non-zero positive values, the left side of Eq. S3 should have a negative value, which indicates that $f(k_h) < 0$. Because the graph of the function has an upward curvature, $k_h$ is between the two roots of the equation $f(\lambda) = 0$. As $\lambda_1$ and $\lambda_2$ are defined to be the macroscopic rate constant for the fast phase and the slow phase, respectively, $\lambda_1$ is greater than $\lambda_2$. Therefore, $\lambda_1$ is greater than $k_h$ and $\lambda_2$ is smaller than $k_h$.

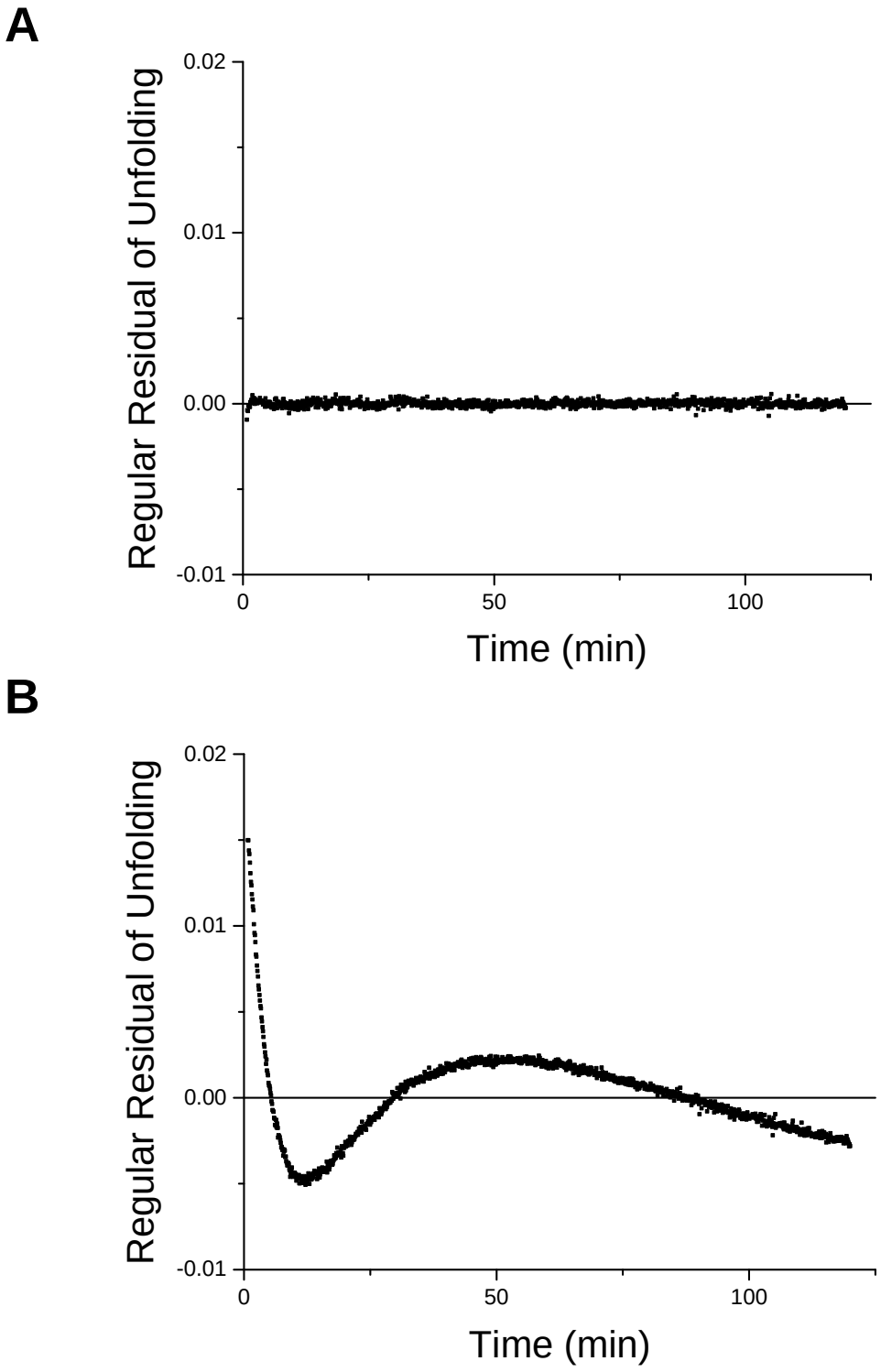



**Figure S1. Residuals from curve-fitting of bR unfolding (A)** The residuals from fitting the unfolding of $bR_F$ at $X_{SDS} = 0.73$ with a double exponential equation. **(B)** The residuals from fitting the unfolding of $bR_F$ at $X_{SDS} = 0.73$ with a single exponential equation.

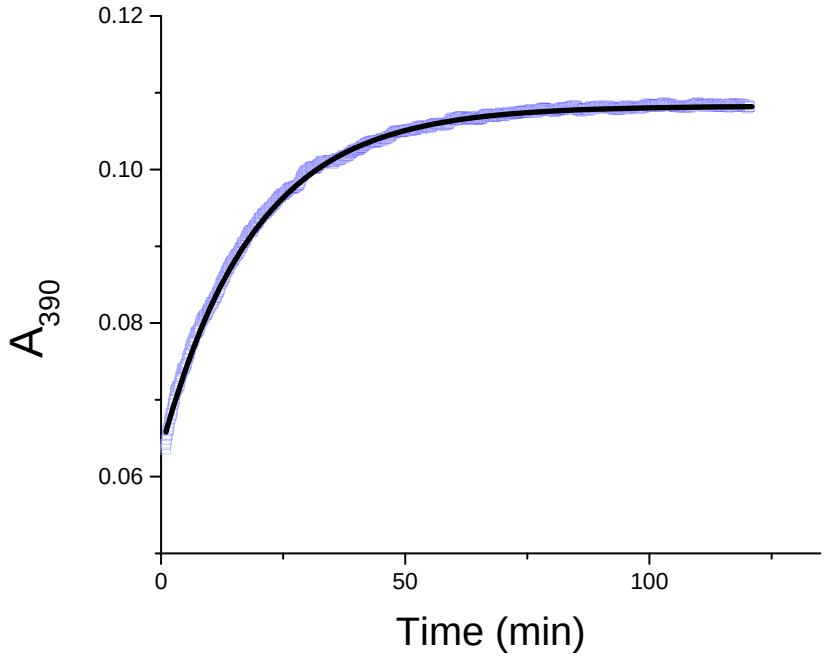



**Figure S2. Kinetics of retinal hydrolysis.** A single exponential fit (black) of the hydrolysis of retinal from $bR_U$ at $X_{SDS}$ = 0.83 as monitored by the increase at $A_{390}$ (blue squares).

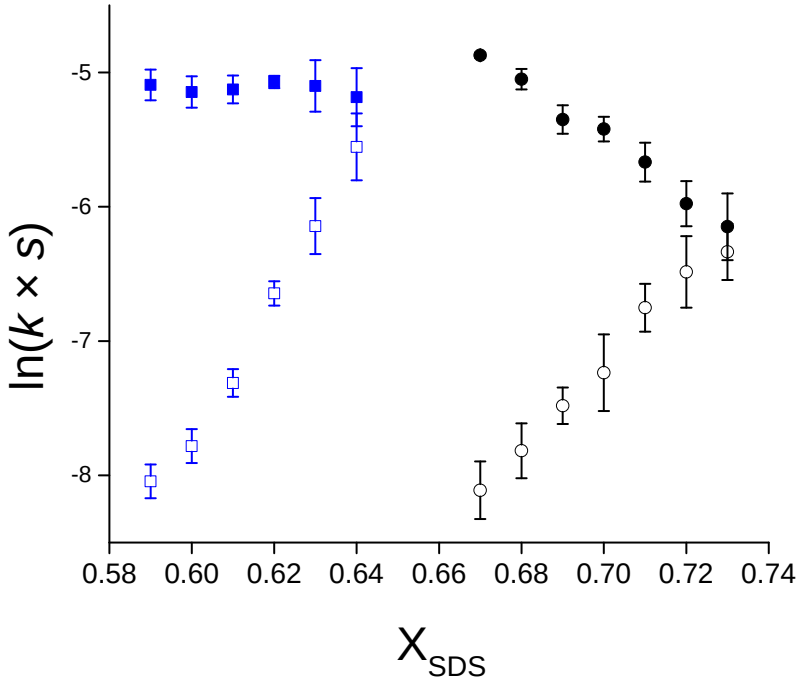



**Figure S3. Folding and unfolding kinetics of I119A bR.** The large shift in the folding slope indicates the change in the folding pathway of I119A bR. The natural log of $k_f$ (filled blue squares) and $k_u$ (open blue squares) of the bR mutant I119A is plotted with the natural log of $k_f$ (filled black circles) and $k_u$ (open black circles) of wild-type bR for reference.

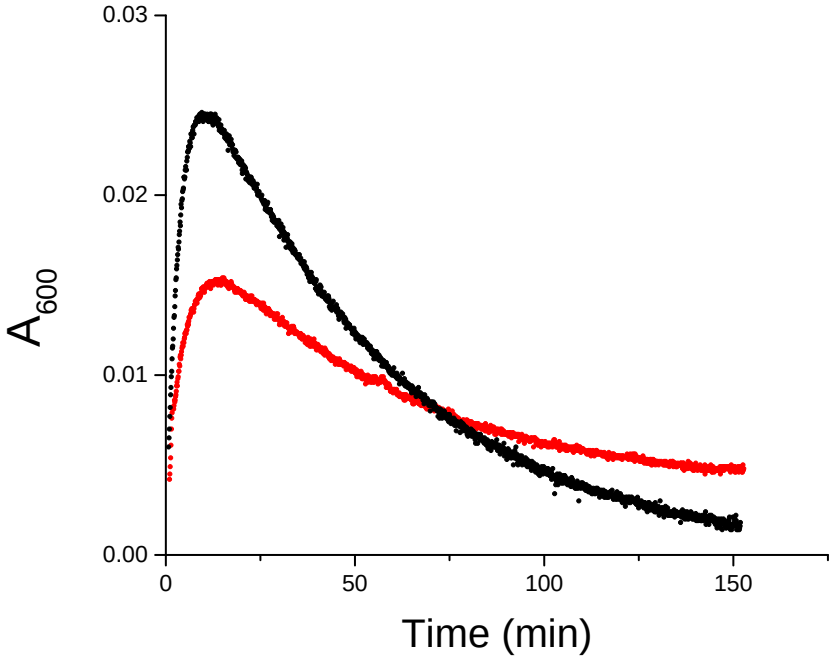
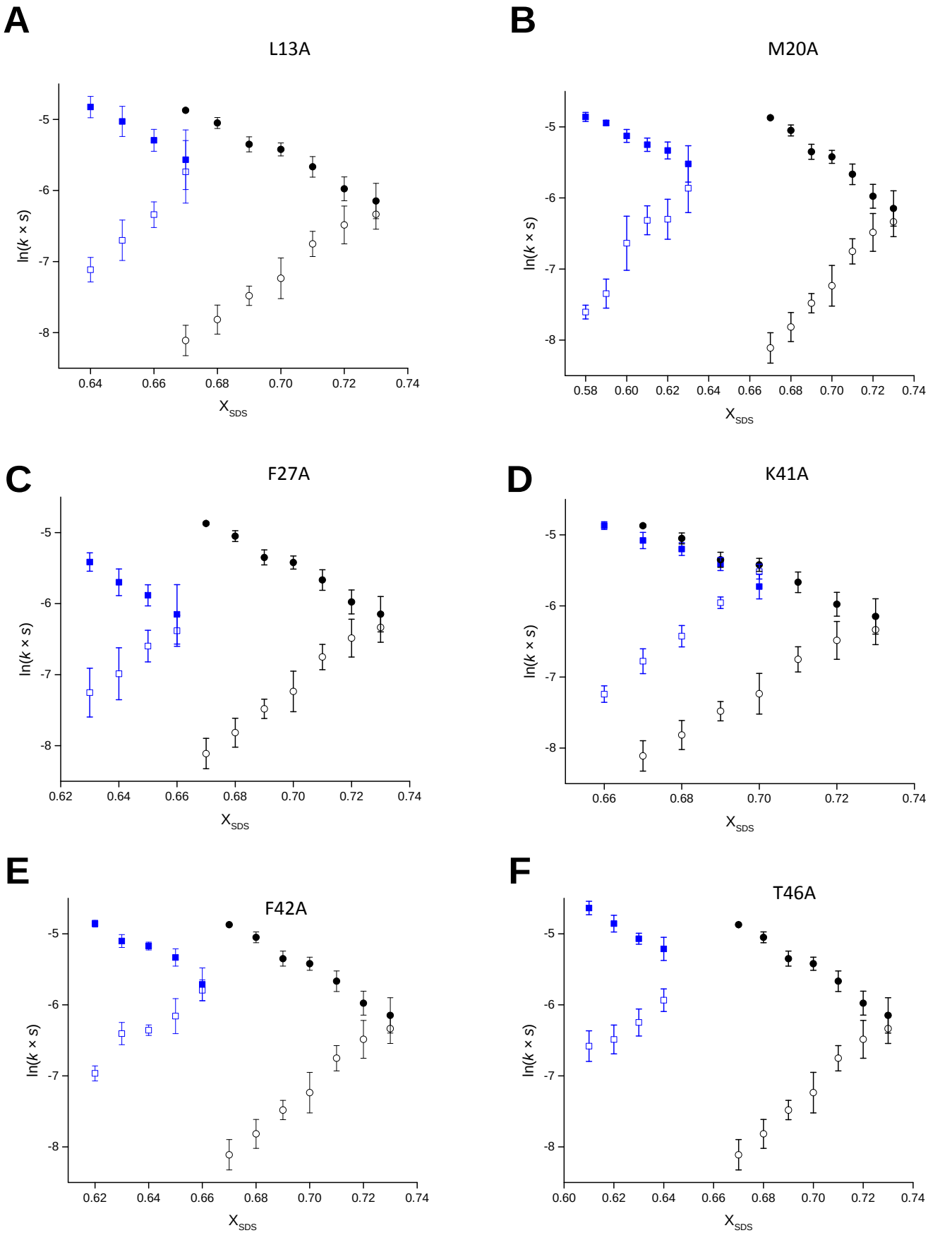
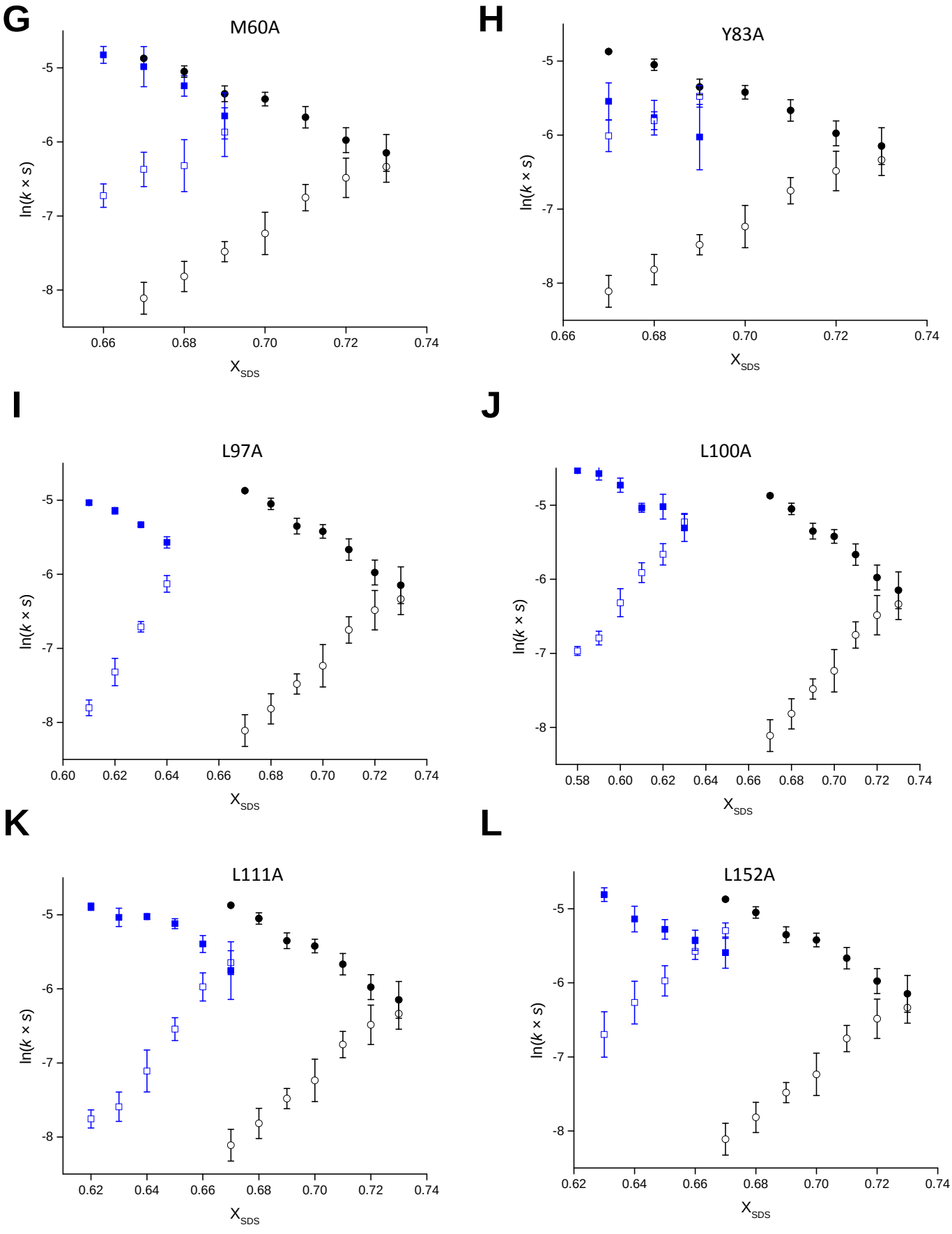



**Figure S4. Refolding of F219A bR.** The change in the kinetic trace shows the change in folding pathway in F219A bR. The refolding trace of the F219 A bR at $X_{SDS}$ = 0.67 (red dots) is plotted with that of wild-type bR at $X_{SDS}$ = 0.73 (black dots) for reference.


A
L13A
B
M20A
C
F27A
D
K41A
E
F42A
F
T46A
ln(k × s)
X_SDS


G
M60A
H
Y83A
I
L97A
J
L100A
K
L111A
L
L152A
ln(k × s)
X_SDS

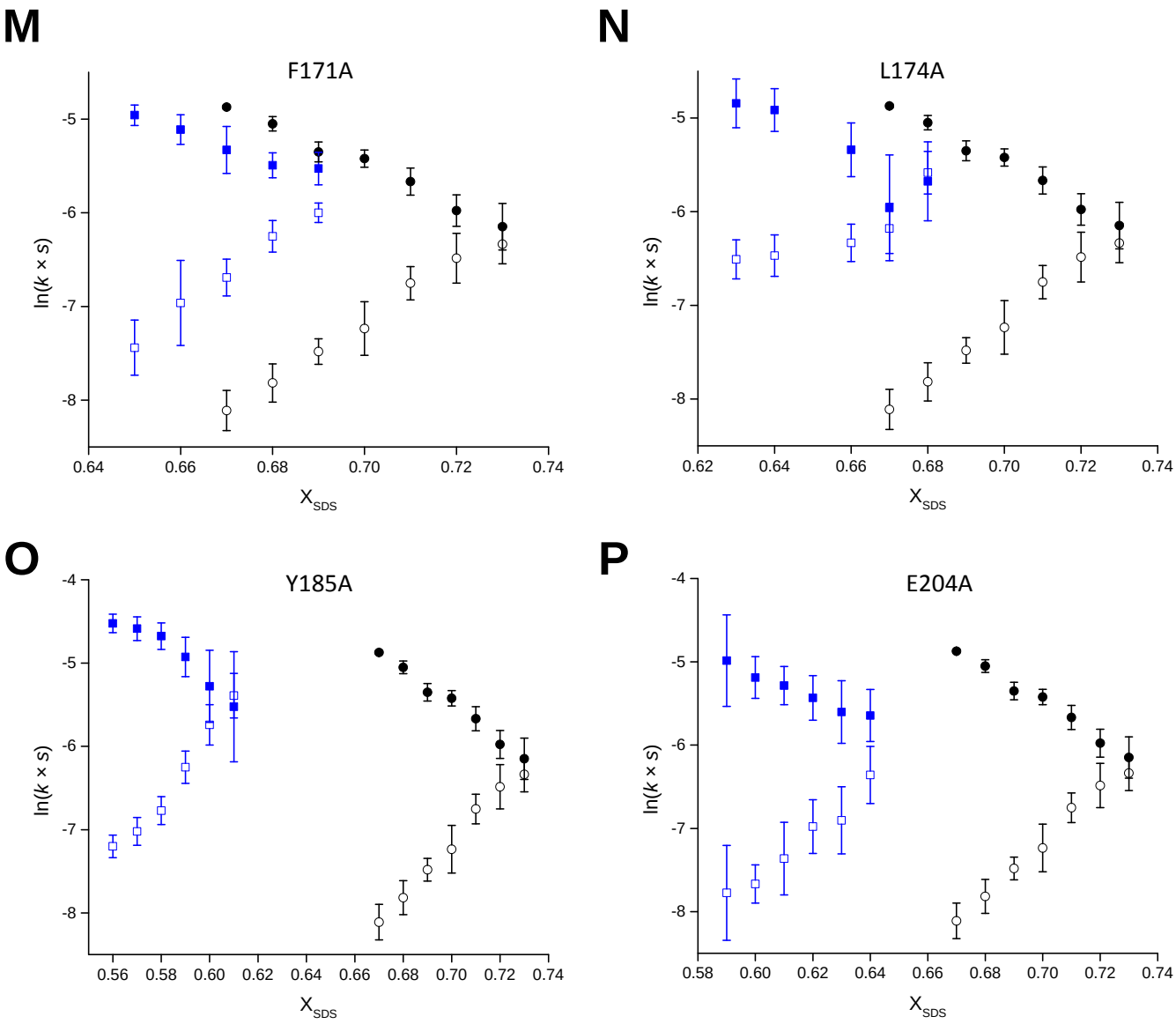



**Figure S5. The extracted rates constants of the bR mutants used for φ-value analysis.** The natural log of $k_f$ (filled blue squares) and $k_u$ (open blue squares) of the bR mutants are plotted with the natural log of $k_f$ (filled black circles) and $k_u$ (open black circles) of wild-type bR for reference.**(A)** L13A **(B)** M20A **(C)** F27A **(D)** K41A **(E)** F42A **(F)** T46A **(G)** M60A **(H)** Y83A **(I)** L97A **(J)** L100A **(K)** L111A **(L)** L152A **(M)** F171A **(N)** L174A **(O)** Y185A **(P)** E204A

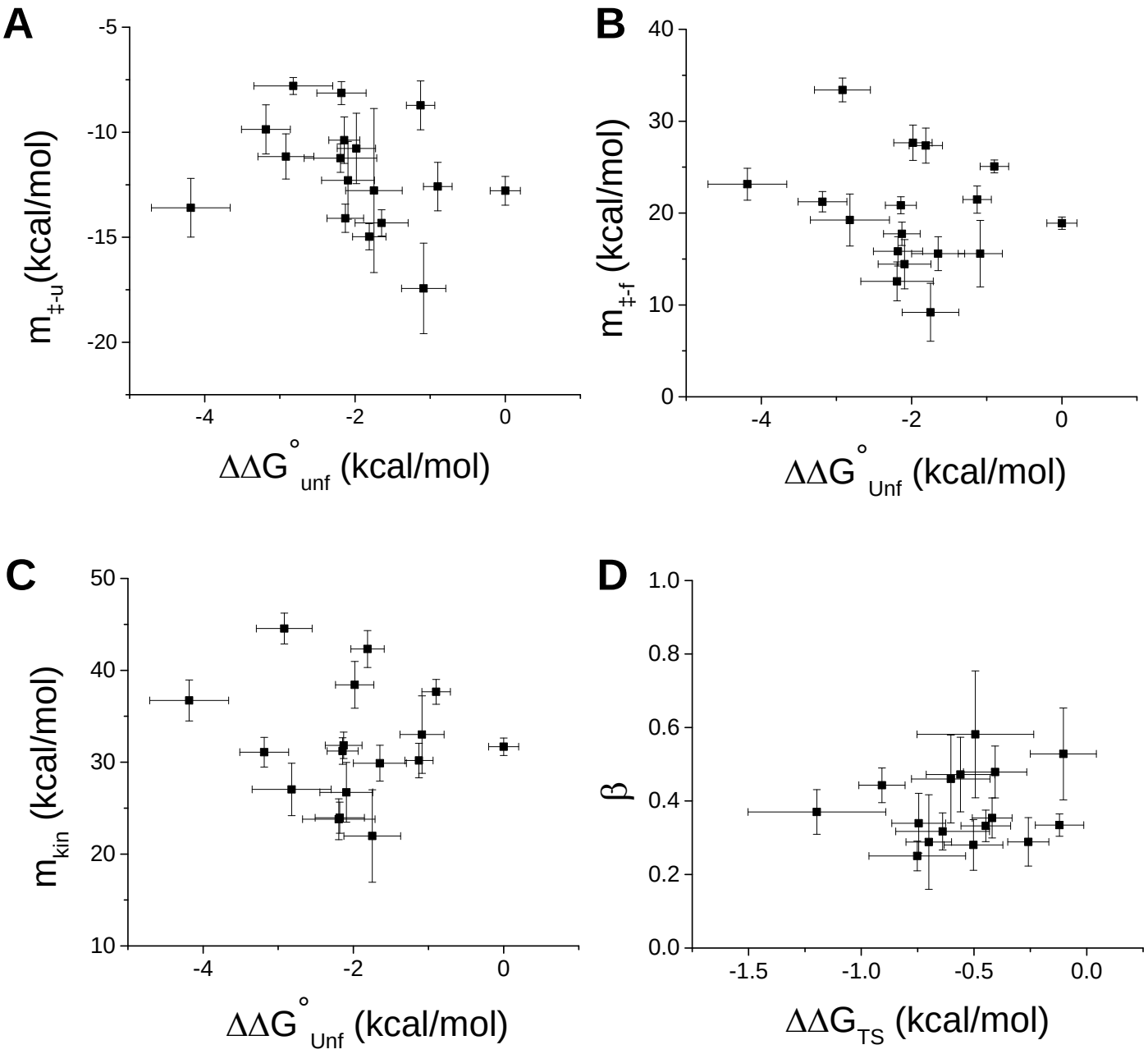



**Figure S6. Lack of dependence of the folding and unfolding behavior of bR on the degree of destabilization upon mutation. (A)** No effect of destabilization on the folding slope **(B)** No effect of destabilization on unfolding slope. **(C)** No effect of destabilization on the kinetic *m*-value **(D)** No correlation between the change in folding rate and β-value.