

## Supplementary Information for

### Unusual Denaturation Trajectory of Bovine Gamma Globulin

#### Studied by Fluorescence Correlation Spectroscopy

Moupriya Nag<sup>†</sup>, Debajyoti Das<sup>‡</sup>, Diptankar Bandyopadhyay<sup>§</sup>, Soumen Basak<sup>†\*</sup>

<sup>†</sup>Chemical Sciences Division, Saha Institute of Nuclear Physics, <sup>‡</sup>Department of Biophysics,  
Molecular Biology and Bioinformatics, <sup>§</sup>Department of Biochemistry, University of Calcutta

\*Corresponding author: Fax: (91)-33-2337 4637.

E-mail address: soumen.basak@saha.ac.in, soumenbasak08@gmail.com

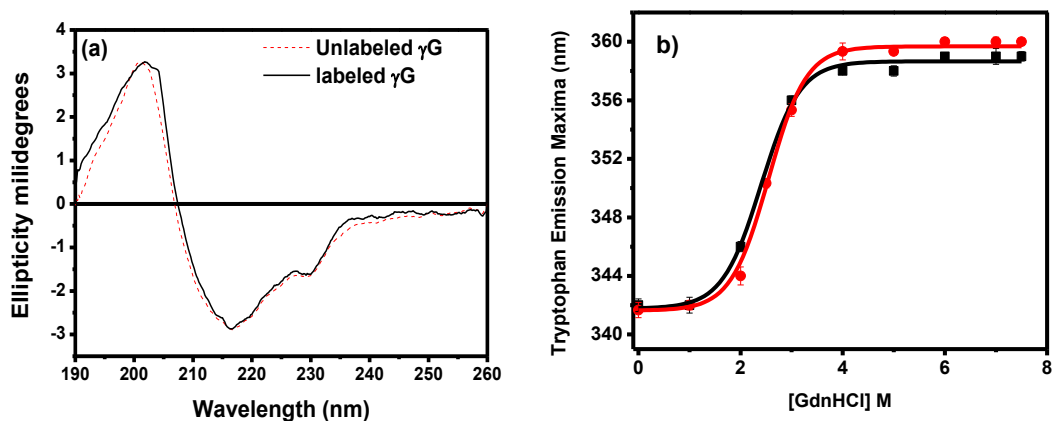


Fig. S1 a) Far-UV CD spectra of  $\gamma$ G in the Rhodamine-labeled and unlabeled states. b) Tryptophan emission maxima (excitation at 295 nm) of labeled (●) and unlabelled (■)  $\gamma$ G in solutions of increasing GdnHCl concentration, superposed with sigmoidal fit of each set of data.

**Table S1. Percentages of secondary structure content of  $\gamma$ G as function of GdnHCl concentration obtained by fitting the CD spectra of Fig. 2(a) with the CDSSTR suite of programs. The RMSD values obtained were <0.03.**

| [GdnHCl] (M) | Helix | Beta sheet | Turns | Random coil |
|--------------|-------|------------|-------|-------------|
| 0            | 3     | 63         | 14    | 20          |
| 1            | 17    | 55         | 10    | 18          |
| 2            | 36    | 43         | 9     | 12          |
| 2.5          | 53    | 35         | 4     | 8           |
| 3            | 36    | 36         | 08    | 20          |
| 4            | 28    | 39         | 11    | 22          |
| 5            | 21    | 29         | 18    | 32          |
| 6            | 13    | 27         | 17    | 43          |

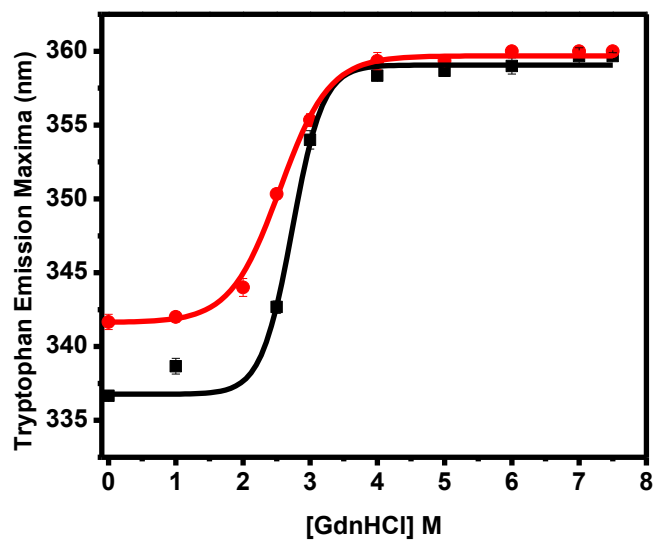


Fig. S2 Unfolding profiles of  $R\text{-}\gamma\text{G}$  measured by fluorescence in absence (●) and presence (■) of 2 M NaCl. Solid lines are sigmoidal fits to the data.

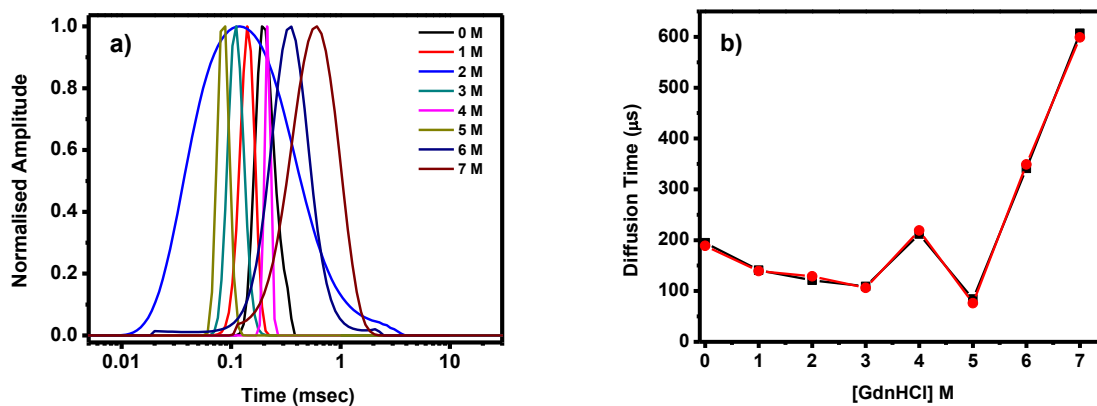


Fig. S3 a) Variation of the maximum entropy profiles of  $R\text{-}\gamma\text{G}$  with GdnHCl concentrations. b) Comparison of diffusion times obtained by fitting the ACF with Eqn. (1) of text (●) with the times at the peak of the distributions obtained from the MEMFCS program (■).

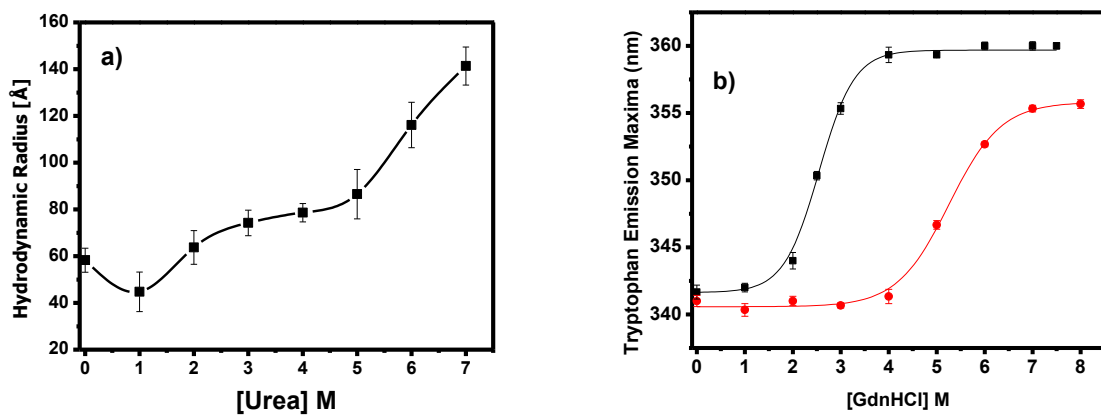


Fig. S4 a) Urea induced denaturation of R- $\gamma$ G studied with FCS. b) Change in wavelength of Trp emission maxima of R- $\gamma$ G in presence of varying concentration of GdnHCl (■) and urea (●) during unfolding.