Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

The Multiple Dissociation Constants of Glutathione Disulfide: Interpreting Experimental pH-Titration Curves with *Ab Initio* MD Simulations

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Supplementary Information

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S1: Convergence

In a metadynamics calculation the choice of the Gaussian height is crucial. The choice must ensure that barrier crossings are achieved for reasonable simulation times and at the same time, ensure that the calculated free energy profiles are properly sampled. To confirm that a Gaussian height of 0.0005 au was adequate to describe the dissociation reaction we had performed calculations for the dissociation of the carboxylic group of the first glutamyl moiety of GSSG for four different Gaussian heights. Free energy profiles for the dissociation of the carboxylic group of the first glutamyl moiety of GSSG were computed using Gaussian heights of 0.0001 au, 0.0005 au, 0.0008 au and 0.001 au are shown in Figure S1a. Convergence of the calculated free energy observed for a Gaussian height 0.0005 au and a Gaussian height 0.0001 au are shown in the inset of Figure S1a. Similar tests were also performed to establish the optimum deposition rates of the Gaussians. The free-energy profiles computed using Gaussian deposition intervals of 20 fs, 30 fs, 40 fs and 60 fs are shown in Figure S1b. Convergence of the calculated free energy for these parameters was observed for a Gaussian deposition interval 40 fs and a Gaussian deposition interval 60 fs as shown in inset of Figure S1b.

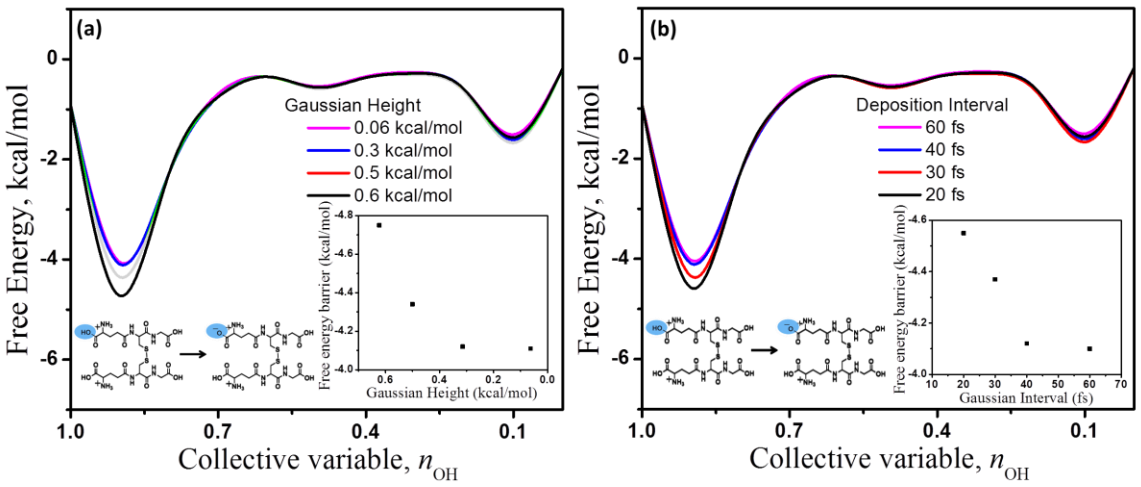

Figure S1: Metadynamics computed free energy profiles for the dissociation of the carboxylic group of the first glutamyl moiety of GSSG for a) different Gaussian heights and b) for different deposition intervals (the Gaussian height is 0.0005 au). The inset in both panels shows the convergence in energies of the first barrier in the free energy profiles.

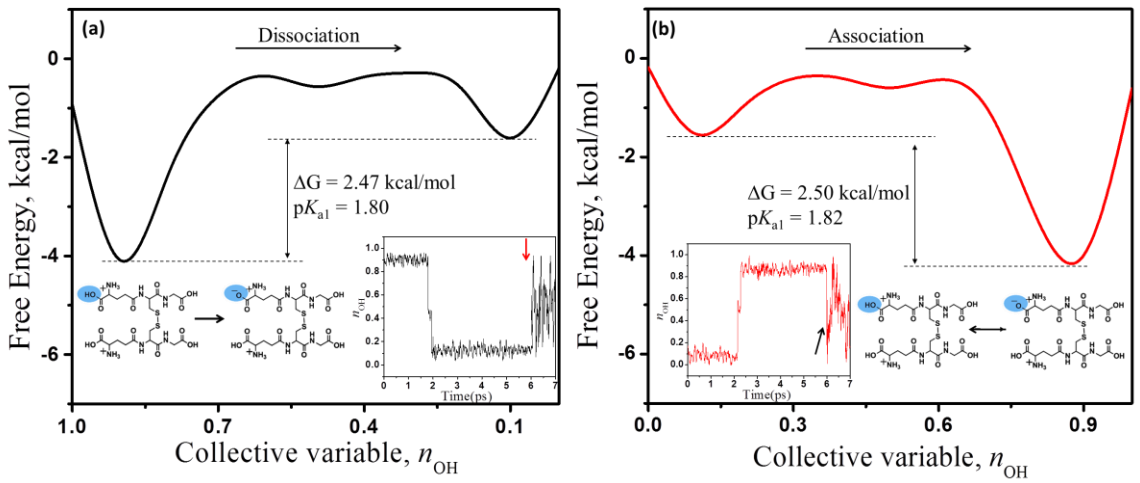

Figure S2: The free energy profiles and trajectories for the dissociation and association of the carboxylic group of the first glutamyl moiety of GSSG initiated from (a) the neutral state and (b) the dissociated anionic state.The arrow indicates the completion of the dissociation reaction.

S2: Dissociation of the second glutamyl carboxylic group (pK_{a2}) of GSSG:

The free energy profile for the dissociation of the second glutamyl carboxylic group (pK_{a2}) of GSSG is shown in Figure S3. The collective variable is n_{OH}; the OH bond length dependent coordination number of the carboxylic group undergoing dissociation (highlighted in Figure S3). In Figure S3 the minima at $n_{OH} \approx 1$ corresponds to the neutral carboxylic group while that at $n_{OH} \approx 0$ to the dissociated carboxylic group. The shallow minima seen in the free energy profiles correspond to a contact-ion pair that subsequently breaks-up to complete the dissociation/deprotonation process. The value of the pK_{a2} estimated from the difference in the free energy values at the minima in the free energy profile (Figure S3) is 2.39.

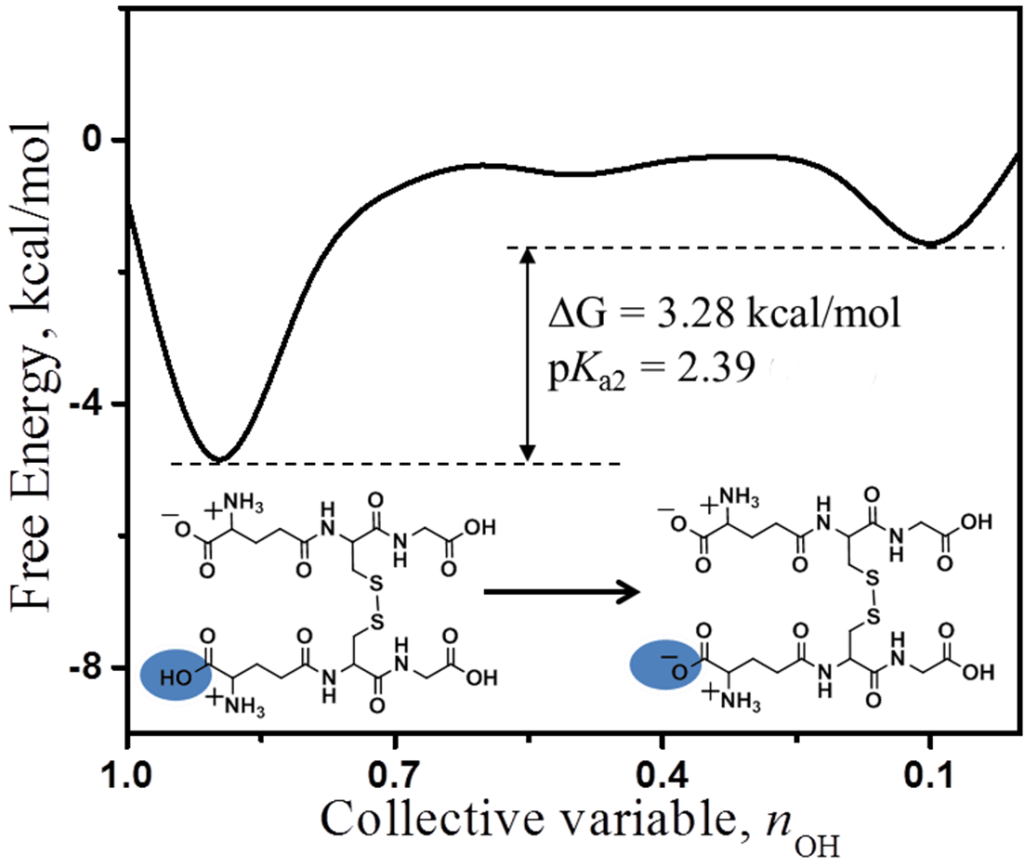

Figure S3: Free-energy profile for the dissociation of the carboxylic group of the second glutamyl moiety of GSSG. The difference in the free-energy values of neutral and dissociated state and the estimated pK_{a2} value are indicated.

S3: Dissociation of the first glycinyl carboxylic group (pK_{a3}) of GSSG:

The free energy profile for the dissociation of the first glycinyl carboxylic group (pK_{a3}) of GSSG is shown in Figure S4. The collective variable is n_{OH}; the OH bond length dependent coordination number of the carboxylic group undergoing dissociation (highlighted in Figure S4). In Figure S4 the minima at $n_{OH} \approx 1$ corresponds to the neutral carboxylic group while that at $n_{OH} \approx 0$ to the dissociated carboxylic group. The shallow minima seen in the free energy profiles correspond to a contact-ion pair that subsequently breaks-up to complete the dissociation/deprotonation process. The value of the pK_{a3} estimated from the difference in the free energy values at the minima in the free energy profile (Figure S4) is 3.29.

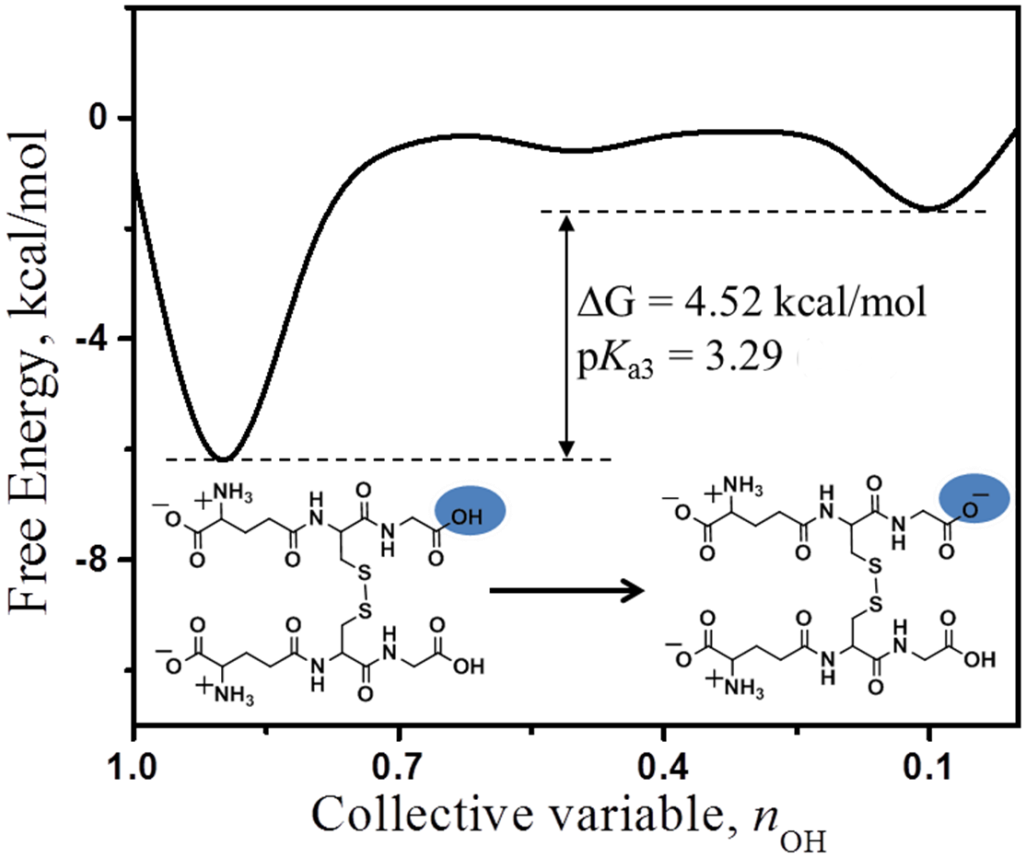

Figure S4: Free-energy profile for the dissociation of the carboxylic group of the first glycinyl moiety of GSSG. The difference in the free-energy values of neutral and dissociated state and the estimated pK_{a3} value are indicated.

S4: Dissociation of the second glycinyl carboxylic group (pK_{a4}) of GSSG:

The free energy profile for the dissociation of the second glycinyl carboxylic group (pK_{a4}) of GSSG is shown in Figure S5. The collective variable is n_{OH}.; the OH bond length dependent coordination number of the carboxylic group undergoing dissociation (highlighted in Figure S5). In Figure S5 the minima at $n_{OH} \approx 1$ corresponds to the neutral carboxylic group while that at $n_{OH} \approx 0$ to the dissociated carboxylic group. The shallow minima seen in the free energy profiles correspond to a contact-ion pair that subsequently breaks-up to complete the dissociation/deprotonation process. The value of the pK_{a4} estimated from the difference in the free energy values at the minima in the free energy profile (Figure S5) is 3.92.

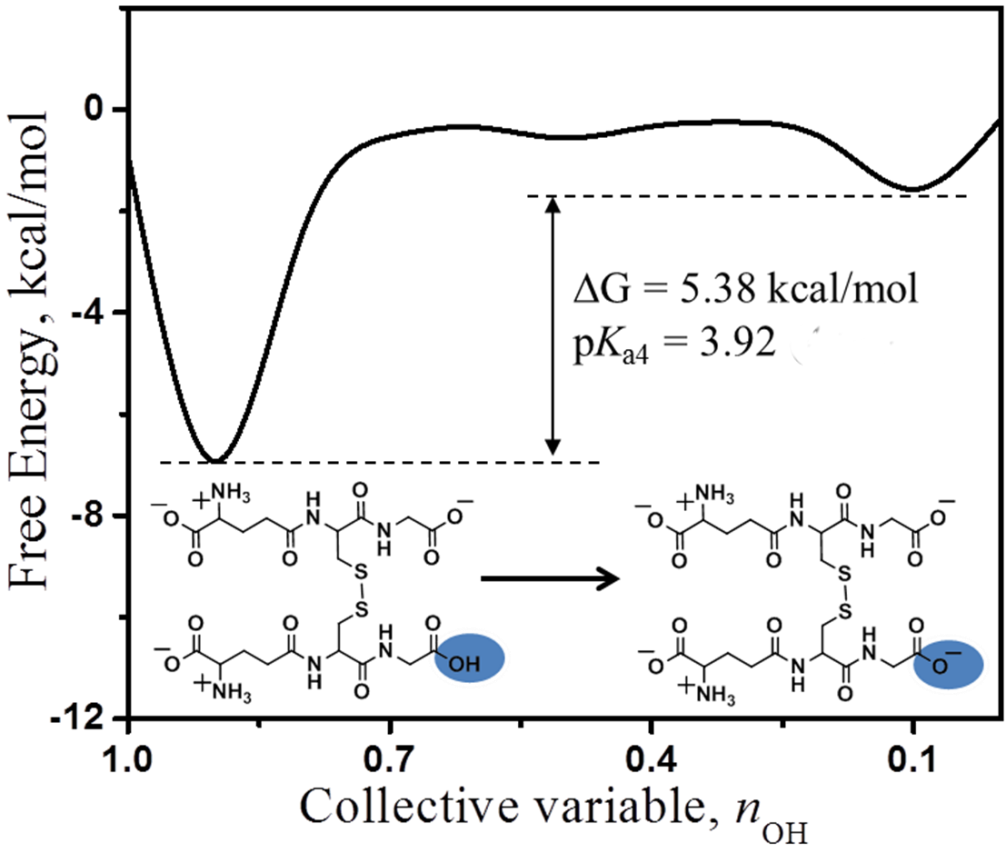

Figure S5: Free-energy profile for the dissociation of the carboxylic group of the second glycinyl moiety of GSSG. The difference in the free-energy values of neutral and dissociated state and the estimated pK_{a4} value are indicated.

S5: Deprotonation of the first glutamyl amino group (pK_{a5}) of GSSG:

The free energy profile for the dissociation of the first glutamyl amino group (pK_{a5}) of GSSG is shown in Figure S6. The collective variable is n_{NH}; the NH bond length dependent coordination number of the amine group undergoing deprotonation (highlighted in Figure S6). In Figure S6 the minima at $n_{NH} \approx 1$ corresponds to the protonated amino group while that at $n_{NH} \approx 0$ to the deprotonated amino group. The shallow minima seen in the free energy profiles correspond to a contact-ion pair that subsequently breaks-up to complete the dissociation/deprotonation process. The value of the pK_{a5} estimated from the difference in the free energy values at the minima in the free energy profile (Figure S6) is 8.94.

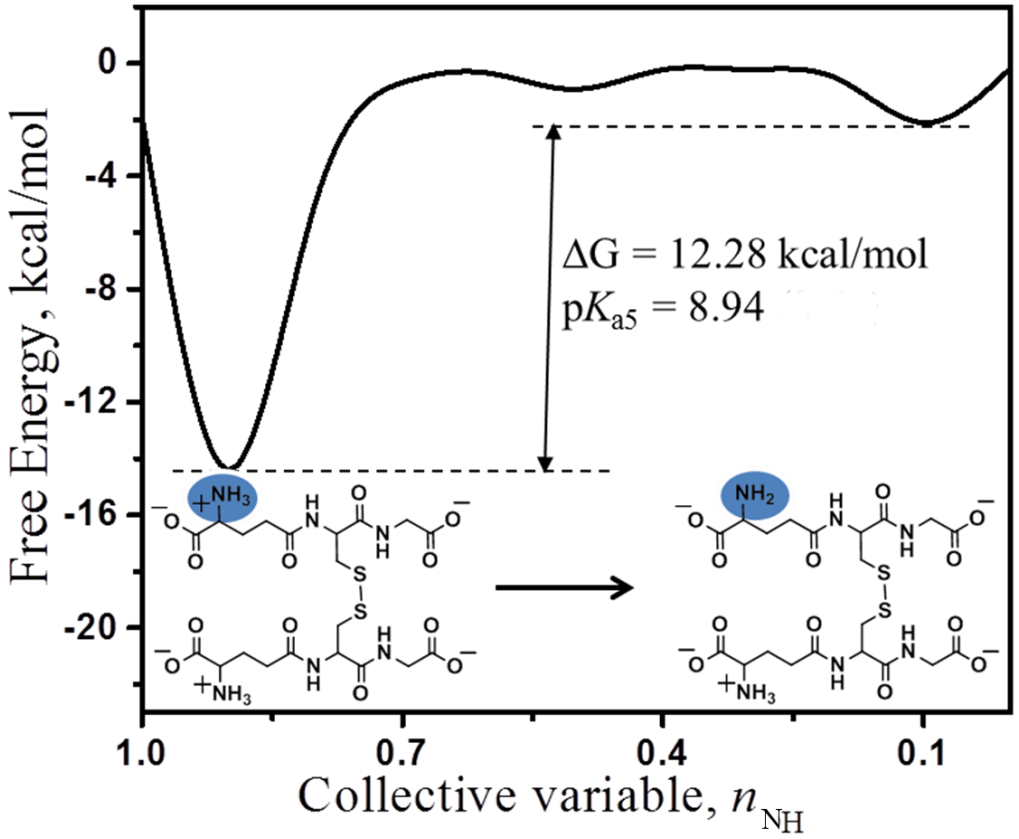

Figure S6: Free-energy profile for the dissociation of the first glutamyl amino group of GSSG.The difference in the free-energy values of deprotonated and protonated state and the estimated pK_{a5} value are indicated.

S6: Deprotonation of the second glutamyl amino group (pK_{a6}) of GSSG:

The free energy profile for the dissociation of the second glutamyl amino group (pK_{a6}) of GSSG is shown in Figure S7. The collective variable is n_{NH}; the NH bond length dependent coordination number of the amine group undergoing deprotonation (highlighted in Figure S7). In Figure S7 the minima at $n_{NH} \approx 1$ corresponds to the protonated amino group while that at $n_{NH} \approx 0$ to the deprotonated amino group. The shallow minima seen in the free energy profiles correspond to a contact-ion pair that subsequently breaks-up to complete the dissociation/deprotonation process. The value of the pK_{a6} estimated from the difference in the free energy values at the minima in the free energy profile (Figure S7) is 9.68.

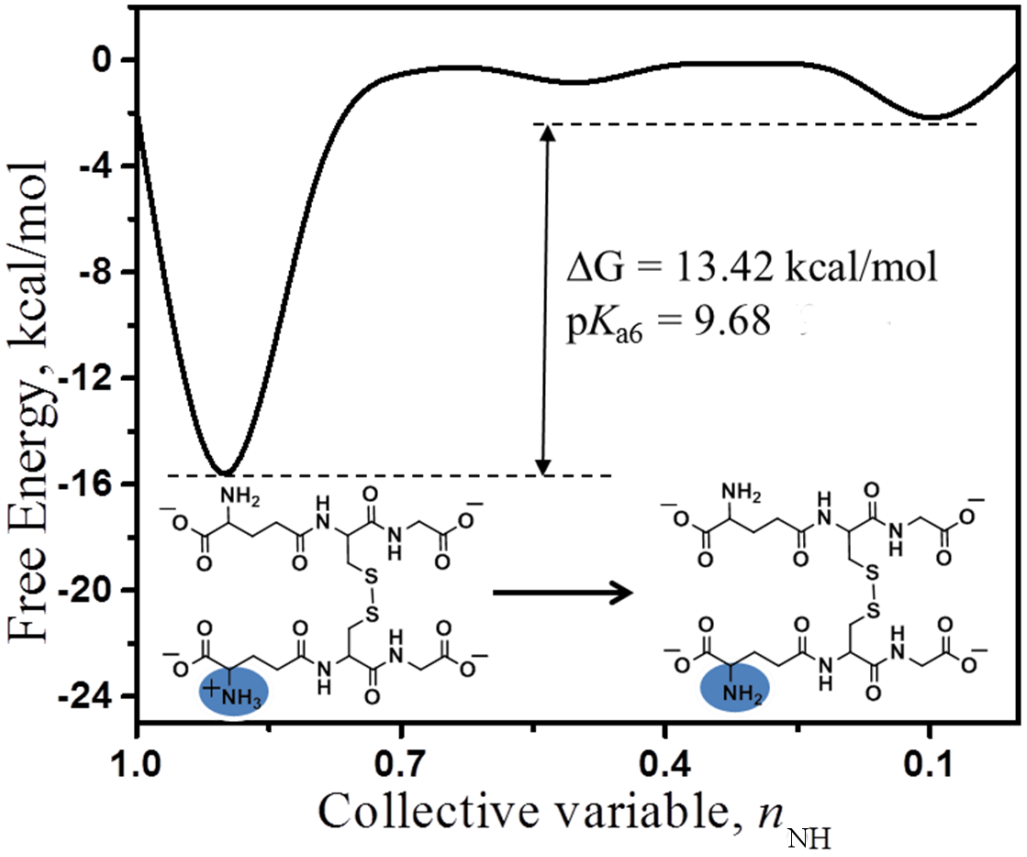

Figure S7: Free-energy profile for the dissociation of the second glutamyl amino group of GSSG.The difference in the free-energy values of deprotonated and protonated state and the estimated pK_{a6} value are indicated.