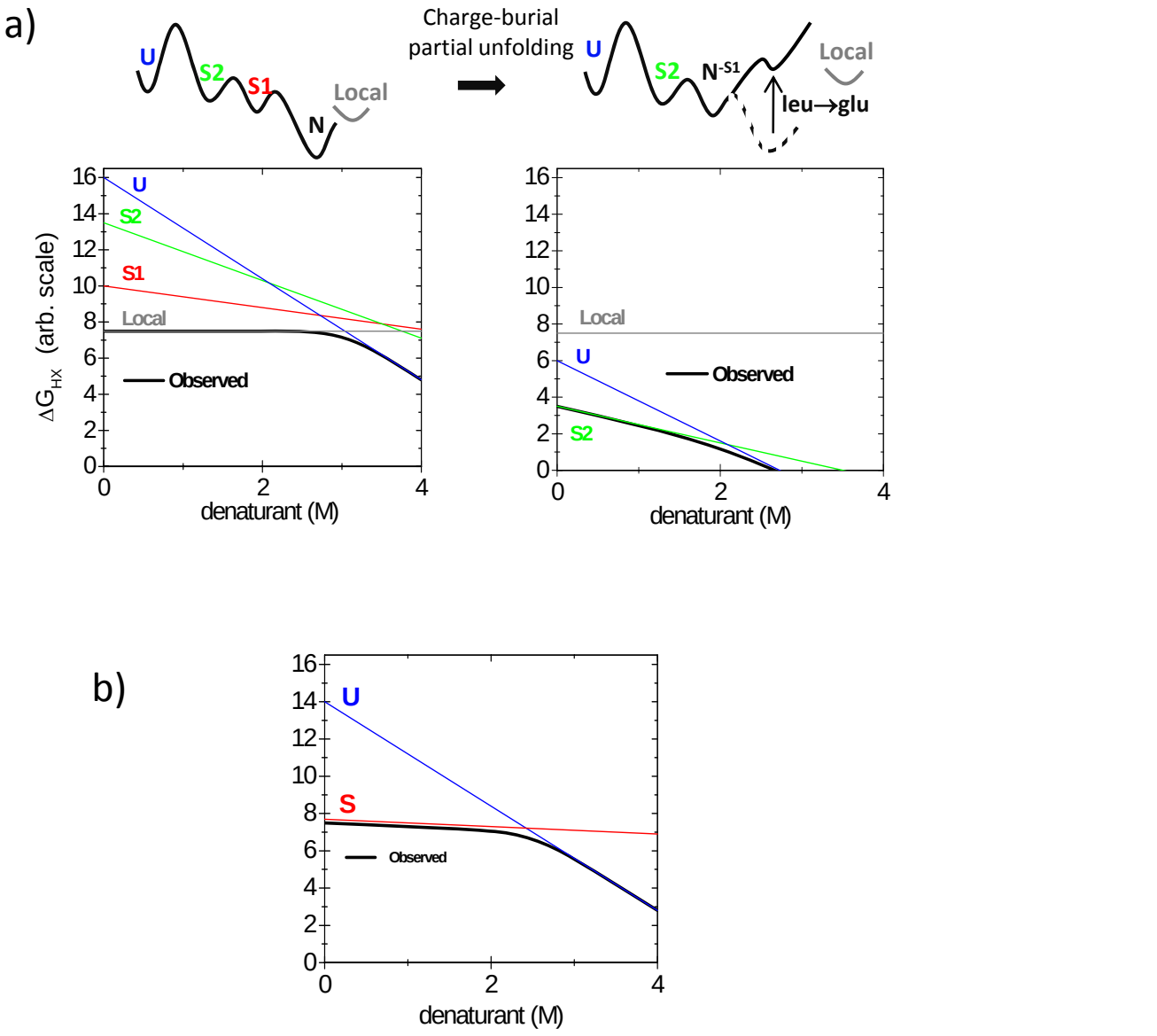
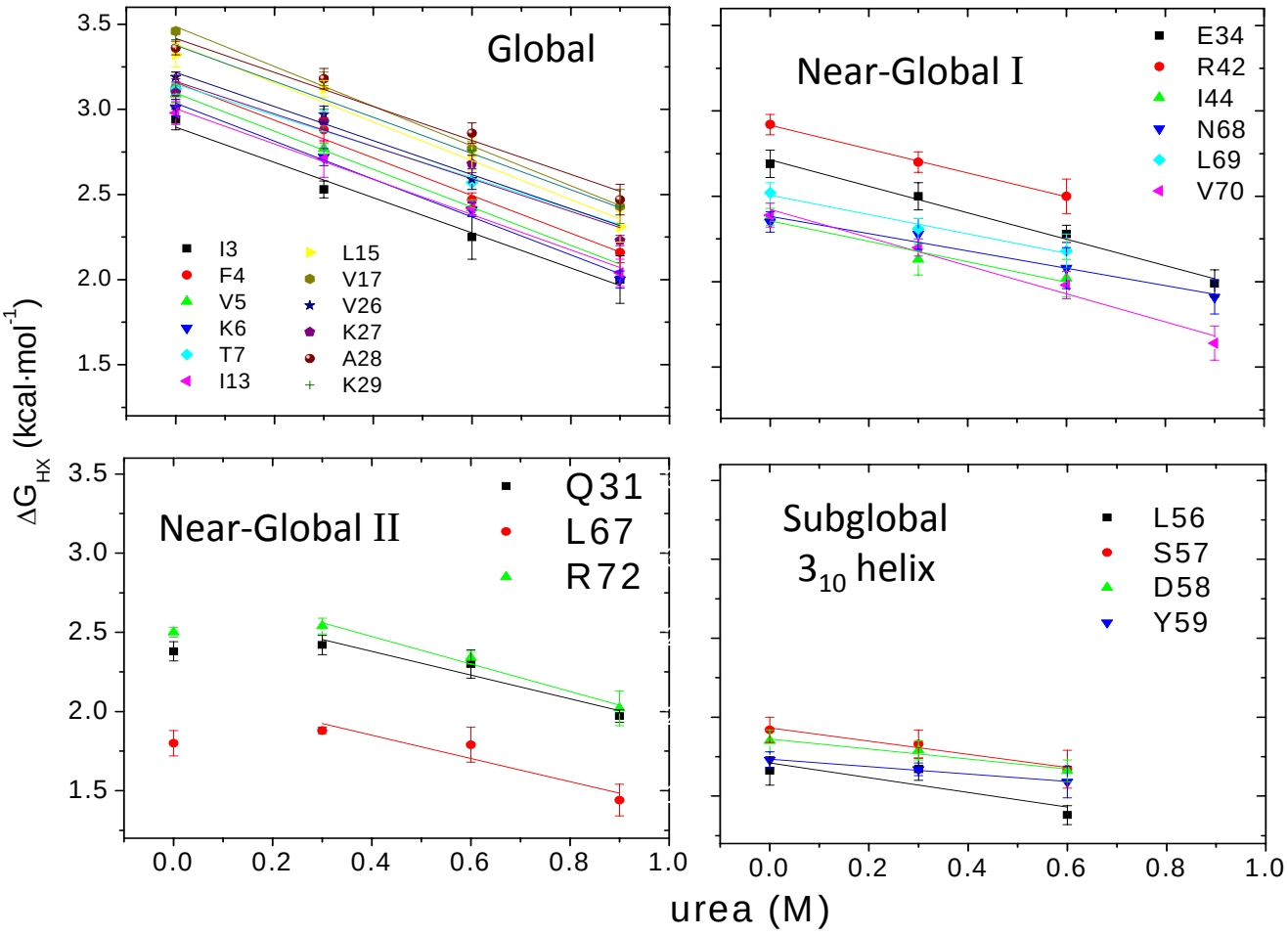
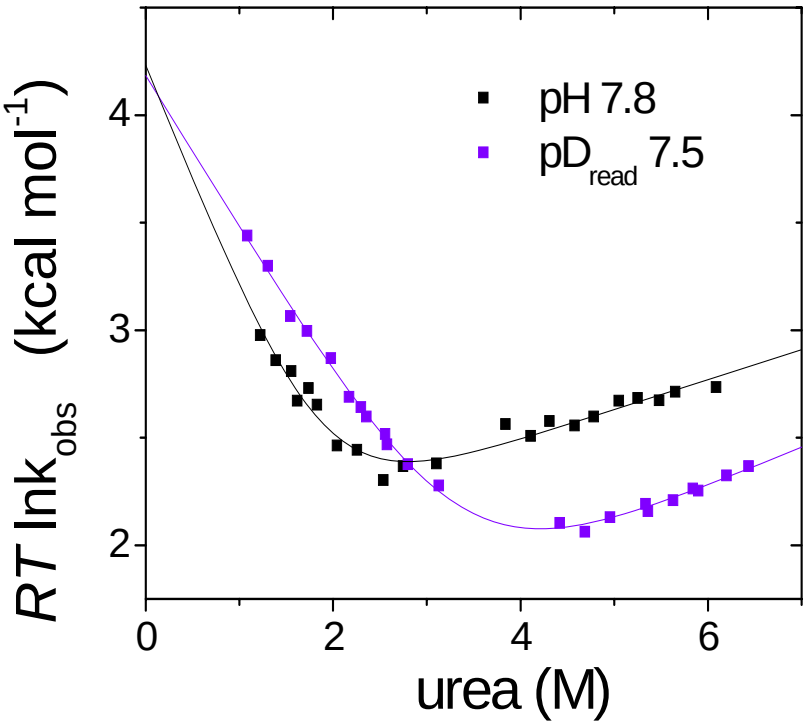


Supplementary Figure 1. NSHX can fail to detect subglobal openings. a) Multiple subglobal unfolding events (S1, S2, green, red) can exist but none of them are detected because their isotherms are masked by the faster local unfolding events (grey) at low denaturant, and by global unfolding events at high denaturant concentration (U, blue). The S1 subglobal opening can be trapped (N^{-S1}) and characterized using the charge-burial strategy (right panel). NSHX can be applied on this trapped PUF to identify subglobal opening S2. Local unfolding events are assumed to be at a fixed energy above the ground state of the system. **b)** If the subglobal opening involves little exposure of surface area, the subglobal unfolding cannot easily be distinguished from a local unfolding event. In this case the structural identity of the subglobal opening can be identified by populating the intermediate using the charge-burial strategy and then identifying which hydrogen bonds are lost using HX at high pH and NMR readout on the N* state where the amide resonances can be assigned.



Supplementary Figure 2. NSHX on UbL50E I state at pD$_{read}$ 7.5. Denaturant dependent isotherms for all measured residues classified according to exchange mechanism.



Supplementary Figure 3. Dependence of UbL50E stability on D_2O level. Comparison of chevrons in H_2O, pH 7.8 (15 mM phosphate, 225 mM NaCl) and 80% D_2O, pD_{read} 7.5 used in the HX studies (extra 10 mM Glu, 10 mM Arg, 80% D_2O). HX buffer stabilizes UbL50E I by ~ 1 kcal mol^{-1}. The K_{eq} determined from the kinetic and equilibrium data agrees with ΔG_{HX} of global unfolding from HX study under the same solvent condition.