Supporting Information

Exploring the pH-dependent structure-dynamics-function relationship of human renin

Shuhua Ma,*,† Jack A. Henderson,‡ and Jana Shen*,‡

† *Department of Chemistry, Jess and Mildred Fisher College of Science and Mathematics, Towson University, Towson, MD 21252*

‡ *Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, MD 21201*

E-mail: sma@towson.edu; jana.shen@rx.umaryland.edu

List of Figures

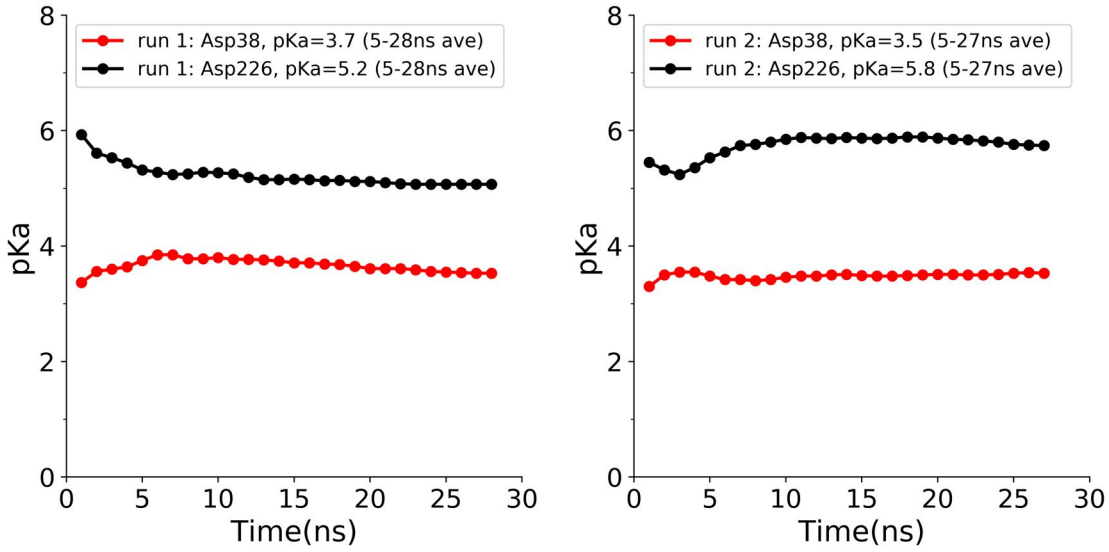

Figure S1: **Convergence of the calculated residue-specific pK$_a$'s of the catalytic dyad in human renin.** Left. Simulations starting from the X-ray structure PDB 2ren (run 1). Right. Simulations starting from the X-ray structure PDB 3sfc (run 2). The data for Asp38 and Asp226 are shown in red and black, respectively.

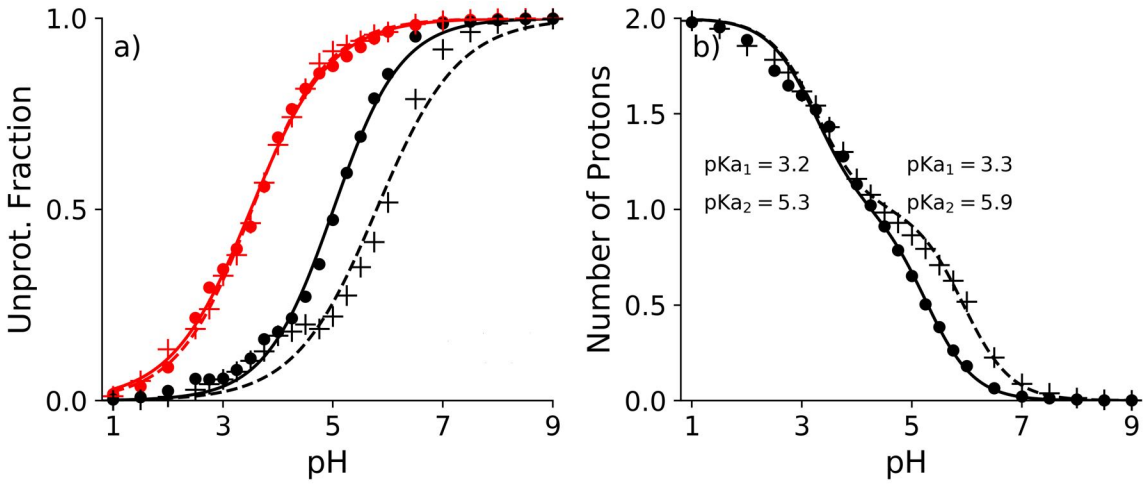

Figure S2: **Microscopic and macroscopic titration curves of the catalytic dyad in human renin.** a) The unprotonated fractions of Asp38 (red) and Asp226 (black) as a function of pH calculated from the simulations with the structures 2ren (run 1, dots) and 3sfc (run 2, crosses). The best fits to the generalized Henderson-Hasselbalch equation are given (solid lines for 2ren and dashes lines for 3sfc). b) The number of protons for the catalytic dyad calculated from the simulations with the structures 2ren (run 1, dots) and 3sfc (run 2, crosses). Data from the first 4 ns per replica in both simulations were discarded in the calculations.

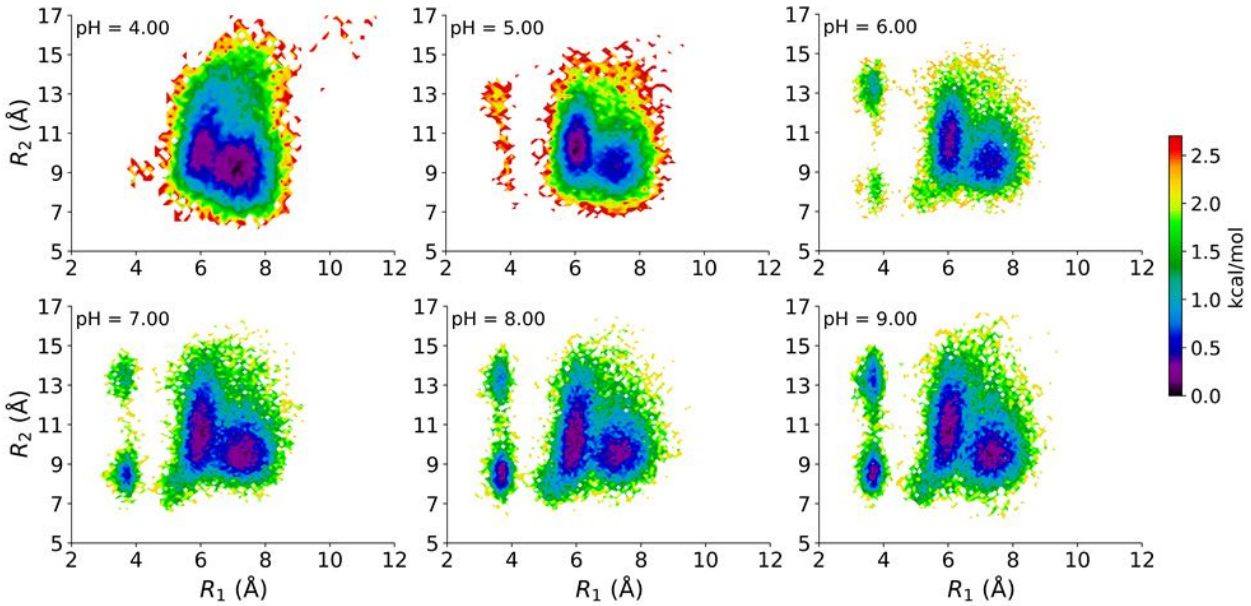

Figure S3: **Conformational dynamics of the flap in human renin at different pH.** Free energy surfaces as a function of the distance between Tyr83:OH and Asp38:CG (R_1) and the distance between Ser84:CB and Asp226:CG (R_2) at pH 4–9. Data from the simulation run 1 were used.

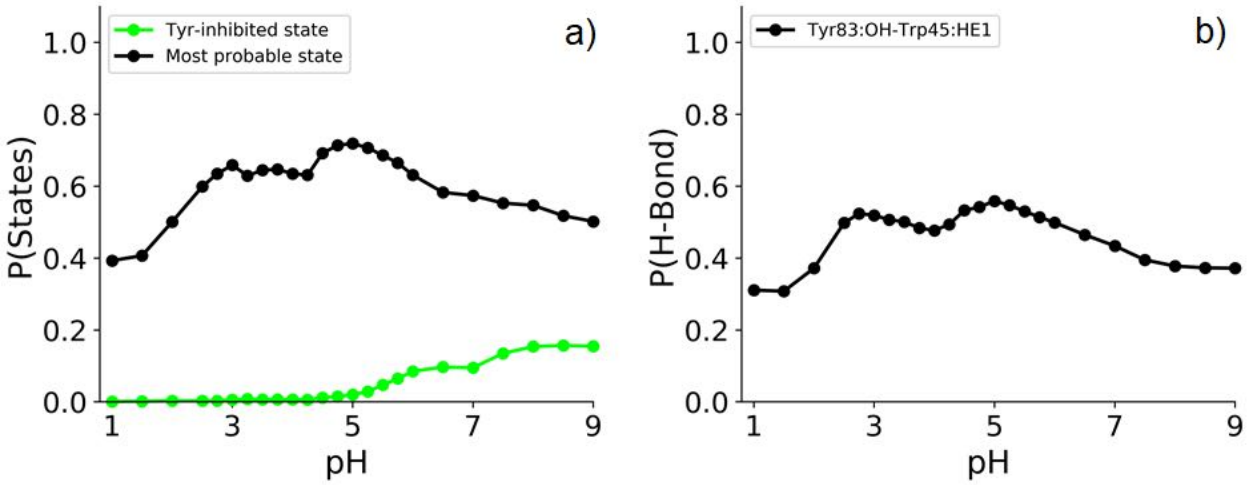

Figure S4: **pH-dependent probabilities of the flap conformational state and a conserved hydrogen bond.** a) Probability of the flap conformational states as a function of pH. Tyr-inhibited state (green) is defined as $R_1 < 5.0$ Å, and the most probable state (black) is defined as 5.0 Å $< R_1 < 7.5$ Å and 7.0 Å $< R_2 < 12.0$ Å, where R_1 and R_2 refer to the Tyr83:OH-Asp38:CG and Ser84:CB-Asp226:CG distances, respectively. b) Probability of the Tyr83:OG-Trp45:HE1 hydrogen bond as a function of pH. Data from the simulation run 1 were used for all plots.

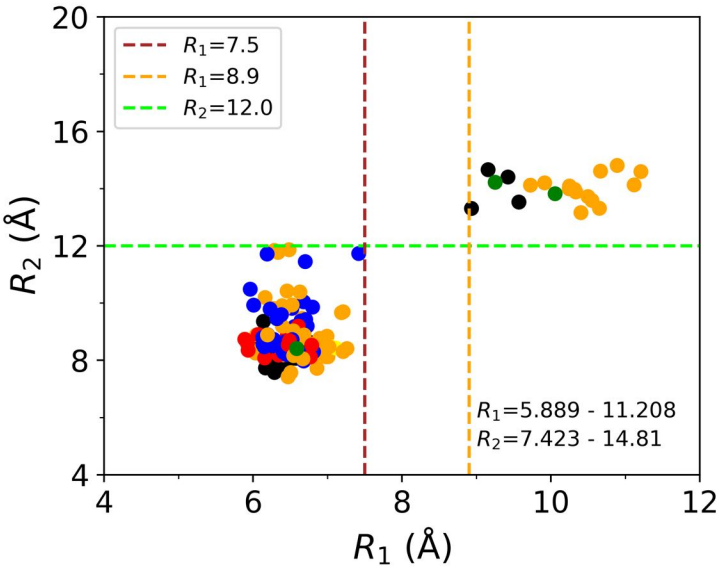

Figure S5: **Flap conformation represented by the crystal structures.** The distance between Tyr83:OH and Asp38:CG (R_1) vs. the distance between Ser84:CB and Asp226:CG (R_2) based on the published apo and holo crystal structures of human renin. Data are color coded according to the crystallization pH condition: red (pH 3-4), orange (pH 4-5), cyan (pH 5-6), green (pH 6-7), blue (pH 7-8.5), and black (pH not provided in the PDB file).

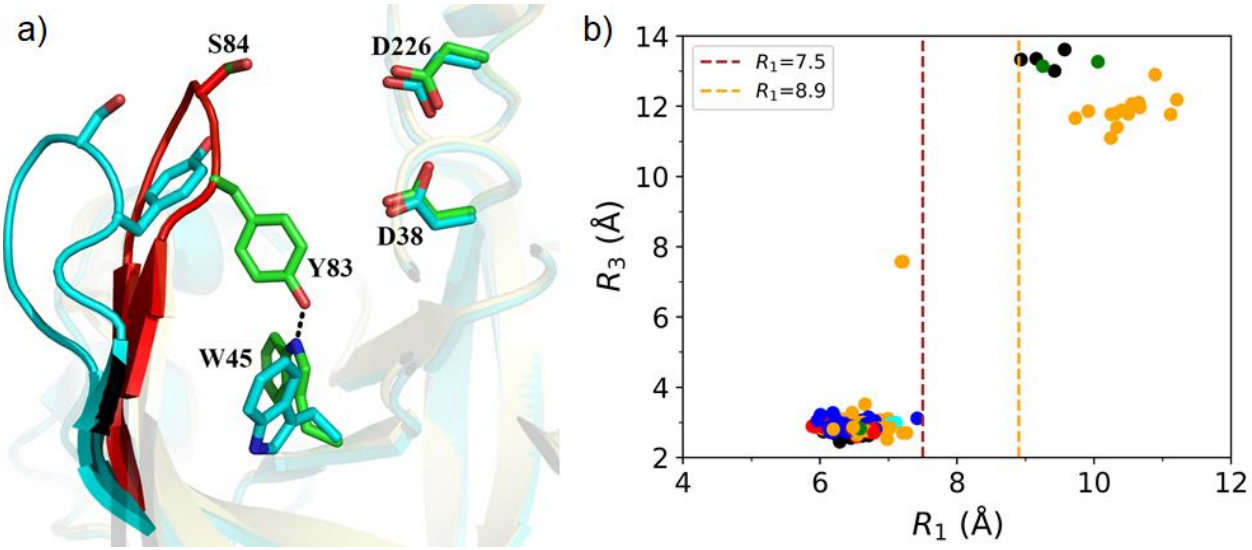

Figure S6: **Further opening of the flap is associated with the breakage of the Tyr83-Trp45 hydrogen bond.** a) A crystal structure of the apo renin (PDB ID: 2bks,[1] chain A) shows that the flap (red) is in the most probable state (see main text) and the Tyr83:OH$\cdots$Trp45:NE1 hydrogen bond is intact. A crystal structure of renin bound to a large inhibitor (PDB ID: 2bks,[1] chain B) shows a widely open flap (cyan) and the absence of the Tyr83:OH$\cdots$Trp45:NE1 hydrogen bond. b) The Tyr83:OH-Asp38:CG distance (R_1) vs. the Tyr83:OH-Trp45:NE1 distance (R_3) shows that the Tyr83$\cdots$Trp45 interaction is disrupted when the flap is widely open. The published apo and holo crystal structures of human renin were used. Data are color coded according to the crystallization pH condition: red (pH 3-4), orange (pH 4-5), cyan (pH 5-6), green (pH 6-7), blue (pH 7-8.5), and black (pH not provided in the PDB file).

References

(1) Powell, N. A.; Clay, E. H.; Holsworth, D. D.; Bryant, J. W.; Ryan, M. J.; Jalaie, M.; Zhang, E.; Edmunds, J. J. Equipotent activity in both enantiomers of a series of ketopiperazine-based renin inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2005**, *15*, 2371–2374.