Towards fast and accurate calculation of protein pK_a values

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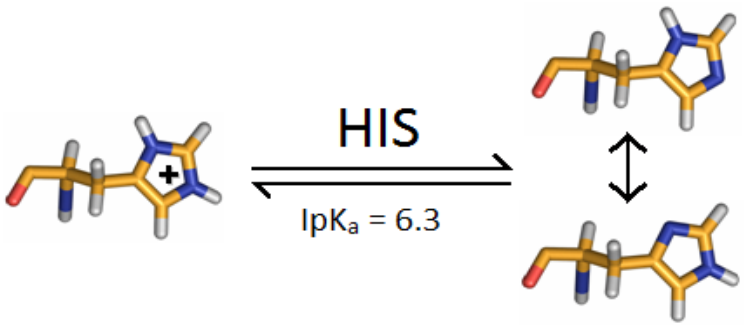
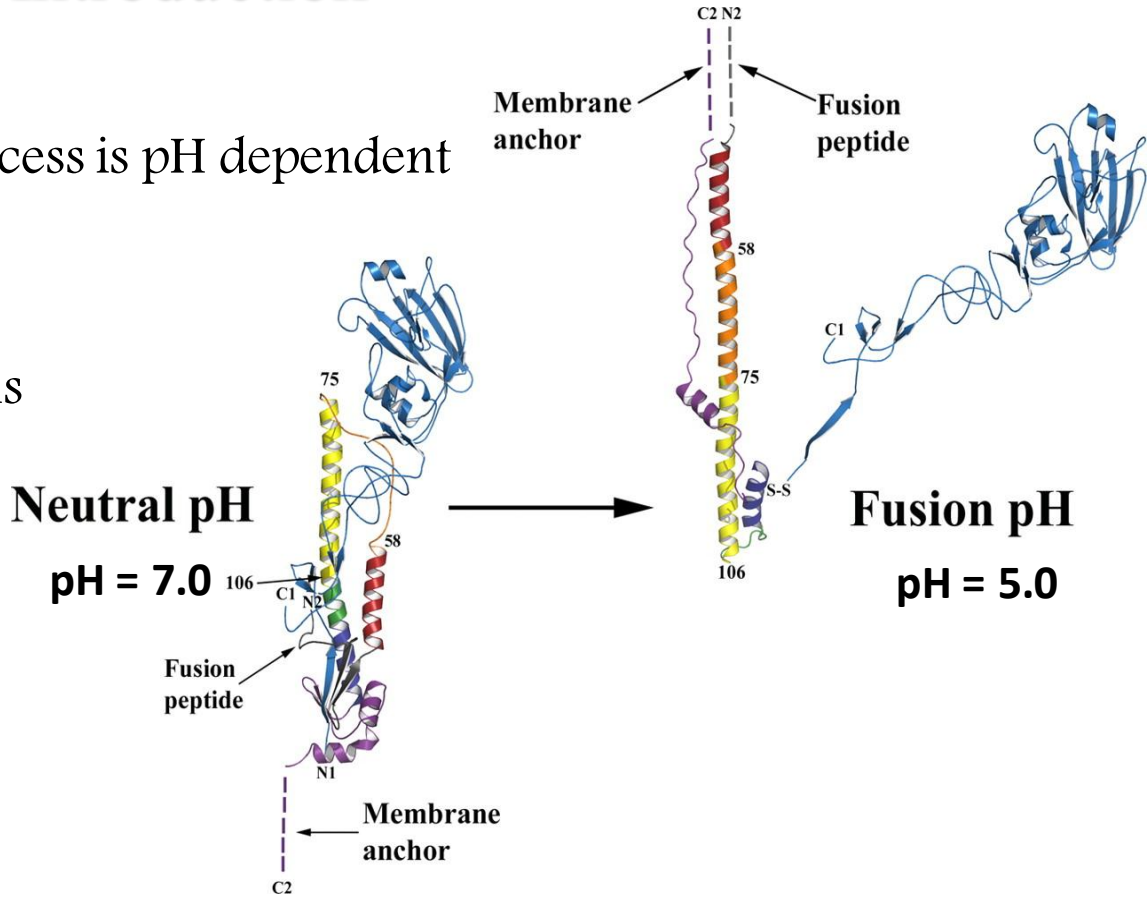
RosettaCon 2011



Introduction

- Virtually every biological process is pH dependent
  - protein folding
  - enzyme catalysis
  - protein-protein interactions
  - pathological conditions



- Influences protonation states of residues in proteins

Russell *et al.* 2008. Proc. Natl. Acad. Sci. USA. 105: 17736 -17741.

pK_a Values For Residues In Proteins

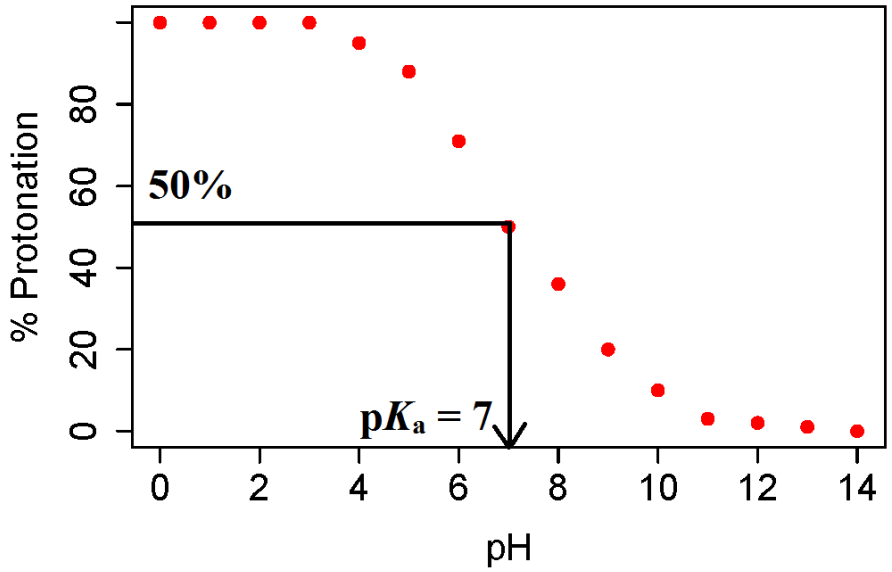
- Acid dissociation constant (pK_a) determines protonation state

$$[HA] \rightleftharpoons [H^+] + [A^-]$$

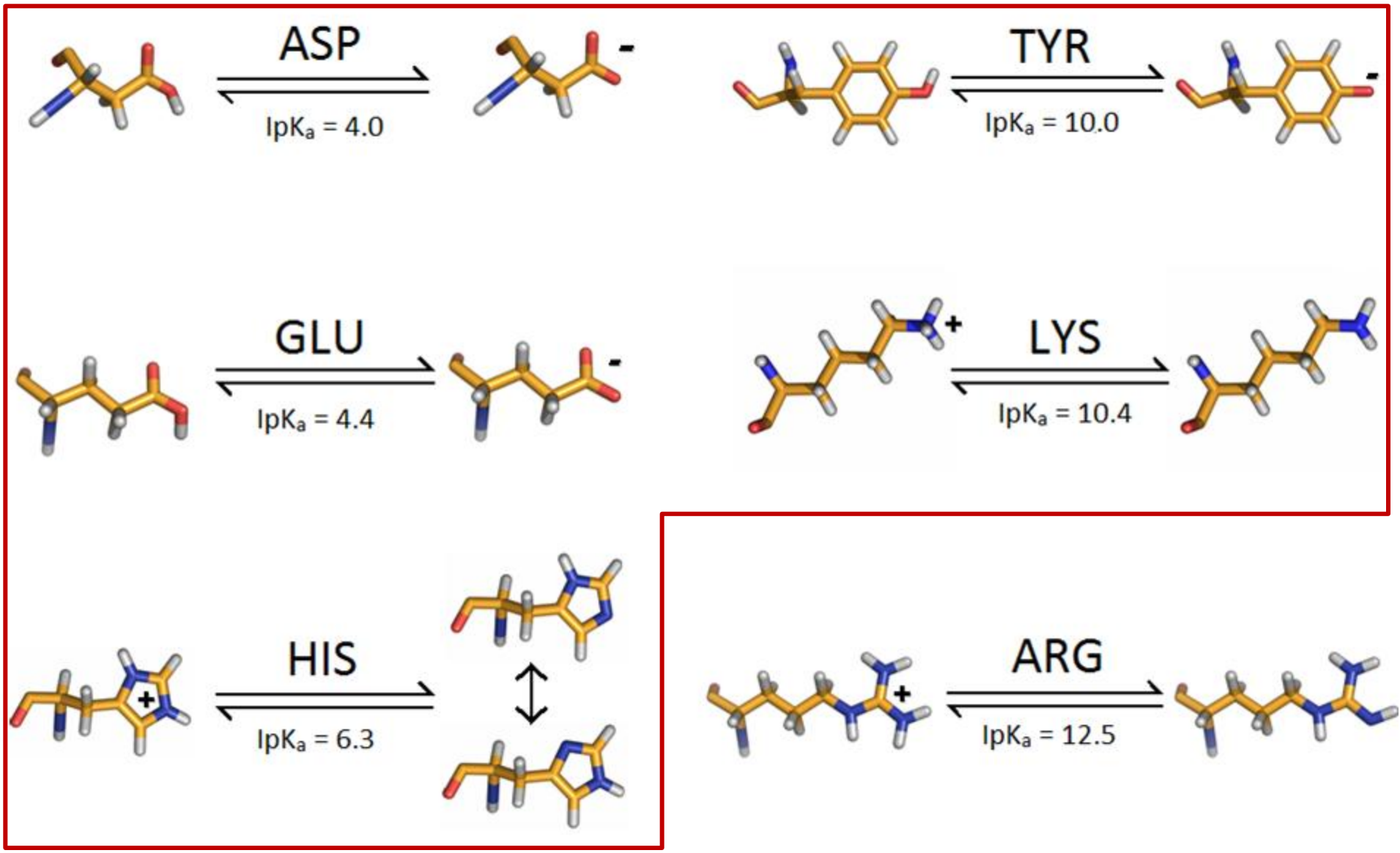
- Henderson–Hasselbalch equation

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

- pH at 50% protonation = pK_a



- Residue pK_a in protein ≠ Intrinsic pK_a in solution

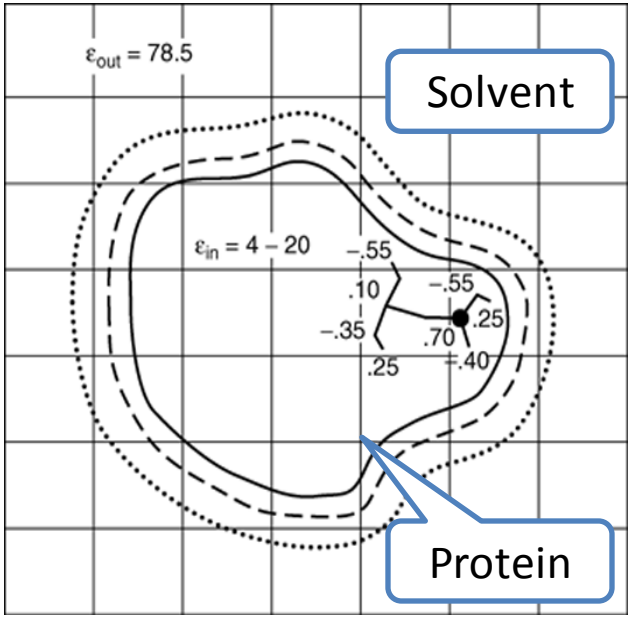

ASP
pK_a = 4.0
TYR
pK_a = 10.0
GLU
pK_a = 4.4
LYS
pK_a = 10.4
HIS
pK_a = 6.3
ARG
pK_a = 12.5

Current pK_a Prediction Methods

- Current methods
  1. continuum electrostatic models (Gunner, Harbury, McCammon etc.)
  2. all-atom MD simulations (Case etc.)
  3. empirical approaches (Jensen, Hellinga etc.)

- Advantages / Disadvantages
  - LPBE models overestimate charge interactions
  - empirical approaches are much faster

- **Rosetta toolset**
  - robust score function
  - varying levels of conformational flexibility
  - relatively computationally inexpensive

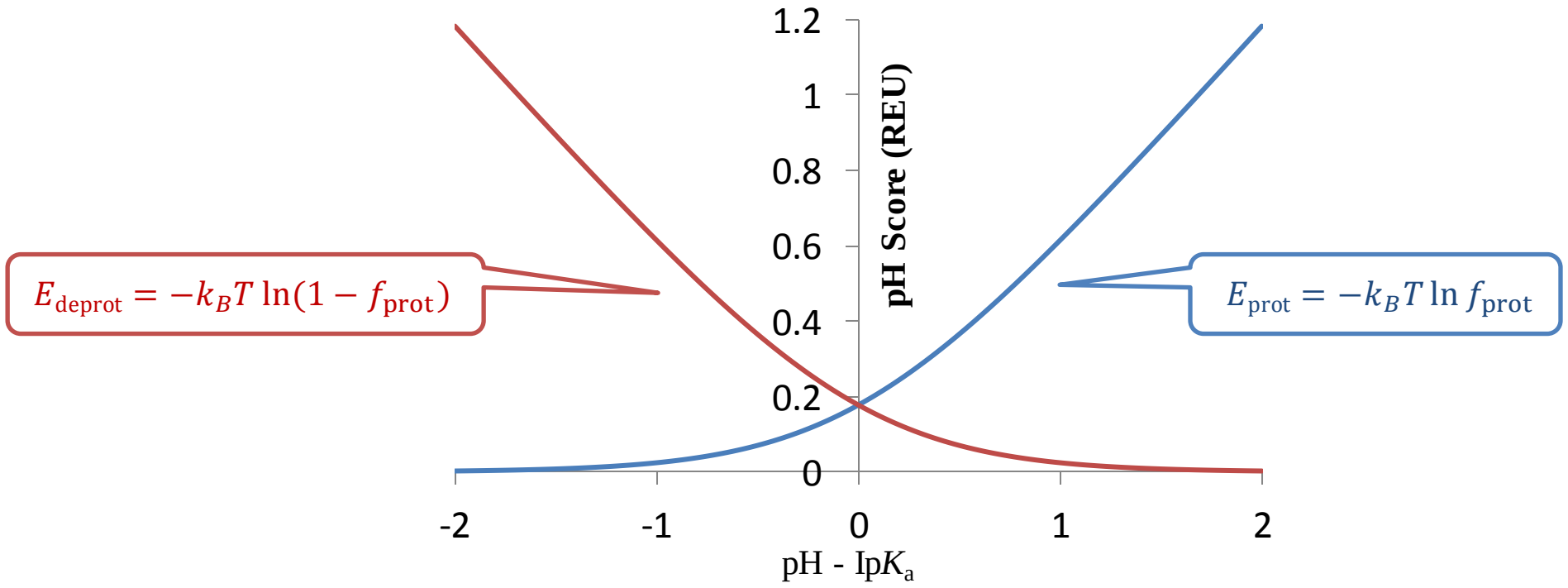


Protein-water system for FDPB calculations

Fitch, C.A., and B. García-Moreno E. 2002. Current Protocols in Bioinformatics

‘pH Score’ Captures Residue Protonation State Changes

- pH score
- Protonation probability $f_{\text{prot}} = \frac{1}{10^{\text{pH}-\text{Ip}K_a+1}}$ (Onufriev et al. 2001)
- $E_{\text{new}} = E_{\text{standard}} + E_{\text{pH}}$



Rotamer Frequency

Starting Structure
(Highly Acidic Environment pH = 1)

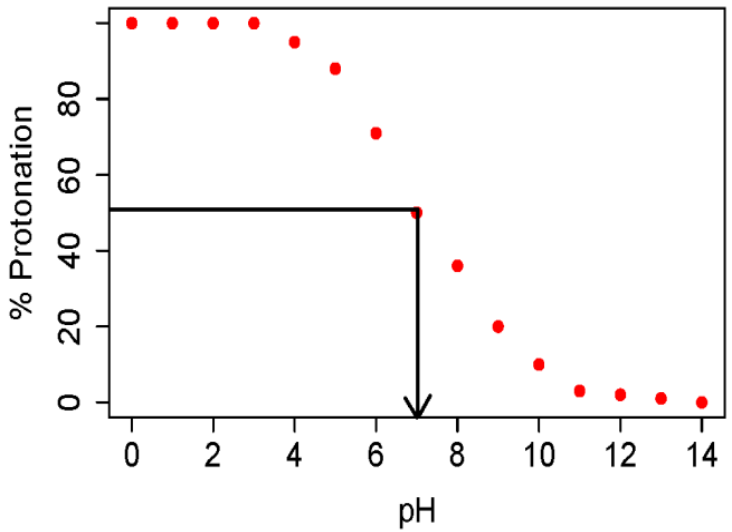
Sample Rotamer Library For Target Residue
(Includes Prot. And Deprot. Variants)

Collect Rotamer Protonation Statistics

Calculate pK_a From Residue Titration Plot

x 14

pH = pH + ΔpH
Titrate Until pH = 14



Starting Structure
(Highly Acidic Environment pH = 1)

Sample Rotamer Library For Target Residue
(Includes Prot. And Deprot. Variants)

Select Lowest Energy Rotamer

Did protonation state change?

NO

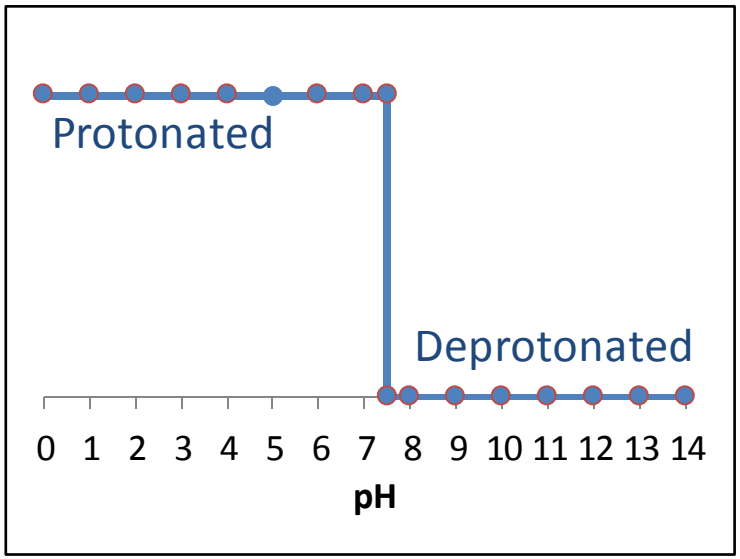
pH = pH + ΔpH

YES

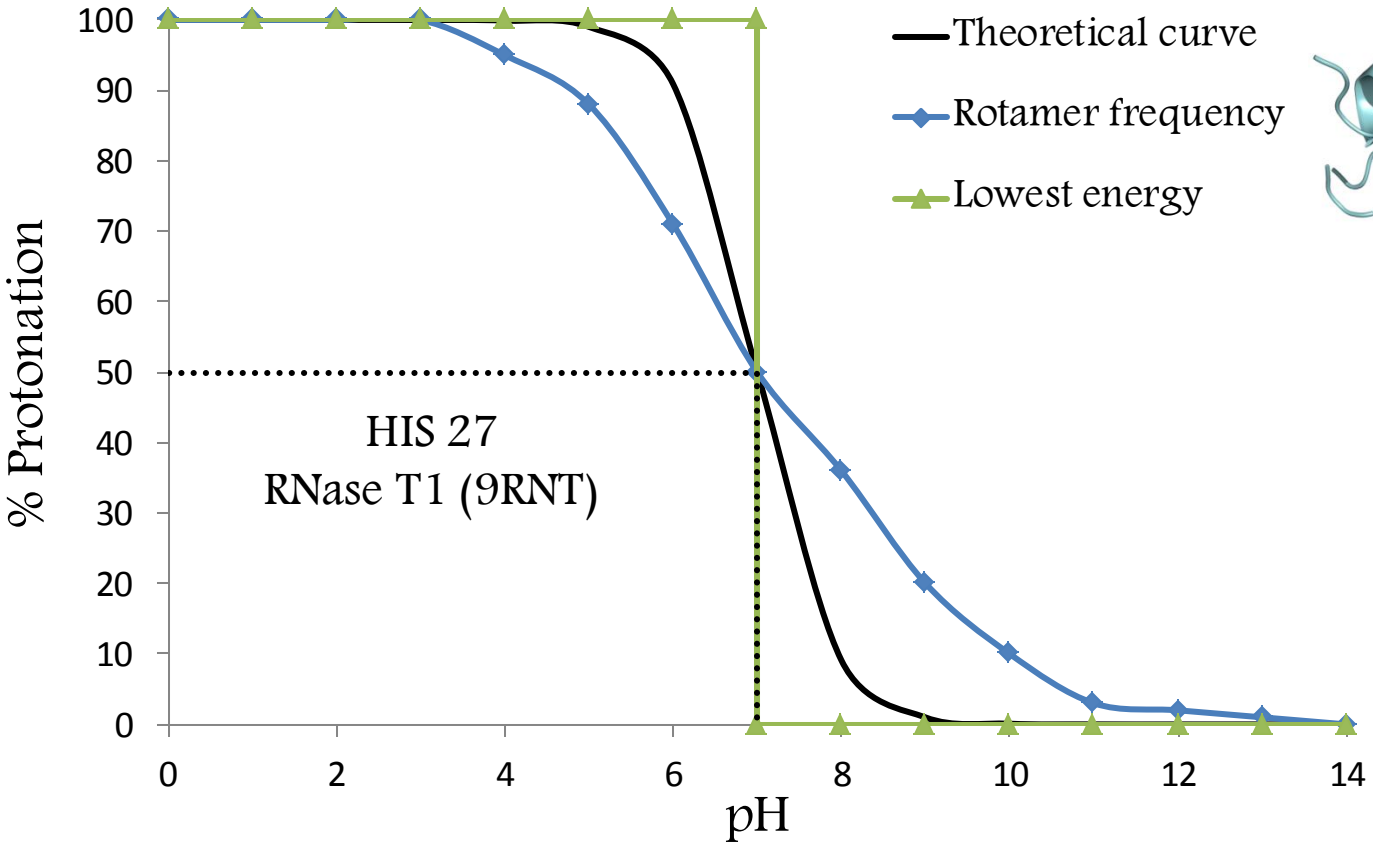
Output pK_a

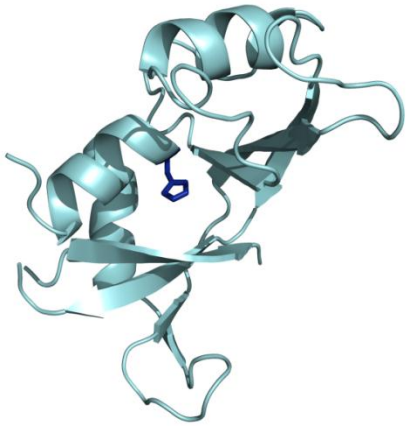
Lowest Energy

Optimize Rosetta Score Function For pK_a prediction

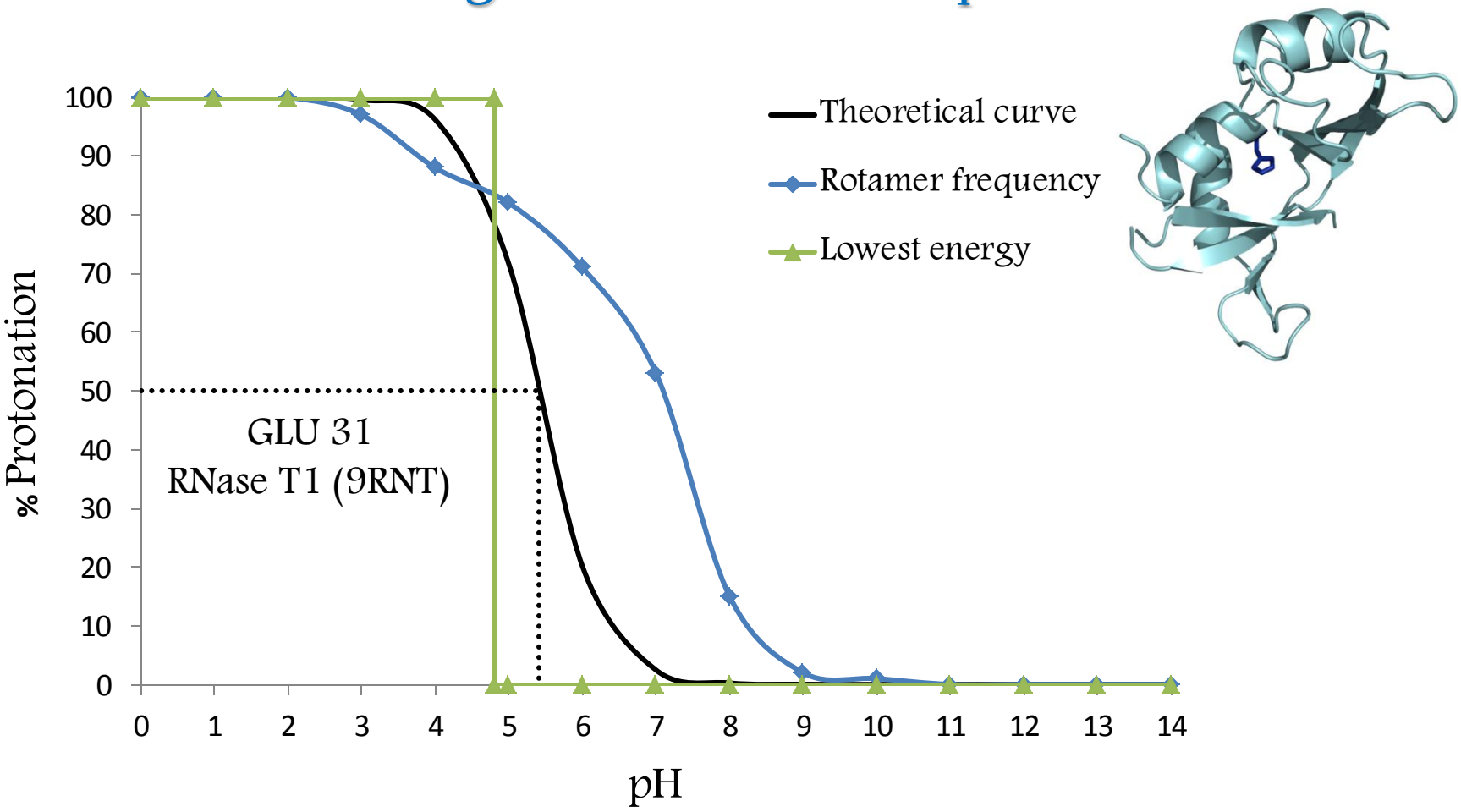


Lowest-Energy Conformation Is Better Than Boltzmann-Weighted Rotamer Frequencies





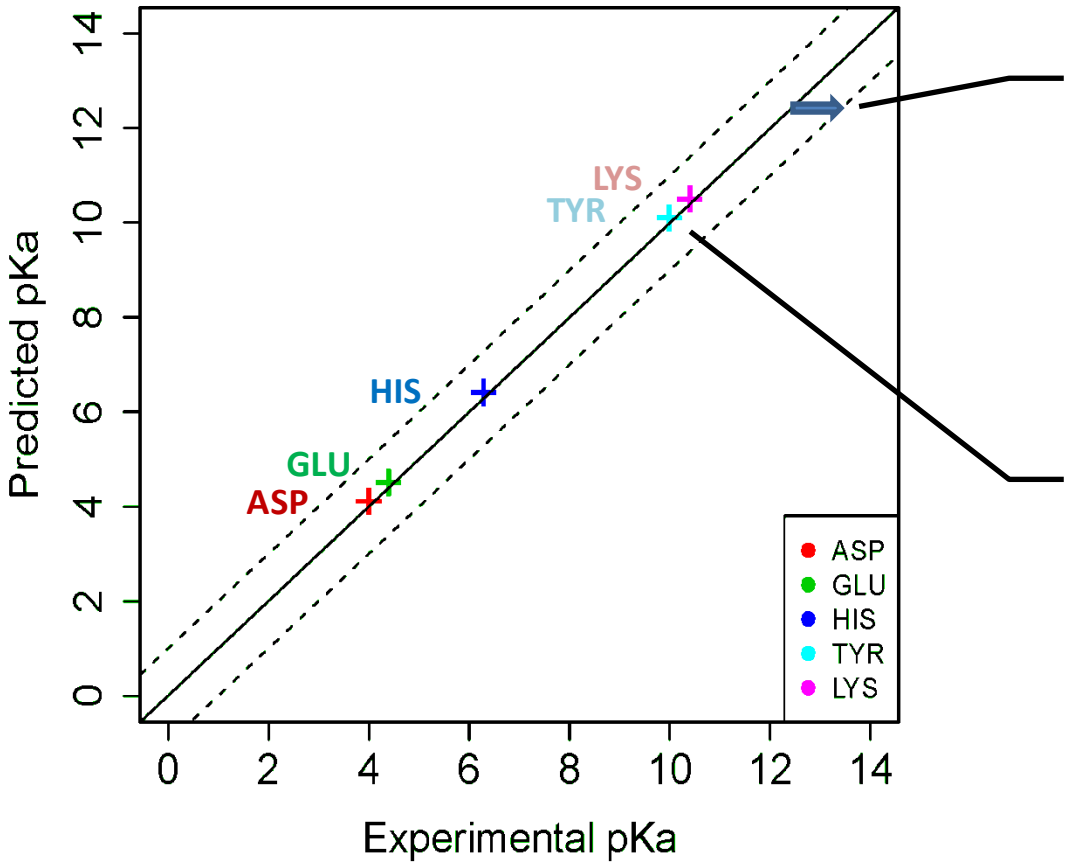
Lowest-Energy Conformation Is Better Than Boltzmann-Weighted Rotamer Frequencies



- Consistent with previous studies of side-chain entropy (Kortemme *et al.* 2002, Hu *et al.* 2006)

pK_a Prediction vs. Experiment

$$E = E_{\text{std}} + E_{\text{pH}}$$

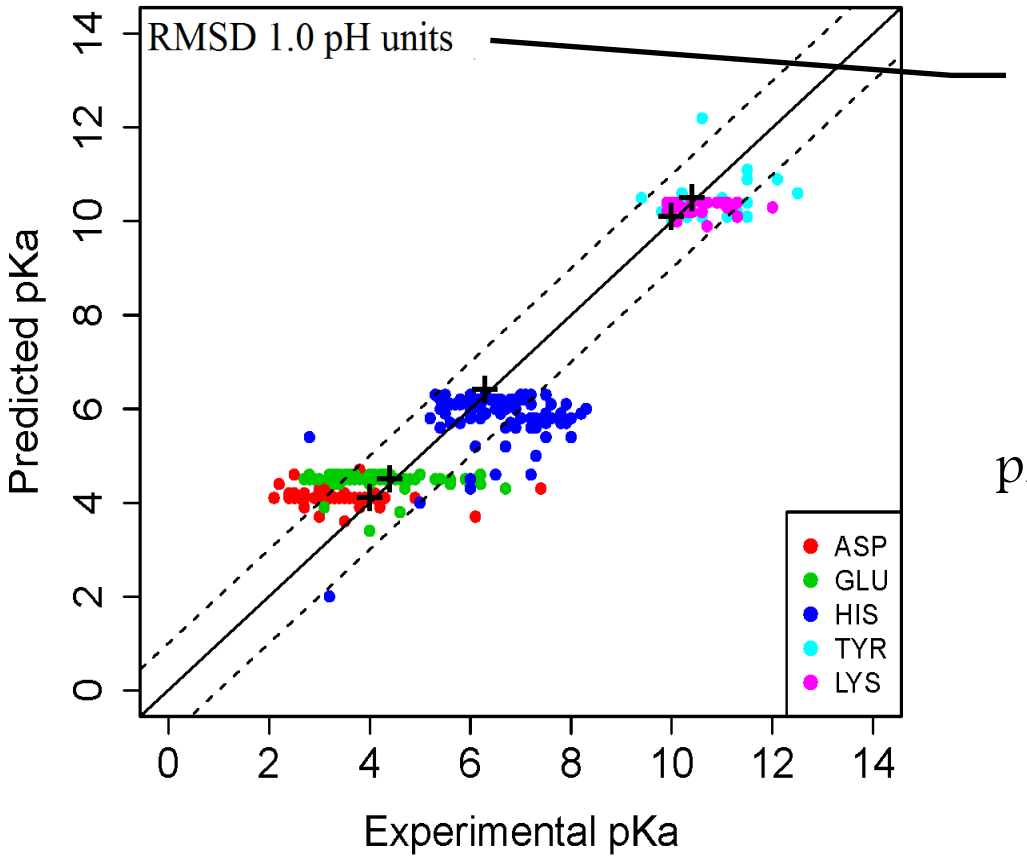


Error range 1 pH unit

Intrinsic pK_a of residue

Rosetta Standard Energy Function Predicts No pK_a Shifts

$$E = E_{std} + E_{pH}$$



RMSD of predicted pK_as from experimental pK_as

pK_a prediction methods (RMSD <1)

- MCCE2 RMSD 0.90 pH units
- PROPKA3 RMSD 0.79 pH units
- Null RMSD 0.92 pH units

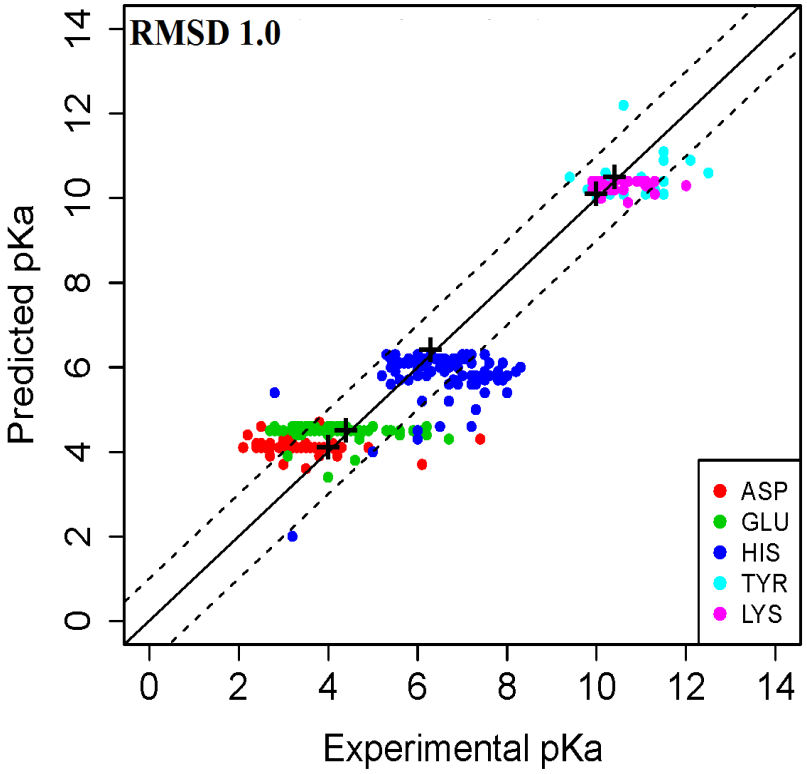
Song *et al.* 2009, Olsson *et al.* 2011

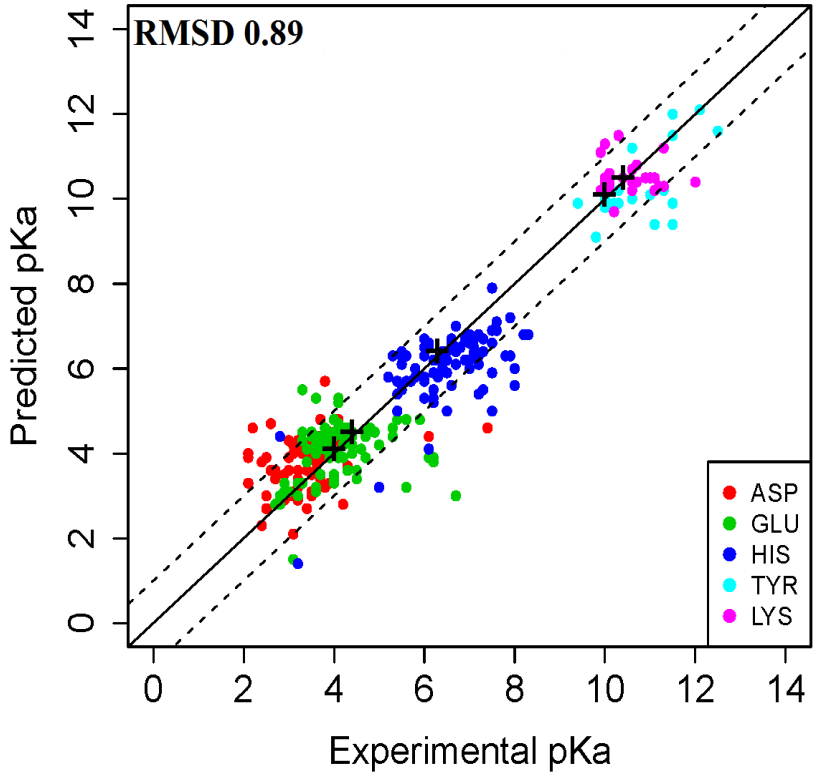
Using Explicit Coulomb Potential Improves pK_a Predictions

(hack_elec E $\propto \frac{q_1 q_2}{\varepsilon r}$; $\varepsilon \propto r$)

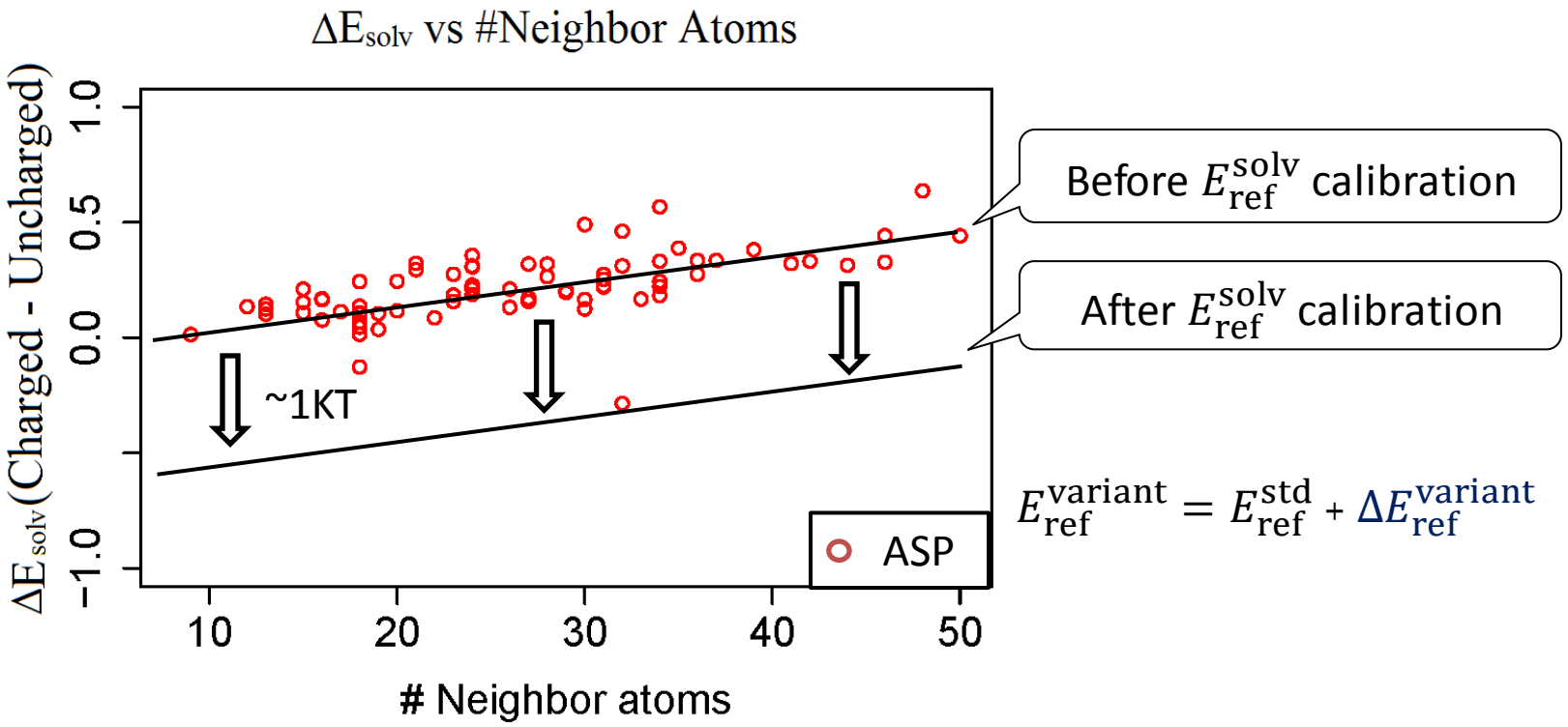
$$E = E_{\text{std}} + E_{\text{pH}}$$

$$E = E_{\text{std}} + E_{\text{pH}} + E_{\text{elec}}$$





Recalibrating LK Solvation Reference Energies For New Residue Protonation Variants

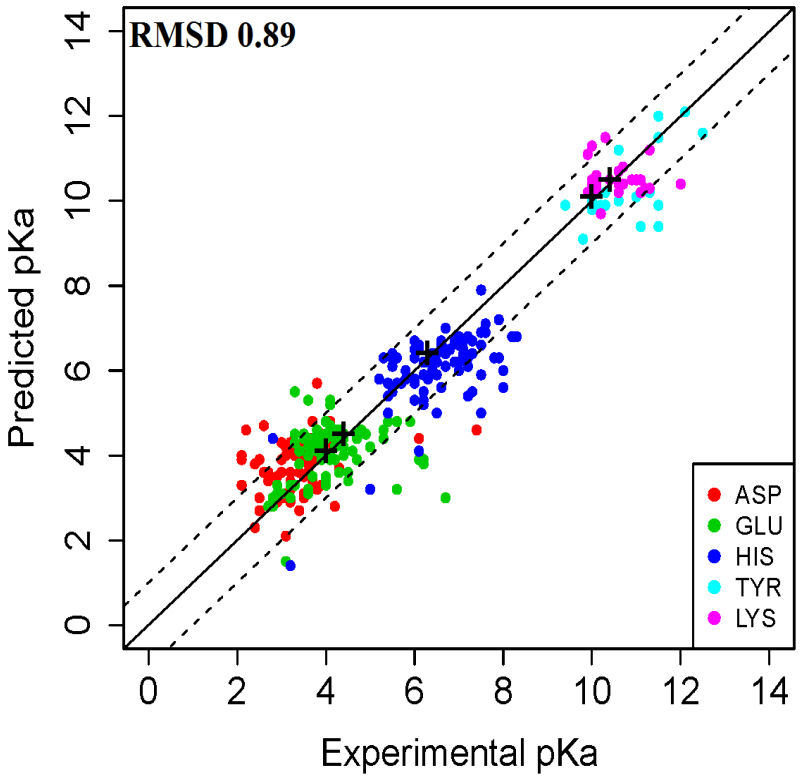


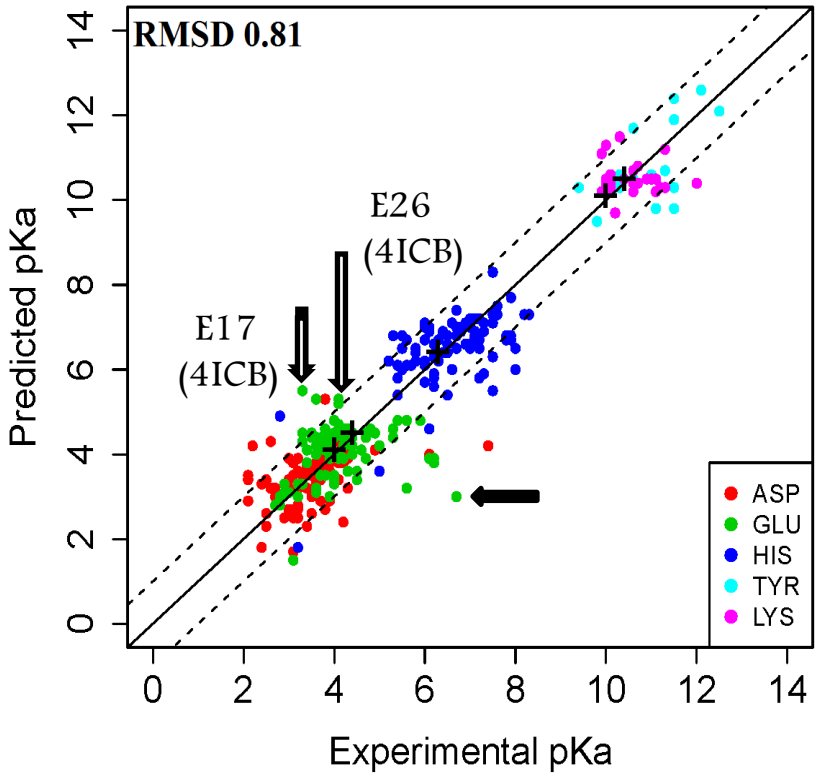
- $E_{charged}^{solv} - E_{uncharged}^{solv} \sim 0$ when exposed to solvent
- $\Delta E_{residue}^{solv} = \sum_{atoms}(\Delta E_{i}^{ref} - \sum_{j \neq i} f_i(r_{ij}) V_j)$ (Lazaridis *et al.* 1999)

Rosetta pH Energy (RPH) Function For pK_a Prediction

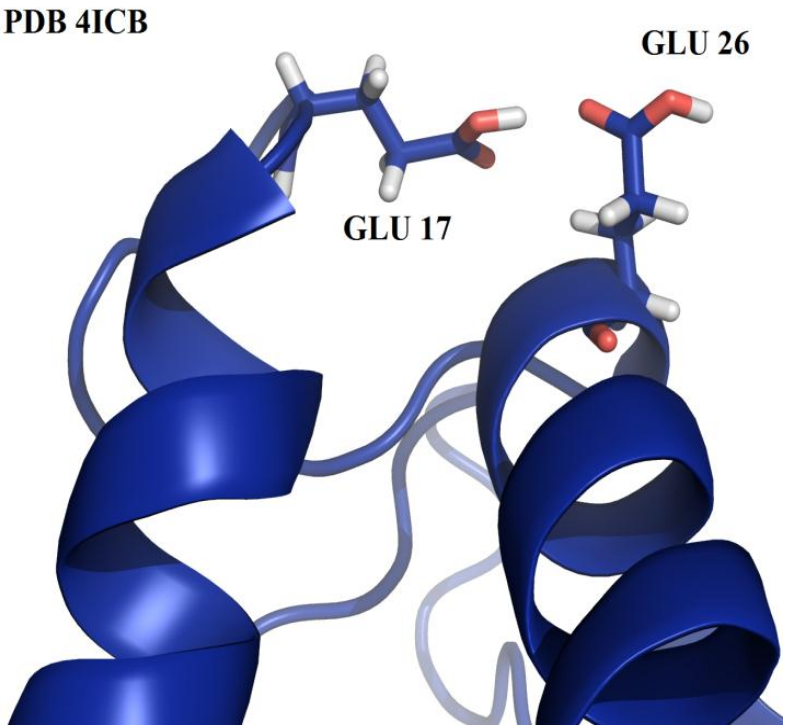
$$E = E_{std} + E_{pH} + E_{elec}$$

$$E = E_{std} + E_{pH} + E_{elec} + E_{ref}$$



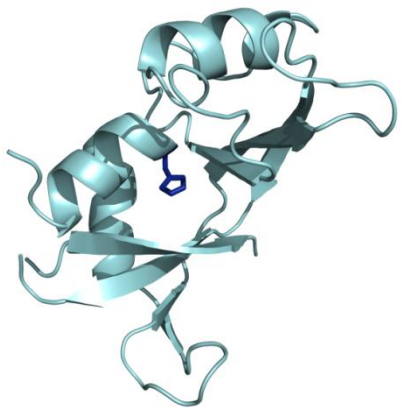


Errors ?

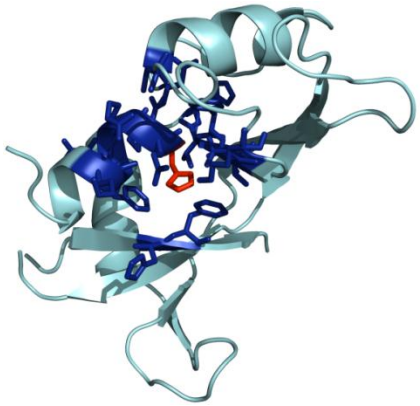


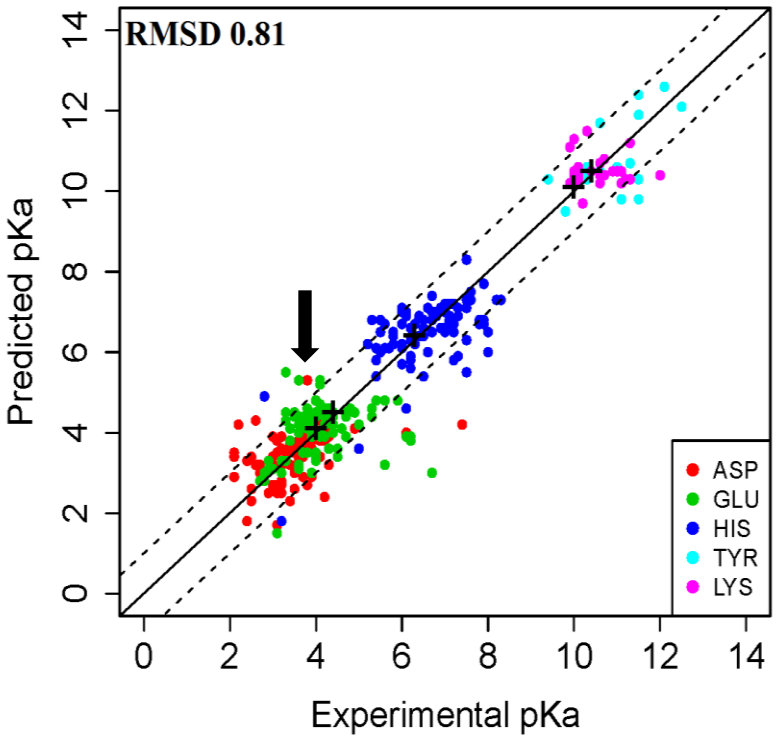
Glu-Glu interaction in Calbindin D9K

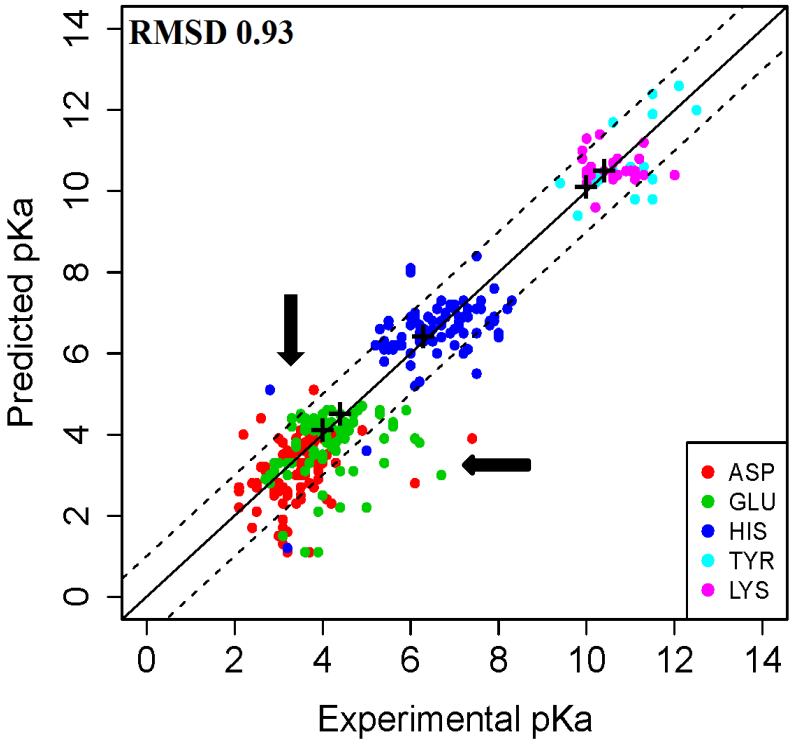
Packing Neighbors (6Å): Some Improvements, But Negates pK_a Shifts



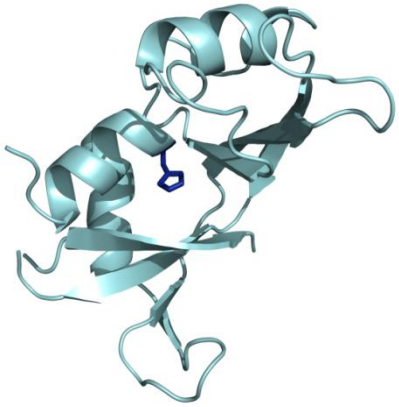
Repack < 6Å



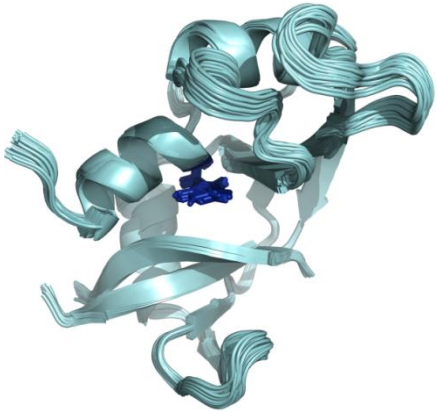


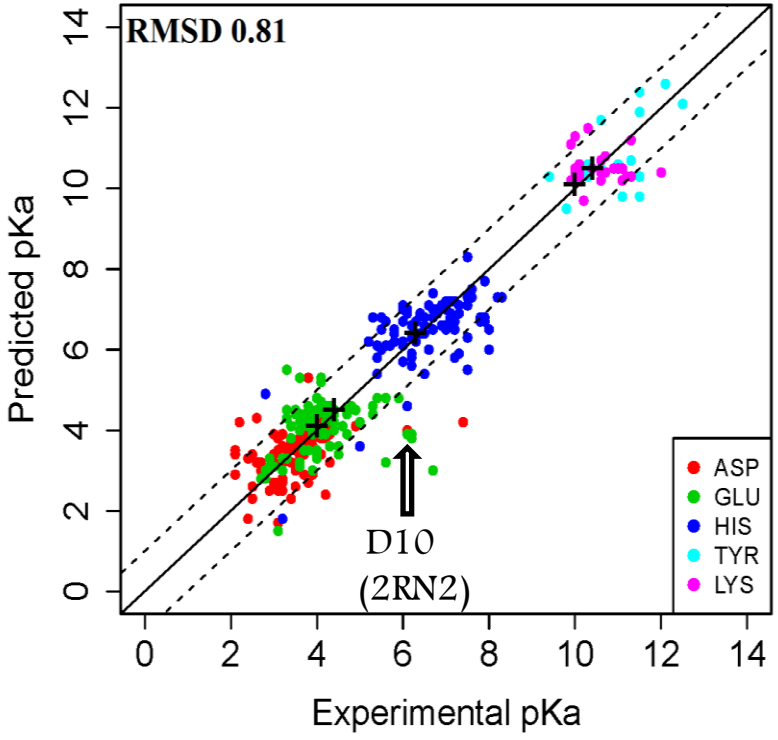


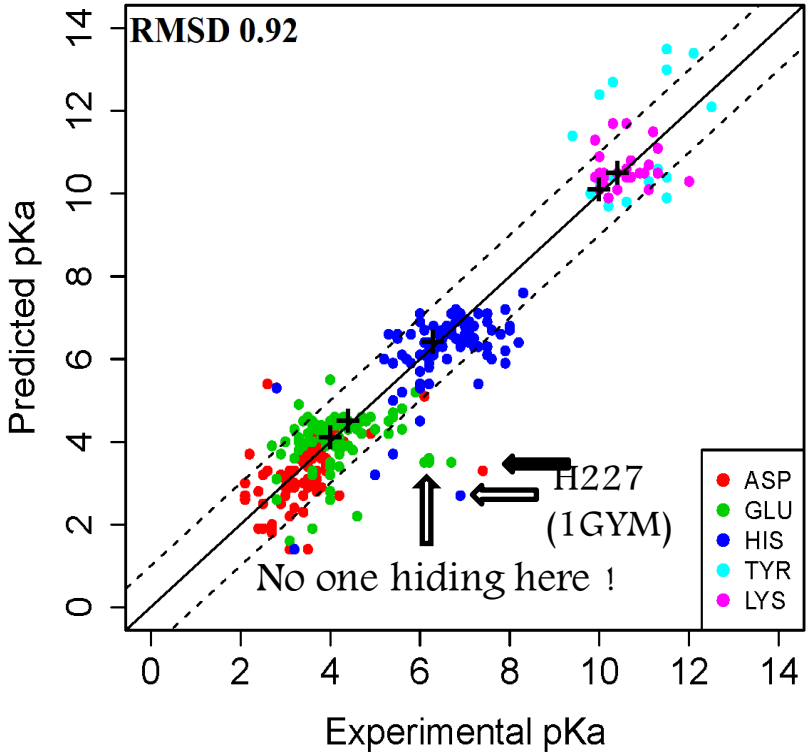
Structural Ensemble : Large Range Of Predictions → Highly Sensitive To Backbone Conformation

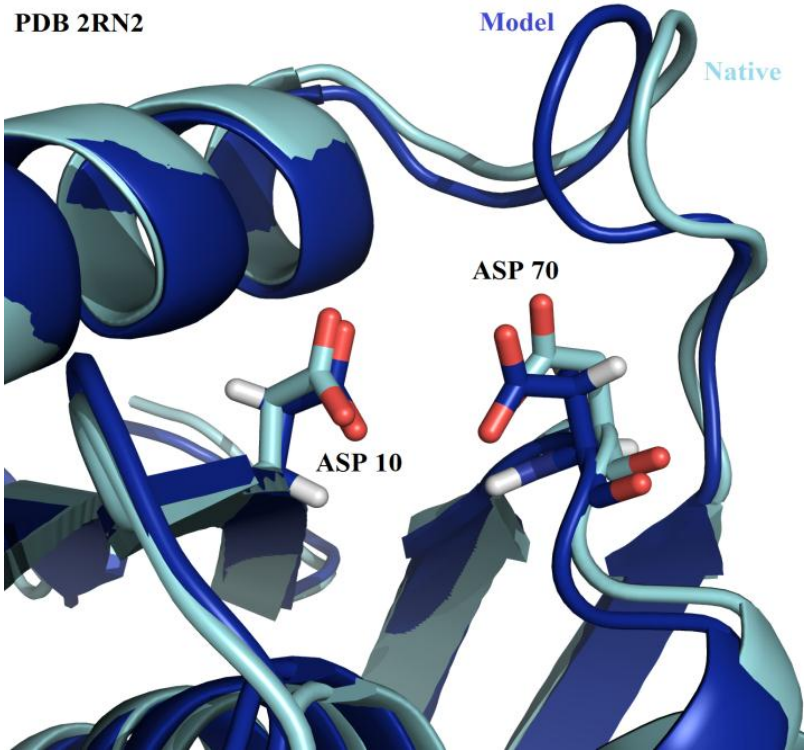


ClassicRelax

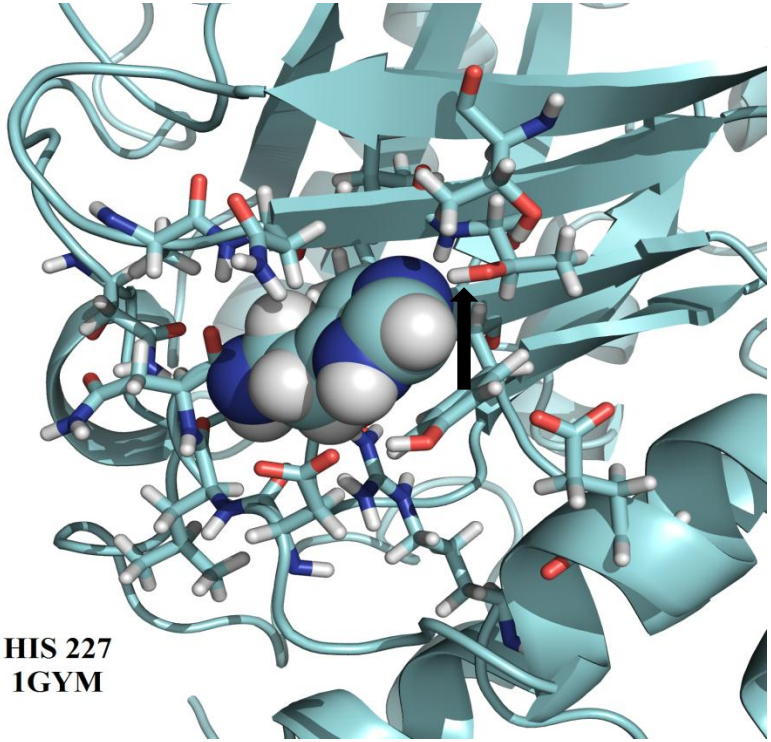








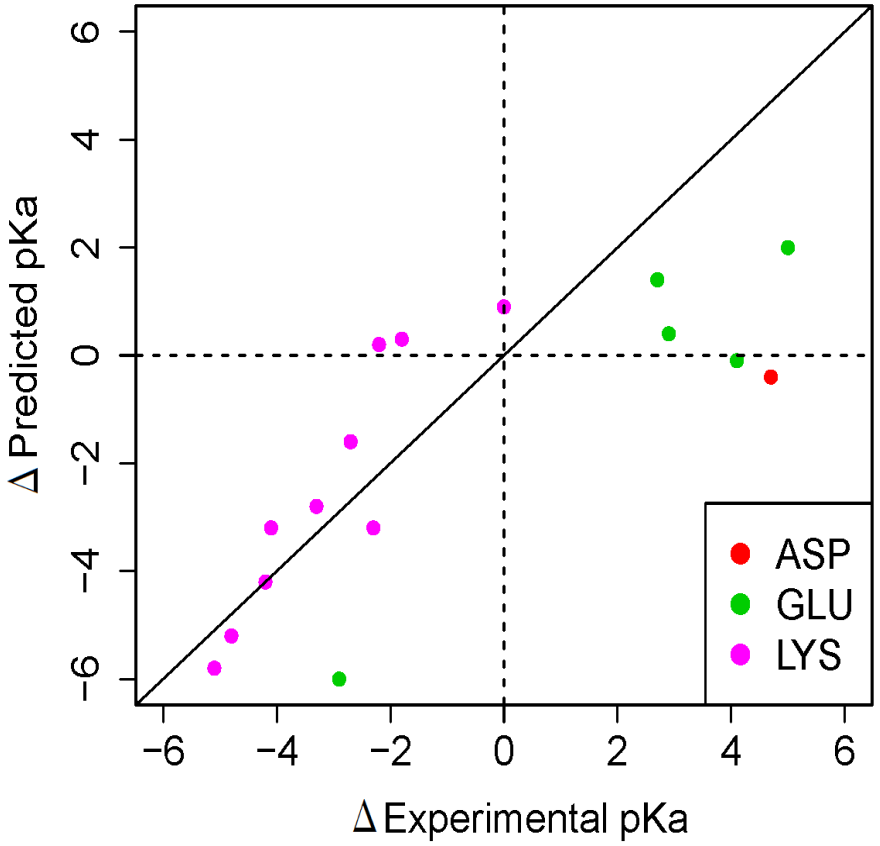
Asp–Asp interaction in RNase H captured with backbone flexibility

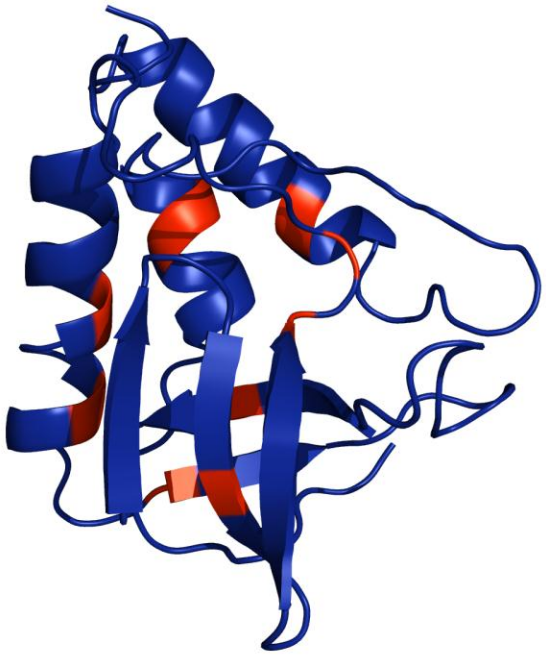


His in Phospholipase C is constrained with no room for a proton

Rosetta Accurately Predicts Some Extreme pK_a Shifts

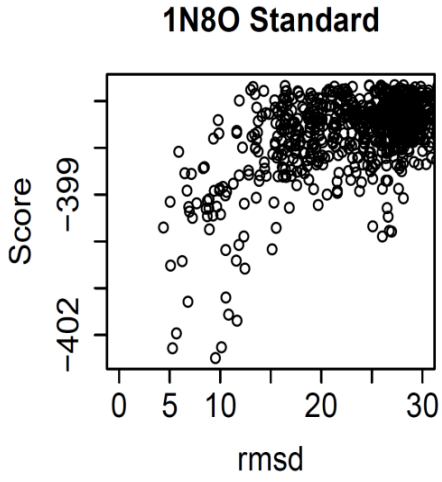
- New mutants of *Staphylococcal nuclease* with dramatic shifts up to 5 pH units (~ 7 kcal/mol)
  [Bertrand Garcia Moreno lab / JHU]

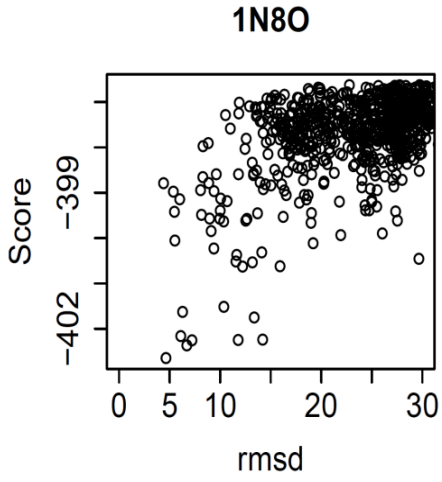


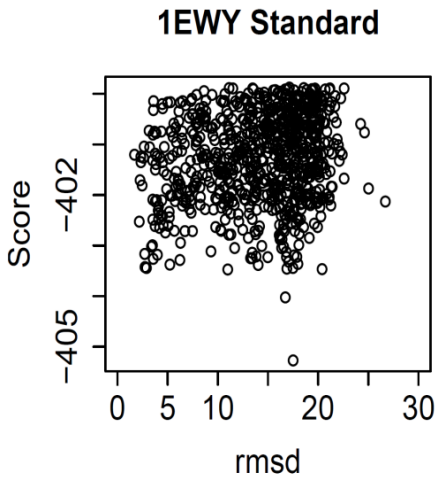


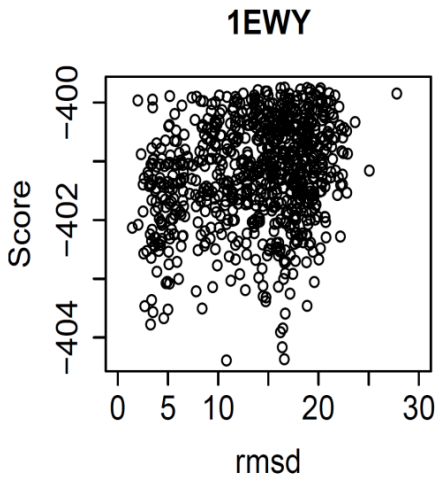
Application to protein-protein docking

- 30% of interface residues ionizable in Docking Benchmark set
- 90% of interfaces – at least one residue changes protonation state (Aguilar *et al.* 2010)
- Docking results with early score function show some improvements (work in progress)









Summary / Future Work

- Explicit electrostatic model is essential for accurate pK_a predictions
- RPH mode predicts pK_as to 0.81 RMSD, comparable to leading methods
- Higher conformational flexibility –
  1. Improves accuracy of some pK_a predictions
  2. Use of pH mode during ClassicRelax to be tested
- **pK_a prediction test can be used to benchmark score functions**
- Protonation states may improve docking, folding, and design

Acknowledgments

- Prof. Jeffrey J Gray
- Prof. Bertrand Garcia Moreno
- Ryan Harrison
- Lab members
- NIH R01 - GM 078221
- Rosetta Community

Thank You

Questions?

Supplemental Data

	Number of χ angles	*Null Model*	*RPH Function*	*Neighbor Repack*	*Ensemble Average*
Asp	2	0.95	0.77	0.97	0.8
Glu	3	0.86	0.92	1.1	0.95
His	2	0.98	0.79	0.84	0.97
Tyr	2	1.1	0.76	0.76	1.3
Lys	4	0.56	0.67	0.66	0.68
All	-	0.92	0.81	0.93	0.92

Work with Docking benchmark set 3.0

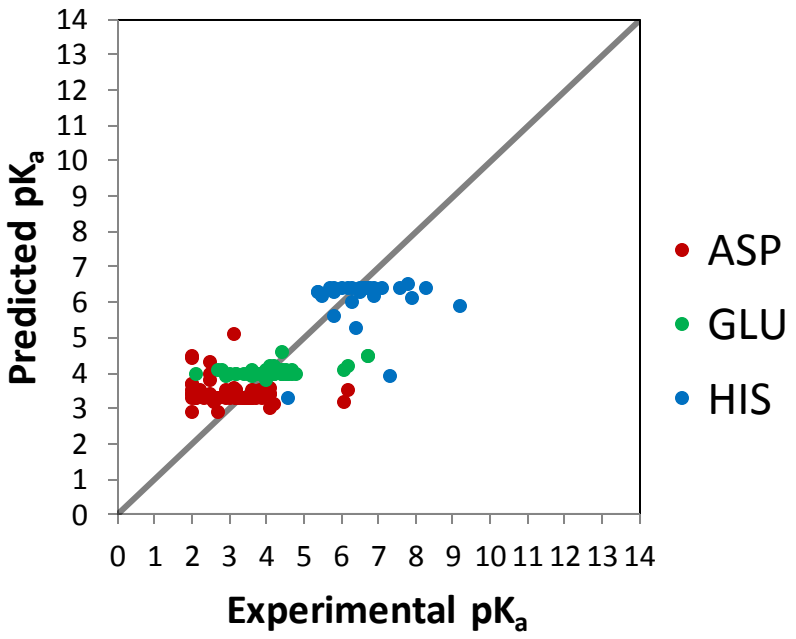
Total number of interface residues (within 5A at the interface)

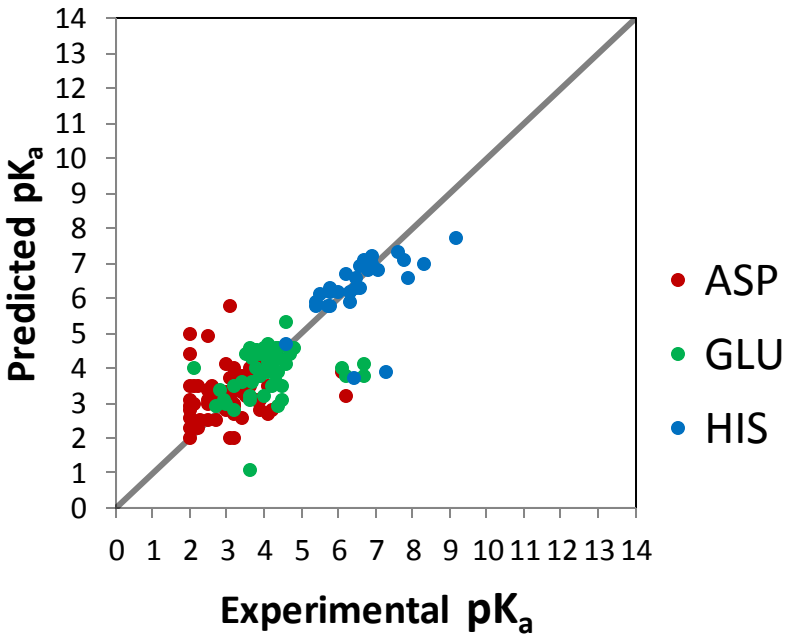
Type	All	Enz-inh	Ab-Ant	Others
ASP	6.4	6.9	6	6.2
GLU	6.8	5	5.6	8.2
HIS	2.9	4.2	2.2	2.5
TYR	6.5	6.6	11	5
LYS	6.2	4.6	4.7	7.5
Chargeable %	28.8	27.3	29.5	29.4
Non-chargeable %	71.2	72.7	70.5	70.6

pK_a shifts at interface

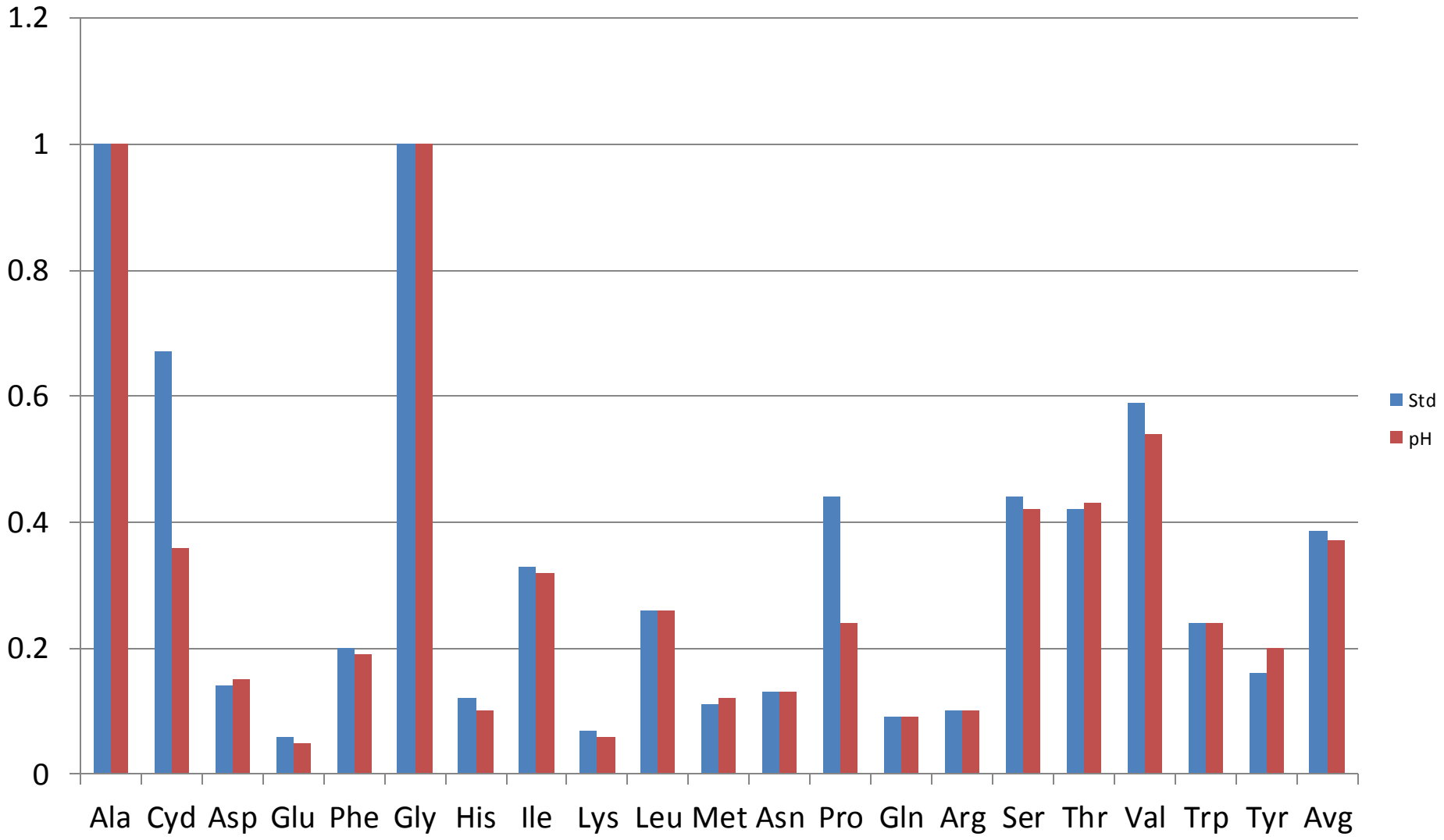
Residue	Total no	Neg_shift	Pos_shift	Avg_shift
ASP	360	233	90	0.72
GLU	381	222	132	0.67
HIS	132	71	55	0.89
TYR	328	143	152	0.69
LYS	330	90	211	0.72

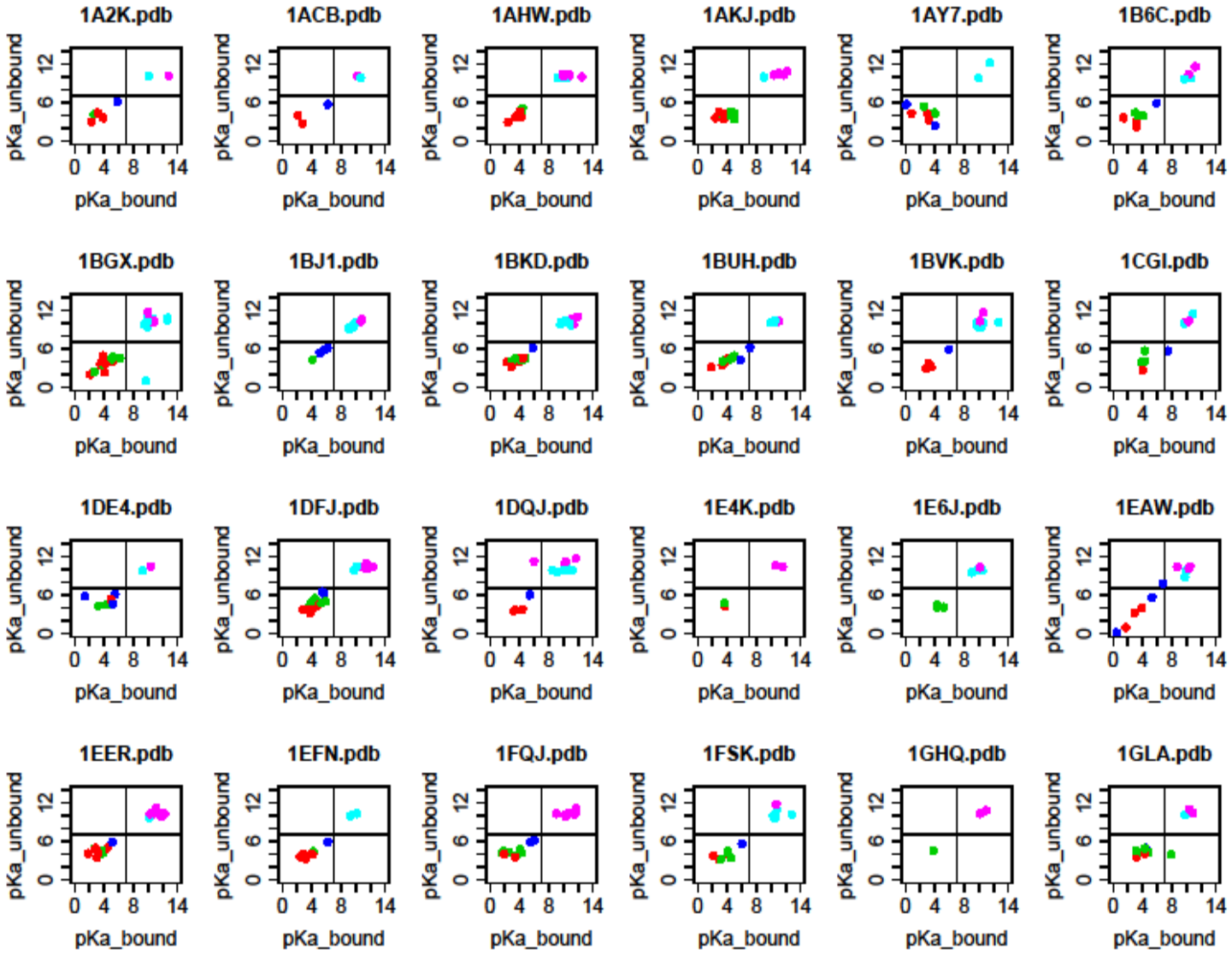
Electrostatic model





Rotamer Recovery

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1BKD.pdb
1BUH.pdb
1BVK.pdb
1CGI.pdb
1DE4.pdb
1DFJ.pdb
1DQJ.pdb
1E4K.pdb
1E6J.pdb
1EAW.pdb
1EER.pdb
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1FQJ.pdb
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pKa_bound
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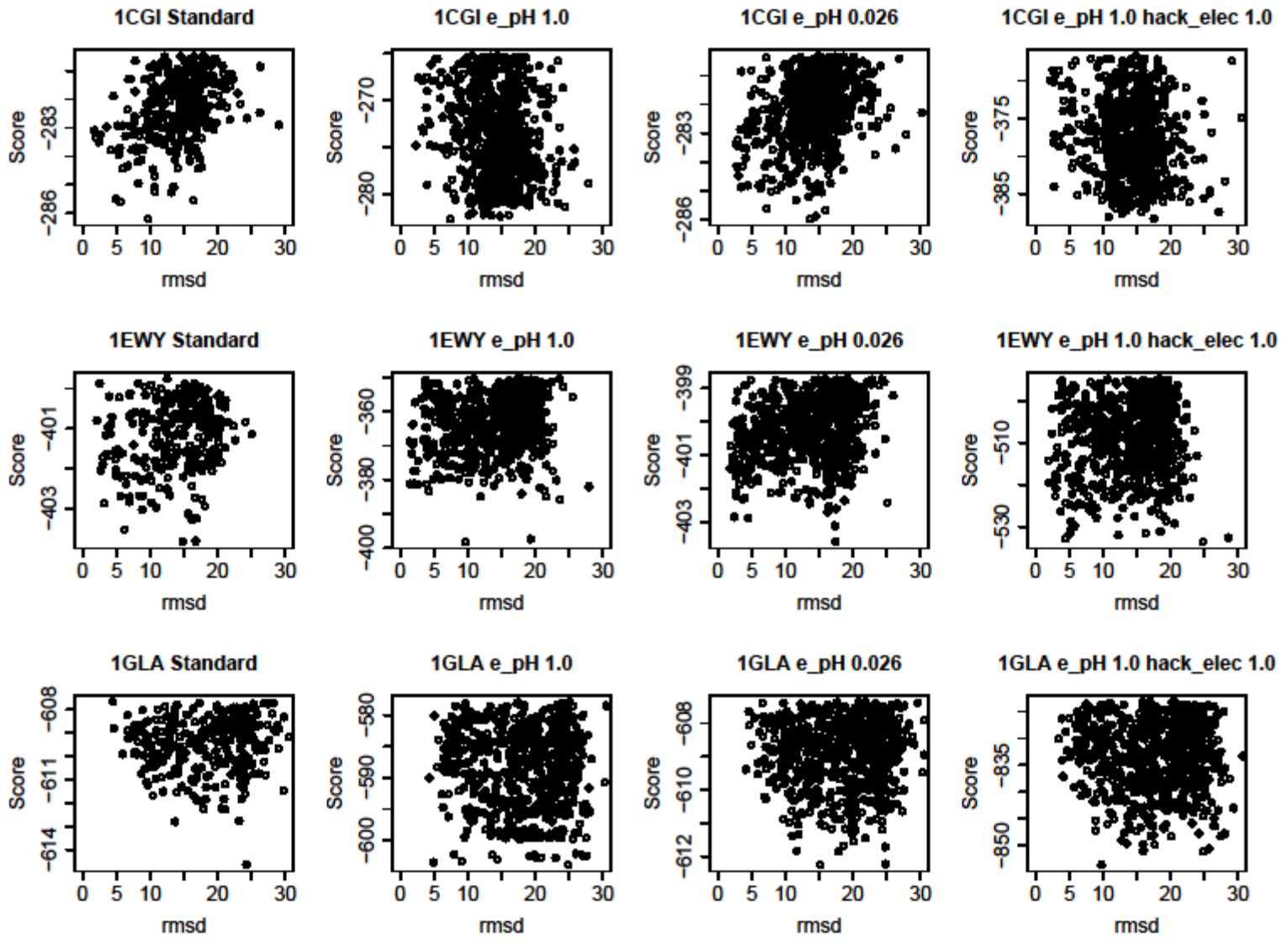
12
0
4
8
14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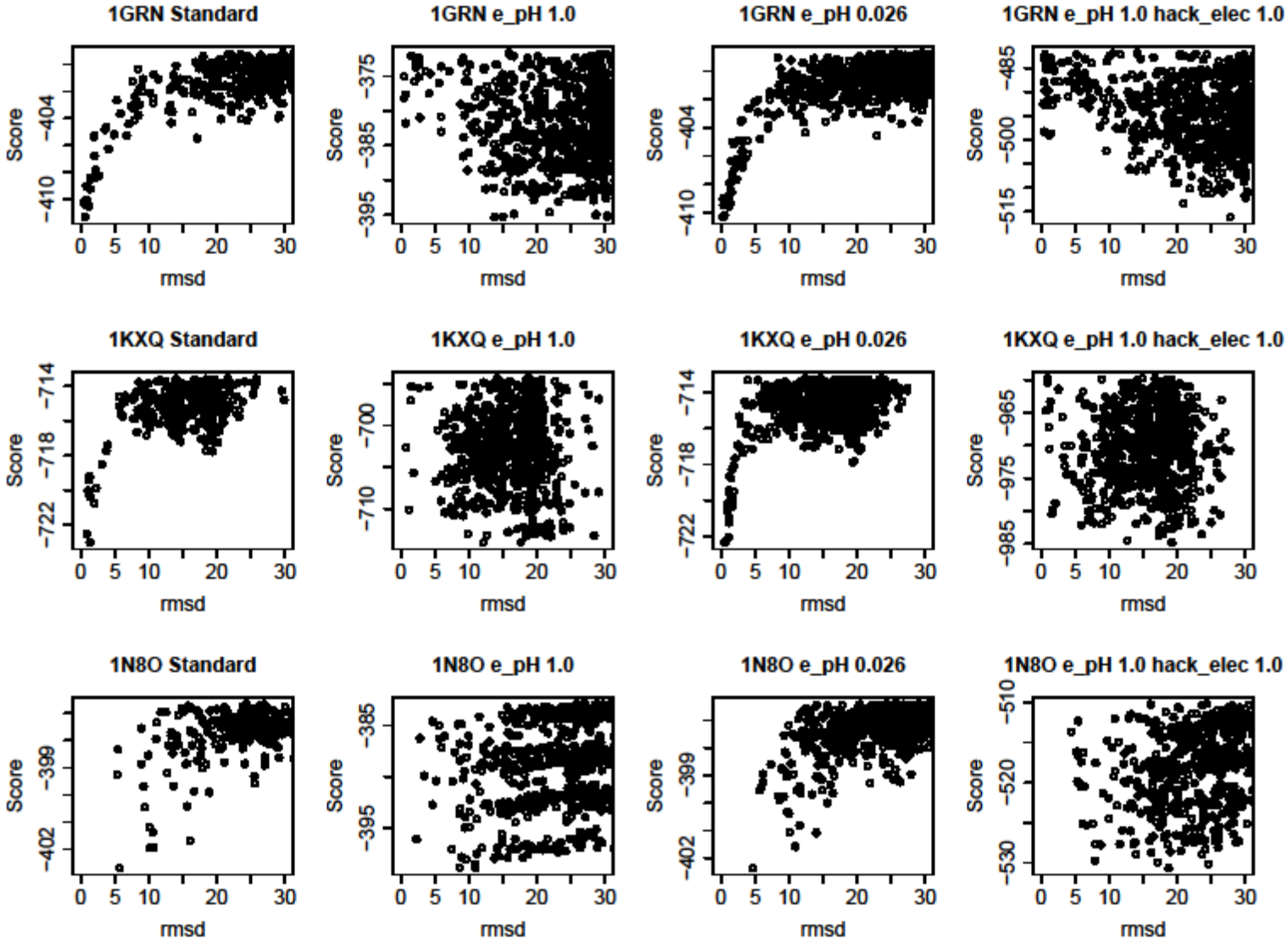
1CGI e_pH 0.026
1CGI e_pH 1.0 hack_elec 1.0
1EWY Standard
1EWY e_pH 1.0
1EWY e_pH 0.026
1EWY e_pH 1.0 hack_elec 1.0
1GLA Standard
1GLA e_pH 1.0
1GLA e_pH 0.026
1GLA e_pH 1.0 hack_elec 1.0
Score
rmsd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1KXQ e_pH 1.0 hack_elec 1.0
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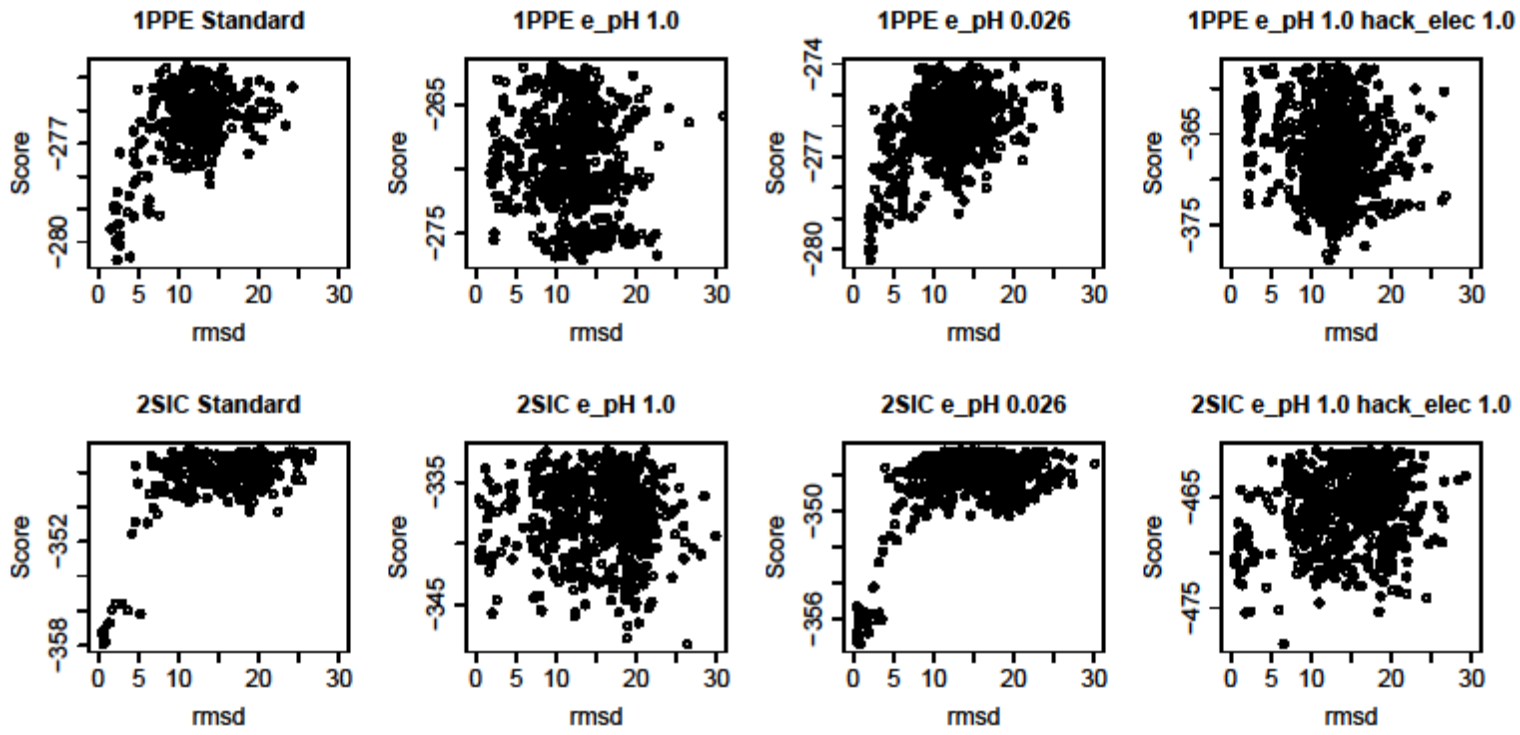
1N8O e_pH 1.0 hack_elec 1.0
Score
rmsd


1PPE Standard
1PPE e_pH 1.0
1PPE e_pH 0.026
1PPE e_pH 1.0 hack_elec 1.0
2SIC Standard
2SIC e_pH 1.0
2SIC e_pH 0.026
2SIC e_pH 1.0 hack_elec 1.0
Score
rmsd

Starting Structure
(Highly Acidic Environment pH = 1)

Sample Rotamer Library For Target Residue

NO

pH = pH + ΔpH

Did protonation state change?

YES

Output pK_a

Select Lowest Energy Rotamer

Calibrate Rosetta Score Function For pK_a prediction

Target Residue

Neighbors (< 6Å)

Backbone

Conformational Flexibility