Computational Prediction of Isoelectric Point and Acidity of Pyridinophane Macrocycle Derivatives using Density Functional Theory

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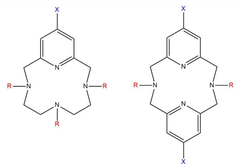
INTRODUCTION



Modes of antioxidant activity[1]:
- Metal binding
- N-oxide formation
- Radical scavenging
- Activation of Nrf2 pathway

→ ROS → Oxidative stress

Why pKa and pI?

Ionization states affect a compound's rate of diffusion through membranes (in this case the Blood-Brain Barrier)

pKa influences:
- Lipophilicity
- Solubility
- Protein binding
- Permeability

} **Directly affect pharmacokinetic characteristics (ADME)[2]**

LONG-TERM GOAL:

Predict pKa and pI values of drug candidates & incorporate into a virtual screening protocol.

OBJECTIVES:

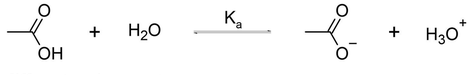
1) Predict various pKa values of H-PyN3 and H-Py2N2 using DFT-computed Gibbs free energies, a linear free energy relationship (LFER), and SMD solvent continuum model with scaled cavity.

2) Predict differences in pI value of any given modified PyN3 structures compared to the desired pI value of OH-Py2N2 using computed protonation energies.

THEORY & METHOD

Computation of pKa requires an appropriate environment and solvent model

Example dissociation reaction of acetic acid:



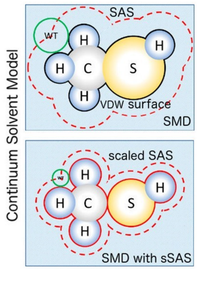
pKa of acetic acid in different environments:

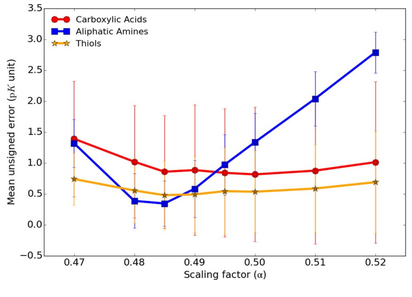
Water	4.76
Gas	>100*

Solvation of the proton in aqueous solution makes the dissociation reaction more energetically favorable

*Value calculated using the Gibbs free energy of the dissociation reaction in Taft et al.[3]

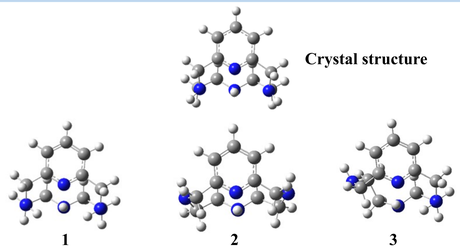
Using a SMD solvent continuum model with a scaled cavity improves error to 0.5 pK units in a previous study with many carboxylic acids, aliphatic amines, and thiols.[4]





Each neutral and protonated species has multiple conformers and protonation sites

For this study, the lowest-energy conformers of each neutral and protonated species was used to compute Gibbs free energy and pKa using the below **linear free energy relation (LFER)**[4]. The conformer ensemble was generated using **CREST conformer analysis**[5].

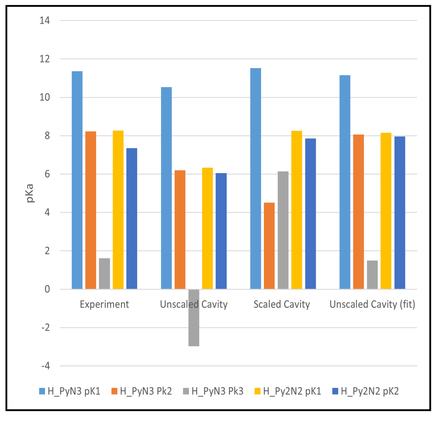


Prediction of pKa values from computed Gibbs free energies:

$$pKa = 627.5095\frac{Hatree}{kcal/mol} \times \frac{\Delta G(A^-) - \Delta G(HA) + \Delta G(H^+)}{RTln(10)}$$

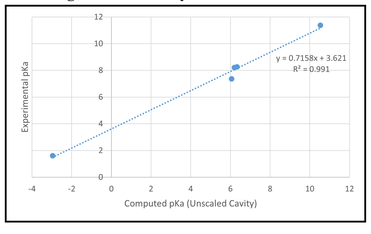
Example conformers	Energy relative to minimum (kcal/mol)
1	0
2	+2.2
3	+2.5

RESULTS

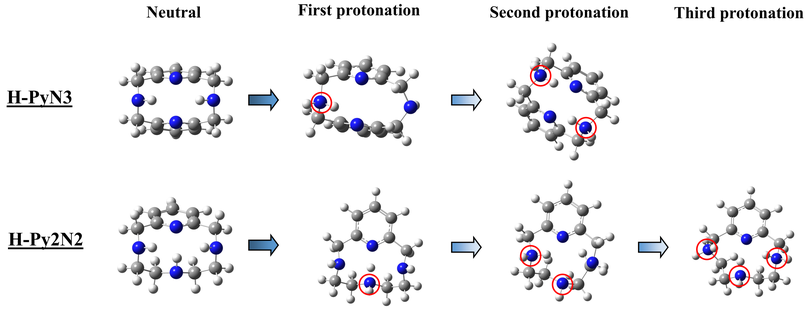


This graph shows the comparison of experimental pKa values and computed pKa values using 3 different methods for H-PyN3 and H-Py2N2 (Unscaled cavity, scaled cavity, and unscaled cavity fit with experimental values)

Linear fit of experimental vs computed pKa values using unscaled cavity:



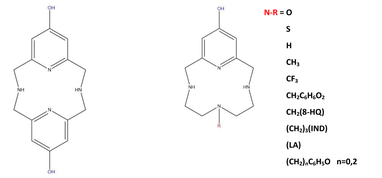
Lowest energy conformations of the most stable neutral and protonated species for H-PyN3 and H-Py2N2 as found by CREST and DFT caluculations



ISOELECTRIC POINT PREDICTION

OH-Py2N2
pI (experiment) = 7.3

OH-PyN3-R
pI = ?

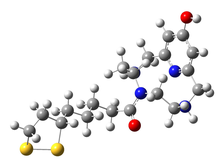


How can we search for OH-PyN3 structures whose pI value is similar or close to 7.3?

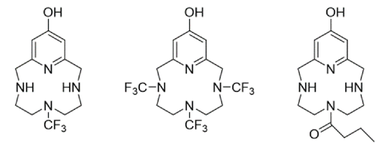
Comparison of protonation energy

LA	11.7
CF3	12.3
S	13.5
O	15.7
8HQ	17.5
Phenol0	17.6
Catechol	18.6
Me	20.5
Indole	21.3
Phenol2	21.4
H	21.7

Tested substituents at the middle R position of OH-PyN3, sorted by error in protonation energies (kcal/mol) from the reference molecule OH-Py2N2



Suggested modified structures of OH-PyN3:



CONCLUSION

SMD solvent model with scaled cavity predicts accurate pKa values of the first protonation (errors ~0.2 pK units), but breaks down for higher charged species due to their higher complexity.

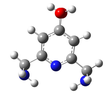
A linear trend can be observed for pKa values computed using the SMD model with unscaled cavity, but with large systematic errors.

Electron-withdrawing groups (Lipoic acid and CF3) are the best choice for substitution at middle R position of OH-PyN3 that would best match the pI value of the reference molecule OH-Py2N2 based on protonation energy.

FUTURE DIRECTIONS

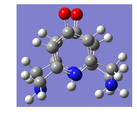
- **pKa study of OH-PyN3 and OH-Py2N2 using SMD solvent model with scaled and unscaled cavity**
- **Adjust the scaling of the cavity to better fit higher charged species or come up with a more sophisticated model**
- **Conformational study of neutral OH-Py2N2, OH-PyN3 and their isomers (keto tautomeric forms, zwitterionic forms)**

Gas phase



Keto tautomer

Zwitterion



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