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13. ABSTRACT *(Maximum 200 Words)*

Matrix metalloproteinases (MMPs) represent an important class of therapeutic targets for the treatment of diseases such as cancer. MMPs play a physiological role in the degradation of structural extra-cellular matrix (ECM) proteins and thus promote angiogenesis, a condition necessary for sustained tumor growth. Consequently, the inhibition of MMP enzymes may serve as disease-modifying agents by preventing ECM degradation and angiogenesis, and ultimately act as anti-cancer agents. In this research, we are using structure-based drug design methodologies in the hopes of finding novel and selective biological inhibitors for MMPs. Specifically, we are developing, refining, and validating the computational protocols and simulations methods used to model MMPs. The focus is on (1) validating the force field parameter sets used in the docking studies by comparing the calculated results with experimental MMP-inhibitor crystal structures, and (2) evaluating which scoring functions are most accurate for estimating MMP affinities. Structure-based design targeting specific MMPs will benefit from these studies by improving the accuracy of predicted binding modes and affinities of compounds prior to purchase or synthesis.

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Introduction

Matrix metalloproteinases (MMPs) are a large family of zinc dependent enzymes that are involved in a number of important biological processes including embryonic development, wound repair, and tissue remodeling.[1-4] Through a highly regulated system MMPs target the structural extra-cellular matrix (ECM) for degradation and promote the formation of new blood vessels (angiogenesis). Since angiogenesis is important for the development and progression of tumor growth and cancer, specific MMP inhibitors may function as effective anti-cancer agents.[1-3]

In this research we are using molecular docking and simulation techniques to develop, validate, and refine computational protocols and simulation techniques that can be used to aid in the development of anti-cancer agents targeting MMPs. Specifically, we are focusing on (1) validating the force field parameter sets used in our docking studies by comparing the calculated results with experimental MMP-inhibitor crystal structures, and (2) evaluating which scoring functions are most accurate for estimating MMP activities (affinities). By modeling known MMP-ligand systems with reasonable accuracy, new, selective, and potent inhibitors can be proposed with greater confidence.

Body

The docking calculations incorporate a classical potential energy expression (force field) consisting of Coulombic and Lennard-Jones terms to compute nonbonded interactions between the ligand and protein atoms separated by a distance r (eq 1).

$$E_{\text{nonbond}} = \sum_{i} \sum_{j>i} \left\{ \frac{q_i q_j e^2}{r_{ij}} + 4\varepsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] \right\} \quad (1)$$

Eq 1 contains the partial atomic charges q and Lennard-Jones radii and well-depths, σ and ε used to compute the pair-wise interaction energy for any given configuration. For docking, eq 1 is often referred to as a scoring function and can be used to rank a set of ligands on a relative basis for the electrostatic and steric complimentarity with a receptor. Given that the accuracy of the calculation results are primarily influenced by the quality

of the force field parameters used to evaluate this energy, parameter set validation is critical.

For the docking calculations, standard protein and ligand nonbonded parameters were assigned from the vdw.defn file distributed with DOCK 4.0.1.[5] Charge assignment for the receptor was performed with the SYBYL[6] implementation of the AMBER force-field.[7] Ligand charges were derived using the AM1-BCC[8] method as implemented in AMBER7.[9] The catalytic zinc ion used for the MMPs was from Stote et al., $\sigma = 1.7$ Å, $\varepsilon = 0.67$ kcal/mol.[10] The Stote model was shown to yield the best overall results out of eight zinc parameter sets tested for docking ligands to thermolysin[11] when compared with experiment. More recently, Molecular Mechanic Poisson-Boltzmann Surface Area (MM/PBSA)[12] calculations have used the Stote zinc model to rank binding energies for six known carboxylate ligands of Stomelysin-1 (MMP-3) in reasonable agreement with experiment.[13]

MM/PBSA methods also include a term to account for ligand desolvation as shown in equation 2. Here, $\Delta G_{solvation}$ is approximated as the sum of polar (electrostatic) and nonpolar (SASA dependant) energies where the difference (Δ) is computed between the isolated complex, ligand, and receptor.

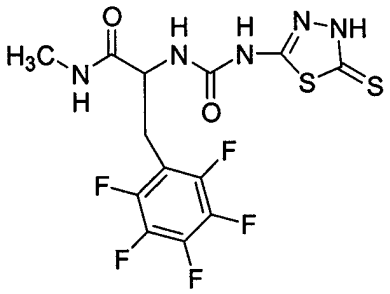
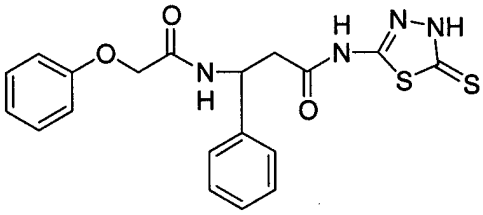
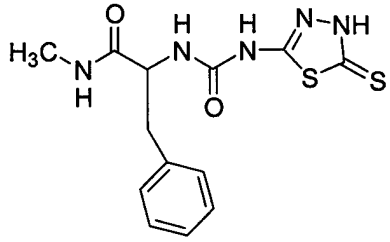
$$\Delta G_{solvation} = \Delta G_{polar} + \Delta G_{nonpolar} \qquad (2)$$

The polar contribution to the total solvation energy is estimated using a continuum finite-difference solution to the Poisson-Boltzmann (PB) equation[14] or can be computed using Generalized Born (GB) methods.[15,16] Nonpolar contributions are estimated by $\Delta G_{nonpolar}$ = SASA*0.00542 A2 + 0.92, where SASA is the total solvent accessible surface area of each isolated state (receptor, ligand, or complex). For docking calculations, including similar solvation terms appears to improve the accuracy of the calculations.[17,18]

To refine and validate our MMP simulation parameters, protocols, and methods, docking calculations were initiated from three MMP-ligand co-complexes to determine if the calculations could regenerate known binding modes of the ligands to the receptors (Table 1). Cross-docking calculations were also pursued to determine if the stromelysin

inhibitors could be placed into the experimental position independent of which ligand went with which receptor (Table 1). All docking calculations were performed with DOCK 4.0.1.[5] To gauge the accuracy of continuum desolvation calculations we have computed free energies of hydration for a test set consisting of 201 neutral and 18 charged organic molecules for comparison with experimental values using two different charge models (Figures 1-8). For these model systems both AM1-BCC/AMBER7[8] and Gasteiger/SYBYL[6] charges were evaluated. The PB calculations were performed with the DelPhi[14] program and the GB calculations were performed with AMBER7.[9] All SASA calculation were performed with the MOL2SURF program as distributed with AMBER7.[9]

Table 1. Cross-docking results for thiadiazole inhibitors to stromelysin (MMP-3). RMSD (Å) values are computed between docked and experimental ligand positions.

Name	Structure	RMSD (Å) PDB number			Activity
		1USN	2USN	3USN	Ki (uM)
PNU-142372 **1USN**		0.68	0.93	5.85	0.018[a]
PNU-141803 **2USN**		1.48	1.48	13.18	0.31[a]
PNU-107859 **3USN**		9.01	8.87	8.25	0.710[b]

[a]Reference[19]. [b]Reference[20].

Calculated vs experimental free energy of hydration
Gasteiger Charges, DelphiPB/molsurf, N= 201 (neutral)

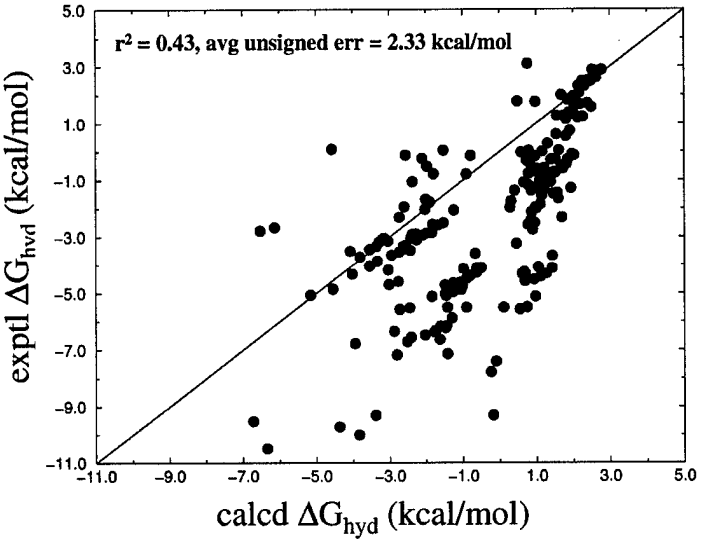


Figure 1.

Calculated vs experimental free energy of hydration
Gasteiger Charges, SanderGB/molsurf, N= 201 (neutral)

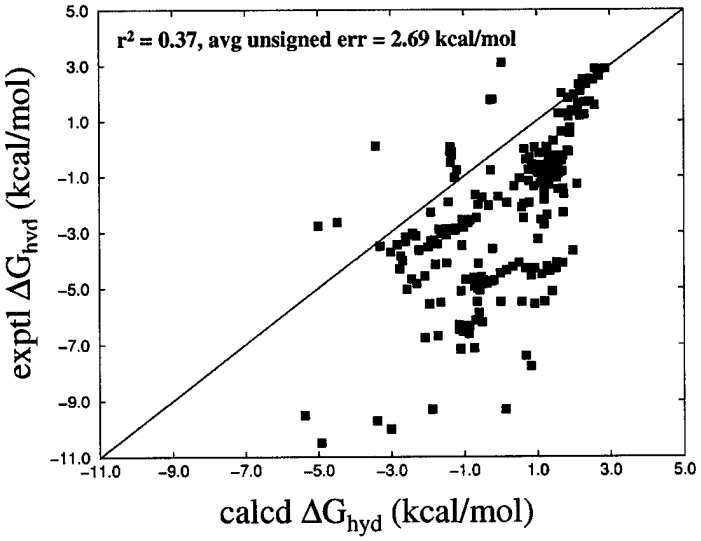


Figure 2.

Calculated vs experimental free energy of hydration
AM1-BCC Charges, DELPHI/molsurf, N= 201 (neutral)

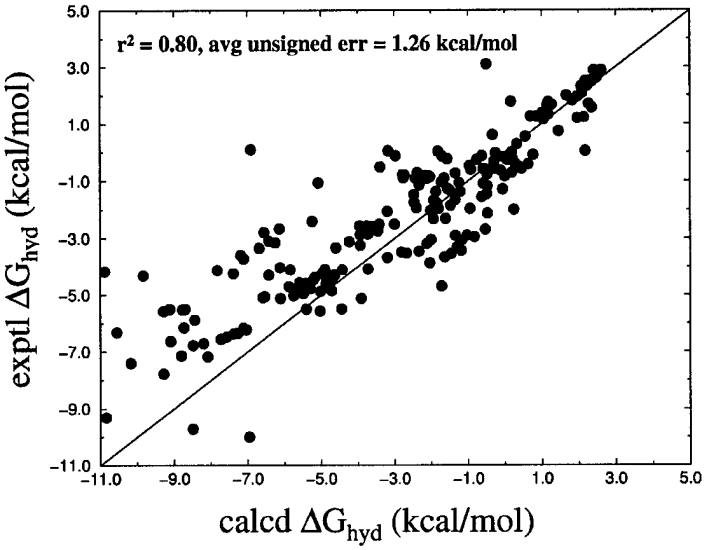


Figure 3.

Calculated vs experimental free energy of hydration
AM1-BCC Charges, SanderGB/molsurf, N= 201 (neutral)

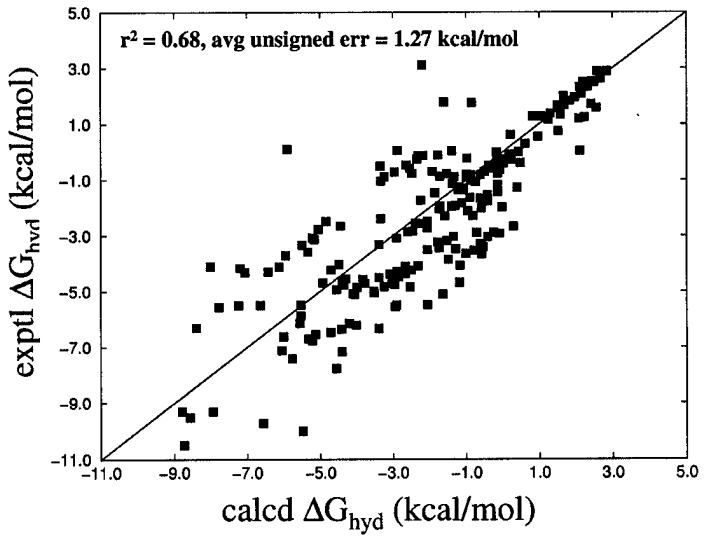


Figure 4.

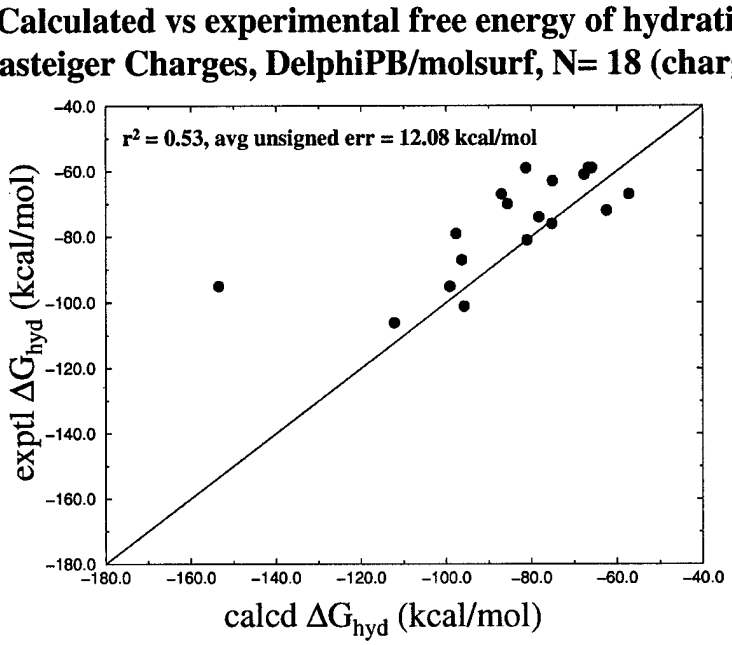


Figure 5.

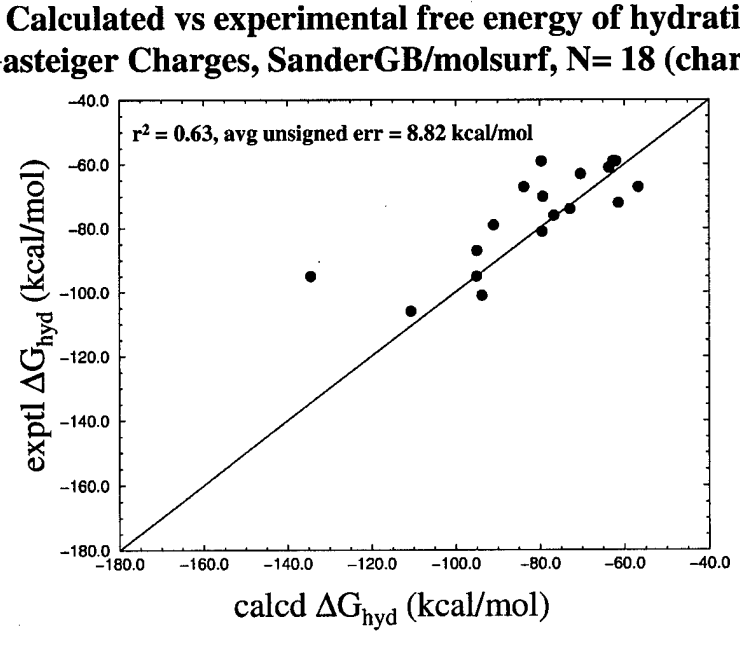


Figure 6.

Calculated vs experimental free energy of hydration
AM1-BCC Charges, DelphiPB/molsurf, N= 18 (charged)

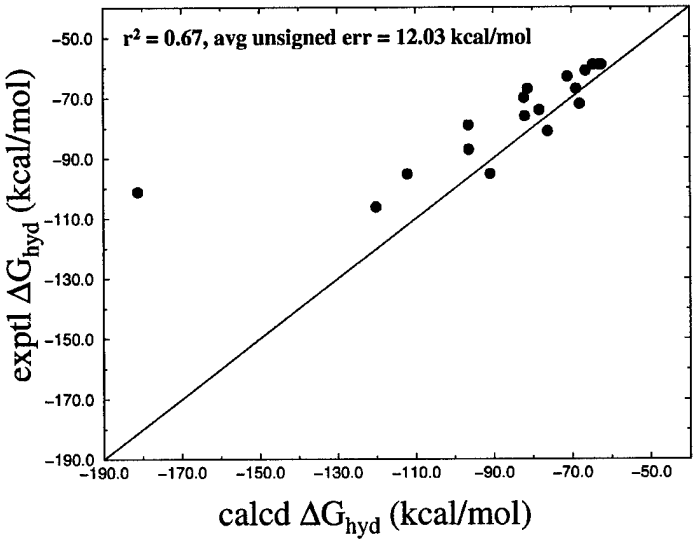


Figure 7.

Calculated vs experimental free energy of hydration
AM1-BCC Charges, SanderGB/molsurf, N= 18 (charged)

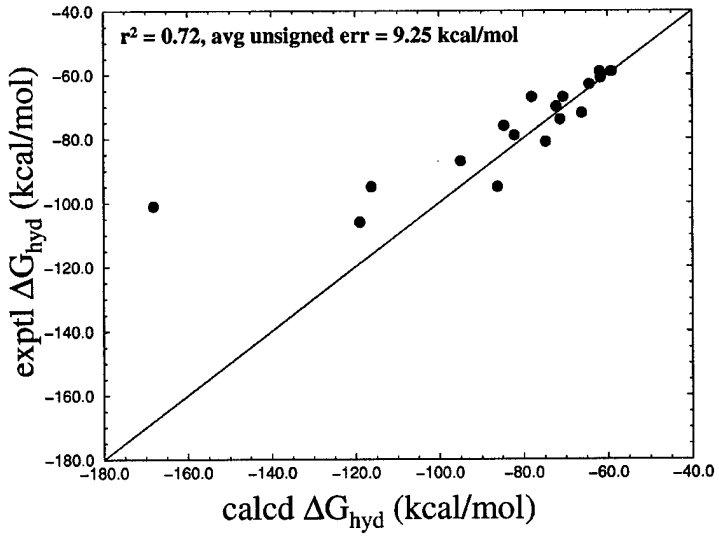


Figure 8.

Key Research Accomplishments

- Performed flexible docking and cross-docking experiments for 5-substituted-1,3,4-thiadozole-2-thiones to stromelysin using AM1-BCC charges for each ligand for comparison with previously reported structural data (Table 1).
- The docking protocols yield binding modes for thiadiazole inhibitors with stromelysin (MMP-3) in reasonable agreement with the crystallographic results (Table 1).
- Compared experimental free energies of hydration with those calculated using the Generalized Born Surface Area (GBSA) and Poisson-Boltzmann Surface Area (PBSA) approach for neutral (N=201) and charged (N=18) data sets using both Gastieger and AM1-BCC[8] charge models (Figures 1-8).
- The GBSA and PBSA calculations yield similar results for neutral (N=201) and charged (N=18) data sets (Figures 1-8)
- The AMBER/AM1-BCC[8] charge model yields considerably better free energies of hydration than does SYBYL/Gasteiger charges for both PBSA and GBSA calculations when compared to experimental values (Figures 1-8).

Reportable Outcomes

- On March 18th, 2001 the current PI Robert C. Rizzo, Ph. D. assumed responsibility for grant DAMD17-00-1-0192 (Modification P00001) from the previous PI Samual Toba, Ph. D.
- The AM1-BCC charges have been incorporated into the databases we use for molecular docking and data-mining calculations.
- Research posters are to be presented at the NIGMS/AIDS-Related Systems Symposium (June 19 - June 21, 2002) and the Gordon Research Conference in Computational Chemistry (June 30 - July 5, 2002) that incorporate aspects of this research.

Conclusions

Consistent with the original Statement of Work objectives we are continuing to develop, refine, and validate computational protocols and simulations methods used to model MMPs. Our focus has been on validating our results against experiment to insure that the computational predictions are efficient and accurate. Flexible cross-docking experiments for 5-substituted-1, 3, 4-thiadozole-2-thione ligands to stromelysin (pdb entries 1USN[19] 2USN[19]) yielded binding modes for the ligands in reasonable agreement with the crystallographic results (Table 1). These calculations provide support for our flexible docking protocols and the choice of the Stote nonbonded zinc model[10] for docking to MMPs. Surprisingly, calculations initiated using the NMR derived complex (pdb entry 3USN)[20] showed significant deviation from experiment and these results are under investigation.

We have confirmed the fact that both GBSA and PBSA methods yield reasonable computed free energies of hydration compared with experiment for a test set of 219 compounds provided a reasonable charge model is employed (Figures 1-8). For these hydration calculations the AMBER/AM1-BCC[9] charge model yielded calculated $\Delta G_{hydration}$ values in good agreement with experiment and significantly outperformed the SYBYL/Gasteiger[6] charges. Validation of the GBSA solvation model[18] implemented in the DOCK5 program[21] is also in progress. In addition, we are currently incorporating the AM1-BCC charges into the databases we use for molecular docking and data-mining calculations such as the National Cancer Institute, (NCI),[22] and Available Chemical Directory (ACD).[23] Anticipated projects that incorporate scaffold design methodologies used to construct and evaluate novel compounds targeting specific MMPs will benefit from these charge parameter and solvation validation studies by improving the accuracy of predicted binding modes and affinities of compounds prior to purchase or synthesis.

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