# Thermodynamics of Binding Site Water Molecules

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## Abstract

When a protein and ligand bind, the water molecules inside the protein's binding site are displaced into the bulk solvent. This displacement is thought to greatly contribute to the free energy change of binding. To predict this change, we implemented the grid inhomogeneous solvation theory (GIST) methods developed by Crystal Nguyen et al. These methods divide the active site into a grid of voxels, bin the water molecules from explicit solvent molecular dynamics simulations into the voxels, and use the distribution of the molecules' positions and orientations to calculate the energetic and entropic contributions of each voxel to the region's free energy. Using GIST, we analyzed the 2012 CSAR competition protein targets and our own target proteins, rationalizing the activity of ligands known to bind to them. We also investigated the correlation between our GIST entropy calculations and the experimental pK values for the CSAR competition ligands, with the goal of improving the affinity predictions of current scoring functions.

## GIST Entropy

$$\Delta S_{sw} = \Delta S^{trans} + \Delta S^{orient}$$

- **Translational entropy per voxel k**

$$\Delta S^{trans}(\boldsymbol{r}_k) \approx k_B \rho^0 V_k g(\boldsymbol{r}_k) \ln g(\boldsymbol{r}_k)$$

$$g(\boldsymbol{r}_k) \equiv \frac{N_k}{\rho^0 V_k N_f}$$

- **Orientational entropy per voxel k**

$$\Delta S^{orient}(\boldsymbol{r}_k) \approx \rho^0 V_k g(\boldsymbol{r}_k) S^{\omega}(\boldsymbol{r}_k)$$

$$S^{\omega}(\boldsymbol{r}_k) = \frac{-k_B}{N_k}\left(-\gamma + \sum_{i=1}^{N_k} \ln \frac{g(\omega_i|\boldsymbol{r}_k)}{N_k}\right)$$

$$g(\omega_i|r_k) = \frac{8\pi}{V_{i^\omega}} \qquad V_{i^\omega} = \frac{4\pi(\Delta\omega)^3}{3}$$

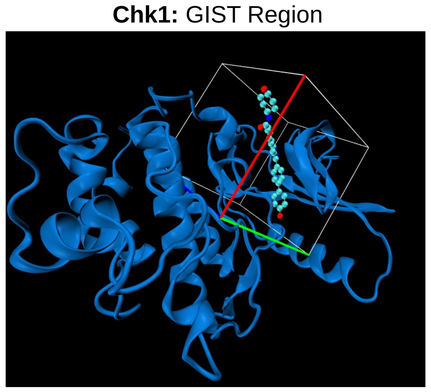
Orientational distribution function

Nearest orientation neighbor term

## Methods

1. Use AMBER12 molecular dynamics package to simulate 4 CSAR targets: erk2, chk1, cdk2 and urokinase
2. Define GIST region around active site
3. Calculate GIST entropy
4. Calculate entropy of waters displaced by ligand
5. Predict smina affinities
6. Fit results to CSAR experimental pK values

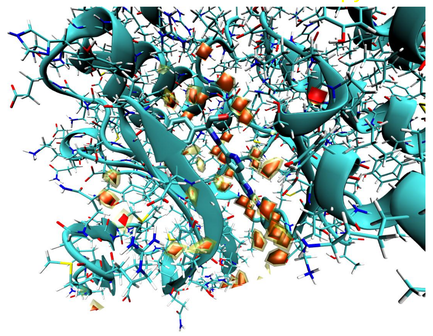
**Chk1:** GIST Region



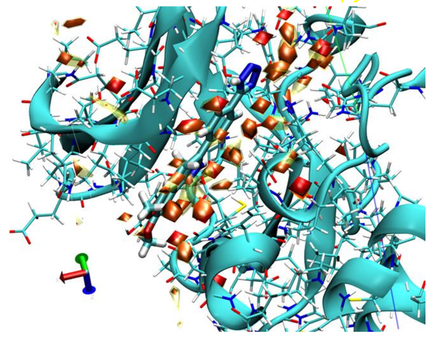
**Entropy Code Snippet:**

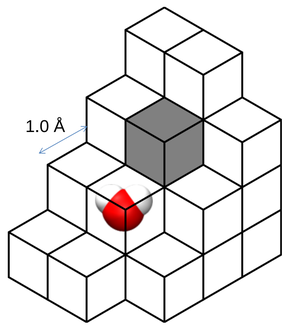
```
# GIST Translational Entropy function
def trans_entropy(voxel, num_frames):


    gr = len(voxel.waters) / (p0 * voxel.volume * num_frames)
    voxel.trans_entropy = kb * p0 * voxel.volume * gr * np.log(gr)
    return voxel.trans_entropy

# GIST Orientational Entropy function
def ori_entropy(voxel, num_frames):
    '''Function to Calculate the GIST Orientational Entropy value'''

    # Calculate the approx orientational distribution function for each
    # water molecule in the voxel
    # Construct kdtree of water molecules in Voxel for nearest neighbor search
    ori_dist_func_sum = np.float64(0)

    gr = len(voxel.eulers) / (p0 * voxel.volume * num_frames)
    if len(voxel.eulers) > 1:
        kdtree = spatial.cKDTree(voxel.eulers, 10)
        for e in voxel.eulers:
            nearest_dist, nearest_index = kdtree.query(e,k=4,p=2)
            i = 1
            delomega = nearest_dist[i]
            if delomega == 0:
                i += 1
                if i >= len(nearest_dist):
                    print 'Could not find non-zero euler angle distribution distance...'
                    print len(nearest_dist), voxel.coords, nearest_dist
                    break
                delomega = nearest_dist[i]

            V = 4*np.pi*(delomega**3)/3
            ori_dist = 8*(np.pi**2)/V
            ori_dist_func_sum += np.log(ori_dist)

    voxel.ori_entropy =  (-kb / len(voxel.eulers))*(-egamma + ori_dist_func_sum)
    voxel.den_ori_entropy = p0 * voxel.volume * gr * voxel.ori_entropy
    return (voxel.ori_entropy,voxel.den_ori_entropy)
```

**Erk2:** Orientational Entropy
Translational Entropy



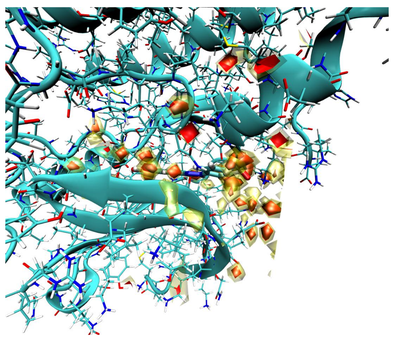
**Chk1:** Orientational Entropy
Translational Entropy



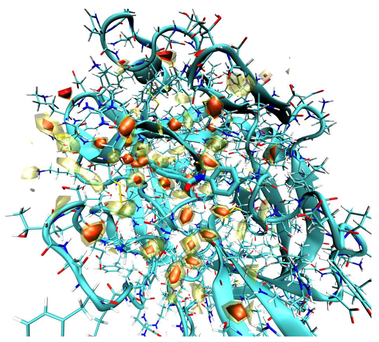



**Above:** Water in voxel
**Right:** Voxels displaced

**Cdk2:** Orientational Entropy
Translational Entropy

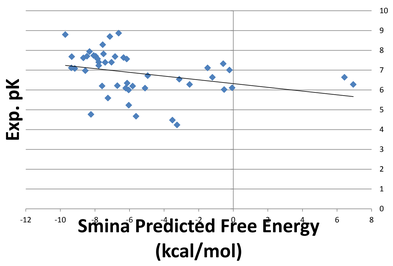


**Urokinase:** Orientational Entropy
Translational Entropy



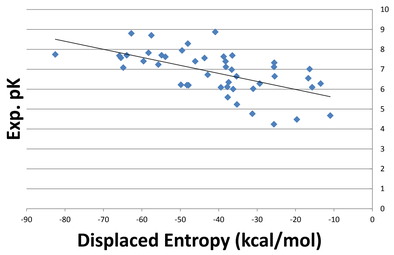
## Results

**Smina vs. Experimental pK**



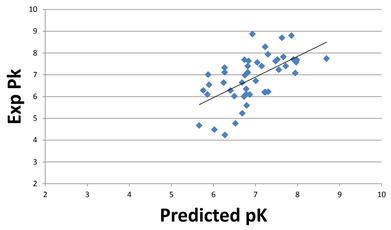

$R^2 = 0.1038$

**Entropy vs. Experimental pK**




$R^2 = 0.3533$

**Entropy and Smina Predicted pK vs. Experimental PK**




$R^2 = 0.3555$

## Conclusion

- The displaced entropy values calculated by GIST correlate well with the experimentally determined pK values from the CSAR competition
- The fit with entropy and smina only marginally better than entropy alone.
- Cross-docking the ligands resulted in non-ideal poses that affected the predicted correlation. Fit with improved poses will be investigated

## Future Work

- Fix smina ligand poses and test
- Cross validate affinity predictions
- Analyze more targets
- Develop more finely grained scoring function
- Optimize MD protocol to minimize simulation time

## References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