# Detection of Protein Binding Sites II

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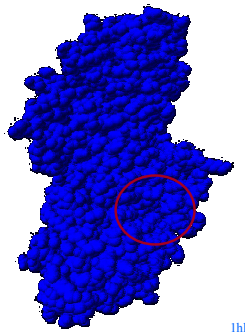
Princeton University
CS597A, Fall 2007

## Introduction

Goal:
- Given a protein structure, predict where a ligand might bind



1hld

## Outline

Introduction

Binding site detection methods
- Geometric, chemical, evolutionary properties, etc.
- Machine learning
- Optimization
- Docking

Evaluation methods

Discussion

## Outline

Introduction

Binding site detection methods
- ➢Geometric, chemical, evolutionary properties, etc.
- Machine learning
- Optimization
- Docking

Previous Lectures

Evaluation methods

Discussion

## Site Properties

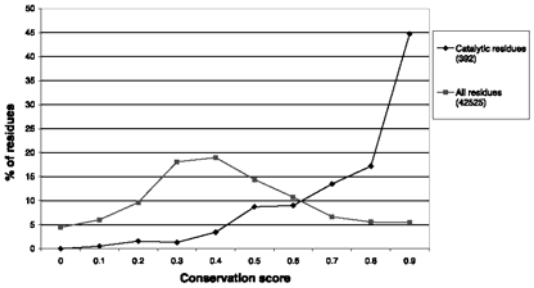
Learned distributions of properties:

| Surface cavity property | Category | Drug-binding cavities | Non drug-binding cavities |
|---|---|---|---|
| Cavity rank[a] | Size | $1.89 \pm 2.07$ | $8.88 \pm 5.4$ |
| Number of residues[b] | Size | $22.8 \pm 14.3$ | $7.31 \pm 5.4$ |
| Number of atoms[c] | Size | $85.0 \pm 62.4$ | $18.7 \pm 21.2$ |
| Smallest moment of inertia[d] | Size/shape | $1.7 \times 10^3 \pm 2.5 \times 10^4$ | $1.2 \times 10^3 \pm 8.3 \times 10^3$ |
| Depth standard deviation[e] | Size/shape | $2.3 \pm 1.1$ (Å) | $0.75 \pm 0.45$ |
| Maximum depth[f] | Size/shape | $10.5 \pm 4.0$ (Å) | $4.75 \pm 1.67$ |
| Average depth[g] | Size/shape | $5.3 \pm 1.9$ (Å) | $3.2 \pm 0.7$ |
| Normalized smallest moment of inertia[h] | Shape | $17.0 \pm 11.7$ | $3.9 \pm 5.3$ |
| Proportion of cavity at depth between [6.5, 6.75][i] | Shape | $0.02 \pm 0.013$ | $0.003 \pm 0.001$ |
| Largest moment of inertia[j] | Size/shape | $1.6 \times 10^5 \pm 8.4 \times 10^4$ | $2.8 \times 10^3 \pm 1.6 \times 10^4$ |
| Average side-chains residual entropy[k] | Rigidity | $-0.41 \pm 0.18$ (kcal) | $-0.55 \pm 0.25$ |
| Average curvature[l] | Shape | $-49.0 \pm 8.3$ | $-57.0 \pm 13.1$ |
| Maximum curvedness[m] | Shape | $6.4 \pm 2.9$ | $4.9 \pm 4.9$ |
| Maximum mean curvature[n] | Shape | $5.3 \pm 2.6$ | $3.5 \pm 4.2$ |
| Curvedness < 0.5[o] | Shape | $0.35 \pm 0.04$ | $0.29 \pm 0.08$ |
| Proportion of proline[p] | Amino acid composition | $0.019 \pm 0.028$ | $0.04 \pm 0.09$ |
| Proportion of cavity with logP between [−1, 0][q] | Hydrophobicity | $0.09 \pm 0.07$ | $0.15 \pm 0.16$ |
| Side-chain residual entropy standard deviation[r] | Rigidity | $0.43 \pm 0.18$ (kcal) | $0.55 \pm 0.17$ |

[Nayal06]

## Site Properties

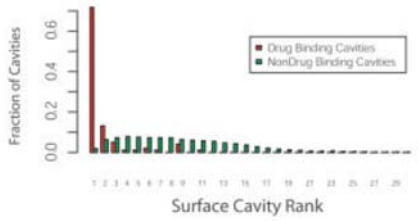
Example: conservation




[Bartlett02]

## Site Properties

Example: cavity rank




[Nayal06]

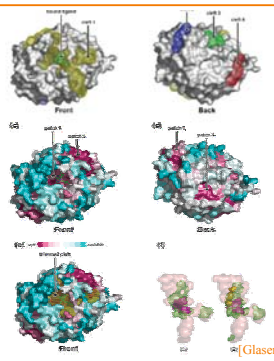
## Combining Multiple Properties

Example:

- Conservation + cavity size

Method:

- Remove portions of cavity (predicted by Surfnet) more than X angstroms from the closest residue with conservation above Y

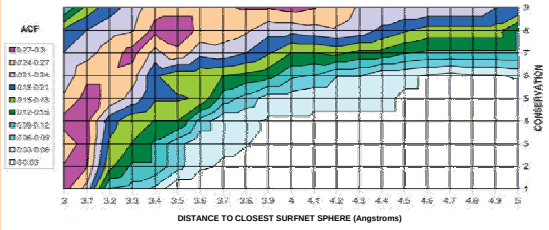


[Glaser06]

## Combining Multiple Properties

Example:

- Conservation + cavity size

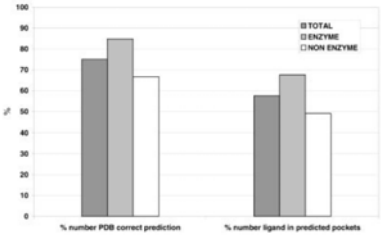



[Glaser06]

## Combining Multiple Properties

Example:

- Conservation + cavity size



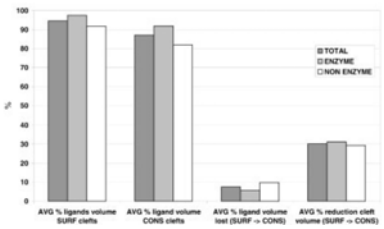

[Glaser06]

## Combining Multiple Properties

Example:

- Conservation + cavity size

"AVG % ligands volume SURF clefts" is the average percentage of ligands volume included in the four biggest clefts produced by the SURFNET program. "AVG % ligand volume CONS clefts" is the average percentage of ligands volume included in the four biggest trimmed clefts. "AVG % ligand volume lost (SURF 3 CONS)" is the average ligand volume lost during the trimming procedure. "AVG % reduction cleft volume (SURF 3 CONS)" is the average volume cleft reduction of clefts including a ligand during the trimming procedure.




[Glaser06]

## Outline

Introduction

Binding site detection methods

- Geometric, chemical, evolutionary properties, etc.
  - ➢Machine learning
- Optimization
- DockingEvaluation methods

Discussion

## Machine Learning

Build classifier to recognize functional residues/sites from multiple properties:

- Depth
- Solvent accessibility
- Propensity
- Conservation
- Hydrophobicity
- Secondary structure type
- Pocket size
- Amino acid
- etc.

## Machine Learning

Example classifiers:

- Naïve Bayes
- Decision trees
- Neural nets
- Support vector machines
- etc.

## Machine Learning [Gutteridge03]

Data type

- Protein residues

Properties

- Many (next slide)

Training set

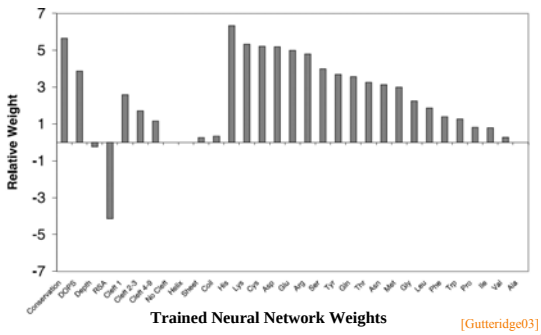
- 159 crystallized proteins
- 55,000 non-catalytic residues, 550 catalytic residues

Classification methods

- Neural network
- Spatial clustering

[Gutteridge03]

## Machine Learning [Gutteridge03]

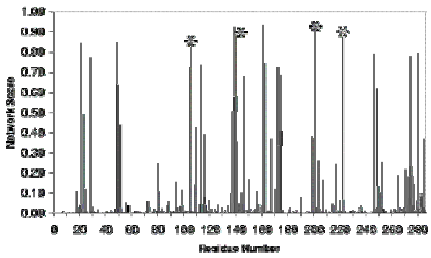



Trained Neural Network Weights

[Gutteridge03]

## Machine Learning [Gutteridge03]

Neural network classifier

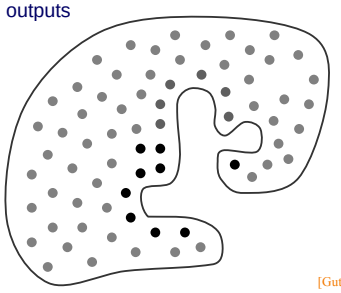



[Gutteridge03]

## Machine Learning [Gutteridge03]

Spatial clustering

- Compute spheres around clusters of nearby residues with high NN outputs



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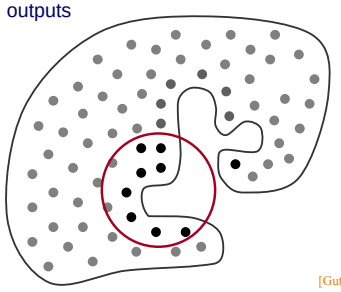
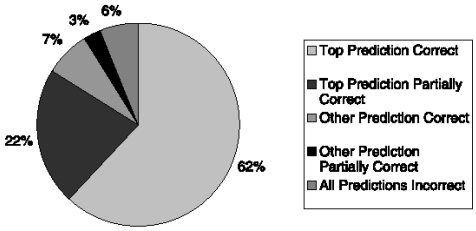


[Gutteridge03]

## Machine Learning [Gutteridge03]

Evaluation

- Sphere overlaps known actives site by > 50%




[Gutteridge03]

## Machine Learning (FEATURE)

Data type

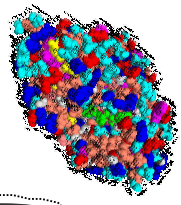
- Points in/around protein

Properties

- Many properties logged in histograms for shells around point (on right)

Classification methods

- Naïve Bayes



[Bagley95]

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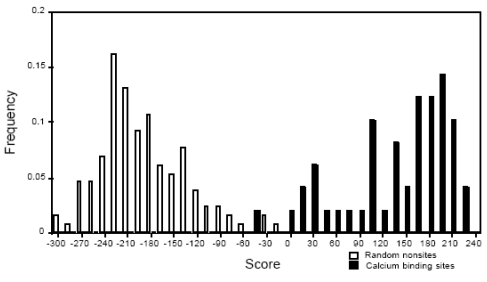



[Bagley95,Wei98]

## Machine Learning [Nayal06]

Data type

- Cavity surfaces

Properties

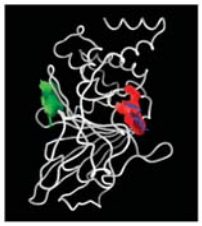
- Many (next slide)

Training set

- 1347 cavities
- 99 non-redundant proteins

Classification methods

- Random forests



[Nayal06]

## Machine Learning [Nayal06]

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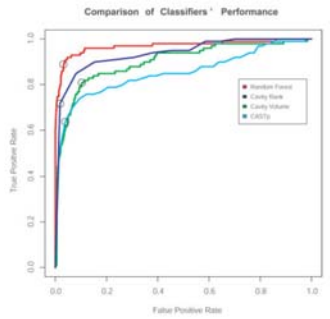



[Nayal06]

## Outline

Introduction

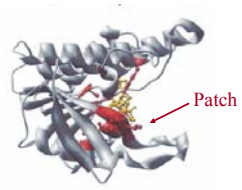
Binding site detection methods
- Geometric, chemical, evolutionary properties, etc.
- Machine learning
- ➢Optimization
- Docking

Evaluation methods

Discussion

## Optimization

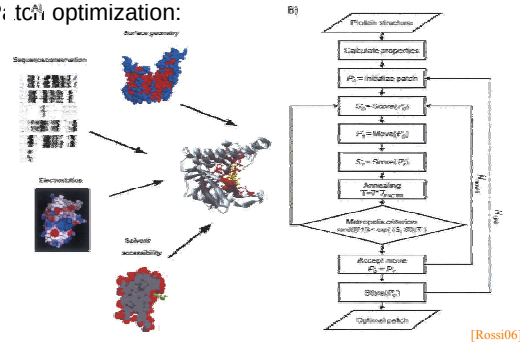
Patch optimization:
- Define patch as set of contiguous residues
- Compute patch properties
- Compute patch score
- If not optimal, grow/shrink patch and iterate




[Rossi06]

## Optimization

Patch optimization:




[Rossi06]

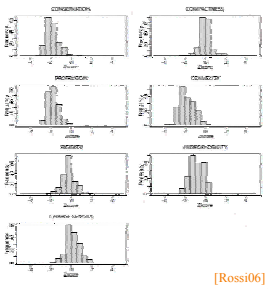
## Site Optimization

Patch properties:
- Z-score in context of training set

$z_p = (value_p - mean_p / \sigma_p$

Patch score:

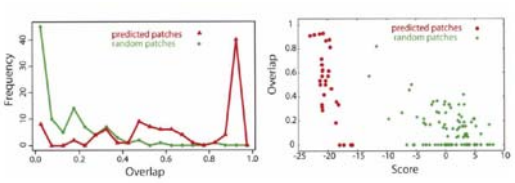
$score = \sum_p w_p z_p$



[Rossi06]

## Optimization

Results:

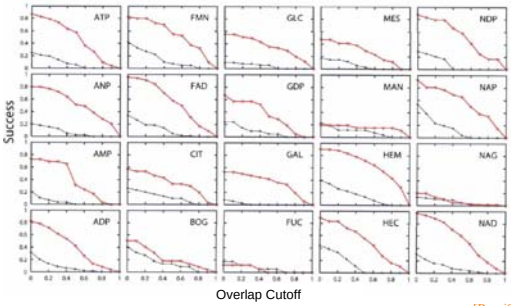



$$\text{Overlap} = \frac{N_1 \cap N_2}{\min(N_1, N_2)}$$

[Rossi06]

## Optimization

Results:




[Rossi06]

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- Optimization
- ➢Docking

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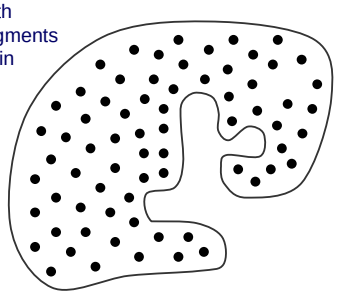
## Docking for Site Prediction

General idea:

- Compute map with distribution of fragments docked into protein



[Miranker91], [Mattos96]

## Docking for Site Prediction

General idea:

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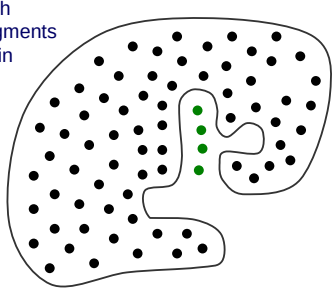

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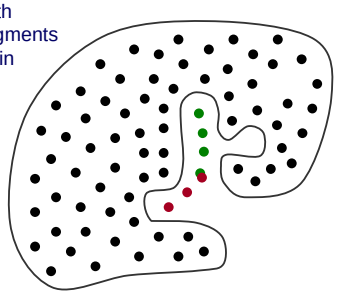

[Miranker91], [Mattos96]

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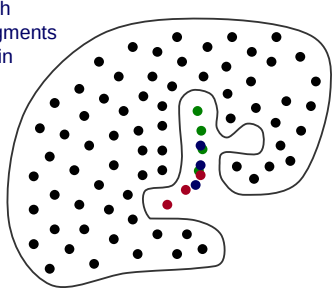

[Miranker91], [Mattos96]

## Docking for Site Prediction

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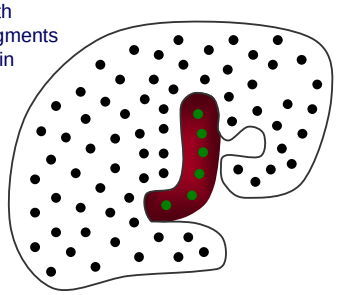
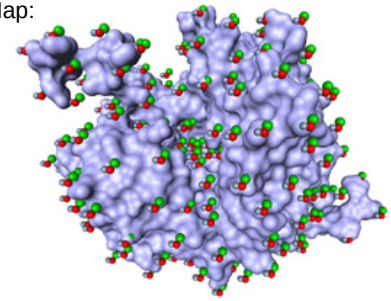


[Miranker91], [Mattos96]

## Docking for Site Prediction

CS-Map:

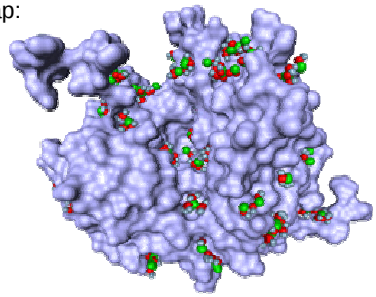


Step 1: Place probes

[Vajda; Silberstein; Landon et al.]

## Docking for Site Prediction

CS-Map:

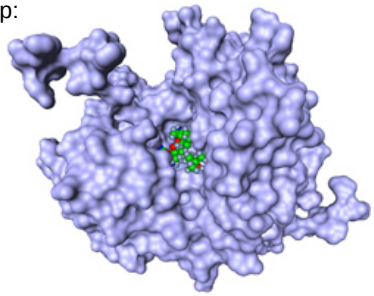


Step 2: Move the probes around to find binding positions

[Vajda; Silberstein; Landon et al.]

## Docking for Site Prediction

CS-Map:

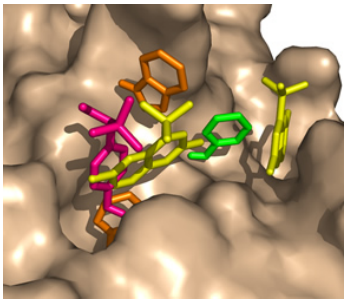


Step 3: Remove high energy clusters of the ligand

[Vajda; Silberstein; Landon et al.]

## Docking for Site Prediction

CS-Map:

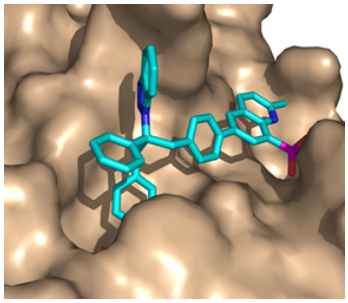


Step 4: Repeat mapping with a number of fragments

[Vajda; Silberstein; Landon et al.]

## Docking for Site Prediction

CS-Map:

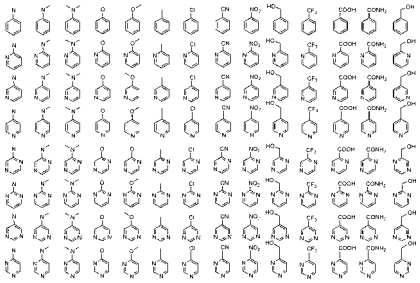


Step 5: Combine fragment into potential ligand molecules

[Vajda; Silberstein; Landon et al.]

## Docking for Site Prediction

CS-Map:

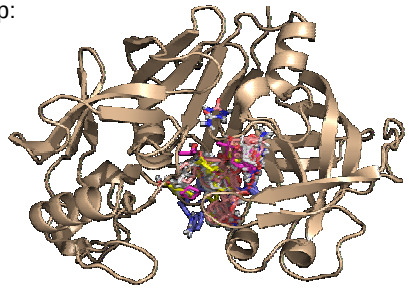


Prototype fragment library

[Vajda; Silberstein; Landon et al.]

## Docking for Site Prediction

CS-Map:

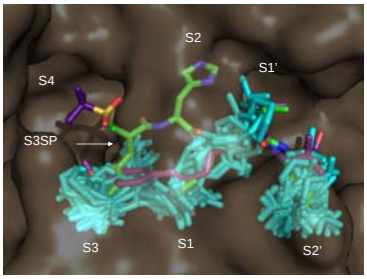



Top two consensus sites for each structure are in binding pocket

[Vajda; Silberstein; Landon et al.]

## Docking for Site Prediction

CS-Map:




Identification of Preferred Binding Modality of Aliskiren using CSMap

[Vajda; Silberstein; Landon et al.]

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Evaluation methods ←

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## General Evaluation Method

Gather a set of PDB files
- Both bound and unbound (with homologues)

Predict binding sites (clefts, pockets)
- Output is usually grid, polyhedron, set of spheres

Report results
- Measure properties of predicted binding sites
- Test how well predictions match bound ligands

## PocketFinder

Liganded-pocket data set

- Consider all protein-ligand complexes from PDB
- Eliminate frequent co-factors (HEM, etc.)
- Eliminate ligands far from protein (>3.5Å)
- Eliminate ligands in seams between assymmetric units
- Eliminate “duplicates” (?)
- 50 < protein residues < 2000
- 6 < ligand atoms
- 2.5Å < resolution

5,616 bound binding sites

[An04]

## PocketFinder

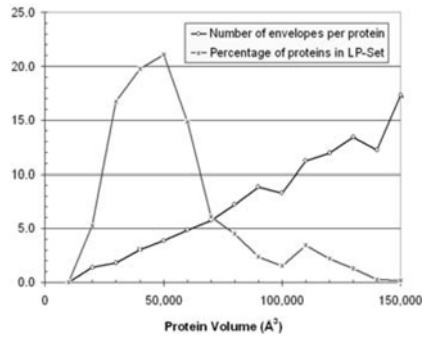
Unliganded-pocket data set

- Align unliganded PDB files with liganded ones
- Single chain proteins
- 95% < sequence identity
- No mutations on surface within 8Å of ligand
- No other ligands within 8Å of ligand
- 2.5Å < resolution

11,510 unbound binding sites

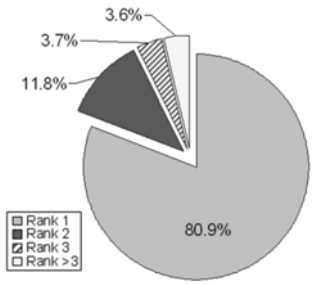
[An04]

## PocketFinder




[An04]

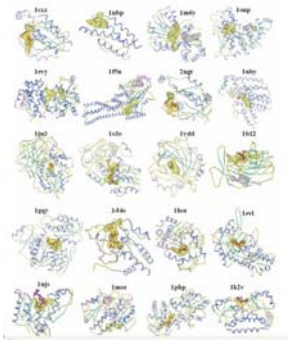
## PocketFinder




Rank of the real binding sites in the predicted putative binding site lists.

[An04]

## PocketFinder



Two largest predicted envelopes (1st:yellow, 2nd:gray)

[An04]

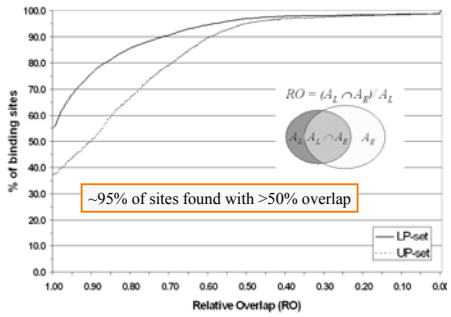
## PocketFinder

Accuracy measured by overlap of protein atoms in contact with ligand and protein atoms in contact with predicted envelope.

$$RO = (A_L \cap A_E) / A_L$$

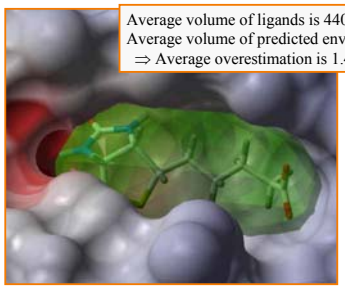
$A_L$ = solvent accessible surface area of protein atoms within 3.5Å of bound ligand
$A_E$ = solvent accessible surface area of protein atoms within 3.5Å of predicted envelope

## PocketFinder




[An04]

## PocketFinder

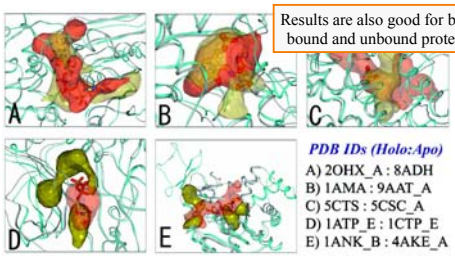


Average volume of ligands is 440Å
Average volume of predicted envelopes is 611Å
⇒ Average overestimation is 1.4x (?)

Biotin-streptavidin binding site predicted with PocketFinder

[An04]

## PocketFinder




Results are also good for both bound and unbound proteins

*PDB IDs (Holo:Apo)*
A) 2OHX_A : 8ADH
B) 1AMA : 9AAT_A
C) 5CTS : 5CSC_A
D) 1ATP_E : 1CTP_E
E) 1ANK_B : 4AKE_A

Effect of conformational changes on predictions for bound (holo) and unbound (apo) proteins
*(enzymes: gray-holo, green-apo)*
*(envelopes: red-holo, yellow-apo)*

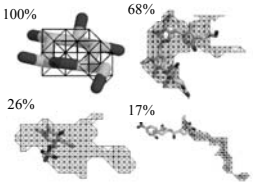
[An04]

## Q-SiteFinder

Test set:
- 134 bound proteins (GOLD test set)
- 35 unbound proteins (homologues to bound proteins)

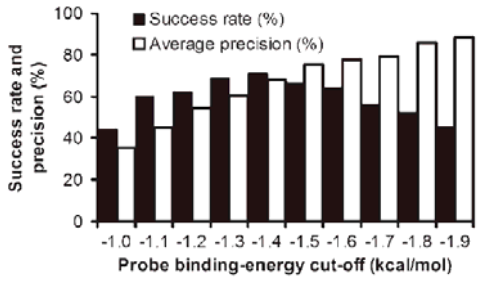
Metric:
- Precision = % predicted site within 1.6Å of ligand

Success:
- Precision >25%



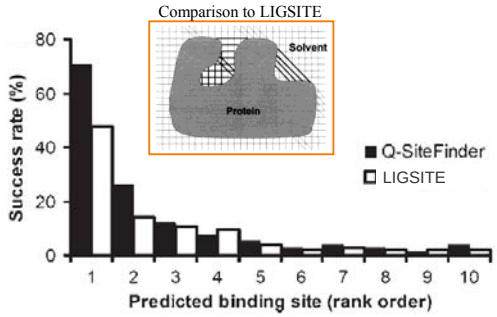

[Laurie05]

## Q-SiteFinder




[Laurie05]

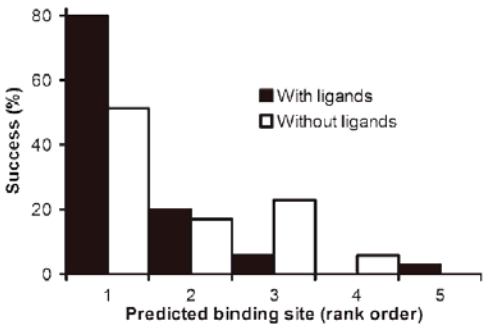
## Q-SiteFinder




Q-SiteFinder cutoff = -1.4 kcal/mol, LIGSITE threshold = 5

[Laurie05]

## Q-SiteFinder




[Laurie05]

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