



# Two Complementary Methods for Quantifying Ligand Binding Site Burial Depth In Proteins: (1.) The "Cutting Plane" Method; and (2.) The "Tangent Sphere" Method

Vicente M. Reyes, Ph.D., Assistant Professor [E-mail: [vmrsbi@rit.edu](mailto:vmrsbi@rit.edu)]  
 Department of Biological Sciences, School of Life Sciences, College of Science,  
 Rochester Institute of Technology, Rochester, NY 14623-5603

## A. Background and Motivation:

Issues Regarding Depth of Ligand Binding Site (LBS) Burial:

1. Which LBS is deeper?



Case 1: small and large proteins with same LBS cavity volume

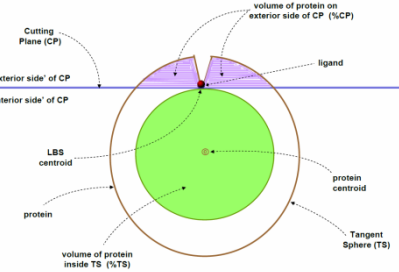
2. What is the significance?



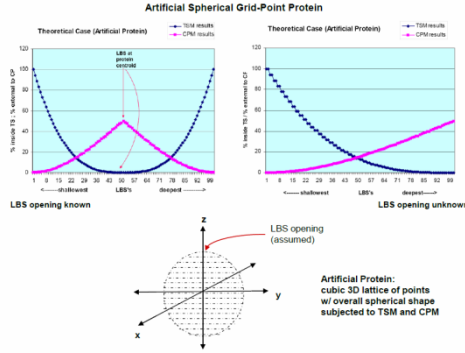
Case 2: same sized proteins with different LBS cavity volume

## B. Introducing the "Cutting Plane" and "Tangent Sphere" Methods

The 'Cutting Plane' and 'Tangent Sphere' Methods

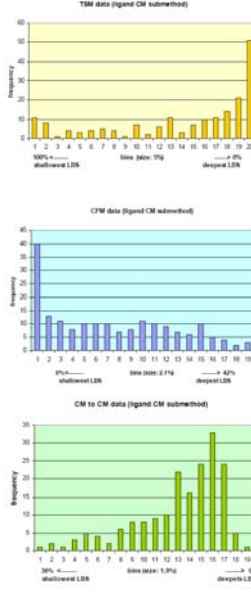


## D. Behavior of the Cutting Plane and Tangent Sphere Methods as Applied to an Artificial Protein in the form of a Spherical Grid of Points

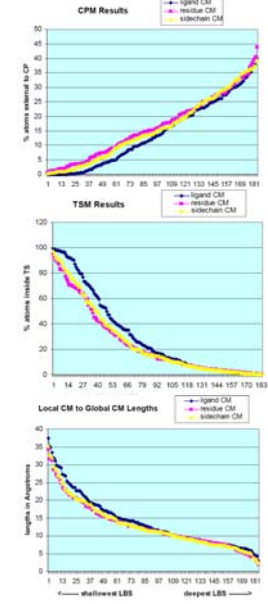


**Abstract.** Methods for quantitative comparison of the depth of burial of the bound ligand is currently lacking (A.). Two complementary (but not redundant on actual proteins) methods, the 'Cutting Plane' and 'Tangent Sphere' methods (CPM and TSM, respectively), are presented to address this void (B.). CPM and TSM are inversely related, as can be seen when applied to an artificial protein in the form of a spherical grid of points (C. and D.), the graph becomes degenerate when the location of the ligand binding site opening relative to that of the bound ligand is unknown, as in the actual implementation of these two methods (D.). CPM and TSM are based on percentages of protein atoms on the external side of the cutting plane and the interior of the tangent sphere (the CPM and TSM indices, respectively), hence are not dependent on protein size (B.). The data set by Laskowski et al. (1996, *Proteins*) was used as test set. (E.). CPM is more specific for deeply bound ligands, while TSM is more specific for shallow bound ones; an auxiliary but not relative method, protein centroid to ligand centroid, is intermediate in sensitivity (F.). CPM, TSM and the auxiliary method were applied to the test set and the results are shown; the inverse relation between CPM and TSM is confirmed, while the auxiliary method behaves similarly as TSM, as expected (G.). CPM and TSM classify the bound ligands in the test set 'shallow', 'medium' and 'deep' in general agreement with visual (manual) classification by Laskowski et al. (1.). See additional note on panel H.

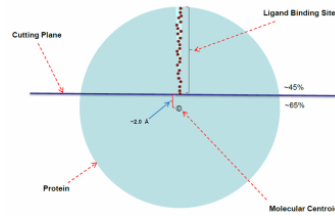
## F. Sensitivity & Specificity of the Cutting Plane and Tangent Sphere Methods



## G. Cutting Plane and Tangent Sphere Methods Applied to the Test Set Using Three Different Sub-methods



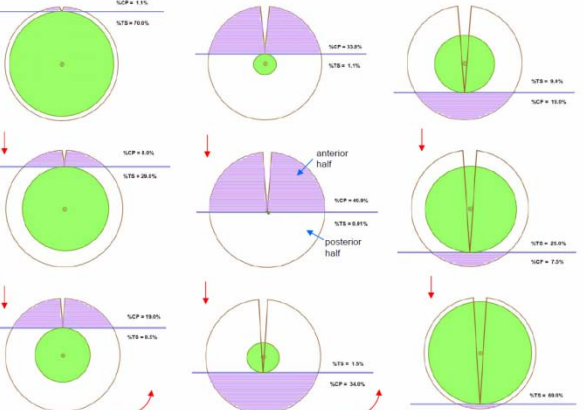
## H. Depth of Burial of Ligands in Laskowski Test Set as Revealed by the CPM & TSM



It is further revealed that ligand burial (at least in the test set) does not get deeper than the protein centroid, but is approx. no closer than 2 Å away from the latter (in the direction of the LBS opening), with the percentage of protein atoms on the external side of the cutting plane (CPM index) approx. no greater than 45% (H.). Meanwhile, the TSM is not sensitive enough to deeply bound ligands, hence no conclusion from TSM can be drawn on this matter.

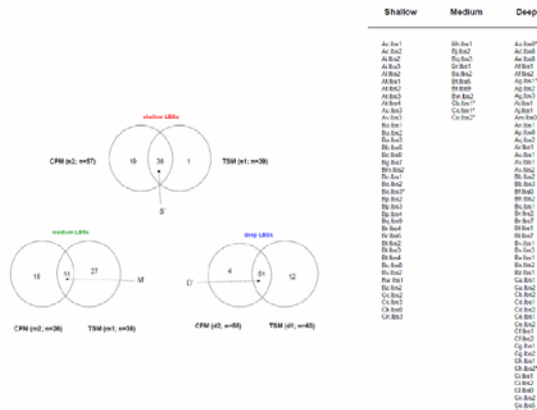
## I. Protein-Ligand Pairs in the Laskowski Test Set Classified as "Shallow", "Medium" and "Deep" by the CPM & TSM

## C. Behavior of the Cutting Plane & Tangent Sphere Methods (CPM & TSM)



## E. The Test Set: Laskowski et al., Proteins, 1996

Structure PDB ID	Shortband designation	LBS # at LBS #	Ligand Name	SCCP	STS
1A01	1A01	1	ATP	100	0
1A02	1A02	1	ATP	100	0
1A03	1A03	1	ATP	100	0
1A04	1A04	1	ATP	100	0
1A05	1A05	1	ATP	100	0
1A06	1A06	1	ATP	100	0
1A07	1A07	1	ATP	100	0
1A08	1A08	1	ATP	100	0
1A09	1A09	1	ATP	100	0
1A10	1A10	1	ATP	100	0
1A11	1A11	1	ATP	100	0
1A12	1A12	1	ATP	100	0
1A13	1A13	1	ATP	100	0
1A14	1A14	1	ATP	100	0
1A15	1A15	1	ATP	100	0
1A16	1A16	1	ATP	100	0
1A17	1A17	1	ATP	100	0
1A18	1A18	1	ATP	100	0
1A19	1A19	1	ATP	100	0
1A20	1A20	1	ATP	100	0
1A21	1A21	1	ATP	100	0
1A22	1A22	1	ATP	100	0
1A23	1A23	1	ATP	100	0
1A24	1A24	1	ATP	100	0
1A25	1A25	1	ATP	100	0
1A26	1A26	1	ATP	100	0
1A27	1A27	1	ATP	100	0
1A28	1A28	1	ATP	100	0
1A29	1A29	1	ATP	100	0
1A30	1A30	1	ATP	100	0
1A31	1A31	1	ATP	100	0
1A32	1A32	1	ATP	100	0
1A33	1A33	1	ATP	100	0
1A34	1A34	1	ATP	100	0
1A35	1A35	1	ATP	100	0
1A36	1A36	1	ATP	100	0
1A37	1A37	1	ATP	100	0
1A38	1A38	1	ATP	100	0
1A39	1A39	1	ATP	100	0
1A40	1A40	1	ATP	100	0
1A41	1A41	1	ATP	100	0
1A42	1A42	1	ATP	100	0
1A43	1A43	1	ATP	100	0
1A44	1A44	1	ATP	100	0
1A45	1A45	1	ATP	100	0
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1A47	1A47	1	ATP	100	0
1A48	1A48	1	ATP	100	0
1A49	1A49	1	ATP	100	0
1A50	1A50	1	ATP	100	0
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1A55	1A55	1	ATP	100	0
1A56	1A56	1	ATP	100	0
1A57	1A57	1	ATP	100	0
1A58	1A58	1	ATP	100	0
1A59	1A59	1	ATP	100	0
1A60	1A60	1	ATP	100	0
1A61	1A61	1	ATP	100	0
1A62	1A62	1	ATP	100	0
1A63	1A63	1	ATP	100	0
1A64	1A64	1	ATP	100	0
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1A69	1A69	1	ATP	100	0
1A70	1A70	1	ATP	100	0
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1A72	1A72	1	ATP	100	0
1A73	1A73	1	ATP	100	0
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1A96	1A96	1	ATP	100	0
1A97	1A97	1	ATP	100	0
1A98	1A98	1	ATP	100	0
1A99	1A99	1	ATP	100	0
1A100	1A100	1	ATP	100	0



Shallow	Medium	Deep
1A01	1B01	1A01P
1A02	1B02	1A02P
1A03	1B03	1A03P
1A04	1B04	1A04P
1A05	1B05	1A05P
1A06	1B06	1A06P
1A07	1B07	1A07P
1A08	1B08	1A08P
1A09	1B09	1A09P
1A10	1B10	1A10P
1A11	1B11	1A11P
1A12	1B12	1A12P
1A13	1B13	1A13P
1A14	1B14	1A14P
1A15	1B15	1A15P
1A16	1B16	1A16P
1A17	1B17	1A17P
1A18	1B18	1A18P
1A19	1B19	1A19P
1A20	1B20	1A20P
1A21	1B21	1A21P
1A22	1B22	1A22P
1A23	1B23	1A23P
1A24	1B24	1A24P
1A25	1B25	1A25P
1A26	1B26	1A26P
1A27	1B27	1A27P
1A28	1B28	1A28P
1A29	1B29	1A29P
1A30	1B30	1A30P
1A31	1B31	1A31P
1A32	1B32	1A32P
1A33	1B33	1A33P
1A34	1B34	1A34P
1A35	1B35	1A35P
1A36	1B36	1A36P
1A37	1B37	1A37P
1A38	1B38	1A38P
1A39	1B39	1A39P
1A40	1B40	1A40P
1A41	1B41	1A41P
1A42	1B42	1A42P
1A43	1B43	1A43P
1A44	1B44	1A44P
1A45	1B45	1A45P
1A46	1B46	1A46P
1A47	1B47	1A47P
1A48	1B48	1A48P
1A49	1B49	1A49P
1A50	1B50	1A50P
1A51	1B51	1A51P
1A52	1B52	1A52P
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1A60	1B60	1A60P
1A61	1B61	1A61P
1A62	1B62	1A62P
1A63	1B63	1A63P
1A64	1B64	1A64P
1A65	1B65	1A65P
1A66	1B66	1A66P
1A67	1B67	1A67P
1A68	1B68	1A68P
1A69	1B69	1A69P
1A70	1B70	1A70P
1A71	1B71	1A71P
1A72	1B72	1A72P
1A73	1B73	1A73P
1A74	1B74	1A74P
1A75	1B75	1A75P
1A76	1B76	1A76P
1A77	1B77	1A77P
1A78	1B78	1A78P
1A79	1B79	1A79P
1A80	1B80	1A80P
1A81	1B81	1A81P
1A82	1B82	1A82P
1A83	1B83	1A83P
1A84	1B84	1A84P
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1A94	1B94	1A94P
1A95	1B95	1A95P
1A96	1B96	1A96P
1A97	1B97	1A97P
1A98	1B98	1A98P
1A99	1B99	1A99P
1A100	1B100	1A100P

## **Two Complementary Methods for Quantifying Ligand Binding Site Burial Depth In Proteins: (1.) The “Cutting Plane” Method; and (2.) The “Tangent Sphere” Method**

**Vicente M. Reyes, Ph.D., Assistant Professor**

**Dept. of Biological Sciences, Rochester Institute of Technology, Rochester, NY 14623**

We describe two complementary methods to quantify the degree of burial of ligand and/or ligand binding site (LBS) in a protein-ligand complex, namely, the 'cutting plane' (CP) and the 'tangent sphere' (TS) methods. To construct the CP and TS, two centroids are required: the protein molecular centroid (global centroid, GC), and the LBS centroid (local centroid, LC). The CP is defined as the plane passing through the LBS centroid (LC) and normal to the line passing through the LC and the protein molecular centroid (GC). The "exterior side" of the CP is the side opposite GC. The TS is defined as the sphere with center at GC and tangent to the CP at LC. The percentage of protein atoms (a.) inside the TS, and (b.) on the exterior side of the CP, are two complementary measures of ligand or LBS burial depth since the latter is directly proportional to (b.) and inversely proportional to (a.). We tested the CP and TS methods using a test set of 67 well characterized protein-ligand structures (Laskowski et al., 1996), as well as the theoretical case of an artificial protein in the form of a cubic lattice grid of points in the overall shape of a sphere and in which LBS of any depth can be specified. Results from both the CP and TS methods agree very well with data reported by Laskowski et al., and results from the theoretical case further confirm that that both methods are suitable measures of ligand or LBS burial. Prior to this study, there were no such numerical measures of LBS burial available, and hence no way to directly and objectively compare LBS depths in different proteins. LBS burial depth is an important parameter as it is usually directly related to the amount of conformational change a protein undergoes upon ligand binding, and ability to quantify it could allow meaningful comparison of protein dynamics and flexibility.