Active Sites of Enzymes are Crowded with Charge

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Abstract

The chemistry of enzymes occurs at active sites that concentrate biological function into functional pockets. **Functional pockets mix catalytic amino acids and substrate in tiny volumes.** Here, we look for biological properties of that small space. We imagine that electric charge plays important roles, because even one charge in a small space produces large electric fields. **To estimate densities of fixed charge, we measure the volume of functional pockets and count 'charged residues' in it.**

We collect locations of functional pockets from enzymes of known structure that catalyze the main six enzymatic reactions. Functional amino acids are identified by their participation in catalysis. We measure the volume of pockets using both solvent-accessible and molecular-surface models. **'Charged residues' are R, K and H (positive); E and D (negative). Charge density is extraordinarily large (~20 Molar on average, often larger).** Mobile counterions for the fixed charge are presumably nearby in high density. Active sites do not resemble the infinitely dilute ideal solutions of classical enzyme kinetics. **Their enormous charge density is comparable to the charge density of solid NaCl**. Different types of enzymes have different charge densities. Hydrolases show the largest values of charge density. Some enzymes have extraordinarily large charge density—phosphoglycerate mutase (PDB = 1O98, density of charge 104 Molar, Molecular Surface), or sulfurtransferase (PDB = 1E0C, 109 Molar, Molecular Surface).

Crowding of charged side-chains and ions produces enormous steric and electrostatic forces in these tiny active sites. The balance of these forces seems likely to be of great importance to enzyme function. Many charged pockets are also found away from active sites. Charged pockets are likely to be involved in many surface interactions. They may be reservoirs of electromechanical energy that can drive conformational changes.

References

Dundas Et Al., NAR 34 (Jul 1, 2006): W116-118.
Porter Et Al., NAR 32 (Jan 1,2004): D129-133.

Acknowledgments

Becas Talentia
El talento elige.

Results

Positive Side Chains (K, R, H) — Negative Side Chains (E,D)

		CDglobal	AC#aa	MS_A^3	CD+	CD-	CD-total
EC1: Oxidoreductases	Mean :	2.80	47	1,552	7.70	4.61	12.31
n = 99	SD:	0.60	21	801	5.40	5.14	7.43
EC2: Transferases	Mean :	3.10	37	1,193	9.49	7.32	16.81
n = 124	SD:	0.45	16	752	7.15	4.14	9.87
EC3: Hydrolases	Mean :	2.70	26	789	11.90	10.66	22.56
n = 212	SD:	0.65	14	684	10.46	9.28	16.39
EC4: Lyases	Mean :	2.80	35	1,076	11.32	7.70	19.02
n = 72	SD:	0.54	19	832	7.45	7.37	11.83
EC5: Isomerases	Mean :	2.90	29	846	13.79	9.64	23.43
n = 43	SD:	0.77	17	713	14.80	7.20	18.77
EC6: Ligases	Mean :	3.00	41	1,233	9.72	8.33	18.04
n = 20	SD:	0.61	20	815	3.61	3.72	6.43
Total	Mean :	**2.82**	34	1066	**10.64**	**8.35**	**18.99**
n = 570	SD	**0.61**	19	794	9.13	7.49	13.79

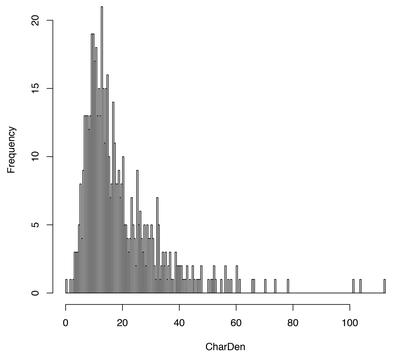
EC#: Enzyme Commission Number based on chemical reaction catalyzed
AC#aa: Number of Amino Acids in the Active Site Pocket
MS_A^3: Molecular Surface Area of the Functional Pocket (Units Angstrom^3)
***n* :** Sample Size
SD: Standard Deviation

CD+: Molar Charge Density (positive)
CD- : Molar Charge Density (negative)
CDt : Total Molar Charge Density

CharDen Distribution

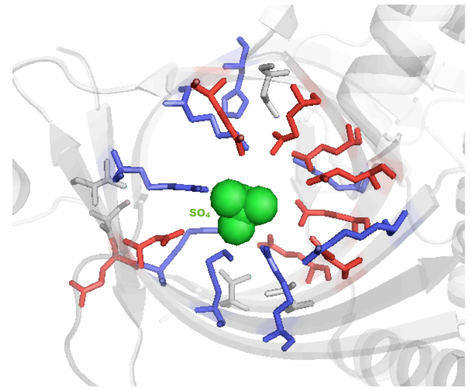
Mean Charge Density = 19 Molar

Frequency distribution of the total CharDen of Active Sites (570 enzymes)



Example of

Large Positive and Negative CharDen



RNA Triphosphatase

(PDB: 1D8H / EC: 3.1.3.33)

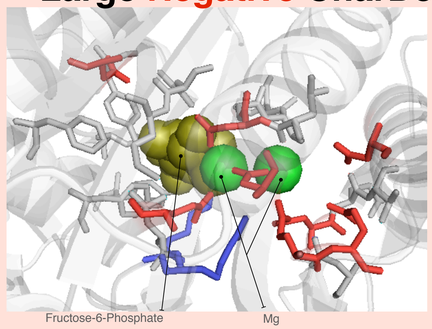
Reaction: 5'-phosphopolynucleotide + H2O = polynucleotide + P

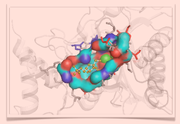
CharDen		
	Positive :	33.2 M
	Negative:	37.3 M
	Total:	70.5 M

Active Site Volume: **400.65 A^3** | **Global Protein CharDen: 2.9 M**

Example of

Large Negative CharDen





Fructose-1,6-BisPhosphatase

(PDB: 1EYI / EC: 3.1.3.11)

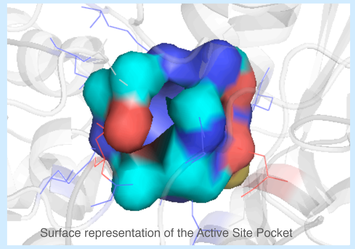
Reaction:
D-fructose 1,6-bisphosphate + H2O = D-fructose 6-phosphate + P

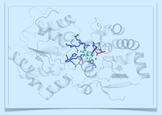
CharDen		
	Positive :	6.3 M
	Negative:	25.3 M
	Total:	31.6 M

Active Site Volume: **526.3 A^3** | **Global Protein CharDen: 3.0 M**

Example of

Large Positive CharDen





Phosphoglycerate Mutase

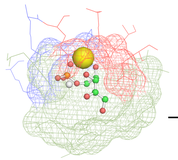
(PDB: 1O98 / EC: 5.4.2.1)

Reaction: 2-phospho-D-glycerate = 3-phospho-D-glycerate

CharDen		
	Positive :	84.7 M
	Negative:	18.8 M
	Total:	103.6 M

Active Site Volume: **176.4 A^3** | **Global Protein CharDen: 3.0 M**

Methods



C h a r D e n
Charge Density

Amount of Charge (Charged Side Chains)
R, K and H (positive); E and D (negative)
Volume (Angstrom^3)

[Units: Molar = Mol / L (mol/dm^3)]

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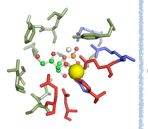
EMBL-EBI
Catalytic Site Atlas
The Catalytic Site Atlas (CSA) is a database documenting catalytic residues in 3D structure of enzymes based on primary literature



http://www.ebi.ac.uk/thornton-srv/databases/CSA/

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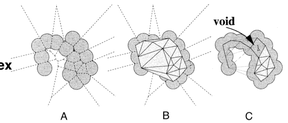
CASTp

Computed Atlas of Surface Topography of Proteins

Binding sites and active sites of proteins and DNAs are often associated with structural pockets and cavities.
CASTp uses the weighted **Delaunay triangulation** and the **alpha complex** for shape measurements. It provides identification and measurements of surface accessible pockets as well as interior inaccessible cavities, for proteins. **We used it to collect the residues held in the same pockets and cavities as the catalytic amino acids.**

http://sts.bioengr.uic.edu/castp/



(A) Voronoi diagrams (B) Convex hull tessellated by Delaunay triangulation (C) Alpha shape