BIOINFORMATICS APPLICATIONS NOTE Vol. 17 no. 3 2001
Pages 284–285

ASEdb: a database of alanine mutations and their effects on the free energy of binding in protein interactions

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Received on October 10, 2000; revised on November 3, 2000; accepted on November 13, 2000

ABSTRACT

Summary: The Alanine Scanning Energetics database (ASEdb) is a searchable database of single alanine mutations in protein–protein, protein–nucleic acid, and protein–small molecule interactions for which binding affinities have been experimentally determined. In cases where structures are available, it contains surface areas of the mutated side chain and links to the PDB entries. It is useful for studying the contribution of single amino acids to the energetics of protein interactions, and can be updated by researchers as new data are generated.

Availability: ASEdb is accessible on the Web at http://www.asedb.org

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Protein interactions with other proteins and with nucleic acids have been widely studied from both structural and energetic perspectives (Janin, 1995, 1997; Jones and Thornton, 1996; Jones *et al.*, 1999; Larsen *et al.*, 1998; McCoy *et al.*, 1997; Nadassy *et al.*, 1999). One technique that has been extremely useful in understanding the principles of protein interactions is alanine scanning mutagenesis followed by the measurement of each mutant's effect on binding (Clackson and Wells, 1995; Wells, 1991). However, a significant challenge in understanding these underlying principles has been the absence of a central repository for this data, which is published in a myriad of journals.

In a protein–protein interface, a small subset of the buried amino acids typically contribute the majority of binding affinity as determined by the change in the free energy of binding ($\Delta\Delta G$) upon mutation of the residue to alanine. Collectively, these energetically important residues are called the hot spot (Bogan and Thorn, 1998; Clackson and Wells, 1995). It has recently been shown that some hot spots of protein interaction show a high propensity for interaction with a variety of partners (DeLano *et al.*, 2000). This suggests that understanding protein hot spots may be useful not only for the analysis of a single protein–protein dimer, but also for determining likely sites of interaction for other binding partners.

To provide a centralized repository for alanine scanning data, we have constructed ASEdb, the Alanine Scanning Energetics database (http://www.asedb.org). ASEdb is a searchable relational database containing current alanine scanning data that can be updated as new data are published. ASEdb contains alanine-scanning mutational analyses of interfaces for which changes in binding energy have been measured (Table 1). Each system is cross-referenced to the publications that contained the experimental data, and to its structure in the PDB (Berman *et al.*, 2000), when available.

In addition, solvent accessible surface areas have been calculated for each mutant side chain. The program NACCESS (Hubbard and Thornton, 1993) was used for this calculation with default parameters. In cases where the dimer structure was available, surface areas were calculated both for the separated monomer and for the complex.

ASEdb contains 2919 mutants of which 1953 have structural data for the monomer and 580 have structural data for the dimer. Mutations which were judged by the authors of the original research paper to have gross effects on protein conformation were excluded from the database.

ASEdb can readily be queried to retrieve the specific types of data required by the user. For example, one can search specifically for mutations that cause a change in ΔG of binding >3.5 kcal/mol and a change in solvent accessible surface area upon binding between 20 and 40 Å^2. The mutants meeting the search criteria can then be viewed in the web browser or saved as a text file for further analysis.

Prior to the construction of the ASEdb relational database, a subset of this data was used to analyze the general properties of hot spots in protein interfaces (Bogan and Thorn, 1998). Several other recent studies

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Table 1. Initial contents of the ASEdb relational database

Interface[a]	PDB code of structure
Dimer structures	
Angiogenin–RNase inhibitor	1a4y
Barnase–Barstar	1brs
BPTI–Chymotrypsin	1cbw
BPTI–Trypsin	2ptc
D1.3–E5.2	1dvf
D1.3–Hen Egg Lysozyme	1vfb
Hen Egg Lysozyme–HyHEL-10	3hfm
hGH–hGHbp	3hhr
Im9–E9 DNase	1bxi
Mcm1–Binding Site DNA	1mnm
Protein A–IgG1	1fc2
RNase Inhibitor–RNase A	1dfj
SEC3–TCR Vb	1jck
Tissue Factor–Fab5G9	1ahw
Tissue Factor–Factor VIIa	1dan
Monomer structures	
Ab4D5-5–p185^{HER2}	1fvc
bFGF–FGFR1b	4fgf
Bovine Profilin I–Rabbit Actin	1pne
CD2–Cd48	1cdc
CD4–gp120	3cd4
Erabutoxin A–AchR	5ebx
Erabutoxin A–Ma2-3	5ebx
hG-CSF–hG-CSFbp	1rhg
hGH–hPRLbp	3hhr
hGH–27 mAbs	3hhr
hIL2–hIL2R$\alpha\beta$	3ink
IGF-1–IGF-1R	2gf1
IGF-1–IGFbp-1	2gf1
IL6–mAb8	1il6
IL4–IL4bp	1rcb
IL8–IL8R	3il8
NT–3-gp75	1nt3
NT–3trkC	1nt3
scTCR Vb–SEC3-1A4	1jck

[a] References for all data are included in the database.

have also used this data. Two groups have analysed high resolution structural data of protein interfaces in the context of binding energetics (Lo Conte *et al.*, 1999; Vaughan *et al.*, 1999). This data has also been used to study solvent (Janin, 1999), electrostatics (Sheinerman *et al.*, 2000), and evolutionary conservation (Hu *et al.*, 2000) at protein interfaces. Finally, the database has recently been used to establish a novel parameter for the analysis of protein structure and stability, residue depth (Chakravarty and Varadarajan, 1999).

In the future, ASEdb will continue to be updated. To keep the database current, submission of new data is encouraged. Mutagenesis and binding data should be from peer-reviewed, published sources and can be submitted directly from the ASEdb web site (http://www.asedb.org). All submitted data will be edited for proper formatting.

ACKNOWLEDGEMENTS

K.S.T. was supported by a Howard Hughes Medical Institute Predoctoral Fellowship. A.A.B. was supported by a National Defense Science and Engineering Graduate Fellowship from the United States Department of Defense and by the Lloyd M. Kozloff Fellowship.

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