VOL.6 NO.2 September 2017 - ISSN 2233 – 1859

Operations Research Society in Bosnia and Herzegovina

Southeast Europe Journal of Soft Computing

Available online: http://scjournal.ius.edu.ba

IUS Soft Computing Research Group

Hydrophilicity of Linear B-Epitopes

M. Can

Faculty of Engineering and Natural Sciences,
International University of Sarajevo International University of Sarajevo,
Hrasnicka Cesta 15, Ilidža 71210 Sarajevo,
Bosnia and Herzegovina
mcan@ius.edu.ba

Article Info

Article history:
Article received on 10 June 2017
Received in revised form 1 August 2017

Keywords:
hydrophilicity/hydrophobicity of B-cell epitopes; Parker's scale, Cornette scale, Doolittle scale

ABSTRACT: Antigenic sites of a protein are those recognized by antibodies. Therefore it is most likely that these sites are accessible or on the surface of the protein, and these regions are probably more mobile than interior regions. Since these sites are on the surface, they are probably hydrophilic. Indeed, algorithms for hydrophilicity and accessibility have been used to predict antigenicity. In this research, the hydrophilicity by Parker's scale, and hydrophobicityby Cornette, and Doolittle scales of linear b-rpitopes is studied on 344121 linear b-eptopes downloaded from *iedb* epitope database. Descriptive statistical analyses revealed that average hydrophilicity of these b-epitopes distributes normally with mean μ=2.1993, standard deviation σ=1.9303, skewness s= - 0.2681, and kurtosis κ=3.1826. It is seen that mean hydrophobicities are also distribute normally. A detailed review is performed to scan available hydrophophilicity/hydrophobicity scales. Altogether 24 scales are listed.

1. INTRODUCTION

Antigenic sites are residues of a native protein that are those recognized by antibodies. It is most probably that these sites are accessible or on the surface of a antigenic proteins. Furthermore these regions are possibly more mobile than interior regions. Since these sites are on the surface, they are probably hydrophilic. Indeed, scores for hydrophilicity, flexibility, and accessibility have been used to predict antigenicity.

At least for the past three decades, new tools are emerged to predict the Antigenic sites, which are called epitopes of antigenic proteins. Prediction of immunogenic epitopes using bioinformatics tools is a challenging task because of the inherent complexity of antigen recognition[1]. At the beginning, to predict continuous B-cell epitopes, a number of algorithms have been developed based on physico-chemical properties of amino acids [2],but their accuracies were very low. The commonly used properties are hydrophilicity [3]flexibility [4], accessibility [5], and turns [6]alongside Levitt secondary structure, accessibility, flexibility, beta turns, polarity, Amino Acid Pair (AAP) antigenicity scale, Amino acid triplet (AAT), Relative position specific amino acid propensity of a dipeptide, Hydropathy, dipeptide, tripeptide and tetrapeptide antigenicity as seen in Table 1.All the prediction calculations are based on the propensity tables for each of the 20 amino acids. Since hydrophilicity/hydrophilicity is one of the main futures to detect b-epitopes, in this article the relation between hydrophilicity/hydrophilicity and antigenicity is studied through 344,121 b-epitopes downloaded from *eidb* database..

Table 1: Comparison of machine learning approaches in epitope prediction [7].

Method	Features used	MLearning Technique
BepiPred [8]	Parker hydrophilicity scale and Levitt secondary structure	Hidden Markov model
ABCPred [9]	Hydrophilicity, accessibility, flexibility, turns, antigenicity, polarity	FF and recurrent NN
Cheng et.al [10]	Hydrophilicity, accessibility, flexibility, turns, antigenicity, Amino Acid Pair (AAP) antigenicity scale	Support Vector Machine(SVM)
BCPred [11]	Hydrophilicity, accessibility, flexibility, turns, antigenicity, Amino Acid Pair(AAP) antigenicity scale	Subsequence kernel based SVM
AAT-fs [12]	Amino acid triplet (AAT) antigenicity scale	Kernel based SVM
BayesB [13]	Relative position specific amino acid propensity of a dipeptide	SVM
LEPS [14]	Hydropathy, accessibility, flexibility, turns, antigenicity, polarity, dipeptide, tripeptide and tetrapeptide antigenicity	Radial Basis Kernel based SVM

2. HYDROPHOBICITY SCALES

Several hydrophobicity scales have been published for various uses. Many of the commonly used hydrophobicity scales are described below. A larger list is given in Tables 3-5.

Kyte-Doolittle scale [15].The Kyte-Doolittle scale is widely used for detecting hydrophobic regions in proteins. Regions with a positive value are hydrophobic. This scale can be used for identifying both surface-exposed regions as well as transmembrane regions, depending on the window size used. Short window sizes of 5-7 generally work well for predicting putative surface-exposed regions. Large window sizes of 19-21 are well suited for finding transmembrane domains if the values calculated are above 1.6. These values should be used as a rule of thumb and deviations from the rule may occur.

Engelman scale [16]. The Engelman hydrophobicity scale, also known as the GES-scale, is another scale which can be used for prediction of protein hydrophobicity. As the Kyte-Doolittle scale, this scale is useful for predicting transmembrane regions in proteins.

Eisenberg scale [17]. The Eisenberg scale is a normalized consensus hydrophobicity scale which shares many features with the other hydrophobicity scales.

Hopp-Woods scale [18]. Hopp and Woods developed their hydrophobicity scale for identification of potentially antigenic sites in proteins. This scale is basically a hydrophilic index where apolar residues have been assigned negative values. Antigenic sites are likely to be predicted when using a window size of 7.

Cornette scale [19]. Cornette et al. computed an optimal hydrophobicity scale based on 28 published scales. This optimized scale is also suitable for prediction of alpha-helices in proteins.

Rose scale [20]. The hydrophobicity scale by Rose et al. is correlated to the average area of buried amino acids in globular proteins. This results in a scale which is not showing the helices of a protein, but rather the surface accessibility.

To standardize hydrophobicity scale tables let us adopt amino acid names, their three letters, and one letter abbreviations as in the Table 2.

Table 2. Amino Acid names, their three letters, and one letter abbreviations

No	Full Name	3 Letter	1 Letter
1	Alanine	Ala	A
2	Arginine	Arg	R
3	Asparagine	Asn	N
4	Aspartate	Asp	D
5	Cysteine	Cys	C
6	Glutamate	Glu	E
7	Glutamine	Gln	Q
8	Glycine	Gly	G
9	Histidine	His	H
10	Isoleucine	Ile	I
11	Leucine	Leu	L
12	Lysine	Lys	K
13	Methionine	Met	M
14	Phenylalanine	Phe	F
15	Proline	Pro	P
16	Serine	Ser	S
17	Threonine	Thr	T
18	Tryptophan	Trp	W
19	Tyrosine	Tyr	Y
20	Valine	Val	V

Janin scale [21]. This scale also provides information about the accessible and buried amino acid residues of globular proteins.

Welling scale [22].Welling et al. used information on the relative occurrence of amino acids in antigenic regions to make a scale which is useful for prediction of antigenic regions. This method is better than the Hopp-Woods scale of hydrophobicity which is also used to identify antigenic regions.

Kolaskar-Tongaonkar [23]. A semi-empirical method for prediction of antigenic regions has been developed. This method also includes information of surface accessibility and flexibility and at the time of publication the method was able to predict antigenic determinants with an accuracy of 75%.

Surface Probability [24]. Display of surface probability based on the algorithm by [Emini et al., 1985]. This algorithm has been used to identify antigenic determinants on the surface of proteins.

Chain Flexibility [25]. Display of backbone chain flexibility based on the algorithm by Karplus and Schulz, (1985). It is known that chain flexibility is an indication of a putative antigenic determinant. Many more scales have been published throughout the last three decades. Even though more advanced methods have been developed for prediction of membrane spanning regions, the simple and very fast calculations are still highly used.

Table 3 Hydrophobicity scales

No	a	Hessa [26]	Doolittle [15]	Hopp-W [18]	Cornette [19]	Eisenberg [17]	Rose [20]	Janin [21]	Engelman [16]
1	A	0.11	1.8	-0.5	0.2	0.62	0.74	0.3	1.6
2	R	2.58	-4.5	3	1.4	-2.53	0.64	-1.4	-12.3
3	N	2.05	-3.5	0.2	-0.5	-0.78	0.63	-0.5	-4.8
4	D	3.49	-3.5	3	-3.1	-0.9	0.62	-0.6	-9.2
5	C	-0.13	2.5	-1	4.1	0.29	0.91	0.9	2
7	E	2.36	-3.5	3	-1.8	-0.74	0.62	-0.7	-8.2
7	Q	2.68	-3.5	0.2	-2.8	-0.85	0.62	-0.7	-4.1
8	G	0.74	-0.4	0	0	0.48	0.72	0.3	1
9	H	2.06	-3.2	-0.5	0.5	-0.4	0.78	-0.1	-3
10	I	-0.6	4.5	-1.8	4.8	1.38	0.88	0.7	3.1
11	L	-0.55	3.8	-1.8	5.7	1.06	0.85	0.5	2.8
12	K	2.71	-3.9	3	-3.1	-1.5	0.52	-1.8	-8.8
13	M	-0.1	1.9	-1.3	4.2	0.64	0.85	0.4	3.4
14	F	-0.32	2.8	-2.5	4.4	1.19	0.88	0.5	3.7
15	P	2.23	-1.6	0	-2.2	0.12	0.64	-0.3	-0.2
16	S	0.84	-0.8	0.3	-0.5	-0.18	0.66	-0.1	0.6
17	T	0.52	-0.7	-0.4	-1.9	-0.05	0.7	-0.2	1.2
18	W	0.3	-0.9	-3.4	1	0.81	0.85	0.3	1.9
19	Y	0.68	-1.3	-2.3	3.2	0.26	0.76	-0.4	-0.7
20	V	-0.31	4.2	-1.5	4.7	1.08	0.86	0.6	2.6

Table 4 Hydrophobicity scales continued.

No	a	Bull [27	Sweet [28]	Abraham [29]	Parker* [30]	Guy [31]	Myazava [32]	Roseman [33]	Wolfenden [34]
1	A	0.61	-0.4	0.44	2.100	0.1	5.33	0.39	1.94
2	R	0.69	-0.59	-2.42	4.200	1.91	4.18	-3.95	-19.92
3	N	0.89	-0.92	-1.32	7.000	0.48	3.71	-1.91	-9.68
4	D	0.61	-1.31	-0.31	10.000	0.78	3.59	-3.81	-10.95
5	C	0.36	0.17	0.58	1.400	-1.42	7.93	0.25	-1.24
7	E	0.97	-0.91	-0.71	6.000	0.95	3.87	-1.3	-9.38
7	Q	0.51	-1.22	-0.34	7.800	0.83	3.65	-2.91	-10.2
8	G	0.81	-0.67	0	5.700	0.33	4.48	0	2.39
9	H	0.69	-0.64	-0.01	2.100	-0.5	5.1	-0.64	-10.27
10	I	-1.45	1.25	2.46	-8.000	-1.13	8.83	1.82	2.15
11	L	-1.65	1.22	2.46	-9.200	-1.18	8.47	1.82	2.28
12	K	0.46	-0.67	-2.45	5.700	1.4	2.95	-2.77	-9.52
13	M	-0.66	1.02	1.1	-4.200	-1.59	8.95	0.96	-1.48
14	F	-1.52	1.92	2.54	-9.200	-2.12	9.03	2.27	-0.76
15	P	-0.17	-0.49	1.29	2.100	0.73	3.87	0.99	0
16	S	0.42	-0.55	-0.84	6.500	0.52	4.09	-1.24	-5.06
17	T	0.29	-0.28	-0.41	5.200	0.07	4.49	-1	-4.88
18	W	-1.2	0.5	2.56	-10.000	-0.51	7.66	2.13	-5.88
19	Y	-1.43	1.67	1.63	-1.900	-0.21	5.89	1.47	-6.11
20	V	0.61	0.91	1.73	-3.700	-1.27	7.63	1.3	1.99

*Parker's scale is the hydrophilicity scale.

Table 5 Hydrophobicity scales continued.

No	a	Wilson [35]	Cowan [36]	Aboderin [37]	Fauchere [38]	Cowan-W [39]	Tanford [40]	Wimley [41]	Moon [42]
1	A	-0.3	0.42	5.1	0.31	0.35	0.62	-0.17	0
2	R	-1.1	-1.56	2	-1.01	-1.5	-2.53	-0.81	3.71
3	N	-0.2	-1.03	0.6	-0.6	-0.99	-0.78	-0.42	3.47
4	D	-1.4	-0.51	0.7	-0.77	-2.15	-0.09	-1.23	2.95
5	C	6.3	0.84	0	1.54	0.76	0.29	0.24	0.49
7	E	-0.2	-0.96	1.4	-0.22	-0.93	-0.85	-0.58	3.01
7	Q	0	-0.37	1.8	-0.64	-1.95	-0.74	-2.02	1.64
8	G	1.2	0	4.1	0	0	0.48	-0.01	1.72
9	H	-1.3	-2.28	1.6	0.13	-0.65	-0.4	-0.96	4.76
10	I	4.3	1.81	9.3	1.8	1.83	1.38	0.31	-1.56
11	L	6.6	1.8	10	1.7	1.8	1.53	0.56	-1.81
12	K	-3.6	-2.03	1.3	-0.99	-1.54	-1.5	-0.99	5.39
13	M	2.5	1.18	8.7	1.23	1.1	0.64	0.23	-0.76
14	F	7.5	1.74	9.6	1.79	1.69	1.19	1.13	-2.2
15	P	2.2	0.86	4.9	0.72	0.84	0.12	-0.45	-1.52
16	S	-0.6	-0.64	3.1	-0.04	-0.63	-0.18	-0.13	1.83
17	T	-2.2	-0.26	3.5	0.26	-0.27	-0.05	-0.14	1.78
18	W	7.9	1.46	9.2	2.25	1.35	0.81	1.85	-0.38
19	Y	7.1	0.51	8	0.96	0.39	0.26	0.94	-1.09
20	V	5.9	1.34	8.5	1.22	1.32	1.8	-0.07	-0.78

3. METHODS

To search the hydrophilicity of linear b-epitopes, a sample of five antigenic peptidesESAT6, PFAL, Menengitis, Poli, and P Vıva, are considered.

3.1 The Five Antigens with Known Linear B-Epitopes

To have an idea about the hydrophilicities of b-epitopes, we have chosen five antigens, whose linear b-epitopes are known through wet lab experiments. Plasmodium Falciparum, Human Polio Virus [43-44], Sabin Strain, Meningitis [46], Plasmodium Vivax [47] and Mycobacterium Tuberculosis [45].

The hydrophilicity of their wet lab reported b-epitopes are computed using Parker, Doolittle, Cornette, and Abraham scores. Results are shown in Table 6.

Table 6 The hydrophilicity of wet lab reported b-epitopes of five antigenic peptides as computed using four different scores.

Anti	Parker	Doolittle	Cornette	Abraham
ESAT	2.38	-0.26	0.28	0.19
PFAL	2.96	-1.12	0.18	-0.01
MEN	2.17	-0.71	0.51	0.06
POLI	1.11	-0.26	0.90	0.35
PVIV	1.32	-0.14	0.78	0.26
AVER	1.99	-0.50	0.53	0.17

To support the claim that linear b-epitopes are essentially hydrophilic, a large scale statistical research is performed on the 344,121 linear b-epitopes of idb database using Parker's hydrophilicity score.

3.1 The Data Set

B-cell epitopes are downloaded from IEDB database[1] that contains 365 076 continuous B-epitopes. After elimination of identical peptides, a data with 344.121 non-redundant entries resulted. The distribution of frequencies of epitopes in their lengths is shown in Table 7.

Table 7. Frequencies of B-Epitopes of given lengths

W Size	Freq	W Size	Freq
6	6862	16	6868
7	5652	17	3462
8	14567	18	3621
9	7953	19	4249
10	19257	20	5914
11	3197	21	2446
12	14128	22	1428
13	5401	23	1454
14	7590	24	1719
15	226459	25	1894

[1] http://www.iedb.org/database_export_v3.php

3.2 Descriptive Statistical Parameters of Hydrophilicity

Hydrophilicity of 344.121 of linear b-epitopes are computed according to Parker, Cornette, and Doolittle hydrophilicity scores. Mean, skewness , kurtosis, and standard deviations are shown in Table 8.

Table 8. Mean hydrophilicity of 344.121 linear b-epitopes according to three different scores all distributes normally

	Parker	Cornette	Doolittle
Mean	2.1993	0.4135	-0.5634
STDV	1.9303	1.0102	0.9696
Skewness	-0.2861	0.1306	0.1609
Kurtosis	3.1826	3.0541	3.6182

It is seen that average hydrophilicity of b-epitopes distribute almost normally with a mean of 2.1993.

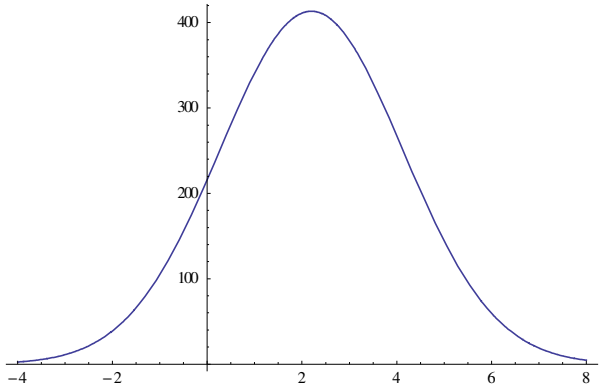

Figure 1. Normal distribution curve with a mean of 2.1993, and standard deviation 1.9303 for Parker's hydrophilicityscale.

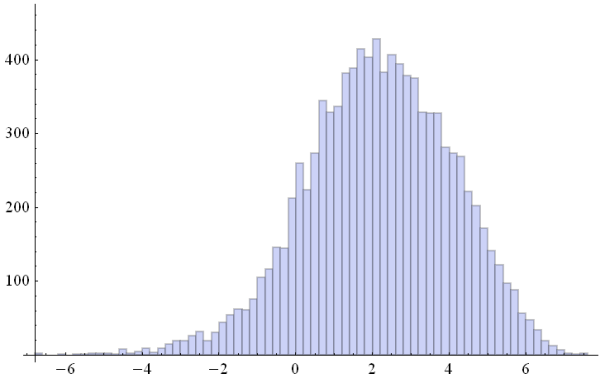

Figure 2. Histogram for average hydrophilicity of randomly chosen 10,000 b-epitopes computed by Parker's hydrophilicityscale.

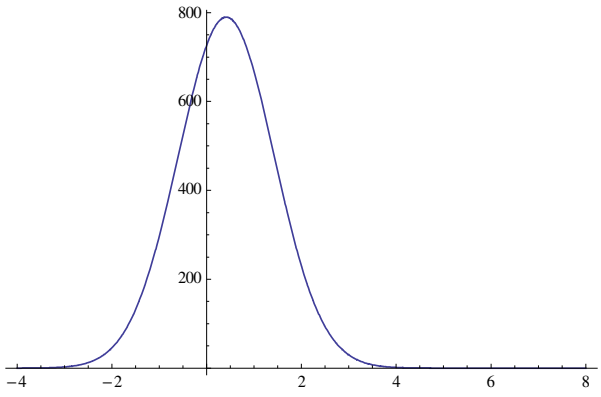

Figure 3. Normal distribution curve with a mean of 0.4135, and standard deviation 1.0102for Cornette's hydrophobicity scale.

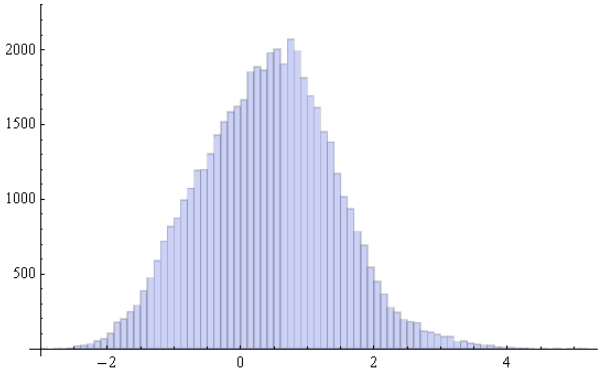

Figure 4. Histogram for average hydrophobicity of randomly chosen 10,000 b-epitopes computed by Cornette's scale.

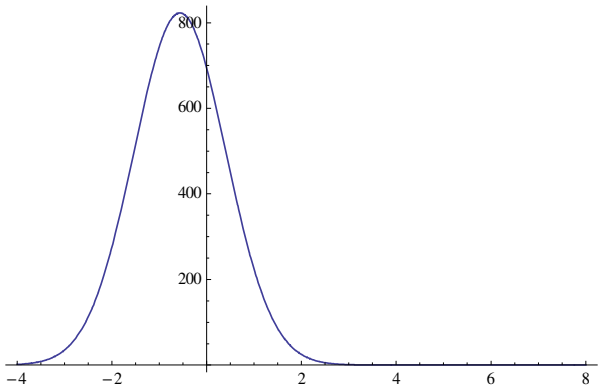

Figure 5. Normal distribution curve with a mean of --0.5634, and standard deviation 0.9696for Doolittle's hydrophobicity scale.

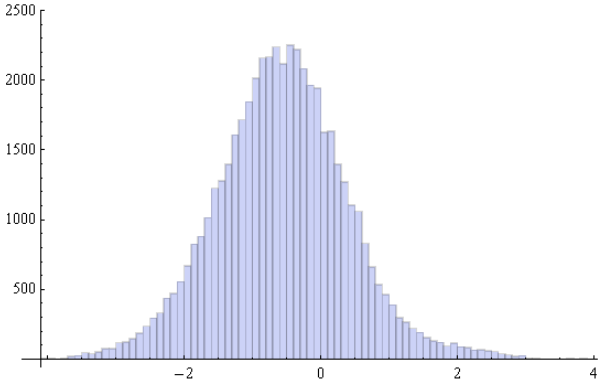

Figure 6. Histogram for average hydrophobicity of randomly chosen 10,000 b-epitopes computed by doolittle's scale.

4. CONCLUSION

For 344.121 B-cell epitopes downloaded from IEDB database. The average hydrophilicities are seen distributed normally with a mean of 2.1993, and standard deviation 1.9303 for Parker's hydrophilicity scale. This result sows that although 25% of b-epitopes are average hydrophobic, they are essentially hydrophilic. Hydrophobicity test performed by Cornette and Doolittle scales showed that b-epitopes are essentially not hydrophobic. However there is a difficulty in using hydrophilicity or hydrophobicity as a validation feature to distinguish epitopes among peptides predicted by insiliko techniques.

REFERENCES

[1] Flower DR. Towards insilico prediction of immunogenic epitopes. Trends Immunol;24:667–674; 2003.

[2] Pellequer JL, Westhof E, Regenmortel MHV. Predicting location of continuous epitopes in proteins from their primary structures. Methods Enzymol;203:176–201; 1991.

[3] Parker JMD, Guo D, Hodges RS. New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and X-ray-derived accessible sites. Biochemistry;25: 5425–5432; 1986.

[4] Karplus PA, Schulz GE. Prediction of chain flexibility in proteins: a tool for the selection of peptide antigen. Naturwissenschaften;72:212,213; 1985.

[5] Emini EA, Hughes JV, Perlow DS, Boger J. Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. J Virol;55:836–839; 1985.

[6] Pellequer J-L, Westhof E, Regenmortel MHV. Correlation between the location of antigenic sites and the prediction of turns in proteins. Immunol Lett;36:83–99; 1993.

[7] KavithaK V, Saritha R, Vinod Chandra S S, Computational Methods in Linear B-cell Epitope Prediction, International Journal of Computer Applications (0975 – 8887) Volume 63– No.12, February 2013 28

[8] Larsen, J., Lund, O., and Nielsen, M., Improved method for predicting linear B-cell epitopes, Immunome Research, vol. 2, article 2, 2006. W24–W29

[9] Saha S, Raghava GP. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins. 2006 Oct 1;65(1):40-8.

[10] Cheng, J., Tegge, AN., and Baldi, P., Machine Learning Methods for Protein Structure Prediction, Ieee Reviews In Biomedical Engineering, Vol. 1, 2008 41

[11] El-Manzalawy Y, Dobbs D, Honavar V (2008) Predicting linear B-cell epitopes using string kernels. Journal of Molecular Recognition 21: 243–255.

[12] Zhang, W., and Niu, Y. Predicting flexible length linear B-cell epitopes using pair wise sequence similarity, Third International Conference on Biomedical engineering and Informatics, 2010.

[13] Lawrence JK Wee1,2, Diane Simarmata1 , Yiu-Wing Kam1 , Lisa FP Ng1,3, Joo Chuan Tong, SVM-based prediction of linear B-cell epitopes using Bayes Feature Extraction, Wee et al. BMC Genomics 2010, 11(Suppl 4):S21

[14] Wang HW, Lin YC, Pai TW, Chang HT (2011) Prediction of B cell linear epitopes with a combination of support vector machine classification and amino acid propensity identification. J Biomed Biotechnol, 2011: 432830

[15] Kyte J., Doolittle R.F,. Amino acid scale: Hydropathicity.J. Mol. Biol. 157:105-132(1982).

[16] Engelman, D. M., Steitz, T. A., and Goldman, A. (1986). Identifying nonpolar transbilayer helices in amino acid sequences of membrane proteins. Annu Rev Biophys Biophys Chem, 15:321-353.

[17] Eisenberg D., Schwarz E., Komarony M., Wall R.

Amino acid scale: Normalized consensus hydrophobicity scale. J. Mol. Biol. 179:125-142(1984).

[18] Hopp T.P., Woods K.R., Amino acid scale: Hydrophilicity. Proc. Natl. Acad. Sci. U.S.A. 78:3824-3828(1981).

[19] Cornette, L, Cease, KB., Margalit, H., Spouge, JL., Berzofsky, JL., and DeLisi, C., Hydrophobicity scales and computational techniques for detecting amphipathic structures in proteins, Journal of Molecular Biology, Volume 195, Issue 3, 5 June 1987, Pages 659-685

[20] Rose, G. D., 1978, Prediction of chain turns in globular proteins on a hydrophobic basis, Nature 272:586–590.

[21] Janin, J. (1979). Surface and inside volumes in globular proteins. Nature, 277(5696):491-492.

[22] Welling, G.W., Weijer,W.J., van der Zee,R. and Welling-Wester,S. (1985) Prediction of sequential antigenic regions in proteins.FEBS Lett., 188, 215–218.

[23] Kolaskar, AS., Tongaonkar, PC,A semi-empirical method for prediction of antigenic determinants on protein antigens, FEBS Lett,vol 276,pp172-174,1990

[24] Emini, EA, Hughes, JV, Perlow, DS, and Boger J. Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. J. Virol. September 1985 vol. 55 no. 3 836-839

[25] Karplus, PA, and Schulz, G.E. Prediction of chain flexibility in proteins – a tool for the selection of peptide antigens, Naturwissenschaft, vol 72, pp 212-213, 1985.

[26] Hessa T, Kim H, Bihlmaier K, Lundin C, Boekel J, Andersson H, Nilsson I, White SH, von Heijne G. Recognition of transmembrane helices by the endoplasmic reticulum translocon. Nature. 2005 Jan 27;433(7024):377-81, supplementary data. Attribute assignment file hhHydrophobicity.txt. In this scale (unlike the first two), more negative values reflect greater hydrophobicity.

[27] Bull H.B., Breese K. Amino acid scale: Hydrophobicity (free energy of transfer to surface in kcal/mole).Arch. Biochem. Biophys. 161:665-670(1974).

[28]Sweet R.M., Eisenberg D. Amino acid scale: Optimized matching hydrophobicity (OMH).J. Mol. Biol. 171:479-488(1983).

[29] Abraham D.J., Leo A.J. , Proteins: Structure, Function and Genetics 2:130-152(1987).

[30] Parker, J. M. R., Guo, D., and Hodges, R. S., New Hydrophilicity Scale Derived from High-Performance Liquid Chromatography Peptide Retention Data Correlation of Predicted Surface Residues with Antigenicity and X-ray-Derived Accessible Sites, Biochemistry 1986, 25, 5425-5432 5425

[31] Guy H.R. Amino acid scale: Hydrophobicity scale based on free energy of transfer (kcal/mole). Biophys J. 47:61-70 (1985).

[32]Miyazawa S., Jernigen R.L. Amino acid scale: Hydrophobicity scale (contact energy derived from 3D data).Macromolecules 18:534-552(1985).

[33] Roseman M.A. Amino acid scale: Hydrophobicity scale (pi-r).J. Mol. Biol. 200:513-522(1988).

[34] Wolfenden R.V., Andersson L., Cullis P.M., Southgate C.C.F. Amino acid scale: Hydration potential (kcal/mole) at 25øC.Biochemistry 20:849-855(1981).

[35] Wilson K.J., Honegger A., Stotzel R.P., Hughes G.J. Amino acid scale: Hydrophobic constants derived from HPLC peptide retention times. Biochem. J. 199:31-41(1981).

[36] Cowan R., Whittaker R.G. Amino acid scale: Hydrophobicity indices at ph 3.4 determined by HPLC. Peptide Research 3:75-80(1990).

[37] Aboderin A.A. Amino acid scale: Mobilities of amino acids on chromatography paper (RF).Int. J. Biochem. 2:537-544(1971).

[38] Fauchere J.-L., Pliska V.E.Amino acid scale: Hydrophobicity scale (pi-r).Eur. J. Med. Chem. 18:369-375(1983).

[39] Cowan R., Whittaker R.G. Amino acid scale: Hydrophobicity indices at ph 7.5 determined by HPLC. Peptide Research 3:75-80(1990).

[40] Tanford C. Amino acid scale: Hydrophobicity scale (Contribution of hydrophobic interactions to the stability of the globular conformation of proteins).J. Am. Chem. Soc. 84:4240-4274(1962).

[41] Wimley WC, White SH. Experimentally determined hydrophobicity scale for proteins at membrane interfaces. Nat Struct Biol. 1996 Oct;3(10):842-8. Attribute assignment filewwHydrophobicity.txt.

[42] Moon CP, Fleming KG. Side-chain hydrophobicity scale derived from transmembrane protein folding into lipid bilayers. Proc Natl Acad Sci USA. 2011 Jun 21;108(25):10174-7, supplementary data. Attribute assignment file mfHydrophobicity.txt. In this scale (unlike the first two), more negative values reflect greater hydrophobicity.

[43] Nomoto A., Omata T., Toyoda H., Kuge S., Horie H., Kataoka Y., Genba Y., Nakano Y., Imura N., Complete nucleotide sequence of the attenuated poliovirus Sabin 1 strain genome." Proc. Natl. Acad. Sci. U.S.A. 79:5793-5797(1982)

http://www.uniprot.org/uniprot/P03301#sequences

[44] Kanduc, D., Fasano, C., Capone, G., Delfino, AP., Calabrò, M., and Polimeno, M., Applying the Concept of Peptide Uniqueness to Anti-Polio Vaccination, Journal of Immunology Research, Volume 2015, Article ID 541282, 8 pages

[45] Yang, Y., Chen, H., Liu, Z., Ma, H., Qin, L., Jin, J., Zheng, R., Feng, Y., Cui, Z., Wang, J., Liu, J., Hu,.Z, A Novel B-Cell Epitope Identified within Mycobacterium tuberculosis CFP10/ESAT-6 Protein, Published: January 7, 2013https://doi.org/10.1371/journal.pone.0052848

[46] Chandra, S., and Singh, TR., Linear B cell epitope prediction for epitope vaccine design against meningococcal disease and their computational validations through physicochemical properties, Netw Model Anal Health Inform Bioinforma (2012) 1:153–159.

[47] Caro-Aguilar, I., Rodríguez, A., Calvo-Calle, JM., Guzmán, F., De la Vega, P., Patarroyo, ME., Galinski, MR., and Moreno, A. Plasmodium vivax Promiscuous T-Helper Epitopes Defined and Evaluated as Linear Peptide Chimera Immunogens, INFECTION AND IMMUNITY, July 2002, p. 3479–3492 Vol. 70, No. 7