

Supplementary Information

Rob C.A. Keller

Section Chemistry, Charlemagne College, Wilhelminastraat 13-15, 6524 AJ Nijmegen,
The Netherlands; E-Mail: rcakeller@kpnmail.nl; Tel.: +0031-243820460; Fax: +0031243820460

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Abstract: The Eisenberg plot or hydrophobic moment plot methodology is one of the most frequently used methods of bioinformatics. Bioinformatics is more and more recognised as a helpful tool in Life Sciences in general and recent developments in approaches recognizing lipid binding regions in proteins are promising in this respect. In this study a bioinformatics approach specialized in identifying lipid binding helical regions in proteins was used to obtain an Eisenberg plot. The validity of the Heliquist generated hydrophobic moment plot were checked and exemplified. This study indicates that the Eisenberg plot methodology can be transferred to another hydrophobicity scale and renders a user-friendly approach which can be utilized in routine checks in protein-lipid interaction and in protein and peptide lipid binding characterization studies. A combined approach seems to be advantageous and results in a powerful tool in the search of helical lipid-binding regions in protein and peptides. The strength and limitations of the Eisenberg plot approach itself are discussed as well. The presented approach not only leads to a better understanding of the nature of the protein-lipid interactions and provides a user-friendly tool for the search of lipid-binding regions in proteins and peptides.

Keywords: Amphitropic proteins; Eisenberg plot; Hydrophobic moment plot; Heliquist; Lipid Binding Regions; Protein-lipid interactions; Transmembrane proteins .

Table S1. Helices included in the Heliquet generated hydrophobic moment plot. Overview of results extracted from the database constructed by Eisenberg *et al.* (1982).

Protein	Sequence	1st residue	<H>	<μH>
<i>Globular:</i>				
Adenylate Kinase (P00571)	PLETVLDMLRDAMVAKVDTS	69(Leu)	0.412	0.375
	PETMTKRLKRGESGRVDD	123(Glu)	-0.026	0.275
	FEKINEGFDLLRSGESIRTIL	353(Glu)	0.214	0.324
	SVDDVFSQVCTHLDTLK	179(Val)	0.467	0.506
Alcohol dehydrogenase (P00327-1)	CLIGCGFSTGYGSAVKVAK	170(Cys)	0.628	0.129
	KSKDSVPKLVADFMKKFAL	202(Gly)	0.557	0.079
	KSKDSVPKLVADFMKKFAL	324(Ser)	0.182	0.303
	FEKINEGFDLLRSGESIRTIL	353(Glu)	0.214	0.324
Carboxypeptidase A (P00730)	HTLDEIYDFMDLLVAEHPQLV	14(Thr)	0.539	0.415
	TNPDGFAFTHSQNRLWRKTR	112(Asn)	0.221	0.236
	SEVEVKSIQVDFVKDHGNFKA	173(Glu)	0.220	0.336
	PDKTELNQVAKSAVEALKSL	215(Asp)	0.062	0.324
	SQIPTAQETWLGVLTIMEH	285(Gln)	0.755	0.365
Chymotrypsin (P00766)	ARVTALVNWVQQTAAAN	230(Arg)	0.507	0.290
HA2 haemagglutinin (P03437)	QDLLEKYVEDTKIDLWSYNAE	77(Glu)	0.282	0.239
Lactate dehydrogenase (P00344)	AVGMACAISILMKDLADEVA	32(Val)	0.569	0.332
	VMEDKLKGEMMDLQHGSF	55(Met)	0.314	0.151
	QQEGESRLNLVQRNVNIFKF	107(Glu)	0.541	0.282
	KFIIPDIVKHSPDCIILVVS	120(Phe)	0.927	0.259
	SGCNLDSARFRYLMGERLGVHS	165(Cys)	0.393	0.137
	TSWAIGLSVADLAETIMKNLCR	249(Trp)	0.593	0.259
	KLKPDEEQQLQKSATTLWDIQK	308(Lys)	0.177	0.260
Myoglobin (P02185)	MVLSEGEWQLVLHVWAKVEAD	1(Val)	0.653	0.242
	ADVAGHGQDILIRLFKSHPETL	20(Asp)	0.417	0.401
	ASEDLKKHGVTVLTALGAIL	58(Ser)	0.399	0.362
	IPIKYLEFISEAIIHVLHSRHP	100(Pro)	0.751	0.434
	GADAQGAMNKALELFRKDI	125(Ala)	0.193	0.448
Myohaem Erythrin (P02247-1)	FYQLDEEHKKIFKGIFDCI	18(Tyr)	0.446	0.442
	NSAPNLATLVKVTTNHFTH	40(Ser)	0.310	0.435
	SEVVPKKMKHKDFLEKIGG	69(Glu)	0.161	0.336
	DAKNVDYCKEVLVNHIGT	93(Ala)	0.423	0.327
Ribonuclease S (P61823-1)	ETAAAKFERQHMDSSSTAASSS	3(Thr)	0.082	0.189
	SNYCNQMMKSRNLTKDRCKPV	24(Asn)	0.036	0.330
Thermolysin (P00800)	YDAPAVDAHYYAGVTYDYYKN	67(Asp)	0.404	0.123
	GIDVVAHELTHAVTDYTAGLIY	137(Ile)	0.439	0.318
	IYQNESGAINEAISDIFGTLVE	160(Glu)	0.424	0.473
	SGIINKAAYLISQGGTHYGVS	235(Gly)	0.454	0.307
	GRDKLGKIFYRALTQYLTPSN	260(Arg)	0.398	0.396
	NFSQLRAAAVQSATDLYGSTS	281(Phe)	0.363	0.276
	TSQEVASVKQAFDAVGK	301(Gln)	0.219	0.218

Table S1. Cont.

Protein	Sequence	1st residue	<H>	<μH>
TMV coat protein (P03571)	ADPIELINLCTNALGNQFQT	20(Pro)	0.594	0.450
	QTQQARTVVQRQFSEVWKPS	38(Gln)	0.243	0.336
	YNAVLDP LVTALLGAFDTRN	74(Ala)	0.558	0.294
	RRVDDATVAIRSAINNLIVE	114(Val)	0.418	0.365
Triose phosphate Isomerise (P00940)	GDKKS LGELIHTLNGAKLSAD	17(Lys)	0.236	0.230
	APSIYLD FARQKLD AKIGVAA	44(Pro)	0.418	0.166
	ESDELIGQKV AHALAEGLGVI	105(Ser)	0.327	0.180
	GITEKVVFEQTKAIADNVKDW	138(Ile)	0.242	0.327
	ATPQQAQEVHEKLRGWLKSH	177(Thr)	0.184	0.466
	VTGGNCKELASQHDVDGFLVG	213(Thr)	0.257	0.059
<i>Membrane:</i>				
Glycophorin (P02724)	ITLIIFGVMAGVIGTILLISYG	12(Ile)	1.133	0.213
Glycoprotein (P03522)	IASFFFIIGLIIGLFLVGIH	51(Ser)	1.264	0.245
Hemagglutinin (P03437)	WISFAISCFLLCVVLLGFI	185(Trp)	1.321	0.216
Hemagglutinin (P03451)	VYQILAIYATVAGSLSLAIM	527(Val)	0.792	0.234
IgM (P01871)	NLWATASTFIVLFLLSLFY	569(Asn)	1.089	0.285
Isomaltase (P07768)	ITLIVLFVIVFHIAIALIAV	10(Ile)	1.434	0.119
M13 coat (P69541)	AMVVVIVGATIGIKLFKKFT	20(Tyr)	0.746	0.207
M13 procoat (P69541)	SLVLKASVAVATLVPMLS	-20(Ser)	0.762	0.204
<i>Surface active:</i>				
δ-Haemolysin (Q512D3)	MAGDIISTIVDFIKLIAETV	1(Met)	0.701	0.578
δ-Haemolysin (P0C1V1)	MAQDIISTIGDLVKWIIDTV	1(Met)	0.689	0.583
Melittin (P01501)	GIGAVLKVLTTGLPALISWIK	1(Gly)	0.759	0.456
Melittin (P01504)	GIGAILKVLATGLPTLISWIK	1(Gly)	0.792	0.477
Cytotoxic peptide (see ref.)	LLQSLLSLLQSLLSLLLQWLK	1(Leu)	0.993	0.545
Diphtheria toxin (fragment B) (P00578)	LVGELVDIGFAAYNFVES	7(Leu)	0.629	0.360

Table S2. Helices included in the Heliquet generated hydrophobic moment plot. Overview of results extracted from the database constructed by Eisenberg *et al.* (1984).

Protein	Sequence	1st residue	<H>	< μ H>
<i>A. Surface region:</i>				
Cecropin A (P01507)	KLFKKIEKVGQNIRDGII	3(Lys)	0.262	0.619
Cecropin B (P01508)	KVFKKIEKMGRNIRNGIV	3(Lys)	0.169	0.635
δ -Haemolysin (Q512D3)	TIVDFIKLIAETVKKFTK	16(Ile)	0.491	0.671
δ -Haemolysin (P0C1V1)	TIGDLVKWIIDTVNKFTKK	16(Ile)	0.514	0.654
Melittin (P01501)	VLKVLTTGLPALISWIKR	12(Gly)	0.756	0.545
Melittin (P01504)	ILKVLATGLPTLISWIKN	12(Gly)	0.811	0.554
Cytotoxic peptide (see ref.)	LLSLLQSLLSLLLQWLKR	12(Leu)	0.927	0.620
Cytotoxic peptide (see ref.)	LQSLLSLLQSLLSLLLQW	2(Leu)	1.024	0.562
<i>B. Transmembrane region:</i>				
Mouse class I H-2d no.1 (P01899)	IVAVLGVLGAMIIIGAVVA	C-43(Met)	0.965	0.264
H-2k (P04223)	VIIAVLVVLGAAIVTGAVV	C-59(Leu)	0.979	0.167
Human class I HLA (P01892)	VGIIAGLVLFGAVITGAVV	C-55(Ile)	0.926	0.238
Mouse class II A $_{\alpha}$ (P01910)	TVVCALGLSVGLVGIVVGTIFI	C-29(Val)	0.905	0.124
A $_{\beta}$ (P01921)	LSGIGGCVLGVIGFLGLGLFI	C-78(Leu)	0.896	0.293
E $_{\alpha}$ (P01904)	NVMCALGLFVGLVGIVVGII	C-38(Val)	0.959	0.216
IgD (delta) (P01882)	GLWPTMCTFVALFLLTLL	C-15(Leu)	1.213	0.291
IgG1 (gamma 1) (P01869)	GLWITITIFISLFLLSVC	C-45(Phe)	1.279	0.188
IgM (mu) (P01871-2)	FIVLFLLSLFYSTTVTLF	C-20(Ile)	1.198	0.122
Human class II DC1 (P01909)	LGLSVGLVGIVVGTVFII	C-29(Val)	1.034	0.169
DR $_{\alpha}$ (P01903)	LGLTVGLVGIIIGTIFII	C-29(Val)	1.147	0.242
DR $_{\beta}$	GVGGFVLGLLFLGAGLFI	C-30(Leu)	1.023	0.151

Table S2. Cont.

Protein	Sequence	1st residue	<H>	<μH>
(P01911)				
Acetylcholine receptor	PLYFVVNVIIIPCLLSFSL	244(Ile)	1.263	0.081
α-subunit:				
(P02710)	MTLSISVLLSLTVFLLVI	278(Thr)	1.160	0.131
	LFTMIFVISSIIITVVVI	304(Phe)	1.257	0.216
	ILLCVFMILICIIGTVSVF	437(Val)	1.337	0.116
β-subunit:	PLFYIVYTHIPCILISIL	243(Phe)	1.335	0.193
(P02712)	MSLSISALLAVTVFLLLL	282(Ala)	1.107	0.073
	LMFIMILVAFSVILSVVVL	313(Ile)	1.271	0.171
	LFLYVFFVICSIGTFSIF	466(Val)	1.271	0.022
γ-subunit:	LFYIINIIAPCVLISSLV	237(Leu)	1.177	0.151
(P02714)	CTLSISVLLAQTIFFLI	271(Leu)	1.154	0.055
	IFVMFVSMLIVMNCVIVL	306(Phe)	1.282	0.115
	CFWIALLLFSIGTLAIFL	471(Ile)	1.328	0.150
δ-subunit:	PLFYVINFITPCVLISFL	247(Leu)	1.215	0.077
(P02718)	MSTAISVLLAQAVFLLLT	276(Glu)	0.939	0.160
	LMFIMSLVTGVIVNCGIV	313(Leu)	1.061	0.221
	MFIITPVMVLGTIFIVM	481(Ile)	1.270	0.127
Bacteriorhodopsin:	IWLALGTALMGLGTLYFLV	15(Leu)	1.076	0.186
(P02945)	FYAITTLVPAIAFTMYLSMLL	42(Phe)	1.051	0.055
	YWARYADWLFTTPLLILLD	93(Leu)	0.890	0.360
	ILALVGADGIMIGTGLVGAL	114(Ala)	0.793	0.147
	VVWAISTAAMLYILYVLFF	136(Val)	1.207	0.133
	LRNVTVVLWSAYPVVWLI	183(Ser)	1.006	0.199
	IETLLFMVLDVSAKVGFGIL	203(Ile)	0.960	0.153
Rhodopsin:	MFLIMLGFPINFLTLYV	49(Met)	1.249	0.182
(P02699)	YILLNLAVADLFMVFGGF	81(Val)	0.992	0.218
	GFFATLGGEIALWSLVVLAI	114(Gly)	0.966	0.239
	IMGVAFTWVMALACAAPP	162(Val)	0.957	0.118
	IYMFVVHFIPLIVIFFC	210(Val)	1.450	0.135
	MVIIMVIAFLICWLPYAG	258(Val)	1.299	0.104
	IFMTIPAFFAKTSAVYNPVIYI	286(Ile)	0.797	0.305