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RESEARCH ARTICLE

Anisotropy across biological membranes: Histidine charges oppose net charge anisotropy

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Considerable interest exists to find unique means to define and assess the inhomogeneity of the transmembrane terrain in terms of structural components such as the properties of the transmembrane proteins as an efficient explanation for vectorial transport, i.e. transport in specified direction. A number of properties of amino acids exhibit significant transmembrane anisotropy, i.e. directionality, when normalized per residue of the aqueous loops. Charge anisotropy in terms of fixed charges was reinvestigated and while the 'positive inwards' rule is obeyed grossly, classes of proteins were seen to show significant distinction among themselves such that receptors exhibit anisotropy 'positive inwards' while ATPases do not. Most interestingly, histidine exhibits transmembrane anisotropy opposite in sign to charge anisotropy among all classes of membrane proteins thus far examined.

DISPOSITION of proteins and amino acids across biological membranes is not symmetric. Certain aspects of this asymmetry are functionally obvious: the receptor proteins with external binding sites would have a larger bulk of the protein facing externally while transport ATPases would have the bulkier active site facing the cytoplasmic aspect. In specific instances, such an anisotropy lends clues to the function, e.g. charge anisotropy of membrane proteins[1]. Charge disposition among membrane proteins was originally described by Von Heijne as 'positive inside' rule which led to interesting possibilities on membrane biosynthesis and insertion of proteins into the membranes[2–4]. A more detailed quantitative analysis significantly amplifying this observation showed that the significant variable is positive inwards and not merely positive inside, based on the available data of confirmed and putative aqueous loops[1]. The direction was consistent with a functional role for this anisotropy such as the direction of proton movements: protons tend to move from the more positive side to the more negative side in biological membranes, which gave the physical basis of Mitchell's conjecture[5] that protons would move vectorially at least in the sense of a fixed direction. The directionality of the pump varies with whether the pump is the primary, the gradient generator, or the secondary, the gradient dissipator. Thus, the secondary pumps should have opposite anisotropy[5,6], do they?

Indeed several specific questions surfaced as a consequence of the above observations: (i) There being a large number of proteins now identified with putative predicted transmembrane topology, does the positive 'inwards' rule remain valid with a much larger sample? (ii) More interestingly, the distribution of internal and

external charges were unrelated for individual proteins, which led to the interesting observation that charge anisotropy conserved larger variance among proteins; simply stated it meant that proteins would differ in their anisotropy rather than tend towards a common value, whose validity also needs to be confirmed among the larger sample of proteins; (iii) Co-transport of electrolytes and nonelectrolytes cannot be explained by transmembrane charge anisotropy alone and it remains to be examined what other properties of amino acids exhibit anisotropies across the membrane and of what magnitude. It would not be possible to guess, *a priori*, which properties would be involved in contributing to vectorial transport since one would imagine that different kinds of proximate forces would be, non-exclusively, involved in the mobility of different solutes, charged or otherwise. While this charge anisotropy would have a direct bearing on the coupled transport of charged species[7], it would by no means account for the degree of varied coupling between electrolytes and/or non-electrolytes seen at the membrane surfaces. (iv) While on a smaller sub-sample, the proton pumps did not differ from other proteins, it is basically unsatisfactory that such structural information is not thus far resolved, say, between primary and secondary pumps. The directionality seen owing to charge anisotropy was consistent with the primary pumps. The directionality of the secondary pumps remains to be accounted for.

We investigated the nature of the trans-bilayer anisotropy for membrane proteins for many amino acid properties[1,2]. We examined whether there exists any preference as to what kind of properties show a more significant anisotropy. We report here unique evidence that histidine anisotropy has a bearing opposite in sign to charge anisotropy, particularly among classes of membrane proteins which also vary in terms of charge anisotropy not in keeping with the 'positive inside' rule. These observations have exciting implications on an electrostatic regulation of transport and related activities at the membrane surface by protonation/deprotonation mechanisms of histidine at physiological pH. A comparison of the preferences with that observed in genetic code suggests the dominant role of long range interactions in proteins.

Materials and methods

A total of 157 non-conflicting and non-repeating integral membrane proteins were obtained. 136 proteins were obtained from SWISSProt Protein Sequence Database (December 1992 release) while 21 were collected from literature. The decreasing order of preference for the sequences was: human, mouse, rat, hamster, rabbit, dog, bovine, pig, yeast, bacteria. These proteins were also classified in the following six non-exclusive groups: 1. ATPases, (Atp, 16 proteins); 2. Cytochromes (Rdx, 15 proteins); 3. Transporters (Tra, 10 proteins); 4. Receptors (Rec, 116 proteins); 5. Proton pump proteins (Ppm, 24 proteins); 6. Non-proton pump proteins (Npm, 17 proteins). This study ignores the structure-based classification adopted by Von Heijne and considers the membrane proteins as a general case first; subclasses are based on their function. No effort was made to identify or distinguish experimentally determined structures as opposed to the predicted topologies for the simple reason that any pattern is particularly meaningful for prediction rather than for confirmation *post facto*.

The putative transmembrane domains were obtained from the sequence data bank and publications, primarily based on hydropathic plots as described before[1,2] and the external and internal segments were assigned consecutively. In view of the debate that exists on the use of hydropathic plots to arrive at transmembrane helices[8–10], extensive simulations using random amino acid sequences and random values for properties were both carried out but did not give any significant correlations as reported here.

Charge anisotropy

Net charge was computed by subtracting the number of negative charges (i.e. acidic amino acid residues of aspartate and glutamate) from the number of positive charges (i.e. basic amino acid residues of lysine and arginine but not histidine) for each of internal and external aqueous loops of integral membrane protein. The algebraic difference between net charge in internal and external regions per protein gives the direction of charges across the bilayer termed as charge anisotropy (CA), i.e.,

CA = Net internal charges – net external charges.

Definition of internal and external charges was based on a strict relationship to the biologically relevant topology as in the previous work[1–3]: for the plasma membrane, the external and internal aspects would be self-evident. The calcium transporting ATPase in the sarcoplasmic reticulum would have active site facing the cytosolic side, which is on the external side of the inside-out lumen of the vesicles. The matrix aspect of the mitochondrion would be inside, while the cytosolic side would be external. Submitochondrial particles would be correspondingly inside-out.

Histidine anisotropy

Assignment of net charge to histidine is complicated since it is the only amino acid residue which gets protonated or deprotonated within the physiological range of pH. It is therefore counted separately. Histidine anisot-

ropy (HA) was calculated simply as: net internal histidine charges – net external histidine charges.

Anisotropy calculations for other amino acid properties

For computation of internal to external anisotropy, 23 different properties of amino acids were considered. The net value for external and internal domains was obtained as the mean property value normalized per residue. Anisotropy was computed per residue for all properties including charge and histidine densities, unlike in the case of charge calculations above, which considered only net charges. Anisotropy value for each property was then determined simply as the algebraic difference between the mean property per residue of internal and external regions. These mean anisotropy values for each protein and property were evaluated for 157 membrane proteins further.

Relationship to genetic code

The absence of certainty with regard to predictions of topology based on hydropathic plots remains a matter of considerable concern. Two alternatives may be considered. To be certain, the analysis could be restricted to only proteins with known topology, thereby severely limiting the sample size as well as losing the essence of the problem, viz. prediction. Alternatively, one could look for the verisimilitude: if the sequence information is generally correct, and the anisotropies reasonably accurate, the ranking of amino acid/properties in terms of their significance should quantitatively match some known ranks of importance of amino acid preference. Here one can use the genetic code to advantage since the assignment of codons to amino acids was shown to have excellent ranking conserving long-range interactions and other properties. Even if all amino acids/properties show anisotropy, it would be logical to consider that the magnitude of such anisotropy for each property would have a bearing on long term structural information of importance, even as long as term evolution *per se*. This problem statement has the additional advantage of being directly testable (using random numbers for properties as also random assignment of amino acids to each value of the property), valuable only if positive. The statement arises from the view that the properties of amino acid, alone and in combination, matter and not the head count of amino acids.

Results

Table 1 shows an updated evaluation of charge anisotropy across biological membranes for all proteins and also class-wise. The proteins are listed in the Appendix. Erythrocytes were taken as an example of a cell type. Essentially the results corroborate the positive inwards rule among all the proteins taken together, with adequate variance within them to justify certain uniqueness of the anisotropy that each protein exhibits[1]. However, the data clearly showed that this 'positive inwards' rule varied with the class of proteins and that ATPases could actually show a reversal of the anisotropy. This observation has little to do with the fact that the active site of ATPases would be the bulkiest among the aqueous loops since the receptors would also exhibit their bulkiness on the external side. Only Swissprot was considered for the database other than the references listed. While some controversy exists regarding the assignment of the topology[11], even when a 10 helix assignment for the calcium ATPase is considered, the fast twitch variant[12] had actually zero charge anisotropy, while the slow variant, SERCA2[13] has a weak inwards anisotropy of 4^+. Thus the primary conclusions of variable anisotropy remained valid for the overall pattern which did not depend on ATPases

Table 1. Net charge of amino acid residues in membrane proteins

Class of proteins	*n*	Internal (*x*) Mean	Internal (*x*) SD	External (*y*) Mean	External (*y*) SD	Anisotropy (*A*) Mean	Anisotropy (*A*) SD	Correlation coefficients *y* vs *x*	*A* vs *y*	*A* vs *x*
All	157	3.00	12.6	–4.53	7.5	7.52	13.4	0.059	–0.473 ***	0.852***
Ppm	24	–1.83	10.4	–1.00	3.8	–0.83	9.1	–0.218	–0.510 *	0.951***
Npm	17	–7.29	14.3	–2.88	3.7	–4.41	12.9	–0.072	–0.313	0.970***
Rec	116	5.50	11.7	–5.50	8.2	11.00	14.2	0.190	–0.4619***	0.784***
Atp	16	–11.63	13.5	–1.63	3.8	–10.00	13.5	0.196	–0.085	0.960***
Rdx	15	2.13	4.5	–2.07	3.4	4.20	5.1	0.243	–0.468	0.744***
Tra	10	–1.40	12.9	–1.60	4.7	0.20	12.2	–0.574	–0.755**	0.970***
Rbc	9	–4.44	12.9	–4.11	5.3	-0.33	17.8	–0.464	–0.704*	0.956***

n, total number of proteins; SD, standard deviation; Classes of integral membrane proteins: All, Total membrane proteins; Ppm, Proton pumps; Npm, Non-proton pumps; Rec, Receptors; Atp, ATPases; Rdx, Cytochromes; Tra, Transporters; Rbc, Erythrocytes; Significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table 2. Property anisotropy of amino acid residues in 157 membrane proteins

Property	Internal (x)		External (y)		Anisotropy (A)	
	Mean	SD	Mean	SD	Mean	SD
Polarity	16.26	3.650	12.23	2.593	0.077	0.081***
Net charge density	0.04	0.035	-0.02	0.004	0.033	0.049***
Chromatographic index	9.17	1.068	9.82	0.751	-0.040	0.072***
Power to form alpha in the middle of helix	1.04	0.110	0.97	0.068	0.028	0.054***
Isoelectric point	6.22	0.602	5.93	0.158	0.036	0.077***
Propensity to form beta-sheet	0.95	0.092	1.00	0.994	-0.034	0.075***
Long range non-bonded energy	0.51	0.047	0.53	0.016	-0.040	0.089***
Entropy of formation	217	218	209	210	0.038	0.094***
Thermodynamic transfer hydrophobicity	1.20	0.169	1.27	0.127	-0.017	0.050***
Protein environment total non-bonded energy	1.71	0.142	1.75	0.026	-0.066	0.212***
Absolute entropy	45.8	46.0	44.6	44.7	0.029	0.096***
Histidine density	0.03	0.095	0.02	0.034	0.012	0.048**
Bulk hydrophobicity	12.5	1.03	12.8	0.187	-0.051	0.210**
Propensity to form alpha-helix	1.00	1.006	0.98	0.034	0.023	0.097**
Beta-sheeting tendency	0.97	0.914	1.01	0.093	-0.001	0.004**
Short and medium range non-bonded energy	1.19	0.098	1.22	0.017	-0.048	0.207**
Bend adopting power	1.06	0.138	1.10	0.109	-0.001	0.006**
Propensity to form beta-turn	1.00	0.999	1.02	1.022	-0.021	0.095**
Bulkness	14.71	1.303	14.90	0.623	-0.002	0.017^{NS}
Power to form alpha at C-terminal	1.04	0.121	1.06	0.062	-0.011	0.076^{NS}
Heat capacity	40.1	3.69	40.5	1.57	-0.004	0.038^{NS}
Power to form alpha at N-terminal	0.96	0.098	0.95	0.063	0.006	0.056^{NS}
Molecular weight	130.6	11.3	129.9	3.86	0.003	0.067^{NS}
pK'	2.09	0.171	2.11	0.035	0.000	0.005^{NS}
Refractive index	16.69	1.777	16.72	1.030	-0.001	0.045^{NS}

Each property is expressed as the mean of values of all residues normalized per residue for each region of the protein[15–17]. The mean and SD (standard deviation) refer to those computed for all proteins for these normalized values. Significance: **, $p < 0.01$; ***, $p < 0.001$. NS, Not significant, tested for non-zero mean using Student's t test[18].

alone. The receptors, with one or several transmembrane helices, carry a charge anisotropy quite opposite to that of ATPases of equally large magnitude which is not located in the binding domain either. The case of erythrocytes is instructive: since the actual charge of a membrane *in situ* is decided by the incidence of copies of each protein, e.g., band 3 protein which is a dominant protein, the positive inside rule manifests itself only when an attempt is made to mimic the copies of the protein to the extent the information is available.

Table 2 shows the properties of amino acids for which the anisotropy was computed and presented in the order of their significance for a non-zero mean, unlike as expected of a random value for a property. Such properties have been exploited to understand the basis of the origin of life and origin of amino acids and, depending on the understanding, combinations of each of these properties have been highlighted to be of significance by different workers in the past. Since the influence of these properties on a specific protein or a specific function remains elusive due to the sheer complexity of the problem, it was considered advisable to test all the readily-available properties for their relative ranking for significance. This objective screening of properties would permit recognition of gross patterns with relative ease without committing *a priori* to the nature of interactions in the functionality of the protein. The essence of anisotropy calculations here involves a comparison of the mean value of the property per residue since all properties exhibit anisotropy due to asymmetric bulkiness of aqueous segments in all the membrane proteins. Charge exhibits anisotropy as net value as well as charge density per residue. Approximately a tenth of the residues carry a charge while the net charge and number of histidines both correspond to a tenth of the total charged residues. It is important to recognize that these calculations rule out trivial objections based on the asymmetric bulkiness of the protein across the membrane since the anisotropy reported here is valid when expressed per residue as well as for the total protein as in Table 1. Table 3 shows the frequency of appearance of charged amino acids in each segment in these membrane proteins. Notably, while the basic amino acids exhibit higher preponderance in internal aqueous loops, histidine incidence was grossly the same internally and externally consistent with earlier reports[2,14]. These results highlight the specificity of the observation that histidine anisotropy counters that of charge in proteins such as ATPases beyond reasonable doubt.

Table 3. Incidence of charged amino acid residues* in external and internal regions of membrane proteins (expressed as percent of total residues in each region of all 157 proteins)

Amino acid	Per cent incidence	
	External	Internal
Aspartate	5.25	5.13
Glutamate	6.14	7.34
Lysine	4.78	7.05
Arginine	4.59	7.12
Histidine	2.50	2.30

*The results were restricted to charged amino acids only since the overall incidence was comparable to the published results[14].

Figure 1 *a–c* shows a plot of histidine display by these proteins and histidine anisotropy as a function of charge anisotropy. The histidine anisotropy was significant in itself as also in its inverse relationship to charge anisotropy. The relationship was even more clear when classes of proteins were considered (Figure 1 *d–f*). Though linear regression for the classes would be inappropriate due to the overlapping classifications, the proteins clearly exhibited an inverse relationship between histidine anisotropy and charge anisotropy and the large scatter was also consistent with an earlier surmise that the proteins maximize variance in the assignment of anisotropy, i.e. each protein occupies its own niche in terms of its anisotropy maximizing its spread[1]. The results were essentially same when net histidine occurrence was replaced with histidine density (i.e. per residue in each segment) (data not given).

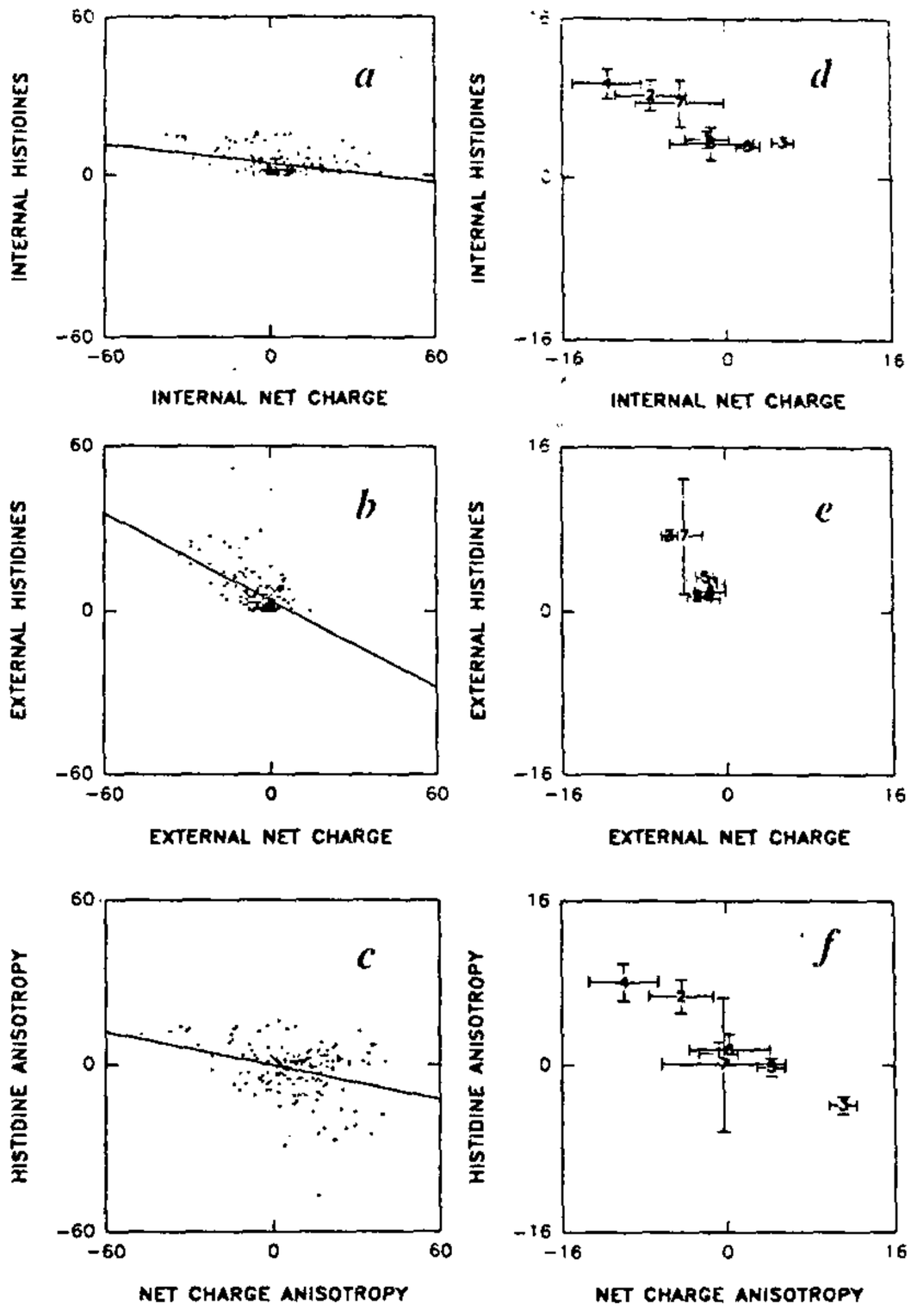

Figure 1. Relationship between histidines and net charges. *a–c*, when all membrane proteins ($n = 157$) were taken together; *d–f*, when specific classes of membrane proteins were considered. Internal cytoplasmic histidines versus internal net charges (*a*, *d*); external cytoplasmic histidines versus external net charges (*b*, *e*); histidine anisotropy versus net charges anisotropy (*c*, *f*); Lines in *a–c* represent linear regressions *vis-à-vis* *a*, $r = -0.9067$; *b*, $r = -0.8356$ and *c*, $r = -0.9649$ (all correlation coefficients were highly significant at $p < 0.00001$). Numbers in *d–f* represent classes of proteins as: 1, Ppm; 2, Npm; 3, Rec; 4, Atp; 5, Rdx; 6, Tra; and 7, Rbc; while horizontal and vertical bars represent standard error around means of each class of proteins.

Discussion

A curious observation of statistical significance with regard to charge anisotropy was that the variance among proteins was conserved among the proteins (see ref. 1). If a particular property was to serve the same purpose among different proteins, such as insertion into the membrane, it would be logical to consider that different proteins would tend to have low variance and not a high variance. This was the essence of the 'positive inside' rule of Von Heijne[2], belied by two aspects of charge anisotropy. Firstly, variance was conserved and not minimized and secondly the anisotropy exhibited definitive relationship to classes of proteins. The same arguments appear to hold good for histidine anisotropy also, which would further highlight the functionality of charge/histidine anisotropy in these proteins.

We examined whether the observed anisotropy among various properties has any order or logic intrinsic to it. One way was to test wherever such properties would be manifest, e.g. as in the genetic code. We have earlier computed the nature of assignment of codons to amino acids and ranked various properties according to the order of their conservation in the face of codon mutations[15–17]. The order of significance in anisotropy among the properties was closely related to the order of conservation observed in the genetic code($r = 0.601$; $n = 23$ properties, $P < 0.001$). Thus the observed anisotropies of properties are of substantive significance functionally and presumably also in terms of evolution. While Nakashima and Nishikawa[14] obtained a comparable incidence of amino acids and differences between the two sides of the membrane, these differences in the incidence of amino acids was not correlated to properties other than charge thus far. Anisotropy was not considered by other workers in the manner considered here. Since long-range interactions were shown to dominate in the as-

signment of codons to amino acids in the genetic code[15,17], comparison of the preference for the assignment of property anisotropy of membrane proteins assumes special significance. For instance, it is now textbook knowledge that the frequency of assignment of codons to amino acids has a direct relationship to the frequency of occurrence of amino acids in proteins across species. Thus long range interactions as with the genetic code playing second only to charge anisotropy in the transmembrane topology would be of functional importance in view of the similarity to the genetic code as well.

Why histidine anisotropy and why is it in a direction opposite to that of charge anisotropy? Clearly, the anisotropy of major magnitude was the net charge and not net histidine charge. This would make histidine more a probe for the electrostatic forces at the interface rather than it being the determinant of the electrostatic forces. Since small changes in the interfacial ionic strength could lead to changes in pH and vice versa, variations in histidine protonation would be a logical consequence of variations of surface pH, which in turn would offer an excellent means for coupling activity of these proteins to ionic strength. Histidine has a role as a charged amino acid as well as an aromatic amino acid while the role of histidine charge in protein function could be evaluated by pH titrimetry, the role of the amino acid itself would require a variety of experiments including titrimetry, chemical modifications and site-directed mutagenesis. If the amino acid is primarily interfacial, an attractive possibility would be the variable charge in the realm of physiological pH such that the uncharged residue could move deeper into the bilayer in the absence of the positive charge. The frequency distribution of the distance of histidines from the interface, external and internal for membrane proteins, was obtained and it could be seen that nearly 50% of all the histidines occurred at the 1st aqueous amino acid and this value reduced with the logarithm of the distance in amino acids from the interface to 50% by the 5th aqueous amino acid from the interface (data omitted, coefficient of correlation, $r = 0.94$, $n = 6$). Thus most histidines would be fairly close to the interface and could participate in interfacial pH changes. Thus the role of such histidines would probably be modulation of activity of membrane proteins as a consequence of interfacial changes of ionic strength/pH and not be determinants of such ionic changes. We are currently investigating the role of electrostatic forces at the interface and their role in membrane stability wherein variable histidine anisotropy could play a significant role. A key input into such investigations would be the remarkable pH dependence near the pK for histidine as well the influence of chemical reagents that destroy the histidine residue such as diethylpyrocarbonate. The electrostatic repulsion at the surface of rabbit sarcoplasmic reticulum (SR) vesicles at physiological pH appear to be influenced little by the carboxyl and amino groups while histidines contribute maximally; further, the contribution of histidine charges was consistent with the predicted topology of the relevant calcium ATPase of the fast and slow twitch muscle SR vesicles (unpublished results). It is hoped that analogous to various sequence specific motifs described thus far, the display of histidines at the surface of the membrane, as a pH-modulable variable, could offer a common explanation for a variety of pH dependent interactions. It is likely as well as testable that a possible role for charge anisotropy with compensating histidine anisotropy could play a role even in biosynthesis of proteins as surmised earlier. Histidine anisotropy could thus offer an additional tool for the topological prediction of membrane proteins in addition to the 'positive inside' rule, while permitting a functional perspective since the positive 'inwards' rule has emphasized the variance in the anisotropy. The relevant experimental and theoretical studies are in progress.

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Appendix

The following table lists all the proteins used in this manuscript and the subclassifications used. The predominant group of proteins are the receptors. The sub classification was overlapping depending on the possible interest in a particular class of proteins.

List of proteins used in the analysis of charge anisotropy

	Protein	Source/code	Class	Number of Amino acids	Helices
1.	5-Hydroxytryptamine 1A receptor (5-HT-1A) (serotonin receptor)	5HTA_HUMAN (P08908)	Rec	421	7
2.	Activin receptor type I precursor (ACTR-I) (EC 2.7.1.-)	AVR1_HUMAN (P27037)	Rec	513	1
3.	Alpha-1A adrenergic receptor	A1AA_HUMAN (P25100)	Rec	501	7
4.	Alpha-2C adrenergic receptor (subtype C4)	A2AC_HUMAN (P18825)	Rec	461	7
5.	Apoptosis-mediating surface antigen FAS precursor (APO-1 antigen)	FASA_HUMAN (P25445)	Rec	335	1
6.	Asialoglycoprotein receptor 1 (hepatic lectin H1) (ASGPR)	LECH_HUMAN (P07306)	Rec	290	1
7.	Asialoglycoprotein receptor 2 (hepatic lectin H2) (ASGPR)	LECI_HUMAN (P07307)	Rec	311	1
8.	Atrial natriuretic peptide receptor A precursor (ANP-A) (ANPRA) (GC-A) (guanylate cyclase) (EC 4.6.1.2)	ANPA_HUMAN (P16066)	Rec	1061	1
9.	Atrial natriuretic peptide receptor B precursor (ANP-B) (ANPRB) (GC-B) (guanylate cyclase) (EC 4.6.1.2)	ANPB_HUMAN (P20594)	Rec	1047	1
10.	B-cell differentiation antigen LYB-2 (CD72)	CD72_HUMAN (P21854)	Rec	359	1
11.	B-cell receptor CD22 precursor	CD22_HUMAN (P20273)	Rec	647	1
12.	Band 3 protein of human erythrocyte; anion-exchange protein (AE1)	BAND3_HUMAN[1]	Tra/Npm/Rbc	911	14
13.	Beta-1-adrenergic receptor	B1AR_HUMAN (P08588)	Rec	477	7
14.	Beta-3-adrenergic receptor	B3AR_HUMAN (P13945)	Rec	402	7
15.	C5A anaphylatoxin chemotactic receptor	C5AR_HUMAN (P21730)	Rec	350	7
16.	Calcium-transporting ATPase plasma membrane 1B (EC 3.6.1.38) (calcium pump) (PMCA1B)	ATCQ_HUMAN (P20020)	Atp/Npm/Rbc	1220	10
17.	Calcium-transporting ATPase plasma membrane 4 (EC 3.6.1.38) (calcium pump) (PMCA4)	ATCR_HUMAN (P23634)	Atp/Npm/Rbc	1205	10
18.	Cannabinoid receptor	CANR_HUMAN (P21554)	Rec	472	7
19.	CD40L receptor precursor (B-cell surface antigen CD40) (BP50) (CDW40)	CD40_HUMAN (P25942)	Rec	277	1
20.	CD44 antigen, epithelial form precursor (CD44E) (phagocytic glycoprotein I) (PGP-1) (hutch-I) (extracellular matrix receptor-III) (ECMR-III)	CD4X_HUMAN (P22511)	Rec	493	1
21.	CD44 antigen, hematopoietic form precursor (CD44H) (phagocytic glycoprotein I) (PGP-1) (hutch-I) (extracellular matrix receptor-III)	CD4H_HUMAN (P16070)	Rec	294	1
22.	Complement receptor type 1 precursor (C3B/C4B receptor) (CD35)	CR1_HUMAN (P17927)	Rec / Rbc	2039	1
23.	D(1A) dopamine receptor	DADR_HUMAN (P21728)	Rec	446	7
24.	Dopamine receptor	D2DR_HUMAN (P14416)	Rec	443	7
25.	Dopamine receptor	D4DR_HUMAN (P21917)	Rec	387	7
26.	Dopamine receptor	D5DR_HUMAN (P21918)	Rec	477	7
27.	Endothelin-1 receptor precursor (ET-A)	ET1R_HUMAN (P25101)	Rec	427	7
28.	ERBB-2 receptor protein-tyrosine kinase precursor (EC 2.7.1.112)	ERB2_HUMAN (P04626)	Rec	1255	1
29.	EVI2 protein precursor	EVI2_HUMAN (P22794)	Rec	232	1
30.	Fibronectin receptor alpha subunit precursor (integrin alpha-F) (integrin alpha-5) (VLA-5) (CD49E)	ITA5_HUMAN (P08648)	Rec	1049	1
31.	Fibronectin receptor beta subunit precursor (integrin beta-1)	ITB1_HUMAN (P05556)	Rec	798	1
32.	FMLP-related receptor I (FMLP-R-I)	FML1_HUMAN (P25089)	Rec	353	7
33.	FMLP-related receptor II (FMLP-R-II)	FML2_HUMAN (P25090)	Rec	351	7
34.	Follicle stimulating hormone receptor precursor (FSH-R)	FSHR_HUMAN (P23945)	Rec	695	7
35.	Glucose transporter protein, erythrocyte/brain	GTR1_HUMAN (P11166)	Tra / Npm	492	12
36.	Glucose transporter, small intestine	GTR5_HUMAN (P22732)	Tra / Npm	501	12
37.	Glucose transporter-like protein, muscle	GTR3_HUMAN (P11169)	Tra / Npm	496	12
38.	Glycophorin C (PAS-2') (glycoprotein beta) (GLPC) (glycoconnectin)	GLPC_HUMAN (P04921)	Rec / Rbc	128	1
39.	Glycoprotein GP34	GP34_HUMAN (P23510)	Rec	183	1
40.	Granule membrane protein 140 precursor (GMP-140) (PADGEM) (CD62) (leukocyte-endothelial cell adhesion molecule 3) (LECAM3)	LEM3_HUMAN (P16109)	Rec	830	1
41.	Granulocyte-macrophage colony-stimulating factor receptor precursor (GM-CSF-R)	GMCR_HUMAN (P15509)	Rec	400	1

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42.	Heat-stable enterotoxin receptor precursor (GC-C) (guanylate cyclase) (EC 4.6.1.2)	HSER_HUMAN (P25092)	Rec	1073	1
43.	High affinity immunoglobulin epsilon receptor alpha-subunit (FCERI) (IGE FC receptor, alpha-subunit)	FCEA_HUMAN (P12319)	Rec	257	1
44.	High affinity immunoglobulin gamma FC receptor I 'A form' precursor (FC-gamma RI) (FCRI) (IGG FC receptor)	FCG0_HUMAN (P12314)	Rec	374	1
45.	High affinity immunoglobulin gamma FC receptor I 'B form' precursor (FC-gamma RI) (FCRI) (IGG FC receptor I)	FCG1_HUMAN (P12315)	Rec	344	1
46.	High affinity interleukin-8 receptor B (IL-8R)	IL8B_HUMAN (P25025)	Rec	355	7
47.	Histamine H2 receptor (gastric receptor I)	HH2R_HUMAN (P25021)	Rec	359	7
48.	Immunoglobulin alpha FC receptor precursor	FCAR_HUMAN (P24071)	Rec	287	1
49.	Inducible membrane protein R2	R2_HUMAN (P27701)	Rec	267	4
50.	Integrin alpha-3 (galactoprotein B3) (GAPB3) (VLA-3)	ITA3_HUMAN (P26006)	Rec	1019	1
51.	Integrin alpha-6 precursor (VLA-6) (integrin ALPHA-E)	ITA6_HUMAN (P23229)	Rec	1073	1
52.	Integrin beta-6 subunit precursor	ITB6_HUMAN (P18564)	Rec	788	1
53.	Integrin beta-7 subunit precursor	ITB7_HUMAN (P26010)	Rec	798	1
54.	Integrin beta-8 subunit precursor	ITB8_HUMAN (P26012)	Rec	769	1
55.	Intercellular adhesion molecule-1 precursor (ICAM-1) (major group rhinovirus receptor) (CD54)	ICA1_HUMAN (P05362)	Rec	532	1
56.	Interferon-alpha receptor precursor (IFN-alpha-REC)	INAR_HUMAN (P17181)	Rec	557	1
57.	Interferon-gamma receptor precursor	INGR_HUMAN (P15260)	Rec	489	1
58.	Interleukin 4 receptor precursor (IL4R)	IL4R_HUMAN (P24394)	Rec	825	1
59.	Interleukin-1 receptor, type I precursor (IL-1R1) (P80)	IL1R_HUMAN (P14778)	Rec	569	1
60.	Interleukin-1 receptor, type II precursor (IL-1R2)	IL1S_HUMAN (P27930)	Rec	398	1
61.	Interleukin-2 receptor alpha chain precursor (IL-2 receptor alpha subunit) (P55) (TAC antigen) (CD25)	IL2A_HUMAN (P01589)	Rec	272	1
62.	Interleukin-2 receptor beta chain precursor (IL-2 receptor) (P70-75) (high affinity IL-2 receptor beta subunit)	IL2B_HUMAN (P14784)	Rec	551	1
63.	Interleukin-7 receptor precursor (IL-7R)	IL7R_HUMAN (P16871)	Rec	459	1
64.	Low affinity immunoglobulin gamma FC receptor II precursor (FC-gamma RII) (FCRII) (IGG FC receptor II) (CD32) (CDW32)	FCG2_HUMAN (P12318)	Rec	311	1
65.	Muscarinic acetylcholine receptor M2	ACM2_HUMAN (P08172)	Rec	466	7
66.	Muscarinic acetylcholine receptor M3	ACM3_HUMAN (P20309)	Rec	590	7
67.	Muscarinic acetylcholine receptor M5	ACM5_HUMAN (P08912)	Rec	532	7
68.	NKG2-A and NKG2-B type II integral membrane proteins	NKGA_HUMAN (P26715)	Rec	233	1
69.	NKG2-C Type II integral membrane protein	NKGC_HUMAN (P26717)	Rec	231	1
70.	NKG2-D type II integral membrane protein	NKGD_HUMAN (P26718)	Rec	216	1
71.	Plasma-cell membrane glycoprotein PC-1 (alkaline phosphodiesterase I (EC 3.1.4.1) / nucleotide pyrophosphatase (EC 3.6.1.9))	PC1_HUMAN (P22413)	Rec	873	1
72.	Platelet membrane glycoprotein IA precursor (collagen receptor) (integrin alpha-2) (VLA-2) (CD49B)	ITA2_HUMAN (P17301)	Rec	1181	1
73.	Platelet membrane glycoprotein IIB precursor (GPIIB) (integrin alpha-IIB) (CD41)	ITAB_HUMAN (P08514)	Rec	1039	1
74.	Probable G protein-coupled receptor EDG-1	EDG1_HUMAN (P21453)	Rec	381	7
75.	Sodium/potassium transporting ATPase alfa-subunit	Pig[9]	Atp / Npm	1013	7
76.	Sodium/potassium-transporting ATPase beta-1 chain (EC 3.6.1.37) (sodium/potassium-dependent ATPase)	ATNB_HUMAN (P05026)	Atp/Npm/Rbc	303	1
77.	Sodium/potassium-transporting ATPase beta-2 chain (EC 3.6.1.37) (sodium/potassium-dependent ATPase)	ATNC_HUMAN (P14415)	Atp/Npm/Rbc	290	1
78.	T-cell surface glycoprotein CD3 epsilon chain precursor (T-cell surface antigen T3/LEU-4 epsilon chain)	CD3E_HUMAN (P07766)	Rec	233	1
79.	T-cell surface glycoprotein CD3 zeta chain precursor (T-cell receptor T3 zeta chain)	CD3Z_HUMAN (P20963)	Rec	163	1
80.	T-cell surface glycoprotein CD5 precursor (lymphocyte glycoprotein T1/LEU-1)	CD5_HUMAN (P06127)	Rec	495	1
81.	Vitronectin receptor alpha subunit precursor (integrin alpha-V) (CD51)	ITAV_HUMAN (P06756)	Rec	1048	1
82.	4F2 cell-surface antigen heavy chain (4F2HC)	4F2_MOUSE (P10852)	Rec	526	1
83.	5-hydroxytryptamine 3 receptor precursor (5-HT-3) (serotonin-gated ion channel receptor)	5HT3_MOUSE (P23979)	Rec	487	4
84.	Activin receptor type IIB precursor (ACTR-IIB) (EC 2.7.1.-)	AVRB_MOUSE (P27040)	Rec	536	1
85.	B-cell-specific glycoprotein B29 precursor	B29_MOUSE (P15530)	Rec	228	1
86.	Basigin precursor (basic immunoglobulin superfamily) (membrane glycoprotein GP42)	BASI_MOUSE (P18572)	Rec	273	1

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87.	Cell surface adhesion glycoproteins LFA-1, CR3 and P150,95, beta-subunit precursor (CD18 antigen beta subunit) (integrin beta-2)	ITB2_MOUSE (P11835)	Rec	770	1
88.	Interleukin-5 receptor precursor (IL5R)	IL5R_MOUSE (P21183)	Rec	415	1
89.	Low affinity immunoglobulin epsilon FC receptor (lymphocyte IGE receptor) (FC-epsilon-RII)	FCE2_MOUSE (P20693)	Rec	331	1
90.	Low affinity immunoglobulin gamma FC receptor II-2 precursor (FC-gamma RII) (FCRII) (IGG FC receptor II beta-2)	FCGX_MOUSE (P08102)	Rec	283	1
91.	Low affinity immunoglobulin gamma FC receptor III precursor (FC-gamma RIII) (FCRIII) (IGG FC receptor alpha)	FCG3_MOUSE (P08508)	Rec	261	1
92.	Natural killer cell surface protein P1-40 (NKR-P1 40)	NK14_MOUSE (P27814)	Rec	220	1
93.	5-Hydroxytryptamine 1C receptor (5-HT-1C) (serotonin receptor)	5HTC_RAT (P08909)	Rec	460	7
94.	5-Hydroxytryptamine 2 receptor (5-HT-2) (serotonin receptor)	5HT2_RAT (P14842)	Rec	471	7
95.	Alpha-2A adrenergic receptor	A2AA_RAT (P22909)	Rec	450	7
96.	Alpha-2B adrenergic receptor	A2AB_RAT (P19328)	Rec	453	7
97.	B2 Bradykinin receptor	BRB2_RAT (P25023)	Rec	396	7
98.	Beta-2-adrenergic receptor	B2AR_RAT (P10608)	Rec	418	7
99.	Calcium-transporting ATPase plasma membrane (EC 3.6.1.38), brain isoform 2 (PMCA2)	ATCQ_RAT (P11506)	Atp / Npm	1198	10
100.	CD44 antigen precursor (phagocytic glycoprotein I) (PGP-I) (hutch-I) (extracellular matrix receptor-III) (ECMR-III) (GP90 lymphocyte homing)	CD44_RAT (P26051)	Rec	364	1
101.	D(1B) dopamine receptor	DBDR_RAT (P25115)	Rec	475	7
102.	Dopamine receptor	D3DR_RAT (P19020)	Rec	446	7
103.	Endothelin B receptor precursor (ET-B) (endothelin receptor non-selective type)	ETBR_RAT (P21451)	Rec	441	7
104.	Glucose transporter protein, liver	GTR2_RAT (P12336)	Tra/Npm/Rbc	522	12
105.	Growth hormone receptor precursor (GH receptor) (serum binding protein)	GHR_RAT (P16310)	Rec	638	1
106.	High affinity immunoglobulin epsilon receptor alpha-subunit (FCERI) (IGE FC receptor, alpha-subunit)	FCE1_RAT (P12371)	Rec	245	1
107.	High affinity immunoglobulin epsilon receptor beta-subunit (FCERI) (IGE FC receptor, beta-subunit)	FCEB_RAT (P13386)	Rec	243	4
108.	Integrin alpha-1 precursor (laminin and collagen receptor) (VLA-1) (CD49A)	ITA1_RAT (P18614)	Rec	1180	1
109.	Kupffer cell receptor	KUCR_RAT (P10716)	Rec	550	1
110.	Lysosome membrane protein II (LIMP II)	LIM2_RAT (P27615)	Rec	477	2
111.	Metabotropic glutamate receptor precursor (GLUGR)	GLGP_RAT (P23385)	Rec	1199	7
112.	Muscarinic acetylcholine receptor M1	ACM1_RAT (P08482)	Rec	460	7
113.	Muscarinic acetylcholine receptor M4	ACM4_RAT (P08485)	Rec	478	7
114.	Neuronal acetylcholine receptor protein, alpha-5 chain precursor	ACH5_RAT (P20420)	Rec	452	4
115.	Potassium-transporting ATPase alpha chain (EC 3.6.1.36) (proton pump) (gastric H^+/K^+ ATPase)	ATHA_RAT (P09626)	Atp / Ppm	1033	8
116.	T-cell surface glycoprotein CD3 delta chain precursor (T-cell receptor T3 delta chain)	CD3D_RAT (P19377)	Rec	173	1
117.	Alpha-1B adrenergic receptor	A1AB_MESAU (P18841)	Rec	515	7
118.	Uncoupler protein	Hamster [10]	Tra / Ppm	304	7
119.	Calcium-transporting ATPase endoplasmic reticulum type (EC 3.6.1.38), class 2 (SERCA2)	ATCE_RABIT (P20647)	Atp / Npm	1042	8
120.	Calcium-transporting ATPase sarcoplasmic reticulum type (EC 3.6.1.38), fast twitch skeletal muscle, adult isoform (calcium pump) (SERCA1)	ATCB_RABIT (P11719)	Atp / Npm	994	8
121.	Potassium-transporting ATPase beta chain (EC 3.6.1.36) (proton pump) (gastric H^+/K^+ ATPase)	ATHB_RABIT (P18597)	Atp / Ppm	291	1
122.	5-Hydroxytryptamine 1D receptor (5-HT-1D) (serotonin receptor)	5HTD_CANFA (P11614)	Rec	377	7
123.	Adenosine A1 receptor	AA1R_CANFA (P11616)	Rec	326	7
124.	Adenosine A2 receptor	AA2R_CANFA (P11617)	Rec	412	7
125.	Sodium/calcium exchanger precursor (Na^+/Ca^{2+}-exchange protein)	NACA_CANFA (P23685)	Atp / Npm	970	11
126.	Alpha-1C adrenergic receptor	A1AC_BOVIN (P18130)	Rec	466	7
127.	Atrial natriuretic peptide clearance receptor precursor (ANP-C) (ANPRC)	ANPC_BOVIN (P10730)	Rec	537	1
128.	Butyrophilin precursor (BT)	BUTY_BOVIN (P18892)	Rec	526	1
129.	Cytochrome B561	C561_BOVIN (P10897)	Rdx / Ppm	273	6
130.	Endothelin B receptor precursor (ET-B) (endothelin receptor non-selective type)	ET1B_BOVIN (P28088)	Rec	441	7
131.	Type-1 angiotensin II receptor (AT1)	AG2R_BOVIN (P25104)	Rec	359	7
132.	Sodium/potassium-transporting ATPase alpha-1 chain (EC 3.6.1.37) (sodium pump) (Na^+/K^+ ATPASE)	ATN1_PIG (P05024)	Atp/Npm/Rbc	1021	8
133.	Cytochrome B6 complex (spinach chloroplast)	Spinach chloroplast[3]	Rdx / Ppm	211	5

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134.	Diacylglycerol cholinephosphotransferase (EC 2.7.8.2) (SN-1,2-diacylglycerol cholinephosphotransferase) (CHOPT)	CPT1_YEAST (P17898)	Rec	407	7
135.	Ethanolaminephosphotransferase (EC 2.7.8.1) (ETHPT)	EPT1_YEAST (P22140)	Rec	391	7
136.	High-affinity glucose transporter HXT2	HXT2_YEAST (P23585)	Tra / Npm	541	12
137.	High-affinity glucose transporter HXT2	HXT2_YEAST (P23585)	Rec	541	12
138.	Inorganic phosphate transporter PHO84	PH84_YEAST (P25297)	Tra / Npm	596	12
139.	Plasma membrane ATPase 1 (EC 3.6.1.35)	ATH1_YEAST (P05030)	Atp / Npm	918	8
140.	Plasma membrane ATPase 2 (EC 3.6.1.35)	ATH2_YEAST (P19657)	Atp / Npm	947	8
141.	Cytochrome B	Monomeric yeast[12]	Rdx / Ppm	385	8
142.	CYO A (cytochrome O terminal oxidase complex)	*E. coli*[13]	Rdx / Ppm	316	2
143.	CYO B (cytochrome O terminal oxidase complex)	*E. coli*[13]	Rdx / Ppm	663	15
144.	CYO C (cytochrome O terminal oxidase complex)	*E. coli*[13]	Rdx / Ppm	204	5
145.	CYO D (cytochrome O terminal oxidase complex)	*E. coli*[13]	Rdx / Ppm	109	3
146.	CYO E (cytochrome O terminal oxidase complex)	*E. coli*[13]	Rdx / Ppm	296	7
147.	EF0 ATPase A subunit	*E. coli*[11]	Atp / Npm	83	2
148.	EF0 ATPase C subunit	*E. coli*[11]	Atp / Npm	79	2
149.	LAC permease	*E. coli*[8]	Tra / Ppm	417	12
150.	Light harvesting complex (LHC IIB polypeptide)	*Rb. sphaeroides*[6]	Rdx / Ppm	233	3
151.	Mitochondrial cytochrome C oxidase (subunit I)	*Rb. sphaeroides*[2]	Rdx / Ppm	565	12
152.	Mitochondrial cytochrome C oxidase (subunit III)	*Rb. sphaeroides*[2]	Rdx / Ppm	261	7
153.	Photosynthetic (light) reaction centre (H subunit)	*Rb. sphaeroides*[5]	Rdx / Ppm	262	1
154.	Photosynthetic (light) reaction centre (L subunit)	*Rb. sphaeroides*[5]	Rdx / Ppm	309	5
155.	Photosynthetic (light) reaction centre (M subunit)	*Rb. sphaeroides*[5]	Rdx / Ppm	282	5
156.	Cytochrome B/C1 complex	*Rhodobacter capsulatus*[4]	Rdx / Ppm	436	9
157.	Bacterial rhodopsin	*E. coli*[7]	Tra / Ppm	211	5

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