GPCR Extracellular Charge Counting

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Abstract

This project aimed to find a set of human GPCR proteins which may possibly interact with defensin proteins. This was done by finding GPCRs which have similar charge distributions to melanocortin receptors which are known to interact with defensins. We found that the melanocortin receptors 1,3, and 4 have unusually negative charges on the distribution of all human GPCRs and that there are 99 other proteins with similarly unusually negative charges.

1 Introduction

Melanocortin receptors 1, 3, and 4 have been shown to interact with defensin ligands, an important protein in the human immune system. This interaction is thought to be specific, but also due to a charge attraction between the cationic defensins and negative charges on the extracellular loops of the transmembrane proteins. Other defensin receptors are still unknown. In order to identify possible candidates for experimental investigation, we found proteins with similarly negatively charged extracellular regions. This was done through aligning all human GPCR proteins to the two GPCRs with known structure and counting charged residues on the three extracellular loops.

2 Methods

2.1 Alignment of Proteins

To obtain meaningful amino acid counts, it was necessary to standardize the protein regions considered despite sequence differences. All human protein sequences, taken from the Swissprot database, were aligned to two different GPCR proteins with known structures (1u19A and 2rh1A) using SAM. Those with E values of less than 1 were found to be GPCRs. Such a high E value was probably sufficient due to the easy prediction of transmembrane regions even given extreme variability in the rest of the proteins. The position and length prediction of transmembrane helices were obtained from TMHMM for the two template proteins and the protein of interest MC4R and compared to confirm that the

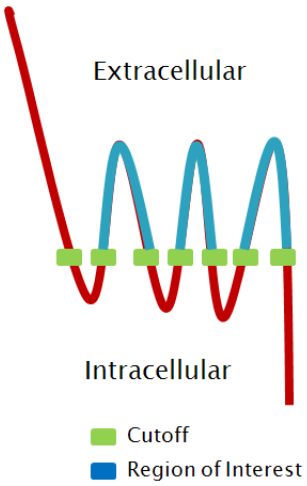

Figure 1: General form of the GPCR proteins and the regions analyzed

alignments were correct. We found that these helices were within a few amino acids of each other in length and position for all three proteins.

2.2 Counting Charged Residues

Both 1u19A and 2rh1A have the typical 7 transmembrane helix structure that characterizes GPCR proteins. By viewing these structures in Rasmol, we could identify which amino acids were extracellular. We made sure to identify cutoff points that included all charged residues within the helix and in the extracellular region, while still at least one helix turn above the charged residues on the inside of the cell. We used a python script to count how many lysine, arginine, histidine, glutamic acid, and aspartic acid amino acids are in the regions of interest. The pH at the cell membrane is unknown, so counts with histidine as charge neutral and positive were recorded.

$$(K + R) - (D + E) = Charge \tag{1}$$

$$(K + R + H) - (D + E) = ChargeH \tag{2}$$

3 Results and Discussion

As expected, the melanocortin receptor proteins' extracellular sections were negatively charged. The counts for both alignments for all five melanocortin receptors were identical and the charge distributions for all human GPCR proteins were very similar for both alignments, as seen in Figure**??**. MC3R and MC4R had a charge count -8, which is two standard deviations away from the

mean. The results for all the melanocortin receptors can be seen in Table **??**. This distribution can be seen to be normal given the parabolic shape on a log y scale. The mean of the distribution is -1.7 and the standard deviation is 3.1. MC3R and MC4R are significantly unusually negative, being over two standard deviations out from the mean. Thirty-five other GPCR proteins with charges this negative or more were identified. Ninety-nine proteins were identified with a charge of -6 or more. Future work can include investigating if any of these proteins identified by charge are likely to also bind to defensins.

Receptor	Charge
1	-6
2	-3
3	-8
4	-8
5	-4

Table 1: Charges of melanocortin receptors calculated by (K+R) - (D+E) = Charge

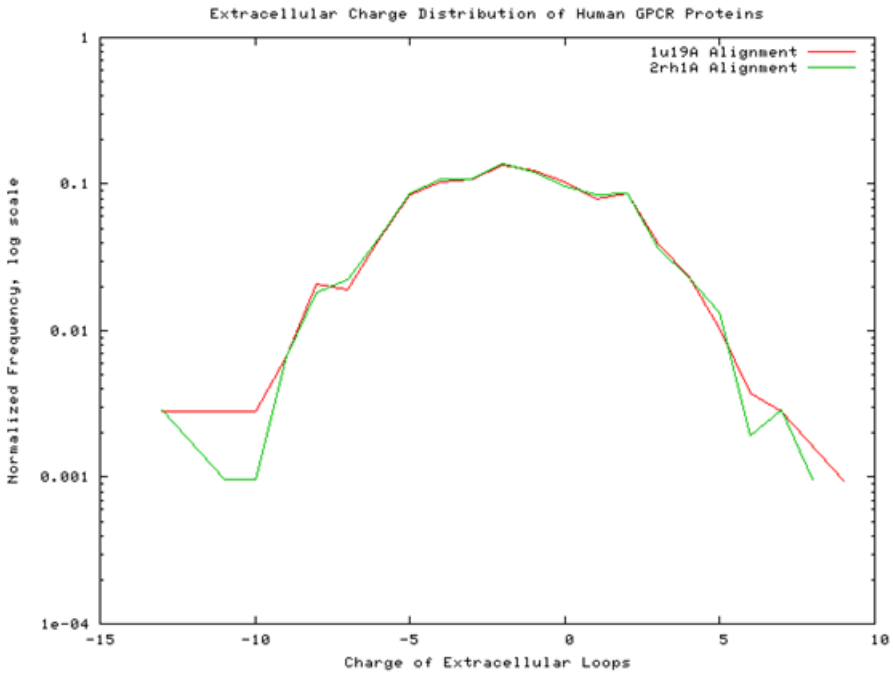

Figure 2: Charge distribution for the sum of all charges on the three extracellular loops of all human GPCR proteins calculated by: (K+R) - (D+E) = Charge. Histidine was considered neutral.