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In-silico Pharmacophore Screening and Structure Based Drug Designing against Migraine Causing Proteins (RAMPs) 1, 2 and 3

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Abstract
Migraine is a chronic neurological disorder characterized by current episodes of several unique throbbing head pain and associated symptoms, such as photophobia. We self understanding of the mechanisms underlying migraine has been hampered by limitations in ascertaining migraine symptoms in animal models. Migraine is a primary headache disorder with almost a genetic basis. Receptor activity-modifying proteins RAMP 1, RAMP 2, and RAMP 3 are unusual accessory proteins that bind with the Calcium gene-related peptide involved in energy homeostasis. Over expression and tension leads to the pain in several parts of the head these proteins are expressed throughout the central nervous system (CNS). The sequence, structure and function of the proteins were deduced by using various bioinformatics tools and databases. Proteins disorder region were identified to reach to the amino acid having a high mutational probability. Binding site was predicted on the basis of repetition of amino acid and proteins were optimized by Sybyl to gain maximum stability. Suitable drug with the respective protein under various parameters of LIPINSKI's rule was identified. The study provide to the organization as there is no special treatment of the disease and about 10% of adults in worldwide suffer from migraines, according to the World Health Organization.

Keywords: Migraines, CGRP, CLR, ECD and RAMPs.

1. Introduction
Migraine is a chronic neurological disorder characterized by recurrent episodes of severe unilateral throbbing head pain and associated symptoms, such as photophobia. Our current understanding of the mechanisms underlying migraine has been hampered by limitations in ascertaining migraine symptoms in animal models. Migraine is a primary headache disorder with almost a genetic basis. Receptor activity-modifying proteins (RAMPs) 1, 2, and 3 are unusual accessory proteins that bind with the Calcitonin gene-related peptide involved in energy homeostasis. These proteins are expressed throughout the central nervous system (CNS) and over expression of these leads to the pain in several parts of the head. Calcitonin gene related peptide (CGRP) has a key role in migraine and recently CGRP receptor antagonists have demonstrated clinical efficacy in the treatment of migraine. However, it remains unclear where the CGRP receptors are located within the CGRP signalling pathway in the human trigeminal system and hence the potential antagonist sites of action remain unknown. Therefore we designed a study to evaluate the localization of CGRP and its receptor components calcitonin receptor-like receptor (CLR) and receptor activity modifying protein (RAMP) 1 in the human trigeminal ganglion using immunohistochemistry and compare with that of rat. Antibodies against purified CLR and RAMP1 proteins were produced and characterized for this study. Trigeminal ganglia were obtained at autopsy from adult subjects and sections from rat trigeminal ganglia were used to compare the immunostaining pattern. The number of cells expressing CGRP, CLR and RAMP1, respectively, were counted. In addition, the glial cells of trigeminal ganglion, particularly the satellite glial cell, were studied to understand a possible relation. We observed immunoreactivity for CGRP, CLR and RAMP1, in the human trigeminal ganglion: 49% of the neurons expressed CGRP, 37% CLR and 36% RAMP1. Co-localization of CGRP and the receptor components was rarely found. There were no CGRP immunoreactions in the glial cells; however some of the glial cells displayed CLR and RAMP1 immunoreactivity. Similar results were observed in rat trigeminal ganglia. We report that human and rat trigeminal neurons store CGRP, CLR and RAMP1; however, CGRP and CLR/RAMP1 do not co-localize regularly but are found in separate neurons. Glial cells also contain the CGRP receptor components but not CGRP. Our results indicate, for the first time, the possibility of CGRP signalling in the human trigeminal ganglion

involving both neurons and satellite glial cells. This suggests a possible site of action for the novel CGRP receptor antagonists in migraine therapy [1].

The receptors for calcitoningene-related peptide (CGRP) and Adriano maudlin (AM) are complexes of the calcitonin receptor-like receptor (CLR) and receptor activity-modifying proteins (RAMP). The CGRP receptor is a CLR/RAMP1 pairing whereas CLR/RAMP2 and CLR/RAMP3 constitute two subtypes of AM receptor: AM1 and AM2, respectively. Previous studies identified Glu74 in RAMP3 to be important for AM binding and potency. To further understand the importance of this residue and its equivalent in RAMP1 (Trp74) we substituted the native amino acids with several others. In RAMP3, these were Trp, Phe, Tyr, Ala, Ser, Thr, Arg and Asn; in RAMP1, Glu, Phe, Tyr, Ala and Asn substitutions were made. The mutant RAMPs were co-expressed with CLR in Cos7 cells; receptor function in response to AM, AM2/intermeddling and CGRP was measured in a cAMP assay and cell surface expression was determined by ELISA. Phe reduced AM potency in RAMP3 but had no effect in RAMP1. In contrast, Tyr had no effect in RAMP3 but enhanced AM potency in RAMP1. Most other substitutions had a small effect on AM potency in both receptors whereas there was little impact on CGRP or AM2 potency. Overall, these data suggest that the geometry and charge of the residue at position 74 contribute to how AM interacts with the AM2 and CGRP receptors and confirms the role of this position in dictating differential AM pharmacology at the AM2 and CGRP receptors [2].

Calcitonin gene-related peptide (CGRP) is a powerful vasodilator that interacts with the autonomic nervous system. A subunit of the CGRP receptor complex, receptoractivity-modifyingprotein1 (RAMP1), is required for trafficking of the receptor to the cell surface and high-affinity binding to CGRP. We hypothesized that up regulation of RAMP1 would favourably enhance autonomic regulation and attenuate hypertension. Blood pressure, heart rate, and loco motor activity were measured by radio telemetry in transgenic mice with ubiquitous expression of human RAMP1 (hRAMP1) and littermate controls. Compared with control mice, hRAMP1 mice exhibited similar mean arterial pressure, a lower mean heart rate, increased heart rate variability, reduced blood pressure variability, and increased bar reflex sensitivity (2.83+/-0.20 versus 1.49+/-0.10 ms/mm Hg in controls; P<0.05). In control mice, infusion of angiotensin II (Ang-II) increased mean arterial pressure from 118+/-2 mm Hg to 153+/-4 and 174+/-6 mm Hg after 7 and 14 days of infusion, respectively (P<0.05). In contrast, Ang-II hypertension was markedly attenuated in hRAMP1 mice with corresponding values of mean arterial pressure of 111+/-2, 119+/-2, and 132+/-3 mm Hg. Ang-II induced decreases in bar reflex sensitivity and heart rate variability, and increases in blood pressure variability observed in control mice were also abrogated or reversed in hRAMP1 mice (P<0.05). Moreover, during the Ang-II infusion, the presser response to the CGRP receptor antagonist CGRP(8-37) was significantly greater (P<0.05) in hRAMP1 mice (+30+/-2 mm Hg) than in control mice (+19+/-2 mm Hg), confirming a significantly greater antihypertensive action of endogenous CGRP in hRAMP1 mice. We conclude that RAMP1 over expression attenuate sAng-II-induced hypertension and induces a protective change in cardiovascular autonomic regulation [3].

Receptors for calcitonin gene-related peptide (CGRP) are composed of the calcitonin-like receptor in association with receptor activity-modifying protein-1 (RAMP1). CGRP is an extremely potent vasodilator and may protect against vascular disease through other mechanisms. We tested the hypothesis that overexpression of RAMP1 enhances vascular effects of CGRP using transgenic mice with ubiquitous expression of human RAMP1. Because angiotensin II (Ang II) is a key mediator of vascular disease, we also tested the hypothesis that RAMP1 protects against AngII-inducedvasculardysfunction.in carotid and basilar arteries in vitro as well as cerebral arterioles in vivo were selectively enhanced in human RAMP1 transgenic mice compared to littermate controls (P<0.05), and this effect was prevented by a CGRP receptor antagonist (P<0.05). Thus, vascular responses to CGRP are normally RAMP1-limited. Responses of carotid arteries were examined in vitro after overnight incubation with vehicle or Ang II. In arteries from control mice, Ang II selectively impaired responses to the endothelium-dependent agonist acetylcholine by ≈50% (P<0.05) via a superoxide-mediated mechanism. In contrast, Ang II did not impair responses to acetylcholine in human RAMP1 transgenic mice. RAMP1 overexpression increases CGRP-induced vasodilation and protects against Ang II-induced endothelial dysfunction. These findings suggest that RAMP1 may be a new therapeutic target to regulate CGRP-mediated effects during disease including pathophysiological states in which Ang II plays a major role [4].

The neuropeptide calcitonin gene-related peptide (CGRP) from the trigeminal ganglion has been established as a key player in the pathogenesis of migraine. In this study, we provide evidence that the responsiveness of neuronal CGRP receptors is strongly enhanced in vitro and in vivo by expression of human receptor activity-modifying protein-1 (hRAMP1), an obligatory subunit of the CGRP receptor. We first demonstrated that activation of CGRP receptors on cultured trigeminal ganglion neurons increased endogenous CGRP mRNA levels and promoter activity. The promoter activation was cAMP dependent and blocked by the antagonist BIBN4096BS [1-piperidinecarboxamide, N-[2-[[5-amino-l-[[4-(4-pyridinyl)-l-piperazinyl]carbonyl]pentyl]amino]-1- (3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-ihydro-2-oxo-3(2H)-quinazolinyl)], a new ant migraine drug. Gene transfer using an adenoviral hRAMP1 expression vector increased the maximal production of cAMP by 1.8 +/- 0.2-fold and decreased the EC50 to 2.3 +/- 0.8 nM from 9.0 +/- 5.9 nM and 15.6 +/- 5.2 nM in uninfected and control-infected cultures, respectively. To establish whether RAMP1 is limiting in vivo as indicated from the culture studies, a transgenic mouse expressing hRAMP1 in the nervous system was generated. After CGRP injection into the whisker pad, the hRAMP1 transgenic mice displayed 2.2 +/- 0.2-fold greater plasma extravasation, which is a measure of neurogenic inflammation. These results demonstrate that RAMP1 is functionally rate limiting for CGRP receptor activity in the trigeminal ganglion, which raises the possibility that elevated RAMP1 might sensitize some individuals to CGRP actions in migraine [5].

Secretin family G protein-coupled receptors (GPCRs) are important therapeutic targets for migraine, diabetes, bone disorders, inflammatory disorders and cardiovascular disease. They possess a large N-terminal extracellular domain (ECD) known to be the primary ligand-binding determinant. Structural determination of several secretin family GPCR ECDs in complex with peptide ligands has been achieved recently, providing insight into the molecular determinants of

hormone binding. Some secretin family GPCRs associate with receptor activity-modifying proteins (RAMPs), resulting in changes to receptor pharmacology. Recently, the first crystal structure of a RAMP ECD in complex with a secretin family GPCR was solved, revealing the elegant mechanism governing receptor selectivity of small molecule antagonists of the calcitonin gene-related peptide (CGRP) receptor. Here we review the structural basis of ligand binding to secretin family GPCRs, concentrating on recent progress made on the structural basis of RAMP-modified GPCR pharmacology and its implications for rational drug design [6].

Two receptor activity-modifying proteins (RAMP2 and RAMP3) enable calcitonin receptor-like receptor (CLR) to function as two heterodimeric receptors (CLR/RAMP2 and CLR/RAMP3) for Adriano maudlin (AM), a potent cardiovascular protective peptide. Following AM stimulation, both receptors undergo rapid internalization through a clathrin-dependent pathway, after which CLR/RAMP3, but not CLR/RAMP2, can be recycled to the cell surface for resensitization. However, human (h) RAMP3 mediates CLR internalization much less efficiently than does hRAMP2. Therefore, the molecular basis of the single transmembrane domain (TMD) and the intracellular domain of hRAMP3 during AM receptor internalization was investigated by transiently transfecting various RAMP chimeras and mutants into HEK-293 cells stably expressing hCLR. Flow cytometric analysis revealed that substituting the RAMP3 TMD with that of RAMP2 markedly enhanced AM-induced internalization of CLR. However, this replacement did not enhance the cell surface expression of CLR, [(125)I]AM binding affinity or AM-induced cAMP response. More detailed analyses showed that substituting the Thr(130)-Val(131) sequence in the RAMP3 TMD with the corresponding sequence (Ile(157)-Pro(158)) from RAMP2 significantly enhanced AM-mediated CLR internalization. In contrast, substituting the RAMP3 target sequence with Ala(130)-Ala(131) did not significantly affect CLR internalization. Thus, the RAMP3 TMD participates in the negative regulation of CLR/RAMP3 internalization, and the aforementioned introduction of the Ile-Pro sequence into the RAMP3 TMD may be a strategy for promoting receptor internalization/resensitization [7].

Adrenomedullins (AM) is a multifaceted distinct subfamily of peptides that belongs to the calcitonin gene-related peptide (CGRP) superfamily. These peptides exert their functional activities via associations of calcitonin receptor-like receptors (CLRs) and receptor activity-modifying proteins (RAMPs) RAMP2 and RAMP3. Recent studies established that RAMPs and CLRs can modify biochemical properties such as trafficking and glycosylation of each other. However there is very little or no understanding regarding how RAMP or CLR influence ligand-induced events of AM-receptor complex. In this study, using pufferfish homologs of CLR (mfCLR1-3) and RAMP (mfRAMP2 and mfRAMP3), we revealed that all combinations of CLR and RAMP quickly underwent ligand-induced internalization; however, their recycling rates were different as follows: mfCLR1-mfRAMP3>mfCLR2-mfRAMP3>mfCLR3-mfRAMP3. Functional receptor assay confirmed that the recycled receptors were resensitized on the plasma membrane. In contrast, a negligible amount of mfCLR1-mfRAMP2 was recycled and reconstituted. Immunocytochemistry results indicated that the lower recovery rate of mfCLR3-mfRAMP3 and mfCLR1-mfRAMP2 was correlated with higher proportion of lysosomal localization of these receptor complexes compared to the other combinations. Collectively our results indicate, for the first time, that the ligand-induced internalization, recycling, and reconstitution properties of RAMP-CLR receptor complexes depend on the receptor-complex as a whole, and not on individual CLR or RAMP alone [8].

Various bioactive peptides have been implicated in the homeostasis of organs and tissues. Adrenomedullin (AM) is a peptide with various bioactivities. AM-receptor, calcitonin-receptor-like receptor (CLR) associates with one of the subtypes of the accessory proteins, RAMPs. Among the RAMP subisoforms, only RAMP2 knockout mice $^{-/-}$ reproduce the phenotype of embryonic lethality of AM$^{-/-}$, illustrating the importance of the AM-RAMP2-signaling system. Although AM and RAMP2 are abundantly expressed in kidney, their function there remains largely unknown. We used genetically modified mice to assess the pathophysiological functions of the AM-RAMP2 system. RAMP2$^{+/-}$ mice and their wild-type littermates were used in a streptozotocin (STZ)-induced renal injury model. The effect of STZ on glomeruli did not differ between the 2 types of mice. On the other hand, damage to the proximal urinary tubules was greater in RAMP2$^{+/-}$. Tubular injury in RAMP2$^{+/-}$ was resistant to correction of blood glucose by insulin administration. We examined the effect of STZ on human renal proximal tubule epithelial cells (RPTECs), which express glucose transporter 2 (GLUT2), the glucose transporter that specifically takes up STZ. STZ activated the endoplasmic reticulum (ER) stress sensor protein kinase RNA-like endoplasmic reticulum kinase (PERK). AM suppressed PERK activation, its downstream signaling, and CCAAT/enhancer-binding homologous protein (CHOP)-induced cell death. We confirmed that the tubular damage was caused by ER stress-induced cell death using tunicamycin (TUN), which directly evokes ER stress. In RAMP2$^{+/-}$ kidneys, TUN caused severe injury with enhanced ER stress. In wild-type mice, TUN-induced tubular damage was reversed by AM administration. On the other hand, in RAMP2$^{+/-}$, the rescue effect of exogenous AM was lost. These results indicate that the AM-RAMP2 system suppresses ER stress-induced tubule cell death, thereby exerting a protective effect on kidney. The AM-RAMP2 system thus has the potential to serve as a therapeutic target in kidney disease [9].

Homology modeling, also known as comparative modeling of protein, refers to constructing an atomic-resolution model of the "*target*" protein from its amino acid sequence and an experimental three-dimensional structure of a related homologous protein (the "*template*"). Homology modeling relies on the identification of one or more known protein structures likely to resemble the structure of the query sequence, and on the production of an alignment that maps residues in the query sequence to residues in the template sequence. It has been shown that protein structures are more conserved than protein sequences amongst homologues, but sequences falling below a 20% sequence identity can have very different structure [10]. Evolutionarily related proteins have similar sequences and naturally occurring homologous proteins have similar protein structure. It has been shown that three-dimensional protein structure is evolutionarily more conserved than would be expected on the basis of sequence conservation alone [11]. The sequence alignment and template structure are then used to produce a structural model of the target. Because protein structures are more conserved than DNA sequences, detectable levels of sequence similarity

usually imply significant structural similarity [12].

The quality of the homology model is dependent on the quality of the sequence alignment and template structure. The approach can be complicated by the presence of alignment gaps (commonly called indels) that indicate a structural region present in the target but not in the template, and by structure gaps in the template that arise from poor resolution in the experimental procedure (usually X-ray crystallography) used to solve the structure. Model quality declines with decreasing sequence identity; a typical model has ~1–2 Å root mean square deviation between the matched C^{α} atoms at 70% sequence identity but only 2–4 Å agreement at 25% sequence identity. However, the errors are significantly higher in the loop regions, where the amino acid sequences of the target and template proteins may be completely different. Regions of the model that were constructed without a template, usually by loop modeling, are generally much less accurate than the rest of the model. Errors in side chain packing and position also increase with decreasing identity, and variations in these packing configurations have been suggested as a major reason for poor model quality at low identity [13]. Taken together, these various atomic-position errors are significant and impede the use of homology models for purposes that require atomic-resolution data, such as drug design and protein–protein interaction predictions; even the quaternary structure of a protein may be difficult to predict from homology models of its subunit(s). Nevertheless, homology models can be useful in reaching *qualitative* conclusions about the biochemistry of the query sequence, especially in formulating hypotheses about why certain residues are conserved, which may in turn lead to experiments to test those hypotheses. For example, the spatial arrangement of conserved residues may suggest whether a particular residue is conserved to stabilize the folding, to participate in binding some small molecule, or to foster association with another protein or nucleic acid.

Homology modeling can produce high-quality structural models when the target and template are closely related, which has inspired the formation of structural genomics consortium dedicated to the production of representative experimental structures for all classes of protein folds [14]. The chief inaccuracies in homology modeling, which worsen with lower sequence identity, derive from errors in the initial sequence alignment and from improper template selection [15]. Like other methods of structure prediction, current practice in homology modeling is assessed in a biennial large-scale experiment known as the Critical Assessment of Techniques for Protein Structure Prediction, or CASP. Clustering algorithms are used to organize this expression data into different biologically relevant clusters. The field of bioinformatics has become a major part of the drug discovery pipeline playing a key role for validating drug targets. By integrating data from many inter-related yet heterogeneous resources, bioinformatics can help in our understanding of complex biological processes and help improve drug discovery [16].

Drug design, sometimes referred to as rational drug design or simply rational design, is the inventive process of finding new medications based on the knowledge of a biological target [17]. The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit to the patient. Drug design frequently but not necessarily relies on computer modeling techniques. This type of modeling is often referred to as computer-aided drug design. Finally, drug design that relies on the knowledge of the three-dimensional structure of the bimolecular target is known as structure-based drug design [18].

2. Materials and Methods: In the following paragraphs, I have briefly enlisted the various bioinformatics databases that have been of critical importance in completing my project work. Biological databases are stores of biological information. Most of these databases are “open source” and are open to all the researches worldwide, thus themselves getting constantly updated according to the latest findings and requirements if any: NCBI, GENECARDS, BLAST, PDB, PubMed [19].

2.1 ProtParam: ProtParam is a tool which allows the computation of various physical and chemical parameters for a given protein stored in SwissProt or for a user entered sequence. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity. ProtParam computes various physico-chemical properties that can be deduced from a protein sequence [20, 21].

2.2 SOPMA: SOPMA is a secondary structure prediction method. SOPMA (Self-Optimized Prediction Method with Alignment) is an improvement of SOPM method. These methods are based on the homologue method of Levin *et al.* SOPMA correctly predicts 69.5% of amino acids for a three-state description of the secondary structure (alpha-helix, beta-sheet and coil) in a whole database containing 126 chains of non-homologous (less than 25% identity) proteins. Joint prediction with SOPMA and a neural networks method (PHD) correctly predicts 82.2% of residues for 74% of co-predicted amino acids [22]

2.3 PHYRE: The ***PHYRE*** automatic fold recognition server for predicting the structure and/or function of your protein sequence. **Phyre** and **Phyre2** (**P**rotein **H**omology Analog **Y** **R**ecognition **Engine**; pronounced as 'fire') are web-based services for protein structure prediction that are free for non-commercial use. Phyre is among the most popular methods for protein structure prediction having been cited over 1000 times [23, 24].

2.4 HH PRED: HHsearch is an open-source software program for protein sequence searching that is part of the free HH-suite software package [25]. **HHpred** is a free protein function and protein structure prediction server that is based on HHsearch and HHblits, another program in the HH-suite package [26]. HHpred and HHsearch are among the most popular methods for protein structure prediction and the detection of remotely related sequences, each having been cited over 500 times**.** The primary aim in developing HHpred was to provide biologists with a method for sequence database searching and structure prediction that is as easy to use as BLAST or PSI-BLAST and that is at the same time much more sensitive in finding remote homologs (27-30).

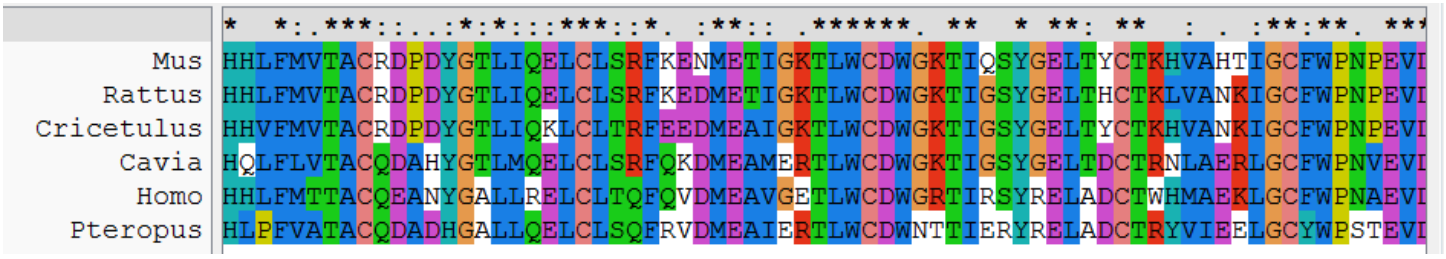
2.5 PROSITE: PROSITE is a protein database [31]. It consists of entries describing the protein families, domains and functional sites as well as amino acid patterns, signatures, and profiles in them. These are manually curetted by a team of the Swiss Institute of Bioinformatics and tightly integrated

into Swiss-Prot protein annotation. PROSITE was created in 1988 by Amos Bairoch [32-35].

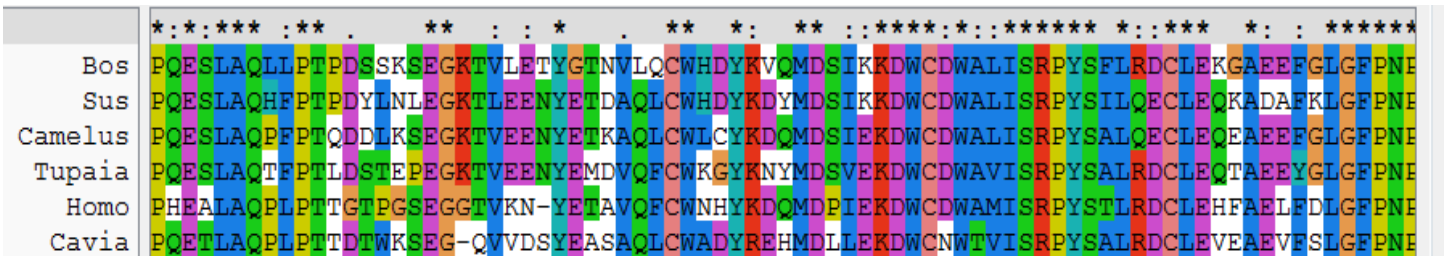
2.6 Hex. Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Hex can also calculate protein-ligand docking, assuming the ligand is rigid, and it can superpose pairs of molecules.

3. Methodology: Methodology includes the process which is responsible for migraine. Within the selected disease for migraine, first we have to search a literature by choosing Receptor activity modifying proteins (RAMPS), to work on the BLAST, phylogenetic analysis-ClustalX, ClustalW, Tcoffee for finding out the similarity with higher model organisms which might be of use during clinical trials in the later stages of drug development. Process includes the primary structure analysis by PROTPARAM, secondary structure prediction by CFSSP, Tertiary structure prediction by HHPRED, CPH and identification of functional region from results of HHPRED, and CPHmodels. Protein associated with the disease was discovered are RAMP1, RAMP2 and RAMP3 proteins are the cause of migraine. The sequence, structure and function of the proteins were deduced by using various bioinformatics tools and databases. The visualization of protein in Rasmol was done by domain analysis via SMART.
The disorder region prediction was done using disEMBL, PRDOS, DISPROT using Q-Site finder, found out all the possible active sites on the requisite domain and the residue layout at each of these sites. By using CAStp, It finds out the specific site of mutation within pocket. The result was matched by Q-site and CASTp, disorder region prediction to localise the target residue in the domain. Migraine is a chronic neurological disorder characterized by recurrent episodes of severe unilateral throbbing head pain and associated symptoms, such as photophobia. Our current understanding of the mechanisms underlying migraine has been hampered by limitations in ascertaining migraine symptoms in animal models.
When the target residue on the domain is confirmed it searched the pubchem database for potential ligant/drug molecules that followed “Lipinski” rule of 5” and validated the potential ligands through the check ring number by using Molergo Virtual Docker, cleaning and optimization of the receptor and ligands takes place by using Sybyl. The neuropeptide calcitonin gene-related peptide (CGRP) from the trigeminal ganglion has been established as a key player in the pathogenesis of migraine. In this study, we provide evidence that the responsiveness of neuronal CGRP receptors is strongly enhanced in vitro and in vivo by expression of human receptor activity-modifying protein-1 (hRAMP1), an obligatory subunit of the CGRP receptor. Docking with the receptor molecule using Argus Lab, Hex 5.0 and Molegro software and it validated the uniqueness of the most potent drug candidate and finalized the ligand.

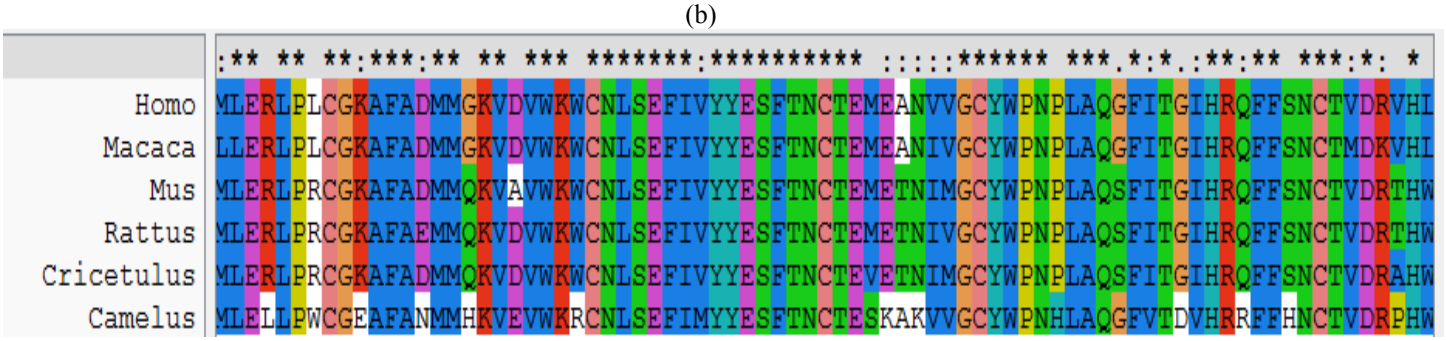
4. Result and Discussion
The calcitonin-receptor-like receptor can function as either a calcitonin-gene-related peptide or an adrenomedullin receptor. The receptors function is modified by receptor-activity-modifying protein or RAMP. RAMPs are single-transmembrane-domain proteins. The calcitonin-receptor-like receptor can function as either a calcitonin-gene-related peptide or an adrenomedullin receptor. The receptors function is modified by receptor-activity-modifying protein or RAMP. RAMPs are single-transmembrane-domain proteins. The calcitonin-receptor-like receptor can function as either a calcitonin-gene-related peptide or an adrenomedullin receptor. The receptors function is modified by receptor-activity-modifying protein or RAMP. RAMPs are single-transmembrane-domain proteins. By using the sequence alignment tool namely BALST, we concluded that query sequence RAMP1 is perfectly identical to the homo sapiens i.e. 100%, whereas *Rattusnorvegicus*, *Cricetulusgriseus*, *Musmusculus*, *Caviaporcellus*, *Pteropusalectoshows* 71%, 70%, 70%, 72% and 65% identity respectively. By using the sequence alignment tool namely BALST, we concluded that query sequence is perfectly identical to the homo sapiens i.e. 100%, whereas *Sus scrofa*, *Bostaurus*, *Camelusferus*, *Tupaiachinensis*, *Caviaporcellusshows* 75%, 77%, 78%, 77% and 71% identity respectively. By using the sequence alignment tool namely blast, we concluded that query sequence is perfectly identical to the homo sapiens i.e. 100%, whereas Macacamulatta, Rattusnorvegicus, Musmusculus, Cricetulusgriseus, Camelusferusshows 96%, 87%, 86%, 90% and 72% identity respectively.
From the CLUSTAL X we infer that the most of the domains are conserved which shows the close relationship among organisms.



(a)



(b)

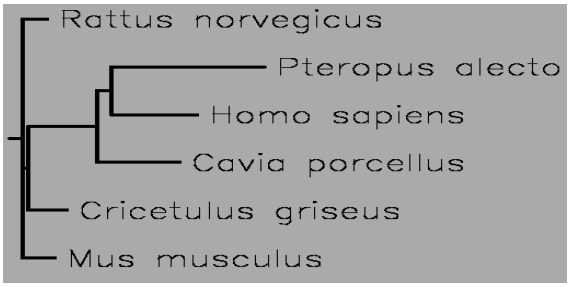


(c)

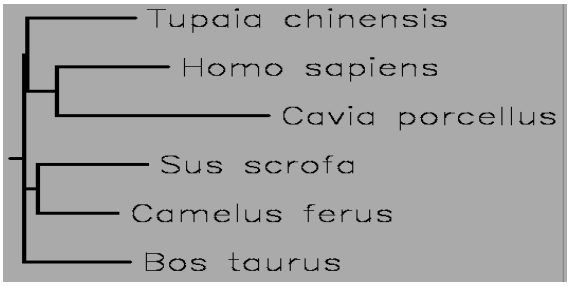
Fig 1: (a), (b) & (c) are showing multiple sequence analysis results for RAMPs 1, 2 & 3. In these figure (:) dot shows that the sequence is highly similar, (**.**) dot shows that the sequence is weak similar, (*) shows that most of the domain are conserved which shows the closed relationship among organism.

4.1 Phylogenetic Analysis

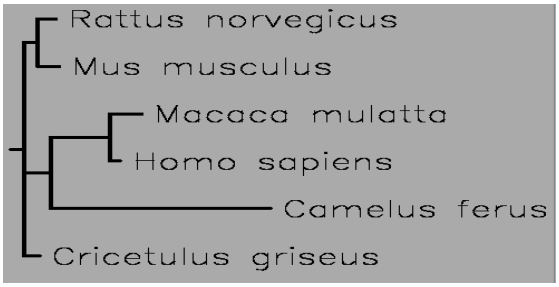
phylogenetic analysis and clustal distance matrix was trying to check the closest organism for RAMP1 RAMP2 AND RAMP3 respectively.



(a)



(b)



(c)

Fig 2: Dendogram of (a) RAMP1 (b) RAMP2 (c) RAMP3

Table 1: (a) Clustal Distance Matrix of RAMP1

		(1)	(2)	(3)	(4)	(5)	(6)
Rattus_norvegicus	(1)	0.000	0.081	0.101	0.264	0.291	0.381
Mus_musculus	(2)	0.081	0.000	0.108	0.277	0.291	0.418
Cricetulus_griseus	(3)	0.101	0.108	0.000	0.277	0.297	0.410
Cavia_porcellus	(4)	0.264	0.277	0.277	0.000	0.284	0.351
Homo_sapiens	(5)	0.291	0.291	0.297	0.284	0.000	0.351
Pteropus_alecto	(6)	0.381	0.418	0.410	0.351	0.351	0.000

Table 1: (b) Clustal Distance Matrix of RAMP2

		(1)	(2)	(3)	(4)	(5)	(6)
Sus_scrofa	(1)	0.000	0.168	0.199	0.208	0.287	0.355
Camelus ferus	(2)	0.168	0.000	0.188	0.187	0.221	0.288
Bos_taurus	(3)	0.199	0.188	0.000	0.195	0.259	0.319
Tupaia_chinensis	(4)	0.208	0.187	0.195	0.000	0.233	0.317
Homo_sapiens	(5)	0.287	0.221	0.259	0.233	0.000	0.290
Cavia_porcellus	(6)	0.355	0.288	0.319	0.317	0.290	0.000

Table 1: (c) Clustal Distance Matrix of RAMP3

		(1)	(2)	(3)	(4)	(5)	(6)
Macaca_mulatta	(1)	0.000	0.041	0.146	0.154	0.121	0.316
Homo_sapiens	(2)	0.041	0.000	0.131	0.138	0.103	0.279
Rattus_norvegicus	(3)	0.146	0.131	0.000	0.038	0.052	0.262
Mus_musculus	(4)	0.154	0.138	0.038	0.000	0.043	0.285
Cricetulus_griseus	(5)	0.121	0.103	0.052	0.043	0.000	0.267
Camelus_ferus	(6)	0.316	0.279	0.262	0.285	0.267	0.000

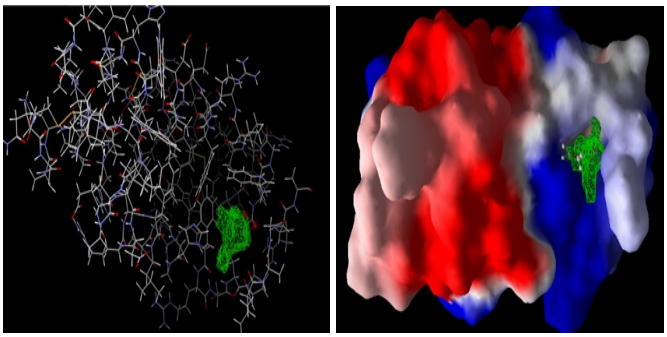
From the above dendogram we infer that the Query and Homo sapiens are 100% similar as the query sequence also belongs to the Homo sapiens. Now we can *say that Cavia_porcellus, Camelus_ferus, Macacamulattaare the closest* organism for RAMP1 RAMP2 AND RAMP3 respectively.

4.2 Primary secondary and tertiary structure analysis

From primary structure analysis we can compare various physio-chemical properties of the proteins. As protein is having the instability index less than 40, this classifies the protein as stable and hydrophillic in nature because GRAVY value of protein (RAMPs) 1, 2 &3 are 0.211, -0.009 and 0.301 respectively that is the protein is α-helix rich. We also infere from the above result the estimated half life of the protein which is found to be 30 hours in mammals and composition of the different amino acids. From secondary structure analysis we can compute that the protein is having maximum percentage of α- helix which is more than β- sheets.

From tertiary structure analysis of query RAMP1, RAMP2 and RAMP3 protien sequence we select a 3N7Pprotien domain id chain R having the maximum similarity with the query and find their function. From PDB, we can find the information about the experimentally – determined structures (3 – D structural data of large biological molecules) of query protein domain i.d. or PDB i.d. that is 3n7p. From the Pubchem server 56 compounds were screened out of 209 which were most likely to be drug against Migraine by Lipinski Rule Of 5.These compounds were further screened on the basis of RING NUMBER. After the screening only 10 compounds were selected that were most likely to be drug against Migraine.

Docking



(a) (b)

Fig 3: (a) and (b) showing Docking result for receptor 3n7p.

After performing the Non - Targeted Docking by Hex docking energy was found for each receptor-ligand complex which is as under:

Table 2: HEX docking result

SN	RECEPTOR	LIGAND(CID)	DOCKING ENERGY
1	3N7P	1220	-220.80
2	3N7P	1355	-193.20
3	3N7P	1809	-194.68
4	3N7P	5011	-210.75
5	3N7P	5202	-1.00
6	3N7P	25681	-19.73
7	3N7P	107992	-45.99
8	3N7P	160796	-312.35
9	3N7P	4048638	-33.64
10	3N7P	5702242	-23.33

5. Summary and Conclusion: Migraine is a common type of headache that may occur with symptoms such as nausea, vomiting, or sensitivity to light. In many people, a throbbing pain is felt only on one side of the head. Protein associated with the disease was discovered are RAMP1, RAMP2 and RAMP3 proteins are the cause of migraine. The sequence, structure and function of the proteins were deduced by using various bioinformatics tools and databases. A disorder region of the protein was identified to reach to the amino acid having a high mutational probability. Binding site was predicted on the basis of repetition of amino acid and proteins are optimized by one to gain maximum stability. To find a suitable drug for dock with the respective protein under various parameters of LIPINSKI's rule. The drug which was most potent to dock the protein and prevent the disease was clobenzorex. Expression profiling and large-scale proteomics have revolutionized biology by generating vast amounts of data about cell state. Genes with significant changes in expression have immediate and widespread interest as markers for diseases, stages of development, and a variety of other cellular phenotypes. To increase the productivity of drug discovery one needs a far deeper understanding of the molecular mechanisms of diseases, taking into account the full biological context of the drug target and moving beyond individual genes and proteins.

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