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RESEARCH ARTICLE

COMPUTATIONAL ANALYSIS FOR IDENTIFICATION OF MOSQUITOCIDAL COMPOUNDS FROM *KALANCHOE PINNATA* TARGETING THE ACETYLCHOLINE ESTERASE OF *CULEX QUINQUEFASCIATUS*

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ABSTRACT

Acetylcholinestrase is the primary cholinestrase in the body an enzyme that catalyses the breakdown of acetylcholine and of some other cholinesters that function as neurotransmitters. Acetylcholinesterase is involved in the termination of impulse transmission by rapid hydrolysis of the neurotransmitter acetylcholine in numerous cholinergic pathways in the central and peripheral nervous systems. The enzyme inactivation, induced by various inhibitors, leads to acetylcholine accumulation, hyperstimulation of nicotinic and muscarinic receptors, and disrupted neurotransmission. Hence, acetylcholinesterase inhibitors, interacting with the enzyme as their primary target, are applied as relevant drugs and toxins. In this present study was computational analysis of potential drugs by the process of molecular docking with important bioactive phytochemicals of the plant *Kalanchoe pinnata* dock with target protein Acetylcholinesterase of mosquitoes by using 11 compounds (Alpha amyrin, Beta amyrin, Benzene, Dioctyl phthalate, 2,3-dihydroxypropyl acetate, N-nonadecanol-1, Pentacosane, 1,2,3- propanetriol, Phthalic acid, Stigmast-5-en-3-ol and Vitamin E)were selected from GC-MS analysis.The binding affinity values of the compounds as Beta amyrin -5.51, Alphaamyrin -5.50,N-nonadecanol-1 -5.18, Phthalic acid -4.79, Stigmast-5-en-3-ol -4.37, Vitamin E -2.97, 1,2,3- propanetriol -2.78, 2,3-dihydroxypropyl acetate -2.67 shows towards AChE.

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INTRODUCTION

Plants have a long history of use in the treatment of many diseases. Plant-derived compounds have played an important role in the development of several clinically useful agents. In the recent years, more emphasis has been placed on identifying plant-derived compounds that can be used as an effective treatment for life-threatening diseases. Acetyl cholinesterase (AChE) is an essential enzyme anchored to the cell membrane close to the cholinergic receptors, which effectively terminates cholinergic transmission by rapid hydrolysis of Ach (Taylor *et al*., 1994). AChE is found both in the peripheral nervous system (PNS) and the central nervous system (CNS).The crystal structure of AChE shows that the catalytic triad, which is formed by serine, histidine and glutamate, is located at the bottom of a narrow 20-A°-deep gorge that penetrates half way into the enzyme and widens close to its base (Sussman *et al*., 1991; Raves *et al*., 1997). The ligand-binding cavity is lined with aromatic residues that account for approximately 40 percent of the cavity surface.

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The entrance of the gorge is termed the peripheral anionic site (PAS) as it was initially believed to contain several negatively charged amino acids due to its preference of binding cationic ligands (Nolte *et al*., 1980). However, the crystal structure indicates that insufficient acidic amino acids are located close to the ligand-binding cavity to support this hypothesis. Instead it has been shown that aromatic residues interact with cationic ligands [Sussman *et al*., 1991; Dougherty and Stauffer, 1990]. A similar interaction pattern can be seen at the catalytic site (CAS), and it is believed that Ach initially binds to the PAS and then rapidly diffuses down to the catalytic site. Compounds that inhibit AChE are very powerful drugs and toxins due to the essential function of the enzyme. In drug discovery programs, AChE inhibitors are of great interest for treatment of cholinergic deficiencies in the PNS (e.g. myasthenia gravis) and CNS (e.g. Alzheimer's disease). In contrast, some of the most dangerous toxins currently known are AChE inhibitors, for example the green mamba (*Dendroaspisangusticeps*) toxin fasciculin, and the nerve agent sarin. In general, most AChE inhibitors mimic ACh by possessing a quaternary amine or a basic nitrogen; hence they are positively charged species at physiological pH and can form cation - aromatic interactions with AChE.

This present work was aimed at the components obtained from the GC-MS analysis of *Kalanchoe pinnata* for their larvicidal activity by docking with Acetyl cholinesterase receptor.

MATERIALS AND METHODS

Insilico Study on Selective Inhibition of Acetylcholinesterase (PDB Id 5HQ3) by several drugs

A computational tool offers the advantage of delivering new drug candidates more quickly and at a lower cost. The present work by computational approach used the following software manipulation of drugs. Molecule inspiration online web service for calculation of drug likeness and bioavailability prediction and molecular docking software.

Docking Process

Docking calculations were carried out using Docking Server (Bikadi and Hazai, 2009). Auto Dock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. The partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Dock explores the conformational space of the ligand using the Lamarkian genetic algorithm (LGA), which is a hybrid of a genetic algorithm (GA) with an adaptive local search (LS) method (Morris *et al*., 1998). In this approach, the ligand's state is represented as a chromosome, which is composed of a string of real-valued genes describing the ligand location (three coordinates), orientation (four quaternions) and conformation (one value for each torsion). The simulation is started by creating a random population of individuals. It is followed by a specified number of generation cycles, each consisting of the following steps: mapping and fitness evaluation, selection, crossover, mutation and elitist selection. Each generation cycle is followed by a local search.

The solutions are scored using energy based scoring function, which includes terms accounting for short-ranged Van Der Waals and electrostatic interactions, loss of entropy upon ligand binding, hydrogen bonding and solvation. Dock requires the receptor and ligand coordinates in MOL2 format. Non polar hydrogen atoms were removed from the receptor file and their partial charges were added to the corresponding carbon atoms. The program Mol2 to PDBQS was used to transform the receptor MOL2 file into the PDBQS format file containing the receptor atom coordinates, partial charges and solvation parameters. The grid maps were centered on the ligand's binding site and were of dimension 61 × 61 × 61 points.

The grid spacing was 0.375 Å yielding a receptor model that included atoms within 22.9 Å of the reference binding site center. The default parameter settings generated by the program were used for docking. For each complex 10 dockings were performed. The initial population was set to 50 individuals; maximum number of energy evaluations was 2.5×105; maximum number of generations was 27,000. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied. The other parameters provided by the default setting were the same as in the followed reference. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools (Morris *et al*., 1998).

Preparation of target proteins

Acetylcholinesterase has been used as target proteins for testing the newly developed docking algorithms in this study. The coordinates of the X-ray crystal structures of these proteins were retrieved from the Protein Data Bank (PDB). All water molecules were removed from the protein structures. After extraction of bound ligands, all hydrogen atoms and the atom charges were assigned to the proteins. Finally, for each protein target, the binding site was defined as the residues around the bound ligand were assigned to the extracted ligand of each protein. The crystal structure of AChE (PDB Id 5HQ3) as PDB file format with resolution of 2.6 Å was retrieved from the protein data bank (PDB) (http://www.rcsb.org). Enzyme structure and mechanism.AChE is a hydrolase that hydrolyzes choline esters. It has a very high catalytic activity - each molecule of AChE degrades about 25000 molecules of acetylcholine (ACh) per second, approaching the limit allowed by diffusion of the substrate. The protein was optimized and energy minimized using the protein preparation wizard of the docking server under the principle of auto dock (http://www.dockingserver.com) was performed. All water molecules and if present, ligands were removed from the proteins for docking studies. Docking was performed for AChE with the ligands as shown in fig .

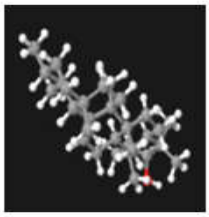
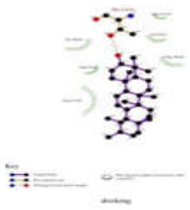
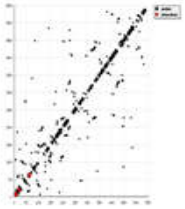
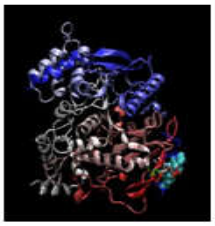
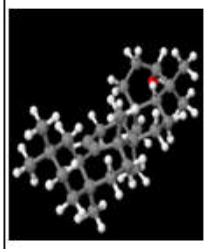
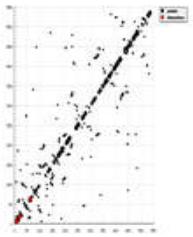
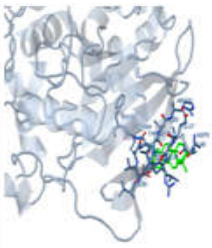
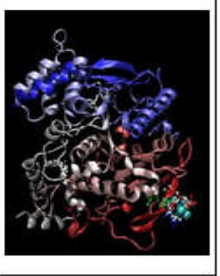
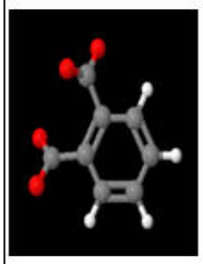
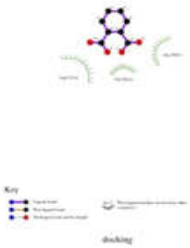
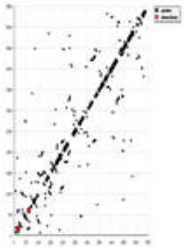
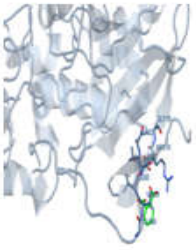
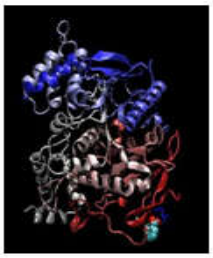
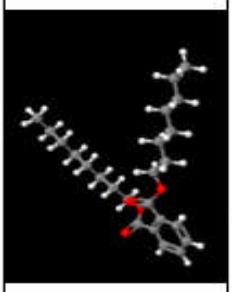
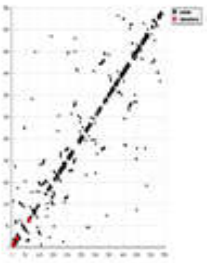
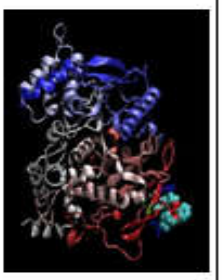
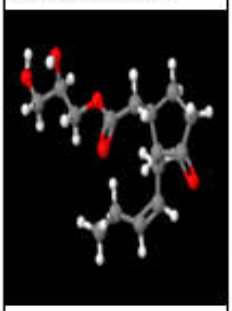
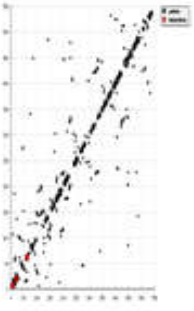
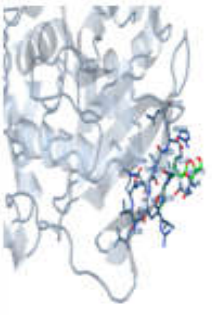
Preparation of Ligand structure

Naturally occurring compounds 11 from *Kalanchoe pinnata* leaf extract were retrieved GC-MS analysis. The structure files were downloaded from the PubChem database (http://www.pubchem.ncbi.nlm. nih.gov). A database of all the compounds was created and energy minimized using ligand preparation module of docking server. Chemical structure was retrieved from Pubchem, Drug Bank (www.drugbank.ca) etc,. The chemical structure was viewed in Chemsketch and its 3D structure was constructed and viewed using 3-D viewer both these programmes are parts of software called ACDLABS version 12.0.The small-molecule topology generator is used for ligand optimization, a tool for high-through put crystallography of protein-ligand complexes which takes input from existing coordinates or various two-dimensional formats and automatically generates coordinates and molecular topologies suitable for X-ray refinement of protein ligand complexes.

RESULTS AND DISCUSSION

Docking Studies of Receptor Ligand Interactions

A recent but alternate method to check out possible inter-action between lead molecules and target enzyme is *insilico* docking. In recent times, the *insilico* approach to drug designing is gaining potential significance in enhancing the process of drug discovery. This approach is an attempt to streamline the lead discovery process by moving as much of the *in vitro* experimentation as possible into the realm of computation, with the help of the ligand (lead molecule) docking and screening algorithms. *Insilico* docking of leads with 3D structures of target enzymes will throw light on whether the target enzyme has docking sites / pockets for the lead. This could be the possible alternate method for validation of drug leads in conjunction with the *in vitro* assays with recombinant enzymes. Among diverse mechanisms of action, the insect enzyme Acetylcholinesterase (AChE) inhibition stands out a promise insecticides control method.

Ligand	2D plot	HB Plot	Ligand receptor interaction	Complex
Alpha amyrin 				
Beta amyrin 				
Benzene 				
Dioctyl phthalate 				
2,3-didihydroxy propyl acetate 				

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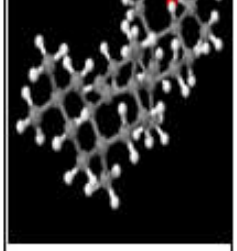
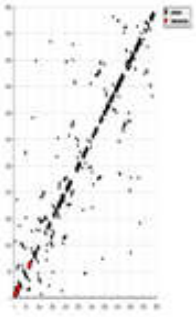
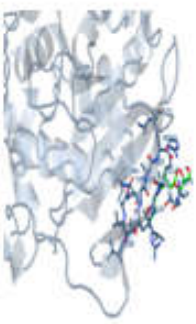
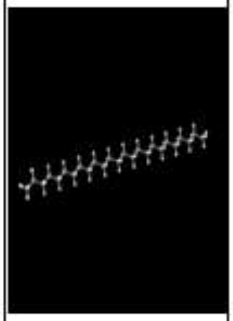
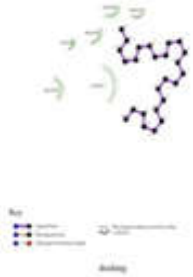
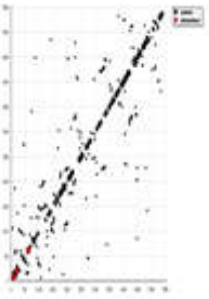
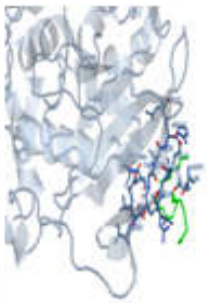
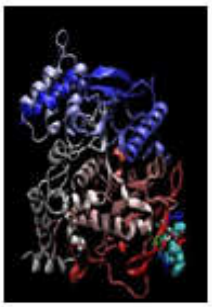
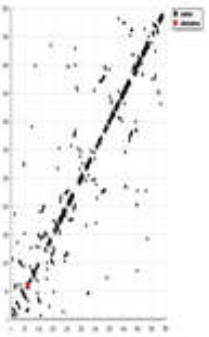
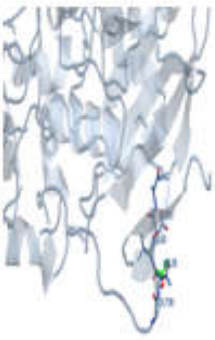
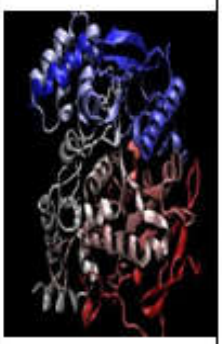
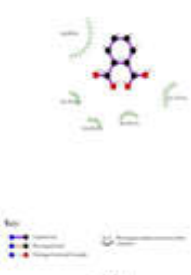
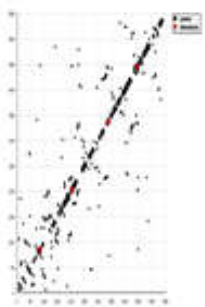
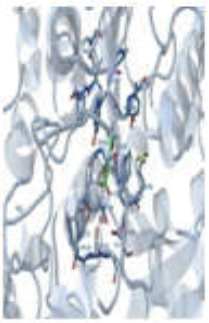
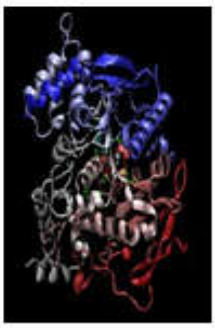
N-nonadecanol-1					
Pentacosane					
1,2,3-propanetriol					
Phthalic acid					

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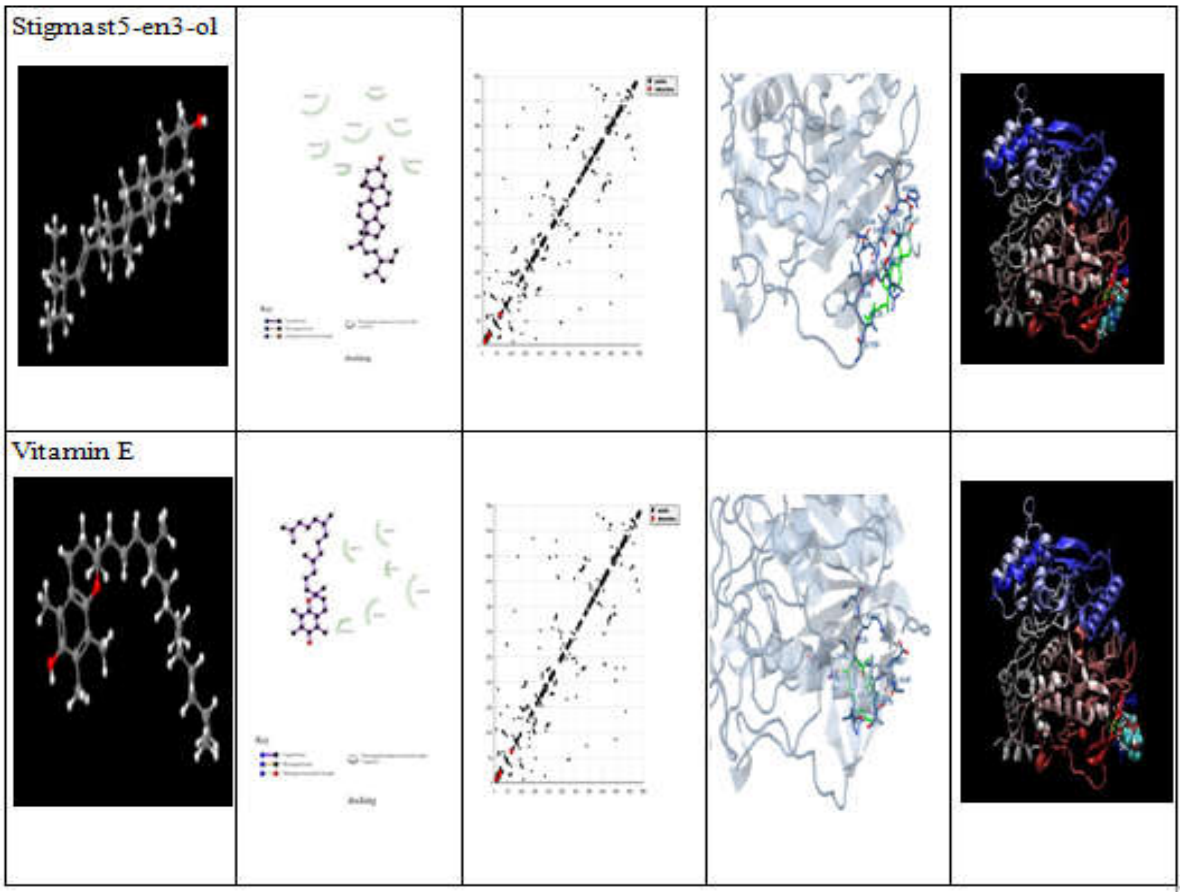

Figure 1. Target Ligand Docked with Acetylcholinestrase

Table 1. Interaction of Acetylcholinesterase with ligands of *Kalanchoepinnata*

Ligand Name	Receptor Name	Docking Score
Alpha amyrin		-5.50 kcal/mol
Beta amyrin		-5.51 kcal/mol
Benzene		-2.25 kcal/mol
Dioctyl phthalate		-0.01 kcal/mol
2,3-dihydroxypropyl acetate		-2.67 kcal/mol
N-nonadecanol-1		-5.18 kcal/mol
Pentacosane	Acetylcholinesterase	-0.25 kcal/mol
1,2,3- propanetriol	(PDB Id 5HQ3)	-2.78 kcal/mol
Phthalic acid		-4.79 kcal/mol
Stigmast-5-en-3-ol		-4.37 kcal/mol
Vitamin E		-2.97 kcal/mol

Inhibitors of this type of mechanism (forex., organophosphate and carbamate chemicals) affect the transmission of nerve impulses accumulating acetylcholine in neuromuscular tissue of insects causing paralysis and then death (Planche *et al*., 2012).Therefore, the discovery of insect AChE inhibitors (AChEIs) is an important task (Casanova *et al.,* 2002; Liu *et al*., 2012) where insecticide design based on natural molecule findings play a key role. In order to explain the differences in bioactivity of the ligands present in *Kalanchoepinnata*, almost 11 ligands (Figure 2) with Acetylcholinesterase (Figure 1), have been subjected to docking calculations using the Auto Dock program (Docking server). *In silico* docking techniques are being used to investigate the complementarity at the molecular level of a ligand and a protein target. As such, docking studies can be used to identify the structural features that are important for binding and for *in silico* screening efforts in which suitable binding partners can be identified. The negative and low value of ΔG indicates strong favorable bonds between the receptor and the ligand inhibitors in their most favorable conformations (Table 1).

The analysis of the docked complexes showed that the inhibitors were located near the active site and were stabilized by hydrogen bonds and pi bonds. The more negative the relative binding, the more potent the binding between the protein and target molecules. It is clear from the above table that both α and β amyrins showed very good values of -5.50 kcal/mol and -5.51 kcal/mol, respectively. N-nonadecanol-1 also showed good docking energies. Phthalic acid and Stigmast-5-en-3-ol also exhibited-4.79 kcal/mol and -4.37 kcal/mol respectively.

The docking of complexes with AChE reveals that all the inhibitor compounds are exhibiting the strongly bonding with active site of enzyme which is showed in Figure 1. Prior to docking, the structures of the complexes were constructed using ChemDraw and geometry optimized force field and saved as Pdb format. The crystal structure of the complex of AChE (PDB) was downloaded from Protein Data Bank. Crystallographic water molecules were removed from the

protein. The structural analysis of docked structures gave significant details about the binding pattern of these complexes.

Molecular interaction of α and β amyrin toward AChE at the anionic site

The calculated binding affinity and 3 Å IE for α and β amyrin when they are accommodated in anionic site are very much different from other binding sites. Both of the compounds are having the highest binding affinity, lower than 6 kcal/mol (Table 2) in this binding pocket despite the absence of hydrogen bond formation. Within the 3 Å binding vicinity, van der Waals interactions are more vital than electrostatics forces to accommodate the ligand in the binding site. The two compounds are found to be most active in this binding site most probably due to the ability in mediating interactions with more residues within 3 Å using a lower energy.

Summary

Mosquito vector population control is a vast and daunting problem faced by populations all over the world especially those in tropical countries. Current study on *Kalanchoe pinnata* bioactive compounds revealed the potential to enhance current control measures, and possibly contribute towards long term control of mosquito populations. In this study Alpha and Beta amyrin present in the extract of *Kalanchoe pinnata* show strong larvicidal activity as can be evidenced by the binding affinity of these compounds towards AChE. The binding study carried out using Auto dock online server suggests that the binding sites could be the possible targets for the compound to interact. The low binding interaction energy implied that α and β amyrins were actually bounded strongly at all sites with the anionic site being the strongest. These findings are consistent with the laboratory results obtained on the AChE assay. Some of the other compounds like N-nonadecanol-1 also showed good docking energies. Phthalic acid and Stigmast-5-en-3-ol also exhibited -4.79 kcal/mol and -4.37kcal/mol respectively. *Kalanchoe pinnata* is an unexplored plant that can be used for its larvicidal activity. A further detailed study of the compounds present in this plant in the purified form will be a break through research in this area.

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