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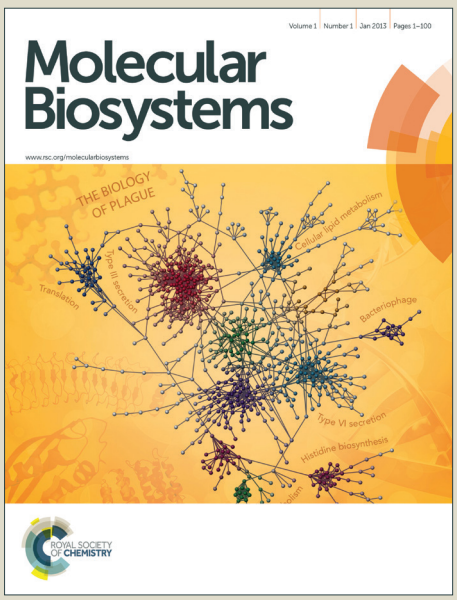

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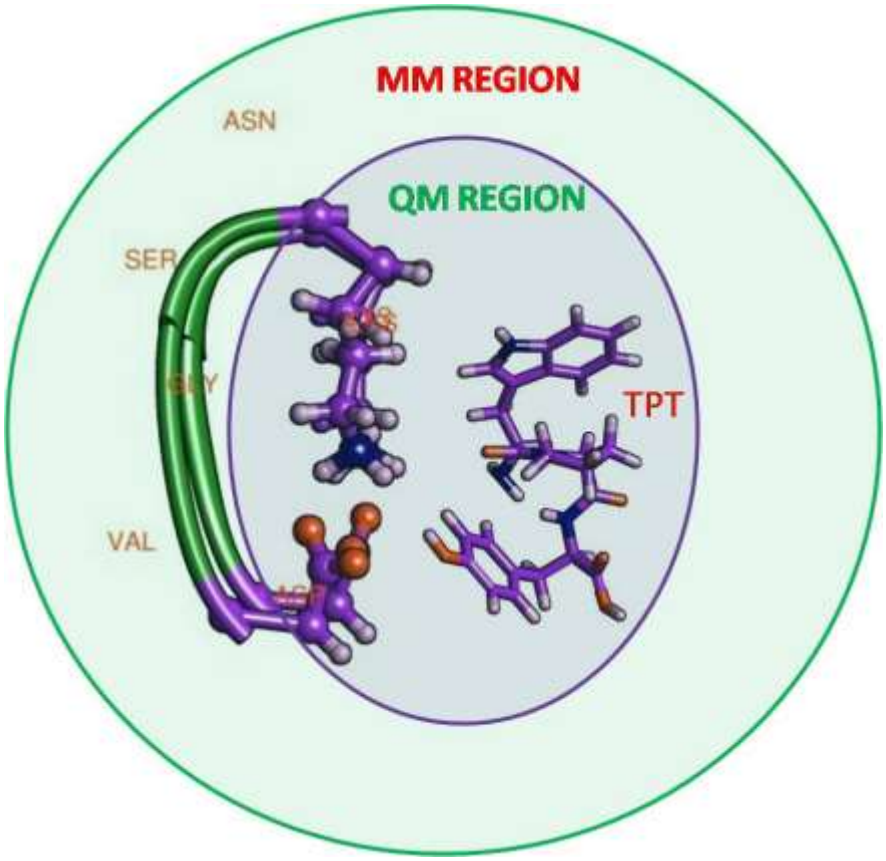

A typical QM/MM approach divides the studied system into a QM core and an MM surrounding, the MM treated part of the Val24-Asn27 is shown in rippon representation, the QM core is highlighted in ball and sticks, where Asp[23], Lys[28] with Morin, TPT and AQ molecule.

Study on the Inter- and Intra-Salt-bridge Mechanism of $A\beta_{23-28}$ Oligomer interaction with small molecules: QM/MM Method

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Abstract

Amyloid β (Aβ) peptides have long been known as a potential candidate for the onset of Alzheimer's disease (AD). The biophysical properties of $A\beta_{42}$ peptides aggregates are of significant importance for the amyloid cascade mechanism of AD. It is necessary to design an inhibitor using small molecules, to reduce the aggregation process in $A\beta_{42}$ peptides. The attention has been given to use the natural products as an anti-aggregation compound, directly targeting Aβ peptide. Polyphenols have been extensively studied as a class of amyloid inhibitors. 9,10-Anthra Quinone (AQ) is abundantly exist in medicinal plants (rhubarb), Trp-Pro-Tyr (TPT) peptide has been found in venom of Black Mamba snake, and Morin molecule is naturally present in wine and green tea, and several other polyphenols derivatives are under clinical trials to develop anti-neurodegenerative drugs. *In vitro* and *in vivo* results strongly suggested that AQ and Morin molecules are potential compounds inhibit the Aβ aggregation, however the detailed understanding of the inhibition mechanism remains largely unknown. The formation of Aβ fibrils and oligomers require a conformational change from α-helix to β-sheet, which was happened due to the formation of a salt-bridge between Asp^{23} and Lys^{28} residues. The present study focused to investigate the salt-bridge mechanism in monomer, dimer and oligomer of $A\beta_{23-28}$ peptide during the interaction of TPT, Morin and AQ molecules. The interaction energy and natural bond orbital analysis have been made using the ONIOM(M05-2X/6-31++G(d,p):UFF) method. The QM/MM studies have been performed to

study the mechanism of salt-bridge during the inhibition process of aggregation of Amyloid β protein. The TPT molecule which bind with Asp^{23} and Lys^{28} residues of Aβ prevent the salt-bridge formation between Asp^{23} and Lys^{28} residues and consequently the chance for the formation of Aβ fibrils get reduced.

Keywords: Amyloid β peptide, Alzheimer's Disease, 9,10-Anthra Quinone, Trp-Pro-Tyr, Morin, Amyloidosis

1. Introduction

Alzheimer's disease (AD) is one of the most common form of senile dementia, associated with memory loss, impaired learning and general cognition.[1] Despite the approval of several drugs for AD, the disease continues to rob millions of people around the world. Even today, there is no effective drug or treatment for the disease, but every year, 7.7 million new peoples get affected by AD.[2] The most important hallmark for the AD is the formation of neurofibrillary tangles and extracellular plaques.[3,4] The plaques are formed by the $Aβ_{40}$, $Aβ_{42}$-peptides (Aβ) of amyloid precursor protein (APP). APP cleavage is driven by two enzymes, β-secretase outside the cell and γ-secretase within the cell. Among the two forms of the Aβ-peptides, $Aβ_{40}$ is present in larger amounts in the brain, but $Aβ_{42}$ is more neurotoxin, which has higher tendency to aggregate.[5-7] The sequence of $Aβ_{42}$ is

Asp-Ala-Glu-Phe-Arg^{5}*-His-Asp-Ser-Gly-Tyr*^{10}*-Glu-Val-His-His-Glu*^{15}*-Lys-Leu-Val-Phe-Phe*^{20}*-Ala-Glu-*<u>Asp^{23}-Val^{24}-Gly^{25}-Ser^{26}-Asn^{27}-Lys^{28}</u>*-Gly-Ala*^{30}*-Ile-Ile-Gly-Leu-Met*^{35}*-Val-Gly-Gly-Val-Val*^{40}**-Ile^{41}-Ala^{42}.**

The amino acids 1-22 and 29-40 indicated in italics, 23-28 underline and 41-42 in bold. The amyloid β-peptide aggregates into oligomers, protofibril, and into plaques, which constitute the characteristic hallmark of Alzheimer's disease. The aggregation of Aβ peptide is not the local phenomena and it has been extended throughout the brain.[1] The understanding

of the Aβ aggregation and its inhibition is clinically important. Indeed, one approach for the development of therapeutic agents in neurodegenerative diseases is use of small molecules which efficiently inhibit the aggregation process. Tremendous efforts have been put in recent years for the development of small molecules which are capable of inhibiting Aβ aggregation process.[8-15] However, not many compounds have been reached to the clinical stage. Unfortunately, currently available drugs, e.g. Aricept and Memantine, usually consider as the best, but it gives an incomplete symptomatic relief.[2]

Atomic structures of oligomers of Aβ, especially their stability and shape have been known for many years.[16,17] The complete understanding of the molecular mechanism of toxicity on Alzheimer's disease and the aggregation process of the Aβ peptide is still a challenging problem. Convertino et al.[18] have performed molecular dynamics simulation to find the influence of two relatively similar tri-cyclic, planar compounds, 9, 10-anthraquinone and Anthracene on the early phase of aggregation of $A\beta_{14-20}$ peptide, and concluded that the hydrophobic residues promote the Aβ self assembly. The simulation shows that Anthraquinone(AQ) interfere more with β-sheet than Anthracene. Scientists have reported that the venom of Black mamba snake is one of the deadliest reptiles in the world, proved to useful to treat the several human disorders and diseases.[19] Recently two molecules have been separated from mamba venom which can reduce the pain of patient as much as potency as morphine. The study of model peptides have shown that turn sequence Trp-Pro-Tyr found in FS2, is one of the major components of black mamba venom. The other toxins subgroups are Calciseptine from Dendroaspis polylepis polylepis, toxin $C_{10}S_2C_2$ from angusticeps and toxin S_4C_8 from Dendroaspis jamesoni kaimosae.[19,20]

Due to the importance of the water model for the stability study of the Asp^{23}-Lys^{28} salt-bridge, the entire $A\beta_{42}$ protofibril structures have been studied.[17,21,22] Lemkul et al.[23] have performed MD simulations, indicated that Morin molecule could disrupt the hydrogen bond

network and hydrophobic packing interactions which stabilize the bend region (Ala21-Leu34). Ladiwala et al.[24] have concluded that the polyphenol myricetin, which is structurally very similar to Morin molecule, could not prevent the aggregation of Aβ peptide. Ono and co-workers[11-14] have published series of papers on natural compounds which are potent inhibitors of Aβ aggregation. Cohen et al.[25] have identified a compound which bind with the misfolded polypeptides and inhibit the formation of oligomeric and fibrillar aggregates. Eventhough many scientific report are available on the above molecules none of these molecules have been successfully reached to clinical stage. We hope, the QM/MM studies will help to understand much better way for the molecular interaction. So we have chosen Morin, Anthraquinone and Black mamba venom molecules and made interaction with Aβ-peptide aggregates. The chemical structure of above molecules is shown in Fig. 1. Since the experimental high-resolution structural characterization of the intermediates in the Aβ aggregation pathway remains a challenging problem, so it is essential to study the different conformations of the molecule over the time period.[26]

The simulation techniques from coarse grain to all-atom with implicit/explicit solvent model have been successfully applied to investigate the structure and dynamics of the selected fragments of Aβ-protein.[22,27-29] A good number of studies have focused to discover the amyloid inhibitors which may prevent or treat amyloidosis disease.[4,8-15,23,27] The experimental investigations have suggested that the structural insight on the nature of Aβ aggregation is elusive and hence the drug designs become difficult.[9] The understanding of binding of small molecules with Aβ would be difficult, when the target peptide undergoes conformational changes. Since the experimental techniques could not provide such informations, the theoretical methods are the best option to the study the molecular interaction. The hybrid ONIOM (QM/MM) techniques is one of the best methods to study the complex molecular interactions.[30] To the best of our knowledge, this is the first time that

hybrid ONIOM technique have been used to characterize the inter and intra salt-bridge mechanism in $A\beta_{23-28}$ monomer and dimers during the interactions with TPT, Morin and Anthraquinone molecules, with a goal of elucidating the mechanism of salt-bridge by which these molecules inhibits the formation of toxic Aβ oligomers.

The previous studies[31] have suggested that Aβ monomer contains three distinct regions, 1. Central hydrophobic (Leu17-Ala21), 2. Loop (Asp23-Lys28), 3. Second hydrophobic (Gly29-Met35). The central hydrophobic and second hydrophobic regions are connected by a loop region, where the salt-bridge is formed between Asp23 and Lys28 residues which contributes to the conformational stability of β-turn(Val24-Glu27), which plays an important role in facilitating the formation of the bend structure in the monomer. The central hydrophobic and second hydrophobic regions are found to exist in β-strand conformation[22], forming two parallel β-sheets whose faces linked by inter sheet hydrophobic interaction between the residues Leu17 and Ile32, Leu17 and Leu34, Val18 and Ile31, and Phe19 and Ala30. The salt-bridge and hydrophobic interaction have contributed to form U-shaped structure (β-strand-bend-β-strand) in the Aβ monomer. The peptides with the U-shaped structure assembled to form salt-bridge between Asp^{23} and Lys^{28} residues.[16] The inter- and intra-molecular salt-bridge plays a significant role in the Aβ-fibril assembly. For instance, the absence of salt-bridge between residues Asp^{23} and Lys^{28} in the loop region destabilise the monomer structure. Further, the hydrophobic interactions between the parallel β-strands get reduced, which may convert the U-shape β-structure into an α-helical (native state) structure, and this process would reduce the rate of aggregation in the Aβ-fibril. The breaking of salt-bridge mechanism is remain an ongoing conflict in the loop region, the experimental evidence is also in favour of the hypothesis that this small region of a protein is responsible for its amyloidogenic behaviour.[32,33] Since owing to the clinical importance, many research groups have been working on the short peptide for the study of Amyloid fibril

formation.[34-36] In the present investigation, we consider the hexamer of the short $A\beta_{23-28}$ peptide as monomer, the two monomers form dimer-i, another two monomer is dimer-ii (Fig. 2). The interaction of the small molecules (TPT, AQ and Morin) affect the inter/intra molecular salt-bridge mechanism in the dimers structure, which is useful to understand their exact effects and mechanisms on the rate of reduction of the Aβ-protein aggregation.

2. Computational Details

The hybrid quantum mechanics/molecular mechanics (QM/MM) method provides a powerful tool to study the molecules in complex environments, such as biomolecules in water. [30,37,38] The principle of QM/MM approach is, treat a small part of the system with the quantum mechanical (QM) method (i.e., a method which describes the electronic structure of molecules) and the rest of the system with molecular mechanical method (i.e., a method which describes the interaction between atoms using simple potential energy function, a "force field") (Fig. 3). Martin Karplus, Michael Levitt and Arieh warshel have laid the foundation for the QM/MM program which has been used to understand and predict the chemical processes. [39]

The QM/MM scheme divides into two approaches known as additive and subtractive. In the additive approach, the total energy of the system is sum of the Quantum Mechanics energy of the QM region (E_{QM}), the Molecular Mechanics energy of the MM region (E_{MM}), and the interaction between QM and MM regions ($E_{QM/MM}$). The additive approach is given in expression (1)

$$E_{tot} = E_{QM} + E_{QM/MM} + E_{MM} \quad (1)$$

The subtractive approach is represented as the Integrated Molecular Orbital Molecular Mechanics (IMOMM), in other words it originally called as ONIOM (Our own N-layered

Integrated molecular Orbital + molecular Mechanics). The subtractive approach is given in equation (2)

$$E_{tot} = E_{real}^{MM} + E_{model}^{QM} - E_{model}^{MM} \quad (2)$$

Where E_{real}^{MM} is the energy of the whole real system which contains both the QM and MM region. E_{model}^{QM} is energy of the QM region calculated at a higher level, and E_{model}^{MM} is the energy of the QM region calculated at the lower Molecular Mechanics level. In the present investigation, the molecules such as Morin, AQ, and TPT molecules interacted with salt bridge region of $A\beta_{23-28}$ peptide, which were treated quantum mechanically and the other parts of peptide treated classically. The QM method allows modelling the electronic rearrangements involve breaking and making the chemical bonds, while the MM treatment allows for the efficient inclusion of the wider environment. Zhao et al.[40] have showed that the global hybrid meta M05-2X functional performs well for many chemical and biological systems.[41-43] The good performance of this functional has been confirmed by studies of organic molecules[44], conjugated addition reaction energies[45], peptides containing amino acids with aromatic ring[46], excited states of stacked nucleo-bases[47], and collagen triple-helix.[48] We considered Asp^{23}- Lys^{28} peptide with TPT, AQ and Morin molecules in the QM level, the interaction energies have been calculated by M05-2X/6-31++G(d,p) level of theory. This hybrid meta exchange-correlation functional derived from the M05 functional, which adds a kinetic energy component to the exchange-correlation function. The interaction energies were calculated using the following equation

$$\Delta E = E(Complex) - E(A\beta_{23-28}) - E(Small\ mol) \quad (3)$$

where, E(Complex) is the energy of the complex structure (Interaction of TPT, AQ and Morin with $A\beta_{23-28}$). $E(A\beta_{23-28})$ *and* $E(small\ mol)$ are energies of the salt-bridge region

of $A\beta_{23\text{-}28}$ peptide and small molecules, where configuration taken from the optimized complex structure (Fig. S1). The many body analysis have been performed for the aforementioned method, even though, many body analyses are not relevant in the case of absence of electron correlation energy, the overall picture of such binding energies of the complexes will give some idea for future investigations. The binding energies were separated by two- and three-body systems as given below

Monomer

$$\Delta^2 E(Mon1 \ldots small\ mol) = E(Mon1 \ldots small\ mol) - E(Mon1) - E(small\ mol) \quad (4)$$

Dimers

$$\Delta^2 E(Mon1 \ldots Mon2) = E(Mon1 \ldots Mon2) - E(Mon1) - E(Mon2) \quad (5)$$

$$\Delta^2 E(Mon3 \ldots Mon4) = E(Mon3 \ldots Mon4) - E(Mon3) - E(Mon4) \quad (6)$$

$$\Delta^3 E(Mon1 \ldots small\ mol \ldots Mon2) = E(Mon1 \ldots small\ mol \ldots Mon2) - E(Mon1) - E(small\ mol) - E(Mon2) \quad (7)$$

$$\Delta^3 E(Mon3 \ldots small\ mol \ldots Mon4) = E(Mon3 \ldots small\ mol \ldots Mon4) - E(Mon3) - E(small\ mol) - E(Mon4) \quad (8)$$

where, small mol= Morin, AQ and TPT molecules, Mon refer to Monomer, $\Delta^3 E$ is the three body energy and $\Delta^2 E$ are the decomposed two-body binding energies between $Mon1$ and $Mon2$, and Mon3 and Mon4 of $A\beta_{23\text{-}28}$ peptide and their interaction with small molecules (TPT, AQ and Morin). Natural bond orbital(NBO)[49] analysis offers useful insights into the donor–acceptor interactions based on the second order perturbation

interactions between filled and vacant orbitals. All electronic structure calculations were performed using Gaussian09 program package.[50]

3. Results and Discussion

3.1. Variation of Salt-bridge (Asp^{23}/Lys^{28}) Distances

The interaction studies of small molecules with Aβ peptide in-vitro[8] and in-vivo[9] may be the useful exercise for the drug discovery. In this work, we made interaction studies on small molecules (Morin, AQ and TPT) with $Aβ_{23-28}$ oligomeric structure as a first step towards the understanding of the structural mechanism by which these compounds inhibit Aβ aggregates. Fig. 4 shows the U-shaped peptide observed in $Aβ_{17-42}$ fibril (Pdb id: 2BEG), studied by solid state NMR and Electron Microscopy.[17] Massman et al.[51] have reported that proposed that this core region of the fibril, comprising residues 17-42, is principally responsible for its stability. The inter molecular salt-bridge formed between the residues Asp^{23} and Lys^{28} in the turn region of amino acids Ser26-Ile31. The U-shaped monomer topology of peptide has been introduced by solvated oligomers of $Aβ_{16-35}$ through modelling and MD simulation.[52] The salt-bridge formation between Asp^{23} and Lys^{28} residues of peptide is driving force for joining the long networks of layered β sheet in the Aβ peptides.[53]

The role of inter- and intra-molecular salt-bridge between Asp^{23} and Lys^{28} residues is important in Aβ peptide. We consider the Asp^{23}, Lys^{28} residues of β-sheet (Fig. 3), for the interaction of small molecules with monomer, dimer-i and dimer-ii structure. The above structures were optimized using ONIOM(M05-2X/6-31++G(d,p):UFF) level of theory. The changes of deformation energy of the monomer and dimers have been monitored during the interaction of small molecules with monomer and dimers (Fig. 5). The average distance of the CO_2^- moiety of Asp^{23} with the NH_3^- moiety of Lys^{28} residues in the intra- and inter-peptide salt-bridge[31] have been calculated and given in the Table2 and Fig. 6. The intra-

peptide salt-bridge distance is found to be 3.03Å in the monomer during the interaction of Morin molecule (Fig. 5b), and it has been increased to 3.09 and 3.10Å during the binding of TPT (Fig. 5d) and AQ (Fig. 5c), respectively. The hydrogen bond interactions of 9,10-anthraquinone with $A\beta_{14-20}$ were augmented by $\pi^{+}\delta^{-}$ interactions between the aromatic rings of 9,10-anthraquinone and carboxyl oxygen of the peptide backbone.[18] The Morin molecule strongly binds with the monomer with interaction energy -460.90kcal/mol, indicated that the Morin can alter the structural arrangement of monomer, capable of interfering with the formation of inter peptide salt-bridge contacts. Anthraquinone molecule weakly binds with the monomer with the energy 15.12kcal/mol, and subsequently TPT molecule binds with the monomer with energy -71.28kcal/mol. These results confirmed that the TPT molecules disrupt more the intra-peptide salt-bridge in monomer when compared with interaction of Morin and Anthraquinone molecules.

The Aβ dimers isolated from neutric and vascular amyloid deposits have been found to be more toxic to neurons in the presence of microglia.[54] Not many studies were available for Aβ dimer structure using experimental and theoretical predictions.[55-62] Multicanonical-multioverlap molecular dynamics[62] have been performed to study the dimerization of $A\beta_{29-42}$ sequence which showed conformational changes from helical to strand structure by step by step method. The formation of the salt-bridge between the residues Asp^{23} and Lys^{28} of different monomers in the dimers is consistent with the experimental (NMR) [16,17] and theoretical results. [52,53,69] These investigations provide the information about the structure and stabilities of the dimers in the Aβ fibrils. Ono et al.[11-14] and Kolandaivel et al.[63,70] have demonstrated the few potential compounds which act as inhibitors for Aβ aggregation. As a first step towards the understanding the structure of $A\beta_{23-28}$ dimers in the oligomers, we have made the optimization for the structures of the dimers with and without interaction of molecules. The first monomer represent as Asp_n^{23} and Lys_n^{28} residues, and second monomer

is Asp_{n-1}^{23} and Lys_{n-1}^{28} residues (Fig. 5e), third and fourth monomers are Asp_{n-2}^{23}, Lys_{n-2}^{28} and Asp_{n-3}^{23}, Lys_{n-3}^{28} (Fig. 5i). We found that the inter-peptide salt-bridge distances are 3.79 and 8.84Å for Asp_n^{23}-Lys_{n-1}^{28} and Asp_{n-1}^{23}-Lys_n^{28} residues respectively. The intra-peptide salt-bridge distances are found as 3.18 and 3.47Å for Asp_n^{23}-Lys_n^{28} and Asp_{n-1}^{23}-Lys_{n-1}^{28} residues, respectively. The present calculation predicted the inter peptide salt-bridge distances as 3.70 and 4.08Å for Asp_{n-2}^{23}-Lys_{n-3}^{28} and Asp_{n-3}^{23}-Lys_{n-2}^{28} respectively, intra salt-bridge distances as 2.89 and 3.37Å for Asp_{n-2}^{23}-Lys_{n-2}^{28} and Asp_{n-3}^{23}-Lys_{n-3}^{28} respectively. It was found that the aggregates have more stable salt-bridge in intra peptide than the inter peptide, which is due to arises of the difference in the salt-bridge distance in the initial aggregates structure. Molecular dynamics simulation predict that the intra peptide salt-bridge population is 42%, higher than inter peptide salt-bridge (24%).[71] These results confirmed that the region Asp^{23}-Lys^{28} becomes a bend structure, which was not folded in the $A\beta_{23-28}$ oligomer.

Morin molecule is a therapeutic candidate, which is found in natural food products, capable of crossing the blood brain barriers, and nontoxic.[72-74] Interaction between natural compounds and α-synuclein with amyloid polypeptide have been studied as a therapeutic compound for Alzheimer disease.[75,76] In general, Morin molecule strongly interacts with Aβ dimers and increased the inter peptide salt-bridge distance as 3.86 and 8.10Å for Asp_{n-1}^{23}-Lys_n^{28} , Asp_n^{23}-Lys_{n-1}^{28} respectively, as well as intra salt-bridge distance as 3.53 and 3.15Å for Asp_{n-1}^{23}-Lys_{n-1}^{28} and Asp_n^{23}-Lys_n^{28}, respectively(Fig. 5f). The molecular dynamics simulation has confirmed that the salt-bridge is formed for the distance less than 4.5Å in the Aβ peptide.[31] Lemkul et al.[15] have reported that if the Morin molecule bound with the bend region, which destabilize the salt-bridge. Consequently, the inter- and intra-peptide salt-bridge distances are increased to 3.79(Asp_{n-3}^{23}-Lys_{n-2}^{28}), 3.9Å (Asp_{n-2}^{23}-Lys_{n-3}^{28}) and 2.89 (Asp_{n-2}^{23}-Lys_{n-2}^{28}), 3.24Å (Asp_{n-3}^{23}-Lys_{n-3}^{28}) respectively(Fig. 5j). The C=O group of the pyridine of Morin molecule makes the hydrogen bond with NH_3 moiety in monomer1, O-H

group of the same molecule interact with CO_2 moiety of monomer2. The O-H group in the benzene ring of Morin molecule interacted with backbone of NH_2 moiety of dimer structure. These interactions distort the salt-bridge in Aβ peptides, when the Morin molecule bound with the dimeric structure, subsequently the β-content was significantly reduced. This result agrees with Lemkul et al,[15] who studied the stability of inter peptide Asp23-Lys28 salt-bridge, with average distance of 3.5±0.4 Å between peptides. If the inter- and intra-peptide salt-bridge distance increases, the peptide gets destabilize which leads to de-aggregation. These results agree with the experimental studies[77], which is shown that dense collection of amyloid fiber is disrupted and produce thinner and shorter fibrils when Morin molecule added into an amyloid polypeptide solution.

The 9,10-anthraquinone(AQ) exists in medicine plant, rhubarb (Rheum rhaponticum) , a plant producing natural anthraquinones. The influence of planar-tricyclic compound on the early phase of ordered aggregation segment of the Aβ peptide promotes oligomerization. Hybrid QM/MM method is used to investigate the aggregation of $A\beta_{23-28}$ in the presence and absence of AQ. According to experimental results[55-57], as well as atomic simulation[58-62], the $A\beta_{23-28}$ segment is chosen because it has a high β-aggregation propensity. Molecular simulation results showed that 9,10-anthraquinones molecule destabilize the inter molecular cross beta strand hydrogen bonds of oligomers through polar interactions with the protein backbones,(i.e) the 9,10-anthraquinone molecule bind with peptide backbone carbonyl oxygen and amide hydrogen via $\pi^{+}\delta^{-}$ interaction and hydrogen bonds, which allow the intercalation of the Anthraquinone molecule into the oligomers.[18] The salt-bridge distances are found to be 3.81 Å(Asp_{n-1}^{23}-Lys_n^{28}), 8.10 Å (Asp_{n-1}^{23}-Lys_n^{28}) and 3.10 Å(Asp_n^{23}-Lys_n^{28}) , 3.49 Å (Asp_{n-1}^{23}-Lys_{n-1}^{28}) respectively for dimer-i during the interaction of 9,10–anthraquinone (Fig. 5g). The inter and intra salt-bridge distances are found to be 3.69 Å (Asp_{n-3}^{23}-Lys_{n-2}^{28}), 3.98 Å (Asp_{n-2}^{23}-Lys_{n-3}^{28}) and 2.95 Å (Asp_{n-2}^{23}-Lys_{n-2}^{28}), 3.29 Å (Asp_{n-3}^{23}-

Lys_{n-3}^{28}), respectively for dimer-ii due to the interaction of 9,10–anthraquinone (Fig. 5k). This molecule interferes with the formation of beta sheet in Aβ peptide, which reduces nearly 33% aggregates formation of Aβ peptides[78]. From the above results, 9,10-anthraquinone molecule is acting as the effective inhibitor for Aβ aggregation because it block the formation of β-sheet structure in $Aβ_{23-28}$ and $Aβ_{14-20}$ segments.[78]

A three dimensional solution NMR structure of toxin FS2, a 60 residue polypeptide was taken from the venom of block mamba snake (Dendroaspis polylepis polylepis).[20] FS2 has a type VIa Cis Proline turn (Trp-Pro-Tyr), which induced toxicity in the venom. We made interaction studies of short peptide (Trp-Pro-Tyr) with $Aβ_{23-28}$ oligomers structure. Due to the interaction, the inter- and intra-salt-bridge distances are found to be 3.77 Å(Asp_{n-3}^{23}-Lys_{n-2}^{28}), 8.12 Å (Asp_{n-2}^{23}-Lys_{n-3}^{28}), and 3.15 Å (Asp_{n-3}^{23}-Lys_{n-3}^{28}), 3.39 Å (Asp_{n-2}^{23}-Lys_{n-2}^{28}) respectively for dimer-ii structure(Fig. 5l), and the same distances are found to be 3.84 Å (Asp_{n-1}^{23}-Lys_{n}^{28}), 7.69 Å (Asp_{n}^{23}-Lys_{n-1}^{28}) and 3.19 Å (Asp_{n}^{23}-Lys_{n}^{28}) , 3.53 Å(Asp_{n-1}^{23}-Lys_{n-1}^{28}) respectively for dimer-i structure(Fig. 5h). These results confirmed that the TPT molecule has attributed a stronger influence to destabilize the inter- and intra-salt-bridge interaction in the dimers compared with the interaction of Morin and AQ molecules. Experimental[16,17] and theoretical[21] studies suggested that salt-bridge has involved in oligomer stability and process of fibril formation of the amyloid β-peptide. Further, it stabilizes the loop region (Asp23-Lys28) and might be responsible for the β-hairpin like conformation of Aβ peptide. We found that the hydrogen bonds formed between the side chains of Asp23 and Lys28 and TPT molecule, the intra peptide salt-bridge Asp23-Lys28 is disrupted, but the inter peptide salt-bridge was completely disappeared and their corresponding distance is 8.29 Å. This result agrees with Triguero et al.[78] work, the oxidation of Met35 caused the loss of the Asp23-Lys28 salt-bridge observed in the Aβ monomer. An interesting observation was made that TPT molecule inhibited Aβ fibril formation more effectively than Morin and 9,10-

anthraquinone interactions. This result might be due to the fact that salt-bridge was completely disappeared in Asp23-Lys28 region, which are present within the β-sheet region of Aβ fibrils. These observations suggest the TPT molecule plays a more significant role to reduce the rate of fibril assembly by promoting inhibiting the polymerization.

3.2. Deformation Energy Analysis

The electrostatic interaction between Asp[23] and Lys[28] residues contribute to the conformational stability of β-turn DVGSNK (23-28), an important structural motif observed in the NMR structure.[53] These peptides are joining together to form dimer, trimer, oligomer and fibrils. The monomers are initially separated in the simulation cell, then it aggregates to form stable dimer through salt-bridge and hydrophobic contacts.[27] The equilibrium distance between Cα atom of Val24 and Asn27 residues provides the stability of the Aβ peptides. In our previous work[31] also, it has been confirmed that the conformations of $A\beta_{42}$ has interaction between the central hydrophobic core LVFFA(17-21) and hydrophobic c-terminus(Met35-Ala42) because of the distance around 4-6.5Å between Cα(Val24)-Cα(Asn27) residues. This result agrees with Luhrs et al,[17] who studied the role of Ile41 and Ala42 residues by increasing the hydrophobic contacts in $A\beta_{42}$ dimers. The electrostatic interaction between Asp23 and Lys28 residues play an important role to produce the deformation (destabilization of the denature) in dimers structure.

The deformation energies of the $A\beta_{23\text{-}28}$ peptide are calculated using the equation

$$\delta_D = E(A\beta_{23-28})^{Complex} - E(A\beta_{23-28})^{isolated} \quad (9)$$

where $E(A\beta_{23-28})^{Complex}$ is the energy of monomer calculated by ONIOM(M05-2X/6-31++G(d,p): UFF) method. The $E(A\beta_{23-28})^{isolated}$ is the optimized monomer structure at the same level of theory. The deformation energy (δ_D) is tabulated in table 1. The

deformation energies of monomer are 45.180, 18.323, and 0.567kcal/mol during the interaction of TPT, Morin and AQ molecules, respectively. The deformation energies of dimer-i are 427.39, 846.18, and 1.44kcal/mol during the interaction of Morin, TPT and AQ molecules, and subsequently the dimer-ii have 31.31, 430.65 and 3.95kcal/mol during the interaction of the same molecule. The higher structural deformation seen in the dimers in the presence of TPT molecule, which could divert oligomeric species of $A\beta_{23-28}$ peptide from higher order aggregated structure. It suggests that the higher deformation found in the dimer structure due to the disruption of the salt-bridge contact between Asp^{23} and Lys^{28} residues. The experimental studies on globular proteins at non zero concentrations have also confirmed the unfolded state of Aβ.[80,81] This deformation confirmed that the denatured state of $A\beta_{23-28}$ monomer and dimers have come to the natural state of the peptide during the interaction of TPT molecule.

3.3. Hydrogen bond Analysis

The stabilization energies based on the interaction between the occupied and the unoccupied orbital shows that there exists an electron delocalized pathway from donor to acceptor atoms between the three molecules and $A\beta_{23-28}$ peptide (monomer and dimers). These interactions are computed by the NBO (Natural Bond Orbital) analysis using the second order perturbation approach. The optimized structure of $A\beta_{23-28}$ peptide and their complexes are shown in Fig. S1. The N1...C2-C3 interaction of dimer-i has stabilization energy 10.17kcal/mol, which is reduced to 1.92kcal/mol, where N1 atom of dimer delocalised to the O156-H155 bond of the Morin molecule. Oxygen and Nitrogen group of (O190, N201) TPT molecule makes hydrogen bond interaction with σ bond of amine and carboxylic group (N135-H146, C80-O82) of dimer, corresponding stabilization energies are 7.94 and 5.58 kcal/mol. Morin molecule (C171=O172) makes interaction with dimer (O158-H157) molecule, their stabilization energy is 3.15kcal/mol. The carboxylic group (C6=O7) of dimer

molecule interacts with σ(O158-H157) bond of Morin with 8.17kcal/mol. The AQ molecule has lesser interaction with the dimer structure, lone pair of the oxygen (O168) of AQ interaction with C2-H10 and N135-H145 bond and their corresponding stabilization energies are 1.02 and 0.39kcal/mol, respectively.

NBO analysis has been performed to investigate the interaction mechanism between peptide and molecules (Morin, AQ and TPT) and results are tabulated in table 3. The interaction distances between Asp_n^{23}(C3=O4) and Lys_n^{28}(N61-H74) residues were increased from 2.25 to 3.18Å, for Asp_{n-1}^{23}(C80=O81) and Lys_{n-1}^{28}(N135-H147) have increased from 2.33 to 2.36 Å during the interaction of TPT molecule. On the other hand, the oxygen (O7) of Asp_n^{23} residue has formed contact with amine (N135-H148) group of Lys_{n-1}^{28} residue, and its stabilization energy is 5.44 kcal/mol, which is reduced to 2.1ab3 kcal/mol due to the interaction of TPT molecule. Subsequently, oxygen (O4) atom of Asp_n^{23} residue has less interaction with amine group (N61-H74) of Lys_n^{28} residue, the energy is 1.96 kcal/mol. This interaction is completely destroyed between Asp^{23} and Lys^{28} due to the interaction of TPT molecules on $A\beta_{23-28}$ dimer. In particular TPT molecules make many interaction with dimer structure, especially lone pair electrons of the atom (O190) makes stronger binding with σ bond of N135-H146(Lys_{n-1}^{28}) and its stabilization energy is 7.94kcal/mol. Consequently, the donor electrons of atom (O190) of TPT molecule have interaction with amine group (N135-H146) of Lys_n^{28} residue with 7.94 kcal/mol. Further, the lone pair electrons of atom(O82) of carboxylic group in Lys_n^{28} residue interacted with TPT molecule (N201-H202) with 5.58 kcal/mol. The oxygen atoms of O81, O82 in Asp_{n-1}^{23} residue bind with σ bond of N201-H202, C199-H200 in TPT molecules and its corresponding stabilization energies are 5.58 and 0.75kcal/mol respectively. These interactions lead to increase the distances and decrease the interaction energies between Asp^{23} and Lys^{28} residues. In addition, Morin molecule interaction has increased the intra molecular interaction (Asp_n^{23}(C6-O7)…Lys_{n-1}^{28}(N135-

H148)) distance from 2.03 to 2.24Å. Lone pair electrons of atoms (O7 and N1) of theAsp_n^{23} residue form the interaction with anti-bond of O157-H158 and O156-H155 bonds in Morin, and their corresponding stabilization energies are 6.54 and 25.09kcal/mol respectively. The lone pair electrons of nitrogen(N1) atom in Asp_n^{23} residue is actively involved for the formation of hydrogen bond with hydrogen(H155) atom of OH group of Morin molecule, the corresponding stabilization energy is 25.09 kcal/mol. During the interaction of TPT molecule with dimer-ii, inter molecular interaction (Lys_n^{28}(N26-H28)…Asp_{n-1}^{23}(C80=O81) distance increased from 1.913 to 7.32Å, and Asp_n^{23}(C5=O6)…Lys_{n-1}^{28}(N119-H120) distance increased from 1.867 to 3.2Å. This shows that the Morin molecule disrupts the intra molecular interaction and TPT molecule has contributed to increase the inter-molecular interaction distance of $A\beta_{23-28}$ peptide oligomers. From these results, it has been noticed that the Morin molecule interacted with dimer and the inter- and intra-molecular interaction get reduced. The other significant result is that the TPT molecule facilitates hydrogen bond formation with Asp^{23} and Lys^{28} residues, play a key role in preventing the formation of electrostatic interaction between Asp^{23} and Lys^{28} in the dimer.

The TPT molecule strongly binds with dimer of $A\beta_{23-28}$ peptide with -82.14kcal/mol. The TPT molecule is able to act not only donor, but also acceptor of inter peptide hydrogen bonds, which has three main consequences 1. The disruption of the inter and intra peptide hydrogen bond, 2. Destabilization of the bend structure and 3. Reduction in β-sheet formation due to the absence of inter-peptide salt-bridge. The 9, 10-anthraquinone molecule could not able to interact with $A\beta_{23-28}$ peptide through hydrogen bond interaction, which apparently not sufficient to perturb the ordered β-architecture. Number of hydrogen bonds between TPT and dimer are shown in Fig. S1, Yamada and co-workers[11] has used thioflavin T assay for electron microscopy study to examine the effect of wine related polyphenols on the formation and destabilization of Aβ peptides. Electrostatic interaction between TPT and dimer is

sufficient to drive the perturbation of $A\beta_{23-28}$ ordered oligomers. This result agrees with Gazit and co-worker[81], who demonstrated that the compound NH_2-D-Trp-Aib-OH is able to inhibit the formation of toxic oligomers in vitro studies using mouse model.

3.4. Stacking Distance

Berhanu et al.[21] have demonstrated that the aggregates form more stable inter chain salt-bridge than intra-chain salt-bridge. The stacking distance between the monomers is around 5.43Å, which has been increased to 6.71Å during the interaction of TPT molecule. When Morin and Anthraquinone molecules have interacted with dimers of $A\beta_{23-28}$ peptides and their stacking distances are 5.257 and 5.583Å respectively. The TPT molecule induce significant changes in stacking distances between $A\beta_{23-28}$ monomers and lead to reduce the inter salt-bridge interaction. This interpreted that the TPT molecule has contributed to destabilisation of aggregation of Aβ peptides.

4. Conclusion

The Inter- and Intra-peptide salt-bridges between the Asp^{23} and Lys^{28} residues are important in the Aβ amyloid fibrils. The electrostatic interaction between Asp^{23} and Lys^{28} residues contributes to the conformational stability of the beta turn (Asp^{23}-Lys^{28}), which is an important structure motif defined by NMR structure.[64] Asp^{23}-Lys^{28} region is playing a major role in facilitating the formation of the bend in the $A\beta_{1-40}$ and $A\beta_{1-42}$ model peptides. The bend is promoting the parallel architecture of long Aβ peptides, which is facilitating the fibrils structures. We have made the following conclusion from the above studies.

1. Salt-bridge helps to stabilize the loop region (Asp^{23}-Lys^{28}), which might be responsible for the β-hairpin like conformation of Amyloid-β peptide. During the interaction of TPT molecule, the loss of the of the Asp^{23}-Lys^{28} salt-bridge observed in

the $A\beta_{23-28}$ oligomer, destabilize the bend structure in the loop region, which facilitate to reduce the hydrophobic contact between parallel β-sheet conformation (Leu17-Ala21, Gly29-Met35). These structural changes could be responsible for reduces the rate of aggregation of the Aβ peptide.

2. We found that binding of TPT molecule with dimers of $A\beta_{23-28}$ peptide produced more structural deformation and it would stimulates the disorder of oligomeric species.
3. The peptide adopts to parallel β-sheet. These peptides are stacking together to form oligomers via hydrophobic contact, salt-bridge interaction and hydrogen bond. We found that the stacking distance between $A\beta_{23-28}$ peptide are increased during the interaction of TPT moiety as compared with interaction Morin and 9,10-Anthraquinone molecules.
4. More number of hydrogen bonds are found between TPT and dimer, not that much found for Morin and 9,10-Anthraquinone molecules. Electrostatic interaction between TPT and dimer are sufficient to drive the perturbation of $A\beta_{23-28}$ ordered oligomers. The strength of the interaction of small molecule with $A\beta_{23-28}$ dimers, is in the following order TPT>Morin>AQ molecule.

The TPT molecule plays a vital role in blocking protein aggregation and preventing amyloid formation and thus can provide essential information towards development of therapeutic molecules against amyloidosis. These findings have provided new insight in the molecular interactions between Aβ peptide and TPT molecule, expected to facilitate the research on the inhibition of Aβ fibrillization.

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Supporting information

The optimized structure of the monomer, dimer-i and dimer-ii with Morin, AQ and TPT molecules are shown in Fig. S1. Prepared input file for dimer structure with and without interaction of AQ, TPT and Morin molecules are given in supporting information.

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Figure Caption

Fig. 1 Chemical structure of a) Morin b) AQ and c) TPT molecules.

Fig. 2 Monomer1 and Monomer2 are joined together to form Dimer-i and Monomer3 and Monomer4 are formed Dimer-ii.

Fig. 3 Principle of QM/MM. A typical QM/MM approach divides the studied system into a QM core and an MM surrounding, the MM treated part of the Val24-Asn27 is shown in ribbon representation, the QM core is highlighted in ball and sticks, where Asp^{23}, Lys^{28} with Morin, TPT and AQ molecules.

Fig. 4 Long networks of layered β sheet of Aβ oligomer, which was taken from protein data bank (pdb id: 2BEG). Asp^{23} and Lys^{28} residue indicated that the yellow color residue, and loop region are rounded with blue line.

Fig. 5 Morin, AQ and TPT molecules interacted with (b-d) monomer, (f-h) dimer-i and (j-l) dimer-ii. The stick of the atom treated in QM region and wire of the atom considered in MM region.

Fig. 6 Inter and intra salt-bridge distance between Asp^{23} and Lys^{28} residues were changed during the interaction of small molecules (Morin, TPT and AQ). Inter salt-bridge represents in rose and orange color and intra salt-bridge is green and purple color lines in the figure. Stacking distances (green arrow mark) are given in the dimer during the interaction of small molecule.

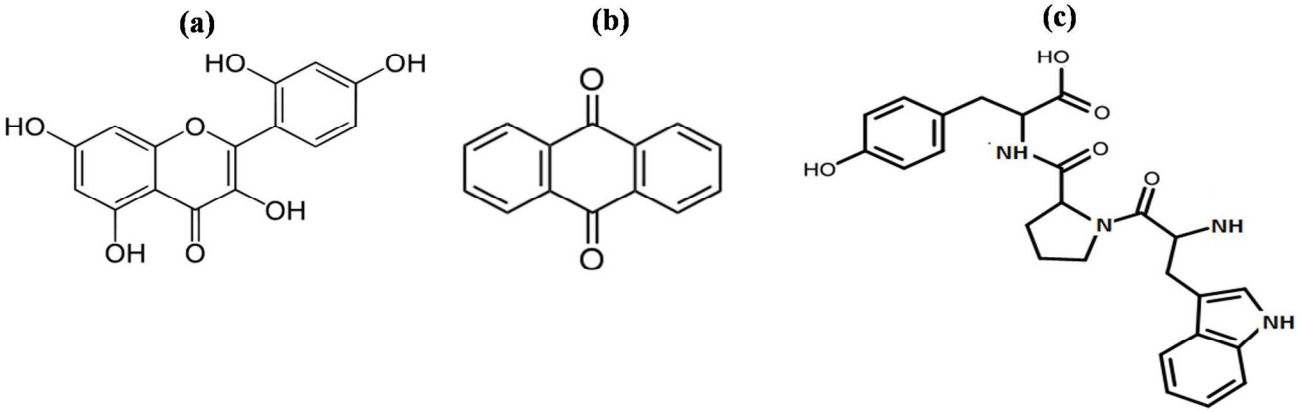

Fig. 1

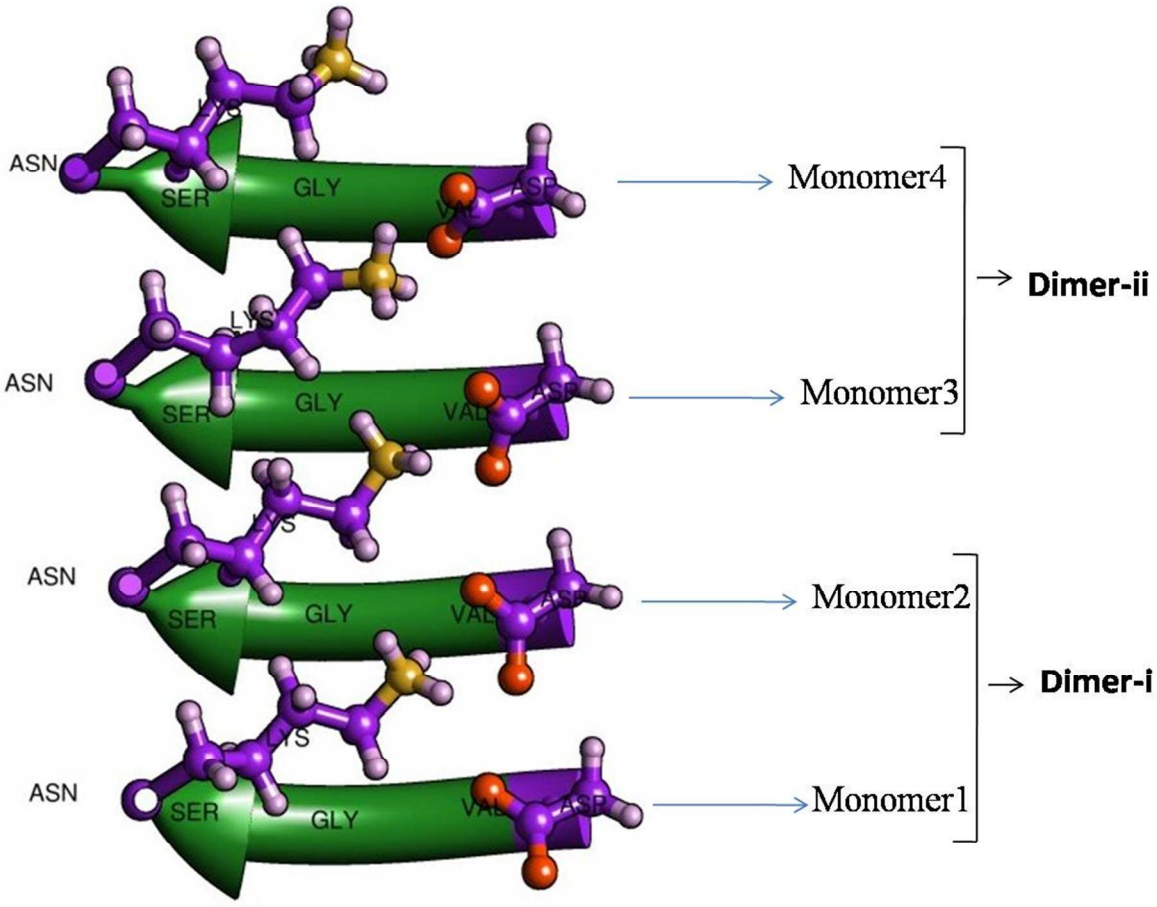

Fig. 2

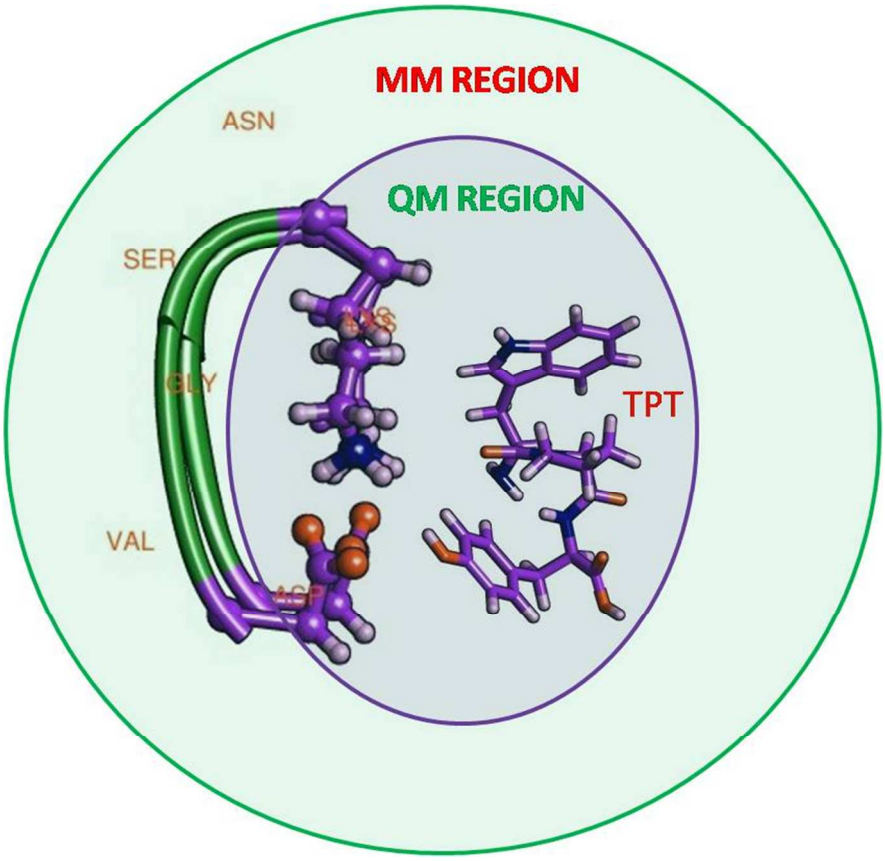

Fig. 3

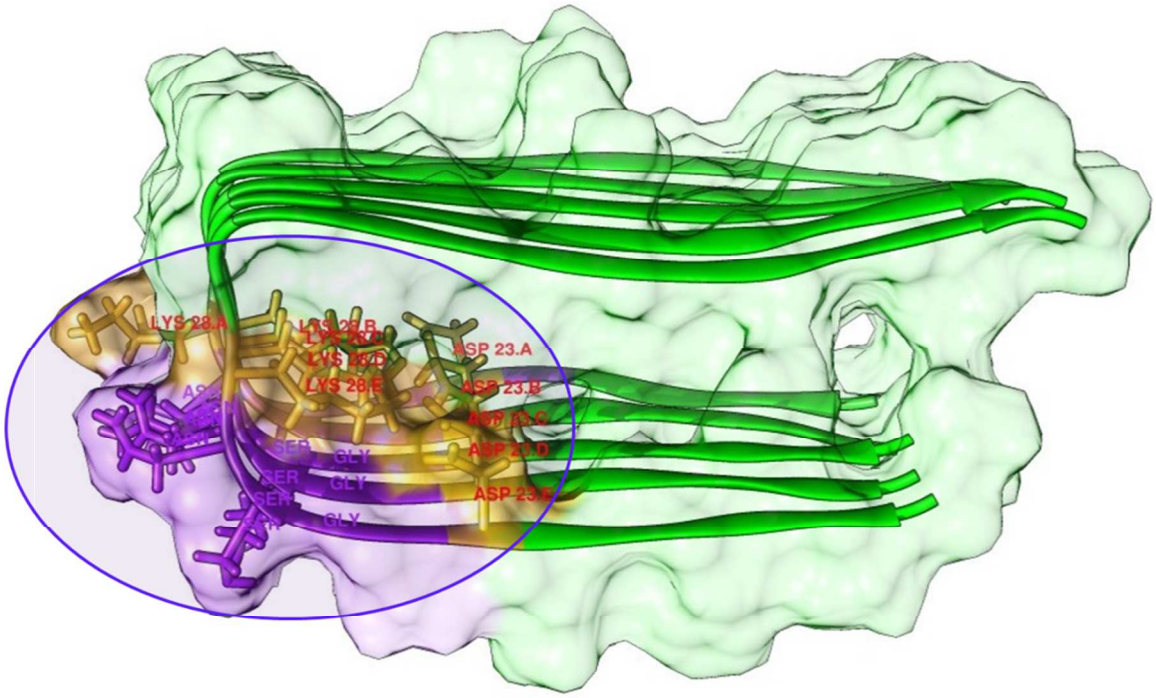

Fig. 4

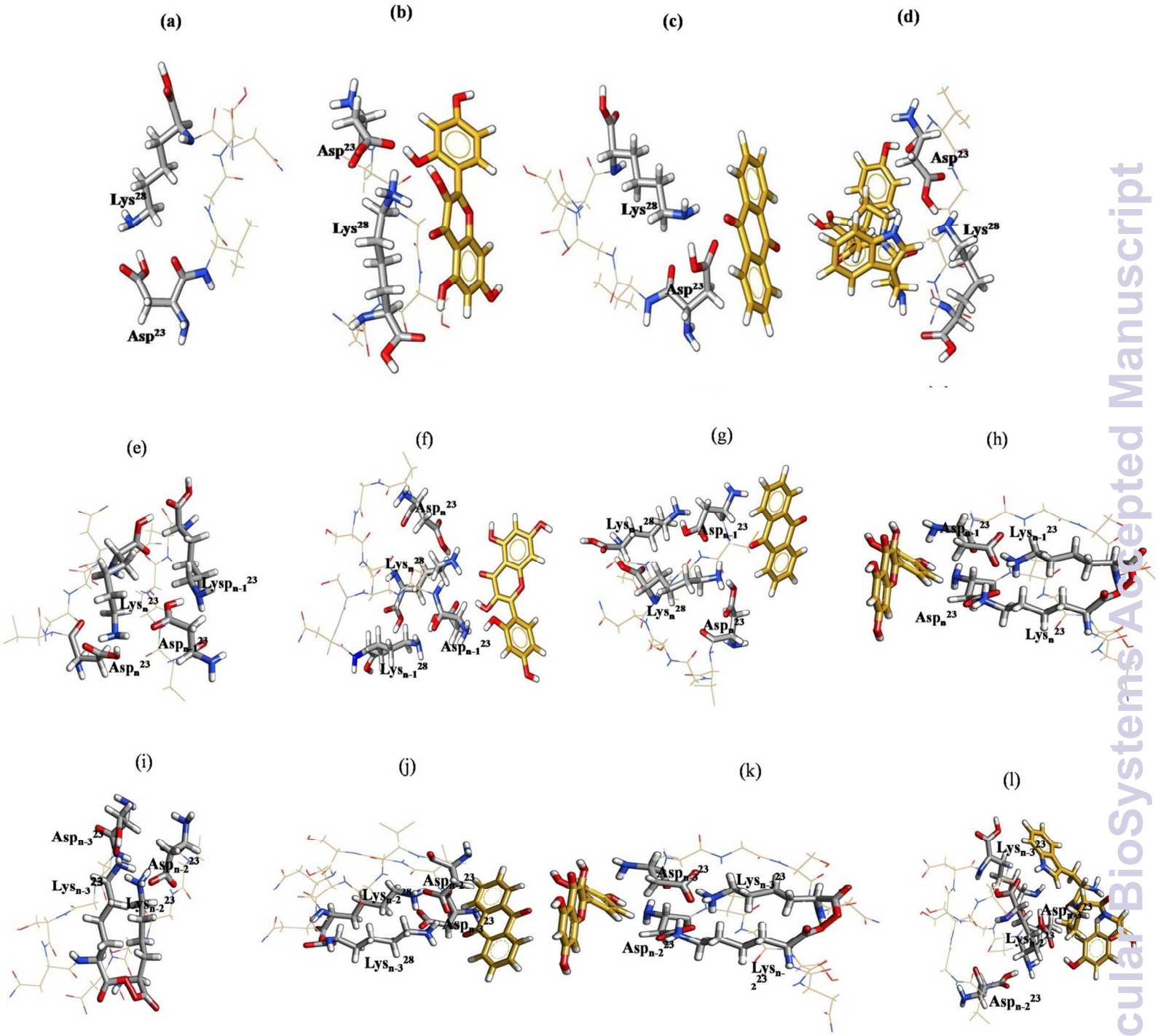

Fig. 5

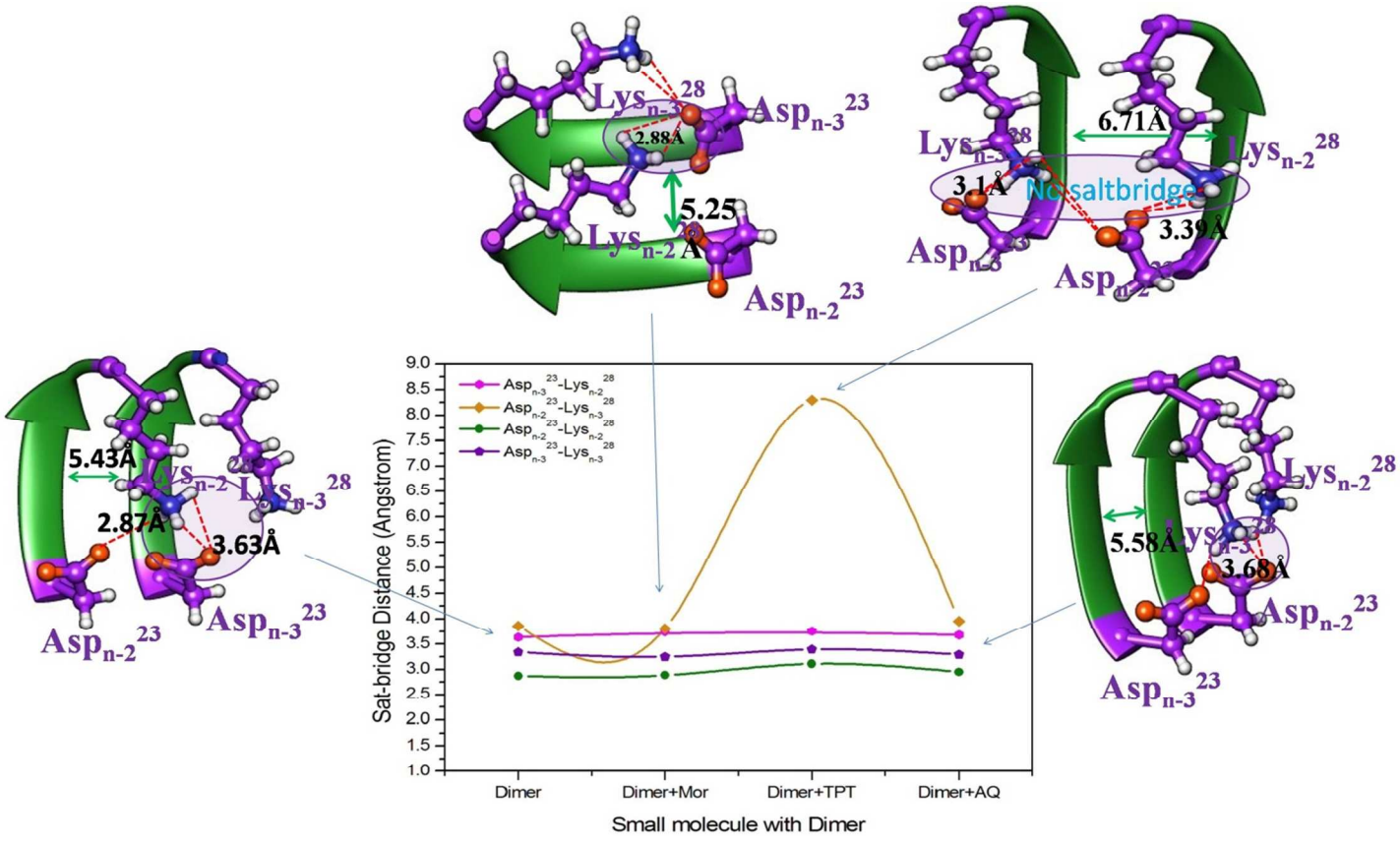

Fig. 6

Table 1: Interaction Energy (ΔE) and Deformation energy(δD) are Calculated for monomer and dimer forms of $A\beta_{23\text{-}28}$ peptide with and without interaction of Morin, TPT, AQ molecules, and the complex structures were optimized at hybrid ONIOM(M05-2X/6-31++G(d, p):UFF) level of theory, where energy(ΔE and δD) was represented in terms of kcal/mol.

	Dimer	Dimer	Dimer...Morin	Dimer...TPT	Dimer...AQ
ΔE	i	-25.41	368.09	-82.14	385.03
	ii	-546.94	-796.17	-253.00	-148.21
δD	i	-	427.39	846.18	1.44
	ii	-	31.31	430.65	3.95
		Mon	Mon...Morin	Mon...TPT	Mon...AQ
ΔE		-	-460.90	-71.28	15.12
δD		-	31.31	45.18	0.56

Table 2: Salt-bridge distances (in Å) are calculated between Asp^{23} and Lys^{28} residues of $A\beta_{23\text{-}28}$ peptide in monomer and dimmers.

Dimer	Salt-bridge	Dimer	Dimer...Morin	Dimer...TPT	Dimer...AQ
i	$Asp_{n-1}^{23} - Lys_{n}^{28}$	3.797	3.860	3.812	3.847
	$Asp_{n}^{23} - Lys_{n-1}^{28}$	8.480	8.104	8.105	7.698
	$Asp_{n}^{23} - Lys_{n}^{28}$	3.183	3.156	3.106	3.197
	$Asp_{n-1}^{23} - Lys_{n-1}^{28}$	3.478	3.532	3.495	3.539
ii	$Asp_{n-3}^{23} - Lys_{n-2}^{28}$	3.707	3.797	3.691	3.779
	$Asp_{n-2}^{23} - Lys_{n-3}^{28}$	4.080	3.901	3.985	8.126
	$Asp_{n-2}^{23} - Lys_{n-2}^{28}$	2.896	2.891	2.957	3.155
	$Asp_{n-3}^{23} - Lys_{n-3}^{28}$	3.370	3.247	3.368	3.396
Monomer		Monomer	Monomer...Morin	Monomer...TPT	Monomer...AQ
	$Asp^{23} - Lys^{28}$	3.114	3.033	3.095	3.106

Table 3: The stabilization energies ($E^{(2)}$) are calculated between $A\beta_{23-28}$ dimer and AQ, TPT, Morin molecules at M05-2X/6-31++G(d, p) level of theory.

Donor	Acceptor	Structure	E(j)-E(i) a.u	F(i, j) a.u	$E^{(2)}$ kcal/mol
Lp (1) O7 (Asp_n^{23})	BD*(1)N135- H148 (Lys_{n-1}^{28})	$A\beta_{23-28}$	1.37	0.77	5.44
		$A\beta_{23-28}$+Morin	1.39	0.05	2.56
		$A\beta_{23-28}$+AQ	1.37	0.06	3.92
		$A\beta_{23-28}$+TPT	0.89	0.04	2.13
Lp (1) O4 (Asp_n^{23})	BD*(1) N61-H74 (Lys_n^{28})	$A\beta_{23-28}$	1.33	0.04	1.96
		$A\beta_{23-28}$+Morin	1.32	0.03	1.15
		$A\beta_{23-28}$+AQ	1.33	0.05	2.41
		$A\beta_{23-28}$+TPT	-	-	-
Lp (1) N1 (Asp_n^{23})	BD*(1) O156-H155 (Morin)	$A\beta_{23-28}$+Morin	0.92	0.13	25.09
Lp (1) O190 (TPT)	BD*(1) N135-H146 (Lys_{n-1}^{28})	$A\beta_{23-28}$+TPT	1.29	0.09	7.94
Lp (1) O7 (Asp_n^{23})	BD*(1) O158-H157 (Morin)	$A\beta_{23-28}$+Morin	1.30	0.08	6.54
Lp (1) O82 (Asp_{n-1}^{23})	BD*(1) N201-H202 (TPT)	$A\beta_{23-28}$+TPT	1.25	0.07	5.58
Lp (1) O172 (Morin)	BD*(1) N135-H146 (Lys_{n-1}^{28})	$A\beta_{23-28}$+Morin	1.37	0.05	3.15
Lp (1) O81 (Asp_{n-1}^{23})	BD*(1) C199-H200 (TPT)	$A\beta_{23-28}$+TPT	1.36	0.02	0.75