Heme prevents amyloid beta peptide aggregation through hydrophobic interaction based on molecular dynamics simulation†

Li Na Zhao, a Yuguang Mu b and Lock Yue Chew* a

Heme, which is abundant in hemoglobin and many other hemoproteins, is known to play an important role in electron transfer, oxygen transport, regulation of gene expression, and many other biological functions. With the belief that the aggregation of Aβ peptides forming higher order oligomers is one of the central pathological pathways in Alzheimer’s disease, the formation of the Aβ-heme complex is essential as it inhibits Aβ aggregation and protects the neurons from degradation. In our studies, conventional molecular dynamics simulations were performed on the 1 Aβ + 1 heme and 2 Aβ + 4 hemes system, respectively, with the identification of several dominant binding motifs. We found that hydrophobic residues of the Aβ peptide have a high affinity to interact with heme instead of the histidine residue. We conclude that hydrophobic interaction plays a dominant role in the Aβ–heme complex formation which indirectly serves to physically prevent Aβ aggregation.

1 Introduction

Alzheimer’s disease (AD) is an irreversible neuronal degenerative disease, which is characterized by extracellular senile plaques and intracellular neuronal fibrillary tangles (NFT). The senile plaques consist of Aβ peptides that are cleaved from the amyloid precursor protein (APP). Aβ may form dimer, trimer and higher order oligomers which are believed to be toxic and are the cause of neuronal apoptosis. 5–12

It is observed that excessive amounts of iron and other metals like copper and zinc tend to concentrate inside or around the senile plaques and the NFT. The presence of ionic zinc, iron and copper is known to facilitate the process of Aβ aggregation. 13–16 Meanwhile, the brain is a very aerobically active organ consuming one fifth of the body’s oxygen. 17 The activation of molecular oxygen together with the reduction of redox active iron, zinc and copper ion may generate detrimental reactive oxygen species (ROS). 18 Hence, the elevated iron deposition found during the earliest stages of AD may generate the most potent ROS hydroxyl radicals and together with the iron–Aβ complex may cause significant oxidative stress. 19 Studies have also shown that Aβ bound iron mediates Aβ toxicity, which can be alleviated by an iron chelator. 17,20–22

Heme is a macromolecule consisting of an iron atom in the center of four substituted pyrrole rings interconnected through methine bridges. It is recognized as a member of the prosthetic group which assists the cytochrome family in electron transfer and oxygen transport in globins. 23,24 Its main biological functions are carried out through redox reactions of the ferrous (Fe2+) state of the heme iron. Heme also plays a significant regulatory role as an intracellular signal transduction messenger in gene expression 25 and ion channels function via the coordination sphere of the iron to a histidine or cystine. 24

It has been proposed that heme tends to bind to one or more intracellular Aβ histidine residues. This decreases the bioavailability of heme, and leads to a deficiency of the functional heme 26,27 which results in oxidative stress, 28 electron transport chain defects 29 and mitochondrial complex IV activity decline. 30 On the other hand, the heme–Aβ 40 complex may cause significant oxidative stress. 29 The Aβ–heme complex can also catalyze the oxidation of serotonin 27 and promote protein nitrotyrosination. 31 It shows a stronger peroxidase activity than heme. 27,31 It has been suggested that both the Aβ–heme peroxidase activity and the binding affinity of heme towards Aβ do not depend on the Aβ aggregation stages. 31

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3cp52354c
There are several identified and putative heme-binding motifs. One is the CxxCH motif in cytochrome c, in which the two cysteine residues form two covalent bonds with the two vinyl groups (−CH=CH₂), while histidine serves as the fifth/proximal axial ligand. Another is HxXXF from the heme chaperone protein CcmE. The flexible C-terminus of CcmE facilitates the formation of a transient covalent complex between histidine and heme.

It has been proposed that the hydrophilic N-terminal of Aβ is the binding domain of heme, which involves Arg, Tyr and the three histidine residues (His-6, His-13 and His-14). In fact, these residues are found to be in the heme-binding pockets. In particular, His-13 and His-14 are believed to be more significant in heme binding and peroxidase activity than His-6. However, the detailed Aβ–heme complex conformation has yet to be determined.

In this article, we investigate the possible Aβ–heme binding motifs through conventional molecular dynamics (MD) simulation for the 1 Aβ + 1 heme system and the 2 Aβ + 4 hemes system respectively. In addition, the detailed atomic-level interaction between Aβ and hemes, and the role of heme in the Aβ oligomerization process are examined for the 2 Aβ + 4 hemes system. We found that the presence of heme physically serves the possible function of an inhibitor to Aβ self-assembly.

2 Simulation setup and methods

The initial structure of Aβ42 was taken from model 1 of the Protein Data Bank (PDB) ID: 1YIT. The sequence of Aβ1–42 is DAEFRHDASY10 EVHHQKLFVF20 AEDVGSNKG30 HGLMVGGVY38. The pKₐ values of the titratable residues were calculated using the H++ server (see ESI,† Table S1). The default internal and external dielectric constants used in the pKₐ value calculation are 6 and 80 respectively. The ionic strength was set to 0.1 M while the pH was set to 7.

2.1 Potential of mean forces

The potential of mean force is to be determined by first constructing a one-dimensional grid with bins that give the minimum distance between the high propensity residues and the Fe ion of heme. The number of sampled conformations that fall into each bin Nᵢ is then computed. The potential of mean force Vpmf is obtained from:

\[ V_{pmf} = -k_B T \log(N_i/N_{max}). \]  

Here k_B is the Boltzmann constant, T is the absolute temperature, and Nmax is the maximum number of the sampled conformations counted in each bin, i.e. the largest N_i.

3 Results

3.1 Aβ–heme complex

In order to uncover the heme-binding Aβ motifs, cluster analysis was performed on each of the last 100 ns of the 20 trajectories.
A Ca rmsd cutoff of 3 Å was used to count the number of neighbors. Then, within this cutoff, Aβ–heme structures with similar configurations are identified as one cluster and their frequency of occurrence counted. More precisely, a total of 10,001 conformations were generated from each trajectory, from which 2814 clusters were identified from the 20 trajectories. The central portion of the 9 most dominant clusters are shown in Fig. 1. The next 9 most populated clusters are given in the ESI† as Fig. S2. The population percentage of the 9 most dominant clusters and the residues that surround the Fe ion are given in Table 1.

In order to identify the residues that surround heme, wrappers were introduced to define the residues within 0.5 nm from the center of mass (COM) of the heme or the Fe ion. From Fig. 2, we observe that there are three possible sets of wrappers. The first set consists of residues Ala-2–His-6, the second set is made up of Gln-15–Phe-20, while the third set is Ala-30–Val-36. By ranking the residues according to their propensity to locate near heme, we detect the following order: Phe having the highest affinity, followed by Val, Ile, Leu, Met, Arg, His, ... (see ESI† Table S2). Remarkably, we observe that the set of residues that have the greatest tendency to lie in the neighborhood of heme are mainly aromatic and hydrophobic residues. This has led us to surmise that the hydrophobic interaction plays a dominant role in the Aβ–heme complex formation.

### Table 1

<table>
<thead>
<tr>
<th>Clusters</th>
<th>Population (%)</th>
<th>Trajectory</th>
<th>Surrounding residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>62.27</td>
<td>traj4</td>
<td>Phe-4 Leu-17 Ile-32 Ile-41</td>
</tr>
<tr>
<td>C2</td>
<td>58.02</td>
<td>traj9</td>
<td>Phe-4 Phe-20 Val-24 Ile-31 Leu-34 Met-35</td>
</tr>
<tr>
<td>C3</td>
<td>54.79</td>
<td>traj15</td>
<td>Phe-4 Arg-5 His-6 Phe-19 Phe-20</td>
</tr>
<tr>
<td>C4</td>
<td>54.37</td>
<td>traj14</td>
<td>Arg-5 Leu-17 Phe-19 Val-24</td>
</tr>
<tr>
<td>C5</td>
<td>51.85</td>
<td>traj16</td>
<td>Phe-4 Arg-5 Ile-31 Leu-34 Met-35</td>
</tr>
<tr>
<td>C6</td>
<td>45.91</td>
<td>traj13</td>
<td></td>
</tr>
<tr>
<td>C7</td>
<td>39.25</td>
<td>traj3</td>
<td>Phe-4 His-6 Ile-32 Gly-33 Met-35</td>
</tr>
<tr>
<td>C8</td>
<td>38.57</td>
<td>traj2</td>
<td>Gln-15 Lys-16 Val-18</td>
</tr>
<tr>
<td>C9</td>
<td>36.60</td>
<td>traj17</td>
<td>Met-35</td>
</tr>
</tbody>
</table>

*The residues that are within 5 Å of the Fe ion.*
3.2 Potential of mean forces

In order to provide a more comprehensive view on how the set of high propensity residues situate near (or interact with) the heme molecule, we plot the potential of mean forces (PMF) between these residues and heme. This is performed through the extensive Aβ–heme configurations that we have obtained from our simulations. Fig. 3 shows the PMFs for the residues His, Phe, Leu, Ile, Val, Arg and Met of Aβ with respect to the Fe ion. The PMF shows that the His-6 has a basin of attraction located around the His-6–Fe minimum distance of 5 Å, which is absent in the case of the His-13 and His-14 residues. For the three histidines, the global minimum is located within the range of 10–17 Å. On the other hand, the global minimum of Phe-4 is located around 5.8 Å; Phe-19 is located around 4.5 Å; and Phe-20 is around 12.6 Å. We observe that Leu-17 and Leu-34 share a similar basin in the range of 7–10 Å. For Ile-32, the global minimum is located around 6.7 Å. As for Ile-31, it is located around 9.7 Å. But for the residue Ile-41, we observe a wide basin of attraction. In the case of Val-24, the first minimum appears around 6 Å, with another basin appearing in the range 11 Å to 23 Å. Note that the first potential barrier of Val-24 corresponds to the basin of Val-18. Arg-5 is observed to have a jagged PMF curve while Met-35 has several minima with its global minimum located around 4.6 Å. In conclusion, we observe that hydrophobic residues such as Phe, Val, Leu, Ile of Aβ tend to situate close to heme through the PMF curves. In particular, Phe-19, Met-35 and His-6 are found to have a high possibility of interacting with the heme group at close distance.

3.3 Hydrogen bond formation between Aβ and heme

We have also obtained information on the hydrogen bond formation between heme and Aβ through the 20 trajectories. We observe that the four N atoms of heme have equal probability of being the hydrogen bond acceptor, while the O atoms of the heme –COOH groups have a much higher chance (more than 3 times) as the hydrogen bond acceptor (see ESI† Table S3). A ranking of Aβ residues in terms of hydrogen bonding affinity from highest to lowest is as follows: Arg-5, Asp-1, His-14, His-6, Lys-16 (see Fig. 4). Interestingly, the highly ranked residues are
observed to be neighbours to the identified residues which locate in close vicinity to heme. For example, Arg-5 is situated next to Phe-4 and we know that Phe-4 has a stronger propensity to form the Aβ–heme complex than Arg-5. Thus, our results show that hydrophobic interaction is the main force that drives the formation of the Aβ–heme complex, while the hydrogen bond interaction serves as an auxiliary force to stabilize the complex. Finally, we notice through our simulation that the Aβ N-terminus has a slight preference to form hydrogen bonds with heme than the C-terminus.

3.4 Aβ secondary structure propensity in the presence/absence of heme

The secondary structure propensity of each residue was examined from the 20 “heme-present” trajectories, 2 “heme-absent” trajectories (see Fig. 5 and ESI† Fig. S3). Four types of secondary structures: β-structure (β-bridge and β-sheet); helix (α-helix and 3-helix); bend & turn; and coil, are used to categorize the secondary structure propensity. From Fig. 5, we can see that the residues His-6, Asp-7, Tyr-10, Glu-11, Val-12, Ala-21, Glu-22 and Asp-23 have a high probability of being involved in the β-structure conformations. On the other hand, the residues Ser-26, Asn-27, Lys-28, Gly-29 and Ala-30 show a strong preference in participating in the helical conformations. By examining the number of residues that adopt the β-structure and the helical structures in our simulation, we see that the presence of heme has the effect of reducing the β-content and increasing the helical-content by 22.1% and 33.6% respectively (see ESI† Fig. S4).

3.5 Aβ–hemes complex

In order to examine the possible heme-binding Aβ motifs during the process of Aβ oligomerization for the 2 Aβ + 4 hemes system, clustering analysis was carried out on the 16 trajectories with a Cα-rmsd cutoff of 3 Å. The central portions of the first 9 dominant clusters are shown in Fig. 6 as well as the population of these 9 dominant clusters. The Aβ residues that surround the Fe ion from heme are given in Table 2.

Our results show that the residues, which are 0.5 nm away from the COM of heme as shown in Fig. 7, display the following
affinity to reside in the neighbourhood of heme from highest to lowest: Phe, Tyr, Arg, His, Ser, Leu, Met. . . On the other hand, the Aβ residues that tend to lie within 0.5 nm of the Fe ions of heme have the following affinity ranking: with Phe having the highest affinity, followed by Tyr, Arg, His, Met, Leu, Ser. . . We see that the Aβ residues that occur in the vicinity of heme and the Fe ions are slightly different for the 2 Aβ + 4 hemes system and the 1 Aβ + 1 heme system. The differences result from interactions between Aβ peptides which affect the Aβ–heme complex motifs. Remarkably, we observe that the residue Phe shows the highest interaction affinity with hemes in both systems.

Furthermore, the hydrogen bond forming propensity of the Aβ residues towards heme were also examined (see Fig. 8).

Our results show that the Aβ residues share similar propensity in hydrogen bond formation in the 2 Aβ + 4 hemes and the 1 Aβ + 1 heme simulations, albeit with the ranking Lys-16, Ser-26, Arg-5, Lys-28, His-6. . . As a final note, we notice a smaller standard deviation error in Fig. 8 as compared to Fig. 4 which is for the 1 Aβ + 1 heme system. This results from the presence of extra heme molecules in the 2 Aβ + 4 hemes system, which can be observed from the ratio of Aβ to heme being 2:1 in this system, versus the 1:1 ratio in the 1 Aβ + 1 heme system.

### 3.6 Heme physically prevents Aβ aggregation

The secondary structure propensity of each residue of the 2 Aβ + 4 hemes system can be determined from Fig. 9. As before, four types of secondary structures are considered to categorize the secondary structure propensity of the system. The four types are: β-structure (β-bridge and β-sheet); helix (α-helix and 3-helix);

### Table 2 The population of the first 9 dominant Aβ-hemes clusters in the last 50 ns

<table>
<thead>
<tr>
<th>Clusters</th>
<th>Trajectory</th>
<th>Surrounding residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3 8</td>
<td>Peptide A: Phe-4 Arg-5 Glu-11 His-14&lt;br&gt;Peptide B: Phe-4 Val-12 Leu-17 Met-35 Val-39</td>
<td></td>
</tr>
<tr>
<td>C4 15</td>
<td>Peptide A: Ala-2 Phe-4 Tyr-10 Leu-17 Phe-19&lt;br&gt;Leu-34 Met-35 Val-36&lt;br&gt;Peptide B: His-13 Leu-17 Phe-19 Ile-31</td>
<td></td>
</tr>
<tr>
<td>C6 15</td>
<td>Peptide A: Leu-17 Phe-19 Leu-34 Met-35&lt;br&gt;Peptide B: His-13 Leu-17 Phe-19 Val-39</td>
<td></td>
</tr>
<tr>
<td>C8 6</td>
<td>Peptide A: Phe-4 Phe-19 Val-24 Gly-25 Ser-26&lt;br&gt;Val-39&lt;br&gt;Peptide B: Leu-17 Phe-19 Ile-32</td>
<td></td>
</tr>
<tr>
<td>C9 6</td>
<td>Peptide A: Phe-4 Tyr-10 Phe-19 Val-39 Ile-40&lt;br&gt;Peptide B: Val-39</td>
<td></td>
</tr>
</tbody>
</table>

* The residues that are within 5 Å of the Fe ion.
bend & turn; and coil. For the sake of clarity, Fig. 9 only displays three of the four types, i.e., the β-structure, bend & turn, and the helix structure. By comparing Fig. 9 with Fig. 5(a), we observe the similar α-structure propensity for the 2 Aβ + 4 hemes system as the 1 Aβ + 1 heme system. For example, the sequence \text{SNKGAI}_{31} is found to be one common helical segment in both systems. By averaging the number of residues adopting the helical-structure and the β-structure every 50 ns (see Fig. 10), one finds that the presence of heme has not led to a significant difference in the secondary structure content of the 2 Aβ + 4 hemes system in comparison to the situation when heme is absent.

The number of contacts between peptide A and peptide B are calculated for all the 32 trajectories. By averaging the number of contacts every 20 ns for the 16 “heme-present” trajectories and “heme-absent” trajectories, we found a lower contact number for the trajectories with the presence of heme in comparison with our group of controls (see Fig. 11(d)). This reduction in contact implies that the presence of heme has prevented the aggregation of Aβ. By extracting the last 10 ns of the 32 trajectories and plotting the averaged contact map (see Fig. 11(a) and (c)), we see that more contacts are formed in the Aβ–hemes without heme than those with heme. In addition, we have plotted in Fig. 11(b) a representative Aβ–hemes complex to illustrate its secondary structure.

### 4 Summary and discussion

The formation of dimer, trimer and higher order oligomers during the process of amyloid β aggregation is believed to be
toxic to the neurons. In order to avert the resulting neuronal apoptosis, there is great interest in the study of the \(\alpha\)-heme complex which plays different roles in the intracellular and extra-cellular space. While the intracellular \(\alpha\)-heme complex is proposed to decrease heme bioavailability which leads to a deficiency of the functional heme,\(^{26,27}\) extracellular \(\alpha\)-heme is reported to inhibit \(\alpha\)\&–aggregation and alleviate \(\alpha\)\&– oligomer's toxicity.\(^{30}\)

Past research has uncovered several \(\alpha\)\&–heme motifs which mainly involve the histidine residues,\(^{27,28,29}\) without any analysis of the detailed secondary structure conformations within the configuration. By means of extensive conventional molecular dynamics simulations, we have identified several binding motifs which indicate that hydrophobic residues have a high tendency to interact with heme. Thus, an \(\alpha\)\&–heme complex may not be histidine-focusing, with the hydrophobic interactions between the heme and \(\alpha\)\&– hydrophobic residues playing a dominant role in the \(\alpha\)\&–heme complex formation as shown in our MD simulations study. However, further experimental as well as QM/MM study is required to provide a more accurate assessment to elucidate whether \(\alpha\)\&–heme complex formation is driven via the interaction with histidine or by hydrophobic interaction. It is important to note that past experiment\(^3\) on the \(\alpha\)\&–heme complex involved peptides such as the \(\alpha\)\&–1–40, \(\alpha\)\&–1–16, \(\alpha\)\&–17–40 and \(\alpha\)\&–10–20, which are mainly non-amyloidogenic, while the toxic \(\alpha\)\&–1–42 has yet to be tested experimentally. On the other hand, recent QM/MM studies\(^4\) on the \(\alpha\)\&–heme complex had only explored a limited number of configurations. Thus, we expect more work to be performed on QM/MM, experiments and MD simulations that aim to fully grasp the detailed interactions between heme and \(\alpha\)\& in the not too distant future.

Through the PMF, we observe that the position of the \(\alpha\)\&–hemic residue has an implicit effect on the binding affinity of heme towards certain residues. In addition, heme physically prevents the \(\alpha\)\& aggregation and it is also found to have an influence on the underlying secondary structure of \(\alpha\)\& during the formation of the \(\alpha\)\&–heme complex, which may explain its inhibitory role in neuronal cell death. The latter conclusion is supported by the simulation of the 2 \(\alpha\)\& + 4 hemes system, which demonstrates explicitly how heme physically prevents \(\alpha\)\& aggregation with an increase in the \(\alpha\)\&–content within the peptides.

**Acknowledgements**

The authors would like to thank Hwee Jin Soh from the High Performance Computing Center for his kind help in the provision of computational support, and Dr See-Wing Chiu from UIUC for his suggestions and review of the preliminary draft. The support of research grants, URC(RG23/11), from Nanyang Technological University and the IDA Cloud Computing Call for Project Proposals 2012 is gratefully acknowledged.

**References**