ABSTRACT: Amyloid β (Aβ) peptides mutated at different positions using a cysteine moiety assemble on Au electrodes using the thiol functionality of cysteine. Self-assembled monolayers (SAMs) of Aβ on Au surfaces can act as abiological platforms that allow the mimicking of fibrils and oligomeric Aβ via the formation of controlled large and small peptide aggregates. These Aβ constructs bind with heme and Cu and exhibit different reactivities. These abiological platforms can also be used to investigate potential drugs that can interact with heme and Cu-Aβ. SAM formation of Aβ mutants allows the study of different morphology and structure as well as behavior changes on binding with different metals and cytochrome c (Cyt c). This review provides a detailed insight into the structure and reactivities of various Aβ aggregated on Au electrodes mimicking the cell membrane.

INTRODUCTION

Self-assembled monolayers (SAMs) generally refer to an organized and ordered molecular arrangement formed spontaneously via adsorption of the constituents from a solution or gas phase usually on solid surfaces and in some cases (e.g., mercury) on liquid surfaces, as well. The resultant crystalline or semicrystalline structures typically consist of the adsorbent surface (substrate), the adsorbate molecules with a well-defined headgroup suitable for binding to the surface, a backbone which determines the ordering, and a terminal or end group which governs the overall nature of the SAM and can be functionalized according to requirements. SAM formation is a particularly good method of surface modification as it provides the advantage of a long shelf life compared to other conventional methods (such as irradiation with a UV/laser/electron beam, plasma, etc.) due to the covalent attachment of molecules in this case. In fact, SAMs have been used in multiple disciplines to study heterogeneous phenomena, with applications ranging from developing modern nanotechnology, molecular electronics, organic electronics, electrochemistry, and even biomimetics. The former few deal with the immobilization of mainly inorganic materials, whereas, in the latter case, biological molecules such as proteins, enzymes, etc. are involved. Use of SAMs to study the reactivity and other aspects of large biomolecules like DNA, cytochrome c (Cyt c), and cytochrome c oxidase (CcO) under heterogeneous conditions is now commonplace as it allows these large molecules to approach an electrode surface even in the absence of any free S atom like anchor.

The amyloid β (Aβ) peptide, in particular, is implicated in the pathogenesis of Alzheimer’s disease (AD), which is a terminal disease of cerebral irregularities estimated to affect approximately 115.4 million people worldwide by 2050. Memory loss and permanent brain disorder are the first signs of this disease. The pathological characteristics of AD include substantial loss of neurons and disruption of synaptic function throughout the brain. That Aβ peptide aggregation is a key factor underlying this disease, is agreed upon almost universally that Aβ is the major constituent of the extraneuronal senile plaques that are typical of AD patients. The β- and γ-secretases sequentially cleave the C-terminus region of the larger APP (amyloid precursor protein) protein resulting in the smaller Aβ peptide consisting of 39–42 amino acids. The Aβ(1–40) and Aβ(1–42) variants are the most abundant fragments. Monomeric Aβ is short-lived and forms soluble oligomers which randomly coalesce to form fibrils and finally plaques in AD affected brains. Earlier, the fibrils were thought to be more toxic, but later, it was found that the soluble intermediates are the main threat in amyloidogenic diseases including AD, Parkinson’s disease, and type II diabetes purportedly due to their membrane permeabilizing ability. Increased presence of...
redox-active metals such as iron (as regulatory heme; concentration ∼30 nM) and copper (0.2−1.7 μM) in the plaques supports their involvement in this disease along with Aβ. These redox-active metals have been found to bind Aβ, which in turn can catalyze the production of harmful reactive oxygen species (ROS; O2−, O2⋅−, and OH⋅). Overproduction of ROS can cause oxidation of the lipid bilayer and extensive oxidative stress to the brain of AD patients.3 Recently, several research groups have reported the active site environment and reactivity of heme-Aβ and Cu-Aβ.4−6 The reactivity of these sites depends on the aggregation state of Aβ. Consequently, the inhibition of their reactivity either by chelation of the metal cofactor or by ROS reduction may also vary according to the aggregation states of Aβ.

Recently, the method of SAM formation has been extended to peptides such as Aβ and amylin, which undergo aggregation and fibril formation under disease conditions, and their self-assembly is influenced by the cell membrane, which is a heterogeneous surface.7 The formation of hydrophobic, hydrophilic, positively charged, and negatively charged SAMs by altering the nature of the end group has been reported. Their impact on the kinetics of Aβ aggregation, structure, and morphology of the various assembly states of the peptide highlights the importance of hydrophobic and electrostatic interactions in the aggregation pathway.5 This mini-review seeks to collect and collate the available information about how the formation of the SAM of Aβ on Au surfaces provides the opportunity to regulate the formation of large and small aggregates of the peptide, which resemble the fibrillar and oligomeric forms of Aβ, respectively. It also offers a consolidated view about how these Aβ constructs bind with heme and Cu and exhibit different reactivities. That these abiological platforms can also be used to investigate potential drugs which can interact with heme and Cu-Aβ and also that the SAM formation by Aβ mutants allow for the study of different morphology and structural as well as behavioral changes on binding with metals and other proteins are reviewed here, as well. This may explain in part the recent shift from the “amyloid cascade hypothesis”, which holds the fibrillar form of Aβ responsible for the AD associated neurotoxicity, toward the “toxic oligomer hypothesis”, which ascribes the same role to the transient oligomeric intermediates in the Aβ aggregation pathway.

FORMATION OF Aβ SAM

Thiol SAMs on a Au/Ag surface have been utilized for different purposes in both chemical and biological fields. Aβ assemblies on a Au electrode have been used to study not only the different physiologically relevant aggregation states of Aβ (Figure 1A) but also the effect of metal binding and for monitoring the interaction of potential drugs with heme and Cu-bound Aβ relevant to AD. The inspection of the amino acid sequence of Aβ reveals that there is no potential group present which can help in SAM formation. Hence, a cysteine moiety is introduced at a different position of the Aβ sequence, which spontaneously assembles on the Au electrode.9−11 Figure 1B schematically represents the SAM formation of Aβ on a Au electrode.

ATOMIC FORCE MICROSCOPY

A cysteine residue is covalently tethered by its thiol group to form a SAM on a Au electrode. This results in the formation of large aggregates of peptides (designated as AβWT) for AβCys(1−16). The atomic force microscopy (AFM) image reveals that AβWT resembles the fibrillar form of the Aβ.
aggregate. Covalently tethered Aβ forms a parallel β-sheet which on dilution with C8SH forms an isolated cluster (AβC8SH) (Figure 2), and on dilution with 1-cysteine in a 1:9 ratio, a homogeneous distribution of small clusters (Aβ1:9) is detected (Figure 3). Such dimensions imply the presence of ~20–30 Aβ monomers aggregated in globular form. Thus, the association of fibrils and large oligomers of Aβ formed in vivo is represented by the formation of the large peptide assemblies, i.e., AβWT, and the small isolated clusters, i.e., AβC8SH and Aβ1:9, on the electrode surfaces under different depositing conditions.9,10 The hydrophilic part of Aβ peptides projects outward from the SAM surface toward the electrolytic solution. This is comparable to an oligomer cross section seen in both in vivo and in vitro situations, which is pseudomicellar in appearance due to the hydrophilic residues projecting outward into the extracellular domain.12,13 Such oligomers involved in fibril formation tend to be short-lived and hence quite unstable in vivo, but upon SAM formation, these oligomers, having the same arrangements, are stable species. Hence, reactivity and toxicities are easy to investigate under these conditions.

In the case of SAM formation by the three Aβ(1–40) mutants G38C, L17C, and Y10C, the presence of cysteine at different positions results in the formation of aggregates having different morphologies (Figure 4). Metal (Cu2+/Zn2+)

![Figure 2](https://pubs.acs.org/journal/acsonf)

**Figure 2.** AFM images of AβCys SAM covered surfaces. 3D topology of (A) wall-like structure and (B) isolated clusters of AβC8SH SAM surfaces. Adapted from ref 10. Copyright 2012 American Chemical Society.

![Figure 3](https://pubs.acs.org/journal/acsonf)

**Figure 3.** Probable arrangements of AβWT (left) and Aβ1:9 (right) on the Au surface as observed in their respective AFM images. Adapted with permission from ref 9. Copyright 2014 Royal Society of Chemistry.

![Figure 4](https://pubs.acs.org/journal/acsonf)

**Figure 4.** Probable schematic representation of (a) G38C, (b) L17C, and (c) Y10C mutants of Aβ(1–40) on Au surfaces. Adapted from ref 11. Copyright 2018 American Chemical Society.

**INTERACTIONS OF Aβ SAM WITH METALS**

The peptide assemblies interact with heme and Cu readily, similar to the solution counterparts, which have been characterized by absorption spectroscopy, surface-enhanced resonance Raman spectroscopy (SERRS), CV, and infrared (IR) spectroscopy.

**Absorption Spectroscopy.** In solution, binding of Aβ to heme results in a Soret band at 392 nm and a shoulder at 363 nm.14 The AβCys peptide assemblies formed on a transparent Au surface also bind heme, showing similar features. On binding of imidazole to different heme-Aβ assemblies, a red shift of the Soret band is observed similar to that of histidine-bound heme-Aβ site9,10 (Figure 5). Despite the coordination has been shown to shorten the height of these assemblies, as evident from the respective AFM images and height distribution profiles. These observed differences in morphology relative to the metal-free analogues point toward a metal-binding induced folding of Aβ, which is further corroborated from the results of cyclic voltammetry (CV) experiments discussed below.11

![Figure 5](https://pubs.acs.org/journal/acsonf)

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respectively (Figure 7). Comparison of the intensities of the heme-Aβ increased proportion of the Mb-type component in the case of respective bands in the solution and surface data shows an spin active site. The SERRS data of the heme-Aβ spin myoglobin (Mb)-type active site and a six-coordinate low-spin heme-Aβ shows the presence of two of the above three components. The other two minor components are a six-coordinate high-spin heme(III)-containing major one has a six-coordinate high-spin heme-Aβ in solution are found to bear a close resemblance to the SERRS data of heme bound to AβC8SH (red), heme-bound AβC8SH in 100 mM imidazole (purple), and reduced heme-bound AβC8SH + CO (cyan) on Au electrodes. Adapted from ref 10. Copyright 2012 American Chemical Society.

fact that absorption data are collected on a monolayer on a transparent electrode surface, i.e., under very dilute condition, distinct absorption features could be observed due to the very high absorption coefficient of heme (ε = 10⁵ mol⁻¹ cm⁻¹). On the contrary, Cu binding to the Aβ SAM could not be monitored via absorption spectroscopy due to the low intensity of the corresponding d-d bands (ε = 10–100 mol⁻¹ cm⁻¹).

Surface-Enhanced Resonance Raman Spectroscopy. Resonance Raman (rR) spectroscopic data on the active site of heme-Aβ in solution are found to bear a close resemblance to the SERRS data of heme bound to AβCys assemblies immobilized on Au electrodes. In the homogeneous phase, a total of three different species are identified, of which the major one has a six-coordinate high-spin heme(III)-containing active site similar to that of horseradish peroxidase (HRP). The other two minor components are a six-coordinate high-spin myoglobin (Mb)-type active site and a six-coordinate low-spin active site. The SERRS data of the heme-AβC8SH SAM show the presence of two of the above three components observed for heme-Aβ in solution. These are the Mb-type and HRP-type six-coordinate high-spin species having their coordination marker bands at 1483 and 1491 cm⁻¹, respectively (Figure 7). Comparison of the intensities of the respective bands in the solution and surface data shows an increased proportion of the Mb-type component in the case of heme-AβC8SH with respect to heme-Aβ in solution. However, the overall similarity between the two species is reflected in the low-energy region as well where the νs band representing the Fe–N(pyrrole) vibration of heme-AβC8SH SAM (348 cm⁻¹) appears at an energy slightly lower than that of heme-Aβ in solution (351 cm⁻¹) possibly due to the higher Fe–N(pyrrole) vibration of the minor low-spin component in the latter. The low-energy region data also show a vibration at ~420 cm⁻¹ in both heme-Aβ (rR) and heme-AβC8SH SAM (SERRS), which may relate to a Fe–OH₂ stretching frequency. All of these imply that the heme moiety remains in a similar environment while being bound to the AβC8SH SAM and Aβ in solution. The SERRS of heme-AβC8SH shows the conversion to a six-coordinate low-spin heme(III) species upon imidazole binding from the major six-coordinate high-spin ferric heme species.15,16 Imidazole binding causes a similar spin change to occur in the case of free heme physisorbed on octanethiol electrode, as well; however, the corresponding SERRS data are quite different from that of imidazole bound to heme-AβC8SH.10

Later, while probing the impact of the aggregation state of Aβ/Cys on heme binding, it emerged that a six-coordinate high-spin heme(III) species is present irrespective of whether heme is bound to large or small peptide clusters (Figure 8). Additionally, a shoulder in the νs₂ region at ~1585 cm⁻¹ is seen, which represents the presence of a six-coordinate low-spin ferric heme species with a greater population in the case of heme-AβC8SH and heme-AβWT compared to heme-AβC8SH due to greater intensity of this shoulder in the former case relative to the latter. Using the SERRS coupled to rotating disc electrochemistry (SERRS-RDE) setup at a reducing potential of −200 mV (vs NHE), the reduced heme active site was investigated, and the resultant SERRS data of both heme-AβWT and heme-AβC8SH provided evidence in support of a high-spin ferrous heme species along with little contributions from the residual high-spin ferric species.9

Cyclic Voltammetry. CV experiments performed in the absence of oxygen with heme-AβWT, heme-AβC8SH, and heme-AβWT show a reversible Fe³⁺/²⁺ redox couple at −130, −150, and −160 mV vs NHE, respectively (Figure 9). In degassed buffer, the CV of Cu-AβWT results in a reversible Cu²⁺/⁻ couple at 370 mV vs NHE. For Cu-AβC8SH, i.e., for the Cu complex with isolated Aβ clusters, the same redox couple appears at 300 mV vs NHE. In the presence of O₂ at pH 7, Cu-AβC8SH shows a quasi-reversible CV at the same potential as well as a weak electrocatalytic oxygen reduction current. On the other hand, heme-AβC8SH shows a huge amount of oxygen reduction current but no Fe³⁺/²⁺ CV.10
When heme and Cu are loaded together, the oxygen reduction current shifts to $-270\text{ mV (vs NHE)}$ (Figure 10). The area under the cathodic current gives a quantitative measure of the amount of redox-active species attached to an electrode and is hence directly proportional to surface coverage by the ions.\textsuperscript{17,18} The surface coverage obtained for large and small heme-A$\beta$ and Cu-A$\beta$ aggregates bears a similar amount of heme and Cu irrespective of aggregation states.\textsuperscript{9} For heme-Cu-A$\beta$, the integrated charges under the Cu$^+$ and Fe$^{2+}$ heme oxidation currents are found to be the same, indicating formation of a 1:1 complex as in the case of heme-Cu-A$\beta$ in a homogeneous medium. It was found that a normal electrode with a 0.5 cm$^{-2}$ cross section can be said to have $0.5 \times 10^{-11}$ moles, i.e., 5 pmol of the active site. An important point to note here is that the electrocatalytic O$_2$ reduction exhibited by the bare Au electrodes is absent when the A$\beta$ SAM is formed on them. Immersion of these surfaces in octanethiol not only affects the aggregation size but also improves the insulation of the electroactive Au surfaces from the conducting solution.

\textbf{IR.} The SAMs of the A$\beta$ mutants G38C, L17C, and Y10C are subjected to UATR-FTIR (universal attenuated total reflection–Fourier transform infrared) spectroscopy before and after metal accumulation in order to determine the impact of metal binding to the peptide assemblies. For all constructs

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**Figure 7.** Solution rR of heme-A$\beta$ (blue) and SERRS of heme-A$\beta$C$_8$SH (red) in the (A) high-energy and (B) low-energy regions. The peaks marked with “*” are either plasma lines or derived from scattering from sample tubes. The $\nu_3$ bands of (C) heme-A$\beta$ and (D) heme-A$\beta$C$_8$SH with fits showing different components. Adapted from ref 10. Copyright 2012 American Chemical Society.

**Figure 8.** Characterization of the heme-modified surfaces by SERRS-RDE experiment. SERRS-RDE data of heme-A$\beta$WT (blue) and heme-A$\beta$C$_8$SH (orange)-modified electrodes in pH 7 buffer under oxidizing potential (i.e., resting state) (a) and under reducing potential in anaerobic pH 7 buffer (b). Adapted with permission from ref 9. Copyright 2014 Royal Society of Chemistry.
prior to metal binding, amide I and amide II bands are observed in the regions of 1600−1700 and 1500−1600 cm$^{-1}$, respectively. Binding of Cu$^{2+}$ to the A$\beta$ assemblies is accompanied by perturbation in the above-mentioned spectral region, which may be assigned to the amino acid residues constituting the peptide’s Cu$^{2+}$ coordination domain. Transmittance at 1265 cm$^{-1}$, which usually corresponds to the C−O stretch of C−OH vibration of a carboxylic acid group, shows a significant decrease in intensity upon Cu$^{2+}$ binding to all of the A$\beta$ constructs (Figure 11). This may be attributed to some carboxylate side chains of A$\beta$ being involved in Cu$^{2+}$ coordination that agrees well with previously proposed apical carboxylate side chain coordination to Cu$^{2+}$.19,20 Zn$^{2+}$ bound to these A$\beta$ assemblies gives rise to similar observations, effectively re-establishing the participation of carboxylate side chains in metal binding.11

REACTIVITY

The reaction of heme(II)-A$\beta$ or Cu(I)-A$\beta$ with O$_2$ produces the two-electron reduced product H$_2$O$_2$ as the four electrons required for complete reduction cannot be provided by the reduced heme or Cu alone. On the other hand, after formation of the A$\beta$ SAM on a Au electrode, the extra electrons necessary for reduction of O$_2$ to H$_2$O can be supplied by the electrode at sufficiently reduced potential. However, ROS formation takes place at intermediate potentials and can be detected in situ under steady-state conditions using rotating ring disc electrochemistry (RRDE). Heme-A$\beta_{WT}$ produces 23.7 ± 0.5% ROS, while heme-A$\beta_{1:9}$ produces 16.3 ± 0.3% at 0 V vs NHE. Heme-A$\beta_{C8SH}$ generates a similar amount of ROS (17 ± 2%) as heme-A$\beta_{1:9}$. This shows that heme bound to large aggregates of A$\beta$ produces greater amounts of ROS than when it is bound to isolated peptide clusters. The scenario gets reversed in the case of Cu-bound A$\beta$ aggregates. The amount of ROS released by Cu-A$\beta_{WT}$ is 19.5 ± 1.5%, whereas 31 ± 1% ROS is produced by Cu-A$\beta_{C8SH}$ at 0 V vs NHE. Thus, Cu bound to small A$\beta$ aggregates is potentially more cytotoxic compared to Cu bound to large aggregates, as the former gives rise to a greater amount of ROS. Heme-Cu-A$\beta_{C8SH}$ produces 23 ± 3% ROS. Here, 100% ROS is not observed because much of the ROS may be converted to H$_2$O due to the readily available electron from the electrode. The impact of Arg5 and Tyr10 on the ROS formation process is also assessed as these residues along with His13 (involved in heme coordination) are absent in rodents who do not suffer from AD, implying their potential significance in AD. As mentioned previously, under homoge-

Figure 9. (a) CV data of heme-A$\beta_{WT}$ (blue) and heme-A$\beta_{1:9}$ (orange)-modified electrodes in deoxygenated pH 7 buffer at a scan rate of 1 V s$^{-1}$. (b) CV data of Cu-A$\beta_{WT}$ (green) and Cu-A$\beta_{C8SH}$ (red)-modified electrodes in pH 7 buffer at a scan rate of 50 mV s$^{-1}$. Adapted with permission from ref 9. Copyright 2014 Royal Society of Chemistry.

Figure 10. CV data of heme-bound, copper-bound, and both heme- and copper-bound A$\beta_{C8SH}$ in air-saturated pH 7 buffer. Adapted from ref 10. Copyright 2012 American Chemical Society.

Figure 11. Particular peak near 1265 cm$^{-1}$ in FTIR data represents −C−O single bond vibration of the −C−OH group for (a) G38C (blue), (b) L17C (violet), and (c) Y10C (dark green) assembly. The corresponding perturbation in transmittance of this particular peak is clearly visible for (a) Cu-G38C (red), (b) Cu-L17C (light green), and (c) Cu-Y10C (light blue) constructs. Adapted from ref 11. Copyright 2018 American Chemical Society.
neous conditions, the reduced metal center of heme(II)-Aβ and Cu(I)-Aβ provides one of the electrons required for H2O2 formation by the two-electron reduction of O2 and the other one comes from Tyr10 of Aβ. When Aβ is immobilized on Au electrodes, similar observations are made as the amount of ROS formed by heme-AβC8SH and Cu-AβC8SH SAMs using the Tyr10Gly mutant of AβCys (7 ± 1 and 13 ± 1%, respectively) is almost half the amount formed when the WT peptide is used. The reduced heme/Cu center and Tyr10 thus provide the two electrons necessary for reduction of O2 to H2O2 in this heterogeneous situation, as well. When Tyr10 is absent, O2 is reduced to O2− by the single electron coming from the reduced cofactor which then undergoes disproportionation to produce half an equivalent of H2O2. A similar phenomenon is seen in the case of heme-Cu-AβC8SH too, where use of the Tyr10Gly mutant reduces the amount of ROS to 7 ± 3% from 23 ± 3% for AβCys9,10 (Table 1). A possible role of the hydrogen bonding Arg5 residue in facilitating the H2O2 production by the Cu-bound, heme-bound, and both heme- and Cu-bound AβC8SH SAMs is evident from the significant decrease in ROS formation (20 ± 5, 9 ± 3, and 11 ± 1%, respectively) on mutating the Arg5 residue. Thus, the aggregation state of Aβ as well as the second sphere residues Arg5 and Tyr10 is found to impact the ROS formation process.

### INTERACTIONS WITH INHIBITORS

8-Hydroxyquinoline (HQ) being an analogue of 5-chloro-7-iodoquinolin-8-ol (clioquinol) can act as a weak Cu chelator. The disappearance of the Cu2+/+ CV is observed when the Cu-AβC8SH SAM is incubated with HQ solution, indicating sequestration of Cu bound to small aggregates of AβCys. However, under similar experimental conditions, the Cu-bound large aggregates of AβCys (AβCysWT) show very little Cu removal. A much higher concentration of HQ and a greater incubation time is necessary to result in Cu chelation from the large Aβ aggregates as compared to the case of small aggregates, implying this chelation process is less favorable in the former case relative to the latter in terms of both kinetics and thermodynamics.9 When the Cu-L17C SAM is incubated with a 15 nM HQ solution, the decrease in transmittance intensity of the peak near 1265 cm−1 in the UATR-FTIR spectrum due to Cu2+ binding gets restored as a consequence of Cu sequestration by HQ (Figure 12). Thus, for the L17C construct, the involvement of a carboxylate side chain in binding Cu is reiterated, utilizing the Cu chelation property of HQ.10

The O2 reduction current displayed by the SAM of heme-AβC8SH does not show much change in the presence of HQ, but when the same is incubated with methylene blue (MB), a substantial decrease in the O2 reduction current is observed (Figure 13).

### Table 1. ROS Produced by Aβ Complexes

| cofactors          | heme | Cu  | heme-Cu |
|--------------------|------|-----|---------|
| AβWT (without diluents) | 23.7 ± 0.5 | 19.5 ± 1.5 | ND |
| AβC8SH (C8SH as diluent) | 17 ± 2 | 31 ± 1 | 23 ± 3% |
| Aβ1.9 (1-cysteine as diluent) | 16.3 ± 0.3 | ND | ND |
| AβC8SH mutant (Tyr10Gly) | 7 ± 1% | 13 ± 1% | 7 ± 3% |
| AβC8SH mutant (Arg5Gly) | 9 ± 3% | 20 ± 5% | 11 ± 1% |

A direct interaction between the heme cofactor and MB occurs, which is confirmed in homogeneous medium by titrating heme-Aβ with MB. Absorption spectroscopy shows changes in the Soret region of heme-Aβ on addition of 1 equiv of MB, while the changes in the Q-band region are obscured by the high intensity charge transfer bands of the latter. The SERRS data provide more conclusive supportive evidence in the form of the ν3 vibration band, showing the presence of only one component after incubation of heme-AβC8SH with MB as opposed to two components being observed for heme-AβC8SH SAM alone. Meanwhile, it was found that heme remains bound to AβC8SH after incubation with MB and has a +3 oxidation state (Figure 14). MB incubation also leads to a simultaneous shift of the ν6 (Fe–N stretch), ν9 (in-plane symmetric pyrrole deformation), and the ν15 (breathing modes) vibrations lying between 670 and 800 cm−1, which are sensitive to axial ligands.10

Similar to the difference in extent of chelation of Cu bound to Aβ assembles by HQ depending on the size of the aggregates, MB too has been found to have a different impact on the heme-bound large aggregates of AβCys relative to heme.

Figure 12. (a) Blue line illustrates the CV response of Cu-L17C construct in pH 7 buffered solution at 20 mV/s using a Ag/AgCl reference and Pt wire counter electrodes. The red line illustrates the same when the construct was incubated with HQ solution for 60 min. (b) Particular peak near 1265 cm−1 in FTIR data representing the C=O single bond vibration of the –C=–OH group is shown in violet for L17C. The corresponding perturbation in transmittance of this particular peak for the Cu-L17C construct and its restoration upon HQ exposure are clearly visible in light green and light blue. Adapted from ref 11. Copyright 2018 American Chemical Society.

Figure 13. CV of heme-AβC8SH (red), heme-AβC8SH + MB after instantaneous addition (green), heme-AβC8SH + MB after 6 h of incubation (blue), and AβC8SH + MB (dark green) in air-saturated pH 7 buffer. Adapted from ref 10. Copyright 2012 American Chemical Society.
bound to isolated small peptide clusters. In fact, the O₂ reduction cannot be inhibited by even 50% when heme-Aβ(1–40) is incubated with 500 μM MB for more than 6 h, whereas for small aggregates, 15 μM of MB is enough to inhibit ROS formation within 10 min and complete inhibition of O₂ reduction can occur within 6 h.

**INTERACTION WITH CYTOCHROME c**

CV experiment performed using the SAM of the three Aβ(1–40) mutants (G38C, L17C, and Y10C) in pH 7 phosphate buffer cannot elicit any redox response. In the presence of Cyt c also the bare Au electrode lacks any CV response. However, under similar reaction conditions, when 100 μM bovine heart Cyt c is present along with the above-mentioned Aβ SAMs, a reversible process is found independent of the Aβ assembly morphology. This corresponds to one-electron oxidation reduction of Cyt c, specifically involving the heme cofactor which represents an Fe³⁺/²⁺ redox couple. Here, it is the Aβ adlayers which make this electron transfer to the Cyt c heme group possible, hinting at an interaction between the peptide and the protein. Incidentally, a previous report of electron transfer from reduced heme-Aβ to oxidized Cyt c involving docking between the two oppositely charged partners also exists. Here too, a similar docking of Cyt c with the Aβ assemblies may be invoked due to complementary charge reciprocation between the two partners as Aβ has an overall charge of −3 while the Cyt c surface around the heme pocket is positively charged.²¹ The carboxylate groups of Aβ are also significant for this electron transfer process, which can be demonstrated using non-amyloid constructs having discrete terminal groups. When the C₃H SAM lacking any carboxylic group is employed, it does not give rise to a CV response in the presence of Cyt c unlike the case of carboxylic SAM, i.e., a SAM composed of 6-mercaptohexanoic acid deposited on Au electrodes where terminal carboxylate groups are present. The interaction of Aβ and Cyt c is purely electrostatic, which is in agreement with previous literature reports of the electrostatic nature of the interaction between Cyt c and heme-Aβ (in vitro study) as well as its other physiologically relevant oxidoreductase partners within the mitochondria of cell.

Cu²⁺ binding to the morphologically different assemblies of G38C, L17C, and Y10C mutants immobilized on Au surface results in the observation of a quasi-reversible Cu²⁺/¹⁺ process (Figure 15). These different E₁/₂ values may be attributed to either the difference in Cu binding sites or to the difference in the environment around the same binding site due to mutation at different positions of Aβ. The surface coverage values compare well with those reported for Cu bound to Aβ₃₅(C₃H)₉ assembles on Au electrodes, and considering the ~10¹⁴ molecules/cm² average surface coverage of thiol SAM, it can be said that the Cyt c sites present on the aforementioned Aβ SAM-modified electrodes are spread thinly. When Cu is bound to these Aβ assemblies, a quasi-reversible redox response possibly corresponding to the Cu²⁺/¹⁺ redox couple is found instead of that corresponding to the Fe³⁺/²⁺ redox couple for heme of Cyt c in case of the metal-free counterparts (Figure 16). However, here, the CV response is slightly different than that when Cyt c is absent. These results thus point toward the interruption of electron transfer to Cyt c from the electrodes due to binding of Cu to Aβ. Another possibility is that the Cu²⁺/¹⁺ redox process overlaps with and envelopes the Cyt c redox response. Hence, similar experiments using redoxinactive Zn²⁺ in place of Cu²⁺ were performed. The absence of a CV response when Zn²⁺-bound Aβ assemblies are present alongside Cyt c verifies that no electron transport to the Cyt c heme can take place from Aβ constructs in the Cu²⁺/Zn²⁺-bound state (Figure 17). To form the Zn-bound Aβ constructs, the Aβ aggregates bearing electrodes are incubated with the Zn²⁺ salt solution for 90 min prior to the CV experiments, implying that complete Zn²⁺ accumulation may take up to 90 min (Figure 18). Hence, CV response of the Fe³⁺/²⁺ couple of Cyt c is monitored at regular time intervals where the experimental setup includes the Aβ constructs, Cyt c, and Zn²⁺.

![Figure 14](https://example.com/figure14)

**Figure 14.** SERRS data of a single monolayer of heme-bound Aβ(C₃H)₉ (red) and heme-Aβ(C₃H)₉-bound MB (blue) on a Ag disk. The black line indicates the difference between the SERRS data of the heme-Aβ(C₃H)₉-MB complex and heme-Aβ(C₃H)₉ (A) High-frequency and (B) Low-frequency regions. Adapted from ref 10. Copyright 2012 American Chemical Society.

![Figure 15](https://example.com/figure15)

**Figure 15.** CV responses of Cu-G38C (orange)-, Cu-L17C (green)-, and Cu-Y10C (light blue)-modified Au electrodes in pH 7 at 20 mV/s using Ag/AgCl as reference and Pt wire counter electrodes. Adapted from ref 11. Copyright 2018 American Chemical Society.
salt. The CV response is observed to gradually diminish with time and vanish completely after a while. A possible explanation for this inhibition of electron transfer from the electrodes to Cyt\(c\) via the A\(\beta\) adlayers on metal binding may be that the carboxylate groups of six amino acids in the peptide chain that impart the negative charges on A\(\beta\) can no longer access the positively charged domain of Cyt\(c\) as a consequence of metal binding, thereby preventing the necessary docking between A\(\beta\) and the protein.\(^{11}\)

**CONCLUSION**

Functionalization or modification of electrode surfaces by introducing different chemical functionalities and screening the property of surface has been an active area of research for a while. The Au electrode surface has been used to study several biological molecules and proteins such as DNA, cytochrome \(c\), and cytochrome \(c\) oxidase. This review collates studies showing A\(\beta\) peptide immobilized on a Au electrode serving as nonbiological platforms that can stabilize metastable and physiologically relevant amyloid aggregates as well as allow the monitoring of the interaction of various potential AD-relevant

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**Figure 16.** Blue, violet, and dark green lines in (a), (b), and (c) illustrate the corresponding CV responses of Cyt\(c\) employing G38C-, L17C-, and Y10C-functionalized Au electrodes in pH 7 at 20 mV/s using Ag/AgCl reference and Pt wire counter electrodes. Further, red, light green, and light blue lines in (a), (b), and (c) indicate the corresponding CV responses of Cu-G38C, Cu-L17C, and Cu-Y10C constructs in the presence of Cyt \(c\) dissolved in pH 7 buffered solution. Adapted from ref 11. Copyright 2018 American Chemical Society.

**Figure 17.** Probable schematic representation of interaction of Cyt\(c\) with (a) G38C, (b) L17C, and (c) Y10C mutants of A\(\beta\)(1–40) on Au surfaces and inhibition of this interaction on metal binding. Adapted from ref 11. Copyright 2018 American Chemical Society.

**Figure 18.** Blue, violet, and dark green lines, respectively, in (a), (b), and (c) illustrate CV responses of Cyt\(c\) employing G38C-, L17C-, and Y10C-modified Au electrodes in pH 7 at 20 mV/s using Ag/AgCl reference and Pt wire counter electrodes. The corresponding light green, light blue, and red lines in (a), (b), and (c) represent CV responses of Zn-G38C, Zn-L17C, and Zn-Y10C constructs. Adapted from ref 11. Copyright 2018 American Chemical Society.
drug candidates with heme and Cu-bound Aβ aggregates. Using the absorption spectroscopy, CV, and SERRS, it is observed that depending on the aggregation state heme-Aβ has a different active site environment, where the population of high-spin ferric active sites is significantly higher in large aggregates and the population of low-spin ferric active sites is higher in small aggregates. It was also found that both large (mimicking the Aβ fibrils) and small (mimicking Aβ oligomers) aggregates of heme and Cu-Aβ produce significant amounts of ROS. Large heme-Aβ aggregates produce a higher amount of PROS than small aggregates, whereas small aggregates of Cu-Aβ produce a higher amount of PROS than large aggregates. At physiological potentials, while Cu-Aβ produces more ROS per mole of O₂ reduced than heme-Aβ, the latter is catalytically more competent than the former at reducing O₂. Inhibition of ROS formation by heme-Aβ or Cu chelation from Cu-Aβ is found to be more efficient for oligomers compared to large fibrils. Limited accessibility of the active site due to steric hindrance in the fibrillar forms may be a possible explanation. This makes the breakdown of the fibrils into oligomers before treatment with Cu/heme targeting drugs imperative.

This review also documents how three Aβ(1−40) mutants with cysteine introduced at different positions when embedded on the Au surface result in aggregates with different morphologies. These Aβ assemblies mediate the transfer of electrons from the electrode to the heme of bovine heart Cyt c. Such an electron transfer is absent in the case of bare gold electrodes. The positively charged domain of Cyt c around heme can likely dock with the negatively charged Aβ through electrostatic interaction. There are no prior reports of direct interaction between Cyt c and Aβ, though several reports have suggested efflux of Cyt c caused by mitochondrial membrane permeabilization by Aβ. Finally, Cu and Zn can arrest the interaction between Aβ and Cyt c, providing an opportunity for the mitigation of possible Aβ-induced ill effects toward mitochondrial Cyt c.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes
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