EGCG attenuates α-synuclein protofibril-membrane interactions and disrupts the protofibril

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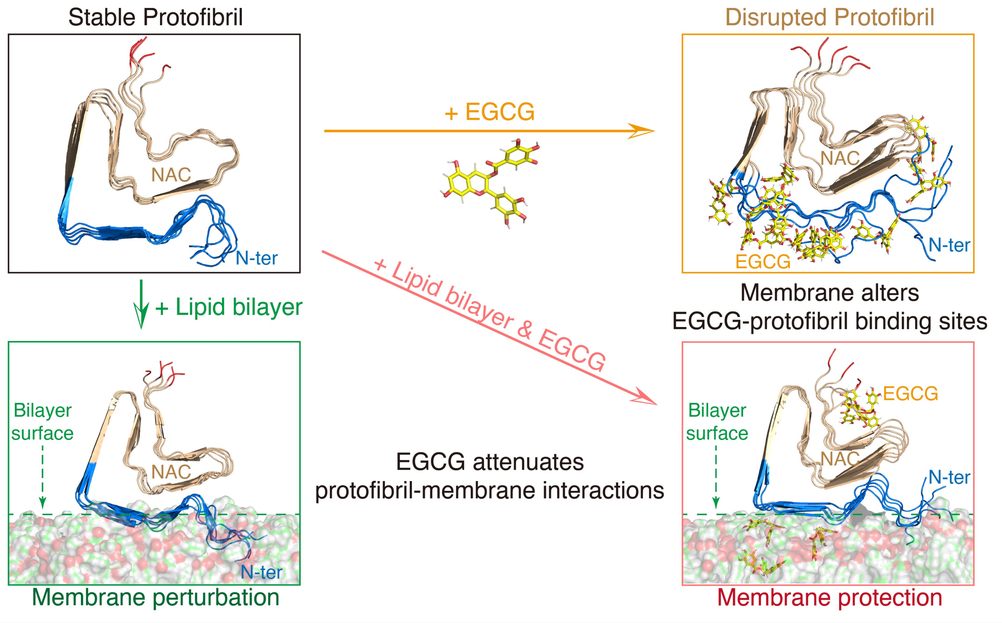
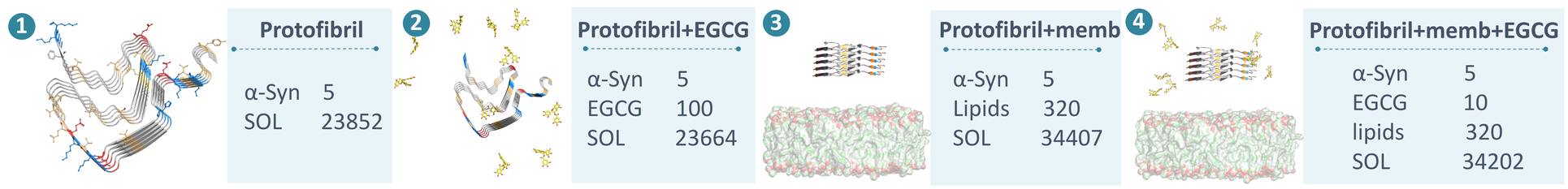
1 | Abstract

The fibrillary aggregates of α-synuclein (α-syn) are closely associated with the etiology of Parkinson's disease (PD). Mounting evidence shows that the interaction of α-syn with biological membranes is a culprit for its aggregation and cytotoxicity. While some small molecules can effectively inhibit α-syn fibrillization in solution, their potential roles in the presence of membrane are rarely studied. Among them, green tea extract epigallocatechin gallate (EGCG) is currently under active investigation. Herein, we investigated the effects of EGCG on α-syn protofibril (an intermediate of α-syn fibril formation) in the presence of a model membrane and on the interactions between α-syn protofibril and the membrane, as well as the underlying mechanisms, by performing microsecond all-atom molecular dynamics simulations. The results show that EGCG has destabilization effects on α-syn protofibril, albeit with a reduced destructive effect in the presence of membrane. Intriguingly, we find that EGCG forms overwhelming H-bonding and cation-π interactions with membrane and thus attenuates protofibril-membrane interactions. Moreover, the decreased protofibril-membrane interactions impede the membrane damage by α-syn protofibril and enable the membrane integrity. These findings provide an atomistic understanding towards the attenuation of α-syn protofibril-induced cytotoxicity by EGCG in cellular environment, which is helpful for the development of EGCG-based therapeutic strategies against PD.



2 | Materials and Methods



- Force Field: Amber99SB-ILDN
- Water model: TIP3P
- Temperature: 310 K
- Simulation time: 1000 ns
- NaCl concentration: 0.15M
- Box size: (10x10x10/16) nm^3

Figure 1. Initial structures of four simulation systems. α-Syn protofibril, EGCG and lipid bilayer are shown as cartoon, sticks and surface, respectively.

3 | Results

EGCG disrupts α-syn protofibril both in solution and at model membrane surface.

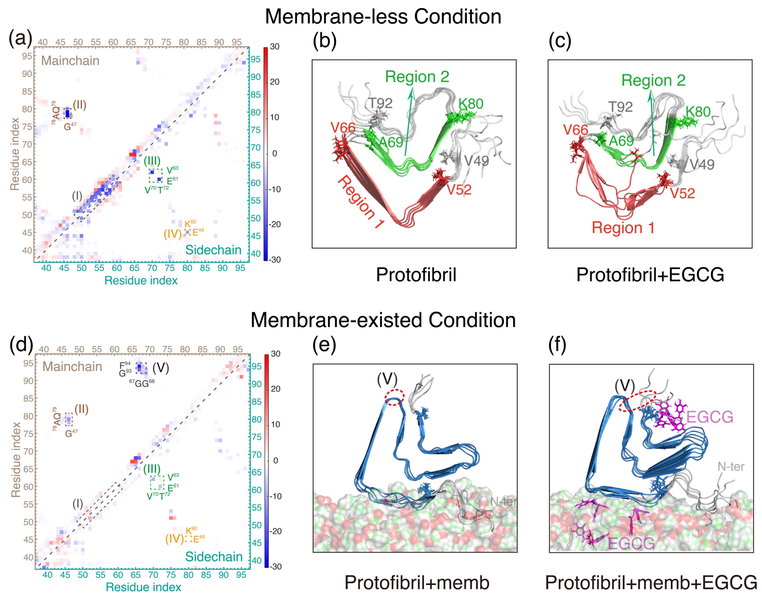


Figure 2. Disruptive effects of EGCG on α-syn protofibril in solution and at membrane surface. Differential residue-residue contact map of α-syn protofibril with/without EGCG in solution (a) and at membrane surface (d). Representative snapshots illustrating the disruptive effects of EGCG on α-syn protofibril in solution (b, c) and at membrane surface (e, f).

EGCG-membrane interactions alter the disruptive modes of EGCG on α-syn protofibril.

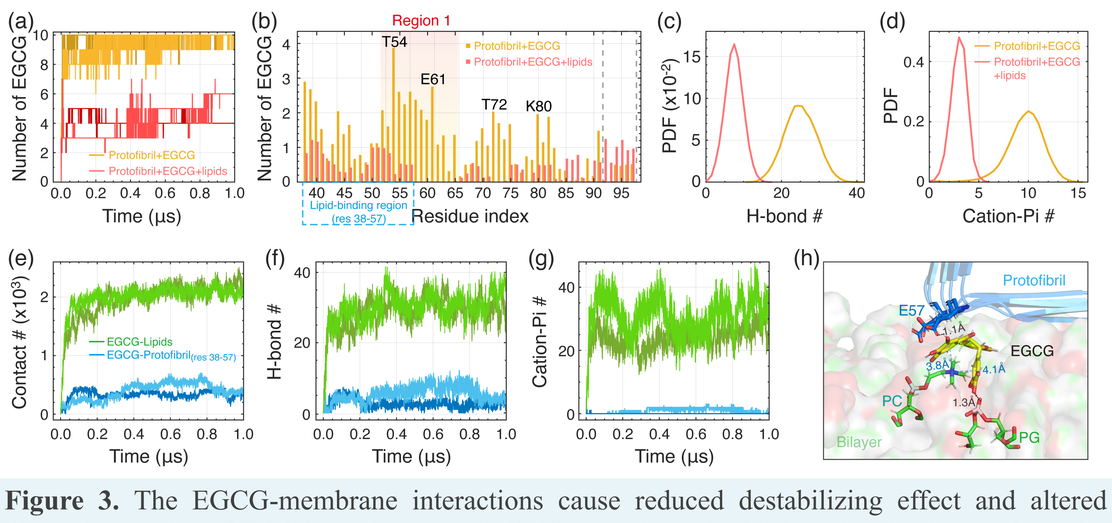


Figure 3. The EGCG-membrane interactions cause reduced destabilizing effect and altered disruptive modes of EGCG on the protofibril. The number of EGCG molecules interacting with α-syn protofibril (a) and with each residue (b) in the absence and presence of membrane. PDF of H-bond (c) and cation-π interaction (d) number. Atomic contacts (e), H-bond (f), and cation-π interaction (g) of EGCG-membrane and EGCG-protofibril. Snapshot (h) illustrating that EGCG is prone to form H-bonding and cation-π interactions with membrane than with α-syn protofibril.

EGCG-membrane interactions attenuate protofibril-membrane binding.

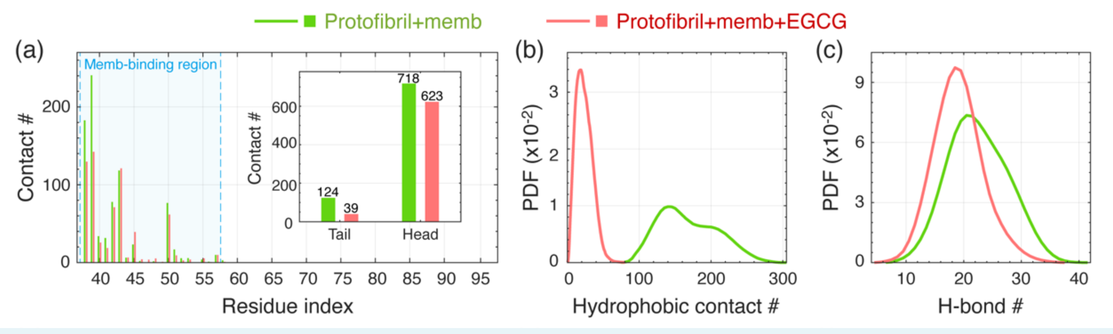


Figure 4. Analysis of α-syn protofibril-membrane interactions in the absence and presence of EGCG. The contact number between membrane and each α-syn residue (a). The inset shows that contact number of the protofibril with the tail and head groups of the lipid bilayer. The PDF of nonpolar contact numbers (b) and H-bond numbers (c) between the protofibril and the membrane.

EGCG suppresses α-syn protofibril-induced membrane perturbation and damage.

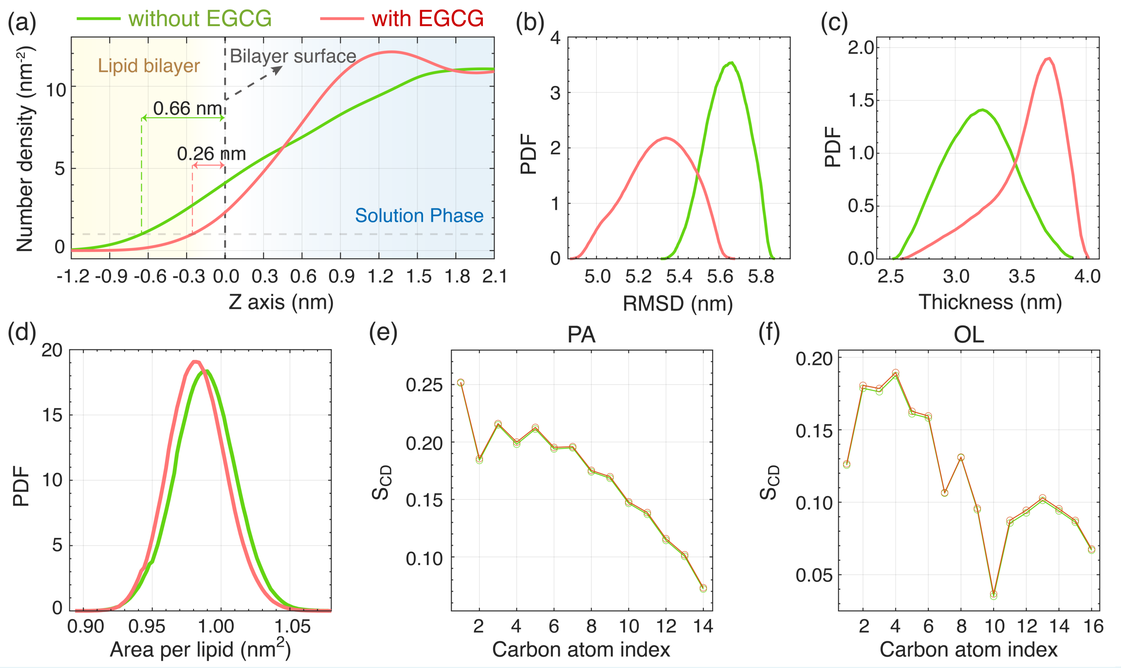


Figure 5. The role of EGCG on the integrity of membrane in the presence of α-syn protofibril. The atom number density profile of α-syn protofibril along the z-axis (a). The PDF of RMSD (b) and thickness (c) of the lipid bilayer. PDF for area per lipid of the membrane in the absence and presence of EGCG (d). S_{CD} of carbon atoms in the PA (e) and OL (f) tail groups of phospholipid molecules.

4 | Conclusions

In the absence of membrane, EGCG has the ability to disrupt α-syn protofibril, especially in the two β-structure regions (residues 52-66 and residues 69-80). In the presence of membrane, EGCG also displays a destabilization effect on α-syn protofibril, albeit to a lesser extent than the membrane-free condition. Interaction analyses reveal that in aqueous solution EGCG disrupts α-syn protofibril primarily by binding to residues 52-66, while in membrane-existed environment, EGGC achieves its destabilization effect mainly by binding the C-terminal residues 92-97. Moreover, we found that EGCG forms overwhelming H-bonding and cation-π interactions with the membrane, weakens the H-bonding and hydrophobic interactions between the protofibril and membrane, and thus leads to the attenuation of the membrane-binding of α-syn protofibril. This would inhibit the α-syn protofibril-induced membrane damage. Our study reveals for the first time at the atomic level the mechanism underlying the protofibril disruption and membrane protection by EGCG. This finding suggests that EGCG could act as a reducer of α-syn protofibril related cytotoxicity and provides realistic insights into the development of future treatments for PD and other synucleinopathies.