D-7	Establishing high-temperature reversible oligomerization strategy and amyloidogenicity of PSD95-PDZ3 variants by reverse engineering					
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[Introduction] The thermal denaturation of most small globular proteins usually exhibits two-state thermal denaturation (N↔D). Unlike typical small proteins, wild-type PSD95-PDZ3, a small third PDZ domain protein of post-synaptic density-95 protein (PSD-95), undergoes a peculiar three-state thermal denaturation (N↔I_n↔D). It forms a reversible oligomerization (RO) at high temperatures relevance amyloidogenicity. We previously reported that a point mutation, F340A, fully inhibits the formation of the high-temperature RO, concurrently amyloidogenesis. Here, we used PDZ3-F340A as a template protein to establish RO and amyloid formation strategy according to a "reverse engineering".

[Materials and methods] We generated the artificial structures using COOT software and calculated the accessible surface area (ASA) by DSSP analysis. We produced three variants (R309L, E310L, and N326L), where we individually mutated residues that are surface exposed in the monomeric structure but buried in the tetrameric structure to a leucine. The PDZ3 variants was mutated and expressed in BL21(DE3) strain of *E. coli*. The physicochemical and biophysical characterizations were observed using several spectrometers including, circular dichroism (CD), differential scanning calorimetry (DSC), Fluorescence spectroscopy and analytical ultracentrifugation (AUC).

[Results and discussion] The secondary structures of all three variants were maintained as examined by circular dichroism. Consequently, PDZ3-F340A N326L produced RO at high temperatures and unfolded according to a three-state thermal denaturation **(Fig.1)**. In contrast, other two variants unfolded according to two-state model as assessed by differential scanning calorimetry. The molar fraction of PDZ3-F340A N326L exhibit intermediate fraction at high temperature similar to PDZ3-wt **(Fig.2)**. The thermodynamic parameters that calculated from CD thermal denaturation corelated to values of intermediate state (N-I_n and N-D) that calculated from DSC data. Moreover, PDZ3-F340A N326L exhibited amyloidogenic at high temperatures as assessed by Thioflavin T fluorescence. It indicated that PDZ3-F340A N326L was a precursor for amyloid fibril formation after seeding at high temperature **(Fig.3)**.

[Conclusion] This reverse engineering experiment demonstrates the ability to control the formation of high-temperature RO and consequent amyloidogenicity by single amino acid mutation. A point mutation strategy can apply for controlling the thermal stability and understanding kinetics of RO and amyloidogenicity in small globular proteins.

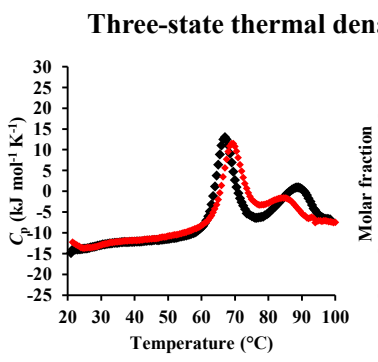

Fig.1 DSC thermogram of

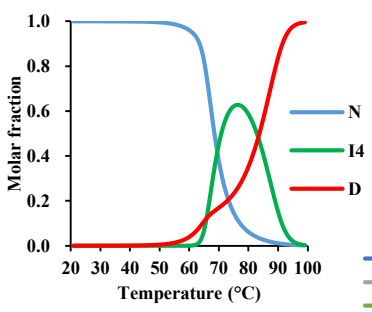

Fig.2 Molar fraction of

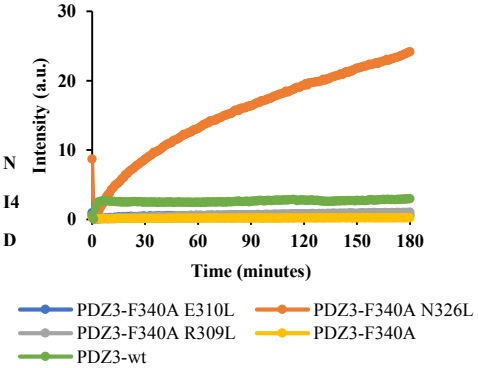

Fig.3 ThT fluorescence of PDZ3