Conference

# The role of stereochemistry in the inhibition of A$\beta$ Amyloid growth and toxicity by silybins.

Michele. F. M. Sciacca,[a,*] Valeria Romanucci,[b] Armando Zarrelli,[b] Irene Monaco,[a] Fabio Lolicato,[c] Natalia Spinella,[d] Clelia Galati,[d] Giuseppe Grasso,[e] Luisa D'Urso,[e] Margherita Romeo,[f] Luisa Diomede,[f] Mario Salmona,[f] Corrado Bongiorno,[g] Giovanni Di Fabio,[b] Carmelo La Rosa,[e] Danilo Milardi.[a]

The self-assembling of the amyloid $\beta$ (A$\beta$) is considered an hallmark in the pathogenesis of Alzheimer's disease (AD). Many efforts have been devoted in designing molecules able to halt disease progression by inhibiting A$\beta$ self-assembly. We combine biophysical, biochemical and computational techniques to investigate the capacity of four optically pure components of the natural product silymarin (silybin A, silybin B, 2,3-dehydrosilybin A, 2,3-dehydrosilybin B) to inhibit A$\beta$ aggregation. TEM analysis demonstrated that all the four investigated flavonoids prevent the formation of mature fibrils, however ThT assays, WB and AFM investigations showed that only silybin B was able inhibit the formation of small protofiber (considered the most toxic species) diverting the aggregation toward the formation of large amorphous aggregates. By using molecular dynamics (MD) simulations we observed that silybin B interacts mainly with the C-terminal hydrophobic segment $^{35}$MVGGVV$^{40}$ of A$\beta$40 and the peptide conformation remains predominantly unstructured along all the simulations. By contrast, silybin A interacts preferentially with the segments $^{17}$LVFF$^{20}$ and $^{27}$NKGAII$^{32}$ of A$\beta$40 which shows a high tendency to form bend, turn, and $\beta$-sheet conformation in and around these two domains. Both 2,3-dehydrosilybin enantiomers bind preferentially the segment $^{17}$LVFF$^{20}$ but lead to the formation of different small-sized, ThT-positive A$\beta$ aggregates. Finally, in vivo studies in a transgenic *Caenorhabditis elegans* strain expressing human A$\beta$ indicated that silybin B is the most effective of the four compounds in counteracting A$\beta$ proteotoxicity. This study underscores the pivotal role of stereochemistry in determining the neuroprotective potential of silybins and points to silybin B as a promising lead compound for further development in anti-AD therapeutics.

[a] *CNR - Istituto di Cristallografia, Via Paolo Gaifami 8, Catania, Italy*
[b] *Department of Chemical Sciences, University of Napoli "Federico II", Via Cintia 4, Napoli, Italy*
[c] *Department of Physics, University of Helsinki, P.O. Box 64, Helsinki, Finlan*
[d] *STMicroelectronics, Stradale Primosole 50, Catania, Italy*
[e] *Dipartimento di Scienze Chimiche, Università degli Studi di Catania, Viale Andrea Doria 6, Catania, Italy*
[f] *IRCCS-Istituto di Ricerche Farmacologiche "Mario Negri", Via Giuseppe La Masa 19, Milano, Italy*
[g] *CNR - Istituto di Microelettonica e Microsistemi, Stradale Primosole 50, Catania, Italy*

† oral communication at 1 st Conference on Crystallography, Structural Chemistry and Biosystems, (Catania) 04-06/10/2021