# Study of conformational changes of Tau protein assembles in presence of synthetic peptides by Atomic force microscopy

*F.Mokhtari[1], Gh. Riazi[1], H. Akbari Moayyer[2], S.A Shahmoradi Zavareh[2], M. R. Lornejad[3]*

1- Bioorganic lab, Institute of biochemistry and Biophysics , University of Tehran , Iran
2- Ara-research Company, Tehran, Iran
3- BioMed-zet Life Science GmbH, Linz, Austria

mokhtari@ibb.ut.ac.ir, info@ara-research.com , Lornejad@googlemail.com

## Aim

Tau protein assembly is a critical mediator in the pathology of AD and many neurodegenerative diseases. Recently many drugs is targeting toxic species of tau aggregates named oligomers.
The aim of this study was investigation of tau protein assembly species (oligomers and fibrils) in the presence some synthetic peptides.

## Background

Numerous neurodegenerative diseases such as Alzheimer are represented by an assembly of Tau protein. Tau is a microtubule (MT) – associated proteins and the main role of tau is modulating MT dynamics for controlling axonal transport in neurons.

During pathogenesis, tau protein aggregates into intracellular oligomeric forms and filamentous or neurofibrillary tangles (NFTs) abnormally. Recent studies indicated that oligomers are toxic forms of Tau in progress of neurodegenerative disease. Tau oligomers might exist in dimmers, multimers and granules.

In this regards, inhibition of tau aggregation has been documented to be a potent therapeutic target in AD and tauopathies. Unfortunately, the current developed synthetic drugs have modest beneficial efficacy with several side effects. Therefore, suggested molecules from natural sources with anti-aggregation properties can be useful in the prevention and treatment of AD.

In other hand, physiochemical, physiological and many properties of peptides opened a new hrizone in drug design and discovery recently,
Based on, Based on, we design and synthesis three short peptides with the ultimate goal to have an impact on tau aggregation.

## Methods

- ***Preparation of Peptides***

The synthesis of peptides were carried out using Wang resin following standard Fmoc strategy. Produced peptides named pn-8, ps-9 and pnos-6. The peptides are analyzed by mass spectrometry and their sequences confirmed with mass spectophometry using LC/MSS Agilent QQQ 6410 **(figure 1)**

- ***Recombinant tau protein expression and purification***

Expression and purification of tau protein done based on our previous work with minor modification(Khalili et al., 2014). Briefly, *E.coli* strain BL21 (DE3) infected with pET-21a vector including human tau 1N/4R gene (*htau34*). Recombinant tau was purified via a succession of Ni-NTA-Agarose precipitation (equilibrated in 10mM HEPES, 100mM NaCl, and 15mM imidazole, pH 7.4) and eluted with 80mM imidazole. The concentration of purified tau was determined using the OD 280 nm with extinction coefficient 7700$M^{-1}cm^{-1}$ and the purity of the protein was verified with SDS-PAGE gel electrophoresis **(figure 2).**

- ***Atomic force microscopy (AFM)***

Tau protein aggregation induced by heparin in 37°C for 7 days. Control samples and treated samples with peptides were monitored by ARA-AFM (AFM manufactured by Ara-Research Company, Tehran, IRAN). AFM observation data were collected for 100 µM samples at similar incubation times and room temperature using a HQ:NSC15/Al BS silicon probe cantilever from Mikromasch Company **(figure 3).**

## Results

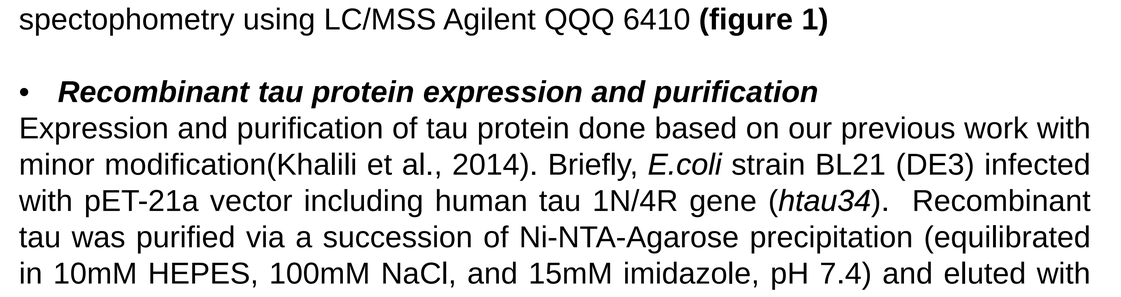


**Figure 1**. Mass spectrometry data of synthesized a) pn-8 and b) ps-9 peptides (range 50-2000) with LC/MSS Agilent QQQ 6410. The observed mass weights confirmed both peptide sequences

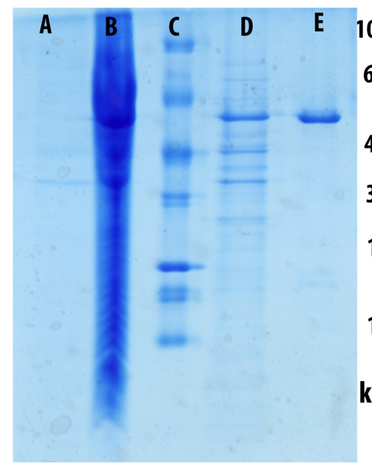


**Figure 2**. Expression and purification of Tau 412 AA (*htau 34*); The samples mixed with SDS- sample buffer and separated on 12% SDS-PEGE gel and stained with Coomassie Brilliant Blue R250. The tau monomer band is in the range of 48 to 63 kDa. A: supernatant of cells, B: After IPTG- induction, C: Markers D: Tau 412 AA (*htau 34*), E: tau after purification with Ni-NTA-Agarose precipitation.

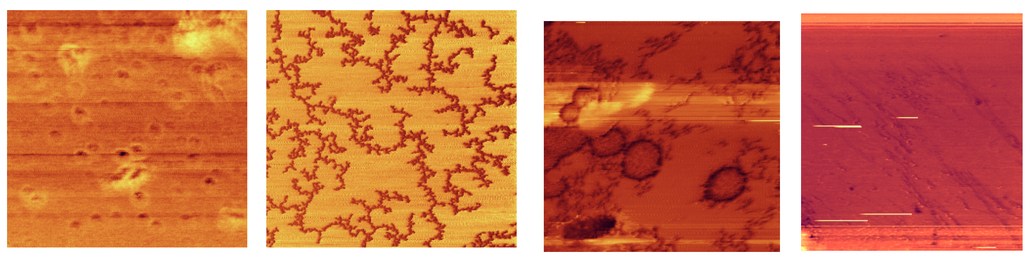


**Figure 3**. AFM images in phase mode. a) Control tau protein , b) Tau+pn-8 c) Tau+ps-9 d) Tau+pnos-6

## Results and Discussion

As shown in Figure 2, the tau protein 412 amino acid (*htau 34*) is the major protein expressed after the induction with IPTG (lane B) compare with before IPTG-induction (lane A) and confirmed a considerable dense band in 48-63 kDa.

Phase images of the samples were processed using Imager software-c1.01 and available in the Figure 3. The results indicate that in the presence of pn-8 tau assembles restricted in oligomer granules and this peptide promotes tau filaments. In addition tau aggregation in the presence of ps-9 and pnos-6 showed conformational changes in comparison to the control.
Therfore, results revealed that tau protein under the fibrillation condition in the presence of pn-8 and ps-9 had enough intencity to aggregate in filbril forms. Pn-8 and ps-9 inhibited the tau toxic forms (oligomers) with $IC_{50}$ of 5µM. Furthermore, this result confirmed with other biochemical techniques ( data not shown )

## Conclusion

Based on our results , tau aggregation in the presence of ps-9 and pnos-6 showed conformational changes and oligomer species as toxic forms reduced significantly, thus these peptides are good candidates for starting some studies for tau- directed drug discovery in therapy of tauopathies and other neurodegeneration diseases.

## Reference:

- Y. Ren and N. Sahara “Characteristic of Tau oligomers”. Front Neurol. 2013; 4:102.
- N. Sahara, J.Avila ““Tau Oligomers,” What We Know and What We Don’t Know”. Front Neurol. 2014; 5: 1.



***Contact address: E-mail: lornejad@zet.or.at**