

Chapter 6

Conclusions and future perspectives

In conclusion, in this thesis are reported significant contributions to the field of AD research on two fronts. Firstly, this research has explored the potential of the identification of large protein aggregates in the CSFs of AD patients and the evaluation of their ability to induce an increase of intracellular Ca^{2+} levels as novel biomarkers for an early diagnosis of AD. Through rigorous biochemical, biophysical and cellular analyses, this study provided compelling evidence for these two novel biomarkers to be able to discriminate CSFs derived from AD patients from control subjects, legitimating their use for AD diagnosis, also in combination with classical biomarkers, to improve their diagnostic accuracy. Secondly, this study underlies the importance of sdAbs raised against A β oligomers as promising tools for the early diagnosis and therapy of AD. By meticulously characterizing their binding properties, demonstrating their efficacy in mitigating A β -induced toxicity *in vitro* and their ability to discriminate between AD CSFs and CSFs derived from age-matched control subjects, this work lays a strong foundation for the advancement of sdAb-based diagnosis and therapy. Despite these promising results, these studies have some limitations. Indeed, it will be important to apply all the studies reported in this thesis to larger and/or other cohorts to corroborate our experimental evidence. It will also be important to assess whether these two novel biomarkers are extendable to plasma samples, usable as prognostic tools, in addition to diagnostic. Finally, it will be important to assess whether they are exploitable for early diagnosis in the preclinical phase of AD, on the grounds that a defective PN is an early event in protein misfolding diseases and a cause of the formation of amyloid plaques and neurofibrillary tangles detectable with classical biomarkers. Additional studies are also required for sdAbs, that should be again tested for their ability to detect A β oligomers in larger cohorts of AD patients, as previously reported. Further investigation

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utilizing animal models or human-relevant cellular models, such as induced pluripotent stem cells (iPSCs), is also crucial. These models offer the distinct advantage of retaining patient-specific genetic backgrounds, enabling the evaluation of these sdAbs in a context that closely mirrors the complexities of human brain cells and facilitates translationally relevant insights.

Altogether, these studies on the research of new putative biomarkers and the testing and engineering of sdAbs as promising tools for an early diagnosis and therapy of AD, represent a crucial step towards a future in which this devastating disease for patients and families, will be only a distant fading nightmare.