A NOVEL MULTIPLEXING ASSAY TO DETECT BOTH β- AND γ-CLEAVAGES OF THE AMYLOID PRECURSOR PROTEIN FROM A SINGLE SAMPLE

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Introduction: Alzheimer’s disease is characterized by the accumulation of extracellular amyloids resulting from the sequential proteolytic processing of the β-amyloid precursor protein (APP) employing β- and γ-secretases.

Aims: To develop a simple and sensitive method to quantify β- and γ-secretase cleavages of APP from the same sample for screening purposes.

Methods: The electrochemiluminescence detection uses SULFO-TAG labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of microplates. In such a multi-array 384-well plate, four spots are spotted onto a single well, two with BSA, one with a capture antibody against Aβ and the other against the β-cleaved ectodomain of APP. The sample and the SULFO-TAG-labeled detection antibody are co-incubated and the detection is made by capturing the light intensity emitted. This allows quantitative determination of Aβ and sAPPβ levels from single sample.

Results: For the first time we show that it is possible to measure sAPPβ and Aβ contemporaneously from a single sample. This method is very sensitive, fast (requires only 4 hours) and highly reproducible. The signal is easily distinguishable from the background. Addition of specific β- or γ-secretase inhibitors dramatically decreased the amount of sAPPβ/Aβ40 and Aβ40, respectively demonstrating the potential of this assay to specifically quantify both amyloidogenic cleavages of APP.

Conclusions: We present a fast method for quantification of β- and γ-cleavage from the same sample in a novel multiplexing assay. The method is reproducible, very sensitive and can be used as a novel read out for screening of inhibitors and modulators.