

INTRA-AMYLOID pH SENSING AND LIGHT-INDUCED FLUORESCENCE ENHANCEMENT OF K114 IN ALZHEIMER'S DISEASE USING HYPERSPECTRAL CONFOCAL MICROSCOPY

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INTRODUCTION

Protein aggregation is a pathological hallmark of Alzheimer's disease (AD) and many other neurodegenerative diseases. Small organic fluorophores such as Congo Red preferentially bind to crossed-beta-sheet-rich deposits and have been used to label amyloid plaques and tau tangles to study structural heterogeneities in histological samples from AD patients. Here, we explore the physicochemical properties of an amyloid-specific dye (trans, trans)-bromo-2,5-bis(4-hydroxystyryl)benzene (K114) [1] on silkworm and tarantula silks (prototypical amyloids), along with an AD (5xFAD) mouse model, to augment quantitative spectral imaging of protein aggregates in tissues.

METHODS AND RESULTS

Using *Bombyx mori* (silkworm) and *Grammostola rosea* (tarantula) silk, as well as 5xFAD mouse brain sections, we examined the effect of pH and excitation intensity on the emission spectrum and intensity of fluorescence of amyloid-bound K114. When bound to beta-sheet-rich assemblies, the emission spectrum of K114 was governed by the nano-pH of the binding pockets within the fibrils, more so than by the pH of the mounting medium. Emission spectrum differed markedly for each type of amyloid which could be explained by a diversity of binding sites and their amino acid composition in amyloid plaques vs silks (Fig 1A). Unexpectedly, exposure to high excitation power caused permanent increases in fluorescence intensity of the amyloid-bound K114, as well as a blue-shift of its emission spectrum (Fig. 1B). The degree of fluorescence enhancement and emission shift depended on the laser power, exposure time, pH and the amyloid type examined.

Both phenomena of nano-pH sensing and increase in fluorescence upon light exposure (photoconversion) were also observed in formalin-fixed human Alzheimer's sections stained with K114, indicating that this may be a general phenomenon characteristic of amyloids. Altogether, our findings show how complex physicochemical interaction of the dye molecules with amyloid aggregates could be used for improved detection and differentiation of protein aggregates.

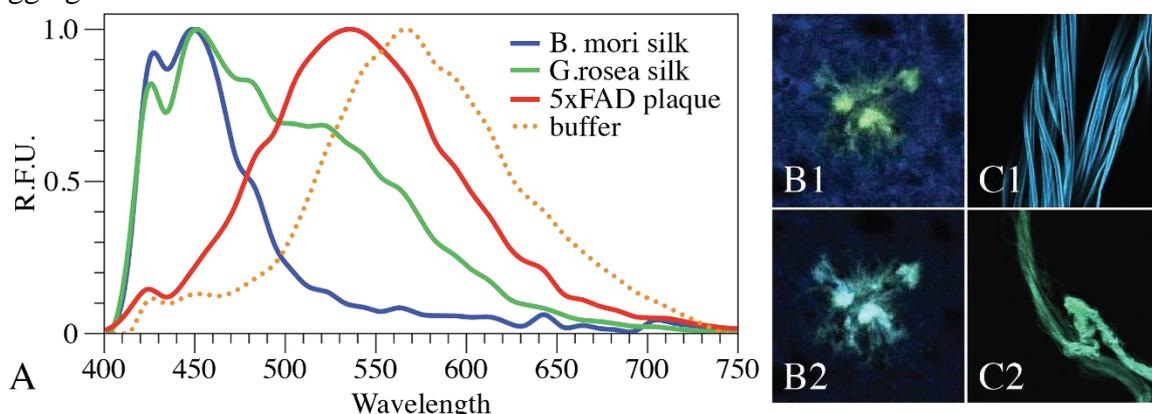


Figure 1. (A) Emission spectra of K114 at pH 11 in various amyloids and free solution. (B1) Truecolour images of 5xFAD amyloid plaque before and after (B2) high power excitation light exposure. (C1) Truecolour images of *Bombyx mori* (silkworm) and *Grammostola rosea* (tarantula) (C2) silk stained with K114 at pH 11.

REFERENCES

[1] Levine, Harry. Mechanism of A (1 - 40) Fibril-Induced Fluorescence of (Trans , Trans) -1-Bromo-2 , 5-Bis (4-Hydroxystyryl) Benzene (K114). *Biochemistry*. 44, 48 pp. 15937-43 (2005).