

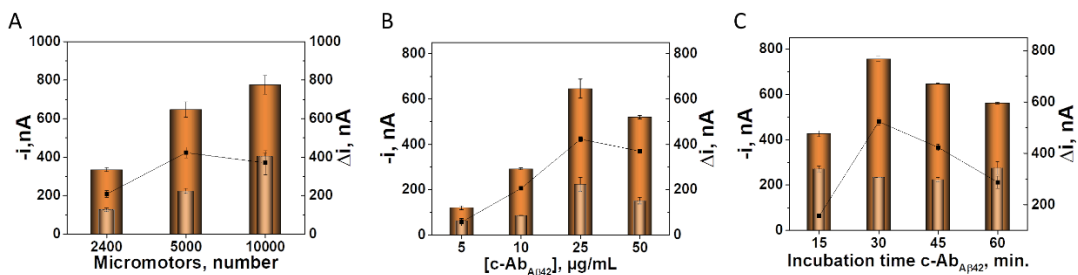
# Micromotor-based electrochemical immunoassays for reliable determination of amyloid- $\beta$ (1-42) in Alzheimer's diagnosed clinical samples.

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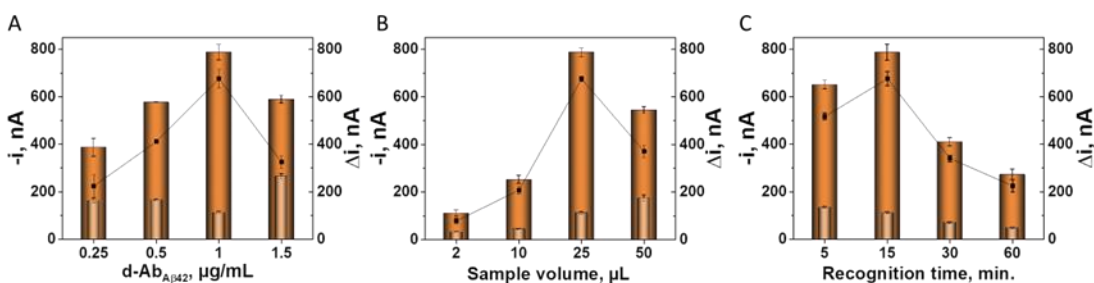
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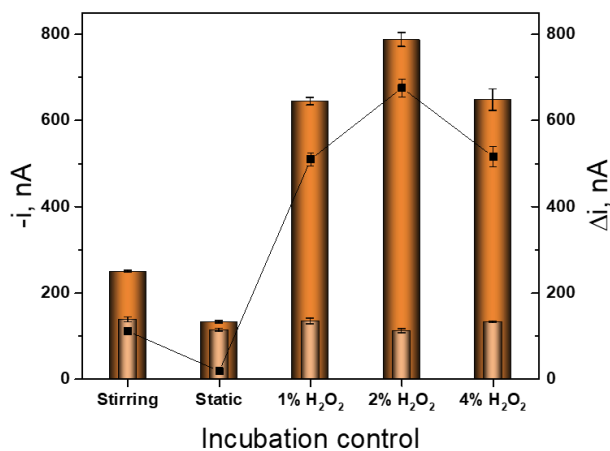
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**Figure S1.** Optimization of the MM functionalized variables: number of MM (A) c-Ab<sub>A $\beta$ -42</sub> concentration (B), c-Ab<sub>A $\beta$ -42</sub> incubation time (C). Dark orange: *on-the-fly* immunoassay (A $\beta$ -42, 50 ng/mL, d-Ab = 1  $\mu$ g/mL). Light orange (controls without A $\beta$ -42, d-Ab = 1  $\mu$ g/mL).



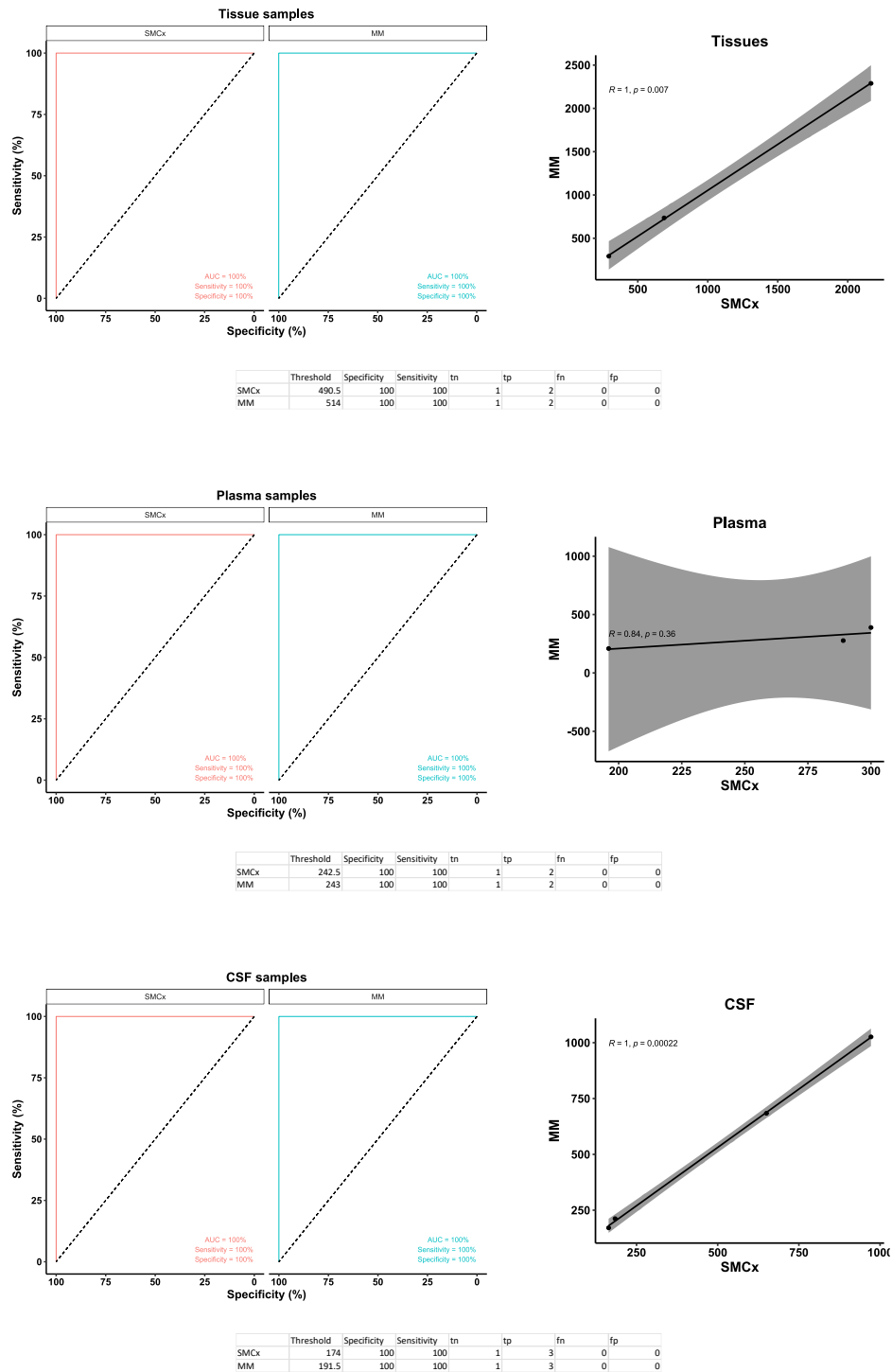
**Figure S2.** Optimization of *on-the-fly* immunoassay variables: d-Ab<sub>A $\beta$ -42</sub> concentration (A), sample volume (B), recognition time (C). Dark orange: *on-the-fly* immunoassay (A $\beta$ -42, 50 ng/mL). Light orange (controls without A $\beta$ -42).



**Figure S3.** Incubation controls for A $\beta$ -42 biosensing (stirring, static, bubble propelled). Conditions: (see Table 1). Dark orange: *on-the-fly* immunoassay (excess of A $\beta$ -42). Light orange (controls without A $\beta$ -42).

**Table S1.** Analytical performance of MM based immunoassay.

Analytical Characteristic	Value
Linear range	0.2-50 ng/mL
r	0.997
LOD	0.06 ng/mL
LOQ	0.2 ng/mL
EC <sub>50</sub>	1.93 ng/mL
RSD intra-assay (%) (n=5)	<5%



**Figure S4.** Diagnostic potential and correlation of the MM and SMCx technology. ROC curves were used to assess the diagnostic potential of the MM in comparison with SMCx. AUC, sensitivity, and specificity of each platform was calculated for the discrimination between healthy samples (controls) and AD patients for brain tissue, plasma, and CSF samples. The threshold in  $\text{pg mL}^{-1}$  to discriminate between groups is depicted in the figure, together with the number of true negative (tn), true positive (tp), false negative (fn), and false positive (fp) samples. Right, correlation between the MM and SMCx results for the quantification of  $\text{A}\beta\text{-42}$  in brain tissue, plasma, and CSF samples. R; Pearson correlation. p, p-value.