

Supplementary information to accompany

**On-chip microtubule gliding assay for parallel measurement of Tau protein
species**

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1. Supplementary tables

Table S1. Microchannels efficiently guide MTs to MT collectors. Out of the total 143 MTs gliding in microchannels, all of them glided towards collector regions without making a U-turn. As we found 8 MTs exiting to microchannels from 18 collectors, 94.5% of MTs remained in the collectors once they entered. MTs were counted in 18 assay regions in three independent devices.

Number of MTs gliding in microchannels from reservoirs.	Number of MTs making U-turn in microchannels.	Number of MTs reached collectors from microchannels.	Number of MTs exiting from collectors to microchannels.
143	0	143	8

Table S2. Overhang structure confines MTs in the assay region. MTs gliding at the periphery of the assay region were evaluated. None of them was able to climb the overhang structure in 1 min, and they kept gliding in the assay region. MTs were counted in 18 assay regions in three independent devices

Number of MT gliding at the periphery of the assay region.	Number of MTs gliding out of the assay region.
42	0

Table S3. Summary of tau proteins evaluated in our study.

Tau protein construct	Length	MW (kDa)	Nos of amino inserts/repeat	Total charge	MT binding function
2N3R	410	42.6	2N/3R	+1.6	Efficient binding ¹
2N4R	441	45.9	2N/4R	+4.8	~3 fold greater binding than 3Rtau ¹
V248L	441	45.9	2N/4R	+4.8	Not reported
G272V	441	45.9	2N/4R	+4.8	Medium loss of binding affinity ²
V337M	441	45.9	2N/4R	+4.8	Medium loss of binding affinity ²
P301L	441	45.9	2N/4R	+4.8	Great loss of binding affinity ²
R406W	441	45.9	2N/4R	+3.8	Mild loss of binding affinity ²

2. Supplementary figure

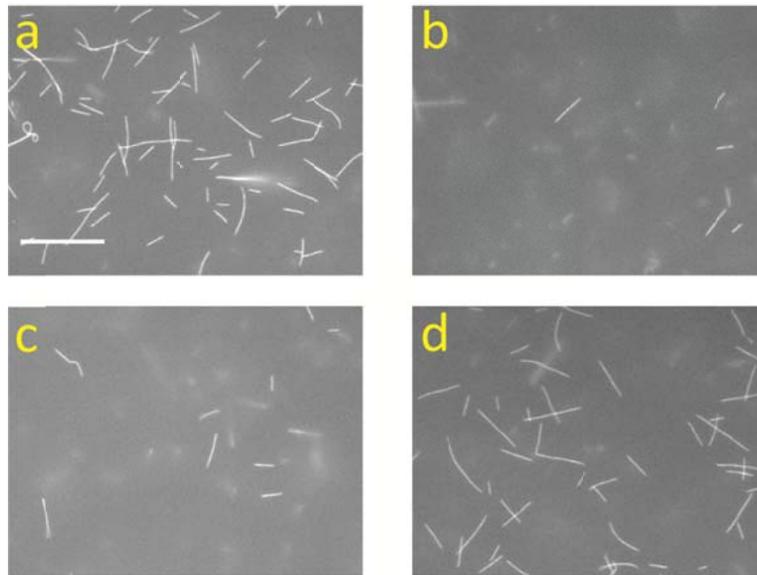


Fig. S1. Fluorescent images of MTs bound to kinesin-coated assay regions. a) no tau-MTs, b) 2N4R-MTs, c) 2N3R-MTs, and d) P301L-MTs. Scale bar, 20 μm .

Reference

1. B. L. Goode, M. Chau, P. E. Denis and S. C. Feinstein, *Journal of Biological Chemistry*, 2000, 275, 38182-38189.
2. M. Hong, V. Zhukareva, V. Vogelsberg-Ragaglia, Z. Wszolek, L. Reed, B. I. Miller, D. H. Geschwind, T. D. Bird, D. McKeel, A. Goate, J. C. Morris, K. C. Wilhelmsen, G. D. Schellenberg, J. Q. Trojanowski and V. M. Y. Lee, *Science*, 1998, 282, 1914-1917.