Supporting information

Real-Time Fast Amyloid Seeding and Translocation (RT-FaST) of α-synuclein using nanopipette

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1. Characterization of Nanopipettes

Table SI-1 : List of nanopipettes used in this work

Pipette number	Experiments	Current at V= 500 mV (nA)	Conductance at NaCl 1M (after L-Dopa coating, nA/V)
1	Control	2	2.83
2	Control	3	1.26
3	Control	0.9	1.16
4	Control	1.5	1.2
5	Control	2.3	1.92
6	Control	2.3	1.77
7	Control	1.3	1.72
8	seeds WT (200 pM)		
9	seeds WT (200 pM)	2.75	1.52
10	Seeds WT (200 pM)	0.9	
11	seeds WT (200 pM)	0.8	4.45
12	seeds WT (200 pM)	0.8	1.93
13	Seeds A53T (200 pM)	1	0.93
14	seeds A53T (200 pM)	1.20	886
15	seeds A53T (200 pM)	0.8	1.27
16	Seeds A53T (200 pM)	1.5	1.23
17	seeds WtT (20 pM)	1.25	1.59
18	seeds WtT (20 pM)	1	1.08
19	seeds WtT (20 pM)	0.6	2.96
20	seeds WtT (2 pM)	1.1	1.42
21	seeds WtT (2 pM)	1.1	1.25
22	seeds WtT (2 pM)		1.15
23	seeds WtT (2 pM)	2.65	0.86

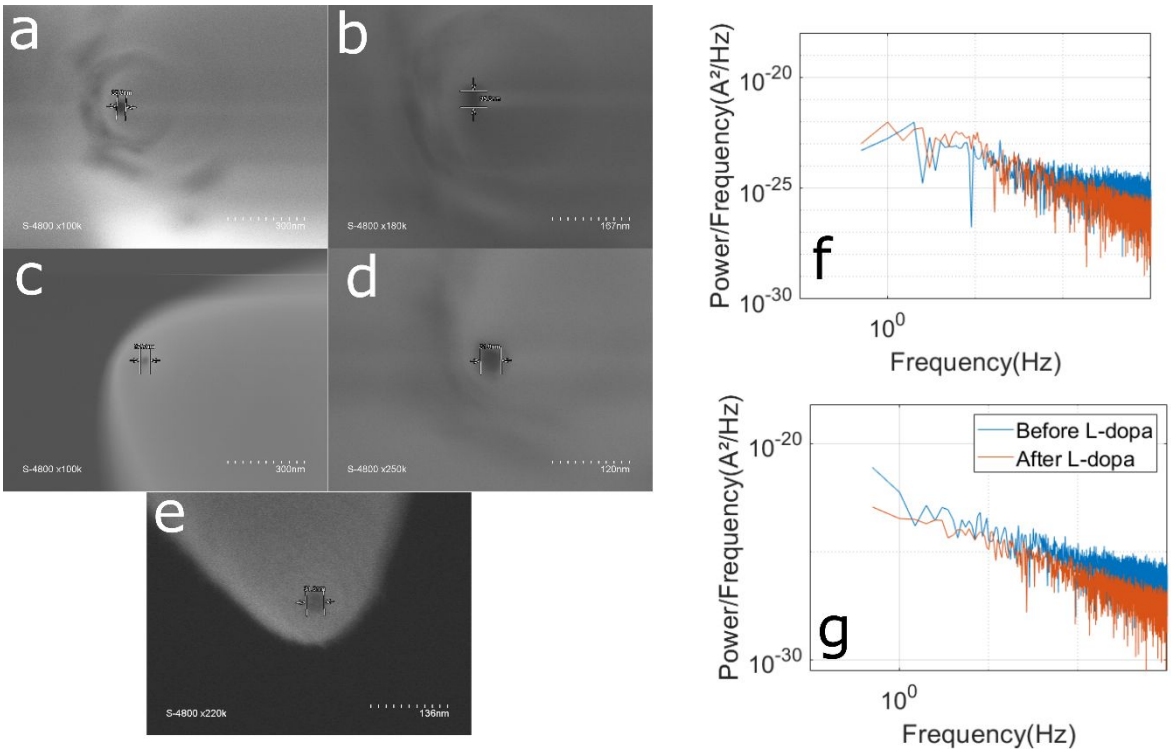

Figure SI-1: MEB images of the tip aperture of five individual nanopipettes show a diameter of (a) 36 nm (b) 35 nm, (c) 38 nm, (d) 32 nm and (e) 32 nm. (f-g) power spectral density of the nanopipette recorded before and after coating with L-DOPA.

2. Characterization of α-synuclein seeds after 180 min incubation at 37°C

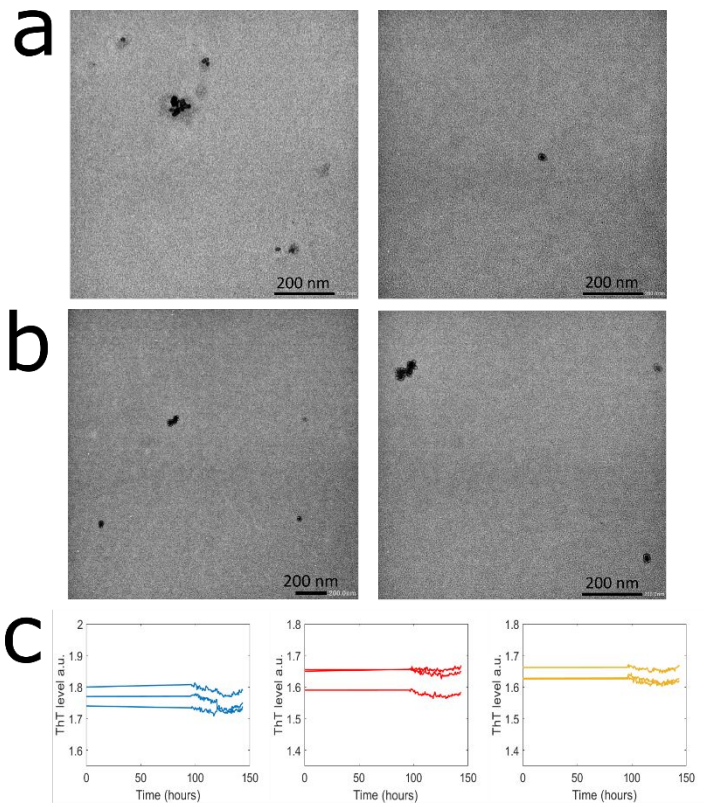

Figure SI-2: TEM images of α-synuclein aggregates WT (a) and A53T (b) used for the seeding in nanopipettes. (c) Kinetic characterization of α-synuclein aggregation at 4 µM 37° C without (blue) and with WT (red) and A53T (yellow) seeds obtained using ThT intercalation. Here, the result does not allow evidencing the presence of β-sheet structure.

3. Additional results for RT-FAST

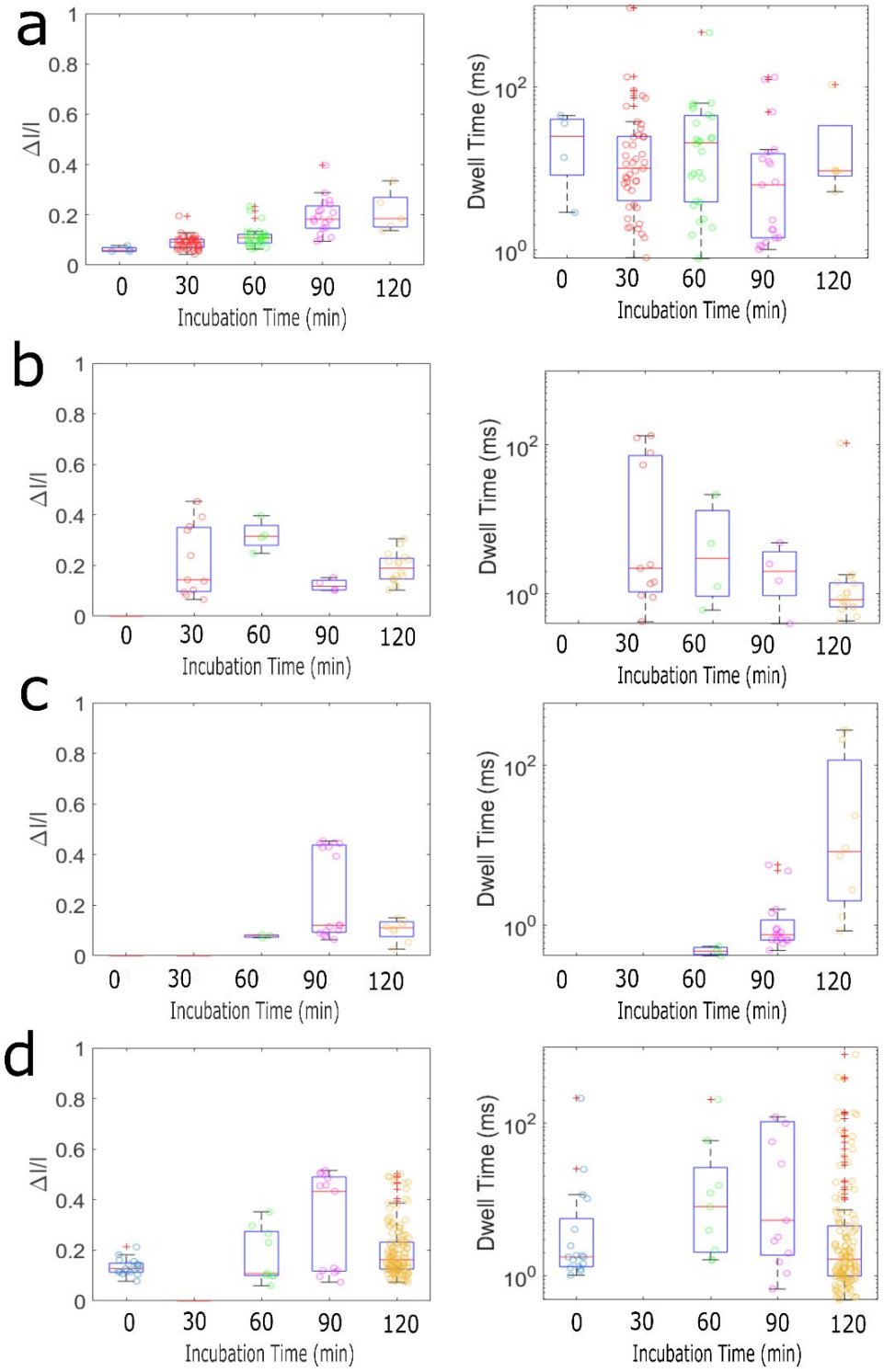

Figure SI-3 : Distribution of amplitude of the current blockade (right) and dwell time (left) recorded for the control as function of incubation time at T =25 °C +/- 2°C. The experiments were performed at V= 500 mV in NaCl 1M, PBS 1X ph 7.4, T= 25°C using (a) pipette N°4, (b) nanopipette N° 5, (c) nanopipette N°6 and (d) nanopipette N°7.

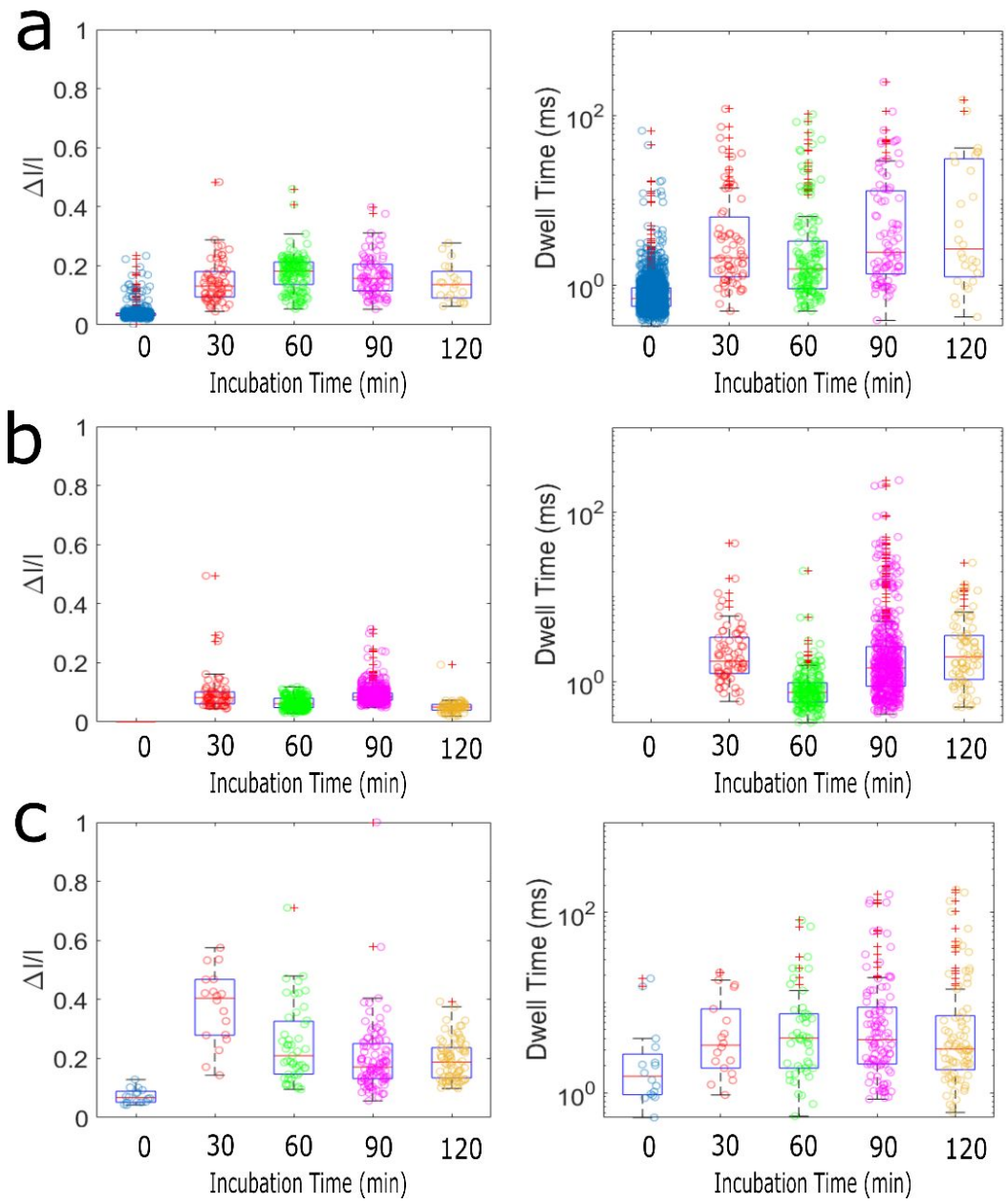

Figure SI-4: Distribution of amplitude of current blockade (right) and dwell time (left) recorded for the control as function of incubation time at T =30°C. The experiments were performed at V= 500 mV in NaCl 1M, PBS 1X ph 7.4, using (a) pipette N°7, (b) nanopipette N°2 and (c) nanopipette N°1

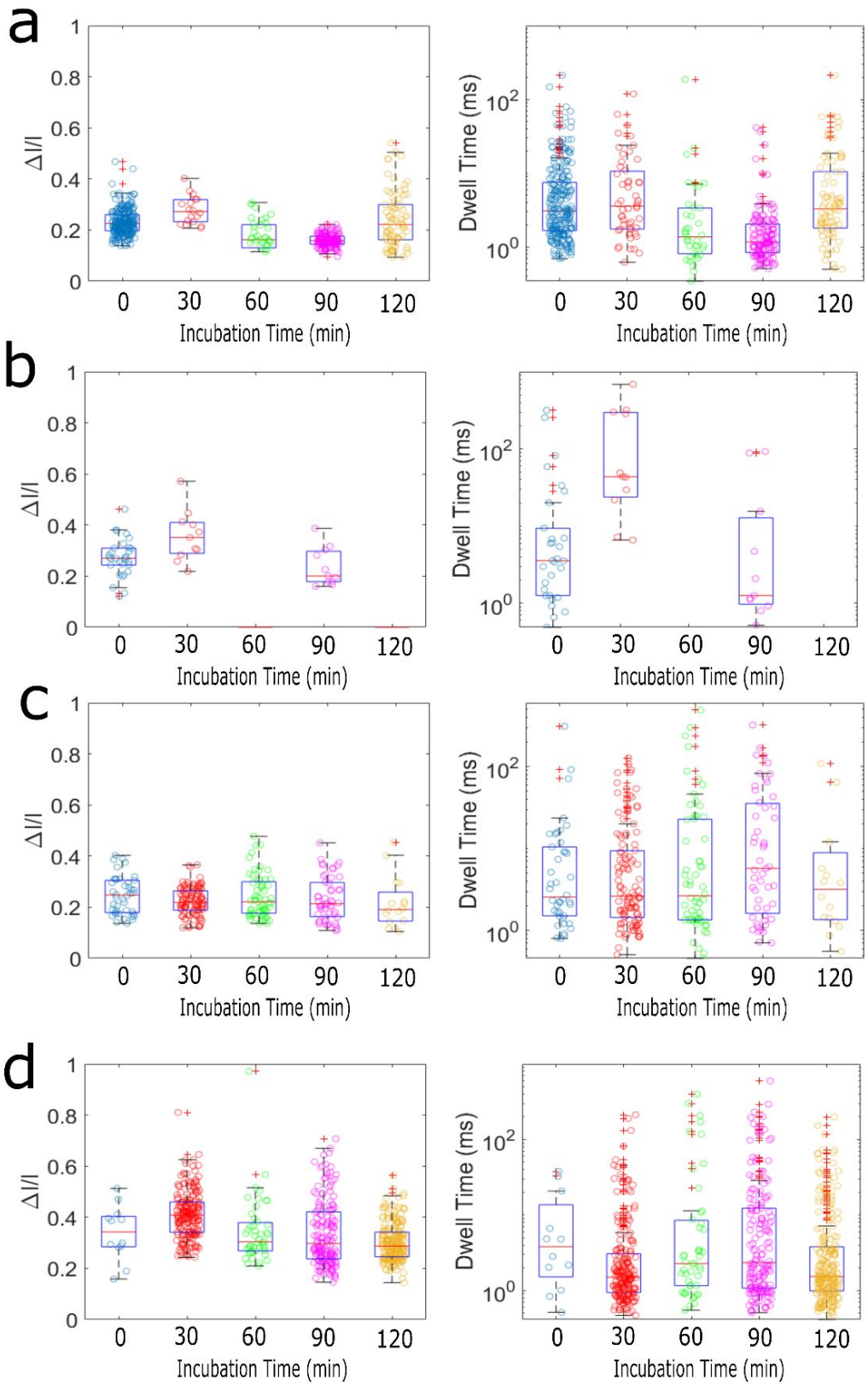

Figure SI-5: Distribution of amplitude of the current blockade (right) and dwell time (left) recorded for the sample seeded with 200 pM of α-synuclein WT as function of incubation time. The experiments were performed at V= 500 mV in NaCl 1M, PBS 1X ph 7.4, using (a) pipette N°9, (b) nanopipette N°10 , (c) nanopipette N°11 and (d) nanopipette N°12.

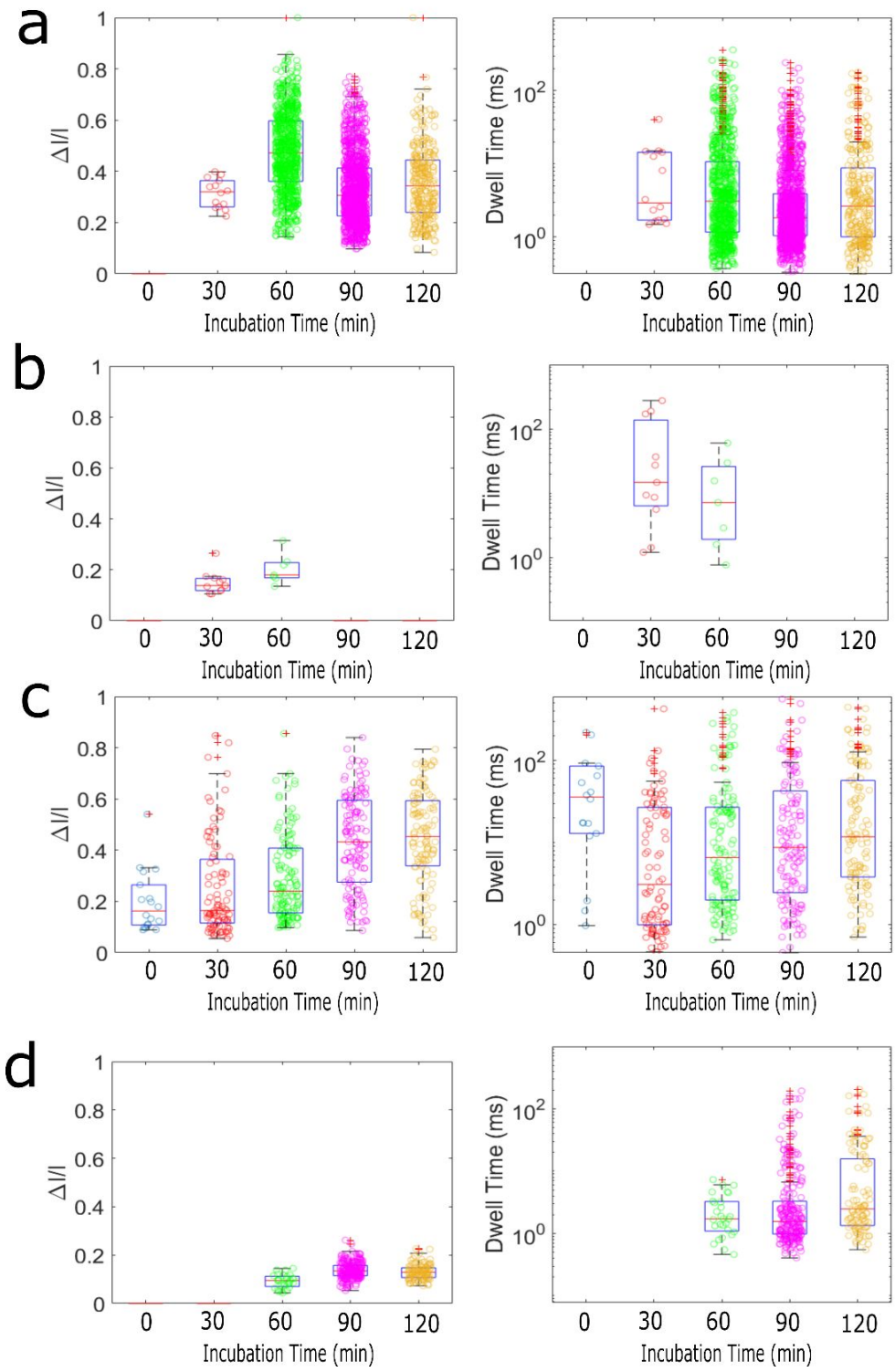

Figure SI-6: Distribution of amplitude of the current blockade (right) and dwell time (left) recorded for the sample seeded with 200 pM of α-synuclein A53T as function of incubation time. The experiments were performed at V= 500 mV in NaCl 1M, PBS 1X ph 7.4, using (a) pipette N°13, (b) nanopipette N°14, (c) nanopipette N°15 and (d) nanopipette N°16.

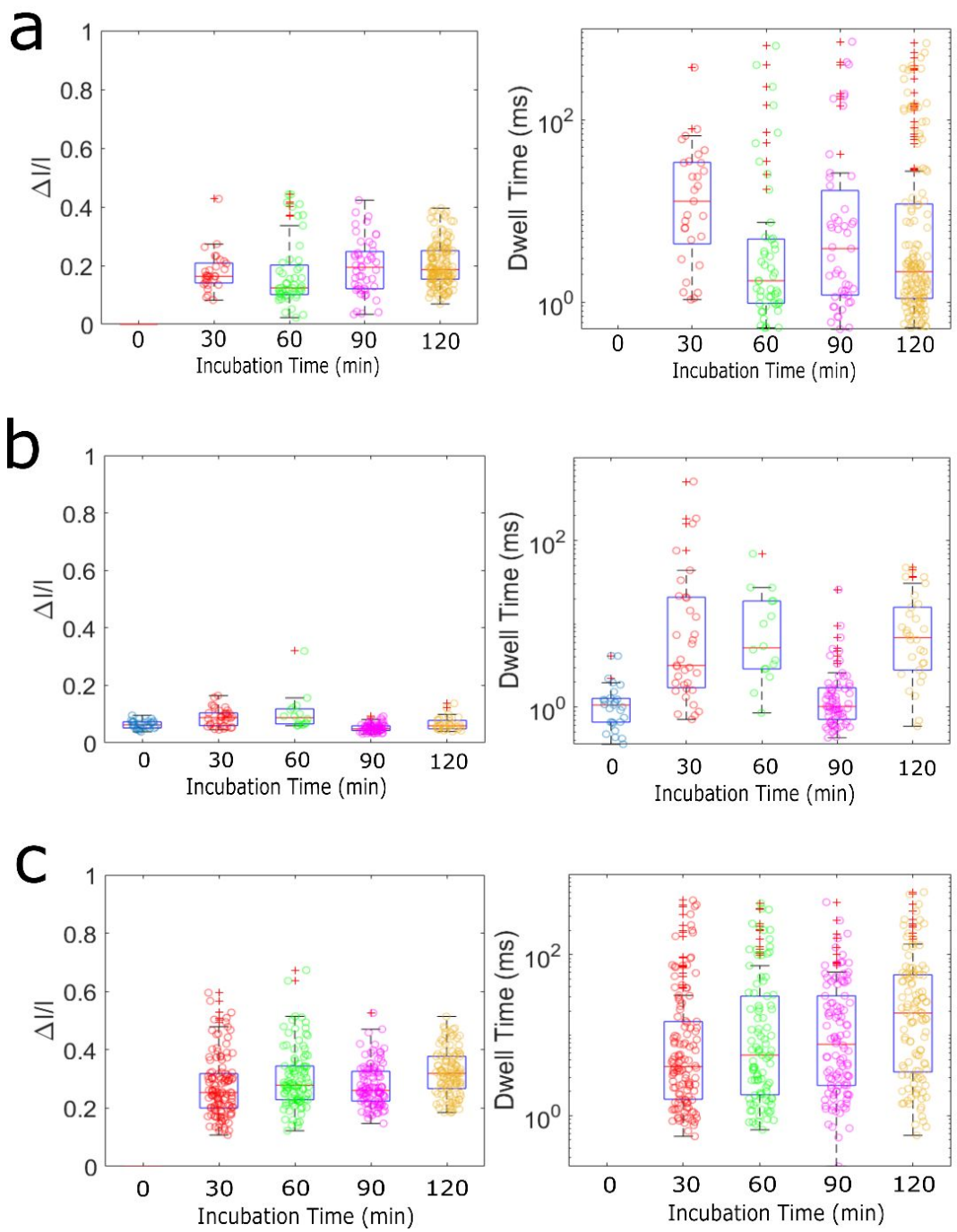

Figure SI-7: Distribution of amplitude of current blockade (right) and dwell time (left) recorded for the sample seeded with 20 pM of α-synuclein WT as function of incubation time. The experiments were performed at V= 500 mV in NaCl 1M, PBS 1X ph 7.4, using (a) pipette N°17, (b) nanopipette N°18 and (c) nanopipette N°19

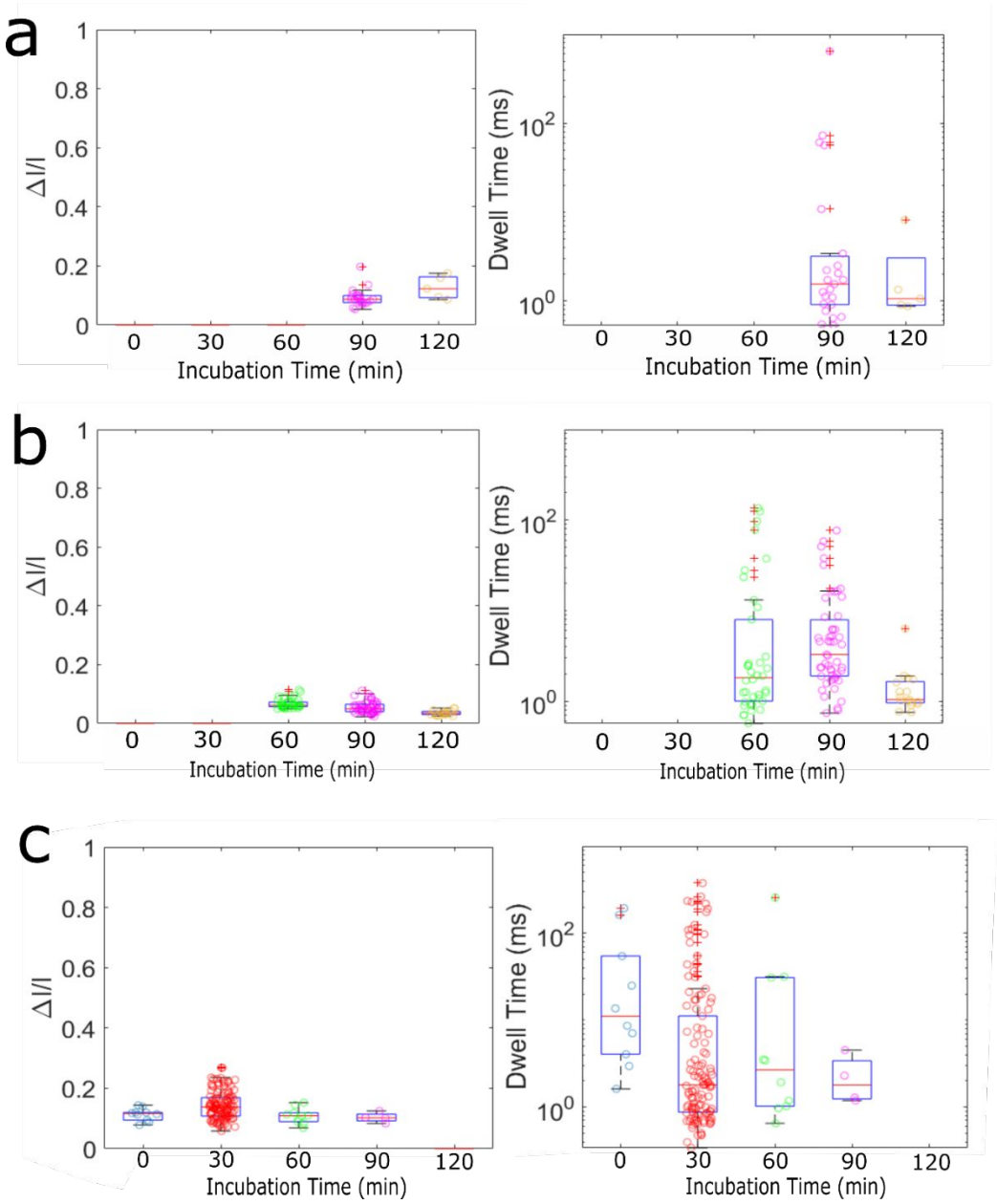

Figure SI-8: Distribution of amplitude of current blockade (right) and dwell time (left) recorded for the sample seeded with 2 pM of α-synuclein WT as function of incubation time. The experiments were performed at V= 500 mV in NaCl 1M, PBS 1X ph 7.4, using (a) nanopipette N° 21, (b) nanopipette N°22 and (c) nanopipette N°23