Electronic Supplementary Material (ESI) for Nanoscale.

Biomolecular interfaces based on self-assembly and self-recognition form biosensors capable to record single molecule binding and release

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Supporting Information S1: The sequence of kinesin 1 (simply called kinesin) heavy chain

The sequence was imported from previous research[1].

10 20 30 40 50

MADLAECNIK VMCRFRPLNE SEVNRGDKYI AKFQGEDTVV IASKPYAFDR

60 70 80 90 100

VFQSSTSQEQ VYNDCAKKIV KDVLEGYNGT IFAYGQTSSG KTHTMEGKLH

110 120 130 140 150

DPEGMGIIPR IVQDIFNYIY SMDENLEFHI KVSYFEIYLD KIRDLLDVSK

160 170 180 190 200

TNLSVHEDKN RVPYVKGCTE RFVCSPDEVM DTIDEGKSNR HVAVTNMNEH

210 220 230 240 250

SSRSHSIFLI NVKQENTQTE QKLSGKLYLV DLAGSEKVSK TGAEGAVLDE

260 270 280 290 300

AKNINKSLSA LGNVISALAE GSTYVPYRDS KMTRILQDSL GGNCRTTIVI

310 320 330 340 350

CCSPSSYNES ETKSTLLFGQ RAKTIKNTVC VNVELTAEQW KKKYEKEKEK

360 370 380 390 400

NKILRNTIQW LENELNRWRN GETVPIDEQF DKEKANLEAF TVDKDITLTN

410 420 430 440 450

DKPATAIGVI GNFTDAERRK CEEEIAKLYK QLDDKDEEIN QQSQLVEKLK

460 470 480 490 500

TQMLDQEELL ASTRRDQDNM QAELNRLQAE NDASKEEVKE VLQALEELAV

510 520 530 540 550

NYDQKSQEVE DKTKEYELLS DELNQKSATL ASIDAELQKL KEMTNHQKKR

560 570 580 590 600

AAEMMASLLK DLAEIGIAVG NNDVKQPEGT GMIDEEFTVA RLYISKMKSE

610 620 630 640 650

VKTMVKRCKQ LESTQTESNK KMEENEKELA ACQLRISQHE AKIKSLTEYL

660 670 680 690 700

QNVEQKKRQL EESVDALSEE LVQLRAQEKV HEMEKEHLNK VQTANEVKQA

710 720 730 740 750

VEQQIQSHRE THQKQISSLR DEVEAKAKLI TDLQDQNQKM MLEQERLRVE

760 770 780 790 800

HEKLKATDQE KSRKLHELTV MQDRREQARQ DLKGLEETVA KELQTLHNLR

810 820 830 840 850

KLFVQDLATR VKKSAEIDSD DTGGSAAQKQ KISFLENNLE QLTKVHKQLV

860 870 880 890 900

RDNADLRCEL PKLEKRLRAT AERVKALESA LKEAKENASR DRKRYQQEVD

910 920 930 940 950

RIKEAVRSKN MARRGHSAQI AKPIRPGQHP AASPTHPSAI RGGGAFVQNS

960

QPVAVRGGGG KQV

Supporting Information S2: Loading of the redox agent onto the kinesin

Kinesin was expressed as described in the manuscript's Materials and Methods[2]. Ferrocene monocarboxylic acid-kinesin conjugates were prepared by dissolving 4 mg ferrocene monocarboxylic acid (FCA, Fisher Scientific, USA) in 800 μL of BRB80 buffer (formed from a mixture of 80 mM piperazine-N,N'-bis(2-ethanesulfonic acid buffer, 1 mM $MgCl_2$ and 1 mM ethylene glycol tetraacetic acid (EGTA), pH 6.8; all reagents were purchased from Fisher Scientific, USA) which contained 5 mM biotin-sulfo-N-Hydroxy succinimide (biotin-sulfo-NHS, Sigma, USA), 2 mM 1-Ethyl-3-(3'-dimethylaminopropyl) carbodiimide (EDC, Fisher Scientific, USA) and 10 μM taxol (Fisher Scientific, USA). The mixture was subsequently incubated for 15 min at room temperature. Upon incubation, 90 μL of 2.8 mg/mL kinesin was mixed with the FCA solution and incubated for 4 h at 4 ^{o}C; the reaction was terminated by adding β-mercaptoethanol (Fisher Scientific, USA, 20 mM final concentration)[3].

Loading of the FCA onto the kinesin was estimated by subtracting the amount of the free FCA in the supernatant of span down FCA-kinesin conjugates from the total FCA being added in the reaction. Specifically, after the FCA-kinesin conjugates were synthesized as described above, the FCA-kinesin conjugates were span at 30,000 rpm for 10 min using an Allegra 64R centrifuge (Beckman Coulter, USA). After the centrifugation, the supernatant was collected and evaluated using fluorescence microscopy (Nikon, USA). The pellet was mixed with BRB80 again and the centrifugation was repeated until there was no kinesin visible in the fluorescence microscopy. The concentration of the free FCA in the supernatant was also evaluated using the electrochemical workstation and a user-performed calibration curve (**Figure S1**).

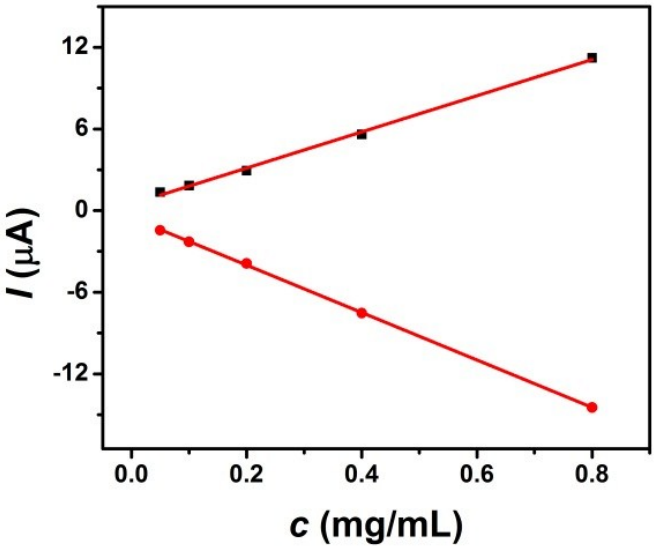

Figure S1: The calibration curve of FCA.

Supporting Information S3: Theoretical evaluation of FCA-kinesin conjugates Immobilized at the user-functionalized electrode interface

Calculations considered the total length of a MT (L_{MT}) as being the overall electrode area (3.14 mm^2) divided by an individual MT diameter (i.e., d=25 nm[4]) as well as that kinesin molecules binding area is about 8 nm at a MT individual binding site[5]. Maximum theoretical numbers of FCA-kinesin conjugates ($N_{FCA\text{-}kinesin\text{-}max}$) on the electrode was thus calculated using:

$$N_{FCA-Kine\sin-\max} = \frac{L_{MT}}{8nm}$$

The full area of the electrode was defined as A_E.

$$A_E = \pi(0.5d)^2 = 3.14\times(0.5\times0.02)^2 = 3.14\times10^{-4}m^2$$

The area that the microtubule occupied on the electrode defied as A_{MT} equaled its cross-section area ($A_{MT\text{-}cross}$). When the MT formed a monolayer on the electrode it was considered that full surface area of the electrode ($A_{MT} = A_E$) was occupied. The length of the microtubule was calculated using the area of the gold electrode divided by the diameter of the microtubule.

$$L_{MT} = \frac{A_{MT}}{d_{MT}} = \frac{A_E}{d_{MT}} = \frac{3.14\times10^{-4} m^2}{25\times10^{-9} m} = 1.256\times10^{4} m$$

$$N_{FCA-Kine\sin-\max} = \frac{L_{MT}}{8nm} = \frac{1.256\times10^{4} m}{8\times10^{-9} m} = 1.57\times10^{12}$$

Supporting Information S4: CV of FCA-kinesin conjugate at the modified electrode before adding ATP.

FCA-kinesin conjugates functionalized gold electrode scanned for 20 cycles with scanning being stopped for 20 sec after finishing each such cycle showed that the peak currents did not change from the first to the last cycle when no ATP was present in solution thus indicating that the number of the immobilized kinesin-FCA conjugates did not change over time (**Figure S2**).

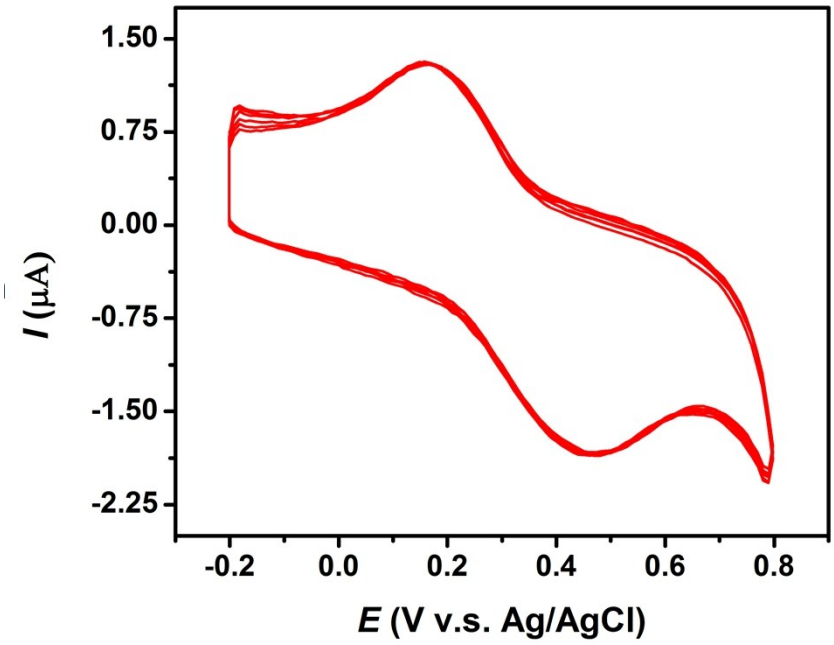

Figure S2: CV of FCA-kinesin conjugate at the modified electrode (Au/MUA /Anti-tubulin Antibody/MT) before adding 250 μM ATP.

Supporting Information S5: CV of FCA-kinesin conjugate at the modified electrode before adding ATP.

FCA-kinesin conjugates dissociation was triggered by the addition of different concentrations of ATP, i.e., 2.5 μM, 250 μM and 2.5 mM respectively. CV analysis performed for a total 10 CV cycles, with the scanning being stopped for 20 sec after finishing each such cycle, are included below (Figure S3). Briefly, the peak current went down with the ATP concentration thus supporting the hypothesis that the binding and unbinding could be specifically controlled by changes in the chemical energy provided to the functional biosensor. This is also supported by previous analysis[6] that has showed that controlling ATP concentrations could lead to differences in the kinesin speed (from 10 nm/s for 1 μM to 550 nm/s for 1000 μM for instance) and thus its subsequent dissociation when encountering the end of a microtubule track.

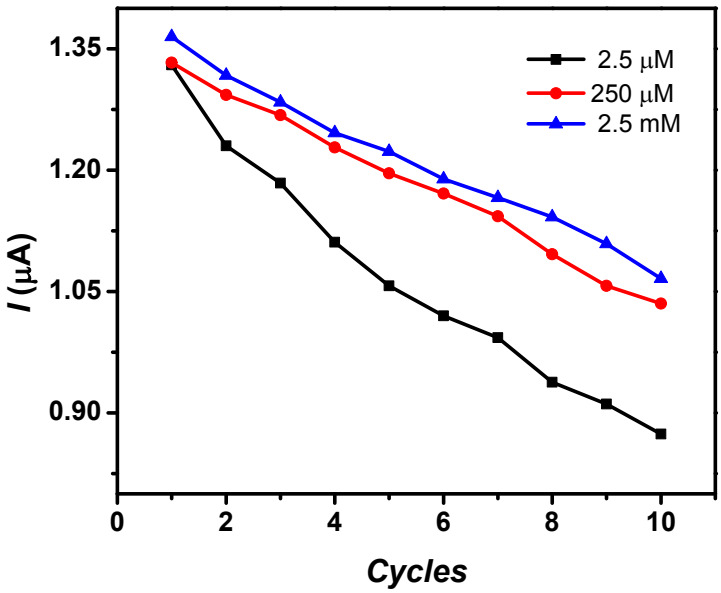

Figure S3 Relationship between peak currents of FCA-kinesin conjugates and CV cycles as measured on a modified electrode and in BRB80 buffer (pH 6.8) containing 10 mM taxol. For such analysis, different concentrations of ATP were used, namely 2.5 μM, 250 μM and 2.5 mM respectively.

References:

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