Label-free Brain Injury Biomarker Detection Based on Highly Sensitive Large Area Organic Thin Film Transistor with Hybrid Coupling Layer

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Figure S1. The interdigitated electrode mask used for fabricating devices (the distance between two neighboring electrodes is 0.25 mm, indicated as short white line)

Electrical properties of transistors and inverter under different conditions
a) Typical output curve of a pentacene OTFT (tested in air) without CYTOP layer

![Output curve of pentacene OTFT without CYTOP layer](image)

b) Typical transfer curve of pentacene OTFT (tested in air) without CYTOP layer

![Transfer curve of pentacene OTFT without CYTOP layer](image)

c) Typical transfer curve of pentacene OTFT (tested in air) with CYTOP layer

![Transfer curve of pentacene OTFT with CYTOP layer](image)

d) Typical transfer curve of pentacene OTFT (tested after 20 minutes in 0.05pbs) with CYTOP and
all the following layers (C44, PS-\textit{block}-PAA, antibody)

e) Typical output curve of 8-3 NTCDI OTFT (tested in air) without CYTOP layer

f) Typical transfer curve of 8-3 NTCDI OTFT (tested in air) without CYTOP layer
g) Typical transfer curve of 8-3 NTCDI OTFT (tested in air) with CYTOP layer

![Typical transfer curve of 8-3 NTCDI OTFT (tested in air) with CYTOP layer](image)

h) Typical transfer curve of 8-3 NTCDI OTFT (tested after 20 minutes in 0.05 PBS) with CYTOP and all the following layers (C44, PS-block-PAA, antibody)

![Typical transfer curve of 8-3 NTCDI OTFT (tested after 20 minutes in 0.05 PBS) with CYTOP and all the following layers (C44, PS-block-PAA, antibody)](image)

i) Typical bulk V-Vg curve of pentacene/8-3 NTCDI inverter under different conditions

**Figure S2.** Electronic prosperities of pentacene, 8-3 NTCDI transistors and inverter under different conditions
Figure S3. AFM image of CYTOP film (above) and CYTOP + C₄₄H₉₀ combined film (below).

Figure S4. Capacitance of combined top layer at different frequency.
Figure S5. Transfer curves of pentacene (left side) and 8-3 NTCDI (right side) devices as a function of pH.

Figure S6. Hysteresis behavior of pentacene and 8-3 NTCDI device in 0.05 PBS
Figure S7. $^1$H NMR spectra of PS-PAA before and after EDC/NHS activation (m:n = 2:1)
Confirming the attachment of anti-GFAP

In order to confirm that the antibody is successfully attached to the device surface by EDC/NHS-based bioconjugation chemistry, we labeled the antibody with rhodamine B, and then attached the labeled antibody on the device surface, using the detailed procedure shown in Scheme S1. First, we activate the carboxyl group on rhodamine B with EDC and NHS, then attach the activated rhodamine B to the antibody by the reaction of N-hydroxysuccinimide on the dye and amine groups on the antibody. Finally, the labeled antibodies were attached to the device surface by EDC/NHS based bioconjugation chemistry. The surface was then gently rinsed with PBS to remove any noncovalently unattached antibody. In Scheme S1 we can see orange fluorescence on sample A and B (both are after the reaction of rhodamine B labeled antibody with EDC/NHS-activated device surface), while no fluorescence on sample C (applying EDC/NHS activated rhodamine B on a control hydroxyethylated device surface).
**Scheme S1.** The synthesis route for Rhodamine-labeled antibody and image of fluorescent labeled antibody attachment on the NHS-treated PS-block-PAA surface (samples A and B), with no attachment on the control surface C.

\[ V_{th} = 2.17, 2.02, 1.52, 1.34, 1.02, 0.79 \text{ V (from left to right)} \]
Figure S8. Representative threshold voltage changes of p- and n-transistor at different GFAP concentrations.

V_{th} = -2.29, -2.48, -3.07, -3.49, -3.58, -4.26 V (from right to left)

Figure S9. Drain current changes of pentacene and 8-3 NTCDI devices at different charge densities (calculated from threshold voltage changes) arising from bound GFAP on device surfaces. The solid lines are guides for the eye.
<table>
<thead>
<tr>
<th>Solutions (all solutions are in 0.05 PBS)</th>
<th>0.05 PBS</th>
<th>0.8 ng/ml GFAP</th>
<th>4 ng/ml GFAP</th>
<th>40 ng/ml GFAP</th>
<th>100 ng/ml GFAP</th>
<th>400 ng/ml GFAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH value</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
</tr>
<tr>
<td>Solutions (all solutions are in 0.05 PBS)</td>
<td>0.05 PBS</td>
<td>40 ng/ml BSA</td>
<td>400 ng/ml BSA</td>
<td>100 µg/ml BSA</td>
<td>300 µg/ml BSA</td>
<td>1 mg/ml BSA</td>
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<tr>
<td>pH value</td>
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<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td>7.6</td>
</tr>
</tbody>
</table>

**Langmuir Model for calculating the affinity constant of Anti-GFAP and GFAP**

\[
\theta = \frac{x}{x_0}
\]

Where \( \theta \) is fractional surface coverage, \( x_0 \) is total available binding sites on the device surface, \( x \) is occupied binding sites;

\[
v_a = k_a c (1-\theta)
\]

\( v_a \) is adsorption rate, \( k_a \) is the rate constant for adsorption;

\[
v_d = k_d \theta
\]

\( v_d \) is desorption rate, \( k_d \) is the rate constant for desorption;

After reach equilibrium statement, \( v_a = v_d \), then we can get:

\[
\theta = \frac{K c}{1+K c}; \quad (K = \frac{k_a}{k_d})
\]

\[
1/x = \frac{1}{x_m K} \frac{1}{1/c + 1/x_m}
\]

**equation S1**

By fitting Figure 1 (b) and (d) into equation S1, then we can get a rough affinity constant value.

**The program for calculating the charge of proteins**

**For example-GFAP**

seq = " MERRRITSAAR RRSYVSSGEM MVGGLAPGRR LGPGTRLSLA RMPPPLPTRV DFSLAGALNA GFKETRASER AEMMELNDRF ASYIEKVRFL EQQNKLALAE LQOQLARQOL VRELEQVLAD GEMASNNM HEAEWYRSDK FADLTDAAR NAELLRQAHK EANDYRRQLQ SLTCDLESLLR GTNESLERQM RQERHSVRE AASYQEALAR LEEEQQSLKD EMARHLQEQYQ DLLNVKLALD IEIATYRKLL EGEENRITIP"
#seq = string.rstrip(uneditedseq, ['"]")

# hydrophobic AA (non polar)
number_G = seq.count("G") # counts the number of each residue
number_A = seq.count("A")
number_V = seq.count("V")
number_I = seq.count("I")
number_M = seq.count("M")
number_F = seq.count("F")
number_W = seq.count("W")
number_P = seq.count("P")

# charged AA
number_E = seq.count("E")
number_D = seq.count("D")
number_R = seq.count("R")
number_K = seq.count("K")
number_H = seq.count("H")
number_C = seq.count("C")

totalAA = number_G+number_A+number_V+number_I+number_M+number_F+
+number_W+number_P+number_E+number_D+number_R+number_K+
+number_H+number_C

pI_E = 4.25 # glutamic acid
pI_R = 12.48
pI_D = 3.86
pI_K = 10.79
pI_H = 6.04
pI_C = 8.33
pI_Y = 10.07

def calculatecharge(pH):
    if pH > 2.5:
        if pH <= 9:
            if pH > pI_E:
                charge_E = -1
            else:
                charge_E = 0
if pH < pI_R:
    charge_R = +1
else:
    charge_R = 0
if pH < pI_H:
    charge_H = +1
else:
    charge_H = 0
if pH > pI_C:
    charge_C = -1
else:
    charge_C = 0
if pH > pI_D:
    charge_D = -1
else:
    charge_D = 0
if pH < pI_K:
    charge_K = 1
else:
    charge_K = 0
netcharge = number_E*charge_E + number_D*charge_D + number_R*charge_R + number_K*charge_K+number_H*charge_H
print netcharge
elif (pH < 2.5):
    charge_H = +1
    charge_K = +1
    charge_R = +1
    charge_D=charge_E = 0
    charge_C = 0
    charge_Y = 0

    netcharge = number_E*charge_E + number_D*charge_D + number_R*charge_R + number_K*charge_K+number_H*charge_H +totalAA*(-1)
    print netcharge
else:
    print "Results are not valid."
    # the amino acid has no net charge on it
return