

Electronic Supplementary Information (ESI)

**Bioelectronic sensor based on canine olfactory nanovesicle-carbon
nanotube hybrid structures for the fast assessment of food quality**

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Supplementary Figures

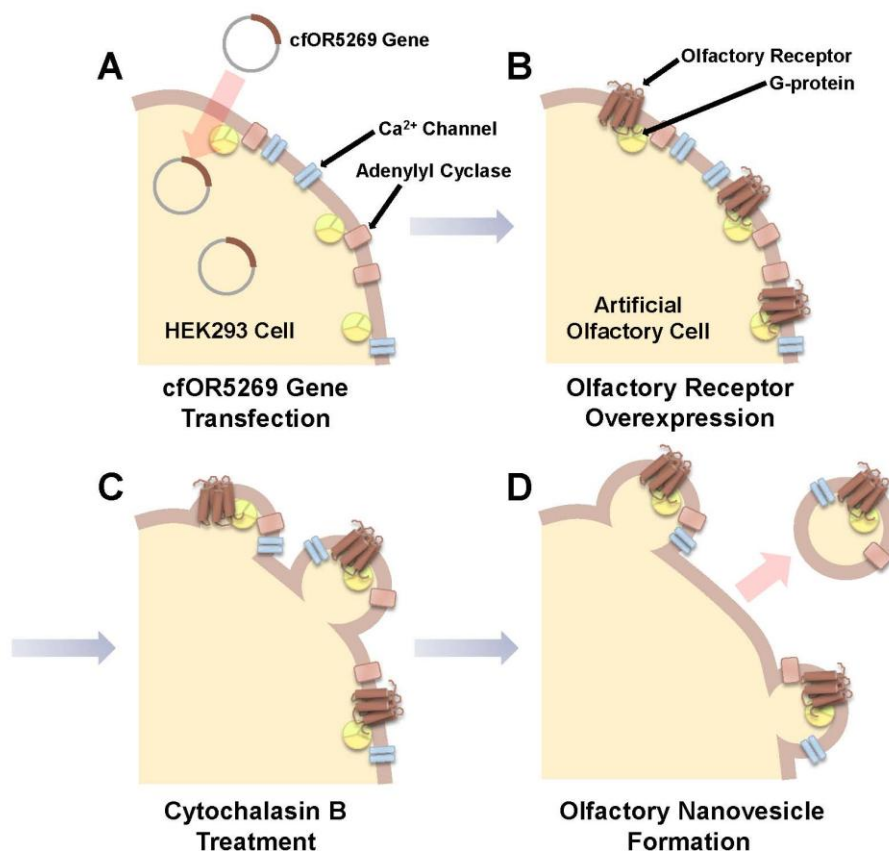


Figure S1. Schematic diagram showing the production method of canine olfactory nanovesicles. (A) Human embryonic kidney (HEK)-293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS) at 37°C in 5% CO₂ environment. The cells were transfected with pIRES plasmid encoding influenza virus leader peptide, cfOR5269, and cMyc epitope using lipofectamine2000 reagent according to the manufacturer's protocol. And then, they were cultivated again for 48 h. (B) cfOR5269 receptors were overexpressed on the cell membrane. (C) Nanovesicles with cfOR5269 were produced from the cfOR5269-expressed cells by incubating the cells with agitation in serum-free DMEM containing cytochalasin B. (D) The nanovesicles were separated from the cells by centrifugation at 500 g for 10 min and then collected by centrifugation at 15,000 g for 30 min. Since the nanovesicles contained cell-derived membrane proteins such as cfOR5269, adenylyl cyclase, Ca²⁺ channels, and cytosolic

components, Ca²⁺ signaling in the olfactory nanovesicles were active as in the parent cells.

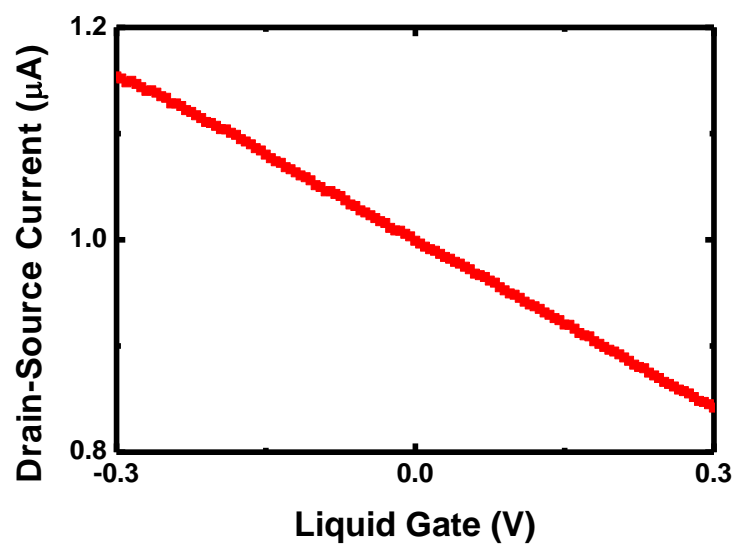


Figure S2. Graph of source-drain current versus liquid gate voltage. The transconductance of this CNT-FET was about $0.52 \mu\text{S}$.

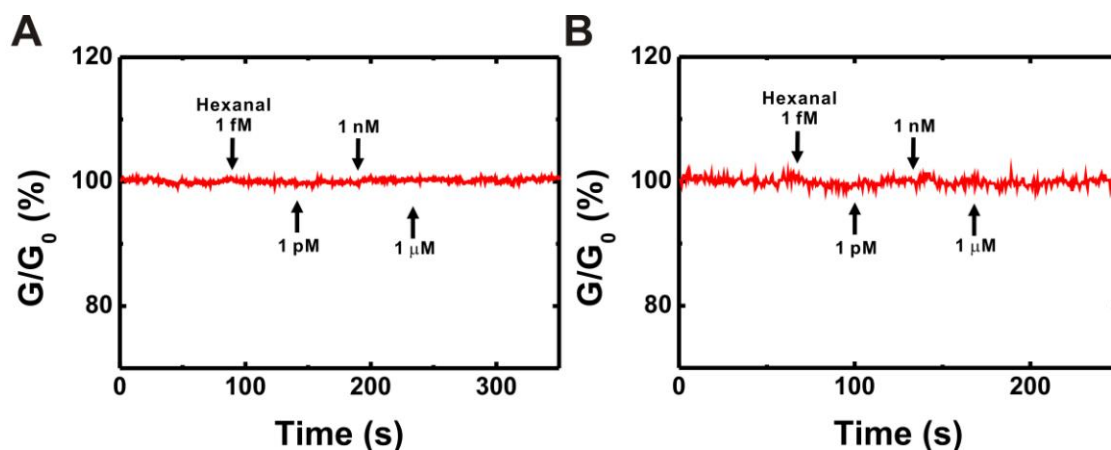


Figure S3. Control experiments for the verification of the proposed sensing mechanism. (A) Real-time conductance measurement of a CNBS device in Ca^{2+} -free PBS after the introduction of hexanal with different concentrations. First, a 9 μ L of PBS was placed on the CNBS. The conductance of the CNT channel in the CNBS was monitored after hexanal treatment. The conductance showed no observable change by the injection of hexanal. (B) Real-time conductance measurement of a CNBS device with nanovesicles *without cfOR5269* after the introduction of hexanal with different concentrations. Other experimental conditions were the same as those in Fig. 3C. There was no significant change in the conductance of its CNT channel.

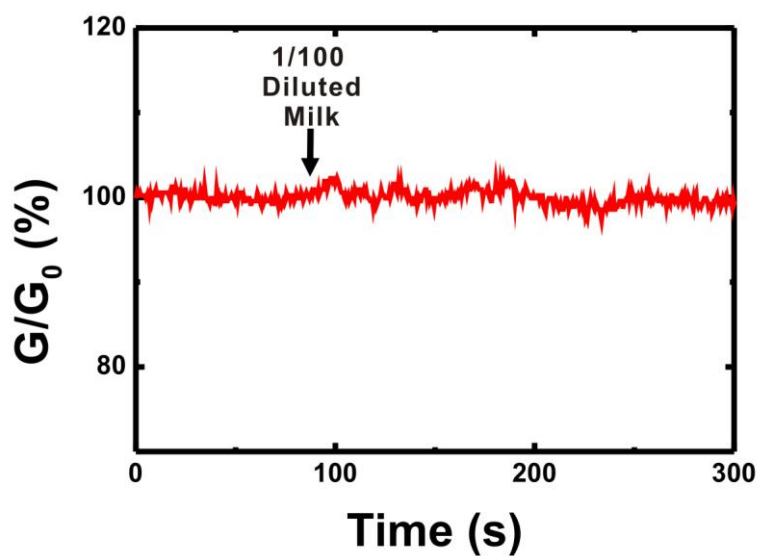


Figure S4. Real-time conductance measurement obtained from a bare CNT transistor after the introduction of milk rotten for 5 days. First, a 9 μL of PBS was placed on the CNT transistor. The conductance of a CNT channel in the CNT transistor was monitored after the introduction of diluted rotten milk. In this control experiment, the addition of the milk exhibited no significant change in the conductance of the CNT transistor.

1. Linear approximation of $\Delta G/G_0$ in terms of C_s

Generally, if $F(x)$ is a continuous and differentiable function of x , we can expand $F(x)$ in a Taylor series format around $x=x_0$ like

$$F(x) = F(x_0) + F'(x_0)(x - x_0) + \frac{F''(x_0)}{2}(x - x_0)^2 + \dots$$

In our sensors, the sensor sensitivity $\Delta G/G_0$ should be a function of the odorant concentration C_s . Thus, we can expand $\Delta G/G_0$ in a Taylor series around $C_s=0$ like

$$\frac{\Delta G}{G_0}(C_s) = f(0) + f'(0)C_s + \frac{f''(0)}{2}C_s^2 + \dots$$

where

$$f(C_s) \equiv \frac{\Delta G}{G_0}(C_s)$$

Since the sensor sensitivity $\Delta G/G_0$ should be 0 when there is no odorant ($C_s = 0$), $f(0) = 0$.

Then,

$$\frac{\Delta G}{G_0}(C_s) = f'(0)C_s + \frac{f''(0)}{2}C_s^2 + \dots$$

If the surface density C_s of the bound odorant molecules to cfOR5269 is small, we can ignore the high order terms of the C_s and approximate the equation like

$$\frac{\Delta G}{G_0}(C_s) \approx f'(0)C_s \equiv kC_s$$

Here, k is a constant representing how sensitively OCBs would respond to the binding of hexanal molecules.