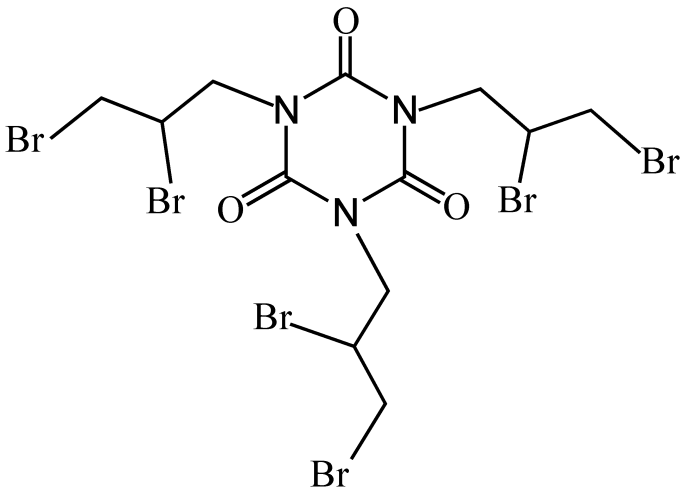
Electronic Supplementary Material (ESI) for Analyst

Supplementary Information

A photoelectrochemical immunosensor for tris (2,3-dibromopropyl) isocyanurate detection with a Multiple Hybrid CdTe/Au-TiO_2 Nanotube arrays

S-1. Chemical structure of TBC sensing molecules



Tris(2,3-dibromopropyl) isocyanurate

CAS: 52434-90-9

Figure S1 Chemical structure of TBC sensing molecules.

S-2. Haptens Synthesis

The synthetic routes of hapten are shown in Fig. S2.

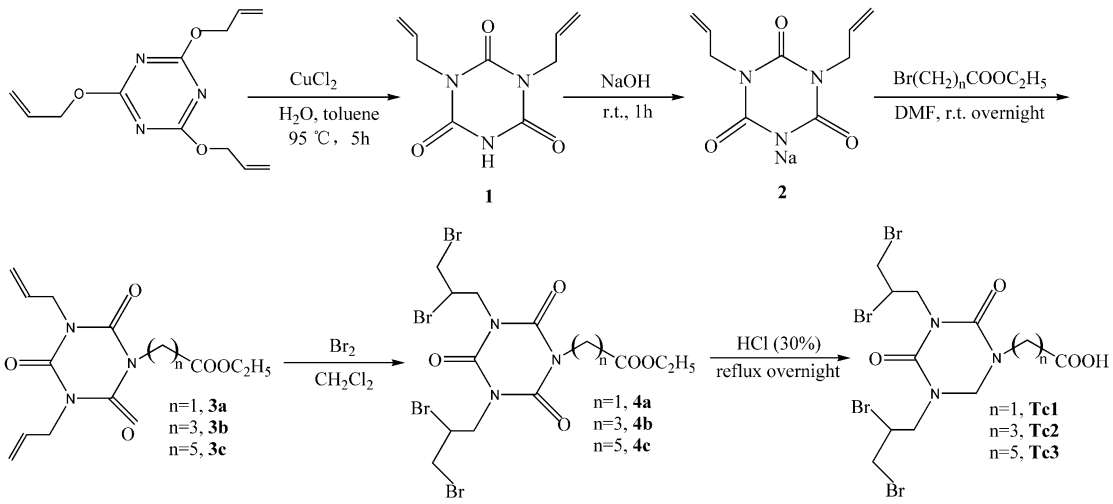


Figure S2 The synthetic route of haptens.

S-2.1 Synthesis of 1,3-diallyl-isocyanurate (1)

The synthesis of compound **1** was followed the Likhterov's method [1]. The mixture of 2,4,6-tri(allyloxy)-1,3,5-triazine (24.90 g, 0.10 mol), water (1.80 g, 0.10 mol), $CuCl_2 \cdot 2H_2O$ (0.767 g, 4.50 mmol) and toluene (35.01g, 0.38 mol) was stirred at 95 ℃ for 5 h, and then cooled to room temperature. After evaporation of solvent, the crude product was obtained by recrystallization from petroleum ether and CH_2Cl_2. By being washed by hydrochloric acid (2 M) and water, the pure compound **1** was obtained (14.5 g). **1**: White solid, m.p. 147-149 ℃; yield: 69%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.47 (d, J = 6.00 Hz, 4H), 5.14 (dd, J = 10.40 Hz & J = 1.20 Hz, 2H), 5.24 (dd, J = 17.20 Hz & J = 1.60 Hz, 2H), 5.82~5.92 (m, 2H), 8.44 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 44.35 (CH_2), 119.23 (CH_2=), 130.55 (CH=), 148.19 (C=O), 149.16 (C=O); MS (EI) *m/z* (%): 209.1 (M^+, 100), 210.2 (M^++1, 12).

S-2.2 Synthesis of monosodium 1,3-diallyl-isocyanurates (2)

The mixture of **1** (2.09 g, 10 mmol), NaOH (400 mg, 10 mmol) and water (4.0 mL) was stirred for 1 h at the room temperature, and then the solvent was evaporated in vacuo to obtain the salt (2.31 g). **2**: White solid, m.p. 202-206 ℃; yield: 100%; ^{1}H NMR (D_2O, 400 MHz) δ 4.41 (d, J = 4.40 Hz, 4H), 5.07 (dd, J = 17.40 Hz & J = 1.20 Hz, 2H), 5.15 (dd, J = 10.40 Hz & J = 1.20 Hz, 2H), 5.86~5.95 (m, 2H); ^{13}C NMR (D_2O, 100 MHz) δ 45.77 (CH_2), 116.97 (CH2=), 134.05 (CH=), 155.78 (C=O), 160.74 (C=O); MS (ESI) *m/z* (%): 208.0 (M-Na^+, 100), 209.0 (M+1-Na^+, 12).

S-2.3 Synthesis of ethyl 2-(3,5-diallyl-2,4,6-trioxo-1,3,5-triazinane-1-yl)acetate (3a)

The mixture of **2** (600 mg, 2.60 mmol), ethyl bromoacetate (435 mg, 2.60 mmol) and DMF (5 mL) was stirred overnight at the room temperature. Then the reaction solution was diluted by water and extracted with CH_2Cl_2. The organic extract was washed by brine and water, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (petroleum ether/ethyl acetate, 5:1 v/v) to yield compound **3a** (613 mg). **3a**: White solid, m.p. 60-62 ℃; yield: 80%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.29 (t, J = 7.20 Hz, 3H), 4.24 (q, J = 7.60 Hz, 2H), 4.50 (d, J = 6.00 Hz, 4H), 4.63 (s, 2H), 5.25 (dd, J = 9.60 Hz & J = 1.20 Hz, 2H), 5.31 (dd, J = 17.60 Hz & J = 1.20 Hz, 2H), 5.83~5.92 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.03 (CH_3), 43.30 (CH_2), 45.03 (CH_2), 61.93 (CH), 118.88 (CH2=), 130.64 (CH=), 148.22 (C=O), 148.49 (C=O), 167.17 (C=O); MS (EI) *m/z* (%): 295.2 (M^+, 32), 296.2 (M^++1, 5).

S-2.4 The synthesis of 3b and 3c were similar to above.

Ethyl 4-(3,5-diallyl-2,4,6-trioxo-1,3,5-triazinane-1-yl)butanoate (3b)**:** White solid, m.p. 55-56 ℃; yield: 74%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (t, J = 6.80 Hz, 3H), 1.88~2.02 (m, 2H), 2.37 (t, J = 7.20 Hz, 2H), 3.96 (t, J = 6.80 Hz, 2H), 4.12 (q, J = 6.80 Hz, 2H), 4.48 (d, J

= 6.40 Hz, 4H), 5.24 (dd, *J* = 10.00 Hz & *J* = 1.20 Hz, 2H), 5.31 (dd, *J* = 17.20 Hz & *J* = 1.20 Hz, 2H), 5.84~5.92 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.14 (CH_3), 22.95 (CH_2), 31.46 (CH_2), 42.34 (CH_2), 44.88 (CH_2), 60.47 (CH_2), 118.96 (CH_2=), 130.88 (CH=), 148.43 (C=O), 148.70 (C=O), 172.59 (C=O); MS (ESI) *m/z* (%): 668.7 ($2M+Na^+$, 100), 669.7 ($2M+1+Na^+$, 29).

***Ethyl 6-(3,5-diallyl-2,4,6-trioxo-1,3,5-triazinane-1-yl)hexanoate (3c)*:** Colorless oil; yield: 70%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (t, *J* = 6.80 Hz, 3H), 1.33~1.41 (m, 2H), 1.63~1.71 (m, 4H), 2.30 (t, *J* = 7.20 Hz, 2H), 3.88 (t, *J* = 7.60 Hz, 2H), 4.12 (q, *J* = 7.20 Hz, 2H), 4.48 (d, *J* = 6.00 Hz, 4H), 5.24 (dd, *J* = 10.40 Hz & *J* = 1.20 Hz, 2H), 5.30 (dd, *J* = 17.20 Hz & *J* = 1.20 Hz, 2H), 5.82~5.92 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.20 (CH_3), 24.44 (CH_2), 26.15 (CH_2), 27.42 (CH_2), 34.07 (CH_2), 42.87 (CH_2), 44.90 (CH_2), 60.23 (CH_2), 118.97 (CH_2=), 130.92 (CH=), 148.48 (C=O), 148.65 (C=O), 173.46 (C=O); MS (ESI) *m/z* (%): 724.7 ($2M+Na^+$, 100), 725.7 ($2M+1+Na^+$, 30).

S-2.5 Synthesis of ethyl 2-(3,5-bis(2,3-dibromopropyl)-2,4,6-trioxo-1,3,5-triazinane-1-yl) acetate (4a)

To the solution of **3a** (400 mg, 1.36 mmol) in CH_2Cl_2 (20 mL) was added Br_2 (542 mg, 3.39 mmol). The reaction solution was refluxed for 2 h and then cooled to room temperature. Saturated $NaHSO_3$ was added to stop the reaction and the mixture was extracted with CH_2Cl_2. The organic extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (petroleum ether/ethyl acetate, 5:1 v/v) to give compound **4a** (787 mg). **4a**: White solid, m.p. 88-89 ℃; yield: 94%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.30 (t, *J* = 7.20 Hz, 3H), 3.69~3.74 (m, 2H), 3.84~3.88 (m, 2H), 4.25 (q, *J* = 7.20 Hz,

2H), 4.44 (d, *J* = 7.20 Hz, 4H), 4.62~4.70 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.09 (CH_3), 33.12 (CH_2), 43.58 (CH_2), 45.97 (CH), 47.66 (CH_2), 62.13 (CH_2), 148.35 (C=O), 148.54 (C=O), 148.60 (C=O), 166.74 (C=O); MS (EI) *m/z* (%): 611.8 (M^++2, 4), 613.8 (M^++4, 13), 615.9 (M^++6, 13), 617.8 (M^++8, 13), 619.8 (M^++10, 4).

S-2.6 The synthesis of 4b and 4c were similar to above.

***Ethyl 4-(3,5-bis(2,3-dibromopropyl)-2,4,6-trioxo-1,3,5-triazinane-1-yl) butanoate (4b)*:**

Colorless oil; yield: 99%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.26 (t, *J* = 7.20 Hz, 3H), 1.99~2.05 (m, 2H), 2.38 (t, *J* = 7.20 Hz, 2H), 3.70~3.76 (m, 2H), 3.84~3.88 (m, 2H), 4.01 (t, *J* = 6.80 Hz, 2H), 4.14 (q, *J* = 7.20 Hz, 2H), 4.43 (d, *J* = 6.80 Hz, 4H), 4.63~4.70 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.15 (CH_3), 22.72 (CH_2), 31.17 (CH_2), 33.21 (CH_2), 42.57 (CH_2), 42.61 (CH_2), 46.11 (CH), 46.15 (CH), 47.33 (CH_2), 47.41 (CH_2), 60.51 (CH_2), 148.52 (C=O), 148.54 (C=O), 148.67 (C=O), 172.49 (C=O); MS (EI) *m/z* (%): 639.0 (M^+, 2), 641.0 (M^++2, 8), 643.0 (M^++4, 11), 645.0 (M^++6, 7), 647.0 (M^++8, 2).

***Ethyl 6-(3,5-bis(2,3-dibromopropyl)-2,4,6-trioxo-1,3,5-triazinane-1-yl) hexanoate (4c)*:**

Colorless oil; yield: 81%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (t, *J* = 7.20 Hz, 3H), 1.34~1.42 (m, 2H), 1.62~1.70 (m, 4H), 2.30 (t, *J* = 7.20 Hz, 2H), 3.70~3.75 (m, 2H), 3.85~3.89 (m, 2H), 3.93 (t, *J* = 7.20 Hz, 2H), 4.12 (q, *J* = 7.20 Hz, 2H), 4.43 (d, *J* = 7.20 Hz, 4H), 4.64~4.71 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.22 (CH_3), 24.41 (CH_2), 26.04 (CH_2), 27.27 (CH_2), 33.17 (CH_2), 34.06 (CH_2), 43.22 (CH_2), 46.08 (CH), 47.38 (CH_2), 47.45 (CH_2), 60.27 (CH_2), 148.53 (C=O), 148.76 (C=O), 148.83 (C=O), 173.41 (C=O); MS (ESI) *m/z* (%): 684.8 (M+NH_4^+, 17), 686.7 (M+2+NH_4^+, 70), 688.7 (M+4+NH_4^+, 100), 690.7 (M+6+NH_4^+,60), 691.8 (M+7+NH_4^+, 43), 693.8 (M+9+NH_4^+, 42), 695.8 (M+11+NH_4^+, 26).

S-2.7 Synthesis of 2-(3,5-bis(2,3-dibromopropyl)-2,4,6-trioxo-1,3,5-triazinane-1-yl) acetic acid (Tc1).

The mixture of **4a** (350 mg, 0.57 mmol) and hydrochloric acid (30%, 10 mL) was stirred overnight at 110 ℃ and allowed to cool to room temperature. The white solid was obtained by filtration, and redissolved with CH_2Cl_2. Then the solution was washed by saturated NH_4Cl and dried over anhydrous Na_2SO_4. After evaporation of solvents, the pure product was obtained (210 mg). **Tc1**: White solid, m.p. 141-143 ℃; yield: 63%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.90~3.95 (m, 2H), 3.97~4.01 (m, 2H), 4.36~4.42 (m, 2H), 4.43~4.49 (m, 2H), 4.62 (s, 2H), 4.62~4.70 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 35.69 (CH_2), 43.91 (CH_2), 48.25 (CH), 49.11 (CH_2), 49.15 (CH_2), 149.61 (C=O), 149.87 (C=O), 168.47 (C=O); MS (ESI) *m/z* (%): 581.7 (M-H$^+$, 21), 583.6 (M+2-H$^+$, 71), 585.6 (M+4-H$^+$, 100), 587.6 (M+6-H$^+$, 63), 589.6 (M+8-H$^+$, 11).

S-2.8 The synthesis of Tc2 and Tc3 were similar to above.

***4-(3,5-bis(2,3-dibromopropyl)-2,4,6-trioxo-1,3,5-triazinane-1-yl)butanoic acid (Tc2)*:**

White solid, m.p. 64-66 ℃; yield: 86%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.98~2.05 (m, 2H), 2.43~2.48 (m, 2H), 3.70~3.76 (m, 2H), 3.84~3.89 (m, 2H), 4.03 (t, *J* = 6.40 Hz, 2H), 4.43 (d, *J* = 7.20 Hz, 4H), 4.54~4.70 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.56 (CH_2), 31.07 (CH_2), 33.23 (CH_2), 42.58 (CH_2), 46.08 (CH), 46.13 (CH), 47.41 (CH_2), 47.48 (CH_2), 148.63 (C=O), 148.66 (C=O), 148.73 (C=O); MS (ESI) *m/z* (%): 645.5 (M+Cl$^-$, 18), 647.5 (M+2+Cl$^-$, 54), 649.5 (M+4+Cl$^-$, 100), 651.5 (M+6+Cl$^-$, 79), 653.5 (M+8+Cl$^-$, 20).

***6-(3,5-bis(2,3-dibromopropyl)-2,4,6-trioxo-1,3,5-triazinane-1-yl) hexanoic acid (Tc3)*:**

White solid, m.p. 52-53 ℃; yield: 78%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36~1.44 (m, 2H),

1.64~1.73 (m, 4H), 2.37 (t, *J* = 6.00 Hz, 2H), 3.70~3.75 (m, 2H), 3.85~3.89 (m, 2H), 3.94 (t, *J* = 7.20 Hz, 2H), 4.43 (d, *J* = 7.20 Hz, 4H), 4.64~4.71 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.13 (CH_2), 25.92 (CH_2), 27.22 (CH_2), 33.17 (CH_2), 33.69 (CH_2), 43.16 (CH_2), 46.05 (CH), 46.10 (CH), 47.39 (CH_2), 47.46 (CH_2), 148.54 (C=O), 148.75 (C=O), 148.83 (C=O); MS (ESI) *m/z* (%): 673.5 ($M+K^+-2H^+$, 18), 675.5 ($M+2+K^+-2H^+$, 52), 677.5 ($M+4+K^+-2H^+$, 100), 679.5 ($M+6+K^+-2H^+$, 72), 681.5 ($M+8+K^+-2H^+$, 31).

S-3. Preparation of immunizing antigens and antibody

S-3.1 Preparation of Immunogens.

The haptens **Tc1**~**Tc3** were conjugated to bovine serum albumin (BSA) as immunogens and to Ovalbumin (OVA) as coating antigens. These conjugated antigens were all prepared by *N*-hydroxysuccinimide (NHS) active ester method similarly as previous reported [2]. Each hapten (20 μmol) was dissolved in dry DMF (0.32 mL) in which NHS (30 μmol) and dicyclohexylcarbodiimide (DCC, 30 μmol) was added. The mixture was stirred overnight at room temperature, and then the precipitate was removed by centrifugation. The solution of the active ester was added slowly to the solution of BSA (53.6 mg) in Phosphate Buffered Saline solution (PBS, 8 mL, 0.05 M, pH 8) with vigorous stirring at 4 ℃. Then this mixture was stirred gently at 4 ℃ for 18 h and dialyzed for 72 h with buffer changed every 12 h. At last, the conjugates were lyophilized and stored at -20℃. UV-vis spectral data were used to confirm the structures of the conjugates and the conjugation density was determined by using the 2,4,6-trinitrobenzene sulfonic acid (TNBS) method [3]. The coating antigens were prepared similarly as above.

S-3.2 Production of polyclonal antibodies.

Each immunogen was immunized to two New Zealand white rabits weighing 1~2 kg as comparisons, and the immunization procedures were followed the protocol reported [4-5]. Briefly, the immunogen solution (0.2 mg in 0.5 mL PBS) was suspended in 0.5 mL Freund's complete adjuvant and injected hypodermically into a New Zealand white rabbit. About 2 weeks later, this rabbit was boosted at 14-day intervals with the same immunogen solution suspended in 0.5 mL Freund's incomplete adjuvant. The rabbit blood samples were collected through an ear vein one week after each booster injection. About 3 months later, the antisera were obtained by centrifugation, stored at -20 ℃and used without further purification.

References

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