Appendix A. Supplementary Material

Photoluminescence detection of 2,4,6-trinitrotoluene (TNT) binding on diatom frustule biosilica functionalized with an anti-TNT monoclonal antibody fragment

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Methods Supplementary material

Biosilica frustule isolation (supplement to section 2.2)

Briefly, two flasks of 21-day-old diatom cell culture suspensions were pooled into 200 mL, centrifuged at 2,000 rpm, and washed twice with deionized water. The cell pellet was transferred into 100 mL 30% v/v hydrogen peroxide solution (H_2O_2) mixed with 200 µL of 35% w/v hydrochloric acid (HCl) and treated at 80 °C with mixing on an orbital shaker at 100 rpm for 24 h. The resulting white biosilica frustules were filtered, washed twice with deionized water and then twice with 100% ethanol, and stored in ethanol. The typical recovery was about 5 mg diatom biosilica after drying in air at room temperature.

Production of scFv (supplement to section 2.3)

Dr. Ellen Goldman, U.S. Naval Research Laboratory, Washington DC, provided the plasmid containing the unmodified anti-TNT scFv. The *E. coli* host was also co-transformed with a plasmid encoding the periplasmic chaperone Skp to mitigate aggregation in the periplasm and increase yield. TurboCells^{TM} BL21 (DE3) Competent *E. coli* transformed with the pET22B+ vector containing the anti-TNT scFv under the T7 promoter were grown overnight at 37 ^{o} C to optical density of 1.0 at 600 nm. Expression of anti-TNT scFv was induced by the addition of IPTG at a concentration of 100 µM, and *E. coli* cultures were transferred to 26 ^{o}C and grown overnight in the dark. Expressed anti-TNT scFv antibody molecules were purified from the

soluble fraction of the *E. coli* lysates using Ni-Affinity Chromatography (Ni Sepharose 6 FF, Fisher Scientific). The column was washed with 5 mM imidazole in 20 mM Tris-HCl (pH 7.9), and the anti-TNT scFv was eluted from the column with 50 mM imidazole in 20 mM Tris-HCl (pH 7.9). The eluent fraction was concentrated through a 10 kDa Amicon filter, run through size exclusion HPLC (BioSep SEC-s2000, Phenomenex, Inc.) and concentrated again through a 10 kDa Amicon filter. Purity was determined by SDS-PAGE.

Photoluminescence spectroscopy (supplement to section 2.5)

The UV laser source was a 337 nm N_2 gas laser (Spectra-Physics VSL, maximum power output: 2.0 mW, peak output power: 40 kW, pulse length: 3 ns, pulse energy: 120μJ, wavelength: 337 nm, repetition rate: 10 Hz, 3 x 8 mm beam dimension). The laser line was filtered by a 337 nm band pass filter, shaped by a 1x 3 mm slit, and aimed at the vertically-mounted diatom frustule thin film sample positioned 45^o to the incident beam. The emitted light was focused by an optical lens positioned 45^o to the sample holder, filtered by a 360 nm cut-off filter, and the filtered spectra were captured by an Acton SP2150 spectra detector (Priceton Instruments,1 mm slit, 600 groves/mm grating blazed for 300 nm) equipped with a PIXIS 100 CCD detector set at 20 s integration time. All measurements were carried out at room temperature within a dark enclosure and optical bench with 3-D locking sample holder to ensure precision of measurement. Spectra were corrected for background signal without sample as well as the glass cover slip blank.

Challenge of scFv-functionalized diatom biosilica with TNT - Nonspecific Adsorption of TNT on Diatom Biosilica (supplement to section 2.6)

The free adsorption of TNT and TNB on non-functionalized diatom biosilica was also measured to verify that the interaction of TNT with the biosilica itself partially quenched the PL response. In these experiments, a suspension of diatom biosilica in water was mixed with TNT or TNB stock solution to final concentration of 0.91 mg/mL biosilica and 0.091 mg/mL TNT ($4.0 \cdot 10^{-4}$ M in 10:1 v/v water:acetonitrile) under gentle mixing for 2 hr, with 10:1 v/v acetonitrile:water serving as the control. A thin film was prepared by pipetting the suspension directly onto the glass substrate as described above, and the PL spectra were measured on the air-dried film.

Synthesis of Alex Fluor 555 labeled trinitrobenzene (supplement to section 2.8)

To prepare Alex Fluor 555 labeled trinitrobenzene (AF-TNB), 1.0 mg aliquot of Alexa Fluor® 555 cadaverine, disodium salt first was dissolved in 500 μL of 0.135 M sodium borate buffer (pH 8.5). Then, 350 μL of 5 wt% 2,4,6-trinitro-benzenesulfonic acid solution (Sigma-Aldrich) and 20 μL of 3 M NaOH were added. The solution was reacted over night at room temperature under agitation, and then loaded on a Supelclean LC-18 SPE column (Supelco), washed with 1:10 v/v borate buffer and eluted with an increasing concentration of methanol in sodium borate buffer. Eluted fractions were desalted and purified on an oligonucleotide purification cartridge (OPC, Applied Biosystems, Foster City, CA) as described by Medintz et al. (2003).

Results Supplementary material

PL spectra from scFv-functionalized diatom biosilica - non specific adsorption of TNT on non-functionalized diatom biosilica (supplement to section 3.3)

To verify that partial quenching of the PL response was due to the interaction of TNT with the diatom biosilica itself, the PL response after free adsorption of TNT on non-functionalized diatom biosilica without washing was also measured, as shown in the Supplementary material (Figure S1). In these experiments, a high concentration of of TNT was used ($4.0 \cdot 10^{-4}$ M) and the biosilica was not washed to maximize the response. The peak PL response was normalized to the no-TNT control. From this experiment, the PL quenching is due to the interaction of TNT with the diatom biosilica.

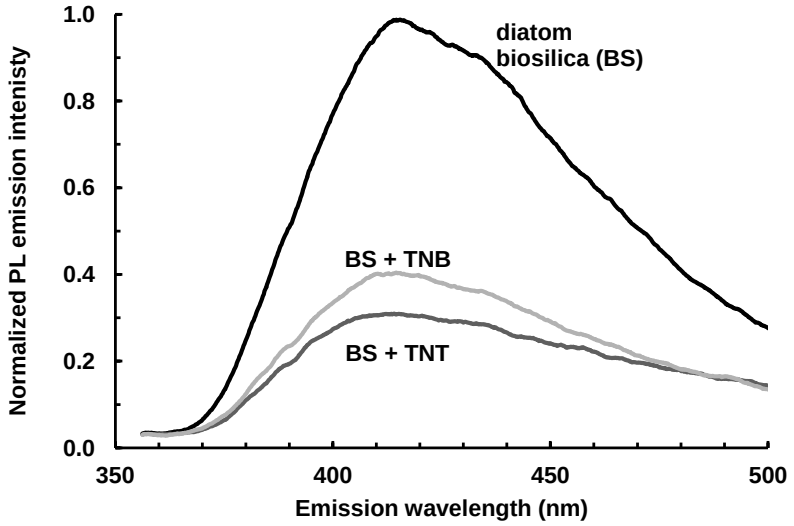

Fig. S1. Comparison of PL emission spectrum from non-functionalized diatom biosilica after nonspecific TNT adsorption with $4.0 \cdot 10^{-4}$ M TNT. All spectra were normalized relative to the non-functionalized diatom biosilica.

References

Medintz, I.L., Goldman, E.R., Lassman, M.E.,Mauro, J.M., 2003. Bioconjugate Chem. 14, 909-918.