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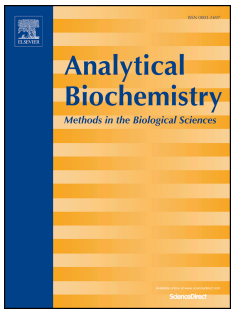

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Near Infrared Optical Biosensor Based on Peptide Functionalized Single-Walled Carbon Nanotubes Hybrids for 2,4,6-trinitrotoluene (TNT) Explosive Detection

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Abstract

A near infrared (NIR) optical biosensor based on peptide functionalized single-walled carbon nanotubes (SWCNTs) hybrids for 2,4,6-trinitrotoluene (TNT) explosive detection was developed. The TNT binding peptide was directly anchored on the sidewall of the SWCNTs using the π-π interaction between the aromatic amino acids and SWCNTs, forming the peptide-SWCNTs hybrids for near infrared absorption spectra measurement. The evidence of the morphology of peptide-SWCNTs hybrids were obtained using Atomic force microscopy (AFM). The results demonstrated that peptide-SWCNTs hybrids based NIR optical biosensor exhibited sensitive and highly selective for TNT explosive determination, addressing a promising optical biosensor for security application.

Keywords: Near infrared, TNT binding peptide, SWCNTs, π-π interaction, TNT analogues

Introduction

Single-walled carbon nanotubes (SWCNTs) are attractive materials for a variety of application due to the intrinsic excellent optical properties such as fluorescence, Raman and absorption spectra[1–3] . Advances in the understanding of their optical properties offered the possibility of using them as solution-phase sensors that respond to analyte adsorption for the qualitative and quantitative monitoring[4–6]. SWCNTs dispersed individually in aqueous medium exhibited strong fluorescence in the near infrared region (NIR) and sharp absorption peak distributions caused by van Hove singularities[7,8]. Solubility of SWCNTs in aqueous medium can be substantially improved through covalent and noncovalent chemical modification [8,9]. Covalent modification suffered changing the properties of SWCNTs because of disruption of the π-electronic states in the sidewalls, while noncovalent modification with less perturbation to the delocalized π electrons could be employed to preserve the desired properties of SWCNTs for a wide range of application in optical or electrical biosensors[4,8,10–13].

Many recent reports have demonstrated the advantages of SWCNTs based electrical biosensor for nitroaromatic explosives or volatile organic compounds (VOCs) detection due to the increased global security issue[14,15,16]. Herein, we developed a near

infrared (NIR) optical sensor based on peptide functionalized SWCNTs hybrids for 2,4,6-trinitrotoluene (TNT) explosive detection. The TNT binding peptide (amino acid sequence ARGYSSFIYWFFDFC), derived from the anti-TNT monoclonal antibody, was synthesized and testified as a promising TNT receptor peptide based on our previous study[17,18]. Enriched in the aromatic amino acid residues (WYF), the TNT binding peptide played an important role in dispersing SWCNTs and non-covalently anchored on the side wall of SWCNTs through π stacking interaction[19,20]. The change of absorbance in near infrared spectra was monitored when the TNT molecule binds to the TNT recognition peptide based SWCNTs hybrids through π-electron-mediated effects and hydrogen bond. The results revealed that the near infrared optical biosensor offered sensitive and selective ~~visible~~ detection for TNT explosive.

Materials and methods

TNT solution dissolved in pure DMF (N,N-dimethylformamide) was obtained from Chugoku Kayaku, Co., Ltd., Kure, Japan. The concentration of the received TNT sample solution was 10000 ppm and was freshly diluted as required before each measurement. Four kinds of TNT analogues, including research and development explosive (RDX) solution dissolved in pure DMF (10000 ppm) were purchased from

Chugoku Kayaku, Co., Ltd., Kure, Japan. 2,4-Dinitrophenyl glycine (DNP-glycine), 2,6-DNT, and 4-nitrobenzoyl-glycyl-glycine were purchased from Tokyo Chemical Industry, Tokyo, Japan. Single-walled Carbon nanotubes with carboxylic acid functionalized (SWCNTs) were purchased from sigma Aldrich, St. Louis, MO, USA. All other chemicals were purchased either from Tokyo Chemical Industry, Tokyo, Japan or Wako Pure Chemical Industries, Tokyo, Japan. All aqueous solutions were prepared by Milli-Q deionized water (18 MΩ) from Milli-Qsystem (Millipore Corporation, Billerica, MA, USA).Then instrument Frontier NIR was purchased from PerkinElmer, Inc. Waltham, MA, USA.

According to our previous work, rationally-designed peptide sequence (ARGYSSFIYWFFDFC) in the heavy chain of the complementarity determining region in the anti-TNT monoclonal antibody was obtained and identified[17,18]. TNT binding peptide anchored the single-walled carbon nanotubes with carboxylic acid functionalized hybrids were optimized by sonicating 5mL DMF solution with 0.2 mg carboxylic acid functionalized SWCNTs and 0.4 mg TNT binding peptide (1 : 2 ratio) for 20 min in an ultrasonic bath (Temperature controlled at 25º with chilled ice). Then the suspension was centrifuged for 40 min at 13200 rpm twice. The final supernatant was very stable with no precipitate observed and directly used for the

further NIR and AFM measurement [12,21,22].

Results and Discussion

Previous investigations have demonstrated that the rational design of TNT binding peptide (amino acid sequence ARGYSSFIYWFFDFC) derived from the anti-TNT monoclonal antibody has high specific binding to TNT molecule than TNT explosive analogues[17,18]. The schematic of peptide synthesis was illustrated in Fig. 1(a). An atomic force microscope (AFM) image of the TNT binding peptide anchored SWCNTs hybrids was shown in Fig. 1(b). Based on the height analysis of the surface morphology of with/without peptide anchored on the side wall of SWCNT on a mica substrate illustrated in Fig. 1(c) and (d), the nodules and light spots on the side wall of SWCNT were peptide unites. The morphology of pristine SWCNT and peptide anchored SWCNT evaluated by AFM and NIR was observed in the supporting information in Fig. 1S. The Fig. 1(d) represented the mechanism of TNT molecule binding to the SWCNTs encapsulated peptide through the π-electron-mediated effects (donor-acceptor character) (the aromatic amino acids W, Y, F) and hydrogen bond (W) based on the previous investigated study [23–25].

It is well known that individually dispersed carbon nanotubes exhibit a series of absorption peaks in the visible and near infrared regions because of quasi

one-dimensionality. To date, the best solvents reported for generating carbon nanotubes dispersions are amides, which can form stable suspensions[7]. Considering of the high percentage of hydrophobic acid consisted of TNT binding peptide and low solubility of SWCNTs, amide solvent *N*,*N*-dimethylformamide (DMF) was used for the TNT binding peptide functionalized SWCNTs hybrids dispersion in this work. The NIR spectra and AFM image results in Fig. 2a showed the well dispersion of the peptide-SWCNTs hybrids through the comparison of the pristine SWCNTs and peptide anchored SWCNTs. The observed NIR spectra features of the peptide-SWCNTs hybrids dispersions in DMF fall into two main categories: the near-infrared absorption peaks near 1160 - 1470 nm, 1710 - 1930 nm, similarly resolved spectrum with the previous study of SWCNTs in DMF dispersions[7], revealing that the individually disperse of peptide-SWCNTs hybrids without disturbing the electronic properties of nanotubes were established. The absorption peak at 1930 nm was used for further concentration estimation.

A solution of 990 μL peptide-SWCNTs hybrids were mixed with 10μL of concentration of series of TNT sample dissolved in DMF for 10 min to 30 min before measurement and the final TNT concentration were 0.01 ppm, 0.1 ppm, 1 ppm, 10 ppm, 100 ppm. Control experiment was conducted by adding 10 μL DMF solution with

peptide-SWCNTs hybrids. The NIR measurements were taken for three times at each sample concentration. The NIR absorption spectra for shown in Fig. 3 exhibited sharp peaks at 1930 nm responds to the variety of TNT concentrations. The results revealed that the NIR absorption intensity at 1930 nm was increased corresponding to the increased concentration of TNT solution in the range of 0.01 ppm ~ 10ppm. The detection of limit (LOD) 0.01 ppm was calculated as 3σ of background solution (0 ppm TNT solution). The results obtained by NIR optical biosensor were compared with previous work summarized in Table S1 in the supporting information. The suspension of peptide-SWCNT hybrids was visibly aggregated when reacted with 100 ppm TNT solution. This phenomenon may be caused by the surface charge changed of peptide-SWCNTs hybrids when the excessive TNT molecule binding to the hybrids surface. The inset of Fig. 2 showed that the absorption curve is gradually saturated with the increase of TNT concentration.

The selectivity of the TNT binding peptide was demonstrated in our previous work by using surface plasmon resonance (SPR) sensor[18]. The TNT analogues, DNP-glycine, 2.6-DNT, RDX and 4-Nitrobenzoyl-glycyl-glycine at the concentration initial concentration 10000 ppm (final concentration 100 ppm) were introduced for NIR measurement. The results in Fig. 3 indicated an average 25% TNT signal response and

the interference binding response to peptide-SWCNTs hybrids as 8% for 4-Nitrobenzoyl-glycyl-glycine, 5% for RDX and DNP-glycine, 9% 2.6-DNT, compared with the peptide-SWCNT NIR response. However, the results in Fig. S2 in the supporting information clearly showed the peptide-SWCNT hybrids have a significantly response for TNT, negligible responses for the four kinds of TNT analogues at the 0.1 ppm concentration. Analysis of the TNT binding response and interference binding response results revealed that acceptable low non-specific binding between TNT analogues and high binging ability to TNT sample as the NIR optical biosensor developed for low concentration detection.

Conclusions

In summary, the TNT binding peptide functionalized SWCNTs hybrids based optical biosensor reflected a significant advantage of highly selective and sensitive detection for TNT explosive target.

Conflicts of Interest: The author declares no conflict of interest.

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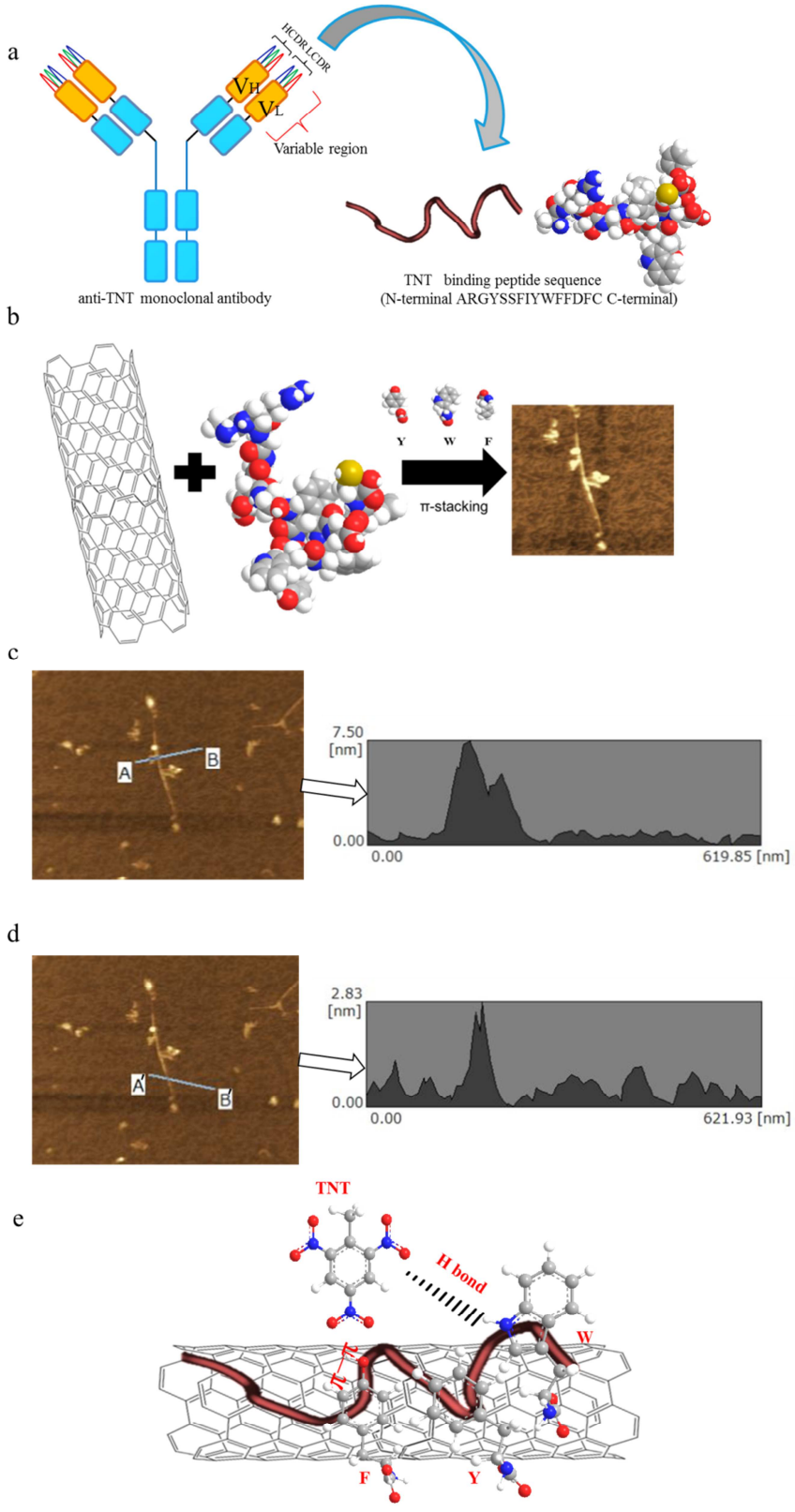
Figure captions

Fig. 1(a) The illustration of schematic of rational design of TNT binding peptide; (b) An Atomic force microscope (AFM) image of the TNT binding peptide anchored SWCNTs hybrids. (c) AFM height analysis of the surface morphology on mica substrate of the TNT binding peptide anchored to the side wall of SWCNTs; (d) The mechanism of TNT molecule binding to the SWCNTs encapsulated peptide.

Fig. 2 The NIR absorption spectra of a series of TNT concentration added into the peptide-SWCNTs hybrids mixture solution for triplicate measurements.

Fig. 3 The NIR absorption spectra of comparison of TNT analogues and TNT added into the peptide-SWCNTs hybrids mixture solution at high concentration (100 ppm)

Fig. 1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VH
VL
Variable region
anti-TNT monoclonal antibody
TNT binding peptide sequence
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b
Y
W
F
π-stacking
c
A
B
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[nm]
0.00
0.00
619.85 [nm]
d
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B
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[nm]
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0.00
621.93 [nm]
e
TNT
H bond
W
F
Y

Fig. 2

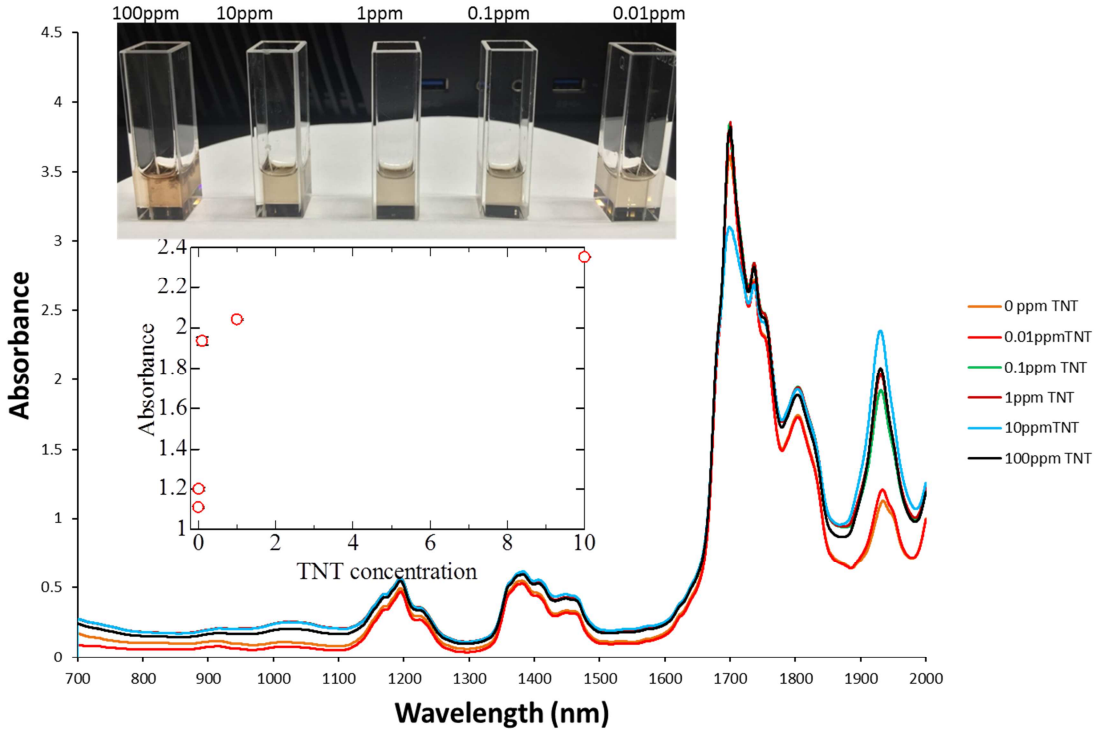
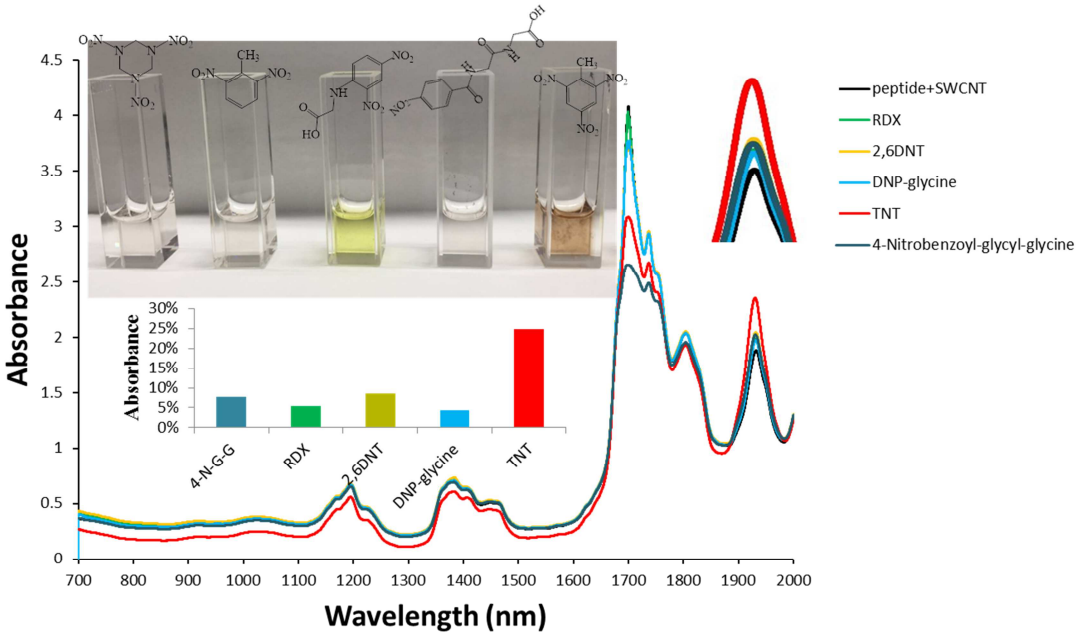

100ppm
10ppm
1ppm
0.1ppm
0.01ppm
Absorbance
Wavelength (nm)
TNT concentration
0 ppm TNT
0.01ppmTNT
0.1ppm TNT
1ppm TNT
10ppmTNT
100ppm TNT

Fig. 3


Absorbance
Wavelength (nm)
peptide+SWCNT
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DNP-glycine
TNT
4-Nitrobenzoyl-glycyl-glycine
4-N-G-G
RDX
2,6DNT
DNP-glycine
TNT

Supporting information

Near Infrared Optical Biosensor Based on Peptide Functionalized Single-Walled Carbon Nanotubes Hybrids for 2,4,6-trinitrotoluene (TNT) Explosive Detection

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Fig. S1

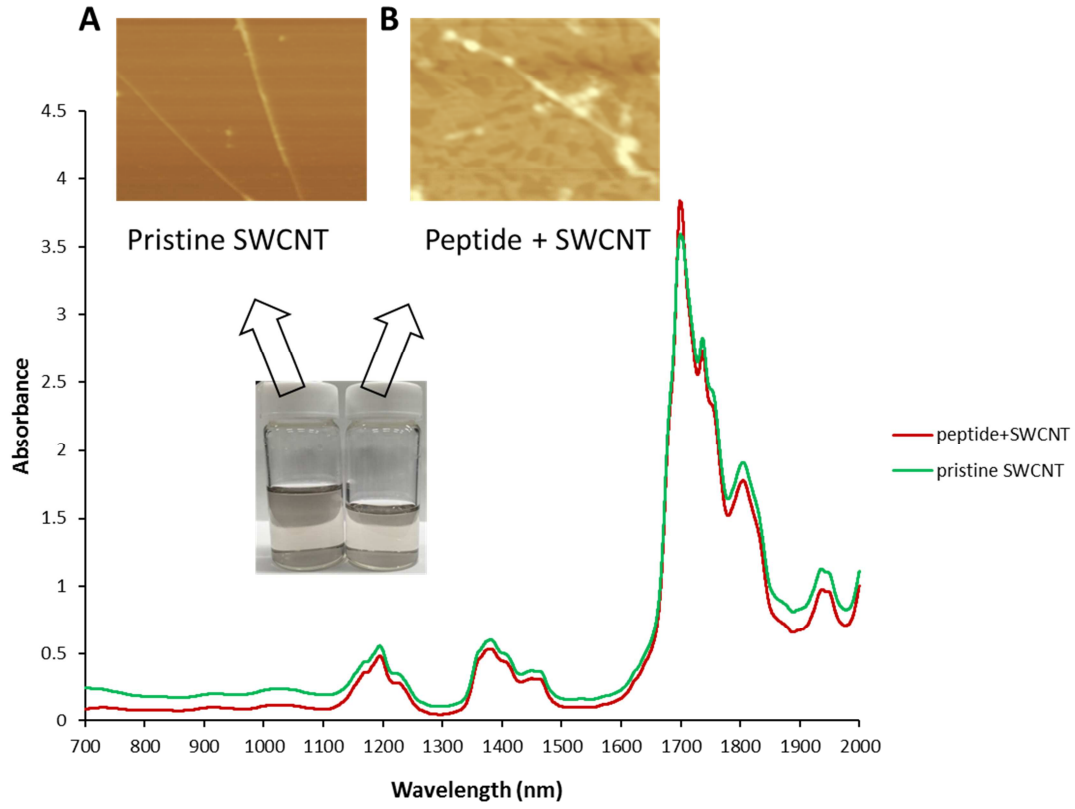

Fig. S1 The NIR absorption spectra and AFM image results of comparison of the pristine SWCNTs and peptide anchored SWCNTs. (A) pristine SWCNT without peptide (B) peptide with SWCNT. The nodules and light spots on the side wall of SWCNT were peptide unites

Fig. S2

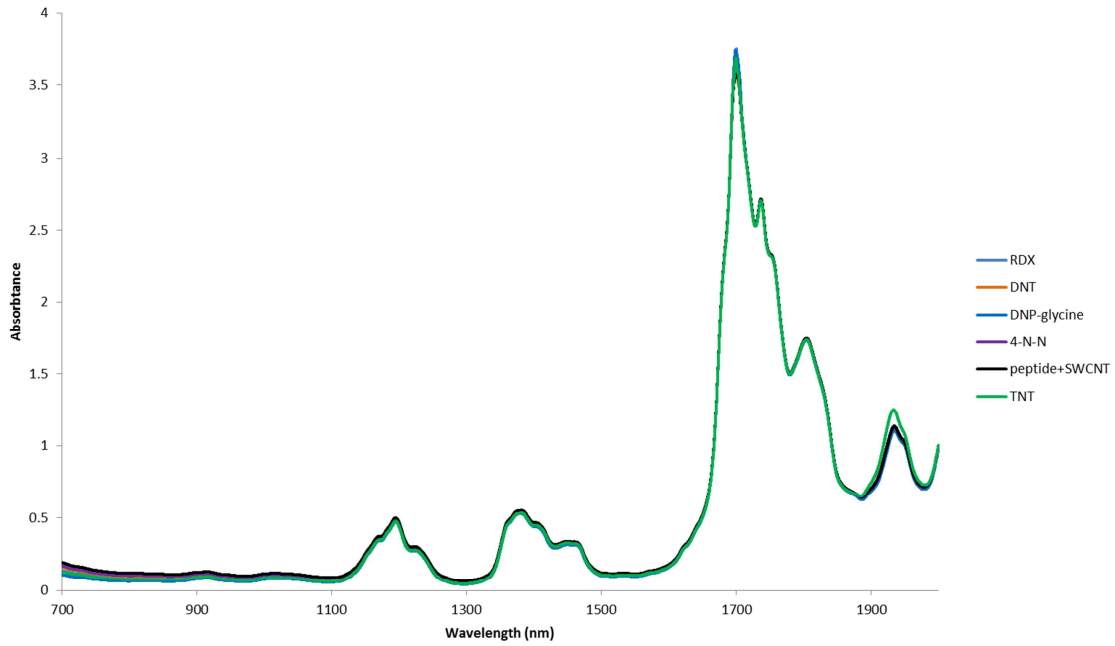

Fig. S2 The NIR absorption spectra of comparison of TNT analogues and TNT added into the peptide-SWCNTs hybrids mixture solution at low concentration (0.1 ppm)

Table S1 peptide based biosensor for TNT detection

Receptor	Sensing devices	Phase	Detection limit	Reference
WHW	CNT-FET	Liquid	4 fM	[25]
WHWQRPLMPVS	Electrochemical impedance spectroscopy (EIS)	Liquid	1 μM	[26]
Oligopeptide	QCM	Liquid	Not mentioned	[27]
WHW	Colorimetric sensor	Gas	0.3 ppm	[28]
VLP-binding peptide	SWV	Liquid	4 ppm	[29]
ARGYSSFIYWFFDFC	SWCNT-NIR	Liquid	0.01 ppm	This work