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Bicomponent β-sheet assembly of dipeptide-fluorophores of opposite polarity and sensitive detection of nitro-explosives†

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Fluorescent hydrogel of two dipeptide-pyrene amphiphiles of opposite polarity is developed through bicomponent antiparallel β-sheet co-assembly. The helical molecular assembly resulted in the formation of fluorescent nanofibers. The sandwich-like interaction of nitroaromatics within the hydrogel matix rendered selective and sensitive detection of toxic nitro-explosives.

Fluorescent peptide hydrogels are emerging as novel tools for the soft materials-based detection of various biological and environmentally relevant analytes. Peptides and proteins are known for their inherent ability to assemble into natural biological architectures through noncovalent interactions driven coiled-coils,[1] β-sheets,[2] β-hairpins,[3] and amphiphilic structures[4]. Dipeptides, the smallest of peptides with diverse functional groups are the versatile building blocks for molecular assembly and hydrogelation.[5] The conjugation of functional aromatic dyes with dipeptides is advantageous to achieve extended aromatic π-stacking interaction and thereby to extract optical signalling properties.[6] However, the design of π-stacking systems that afford special arrangement for the intercalation of guest of interest is a challenging task. This requires either known specific complementary interactions (e.g., donor-acceptor), or choice of molecules or guests which disturb the self-sorting phenomena to form co-assembly.[6] In this context, a pre-programmed assembly can capture small aromatic molecules via intercalation which result in wide range of applications viz., toxic analyte detection and pollutant removal among others.

Fluorescent hydrogels with suitably pre-organised molecular ordering can be used as platform to develop chemical sensor systems and devices. Such a chemical sensor system capable of detecting explosives is sought after, due to their harmful effects on the environment and human health.[7] Nitroaromatic compounds (NACs), particularly trinitroaromatics are the known explosives widely used in military, mining as well as unlawful activities.[8] The NACs are known toxic pollutants present in air, water and soil. Exposure to NACs leads to severe adverse effects on human health, mainly targeting the circulatory system, liver, spleen, and immune system. Moreover, NACs and their (bio)degradation products are mutagenic and carcinogenic in nature.[9] Therefore, sensitive and reliable chemical sensors for the detection of NAC explosives are in high demand to protect human health, environment and security related issues. Among several approaches, chemical sensors based on fluorescence (FL) response are widely used to detect NACs.[7,10] In case of FL-based sensors, electron transfer from excited fluorophore to acceptor guest (NACs) results in FL change, which can be visually detected and monitored quantitatively. FL technique is straight forward, sensitive and reliable for the detection of numerous analytes. Therefore, suitably preorganised fluorophore self-assembly can be developed to detect NACs by monitoring the FL response.

In this communication, we report a novel and efficient florescent soft sensory system for the detection of NACs. Two dipeptide (Ala-Ala) amphiphiles were designed and synthesised with pyrene conjugated at C-terminus (TGM-82) and N-terminus (TGM-83) respectively, this results in dipeptide amphiphiles of opposite polarity (Fig. 1, Scheme S1 and S2, ESI†). TGM-82 and TGM-83 were envisioned to undergo bicomponent molecular assembly to form 1D nanofibers which further entangle into 3D network and form fluorescent gel in aqueous solution (Fig. 1 and Fig. S3). The bicomponent assembly was effected by adding 1:1 molar ratio of TGM-82 and TGM-83 into aqueous solution at pH 7.4 (Fig. S1a). The solution transformed into the hydrogel state at 2.0 mM total concentration owing to the balanced hydrophilic-hydrophobic interactions that control the physical state of the system. Notably, the individual components (TGM-82 or TGM-83) failed to form hydrogels under similar conditions. This in fact supports our design strategy of customised molecular ordering

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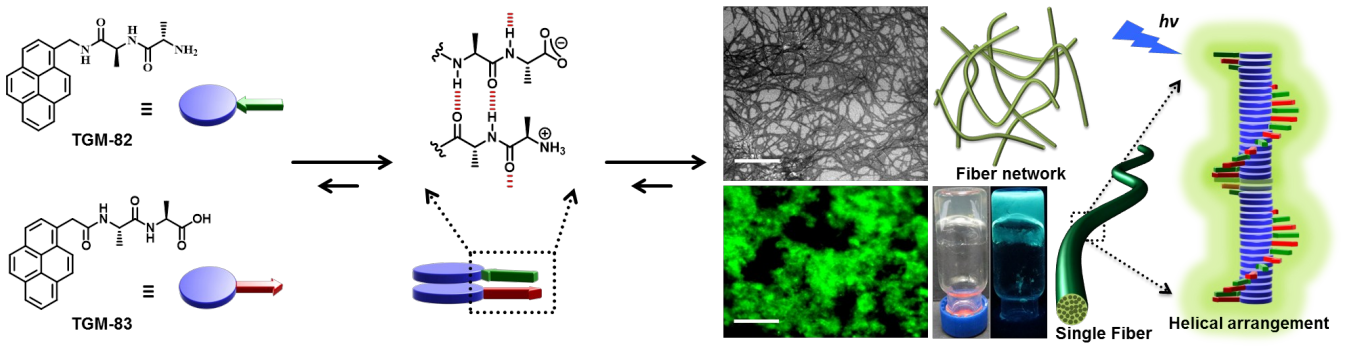

Fig. 1 Chemical structures of pyrene conjugated dipeptide amphiphiles (TGM-82 and TGM-83, and their bicomponent assembly supported by antiparallel β-sheet formation via non-covalent interactions. FESEM and fluorescent optical microscopy images of 1D nanofibers in the fluorescent hydrogel (inverted gel vial). Schematic representation of the helical bicomponent assembly of TGM-82 and TGM-83 in nanofiber. Scale bar: 1 μM and 50 μM for FESEM and FL images, respectively.

via hydrogen bonding, ionic (among carboxylate and ammonium terminals) and aromatic π-π interactions. The main advantage of the design is that it provides a highly programmable 3D microenvironment to study π-electron deficient NACs detection via photo-induced electron transfer (PET) mechanism. Accordingly, we prepared a fluorescent hydrogel that contains assemblies of high stiffness to observe the effect of guest diffusion into the matrix. This is particularly useful in developing fluorescent thin-film or chip-based device to effectively allow NACs diffusion and their co-assembly within the hydrogel.

The bicomponent co-assembly of TGM-82 and TGM-83 at 1:1 stoichiometry showed strong pyrene-excimer band at λ_{em} = 485 nm (Fig. 2a, Fig. S1b, ESI†). The excimer emission is attributed to effective cofacial π-π interactions of the pyrene chromophores in the bicomponent assembly state.[11] The ionic interaction between the ammonium ($-NH_3^+$) and caroxylate ($-COO^-$) terminals is assumed to play a key role in the assembly of TGM-82 and TGM-83. The ionic (COO^- : NH_3^+) pair is possibly the best-known intra- and intermolecular zwitterionic interactions in biological systems, especially among amino acids. Therefore, the assembly formation between TGM-82 and TGM-83 was studied at different pH (1 to 12, Fig. S2, ESI†). The maximum excimer band intensity was observed at pH 7-9, which confirmed possible electrostatic interaction among the components. In addition, noncovalent interactions viz., hydrogen bonding and aromatic π-π interactions assisted the molecular ordering, which is responsible for the observed high excimer emission. However, the bicomponent molecular system (TGM-82 and TGM-83) under pH< 7 is dominated by–NH_3^+ and pH> 9 by $-COO^-$. This reduced the possible ionic attraction between the components under highly acidic or basic conditions.

The hydrogel was prepared at 2.0 mM total concentration of the dipeptide amphiphiles at pH 7.4 and the gel formation was confirmed by vial inversion experiment. The hydrogel showed blue-green FL under the UV light (λ_{ex} = 365 nm) (Fig. 1). Supramolecular gels are viscoelastic semi-solid materials that can store or dissipate energy, and was assessed by oscillatory rheology. The gel state is characterised by storage modulus, G′ (ω) and loss modulus, G′ ′ (ω), where ω is the angular frequency.[12] The dynamic frequency sweep experiment performed at 1% constant strain showed a wide linear viscoelastic region (LVR) of frequency and considerably higher modulus value (G′/G′′ ~ 10) (Fig. 2b). The strain plot of the hydrogel at 1 Hz constant frequency revealed critical strain value γ = 36·2% (Fig. S4, ESI†). This signifies higher stiffness opposing the monotonic collapse of the hydrogel to a quasi-liquid state. Therefore, low yield stress (σ*, 5·4 Pa) combined with high stiffness (G′/G′′) show the low compactness of the hydrogel and characterises it as a soft elastic material.

Circular dichroism (CD) and Fourier transform infrared (FTIR) analysis revealed the formation of antiparallel β-sheet structures of the 1:1 complex at 25 °C (Fig. 2c,d). The CD spectra of the independent components (TGM-82 and TGM-83) and their 1:1 complex were studied. The independent components did not exhibit any significant spectral features. However, the CD spectrum of 1:1 complex showed two positive (355 nm and 338 nm) and a negative (287 nm) signals in the pyrene-chromophore absorption regions (Fig. S1a, ESI†)

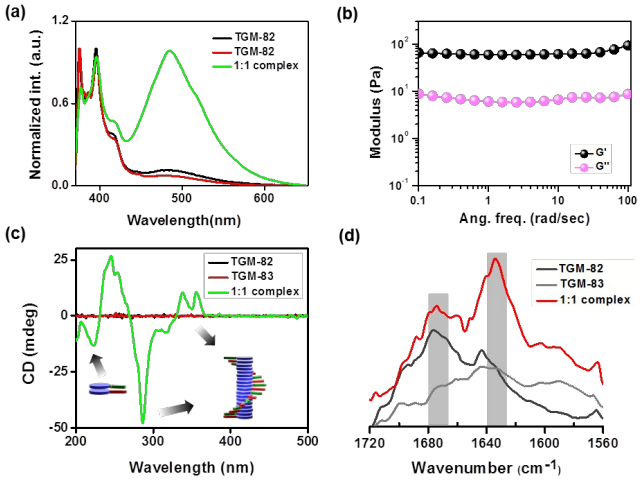

Fig. 2 (a) FL spectra of TGM-82, TGM-83 and their 1:1 complex at 0.25 mM total concentration; (b) Frequency sweep experiment in oscillatory rheology of the hydrogel at 2.0 mM total concentration and at 25 °C; (c) CD spectra of the complex at 0.25 mM total concentration and at 25 °C; and (d) FTIR spectra of TGM-82, TGM-83 and xerogel of 1:1 complex.

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which suggest the formation of right-handed (*P*-type) helical bicomponent organisation. A negative CD band at 220 nm is consistent with β-sheet structure formation. The CD study revealed the formation of ordered molecular organisation possibly through bicomponent β-sheet structure of dipeptide components, in addition to aromatic and ionic interactions. To ascertain this notion, we performed FTIR analysis of the independent components and their 1:1 complex. The spectrum of bicomponent assembly exhibited strong bands at 1634, 1675 cm^{-1} and a shoulder band at 1685 cm^{-1}, which corresponds to antiparallel β-sheet structure.[13] Further, the contribution of aromatic π-π interaction to the bicomponent co-assembly was evident from the hypochromic shift in the absorption spectra (Fig. S5, ESI†). Overall, the noncovalent interactions driven bicomponent antiparallel β-sheet assembly of dipeptide amphiphiles with opposite polarity resulted in the formation of nanofibers of high aspect ratio, and their entangled 3D network in aqueous solvent produced hydrogels.

The hydrogel was subsequently used as soft template for the selective and sensitive detection of NACs via change in the FL intensity of the excimer emission. The detection experiment was carried out in the presence of a number of analytes. Detection of the NACs was achieved by monitoring the FL change($I_o/I \times 100\%$) under the UV light (λ_{ex} = 365 nm) (Fig. 3a). The NACs, viz., trinitrobenzene (TNB) and trinitrotoluene (TNT) were found to show highest FL change (quenching of excimer emission at λ_{em} = 485 nm) due to their low lying HOMO energy level (Fig. 3c). The quenching mechanism could be ascribed to the PET from excited pyrene to the electron-deficient NACs (Fig. 3b).

The non-NACs viz., benzene, toluene were unable to induce significant FL change (Fig. 3d). The sensing efficiency was found to be below 10% for all non-NACs. However, NACs exhibited appreciable to very high FL quenching and simple nitrobenzene (NB) or nitrophenol (NP) showed ~50% efficiencies. The highest efficiencies of 90%, 94.2% and 97% were observed for TNT, Cl-DNT and TNB, respectively. The quenching efficiency increased with the electron-acceptor ability of the NACs. Cl-DNT and TNB with electron withdrawing groups (−Cl and $-NO_2$) symmetrically positioned in the aromatic core, showed relatively higher efficiency compared to TNT with an electron donating group ($-CH_3$) in the core. The overall efficiency for NACs was found to be in the order: TNB>Cl-DNB>TNT>DMB>NB. The electron-deficient NACs bind to electron-rich pyrene through donor-acceptor interaction in the ground state. Upon photo-excitation, the excited state electrons tunnel into the low lying LUMO of the acceptors causing non-radiative decay that led to FL-OFF state. The lifetime (τ) of the excited electron (exciton) does not change its value in the absence and presence of TNT (Fig. S7, ESI†) revealing static quenching interactions.[14] A "sandwitch-like" complex (D-A-D) formation is considered based on the above discussion, which is responsible for FL change in the system. The efficient long-range exciton migration along the extended and π-

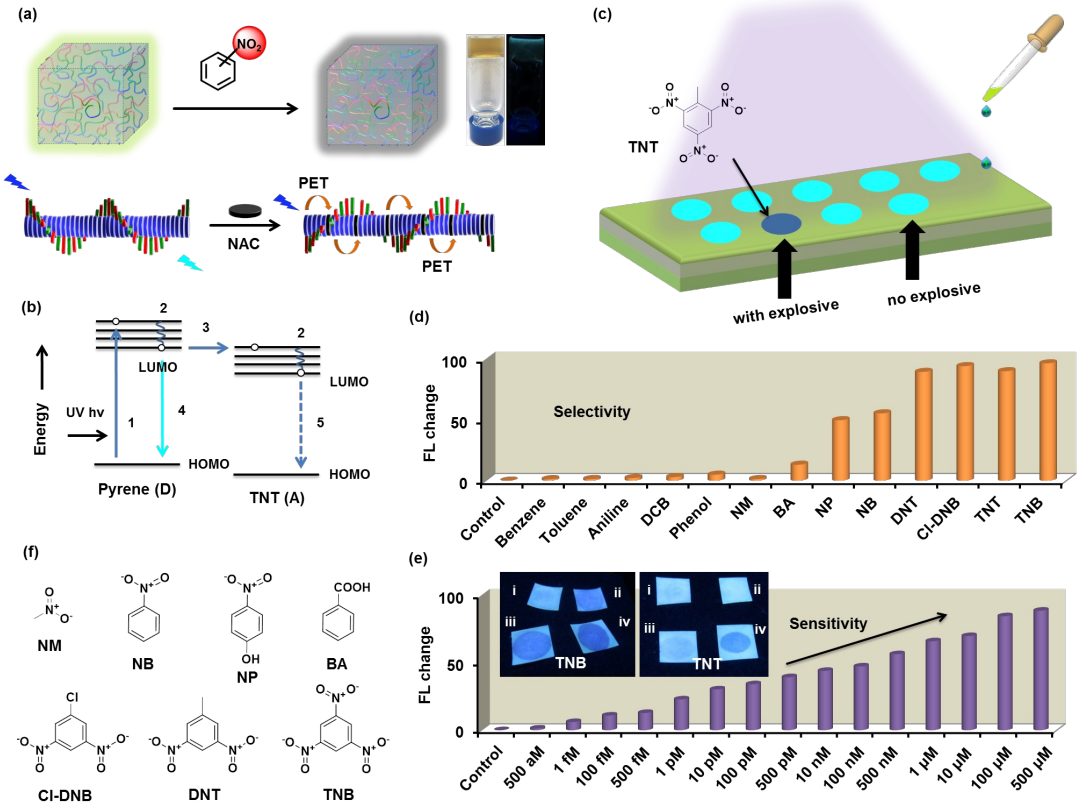

Fig. 3 Selection and detection of NACs via FL quenching: (a) Schematic illustration of the hydrogel-templated detection of the analytes via PET mechanism. Proposed "sandwich-like" conformation of pyrene-NAC-pyrene within the helical nanofiber and the potential intercalative interactions; (b) Energy profile diagram for the PET mechanism (1-5: excitation, vibrational relaxation, electron transfer, radiative decay, and non-radiative decay, respectively ; (c) Experimental set up for the bicomponent co-assembly coated film development NACs detection; (d-e) The selectivity of different analytes and sensitivity of TNT was measured as a function of FL change (%Quenching), inset: film-based NACs detection, i-iv: 100 pM, 100 nM, 1 μM, and 100 μM, respectively; (f) The chemical structures of the analytes: nitromethane (NM), NB, NP, benzoic acid (BA), chloro-dinitrobenzene (Cl-DNB), dinitrotoluene (DNT), TNB, and TNT.

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conjugated nanofibers is assumed to function similar to 'molecular wires' (Fig. 3a).[15] Further, we estimated the lowest detection limit (LOD) for TNB and TNT with the highest FL quenching. A concentration dependent study in a range of 10-500 μM was performed and LOD for TNB and TNT detection was found to as 13.4 and 17.8 μM in solution, respectively (Fig. S9, ESI†).

The visual detection of NAC explosives at extremely low concentrations was achieved in a film-based experiment. The NACs have high vapour pressure and can contaminate the surroundings. We designed a film-based sensory system by quartz-coating with bicomponent assembly-system of dipeptide amphiphiles at a total concentration of 250 μM. The fluorescent films responded to various NACs and turned black, as observed under UV light. The dark spot on the fluorescent film under the UV light is established as a tool for visual detection of explosive. This fluorescent thin film-based method achieved superior detection (5 fold intensity change) at concentration as low as 5 nM (1 ppb) and 100 nM 27 ppb) for both TNB and TNT, respectively (Fig. S10, ESI†).

In summary, a fluorescent hydrogel formed by the bicomponent assembly of two dipeptide-amphiphiles of opposite polarity (TGM-82 and TGM-83) is developed for the selective and sensitive detection of NACs, including the most challenging ones such as nitrophenol, trinitrobenzene and trinitrotoluene. The antiparallel β-sheet induced co-assembly of the dipeptides directed the helical organisation of pyrene chromophores that provide a suitable microenvironment to intercalate guest NACs. The sensing mechanism of NACs is attributed to the proposed pyrene-NAC-pyrene "sandwich-like" interaction that allowed efficient long-range exciton migration. The sensitivity was amplified by developing thin film-based detection platform, which also facilitated quick and reliable visual detection of NACs. Overall, we presented a novel design strategy and detailed study that provides a new insight into the design of peptide-based explosive sensors, which are likely to be useful in protecting human health, environment and security related issues.

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Conflicts of interest

Authors declare no conflicts of interest.

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Graphical Abstract

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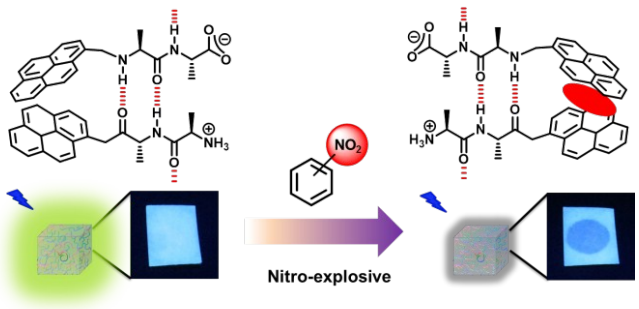

Fluorescent hydrogel formed by the bicomponent β-sheet co-assembly of dipeptide-pyrene amphiphiles of opposite polarity provide a 3D microenvironment to detect toxic nitro-explosives.