Journal of Materials Chemistry

Cite this: *J. Mater. Chem.*, 2012, **22**, 735

www.rsc.org/materials

PAPER

Bifunctional catechol based linkers for modification of TiO_2 surfaces†

Bianca Geiseler and Ljiljana Fruk*

Received 21st June 2011, Accepted 11th October 2011
DOI: 10.1039/c1jm12863a

Bifunctional linkers for modification of TiO_2 nanoparticles were prepared containing a catechol group for TiO_2 surface attachment and a maleimide and alkyne group for Michael addition and Cu catalysed Huisgen cycloaddition respectively. Peptide and fluorophore functionalised TiO_2 NPs were prepared, different purification methodologies were explored and conjugates were characterized using a range of methods.

Introduction

Biofunctionalisation of nanoparticles has been an important topic ever since the great potential of different nanoparticle classes was recognised. Stable biohybrids with preserved nanoparticle and biomolecular function have already found applications in bioimaging,[1] biosensors design[2,3] and nanomedicine[4–6] and there are ongoing efforts to widen the application scope. Semiconducting nanoparticles, with characteristic band gaps determined by the crystallite boundaries, which lead to their size dependent properties, have been thoroughly investigated in the past two decades.[7–9] TiO_2 is a large gap semiconductor and the TiO_2 nanoparticles have found numerous applications in the design of novel photovoltaic devices[10–12] and gas sensors,[13] as a powerful photocatalyst[14–18] and in pollutants removal.[19–22] Depending on the surface modification, they can be photosensitized by the use of UV or visible light and the generated electron–hole (e^-/h^+) pairs can be exploited for various processes at the particle interface.[23] For example, the ability of TiO_2 to induce the production of radical oxygen species (ROS) such as hydroxyl radical, superoxide anion and hydrogen peroxide in aqueous environment upon irradiation[24–26] has attracted attention of biochemists and cell biologists interested in harvesting this ability to use ROS for photodynamic therapy[27] or the study of cell inactivation.[28,29] Recently, the investigation of plant cells in the presence of nano TiO_2 modified with Alizarin Red S has given more insight into the nanoparticle cell uptake and distribution, although without the focus on the qualitative mechanism of action.[30] In particular for biochemical applications, the design of stable NP hybrids, which can withstand the cell uptake without restructuring or dissolution, for example, without unwanted biomolecule removal, ligand exchange, biomolecule or nanoparticle inactivation, is crucial.[31] Thus, it is important that the chemistry, on which the hybrid design is based, enables mild, stereospecific and stable attachment of the molecule of interest to the NP surface. Different functional groups such as phosphates,[32] carboxylic[33–35] and hydroxyl groups[36] have been shown to have affinity for the TiO_2 NP surface. The coordination sphere of the surface metal atoms in small TiO_2 NPs (smaller than 20 nm) is incomplete and therefore exhibits high affinity for oxygen containing ligands.[37] Thus, photoactive TiO_2 biohybrids were prepared by adsorbing harvesting dyes and enzymes through the phosphates and carboxylic groups to enable CO_2 reduction[38] and hydrogen production[38,39] using visible light irradiation. In addition, it has been known that enediols, such as catechols, bind particularly strongly to the TiO_2 surface and show a huge stabilising effect through bidentate chelating and improved surface coverage.[23,40,41] Naturally occurring enediol, dopamine (DA), forms a stable charge transfer complex with TiO_2 where the light absorption at 440 nm results in excitation of an electron from the ligand directly to the conduction band.[40,42,43] This charge transfer formation ability was used to investigate DNA–TiO_2 hybrids, in particular the photocatalytic cleavage of DNA under UV irradiation,[40,44] and used to design *in vivo* light activatable nucleic acid endonuclease.[45] DNA was covalently attached to the primary amino group of dopamine through 1-ethyl-3-(3-dimethylaminopropyl carbodiimide) (EDC)/*N*-hydroxysuccinimide (NHS) coupling.[46] However, EDC coupling usually requires a huge excess of reagents which need to be removed prior to surface immobilisation and EDC has been shown to interfere with other functional groups present in complex biomolecules.[47] When protein binding is concerned, there is also often no control over the stereospecificity of modification, which can in turn lead to the loss of control over the orientation and activity of the attached protein. Click methodology has been recently adapted as a new tool to enable facile attachment of the biomolecules[48,49] and in the wake of huge interest in the design of TiO_2 biohybrids, in particular those containing peptide and protein moieties, we have focused our efforts on the design of different catechol based bifunctional linker systems, which enable mild, facile and stable attachment of molecules of interest to the TiO_2 surface.

Karlsruhe Institute of Technology, DFG—Centre for Functional Nanostructures, 76131 Karlsruhe, Germany. E-mail: ljiljana.fruk@kit.edu
† Electronic supplementary information (ESI) available: TEM images, HPLC chromatograms, MALDI data and details on the click reaction in solution. See DOI: 10.1039/c1jm12863a

Furthermore, we have noticed that there is an experimental gap concerning the purification and characterisation of the TiO_2-biohybrids and for numerous applications, it is of seminal importance to have a fast and reliable purification method to afford pure nano conjugates.

Herewith, we present the syntheses of different catechol based linkers, which are able to bind to the TiO_2 NP surface. These linkers have various groups available for binding of corresponding biomolecules. The maleimide function was chosen because of its high affinity for thiol containing molecules such as cysteines[50] and thus, dopamine maleimide was prepared and used for attachment of thiolated peptides onto the TiO_2 NPs. In addition, taking into account numerous uses of copper catalysed Huisgen cycloaddition in biofunctionalisation,[48] the alkyne modified 3,4-dihydroxyphenylacetic acid (DOPAC) was synthesised to enable nanoparticle modification through additional click methodology.

Experimental section

Chemicals and instruments

All chemicals were purchased from *Sigma Aldrich* and used without further purification. The peptide with the *C*-terminal carboxyl group and the *N*-terminal fluorescein group was purchased from *PANATecs* GmbH (Germany). Double deionized water was used throughout the experiments. The fluorescence spectra were recorded using a CARY ECLIPSE spectrofluorometer (*Varian*) with a quartz cell and UV/Vis absorption spectra using a CARY50 UV/Vis spectrophotometer (*Varian*). The TEM images were obtained on a Philips CM200 FEG/ST electron microscope. The HPLC purification was performed using an *Agilent* 1200 series HPLC system. The size exclusion chromatography was performed using a *GE Healthcare Äktaexplorer*. Dynamic light scattering (DLS) data were obtained using a *Malvern* Zetasizer Nano instrument.

Synthesis of the nanoparticles

TiO_2 nanoparticles were prepared using a protocol by Wang *et al.*[51] with slight modification. In a typical synthesis, 0.2 mL titanium(IV) chloride was added to 6 mL of diethylene glycol (DEG) under vigorous stirring and inert atmosphere at 60 °C. White precipitate was immediately formed after which the suspension was heated at 75 °C until the solution became clear. Then, 0.1 mL ddH_2O was injected into the solution and the solution was refluxed at 160 °C for 6 h. After cooling to the room temperature, 15 mL acetone was added to aid the NP precipitation. The NP solution was centrifuged at 3500 rpm for 30 minutes and washed several times with acetone to remove residual surfactants. The TiO_2 NPs were characterised by transmission electron microscopy (see ESI, Fig. S1†).

Synthesis of the linkers

1-(3,4-Dihydroxyphenethyl)-1*H*-pyrrole-2,5-dione 1. Dopamine hydrochloride (1.00 g, 5.27 mmol) in 30 mL saturated $NaHCO_3$ solution was treated at 0 °C under stirring with *N*-methoxycarbonylmaleimide (0.817 g, 5.27 mmol). After 10 min the solution was diluted with 100 mL water and stirred at room temperature for 40 min. The solution was acidified to pH 1–2 with concentrated H_2SO_4 and extracted three times with 10 mL of ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by silica gel chromatography (CH_2Cl_2/MeOH 20 : 1) to give **1** as a yellow solid.

Yield: 65%. ^{1}H NMR (250 MHz, CD_3OD, δ): 6.74 (s, 2*H*, maleimide), 6.15–6.66 (m, 3H, Ar *H*), 3.60–3.67 (m, 2H, CH_2NH), 2.70 (t, $^3J = 7.1$ Hz, 2H, CH_2Ar); ^{13}C NMR (100 MHz, CD_3OD, δ): 34.72, 40.35, 116.23, 116.94, 121.03, 130.73, 134.50, 145.01, 146.31, 172.42; HRMS (FAB) calcd for $C_{12}H_{11}NO_4$ [M + H]$^+$: 234.0688; found: 234.0765; FT-IR (ATR) ν_{max}: 3457, 2952, 1683, 1605, 1516 cm^{-1}.

2-(3,4-Dihydroxyphenyl)-*N*-(prop-2-yn-1-yl)acetamide[52] 2

(a) Protection of acetic acid—(3,4-dihydroxyphenyl)acetic acid methyl ester. Five drops of concentrated H_2SO_4 were added to a solution of (3,4-dihydroxyphenyl)acetic acid (1.50 g, 8.93 mmol) in MeOH (150 mL) and the reaction was refluxed in the dark and under an argon atmosphere for 2 h. The solvent was removed, the solid was dissolved in EtOAc and washed with saturated $NaHCO_3$. The aqueous phase was extracted with EtOAc, and the combined organic extracts were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure to give the ester as yellow oil.

Yield: quantitative. ^{1}H NMR (250 MHz, $CDCl_3$, δ): 6.80–6.84 (m, 2H, Ar *H*), 6.72 (d, $J = 2$ Hz, 1H; Ar *H*), 5.81 (br, 2H, O*H*), 3.77 (s, 3H, CH_3), 3.58 (s, 2H, CH_2); ^{13}C NMR (60 MHz, $CDCl_3$, δ): 174.23, 143.97, 143.25, 126.05, 121.81, 116.56, 115.68, 52.59, 40.51.

(b) Protection of the catechol—2-(2,2-dimethylbenzo[1,3]dioxol-5-yl)acetic acid methyl ester[52]. 2,2-Dimethoxypropane (9.88 mL, 80.38 mmol) and *p*-toluenesulfonic acid (0.28 g, 1.48 mmol) were added to a solution of (3,4-dihydroxyphenyl)acetic acid methyl ester (1.67 g, 9.17 mmol) in anhydrous $CHCl_3$, and the solution was refluxed in the dark under an argon atmosphere for 8 h. The reaction mixture was neutralized with $NaHCO_3$ and the resulting aqueous phase was extracted 3 times with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and evaporated under reduced pressure. The crude residue was purified using column chromatography by elution with cyclohexane/Et_2O (9 : 1, v/v) to give pure 2-(2,2-dimethylbenzo[1,3]dioxol-5-yl)acetic acid methyl ester as red oil. Yield: 72%. ^{1}H NMR (250 MHz, $CDCl_3$, δ): 6.60–6.68 (m, 3H, Ar *H*), 3.69 (s, 3H, CH_3), 3.52 (s, 2H, CH_2), 1.66 (s, 6H, $C(CH_3)_2$); ^{13}C NMR (60 MHz, $CDCl_3$, δ): 172.31, 147.70, 146.67, 126.94, 121.80, 118.03, 109.55, 108.15, 52.05, 40.92, 25.94.

(c) Acid deprotection—methyl-2-(3,4-dihydroxyphenyl)acetate[53]. To the catechol and acid protected *2-(2,2-dimethylbenzo[1,3]dioxol-5-yl)acetic acid methyl ester* (1.47 g, 6.62 mmol) dissolved in methanol/water (1 : 1, v/v), LiOH (0.320 mg, 13.2 mmol) was added and the reaction mixture was stirred at room temperature overnight. The organic solvent was evaporated under reduced pressure and the aqueous phase was adjusted to pH 5–6 and extracted three times with EtOAc. The combined organic extracts were dried over Na_2SO_4 and evaporated under reduced pressure to give methyl 2-(3,4-dihydroxyphenyl)acetate as yellow oil. Yield: 87%. ^{1}H NMR (300 MHz,

$CDCl_3$, δ): 6.65–6.68 (m, 3H, Ar *H*), 3.54 (s, 2H, CH_2), 1.66 (s, 6H, $C(CH_3)_2$); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 172.83, 146.64, 145.48, 127.98, 121.77, 117.67, 109.51, 107.64, 40.18, 25.47.

*(d) Propargylamine coupling to the deprotected acid group—2-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-N-(prop-2-yn-1-yl) acetamide*[54]. In the solution of *methyl-2-(3,4-dihydroxyphenyl) acetate* (1.26 g, 5.78 mmol) in 10 mL DMF, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) (1.33 g, 6.93 mmol), 1-hydroxybenzotriazole (HOBt) (390 mg, 2.90 mmol) and propargylamine (0.370 mL, 5.78 mmol) were added. The mixture was stirred overnight, the solvent was evaporated under reduced pressure and the crude product was purified on silica gel by elution with cyclohexane/ethyl acetate (5 : 2 → 1 : 1, v/v) to give 2-(2,2-dimethylbenzo[*d*][1,3]dioxol-5-yl)-*N*-(prop-2-yn-1-yl)acetamide as a white solid. Yield: 90%. ^{1}H NMR (300 MHz, DMSO-d_6, δ): 8.39 (br, 1H, N*H*), 6.62–6.73 (m, 3H, Ar *H*), 3.82–3.854 (m, 2H, $NHCH_2$), 3.33 (s, 2H, $ArCH_2$), 3.10–3.11 (m, 1H, C*H*), 1.61 (s, 6H, $C(CH_3)_2$); ^{13}C NMR (60 MHz, DMSO-d_6, δ): 169.95, 146.61, 145.38, 128.94, 121.34, 117.59, 109.05, 107.63, 81.06, 72.92, 41.51, 27.86, 25.45; HRMS (FAB) calcd for $C_{14}H_{15}NO_3$ $[M + H]^+$: 246.1051; found: 246.1131; FT-IR (ATR) ν_{max}: 3281, 3251, 3066, 1666, 1644, 1380 cm^{-1}.

*(e) Synthesis of 2-(3,4-dihydroxyphenyl)-*N*-(prop-2-yn-1-yl) acetamide* ***2***. 2 mL degassed chloroform, 0.625 mL TFA and 0.025 mL water were added to the solution of acetonide 2-(2,2-dimethylbenzo[*d*][1,3]dioxol-5-yl)-*N*-(prop-2-yn-1-yl)acetamide (50.0 mg, 0.204 mmol) under argon and the mixture was stirred for 3 h at room temperature. The solvents were evaporated under reduced pressure to give 2-(3,4-dihydroxyphenyl)-*N*-(prop-2-yn-1-yl)acetamide **2** as colorless oil.

Yield: quantitative. ^{1}H NMR (300 MHz, DMSO-d_6, δ): 8.35 (t, $J = 5$ Hz, 1H; N*H*), 6.61–6.66 (m, 2H, Ar *H*), 6.49 (dd, $J = 2$ Hz, 1H; Ar *H*), 3.82–3.84 (m, 2H, $NHCH_2$), 3.22 (s, 2H, $ArCH_2$), 3.08 (t, $J = 3$ Hz, 1H, C*H*); ^{13}C NMR (400 MHz, DMSO-d_6, δ): 170.36, 144.76, 143.71, 126.67, 119.71, 116.31, 115.21, 81.15, 72.89, 41.41, 27.88; HRMS (FAB) calcd for $C_{11}H_{12}NO_3$ $[M + H]^+$: 206.0739; found: 206.0815; FT-IR (ATR) ν_{max}: 3268, 2125, 1642, 1357 cm^{-1}.

3-Azidocoumarin. 3-Azidocoumarin was synthesised according to the literature.[55]

Yield: 37%. ^{1}H NMR (250 MHz, DMSO-d_6, δ): 10.5 (s, 1H, O*H*), 7.60 (s, 1H, Olefin *H*), 7.46–7.50 (m, 1H, Ar *H*), 6.76–6.83 (m, 2H, Ar*H*); ^{13}C NMR (75 MHz, DMSO-d_6, δ): 161.0, 158.0, 153.4, 128.7, 128.5, 121.8, 114.5, 112.0, 102.7.

Linker modified peptide ***3***. The fluorescent peptide (2.95 mg, 3.78 μmol) was dissolved in 200 μL water, followed by addition of dopamine maleimide **1** (2.65 mg, 11.1 μmol) in 200 μL acetonitrile. The reaction mixture was stirred overnight at room temperature. The crude product was further purified by HPLC using a linear gradient of B over 33 min (A: H_2O + 0.1% TFA, B: CH_3CN + 0.1% TFA) to collect 3.00 mg of the linker peptide in 78% yield (see ESI, Fig. S2a†). The presence of the modified peptide was confirmed by MALDI measurements (see ESI, Fig. S2b†).

Surface modification of TiO_2 nanoparticles. TiO_2 nanoparticles (1.00 mg) were dissolved in 200 μL water, followed by addition of the peptide linker compound **3** (3.00 mg) in 200 μL water. After five seconds, a color change from yellow to orange was observed indicating the formation of the charge transfer complex. The reaction mixture was further stirred overnight at 22 °C. The crude product was purified by size exclusion chromatography.

Click reaction on the NP surface. 0.50 mg TiO_2 NP (in 200 μL H_2O) and linker **2** (30.0 mg, 0.123 mmol) were stirred overnight to afford alkyne modified TiO_2-**2**. After purification by centrifugation (3×), 3-azidocoumarin (25.0 mg, 0.123 mmol) was added followed by 1 M freshly prepared solution of sodium ascorbate (24.5 μL, 0.0246 mmol) and 7.5% copper(II) sulfate pentahydrate solution (in water, 20.5 μL, 0.00615 mmol). The reaction mixture was stirred overnight in the dark at room temperature. After purification by centrifugation (3×), the residue was dissolved in DMSO to perform UV-Vis, fluorescent and DLS measurements.

Results and discussion

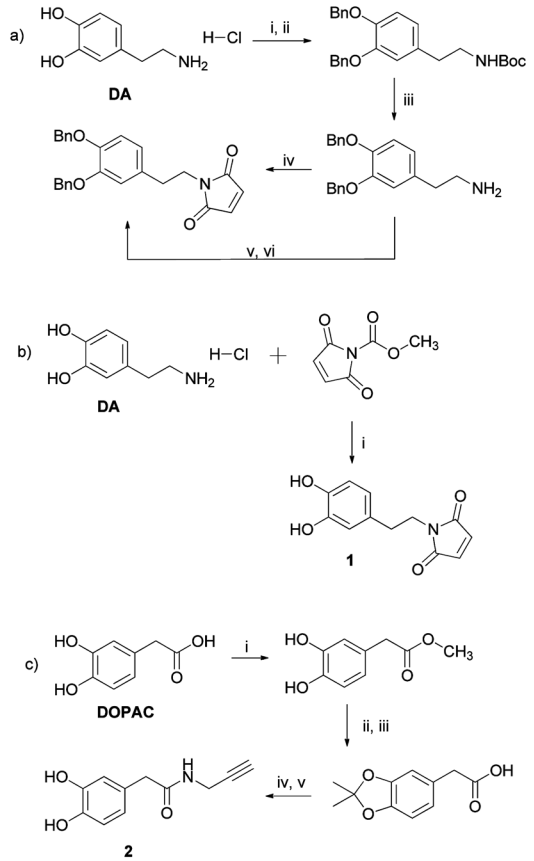
Synthesis of dopamine derivatives

Different strategies were explored to prepare the dopamine maleimide linker **1**, as obtaining this bifunctional linker in high yield was not as straightforward as expected. Widely reported amine derivatisation with maleic acid anhydride and subsequent ring closure were not successful in our hands.[56] However, after the protection of catechol moieties with the benzyl group and treatment with sodium acetate and acetic acid in the absence[57] and presence of a catalytic amount of cobalt(II) naphthenate[58] the desired maleimide was obtained in rather low yield (Scheme 1a). However, deprotection of benzyl group using H_2, Pd/C to obtain free catechol, which is crucial for TiO_2 binding, was not possible without the destruction of the maleimide ring. Therefore, we have used a milder approach based on the work of Keller *et al.* where a method for synthesis of *N*-alkoxycarbonylmaleimides and their use to convert amino acids to maleimido acids in aqueous solution were reported.[59] We used *N*-methoxycarbonylmaleimide for direct coupling to the dopamine hydrochloride, without any catechol protection and in $NaHCO_3$ solution at 0 °C (Scheme 1b), and dopamine maleimide was obtained in 65% yield.

Bifunctional catechol-alkyne linker **2** was prepared using 3,4-dihydroxyphenylacetic acid (DOPAC) as a precursor. Both carboxyl and catechol groups needed to be protected in order to enable successful propargylamine coupling (Scheme 1c). First, the carboxyl group and then the catechol group were protected to obtain fully protected DOPAC. After ester deprotection, propargylamine was reacted with the carboxyl group using an HOBt and EDC coupling strategy and catechol alkyne was obtained in a 90% yield (Scheme 1c). The deprotection of the catechol was performed with 25% trifluoroacetic acid in chloroform to obtain **2** in quantitative yield.

Preparation and modification of TiO_2 nanoparticles

TiO_2 nanoparticles were synthesised by a sol–gel method using Ti(IV) chloride as a precursor and diethylene glycol (DEG) as



a) i: Boc_2O, Et_3N, MeOH, 99%; ii: Benzyl bromide, DMF, K_2CO_3, 63%; iii: TFA/CH_2Cl_2, quant.; iv: Maleic anhydride, cobalt naphthenate, DMA; v: pyridine, maleic anhydride, quant.; vi: Sodium acetate, acetic anhydride. b) i: $NaHCO_3$,65%. c) i: MeOH, H_2SO_4, quant.; ii: *p*-TsOH, DMP, 72%; iii: LiOH, H_2O/MeOH, 87%; iv: Propargylamine, EDC, HOBt, DMF, 90%; v: 25%TFA, $CHCl_3/H_2O$, quant.

Scheme 1 Preparation of (a) dopamine maleimide **1** using (i) maleic anhydride, (ii) sodium acetate/acetic anhydride and cobalt naphthenate; (b) dopamine hydrochloride and *N*-methoxycarbonylmaleimide; (c) 3,4-dihydroxyphenylacetic acid and propargylamine **2**.

a capping agent.[60] The reaction was performed under mild conditions and water soluble 5 nm spheric anatase NPs were obtained (see ESI, Fig. S1†). Since the surface of the NPs is stabilized with weakly bound diethylene glycol, it can be replaced by catechols through a ligand exchange reaction.

TiO_2 NPs were modified by addition of 12 equivalents of dopamine hydrochloride (DA), dopamine maleimide **1** and catechol alkyne **2**, and the attachment of the catechol is easily observed as the formation of the charge transfer complex results in the immediate change of colour (Fig. 1a). Additionally, the catechol binding was confirmed by the absorption band shift in UV-Vis spectra (Fig. 1b).

From the UV-Vis spectra, the bandgap energy for every type of NP can be calculated as E (eV) = 1240/λ_g (nm), where λ_g is the band gap wavelength, which was obtained from the spectra (it is the wavelength between 300 and 400 nm at which a trend line in the UV-Vis spectrum defined by the linear region of decreasing absorbance crosses the horizontal axis).[61] Band gap determines the energy necessary to create electron–hole pairs and it is therefore very important for the determination of the

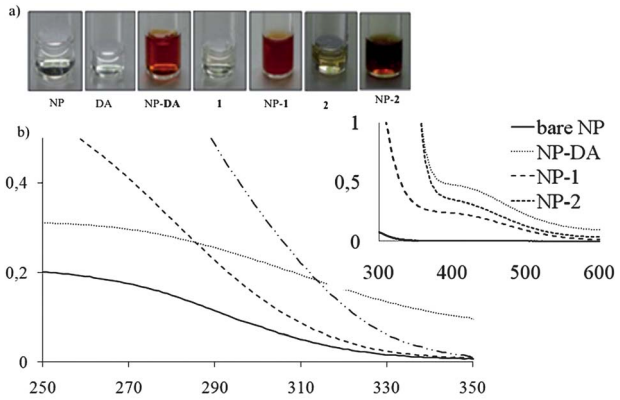


Fig. 1 (a) The change of color upon the charge transfer complex formation between the catechol ligands and TiO_2 NPs; (b) UV-Vis spectrum of the TiO_2 NPs modified with **DA**, **1** and **2**. The charge transfer complex absorbance is shown in the inset.

photocatalytic abilities of the semiconductors—*i.e.*, large bandgaps only allow absorbance of very energetic UV light while the shifts towards smaller bandgaps indicate the possibility of the activation at lower energies (larger wavelengths). As it can be seen from the Table 1, the decrease of the bandgap energy can be observed upon the ligand addition, with TiO_2 modified with maleimide **1** (NP-**1**) showing the biggest shift of 0.4 eV in comparison to bare TiO_2. Our shift values are much smaller than reported previously,[40,62,63] but the band gap data reported till now concerned particles prepared without the water soluble capping agent, which might affect initial values. However, the broad absorbance peak centred around 440 nm with the tail up to 590 nm characteristic of charge transfer complex is observed in all conjugates (Fig. 1b).

Peptide–TiO_2 conjugate preparation using dopamine maleimide 1

To prove that our bifunctional linkers can be used both for TiO_2 attachment through catechols and for additional attachment of the molecules of interest, we have first used linker **1** to introduce a model peptide to the surface of TiO_2. Peptides are important for intracellular transport[64] and have been shown to take part in the cancer growth suppression.[65] Therefore the preparation of the TiO_2–peptide conjugates could enable the future use of these NPs for intracellular applications. Model peptide KGGGC was labelled with 5-carboxyfluorescein on the *C*-terminus and 3 equivalents of dopamine maleimide **1** were added to enable the cysteine modification (Fig. 2a). The resulting dopamine–peptide

Table 1 Band gap energy and the charge transfer complex absorption maxima of modified TiO_2 NPs with different ligands and the charge transfer complex maxima[a]

Sample	λ_g/nm	E_g/eV	λ_{ct}/nm
Bare NP	322	3.85	—
NP-**DA**	331	3.75	389
NP-**1**	359	3.45	381
NP-**2**	340	3.65	395

[a] g: bandgap; ct: charge transfer.

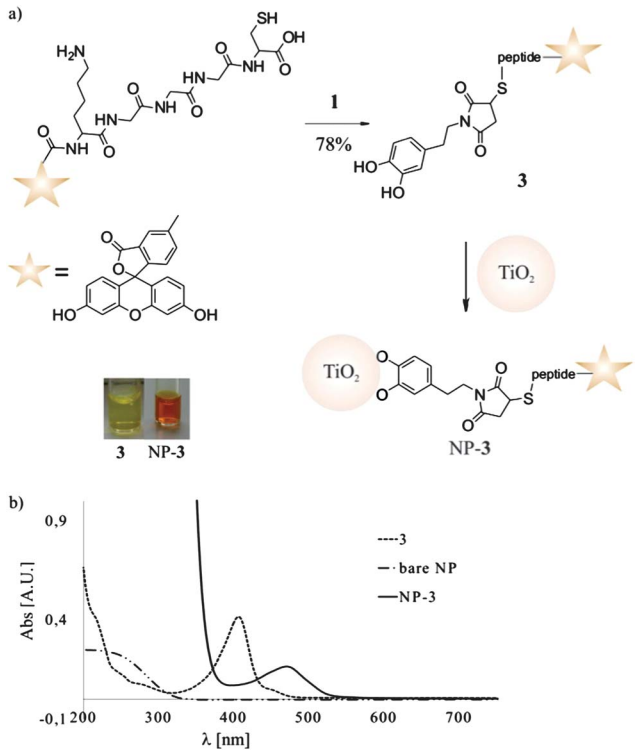


Fig. 2 Peptide modified TiO_2 NPs. (a) Preparation of dopamine modified peptide **3** and the attachment to the TiO_2 surface and (b) UV-Vis spectra of NP-**3** showing charge transfer complex absorbance.

3 was purified using high-performance liquid chromatography (HPLC), and the presence of expected compound is confirmed by mass spectrometry (MALDI) (see ESI, Fig. S2†).

The purified peptide **3** was added to the TiO_2 nanoparticles in water and an immediate change of colour to orange was observed indicating the charge transfer formation and the surface modification (Fig. 2a and b).

However, often the real challenge in preparation of nanoparticle–biomolecule conjugates lies in the purification of the resulting biohybrids. Conventional removal by centrifugation leads to the incomplete removal of the unmodified species and the loss of product through extensive wash steps. Therefore automated size exclusion chromatography using a Superdex 200 10/300 GL column was performed to purify TiO_2–peptide conjugate NP-**3**. As can be seen in Fig. 3 three main peaks can be observed. Peaks 2 and 3 clearly belong to the peptide indicating the formation of disulfide bridged peptide dimers. There are no unmodified TiO_2 present, but instead large peak 1 is obtained which was collected and TEM measurements confirmed the presence of NPs.

In earlier publications it was mentioned that carboxylic acids of rhodamine dye as well as fluorescein can bind to the TiO_2 surface under certain conditions.[35] Therefore, control experiments were performed where the unmodified peptide was added to the TiO_2 and in that case, no peak corresponding to the peptide–TiO_2 was observed (data not shown). In addition, the peptide attachment was confirmed by dynamic light scattering (DLS) where a clear increase of hydrodynamic radius can be observed upon peptide addition (Table 2). A large increase for

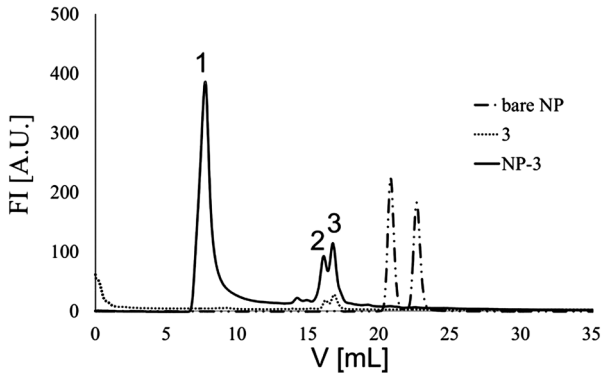


Fig. 3 Size exclusion chromatogram of the modified NP-**1** and corresponding controls monitored at 280 nm.

NP-**3** containing attached peptide indicates that there might be some aggregation in the solvent combination used for DLS measurements although no aggregation was observed in TEM images of NP-**3** after size exclusion chromatography. Controls in which unmodified peptide was mixed with bare TiO_2 NPs alone and TiO_2–DA showed no protein binding, which clearly indicates that the presence of bifunctional linker is necessary to enable the nanoparticle modification.

Modified NPs were also characterized by gel electrophoresis using SDS/polyacrylamide gels. As shown in Fig. 4, the modified nanoparticles did not enter the gel (changing the gel percentage did not alter the result) as indicated by the fluorescent band in lane 3 where NP-**3** was added. The band in lane 3 was cut out and after the gel removal, TEM images were obtained clearly showing the presence of NPs (see ESI, Fig S3†), which additionally confirmed that the conjugate was obtained; if no other method was available, gel electrophoresis can be used for hybrid purification. Our initial attempts to bind thiolated peptide onto the maleimide modified TiO_2 were unsuccessful, most probably due to the pH of the TiO_2 surface and ongoing work is focused on resolving this issue.

Coumarin–TiO_2 conjugate preparation using Cu-catalysed click methodology

In a proof of concept reaction, Huisgen cycloaddition between NP-**2** and 3-azidocoumarin dye was performed (Fig. 5a). 3-Azidocoumarin is often used to monitor the click chemistry progress and conditions because of the change in fluorescence upon reaction due to the formation of strongly fluorescent product from a nonfluorescent coumarin precursor.[66,67]

Table 2 Hydrodynamic radius of modified TiO_2–NPs

Sample	Radius/nm
Bare NP	5.13 ± 0.0500
NP-**1**	34.2 ± 0.395
NP-**2**	20.0 ± 3.18
NP-peptide	4.20 ± 0.246
NP-**3**[a]	62.3 ± 2.05

[a] After purification with size exclusion chromatography.

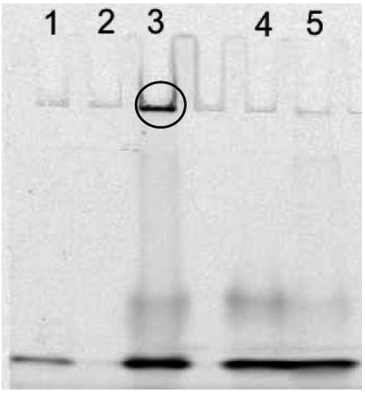


Fig. 4 Gel electrophoresis using 12% SDS-PAGE of the attachment of **3** onto the NP surface. Lane 1: purified peptide linker **3**; lane 2: bare NPs; lane 3: NP-**3**; lane 4: unmodified peptide; lane 5: NP + unmodified peptide.

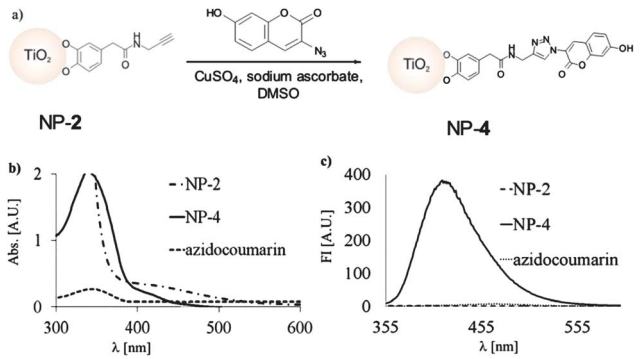


Fig. 5 (a) Scheme of the click reaction of 3-azidocoumarin on the TiO_2 surface; (b) UV-Vis and (c) fluorescence spectra of the reaction product (NP-**4**) and corresponding controls.

3-Azidocoumarin, sodium ascorbate and copper sulfate were added to TiO_2 NPs modified with alkyne linker (NP-**2**) and allowed to react overnight. After purification by centrifugation, fluorescence measurements were performed (Fig. 5c). As mentioned earlier, azidocoumarin itself shows very weak fluorescence and the click product shows strong fluorescence when 348 nm excitation is used (emission at 413 nm). Indeed, the strong fluorescence was observed (Fig. 5c) and in addition, UV-Vis spectra (Fig. 5b) confirmed the presence of TiO_2–coumarin conjugate NP-**4** for which the bandgap energy shift to 3.20 eV is calculated. To confirm additionally that the reaction takes place on the TiO_2 surface, dopamine alkyne **2** was first reacted with 3-azidocoumarin in the solution and the click product was added to the TiO_2 NPs (see ESI, Scheme S1†). Corresponding UV-Vis and fluorescence spectra are identical to the ones obtained by the direct reaction on the nanoparticle surface. In addition, DLS data show a clear change in the hydrodynamic radius (see ESI, Table S1†).

Conclusion

In conclusion, catechol based linkers with different functional groups were synthesised which form a stable charge transfer complex with a TiO_2 NP surface and enable additional click attachment of molecules of interest. A short peptide was attached to the TiO_2 using maleimide attachment *via* Michael addition and the NP conjugate was purified both by size exclusion chromatography and gel electrophoresis. In addition, dopamine alkyne was designed and click chemistry was performed using 3-azidocoumarin as a reaction reporter. We believe that both linkers could find an application both in material science for NP modification and in the other fields such as in the synthetic and polymer chemistry and bioengineering, in which relatively recent discovery of the mussel inspired polydopamine adhesive films[68] and their uses for bio applications[69] have attracted lots of interest in the wider scientific community.

Acknowledgements

This work was supported by KIT Excellence Initiative 2006–2011, grant A5.7. We would like to thank Anna Petri for the help with dopamine alkyne synthesis, Andreas Lefarth and Philipp Müller from Laboratory for Electron Microscopy, KIT for their help with TEM imaging and Marko Miljevic for his help with DLS measurements.

References

1 H. S. Mader, P. Kele, S. M. Saleh and O. S. Wolfbeis, *Curr. Opin. Chem. Biol.*, 2010, **14**, 582.
2 M. C. Daniel and D. Astruc, *Chem. Rev.*, 2004, **104**, 293.
3 O. R. Miranda, B. Creran and V. M. Rotello, *Curr. Opin. Chem. Biol.*, 2010, **14**, 728.
4 I. L. Medintz, H. Mattoussi and A. R. Clapp, *Int. J. Nanomed.*, 2008, **3**, 151.
5 R. Banerjee, Y. Katsenovich, L. Lagos, M. McIintosh, X. Zhang and C. Z. Li, *Curr. Med. Chem.*, 2010, **17**, 3120.
6 R. Seigneuric, L. Markey, D. S. Nuyten, C. Dubernet, C. T. Evelo, E. Finot and C. Garrido, *Curr. Mol. Med.*, 2010, **10**, 640.
7 A. P. Alivisatos, *Science*, 1996, **271**, 933.
8 I. L. Medintz, H. T. Uyeda, E. R. Goldman and H. Mattoussi, *Nat. Mater.*, 2005, **4**, 435.
9 J. Tang and E. H. Sargent, *Adv. Mater.*, 2011, **23**, 12.
10 S. H. Lee, Y. Park, K. R. Wee, H. J. Son, D. W. Cho, C. Pac, W. Choi and S. O. Kang, *Org. Lett.*, 2010, **12**, 460.
11 W. J. Youngblood, S. H. Lee, K. Maeda and T. E. Mallouk, *Acc. Chem. Res.*, 2009, **42**, 1966.
12 N. K. Subbaiyan, C. A. Wijesinghe and F. D'Souza, *J. Am. Chem. Soc.*, 2009, **131**, 14646.
13 A. Tricoli, M. Righettoni and A. Teleki, *Angew. Chem., Int. Ed.*, 2010, **49**, 7632.
14 N. M. Dimitrijevic, B. K. Vijayan, O. G. Poluektov, T. Rajh, K. A. Gray, H. He and P. Zapol, *J. Am. Chem. Soc.*, 2011, **133**, 3964.
15 S. Sato, T. Morikawa, S. Saeki, T. Kajino and T. Motohiro, *Angew. Chem., Int. Ed.*, 2010, **49**, 5101.
16 H. Zhou, X. Gan, J. Wang, X. Zhu and G. Li, *Anal. Chem.*, 2005, **77**, 6102.
17 S. Sajjad, S. A. Leghari, F. Chen and J. Zhang, *Chem.–Eur. J.*, 2010, **16**, 13795.
18 Y. Shiraishi, M. Ikeda, D. Tsukamoto, S. Tanaka and T. Hirai, *Chem. Commun.*, 2011, **47**, 4811.
19 C. Chen, W. Ma and J. Zhao, *Chem. Soc. Rev.*, 2010, **39**, 4206.
20 D. Zhao, C. Chen, Y. Wang, W. Ma, J. Zhao, T. Rajh and L. Zang, *Environ. Sci. Technol.*, 2008, **42**, 308.
21 B. Wen, Y. Li, C. Chen, W. Ma and J. Zhao, *Chem.–Eur. J.*, 2010, **16**, 11859.
22 S. O. Obare, T. Ito and G. J. Meyer, *J. Am. Chem. Soc.*, 2006, **128**, 712.
23 T. Rajh, L. X. Chen, K. Lukas, T. Liu, M. C. Thurnauer and D. M. Tiede, *J. Phys. Chem. B*, 2002, **106**, 10543.
24 A. Mills and S. Le Hunte, *J. Photochem. Photobiol., A*, 1997, **108**, 1.
25 M. R. Hoffmann, S. T. Martin, W. Y. Choi and D. W. Bahnemann, *Chem. Rev.*, 1995, **95**, 69.

26 W. Li, N. Gandra, E. D. Ellis, S. Courtney, S. Li, E. Butler and R. Gao, *ACS Appl. Mater. Interfaces*, 2009, **1**, 1778.
27 J. W. Seo, H. Chung, M. Y. Kim, J. Lee, I. H. Choi and J. Cheon, *Small*, 2007, **3**, 850.
28 J. Joo, S. G. Kwon, T. Yu, M. Cho, J. Lee, J. Yoon and T. Hyeon, *J. Phys. Chem. B*, 2005, **109**, 15297.
29 S. Han, S. H. Choi, S. S. Kim, M. Cho, B. Jang, D. Y. Kim, J. Yoon and T. Hyeon, *Small*, 2005, **1**, 812.
30 J. Kurepa, T. Paunesku, S. Vogt, H. Arora, B. M. Rabatic, J. Lu, M. B. Wanzer, G. E. Woloschak and J. A. Smalle, *Nano Lett.*, 2010, **10**, 2296.
31 A. E. Nel, L. Madler, D. Velegol, T. Xia, E. M. Hoek, P. Somasundaran, F. Klaessig, V. Castranova and M. Thompson, *Nat. Mater.*, 2009, **8**, 543.
32 C. T. Chen and Y. C. Chen, *Anal. Chem.*, 2005, **77**, 5912.
33 Q. Wang, C. Chen, D. Zhao, W. Ma and J. Zhao, *Langmuir*, 2008, **24**, 7338.
34 H. P. Wu, T. L. Cheng and W. L. Tseng, *Langmuir*, 2007, **23**, 7880.
35 D. E. Mekkawi and M. S. A. Abdel-Mottaleb, *Int. J. Photoenergy*, 2005, **7**, 95.
36 H. Huang, J. Zhou, H. Liu, Y. Zhou and Y. Feng, *J. Hazard. Mater.*, 2010, **178**, 994.
37 L. de la Garza, Z. V. Saponjic, N. M. Dimitrijevic, M. C. Thurnauer and T. Rajh, *J. Phys. Chem. B*, 2006, **110**, 680.
38 T. W. Woolerton, S. Sheard, E. Reisner, E. Pierce, S. W. Ragsdale and F. A. Armstrong, *J. Am. Chem. Soc.*, 2010, **132**, 2132.
39 E. Reisner, J. C. Fontecilla-Camps and F. A. Armstrong, *Chem. Commun.*, 2009, 550.
40 T. Rajh, Z. Saponjic, J. Q. Liu, N. M. Dimitrijevic, N. F. Scherer, M. Vega-Arroyo, P. Zapol, L. A. Curtiss and M. C. Thurnauer, *Nano Lett.*, 2004, **4**, 1017.
41 B. Malisova, S. Tosatti, M. Textor, K. Gademann and S. Zurcher, *Langmuir*, 2010, **26**, 4018.
42 N. M. Dimitrijevic, E. Rozhkova and T. Rajh, *J. Am. Chem. Soc.*, 2009, **131**, 2893.
43 M. C. Thurnauer, T. Rajh and D. M. Tiede, *Acta Chem. Scand.*, 1997, **51**, 610.
44 T. Tachikawa, Y. Asanoi, K. Kawai, S. Tojo, A. Sugimoto, M. Fujitsuka and T. Majima, *Chem.–Eur. J.*, 2008, **14**, 1492.
45 T. Paunesku, T. Rajh, G. Wiederrecht, J. Maser, S. Vogt, N. Stojicevic, M. Protic, B. Lai, J. Oryhon, M. Thurnauer and G. Woloschak, *Nat. Mater.*, 2003, **2**, 343.
46 J. Liu, L. de la Garza, L. Zhang, N. M. Dimitrijevic, X. Zuo, D. M. Tiede and T. Rajh, *Chem. Phys.*, 2007, **339**, 154.
47 Z. Grabarek and J. Gergely, *Anal. Biochem.*, 1990, **185**, 131.
48 M. D. Best, *Biochemistry*, 2009, **48**, 6571.
49 L. Moni, S. Rossetti, M. Scoponi, A. Marra and A. Dondoni, *Chem. Commun.*, 2010, **46**, 475.
50 S. S. Ghosh, P. M. Kao, A. W. McCue and H. L. Chappelle, *Bioconjugate Chem.*, 1990, **1**, 71.
51 D. J. Wang, P. Wang, H. Y. Li, T. F. Xie, H. Z. Wang and Z. L. Do, *J. Colloid Interface Sci.*, 2007, **314**, 337.
52 A. Gambacorta, D. Tofani, R. Bernini and A. Migliorini, *J. Agric. Food Chem.*, 2007, **55**, 3386.
53 P. B. Messersmith, Z. Q. Liu and B. H. Hu, *Tetrahedron Lett.*, 2010, **51**, 2403.
54 R. J. Amir, E. Danieli and D. Shabat, *Chem.–Eur. J.*, 2007, **13**, 812.
55 K. Sivakumar, F. Xie, B. M. Cash, S. Long, H. N. Barnhill and Q. Wang, *Org. Lett.*, 2004, **6**, 4603.
56 D. H. Rich, P. D. Gesellchen, D. Tong, A. Cheung and C. K. Buckner, *J. Med. Chem.*, 1975, **18**, 1004.
57 L. Fruk and D. Graham, *Heterocycles*, 2003, **60**, 2305.
58 D. Graham, L. Fruk and W. E. Smith, *Analyst*, 2003, **128**, 692.
59 O. Keller and J. Rudinger, *Helv. Chim. Acta*, 1975, **58**, 531.
60 P. Wang, D. J. Wang, H. Y. Li, T. F. Xie, H. Z. Wang and Z. L. Do, *J. Colloid Interface Sci.*, 2007, **314**, 337.
61 M. Ziolek, I. Tacchini, M. T. Martinez, X. Yang, L. Sun and A. Douhal, *Phys. Chem. Chem. Phys.*, 2011, **13**, 4032.
62 T. Rajh, N. M. Dimitrijevic, Z. V. Saponjic, D. M. Bartels, M. C. Thurnauer and D. M. Tiede, *J. Phys. Chem. B*, 2003, **107**, 7368.
63 A. Jankovic, Z. Saponjic, E. Dzunuzovic and J. Nedeljkovic, *Nanoscale Res. Lett.*, 2010, 81.
64 B. R. Liu, Y. W. Huang, H. J. Chiang and H. J. Lee, *J. Nanosci. Nanotechnol.*, 2010, **10**, 7897.
65 E. Kondo, T. Tanaka, T. Miyake, T. Ichikawa, M. Hirai, M. Adachi, K. Yoshikawa, K. Ichimura, N. Ohara, A. Moriwaki, I. Date, R. Ueda and T. Yoshino, *Mol. Cancer Ther.*, 2008, **7**, 1461.
66 M. S. Schiedel, C. A. Briehn and P. Bauerle, *Angew. Chem., Int. Ed.*, 2001, **40**, 4677.
67 D. J. Yee, V. Balsanek and D. Sames, *J. Am. Chem. Soc.*, 2004, **126**, 2282.
68 H. Lee, S. M. Dellatore, W. M. Miller and P. B. Messersmith, *Science*, 2007, **318**, 426.
69 S. H. Yang, S. M. Kang, K.-B. Lee, T. D. Chung, H. Lee and I. S. Choi, *J. Am. Chem. Soc.*, 2011, **133**, 2795.