# Supplementary Material

# Playing the surface game - Diels Alder reactions on diamond nanoparticles

Gerald Jarre,<sup>a</sup> Yuejiang Liang,<sup>a</sup> Patrick Betz,<sup>a</sup> Daniel Lang,<sup>a</sup> and Anke Krueger<sup>a,b</sup>

# 1. General Chemicals and Methods

Detonation diamond has been purchased from Gansu Lingyun Corp. (China). All other chemicals have been purchased from Aldrich and Fluka and have been used without further purification. Solvents have been dried according to standard procedures.

The following apparatus have been used for the experimental work:

## Sonication:

```
Ultrasound bath: Bandelin Sonorex Digitec Typ DT52 (max. 80 W, 35 kHz)
```

## Centrifuges:

\_

- Hettich EBA 21 Typ 1004

## *FT-IR*:

- Jasco FT/IR-410 equipped with an ATR unit
- *Perkin-Elmer* 1600 Series with home-made vacuum cell (KBr pellet). Samples for measurements using the vacuum cell were heated at 100 120 °C for 2 h *in vacuo* in order to remove most of the water adsorbed at the diamond surface.

## UV/Vis: Perkin-Elmer Lambda 14

## Thermogravimetry (TGA): - Perkin Elmer STA 6000

Measurements were performed in high purity nitrogen with a heating rate of 4  $^{\circ}$ C min<sup>-1</sup> (ca. 20 mg of sample were used for an analysis).

- Netzsch ST2010

Measurements were performed in high purity nitrogen with a heating rate of 4  $^{\circ}$ C min<sup>-1</sup> (ca. 30 mg of sample were used for an analysis).

Elemental analysis: Euro Vector Euro EA 3000 Series

Particle size: Photal FPAR-1000 (dynamic light scattering, Marquardt method)

Stable dispersions for particle size measurements were obtained after centrifugation (15 min at 15000 RPM). Values of particle sizes are based on the weight converted distribution.

## NMR: Bruker AC 200

Fluorescence Microscopy: Zeiss Axiovert 200M (Software: Axiovision)

Samples for fluorescence microscopy were prepared by spin coating of an aqueous diamond suspension on a glass substrate.

Fluorescence Spectroscopy: Photon Technology International QuantaMaster Model QM-2000-4

*Zetapotential*: *Brookhaven* ZetaPlus *Tube furnace*: *Thermolyne* Type 21100

## 2. Syntheses

3,4-bis(bromomethy)benzoic acid(9)



A mixture of 4.5 g 3,4-dimethylbenzoic acid, 14.5 g NBS and 0.5 g benzoyl peroxide was refluxed for 2 h in 250 ml of CCl<sub>4</sub> under nitrogen. The formed succinimide was removed by filtration and the solvent evaporated. The crude product was recrystallized out of methanol. 5.4 g (yield 58 %) of a white solid was obtained as the product. Drying *in vacuo* yielded the product as colourless crystals.

Yield: 5.4 g (58 %).

<sup>1</sup>**H-NMR** (200 MHz, CDCl<sub>3</sub>, TMS, 25 °C):  $\delta = 8.12$  (d, <sup>3</sup>J = 1.7 Hz, 1H), 8.07 (dd, <sup>3</sup>J = 8.1 Hz, <sup>3</sup>J = 1.7 Hz, 1H), 7.5 (d, <sup>3</sup>J = 8.0 Hz, 1H), 4.69 (s, 2H), 4.68 (s, 2H) ppm.

## A: General procedure for thermal annealing of nanodiamond

The diamond material was placed in a tube furnace and heated *in vacuo* ( $< 10^{-3}$  mbar). The heating rate was 100 K min<sup>-1</sup> and the furnace was allowed to cool to room temperature within 2 h (while maintaining the vacuum).

## B: General procedure for arylation of nanodiamond via Diels-Alder-reaction

Thermally annealed nanodiamond was suspended in a solution of  $\alpha, \alpha'$ -dibromo-o-xylene, 18crown-6 and potassium iodide in dry toluene. The suspension was heated for 72 h under nitrogen and reflux. After cooling the diamond particles were isolated by centrifugation. The precipitate was washed repeatedly with acetone, water and dichloromethane in consecutive washing / centrifugation cycles. Ultrasonic treatment was used in every cycle in order to redisperse the diamond and remove adsorbed impurities. After washing, the sample was dried at 70 °C *in vacuo*.

## C: General procedure for nitration of arylated nanodiamond

Aryl functionalized nanodiamond was suspended in a mixture of conc. sulphuric acid and 65 % nitric acid. The suspension was stirred for 20 h at room temperature. After cooling the diamond particles were isolated by centrifugation. The precipitate was washed repeatedly with water until the supernatant became neutral and subsequently with acetone in consecutive washing / centrifugation cycles. Ultrasonic treatment was used in every cycle in order to

redisperse the diamond and remove adsorbed impurities. After washing, the sample was dried at 70 °C *in vacuo*.

#### D: General procedure for sulfonation of arylated nanodiamond

Aryl functionalized nanodiamond was suspended in chlorosulfonic acid and heated to 50 °C for 20 h. After cooling the reaction mixture was poured on ice, and the diamond particles were isolated by centrifugation. The precipitate was washed repeatedly water until the supernatant became neutral. Following, the diamond particles were suspended in 1 N NaOH and stirred for 30 min. The mixture was neutralized with conc. hydrochloric acid and the diamond particles were isolated by centrifugation. The precipitate was washed repeatedly with water until the supernatant became neutral and acetone in consecutive washing / centrifugation cycles. Ultrasonic treatment was used in every cycle in order to redisperse the diamond and remove adsorbed impurities. After washing, the sample was dried at 70 °C *in vacuo*.

## E: General procedure for reduction of arylated nanodiamond after sulfonation

To a suspension of sulfoarylated nanodiamond in dry benzene triphenylphosphine and iodine were added. The suspension was heated for 20 h under nitrogen and reflux. After cooling the diamond particles were isolated by centrifugation. The precipitate was washed repeatedly with toluene, acetone, sat. sodium thiosulfate solution, water and dichloromethane in consecutive washing / centrifugation cycles until no impurities could be detected by TLC. Ultrasonic treatment was used in every cycle in order to redisperse the diamond and remove adsorbed impurities. After washing, the sample was dried at 70 °C *in vacuo*.

## F: General procedure for Oregon Green<sup>®</sup> 488 labelled nanodiamond

Oregon Green<sup>®</sup> 488 maleiimide (7) was dissolved in DMF and added to thioarylated nanodiamond dispersed in DMF and PBS buffer (pH = 7.2). The suspension was stirred in the dark for 20 h under nitrogen at room temperature. The diamond particles were isolated by centrifugation. The precipitate was washed repeatedly with DMF until there was no dye detectable by UV / Vis spectroscopy in the supernatant after centrifugation. Ultrasonic treatment was used in every cycle in order to redisperse the diamond and remove adsorbed impurities. After washing diethyl ether the product was dried *in vacuo* and stored under argon at -30 °C.

#### G: General procedure for grafting nanodiamond with benzoic acid via Diels-Alder-reaction

Thermally annealed nanodiamond was suspended in a solution of 3,4-bis(bromomethyl)benzoic acid, 18-crown-6 and potassium iodide in 15 ml of dry toluene. The suspension was heated for 72 h under nitrogen under reflux. After cooing the diamond particles were isolated by centrifugation. The precipitate was washed repeatedly with acetone, water and dichloromethane in consecutive washing / centrifugation cycles. Ultrasonic treatment was used in every cycle in order to redisperse the diamond and remove adsorbed impurities. After washing, the sample was dried at 70 °C *in vacuo*.

#### Analytical data

Thermally annealed detonation nanodiamond (1a)



1a

According to general procedure A (annealing temperature: 1000 °C; amount of DND: 1.0 g). **FT-IR** (vacuum cell):  $\tilde{\nu}$  = no specific absorption. **Elemental analysis**: N: 2.0 %, C: 93.9 %, H: 0.3 %. **Particle size**: a stable dispersion could not be obtained.

#### Thermally annealed detonation nanodiamond (1b)



#### 1b

According to general procedure A (annealing temperature: 750 °C; amount of DND: 1.0 g).

**FT-IR** (vacuum cell):  $\tilde{v}$  = no specific absorption.

Elemental analysis: N: 2.1 %, C: 95.1 %, H: 0.7 %.

Particle size: a stable dispersion could not be obtained.

Arylated detonation diamond (2a)



2a

According to general procedure B. Amount of reagents and solvent as follows: 600 mg of thermally annealed DND (1a), 2.34 g (9.00 mmol) of  $\alpha,\alpha$ '-dibromo-o-xylene, 3.60 g (13.5 mmol) of 18-crown-6, 9.93 g (60 mmol) potassium iodide, 30 ml of dry toluene.

#### Yield: 444 mg.

**FT-IR** (vacuum cell):  $\tilde{\nu} = 3062, 2926, 2985, 1592, 1546, 1494, 1460, 1382, 1218, 1156, 1116, 754 cm<sup>-1</sup>.$ 

**Elemental analysis**: N: 2.1 %, C: 89.6 %, H: 1.1 %.

**Surface loading**: (calculated from TGA):  $0.28 \text{ mmol g}^{-1}$ .

 $(\Delta m \ 140 - 415 \ ^{\circ}C: 2.9 \ \%; \ fragment: C_8H_8 \ (104 \ g \ mol^{-1})$ 

**Particle size:** (H<sub>2</sub>O): 10 % < 30 nm, 50 % < 41 nm, 90 % < 65 nm.

(Methanol): 10 % < 27 nm, 50 % < 36 nm, 90 % < 56 nm.

(CH<sub>2</sub>Cl<sub>2</sub>): 10 % < 39 nm, 50 % < 50 nm, 90 % < 6 $\beta$  nm.

(Acetone): 10 % < 90 nm, 50 % < 125 nm, 90 % < 202 nm.

Zeta potential: 20.4 mV.

UV (water):  $\lambda = 225, 261$  nm.

#### Arylated detonation diamond (2b)



According to general procedure B. Amount of reagents and solvent as follows: 100 mg of thermally annealed DND (**1b**), 390 mg (1.50 mmol) of  $\alpha,\alpha$ '-dibromo-o-xylene, 600 mg (2.25 mmol) of 18-crown-6, 1.66 g (10 mmol) potassium iodide, 10 ml of dry toluene. Yield: 49 mg.

**FT-IR** (vacuum cell):  $\tilde{\nu} = 2946, 2880, 1722, 1608, 1460, 1432, 1210, 1156, 1120, 752 cm<sup>-1</sup>.$ 

Elemental analysis: N: 2.0 %, C: 88.4 %, H: 0.8 %.

**Surface loading**: (calculated from TGA): 0.16 mmol  $g^{-1}$ .

 $(\Delta m \ 140 - 460 \ ^{\circ}C: 1.7 \ ^{\circ}; fragment: C_8H_8 \ (104 \ g \ mol^{-1})$ 

**Particle size:** (H<sub>2</sub>O): 10 % < 6 nm, 50 % < 8 nm, 90 % < 22 nm.

(Methanol): 10 % < 33 nm, 45 % < 36 nm, 90 % < 73 nm.

(Acetone): 10 % < 81 nm, 50 % < 125 nm, 108 % < 710 nm.

#### Zeta potential: 39.8 mV.

UV (water):  $\lambda = 263(s)$  nm.

#### Nitration of arylated diamond (2a) to (4a)



According to general procedure C. Amount of reagents and solvent as follows: 364 mg of arylated diamond (**2a**), 7 ml of conc. sulphuric acid, 5 ml of 65 % nitric acid.

Yield: 292 mg.

**FT-IR** (vacuum cell):  $\tilde{v} = 2956, 2926, 1722, 1656, 1598, 1542, 1350, 1220, 1214, 1154, 1114, 846, 824, 744 cm<sup>-1</sup>.$ 

Elemental analysis: N: 2.4 %, C: 88.6 %, H: 0.6 %.

**Surface loading:** (calculated from TGA): 0.29 mmol  $g^{-1}$ .

 $(\Delta m \ 120 - 330 \ ^{\circ}C: 4.4 \ \%; \ fragment: C_8H_7NO_2 \ (149 \ g \ mol^{-1})$ 

**Particle size:** (DMSO): 10 % < 39 nm, 50 % < 53 nm, 90 % < 84 nm.

Zeta potential: -3.4 mV.

Nitration of arylated diamond (2b) to (4b)



According to general procedure C. Amount of reagents and solvent as follows: 83 mg of arylated diamond (**2b**), 4 ml of conc. sulphuric acid, 3 ml of 65 % nitric acid.

Yield: 58 mg.

**FT-IR** (vacuum cell):  $\tilde{\nu} = 2956, 2928, 2870, 1712, 1656, 1588, 1432, 1350, 1220, 1156, 1116, 884, 842, 760 cm<sup>-1</sup>.$ 

**Elemental analysis**: N: 2.2 %, C: 88.1 %, H: 0.9 %.

**Surface loading**: (calculated from TGA): 0.14 mmol  $g^{-1}$ .

 $(\Delta m \ 120 - 330 \ ^{\circ}C: 2.1 \ \%; \ fragment: C_8H_7NO_2 \ (149 \ g \ mol^{-1})$ 

**Particle size:** (DMSO): 10 % < 63 nm, 50 % < 86 nm, 90 % < 135 nm.

Zeta potential: 8.2 mV.

#### Attempt of nitration of annealed detonation diamond (1a), for comparison

According to general procedure C. Amount of reagents and solvent as follows: 100 mg of anneald diamond (1a), 7 ml of conc. sulphuric acid, 5 ml of 65 % nitric acid. Yield: 96 mg **FT-IR** (vacuum cell):  $\tilde{v} = 2926, 2880, 1722, 1606, 1432, 1116, 1054, 862, 772 cm<sup>-1</sup>.$  **Elemental analysis**: N: 2.2 %, C: 92.3 %, H: 0.7 %.

Surface loading: no mass loss step in the relevant region was observed.

Particle size: a stable suspension could not be obtained.

Zeta potential: 13.5 mV.

Sulfonation of arylated diamond (2a) to (5a)



According to general procedure D. Amount of reagents and solvent as follows: 468 mg of arylated diamond (**2a**), 5 ml of chlorosulfonic.

Yield: 337 mg.

**FT-IR** (vacuum cell):  $\tilde{\nu} = 2956$ , 2934, 2886, 1722, 1602, 1198, 1126, 1036, 814, 742, 688 cm<sup>-1</sup>.

Elemental analysis: N: 1.9 %, C: 91.1 %, H: 0.8 %, S: 1.0 %.

Surface loading:(calculated from TGA): 0.32 mmol  $g^{-1}$ .<br/>(calculated from change in sulphur): 0.30 mmol  $g^{-1}$ .<br/>( $\Delta m \ 270 - 330 \ ^{\circ}C$ : 6.0 %; fragment:  $C_8H_8O_3S$  (184 g mol<sup>-1</sup>)

**Particle size:** (water): 10 % < 46 nm, 50 % < 61 nm, 90 % < 99 nm.

Zeta potential: -21.3 mV.

UV (water):  $\lambda = 224$ , 273(s) nm.

## Sulfonation of arylated diamond (2b) to (5b)



According to general procedure D. Amount of reagents and solvent as follows: 300 mg of arylated diamond (**2b**), 5 ml of chlorosulfonic acid.

Yield: 216 mg.

**FT-IR** (vacuum cell):  $\tilde{v} = 2950, 2875, 1706, 1600, 1462, 1432, 1364, 1200, 1116, 1036, 860, 833, 768 cm<sup>-1</sup>.$ 

Elemental analysis: N: 2.2 %, C: 86.6 %, H: 0.9 %, S: 0.4 %.

Surface loading:(calculated from TGA): 0.12 mmol g $^{-1}$ .<br/>(calculated from change in sulphur): 0.13 mmol g $^{-1}$ .<br/>( $\Delta m 160 - 460 \ ^{\circ}C$ : 2.2 %; fragment: C $_8H_8O_3S$  (184 g mol $^{-1}$ )

**Particle size:** (water): 10 % < 31 nm, 50 % < 41 nm, 90 % < 61 nm.

Zeta potential: -12.7 mV.

UV (water):  $\lambda = 225(s)$ , 272(s) nm.

## Attempt of sulfonation of annealed detonation diamond (1a) for comparison

According to general procedure E. Amount of reagents and solvent as follows: 100 mg of annealed diamond (1a), 5 ml of chlorosulfonic acid.

Yield: 58 mg.

**FT-IR** (vacuum cell):  $\tilde{\nu} = 2946, 2924, 2846, 1700, 1638, 1116, 1050 \text{ cm}^{-1}$ .

Elemental analysis: N: 2.2 %, C: 92.2 %, H: 0.8 %.

Surface loading: no mass loss step in the relevant region was observed.

Particle size: a stable suspension could not be obtained.

Zeta potential: 11.9 mV.

## Reduction of the sulfonic group of (5a) to( 6a)



#### 6a

According to general procedure E. Amount of reagents and solvent as follows: 287 mg of arylated diamond carrying sulfonic functional group (**5a**), 2.60 g (10.0 mmol) of triphenylphospine, 0.30 g (1.17 mmol) of iodine, 5 ml of dry benzene. Yield: 267 mg.

**FT-IR** (vacuum cell):  $\tilde{\nu} = 3062, 2256, 2856, 1712, 1598, 1194, 1160, 1118, 1036, 830 cm<sup>-1</sup>.$ 

Elemental analysis: N: 2.0 %, C: 85.6 %, H: 0.8 %, S: 0.9 %.

**Surface loading:** (calculated from TGA):  $0.29 \text{ mmol g}^{-1}$ .

(calculated from change in sulphur): 0.29 mmol  $g^{-1}$ .

 $(\Delta m \ 150 - 485 \ ^{\circ}C: 4.0 \ \%; \ fragment: \ C_8H_8S \ (136 \ g \ mol^{-1})$ 

**Particle size:** (DMSO): 10 % < 56 nm, 50 % < 76 nm, 90 % < 127 nm.

Zeta potential: 9 mV.

## Reduction of the sulfonic group of (5b) to (6b)



According to general procedure E. Amount of reagents and solvent as follows: 149 mg of arylated diamond carrying sulfonic functional group (**5b**), 2.30 g (5.0 mmol) of triphenylphospine, 0.150 g (0.585 mmol) of iodine, 5 ml of dry benzene. Yield: 134 mg. **FT-IR** (vacuum cell):  $\tilde{\nu} = 3058$ , 2954, 2864, 1716, 1600, 1206, 1156, 1116, 1038, 838 cm<sup>-1</sup>. **Elemental analysis**: 2.3 %, C: 87.4 %, H: 0.8 %, S: 0.4 %. **Surface loading:** (calculated from TGA): 0.14 mmol g<sup>-1</sup>. (calculated from change in sulphur): 0.13 mmol g<sup>-1</sup>. ( $\Delta m \ 170 - 320 \ ^{\circ}$ C: 1.9 %; fragment: C<sub>8</sub>H<sub>8</sub>S (136 g mol<sup>-1</sup>) **Particle size:** (DMSO): 10 % < 8 nm, 14 % < 41 nm, 90 % < 38 nm.

Zeta potential: 15.2 mV.

Oregon Green<sup>®</sup> labelled detonation diamond (8a)



**8**a

According to general procedure F. Amount of reagents and solvent as follows: 4 mg of arylated diamond carrying thiol functional group (**6a**), 2 mg of Oregon Green<sup>®</sup> 488 maleiimide (**7**), 2.5 ml of DMF, 2.5 ml of PBS buffer. **UV** (DMF):  $\lambda = 474$ , 510 nm.

## OregonGreen<sup>®</sup> labelled detonation diamond (8b)



8b

According to general procedure F. Amount of reagents and solvent as follows: 10 mg of arylated diamond carrying thiol functional group (**6b**), 3 mg of OregonGreen<sup>®</sup> 488 maleimide (**7**), 3 ml of DMF, 3 ml of PBS buffer.

**FT-IR** (ATR):  $\tilde{v} = 2979, 2940, 2880, 1718, 1647, 1599, 1558, 1539, 1490, 1434, 1373, 1245, 1176, 1122, 1099, 1040, 952, 877, 840, 794, 765 cm<sup>-1</sup>.$ 

**Surface loading Oregon Green**<sup>®</sup> **488:** (calculated from TGA): 0.05 mmol g<sup>-1</sup>.

 $(\Delta m \ 170 - 280 \ ^{\circ}C: 1.4 \ \%; \ \Delta m \ 280 - 480 \ ^{\circ}C: 3.0 \ \%).$ 

UV (water):  $\lambda = 481$ , 517 nm.

**Fluorescence** ( $\lambda_{Ex}$  = 480 nm, 20 °C, DMF):  $\lambda_{max}$  = 550 nm.

Benzoic acid modified detonation diamond (3a)



According to general procedure G. Amount of reagents and solvent as follows: 155 mg of thermally annealed DND (1a), 0.920 g (3.00 mmol) 3,4-bis(bromomethyl)benzoic acid, 1.20 g (4.50 mmol) 18-crown-6, 4.90 g (30 mmol) potassium iodide, 15 ml of dry toluene.

Yield: 112 mg.

**FT-IR** (vacuum cell):  $\tilde{\nu} = 3062, 2956, 2922, 2854, 1728, 1612, 1580, 1426, 1372, 1262, 1210, 1180, 1102, 1006, 910, 844, 760 cm<sup>-1</sup>.$ 

Elemental analysis: N: 1.3 %, C: 77.2 %, H: 2.3 %.

**Surface loading**: (calculated from TGA):  $1.82 \text{ mmol g}^{-1}$ .

 $(\Delta m \ 160-470 \ ^{\circ}C: 26.9 \ \%; \ fragment: \ C_9H_8O_2 \ (148 \ g \ mol^{-1})$ 

**Particle size:** (DMSO): 10 % < 35 nm, 50 % < 47 nm, 90 % < 75 nm.

Zeta potential: -39.7 mV.

Benzoic acid modified detonation diamond (3b)



According to general procedure G. Amount of reagents and solvent as follows: 175 mg of thermally annealed DND (**1b**), 0.920 g (3.00 mmol) 3,4-bis(bromomethyl)benzoic acid, 1.20 g (4.50 mmol) 18-crown-6, 4.90 g (30 mmol) potassium iodide, 15 ml of dry toluene. Yield: 165 mg.

**FT-IR** (vacuum cell):  $\tilde{\nu} = 3062, 2926, 1724, 1610, 1580, 1426, 1372, 1262, 1210, 1180, 1104, 1004, 968, 912, 844, 762 cm<sup>-1</sup>.$ 

**Elemental analysis**: N: 1.8 %, C: 83.8 %, H: 1.8 %.

3,4-bis(bromomethy)benzoic acid (9)



A mixture of 4.5 g 3,4-dimethylbenzoic acid, 14.5 g NBS and 0.5 g benzoyl peroxide was refluxed for 2 h in 250 ml of  $CCl_4$  under nitrogen. The formed succinimide was removed by filtration and the solvent evaporated. The crude product was recrystallized from methanol. Drying *in vacuo* yielded the product as colourless crystals.

Yield: 5.4 g (58 %).

<sup>1</sup>**H-NMR** (200 MHz, CDCl<sub>3</sub>, TMS, 25 °C):  $\delta = 8.12$  (d, <sup>3</sup>J = 1.7 Hz, 1H), 8.07 (dd, <sup>3</sup>J = 8.1 Hz, <sup>3</sup>J = 1.7 Hz, 1H), 7.5 (d, <sup>3</sup>J = 8.0 Hz, 1H), 4.69 (s, 2H), 4.68 (s, 2H) ppm.

*Table 1*: Summary of analytical data of arylated detonation diamond and functionalised derivatives.

sample	annealing temperature / °C	particle size / nm <sup>a)</sup>	ξ-potential / mV <sup>b)</sup>	mass loss / % <sup>c)</sup>	surface loading / mmol g <sup>-1</sup>
	1000 ( <b>1a</b> )	-	-	-	-
	750 ( <b>1b</b> )	-	-	-	-
	1000 ( <b>2a</b> )	41 <sup>d)</sup>	20.4	2.9	0.28
	750 ( <b>2b</b> )	8 <sup>d)</sup>	39.8	1.7	0.15
ОСОН	1000 ( <b>3a</b> )	47 <sup>e)</sup>	-39.7	26.9	1.82
	750 ( <b>3b</b> )	52 <sup>e)</sup>	-26.0	10.6	0.72
NO <sub>2</sub>	1000 ( <b>4a</b> )	53 <sup>e)</sup>	-3.4	4.4	0.29
	750 ( <b>4b</b> )	86 <sup>e)</sup>	8.2	2.1	0.14
SO <sub>3</sub> H	1000 ( <b>5a</b> )	61 <sup>d)</sup>	-21.3	6.0	0.30
	750 ( <b>5b</b> )	41 <sup>d)</sup>	-12.7	2.2	0.13
SH	1000 ( <b>6a</b> )	76 <sup>e)</sup>	9.0	4.0	0.29
	750 ( <b>6b</b> )	14 <sup>e)</sup>	15.2	1.9	0.23

a) 50% value by weight; b) measured in .water (pH 5.8); c) of the relevant mass loss step in TGA; d) supernatant in water after centrifugation at 15000 rpm for 15 min; e) supernatant in DMSO after centrifugation at 15000 rpm for 15 min;

## 3. Spectroscopic data

## 700 600 500 counts (a.u.) 400 300 DND, 1000°C, 2h 200 DND, 750°C, 2h 100 DND, pristine 0 10 30 50 70 90 2θ(°)

Fig. 1S. XRD spectra of pristine detonation nanodiamond (DND, black), DND annealed at 750°C for 2 h (green), and DND annealed at 1000 °C for 2 h (red). It is visible from these data that only at 1000 °C a small signal for graphitic carbon (arrow) appears. No graphitic carbon is observed for the sample annealed at 750 °C. The size reduction of the diamond signal in the case of **1a** (DND annealed at 1000 °C) is due to the formation of amorphous, disordered carbon. The crystallite size calculated from the halfwidth of the diamond signals remains unchanged after the annealing step.

#### XRD data:

#### **FT-IR Spectroscopy**



*Fig. 2S:* FTIR data of nanodiamond: a) pristine, b) after thermal annealing at 1000 °C (1a), c) after arylation (2a), d) nitration (4a), e) sulfonation (5a), f) reduction to thiol (6a), and g) benzoic acid functionalization (3a). The 1,2,4-substitution pattern of 4a and 5a has been established by comparison of the IR fingerprint region with the literature. [S1, S2]



*Fig. 3S:* FTIR data of nanodiamond: a) pristine, b) after thermal annealing at 750 °C (**1b**), c) after arylation (**2b**), d) nitration (**4b**), e) sulfonation (**5b**), f) reduction to thiol (**6b**), and g) benzoic acid functionalization (**3b**).



*Fig. 4S*: FTIR-Spectra of a) pristine, b) after annealing (1a), c) control reaction of sulfonation and d) sulfonation of arylated nanodiamond (5a).



Fig. 5S: FTIR-Spectra of a) pristine, b) after annealing (1a), c) control reaction of nitration and d) nitration of arylated nanodiamond (4a).



Fig.6S: FT-IR of a) pristine, b) after annealing (1b), c) thioarylated nanodiamond (6b) and d) Oregon Green<sup>®</sup> labelled nanodiamond (8b).

## Thermogravimetry



*Fig.7S:* Thermograms of nanodiamond. First row (from left to right): pristine DND, after thermal annealing (1a), after arylation (2a); second row: after control nitration reaction, after nitration (4a), after control sulfonation reaction; third row: sulfonation (5a), reduction to thiol (6a), and benzoic acid functionalization (3a).



*Fig. 85:* Thermograms of nanodiamond. First row (from lefto to right): pristine, after thermal annealing (1b), after arylation (2b); second row: after control nitration reaction, after nitration (4b), after control sulfonation reaction; third row: sulfonation (5b), reduction to thiol (6b), and benzoic acid functionalization (3b).

#### **UV/Vis Spectroscopy**



*Fig. 9S:* Comparison of UV/Vis spectra of arylated (2) (top) and after sulfonation (5) (bottom) detonation diamond annealed at 750 °C (left graphs) and at 1000 °C (right graphs).



Fig. 10S: UV/Vis spectra a) of OregonGreen labelled nanodiamond. (8a), b) of Oregon Green (7), and c) of supernatant solvent after centrifugation of 8a.



Fig. 11S: UV/Vis spectra a) of Oregon Green labelled nanodiamond (8b), b) Oregon Green (7), and c) of supernatant solvent after centrifugation of 8b.

*Table 2*: UV absorption maxima and shift of OregonGreen labelled nanodiamond (8) compared to free dye 7. The shift results from the covalent grafting of the dye molecule to the electron-deficient nanoparticle.

sample	$\lambda_{1 \max}$ (nm)	$\lambda_{2max}$ (nm)	$\Delta shift_{max1}$ (nm)	$\Delta shift_{max2} (nm)$
7	524.4	488.9	-	-
<b>8</b> a	509.9	474.5	14.5	14.4
8b	517.2	481.7	7.3	7.2

#### **Column Chromatography**



*Fig. 12S:* Chromatography of the Oregon Green<sup>®</sup> labelled nanodiamond (**8b**) (left) and pure Oregon Green<sup>®</sup> maleiimide (7, right): stationary phase: alumina, solvent: DMF. No elution from the column is observed for the free dye. Only the conjugate is mobile in this chromatography.

#### **Fluorescence Microscopy**



Fig 13S: Fluorescence microscopic picture of Oregon Green<sup>®</sup> labelled nanodiamond (8b): left: overlay, center: bright-field image, right: fluorescence.

#### **Supplementary references**

- [S1] M. Hesse, H. Meier, B. Zeh, Spektroskopische Methoden der organischen Chemie, 5<sup>th</sup> ed., Georg Thieme Verlag, Stuttgart New York, 1995.
- [S2] A. D. Cross, *Introduction to Practical Infra-red Spectroscopy*, Butterworths Scientific Publications, London 1960.