Poly(ethylene glycol) Adlayers Immobilized to Metal Oxide Substrates Through Catechol Derivatives: Influence of Assembly Conditions on Formation and Stability

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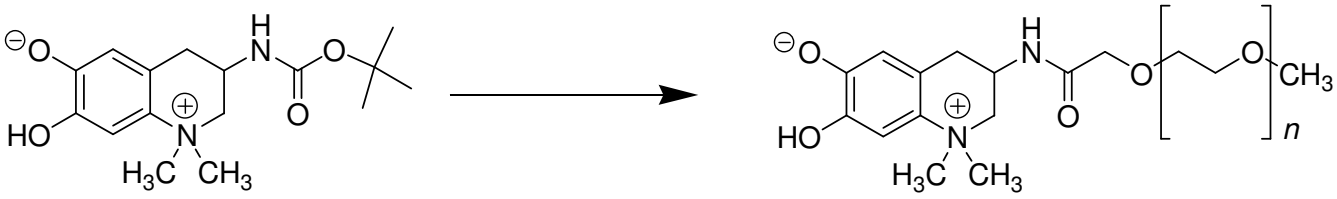
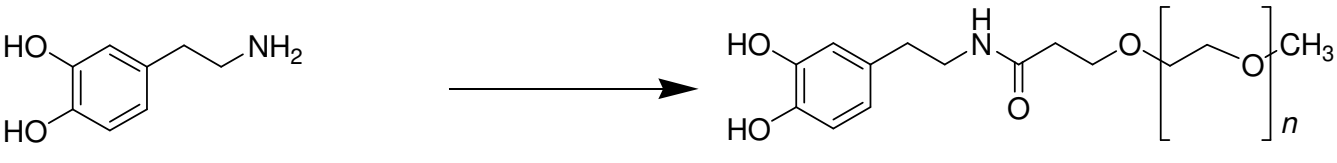
1. Synthesis of compound **1**



i **1**

Boc-Anacat (**i**) (Prepared according to Gademann et al.[1]) (6.8 mg, 22 µmol) was dissolved at 0 °C (ice-bath) in HCl (4 M) in dioxane (0.5 mL). The reaction mixture was stirred for 1.5 h at 0 °C, whereas after 30 minutes a yellow-orange precipitate formed, and for 10 minutes at RT under Ar. The solvent was removed under reduced pressure and the resulting off-white powder was dried under high vacuum. The residue was suspended in DMF (1 mL) and CH_2Cl_2 (1 mL) and N-methylmorpholine (50 µL) was added. Additional DMF (1 mL) was added and the reaction mixture was stirred for 15 minutes until an orange-yellow solution resulted. mPEG-ASA (50 mg, ca. 10 µmol) was added in one portion and the orange-yellow solution was stirred at RT under Ar and under the exclusion of light for 18 h. The reaction mixture was then filtered, and diluted with 50 mL diethylether. The solution was allowed to stand for 30 minutes at 4 °C, after which a white precipitate formed. The precipitate was filtered and washed extensively with diethylether. The product was dried under high vacuum to give **1** (33.2 mg, ca 6.6 µmol, 66 %) orange solid. Elemental Analysis for $C_{242}H_{477}N_2O_{118}$ (MW: 5304) in weight %, found (calculated): C: 53.63 % (54.80 %), H: 8.90 % (9.08 %), N: 0.69 % (0.53 %), O: 35.56 % (35.59 %).

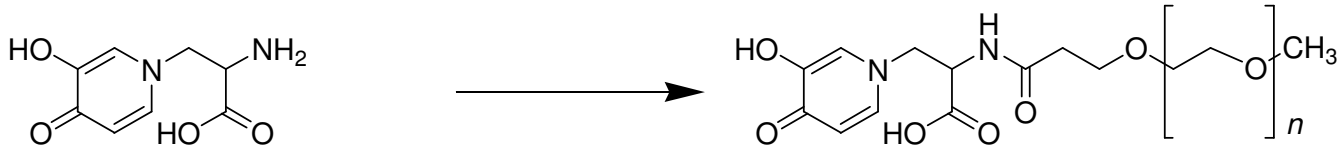
2. Synthesis of compound **2**



ii **2**

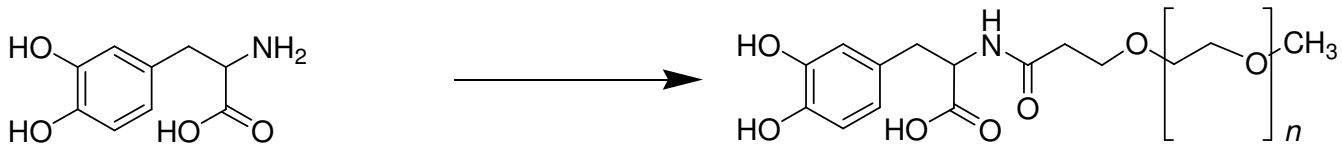
Dopamine hydrochloride (**ii**) (50 mg, 0.26 mmol) and N-methylmorpholine (50 µL) were dissolved in Ethanol (20 mL). mPEG-ASA (1.34 g, ca. 0.26 mmol) dissolved in 5 mL chloroform was added and the mixture was stirred at room temperature for 2.5 h. The crude reaction mixture was evaporated to about 15 mL on a rotary evaporator and diethylether (40 mL) was added. The solution was allowed to stand for 30 minutes at 4 °C, and then at -20 °C over night, after which a white precipitate formed. The precipitate was filtered and washed extensively with diethylether. The product was dried under high vacuum to give **2** (1.26 g, ca 0.24 mmol, 92 %) as a white solid. Elemental Analysis for $C_{240}H_{473}N_1O_{118}$, m.w.: 5262 in weight %, found (calculated): C: 54.40 % (54.78 %), H: 8.83 % (9.08 %), N: 0.29 % (0.27 %), O: 36.11 % (35.88 %).

3. Synthesis of compound **3**



Mimosine (**iii**) (10 mg, 0.05 mmol) was dissolved in sodiumborate buffer (5 mL, 50 mM, pH 8.4). mPEG-ASA (252 mg, ca. 0.05 mmol) was added and the resulting mixture was stirred at room temperature for 16 h after which time a HCl solution (0.5 mL, 2 N) was added. The compound was extracted into chloroform and the combined organic phases were dried with $MgSO_4$. After reducing the solvent to 5 mL on a rotary evaporator, diethylether (40 mL) was added. The solution was allowed to stand for 30 minutes at 4 °C, and then at -20 °C over night, after which a white precipitate formed. The precipitate was filtered and washed extensively with diethylether. The product was dried under high vacuum to give **3** (220 mg, ca 0.04 mmol, 85 %) as a white solid. Elemental Analysis for $C_{240}H_{473}N_2O_{120}$, m.w.: 5303 in weight %, found (calculated): C: 54.23 % (54.32 %), H: 9.04 % (8.97 %), N: 0.32 % (0.53 %), O: 36.19 % (36.18 %).

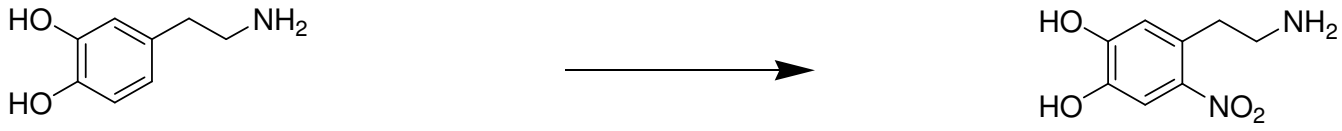
4. Synthesis of compound **4**



iv **4**

L-DOPA (**iv**) (3.9 mg, 0.02 mmol) was dissolved in sodiumborate buffer (4 mL, 50 mM, pH 8.4) under nitrogen. mPEG-ASA (100 mg, ca. 0.02 mmol) was added and the resulting mixture was stirred at room temperature for 24 h after which time a HCl solution (0.5 mL, 2 N) was added. The compound was extracted into chloroform and the combined organic phases were dried with $MgSO_4$. After reducing the solvent to 5 mL on a rotary evaporator, diethylether (40 mL) was added. The solution was allowed to stand for 30 minutes at 4 °C, and then at -20 °C over night, after which a white precipitate formed. The precipitate was filtered and washed extensively with diethylether. The product was dried under high vacuum to give **4** (90 mg, ca 0.017 mmol, 87 %) as a white solid. Elemental Analysis for $C_{241}H_{474}N_1O_{120}$, m.w.: 5302 in weight %, found (calculated): C: 54.52 % (54.56 %), H: 8.84 % (8.99 %), N: 0.28 % (0.26 %), O: 36.18 % (36.19 %).

Nitrodopamine (**v**)

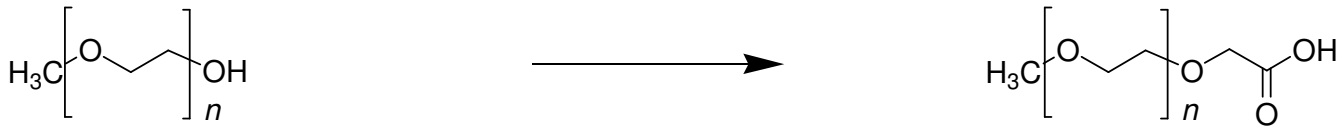


ii **v**

Dopamine hydrochloride (**ii**) (1.90 g, 10 mmol) and sodium nitrite (1.52 g, 22 mmol) were dissolved in water (25 mL) and cooled down to 0 ºC. Sulfuric acid (17.4 mmol in 10 mL water) was added slowly to the mixture and a yellow precipitate was formed. After stirring at room temperature overnight the

precipitate was filtered and recrystallized from water. The product was dried under high vacuum to give nitrodopamine[2, 3] (**v**) as the hemi-sulfate salt 6-nitro-3-hydroxytyramine hemi-sulfate salt), (1.389 g, 58 %). 1H-NMR (D_2O, 300 MHz, ppm): 7.62 (s, 1H nitrodopamine), 6.83 (s, 1H nitrodopamine), 3.24 (t, 2H CH_2), 3.12 (t, 2H CH_2). Elemental Analysis for $C_{16}H_{22}N_4O_{12}S$ in weight% found (calculated): C 38.01 (38.87); H 4.48 (4.48); N 11.10 (11.33); S 6.89 (6.49).

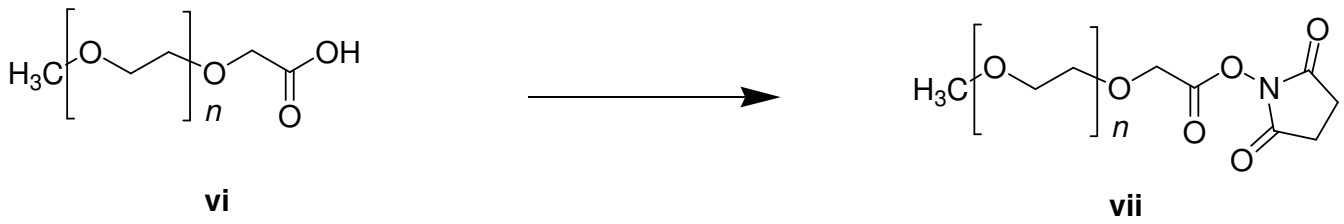
mPEG-COOH (**vi**)



mPEG-OH **vi**

mPEG-OH (50 g, 10 mmol; Fluka, m.w.: 5000 in weight %) and KOH (14 g, 0.26 mmol) were dissolved in 250 mL water. $KMnO_4$ (4.74 g, 30 mmol) was added and the reaction mixture was stirred over night. The resulting brown suspension was filtered over celite and 44 mL of concentrated HCl (37%) was added. The product was extracted into chloroform followed by drying with $MgSO_4$. After reducing the volume by evaporation, diethylether was added until the solution became turbid. The solution was stored in the fridge over night and the precipitate mPEG-COOH (**vi**) was filtered and dried under high vacuum (33.8 g, 6.76 mmol, 68 %). 1H-NMR ($CDCl_3$, 300 MHz, ppm): 4.19 (s, 2H, O-CH_2-COO), 3.57-3.9 (m, -CH_2CH_2O-), 3.4 (s, 3H, -OCH_3).

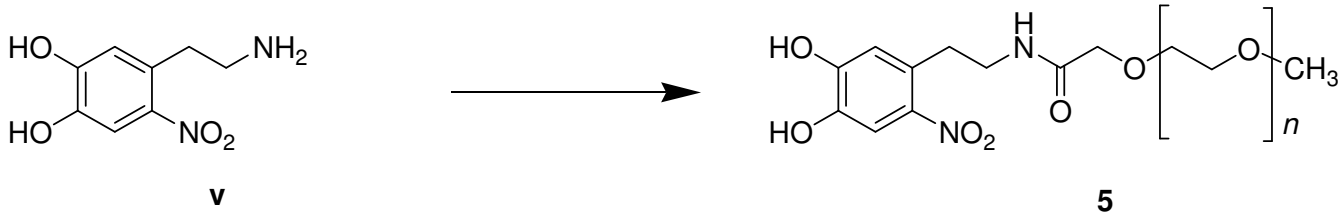
mPEG-ASA (**vii**)



vi **vii**

mPEG-COOH (**vi**) (33.8 g, 6.76 mmol) in CH_2Cl_2 (200 mL) with addition of 13 g molecular sieves (3 Å spheres, Fluka) was stirred over night in CH_2Cl_2 (200 mL) to remove residual water. 1,3-dicyclohexylcarbodiimide (DCC; 2.09 g, 10.14 mmol) and N-hydroxysuccinimide (NHS; 1.17 g, 10.14 mmol) were added and the suspension was stirred over night. Resulting N,N-dicyclohexylurea (DCU) was filtered off over celite. The volume of the solvent was reduced to about 50 mL with a rotary evaporator and then diethylether added until the solution became turbid. The solution was stored in the fridge over night and the resulting precipitate filtered and dried at high vacuum to give 30.65 g of mPEG-ASA (**vii**) (6.13 mmol, 91 %). 1H-NMR ($CDCl_3$, 300 MHz, ppm): 4.55 (s, 2H, O-CH_2-COO), 3.57-3.9 (m, -CH_2CH_2O-), 3.4 (s, 3H, -OCH_3), 2.89 (s, 4H, succinimide).

mPEG-nitrodopamine (**5**)



v **5**

Nitrodopamine (**v**), (30 mg, 0.1 mmol) and N-methylmorpholine (20 mL) were dissolved in a mixture of ethanol (10 mL) and chloroform (2 mL). mPEG-ASA (500 mg, 0.1 mmol) was added and the mixture stirred at room temperature over night. The crude reaction mixture was filtered over celite, washed with chloroform and the clear yellow solution evaporated to dryness. The product was dried under high vacuum to give 428 mg (0.08 mmol, 80 %) as a yellow-greenish solid. 1H-NMR ($CDCl_3$, 300 MHz, ppm): 7.70 (s, 1H, nitrodopamine), 6.83 (s, 1H, nitrodopamine), 3.98 (s, 2H, O-CH_2-CON), 3.57-3.9

(m, -CH_2CH_2O-), 3.4 (s, 3H, -OCH_3). Elemental Analysis for $C_{240}H_{470}N_2O_{120}$, m.w.: 5000, in weight % found (calculated): C: 54.05 % (54.35 %), H: 8.98 % (8.93 %), N: 0.52 % (0.53 %), O: 36.15 %, (36.20 %).

Adsorption results as a function of polymer concentration:

In order to reduce the amount of polymer needed and to reduce the immersion time to equilibrate the surfaces for subsequent protein resistance tests, polymer adsorption as function of polymer concentration was investigated for **1**, **2** and **5** (Figure 1). Furthermore desorption/stability properties of adsorbed polymer 2 incubated in physiological buffer (Figure 2) were tested.

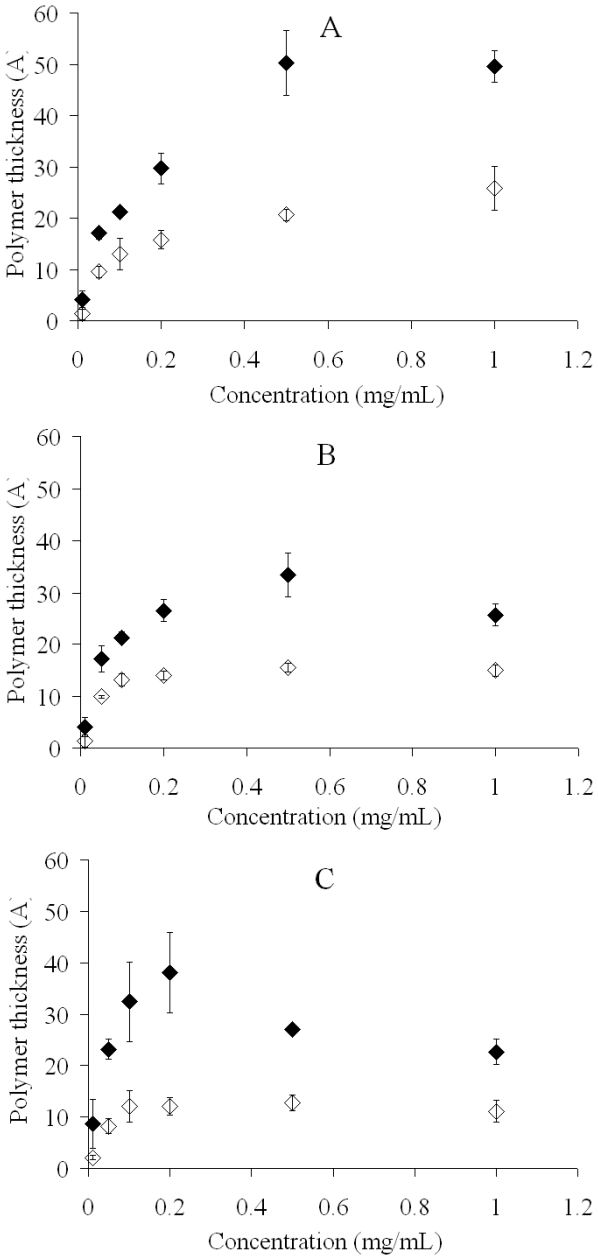


Figure 1 Adlayer thickness of (A) PEG-anacat (**1**), (B) PEG-dopamine (**2**) and (C) PEG-nitrodopamine (**5**) as a function of polymer concentration; First (◆) directly after adsorption (4 h, 50 °C, pH 6, 0.1 M MOPS buffer 0.6 M NaCl and 0.6 M K_2SO_4); Further (◇) after immersion in physiological buffer solution for 24 hours.

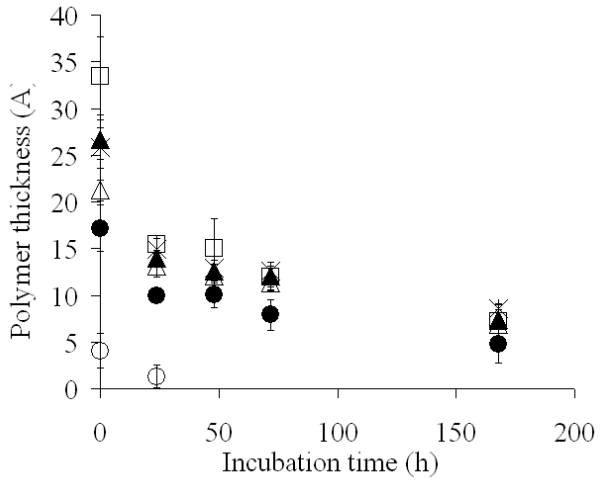


Figure 2 Ellipsometric thickness values of PEG-dopamine (**2**) layers deposited from different concentrations (1 mg/mL (∗), 0.5 mg/mL (□), 0.2 mg/mL (▲), 0.1 mg/mL (△), 0.05 mg/mL (●) and 0.01 mg/mL (○)) as a function of incubation time in physiological buffer solution. Polymer **2** was previously adsorbed during 4 h on TiO_2 at pH 6 at 50 °C.

Additional prove of polymer adsorption of samples modified at all conditions described above was performed by XPS. Ratio of C(PEG)/Ti as a function of polymer thickness confirms that the adlayer thickness measured by VASE corresponds to the polymer adsorbed (and not a possible contamination) (Figure 3).

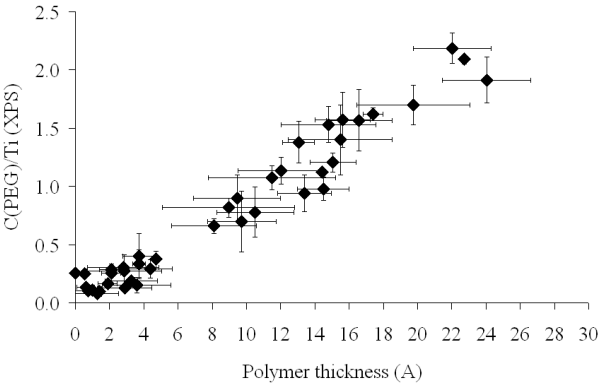


Figure 3. C(PEG)/Ti ratio (signals measured by XPS) as a function of polymer thickness (VASE).

References:

1 Gademann, K., *Chembiochem* **2005**, 6, (5), 913-919.

2. Napolitano, A.; Dischia, M.; Constantini, C.; Prota G., A., *Tetrahedron* **1992,** 48, (39), 8515-8522.

3. Ranganathan, S.; Tamilarasu, N., *J. Indian. Chem. Soc.* **1999,** 76, (11-12), 727-731.