Supporting Information

AFM-based Thermal Lithography of P*t*BA Block Copolymer Films for Bioconjugation

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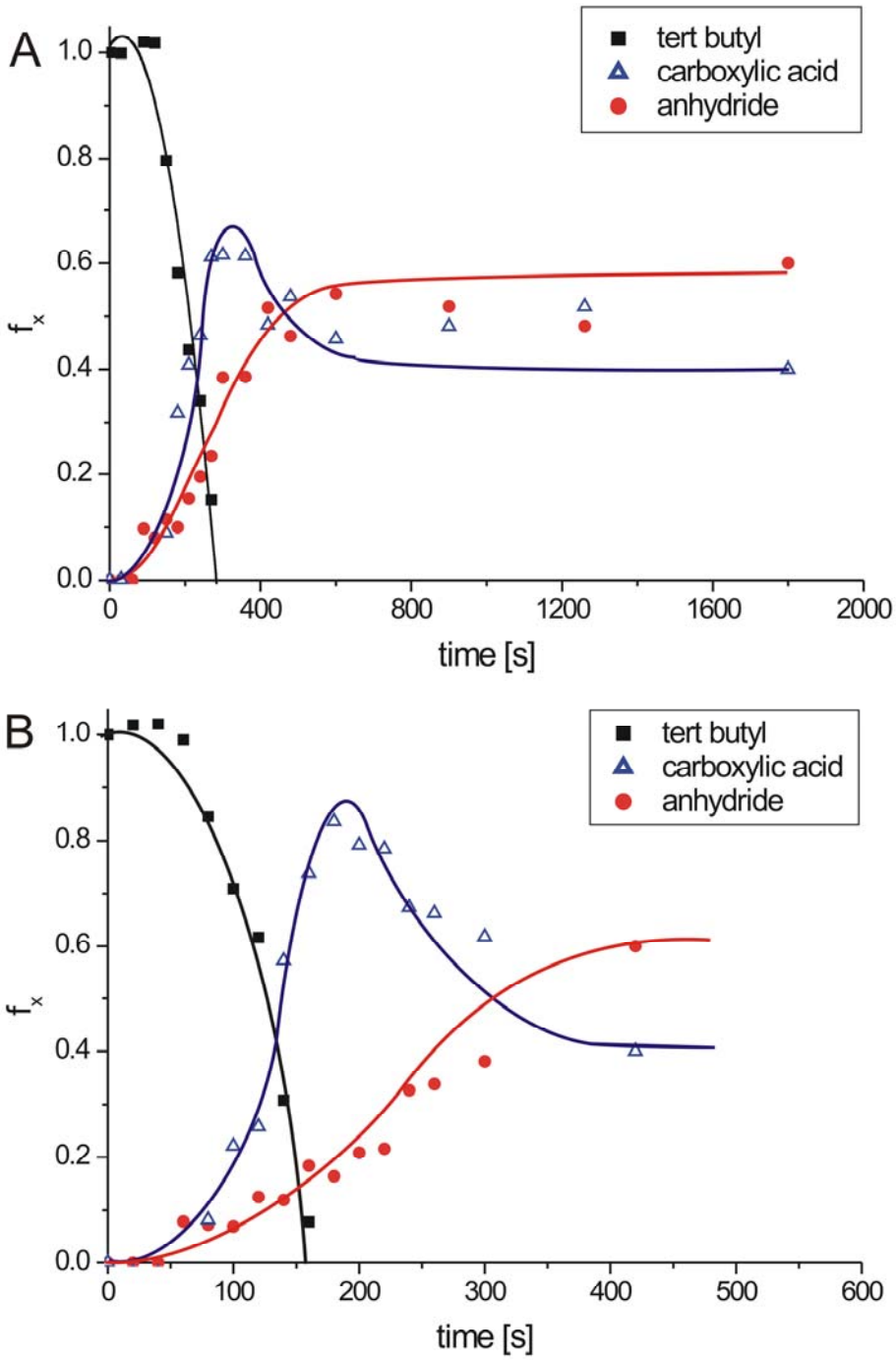


Figure S-1. Fraction of tBA, carboxylic acid and anhydride groups as a function of thermolysis times for thermolysis at (a) 230 ℃ and (b) 240 ℃.

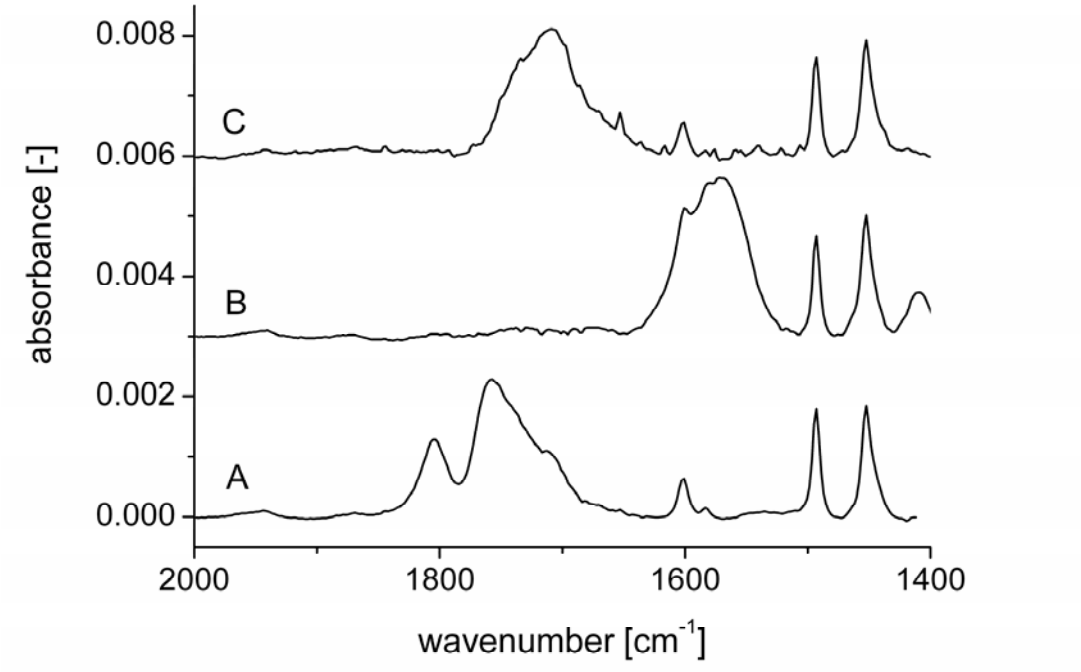


Figure S-2. Transmission FTIR spectra of thin PS-b-PtBA films: (A) after thermolysis for 240 seconds at 250°C, (B) after being subsequently incubated overnight in PBS buffer (pH 7.4), and (C) after subsequent immersion in 3 M HCl for 10 minutes. In spectrum (A) the $\nu_{C=O}$ of the anhydride groups at 1752 cm^{-1} and 1804 cm^{-1} as well as a shoulder at 1709 cm^{-1}, attributed $\nu_{C=O}$ of carboxylic acid groups absorbances, are clearly visible. Hydrolysis of the anhydride groups in PBS buffer followed by deprotonation of the formed carboxylic acid groups was evidenced in (B) by the presence of the absorbances of $\nu_{C=O}$ of the carboxylate ions at 1570 cm^{-1} and 1409 cm^{-1}. No absorbance of $\nu_{C=O}$ of the tert butyl ester at 1733 cm^{-1} is present indicating 100% conversion of tert butyl ester to carboxylic acid groups. Protonation of the carboxylate ions in 3 M HCl (10 minutes) yields carboxylic acid groups which give rise to a maximum $\nu_{C=O}$ of carboxylic acid groups at 1709 cm^{-1} in spectrum (C). In order to calculate the conversion of carboxylic acid groups to anhydride we determined the peak height of $\nu_{C=O}$ of carboxylic acid groups at 1709 cm^{-1} for spectrum (A) (after spectral deconvolution) and (C) with respect to the PS $\nu_{s(C-C)}$ absorbances at 1455 cm^{-1} in the same spectrum. The conversion of carboxylic acid groups to anhydride groups was found to be approximately 60%. This fraction was used as correction factor in equation 2 for the calculation of the fraction of anhydride groups for all thermolysis temperatures.

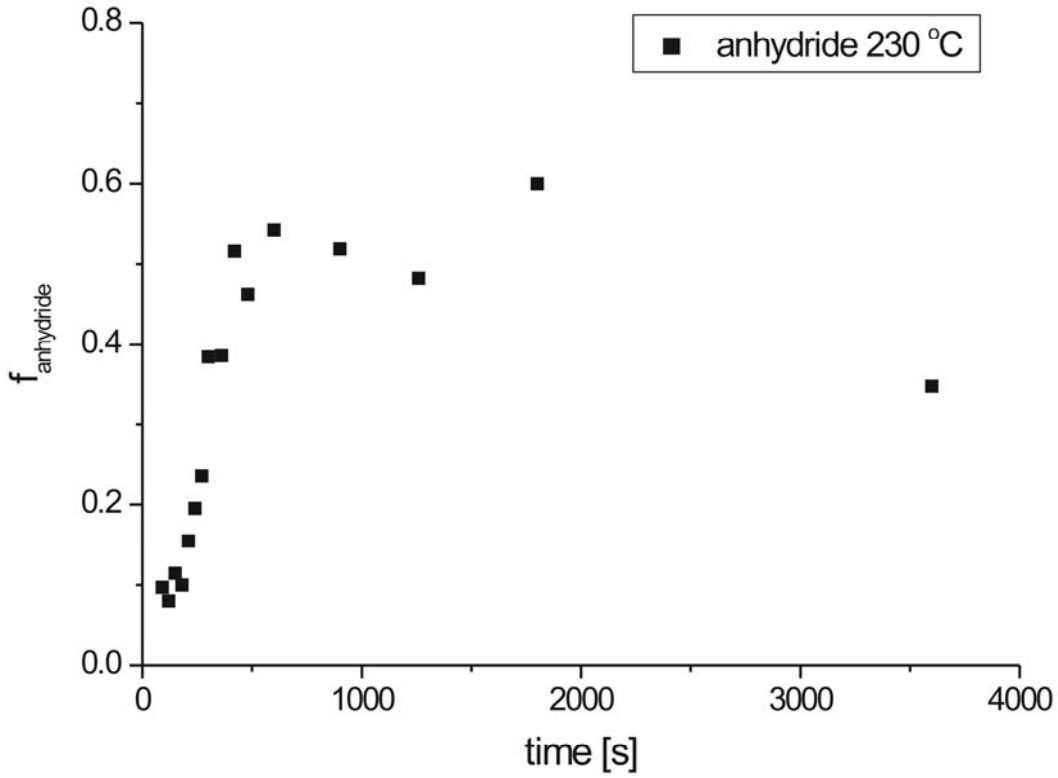
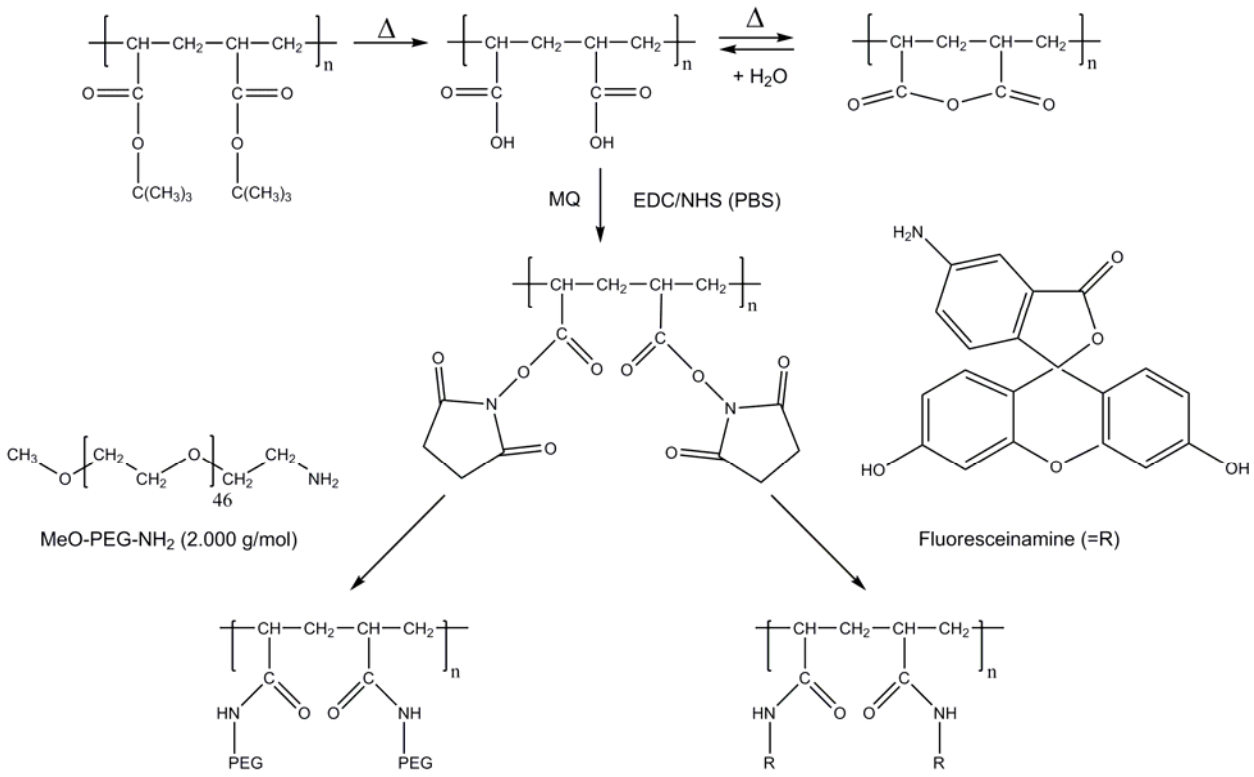


Figure S-3. Fraction of anhydride as a function of thermolysis time at 230℃ determined by FTIR according to $f_{anhydride} = 0.6\ (\nu_s(C=O)_{1755} / \nu_s(C\text{-}C)_{1455})_t / (\nu_s(C=O)_{1755} / \nu_s(C\text{-}C)_{1455})_{t0}$. *The decrease in anhydride fraction for longer thermolysis times is attributed to decarboxylation. This behavior was also observed for thermolysis carried out at 240℃ and 250℃ (data not shown).*



Scheme S-1: Either MeO-PEG$_{45}$-NH$_2$ or fluoresceinamine were covalently immobilized on the thermolyzed PS-b-PtBA films. After thermolysis the films were immersed first for 1 hour in Milli Q water to hydrolyze the anhydride groups followed by activation of the carboxylic acid groups via EDC/NHS chemistry.

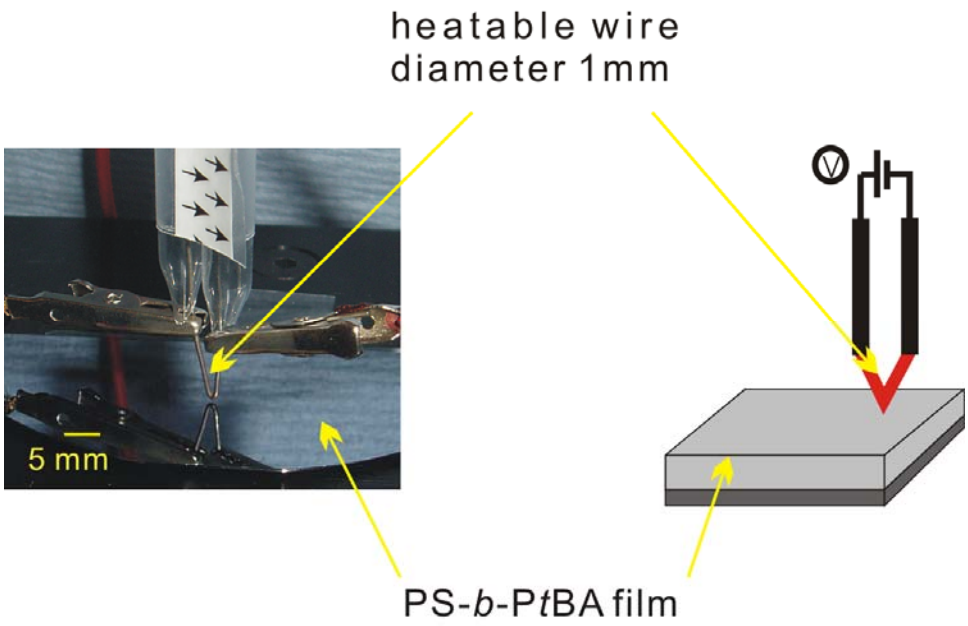


Figure S-4. *Photograph and schematic of the heatable wire setup used.*

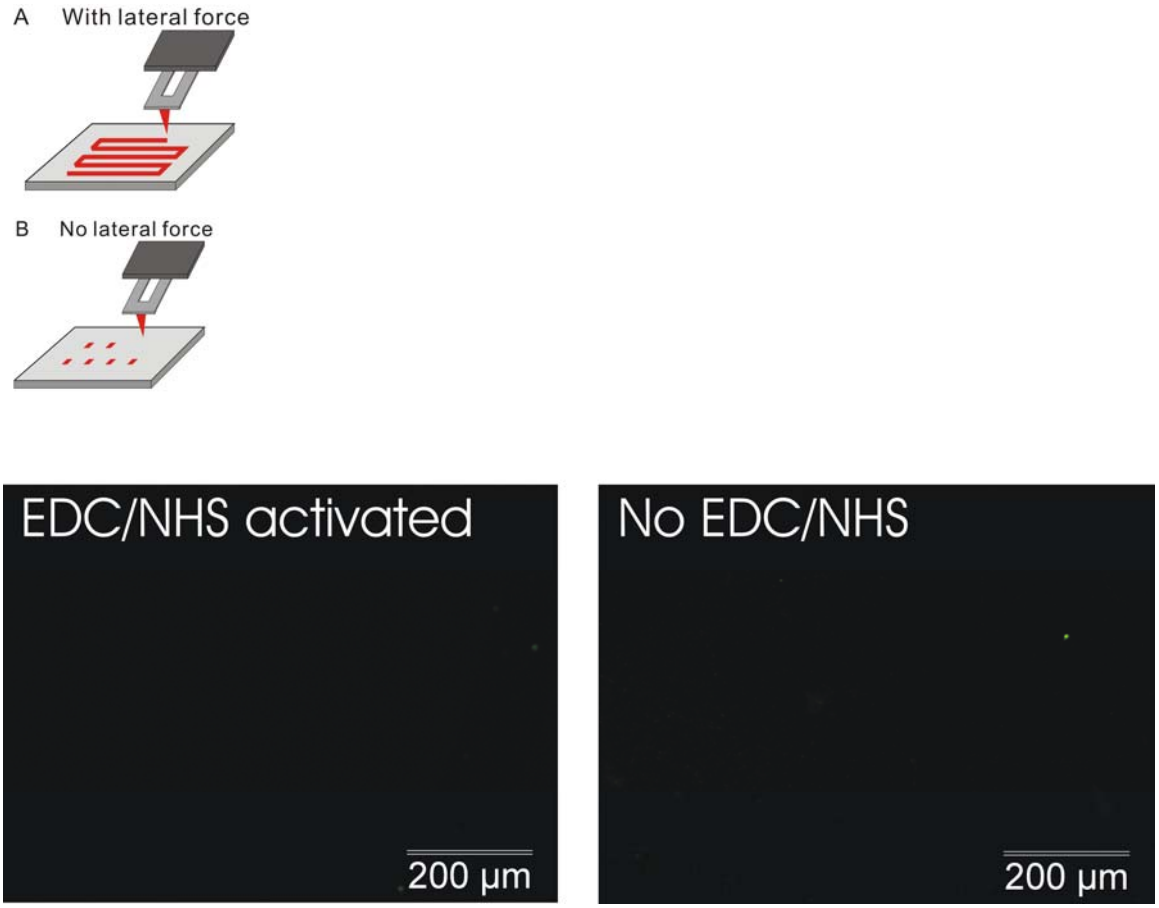


Figure S-5. Fluorescence microscopy images of two control experiments for the two thermal scanning probe lithography approaches (depicted schematically above). For the data shown on the left, two rows of square-shaped areas of 15 µm x 15 µm (with a lateral spacing of 30 µm) were scanned according to the two approaches with two probe temperatures that are below the thermolysis threshold, namely room temperature and 150ºC, respectively. Then the sample was activated using EDC/NHS (1 hour, followed by immersion in fluoresceinamine solution (1 hour); subsequently the sample was thoroughly rinsed with MilliQ water and dried with nitrogen. In the right image two rows of square-shaped areas of 15 µm x 15 µm (with a lateral spacing of 30 µm) were scanned according to the two approaches with three different probe temperatures: room temperature, 150ºC and 265ºC, respectively. No activation of carboxylic acid groups with EDC/NHS was performed prior to immersing the sample in a fluoresceinamine solution. No fluorescence originating from fluoresceinamine was observed in the patterned areas. Hence physisorption of fluoresceinamine as a result of topographic and chemical surface changes is absent.