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# Determination of force-free wet adhesion of mussel-inspired polymers to spin labeled surface

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ABSTRACT

Hydration repulsive forces oppose the adhesive interactions, especially in the force-free conditions. Here, we studied spontaneous wet adhesion of 3,4-dihydroxyphenylalanine (DOPA) functionalized poly(ethylene glycol) (PEG) polymers inspired by marine mussels. Using electron paramagnetic resonance (EPR) spectroscopy, we can monitor spontaneous adhesion of DOPA containing polymer to suspended spin labeled hydrophobic polystyrene nanobeads at molecular level. The surface coverage up to 82% is obtained from EPR measurements. However, in the force-free condition, EPR measurements do not show any detectable DOPA based adhesion to hydrophilic silica nanobead.

## 1. Introduction

Marine mussels have exceptional underwater adhesive abilities. Their adhesive mechanisms have become the focus of much attention for designing artificial wet adhesives. Mussels attach to various types of surfaces by seven known mussel foot proteins (Mfps). All Mfps are containing posttranslationally modified DOPA which plays a critical role in the adhesion of Mfps to wet surfaces [1,2].

In order to achieve the wet adhesion, mussels apply force to disturb the hydration layers and/or mussels dry the surface using Mfps [3]. Similarly, most of the studies e.g. surface force apparatus (SFA) and atomic force microscope (AFM) measurements were applied with external force to achieve durable adhesion in water [4,5]. However, various spectroscopic techniques can measure how well Mfps adhere to surfaces under force-free conditions [6–9]. All of these techniques have provided invaluable information about the molecular mechanism of mussel adhesion, which is achieved mainly by DOPA.

DOPA molecules have been incorporated into several polymers, including PEG, to obtain wet adhesive synthetic materials [10–13]. Here, we employed the EPR spectroscopy to assess the adhesion of a DOPA modified 4-armed PEG polymer (Fig. 1(A)) in the force-free condition. As model surfaces we prepared spin labeled hydrophobic polystyrene (SL-PS) and hydrophilic silica (SL-$SiO_2$) nanobeads suspended in solution (Fig. 1(B)). Furthermore, we can calculate fraction of the nanobead surface covered by adhesives using EPR.

## 2. Experimental

The synthesis of PEG-(N-Boc-L-DOPA)$_4$ and PEG-(Trp)$_4$ were prepared as described in published procedures and explained in supplementary file [14–16]. For PEG-(N-Boc-L-DOPA)$_4$, briefly, the amine group of L-DOPA was protected with tertbutyloxycarbonyl (Boc) group and then the product of N-Boc-L-DOPA was reacted with PEG-$(NH_2)_4$ (10 kDa). The DOPA-modified PEG molecules were purified by precipitation in cold diethyl ether and examined using $^1$H NMR spectroscopy, UV–vis spectroscopy and ninhydrin test (Figs. S1–S3). For PEG-(Trp)$_4$, briefly, PEG-$(OH)_4$ (10 kDa) and p-nitrophenyl chloroformate were mixed to obtain PEG-(p-nitrophenyl carbonate)$_4$. Next, tryptophan and PEG-(p-nitrophenyl carbonate)$_4$ were mixed to get PEG-(Trp)$_4$ [16]. $^1$H NMR data are available in SI (Fig. S4).

SL-PS and SL-$SiO_2$ nanobeads were prepared according to previous study [9]. Amine modified polystyrene and silica nanobeads (∼60 nm) were mixed with 4-carboxy-Tempo and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide in MES buffer (pH 3.0) for one day at room temperature (RT). The resulting SL-PS and SL-$SiO_2$ were purified in centrifuge concentrator with washing several times with MES buffer. SL-PS and SL-$SiO_2$ were mixed with different concentrations of PEG-$(NH_2)_4$ and PEG-(N-Boc-L-DOPA)$_4$

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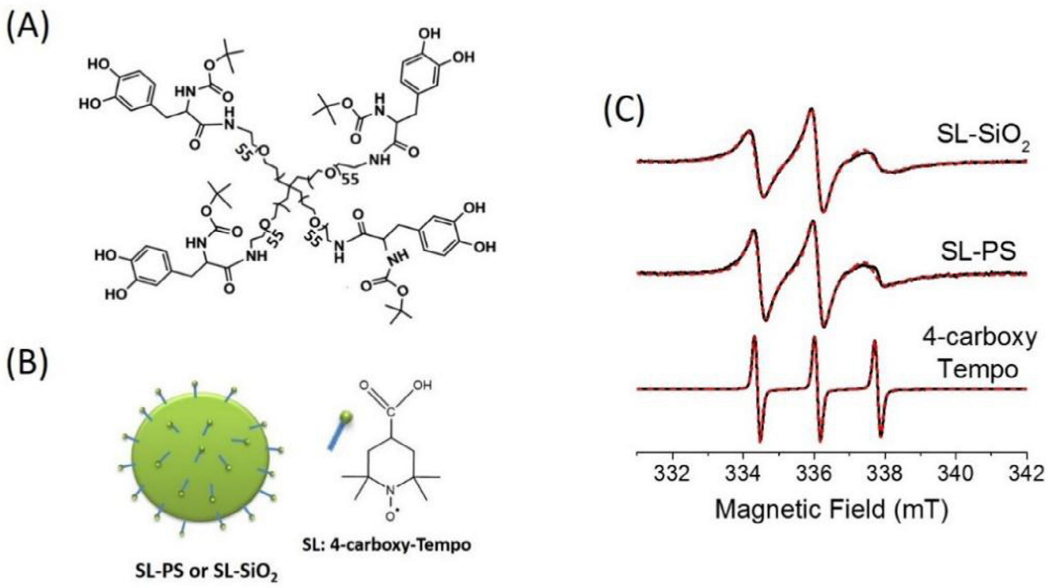

**Fig. 1.** (A) Chemical structure of the used PEG-(N-Boc-L-DOPA)$_4$. (B) SL-PS or SL-SiO$_2$ nanobeads. Pins represent SL, 4-carboxy-Tempo. (C) EPR spectra of 4-carboxy-Tempo, SL-PS and SL-SiO$_2$ (black) and their simulations (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

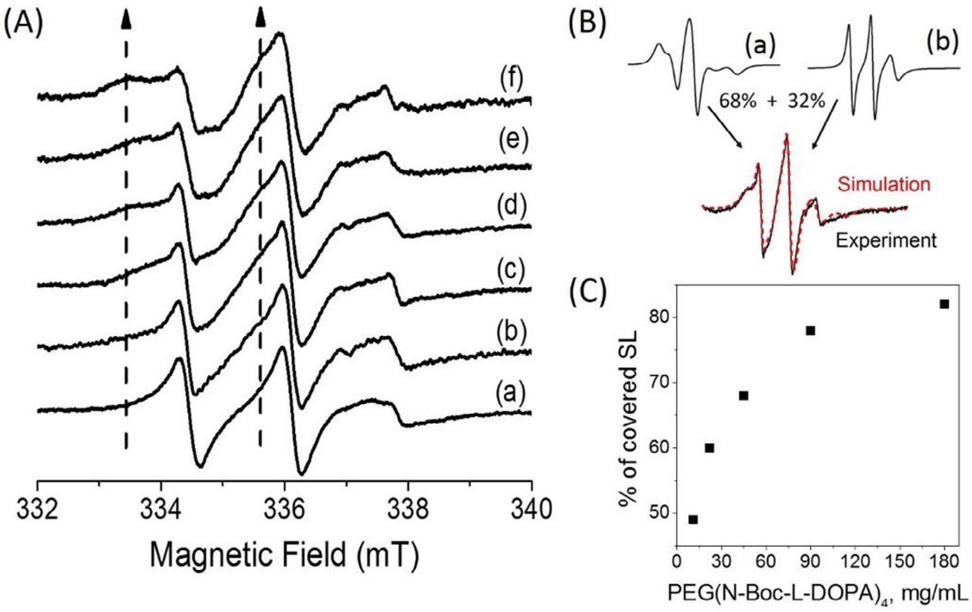

**Fig. 2.** (A) EPR spectra of SL-PS before (a) and after addition of PEG-(N-Boc-L-DOPA)$_4$ at different final concentrations: 11 mg/mL (b), 22 mg/mL (c), 45 mg/mL (d), 90 mg/mL (e), and 180 mg/mL (f). (B) Simulations of EPR spectra of covered (a) and uncovered (b) spin labels on PS with appropriate proportions produce the experimental result (with 45 mg/mL PEG-(N-Boc-L-DOPA)$_4$). (C) Determination of the percentage of covered SL on PS upon addition of the PEG-(N-Boc-L-DOPA)$_4$.

with a volume ratio of 1:1 in MES buffer at pH 3.0. Acidic medium was used to avoid DOPA oxidation.

X-band EPR measurements were done using a CMS 8400 (Adani) benchtop spectrometer at RT. All spectra were normalized to the intensities of the middle signals and also they were simulated using the Matlab-based Easyspin 4.5.5 software package [17].

## 3. Results and discussion

Fig. 1(C) shows EPR spectra of SL-PS and SL-SiO$_2$ in solution with their corresponding simulations. For comparison, reference spectrum of 4-carboxy-Tempo (spin label) is displayed. In solution, rotational dynamics of spin labels can be studied by the analysis of EPR line shapes [18,19]. At RT, the 4-carboxy-Tempo possesses sharp three-line signals with a rotational correlation time $\tau_R = 2.0$ ps, signatures of freely tumbling motion. However, EPR line shapes of SL-PS and SL-SiO$_2$ consist of broad hyperfine lines stemming from restricted rotational motion, with $\tau_R$ 2.8 ns and 2.9 ns, respectively. These results show that binding of spin labels to the surface of nanobeads restricts the rotational freedom and increases the rotational correlation time range from the ps to the ns range.

Fig. 2(A) shows EPR spectra of SL-PS before and after addition of PEG-(N-Boc-DOPA)$_4$ with different concentrations from 11 mg/mL to 180 mg/mL. Explicitly, a second type of signal with a longer rotational correlation time is observed upon addition of