Supporting information

Covalent Immobilization of active Lysozyme on Si/glass

surface using alkoxy Fischer carbene complex on SAM

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A. Synthesis of double bond terminated Fischer carbene complex:¹

(a) Synthesis of 1-bromo-4-(but-3-enyloxy)benzene:

Small pieces of sodium (69 mg, 3 mmol) was added to absolute ethanol (4 ml) and stirred for 30 min. A solution of *p*-bromophenol (519.03 mg, 3 mmol) in 2 ml absolute ethanol was added and stirred for 30 min. 4-Bromo-1-butene (445.5 mg, 3.3 mmol) was added very slowly by a syringe. The solution was refluxed for 3 h. After evaporating the solvent, 5 ml of water was added and extracted with ethylacetate. The ethylacetate layers were collected, dried over Na_2SO_4 and concentrated. Flash column chromatography with 5% ethylacetate/petroleum ether afforded 350 m g (55%) of compound (A).

¹H NMR (CDCl₃) δ_{H} : 2.45-2.60 (m, 2H), 3.99 (t, 2H, J = 7.5 Hz), 5.10-5.23 (m, 2H), 5.81-6.01 (m, 1H), 6.75-6.83 (m, 2H), 7.02-7.23 (m, 2H); ¹³C NMR (CDCl₃) δ_{C} : 33.48, 67.48, 113.80, 116.40, 117.07, 130.57, 134.12, and 159.59. Analysis: Calculated (C = 52.89, H = 4.88) and Observed (C = 52.91, H = 4.89).

(b) Synthesis of the Fischer carbene:

Following a reported procedure, in a two-necked round bottom flask, compound A (228 mg, 1 mmol) was dissolved in dry ether and the solution was cooled to 0 $^{\circ}$ C under argon. ⁿBuLi (0.78 ml, 1.1 mmol) was added to it drop wise, and the mixture was stirred for 30 min at 0 $^{\circ}$ C. Then temperature was raised to room temperature and stirring was continued for additional 15 min. The lithiated product was transferred to a suspension of tungsten hexacarbonyl (352 mg, 1 mmol) in dry ether (2 ml) at 0 $^{\circ}$ C

under argon atmosphere in another round bottom flask. After stirring for 10 min at 0 $^{\circ}$ C, the reaction mixture was allowed to attain room temperature and stirring was continued for 30 min. The solvent was evaporated followed by addition of 2 ml of degassed distilled water. Meerwein's salt (1M solution in DCM) (0.97 ml, 0.97 mmol) was added to it in one portion. The complex was extracted with pet ether and filtered through a pad of silica. Further purification was done by flash column chromatography (10% ethyl acetate / petroleum ether) afforded 260 mg (65%) of the pure product.

¹H NMR (CDCl₃) $\delta_{\rm H}$: 1.71 (t, 3H, *J*= 7.5 Hz), 2.58 (q, 2H, *J*= 6 Hz), 4.11 (t, 2H, *J*= 6 Hz), 4.98-5.24 (m, 4H), 5.81-6.00 (m, 1H), 6.89-6.94 (m, 2H), 7.86-7.90 (m, 2H). ¹³C NMR (CDCl₃) $\delta_{\rm C}$: 14.94, 33.38, 67.54, 79.52, 113.78, 114.60, 117.37, 131.92, 133.87, 146.92, 163.27, 197.77, 203.10, 311.01. Analysis: Calculated (C = 40.93, H = 3.05) and Observed (C = 40.95, H = 3.07).

B. Characterization of Trichloro(undec-10-enyl)silane:

Synthesized as reported in literature.^{2,3} ¹H NMR (CDCl₃) $\delta_{\rm H}$: 1.28 – 1.52 (m, 16H), 1.88 – 2.06 (m, 2H), 4.89 – 5.01 (m, 2H), 5.72 – 5.83 (m, 1H). ¹³C NMR (CDCl₃) &: 22.36, 24.47, 29.04, 29.18, 29.34, 29.58, 31.93, 33.87, 114.17, 139.07. Analysis: Calculated (C = 45.92, H = 7.36) and Observed (C = 45.87, H = 7.35).

C. FT-IR spectroscopy measurements:

Surface modification of activated SiO₂ was carried out following the procedure as depicted in Scheme 1 and the SiO₂ was centrifuged, thoroughly washed with ethanol and dried. FT-IR of modified silica powder was obtained using KBr pellets. FT-IR transmission spectra of powdered silica coated with monolayer of 10-undecenetrichlorosilane shows two important peaks corresponding to antisymmetric and symmetric stretch for methylene at 2933 cm⁻¹ and 2861 cm⁻¹. After cross-metathesis with the Fischer carbene complex on SAM two new bands appeared at 2068 cm⁻¹ and 1942 cm⁻¹(CO). Then aminolysis of the terminal carbene with pyrenemethylamine the absorption peak for W-CO stretching shows a shift to 2061 cm⁻¹ and 1927 cm⁻¹.







D. ATR-IR spectroscopy measurements on Silicon wafers:

The same experiments were carried out on silicon wafers and the surfaces were monitored by Attenuated Total Reflection (ATR-IR) spectroscopic measurements. The alkene terminated 10-undecenetrichlorosilane monolayer shows strong absorption bands at 2950 cm⁻¹ and 2839 cm⁻¹.



After cross-metathesis reaction with alkene tethered Fischer carbene complex two new characteristic peaks for W-CO stretching appeared at 2065 cm^{-1} and 1942 cm^{-1} .



After aminolysis of the terminal carbone with lysine groups of BSA and enzyme lysozyme, the absorption peak for W-CO stretching shows a shift to 1922 cm^{-1} .



E. SEM measurements:

The HRSEM picture of the immobilized lysozyme on surface.



F. Enzymatic activity of the immobilized lysozyme:

Fluorescence microscopy image of bacteria on control surface (without lysozyme) stained with fluorescent cell viability marker (Live/Dead dye).



Fluorescence microscopy image of *M. Lysodeikticus* on contol slide (without lysozyme) stained with LIVE/DEAD marker after 2 hr.

G) AFM image:

AFM image of the control surface (alkene terminated SAM dipped in lysozyme solution) after extensive washing with buffer and milli-Q water.



H) NMR spectra:









I) References:

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