

## Supporting information

# A new bio-active surface for protein immobilisation via copper free “Click” between azido SAM and alkynyl Fischer carbene complex

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**General.** All chemicals were purchased from Sigma-Aldrich. NMR experiments were carried out on a Bruker Avance 300 MHz spectrometer. FT-IR spectra were obtained using a Shimadzu FTIR-8300 spectrophotometer. ATR-IR spectra were recorded on Nicolet 380 spectrometer with Smart SAGA. Contact angles were measured with a digidrop from GBX (Romans sur Isère, France). An ellipsometer (Sentech SE 500) operating at a 70° incidence angle was used to measure the thickness of self-assembled monolayers (SAMs) on silicon wafers.

### **A. Synthesis of Phenylacetylenylethoxycarbene-pentacarbonyl tungsten (complex 1):<sup>1</sup>**

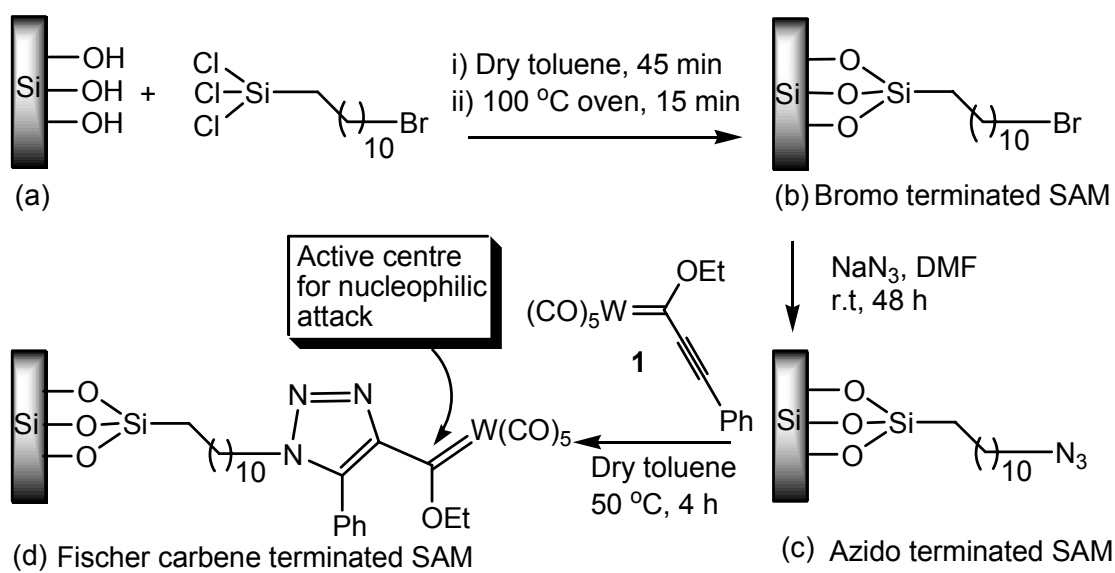
In a 100 ml two neck r. b. flask, 3.06 gm phenylacetylene (30 mmol) was taken in 30 ml dry ether under Argon. The stirred solution was cooled to -50 °C and 19 ml of <sup>n</sup>BuLi in hexane (1.6 M, 30 mmol) was added drop wise. Stirring was continued for 1 h. In another 100 ml r. b. flask, 10.5 gm (30 mmol) of W(CO)<sub>6</sub> was taken in 30 ml dry ether under Ar and the suspension was cooled to -50 °C. The phenylacetylide solution was then added to the tungsten hexacarbonyl suspension. The temperature was raised to 0 °C and stirred for 1 h. The ether was evaporated and the salt dissolved in minimum

amount of ice-cold water, to which 30 ml of Meerwin's salt solution (1 mM, 30 mmol) was added. The resulting dark red solution was extracted with petroleum ether (4 x 50 ml), concentrated and purified by flash column chromatography (pet ether: acetone 90:10) under N<sub>2</sub>. Yield 76%, 10.9 gm.

<sup>1</sup>H NMR (300 MHz; CDCl<sub>3</sub>) δ<sub>H</sub>: 1.54 (t, 3H, -OCH<sub>2</sub>CH<sub>3</sub>), 4.64 (q, 2H, -OCH<sub>2</sub>CH<sub>3</sub>), 7.38 – 7.57(m, 5H, arom); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ<sub>C</sub>: 14.3, 58.3, 90.3, 126.1, 128, 136.4, 216, 225.5, 315.3 ; IR ν<sub>max</sub>/cm<sup>-1</sup> (KBr): 1936.5, 2067.4, 2157.7 cm<sup>-1</sup>.

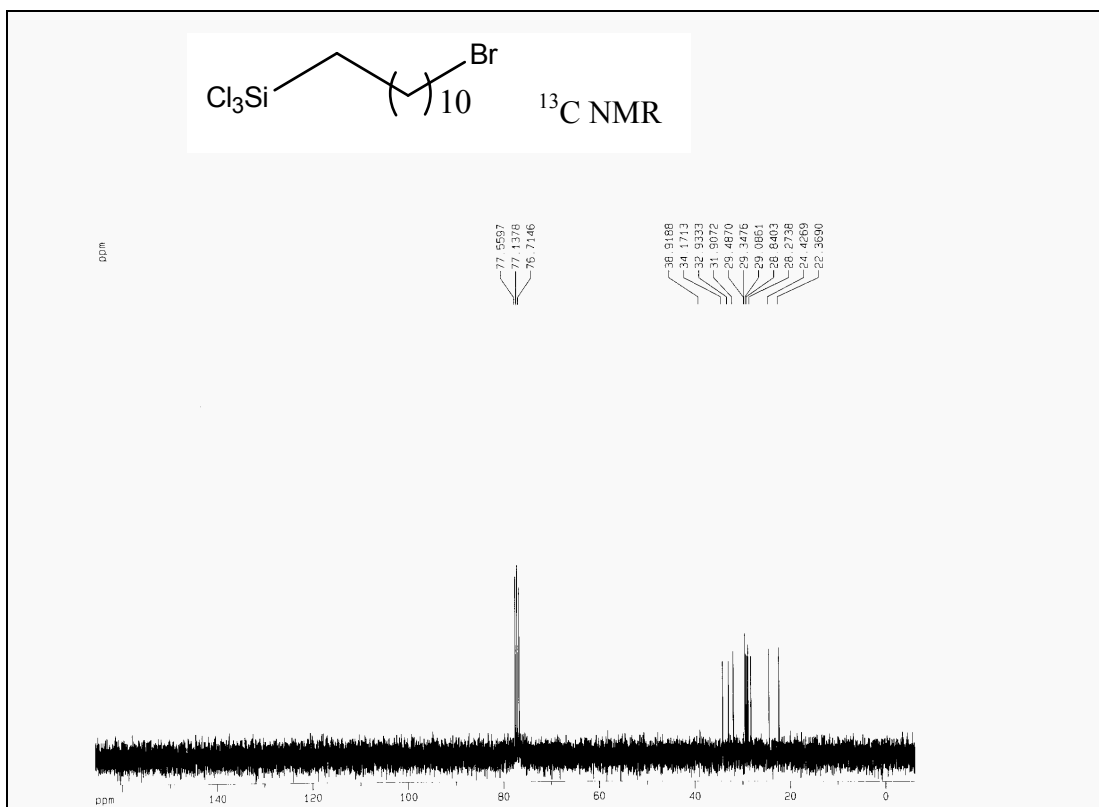
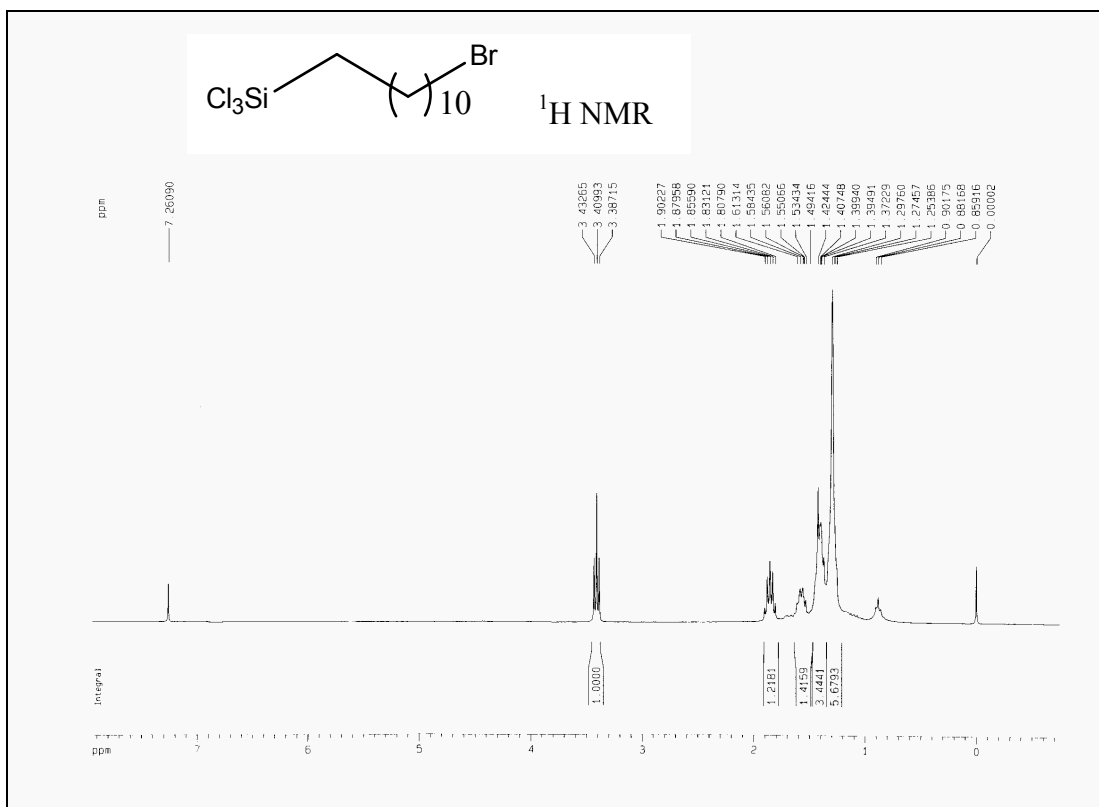
## B. Preparation and characterization of Fischer carbene terminated SAM on glass/Si surface:

*Scheme 1: Introduction of Fischer carbene function on SAM via copper-free 'Click' reaction.*



A) *Preparation of 11-bromo-undecyl-trichloro-silane from 11-bromo undec-1-ene:*<sup>2</sup>

In a 10 mL pressure bottle with magnetic stirring bar, 11-bromo-undec-1-ene (1.0g, 4 mmol), HSiCl<sub>3</sub> (4 mL, 40 mmol) and 0.02 mol% Spier's catalyst (20ml of 4% solution H<sub>2</sub>PtCl<sub>6</sub> in dry isopropanol) were placed under argon. The bottle was sealed and stirred at 60 °C for 24 h. After completion of reaction, excess HSiCl<sub>3</sub> was removed under vacuum and the pure product was distilled (60-65 °C/ 40 mm Hg); yield 0.98g, 67%. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ<sub>H</sub>: 1.27 (brs, 10H, -CH<sub>2</sub>-), 1.40-1.49 (m, 6H, -CH<sub>2</sub>-), 1.57 (m, 2H, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Br), 1.85 (p, 2H, -CH<sub>2</sub>CH<sub>2</sub>Br), 3.40 (t, 2H, -CH<sub>2</sub>Br). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ<sub>C</sub> : 22.36, 24.42, 28.27, 28.84, 29.08, 29.34, 29.48, 31.9, 32.93, 34.17, 38.91.



B) *Silanization of glass/Si surface with 11-bromo-undecyl-trichloro-silane:*

i) Cleaning and activation of Si/glass surface:<sup>3</sup>

To obtain an activating OH surface group on which the self assembly can take place, the glass slides or the Ge-doped silicon wafers were sequentially cleaned with ethanol and double distilled water, oxidized by a mixture of concentrated H<sub>2</sub>SO<sub>4</sub> and 40% aq. H<sub>2</sub>O<sub>2</sub> (70:30 v/v, known as ‘piranha solution’) at 60 °C, washed with water, and dried under N<sub>2</sub>. The active surface becomes highly hydrophilic as evident from its very low water contact angle.

ii) Preparation of SAM with 11-bromo-undecyl-trichloro-silane (BUTS):

The activated glass slides or Si wafers were immersed in a 1% v/v solution of BUTS in dry toluene under Ar atmosphere and stored in refrigerator for 45 min. The slides were then rinsed thoroughly with dry toluene and ethanol. The glass slides coated with SAM were baked in oven at 100 °C for 10 min.

C) *Surface modification by S<sub>N</sub>2 reaction with Sodium azide:*

Nucleophilic displacement of bromide by azide on SAM was carried out in DMF with sodium azide. The slides/wafers were immersed in a saturated solution of NaN<sub>3</sub> in DMF and stirred for 48 h at room temperature. Then it was washed with distilled water and dried under N<sub>2</sub>.

D) *Grafting of Fischer carbene complex 1 on surface by Cu-free ‘Click’ chemistry:*

The azide terminated monolayers were subsequently treated with Fischer carbene complex **1** (phenylacetyleneethoxycarbene-pentacarbonyl tungsten) (10 mM in dry toluene) under argon atmosphere with stirring at 50 °C for 4 h. It was then washed with toluene followed by ethanol and dried under argon.

E) *Characterization of SAM modifications:*

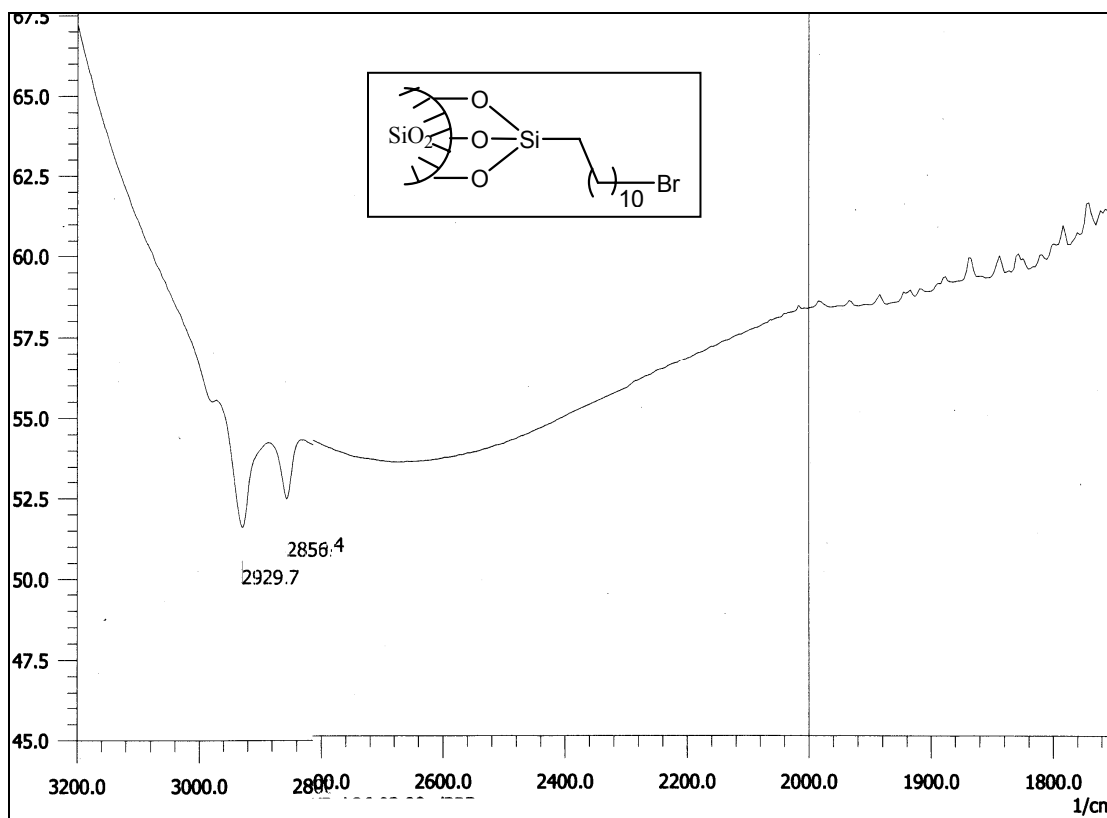
**i) Thickness and wetting measurements:**

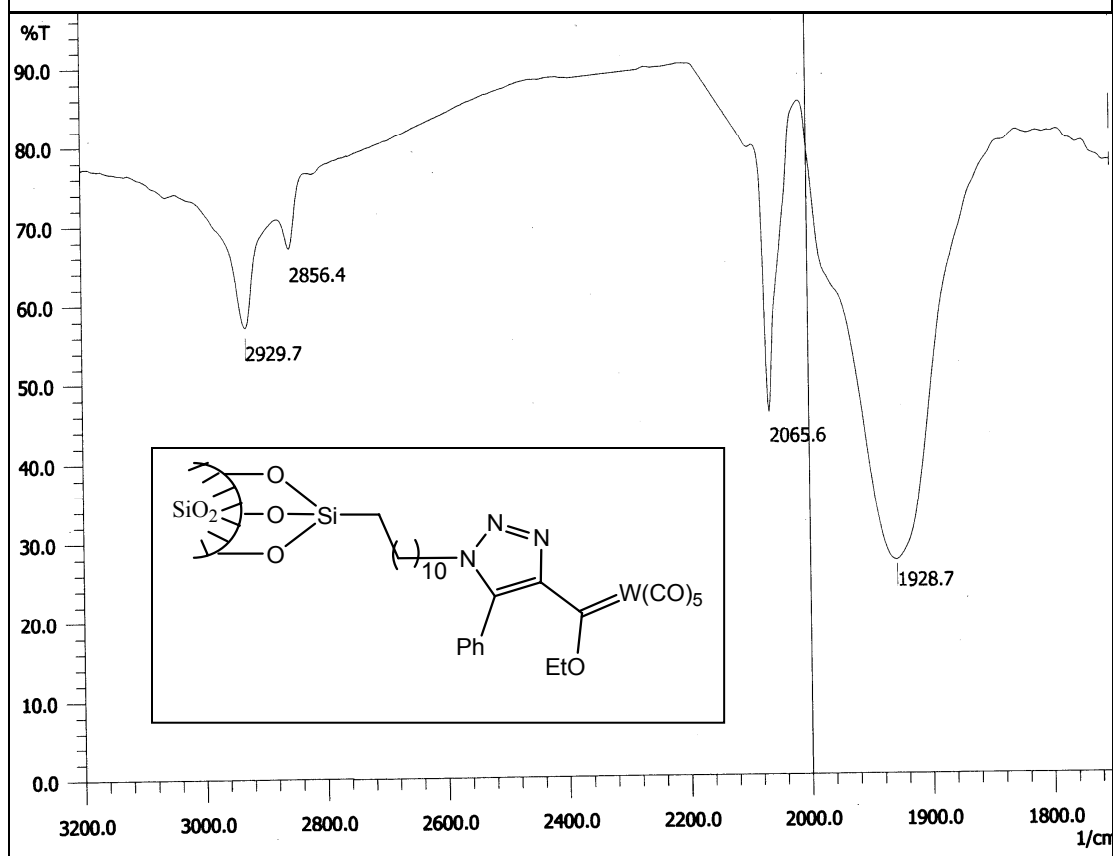
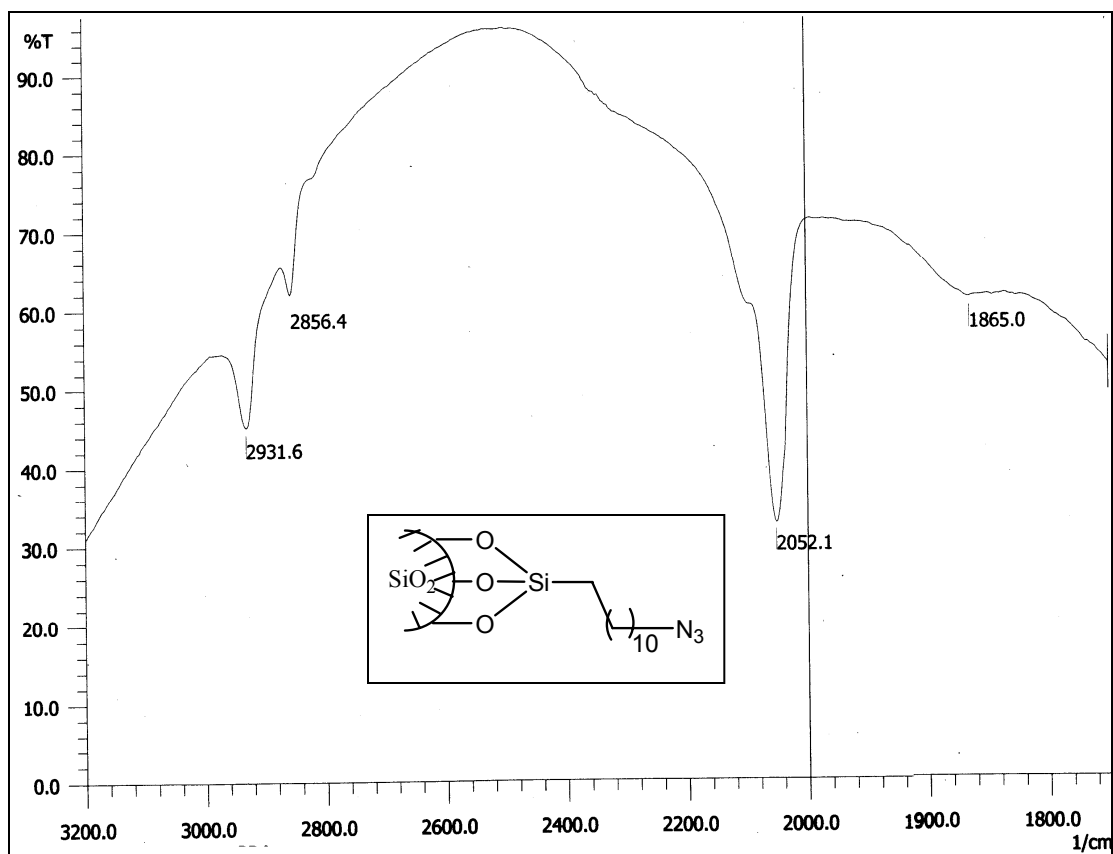
Table 1:

Surface	Thickness (nm)	Contact Angle (°)
Bromo terminated SAM (b)	1.81	103.8±3.5
Azide terminated SAM (c)	1.71	94.1±2.2
Fischer carbene terminated SAM (d)	2.37	91.1±1.1

## ii) FT-IR spectroscopy measurements:

Surface modification of powdered SiO<sub>2</sub> was carried out following the procedure as depicted in Scheme 1 and the resulting powder was centrifuged, thoroughly washed with ethanol and dried. Infrared transmission spectra of modified silica powder were obtained from KBr pellets.<sup>4</sup> FT-IR transmission spectra of powdered silica coated with monolayer of 11-bromo-undecylsiloxane shows IR stretching bands at:  $\nu_{\max}/\text{cm}^{-1}$  2929 cm<sup>-1</sup> (CH) and 2856 cm<sup>-1</sup> (CH<sub>2</sub>). After transformation from bromo to azide terminal group of SAM shows one additional strong absorption band at:  $\nu_{\max}/\text{cm}^{-1}$  2051 (N<sub>3</sub>). The Fischer carbene grafting by 'click' cycloaddition was evidenced by complete disappearance of  $\nu_{\text{N}_3}$  peak and the growth of IR signal at:  $\nu_{\max}/\text{cm}^{-1}$  2065.6 and 1928.7 (CO).





F) *Reaction between Fischer carbene grafted SAM and 1-pyrenemethyl amine:*

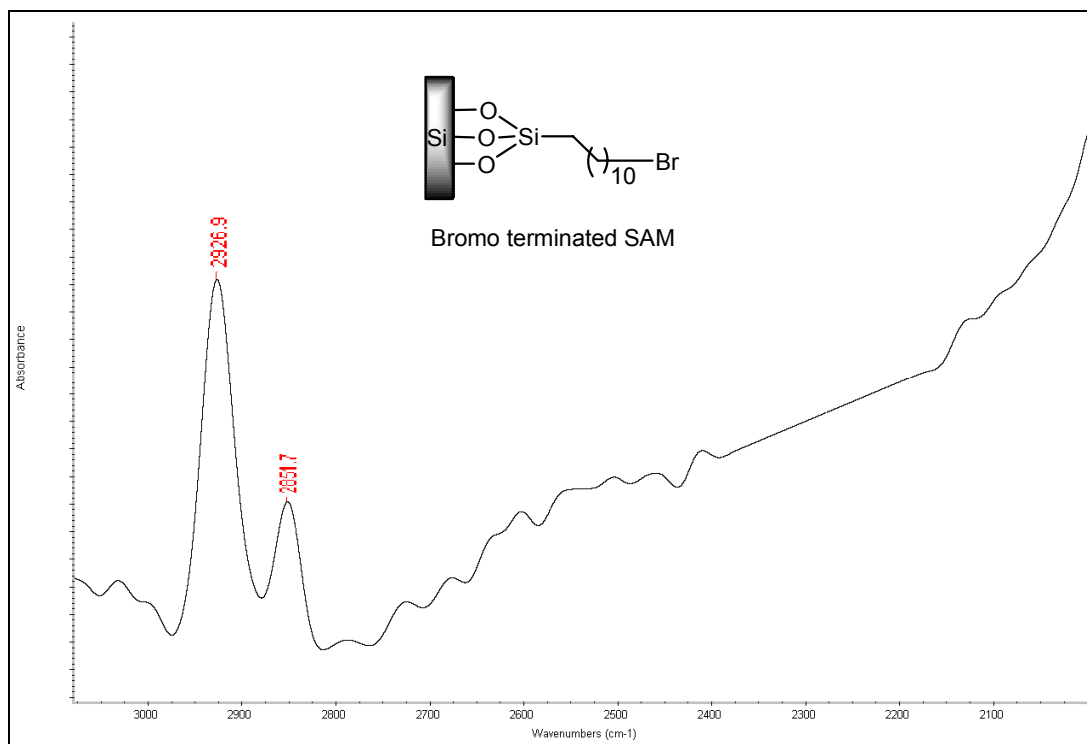
First, the 1-pyrenemethylamine hydrochloride salt was neutralized with dil. NaOH solution (10%) and then the amine was extracted with DCM, dried ( $\text{Na}_2\text{SO}_4$ ) concentrated under vacuum to yield yellowish white solid compound **2**. Then the 1-pyrenemethyl amine was dissolved in absolute EtOH (1 mol %) and the glass slides covered with Fischer carbene moieties were dipped into this solution for 10 min in dark. The slides were then washed thoroughly with EtOH to get rid from unreacted pyrenyl compound which were then examined under fluorescent microscope and by fluorescent spectroscopy.

G) *Immobilisation of protein Bovine Serum Albumin (BSA) on SAM:*

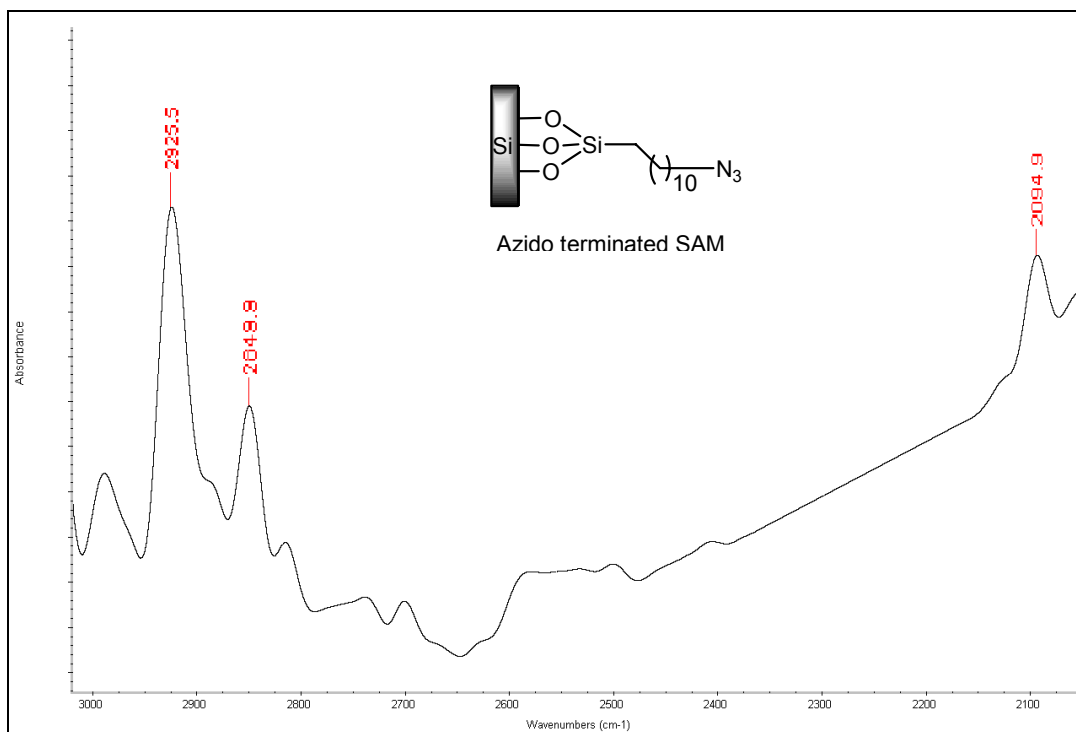
The Fischer carbene grafted Si wafers or glass slides were then immersed in 1  $\mu\text{M}$  BSA solution in borate buffer (pH 9.5) of for 10 min. It was thoroughly washed with borate buffer solution (twice), then with milli-Q water (four times) and dried under  $\text{N}_2$ .

**iii) ATR-IR spectroscopy measurements on Silicon wafers:**

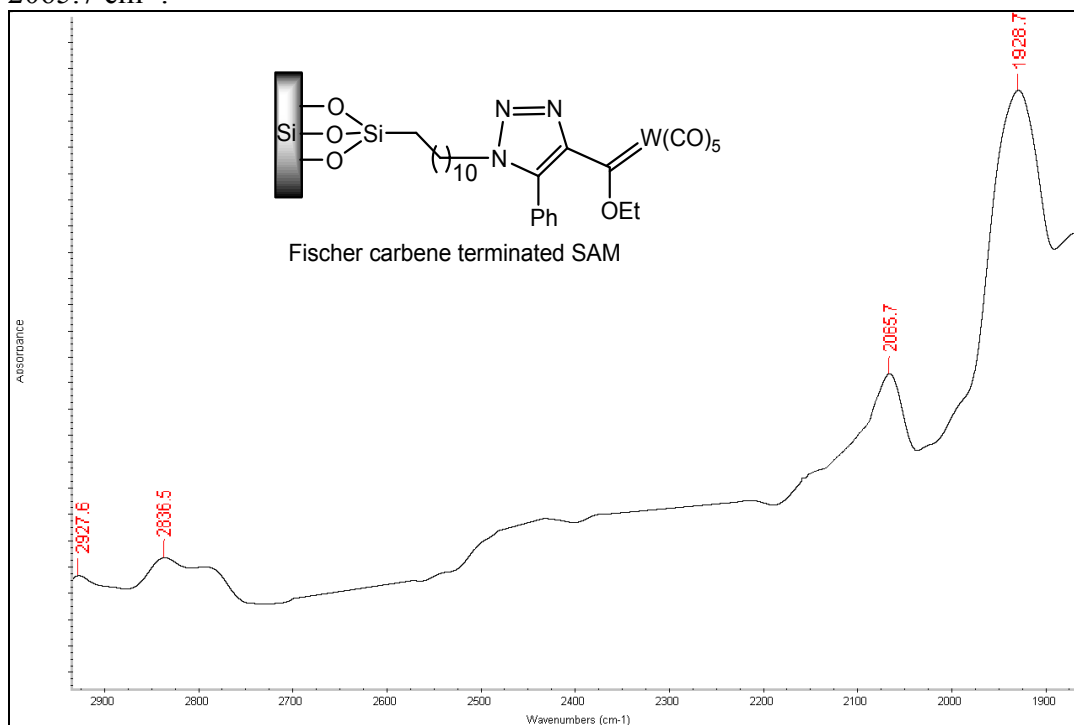
The same experiments were carried out on silicon wafers and the surfaces were characterized by Attenuated Total Reflection (ATR-IR) spectroscopic measurements. The bromo terminated undecylsilane monolayer shows strong absorption bands at 2926.1 and 2851.7  $\text{cm}^{-1}$ .



The azide monolayer on Si surface shows an absorption band at  $2094.9\text{ cm}^{-1}$  due to  $\text{N}_3$  stretching.

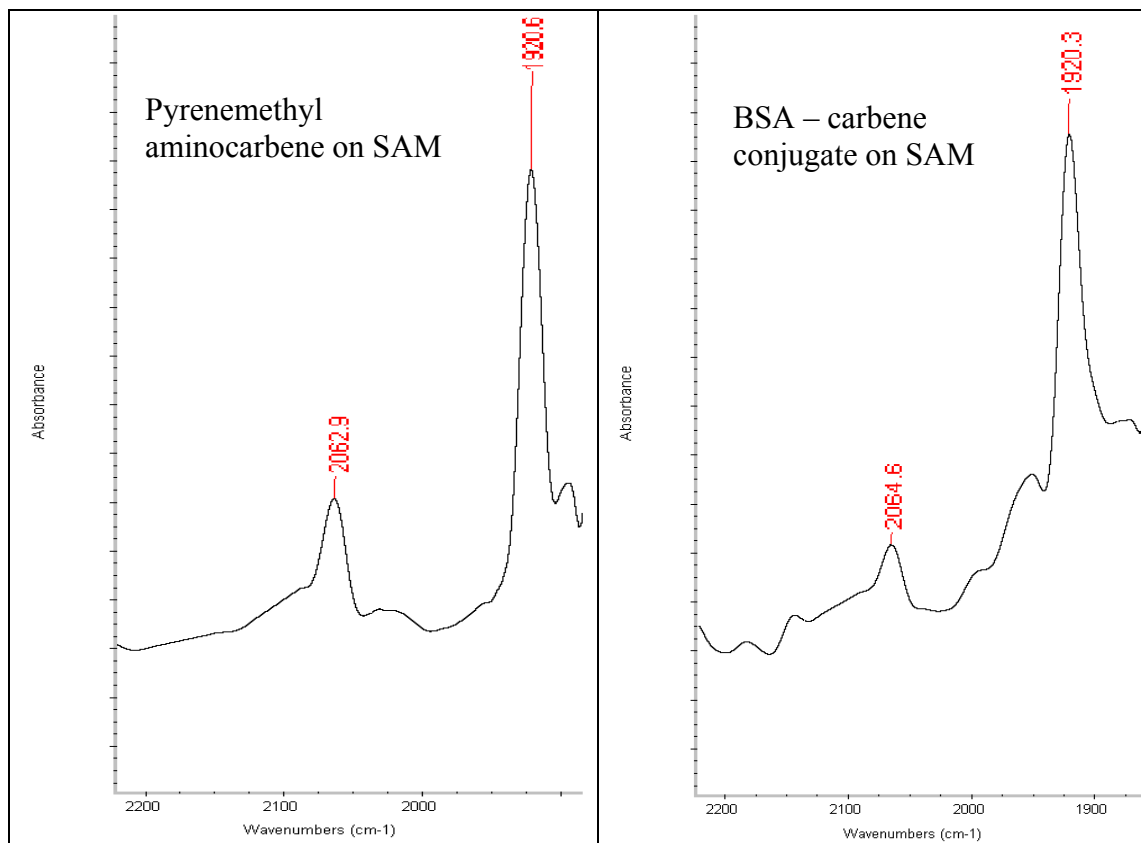


After 'click' reaction with alkynyl Fischer carbene complex the peak for  $\text{N}_3$  disappeared and characteristic peaks for W-CO stretching appeared at  $1928.7$  and  $2065.7\text{ cm}^{-1}$ .





After aminolysis of the terminal carbene with pyrenemethyl amine and lysine groups of protein BSA, the absorption peak for W-CO stretching shows a shift of about  $8\text{ cm}^{-1}$ . The bands for amino carbene now appeared at  $1920.6$  and  $1920.3\text{ cm}^{-1}$  for pyrenemethyl and BSA conjugate respectively.



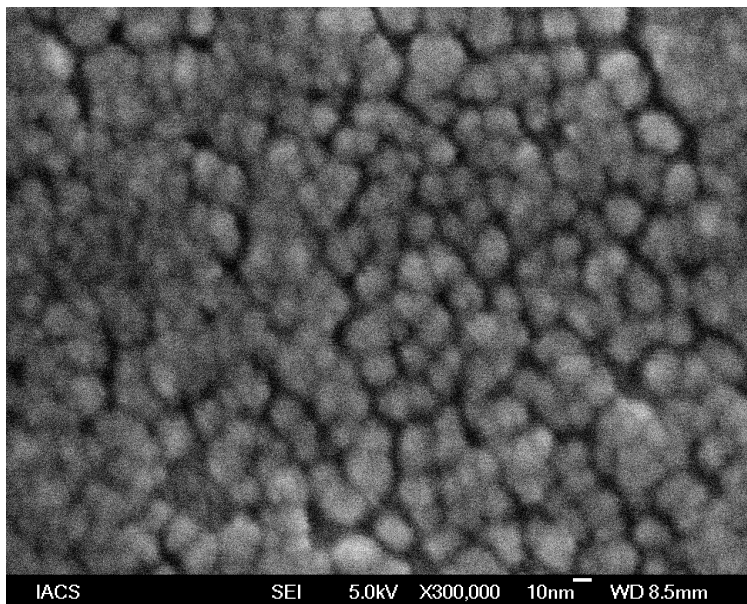
#### iv) AFM measurements:

For AFM measurements the surface modification was carried out on Ge-doped Si wafers polished at one side.

Data acquisition: Atomic Force Microscopy was carried out with AFM (diCP-II) to observe the topology of the wafer surface before and after protein immobilisation. The microscope was operated in non contact mode under ambient condition (set oscillation frequency 300 kHz) using cantilevers of Veeco (model no. RTESPA-M) (1-10 ohm cm phosphorous (N) doped Si) of length 115-135  $\mu\text{m}$ , width 30-40  $\mu\text{m}$ , thickness 3.5-4.5  $\mu\text{m}$ , spring constant 20-80N/m and frequency 245-287 Hz, coating only back side (50 +/- 10 nm Al) and radius of curvature was  $\leq 20$  nm. The scanning was performed within 1  $\mu\text{m}$  x 1  $\mu\text{m}$  with scan rate 0.5-0.6 Hz. Off-line procedures such as line, section, area and particle analysis were performed using DI Company SPMLab Analysis software.

**v) SEM measurements:**

High Resolution Scanning Electron Microscopy (HRSEM) picture was taken in JEOL (JSM-6700F) Field Emission Scanning Electron Microscope of the same sample.



**References:**

1. (a) E. O. Fischer, F. R. Kreissl, *J. Organomet. Chem.*, 1972, **35**, C47; (b) F. R. Kreissl, E. O. Fischer, C. G. Kreiter, *J. Organomet. Chem.*, 1972, **57**, C9.
2. H. Grisar, Y. Cohen, D. Aurbach, C. N. Sukenik, *Langmuir*, 2001, **17**, 1608.
3. J. J. Cras, C. A. Rowe-Taitt, D. A. Nivens, F. S. Ligler, *Biosensors & Bioelectronics*, 1999, **14**, 683.
4. T. Lummerstorfer, H. Hoffmann, *J. Phys. Chem. B*, 2004, **108**, 3963.