ANALYSIS OF ANCHOR MOLECULES FOR TETHERED BILAYER LIPID MEMBRANE FORMATION ON NOVEL SERS ACTIVE BIOSENSOR

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INTRODUCTION

Surface enhanced Raman spectroscopy (SERS) is a powerful vibrational spectroscopy technique that allows highly sensitive structural detection of low concentration analytes and is applicable for studies of model systems on roughened electrodes [1]. The active area of our SERS substrates is formed using an ultra-short pulse laser on a soda-lime glass substrate. The resulting surface structure features stochastic nanopattern, which meets good resonance characteristics for various excitation wavelengths and adsorbed analyte molecules. The tethered lipid bilayers (tBLMs) (Fig. 1) are considered as perspective experimental platforms for membrane biosensors and may be suitable for a broad spectrum of biophysical experiments such as peptide/membrane interactions, protein/membrane interactions, lipid phase transitions and others. Attached to a SERS active surface, tBLMs may allow monitoring of biologically relevant events with Raman spectroscopy. It is known that structure of the self-assembled monolayers (SAMs) used to anchor phospholipid bilayers to surfaces affects the functional properties of the tethered bilayer membranes (tBLMs) [2].

The aims of this study are to test new surface enhanced Raman scattering (SERS) substrates coated with silver to determine their suitability to analyse self-assembled monolayer (SAM) of anchor molecules for tBLM formation on roughed surface.

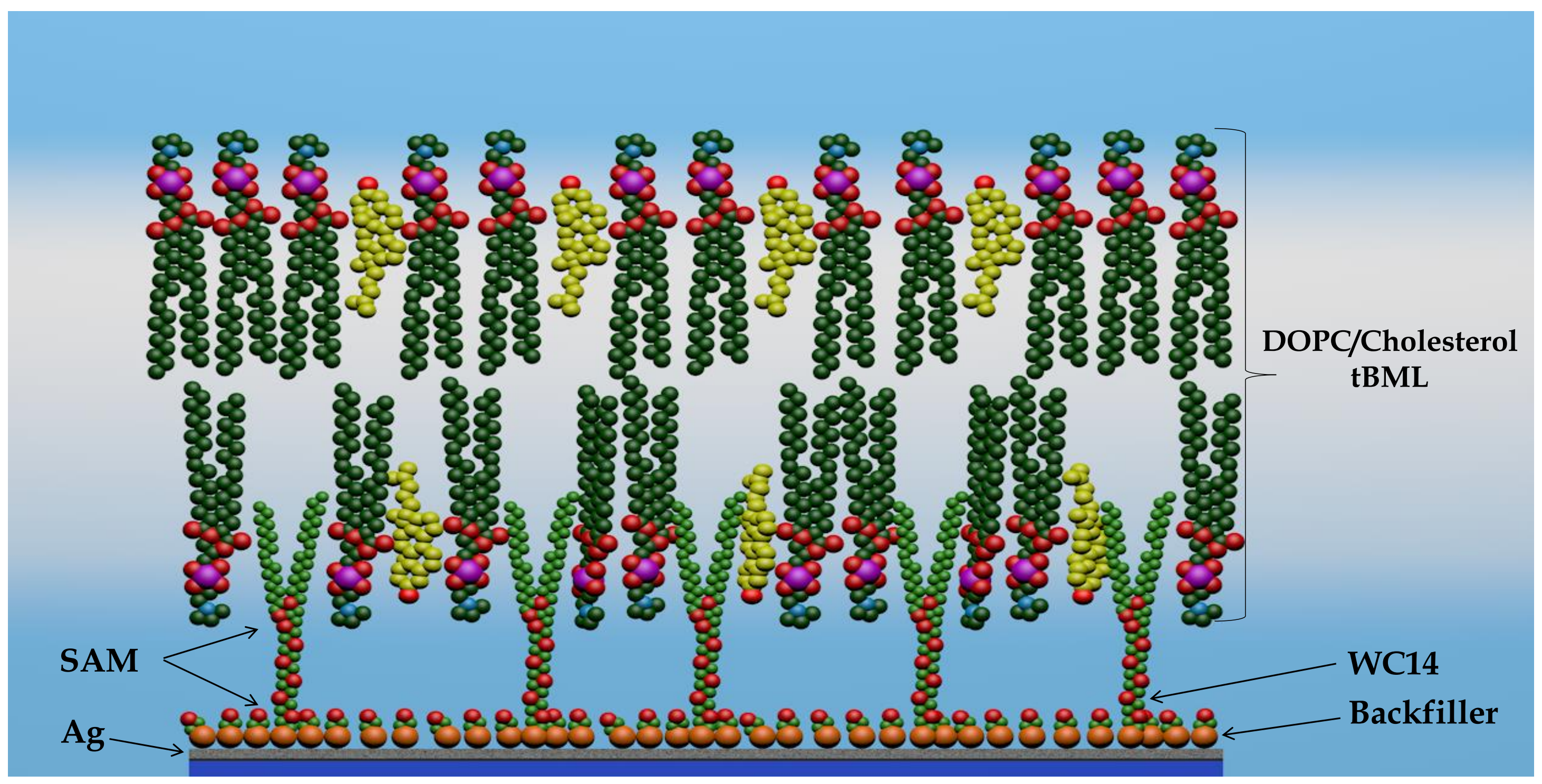

Figure 1. Schematic representation of tethered bilayer lipid membrane (tBLM) on a silver surface.

ANCHOR MOLECULES

Two types of anchor molecules were chosen to form SAMs on nanostructured silver surface - long strand thiolipid Wilma's compound (WC14) and three different short strand backfillers 2-mercaptoethanol (2-ME), 3-mercapto-1-propanol (3-M-1-P) and 4-mercapto-1-butanol (4-M-1-P) (Fig. 2).

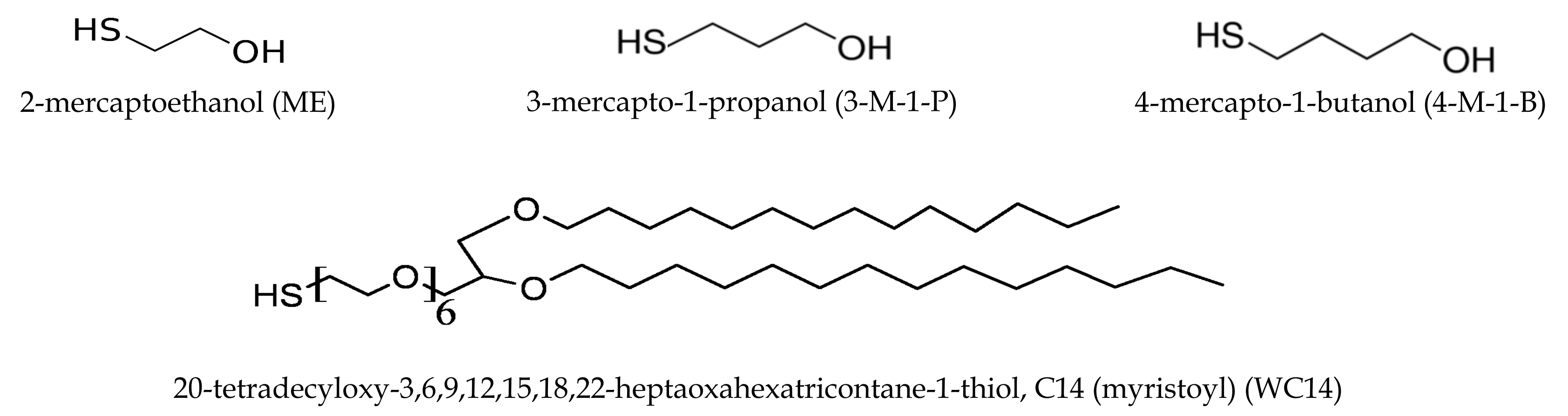

Figure 2. Structure of anchor molecules.

We received a good quality SERS spectra of anchor molecules adsorbed on nanostructured silver surface (Fig. 3). Vibrational peaks assignment showed in a table 1 below.

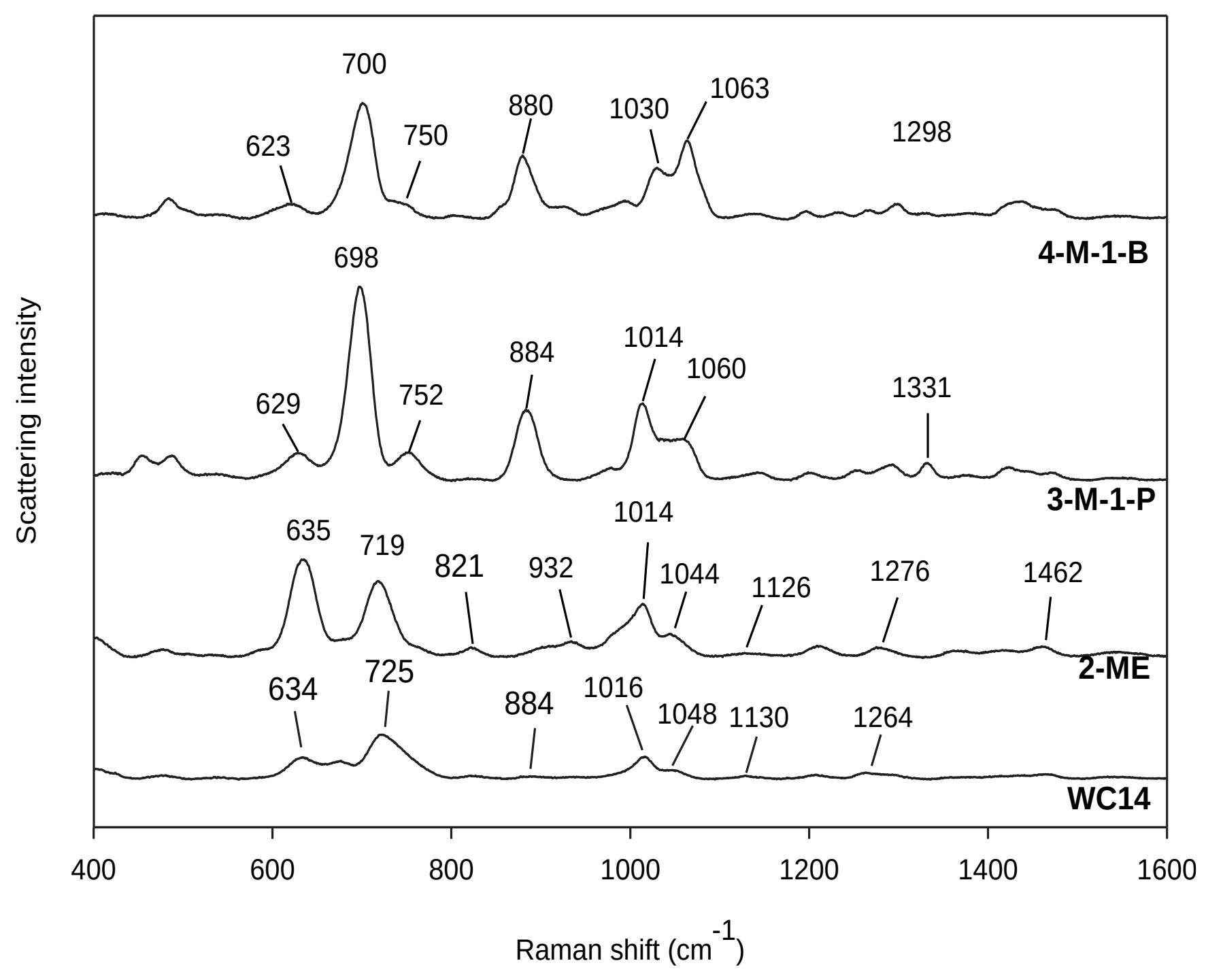

Figure 3. SERS spectrum of anchor molecules adsorbed on nanostructured Ag surface.

Wavenumber: cm^{-1}				
WC14	2-ME	3-M-1-P	4-M-1-P	Assignment
634	635	629	623	ν(C–S)$_G$
725	719	698	700	ν(C–S)$_T$
n.a.	821	752	750	δr(CH$_2$)$_G$
884	n.a.	n.a.	n.a.	δr(CH$_3$)$_T$; δr(CH$_2$)
n.a.	n.a.	884	880	δr(CH$_2$)
n.a.	932	n.a.	n.a.	δr(CH$_2$)
1016	1014	1014	1030	ν(C–C); ν(O–C)
1048	1044	1060	1063	νs(C–C)$_T$; νs(C-O)$_G$
1130	1126	n.a.	n.a.	ν(C–C)$_T$; νas(C–O–C)
1264	n.a.	n.a.	n.a.	δt(CH$_2$) EO chain; δw(CH2)
n.a.	1276	n.a.	1298	δw(CH$_2$)
n.a.	n.a.	1331	n.a.	δw(CH$_2$)
n.a.	1462	n.a.	n.a.	δs(CH$_2$)

Abbreviations: n.a. - not applicable; ν – stretching; νas - asymmetric stretching; νs - symmetric stretching; δ - deformation; t - twist; wag - waging; r - rock; T - trans; G - gauche.

Table 1. Vibrational assignments for anchor molecules.

MIXED SAMs

Since WC14 molecules by themselves form too tight SAM to achieve a functional tBLM, it is very important to carefully choose short strand backfiller molecules that determines a softer SAM for biologically relevant tBLM.

Mixed SAMs on the roughened Ag surface was formed from ethanolic solutions containing 50% long strand anchor WC14 and 50% different short strand backfiller: 2-ME, 3-M-1P, 4-M-1B.

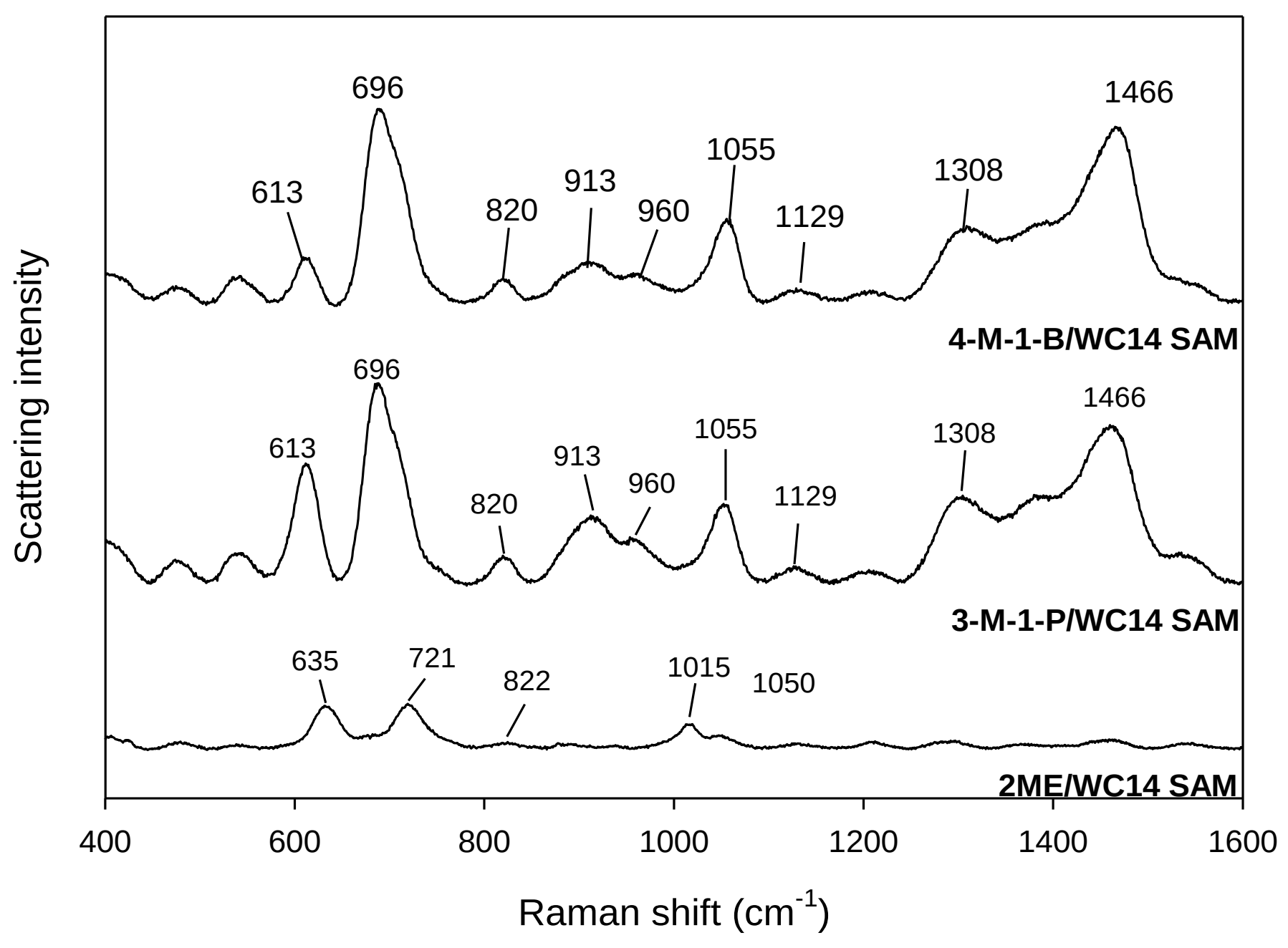

Figure 4. SERS spectrum of mixed SAMs formed on nanostructured Ag surface.

Wavenumber: cm^{-1}			
2-ME/WC14 SAM	3-M-1-P/WC14 SAM	4-M-1-P/WC14 SAM	Assignment
635	613	613	ν(C–S)$_G$
721	696	696	ν(C–S)$_T$
822	820	820	δr(CH$_2$)$_G$
n.a.	913	913	δr(CH$_2$)
n.a.	960	960	δr(CH$_2$)
1015	n.a.	n.a.	ν(C–C); ν(O–C)
1050	1053	1055	νs(C–C)$_T$; νs(C-O)$_G$
n.a.	1130	1129	ν(C–C)$_T$; νas(C–O–C)
n.a.	1303	1308	δw(CH$_2$)
n.a.	1460	1466	δs(CH$_2$)

Abbreviations: n.a. - not applicable; ν - stretching; νas - asymmetric stretching; νs - symmetric stretching; δ - deformation; t - twist; w - waging; r - rock; T - trans; G - gauche.

Table 2. Vibrational assignments for SERS peaks of mixed SAMs.

Figure 4 shows SERS spectra obtained from mixed SAMs. Beyond other assigned vibrational peaks, bands at 1129 cm^{-1} assigned specifically to C-C and C-O-C bonds symmetric stretching of WC14 molecule (Table 2) proves, that heterogeneous SAMs were formed.

Further analysis of SERS spectra revealed, that by choosing backfiller molecules of different length we can regulate the arrangement of long chain anchor molecules on the surface. Increasing length of short strand molecules results in the higher intensity of peaks 721 cm^{-1} and 696 cm^{-1} assigned to C-S bond stretching in trans conformation (table 2) in comparison with C-S bond stretching in gauche conformation at 635 cm^{-1} and 613 cm^{-1}. The longer chain of the backfiller molecule leads to easier transition of WC14 from gauche to trans conformation (Fig. 6, B and C).

tBLM FORMATION

SAM containing WC14 molecules and 4-M-1-B as a backfiller was chosen for tBLM formation by multilamellar lipid vesicle fusion. The analysis of SERS spectra obtained after tBLM formation indicates the new band at 886 cm^{-1} assigned to CH$_3$ vibrational mode of WC14 in trans conformation (Fig. 5). This means that WC14 changes its orientation after vesicle fusion by straightening the hydrophilic part of the molecule and withdrawing the hydrophobic part from the surface (Fig. 6, D).

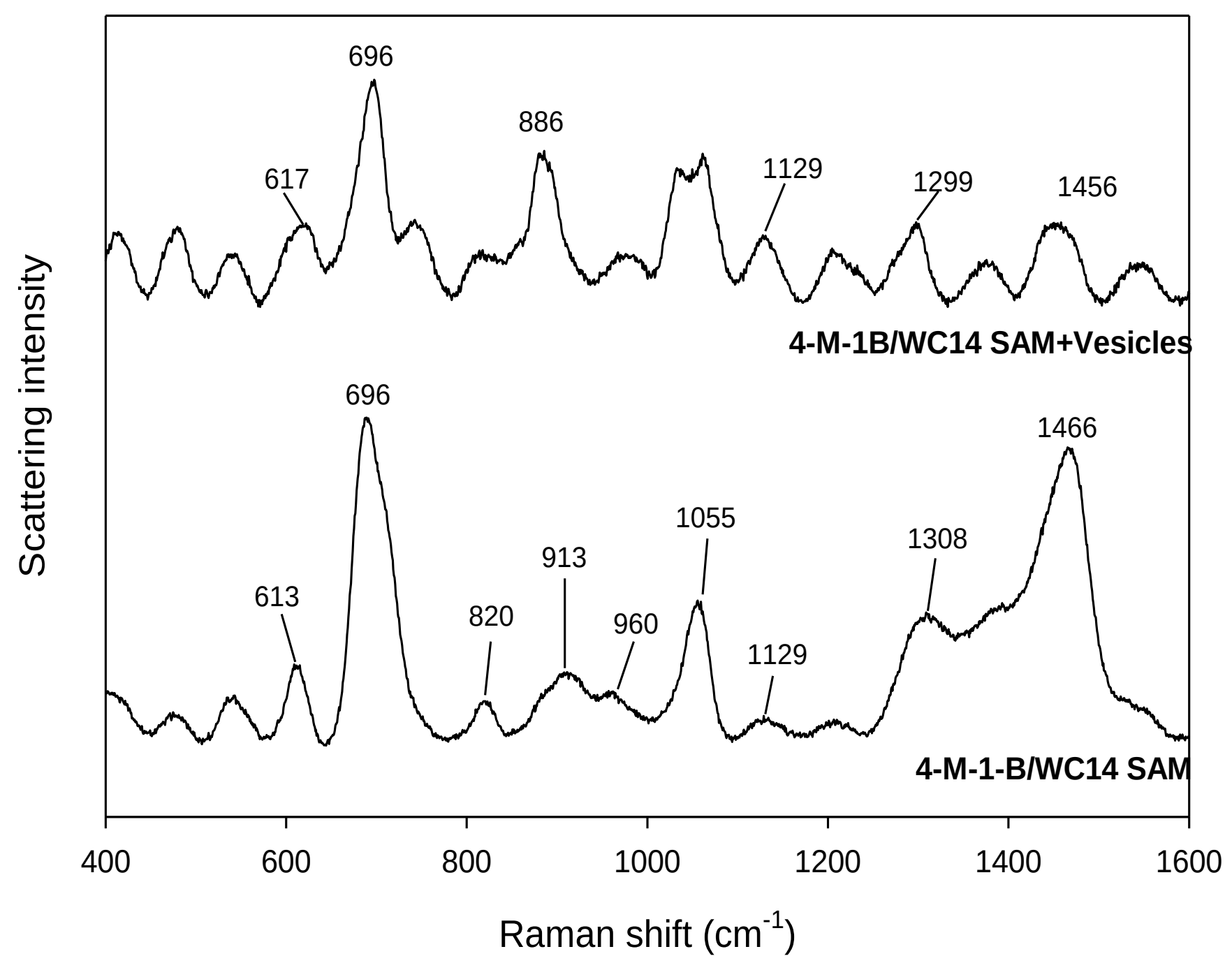

Figure 5. Comparison of mixed WC14/4-M-1-B SAM SERS spectrum before and after tBLM formation.

Wavenumber: cm^{-1}		
4-M-1-P/WC14 SAM	4-M-1-B/WC14 SAM+ Vesicles	Assignment
613	617	ν(C–S)$_G$
696	696	ν(C–S)$_T$
820	n.a.	δr(CH$_2$)$_G$
n.a.	886	δr(CH$_3$)$_T$
913	n.a.	δr(CH$_2$)
960	n.a.	δr(CH$_2$)
1055	n.a.	νs(C–C)$_T$; νs(C-O)$_G$
1129	1129	ν(C–C)$_T$; νas(C–O–C)
1308	1299	δw(CH$_2$)
1466	1456	δs(CH$_2$)

Abbreviations: n.a. - not applicable; ν - stretching; νas - asymmetric stretching; νs - symmetric stretching; δ - deformation; t - twist; w - waging; r - rock; T - trans; G - gauche.

Table 3. Vibrational assignments for SERS bands of mixed SAM before and after vesicle fusion.

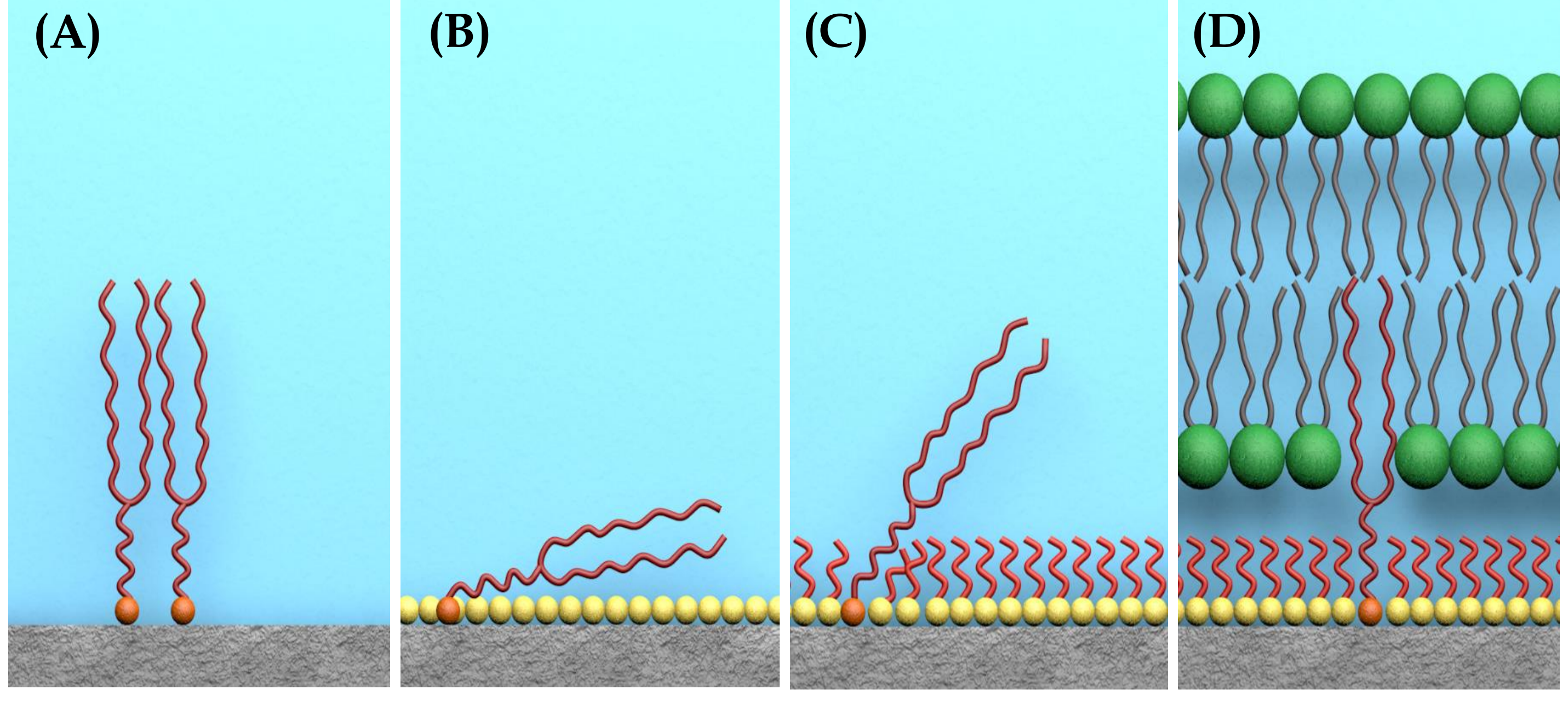

Figure 6. Orientation of a long strand anchor molecules (WC14) on the Ag surface: (A) 100% WC14; **(B)** WC14 diluted with 2-ME; **(C)** WC14 diluted with 4-M-1-P; **(D)** Orientation of WC14 molecules after phospholipid vesicle fusion.

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