

## Peptide containing vesicles trapped with Raman tweezers – Vpu<sub>1-32</sub> from HIV-1

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### Introduction

Viruses can modify the intracellular milieu of an infected cell for their own benefit by altering factors such as pH or electrochemical gradients which drives protein production and manipulates membrane permeability. For this purpose some viruses encode small membrane proteins of around 100 amino acids which enable the flux of ions and substrates across lipid membranes and are called ion channel forming proteins<sup>1</sup>. It is our goal to functionally analyze these systems for the development of novel drug therapies with the current focus on the auxiliary protein Vpu (virus protein 'u') of human immunodeficiency virus type-1 (HIV-1) and its interaction with a putative blocker<sup>2</sup>.

Vpu is an 81 amino acid protein which is known to self-assemble in a lipid bilayer showing channel activity. This mechanism is proposed to amplify the release of progeny viruses at the site of the cell membrane (budding). Its second role is to interact in the endoplasmic reticulum with the viral receptor protein CD4 to initiate its degradation within the cell. Whilst this role is due to its cytoplasmic part, self-assembly and channel formation is related to its single transmembrane part. Recently a drug has been found that blocks channel activity of Vpu. As structural data is readily available for Vpu it can be investigated in a combined computational and experimental approach<sup>3-4</sup>.

Raman spectroscopy is a powerful tool to investigate proteins in aqueous solution. The sensitivity of the technique is based on the change of the polarizability of bonds during vibration. This makes it extremely sensitive for monitoring aromatic residues and has been widely used in the field of protein structure analysis. Docking studies have shown that the blocker cyclohexamethylene amiloride is interacting with a tryptophan (Trp-23), which is located in the TM region of Vpu<sup>2</sup>. Using Raman spectroscopy together with optical tweezers, it enables to investigate the TM region of Vpu<sub>1-32</sub> in vesicles – membrane-like environment.

The investigations focus on the protocol to identify the characteristic bands for tryptophan in Vpu as a lyophilized powder and in DMPC vesicles.

### Method

#### Peptide Synthesis

Vpu<sub>1-32</sub> (MQPIPIVAIV ALVVAIITAI VVWSIVIIIEY RK) was synthesized on a Pioneer Synthesizer from Applied Biosystems Instruments using Fmoc chemistry (Lam, Fischer, Watts, Fischer unpublished results). Peptide was characterized by MALDI-MS, HPLC and peptide sequencing. The peptide was then purified by HPLC using reverse-phase column and the elution peak was then again characterized by MALDI-MS.

#### Vesicles preparation

Lipids (DMPC, 8mg) was first dissolved in chloroform (2ml) in a round bottom flask, the solvent were then removed by rotary evaporation and then dried further under the vacuum pump for 2 hours. Samples were then hydrated with 1ml MilliQ water,

freeze-thawed and vortexed several times to obtain LMVs. The sample was then dilute to 100 times and lipid vesicles made by extrusion with a 1µm pore size filter. Samples were extruded 10 times and then stored at 4°C.

To incorporate the vesicles into Vpu<sub>1-32</sub>, the samples were prepared in a similar fashion to above. Vpu<sub>1-32</sub> was dissolved into trifluoroethanol and added to the DMPC in chloroform with 1 peptide: 30 DMPC molar ratio.

#### Raman spectra

The vesicles were optically trapped using a laser wavelength of 514.5 nm, the backscattered Raman signal was then collected using the same objective lens used to form the optical trap. The signal was directed into an Acton SP500 spectrograph and imaged onto a CCD after removing the laser line with a holographic notch filter. Typically the laser powers at focus were 10 to 20 mW and collection times were of the order of 60 seconds.

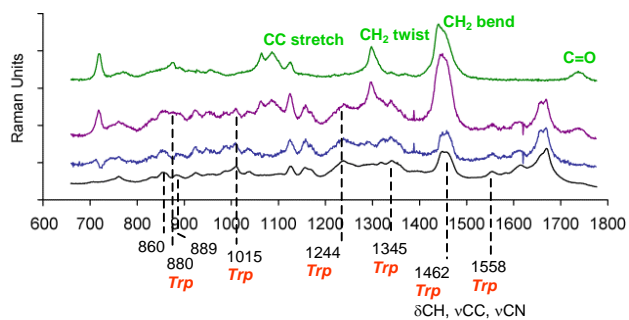
### Results and discussions

The vesicles were ~1 µm in diameter which is sufficient to be trapped in the Raman tweezers (Figure 1).



**Figure 1.** A DMPC vesicle trapped in the focus of an optical Raman tweezer and used for data collection (left) and a vesicle containing Vpu<sub>1-32</sub> (right).

These vesicles give rise to a typical lipid spectrum as shown in Figure 2, top trace. The characteristic bands observed are the C-C skeletal stretching vibrations in the range 1050 – 1130 cm<sup>-1</sup> and the CH<sub>2</sub> twist and CH<sub>2</sub> bend at 1296 cm<sup>-1</sup> and 1445 cm<sup>-1</sup> respectively<sup>5</sup>. DMPC, Vpu<sub>1-32</sub> gives discernable Raman features (Figure 2, second trace from top). The resultant spectra clearly demonstrate that Raman spectroscopy can be used to observe the peptide. The ability of the tweezers to trap the vesicle enables the concentration density of the peptide to be seen under non-resonant Raman conditions. The non-resonant nature ensures no photo degradation of the sample occurs. From the two traces a difference spectra can be obtained (third trace) which leads to the spectra of the Vpu<sub>1-32</sub> peptide in the DMPC vesicles. Characteristic bands of the indole ring system are observed around 880 (W17), 1015 (W16), 1345 (W7) and 1558 cm<sup>-1</sup> (W3)<sup>6</sup>. For comparison Raman spectra of pure Vpu<sub>1-32</sub> is found on the lowest trace verifying the bands found in the difference spectra.



**Figure 2.** Raman spectra (514.5 nm) of DMPC vesicles (green) and DMPC vesicles with Vpu<sub>1-32</sub> (magenta). Difference spectra (blue) of the first (green) and the second spectra (magenta). Spectra of powdered Vpu<sub>1-32</sub> (black).

### Conclusion

The technique of optical trapping a vesicle containing peptides has been demonstrated. This technique is promising in as much it enables the investigations under almost realistic membrane conditions as the curvature of the vesicles in comparison to the dimensions of a peptide bundle mimics a planar environment for the peptide. The results suggest that within a single vesicle sufficient peptide can be hosted to derive good quality Raman spectra under non-enhancement conditions.

### Acknowledgement

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