NEMS-FTIR PROTEIN ANALYSIS WITH EMILIE™

Picogram sensitivity for dry protein film analysis

October 17, 2024

1 INTRODUCTION

Fourier-Transform Infrared (FTIR) spectroscopy is routinely used for the quantification and characterization of proteins and other biological materials. However, low sensitivity combined with the overlap between a protein's amide I band (1700-1600 cm^{-1}) and water's HOH-bend (1643 cm^{-1}) can make the analysis of very small amounts of proteins challenging with conventional FTIR instrumentation. Here we demonstrate that nanoelectromechanical system (NEMS) - FTIR with EMILIE™ allows for highly sensitive analysis of proteins, offering spectral information from the Amide I, II, and III bands.

2 MATERIAL AND METHODS

2.1 Sample preparation

Bovine Serum Albumin (BSA) (Ref. A7906-10G, Sigma-Aldrich) and ubiquitin (ubiquitin from bovine erythrocytes, U6253-5MG, Sigma-Aldrich) solutions (1000, 500, 250, 100, 50, 25, 10, 5, and 2.5 µg/ml) were prepared in UPLC-MS grade water (Ref: W81, ThermoFisher).

2.2 Sample deposition

20 nL sample aliquots were deposited on the surface of the EMILIE™ nanomechanical sampling and sensing chips using a nanodrop dispensing tool (PIPEJET™, Hamilton Freiburg GmbH) corresponding in amounts varying between 50 pg and 10 ng of protein deposited. Samples were prepared in triplicate and method blanks in sextuplet. The EMILIE™ nanomechanical sampling and sensing chips were allowed to dry in a controlled, dry environment to ensure consistency. BSA samples containing ethylene glycol were dried at 4° C for 24 hours.

2.3 NEMS-FTIR measurements and data processing

FTIR spectra were recorded using EMILIE™ in conjunction with a commercially available FTIR spectrometer (Vertex 70, Bruker, USA). Measurement parameters are listed in Table 1.

EMILIE	
Chip temperature	30° C
Drive voltage	50-300 mV
Resonance mode	2
Output Range	±4882 Hz
FTIR	
Resolution	4 cm^{-1}
Aperture	6 mm
Stabilization delay	30 ms

Table 1: Measurement parameters

Each spectrum acquired with EMILIE™ was power-corrected by dividing the measured spectrum by the spectrum of the light source as measured with the spectrometer's internal detector. A Savitzky-Golay filter [1] with a second-order polynomial and a window of 20 data points was applied to the data.

3 RESULTS

The resulting NEMS-FTIR spectra for BSA and ubiquitin are shown in Figure 1&2, respectively. The spectral features of BSA's amide I (1665 cm^{-1}), amide II (1536 cm^{-1}), and amide III (1400 cm^{-1}) bands are clearly identifiable. Unlike in the native, hydrated, protein, the amide I band is larger than the amide II band, a characteristic of low hydration BSA thin films [2].

Limits of detections (LoD, 3σ) for BSA and ubiquitin were evaluated at 167 and 208 pg, respectively (Table 2 and Table 3).

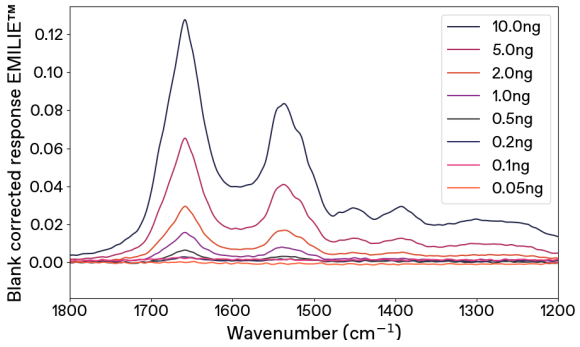

Figure 1: Average (N=3) NEMS-FTIR spectra for various amounts of BSA deposited on the EMILIE™ nanomechanical sampling and sensing chip.

Amide Band	Wavenumber [cm^{-1}]	LoD [pg]
Amide I	1665	208
Amide II	1536	291
Amide III	1400	633

Table 2: LoD for BSA measured with EMILIE™.

These results are consistent with the LoD estimated for BSA analysed by NEMS-FTIR from theory. For an estimated noise equivalent power $NEP \approx 4$ pW/Hz$^{1/2}$, and a low-noise thermal light source with a power of 100 nW and a focal beam diameter of 1 mm^2 such as in an FTIR, the LoD of BSA (molecular mass of 66.5 kDa and an absorption cross-section of 7.3×10^{-20} m^2 at a wavelength of 6 µm [3]), an estimated LoD (3σ) of 180 pg for an integration time of 1 second is estimated [4].

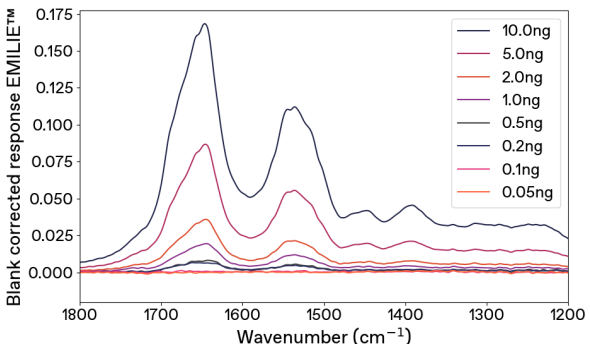

Figure 2: Average (N=3) NEMS-FTIR spectra for various amount of ubiquitin deposited on the EMILIE™ nanomechanical sampling and sensing chip.

Amide Band	Wavenumber [cm^{-1}]	LoD [pg]
Amide I	1661	167
Amide II	1544	336
Amide III	1396	944

Table 3: LoD for ubiquitin measured with EMILIE™.

4 BENEFITS OF USING EMILIE™

- Picogram sensitivity
- No water interference
- Rapid sample deposition via dropcasting
- Rapid identification and quantification
- Spectral information from Amide I, II, and III bands

REFERENCES

[1] Abraham Savitzky and Marcel JE Golay. Smoothing and differentiation of data by simplified least squares procedures. Analytical chemistry, 36(8):1627–1639, 1964.

[2] Grdadolnik J and Maréchal Y. Bovine serum albumin observed by infrared spectrometry. i. methodology, structural investigation, and water uptake. Biopolymers, 62(1):40–53, 2001.

[3] Andreas Schwaighofer, Christopher K Akhgar, and Bernhard Lendl. Broadband laser-based mid-ir spectroscopy for analysis of proteins and monitoring of enzyme activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 253:119563, 2021.

[4] Invisible-Light Labs. Nanomechanical photothermal spectroscopy, an introduction to the EMILIE™ technology, 2024.