Patient-specific longitudinal assessment of myeloma therapy by detection of intermolecular β-sheet-structure formation

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Multiple myeloma therapy efficacy is conventionally assessed by whole cell-population methods such as Fluorescence-Activated Cell Sorting and Western Blot assays or serum analysis and flow cytometry of bone marrow aspirates and biopsies. However, these methods require a large number of cells, which results in limited applicability for longitudinal evaluation of patient-specific therapeutic response. **Label-free** monitoring of treatment at **single-cell** levels would avoid the need of using large patient cell populations for longitudinal evaluation. Here, we present a unique technology that exploiting the mechanistic action of proteasome inhibition in synergy with label-free protein-structure specificity of **mid-infrared optoacoustic microscopy**, facilitates longitudinal evaluation of myeloma treatment exploring patients' heterogeneous response. Specifically, we use intermolecular β-sheet formation as an endogenous biomarker for cell viability during proteasome inhibition therapy. Aiming to promote **personalized medicine** in myeloma therapy, detection of intermolecular β-sheet structure was applied to assess drug-treatment performance in myeloma patients.

Mid-infrared Optoacoustic Microscopy

Mid-infrared Optoacoustic Microscopy (**MiROM**) combines the high spectral specificity of mid-infrared excitation with the positive contrast nature of **optoacoustic sensing**.

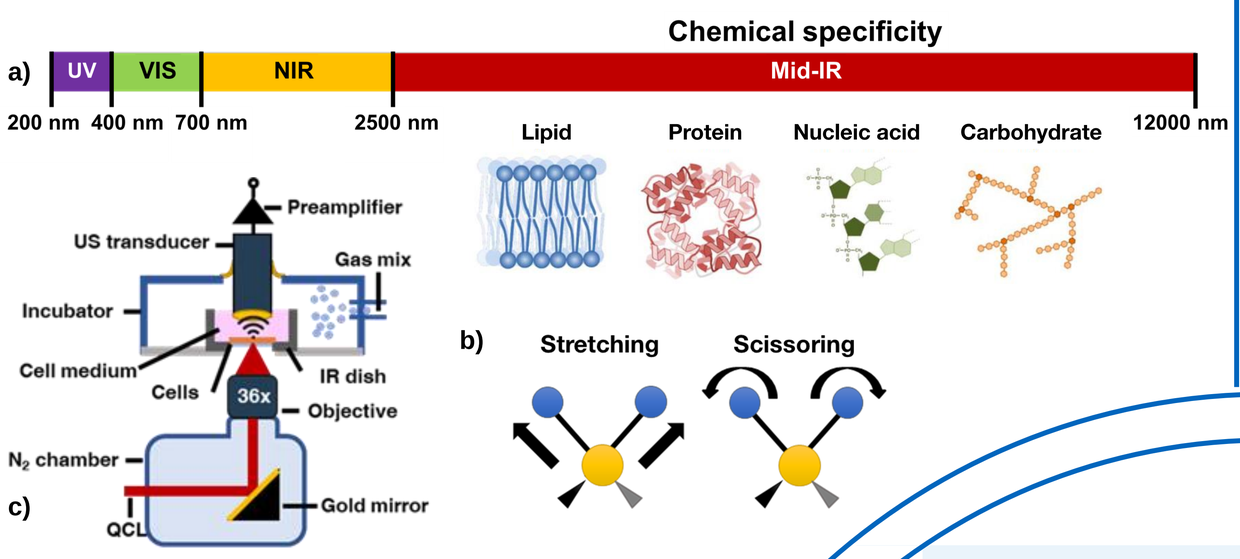


Figure 2. Method. a) Light absorption spectral range. Mid-infrared (Mid-IR) spectroscopy is specific for biomolecular detection. b) Specific molecular vibrations induced by mid-IR excitation. c) Schematic representation for **MiROM**. QCL: Quantum Cascade Laser. US: Ultrasound. IR: InfraRed.

Label-free monitoring of myeloma cells therapy

MiROM can detect misfolded proteins, rich in **intermolecular β-sheet structures** at 1620 cm^{-1}, and can use this absorption band as intrinsic marker to **assess myeloma drug therapy.**

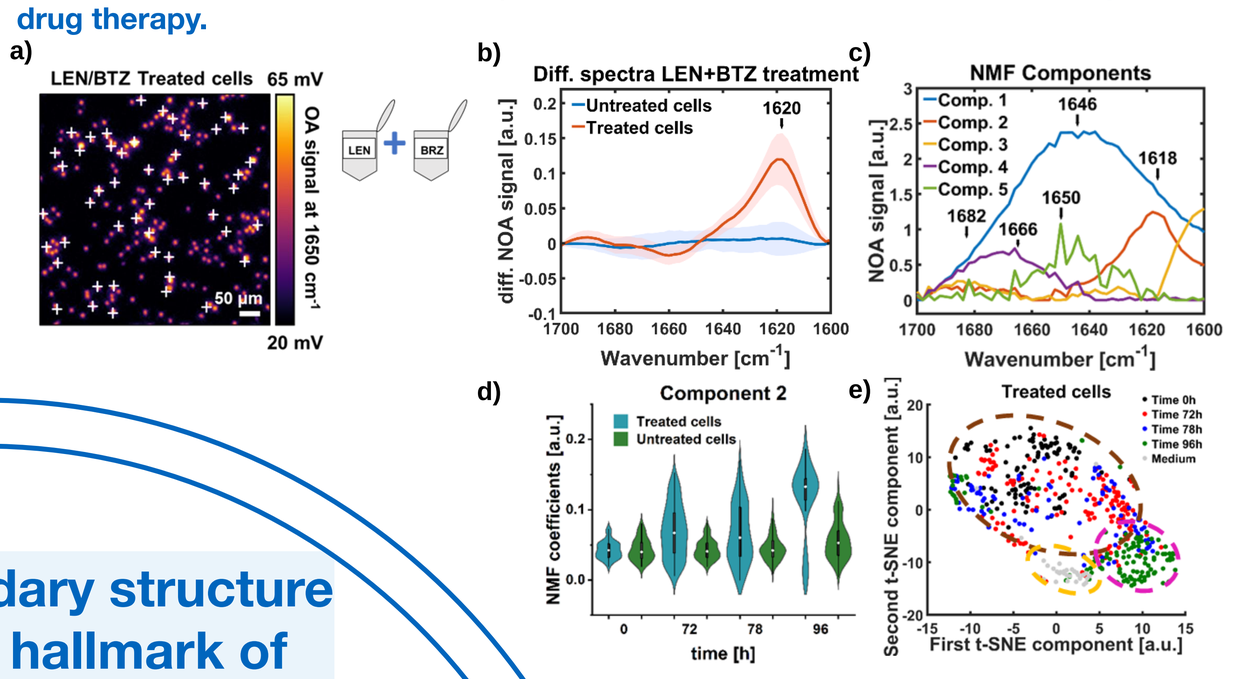


Figure 3. Monitoring protein misfolding in myeloma cells. a) LEN/BTZ treated myeloma cells. b) Differential spectra of **LEN/BTZ** treated myeloma cells (red line) and untreated cells (blue line). The band at 1620 cm^{-1} is assigned to **intermolecular β-sheet** assigned to misfolded proteins. c) Non-negative Matrix Factorization (**NMF**) components extracted from spectral data in (b). d) Violin plots showing the time evolution coefficients of NMF component 2. e) t-Distributed Stochastic Neighbor Embedding (**t-SNE**) map representing the distribution of the 5 components identified in LEN/BTZ treated and untreated myeloma cells. f) Differential spectra of **BTZ** treated myeloma cells (red) show the intermolecular β-sheet band at 1620 cm^{-1}. g) Differential spectra of **LEN** treated myeloma cells (red) show the intermolecular β-sheet band. i) Differential spectra of doxorubicin (**DOX**) treated myeloma cells (red) do **not** show the intermolecular β-sheet band. Untreated cells in blue.

Protein secondary structure detection as hallmark of myeloma therapy efficacy

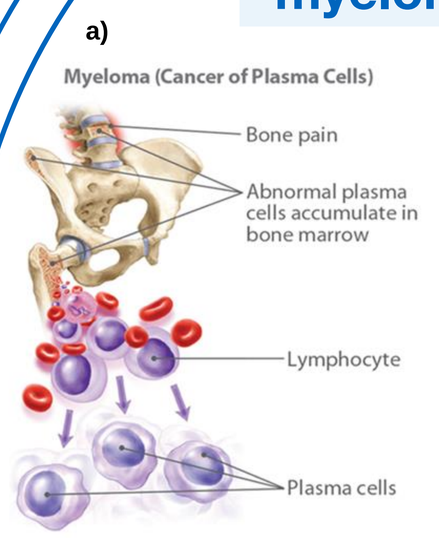
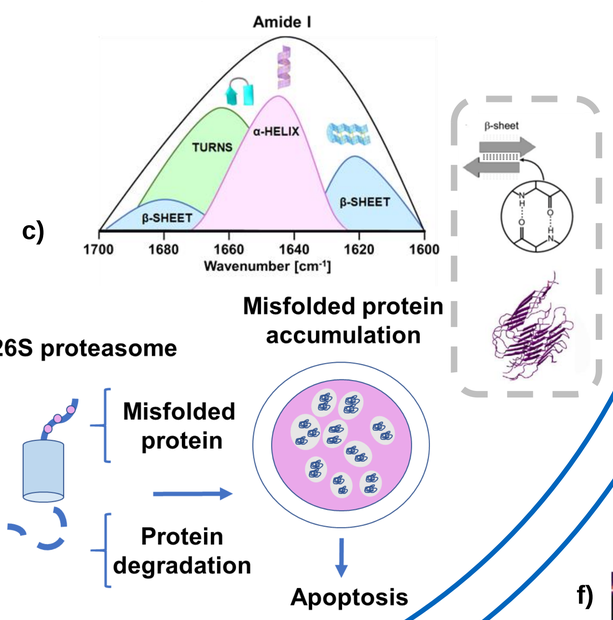


Figure 1. Introduction.
a) Abnormal plasma cells caused **multiple myeloma.*** b) LEN/BTZ therapy generates accumulation of **misfolded proteins** in myeloma cells. c) Misfolded proteins are rich of intermolecular β-sheet structures detectable in the amide I mid-infrared spectra.



Assessment to therapy response in LEN/BTZ sensitive and resistant myeloma patients

MiROM assesses myeloma therapy response at **single-cells** level in patients samples.

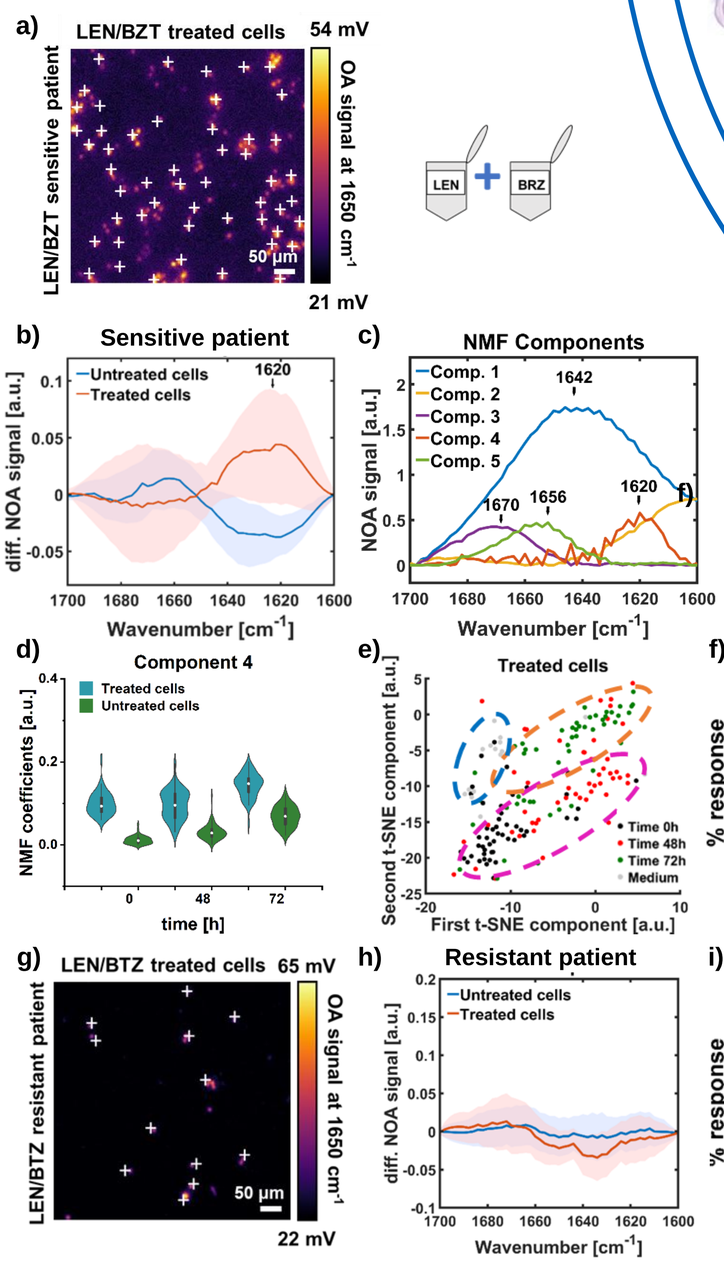
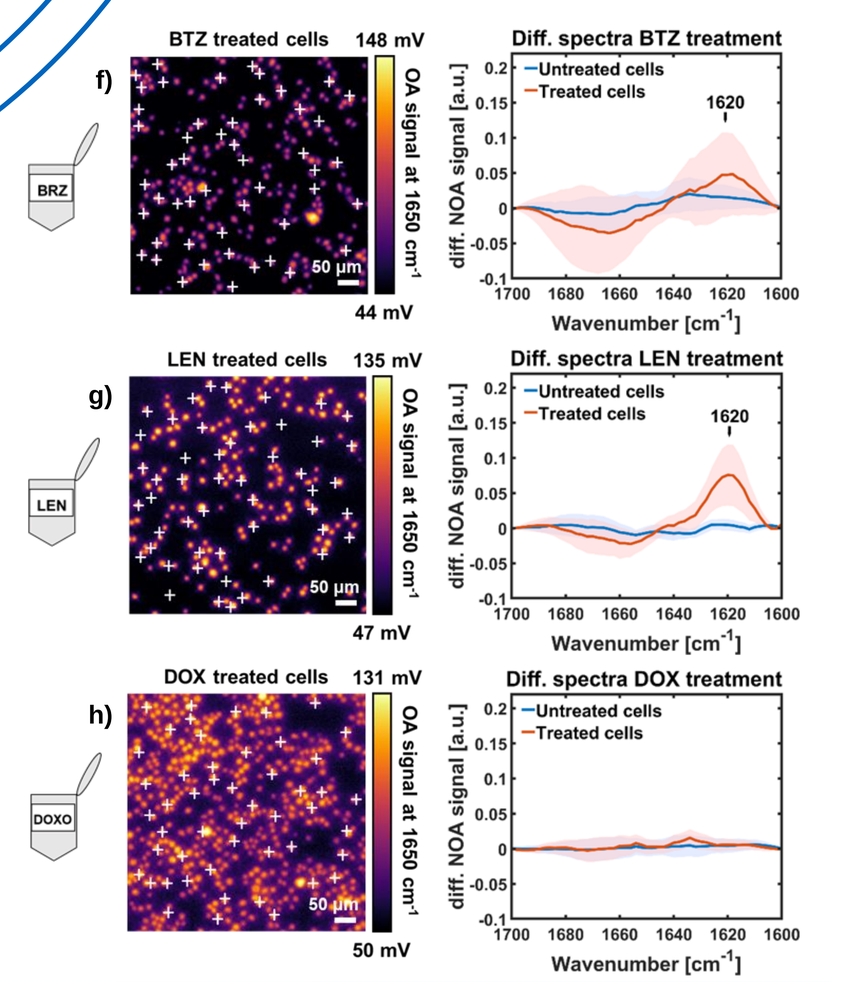


Figure 4. Monitoring protein misfolding in LEN/BTZ sensitive or resistant patients. a) LEN/BTZ treated myeloma cells biopsied from LEN/BTZ **sensitive patient.** b) Differential spectra of LEN/BTZ treated (red) and untreated (blue) patient myeloma cells in (a). The band at 1620 cm^{-1} indicate presence of **misfolded proteins**. c) NMF components extracted from spectral data in (b). d) Violin plots showing the time evolution of NMF component 4. e) t-SNE map representing the distribution of the 5 NMF components. f) **Percentage response** (%) of LEN/BTZ sensitive patients' cells analyzed from 10 independent patients. g) LEN/BTZ treated myeloma cells biopsied from LEN/BTZ **resistant patient**. h) Differential spectra of LEN/BTZ treated (red) and untreated (blue) patient myeloma cells in (g). i) Percentage response of LEN/BTZ resistant patients' cells.



Key publications

- * Hideshima T. *et al,* Molecular Cancer Therapeutics, 2011
- Pleitez M.A., ... Gasparin F. *et al.* (2020)*, Nat. Biotechnol.,* 38, 293-296.
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