Real-time tracking of free radicals as biomarkers in cell-based drug potency assays at subcellular location

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Free radical tracking & drug potency determination

We monitor magnetic interference of radical/paramagnetic species with subcellular resolution in biological samples (cells to tissues) using a fluorescent nanodiamond (FND) as a quantum sensor, detecting free radicals and Reactive Oxygen Species (ROS) in a 50nm radius around the particle in real time with high sensitivity (1 - 10 nM). Drug potency is determined based on free radical kinetics, their dynamic change, and before/after treatment.

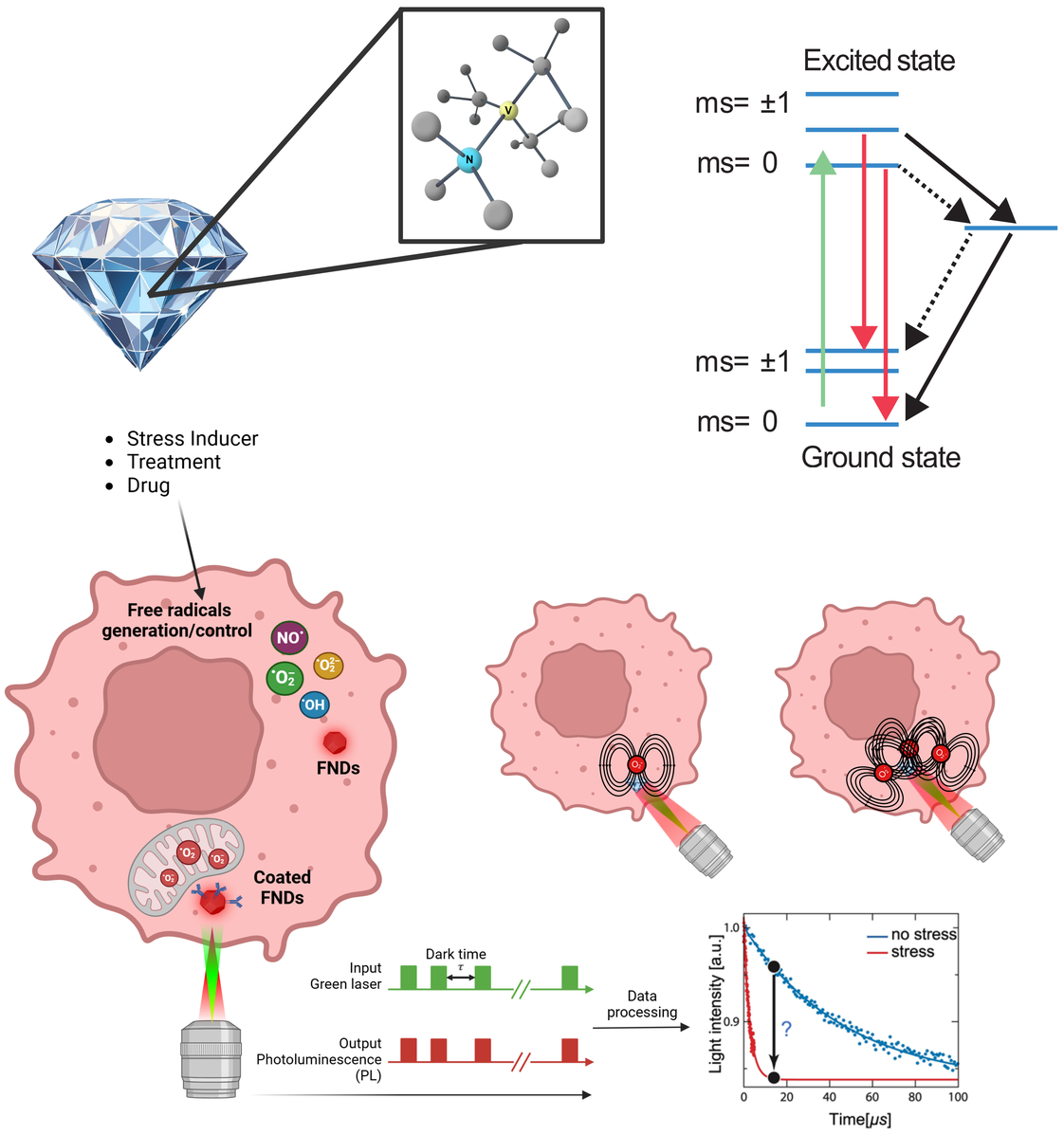


Figure 1: Quantum sensing method. An NV-Center is a designed impurity in the diamond lattice. Carbon is replaced with Nitrogen. This leads to 2 unbound electrons in a Vacancy next to the Nitrogen. Excitation of the FND by a laser of 550nm leads to emission in the 650 nm – 800 nm range. The magnetic interference from free radicals (paramagnetic species) interferes with the decay of the two vacancy-excited electrons back to the ground state & emission of photons. The difference in decay rate can be monitored via Spin-Lattice relaxation (T1 Relaxometry). The T1 relaxation time of the nanodiamond correlates with the number of free radicals around the FND. FNDs do not photobleach Enables prolonged experiments (1).

Drug potency assay for ONC-201

The imipridone, ONC-201 is a small molecule activator of the mitochondrial caseinolytic protease P (ClpP agonist) depleting mitochondrial DNA and inducing integrated stress response, currently undergoing clinical evaluation across multiple cancer types. However, there are no omics technologies available for direct measurement of mitochondrial stress response in MDA-MB-230 cells.

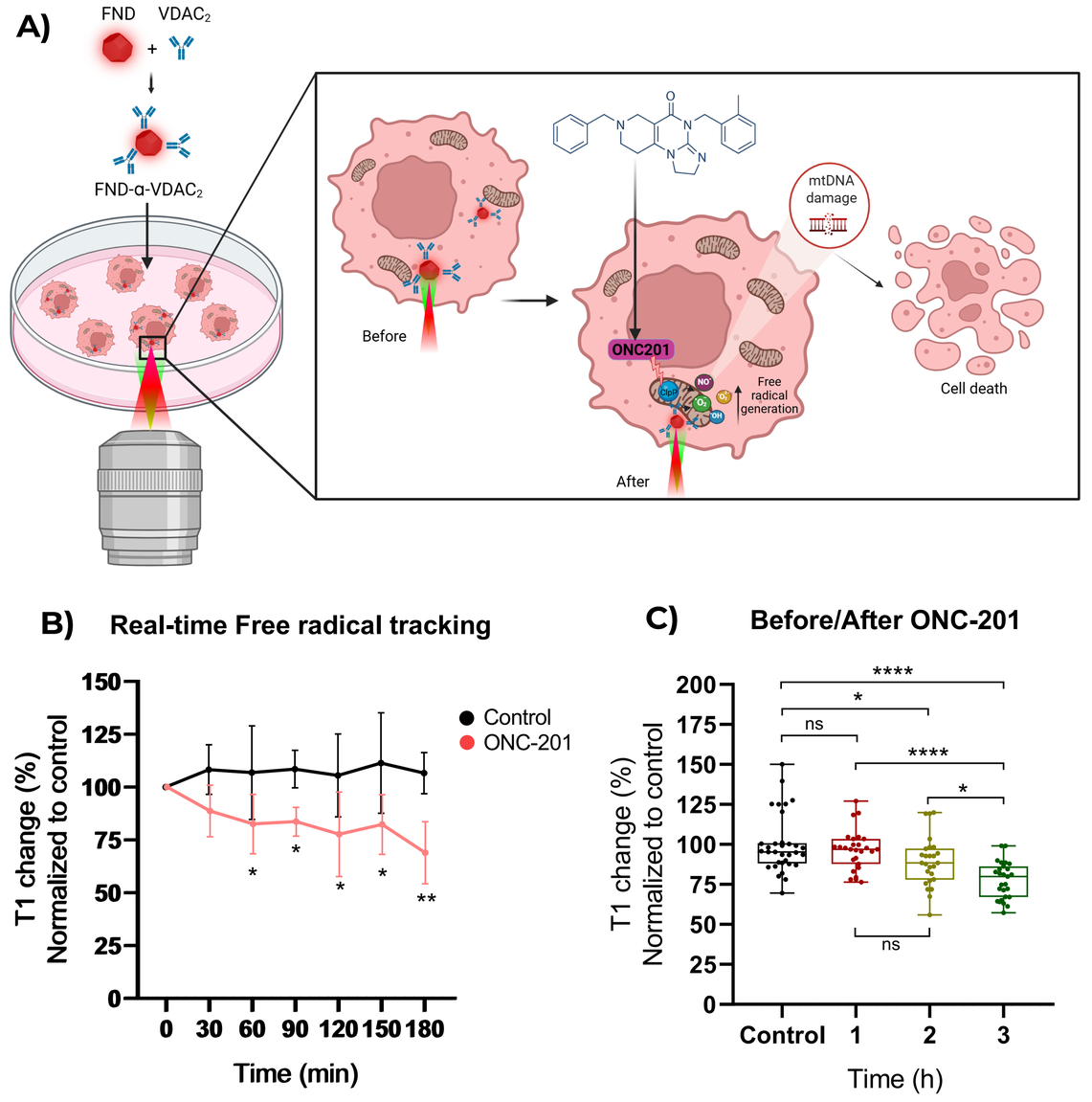


Figure 3. Mitochondrial real-time free radical tracking and T_1 change percentage before and after ONC-201 treatment in MDA-MB-231 breast cancer cells. Controls were measured first in cells grown in medium before the addition of ONC-201 (3 μM). (A) Methodology. (B) T_1 values were recorded every 30 minutes to observe the dynamic change comparing the same particle before/after addition. Each curve represents the average of three independent particles (one particle/cell per plate) and three measurements per time point normalized to time 0. (C) T1 measurements after 1, 2, and 3 h of treatment with ONC-201, each point per group represents one T1 relaxation time repetition of different FND-anti-VDAC2 particles. The experiment was repeated in 9 particles per group from 3 independent plates (3 particles per plate) and at least three repetitions per particle. Significance between groups was analysed in by one-way ANOVA with Fisher's LSD post-hoc test for (B) *p ≤ 0.04, **p ≤ 0.0015, and with Tukey´s post-hoc test for (C) *p 0.03, ****p ≤ 0.0001, ns=non-significant. Error bars represents SD.

Fluorescent nanodiamonds in breast cancer cells

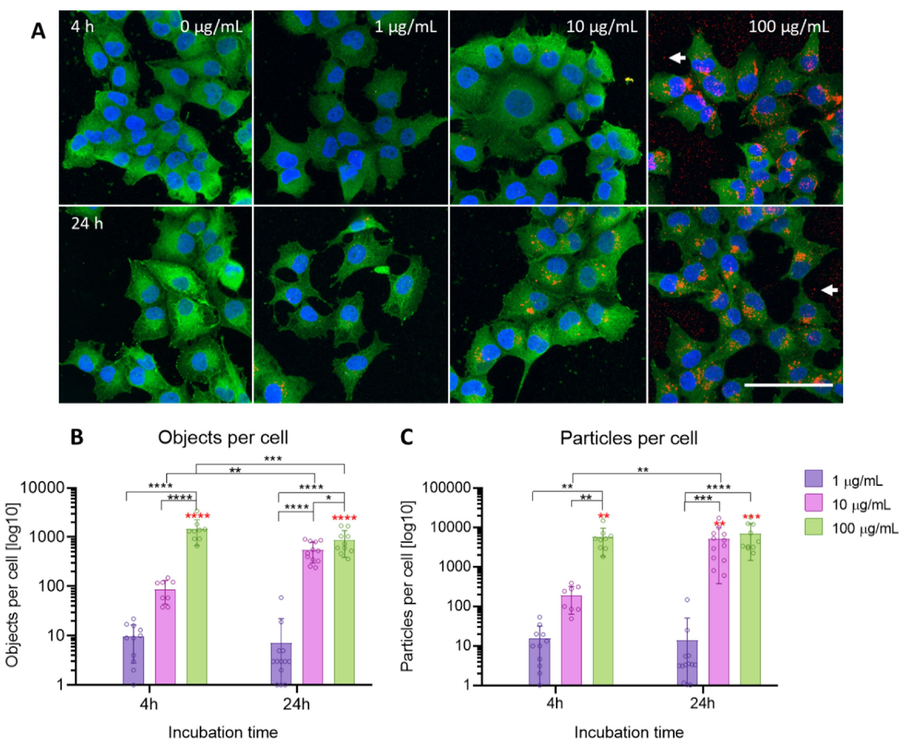
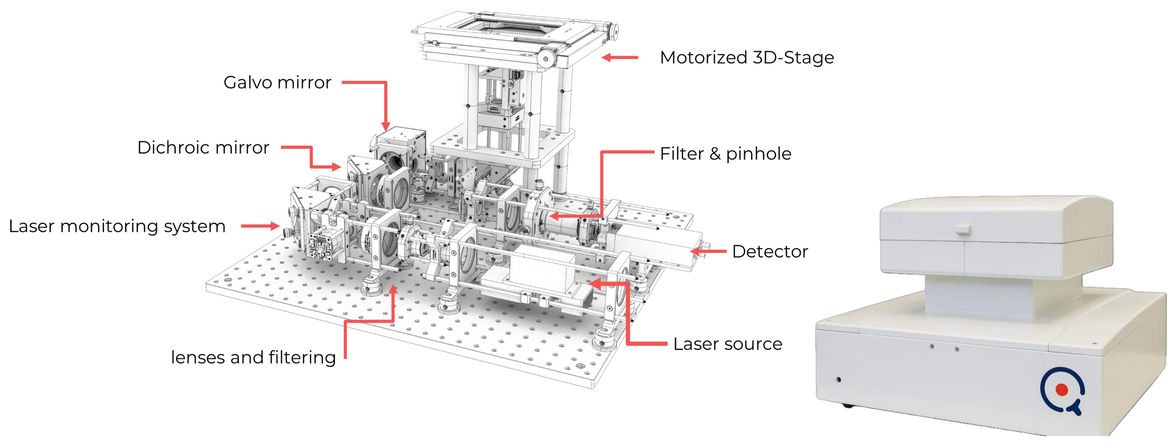


Figure 2. FND internalization in human MDA-MB-231 breast cancer cells. (A) Representative confocal images of cells exposed to different concentrations of 70 nm FNDs for 4 and 24 h (blue: DAPI, green: FITC-Vinculin, red: FNDs, white arrows: extracellular FNDs, scale bar: 30 μm). (B) objects per cell and (C) particles per cell (an object can consist of more than one particle). For each condition, FNDs in at least 20 cells were counted. Error bars represent SD. Statistical significance was analysed by two-way ANOVA with Fisher's LSD post hoc, **p < 0.01, ***p < 0.001, ****p < 0.0001 (red stars: comparison against 0 μg/mL control in each time point). Images and data reproduced with permission from (2)

Quantum Nuova for free radical detection



- Modified Confocal Microscope – Specialized in measuring free radical generation/neutralization
- Quantum Nuova measures emission of FNDs in real-time
- Monitor responses of cell phenotypes/heterogeneity vs external stimuli in ROS
- Measuring real-time drug potency
- Keeps cells alive for further analysis with other cell-based techniques.

References

1. Mzyk A, et al. Acc. Chem. Res. 55, 24, (2022), 3572–3580
2. Reyes-San-Martin, et al. Carbon. Vol 228 (2024). 119405.
3. Bucher, et al. AAPS Journal. Vol 27. Article 109 (2025)