**Dynamic Nuclear Polarization NMR Below 6 Kelvin within Human Cells Using Fluorescent Trimodal Polarizing Agents**

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Nuclear magnetic resonance (NMR) is exquisitely suited to determine atomic level structural detail of biomolecules within intact cells. However, the concentration of the target biomolecule of interest is reduced compared to in vitro preparations, leading to a concomitant reduction in NMR sensitivity. Previous in-cell NMR experiments typically involve increasing the concentration of target proteins beyond their endogenous levels to recover sufficient NMR sensitivity and resolution. Yet, such perturbations are detrimental to retaining the endogenous interplay of pathways and biomolecular interactions. The foremost challenge of characterizing biomolecular structures with in-cell NMR is therefore weak NMR signals. Strategies to increase NMR sensitivity we apply include access to high static magnetic fields, cryogenic sample temperatures, and transfer of spin polarization.

Our preliminary results characterize sensitivity enhancement of $^{13}C$ enriched HEK293F cells using newly designed and synthesized fluorescent trimodal polarizing agent, TotaFAM (Figure 1). It's composed of three moieties: fluorescein—FAM, DNP polarizing agent—TotaPol, and cell penetrating peptide—TAT (47-57). Conjunction of FAM allows us to confirm localization of the polarizing agents and correlate subcellular localization to NMR spectra. Binitroxide Totapol affords access to the cross-effect DNP mechanism which provides high NMR signal enhancement at magnetic fields suitable for high-resolution NMR spectroscopy. Eleven residues of the HIV-TAT protein link the Totapol moiety to the fluorescein fluorophore and facilitates the uptake of the polarizing agents into HEK293F cells. We confirmed that TotaFAM was uptaken by HEK293F cells and achieved sensitivity enhancement over 60. This, to our best knowledge, is the best enhancement ever reported on human cells.

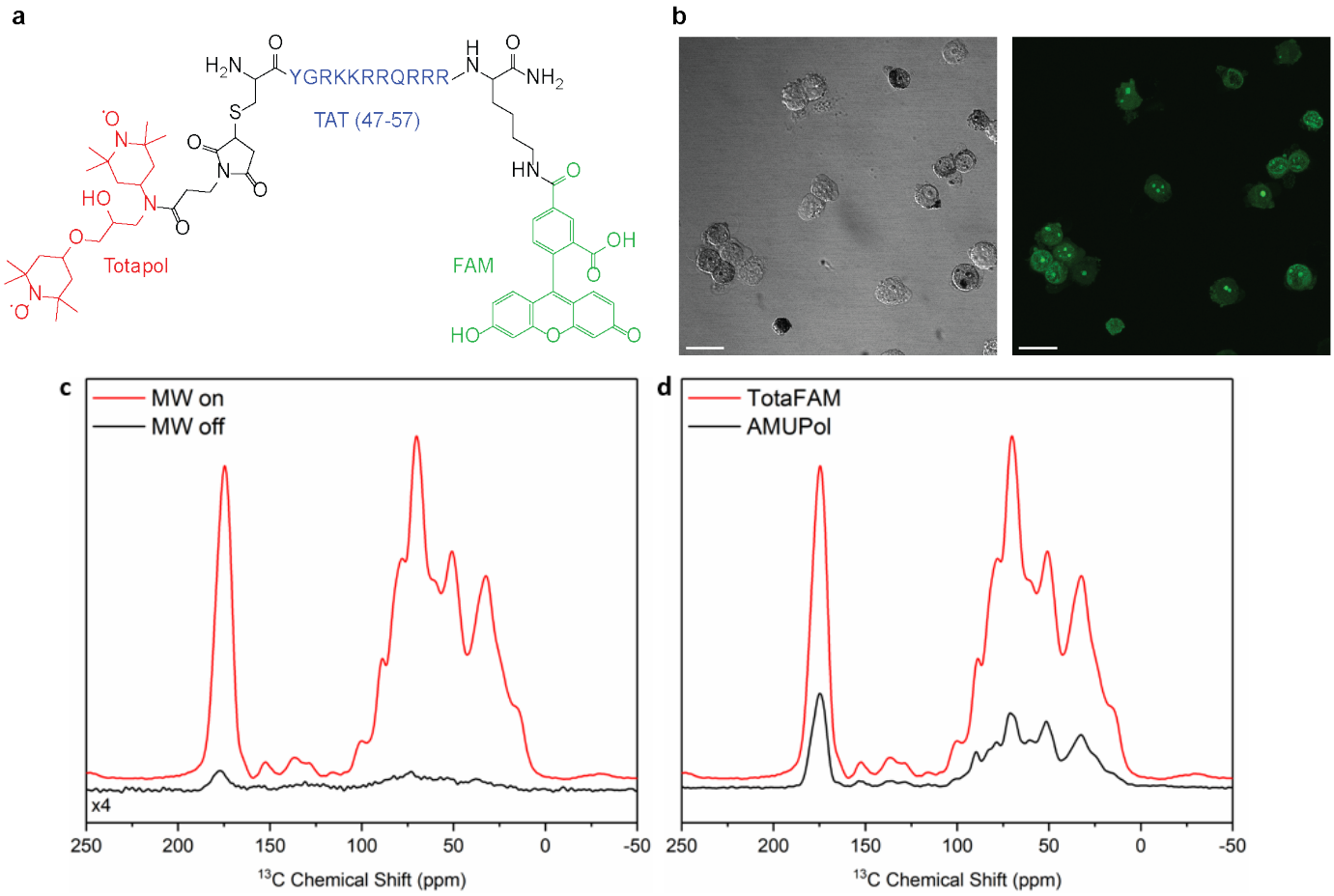


***Figure 1.*** Structures of TotaFAM (a) with three moieties highlighted. (b) Fluorescent microscopy confirms cellular uptake through fluorescein detection at 515 nm. (c) are spectra of $^{13}C$ enriched HEK293F cells mixed with 2.7 mM TotaFAM showing an enhancement if 63. Black spectra represent spectrum with no microwave irradiation and red spectrum have microwave irradiation present. (d) Spectra comparison of HEK293F cells with 2.7 mM TotaFAM (red) and with 2.7 mM AMUpol (black).