

# Looking at Live Cells by High-Resolution, High-Field NMR

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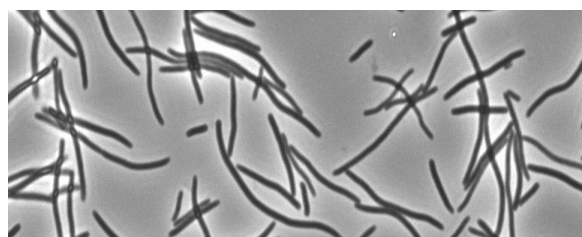
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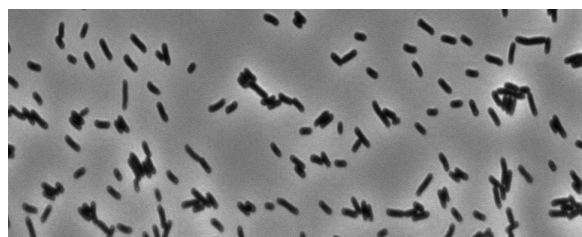
Cell metabolism can change drastically due to naturally occurring fluctuations in internal cell state and in environmental conditions. Genetic engineering also often results in modifications to cell metabolism in potentially undesired and unanticipated ways that can lead to cell stress, loss of function, and loss of viability. An important component of Systems Biology is to gain an understanding of such changes and their relationships to the dynamics of genetic and protein networks. The main requirement for performing such studies is the ability to probe the state of the cells. However, existing approaches to probing cell state suffer from a variety of limitations since no current approach can examine the dynamic state of a large number of cell components *simultaneously*, in *real-time*, within *living cells*. We are now exploring the use of NMR for such purposes.

Nuclear magnetic resonance (NMR) is a quantitative non-destructive technique reporting at the molecular level. As such, it may be very informative about differential cell biochemistry even if the changes have not reached the level of observable differentiation in the phenotype. We have started an NMR study to distinguish and characterize intact, live cells of various behavior and origin, as well as looking at carefully prepared cell extracts, using both more conventional tube-based, as well as capillary flow NMR techniques for very small quantity samples.

Our pilot studies have been conducted on selected bacterial cell cultures. We transformed *Escherichia coli* cells with a plasmid that overexpresses a recombinant protein, the Yellow Fluorescent Protein. This overexpression likely places a metabolic stress on the cells, and they become filamentous after several hours of growth, as seen in the microscope image in Figure 1(a). After additional growth, the cells return to their normal rod shape, as can be seen in Figure 1(b).



(a) Initial observation of cells



(b) Same cells after 4 hours

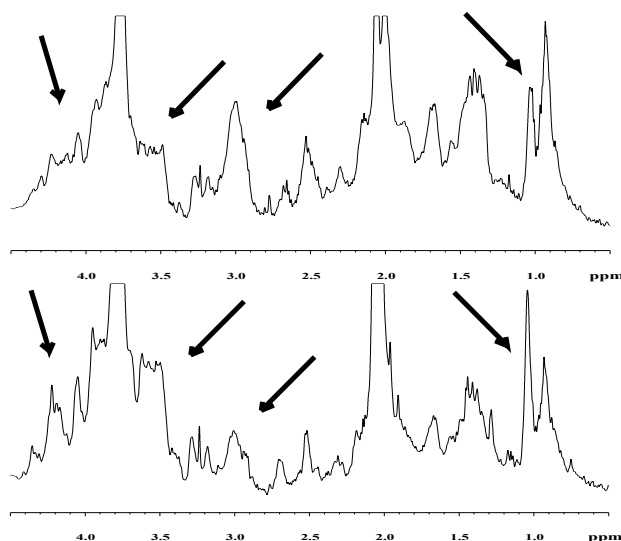


Figure 2: Representative <sup>1</sup>H-NMR spectral segments of cell samples with marked differences

Figure 1: Filamentous cells which then mutate to normal looking cells

We have conducted several consecutive experiments on various cell samples. Simple <sup>1</sup>H NMR spectra show specific changes between samples (representative segments are shown on Figure 2), which seem to be diagnostically correlated with differential origin and state of these cells, which we'll demonstrate on the poster. Cell extracts are also prepared with as little biochemical or chemical bias introduced as possible. Such cell extracts can be studied at very low absolute amounts also taking advantage of capillary flow NMR. We'll also discuss possible future extensions of such NMR studies.