

UROP Summaries

Elucidating EGFRvIII Cellular Signaling Networks: A Quantitative Approach

September 2005 – Present

Division of Biological Engineering, White Laboratory

Principal Investigator: Professor Forest M. White

Supervisor: Paul Huang

Zachary Brewer, Class of 2007

Majors: Chemical Engineering and Biology

EGFRvIII is a truncated mutant of the epidermal growth factor receptor (EGFR), which is implicated in the progression of many cancers, including breast, ovarian and brain. EGFRvIII lacks the extracellular ligand binding domain encoded by exon 2-7 of the EGFR gene and thus is unable to bind EGF. Despite the lack of a ligand binding domain, it has been shown that EGFRvIII is constitutively phosphorylated and able to activate downstream signaling cascades at 10% of the level of the wildtype receptor. The basis of signal initiation is unclear but various studies have attributed this to homodimerization or heterodimerization with other members of the ErbB family. While much work has been done to elucidate the pathways involved in EGFRvIII receptor signaling, the global map of the signaling network activated by this mutant receptor is still very much incomplete, making it difficult to assess downstream components involved in EGFRvIII-mediated tumor progression. There is a strong need to develop a clearer picture of the signal transduction events originating from EGFRvIII in order to develop effective targeted therapies.

Our laboratory has previously developed a mass spectrometric approach to quantitatively measure tyrosine phosphorylation on specific sites on a large number of key signaling proteins involved in the canonical EGFR signaling pathway in a multiplexed manner (Figure 1). This approach has been extended to the EGFRvIII receptor and has allowed us to obtain a systems view of immediate early signaling networks initiated by the activation of the EGFRvIII receptor. Initial studies were performed on a model system of 184A1 human mammary epithelial cell lines (HMEC) stably transfected

to express EGFRvIII. This research has now been extended to tumorigenic U87MG glioblastoma cell lines expressing a series of EGFRvIII levels by retroviral infection. The comparison of the signaling pathways in these two cell lines will allow us to evaluate the extent of cell-type specific variation in the tyrosine phosphorylation induced by EGFRvIII.

In this study, we have mapped the temporal profile of tyrosine phosphorylation in these cell lines upon stimulation with EGF. We have identified and quantified many critical signaling proteins which are differentially tyrosine phosphorylated as a function of increasing EGFRvIII levels. Together with phenotypic measurements, such quantitative signal transduction data has shed light on the contribution of EGFRvIII signaling to tumor-associated phenotypes such as cell migration, proliferation, and invasion. We anticipate that such mass spectrometric and phenotypic data used in conjunction with computational tools will allow us to predict how such tumor-associated cell responses are governed by signal transduction pathways downstream of the EGFRvIII receptor. In addition, the application of this technique to subcutaneous xenografts of U87MG cells expressing EGFRvIII is currently underway in our laboratory.

Nanoparticle Drug Delivery to the Central Nervous System

September 2005 - Present

Division of Biological Engineering, MIT and the Day Laboratory for Neuromuscular Research, Massachusetts General Hospital

Principal Investigators: Professor Robert Langer and Dr. Robert Brown

Supervisor: Seth Townsend

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Major: Brain and Cognitive Sciences

Amyotrophic lateral sclerosis (ALS; Lou Gehrig's Disease) is a neurodegenerative disease affecting motor neurons, causing progressive paralysis and, in most cases, death. Although progress has been made in understand-

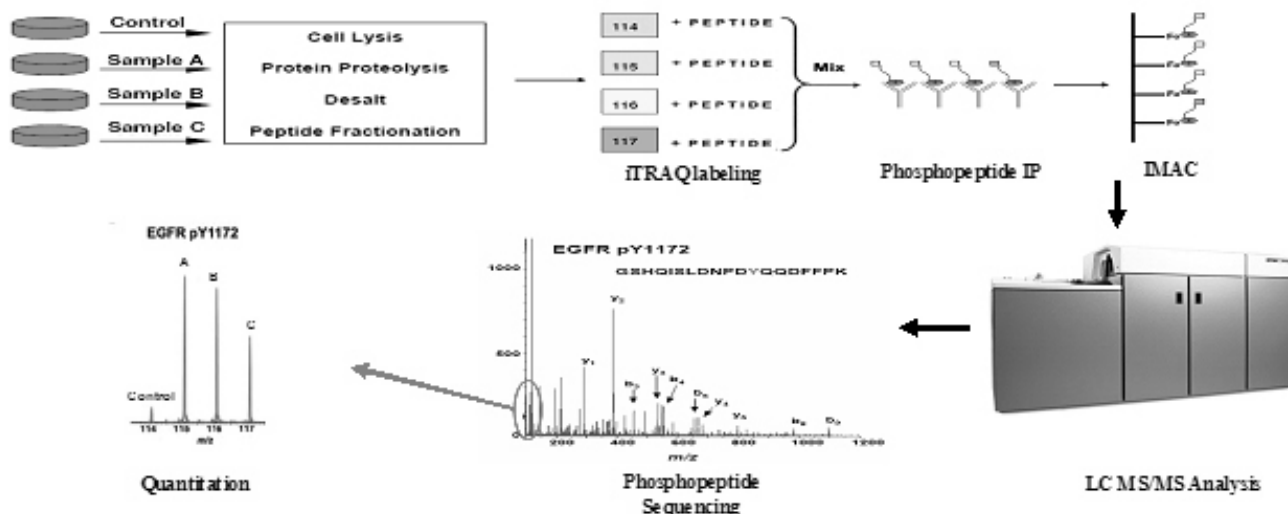


Figure 1. Overview of mass spectrometry strategy for quantification of phosphotyrosine peptides.

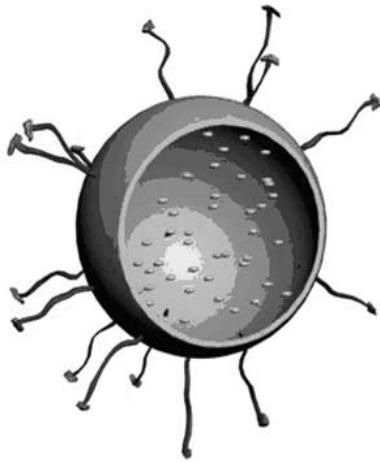


Figure 1. Schematic model (not to scale) of a biodegradable nanoparticle encapsulating a therapeutic. Polyethylene glycol polymers decreasing systemic clearance are in purple, and the compound targeting the central nervous system is attached in green.

ing the etiology of the disease (1), there is still no effective treatment and no single test or procedure that can establish a diagnosis. Most candidate drugs fail in clinical trials, a fact that is largely thought to be a consequence of targeting the disease only once symptoms are visible in its later stages (2), as well as the inability to deliver therapeutics efficiently to their sites of action.

The project involves developing a noninvasive diagnostic and nanoparticle drug delivery system for the treatment of ALS in a collaborative effort led by Dr. Robert Langer (Institute Professor, MIT) and Dr. Robert Brown (Professor of Neurology, Harvard Medical School, and Director of the Day Laboratory for Neuromuscular Research, MGH). To achieve this, we have synthesized novel biodegradable polymers and developed techniques enabling specific surface conjugation of proteins to nanoparticles, in order to target the particles to the central nervous system for delivery of encapsulated therapeutics and new diagnostics for ALS.

The project is motivated by the belief that once the crucial link of being able to deliver any drug to any desired cell is made (3), the full potential of molecular biology, genetics and modern drug design can be fulfilled. By creating a modular vehicle for candidate drugs for ALS, which enables specific targeting of motor neurons, we hope to develop better diagnostics and treatments for this disease.

References:

1. Brown RH, Robberecht W. Amyotrophic Lateral Sclerosis: Pathogenesis. *Seminars in Neurology*. 21(2): 131-139 (2001).
2. Karitzky J, Ludolph AC. Imaging and neurochemical markers for diagnosis and disease progression in ALS. *Journal of the Neurological Sciences*. 191: 35-41 (2001).
3. Langer R. Drug Delivery: Drugs on Target. *Science*. 293(5527): 58-59 (2001).

Force Plate Design and Construction on Small-Radius Human Centrifuge

February 2006 - August 2006

Division of Aeronautics & Astronautics - Man Vehicle Laboratory (MVL)

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Major: Mechanical Engineering

Over the spring and summer of 2006, the small-radius human centrifuge at the MIT Man Vehicle Laboratory (MVL) has been extensively modified. MVL has long been dedicated to addressing many of the human-related issues of space travel, namely, muscle atrophy and bone loss in long-term missions. Since the discovery of these effects, researchers have conjured few remedies able to reduce or decelerate their rate. Some existing methods include rigorous exercise, lower-body negative pressure suits and resistive garments. However, these countermeasures are insufficient in reconstructing the effects of gravity on Earth (1g). The current method used on missions is exercise, which is both time-consuming and inadequate in the treatment of muscle deterioration.

At MVL, researchers have developed a human centrifuge that can simulate the effects of gravity by spinning test subjects on a horizontal apparatus. The centrifuge provides a centripetal force comparable to that felt on Earth and allows astronauts to move with normal resistance.

This past spring, MVL proposed to redesign the foot plates, devices on the centrifuge responsible for measuring a subject's "artificial weight." The footplates used previously were approximately three inches thick and were therefore redesigned to measure less than one inch in thickness to better accommodate taller subjects. The principle used in both designs is essentially the same: four string gauges mounted between two aluminum plates measure the force applied at the feet of the subject.

A significant amount of time was spent on not only the design, but also the construction and assembly of the new foot plates. The production of each plate gave students the opportunity to familiarize themselves with much of the machinery in the Aeronautics & Astronautics machine shop, including the mill, lathe, and water jet.

This past summer, students were also trained to run subjects on the centrifuge and analyze the experimental effects of artificial gravity. Many subjects reported a tumbling or spinning sensation when asked to turn their head from the nose-up position to the ear-down position. Occasionally, these sensations were intense enough to induce motion sickness. Students were responsible for quantifying subjects' sensations and motion sickness, as well as analyzing data through I-SCAN, a program that tracked eye movements in each subject.

Neural Signal Processing for Motor Control Experiments

February 2006 – Present

Department of Brain and Cognitive Sciences

Supervisors: Andrew Richardson, Simon Overduin

Faculty Supervisor: Emilio Bizzi

Jeff Moore, Class of 2006

Majors: Brain & Cognitive Sciences, Electrical Engineering

Background

Several experiments at the Bizzi Lab are aimed at understanding how the central nervous system controls motor planning, execution, and learning. In one experimental paradigm, a monkey uses its own arm to manipulate a robotic arm (manipulandum) that moves a cursor on a computer screen. The monkey is trained to move the cursor toward targets which appear on the screen; however, during some trial movements the monkey's task is complicated by force fields which impede the motion of the manipulandum. Throughout these experiments, there are several microelectrodes

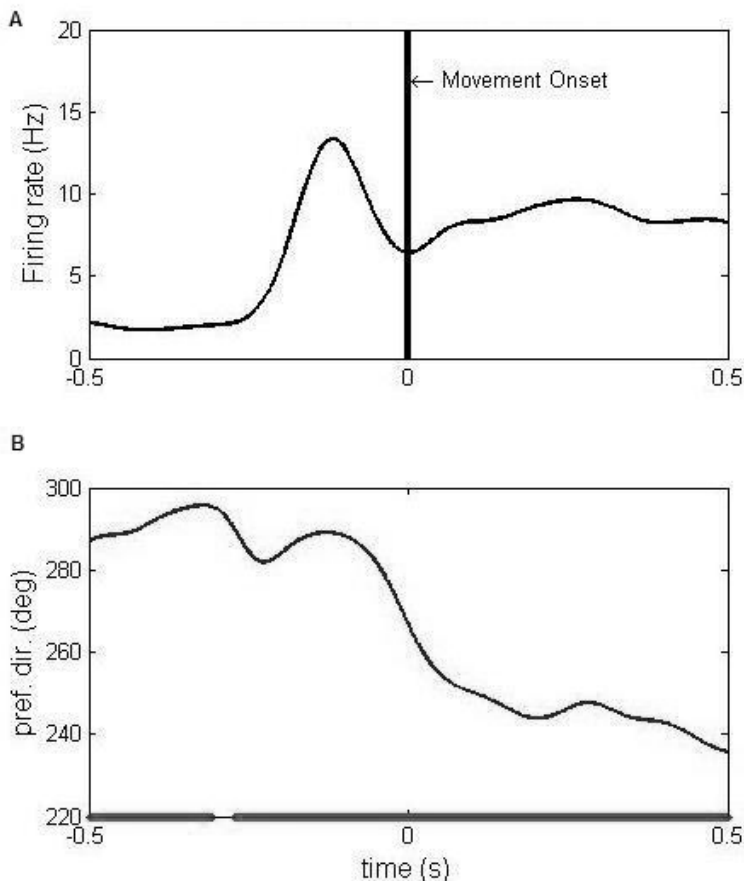


Figure 1. Monkey M1 cell firing pattern during directed arm movements. Spikes are synchronized with movement onset time and smoothed. (A) Mean "instantaneous" firing rate, over all trials in all directions. (B) "instantaneous" preferred direction of the same cell. In this example, no external forces were applied. Red line indicators significant directional tuning.

implanted in various areas of the monkey's cerebral cortex, including the primary motor cortex.

Project Summary

This UROP project involved analyzing the voltage signals recorded during the experiment from individual neurons in the primary motor cortex to determine how the firing properties of the neurons change as the monkey moves its arm. For this analysis, relevant time-varying signals were computed via a MATLAB routine, including the "mean firing rate" of each cell synchronized with a behavioral event, and the magnitude and direction of the "directional tuning vector."

The "mean firing rate" is defined as the number of action potentials, or "spikes," generated per second. In order to determine how the firing rate varies with time, the cell potential was modeled as an impulse train by placing an impulse at each time sample in which a spike was detected. This impulse train was then convolved with a Gaussian "smoothing" filter, producing a continuous, integrated waveform. These continuous rate waveforms were then synchronized to the time of movement onset and averaged over all trials (Figure 1a). The particular cell in Figure 1 shows a burst of increased activity near when the monkey began moving, and may play a role in commanding the muscles to move, and/or processing proprioceptive feedback from the motion.

The computation of the directional tuning vector is slightly more complicated. First, it has been shown that neurons in the primary motor cortex

respond maximally to movements in a particular, "preferred" direction¹. By comparing the relative firing rates related to motion toward the different target directions, it is possible to compute this preferred direction. Using the value of the average "Gaussian smoothed" firing rate in each direction at each time sample, we computed the preferred direction of each cell at each time step, synchronized with movement onset (Figure 1b). We also computed whether this preferred direction was significant at each time sample ($p=0.01$), using a random shuffling/bootstrapping test².

In the cell in Figure 1, there is a significant, large shift in preferred direction near the time of movement onset. At this point it is unclear what a shift in directional tuning around movement onset might mean in terms of the cell's functional role. Thus, the next step would be to determine whether other physiological signals (i.e., local field potentials and/or electromyography signals) show related directional tuning shifts, and whether these shifts are dependent on the external manipulandum forces.

References:

- Alexander GE, Crutcher MD. "Preparation for movement: neural representations of intended direction in three motor areas of the monkey." *Journal of Neurophysiology* 64(1990):133-150.
- Scott S, Kalaska JF. "Reaching movements with similar hand paths but different arm orientations. I. Activity of individual cells in motor cortex." *Journal of Neurophysiology* 77(1997):826-852.

Hypoxia-targeting compounds for Boron Neutron Capture Therapy

June 2006 – Present

Department of Nuclear Science and Engineering

Boron Neutron Capture Therapy – MIT Nuclear Reactor Lab

Principle Investigator: Dr. Otto Harling

Supervisor: Dr. Peter Binns

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Majors: Nuclear Science and Engineering, Biology

Boron Neutron Capture Therapy (BNCT) is a biochemically targeted form of radiotherapy. In BNCT, a compound labeled with the stable isotope boron-10 is intravenously administered, and tumor cells selectively uptake boron-10 at higher concentrations than healthy cells. The disease site is thus irradiated with low energy neutrons that are captured by the boron-10. The newly created and short-lived boron-11 nuclei are unstable isotopes; as a result, they fission by β^- -decay, each emitting an alpha particle while converting to lithium-7. The lithium-7 charged particles travel only approximately 12-14 μm in tissue, equivalent to the approximate diameter of a cell. Thus energy deposition (i.e. absorbed dose) is restricted primarily to those the cells that take up boron.

One of the problems currently facing BNCT is the need to attain a suitable boron-10 concentration in tumor cells while minimizing the isotope's concentration in healthy cells. Additionally, the current drug delivery molecule, boronophenylalanine-fructose (BPA-F), a boronated amino acid analogue, allows tumor regeneration to occur. One of the reasons postulated for tumor regeneration is that the diffusion of BPA-F is limited to only well-vascularized regions of tumor, leaving some portions of the tumor cell population boron-free. A necessary requirement for effective BNCT is to target less well-vascularized areas within the tumor, such as hypoxic regions, by developing a new boron delivery compound.

