

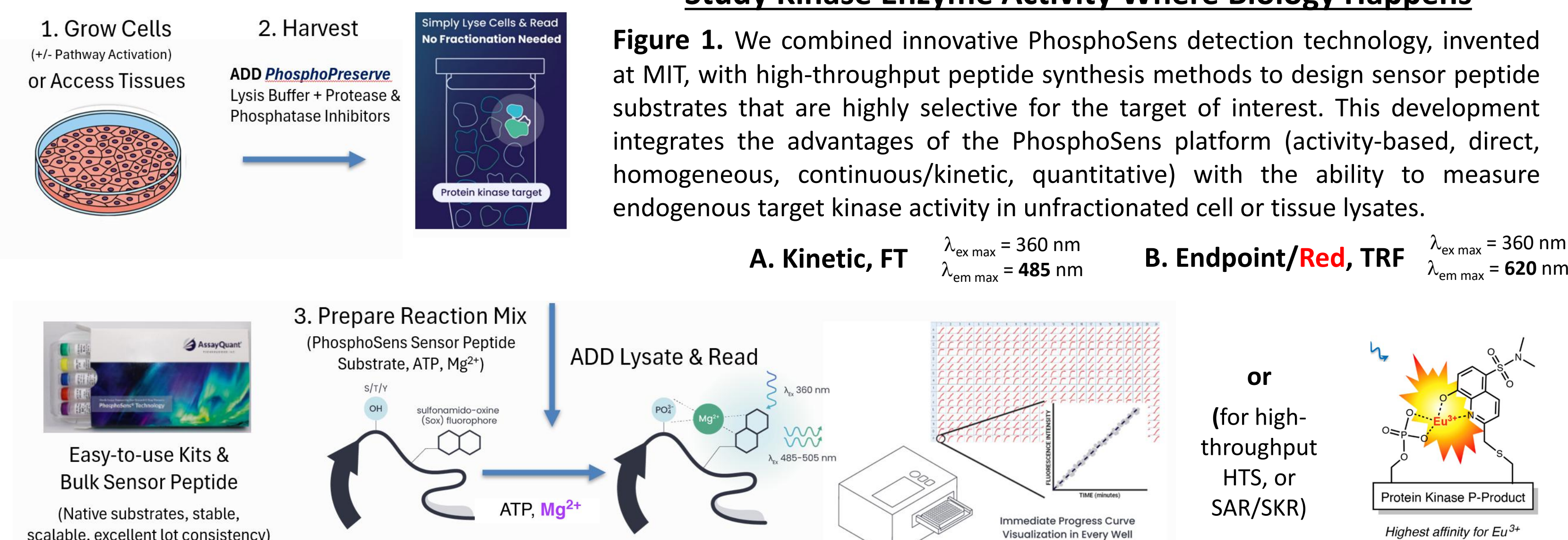


Erik Schaefer¹, Susan Cornell-Kennon¹, Venkatesh Nemmara¹, Khanh huynh¹, Daniel Urul¹, Ashleigh Homon-Rahall¹, Nicole Tompkins¹, Eric Berg¹, and Barbara Imperiali^{1,2}. ¹AssayQuant Technologies, Inc. • 260 Cedar Hill Street • Marlborough, MA 01752 • USA • www.assayquant.com; erik.schaefer@assayquant.com; ²Departments of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge, MA. Program supported by an NIH/NCI Phase II SBIR (ES is PI).

ABSTRACT & INTRODUCTION

The kinome features 538 protein kinases implicated in ~400 diseases. These enzymes form complex signaling networks that are regulated both positively and negatively. By combining high-throughput sensor peptide synthesis and screening with on- and off-target assays, we developed selective sensor peptide substrates to directly quantify the activity of 21 kinases in unfractionated cell lysates. We will demonstrate this method's utility for measuring pathway regulation, inhibitor potency, and as functional biomarkers. Our initial development has successfully targeted kinase activity for AKT1-3, GSK3 α/β , DNA-PK, CDK1-3, 5, c-Kit D816V, multiple MAPKs, including pro-growth (ERK1/2) and stress (JNK, p38) pathways, and ZAP70. We used these assays to measure kinase activity in lysates from multiple cell models and in response to different treatments.

The PhosphoSens® Lysate Kinase Activity Assay Workflow



RESULTS & DISCUSSION: MAP Kinase (MAPK) Pathways Cell Lysate Activity Assays

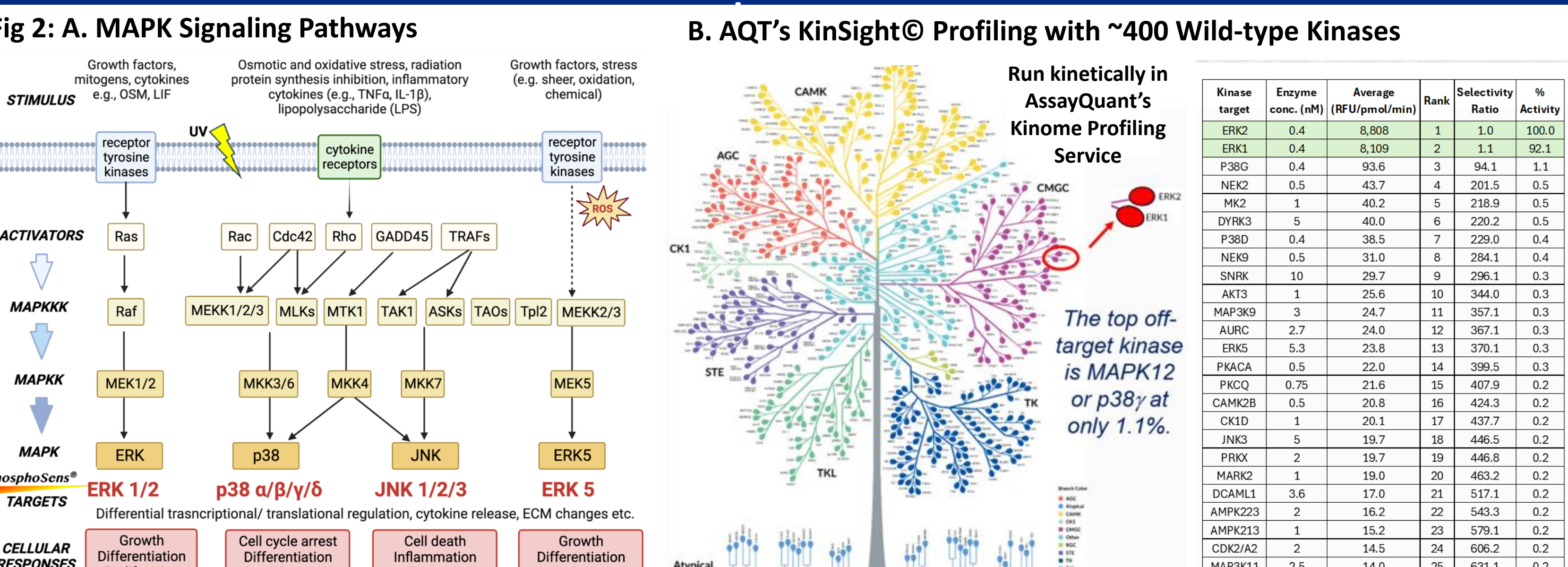


Figure 2-A. MAPKs execute major cellular signaling pathways (Figure adapted from Tomida, 2015). The most actively studied MAPKs are associated with four pathways: 1) Extracellular-signal-regulated kinase 1 and 2 (ERK1/2), 2) c-Jun N-terminal kinase (JNK1-3), 3) p38 $\alpha/\beta/\gamma$, and 4) ERK5, where different stimuli activate these individual MAPK subfamilies; mutations and copy number variations identified in human cancers are promising drug targets. Although pTyr phosphorylation is often measured with phospho-specific antibodies as a surrogate for activation, this does not always correlate with activity, underscoring the urgent need for quantitative activity-based assays compatible with cell lysates and tissue homogenates. **B. AQT's KinSight® profiling results** with >400 wild-type kinases highlight the exquisite selectivity of the AQT1076 sensor peptide for ERK1/2. **C. Lysate quantitation:** AQT1076 enables sensitive quantitation of ERK1/2 activity in crude lysates from NIH3T3 cells $\pm$ PDGF. The signal is eliminated with the selective ERK1/2 inhibitor (SCH722984) or heat inactivation of the lysate. **D. AQT1107 Control versus AQT1076 + purified ERK2:** The ERK2 protein (0.4 nM) fully phosphorylates the AQT1076 sensor peptide substrate (15 µM) by 240 minutes, with the signal converging with that obtained with the AQT1107 phosphopeptide positive control (15 µM). AQT1107 allows for absolute quantitation (moles of product/min) for comparison.

Figure 3-A. ERK1/2 Activity in Crude Cell Lysates from Multiple Cell Lines with the AQT1076 Sensor Peptide

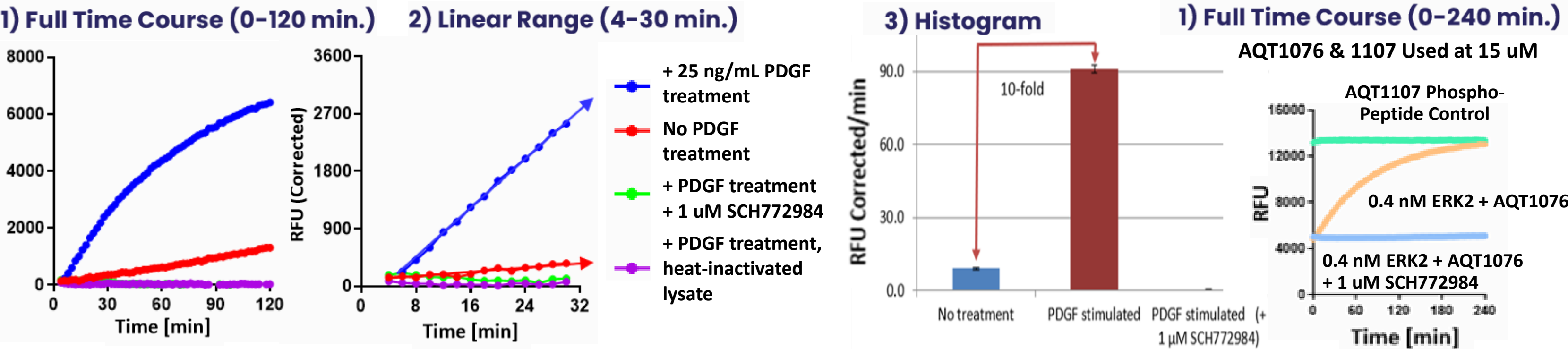


Figure 3-C. Titration of HeLa cells (5K to 160K cells/well) grown for 48 hours, serum-starved overnight, then treated with +/- anisomycin (stress stimulus). JNK activity increased up to 22-fold, with strong induction regardless of cell density, and was completely inhibited by the JNK-IN-8 inhibitor (not shown). p38 activity increased 6.2-fold at the lowest cell density but became increasingly constitutively activated at higher cell densities, highlighting differential regulation of these MAPK stress pathways in HeLa cells.

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