Peptide microarrays for detailed, high-throughput substrate identification, kinetic characterization, and inhibition studies on protein kinase A

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Study Design
A PamChip® containing 140 serine/threonine peptides was used for real-time detection of protein kinase A (PKA) for substrate identification and kinetic characterization of PKA (figure 1). Figure 2 shows a time course of peptide phosphorylation over 50 minutes. In this assay the IC50 of three PKA inhibitors, AMP–PNP, staurosporin, and PKA inhibitor peptide, was investigated as a function of ATP conc. (0.5 – 4.0 times Km) and CREB peptide conc. (0.22 -3.3 times Km) (table 1).

Key Findings
In comparison to singleplex kinase activity assays, data quality was high, variation in assay conditions and reagent consumption were reduced considerably. PKA was shown to phosphorylate many peptides containing known PKA phosphorylation sites as well as some new substrates. Data in table 1 shows that staurosporine is a full ATP competitive inhibitor whereas AMP-PNP has a different inhibition mechanism.

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The kinetic readout gives high-quality data; the simultaneous incubation eliminates differences in medium composition and experimental conditions.

Background
As a first step toward understanding the behavior of kinase inhibitors in a cellular context, and ultimately in vivo, the effect on the target kinase is usually studied in vitro. Using standard kinase assays, such an approach is laborious and time-consuming, whereas the use of a peptide microarray makes such investigations feasible in a short time frame while using very small amounts of reagents such as recombinant expressed kinases.

Conclusion
PamChip® STK peptide arrays allow profiling of PKA and are used in potency determination of PKA inhibitors as function of peptide substrate. STK arrays allow identification of peptide substrates for STK kinases that are still uncharacterized.

References: