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Absolute Quantification of Yeast Kinases by LC-MS/MS using QconCAT and MRM Technologies

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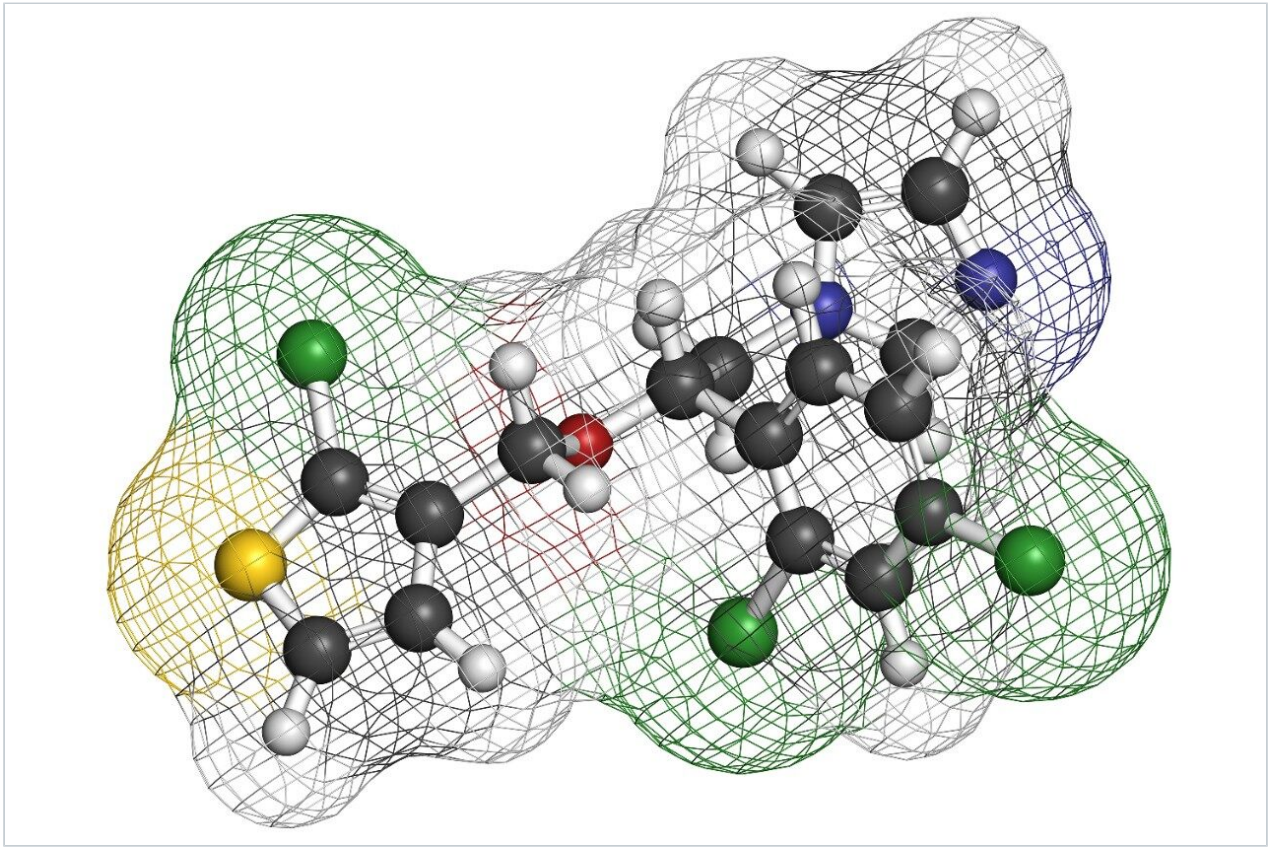

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Abstract

This application note presents the application of label-mediated targeted mass spectrometry to quantify a range of kinases from yeast, spanning a five-order dynamic range.

Benefits

Nanoscale separations are combined with quantitative MRM mass spectrometry to accurately determine absolute yeast protein amounts over a wide dynamic range using isotopically labelled standards. More peptides and proteins are more easily quantified and data analysis is more straightforward using elevated analyzer resolution settings and a high sensitivity triple quadrupole mass spectrometer.

Introduction

Absolute protein quantification by LC-MS/MS is an important tool in assay development and creating data for systems modeling. Enabling predictive biology is one of the primary goals of many system biology studies, achieving detailed knowledge of the cellular constituents, their quantities, dynamics, and interactions. This information can be subsequently embedded in mathematical models that permit simulation of cellular state changes, testable by experiment, and leading to biological process definitions.[1] However, this requires accurate baseline values for the cellular quantities of proteins. The large dynamic range of a proteome is the most challenging barrier to LC-MS/MS-based protein quantification.

This application note presents the application of label-mediated targeted mass spectrometry to quantify a range of kinases from yeast, spanning a five-order dynamic range. QconCAT[2] technology was used to create isotopically-labeled internal standard peptides for 138 target proteins and quantification was performed by time-scheduled MRM tandem quadrupole mass spectrometry, investigating the sensitivity of different platforms, assay specificity, and quantitation dynamic range.

Experimental

Sample preparation

Several yeast kinase QconCATs were designed to contain two isotopically labelled peptides for each of the targeted proteins and tryptically codigested with a native yeast strain as shown in Figures 1 and 2, respectively. Figure 1 illustrates the QconCAT principle whereby a synthetic gene is designed to encode proteotypic peptides of the sample protein mixture. Summarized in Figure 2 are the design of a quantitation concatamer and the high-throughput MRM quantitation workflow. Yeast kinases, as shown in Figure 3, span the complete yeast abundance distribution range in terms of number of copies/cell.

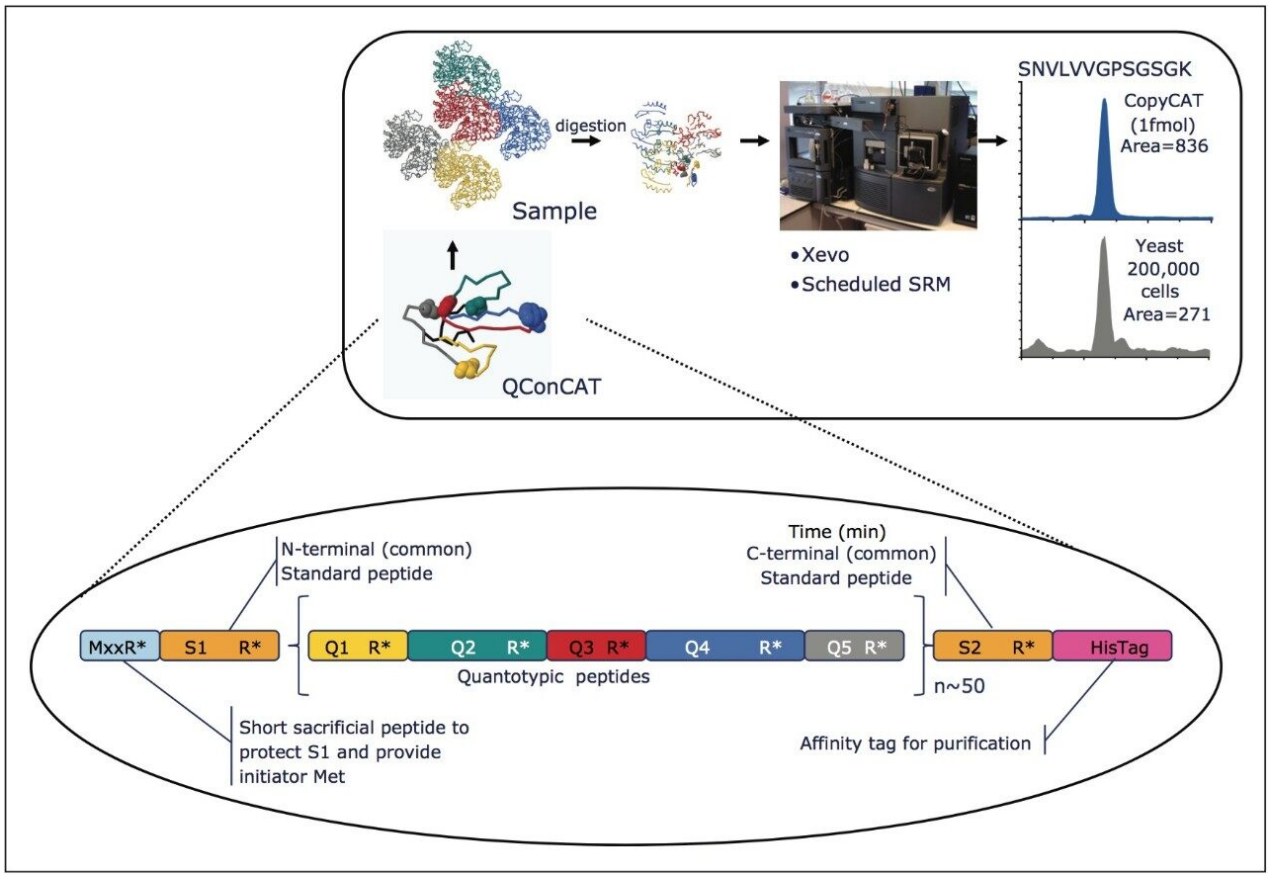

Figure 1. The principle of QconCAT technology.

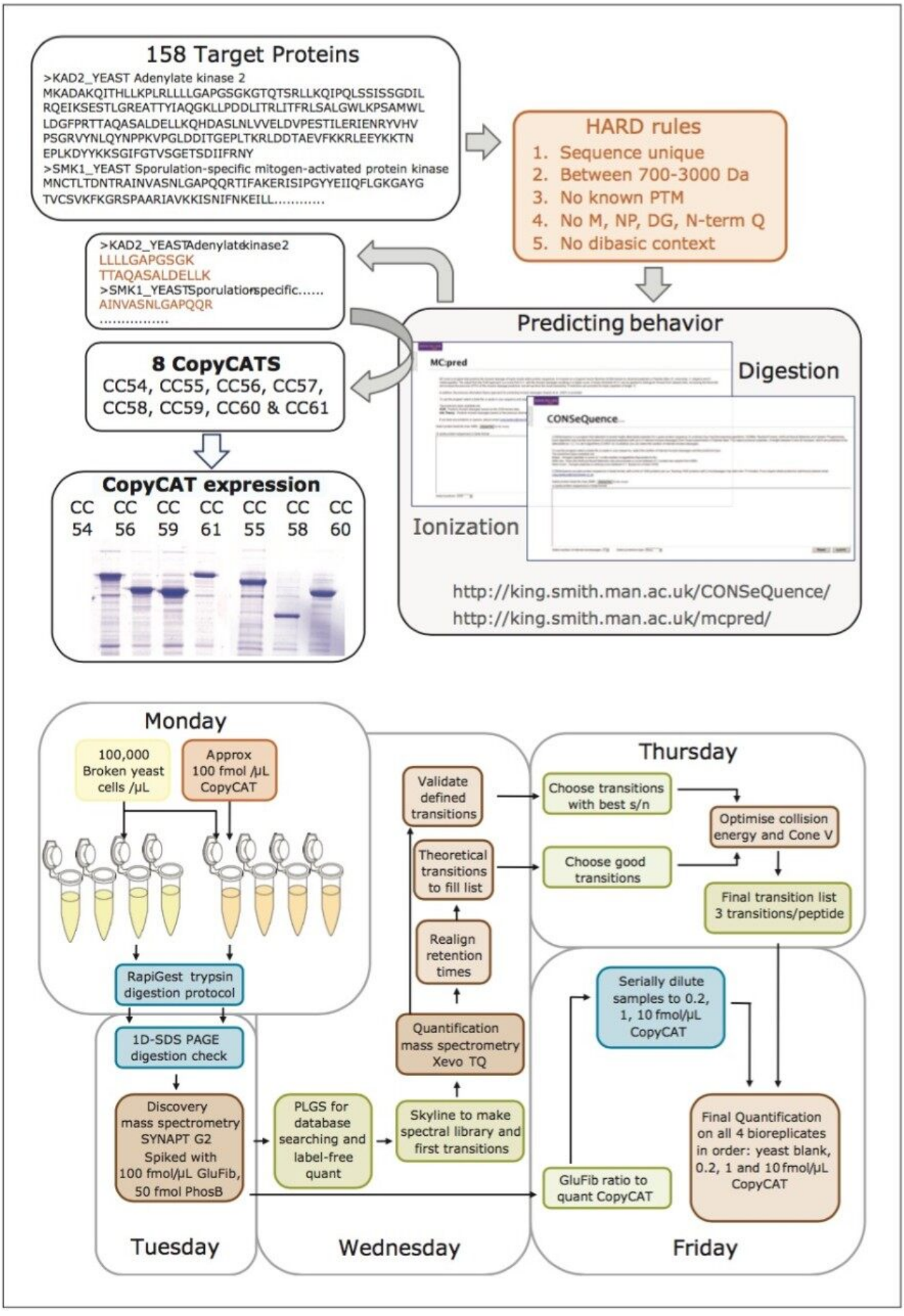

Figure 2. Design of a quantitation concatamer (QconCAT) and high-throughput MRM quantitation workflow.

LC-MS/MS conditions

- Equivalent mammalian range would be to 1000 to 35,000,000 CPC

- Deciding factor in quantification success is peptide choice
  - Still unpredictable parameters
- If predicting quantotypic peptides, use multiple peptides to infer protein abundance (at least N=3)
  - Many ways for a peptide to produce incorrect protein quantification
- Xevo TQ-S shows a significant increase in performance over Xevo TQ
  - More and better quantifications, more straightforward data analysis

References

1. Brownridge P et al. Global absolute quantification of a proteome: Challenges in the deployment of a QconCAT strategy. Brownridge P *et al*. *Proteomics*. 2011 Aug;11(15):2957-70.
2. Beynon RJ, Doherty MK, Pratt JM, Gaskell SJ. Multiplexed absolute quantification in proteomics using artificial QCAT proteins of concatenated signature peptides. *Nat Methods*. 2005 Aug;2(8):587-9.
3. Giles K and Gordon D. A new conjoined RF ion guide for enhanced ion transmission. ASMS 2010 poster (described in Travelling Wave ion mobility, *Int J Ion Mobility Spectrom*, 2013; 16(1):1-3.
4. MacLean B *et al*. Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics*. 2010 Apr 1;26(7):966-8.
5. Reiter L *et al*. mProphet: automated data processing and statistical validation for large-scale SRM experiments. *Nat Methods*. 2011 May;8(5):430-5.

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