**ProteoMMX 4.0 Abstract**

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**Title:**  The Leaky-TAP: Does epitope-tagging perturb protein concentrations in quantitative proteomics?

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**Introduction (max 100 words):**

Epitope tagging, where a tag is fused to a protein of interest to support immunoprecipitation via a monoclonal antibody has become widely used in proteomics, supporting genome-wide studies of protein location, interactions and quantification. However, no systematic study has assessed whether such a non-endogenous protein tag has a marked effect on the cellular concentration of the tagged protein or any off-target effects on any other proteome components. We address this by comparing TAP-based quantitative Western analyses with mass spectrometry based methods using a targetted SRM-SILAC approach for a subset of selected yeast proteins, and SILAC to compare tagged/untagged total proteomes.

**Methods (max 150 words):**

We targeted 21 yeast proteins for analysis, representative of protein classes which could have major effects on cellular proteome concentration including 7 chaperones, 7 translation factors and 7 transcription factors. TAP strains were obtained from the standard collection, cultured in quadruplicate in YPD at 30oC prior to protein extraction and quantitative analysis using Protein A from *Staphylococcus aureus* (SIGMA UK) as a standard. In parallel, untagged endogenous yeast was grown in heavy-labelled medium prior to extraction and targeted SRM analysis in combination with the tagged strain on a XevoTM TQS triple quad MS (Waters). Finally, a SILAC experiment on each tagged/wild type yeast pair was carried out on the Q-Exactive(?) to quantify any changes in the global proteome induced by tags, processing data via Maxquant and MSStats.

**Results (max 200 words):**

We compared our quantitative western blots with the previously published “gold-standard” undertaken by Ghaemmghammi and colleagues (*Nature* **425**, 737-741), which we attempted to replicate as a control. Good agreement between our protein abundance estimates was observed, (correl?) as well as with an independent determination we conducted using QconCATs and SRM. (we see good agreement). SRM SILAC shows most proteins are not affected at the local level, however we see larger changes at the global proteome level. Individual points to note – CCT4 is a large perturbation, TFs were generally hard to spot (low abundance). Cell volume changes

TEF1/2 curiosity.

**Significance (max 50 words):**

TAP tagging generally has a low, relative effect on protein level order within proteomes but care should be taken for absolute.

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