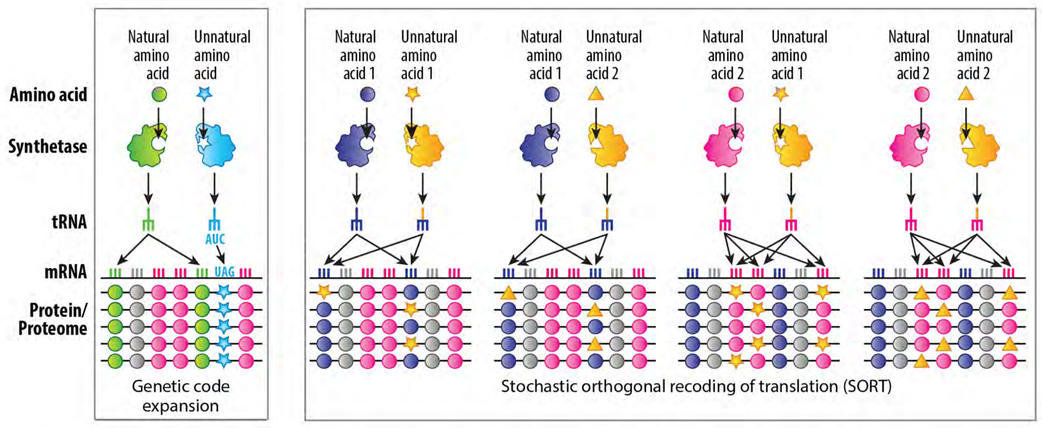
Proteome labeling and protein identification in specific tissues and at specific developmental stages in an animal

T S Elliott[1,4], F M Townsley[1,4], A Bianco[1,4], R J Ernst[1], A Sachdeva[1], S J Elsässer[1], L Davis[1], K Lang[1], R Pisa[1,2], S Greiss[1], K S Lilley[3], J W Chin[1,2]

[1] Medical Research Council Laboratory of Molecular Biology, Cambridge, England, UK. [2] Department of Chemistry, University of Cambridge, Cambridge, England, UK.

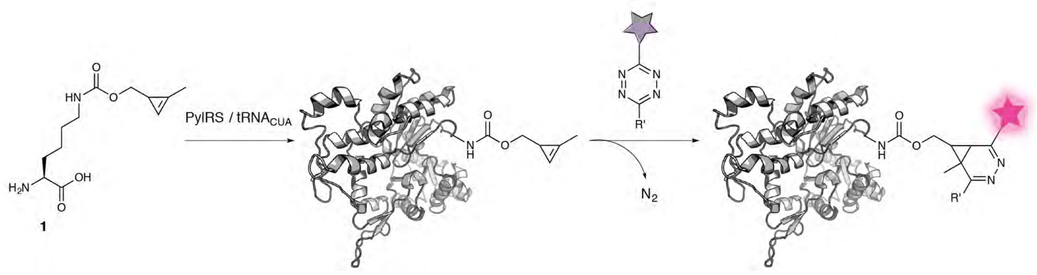
[3] Department of Biochemistry, University of Cambridge, Cambridge, England, UK. 4These authors contributed equally to this work.

1



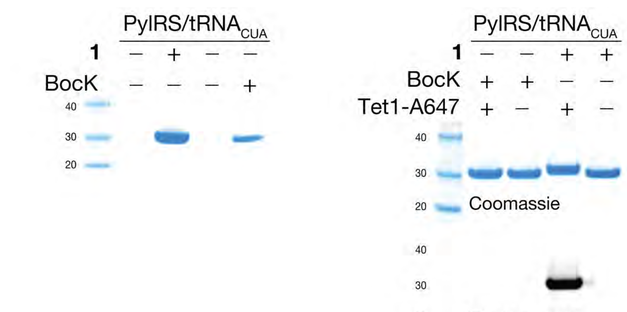
Genetic code expansion using an orthogonal tRNA/Aminoacyl tRNA Synthetase pair (left panel) and Stochastic orthogonal recoding of translation (SORT)

2



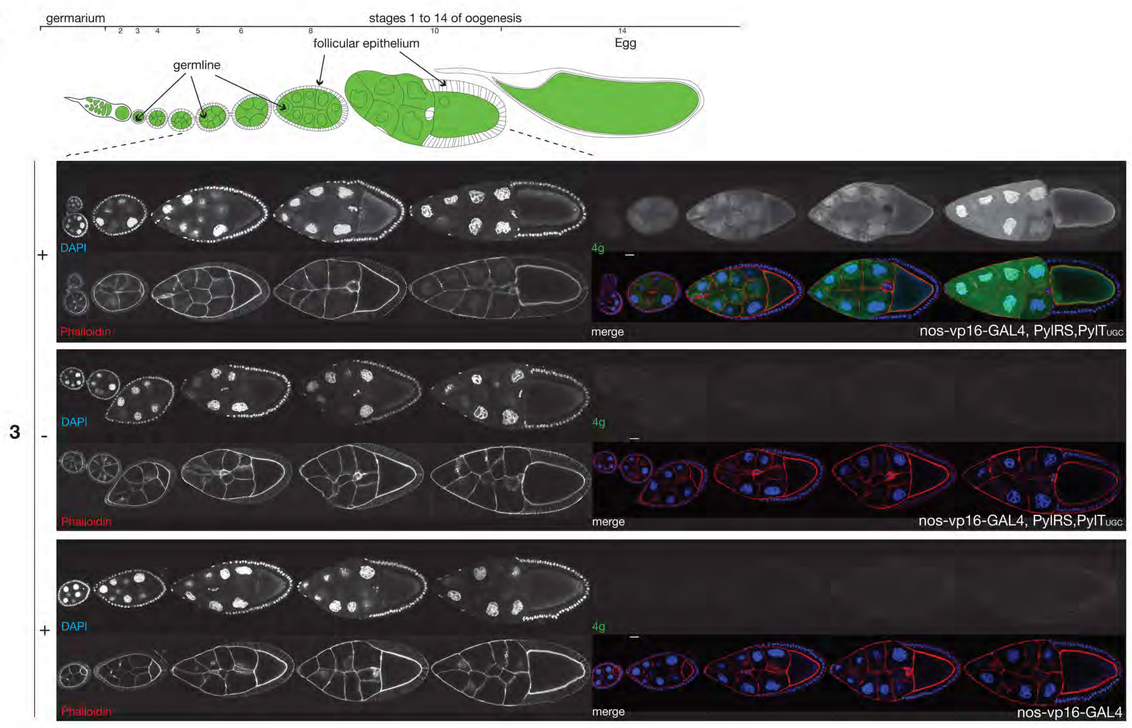
The inverse electron demand Diels-Alder reaction between 1,4-disubstituted tetrazines and a strained alkene enables rapid bio-orthogonal labelling of proteins.

3



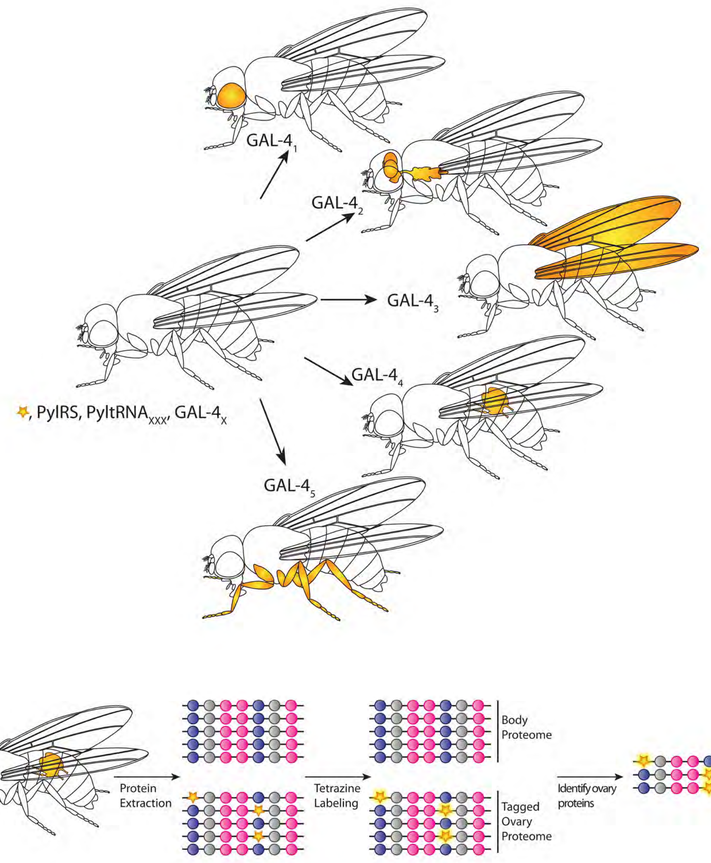
(Top Panel) Site specific incorporation of **1** into proteins in response to an amber codon.
(Bottom panel) Proteome labeling in response to diverse sense codons via SORT-M in *E. coli* (bottom panel). Tet1-A647 is a tetrazine conjugated to the fluorescent dye Alexa647. BocK is a PylRS substrate.

4



SORT-M enables selective imaging of proteins synthesized within the germ cells of the fly ovary from stage 5 onwards.

5



(Top panel) Tissue specific and developmental stage specific tagging of proteomes in *Drosophila melanogaster* using SORT. The GAL-4 expression system allows control of PylRS expression in specific tissues and at specific developmental stages.
(Bottom panel) A fly expressing PyltRNA$_{UGC}$ (U6 promoter), PylRS (under GAL-4 control) is fed normal food ,supplemented with unnatural amino acid (yellow star, **1**). The unnatural amino acid is incorporated into the proteome, only in tissues where GAL-4 is active (yellow ovary). This allows for identification of ovary proteins without dissection

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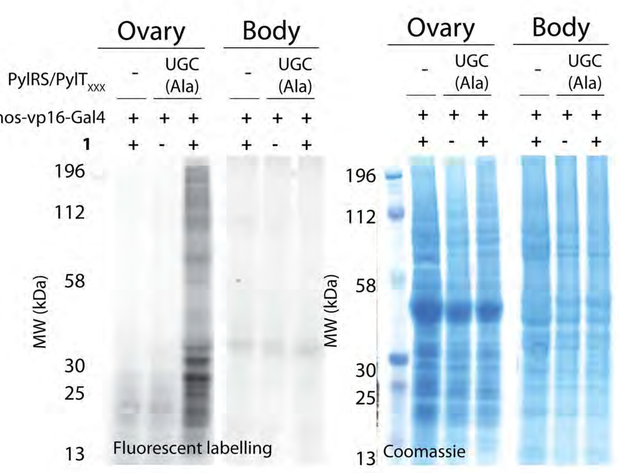
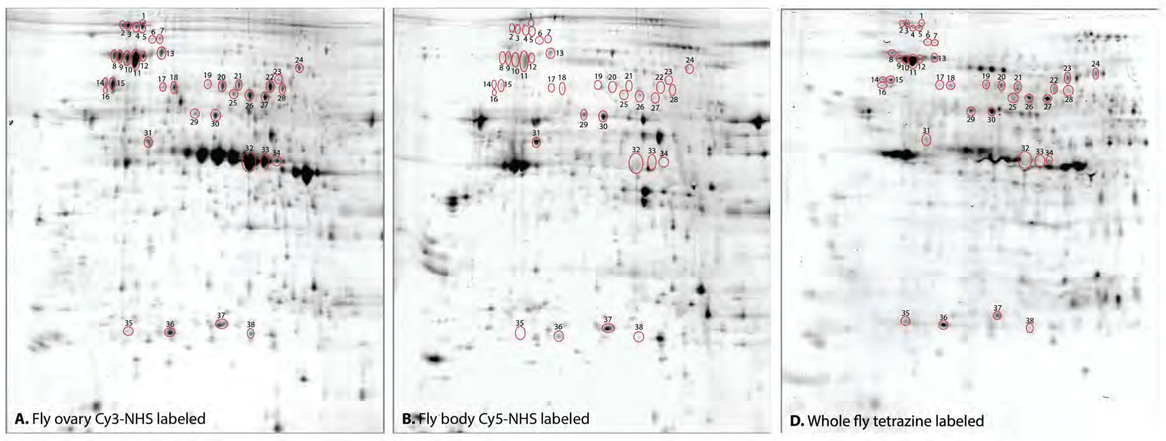
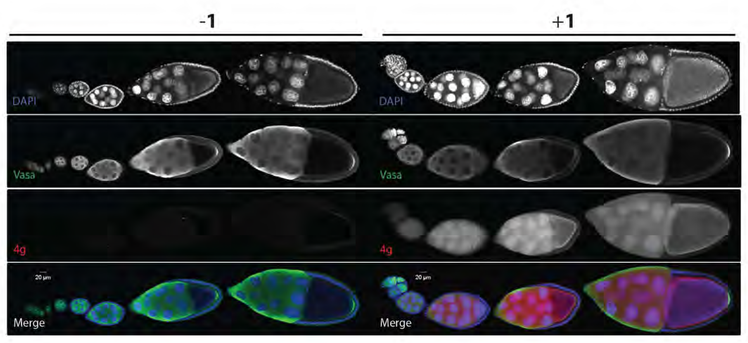


Tissue specific proteome labelling with SORT-M demostrated for the fly ovary.

7





Tissue- and developmental stage–specific proteome labeling. Proteins identified by mass spec from 2D gel electrophoresis are validated *in vivo* via immunostaining.

References

Proteome labeling and protein identification in specific tissues and at specific developmental stages in an animal.
Elliott TS, Townsley FM, Bianco A, Ernst RJ, Sachdeva A, Elsässer SJ, Davis L, Lang K, Pisa R, Greiss S, Lilley KS, Chin JW. *Nat Biotechnol.* 2014 May;32(5):465-72.

Genetic code expansion and bioorthogonal labelling enables cell specific proteomics in an animal.
Elliott TS, Bianco A, Chin JW. *Curr Opin Chem Biol.* 2014 Aug;21C:154-160.

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