Site Specific Genetic Incorporation of Azidophenylalanine in *Schizosaccharomyces pombe*

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The diversity of protein functions is impacted in significant part by the chemical properties of the twenty amino acids, which are used as building blocks for nearly all proteins. The ability to incorporate unnatural amino acids (UAAs) into proteins in a site specific manner can vastly expand the repertoire of protein functions and also allows detailed analysis of protein function. In recent years UAAs have been incorporated in a site-specific manner into proteins in a number of organisms. In nearly all cases, the amber codon is used as a sense codon, and an orthogonal tRNA/aminoacyl-tRNA synthetase (RS) pair is used to generate amber suppressing tRNAs charged with the UAA. In this work, we have developed tools to incorporate the cross-linking amino acid azido-phenylalanine (AzF) through the use of bacterial tRNA Tyr and a modified version of TyrRS, AzFRS, in *Schizosaccharomyces pombe*, which is an attractive model organism for the study of cell behavior and function. We have incorporated AzF into three different proteins. We show that the majority of AzF is modified to amino-phenyl alanine, but protein cross-linking was still observed. These studies set the stage for exploitation of this new technology for the analysis of *S. pombe* proteins.

Twenty canonical amino acids are used as building blocks to construct proteins in all organisms. The sequences of amino acids and their chemical properties give rise to the large array of activities encoded in proteins. Interestingly, some non-canonical amino acids, like pyrrolysine, selenocysteine, exist in living systems and are incorporated into proteins through endogenous translation systems¹⁻³. Large arrays of unnatural amino acids have been developed through chemical approaches. Incorporation of these amino acids at precise locations in proteins should lead to novel ways of studying protein function as well as improved biotechnological applications.

Addition of new building blocks to the natural amino acids requires a unique codon which has not been assigned to any natural amino acid⁴. In addition, an orthogonal tRNA/aminoacyl-tRNA synthetase pair, which does not cross-react with any endogenous translation components has to be introduced into host cells to incorporate the unnatural amino acid(s) at specified locations⁴⁻⁶. Genetic code expansion and protein functional studies have been achieved in many organisms and was first described in *Escherichia coli⁷,⁸*. Now more than 80 unnatural amino acids with novel chemical, physical, and biological properties⁸ have been successfully incorporated into eukaryotic model organisms or cell lines in response to the amber codon TAG, including in *Saccharomyces cerevisiae⁹*, *Candida albicans¹⁰*, *Caenorhabditis elegans¹¹*, mammalian somatic cells and stem cells¹²,¹³.

Fission yeast *Schizosaccharomyces pombe* is an attractive model for understanding molecular mechanisms governing cell growth, division, and polarity. *S. pombe* is amenable to experimental manipulation.
using methods of genetics, molecular biology, biochemistry, genomics, and high resolution imaging. To expand the repertoire of technologies that can be used in fission yeast to study protein function, we sought to develop experimental approaches to expand the fission yeast genetic code and thereby to facilitate genetic incorporation of unnatural amino acids at specified locations into fission yeast proteins.

In this study, we expanded the genetic code of S. pombe. We have engineered S. pombe cells with an orthogonal E. coli tyrosyl-tRNA synthetase (TyrRS)/E. coli tRNA Tyr\textsuperscript{E} pair, enabling site specific incorporation of natural and unnatural amino acids through amber codon suppression. We show that the cross-linking nitrene generating amino acid azido-phenylalanine (AzF) can be introduced into three different proteins in fission yeast. We also show the ability of glutathione S-transferase containing AzF at position 52 (the dimerization site) to generate covalently linked multimers upon UV exposure. Our studies introduce a new and powerful approach to study function of fission yeast proteins.

Results
Expression and functionality of the orthogonal pair in S. pombe. Incorporation of the unnatural amino acids at a specified location within a protein of interest is achieved by the availability of 3 elements: 1. a codon that does not encode any natural amino acid and can encode the unnatural amino acid when present in the mRNA. 2. a tRNA that base-pairs with this codon and 3. an aminoacyl-tRNA synthetase that charges the tRNA with the unnatural amino acid\textsuperscript{9}. It is also important that the tRNA and the tRNA synthetase are orthogonal. The amber codon (UAG) has been developed as a sense codon for the incorporation of several unnatural amino acids into proteins in recent years. It has been established that the tRNA for tyrosine (tRNA Tyr\textsuperscript{E}) from Escherichia coli or Bacillus stearothermophilus and the tyrosyl-tRNA synthetase (TyrRS) from E. coli function as an orthogonal pair in many eukaryotes\textsuperscript{12,14}. Furthermore, mutant versions of E. coli/B. stearothermophilus (tRNA Tyr\textsuperscript{E}) with mutations in the anti-codon loop that base-pair with amber codon and aaRS that can charge these tRNAs with tyrosine or UAs have been generated\textsuperscript{9}. We therefore chose to use this orthogonal tRNA Tyr\textsuperscript{E} and TyrRS pair for genetic code expansion studies in fission yeast.

To expand the genetic code of S. pombe, we first designed a system to express tRNAs. It is known that an S. pombe tRNA\textsuperscript{E} is co-transcribed with tRNA Met\textsuperscript{S} (Fig. 1A Construct 1). Previous work has also led to the identification of sup3–5 as an opal suppressing tRNA Met\textsuperscript{S}, which is known to suppress the adenine auxotrophic mutant ade\textsuperscript{6–704} (Fig. 1A Construct 2)\textsuperscript{7,18}. We designed two DNA constructs in which the tRNA Met\textsuperscript{S} was replaced with tRNA Tyr\textsuperscript{E} from B. stearothermophilus (Fig. 1A Construct 3) or E. coli (Fig. 1A Construct 4). All DNA constructs described in Fig. 1A constructs 2, 3, and 4 also carried the marker gene his\textsuperscript{5}, into which an amber codon had been inserted in place of the codon for Tyr at position 63. The three DNA constructs were introduced into an ade\textsuperscript{6–704} his\textsuperscript{5}Δ strain also expressing E. coli TyrRS (Fig. 1B Strain 1) and selected for growth on medium lacking adenine. Transformants thus obtained were tested for their ability to grow on medium lacking histidine. We found that expressing B. stearothermophilus or E. coli tRNA Tyr\textsuperscript{E}, but not strains expressing S. pombe tRNA Met\textsuperscript{S} were capable of growth on medium lacking histidine (Fig. 1C). The suppression efficiency for the heterologous pair, B. stearothermophilus tRNA Tyr\textsuperscript{E} and E. coli TyrRS was significantly lower than the suppression by E. coli tRNA Tyr\textsuperscript{E} and E. coli TyrRS\textsuperscript{12,19,20}. These observations established that the bacterial tRNA Tyr\textsuperscript{E}·TyrRS functioned as an orthogonal pair in fission yeast where they functioned as an amber suppressor. We chose to work with E. coli tRNA Tyr\textsuperscript{E} in the rest of the study.

To incorporate unnatural amino acids through the expanded genetic code, we employed the mutated E. coli TyrRS designated as E. coli AzF, capable of charging E. coli tRNA Tyr\textsuperscript{E} with unnatural amino acid azido-phenylalanine (AzF)\textsuperscript{9,10}. In this experiment, the construct (Fig. 1A Construct 4) carrying the marker for yeast transformation (sup3–5) and expressing E. coli tRNA Tyr\textsuperscript{E} carrying CUA in the anti-codon position was introduced into ade\textsuperscript{6–704} his\textsuperscript{5}Δ fission yeast strains expressing either E. coli TyrRS (Fig. 1B Strain 1) or E. coli AzF (Fig. 1B Strain 2). The construct also carried a his\textsuperscript{5} selectable marker into which a UAG stop codon was introduced. Note that all transformants grew on medium lacking adenine, suggesting the existence of plasmids in the strains (Fig. 1D). The suppression of the UAG codon in his\textsuperscript{5}, and the resulting growth on the medium lacking histidine, was observed when cells expressed E. coli tRNA Tyr\textsuperscript{E} and E. coli TyrRS, but not in cells carrying the E. coli AzF and E. coli tRNA Tyr\textsuperscript{E} (Fig. 1D). Importantly, cells expressing E. coli AzF and E. coli tRNA Tyr\textsuperscript{E} were able to grow only on medium lacking histidine but supplemented with AzF, confirming that AzF was genetically incorporated in a mechanism in which the UAG stop codon was read by the translation machinery as a codon for AzF (Fig. 1D).

Optimization of suppression of E. coli tyrosine tRNA pair in fission yeast His\textsuperscript{5}p. Effective application of unnatural amino acids to study proteins functions in fission yeast cells is often challenging due to inefficient non-sense suppression and unnatural amino acid incorporation. Since an increase in the expression of the desired mRNA using a strong promoter led to a more robust suppression in budding yeast, we tested if this effect could be recapitulated in fission yeast\textsuperscript{21,22}. In this experiment, we designed one new construct in which the expression of the gene of interest is driven by the strong inducible nmt1-mini promoter (Singh et al., unpublished observations), which contains 400 bp of nmt1 promoter (Fig. 2A Construct 3). The gene we tested was a his\textsuperscript{5} mutant with an amber codon in place of the codon for Tyr63. Both E. coli tRNA Tyr\textsuperscript{E} and targeted gene his\textsuperscript{5} are encoded on a pNO13 plasmid (Fig. 2A). Spot
assays showed that all transformants grew on medium lacking adenine (Fig. 2B). Suppression of the UAG stop codon in his5 and thereby growth on medium lacking histidine was observed when cells expressed E. coli TyrRS and E. coli tRNATyr (Fig. 2B row a2 and a3), and in cells carrying the E. coli tRNA\textsuperscript{59} and sup3-5. However, suppression was observed in AzFRS expressing cells only when AzF was supplemented in the medium. As expected, suppression was not observed in cells expressing E. coli TyrRS or E. coli AzFRS in the absence of E. coli tRNATyr (Fig. 2B row a1 and b1). Importantly, suppression of the UAG stop codon in his5 is stronger when his5 expression was under control of the nmt1-mini promoter (Fig. 2B row a3 and b3), compared to the native his5 promoter (Fig. 2B row a2 and b2). Through these experiments we established that overexpression of the mRNA of interest (carrying the UAG codon) increased the efficiency of growth in medium lacking histidine, potentially due to the increase in the overall level of the suppressible mRNA.

Visualization of the suppression of E. coli tyrosine tRNA pair in vivo and in vitro. We next investigated the ability of tRNA\textsuperscript{59}, TyrRS and AzFRS to suppress amber codon mutations in mRNA encoding a synthetic peptide fused between myosin II light chain (Rlc1p) and GFP and in mRNA encoding Glutathione-S-Transferase (GST) in place of the codon for Gly at position 6 (Fig. 3A).

To investigate suppression of amber stop codon in vivo, a DNA molecule in which one amber stop codon TAG was placed between rlc1 and GFP was introduced into an ade6–704 strain expressing either TyrRS or AzFRS and GFP fluorescence was monitored. In the strain expressing TyrRS, GFP fluorescence in the actomyosin ring was observed in the presence or in the absence of AzF in the medium. However, in the strain expressing the AzFRS, GFP fluorescence was detected in the ring only when cells were grown on medium containing AzF (Fig. 3B).
the strain expressing TyrRS in the absence of AzF was not significantly different from the growth in the presence of AzF.

Next we checked suppression when a UAG codon was introduced into RNA encoding a third reporter protein, Glutathione-S-Transferase, GST. Again, suppression was observed in the presence and absence of AzF when TyrRS was expressed. However, full length GST was not detected in the cells expressing AzFRS when cultured in the absence of AzF, but was present when cells were grown in the presence of AzF (Fig. 3D). Presently, we do not understand why the suppression is reduced in the presence of AzF when TyrRS and tRNA Tyr are used. It is possible that AzF and Tyr may compete for a similar binding site on TyrRS, which might affect the incorporation of Tyr in the presence of AzF, notwithstanding the fact that tRNA Tyr can only be charged with tyrosine. Collectively, our studies have shown efficient non-sense suppression within 3 mRNAs and also that the unnatural amino acid AzF can be introduced in a site-specific manner into fission yeast proteins.

Azido-phenylalanine incorporation in response to an amber stop codon. To further verify site-specific incorporation of AzF into the protein of interest and to determine its occupancy at the amber position (position 6) rather than any other amino acid, the GST protein purified from cells expressing TyrRS, GST (G6Y), and from cells expressing AzFRS, GST (G6AzF), were analyzed by nano LC-ESI-MS/MS after digestion with trypsin. The GST peptide (SPILYWK) that incorporates tyrosine at position 6, was readily identified by mass spectrometry from cells expressing TyrRS as a 2+ ion of 535.29 m/z (Fig. 4 top). Curiously, despite the AzF-dependent suppression of the UAG codon in GST protein upon expression of AzFRS and tRNA Tyr are used, we were unable to detect the expected mass increase of 131 Da from the wild type GST peptide SPILYWK (Supplemental Fig. 1A) that would represent incorporation of AzF. However, we identified a 2+ ion of 534.80 m/z that fragmented to match peptide SPILYFKW with the F modified by an additional 15 Da of mass. The position of the F +15 Da modification is supported by y ions 4, 5, 6, 7 (Fig. 4 bottom). It is known that AzF can be reduced to p-amino-L-phenylalanine (AmF) during sample preparation.9,10,23. We suggest that AzF at position 6 was reduced to AmF, which would be consistent with the molecular weight observed (Supplemental Fig. 1B). Taken together, we conclude that AzF is site specifically incorporated in response to an amber codon into S. pombe proteins and is readily converted into AmF.

Covalent cross-linking in GST upon incorporation of AzF. Azido-phenylalanine is a nitrene generating cross-linking amino acid, whose reactive nitrenes can be generated by exposure of the protein with ultraviolet light. Although the bulk of AzF appeared to be converted to AmF, we reasoned that a
fraction of AzF might still be present in the mixture and might be able to covalently cross-link with interacting proteins. To develop this system further, we generated GST with a UAG codon in place of the codon for Phe52, a site known to be involved in GST-dimerization. UAG codon suppression was achieved upon expression of TyrRS and AzFRS, in which case suppression required the presence of AzF in the medium (Fig. 5A). Upon exposure to UV, GST carrying AzF at Phe52, but not GST carrying tyrosine at Phe52, was converted into covalently linked multimers that ran slower on SDS-PAGE (Fig. 5B). These experiments established that despite the conversion of the majority of AzF to AmF a fraction of GST may contain AzF which aids in UV cross-linking.

Discussion
In this study, we have developed the tools to introduce the unnatural amino acid AzF in a site-specific manner into Schizosaccharomyces pombe proteins. We have achieved this by the generation of S. pombe strains expressing orthogonal pairs of bacterial tRNA (that base pair with the UAG non-sense codon) and aminoacyl-tRNA synthetases (for natural or unnatural amino acids). Using this approach we have succeeded in introducing tyrosine and the nitrene-generating unnatural amino acid, azido-phenyl alanine.

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Figure 3. Detection of unnatural amino acid incorporation by Western blot and fluorescence imaging. (A) Schematic representation of mutation sites in Rlc1p-GFP and Glutathion-S-transferase (GST). (B) Top: S. pombe cells expressing E. coli TyrRS and Rlc1p-TAG-GFP from a plasmid (MBY 8965) were grown at 30°C in the presence or absence of 1 mM azido-phenylalanine to exponential growth phase and live cells were imaged using Andor Spinning Disk. 5 μm. Bottom: S. pombe cells expressing E. coli AzFRS and Rlc1p-TAG-GFP from a plasmid (MBY 8966) were grown at 30°C in the presence or absence of 1 mM azido-phenylalanine to exponential growth phase. Cell were imaged using Andor Spinning Disk. 5 μm. Please refer to Supplemental Table S1 for the strain numbers. (C) AzF has no adverse effect on cell growth and cell cycle progression. The E. coli TyrRS cells expressing Rlc1p-TAG-GFP in the absence or presence of AzF were quantified. The relative proportions of mononucleated cells (shown as blue bars), mononucleated cells with actomyosin ring (shown as red bars), binucleated cells (shown as purple bars), and binucleated cells with actomyosin ring (shown as green bars) were quantitated. The mean relative proportion of each category from three independent experiments is shown (N > 60 for each strain in each experiment). (D) The cell extract were prepared from E. coli TyrRS strain expressing GST (G6stop)-6xHIS (MBY 10031) in the AzF+ and AzF− medium, respectively. The same as E. coli AzFRS strain expressing GST (G6stop)-6xHIS (MBY 10026). In total, all the four samples were purified with Ni-NTA, respectively and detected with α-GST antibodies. Please refer to Supplemental Table S1 for the strain number.
(AzF) into three proteins (GST, His5p, and a synthetic linker peptide). We did not observe any major deleterious phenotypic effects in cells carrying non-sense suppressor tRNA and aaRSs. Thus non-sense suppression based on introduction of unnatural amino acids can be used to study fission yeast protein structure and function. This work has also established that the \textit{E. coli} and \textit{B. stearothemophilus} tRNATyr and \textit{E. coli} TyrRS function as an orthogonal pair in fission yeast.

Although non-sense suppression was only observed in the presence of all three factors, 1. tRNA\textsuperscript{Tyr}, 2. AzFRS, and 3. AzF in the growth medium, we were unable to detect AzF in mass spectrometric analysis. Rather we found a peak consistent with conversion of AzF to amino-phenylalanine (as described in previous work\textsuperscript{9,10,23}). The conversion of AzF to AmF reduced the utility of the current system we have developed. Additional work is required to prevent reduction of AzF to AmF, so that the system can be used in protein-protein interaction studies. We have detected covalent cross-links between GST
molecules, suggesting that despite our inability to detect an AzF peak in mass spectrometry, a fraction of GST should contain unreduced bona fide AzF.

In western blot analysis, we found that the amount of GST was less in non-sense suppressed strains compared to the level of GST expressed from mRNA that did not have an internal non-sense codon (data not shown). This is consistent with previous work that non-sense suppression is an inefficient process. In addition, non-sense mediated decay of mRNA might also affect transcript abundance, as has been shown in C. elegans and S. cerevisiae. Consistently, an increase in non-sense suppression was observed in S. cerevisiae, strains defective in the NMD pathway. Development of such strains should increase the non-suppression efficiency in fission yeast.

Site-specific incorporation of AzF has been achieved in the budding yeasts S. cerevisiae and C. albicans. However, the biology of S. pombe allows for the investigation of a number of cell biological questions (such as cytokinesis and RNA interference) that are not easily tackled in budding yeasts. Our work on incorporation of site-specific incorporation of AzF, thus fills an important gap and opens up a new avenue to study fission yeast protein function. Further refinement of this method is necessary to allow full utilization of this approach to investigate protein function.

**Methods**

**Media and growth conditions.** Cells were grown and maintained in the yeast extract medium (YES) or Edinburgh minimal medium with appropriate supplements, as described in Moreno et al. (1991). YPD medium containing 1% yeast extract (Gibco-BRL), 2% peptone (Gibco-BRL) and 2% glucose was mainly used for mating and sporulation. All experiments were done at 30°C which is the optimal growth temperature for tRNA-Tyr-TyrRS expressing fission yeast strains, unless indicated otherwise. To suppress the stop codon in rlc1-TAG-gfp, which was expressed under nmt1 promoter control, cells were grown in thiamine containing medium (repressing condition) overnight, followed by three washes with medium lacking thiamine and growth for another 6 h in medium lacking thiamine but supplemented with unnatural amino acid before imaging.

**Plasmid construction.** All the polymerase chain reactions are performed on a T100 Thermal Cycler from BIO-RAD. Inverse PCR based plasmid construction method was used to generate tRNA expression constructs as well as mutations on the gfp and rlc1-gfp. For inverse PCR, two primers were designed to introduce insertion fragment. Each primer was ~50 bp in length and contained ~25 bp overlap with the plasmid backbone sequence. A standard PCR was performed firstly to generate the insertion fragment. 500 ng of the purified PCR product was used as primer in a 50 μL inverse PCR mixture which contained 30 ng of plasmid template, 5 μL of dNTP, 5 μL of Pfu buffer, 1 μL Pfu and ddH2O made up to 50 μL. For site-directed mutagenic PCR, most steps were the same as in the inverse PCR. The only difference was the targeted mutation was designed into the primers and located into the middle of the overlap region of forward primer and reverse primers.

**S. pombe Lithium Acetate transformation.** To transform plasmids into fission yeast cell, 20 mL overnight culture (a concentration of 0.5–1 × 10^7 cells/ml (OD595 ≈ 0.5)) was harvested at 3,000 rpm for 1 min and washed with 1 mL LiAc/TE buffer (made from 100 mM lithium acetate pH 7.5, 10 mM Tris–HCl and 1 mM EDTA pH 7.5). After suspending cells in 100 μL LiAc/TE buffer, 2 μL SSDNA (sonicated salmon sperm carrier DNA, Stratagene) and 2 μg DNA fragments (linearized) or plasmids were added to the suspended cells and incubated at room temperature for 15 min. Next, 240 μL of PEG/LiAc/TE (40% w/v PEG 4000 in 100 mM LiAc, 10 mM Tris–HCl, 1 mM EDTA) was added to cells followed by gentle mixing. After incubating them at 30°C for 45–60 min, 43 μL of dimethyl sulfoxide (DMSO) was added to the cells followed by gentle shaking. Cells were heated at 42°C for 5 min and rinsed with sterile water. Cells were resuspended in 100 μL water and plated on the selective agar plates.

**Western Blot.** Cells were lysed by glass bead disruption and eluted with lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 5 mM EDTA, 1 mM PMSF, supplemented with protease inhibitors (Complete EDTA-free; Roche Diagnostics, Basel, Switzerland)). The lysate was clarified by centrifugation at 200 g. The supernatant was heated at 100°C for 5 minutes after the addition of 2× Laemmli sample buffer and dithiothreitol. For western blotting, equal volumes of each sample were loaded on SDS-polyacrylamide gels of appropriate concentration and run at a constant current of 20 mA for 80 min. Note that the maximum voltage was lower than 200 V. After SDS-PAGE, proteins were blotted to PVDF (Polyvinylidene fluoride) membranes at a constant voltage of 90 V for 100 min at 4°C. Transfer buffer used was 25 mM Tris–HCl pH 8.3, 192 mM glycine, 10% v/v methanol. Membranes were incubated with appropriate concentration of primary antibodies in blocking buffer (5% w/v milk, 0.05% w/v Tween-20 in TBS or PBS) at room temperature for 3 h or 4°C overnight. After washing the blots thrice with PBST or TBST with gentle agitation, secondary antibodies were added and incubated at room temperature for 1 h. Signals were detected using the ECL approach.

**Glutathione S-transferase purification.** Cell extract preparation for the glutathione S-transferase purification was as described for the western blot experiments. The extracts were incubated for 1–2 h at
4 °C with 20 μL of pre-swollen glutathione-sepharose 4B (GE healthcare) beads equilibrated with lysis buffer (150 mM NaCl, 50 mM Tris pH 8.0, 10% glycerol, complete protease inhibitors and 1.5 μg/mL of PMSF). The supernatants were removed after centrifugation at a speed of 1,000 rpm for 2 min. The beads were washed with PBS containing protease inhibitors twice and lysis buffer containing 0.1% Triton-X100 once. The final elution buffer was 50 mM Tris/HCl, 10 mM reduced Glutathione, pH 8.0. Two fractions were collected.

**His-tag purification.** Cell extracts from 300 mL cell for the His purification was prepared as described for the western blot experiments. The extracts were incubated for 1–2 h at 4 °C with 300 μL of pre-washed HisPur Ni-NTA Resin (Thermo Scientific) equilibrated with lysis buffer (150 mM NaCl, 50 mM Tris pH 8.0, 10% glycerol, complete protease inhibitors and 1.5 μg/mL of PMSF). The supernatants were removed after spinning down at a speed of 2,000 rpm for 2 min. The beads were washed with lysis buffer with 20 mM imidazole thrice. The final elution buffer was 500 mM imidazole.

**GST UV Crosslinking.** Purified GST proteins containing unnatural amino acid AzF or tyrosine were used in this experiment. The concentration of purified proteins was estimated and diluted to equivalent levels. 20 μL of each sample was loaded on parafilm, which was kept on an icebox. Samples were exposed to UV (254 nm) in UV crosslinker CL-1000 (VWR) for 5 min and collected into a microfuge tube.

**Spinning Disk Microscopy.** Spinning disk images were captured by Andor spinning disk. Andor spinning disk using a Nikon ECLIPSE Ti-E microscopy equipped with a Plan Apo Vc 100×/1.40 NA. Object lens, a Yokogawa CSU-X1 unit spinning disk system, Andor iXon Ultra EMCCD (16 μm/pixl) camera with 2× adapter for CSU-X1 and Andor iQ3 software. 405, 488, 561 and 640 nm solid state diode laser were used for excitation. All images were processed and analyzed by using ImageJ (http://rsb.info.nih.gov/ij/) software. Graphs in this paper were made by Excel.

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**Author Contributions**

N.S. and N.S.S. contributed equally to this paper. N.S. and M.K.B. wrote the main manuscript text. N.S. and N.S.S. prepared Figs 1, 2 and 5. N.S. prepared Fig. 3. N.S., S.E.S. and A.M.E.J. prepared Fig. 4. All authors reviewed this manuscript.

**Additional Information**

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Corrigendum: Site Specific Genetic Incorporation of Azidophenylalanine in *Schizosaccharomyces pombe*

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This Article contains errors in the Acknowledgements section:

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should read:

“We express our sincere gratitude to the WPH Proteomics Research Technology Platform for the contribution to the mass spectrometry analysis. In addition, we would like to express our thanks to Prof. Thomas Sakmar (Rockefeller University) for providing us the tRNA-synthetase pair. In the end, we would like to thank all the MKB laboratory members for discussion. This work was supported by research funds from National University of Singapore, Wellcome Trust (WT101885MA), The Royal Society, Temasek Life Sciences Laboratory and University of Warwick.”

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