

Mass Spectrometric Investigation of Ribosome Gymnastics

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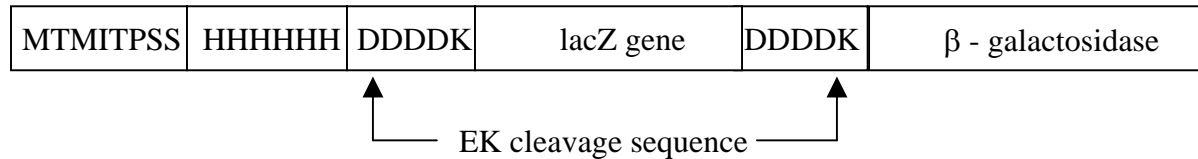
ABRF 2005, Savannah GA

Introduction

- Peptide synthesis is conventionally pictured as the orderly, sequential addition of amino acids to a growing peptide chain dictated by the sequence of triplets in the messenger RNA. However, several alternatives to this linear progression are possible when a particular aminoacyl-tRNA, becomes limited. These alternatives include noncognate aminoacyl binding, frameshifting, peptidyl-tRNA release and bypassing over many nucleotides downstream [1].
- In bypassing, the ribosome:peptidyl-tRNA complex shifts at least a few dozen nucleotides in the 3' direction, “taking off” one codon upstream of a *hungry codon* and “landing” at another codon recognized by the tRNA portion of the peptidyl-tRNA. The result is a protein which lacks the amino acids encoded in the bypassed region of the mRNA, between the two sites.
- Here we report a novel mass spectrometry-based strategy that can be used to investigate ribosome bypassing.
- Starvation-induced bypassing requires synonymous takeoff and landing triplets and the probability of the event decreases when the spacing between triplets increases. In addition to starvation-induced bypassing, we are demonstrating here that spontaneous ribosome bypassing can also occur in growing cells.

Design of the Experiment

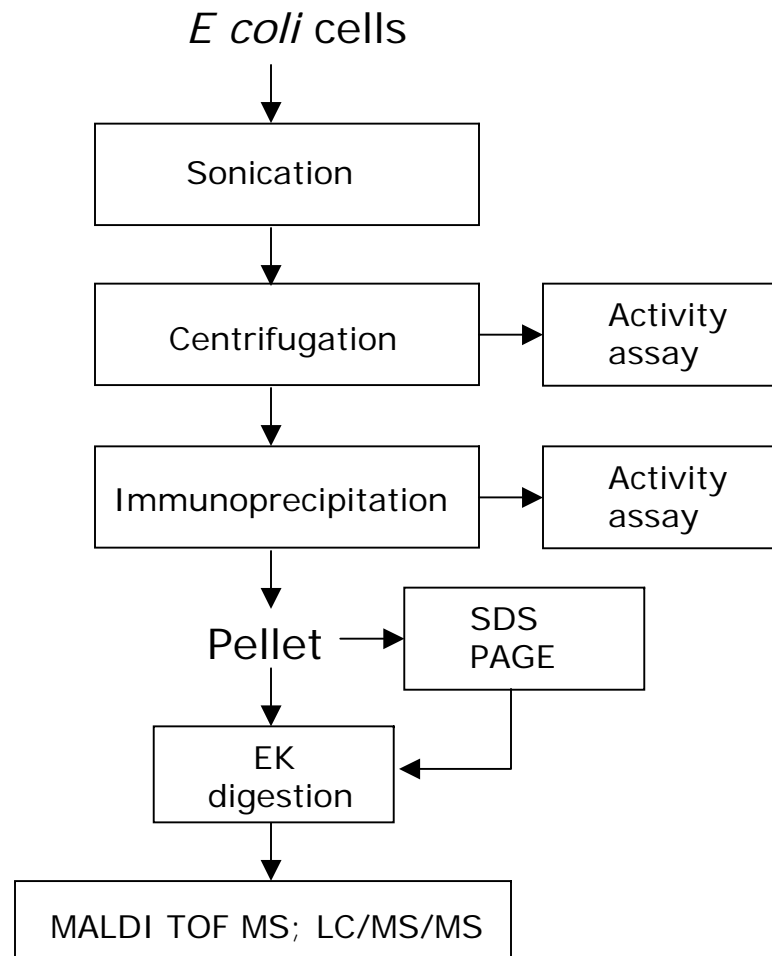
- We inserted the recognition sequence of enterokinase (EK) at two sites near the 5' end of an engineered *lacZ* gene [1], flanking a synthetic sequence which dictates a bypassing or frameshifting event necessary to synthesize active β -galactosidase in *E. coli*.



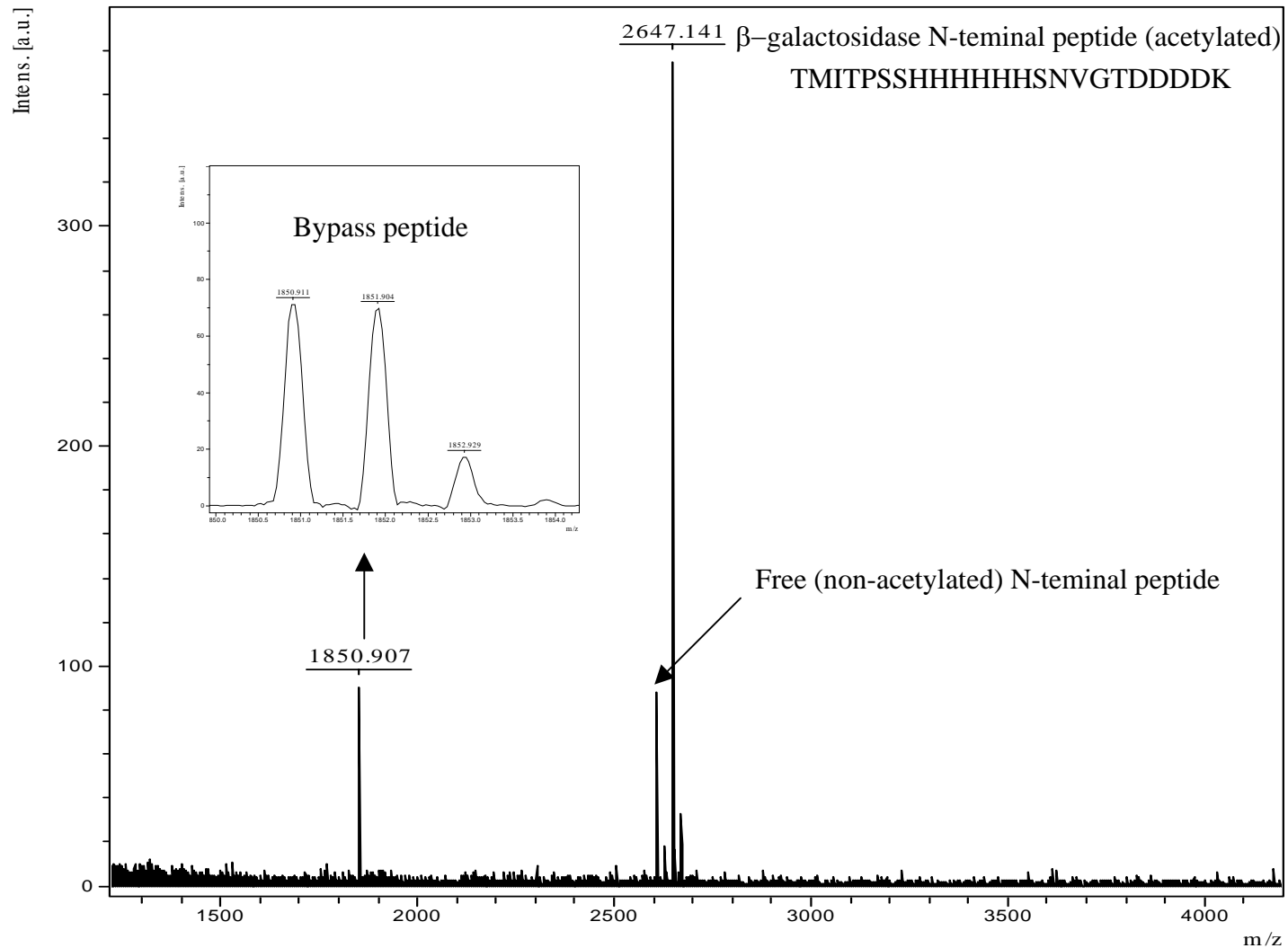
- The *lacZ* β -galactoside was purified by immunoprecipitation [2] and digested with EK in-gel or in solution. Peptides released were analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS) and by nanoscale liquid chromatography/ electrospray ionization tandem mass spectrometry (nanoLC ESI-MS/MS) to identify translational anomalies.

1. Gallant et al. (1998) *Proc. Natl. Acad. Sci. USA* 95, 13771-13776.
2. Gallant et al. (1996) *J Mol. Biol.* 256, 676-684.

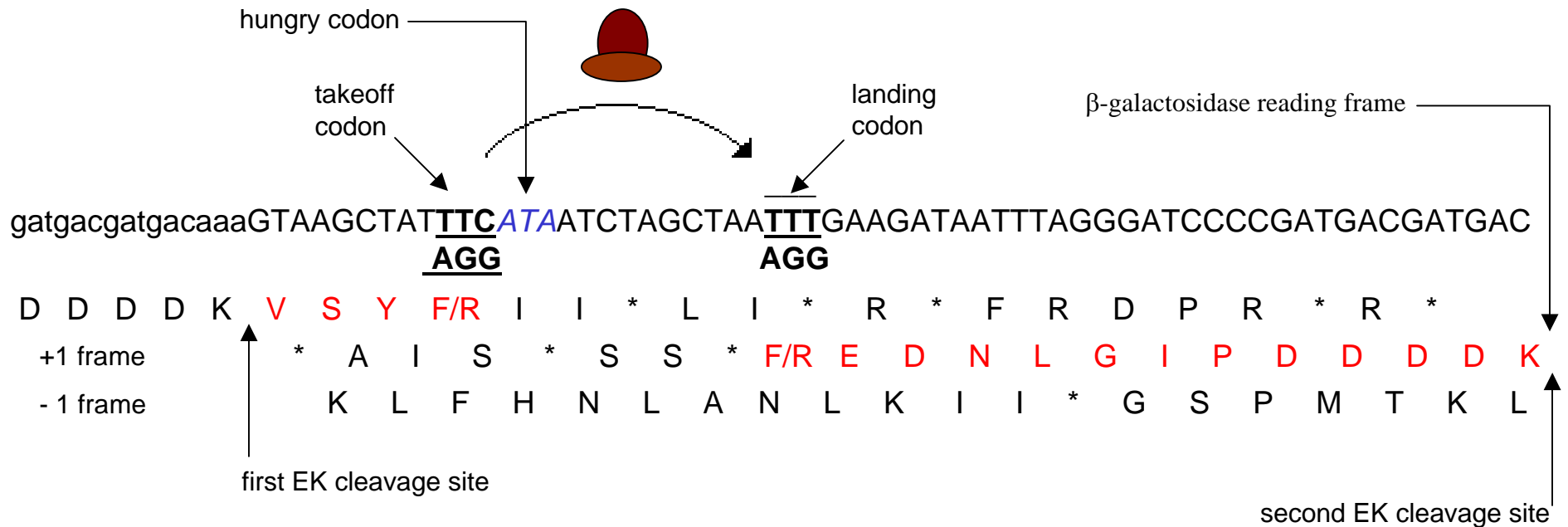
Experimental Protocol



MALDI Spectrum of the Digested Immunoprecipitate



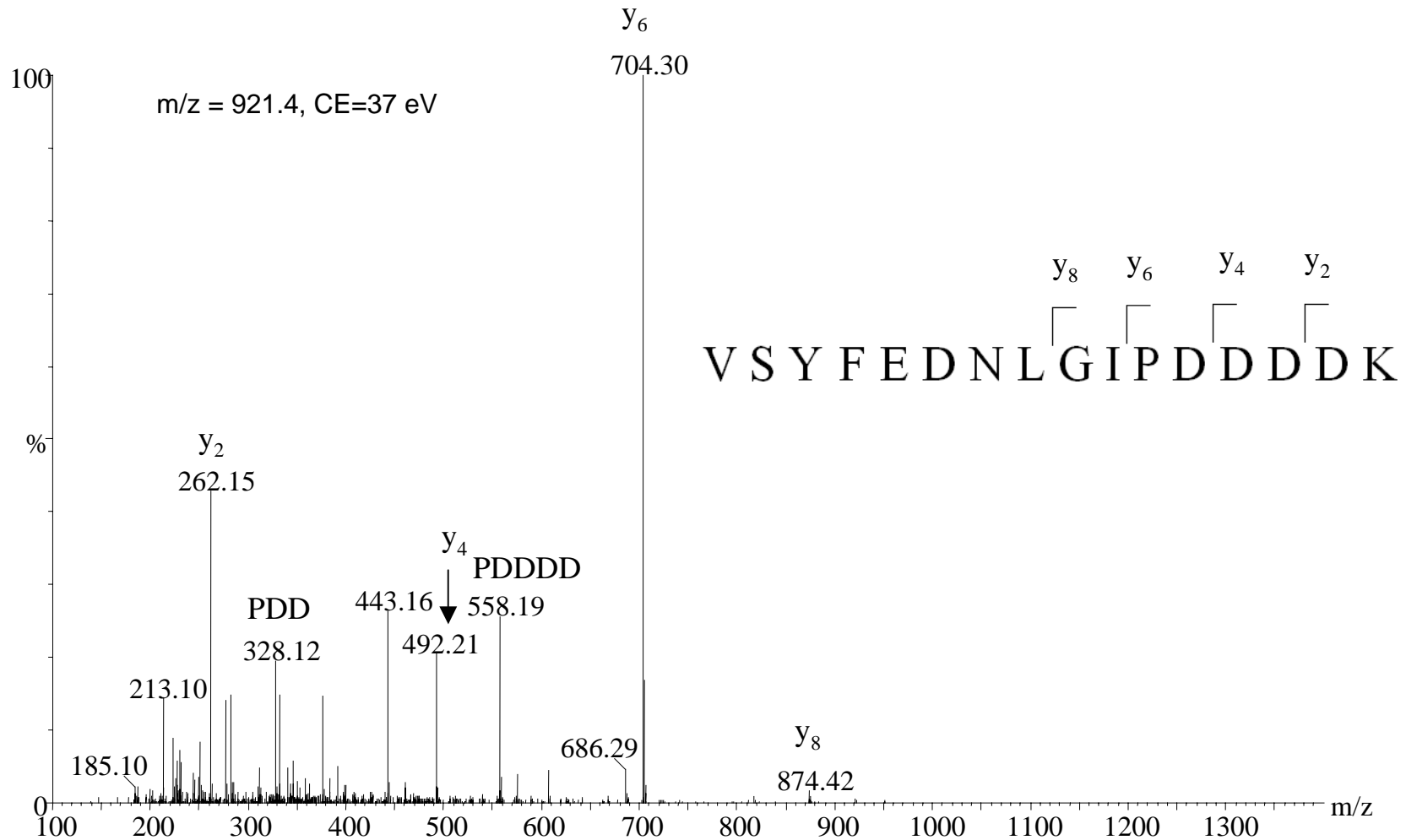
Ribosome Jumping over 16 Nucleotides



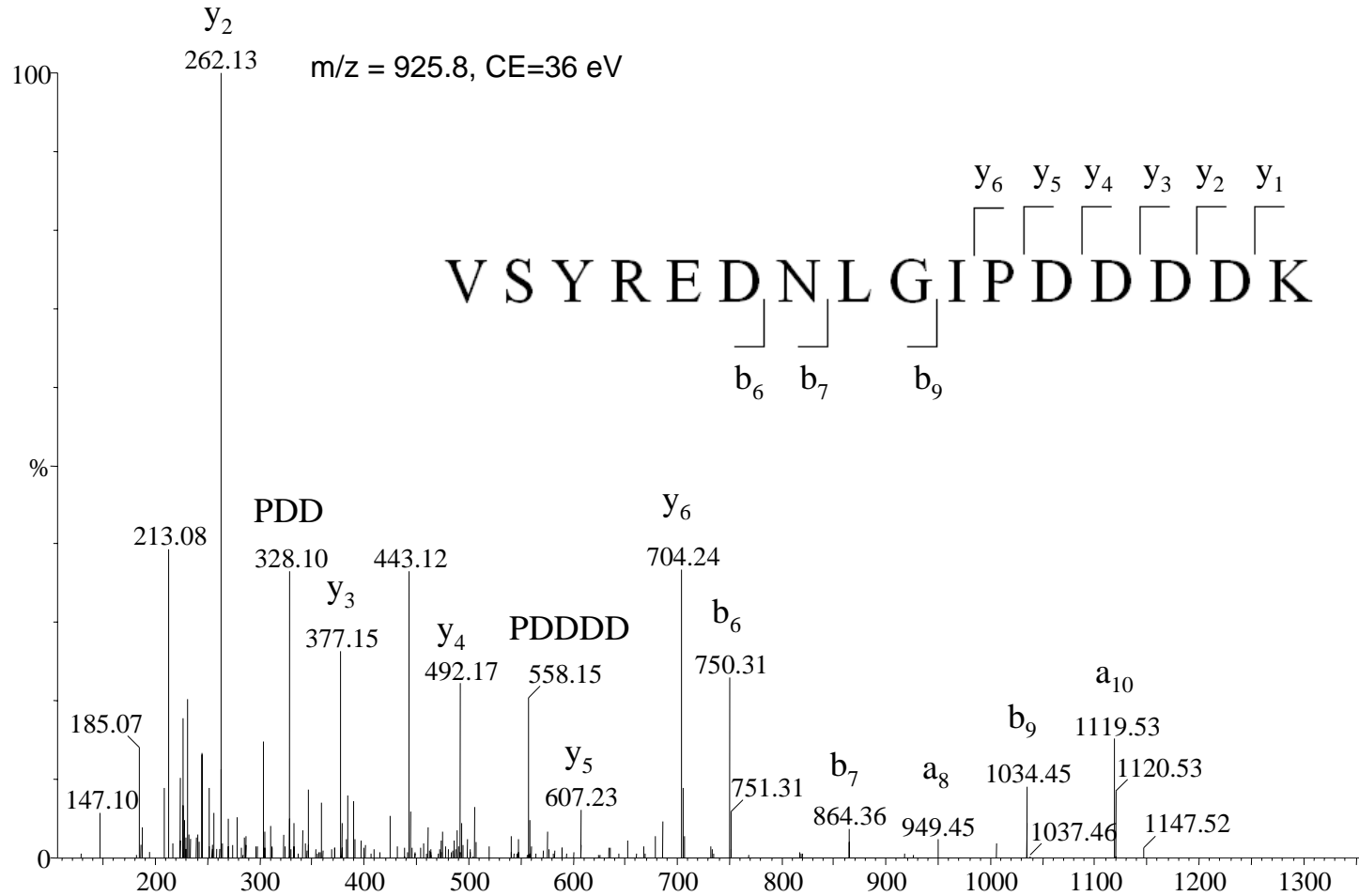
The mass spectrometric approach was tested on a ribosome “jump” of 16 nucleotides previously reported [1]. EK treatment released only two peptides, an N-terminus acetylated peptide and a bypass peptide (either VSYFEDNLGIPGDDDK or VSYREDNLGIPGDDDK). Ribosomes landed in the β -galactosidase reading frame (+1) at a triplet (TTT or AGG) synonymous to the takeoff triplet.

1. Gallant et al. (1998) *Proc. Natl. Acad. Sci. USA* 95, 13771-13776.

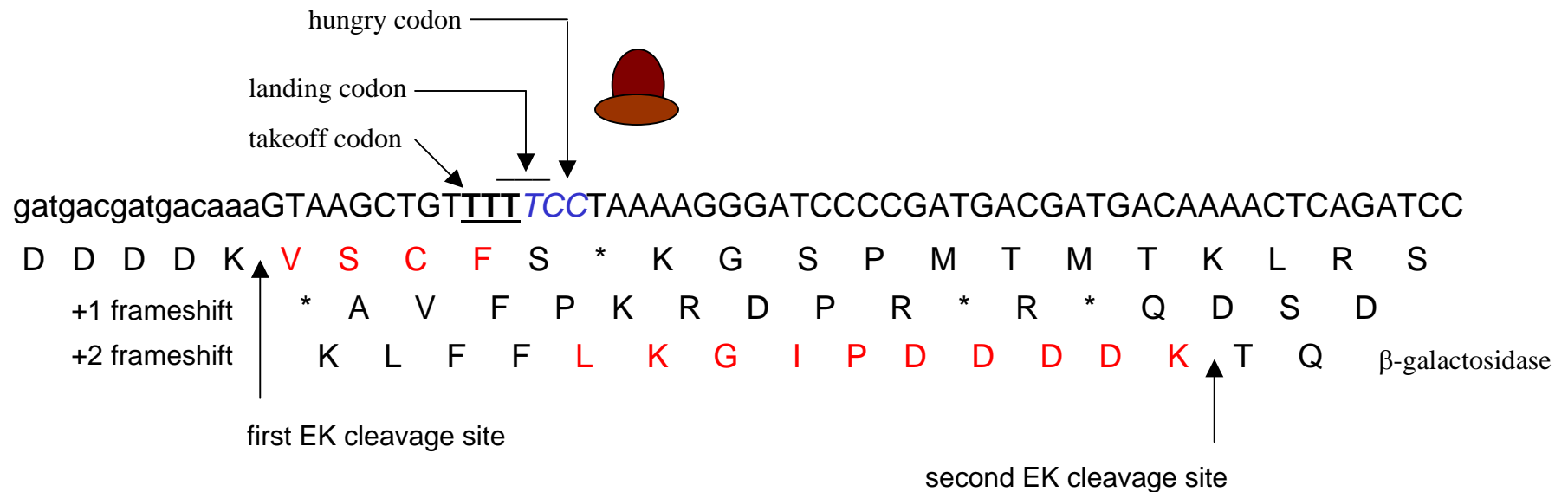
MS/MS Spectrum of a Bypass Peptide Detected in Cells Limited for Isoleucyl t-RNA



MS/MS Spectrum of a Bypass Peptide Detected in Non-starved Cells

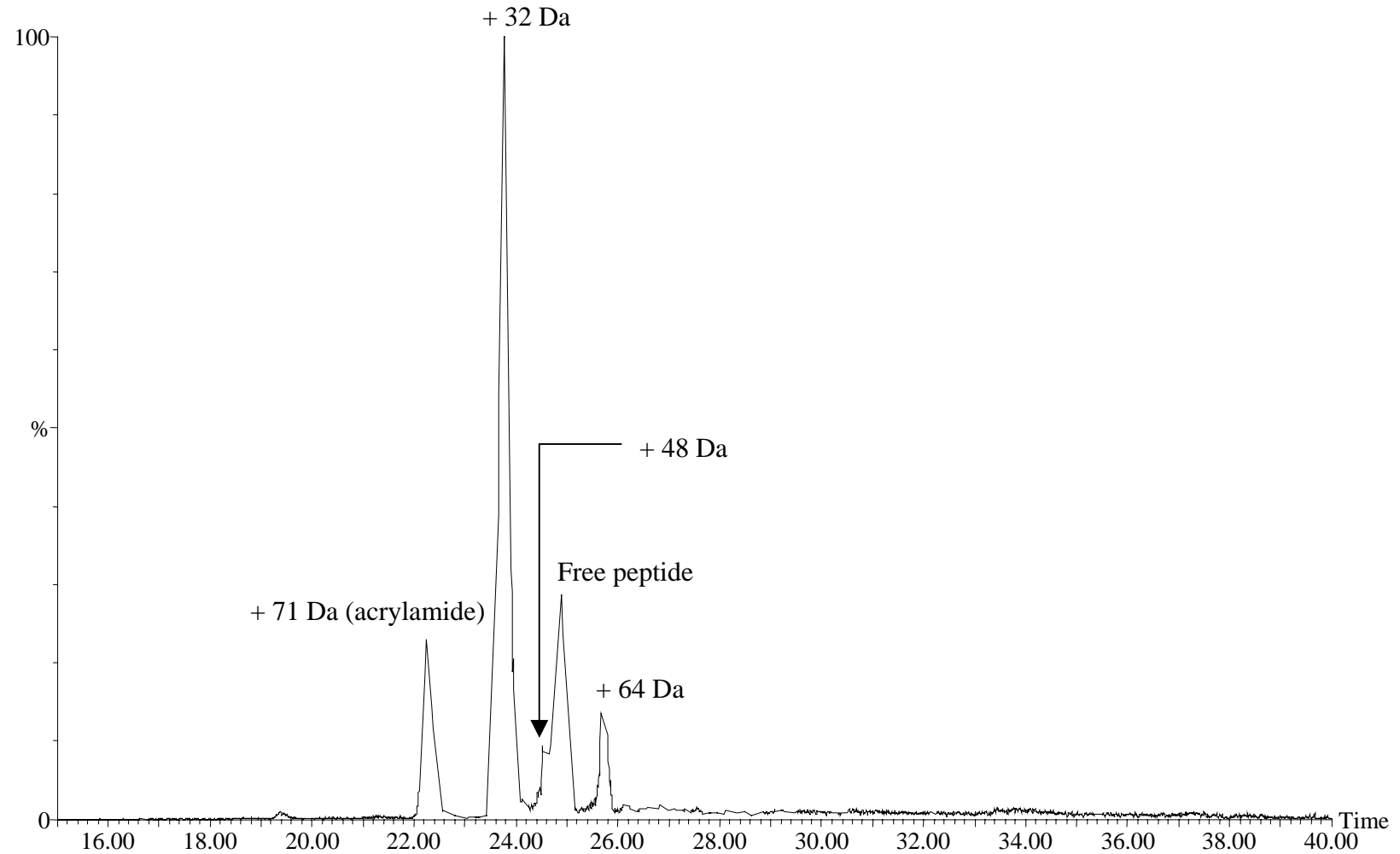


Ribosome Hopping over 2 Nucleotides

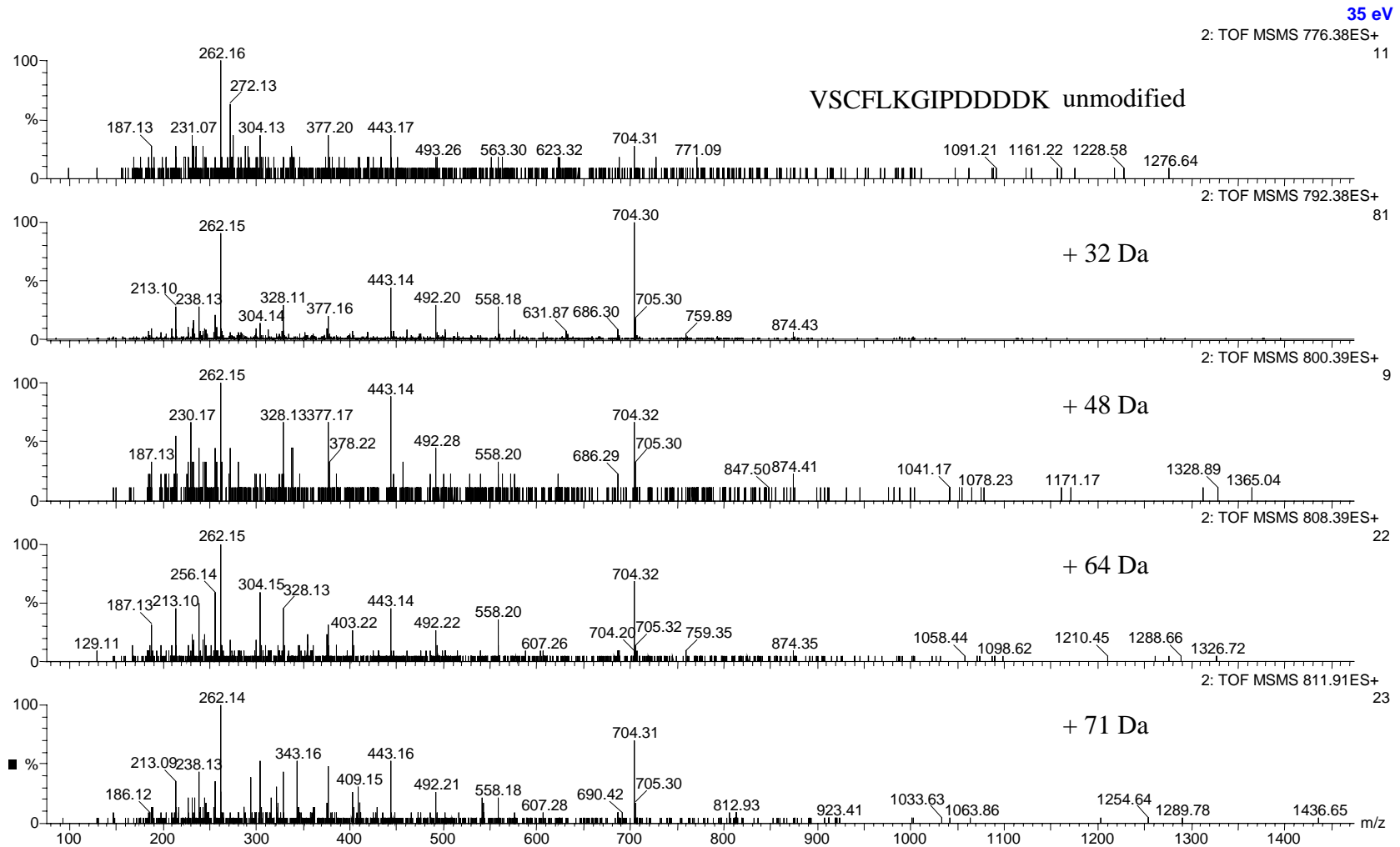


The ribosome “slipped” over 2 nucleotides in serine-starved cell. Several varieties of the bypass peptide (VSCFLKGIPDDDK) were detected in this case because of the reactive cysteine. The amino acid sequence of the peptide indicates that ribosomes, which paused due to a hungry serine codon (TTC) in the A-site, shifted from TTT to the (+2) β -galactosidase reading frame, landing at the synonymous TTC(overlined) triplet. The fragment (VSCFFLKGIPDDDDK) expected from the (-1) frameshift event was not detected.

Chromatographic Separation of the Modified Bypass Peptides



MS/MS Spectra of Modified Bypass Peptides



Conclusions

- Enterokinase cleavage of the crude immunoprecipitate provided very clean samples for both MALDI and LC/MS/MS.
- In most of these experiments, the EK treatment released only two peptides, an N-terminal acetylated peptide and a bypass peptide.
- The method can easily detect multiple translational anomalies, even from complex peptide mixtures, because all peptides of interest share the same C-terminal EK recognition sequence (DDDDK), which produces a specific y-type fragmentation pattern. In the case on multiple bypass peptides, this approach can be used to estimate the probability of occurrence of each bypass event.
- In addition to starvation-induced bypassing, we have demonstrated here that spontaneous ribosome bypassing can also occur in growing cells.
- This methodology will be employed to investigate multiple products of translation of the HIV-1 frameshift in *E.coli* cells.

Acknowledgements

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- Our lab manager Bill Howald for allowing me to spend more time on this research project.

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