# SunRiSE – measuring translation elongation at single-cell resolution by means of flow cytometry

R. J. Argüello[1], M. Reverendo[1], A. Mendes[1], V. Camosseto[1], A. G. Torres[3], L. Ribas de Pouplana[3,4], S. A. Van de Pavert [1], E. Gatti[1,2], P. Pierre[1,2]

1 — Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université, Inserm, CNRS, France
2 — Department of Medical Sciences & iBiMED and Ilidio Pinho Foundation, University of Aveiro
3 — Institute for Research in Biomedicine (IRBBarcelona), The Barcelona Institute of Science and Technology, Parc Científic de Barcelona, Spain
4 — Catalan Institute for Research and Advanced Studies (ICREA), P/ Lluis Companys Barcelona, Spain

With this work, the group of Doctor Philippe Pierre proposes a new methodology of analysis of the protein synthesis that studies the elongation phase. The levels of protein synthesis control the folding of the proteins as they are produced and contribute to the stability of the messenger RNAs. Many factors regulate translation elongation, including tRNA levels, codon usage and phosphorylation of eukaryotic elongation factor2 (eEF2). Current methods to measure translation elongation lack single-cell resolution, require expression of multiple transgenes and have never been successfully applied ex vivo. The relative contribution of the different environmental and endogenous factors on translation elongation in cells directly isolated from human blood or mice tissues is poorly understood and is still methodologically demanding. Approaching these questions requires a technique with high-throughput potential and that measures translation elongation rates under physiological conditions in non-abundant and non-transformed cells ex vivo. Reduced manipulation and applicability to complex mixes of cells should also be a requirement for such a method. Here, we show, by using a combination of puromycilation detection and flow cytometry (a method we call 'SunRiSE'), that translation elongation can be measured accurately in primary cells in pure or heterogenous populations isolated from blood or tissues. This method allows for the simultaneous monitoring of multiple parameters, such as mTOR or S6K1/2 signaling activity, the cell cycle stage and phosphorylation of translation factors in single cells, without elaborated, costly and lengthy purification procedures.

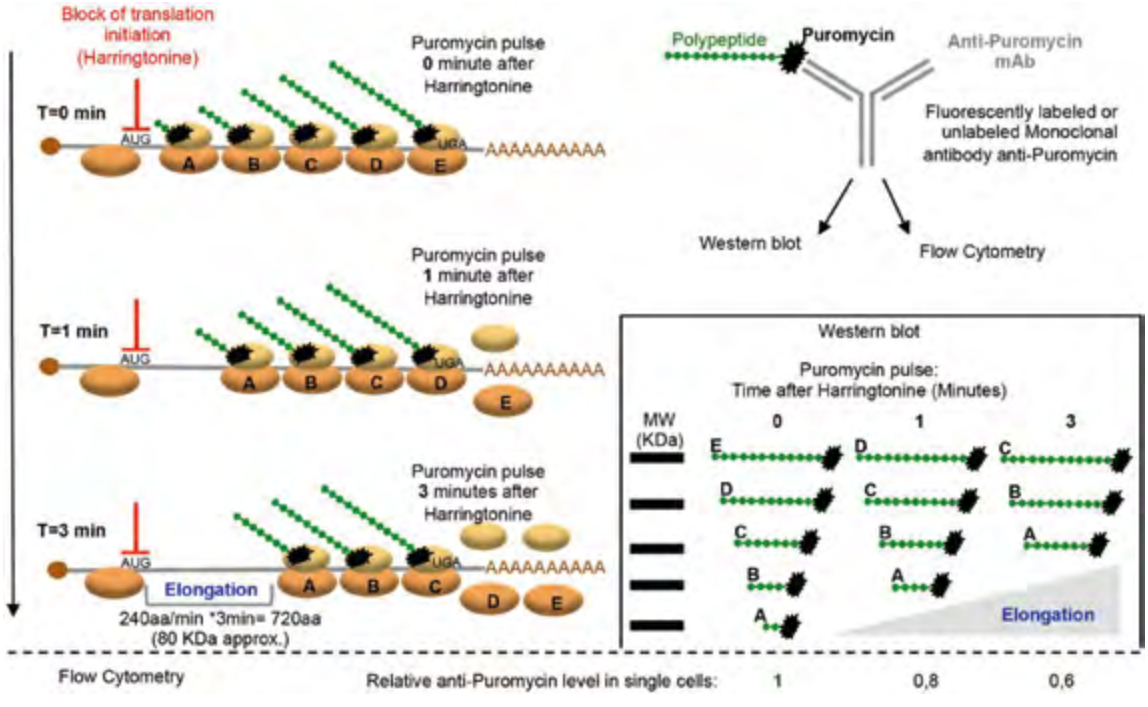


**FIGURE 1**

Schematic representation of the SunRiSE protocol and results. Cells are seeded in culture wells, and translation initiation is blocked by addition of harringtonine at time 0. Puro is added at different times after inhibition of translation initiation and is incorporated into nascent peptides by elongating ribosomes (denoted A–E). After a certain time of harringtonine treatment, pre-engaged ribosomes continue to elongate, and thus ribosomes that were bearing short peptides at T0, are associated with longer peptides after 240 s of treatment. Eventually translating ribosomes run-off from mRNAs, and the gap between the last initiating ribosomes and the translation initiation site will directly correlate with the elongation rate of the ribosomes, and therefore inversely correlate to the amount of puro incorporation. Puro incorporation is revealed by immunodetection through immunoblotting and flow cytometry, allowing a measure of puro incorporation decay (which correlated to the translation elongation rate) in bulk or at single-cell resolution. MW, molecular mass; aa, amino acids.