Protein synthesis, also called translation, is a process of gene expression by which amino acids are linearly arranged into proteins. It involves ribosomes, transfer RNAs, messenger RNAs and a thousand of regulatory factors constituting a riboproteome. Cell-free protein synthesis systems (CFPS) have been widely used to express proteins and to explore gene expression pathways. Promega supplies one of the only commercial mammalian CFPS, the rabbit reticulocyte lysate (RRL Promega- #. L4151), a key component widely used for biotechnology and research applications. To explore the cell specific ribosome, Human-derived CFPS are in top demand in the scientific community but remains excruciatingly difficult to obtain and are not available commercially.

The last four years, we developed an innovative *in vitro* translation assay that relies on the hybrid assembly of exogenous ribosomes isolated from human cells with the RRL depleted of its endogenous ribosomes. Hybrid RRL systems maintain translational properties of the original ribosome cell types – cell stress, while delivering protein expression levels similar to RRL.

We exploited this new method to understand the viral subversion of ribosomes. All viruses are dependent on the host cell translational apparatus to express their own proteins. This creates a competitive situation between viral and cellular messengers which are often bypassed by viral strategies to ensure preferential use of ribosomes for viral mRNAs. We applied the hybrid RRL biotechnology to study human cell line derived ribosome in presence or absence of regulatory factors to screen virus mRNAs dependence for replication. We obtained a series of important results. For example, we evidenced a series of viral mRNA like Hepatitis C virus (HCV) RNA that were insensitive to eukaryotic translation initiation factor 3 (eIF3) depletion. As HCV dispense a strict hepatocyte tropism, we first worked *in vitro* with hepatocyte derived ribosomes into the hybrid RRL system. Those results were validated in hepatocyte infected cells to demonstrate that eiF3 was not required for viral proliferation. The *in vitro* approach led us to go deeper in the mechanism by which HCV get advantage under conditions of low eIF3 expression in infected human cells.