Inhibitors of eIF6-ribosome interaction

A large group of authors represented by Stefano Biffo as corresponding author has submitted a manuscript describing a rapid screening assay for identifying chemical inhibitors of interaction between eIF6 and the ribosome. eIF6 has two basic functions as a ribosome biogenesis factor (apparently not relevant to the assay, see Fig 8) and as a translation factor which prevents the 40S and 60S ribosomal subunits from forming inactive 80S subunits without an associated mRNA. Even though eIF6 thus has a general function in translation, it appears to be involved in differentiation of the rate with which different mRNAs associate with the ribosome. As such, eIF6 has also been implicated in the mechanism for various human diseases. It is therefore important to understand the precise mechanism of eIF6 function and to identify drugs of potential clinical manipulation of the interaction.

The current manuscript describes an assay for rapid screening of chemicals that can modify the interaction of eIF6 with the ribosome. As such this is an important manuscript. However, a number of improvements are required in order to give the manuscript its proper impact. In my opinion, that the following issues must be addressed prior to acceptance.

1. The assay is described in Fig 1A as a FRET assay that brings biotin-tagged ribosomes and His-tagged eIF6 together with beads for primary and secondary light emission. If eIF6 is associated with the ribosome, the energy transfer is active, but if the interaction is inhibited by chemicals, ribosome and eIF6 are not close enough for the energy transfer and the secondary light signals declines. This is a clever assay, but it is not straight forward for a non-aficionado to connect the picture in Fig 1A with the microtiter-plate assay described in Materials and Methods. Where is the microtiter dish in Fig 1A? In contrast the plate is clearly relevant for the ELISA assay in Fig 3. This should be clarified.
2. Line 38: Do the authors mean "**differentially** decoded"?
3. Lines 44-45 and other places: Please define abbreviations
4. Line 60: "On this line" is awkward. "Along these lines"?
5. Line 142 and other places: I presume that the authors mean nmol, (nano-mole) i.e. amount of the compound, not nM, (nano-molar), i.e. the concentration of the compound.
6. Line 142: It appears that the text should be "**biotinylated** ribosomes". Please clearly indicate where tagged and non-tagged compounds are used.
7. Line 215: "confirmed the anticipated behavior". Refer to specific results.
8. Fig 1D: header should be "non-biotinylated **ribosome**-eIF6 competition assay" or even "non-biotinylated **ribosome**-**His-tagged** eIF6 competition assay"
9. Line 243 and Fig 2A: What is the meaning of a negative value on the X axis? Compounds strengthening the FRET signal?
10. Lines 304-5: What are the statistics for comparing the results for sixty-1 vs -4?
11. The evaluation of the value of the assay depends highly on the understanding of the biochemical mechanism behind the changes in the FRET result. The molecular-biological/biochemical analysis of the three compounds (Figs 4-7) clearly show that the mechanism behind the FRET results is different between the compounds. This may also be the reason for showing the chemicals bound to eFI6 OR to ribosomes (Fig 3, right column of cartoons). There is no discussion of this important aspect. Please provide a discussion as this would be important for interpreting the results and improving the assay.