Review of: "[Review Article] Melatonin, ATP, and Cataracts: The Two Faces of Crystallin Phase Separation"

Hayet Belkacemi[1]

1 Université de Béjaia

Potential competing interests: No potential competing interests to declare.

Reviewer 2 : HB

This study is very original and interesting but it need a revision.

I think this study could contribute better as a chapter in a book in a medical field.

In order to improve the structure of the article, it is necessary to better clarify certain points raised throughout the sections, to clarify the content of the study.

Recommendations are therefore cited below.

Recommendations :

1. **Graphical abstract:**

Is good but requires some modifications on the diagram of the graphical abstract. In particular for the illustration of the eyeball on the right must also be different to represent the anomaly created by the effect of the cataract, on the deviation of the beam within the crystallin globe, which must be of direction not focused on the retina, in order to symbolize the optical defect created in the eye.

1. **The abstract:**

-It must be brief, short and concise, also to be focused on the objective by recalling the most relevant results obtained.

- in the sixth line you wrote: "..**prevents amorphous crystalline condensates from transitioning into amyloidogenic fibrillar aggregates**…"

The meaning could be more precise, if you write "..prevents**the crystallin-amorphous condensates transition into** amyloidogenic fibrillar aggregates.."

3- The manuscript:

- **In Introduction**:

- in the second paragraph and sixth line, you write: "..**the native tertiary structural state**.."

You must specify if it is about the structure of proteins, in which case you can write: "..the native tertiary structural state **of proteins**..."

On page 4 and in the first line:

- **At section 2, in the second paragraph the 8th line, you write: "…**crystallins can maintain their native tertiary structures under stressful conditions such as temperatures as high as 60 °C…."

However, according to work carried out on the stability of proteins, it is very possible that they also transform into the quaternary structure favored at higher temperatures such as 60°C for example. Which can degrade the nature and the property of crystallinity, in this case optical.

In the Sahara for example, the number of cases of cataracts is higher, knowing that the temperature conditions are also high and often exceed 40°C.

- **At subsection 2.1 on page 4: you may rewrite the title without details, like this:** "Lens αA-Crystallin Against Aberrant Protein Aggregation "
- On the 3rd line of the same paragraph, you must specify the size of the proteins when their dimensions are larger.

On page 5:

- **at second line** You write: "….susceptibility of crystallins to entropically-driven phase separation with decreased hydrophobicity …"

Can you explain and precise why the change in entropy correlates with the decrease in hydrophobicity and in relation to which endogenous molecule?

- **in subsection 2.2:** You need to rewrite the entire paragraph from the first line to the 5th

" **A study ……low critical temperature** " because it is too long and not clear enough.

- **in subsection 2.3 and the 3rd line**, you raised the effect of physiological conditions. Can you specify them?

On page 19:

- **In subsection 6.3.2, at the first line you write :** "the solubilizing effect of ATP in aqueous solutions at neutral and **elevated pH**"

Can you specify the pH range, basic and why?

- At the 4th line: "..**ATP can antagonize the crowding-induced destabilization effect**."

In this case, can you give details on the type of ATP target receptor sites existing on the fibril proteins. If ATP plays the role of antagonist, with which agonist could it compete, or does ATP have just an amphiphilic character, which makes it a

good mediator of the solubilization of lens proteins?

On page 20:

- In subsection 6.5, at the 4th line you write: “.**high levels of melatonin** failed to reproduce similar results exist. Even though melatonin disaggregated preformed tau fibrils in a dose-dependent manner where 0.1 mM and 5 mM melatonin..”

Is it possible to specify here the level of toxicity of melatonin, as this could constitute a limitation to its excessive use for protein solubilization?

On page 21:

- In subsection 6.5, the 3rd paragraph and the 1st line you write:

“**An analysis** of primary neuronal cells exposed to solutions containing α-syn pretreated with different concentrations…”

What types of analysis or methods used here? Specify.

- **In subsection 6.6, at the 5th and 6th lines, you write: “ ..** amyloid aggregation that steers an amorphous aggregate…”

How to control the transition to the amorphous state of aggregates, is there a more appropriate method to detect it.

On page 22:

- **At Figure 2:** Add a legend at the bottom of Figure 2 that specifies the nature of the chemical elements by associating them with different colors respectively (red for oxygen, blue for nitrogen, etc.)

On page 23:

- **At the 1st line of the 1st paragraph, you write :”** intramolecular hydrogen bonding that restricts proton motions that result in stronger hydrogen bonding.”

You must specify here which functional groups of either ATP or melatonin would be involved in hydrogen bonds with water molecules. Therefore, could there be hydrogen bridges between ATP and melatonin? This would strengthen the association between the two endogenous molecules and the stability of the complex formed.

- **At 6.6.2 section, you mentioned**:

- In the subtitle : ‘**IOP**”

You must first give its meaning in full terms before the abbreviation, for example, "Intraocular pressure (IOP)," and give a definition of this effect in the paragraph.

Melanopsin should be added to the title, thus: **"The complex effects of ATP/Melatonin/Melanopsin on IOP and**

hydrostatic pressure", since melanopsin is very important in the regulation of ATP and melatonin concentrations produced.

Do these three endogenous molecules have the same type of receptor, and which one?

On page 25, at the conclusion:

Can you consider the cost of a drug based on the two active ingredients, ATP and melatonin, if such treatment for cataracts and glaucoma would be considered in the future? Comment and add to the conclusion, which, in my opinion, as written, does not present all the objectives of the study.