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# Application of a fluorescent indicator yAT1.03 for ATP level measurements in living yeast

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Adenosine-5'-triphosphate (ATP) is the main energy source in all living organisms and is involved in many cellular processes. A variety of fluorescent indicators has been developed to monitor ATP levels within living cells. Many of these indicators are recombinant protein fusions composed of the $\epsilon$ subunit of *Bacillus* sp. ATP synthase flanked by one or two fluorescent proteins. The reversible binding of an ATP molecule to the $\epsilon$ subunit results in the conformational changes in the latter altering the fluorescent response of the reporter. In case of FRET-based sensors (ATeam family), the ATP binding increases the efficiency of the energy transfer between the donor and acceptor proteins. These sensors are widely used in microscopy studies to visualize the changes in ATP concentration at a single-cell level.

In this work, we used two techniques to monitor the changes of the intracellular ATP level in baker's yeast *Saccharomyces cerevisiae* in response to known growth substrates and inhibitors. We assessed the ATP level within the intact cells by spectrofluorometry using yAT1.03, a version of FRET-based ATP fluorescent indicator optimized for yeast [1]. The results obtained were verified by measuring the ATP concentration in the cell lysates by firefly luciferase reaction. We have shown that yAT1.03 provides an adequate qualitative response to ATP depletion in living yeast cells. The fluorometric approach using yAT1.03 seems to be a fast and effective tool for screening the effect of various compounds on intracellular ATP level in yeast.

## References

1) Botman D., van Heerden J.H., Teusink B. An Improved ATP FRET Sensor For Yeast Shows Heterogeneity During Nutrient Transitions // ACS Sens. 2020. Vol. 5, № 3. P. 814–822.