

## Regulation of glycolytic oscillations by the dynamics of intracellular water

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We have explored the coupling of dynamics of intracellular water with metabolism in yeast cells. Using the polarity sensitive probe 6-acetyl-2-dimethylaminonaphthalene (ACDAN) we could show that glycolytic oscillations in the yeast *S. cerevisiae* BY4743 wild type strain are coupled to the Generalized Polarization (GP) function of ACDAN, which measures the ability of intracellular water to reorient in response to the excited state dipole of the probe. We analyzed the oscillatory dynamics in the wild type and 24 mutant strains with mutations in glycolytic, mitochondrial and vacuolar enzymes/ATPases, as well as proteins involved in actin polymerization and microtubule formation. Using fluorescence spectroscopy, we measured the amplitude and frequency of the metabolic oscillations and linked them to the ACDAN GP in the resting state of all 25 strains. The results show that there exist a lower and an upper threshold of ACDAN GP beyond which oscillations do not occur. The transition from steady state behavior to oscillation could be described by two supercritical Hopf bifurcations. This critical GP range is also phenomenologically linked to the occurrence of oscillations when cells are grown at different temperatures. Furthermore, the link between glycolytic oscillations and the ACDAN GP value also holds when ATP synthesis or the integrity of the cell cytoskeleton (actin polymerization) is perturbed. Our results represent the first demonstration that the dynamic behavior of a metabolic process can be, directly or indirectly regulated by a cell-wide physical property: the dynamic state of intracellular water, which represents an emergent property of the cytosol. We hypothesize that our results may be explained using the Association-Induction hypothesis developed by G.N. Ling. According to this hypothesis the association of some central metabolites (e.g. ATP) with fibrillar proteins causes conformational changes, which, in turn, modulate the binding affinity of particular ions for proteins and hence also affect the patterns of hydrogen bonding of water.

We have developed a minimal mathematical model that can explain and simulate our experimental data. This model uses that the enzyme *phosphofructokinase* (PFK) seems to play an important role in the mechanism responsible for the oscillations. Using the Yang-Ling isotherm model we have modeled the binding of the substrate ATP and the activator ADP to PFK. We further assume that the dissociation constants for binding of ATP and ADP are proportional to the rotational correlation time ( $\tau$ ) of intracellular water and that the change in  $\tau$  depends on the activity of intracellular ATP. Simulations of the model show oscillations in ATP coupled to oscillations in  $\tau$ . ATP and  $\tau$  oscillate almost in phase, consistent with the experimental observations. Furthermore the oscillations only occur in a small region of resting  $\tau$  values. Outside this narrow region the model exhibits only steady-state behavior. As in the experimental system the transition from steady-state to oscillation takes place through two supercritical Hopf bifurcations.