Project description for the application of a scholarship from the "International Graduate School in Molecular Medicine Ulm" for the years 2013-2016 by Nils Johnsson, Institute of Molecular Genetics and Cell Biology, Ulm University.

Title: Differential segregation of cellular components and organelles during replicative ageing of yeast cells.

The baker's yeast *Saccharomyces cerevisae* is a favored model organism for investigating molecular aspects and causes of ageing. Yeast cells divide by growing a bud. The cell division is highly asymmetric. Due the selected distribution of factors responsible for the ageing of the cell, the daughter cells are always born young whereas mother cells actively keep the age-contributing factors and become older during each completed cell cycle. Mother cells die after giving birth to about 30 daughters. The search for ageing factors did not reveal one single determinant but suggested that different factors contribute additively to the ageing of the cell. In this proposal we aim to systematically investigate organelles, proteins and membranes for their asymmetric distribution according to their age and thus try to identify further factors contributing to cellular ageing.
Specifically we propose:

1. Based on our SNAP-tag based method of specifically labeling the cell surface of yeast cells with small-molecule compounds, we will devise a procedure to enrich and isolate very old and very young haploid cells of opposite mating types.
2. By mating old with very young haploid cells we will create diploids with a cytosol that consist of an equal mix of old and young components.
3. We will follow the mating of old with young cells on-line by time-lapse fluorescence microscopy. By labeling the organelles/proteins/membranes of old cells with mCherry and those of young cells with GFP we can specifically follow the distribution of the old and young components during the first cell cycle and the generation of the first diploid daughter. Components indicating ageing will be revealed by their non-equal distribution of their mCherry and GFP-versions in the daughter cell. Specifically, we would expect to see a high ratio of green to red fluorescence of those organelles/proteins that are actively retained in the mother cell due to their comparably old age.
4. We will follow this strategy in different yeast deletion strains to quantitatively evaluate the impact of the deleted genes on the age-specific retention of the identified components.
5. We will measure protein-protein complexes with high spatial and temporal resolution in living single cells to compare the interactions between the receptors of ageing-relevant cargos and their transporters in old and young cells.
6. By manipulating these interactions we aim to create yeast mutants that do not retain the ageing factors in the mother cells. These mutants will help to evaluate whether these factors not only indicate but also induce premature ageing of daughter cells.

We strongly expect that our novel search for components that affect ageing and the mechanisms of their retention will open new ways of studying ageing in higher eukaryotes.