Endocytosis of membrane proteins in yeast requires α-arrestin-mediated ubiquitylation by the ubiquitin ligase Rsp5. Yet, the diversity of α-arrestin targets studied is restricted to a small subset of plasma membrane (PM) proteins and the regulation of trafficking functions of α-arrestins remains largely focused on carbon and amino acid metabolism. Our lab performed quantitative proteomics to identify new targets of 12 α-arrestins and gained insight into a role of Art2 and Art9 α-arrestins in thiamine (vitamin B1) homeostasis. Indeed, in cycloheximide (CHX)-treated conditions, the thiamine transporter Thi7 was 4- and 11-fold more abundant at the PM in art2Δ and art9Δ strains compared to a wild-type strain, respectively. Fluorescence microscopy and western blot analyses confirmed that Art2 is required for thiamine- and CHX-induced, Rsp5-dependent, endocytosis of the three thiamine transporters Thi7, Nrt1 and Thi72.

In this study, we demonstrated that endocytosis of Thi7 requires Art2-dependent ubiquitylation of its cytosolic C-terminal tail. To address the underlying mechanism of Thi7 endocytosis in response to thiamine transport, we developed a genetic screening to isolate transport-defective Thi7 mutants, which impaired thiamine-induced endocytosis. Two mutants showed a strong transport defect: Thi7N585K and Thi7M599R. Then, we generated three-dimensional models of native and mutated Thi7 and simulated thiamine docking to predict the thiamine binding site. Eight mutants appeared to result from mutations of residues forming/surrounding the thiamine binding pocket: Thi7C596R, Thi7D626G, Thi7N113K, Thi7M247I, Thi7P258Q, Thi7N585K, Thi7V588F and Thi7M599R. Therefore, their transport defects might be explained by disturbances localized in the binding pocket that prevent interaction and stabilization of thiamine. Then, co-expression of inactive mutants with wild-type Thi7 revealed that both transporter conformation and transport activity are important to induce endocytosis. We also provided evidence that Art2-mediated Thi7 endocytosis requires the Sit4 phosphatase, involves the TORC1 complex but is not inhibited by the Npr1 kinase.