Peroxisomes play crucial roles in dormancy breaking, lipid metabolism, synthesis of plant hormones, photorespiration and redox homeostasis. As peroxisomes contain no DNA all proteins must be imported from the cytoplasm and the metabolic function of peroxisomes will be determined by the enzymatic complement of the organelle. Analysis of mutants in peroxisomal enzymes or in biogenesis of the organelle demonstrate that correct functioning of peroxisomes is essential for all stages of the plant life cycle from germination to seed set. Because peroxisomes play such vital roles in multiple biochemical processes, biogenesis mutants often have very strong developmental phenotypes, making cellular and biochemical studies difficult. As a complementary approach we have been using small molecules as tools to probe different aspects of peroxisome biogenesis and positioning. An advantage of this approach is that there is a greater degree of experimental control in terms of timing of application and dosage of the small molecule and the possibility of ‘wash out’ experiments to reverse effects. This approach has yielded molecules with differential effects on protein import pathways and peroxisome location within the cell.