This research used live cell imaging to report on the disassembly and subsequent recovery of the Golgi apparatus in tobacco leaf epidermal cells and Arabidopsis thaliana during Brefeldin A (BFA) treatment. BFA, a macrocyclic macrotone, is a fungal drug commonly used in vesicle-mediated protein trafficking research for its ability to disturb the guanine-nucleotide exchange factors (GEFs) function resulting in a blockage of protein trafficking. To observe the effect of the drug on the plant Golgi apparatus and to reveal more about the related protein trafficking mechanism, different concentrations of the BFA were used and tested on tobacco leaf epidermal cells and multiple tissues including leaf, hypocotyl and root tissue of A. thaliana. The Golgi related proteins of interest included a small GTPase ADP ribosylation factor (ARF), trans specific sialyltransferase (ST), a cis/medial specific part of a β1,2-N-acetylglucosaminyl-transferase-I (GnTI), and a post-Golgi compartment protein (PS1). Those proteins were fluorescently tagged using green fluorescent protein (GFP) and monomeric red fluorescent protein (RFP) and underwent 3 hr or overnight BFA incubation. The results have confirmed previously described disruptive effect of the BFA on the Golgi apparatus and protein trafficking in general to be time and dose dependent and even tissue specific in A. thaliana. For the ARF-GFP activity in tobacco, the protein retreat from the Golgi into the cytosol was seen after just 60 min incubation with 25µg/mL BFA and indicated the Golgi recovery in longer overnight samples only. In the case of PS1-GFP in tobacco, the Golgi disruption was recorded after 60 min BFA incubation with the protein locating to vacuoles. The reappearance of Golgi bodies occurred after 210 min drug treatment. For ST-GFP, the results from tobacco showed slower disassembly of the Golgi apparatus and the protein retreat into the endoplasmic reticulum (ER) after 90 min of 25µg/mL BFA treatment but much faster progression of Golgi depletion was recorded in ST-GFP expressing roots of A. thaliana. On contrary, the Golgi disassembly in the leaves and hypocotyl of A. thaliana were recorded after 120 min drug incubation and the Golgi recovery was the fastest in hypocotyl with just 210 min after the BFA incubation initiation, showing the diverse effect of BFA across Arabidopsis tissues.

To assess the manner in which Golgi recovery occurs further, A. thaliana expressing both cis/medial located GnTI-mRFP and trans located ST-GFP markers was used in additional BFA treatment experiment. The results of this experiment suggested that the reassembly of the Golgi apparatus happens in cis to cis/medial and trans manner.

Acknowledgement: The authors would like to thank Dr Anne Osterrieder and Dr John Runions, both from Oxford Brookes University, for their help during the research.
Fig. 1: Wild-type tobacco epidermal cells expressing ST-GFP. Micrographs show three BFA [25 µg/mL] treatment times: 0, 120 min treatment and overnight (20 hrs). The ER and the nuclear envelope are clearly visible at 120 min of BFA treatment but no ER is seen before and overnight samples - showing the recovery ability of ST proteins. Scale bars = 20 µm.

Fig. 2: A. thaliana leaves coexpressing cis located GnTI-mRFP (red) and trans located ST-GFP (green) treated with BFA [25 µg/mL] for 150 min. The merged micrograph shows that the ER proteins come from different parts of the Golgi apparatus. The reassembly of the Golgi apparatus happens in cis to cis/medial and trans manner. Scale bars = 20 µm.