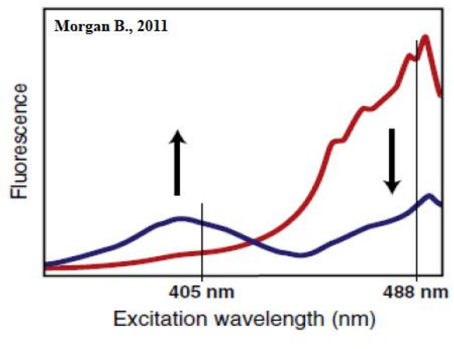
# Determination of the topology of microsomal 17β-hydroxysteroid dehydrogenase enzymes using redox-sensitive green-fluorescence protein fusions

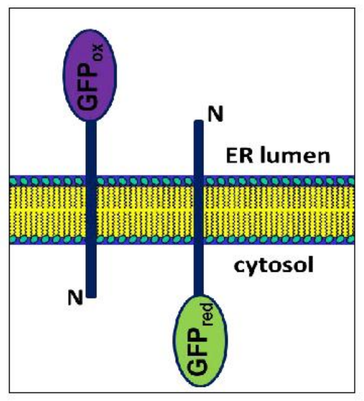
**Maria Tsachaki[1], Julia Birk[1] and Alex Odermatt[1]**

[1] Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland

## Introduction and objectives

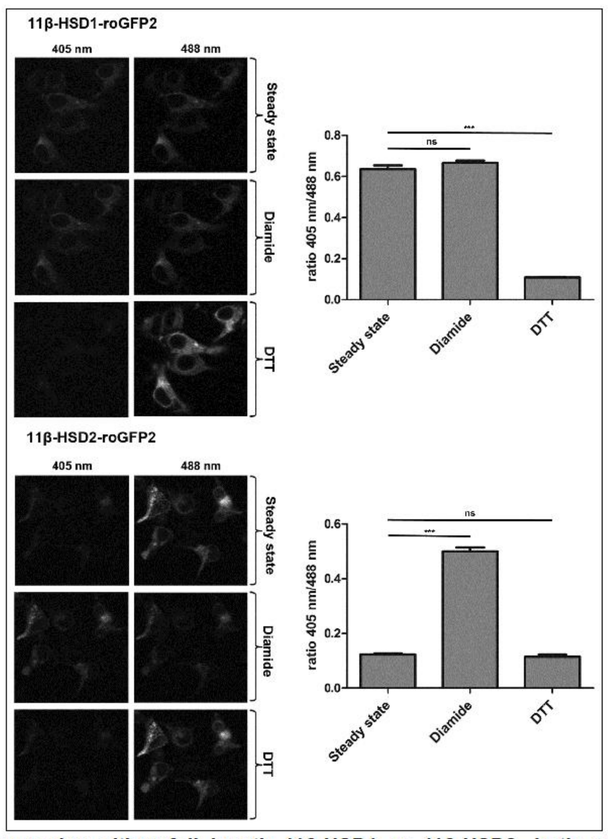
**Membrane proteins of the endoplasmic reticulum (ER) are involved in a wide array of essential cellular functions. Identification of membrane protein topology can provide important insights into their mechanism of action and biological roles. This is particularly important for membrane enzymes, since their topology determines the subcellular site where a biochemical reaction takes place and the dependence on luminal or cytosolic co-factor pools and substrates. We have employed a method for defining the topology of ER membrane proteins in living cells, based on fusion of the respective protein with redox-sensitive green fluorescent protein (roGFP). We validated the method using microsomal 11β-HSD proteins whose topology has been resolved, and comparing with an independent approach. We then implemented the roGFP method to determine the membrane topology of six microsomal members of the 17β-hydroxysteroid dehydrogenase (17β-HSD) family.**



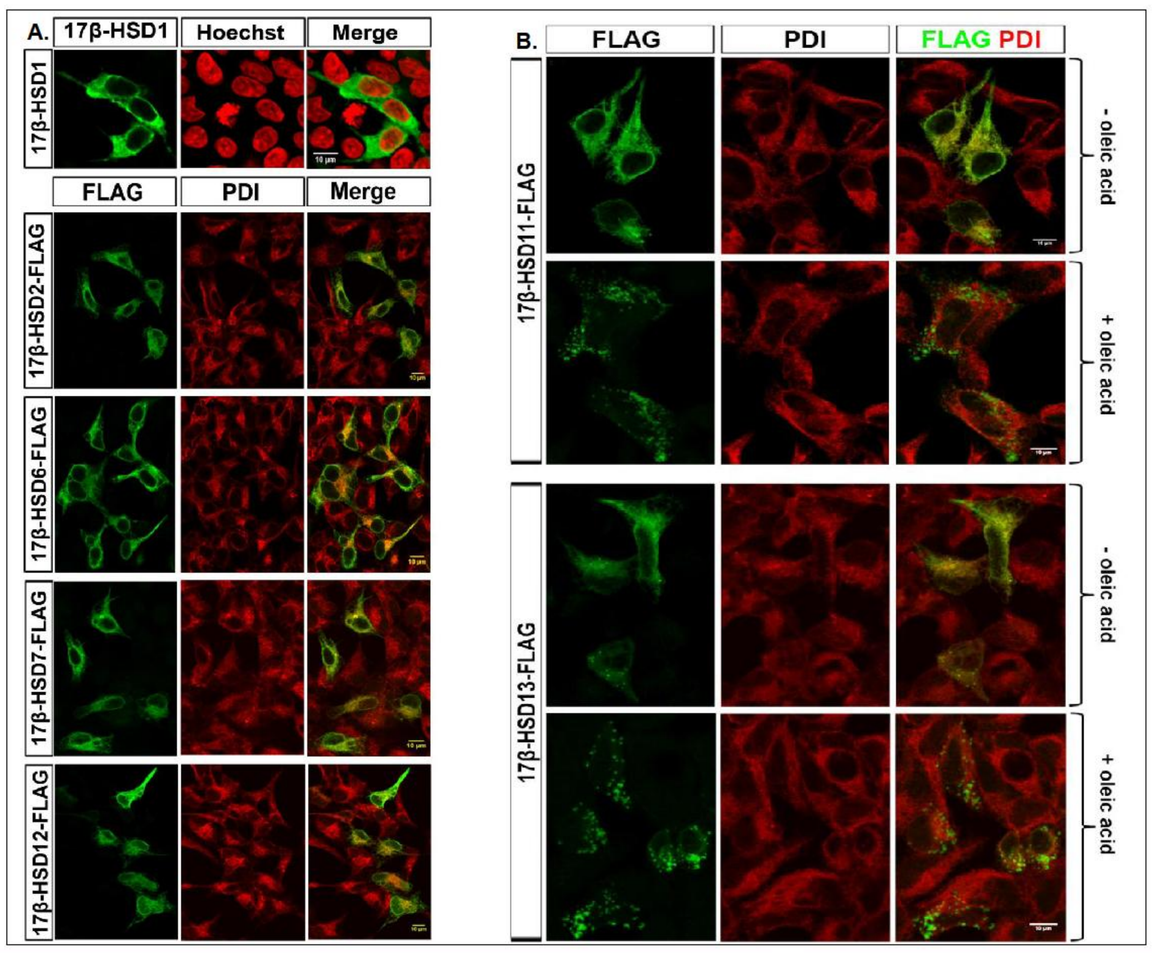

## Results

### The roGFP method for topology determination- Proof of concept




**HEK-293 cells expressing either full length 11β-HSD1 or 11β-HSD2, both containing C-terminal roGFP2, were imaged by laser-scanning confocal microscopy after excitation at 405 nm and 488 nm at steady state and upon sequential addition of diamide and DTT. Results shown in the bar graphs represent mean ± S.D. from three independent experiments, after analysis of 8-10 cells per experiment.**

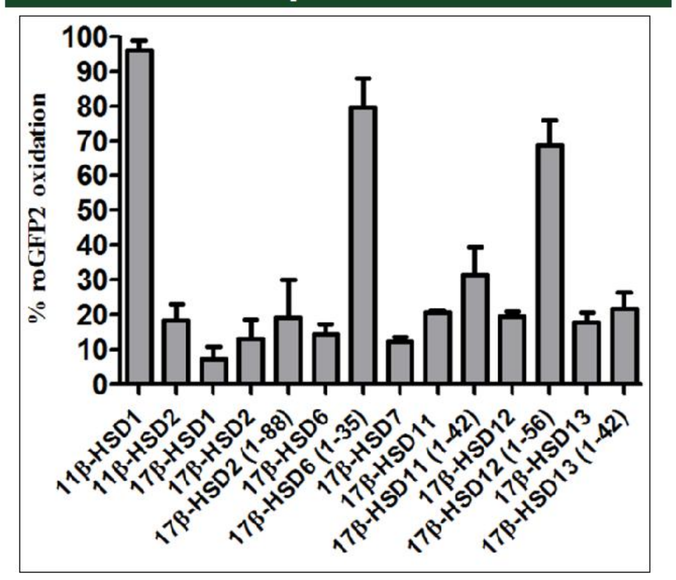
### Subcellular localization of the 17-βHSD enzymes




**A. 17β-HSD1, 17β-HSD2-FLAG, 17β-HSD6-FLAG, 17β-HSD7-FLAG and 17β-HSD12-FLAG were expressed in HEK-293 cells. 17β-HSD1-transfected cells were stained with rabbit anti-17β-HSD1 antibody and Hoechst-33342 dye for nuclear staining. The remaining transfected cells were stained with anti-mouse PDI and anti-rabbit FLAG. Representative confocal images of the localization of proteins are shown (anti-17β-HSD1: green, Hoechst-33342: red, FLAG: green, PDI: red).**

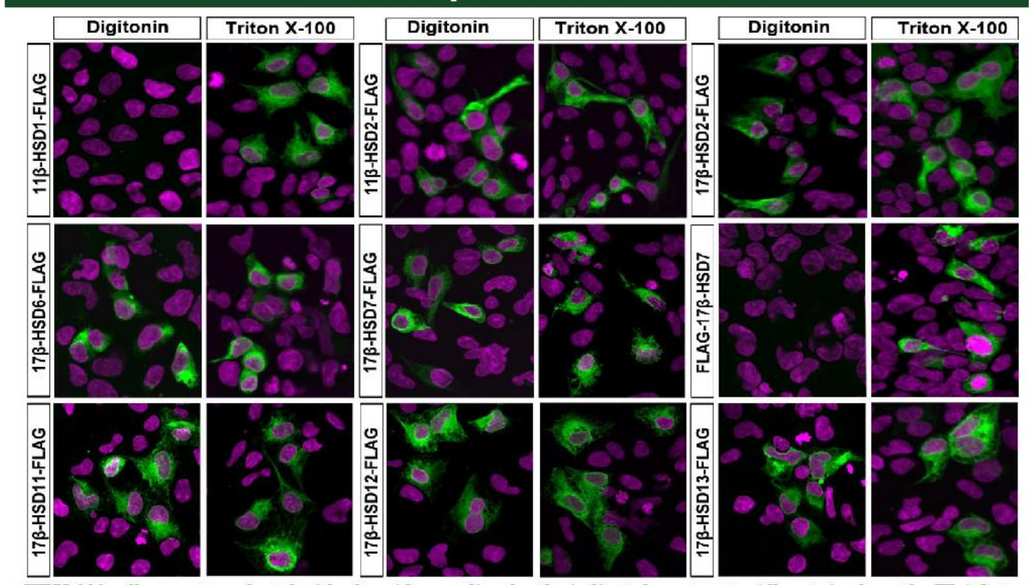
**B. HEK-293 cells were transfected with constructs encoding for 17β-HSD11-FLAG and 17β-HSD13-FLAG and left untreated or treated with oleic acid for 16 h to trigger formation of lipid droplets. At 48 h post-transfection cells were immunostained with rabbit anti-FLAG antibody and mouse anti-PDI antibody. Representative confocal images of the immunostainings are shown (FLAG: green, PDI: red).**

### Determination of the topology of 17β-HSDs



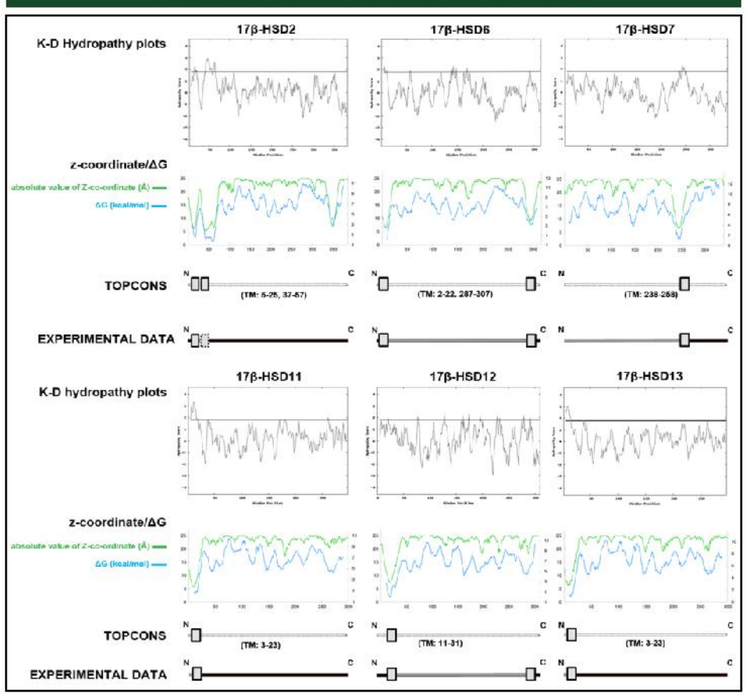

**roGFP2 fusions of the indicated enzymes were expressed in HEK-293 cells. Samples were imaged 48h post-transfection after excitation with 405 and 488 nm lasers at steady state, upon addition of diamide and DTT. The percentage of oxidation of roGFP2 was calculated and plotted (mean ± S.D. from three independent experiments).**

### Validation of the roGFP2 fusion method using selective permeabilization



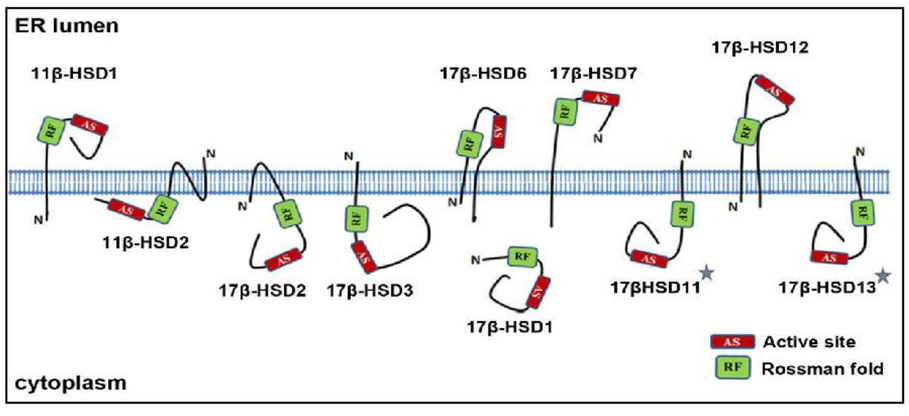

**HEK-293 cells were transfected with plasmids encoding for the indicated constructs. All proteins bear the FLAG tag at the C-terminus, except for FLAG-17β-HSD7, which bears an N-terminal FLAG. At 48 h post-transfection cells were fixed, permeabilized with digitonin or Triton X-100 and stained with anti-FLAG and Hoechst-33342 dye. Fluorescence was analyzed using confocal laser-scanning microscope (FLAG: green, Hoechst-33342: magenta).**

### *In silico* analysis of 17β-HSD structural architecture




## Conclusions

- **We describe a method for the identification of ER membrane protein topology in mammalian cells.**
- **Using this approach we determined the topology of 6 members of the 17β-HSD family of enzymes. Many 17β-HSDs have been implicated in different forms of sex-specific cancer and 17β-HSD inhibitors have attracted considerable interest as therapeutic targets. Appropriate access of such inhibitors to the respective intracellular compartment needs to be considered in drug development.**
- **Knowledge of the intracellular location of the catalytic site of these enzymes will enable future studies on their biological functions and roles in human diseases.**




## References

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Maria Tsachaki

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