Proper functioning of the intestines necessitates a sophisticated, fine-tuned crosstalk between specialized cell types which integrate the nervous and hormonal signals. Sex hormones, particularly estrogens, and their fluctuation with age and critical phases such as puberty and menopause, might induce epigenetic changes that contribute to the differential pathogenesis and epidemiology of **inflammatory bowel disease (IBD)**. IBD is characterized by non-infectious chronic inflammation of the gastrointestinal tract, which causes a significant deterioration of life quality.

Several exact molecular pathways involved in the pathogenesis of IBD were outlined, including the role of stress-responsive transcription factor NRF2 (nuclear factor erythroid 2-related factor 2). Notably, NRF2 transcriptional activity significantly deteriorates with age and NRF2 gene polymorphism are associated with IBD. Accordingly, our studies showed that **NRF2 deficiency triggers IBD-like changes in the function of the colon of female mice, and it can be mitigated by modulation of estrogen signaling**. Furthermore, more general studies showed that histological outcomes associated with induced colitis are more severe in females compared to male mice, and 17β-estradiol treatment to male mice ameliorated intestinal damage pointing a significant role of sex-hormones in IBD development and progression. Therefore, **NRF2 knockout mice may be a useful model to study the ethology of IBD** and its relapses episodes without using harmful drugs to induce colitis.

Until now, our research focused on clear depiction of abnormal phenotype and function of colon in NRF2 KO mice. We have not addressed the main causative mechanisms of the impairment, including the major affected cell types. Therefore, in this project, we aim to **verify if the epithelial cells are the major driver of the defective function**, and through combination of unique mouse models and high throughput analyses, we want to depict the major molecular mechanisms explaining our phenotype. In parallel, **through mechanistic studies, we will comprehensively address the most plausible causes of anomalous phenotype,** including microbiota composition, neuro- and endocrine modulation. Importantly, available data pinpoint the crucial role of both NRF2 and estrogens in these regulatory mechanisms. Still, no one up to now has analyzed their significance in NRF2-related experimental settings.

Notably, we could partially rescue the functional impairment in NRF2 KO by exogenous administration of estradiol. At the same time, we found that estradiol dosage must be carefully finetuned, as in wild-type animals we observed excessive intestinal motility upon treatment. **It implies that understanding the reciprocal relation of NRF2 and estrogen signaling is necessary to address the therapeutical relevance of estrogens and explain the adverse effect of drugs impacting estrogen level (such as contraceptives). We hypothesize that modulation of estrogen signaling could alleviate the IBD-like symptoms.** In the frame of the project, **leveraging state-of-art models**, we would like to comprehensively address the role of estrogens to provide a broad therapeutical perspective.

In whole, we aim to explore the premise that local or global NRF2 TA regulates intestine function via modulation of endocrine and/or neurocrine factors and/or gut microbiota and if targeting those processes, under NRF2 TA deficiency, may change IBD's development and/or progression. The main hypothesis will be verified by **four interrelated research questions: I)** Is intestinal epithelium a driver of changes in intestinal structure and may it influence intestinal functionality? **II)** Are estrogens the key modulators of intestinal function under NRF2 deficiency? **III)** Are there any other processes which may be involved in changed gut function under NRF2 deficiency? **IV)** May NRF2-estrogen pathway be a possible target for IBD treatment?

This project will combine *in vivo* research on mice, using the gold-standard models of IBD and assessment of intestinal function. If rationale, we will perform 'rescue experiments' to provide detailed mechanistic result. Moreover, we will use human and mice intestinal organoids to verify if targeting estrogen receptor signaling may change IBD's development and/or progression under NRF2 deficiency, which increases translational potential of the project.

Predicted outcome of the proposed study is an indication of the origin of morphological changes of the intestines upon the lack of NRF2 and evidencing mechanisms of protection of NRF2 inhibition against gut dysfunction. The ultimate goal of this study is to characterize molecular background of the inflammatory bowel diseases which are **chronic** and oftentimes progressive inflammatory diseases of the bowel with periods of acute or subacute exacerbations that affects nearly **3.9 million females** and nearly 3.0 million males are living **with IBD worldwide**. This is important for health-care delivery systems and economies because **standard care** for IBDs, particularly immunotherapies, is **extremely costly**.