	GL leader	**Jiří Nováček, PhD**
1	**CEIPEX RESEARCH TOPIC LEVEL2**	**Structural Biology: protein structure and dynamics; protein-DNA interactions**
2	**RESEARCH GROUP**	**Cryo-Electron Microscopy and Tomography Core Facility**
3	**TOPICS/FOCUS**	Advancing Time-Resolved Cryo-EM to Elucidate Insulin Receptor Inhibition Mechanisms
4	**SUMMARY**	The proposed project will aim to provide insight into the activation mechanism of the insulin receptor (IR), a key regulator of glucose homeostasis and a prototypical receptor tyrosine kinase. Despite the availability of high-resolution structures of apo and fully bound IR, the sequence and timing of intermediate conformational states remain poorly understood. We will implement a comprehensive time-resolved cryo-electron microscopy (trEM) workflow to capture structural snapshots from receptor activation on both microsecond and millisecond timescale. For that, we will first adapt an integrated cryo-fluorescence microscope, developed in the CEITEC cryo-EM core facility (CEMCOF), into a microsecond-resolved cryo-sample preparation platform. This will enable precise triggering (e.g., pH or photochemical stimulus), localized laser melting, and revitrification of the sample. The system will be calibrated using a pH-dependent conformational transition of a virus from the Picornaviridae family, a process with well-characterized microsecond-scale dynamics. Concurrently, we will benchmark a time-resolved cryo-EM plunger, currently under development at CEMCOF, which will utilize mix–spray–plunge vitrification >100ms time scale and calibrate its performance using a well-studied case of bacterial ribosomal subunits association. These two complementary approaches will then be applied to study the insulin receptor activation process at multiple temporal resolutions. By capturing intermediate states during insulin binding and receptor conformational changes, we aim to reconstruct the sequence of structural events that underlie IR activation. The project will not only advance mechanistic understanding of insulin signaling but will also establish a validated infrastructure for time-resolved cryo-EM as a service to the broader research community.
5	**RG WEBPAGE/CONTACT**	https://cryo.ceitec.cz/