**FUNDING PROGRAM FOR NEXT GENERATION WORLD-LEADING RESEARCHERS**

| **Project Title**: Initiation and development of *in vivo* structural proteomics | |
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**1. Background of research**

In living cells, proteins function in an environment where they interact with various intracellular molecules and are subject to extreme molecular crowding that makes the cellular environment difficult to replicate *in vitro*. While *in vitro* methods of structure determination have made very valuable contributions to understanding the functions of many proteins, *in vivo* observations of 3D structures, structural changes, dynamics or interactions of proteins are required for the explicit understanding of the structural basis of their functions inside cells. Its non-invasive character and ability to provide data at atomic resolution make NMR spectroscopy ideally suited for the task. In-cell NMR spectroscopy yields multi-dimensional NMR spectra of macromolecules in living cells. For the first time we have determined the 3D structure of a protein exclusively on the basis of information obtained in living cells.

**2. Research objectives**

This research project focuses on the development of the in-cell NMR technique as a robust tool for the direct observation of three-dimensional structures, dynamics and interactions of various proteins in living cells.

**3. Research characteristics (incl. originality and creativity)**

In-cell NMR is receiving attention both at home and abroad as a breakthrough technique for investigating intracellular behaviours of proteins in detail. Since the report of the world's first 3D protein structure in living cells, our research group is contributing to leading-edge researches of this filed.

**4. Anticipated effects and future applications of research**

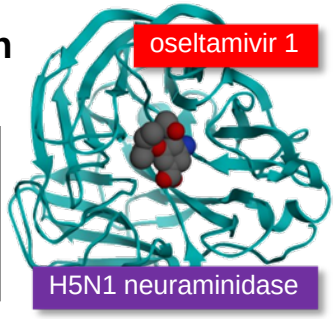
This research project opens new avenues for the investigation of proteins' conformations at atomic resolution and how they change in response to biological events in living environments.

The expected outcome of this research can have spillover effects on drug discovery and state-of-the-art medical technologies, since in-cell NMR-based techniques can be utilised as tools for efficient screening of new drags, validations of the effect of drags on our cells, etc.

# Structural Biology

**Understanding of the functions of biomacromolecules based on their 3D structures**

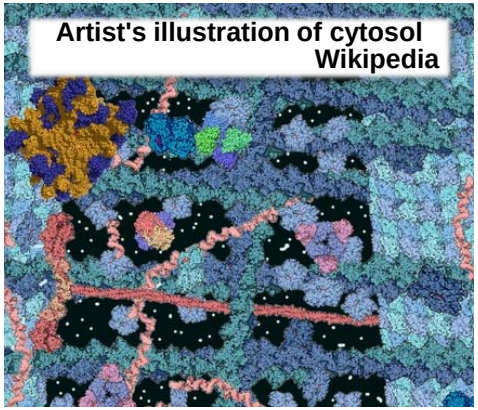
**e.g. 3D structures of neuraminidases led to the discovery of "oseltamivir"**
*Nature* **443**, 45-49 (2006)




**While *in vitro* methods of structure determination have made very valuable contributions, *in vivo* observations are required for the explicit understanding of the structural basis of their functions inside cells.**

# Intracellular environment

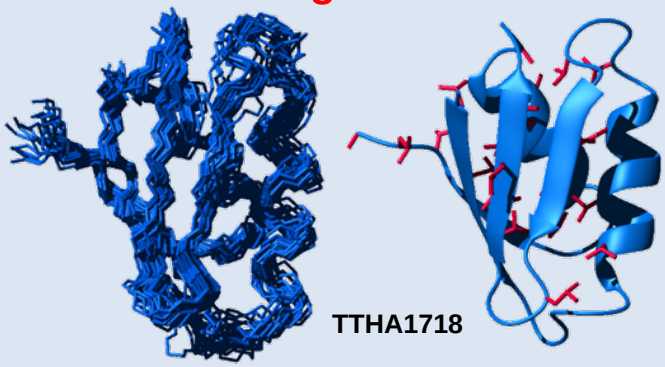
- **Macromolecular crowding**
- **Cytoskeleton divides the cytosol into a network of narrow pores.**
- **Dynamic and non-equilibrium state**
- **Crowding effect can make molecules in cells behave in different ways than in test-tube assays.**




**There were no tools to investigate proteins "at work" in a living environment at atomic resolution.**

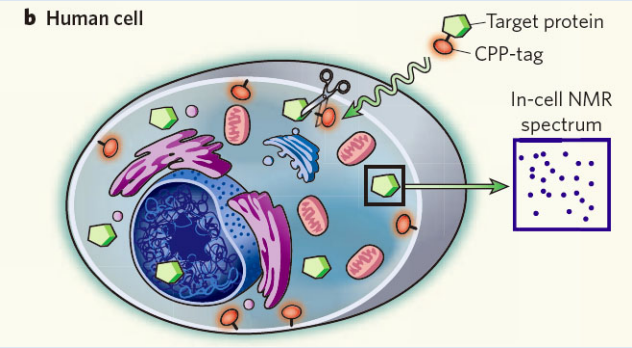
## In-cell NMR: a new tool for investigating structure and dynamics of proteins at work inside cells

**The world's first 3D protein structure in living cells**




Sakakibara, D. et al. *Nature* **458**, 102-105 (2009)

**High-resolution multi-dimensional NMR spectroscopy of proteins in human cells**




Inomata, K. et al. *Nature* **458**, 106-109 (2009)

# Development of the in-cell NMR technique as a robust tool for the direct observation of three-dimensional structures, dynamics and interactions of various proteins in living cells

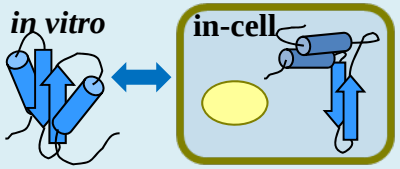
**Observation of detailed structural changes of proteins induced by intracellular environment.**

**Structure determination of intrinsically disordered proteins (IDPs) inside living cells.**

**Investigation of protein dynamics and folding inside living cells.**

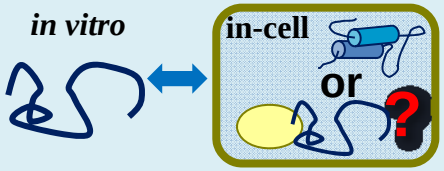
**Monitoring of protein-ligand and protein-protein interactions inside living cells at atomic resolution.**

## Structural changes?



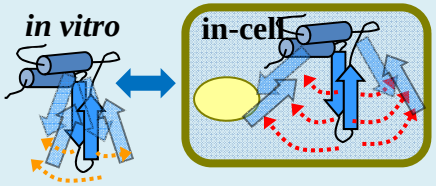

**Understanding of the effects of molecular crowding on protein conformation**

## Folded or disordered?



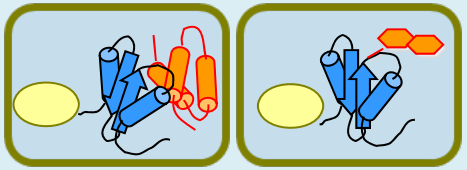

**Understanding of the structure-function relationship of IDPs**

## Difference in dynamics?




**Understanding of the effects of molecular crowding on protein folding mechanism**

## Interactions



**Experimental data for bioinformatics and simulation of intracellular responses**

# Future perspectives

**In-cell NMR-based techniques can be utilised as tools for efficient screening of new drags, validations of the effect of drags on our cells, etc.**

**The expected outcome of this research can have spillover effects on drug discovery and state-of-the-art medical technologies.**

**In-cell NMR observation of specific complexes of FKBP12 with extracellularly administered immunosuppressants in HeLa cells**