A Fluorescence-Based Technology to Identify Novel Therapeutics

Doctorate Program in Industrial Innovation

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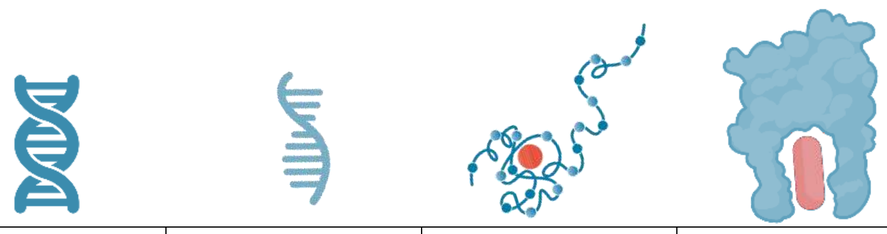
SIBYLLA Fold the future

BACKGROUND



DNA	RNA	PPI-FIT	Folded proteins
Single use, irreversibly modify a gene (usually with an unwanted mutation) or engineered cells	Antisense oligonucleotides, modify protein synthesis	Targeting a folding intermediate, inhibiting the physiological protein to fold and function	Targeting the 3D structure of known protein to modify its activity or interactions
Off-targets, not every tissue can be modified	Invasive and repeated administration	Still to be demonstrated	non-specific interaction, difficulty to target a large surface
Requires a known sequence!		**Requires a known structure!**	

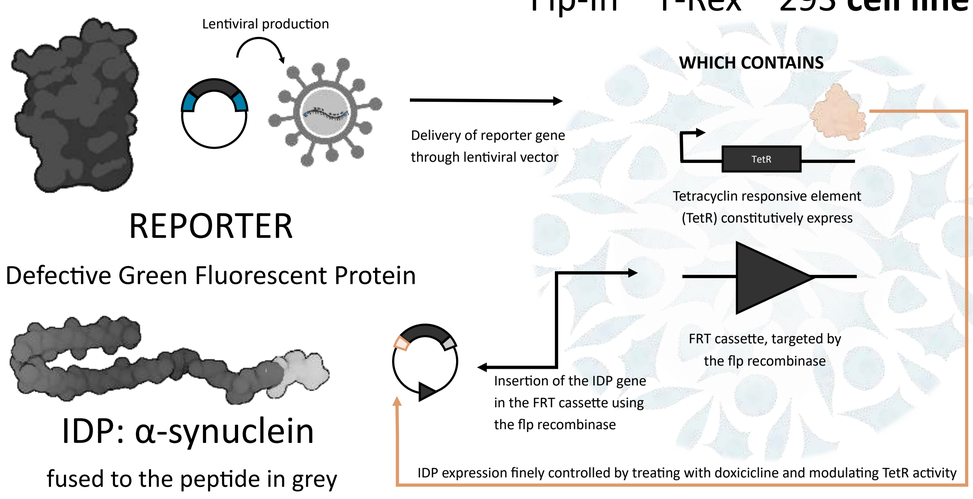
Intrinsically disordered proteins (IDPs) are a class of proteins **lacking a well-defined three-dimensional structure** under physiological conditions[2]. This peculiarity allows them to interact with a wide range of partners and play crucial roles in many cellular processes. However, this increased flexibility makes defining a probable binding site to screen compounds difficult, whereas folded protein structure can be an useful tool in the *in silico* screening for the drug discovery pipeline.

IDPs count for **32% of the human proteome** and another 19% of proteins have more than a third of their sequence as intrinsically disordered regions[3]. For decades trying to target these proteins pharmacologically has been a challenge. Still, their essentiality in various diseases, including **cancer, neurodegenerative disease, viral infections, and cardiovascular diseases**, makes them interesting targets to unravel.

We propose a **fluorescent cell-based technology** that will be used in a high-content screening to screen a fragment-based library and detect modulations in the behavior and concentration of the IDP under study. The first protein tested will be **α-synuclein**, an IDP that is involved in **Parkinson's disease**, it's the main component of Lewy Bodies and thought to play a role in the regulation of synaptic vesicle trafficking.

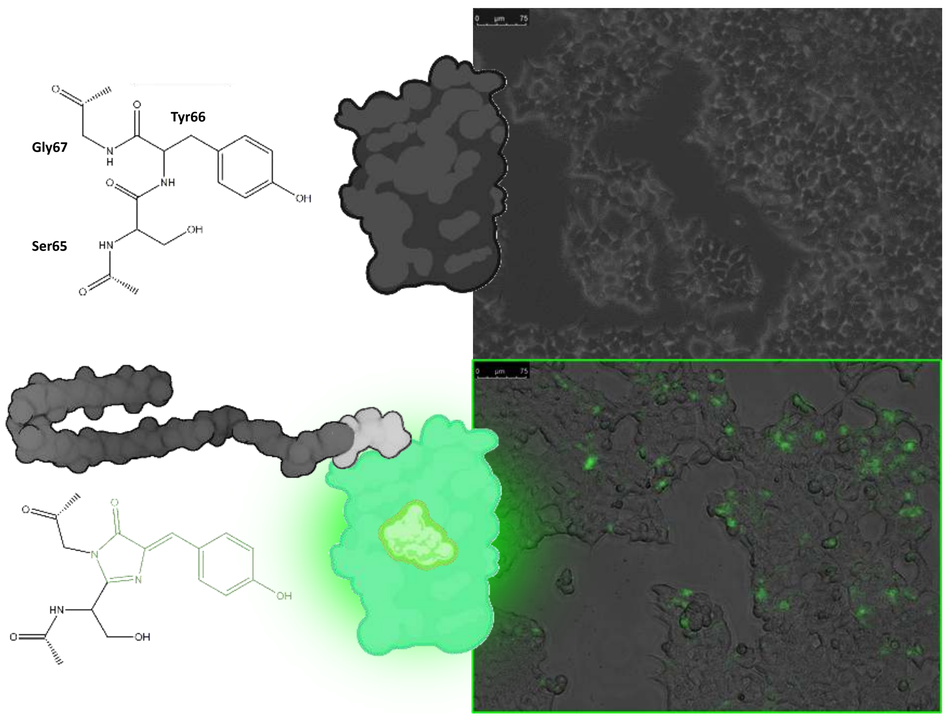
STRATEGY

CONSTRUCTS



We will use Flp In T-Rex™ HEK293 cell line[4] for their built-in **FRT cassette** to easily insert the IDP construct with the Flp recombinase. To insert the reporter defective fluorescent protein, we will use a **lentiviral vector** to have an high efficiency insertion in the cell genome.

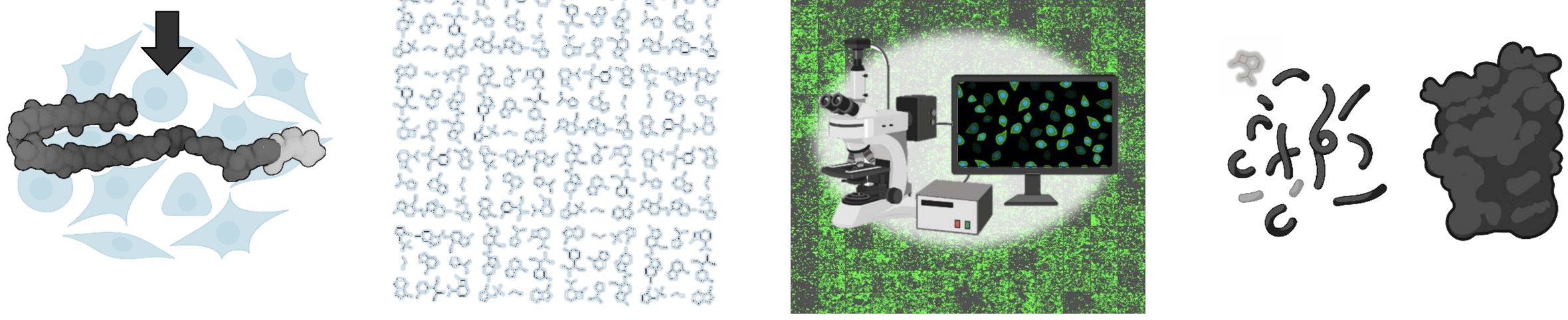
MECHANISM



When **only the reporter** defective fluorescent protein is expressed, **no fluorescence** can be spotted with the microscope.

When the **IDP expression is induced**, the small peptide fused to the IDP will covalently bond with the reporter, **triggering the catalysis** of the fluorophore for easy detection by live microscopy.[5]

HIGH CONTENT SCREENING WORKFLOW



INDUCIBLE IDP CELL LINE → FRAGMENT BASED DRUG-DISCOVERY → HIGH CONTENT SCREENING → EXPECTED RESULTS

The IDP upstream of the start codon has two tetracycline response elements, allowing **the control of its expression** treating with doxycycline, thanks to the T-Rex system. The reporter is constitutively expressed

Fragment libraries are composed of **highly soluble, small-sized organic molecules**.[6] These advantages increase the chances of finding fragments targeting the IDP, that later can be combined to form an optimize ligand.

With high content screening (HCS), it is possible to **discriminate different phenotypes** by **imaging subcellular events**.[7] This versatility significantly increases the possibility of correctly identifying a relevant pharmacophore.

If the molecule affects the proteostasis of the IDP, we will see a change in fluorescence. Further secondary analysis will exclude indirect effects. Hopefully, we will find molecule(s) **that interact directy with the IDP** that we can use to further optimization.

CONCLUSIONS and FUTURE PERSPECTIVES

This technology could be a novel imaging-based paradigm to study IDPs and be used in the drug discovery process. We chose to start with α-synuclein as our first target, and by the end of the year, we could start collecting some data. In the future, we could both characterize the pharmacophores we found in this screening or explore other targets.

BIBLIOGRAPHY

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