evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

*Characterization and Isolation of GFP Protein

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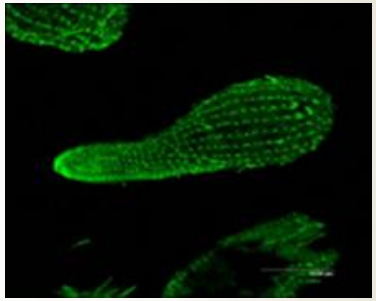
Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik

*GFP protein

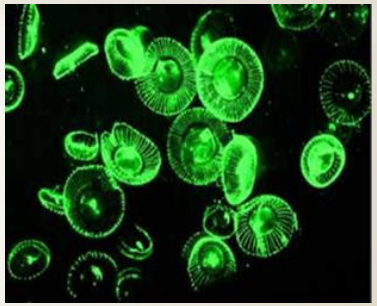
* Osamu Shimomura got the Nobel Prize in Chemistry in 2008, for the discovery and development of the green fluorescent protein (GFP)*(Shimomura O,Jhonson F, Saiga Y, 1962)*.



* GFP was isolated from the jellyfish *Aequorea victoria*

* GFP is commonly used as molecular imaging tool in biology, chemistry, genetics and medicine *(Phillips G, et al, 2001)*.

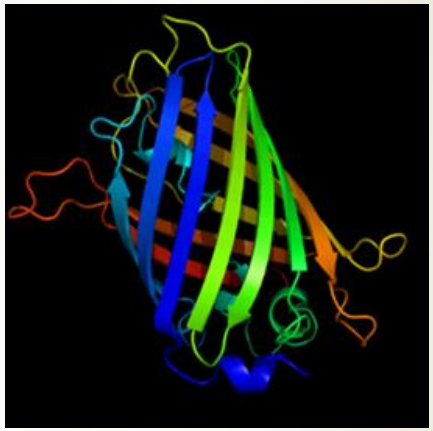
* The GFP gene has been introduced and expressed in many bacteria, yeast, fungi, fish, plant and mammalian cells.



* The enormous flexibility as non-invasive marker in living cells allows for numerous applications.

Structure of protein

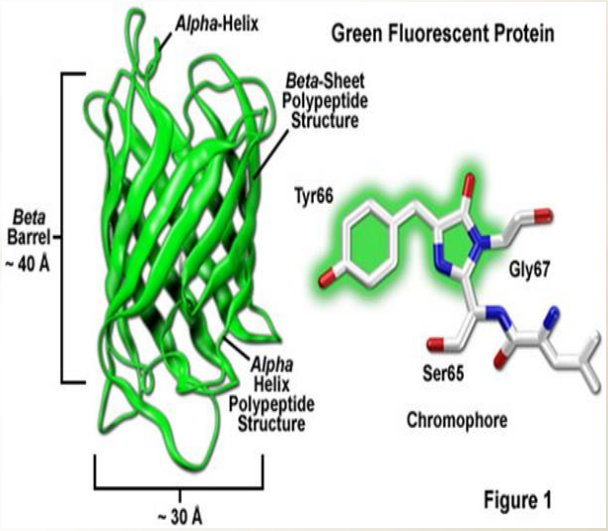
* This protein is composed of 238 amino acid residues. The excitation peak is at 395 nm and the emission peak is at 488 nm.



* GFP has a beta barrel structure, consisting of 11 ß-sheets with 6 alpha helix inside *(Tsien R, et al,1998)*

* The fluorophore or chromophore is a tripeptide consisting of the residues Ser65-Tyr66-Gly67 , which is post-translationally modified to highly fluorescent p-HBI structure *(Tsien R, et al,1998)*.

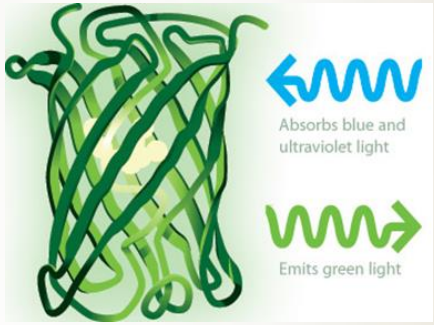
* The fluorophore is located in the middle of the beta-barrel and is the responsible of the fluorescence.

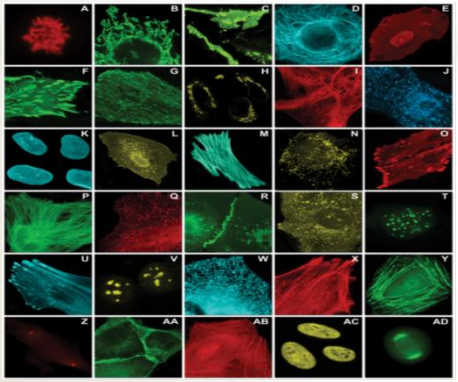


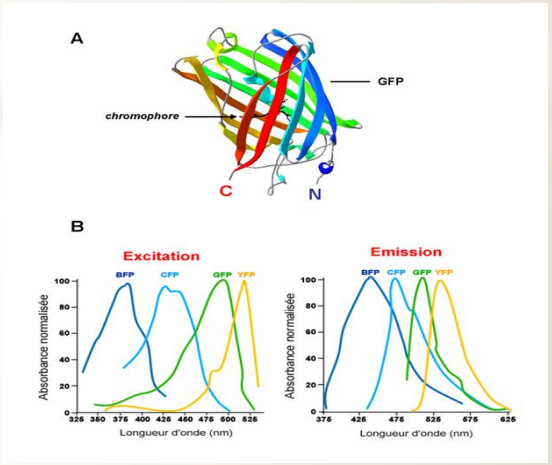
*GFP Types

* Mutagenesis of GFP has resulted in fluorescent proteins that range in colour from blue to yellow *(Hein R et al,1995)*.
* GFP-family members may be divided into seven classes based upon their emission maxima:

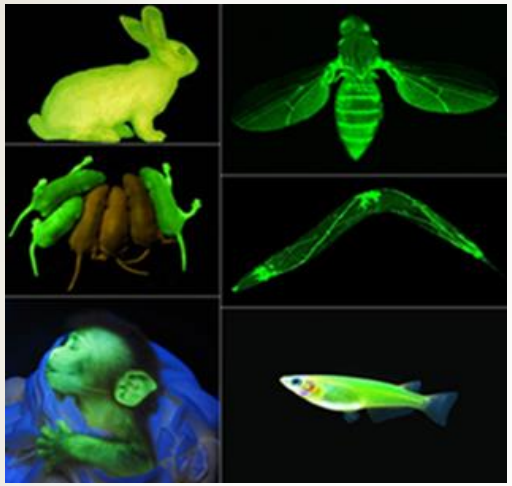
- ✓ **Blue fluorescent protein** (BFPs; λem = 440–470 nm
- ✓ **Cyan fluorescent protein** (CFPs; λem = 471–500 nm),
- ✓ **Yellow fluorescent protein** (YFPs; λem = 521–550 nm)
- ✓ **Green flourescent protein** (GFPs; λem = 501– 20 nm)
- ✓ **Orange flourescent protein** (OFPs; λem = 551–75 nm)
- ✓ **Red flourescent protein** (RFPs: λem = 576– 610 nm),
- ✓ **Far-red flourescent protein** (FRFPs; λem = 611–660 nm).

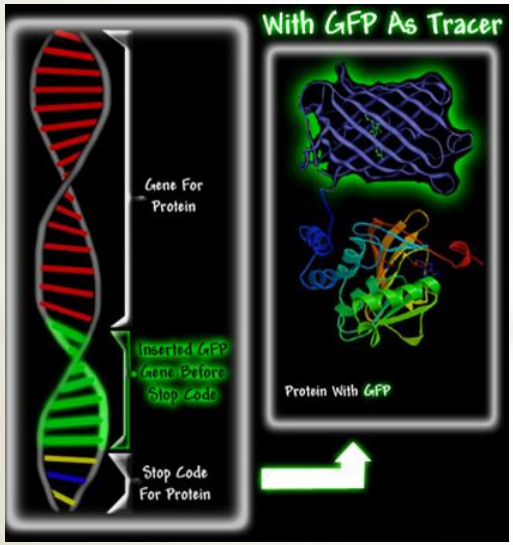






Fluorescence Microscopy

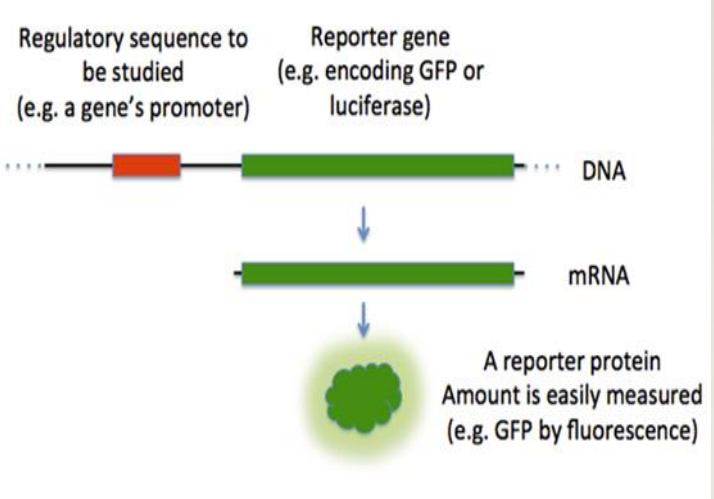


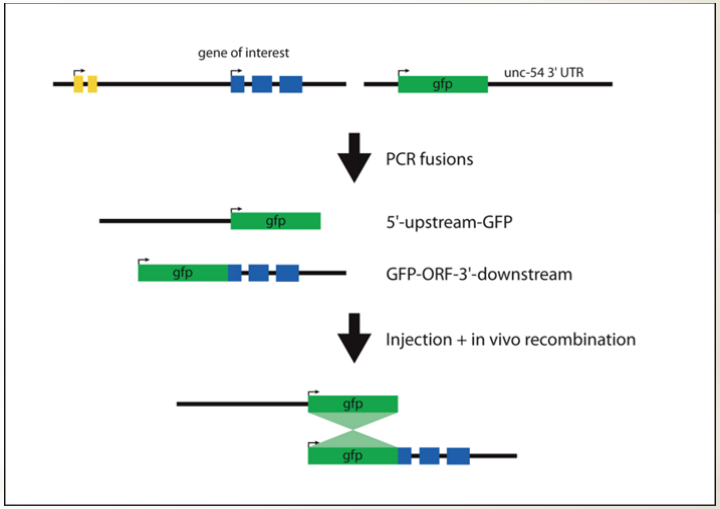


- The GFP and its derivatives has redefined Fluorescence Microscopy *(Chudakov D, et al,2005)*.
- When expressed in mammalian cells fluorescence from GFP is typically distributed into the cytoplasm and nucleus. Also is possible locate in mitochondria, secretory pathway, plasma membrane and cytoskeleton *(Yuster R, et al, 2005)*.
- The enormous flexibility as a non-invasive marker in living cells allows for numerous applications such as a cell lineage tracer, reporter of gene expression and a protein-protein interactions.

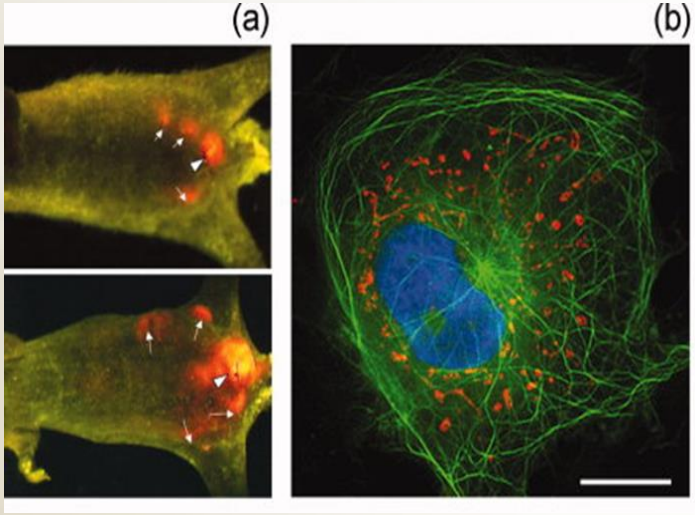
Other interesting uses of GFP

- Sensors of neuron membrane potential
- Tracking of the infection of some virus
- Protein folding and protein transport
- Estimation rates of gene expression
- Cryobiology
- Co-transfection in mammalian cells





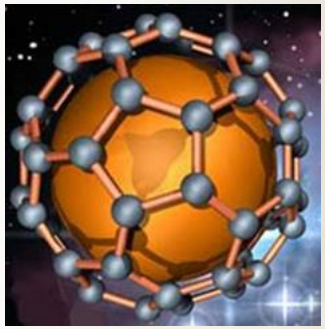
Elliott G,et al,2000



Applications of GFP-like proteins.
(a) Tumor progression in mice using DsRed-2
(b) Multicolor imaging in HeLa cells

GFP as biosensor

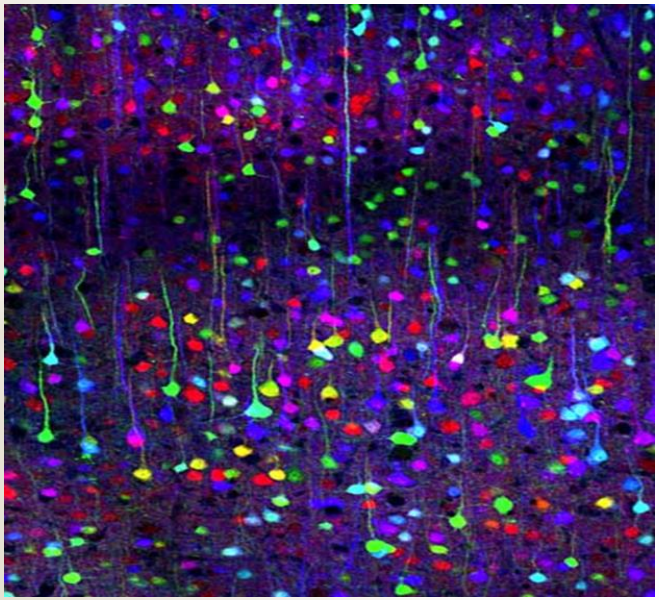
* GFP is a biomolecule with great stability, versatility and capacity to be readily mutated which make that this protein will be highly promising in the field of biosensors *(Barondeau DP, et al, 2002)*



* Biosensors based on GFP fusion proteins are tools for observing real-time events within living cells.

* Fluorescence Resonance Energy Transfer (FRET) could use GFP as an acceptor and Quantum dots (QDs) as donors. Will be possible use in areas such as the elucidation of biological molecules and their interactions, in vitro or in vivo assays.

*Our results



Laboratoř Metalomiky a Nanotechnologií
Laboratory of Metallomics and Nanotechnologies

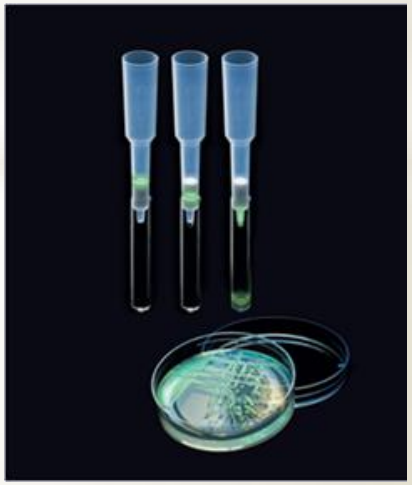
*GFP in Biotechnology

* The pGLO plasmid is a plasmid used in biotechnology as a vector for creating genetically modified organisms.

* The plasmid contains the green fluorescent protein (GFP) and the ampicillin resistance gene. The GFP gene is expressed in the presence of arabinose.

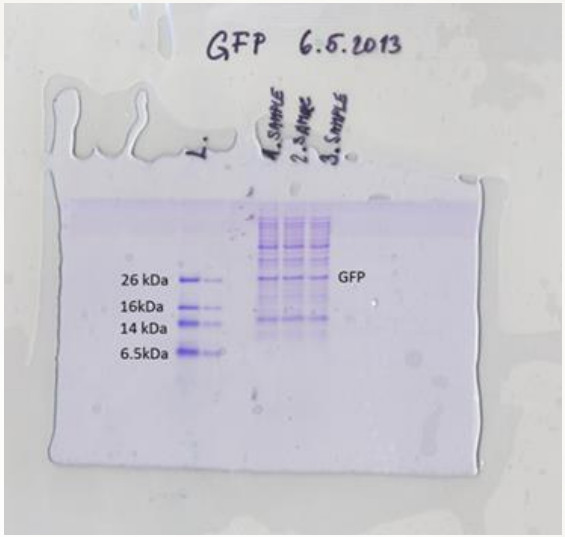
* GFP protein was purified from the bacterial lysate using hydrophobic interaction chromatography (HIC) columns (Macro-Prep® Methyl HIC Column, Biorad) (Markéta Komínková)

pGLO™ Bacterial Transformation Kit (Biorad)

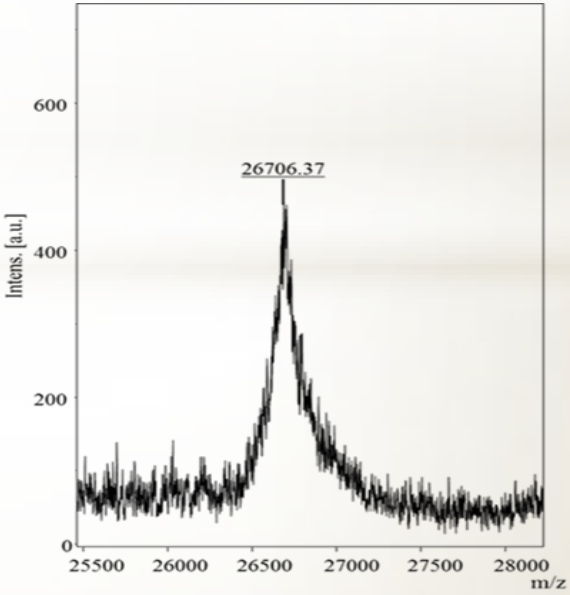


*GFP Characterization

* After protein isolation, GFP was characterized by gel electrophoresis, MALDI-TOF, and fluorescence imaging.



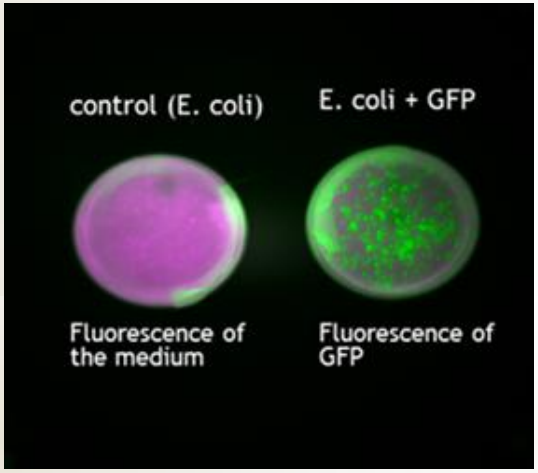
* The result in acrylamide gel and spectra by MALDI-TOF were corrects, the expected atomic mass of the full GFP was 26kDa in both methods (Miguel Angel Merlos).

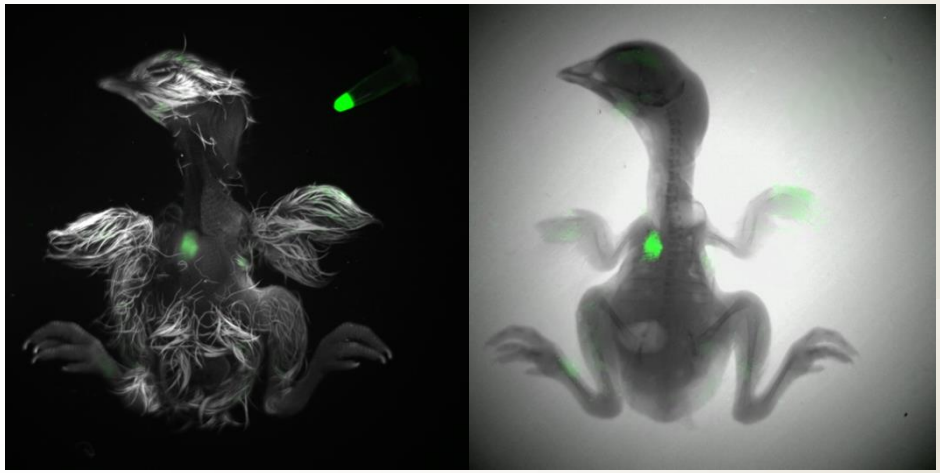


Fluorescence Detection

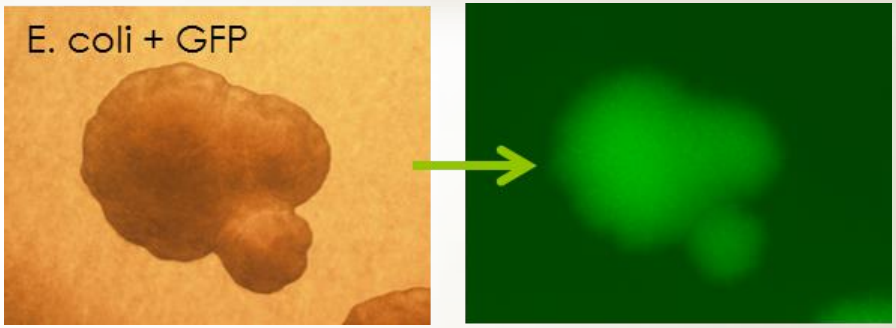
* It was possible detection of GFP protein by In-vivo Xtreme and by Flourescence microscopy (Iva Blažková).

In-vivo Xtreme





Flourescence Microscopy

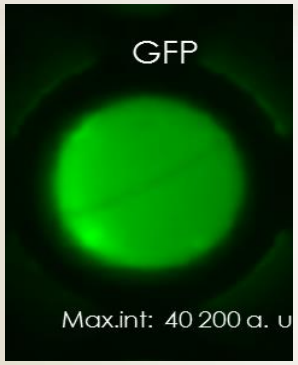


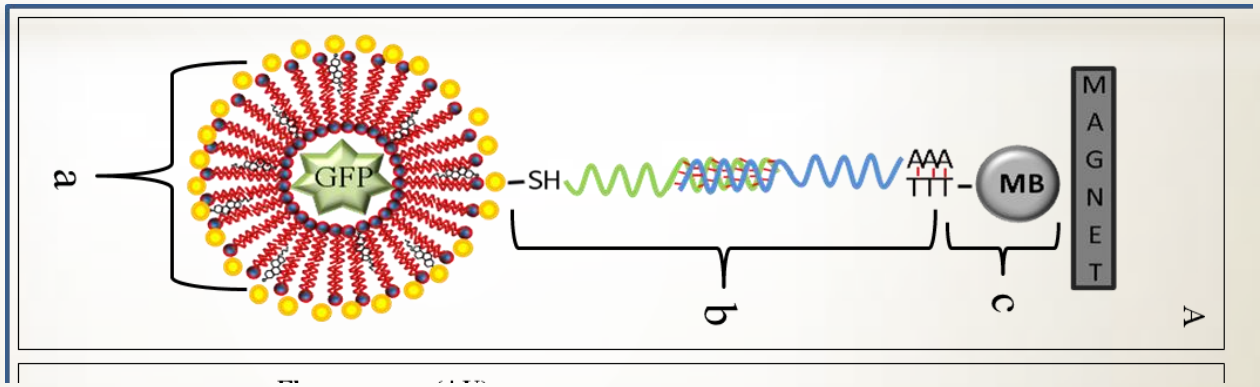
*GFP in liposome

* Liposomes are artificially-prepared microscopic particles formed by an lipid bilayer that encloses an aqueous compartment which are able to carry various types of compounds *(Akbarzadeh A, et al, 2013)*.

* The aim of this work was to propose and develop a modular nano device consisting of oligonucleotides and gold nanoparticles (AuNPs) modified liposomes that enclose GFP. Liposomes were isolated using magnetic microparticles *(Martins S, et al, 2007)*.

* The functionality of the procedure was verified using fluorimetry. The next step will be apply these liposome to into the human fibroblasts cell line (Lukas Nejdl and Iva Blažková).





Mendel University in Brno

*Thank you for your attention
Děkuji vám za pozornost ☺

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