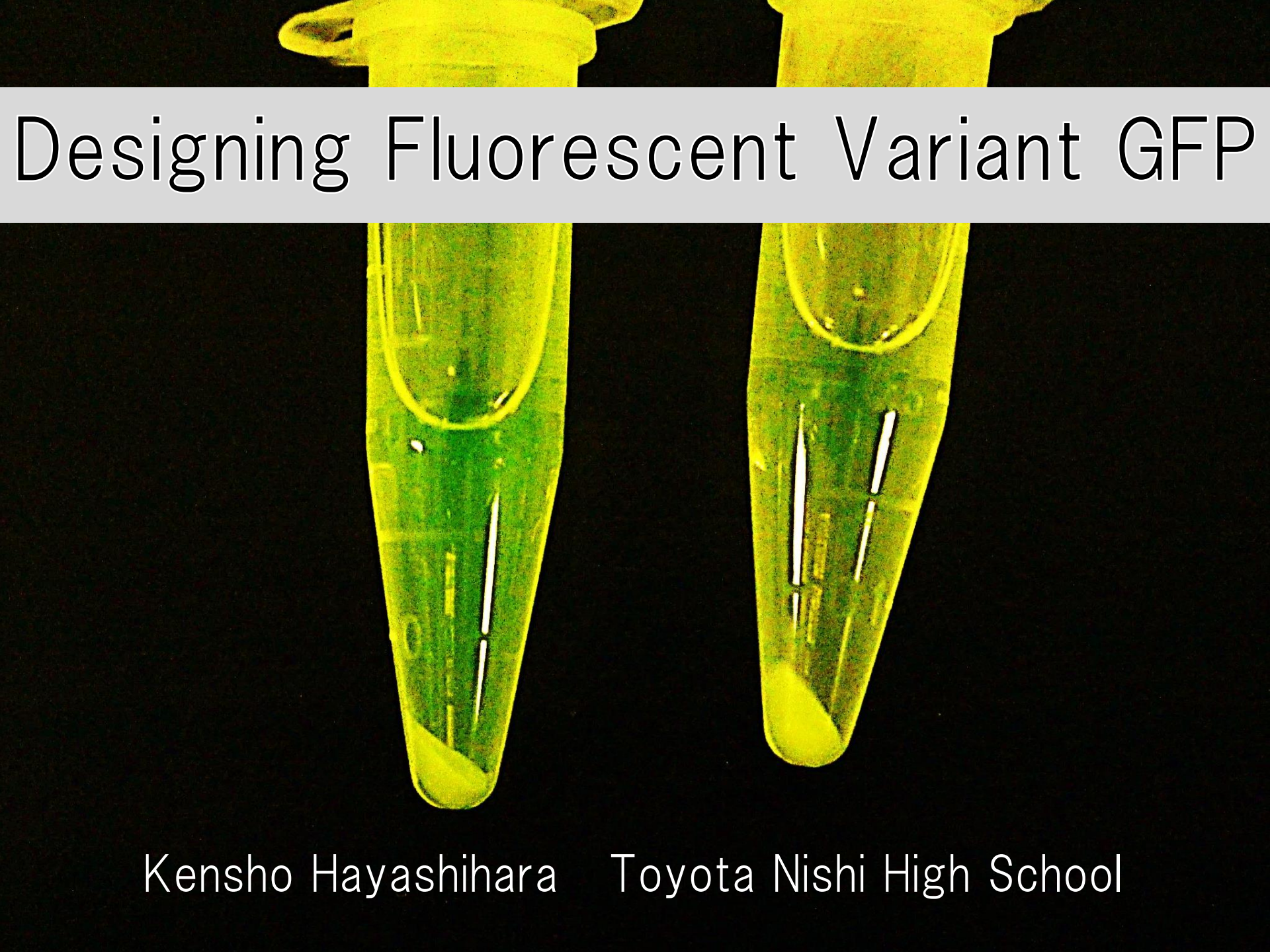
Designing Fluorescent Variant GFP



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- Hypothesis
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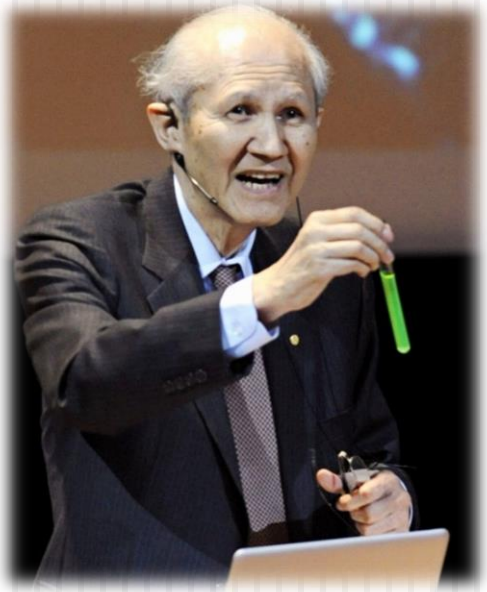
What is GFP?

What is GFP?

- GFP (Green Fluorescent Protein)
- Protein from *Aequorea victoria*
- Prof. Osamu Shimomura won a Nobel prize in 2008 for its discovery and development



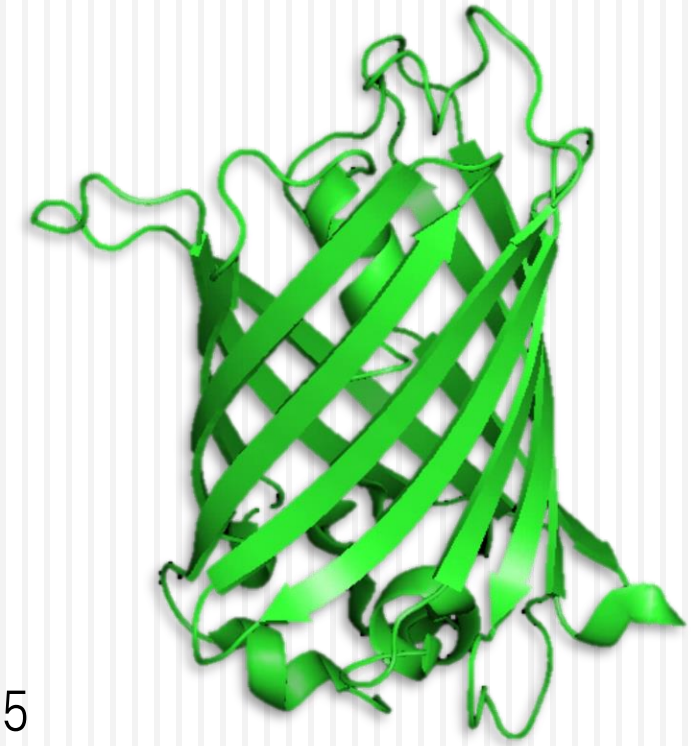
Uses of GFP

- Traces protein's movement
- Able to provide fluorescence on specific protein on the target cell

Motivation

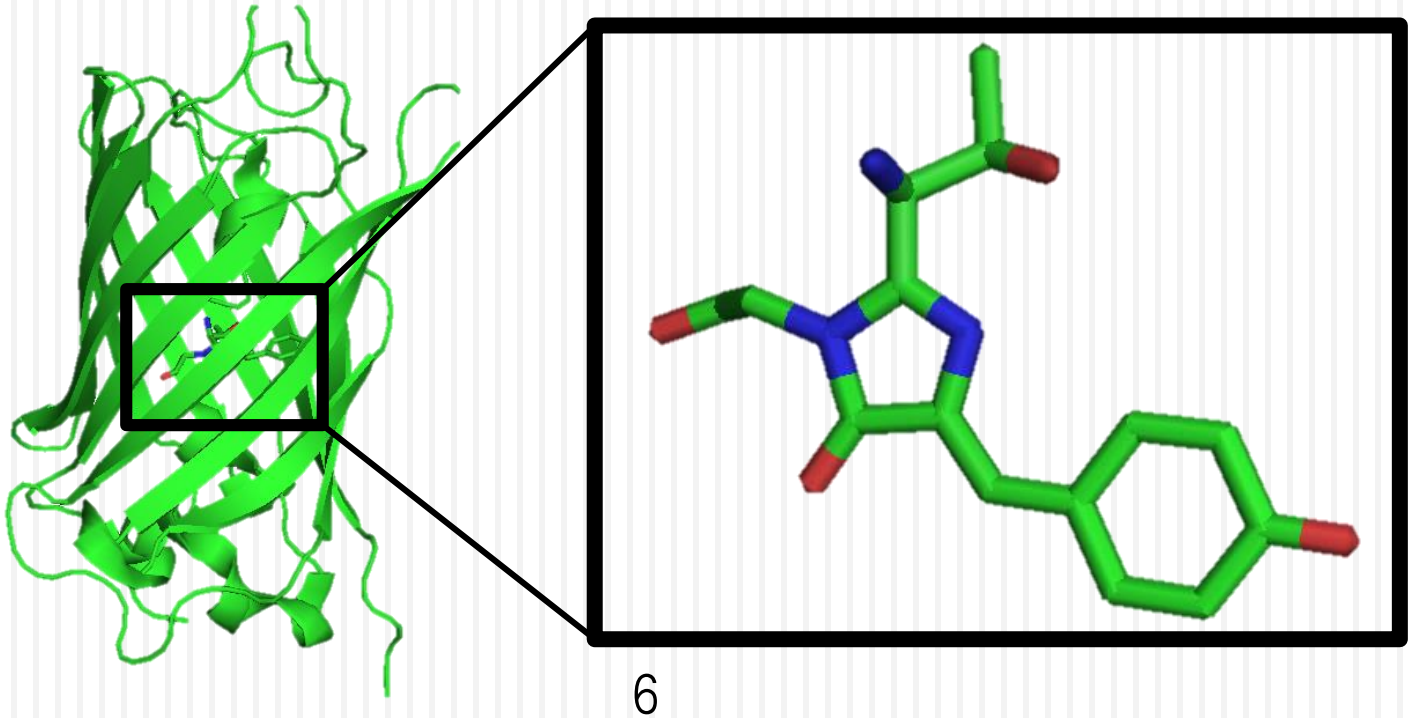
- Knew the structure of GFP
- Practical use for medical care and researches.

Steric structure of GFP
(PDB ID:2B3B)



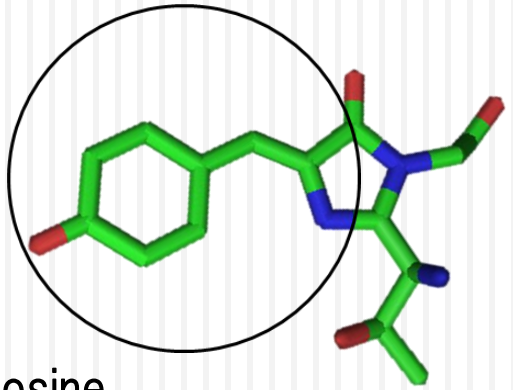
Purpose

- Replace the amino acid
- Create the mutant GFP



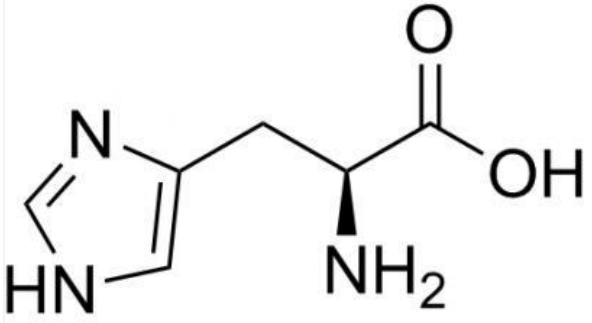
Hypothesis 1

Change Tyrosine into Histidine



Tyrosine

Pi-bond & double bond decreases

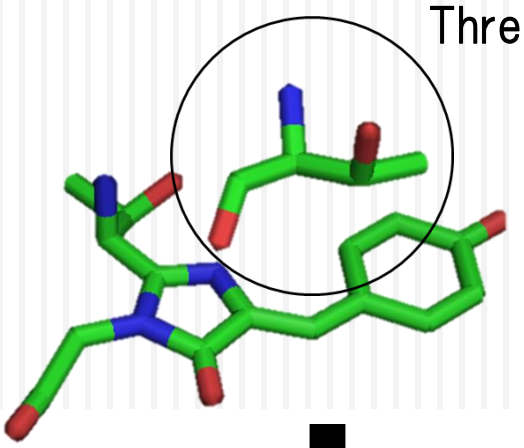


Histidine

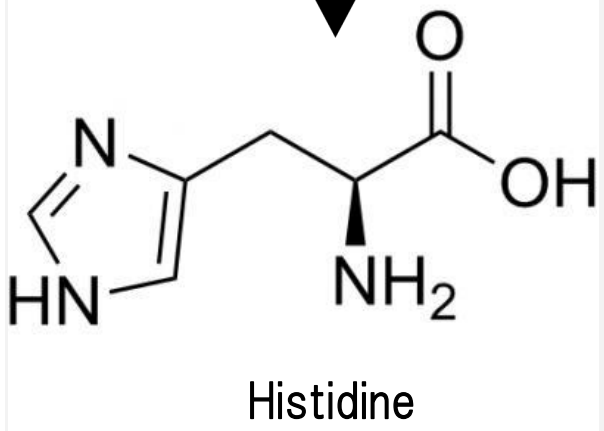
Fluorescence change to shorter wavelength

Hypothesis 2

Change Threonine into Histidine



Cyclic structure near the side chain of Histidine interacts to Tyrosine



Histidine

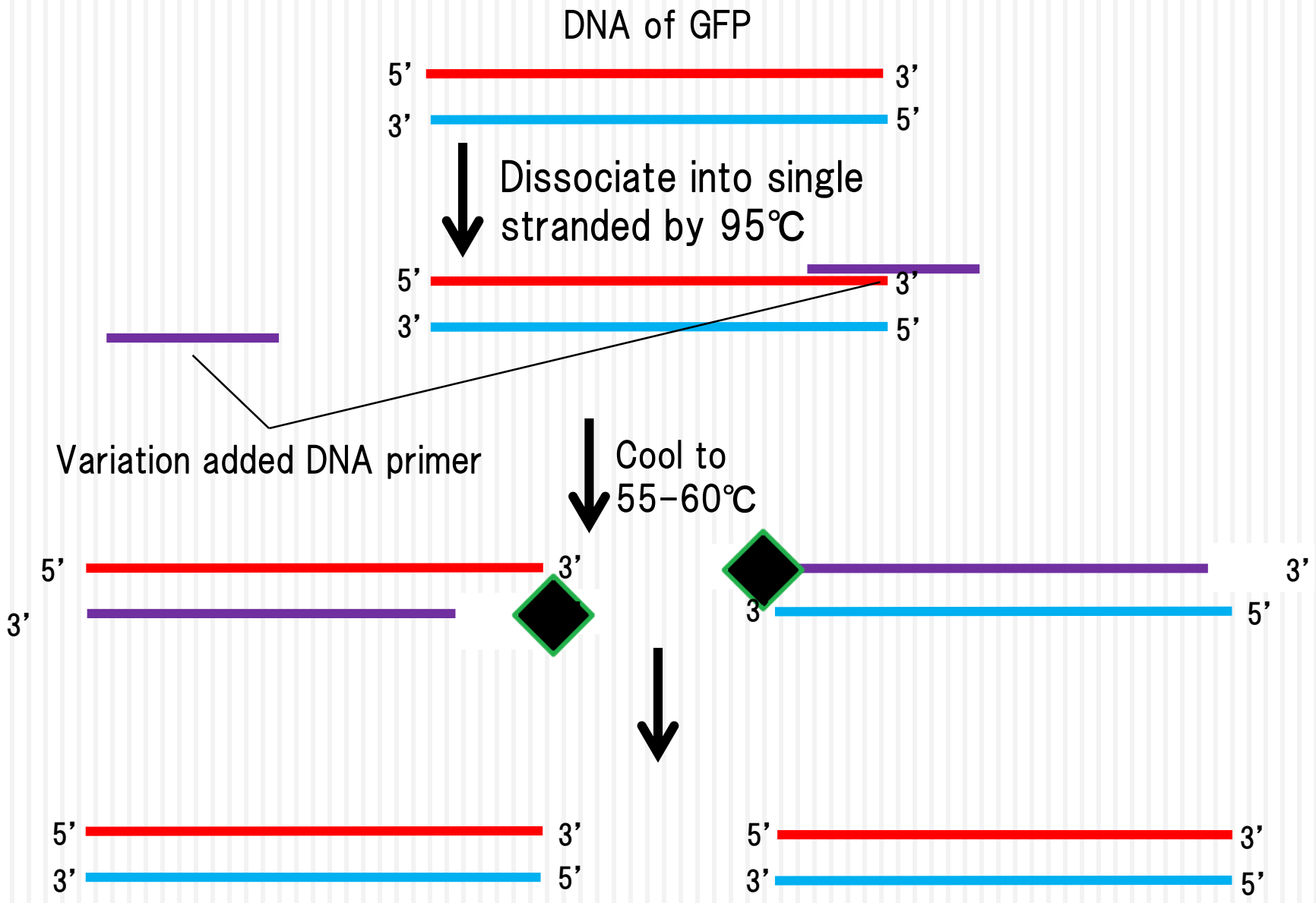
Fluorescence change to longer wavelength

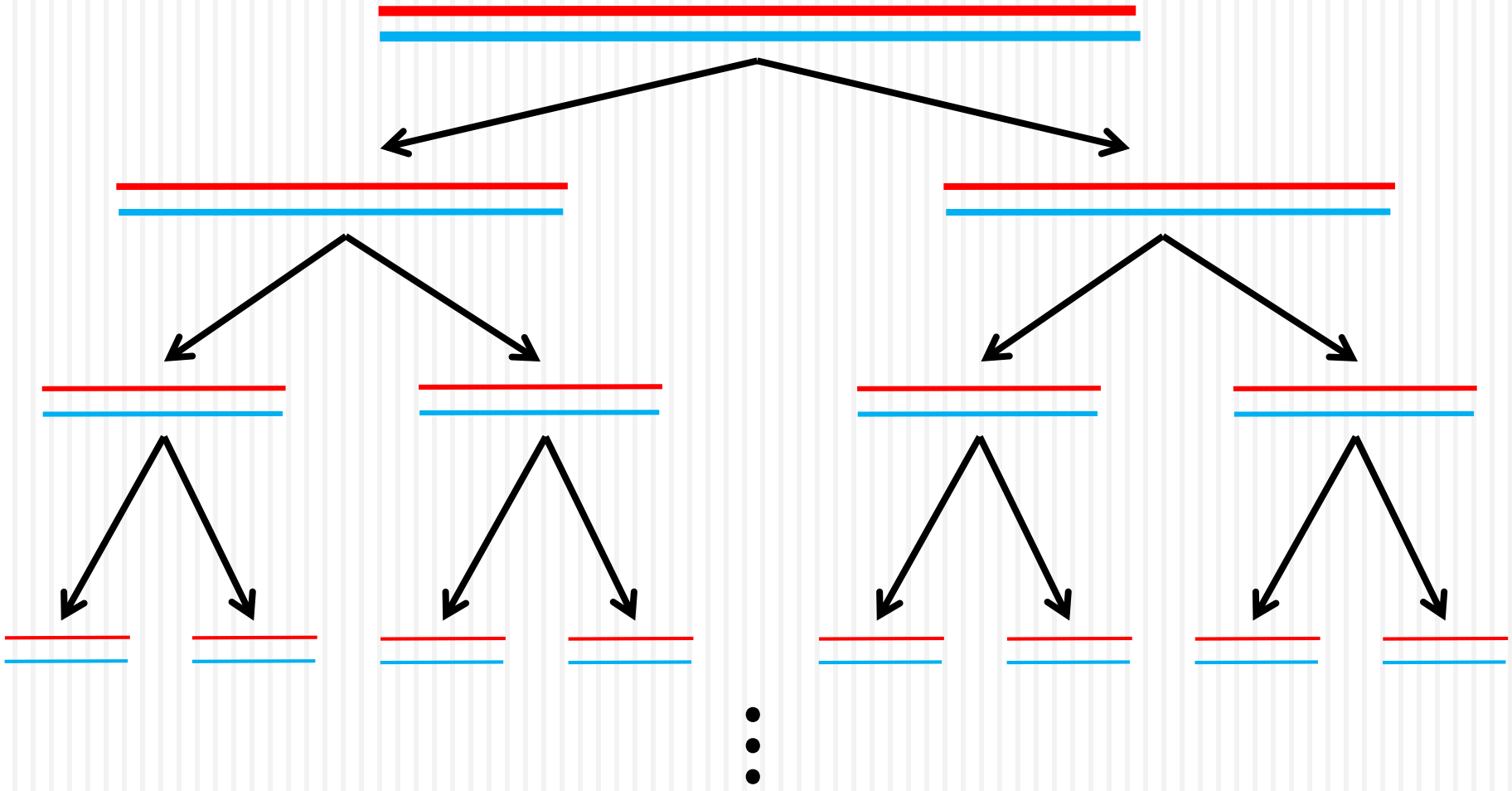
Experiment

1. Synthesis mutant GFP's DNA by PCR method
2. Insert mutant GFP to bacteria to culture
3. Repeat centrifuge and breaking the dissolved deposit and obtain GFP 1&2
4. Measure fluorescence spectra
5. Compare with wild GFP

PCR Method

- PCR (Polymerase Chain Reaction)
- Developed by Kary Mullis in 1980s
- Specific sequence accumulates in billions of copies

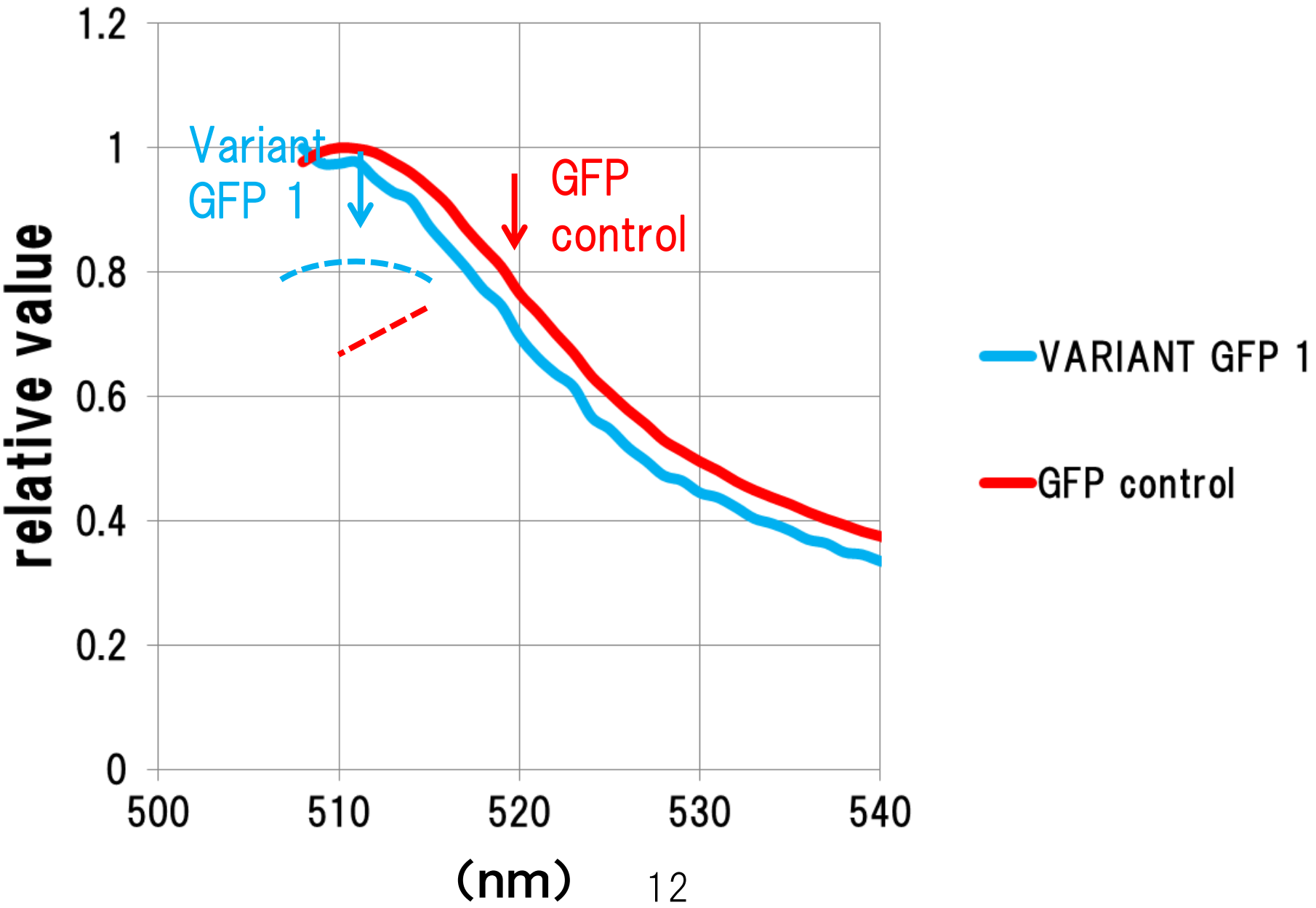

DNA of GFP
5'
3'
3'
5'
Dissociate into single stranded by 95℃
5'
3'
3'
5'
Variation added DNA primer
Cool to 55-60℃
5'
3'
3'
3'
3'
5'
5'
3'
3'
5'
5'
3'
3'
5'



$2^n =$ (number of DNA copies)

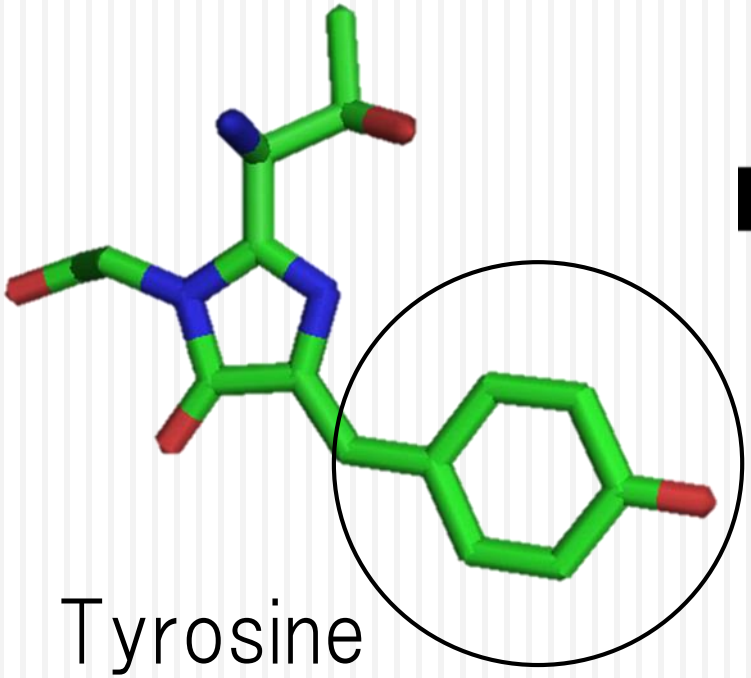
$n = 40 \longrightarrow 2^n \fallingdotseq 1{,}000{,}000{,}000{,}000$copies

Comparison between GFP1 and wild GFP

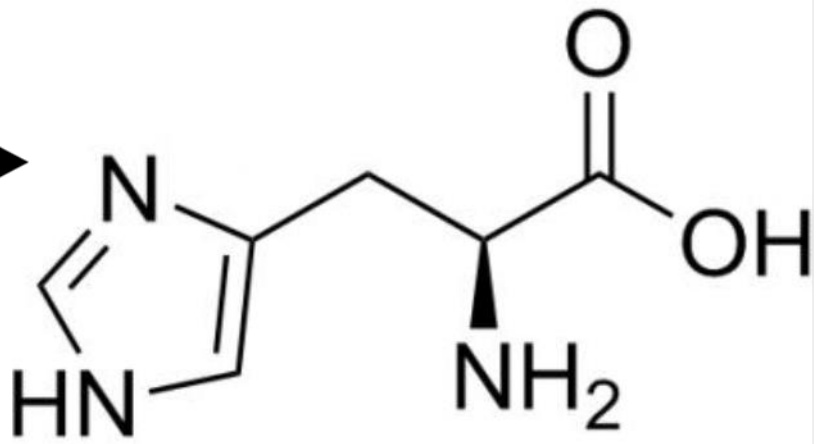


Conclusion 1

- Succeeded in creation of short wavelength GFP

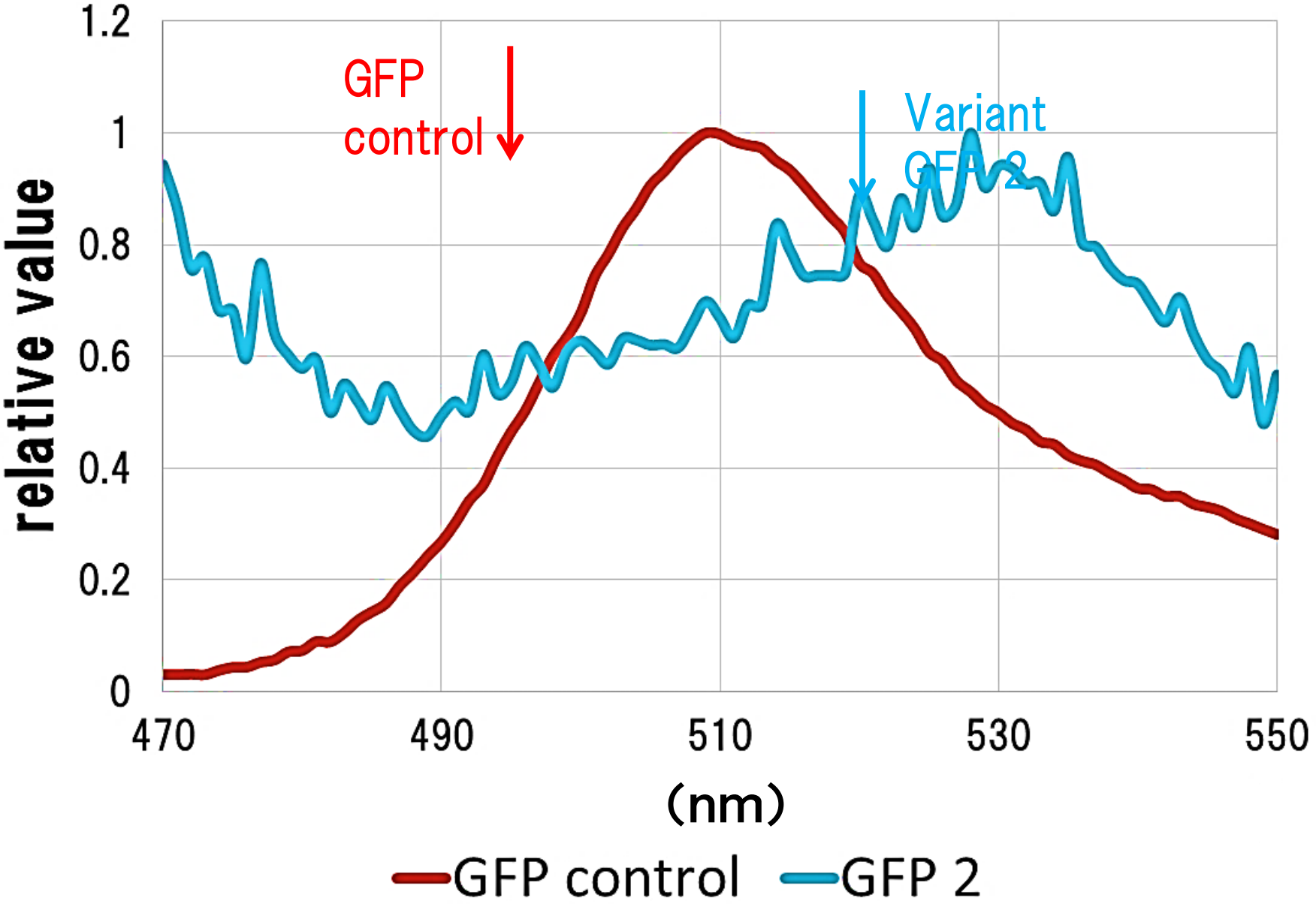


Tyrosine



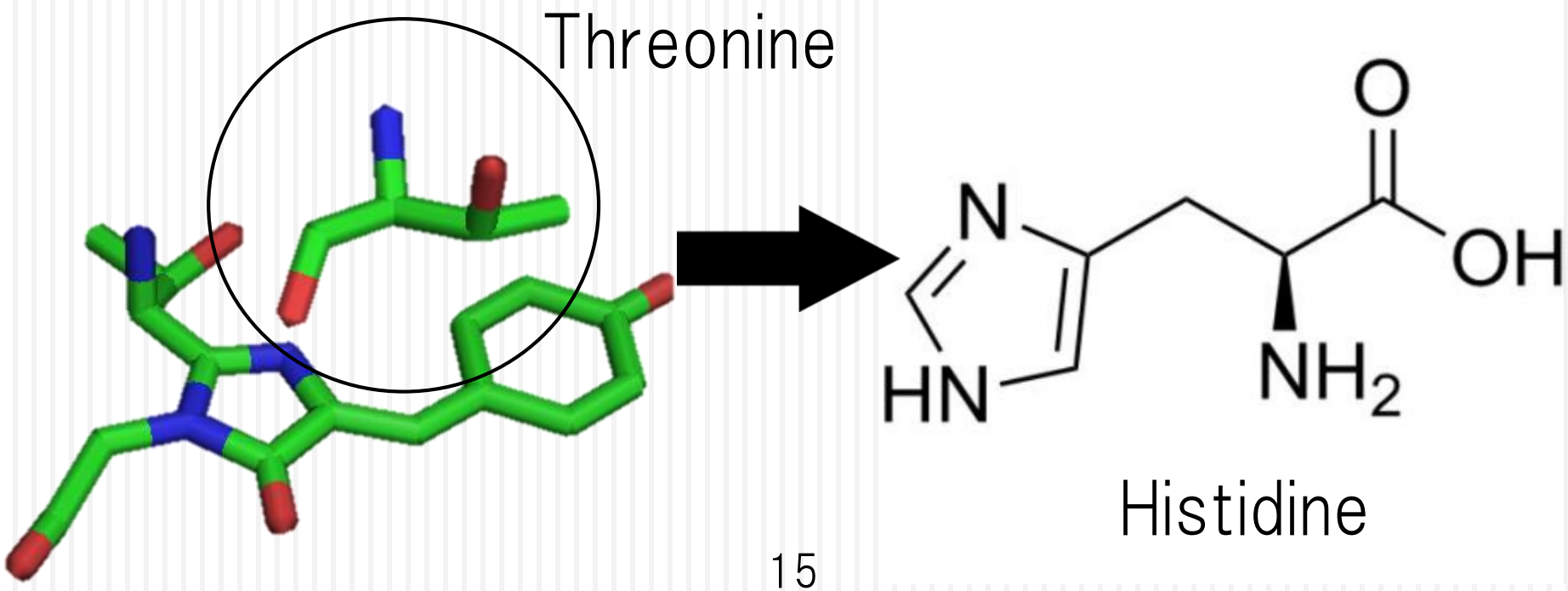
Histidine

Comparison between GFP2 and wild GFP



Conclusion 2

- Succeeded in creation of long wavelength GFP



Future Work

- Challenge creating a new GFP having both properties of what we have made
- Analyze the base sequence of GFP's DNA to check whether the change is occurred correctly

Acknowledgements

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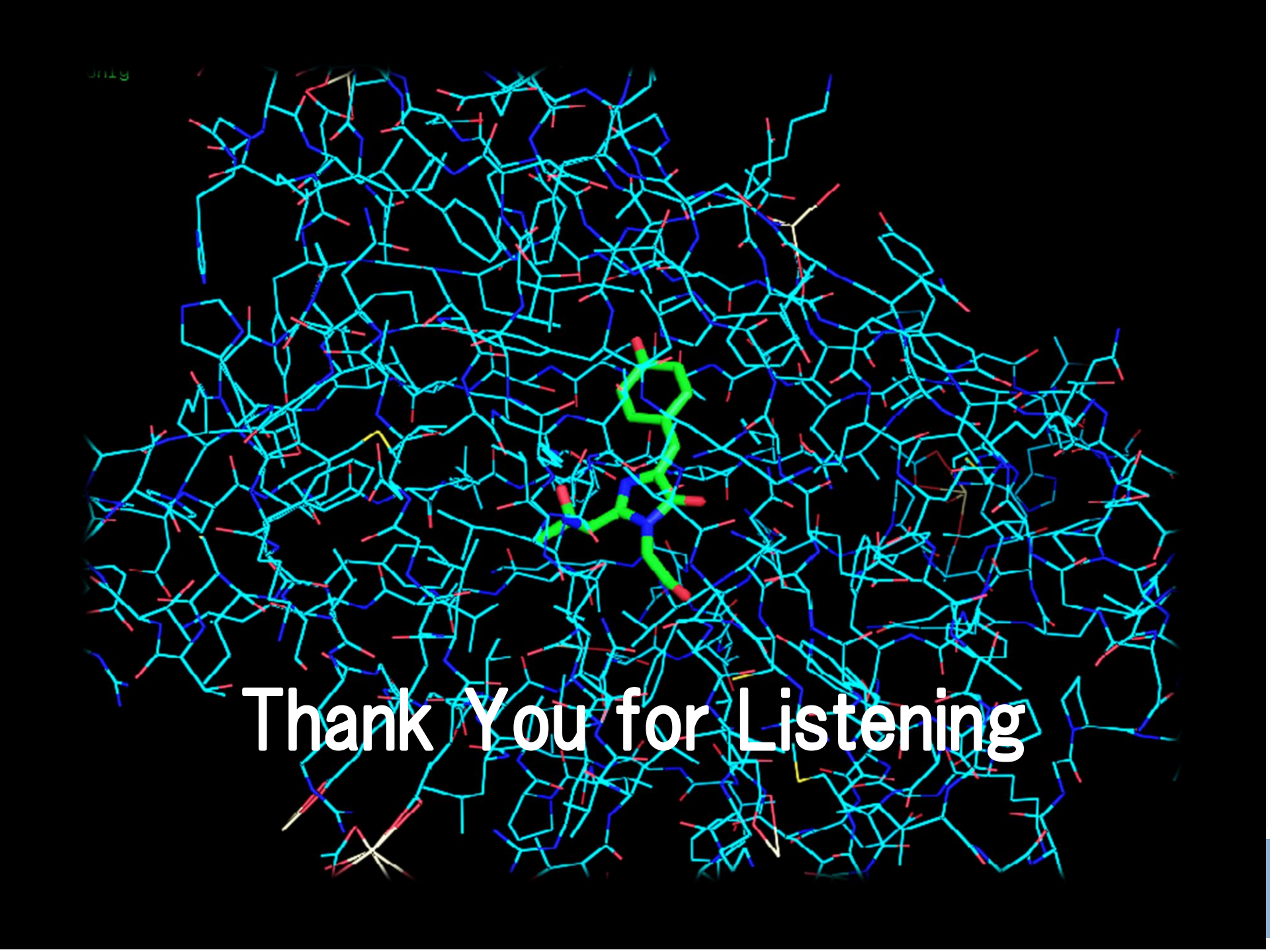
Thank You for Listening