Photoactivated RNAi with Caged 2'Fluoro-RNA in Cells and Tissues

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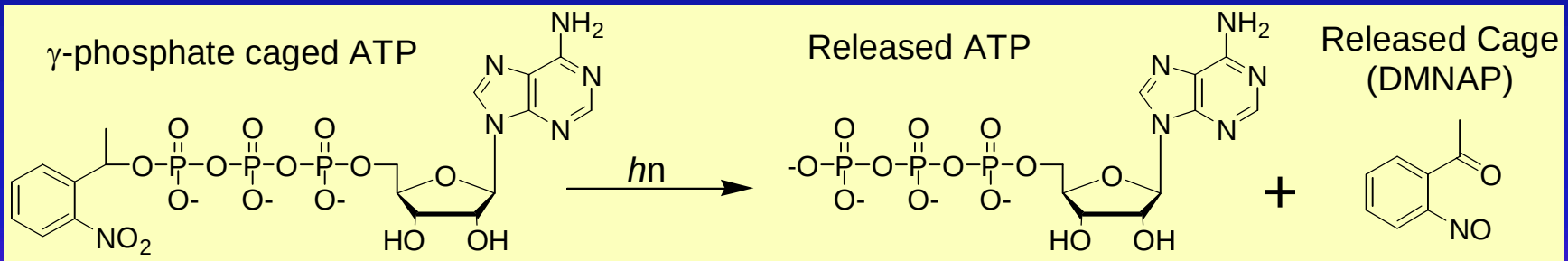
Project Overview

- Photo-control of RNA interference
- FNAs : stable RNA analogs
- Adds controllable trigger to gene knockdown
- Also adds resistance to enzymatic digestion
- Demonstrated in:
  - Cell culture GFP assay
  - Zebrafish embryo GFP and development assay

Caged Molecules

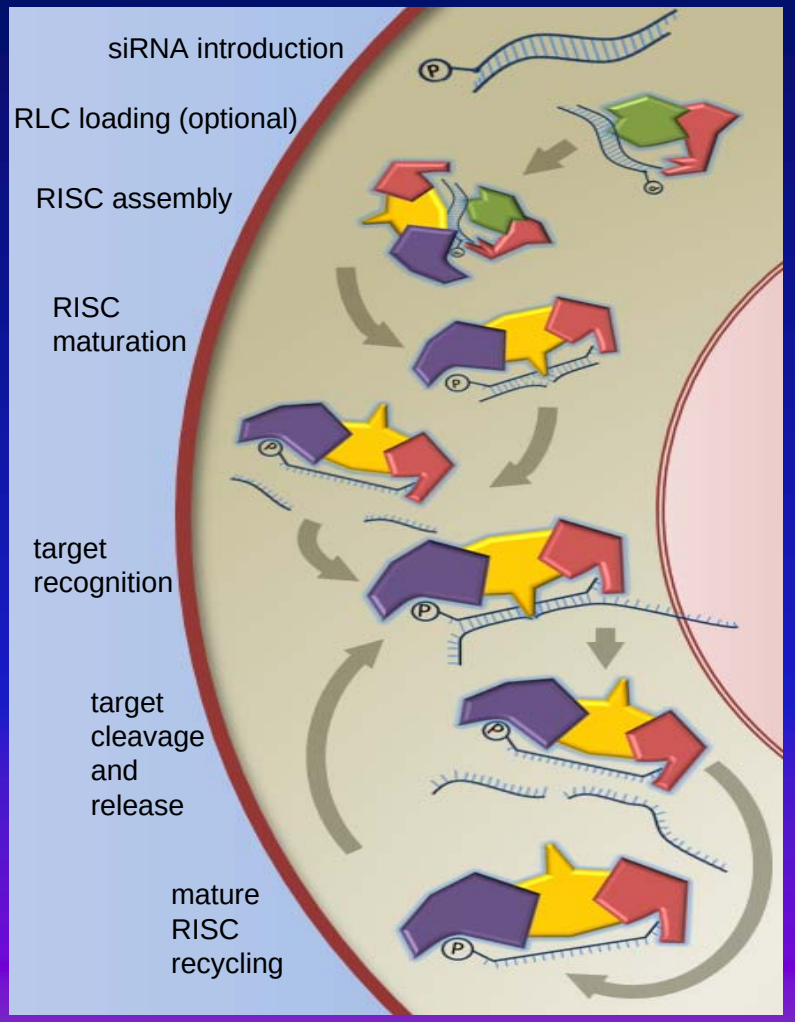
- Photo-activation of biological activity
- Study time course of cellular responses



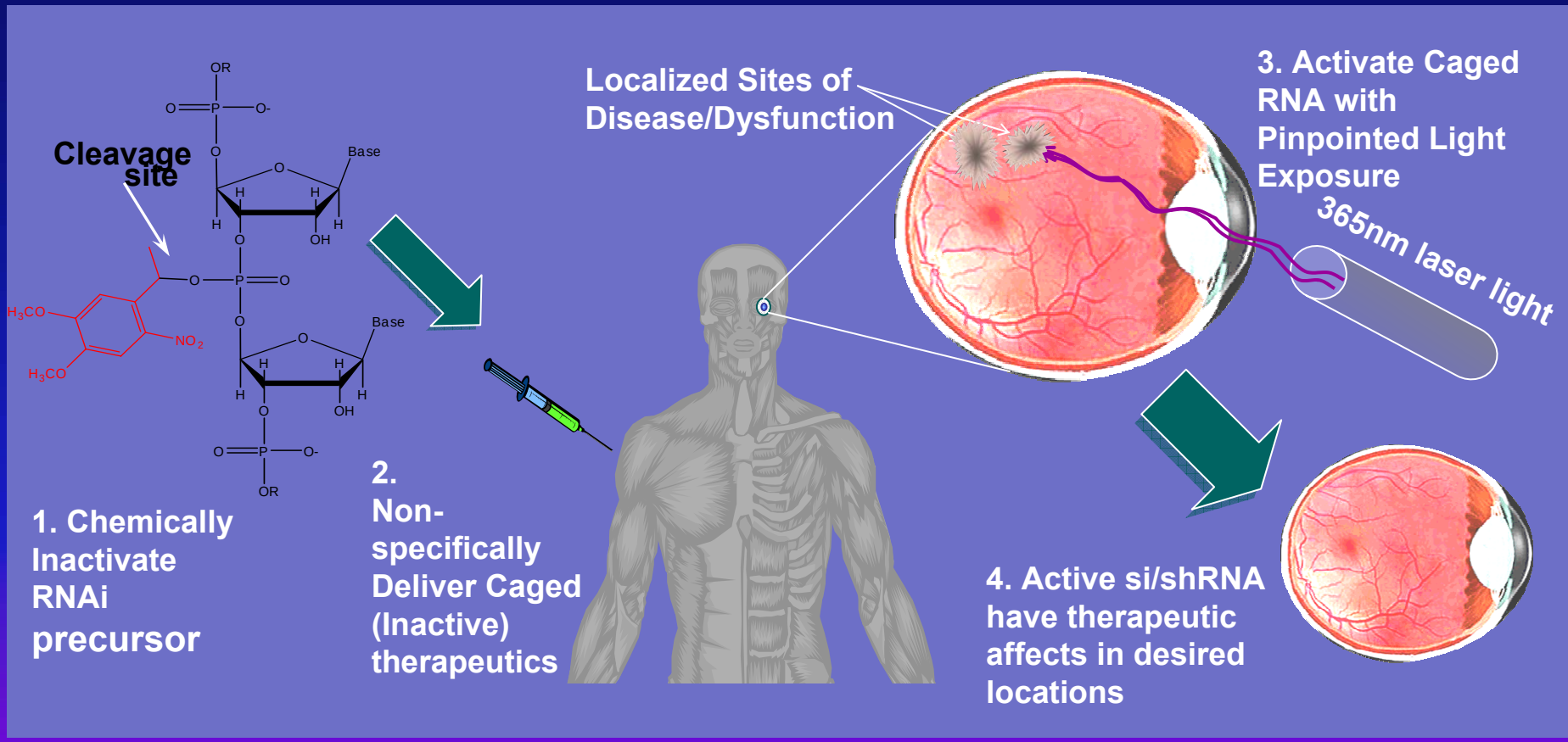
Kaplan, J. H., B. Forbush, et al. (1978). Biochemistry **17**(10): 1929-1935.

RNA Interference

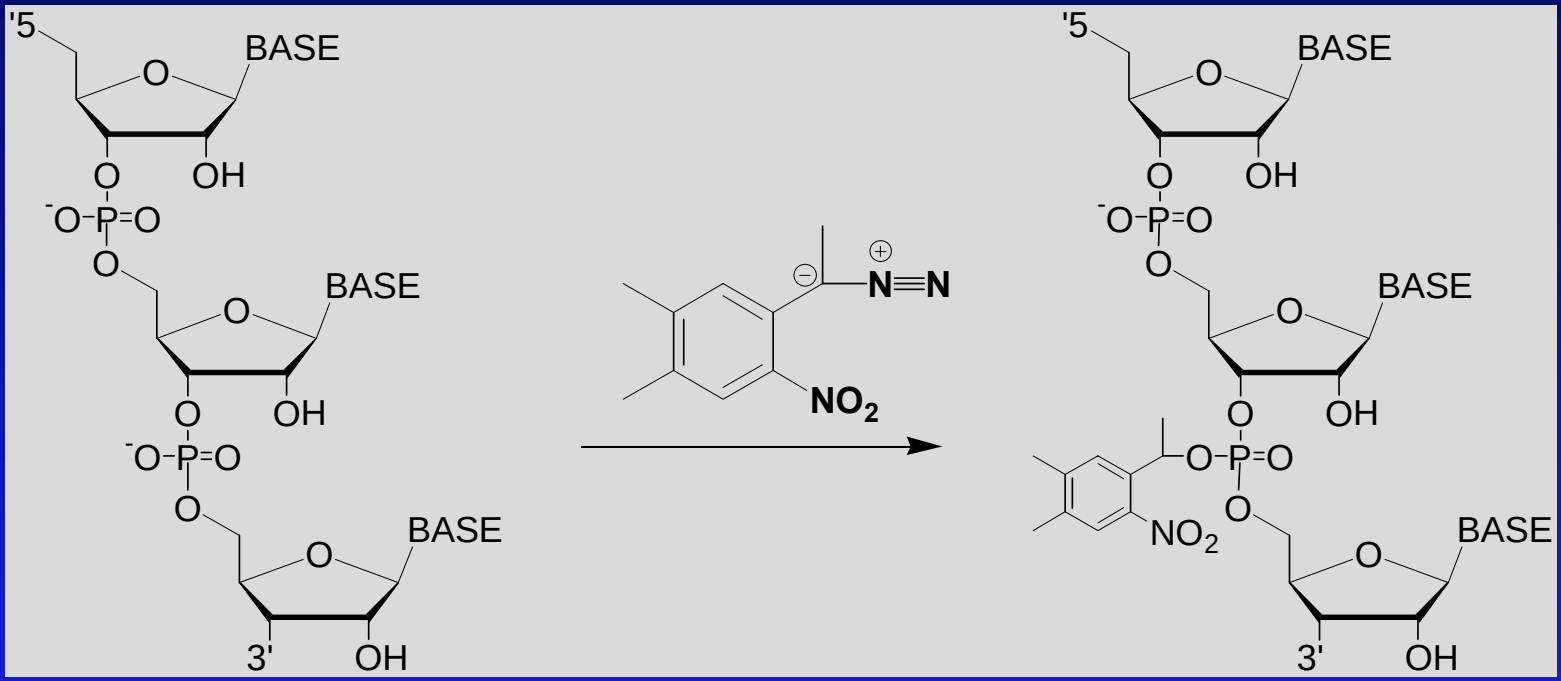
- Post-transcriptional gene silencing from small regulatory RNAs
- More effective than antisense agents
- Endogenous miRNAs are critical in gene regulation and development
- 2006 Medicine Nobel to A. Fire and C. Mello for discovery of RNAi
- Needs control for mechanistic studies, targeting for therapeutics



Therapeutic Application



Initial Studies: Caging Protocol



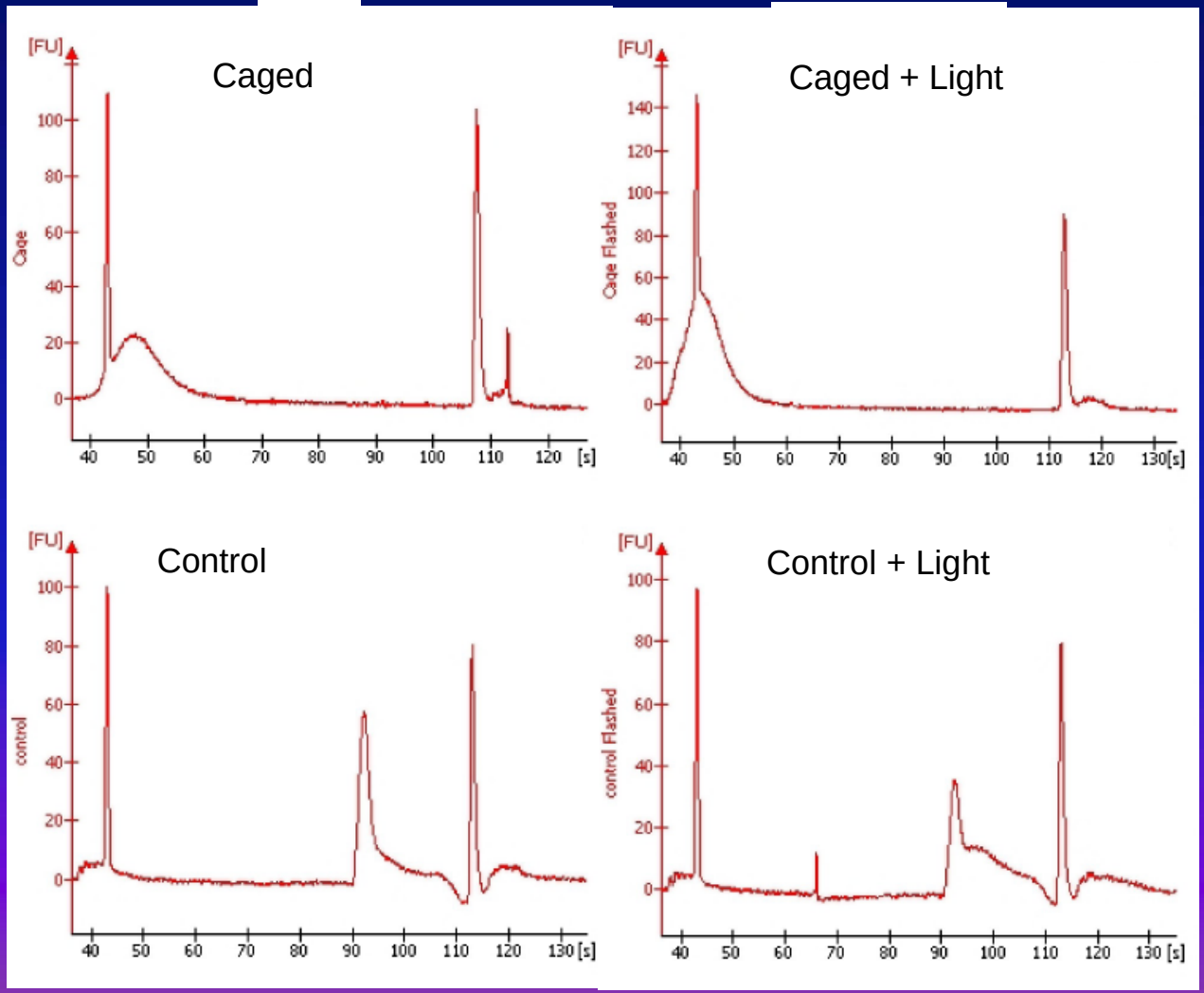
Parameter Study

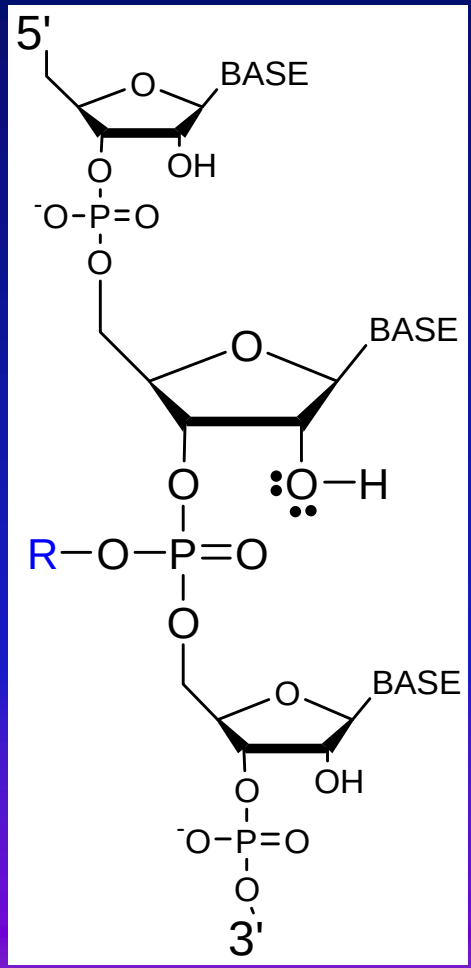
- Cage concentration
- Temp, time of rxn
- Solvent, pH conditions

Evaluation Study

- Cage attachment
- Oligo purification

Initial Studies: 700bp dsRNA Caging





Confirmation of Caged 2'FNA

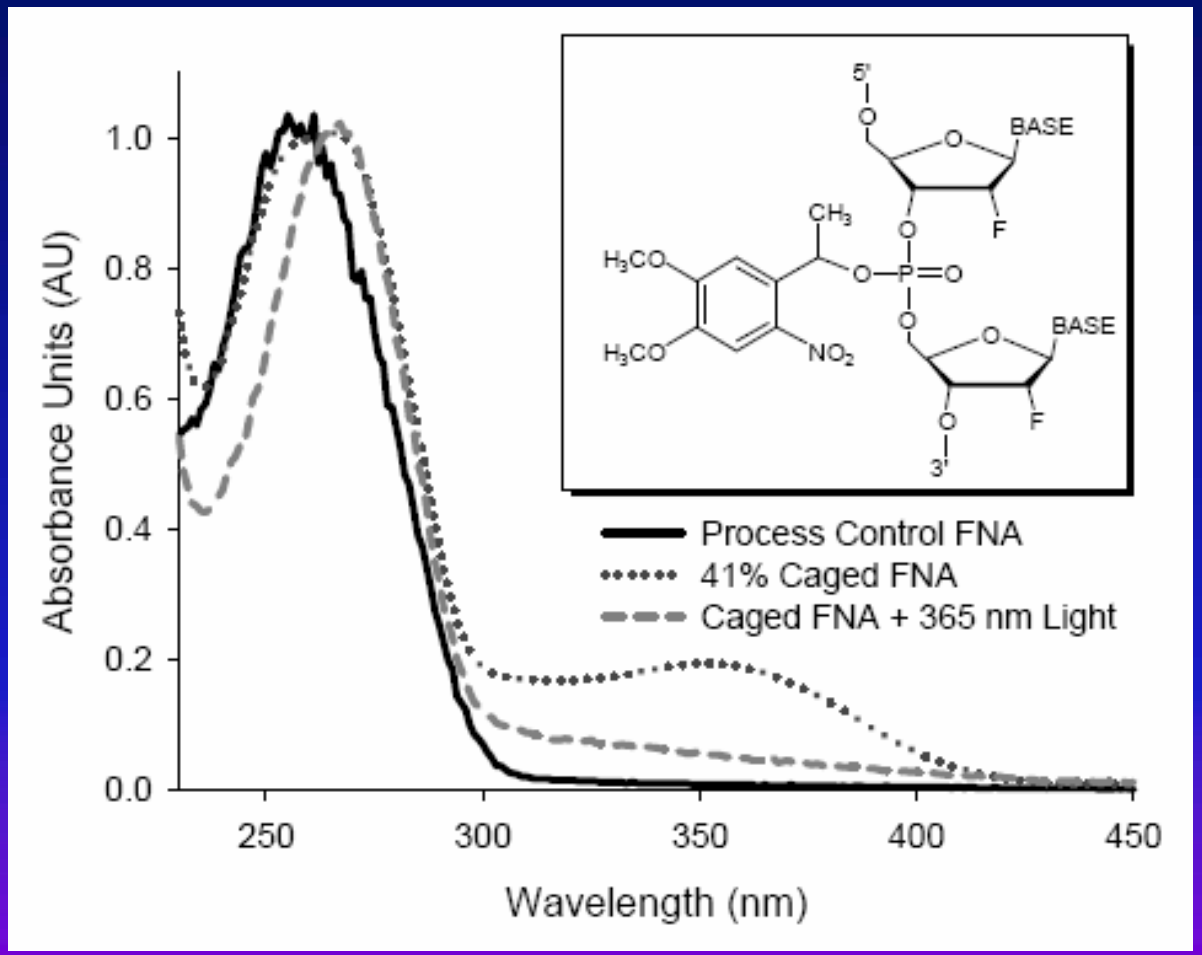
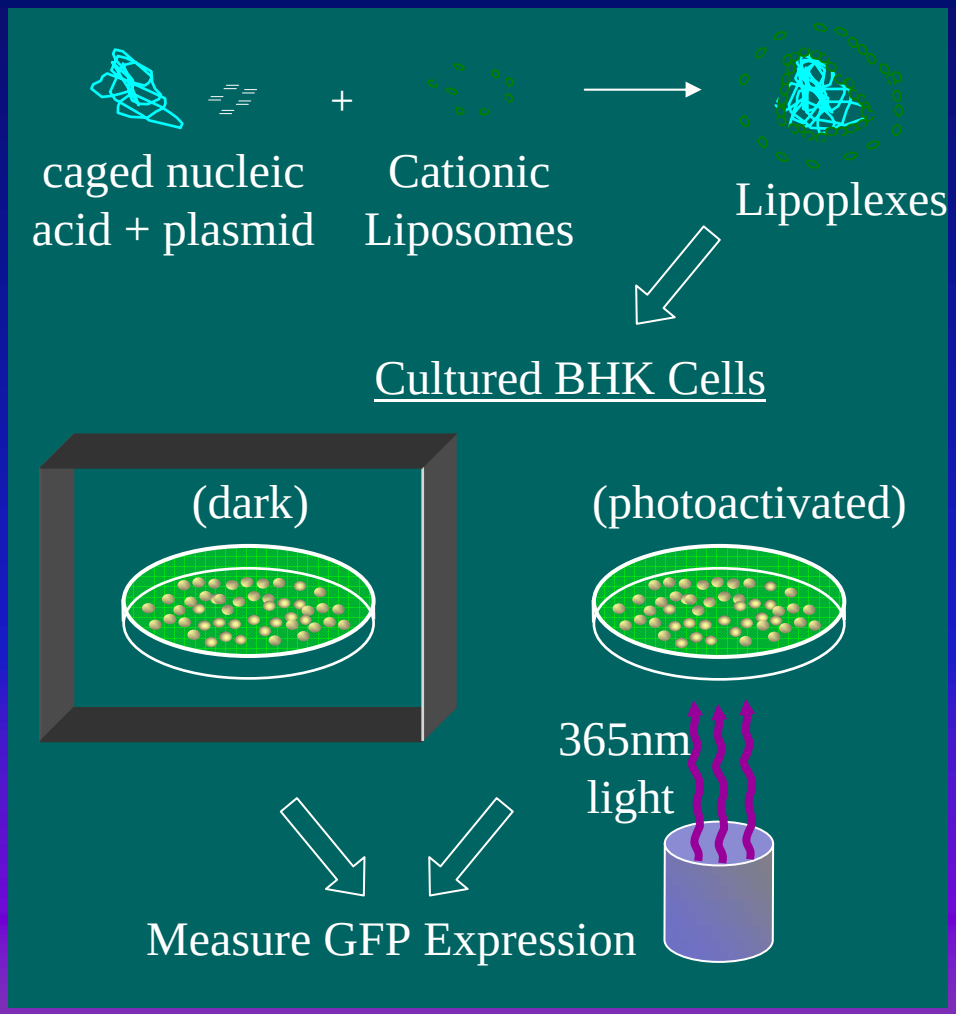
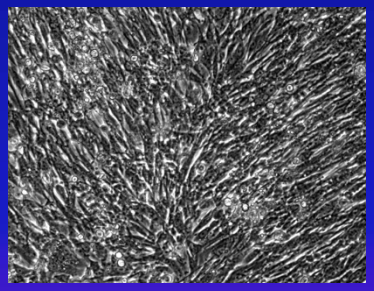
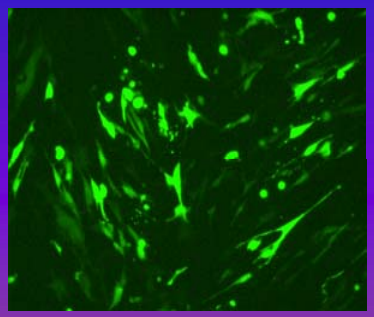


Photo-induced RNAi Experiments

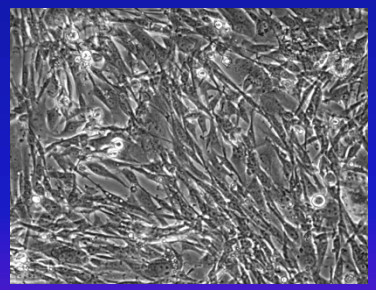


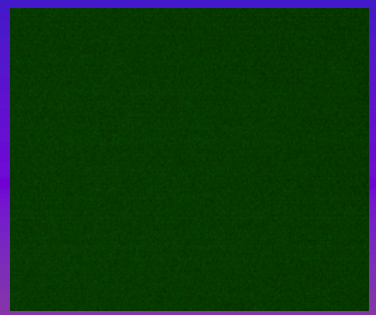
Caged siFNA



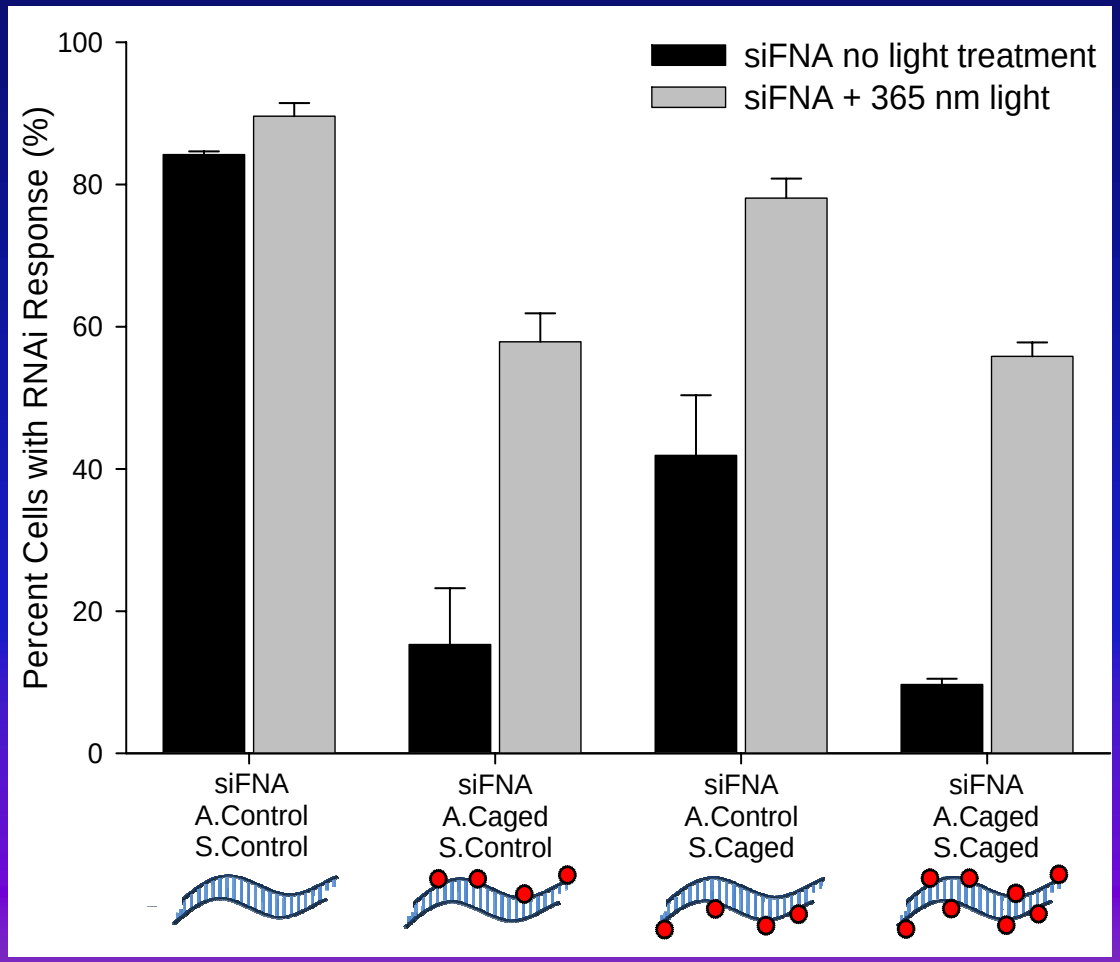


Uncaged siFNA





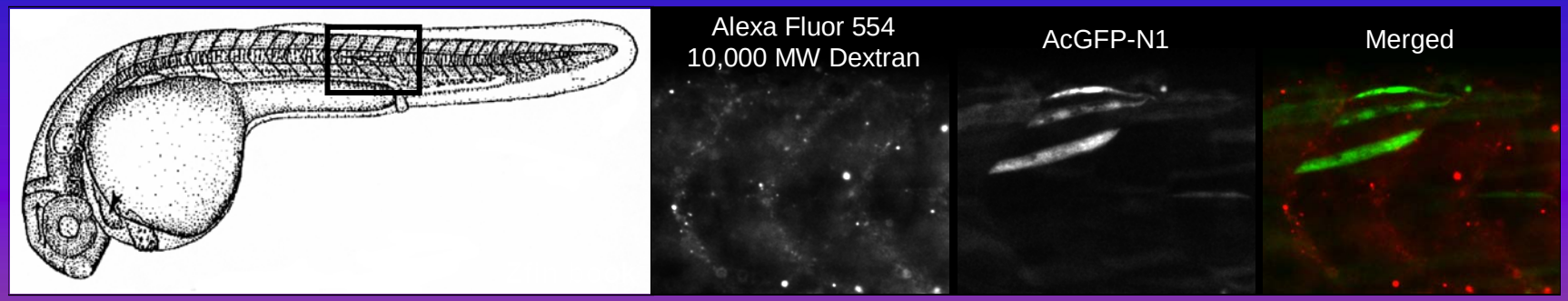
RNAi with caged siFNAs



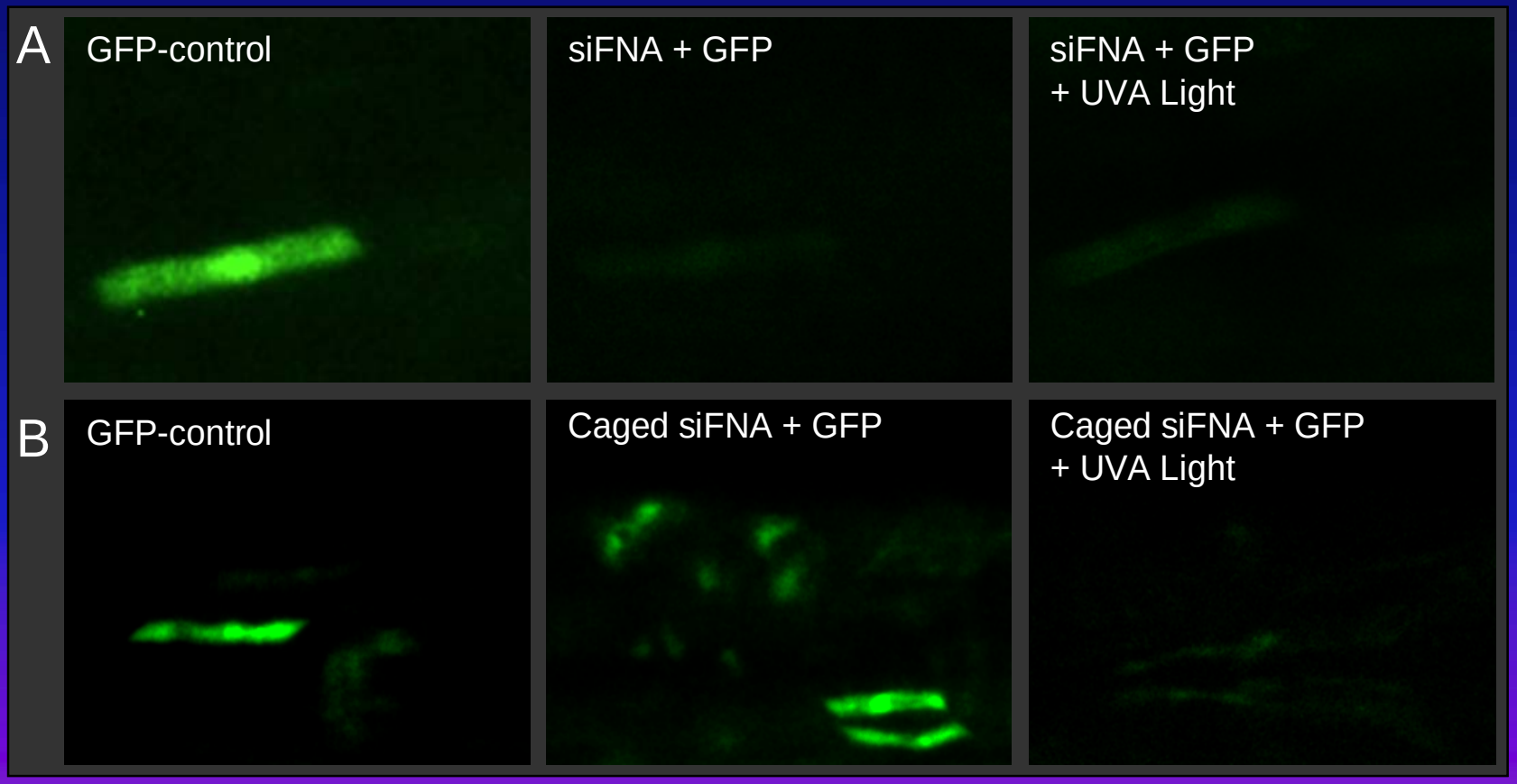
- Mix and Match
  - Control Antisense
  - Control Sense
  - Caged Antisense
  - Caged Sense
- Caged
  - ↓ RNAi activity
- Photoexposed
  - ↑ RNAi activity

Zebrafish Injections

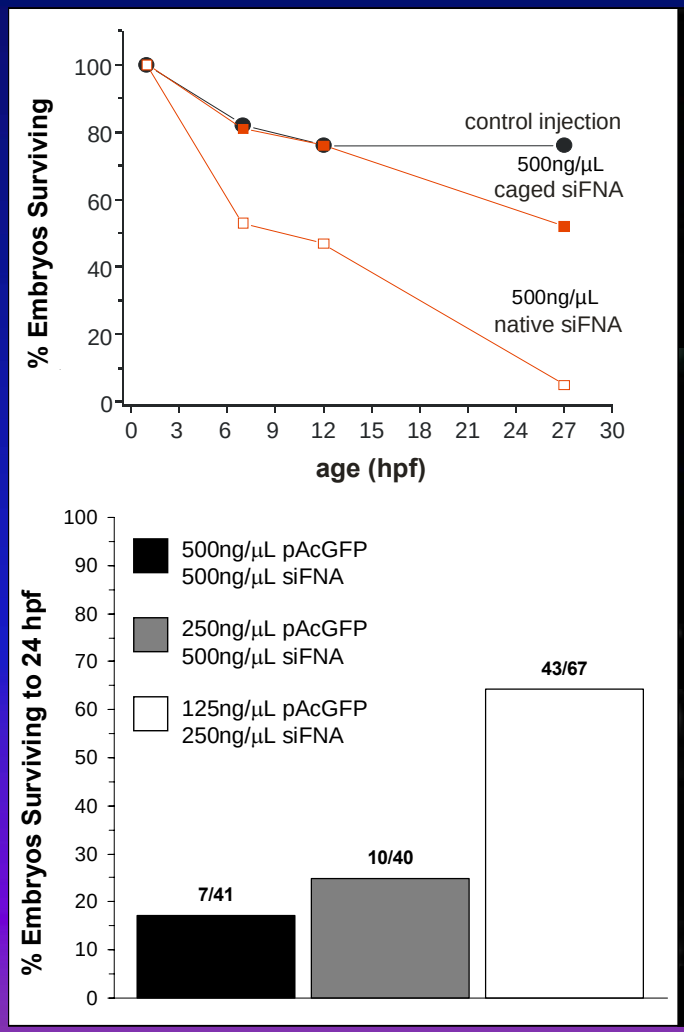
- Co-injection of all reagents
  - Plasmid
  - siFNA (control or caged)
  - Tracking dye (conjugated dextran)
  - Phenol red
- 28 hpf evaluation
  - Fixed and evaluated by microscopy
  - Tracking dye used to select good injections
  - Each experiment had GFP control fish



GFP Knockdown: Native and Caged siFNAs



Toxicity: Native and Caged siFNAs



- siFNA titration shows toxicity
- Caging reduces the observed toxicity
- Characterized by delayed development

Summary of Caged siFNA Results

- Cage protects from nuclease digestion
- Cell culture
  - Cage blocks RNAi activity
  - Caging antisense strand is more effective
  - Photo-exposure partially restores activity
- Fish embryos
  - Cage blocks RNAi activity
  - Photo-exposure partially restores activity
  - Cage can protect biological system from toxicity of high effector concentration

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