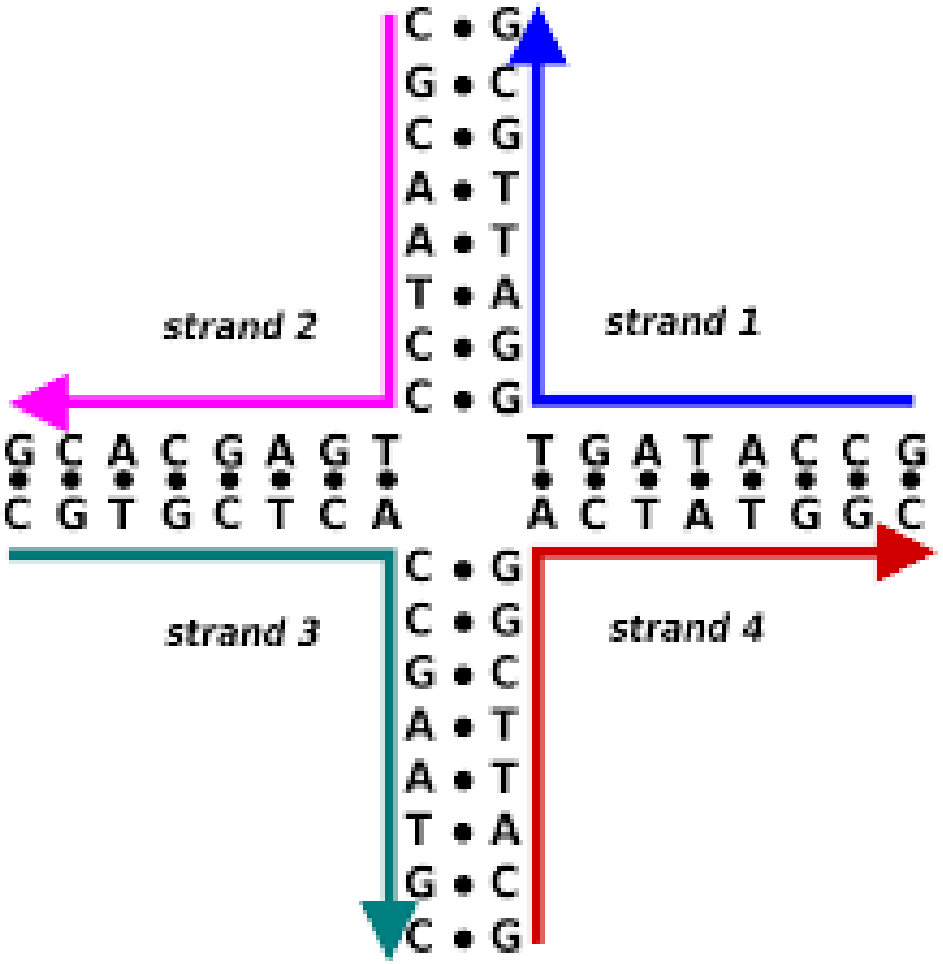
BT 601: Analytical Biotechnology

-Prof. Siddhartha Sankar Ghosh

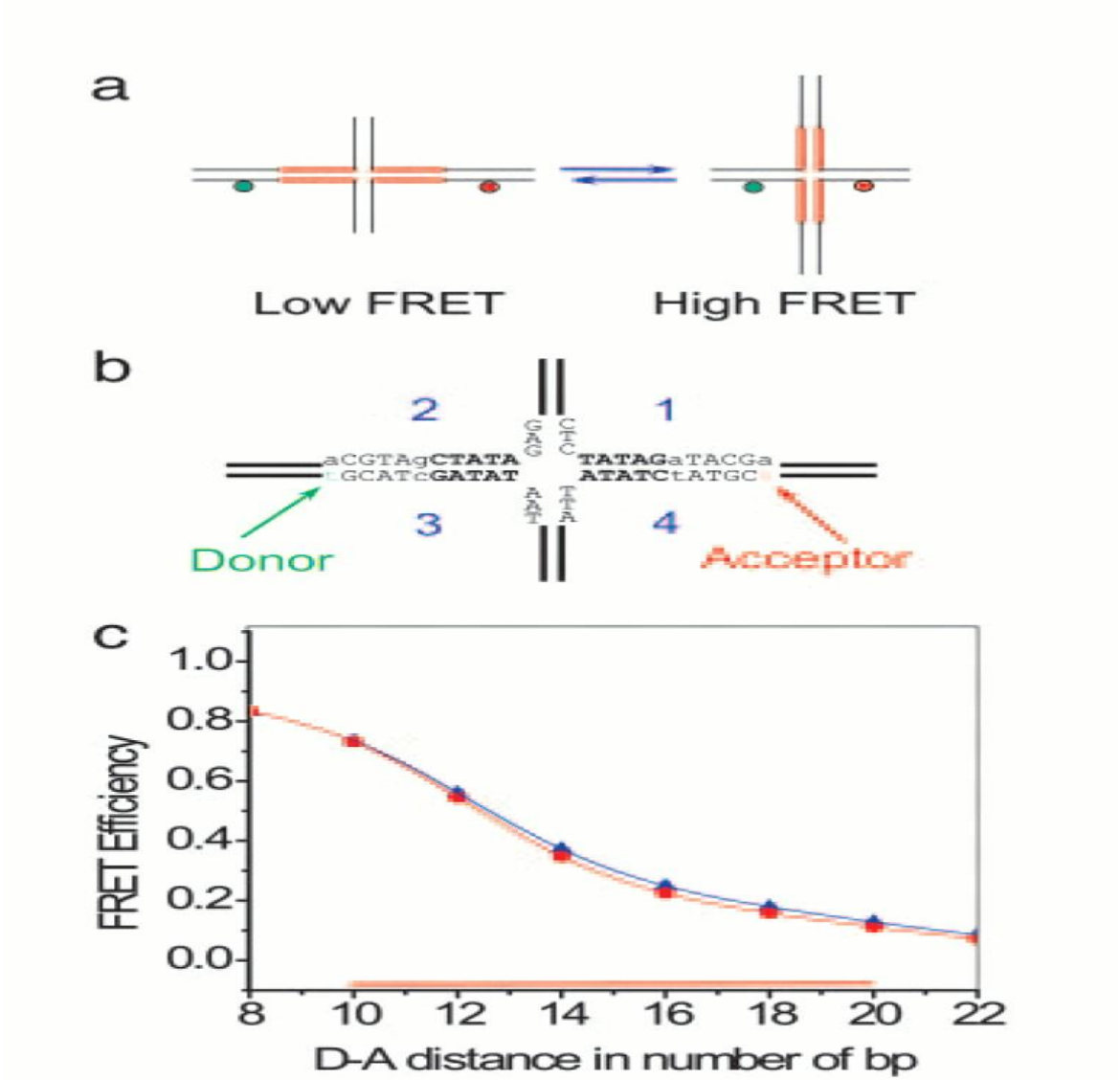
Lec-13

Computing in mammalian cells with nucleic acid strand exchange

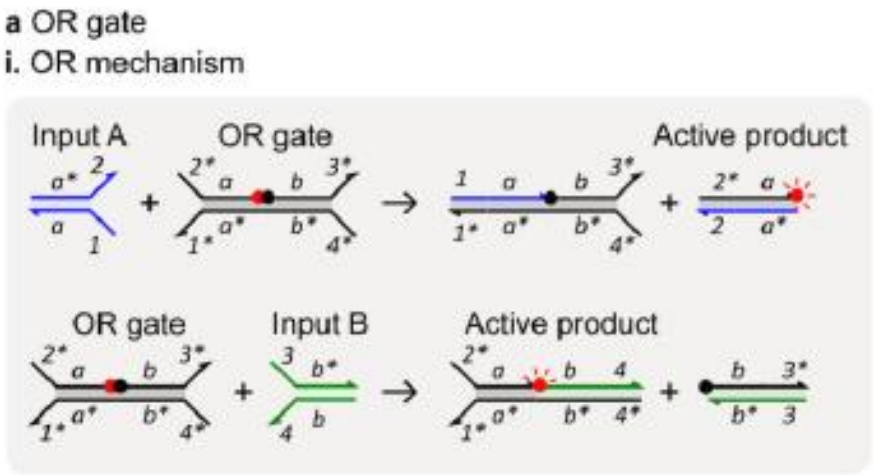
Four Point Junction of DNA

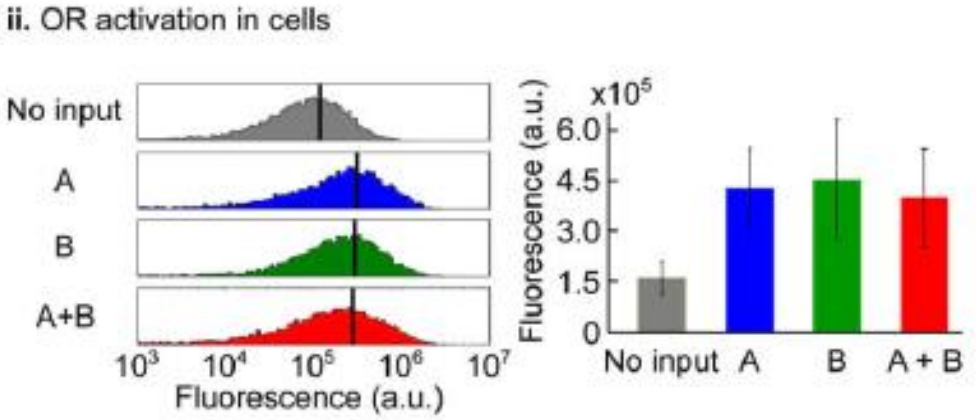


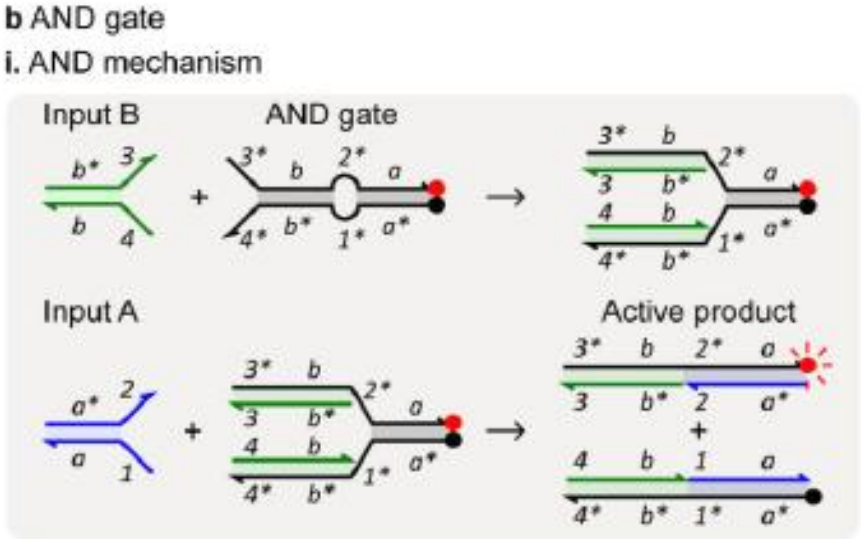
Low to High FRET

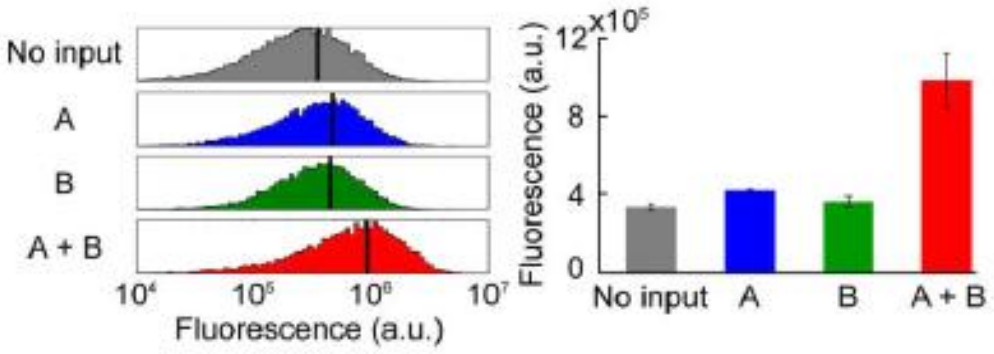


Strand exchange-based OR and AND logic gates work in mammalian cells

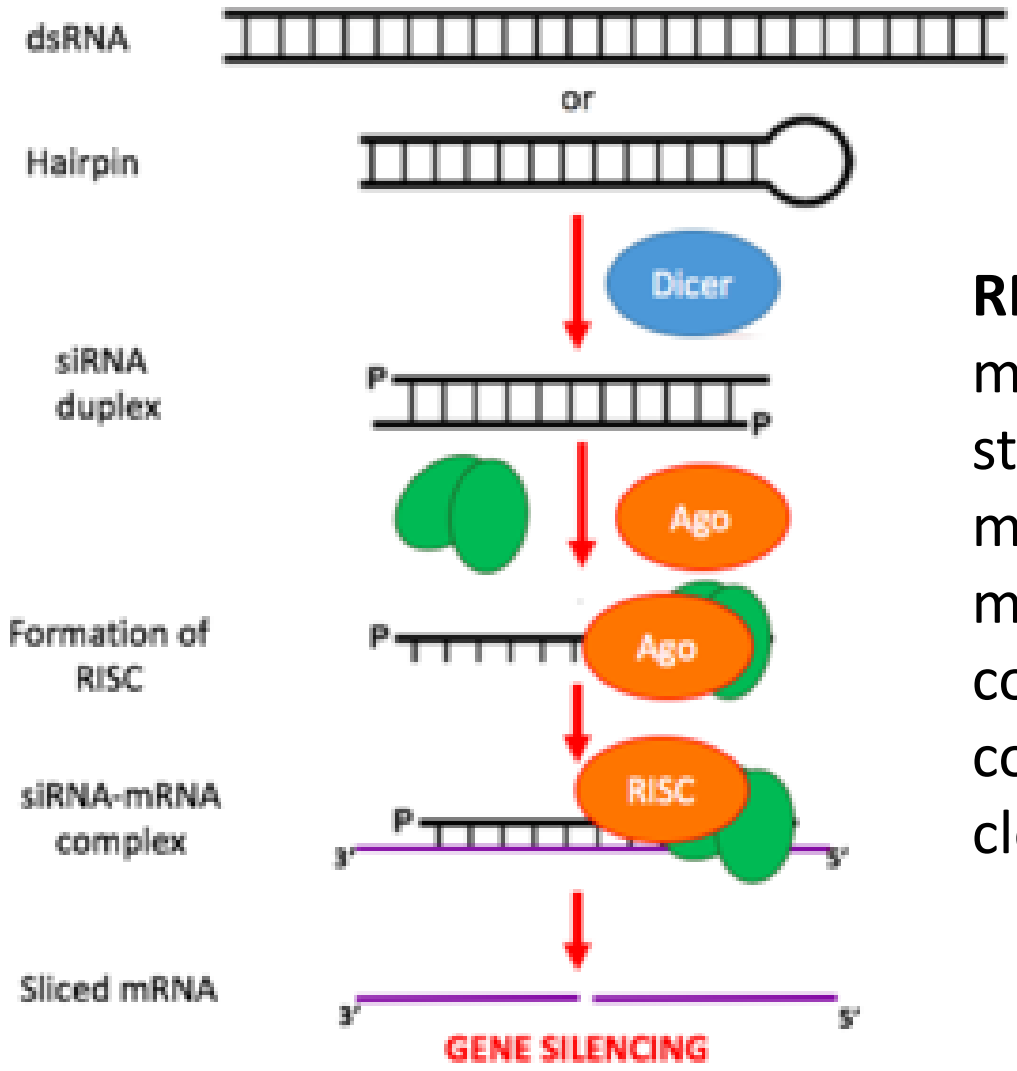






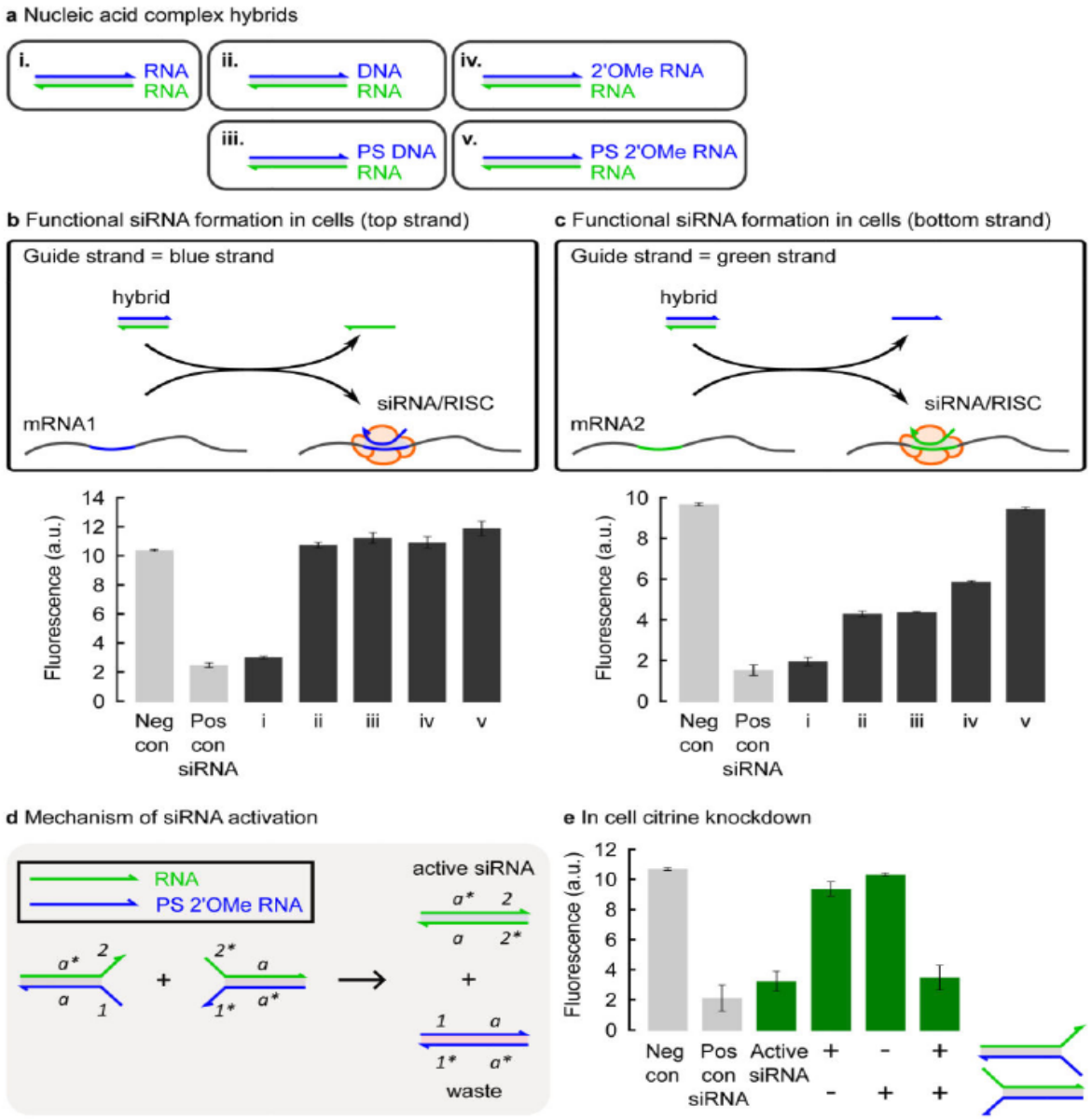


SiRNA



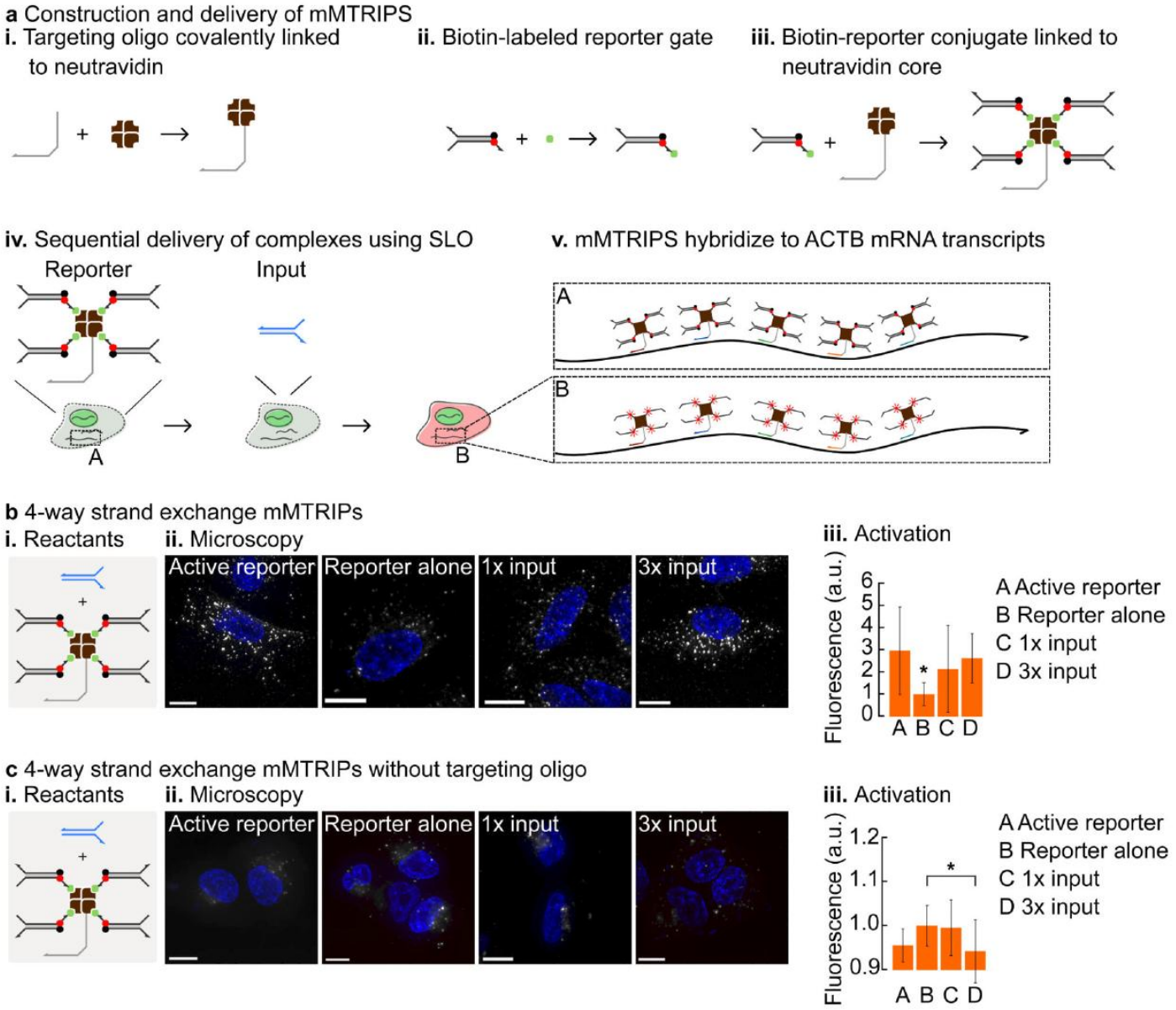
RNA-induced silencing complex, or RISC, is a multiprotein complex that incorporates one strand of a small interfering RNA (siRNA) or micro RNA (miRNA). RISC uses the siRNA or miRNA as a template for recognizing complementary mRNA. When it finds a complementary strand, it activates RNase and cleaves the RNA.

A functional siRNA can be activated through 4-way strand exchange

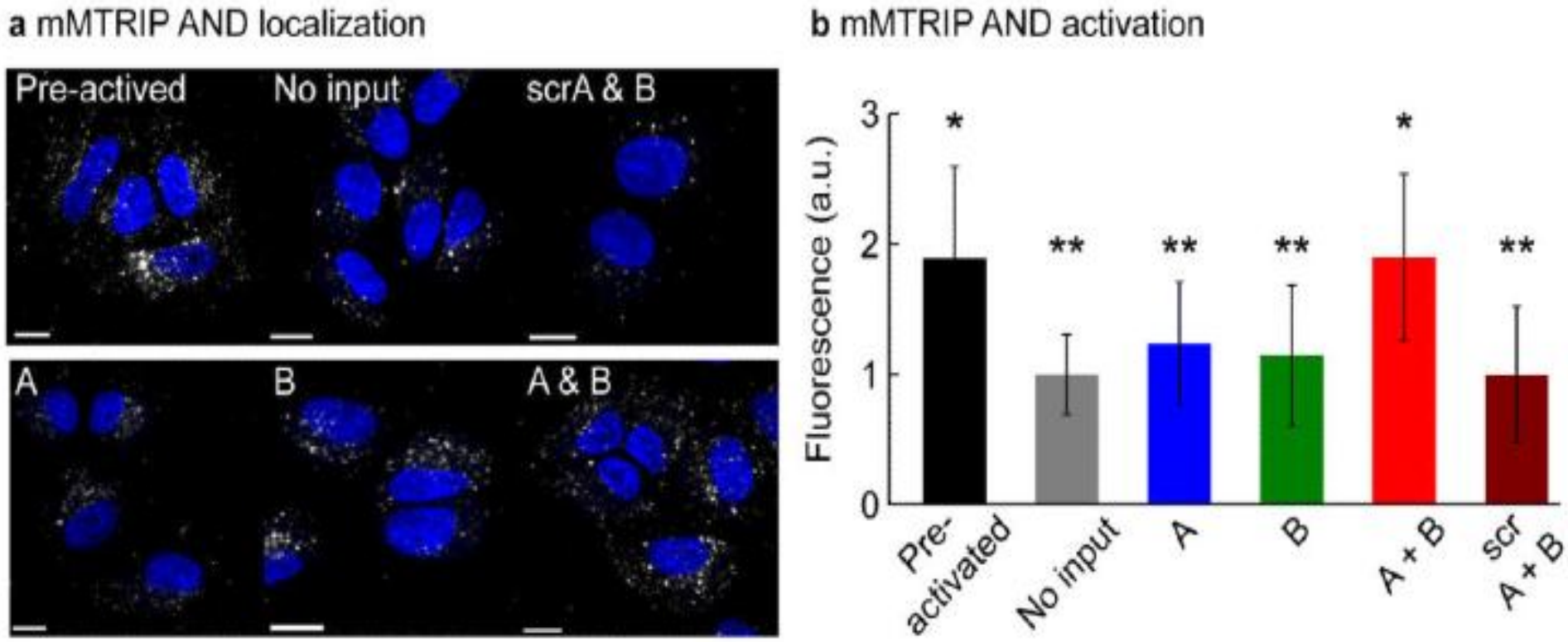


Endogenous mRNA and multiply-labeled, tetravalent imaging probes (mMTRIPS) can serve as scaffolds for strand exchange reactions

Streptolysin-O (SLO) mediated reversible permeabilization



ACTB mRNA-scaffolded mMTRIP AND logic gates work in cells



BIOLOGICAL FLUOROPHORES

1. Amino acids: Aromatic amino acids **tryptophan** (Trp), **tyrosine** (Tyr), and **phenylalanine** (Phe) are the most important **intrinsic biological fluorophores**.

2. Nucleotides: Nicotinamide adenine dinucleotide in its reduced form, **NADH** and the **flavin adenine dinucl**eotide in its oxidized form, **FAD** are fluorescent in the visible region of the electromagnetic spectrum.

3. Proteins: Proteins are fluorescent due to the presence of **aromatic amino acids that fluoresce in the near UV region.**

Certain proteins, however, do fluoresce in the visible region. **Green fluorescent protein (GFP),** for example, fluoresces in the **green region of the electromagnetic spectrum**.

Figure: PDB structure of Green fluorescent protein, abbreviated as GFP. It was discovered by Shimomura and coworkers in 1962. Protein was isolated from the jellyfish, Aequorea victoria, that glows in the dark.

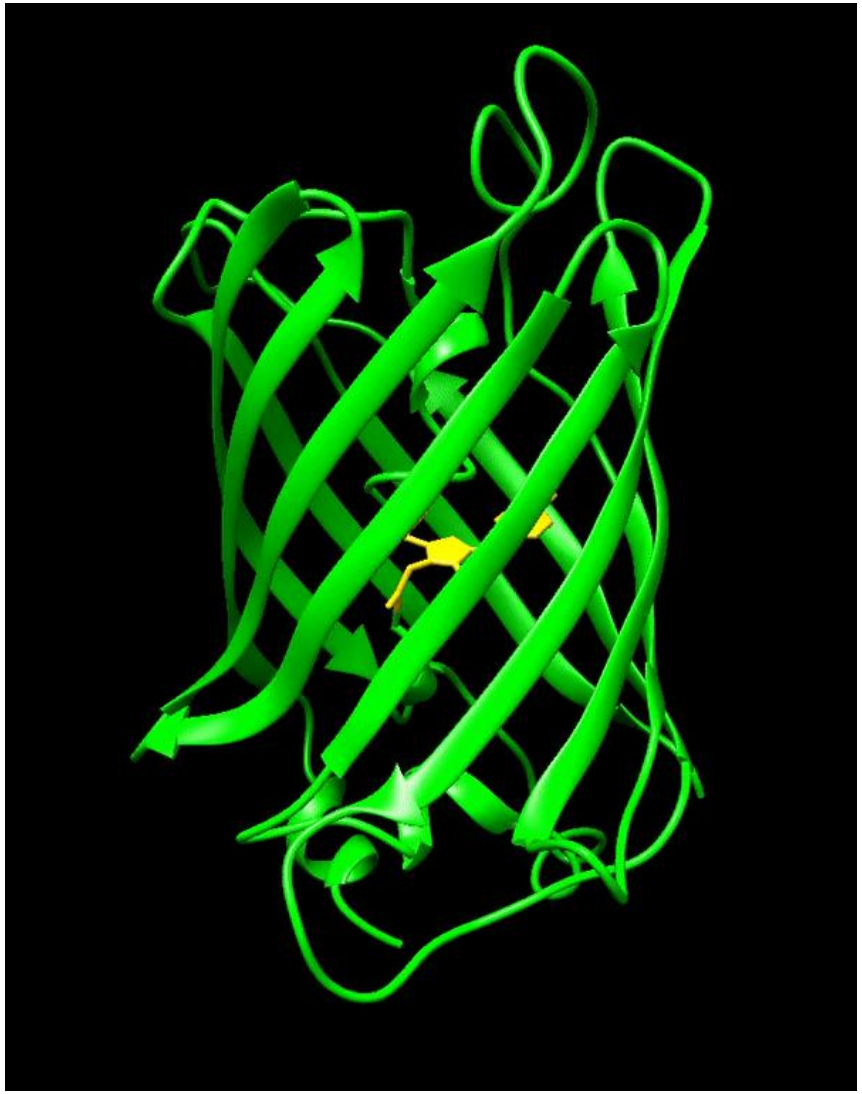


Figure: GFP 3D structure and zoom in to fluorescent chromophore.

Protein folding

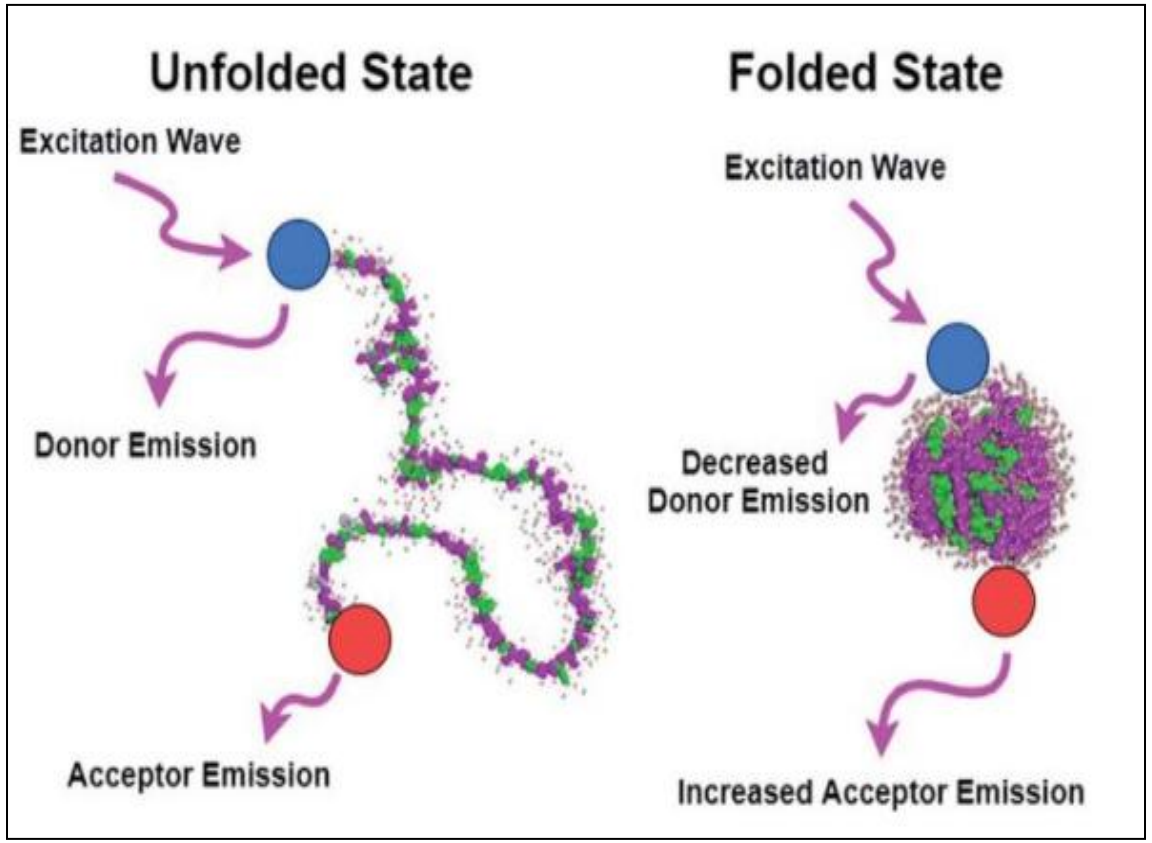


Figure: Protein folding and unfolding detection with FRET.

THANK YOU