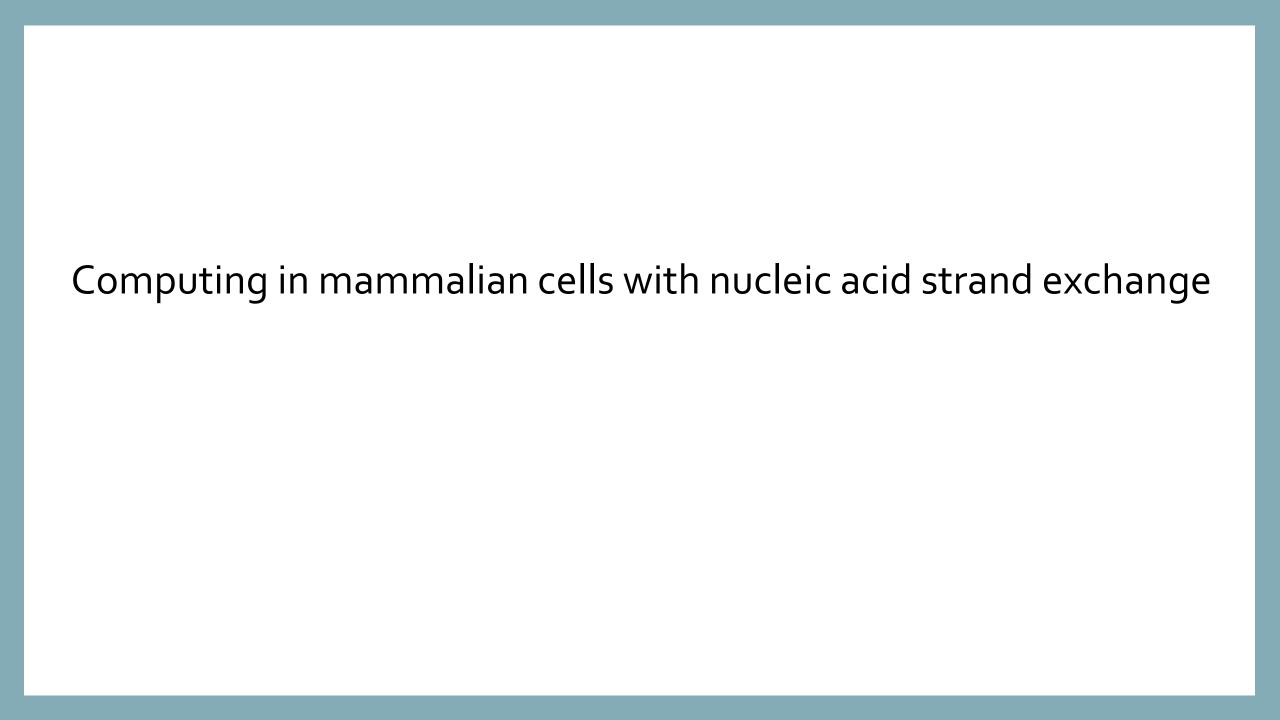
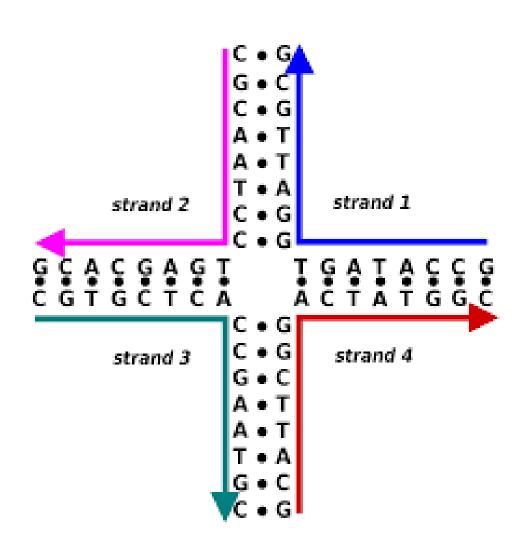
BT 601: Analytical Biotechnology

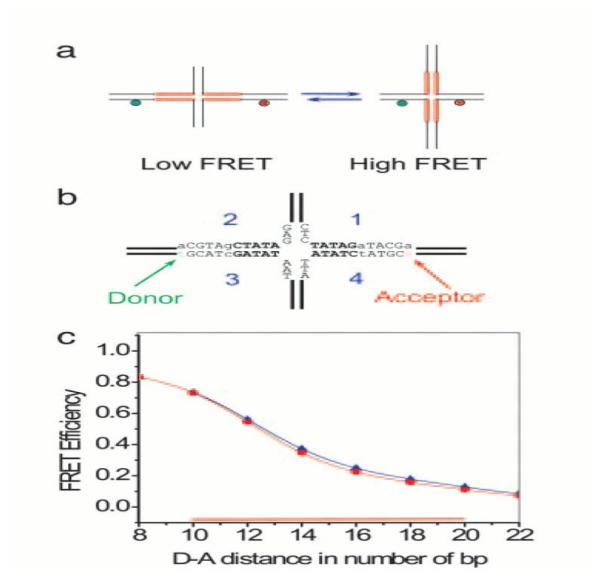
-Prof. Siddhartha Sankar Ghosh



Four Point Junction of DNA

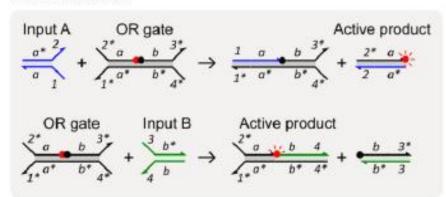


Low to High FRET

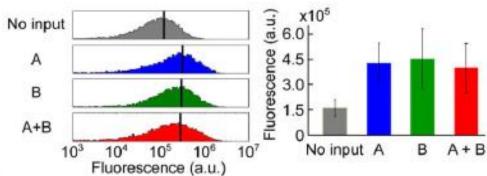


Strand exchange-based OR and AND logic gates work in mammalian cells

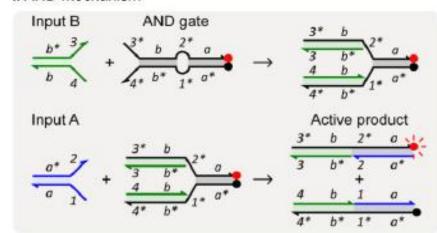
- a OR gate
- i. OR mechanism



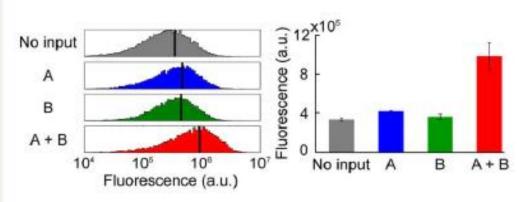
ii. OR activation in cells



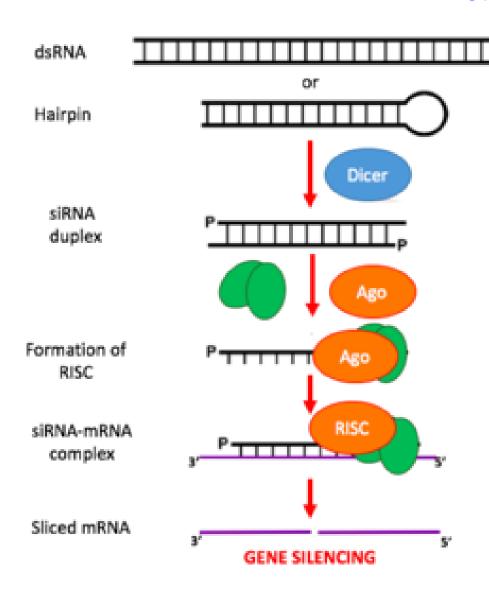
- b AND gate
- i. AND mechanism



ii. AND activation in 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

a Nucleic acid complex hybrids RNA DNA 2'OMe RNA PS DNA RNA PS 2'OMe RNA b Functional siRNA formation in cells (top strand) c Functional siRNA formation in cells (bottom strand) Guide strand = green strand Guide strand = blue strand hybrid hybrid siRNA/RISC siRNA/RISC mRNA1 mRNA2 Fluorescence (a.u.) Fluorescence (a.u.) 10 Neg Pos ii Neg Pos iii con con con con siRNA siRNA d Mechanism of siRNA activation e In cell citrine knockdown Fluorescence (a.u.) active siRNA RNA 10 PS 2'OMe RNA 6 Pos Active + con siRNA waste siRNA

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

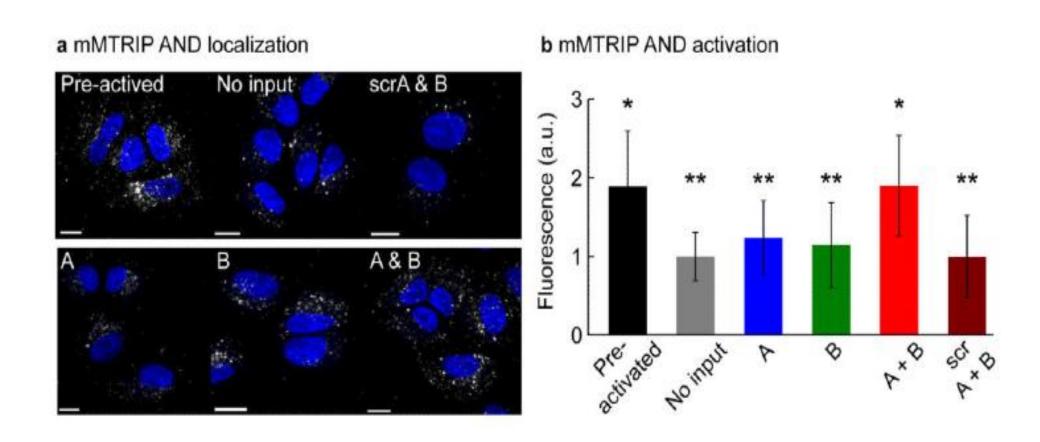
a Construction and delivery of mMTRIPS i. Targeting oligo covalently linked ii. Biotin-labeled reporter gate iii. Biotin-reporter conjugate linked to to neutravidin neutravidin core iv. Sequential delivery of complexes using SLO v. mMTRIPS hybridize to ACTB mRNA transcripts Input Reporter **b** 4-way strand exchange mMTRIPs iii. Activation i. Reactants ii. Microscopy Fluorescence (a.u.) Active reporter Reporter alone 1x input A Active reporter B Reporter alone C 1x input D 3x input ABCD c 4-way strand exchange mMTRIPs without targeting oligo ii. Microscopy iii. Activation i. Reactants 2.1 (a) A Active reporter Active reporter Reporter alone 1x input 3x input B Reporter alone C 1x input D 3x input

ABCD

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 dinucleotide 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.

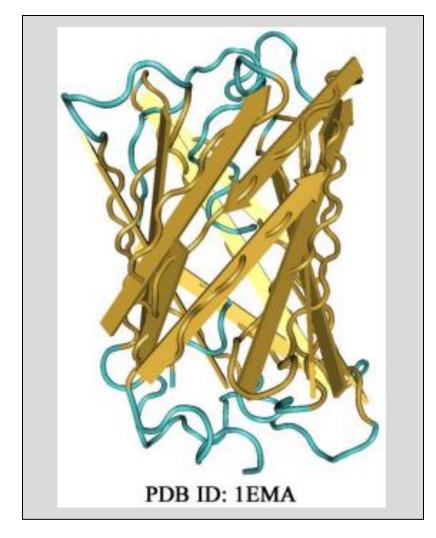


Figure: PDB structure of Green fluorescent protein, abbreviated as GFP. It was discovered by Shimomura and coworkers in 1962. Protein was isolated from the jellyfish, Aequorea victoria, that glows in the dark.

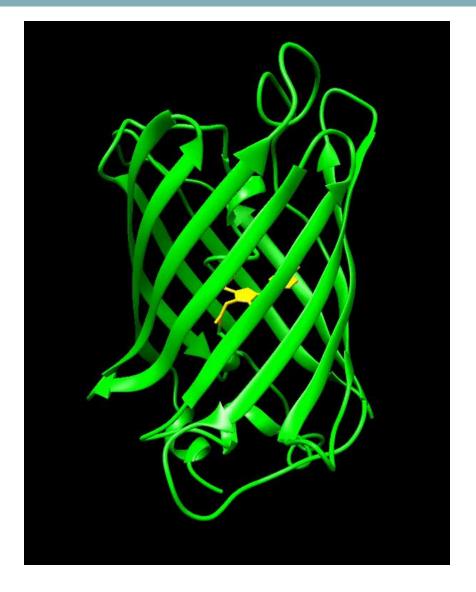


Figure: GFP 3D structure and zoom in to fluorescent chromophore.

Protein folding

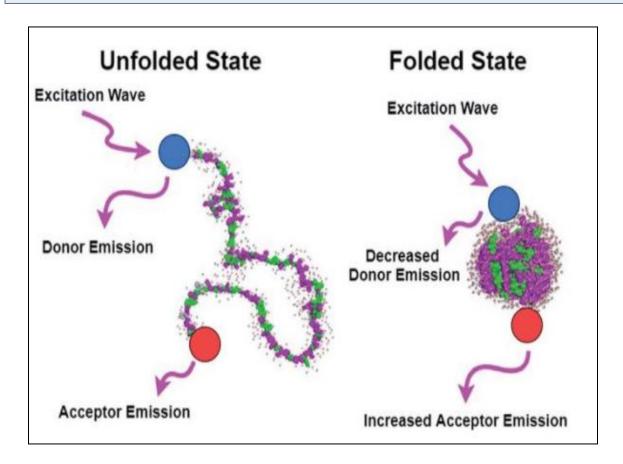


Figure: Protein folding and unfolding detection with FRET.

THANK YOU