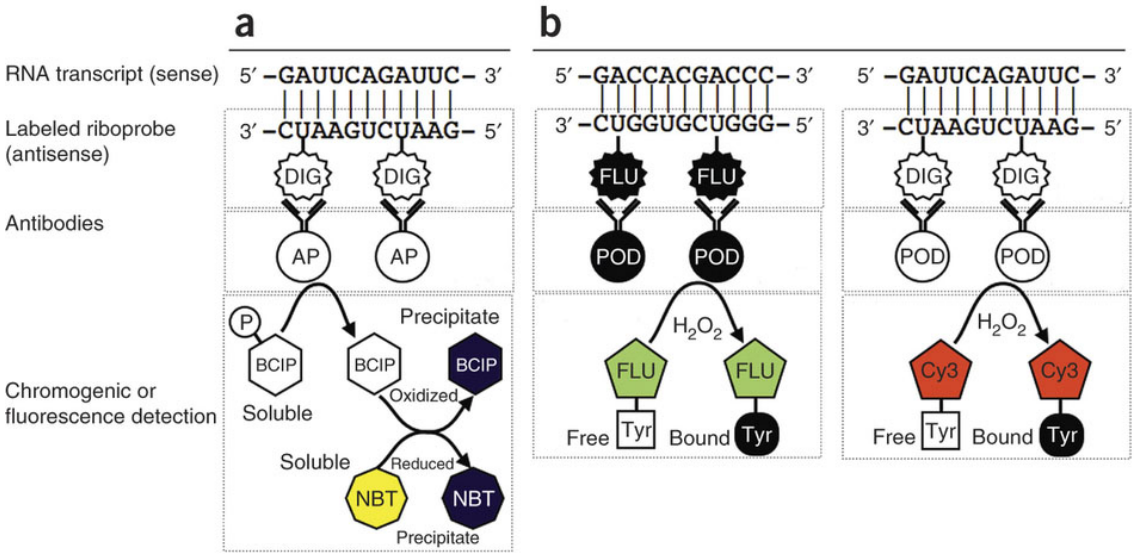
Ingrid Lekk

The magic behind in situs



1. Probe labelling

In order to have a detectable probe, it needs to be synthesised with nucleotides that are tagged with a label that can be detected with an antibody. The label is a hapten of some sort (a small molecule with high antigenity) conjugated to uracil nucleotides. Three main labels are used:

1. DIG (digoxigenin) – a steroid from plants
2. FLUO (fluorescein) – a synthetic organic compound
3. DNP (dinitrophenol) – the name says what it is…

2. Antibodies

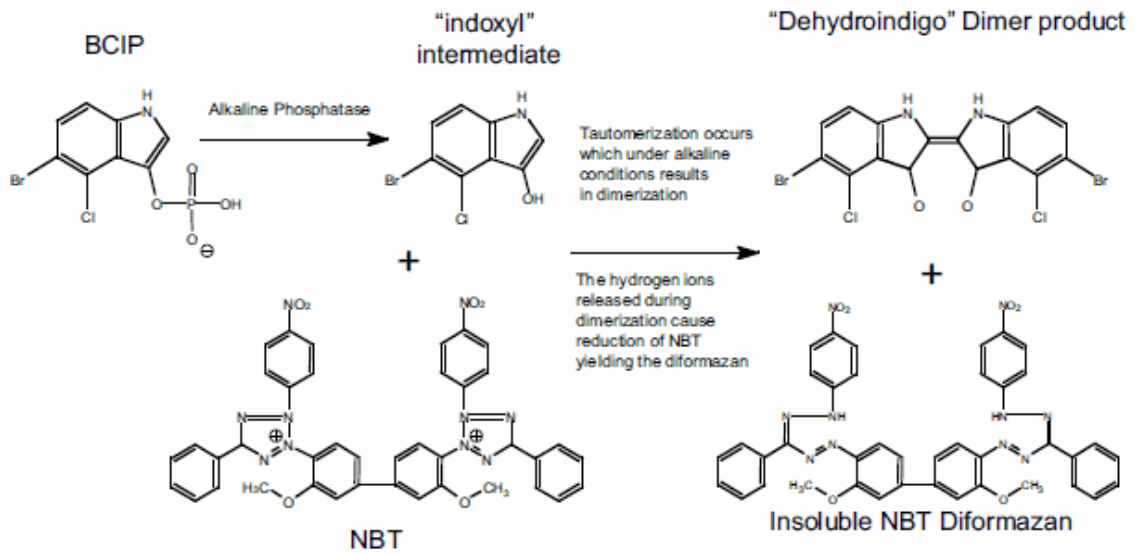
To detect the label in the probe bound to the complementary transcript, two main antibody types can be used differing in the enzyme that is conjugated with the antibody.

1. Alkaline phosphatase (AP) conjugated antibody. AP uses BCIP or Fast Red as a substrate (see next section)
2. HRP/POD/HRPO (horseradish peroxidase) conjugated antibody. HRP catalyses tyramide reaction (can for example be used with fluorescein, Cy3, Cy5).

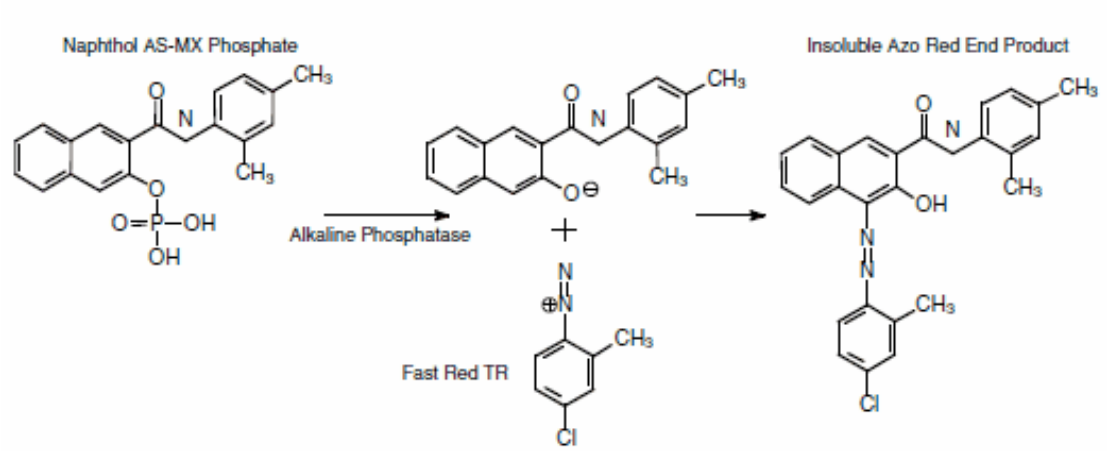
3. Substrates/dyes

Depends on the enzyme the anibody was conjugated with. One can either do a colorimetric or fluorescent in situ:

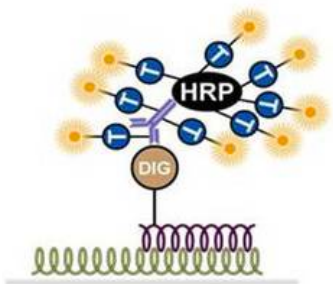
1. BCIP/NBT (5-Bromo-4-chloro-3-indolyl phosphate/nitroblue tertrazolium) – BCIP is an artificial chromogenic substrate to AP that is oxidized by NBT. The reaction gives a dark blue precipitate.



2. Fast red/Naphtol phosphate (4-Chloro-2-methylbenzenediazonium/ 3- Hydroxy-2-naphthoic acid 2,4-dimethylanilide phosphate) – substrate to AP that gives a red precipitate as an end product of the reaction for colorimetric in situ. Fast red can also be used as a fluorescent label (excitation around 560nm, emission at around 600nm).



3. Cyanin dyes – fluorescent synthetic dyes with different excitation/emission spectra depending on the structure. Used as conjugates to tyramids which are phenolic compounds activated by HRP. Upon activation, tyramide forms a highly reactive (short-lived) intermediate that binds to tyrosin residues in close proximity. The whole thing is called TSA (tyramide signal amplification – as many tyramids react close to HRP-conjugated probe). (TSA system can also be used with other labels not just fluorescent dyes).



Kit	Excitation wavelength	Emission wavelength
Fluorescein	494 nm	517 nm
Cyanine 3	550 nm	570 nm
Cyanine 5	648 nm	667 nm