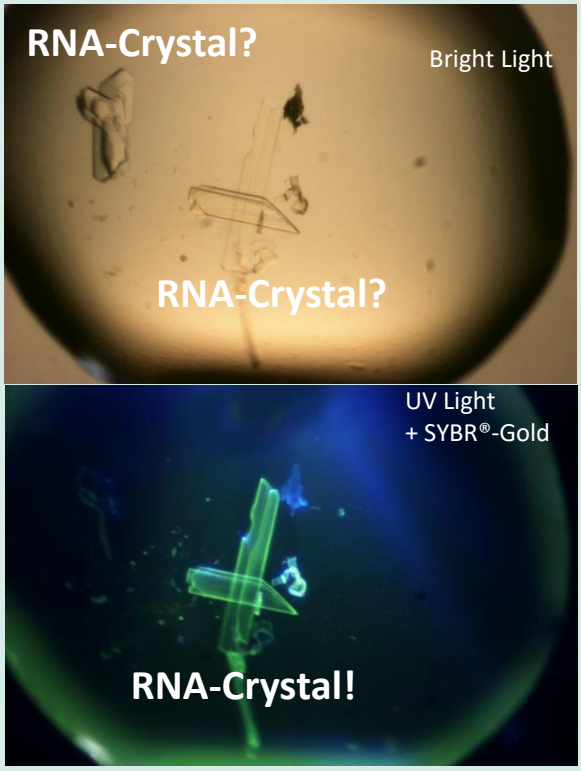
Immediate identification of nucleic acid content in protein crystals

In protein crystallization, intrinsic UV-fluorescence imaging is widely used for crystal
identification. However, if the target is a protein-nucleic acid complex (aptamer), the presence of RNA or DNA, embedded in the crystal lattice cannot be determined based intrinsic fluorescence imaging, applying vis cameras. The reason is that protein UV-fluorescence is mainly generated by tryptophane with an fluorescence maximum at around 360 nm while the excitation wavelength's maximum is around 280 nm. Nucleic acids on the other hand have a shorter excitation maximum than tryptophane. Transmission properties of optics and disposable materials as well as the availability of light sources for emitting this part of the spectrum hinder a practical use of UV-light imaging based on nucleic acid's intrinsic fluorescence. However, there's a simply and elegant method to solve the problem:

Label-free fluorescence of nucleic acids
can be achieved by applying a nucleic acid specific, non-covalently binding fluorescence dyes (in this case SYBR® GOLD was used). Ín order to apply the dye, the crystallization well has to be opened and a tiny amount of the dye has to be added to the crystal solution.

After incubation of 5 to 15 min, even a very low dosage of the nucleic specific fluorescence dye generates a clearly visible and highly specific fluorescence signal with low background of the surrounding solution or plate material.

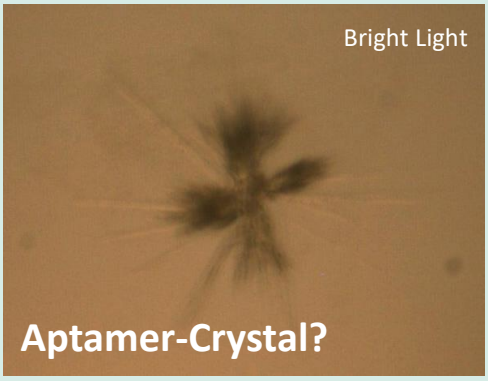


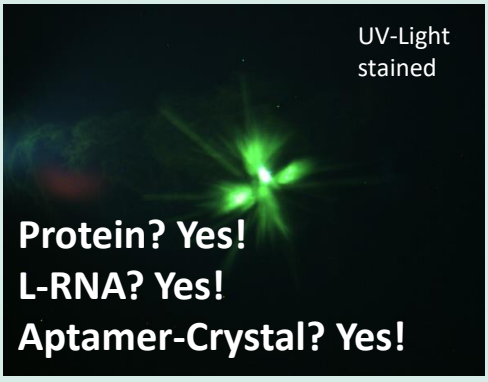
Pure DNA/RNA-Crystals
In this example, a short hexameric RNA-fragment was used. Sodium cacodylate was used as precipitant for crystallization.
Crystals with similar morphologies appeared after 6-8 days. Such crystals could be easily distinguished to be Na-cacodylate and hexameric RNA.

Non-sequence specificity
SYBR®GOLD
staining is non-sequence specific. Even short RNA-segments of 6 bases can be stained specific.

Identification of Protein-Nucleic Acid-Complex-Crystals:
Non-covalent nucleic acid specific fluorescence staining also works, when a RNA/DNA-fragment has been crystallized in form of a complex with a protein in a co-crystallization approach.

Before staining, crystals were illuminated via UV-light. Once crystals have been identified to be proteinogenic, due to its bluish tryptophane fluorescence, the next step was to apply SYBR®GOLD in a very low dose. When the crystals were illuminated via UV-light again, after incubation of a few minutes, the color of the fluorescence light has changed from bluish to greenish. This is a reliable indicator for the presence of nucleic acid in a crystal.





RNA and DNA Aptamers + L-RNA-Aptamers: The specificity of SYBR®GOLD to nucleic acids is also provided when L-nucleotides were used as a ligand. This non-enantiomer-specific staining can be used to identify the presence of highly stable "Spiegelmers" in a crystal, as shown in this example.

Same Light Source can be used since the same UV-Light spectrum that excites intrinsic tryptophane florescence is also capable to excite SYBR®GOLD's fluorescence. Therefore XtalLight 100, SpectroLight 600 and SpectroQ can be directly used for aptamer-crystal-identification.