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Iodination of protein crystals for fun and profit

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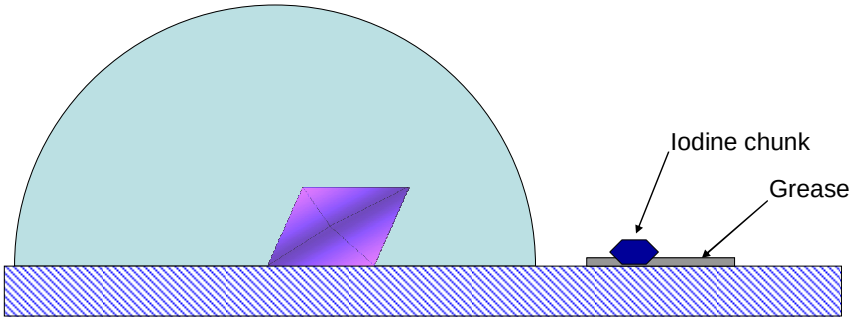
The idea of iodination in-crystal has been explored by such venerable authorities as Kretsinger, Wykoff, Matthews and Paul Sigler (1970). Graham & Butler used iodination as means to mark specific residues in tobacco mosaic virus coat in 1977. More recently crystal structures were solved using iodo-tyrosine scattering as the sole source of phasing information (Ghosh, 1999). The two methods presented below are convenient modifications of traditional iodination techniques – since nothing is new under the sun, I am sure that other people have independently developed and used this technique before.

The first method relies on the ability of elemental iodine (I_2) to rapidly sublime at room temperature – therefore iodination of protein crystals may be performed solely by means of vapor diffusion, thus bypassing any potentially harmful manipulation or dilution of the crystallization drop. The process is illustrated and described below:

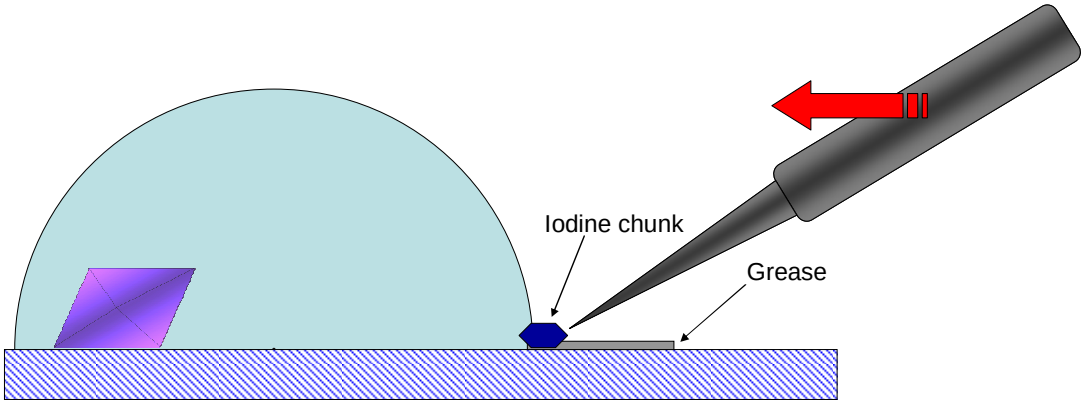


Set-up:

1. Obtain a small amount of solid iodine. Short-term storage (hours) of iodine crystals in plastic containers is acceptable – however long-term storage (days) usually results in iodine evaporation through the walls of the container (which can acquire a lovely yellow or purple hue due to I_2 dissolved in the plastic). Crush iodine with the spatula if necessary.
2. Locate suitable drops with crystals, open the crystallzation experiment and quickly dab a tiny amount of grease on the crystallization device (cover slip or tray surface) about 1 mm away from the edge of the drop.
3. Embed 1-2 tiny (~0.1 mm) iodine crystals into the grease next to the drop, taking care to leave most of the iodine surface uncovered.
4. Re-seal the crystallization experiment and place it back into its incubator overnight.

Outcome: Iodination can take 8-24 hours depending on the crystallization conditions, the amount of iodine used, the temperature, and other factors. It is highly adviseable to collect test diffractograms on sample crystals every 6-8 hours. Iodine crystals should disappear overnight – you can add more if you feel that the process is not complete. As iodine migrates into the drop (and generally distributes itself throughout your experimental set-up) your protein crystals may retain their original color (if they have any), or they may acquire a yellow hue – neither is a reliable signal that iodination is successful. Changes in unit cell dimensions are a good hint that something interesting is going on – but of course the final proof is in the data.

If your initial attempts are not successful, you may want to try adding a microscopic amount of 1M KI solution to your drop (enough to have 1-5 mM final concentration), then try again - or proceed with the the next technique:



Set-up:

1. Obtain a small amount of solid iodine as well as a few microliters of 1M potassium or sodium iodide solution.
2. Locate suitable drops with crystals, open the crystallzation experiment and quickly dab a tiny amount of grease on the very edge of the drop. If you want to speed up the process even more, you can add a tiny amount of KI or NaI solution to the drop (you can use a whisker or a seeding needle dipped into the solution to transfer a microscopic droplet). Aim for 1-5 mM final concentration of iodide.
3. Embed 1-2 tiny (~0.1 mm) iodine crystals into the grease and gently push them into the crystallization drop using an acupuncture needle, whisker, or micro-tool. Try to use the side of the drop that faces away from the best-looking crystals.
4. Re-seal the crystallization experiment and place it back into its incubator overnight.

Outcome: As in the first method – iodination usually takes many hours to complete. This is a fairly violent way of iodinating protein crystals – so you can expect some of the crystals not to survive, which is why I recommend positioning iodine pieces further away from the best protein crystals.

Useful considerations and tidbits:

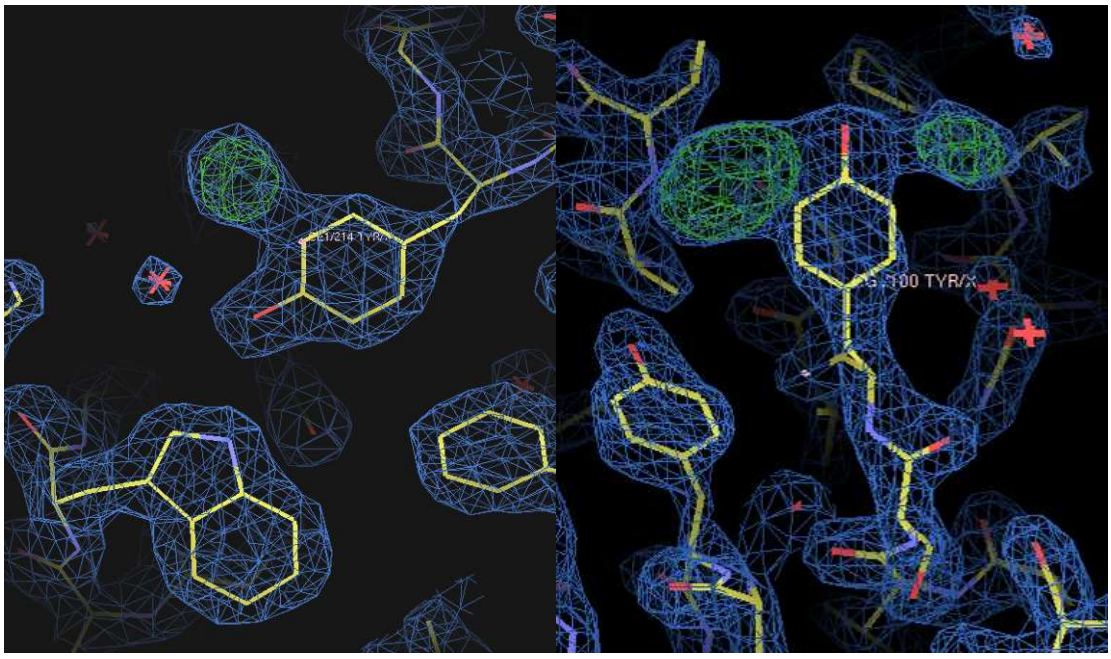
- This method works better when the support for the drop is a glass cover slip, because iodine readily diffuses through many plastics. If you are using plastic devices for crystallization (i.e. NeXtal/Qiagen “EasyXtal™” or multiwell plates) you may have to experiment with somewhat larger amounts of iodine; or try the second method. Likewise, tape-covered experiments may need serious tweaking.

- The small ammount of iodide that you may want to add to the drop acts as a carrier for I_2 molecules (as I_3^- ions and polyiodide). Its presence tends to facilitate iodination since iodine is not very soluble in water.

- Iodination of tyrosine residues is pH-dependent, with best results obtained above pH 5.5. If your crystallization condition pH is low you may still succeed, however it probably takes longer incubation and more iodine.

- If your crystallization pH is above 8 you have a decent chance to observe iodination both tyrosine and histidine residues.

- Tyrosines may bear either one or two iodine atoms (in *ortho*- positions with respect to the hydroxyl). Histidines are usually mono-iodinated, although doubly substituted derivatives may also form.



Iodine anomalous difference density peaks – on the left only one *ortho*- position was exposed (the other one is protected by the protein core). On the right both positions are modified.

- The presence of thiol-based reducing agents in the drop may be an issue as they tend to eat up the iodine.

- Don't be surprised if you encounter bound I_2 molecules in your crystals as well as I^- I_3^- and poly-iodide ions. Molecular iodine is fairly hydrophobic and will gravitate towards suitably greasy areas of protein.

- If you cannot easily or quickly obtain solid iodine – you can make a small amount of I_2 out of sodium or potassium iodide by slowly adding ~1ml of concentrated sulphuric acid to 100-200 mg of the salt (under the hood, in an open Pyrex glass vessel, wearing appropriate protective gear!). Tiny black crystals of elemental iodine will form – wash these iodine crystals carefully (many times) with cold distilled water (which will dissolve the unreacted iodide and the acid), once with dilute bicarbonate solution (to neutralize traces of acid), with water one last time - then quickly dab the iodine crystals dry on a piece of clean paper towel. *Observe all appropriate safety precautions when handling concentrated acid and elemental iodine* (the former will burn you, the latter will viciously stain you).

References:

Ghosh D, Erman M, Sawicki M, Lala P, Weeks DR, Li N, Pangborn W, Thiel DJ, Jörnvall H, Gutierrez R, Eyzaguirre J. Determination of a protein structure by iodination: the structure of iodinated acetylxylan esterase. Acta Crystallogr D Biol Crystallogr. 1999 55(4):779-84

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