Silver Staining – Protein Gels

NOTE 1: Done on SDS-PAGE (1D or 2D) resolved protein gels

NOTE 2: Requires clean equipment (fingerprints will show up and make your gels look like crap).
Extreme clean: soak plates (an hour or so), combs and spacers (minutes only - they will dissolve) in 80% HCl (**CAREFUL : spill on yourself = burn**). Rinse well with ddH_2O before use.

NOTE 3: Ensure the gels are complete immersed and shaking in each solution and wash. If doing two gels in the same box ensure that they're not stuck together at any stage.

Protocol -

1. Run SDS-PAGE gel as appropriate to ensure optimal separation of your complex. Carefully disassemble, cut off stacking, and fix gel for 1 hour with gentle shaking .

Fix: 50ml Methanol
12ml Glacial Acetic Acid
50µl formaldehyde
to 100ml with ddH_2O

2. While shaking make :
(i) 50ml 0.1% $AgNO_3$ / 25µl formaldehyde (in a foil wrapped falcon, chill on ice)
(ii) 50ml 0.02% NaThiosulfate (at RT^o)
(iii) 50ml 2% Na_2CO_3

3. Rinse gel with 50% MeOH (12 min)
Rinse gel with ddH_2O (2 x 5min)

4. Immerse gel in 40ml 0.02% NaThiosulfate for 60s EXACTLY with gentle shaking

5. Rinse gel with ddH_2O (2 x 1min)

6. Immerse gel in 50ml chilled 0.1% $AgNO_3$ / formaldehyde solution. Cover with foil and incubate for 25 min with gentle shaking in the cold room.

7. Rinse gel with ddH_2O (3 x 20s)

8. Add developing solution:
50ml 2% Na_2CO_3
1ml 0.02% Na-Thiosulfate
25µl formaldehyde
Immerse gel and shake at RT^o until bands appear ($\geq$ 5min)

9. Stop reaction by removing developer and swirling in Fix solution for 5 min. Rinse gel with ddH_2O (3 x 1min). **Photograph gel ASAP for records**.