Protein Dimerization and Aggregation

Unexpected high molecular weights on western blots have observed for many proteins. They are may be caused by dimerization and aggregation.

Preparation of Whole Cell Lysate

Lysis buffer:
1% Triton X-100
0.5% Nonidet P-40
150mM NaCl (58.44g/mol)
10mM Tris (121.14g/mol), pH 7.4
2mM EDTA (372.2g/mol)
1mM EGTA (380.4g/mol)
Protease inhibitors (83μg/ml aprotinin, 30 μg/ml leupeptin, 1mg/ml Pefabloc, 50μg/ml calpain inhibitor, 50μg/ml bestatin and 5μg/ml pepstatin.

It is also important to add 100mM iodoacetamide (Sigma I1149-5G, MWT 184.96g/mol stored at 4C) to the lysis buffer to block any non-specific sulfide link that might form between free cystines during the lysis and extraction of the proteins (i.e. avoid aggregate formation). Iodoacetamide will block thiol of Cys , thus, if aggregate is still appears after iodacetamide addition to the lysis buffer, this means that the cys-cys bond is SPECIFIC.

Procedure:
(1) Prepare the lysis buffer with and without 100mM Iodoacetamide (to examine the non specific disulfide link).
(2) Cells are washed twice with PBS
(3) Cells are solubilized with the lysis buffer O/N at 4C using end-over-end rotor.
(4) Spin the lysate at 15,000rpm for 15min at 4C (to remove insoluble stuff).
(5) The supernatant is considered as a total cell lysate
(6) Determine the protein concentration in the sample (use Lowery method).

Thus, to prepare 50ml Lysis buffer (don't add iodoacetamide)
500μl Triton X-100
250μl NP-40
0.438g NaCl
0.06g Tris (pH 7.4)
0.0372g EDTA
0.019g EGTA
Add the protease inhibitor cocktail (Sigma P-8340) as 1:100 (i.e. 10μl of the protease for each 1ml of the lysate). Add 0.0925g of Iodoacetamide for 5ml of the lysis buffer to get 0.1M .