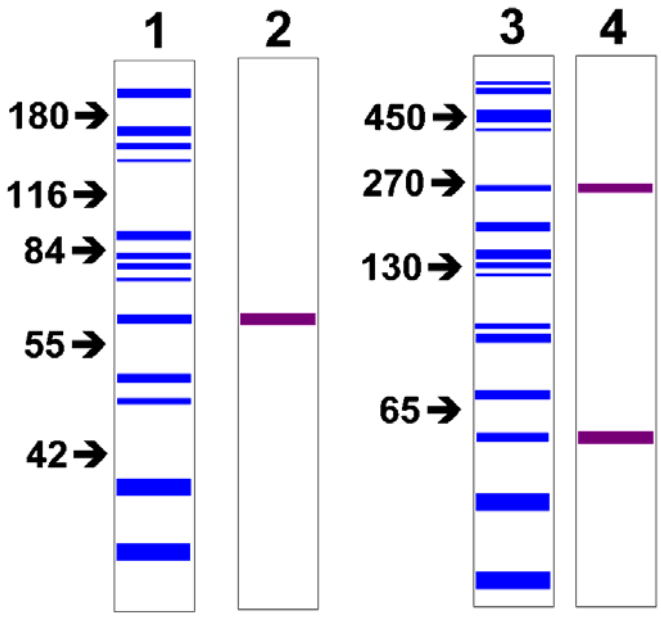
Homework #7

Immune serum raised against a purified protein is used to carry out immunoblotting following SDS gel electrophoresis (in presence of β-mercaptoethanol) or non-denaturing gel electrophoresis (same buffers as SDS gels, but no SDS or mercaptoethanol). You analyze the gels by Coomassie blue staining and Western blotting (see Figure and Legend). Give at least two possible explanations or interpretations for the observed results in the two gel systems (i.e. one band vs. two bands). What techniques or procedures would you carry out to distinguish between your hypotheses? Explain the rationale for the experiment(s) and/or give anticipated results. Be sure to give sufficient detail to demonstrate that the hypothesis/explanation is being tested.



Legend. For SDS-PAGE the sample was mixed with sample buffer containing SDS and β-mercaptoethanol and boiled before being electrophoresed on a 10% polyacrylamide SDS gel (lanes 1 and 2). For non-denaturing electrophoresis the sample was directly applied to a 6% polyacrylamide gel (lanes 3 and 4). Shown is the result of staining the gel with Coomassie blue (lanes 1 and 3) and the result of the immunoblotting assays for both types of electrophoresis (lanes 2 and 4). The arrows indicate the migration position of proteins with known molecular weights (in kDa). Numerous proteins/polypeptides are detected by Coomassie blue staining in both electrophoresis systems, whereas only a single or two proteins/polypeptides are detected by the antibody following SDS gel electrophoresis or non-denaturing gel electrophoresis, respectively.