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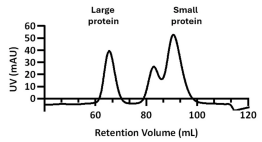
Size Exclusion Chromatography



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We use this protocol and it's working

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Abstract

Size-exclusion chromatography (SEC) is a method for separating proteins according to their size. To achieve this, a protein sample is applied to a column that is tightly packed with porous beads.
The size separation is determined by the thickness of the hydration shell of the proteins in a solvent. Small molecules can penetrate the pores, while large molecules cannot and are eluted first from the column. Smaller proteins follow, whereby the elution time is inversely proportional to the protein size.
We used this method to investigate whether proteins form a stable complex. The individual complex partners have a characteristic elution profile. If there is a stable interaction, they are expected to co-elute after mixing.

Sample Preparation

1 Mix the two purified interacting proteins in appropriate buffer with the appropriate ratio.

In our case: Protein 2 LRRK2^{RCKW} + Protein 2 DARPin E11 with a ratio of 1:5 in 2 mL buffer

Note

Buffer can be any buffer your proteins of interest behave well.

Sample Analysis

2 2 mL sample were subjected to a S200 gel filtration column combined with an ÄKTA XPress system in running buffer: 20 mM HEPES pH 7.4, 500 mM NaCl, 2.5 mM MgCl2, 20 µM GDP, 0.5 mM TCEP, 0.5%
glycerol

3 Collect flowthrough fractions (e.g. 3-mL)

4 Analyse collected fractions by SDS PAGE and Coomassie stain or immunoblot analysis.