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Purification and analysis of SKP1-FBXO7 complexes

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We use this protocol and it's working

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Abstract

Protocol for the biochemical purification and analysis of SKP1-FBXO7 complexes

Guidelines

Please wear appropriate PE while performing the experiment.
Please familiarise yourself with the laboratory safety rules and guidelines and follow these while performing the experiment.

Materials

Buffer A for size exclusion chromatography (SEC):
- 25mM HEPES pH7.5 (KOH)
- 150 mM NaCl
- 1mM DTT

TB medium

Troubleshooting

Preparatory note

1 All constructs were prepared utilizing standard molecular biological techniques and verified by sanger sequencing.

The cDNAs coding for HsFbxo7-129-398 and Skp1 preceding a second RBS were cloned into pGEX4T1 as previously described for other substrate receptor/Skp1 complexes (Schulman et al. 2000) (pGEX4T1-TEV-HsFbxo7-129-398/HsSkp1).

The cDNA coding for HsPI31-1-151 (with N-terminal TEV cleavable His8-tag) was cloned into pRSF1b (pRSF1b-His8-TEV-HsPI31-1-151).

For co-expression of Fbxo7/Skp1/PI31 complexes, both plasmids were co-transformed into *E. coli* BL21 Rosetta (DE3).

Molecular biological methods and protein expression

2 Grow *E.coli* cultures in Terrific Broth (TB) medium at 37°C. 1d
At OD600 of 0.8, induce expression with 0.5mM IPTG.
Continue growing the *E.coli* culture for 16h at 18°C.

3 Purify FBXO7/Skp1/PI31 complexes by sequential standard GST- and His-affinity chromatography. 1d
Cleave affinity tags by incubation with TEV protease at 4°C for 16h.

4 Further purify complexes by preparative size exclusion chromatography (SEC) in buffer A (25mM HEPES pH7.5 (KOH), 150 mM NaCl, 1mM DTT; for details please see next section) on a Superdex 200 Increase 10/300 GL column.

5 Pool fractions of interest, aliquoted and snap freeze in liquid N2.
Store fractions at -80°C until further usage.

6 HsCul1-1-410 was expressed as GST-fusion protein and purified as described previously (Hopf et al., 2022).

Analytical size exclusion chromatography (SEC) 30m

7 Analytical SEC was carried out on an ÄKTApure system (GE Healthcare) equipped with a Superdex 200 Increase 10/300 GL column (Cytiva), in buffer A (25mM HEPES pH7.5 (KOH), 150 mM NaCl, 1mM DTT).

8 Preincubate samples at 37°C for 10 min before loading.

Samples:
HsCul1-1-410,
HsFbxo7-129-398/HsSkp1/HsPI31-1-151
HsFbxo7-129-398/HsSkp1/HsPI31-1-151 + HsCul1-410

9 Apply samples (100 µl at a concentration of 45 µM) on column.
Set flow rate to 1 ml/min. 30m

10 Record UV absorbance at 280 nm and collect fractions of 200 µl volume.

11 Analyze fractions by SDS-PAGE.

Protocol references

Ref1: Schulman et al., 2000,PMID:**11099048**
Ref2: Hopf et al., 2022,PMID:**35982156**