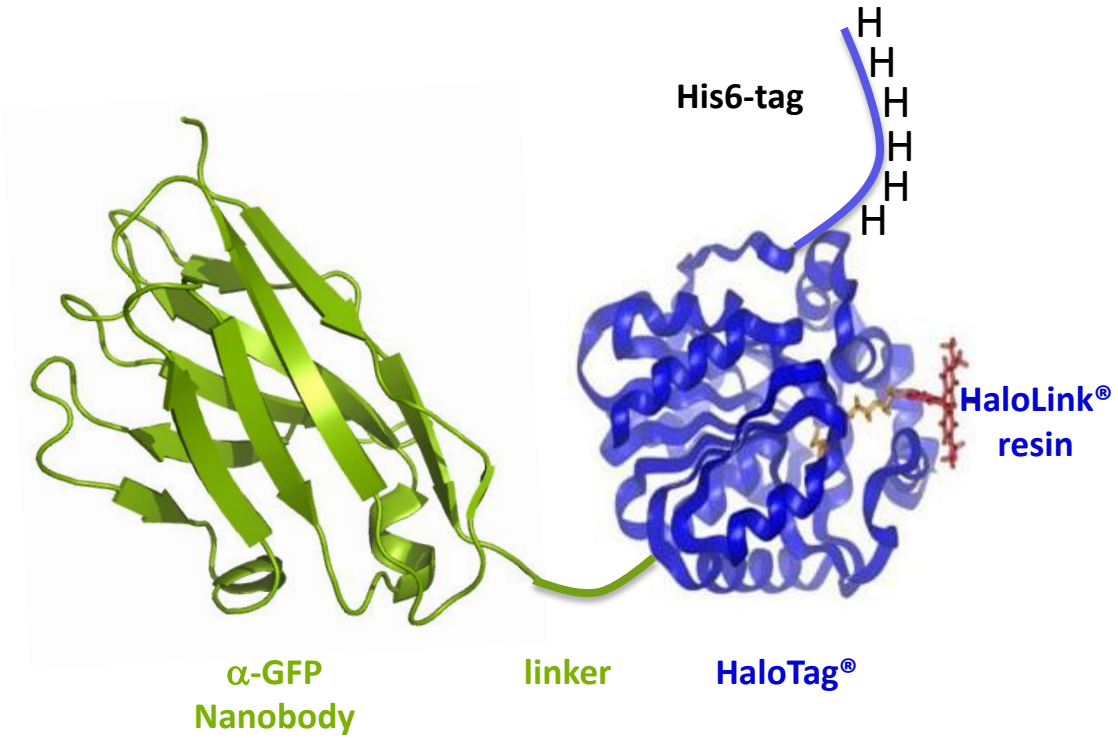
Production of magnetic GFP affinity beads



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Plasmid pOPINE GFP nanobody was a kind gift from Brett Collins (Addgene plasmid #49172); ***Structural and thermodynamic analysis of the GFP:GFP-nanobody complex****.* *Kubala MH, Kovtun O, Alexandrov K, Collins BM. Protein Sci. 2010 Dec;19(12):2389-401. doi: 10.1002/pro.519. 10.1002/pro.519* PubMed 20945358

The α-GFP nanobody structure was rendered using Pymol software (Schrödinger, Inc.). HaloTag and HaloLink are trademarks of Promega corporation. HaloTag image credit: By Dorsa.semsar - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=47123227

- **the isolated α-GFP nanobody binds to GFP with a K_d of ~ 1 nM [1]**
- **α-GFP nanobody binds to GFP and YFP but not to CFP or red-shifted fluorescent proteins [1]**
- **the HaloTag® allows covalent linkage to HaloLink® resins [2]**
- **His6-tag enables straight-forward purification of the fusion protein from *E. coli***

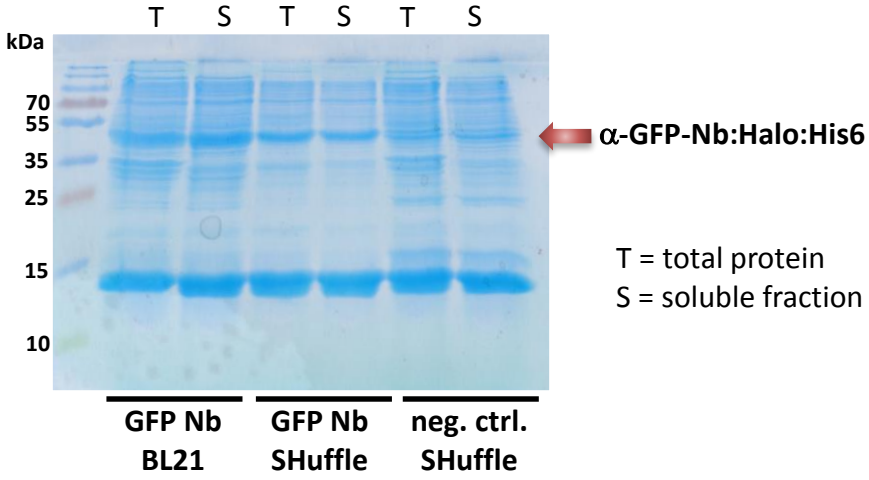
[1] **Structural and thermodynamic analysis of the GFP:GFP-nanobody complex.** Kubala MH, Kovtun O, Alexandrov K, Collins BM. Protein Sci. 2010 Dec;19(12):2389-401. doi: 10.1002/pro.519. 10.1002/pro.519 PubMed 20945358

[2] **HaloTag and HaloLink are registered trademarks of Promega Corporation**

Expression and cell lysis

- the fusion protein can be expressed in *E. coli* BL21 (DE3) or SHuffle® T7 Express [1] (DE3) cells
- expression plasmid is available from Addgene (#111090)
- grow *E. coli* in LB + Amp at 37 °C until OD_{600} reaches 0.8-1
- shift temperature to 18 °C (continuous shaking)
- induce expression by adding 0.5 mM IPTG
- protein expression o/N at 18 °C
- harvest cells by centrifugation (15 min., 5000 x *g*)

Pause point -> cell pellet can be stored at -20 or -80 °C

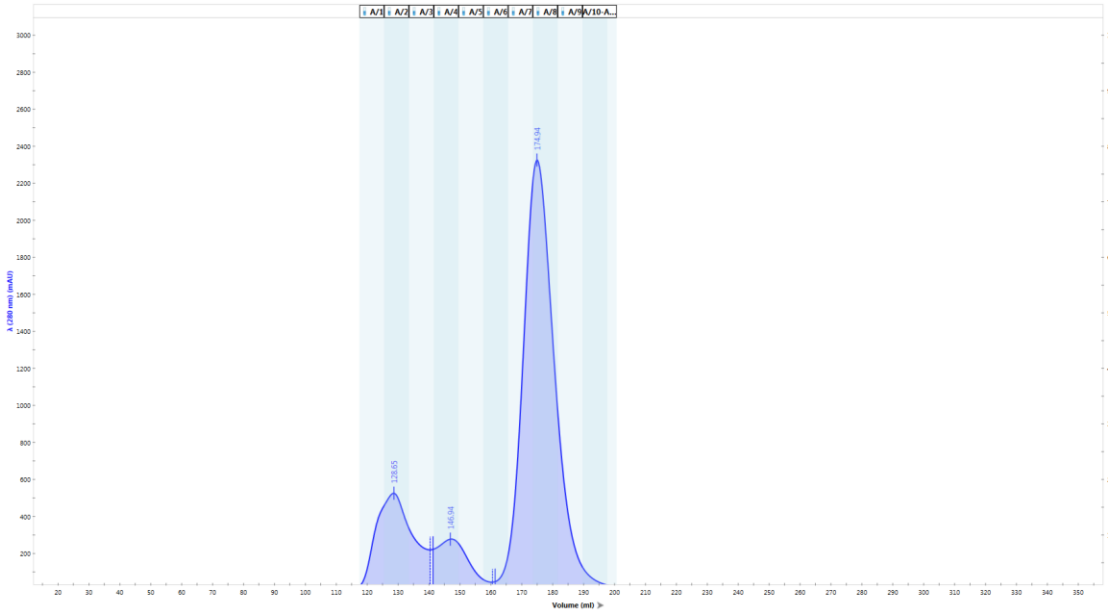


- cell lysis by Lysozyme and sonication in lysis buffer (2% of initial culture volume; see last slide for buffer composition)
- 20 min. / 30.000 x *g* / 4 °C spin to obtain soluble fraction

[1]SHuffle is a registered trademarks of New England Biolabs

Purification

- apply soluble fraction onto an IMAC column that has been pre-equilibrated with buffer A1
- wash IMAC column with 10-15 column volumes of buffer A1
- elute with three column volumes of buffer B1
- either directly proceed to the binding reaction or perform an additional purification step by size exclusion chromatography in A4 buffer (size exclusion step is not strictly required, see slide 6)
- on a S75 size exclusion column we usually observe some aggregates followed by a defined peak corresponding to the 49 kDa fusion protein



Pause point -> aliquots of purified protein can be flash-frozen in liquid N_2 and stored at -80 °C

Coupling the fusion protein to magnetic beads

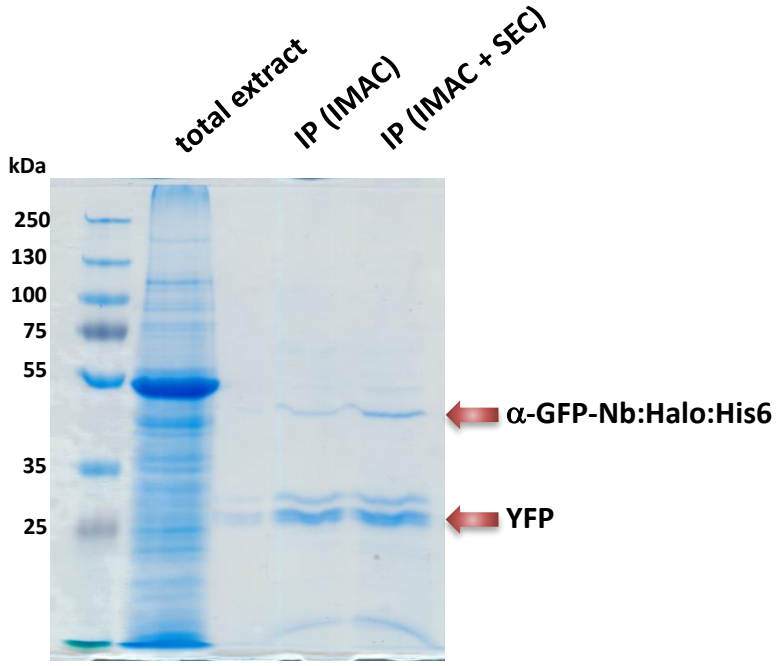
- **250 µl of Magne® HaloTag® Beads bind >1 mg of Halo-tagged protein [1]**
- **wash 250 µl of the beads in A4 buffer and collect them using a magnetic rack**
- **dilute 1.5 mg of α-GFP-Nb:Halo:His6 protein in 2 ml A4 and add the protein solution to the beads**
  (save a few µl as 'input' sample)
- **incubate on a rotating wheel for 2h at 4 °C**
- **collect the beads on the magnetic rack and take a sample from the supernatant**
  (you should observe a depletion compared to 'input' sample from above on a Coomassie gel)
- **wash the beads 3 times with 2 ml A4**
  (the supernatant from the last wash should no longer contain unbound α-GFP-Nb:Halo:His6 protein)
- **resuspend the beads in 250 µl of storage buffer**

Pause point -> The beads are stable for at least 4 weeks at 4 °C, we have not tested longer times of storage

[1]Magne and HaloTag are registered trademarks of Promega Corporation

Comparison of 1-step vs 2-step purification

- α-GFP-Nb:Halo:His6 either directly coupled to magnetic beads after IMAC or with intermediate size exclusion chromatography step
- We used both types of beads to purify YFP from an *N. benthamiana* protein extract



buffer composition

Lysis buffer:
50 mM Tris–HCl pH 8.0
0.3 M NaCl
20 mM imidazole
5 % (v/v) glycerol
50 mM glycine
[before use add 0.25 % (w/v) polyethyleneimine and
EDTA-free protease inhibitors]

A1 buffer:
50 mM Tris-HCl pH 8.0
0.3M NaCl
20 mM imidazole
5% (v/v) glycerol
50mM glycine

B1 buffer:
same as A1 but with 0.5M imidazole

A4 buffer:
20 mM HEPES pH 7.5
0.15 M NaCl

storage buffer:
50 mM HEPES pH 7.5
0.15 M NaCl
15% (v/v) glycerol
0.05% NaN_3