Overexpression of tagged NOX and peptides

Cultivation

20 ml of 2xYT Media with 100 μg/ml Ampicillin were inoculated 1/500 with overnight cultures and incubated at 37 °C at an agitation of 200 rpm. At an OD_{600} of 0.5- 0.6 the cultures were induced with 0.5 mM IPTG for the peptides or 50 ng/ml (NOX empty, NOX C1) and 100 ng/ml (NOX C1) of Tetracyclin for NOX. The cultures were further cultivated for 2 hours, followed by centrifugation (3500 g, 5 min, 4 °C), washing in 5 ml R-Buffer (50 mM Tris, 100 mM NaCl, 5% v/v glycerol, pH 7.5) and finally resuspended in 1 ml of the same buffer, while working on ice. The cells were lysed by pulse sonification (10 seconds sonification, 10 seconds pause) at an amplitude of 20 % for 2 minutes (total sonification time). The lysates were then centrifuged for 15 minutes at 16,060 g and 4 °C resulting in a clear supernatant and a pellet.

SDS-PAGE

The different fractions (pre-induction, taken at OD_{600}=0.5 just before induction; after induction= 2 hours after induction; P = pellet of the lysed cells; SN = supernatant of the lysed cells) were analysed by SDS-PAGE on a 12.5 % polyacrylamide gel. The gel ran for approximately 1:15 hours at 170 V until the dye leaked out of the gel. The gel was then stained with Coomassie.

Dilution of the fractions:

Constructs of the peptides: 5 μl of the fractions were diluted with 15 μl Buffer + 4 μl 6x Laemmli Loading Dye and incubated for 10 minutes at 95° C

NOX-constructs: 4 μl of 6x Laemmli Loading Dye were added to 20 ml of fractions and incubated for 10 minutes at 95°C.

Media & Buffer

2xYT Media	For 1 liter: 16g peptone, 10g yeast, 10g NaCl
R-Buffer (resuspension)	50 mM Tris, 100 mM NaCl, 5% v/v glycerol, pH 7.5
THB Media	Todd-Hewitt Broth, was prepared from OXOID THB mixture (CMO189), but can otherwise be prepared from: Heart Infusion (dehydrated) 3.1 g Yeast Enriched Peptone 20 g Dextrose 2 g Sodium Chloride 2 g Disodium Phosphate0.4 g Sodium Carbonate 2.5 g

Protocol generously provided by the lab

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