# PHD2 immobilisation using magnetic beads

For the immobilisation of the target protein PHD2-bio, streptavidin magnetic Dynabeads M 280 Streptavidin (Life Technologies) were used. For 1 cycle of selection 100 µg (10 µL of beads slurry) was incubated with approximately 40 pmol PHD2-bio at slow rotation at 4ºC for 30 min; with approximately 25 pmol PHD2 being immobilised on the beads. For the immobilisation of the target protein PHD2-bio, streptavidin magnetic Dynabeads M 280 Streptavidin (Life Technologies) were used.

# PHD2-Activity Confirmation

Activity assay was initiated by mixing 50 µL (10 µM immobilised PHD2, and 600 µM 2OG in 100 mM NaCl and 50 mM Tris-Cl at pH 7.5) and 50 µL (50 µM CODD, 1 mM L-Asc, and 100 µM Fe(ii) and in 100 mM NaCl and 50 mM Tris-Cl at pH 7.5).

The reaction was incubated for 1 h at 37ºC. The beads were removed from the solution. Activity of PHD2 was confirmed by observing a +16 Da mass shift on MALDI spectrum.

# Selection

Selection against PHD2 from the mRNA-cyclic-peptides library was carried using a protocol previously described in details (Reference\_\_\_). The original cDNA library was transcribed into mRNA and ligated to puromycin using T4 ligase. Translation was performed using Met (—) FIT system and ClAc-D-Tyr-tRNAfMetCAU, used to initiate the peptide chain and to enable post-translational cyclisation with Cys residue in a peptide, thus enabling an intramolecular thioester.

To produce c-DNA-mRNA-cyclic-peptides library, the mRNA part of thus generated fusion was reverse transcribed using MMLV RNase H (—) (Promega). 100 mM Tris-HCl (pH 7.5) supplied with 200 mM NaCl and 0.1% tween was used as 2 × selection buffer (100 mM NaCl, 50 mM Tris-HCl at pH 7.5, and 0.1% Tween-20). 0.2% BSA in 2 × selection buffer was used as 2 × blocking solution.

To block active streptavidin, 1 µL 500 µM biotin was added to the beads and they were incubated for another 30 min. For a dummy incubation two 5 µL of bead slurry aliquots were used: one was incubated with 1 µL 500 µM biotin in 100 µL selection buffer for 30 min, another was incubated in 100 µL selection buffer for 30 min.

After incubation both aliquots were washed 3 times using 1 × selection buffer and combined together. Prior to the incubation with the immobilised PHD2, c-DNA-mRNA-cyclic-peptides library was diluted with equal volume of 2 × blocking solution.

# SPR Analysis

KD of the cyclic-peptides complexes with PHD2 were estimated using a Biacore T200 machine (GE Healthcare) equipped with Sensor Chip SA. PHD2-bio immobilisation on the chip Biotin CAPture Kit was performed following the standard protocol. The running buffer was 50 mM Tris-HCl (pH 7.5) supplied with 100 mM NaCl and 0.05% tween, and 0.1% DMSO. Complex formation was tested by injecting varying concentrations (4 nM to 1 024 nM) at a flow rate of 30 μl min−1 and measured by single-cycle kinetics method. All data were fitted to the standard 1:1 binding model.

# Peptide Synthesis

Peptide synthesis was performed using Biotage® Syro Wave™. For a single peptide synthesis 48 mg NovaPEG Rink Amide resin was used. Protected amino acids and solvents were measured according to the standard protocol. After final de-protection step of Fmoc-synthesis 0.2 M ClAc-NHS (MW = 174 g・mol-1) solution in DMF, was added. The procedure was carried out twice for 30 min using 600 µL for per peptide per repeat. Resin was washed 5 times with DMF and 5 times with CH2Cl2; afterwards it was dried under vacuum for 30 min. For de-protection TFA containing TIS, EDT and H2O 2.5% (v/v) each was used. This cocktail was added to each sample to cover the resin (∼2 mL per sample) and mixed gently. Samples were incubated at RT with constant mixing for 1.5 hours. Supernatant was collected and the resin was washed twice using 1 mL TFA. The washes were combined with collected supernatant. The samples were concentrated for 30-60 min using centrifugal evaporator at 40ºC. Peptides were precipitated by addition of of 10 mL ice-cold Et2O and following manual centrifugation. Peptides have been washed 3 times using 5 mL ice-cold Et2O and dried briefly (∼5 min) in the centrifugal evaporator at 20ºC. Peptides were reconstituted in 500 μL DMSO, followed by addition of 5 mL MeCN. For cyclisation, the peptide solution was alkalised using 20 μL TEA; and the mixture has been incubated at 20ºC for 1 hour at constant mixing. To quench the reaction the mixture has been quenched using 50 µL FA. Cyclisation was confirmed using MALDI-MS and α-CHCA matrix. HPLC purification Peptides were purified using Aeris PEPTIDE column, 250 x 21.2 mm, C18 5 μm, 100 Å, which was operated at 20 mL･min-1. For separation, linear gradient 10 mM ammonium formate and 30 mM formic acid in 5% MeCN to 10mM ammonium formate and 10 mM formic acid 95% MeCN over 40 min was used. 5 mL sample was loaded on the column. Peptide fractions were dried from MeCN for 60-90 min using centrifugal evaporator at 40ºC and lyophylised using freeze-drying. The resulting peptides were reconstituted in DMSO. The purity was confirmed using MALDI-MS and α-CHCA matrix.

# NGS Data Analysis

NGS data were analysed using python script available on <https://github.com/NikitaLoik/mRNADisplayResults_Analysis>.