To facilitate the search for peptide ligands with useful properties — drug-like compounds, protein inhibitors, protein activators, or co-crystallisation chaperons — DNA-encoded peptide libraries were introduced. To select protein ligands from highly diverse peptide libraries, selection algorithm is organised in cycles: (1) incubation of a DNA-tagged peptide library with a target protein, (2) recovery of DNA-tagged ligands, and (3) regeneration of a less diverse library from the recovered DNA tags. Selection cycles are repeated until library diversity reduces sufficiently to make its analysis possible. Identified peptides are subjected to verification of their complex formation with the target protein and identification of their potentially useful properties. Selection is termed ‘successful’ if amongst thus recovered peptides, some demonstrate high affinity towards the target protein (kD < 10-7 M).

Based on such selection algorithm, in the last three decades, phage display and mRNA display were developed. These are of particular interest because they enable selection from highly diverse DNA-encoded peptide libraries (approximately 109 for phage display to 1012 for mRNA display). Successful selections using display techniques produced a number of useful peptide ligands. Unfortunately, many selections are failing to produce a ligand for a target protein. This has most commonly been attributed (1) to interference of the target protein with the phage, cDNA or mRNA part of a ligand or (2) to individual cDNA sequences amplification efficiencies. While it may be impossible to eliminate the undesirable interference of a ligand with the target protein or to alter the amplification efficiencies of ligands, it is hoped that alternative approach to the selection-results analyses may uncover the ligands with higher affinity towards target protein, thus increasing the rate of successful ligand discoveries. The analysis of display results is majorly based on two assumptions: (1) the recovery of a peptide ligand in the process of selection is directed by and is proportional to its affinity towards a protein target, and (2) the DNA tags do not affect the outcome of selection process. To date, these assumptions have not been validated.

A peptide-ligand recovery is thought to be regulated by its ability to form a complex with a target protein; and for the sake of the analysis, ligand recovery is taken directly proportional to its affinity towards target protein (*i.e.* ligand-target complex KD value). Thus, it is often concluded that peptides recovered in higher proportion have higher affinity towards target protein. However, without verification of the mechanism of selection this conclusion may result in less enriched peptides, yet having lower KD, being disregarded.

Recovery of structurally similar peptide ligands is often viewed as evidence for a particular consensus sequence being favoured in the selection, *i.e.* a complex between target protein and such consensus peptide ligand having lower KD. Often based on the recovered peptide sequences, a consensus-sequence is constructed, and such peptide is further tested for useful properties. However, if the structurally similar peptides recovered in a selection are genetically related (*i.e.* are mutants of one prevalent peptide), rather than accidentally present in the original library, such approach would produce a heavily biased peptide sequence, which may not have high affinity towards the target protein.

This paper provides detailed analysis of a successful selection against human recombinant PHD2 from a cyclic-peptide library performed using RaPID system, a version of mRNA display based on (1) the flexible in vitro translation (FIT) of an mRNA library, (2) mRNA-to-peptide ligation using puromycin, and (3) peptide cyclisation using the non-proteinogeinc amino acids incorporated into the peptide chain. This work verifies the effect of the cDNA tags on the selection results and introduces a robust approach to the selection-results analysis. In particular, this paper suggests a thermodynamic mechanism of selection that informs on to which extent the selection process is defined by the ligand-to-target affinity, highlights potential limitations of mRNA display and indicates potential ways of protocol improvements.