De novo designed parallel heterodimeric coiled coil peptide pairs with high affinity for use in mammalian cells

P-05.2-41

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Coiled coil (CC) peptides represent attractive building blocks for assembling modular protein structures. We designed three sets of orthogonal CC forming peptides with low Kd's. CC dimer formation is based on interactions between two alpha helices assuming a twisted super-coiled structure with a heptad repeat periodicity. Our group previously designed several orthogonal CC peptide pairs with Kd in the micro molar range. In order to extend the toolbox of CC-peptide we designed a set of tighter binders that would be more suitable for in vivo application. The original pairs were modulated by the number of asparagine residues along the hydrophobic seam. At the b, c and f position, alanine residues were placed in order to increase the stability of CC pairs. Furthermore, we tuned the stability of CCs by introducing a polar glutamine residue at the f position in all heptads or by introducing a second asparagine instead of isoleucine at the a position of the first or fourth heptad. The Asn at the a position in the fourth heptad reduced the thermal stability and reduced the likelihood of homodimers formation. Formation of CC dimers in vivo was measured by reconstitution of split luciferase fused to orthogonal peptides and split transcription factor, based on TALEN DNA binding domain and VP16 activation domain fused to coiled coil peptide pairs. Luciferase and TALEN activity was proportional to the stability of the coiled coil pairs. Peptide displacement of the reconstituted split luciferase could be controlled by the CC building blocks with the increased stability. Luciferase assay confirmed the traits of in vitro coiled coil characterization. The set of orthogonal peptides with different thermal stability of CC's extends the toolbox of peptides that can be used as regulators of enzymatic activity or transcription regulators.