β-Sheet mimetics that Target the Transcriptional Coactivator β-Catenin

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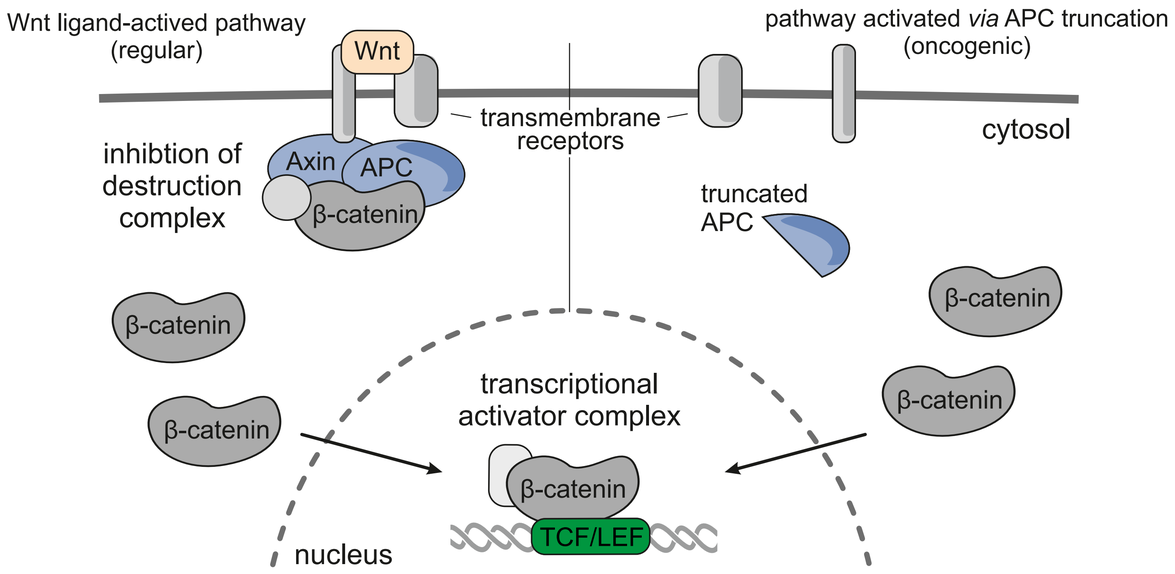
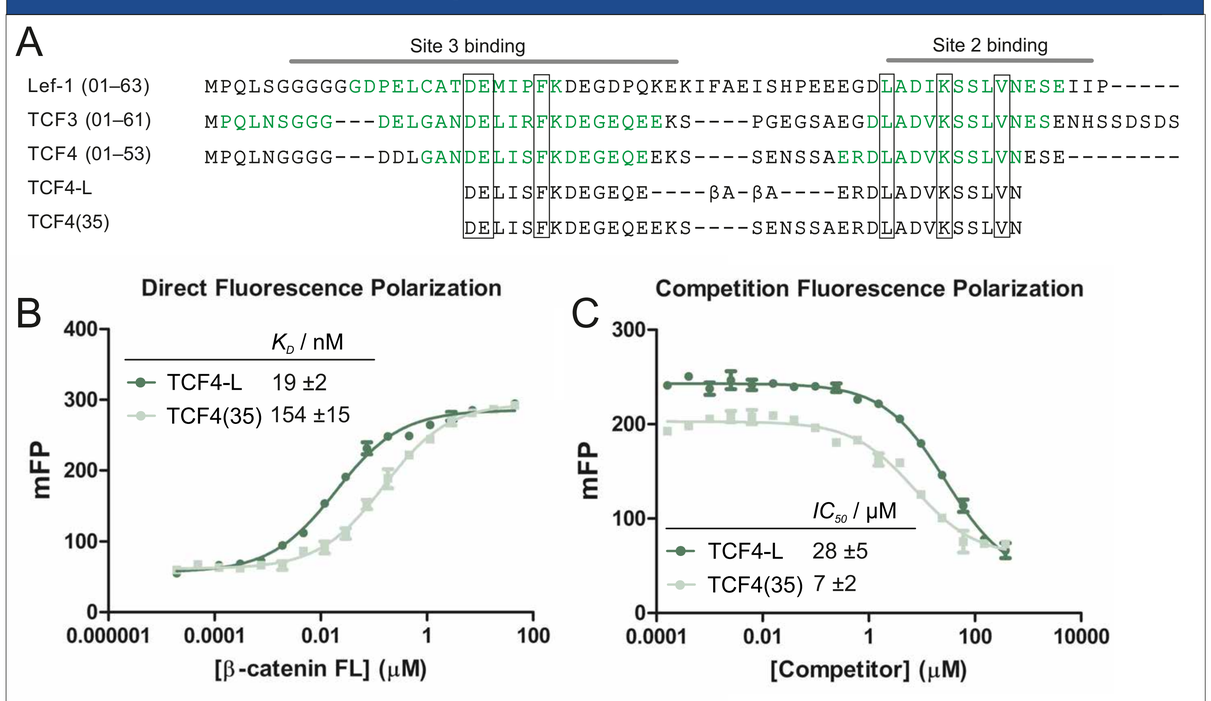


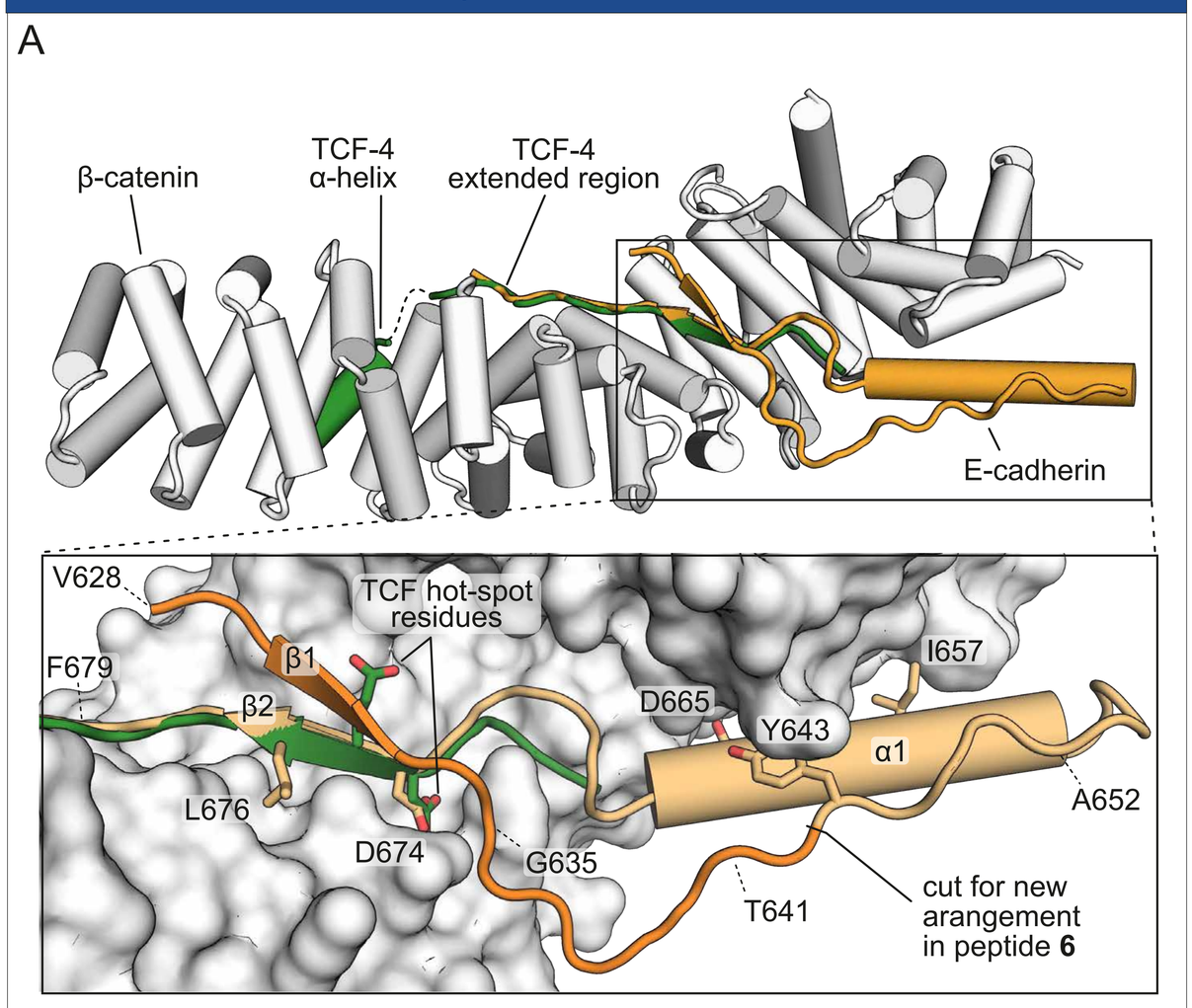
Protein complexes are defined by the three-dimensional structure of participating binding partners. Knowledge about these structures can facilitate the design of peptidomimetics which have been applied for example, as inhibitors of protein–protein interactions (PPIs). Even though β-sheets participate widely in PPIs, they have only rarely served as the basis for peptidomimetic PPI inhibitors,inparticular when addressing intracellular targets. Here,wepresent the structure-based design of β-sheet mimetics targeting the intracellular protein β-catenin, acentral component of the Wnt signaling pathway. Based on aprotein binding partner of β-catenin, a macrocyclic peptide was designed and its crystal structure in complex with β-catenin obtained. The presented design strategy can support the development of inhibitors for other β-sheet-mediated PPIs.

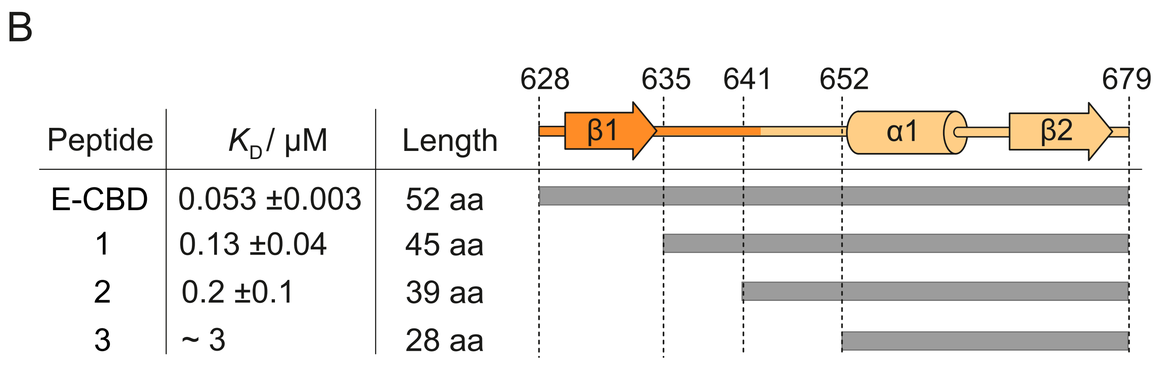
1 TCF Derived Tracer Peptide



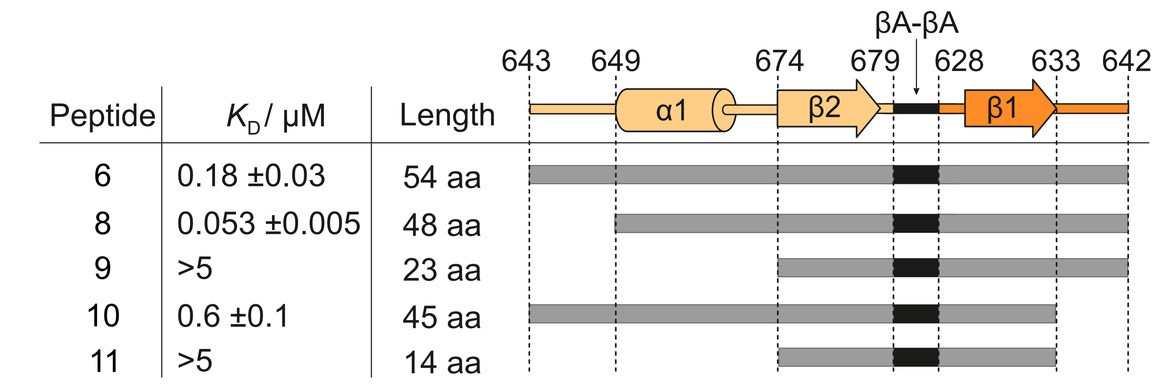
(A) Sequence alignment of the CBD's of Lef-1 (PDB ID 3ouw), TCF3 (PDB ID 1g3j), TCF4 (PDB ID 2gl7) and TCF4-L and TCF4(35). In green the resolved residues by X-ray. Framed the crucial residues for binding. (B) FP-binding assay of TCF4-L and TCF4(35) towards β-catenin. K_D-values were obtained from FP measurements (n=3, ± std. error). (C) FP competition assay with TCF4-derived tracer peptides c = 10 nM) and full-length β-catenin (c = 250 nM). Competitor concentration was varied (c = 1.6×10^{-4}–375 μM, n=2, ± std. error)

2 E-Cadherin-Derived Peptides





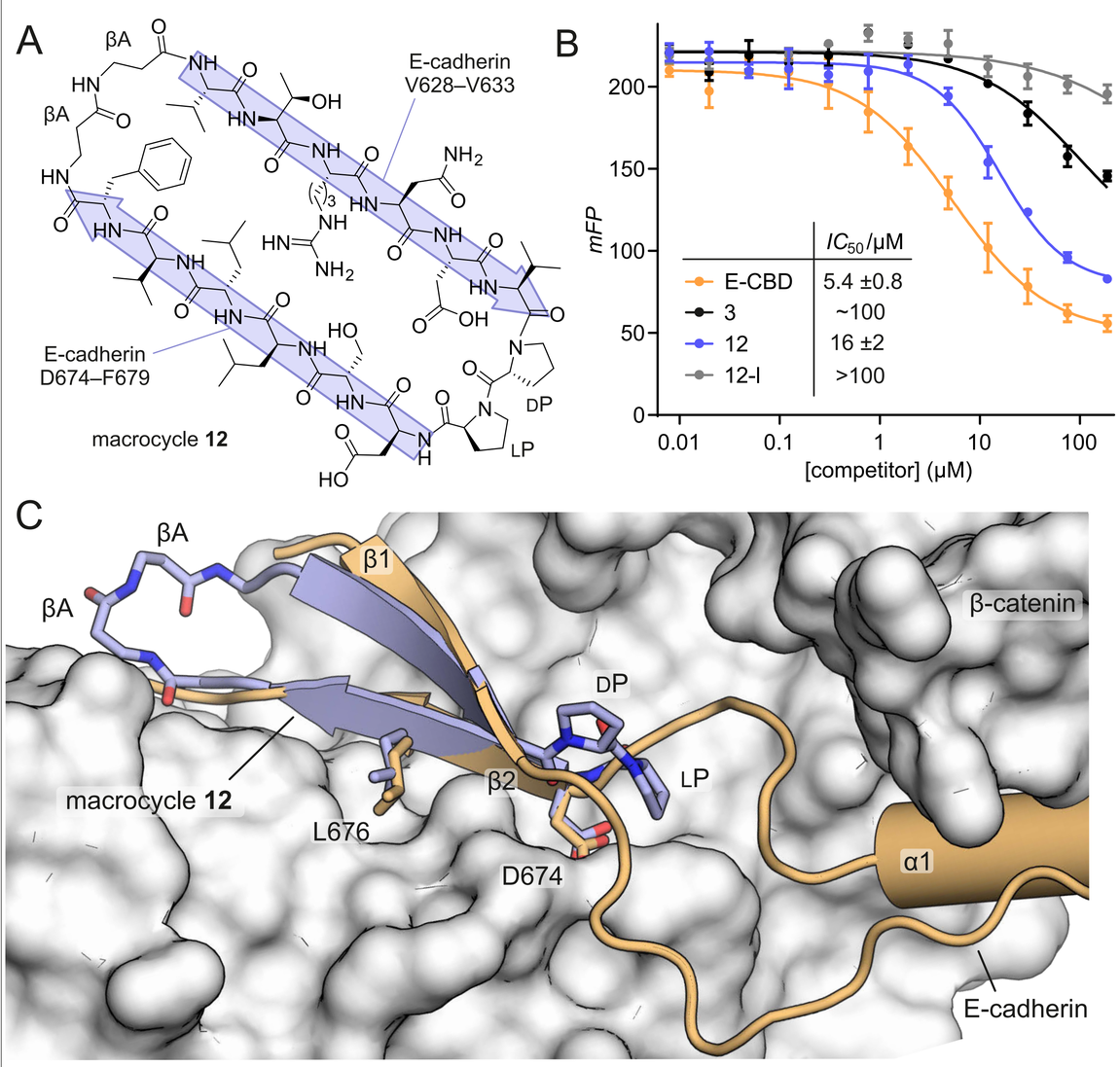
Peptide	K_D / μM	Length
E-CBD	0.053 ±0.003	52 aa
1	0.13 ±0.04	45 aa
2	0.2 ±0.1	39 aa
3	~ 3	28 aa



Peptide	K_D / μM	Length
6	0.18 ±0.03	54 aa
8	0.053 ±0.005	48 aa
9	>5	23 aa
10	0.6 ±0.1	45 aa
11	>5	14 aa

(A) Top: Crystal structure (PDB ID 2gl7) of β-catenin (grey) in complex with the CBD of TCF-4 (green) and superimposed with the CBD of E-cadherin (orange, PDB ID 1i7x, chain B). Bottom: Close-up of indicated region with b-catenin in surface representation (grey). Hot-spot amino acids of E-CBD (D665, I657, Y643, D674, L676) and TCF (D16, E17) are shown as stick. Terminal amino acids of peptides E-CBD, 1, 2 and 3 are indicated. Position where sequence of E-CBD was opened to generate peptide 6 is indicated. (B) Peptide affinities and lengths are shown including the visualization of involved peptide regions. K_D-values were obtained from FP measurements (n=3, ± std. error). (C) Table shows peptides derived from 6. The β-alanine loop (βA-βA) is highlighted in black. Measurements in analogy to Figure 1b..

3 A Cyclic Peptide Inhibits the β-Catenin/TCF4 Interaction



(A) Chemical structure of the macrocycle 12. Amino acids of β-strands β1 and β2 are highlighted in blue. (B) FP competition assay with TCF-4-derived tracer peptide c = 10 nM) and full-length β-catenin (c = 250 nM). Competitor concentration was varied (c = 7.9×10^{-3}–188 μM, n=3, ± std. error). (C) Crystal structure of β-catenin (grey, surface representation) in complex with peptide 12 (blue, cartoon representation, PDB ID 7ar4). The hot spot residues as well as βA-βAand the DP-LP turn are shown in stick representation (blue). 12 is superimposed with the CBD of E-cadherin (orange, cartoon representation, PDB ID 1i7x). Residues L676 and D674 are shown explicitly.

4 Conclusions

– This is the first crystal structure of a synthetic ligand bound to this site of β-catenin.

– A derivative peptide of **12** showed activity in Wnt reporter gene assay.

– Outlook is to improve cellular uptake further.

References

[1] M. Pelay-Gimeno, A. Glas, O. Koch, T. N. Grossmann, *Angew. Chem. Int. Ed.* **2015**, *54*, 8896–8927.

[2] H. Clevers, R. Nusse, *Cell* **2012**, *149*, 1192–1205.

[3] M. Wendt, R. Bellavita, A. Gerber, N-L. Efrem, T. van Ramshorst, N. M. Pearce, P. R. J. Davey, I. Everard, M. Vazquez-Chantada, E. Chiarparin, P. Grieco, Sven Hennig, T. N. Grossmann, *Angew. Chem. Int. Ed.* **2021**, 60, 13937–13944.

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