The interaction of β-catenin with T-cell factor (Tcf) 4 plays a central role in the Wnt signaling pathway and has been discussed as a possible site of intervention for the development of anti-cancer drugs. In this study, we performed Ala-scanning mutagenesis of all Tcf4 residues in the Tcf-β-catenin interface and studied the binding energetics of these mutants using isothermal titration calorimetry. Binding of Tcf4 was found to be highly cooperative. Single site mutations of most Tcf4 residues resulted in a significant reduction in binding enthalpies but in similar binding constants as compared with wild type Tcf4. Interestingly, this was also true for residues that are disordered in the reported crystal structures. The mutation D16A caused the largest reduction in binding constant (50-fold) accompanied by a large unfavorable entropic contribution to the binding. Single site mutations of the hydrophobic residues Leu41, Val44, and Leu48 resulted in a significant reduction in binding enthalpies, which were largely compensated for by unfavorable entropic contributions to the binding. Other mutations that significantly reduced Tcf4 binding constants were D11A and alanine mutations of the hydrophobic residues Leu41, Val44, and Leu48. The measured thermodynamic data are discussed with the available structural information of Tcf-β-catenin crystal structures and allow us to propose possible sites for development of Tcf antagonists.

β-Catenin is a crucial component of the cell adhesion machinery as well as an intracellular mediator of the Wnt signaling pathway. These diverse functions are realized through interaction with various binding partners. In its signaling role, cytosolic β-catenin interacts with proteins of the Tcf/Lef family of DNA-binding proteins. In the absence of Wnt signaling, target genes are maintained in a quiescent state by Tcf proteins (1, 2). When Wnt signaling is activated, cytosolic β-catenin is stabilized and translocated into the nucleus, where it functions with Tcf proteins as a transcriptional activator for a large number of target genes (3–8). Transcriptional activation of β-catenin-Tcf target genes is a hallmark of colorectal cancer cells, and the constitutive activation of some of these genes is essential for creating and maintaining the malignant phenotype (9, 10). Consequently, the β-catenin-Tcf complex has emerged as an attractive drug development target.

The first 53 amino acids in Tcf4 constitute the catenin-binding domain (CBD) of this protein. The N-terminal sequence stretch of Tcf4 is highly acidic and is not folded in solution (11). The CBD is necessary and sufficient for high affinity interaction with β-catenin (4, 5, 8, 11). Several crystal structures have been determined to date; one structure is available for the Xenopus xTcf3-β-catenin complex, and two structures have been determined for human Tcf4-β-catenin complexes (12–14).

Structural alignments of the two human Tcf4-β-catenin complexes and the homologous structure of xTcf3-β-catenin revealed a number of surprising conformational differences, in particular in the N-terminal and the extended regions of Tcf, indicating a high degree of molecular plasticity. Because the Tcf4 ligand is not implicated in crystal packing in any of the published structures, the observed differences might be a result of changes in solvent conditions (12–14).

Structural data as well as co-immunoprecipitation assays have suggested that the main interaction points for the recognition of β-catenin by Tcf are two regions of highly positive electrostatic potential. These so-called charge buttons are formed by the β-catenin residues Lys135, Arg160, His170, Asn208, and Lys209 as well as Lys120 that interact with the xTcf3 residues Asp16, Glu17, and Glu24, respectively (12). The importance of the first charge button for Lef1 binding has been described previously in a mutagenesis study on β-catenin (15). In addition, several hydrophobic contacts such as those formed by the Tcf residues Phe21 and Val18 have been reported to be important for Tcf4 binding to β-catenin (16). Calorimetric, intrinsic fluorescent quenching and surface plasmon resonance binding data have shown that Tcf4 binds to β-catenin armadillo with a binding constant of $3 \pm 2 \times 10^{6}$ mol$^{-1}$ at 20 °C. The interaction is strongly favored by a large negative binding enthalpy that is strongly temperature-dependent and gives rise to a heat capacity change ($\Delta C_{p}$) of $-1.5$ kcal/mol K$^{-1}$ (11).

To define interaction hot spots on Tcf4 that may be mimicked by low molecular weight antagonists and to understand the interactions with β-catenin, we mutated all residues in Tcf that make significant contacts with β-catenin. The binding of the Tcf mutants was subsequently studied by isothermal titration calorimetry, which allowed not only the determination of binding constants in solution but also gave insight into the binding energetics of the Tcf4 mutants.

Our study defined residue Asp16 as the most important interaction point of Tcf4. The mutation D16A results in a large decrease in binding enthalpy and binding constant. Single site mutations of Tcf4 residues interacting with the second charge...
C-terminal helix of the Tcf-CBD.

In addition, an important cluster of hydrophobic residues for disordered in crystal structures might significantly contribute was introduced between the CBD and the C-terminal His6 tag. Site-

56) was judged to be pure after affinity chromatography as assessed by SDS-

(PHarmacia) and a standard protocol (Novagen). The proteins were

grown for 3 h and harvested by centrifugation. Cell pellets were resus-

pended and lysed in PBS (Sigma) using a French press. The recombi-

nantly expressed His6-tagged proteins were affinity purified using nickel-agarose matrices, and the integrity of the fusion proteins was confirmed with an antibody against the C-terminal His6 tag.

Calorimetric measurements were performed using a high-precision isothermal titration calorimeter (MicroCal). Samples were extensively

dialyzed against PBS, 1 mM DTT. Heats of dilution were measured in blank titrations by injecting the protein into the buffer used in the particular experiment and subtracted from the binding heats. Thermodynamic parameters were determined by non-linear least squares methods using routines included in the Origin software package (MicroCal). Due to the large binding enthalpy of the interaction, a protein concentration in the calorimeter of 2 μM was sufficient to obtain a good signal-to-noise ratio and to reduce ‘c-values’ (c = K_P [P] N; [P] = protein concentration; N = stoichiometry) below 500. Measured thermodynamic values are usually a function of solvent conditions. To indicate this, observed binding enthalpy and entropy changes are abbreviated as ΔH° and ΔS°, respectively throughout this publication.

RESULTS

In this study we mutated all Tcf4-CBD residues that make significant contacts with β-catenin to alanine and studied the binding of these mutants using isothermal titration calorimetry. The results were analyzed on the basis of the published structures of Tcf-β-catenin complexes. The studied protein domain of Tcf4 contained residues 1–56, which have been shown to be sufficient for high affinity binding to β-catenin (11, 16). To determine the protein concentration spectroscopically and facilitate purification, we introduced a tryptophan residue and a His$_6$ tag at the C terminus of Tcf4-CBD. These additional C-terminal residues did not alter binding affinities for β-catenin (Table I). In this study we noted that the slightly different solvent conditions used (PBS, 1 mM DTT) result in somewhat smaller binding enthalpies than reported in an earlier study in which a buffer of 50 mM sodium phosphate, pH 7.4, 100 mM NaCl, and 2 mM DTT was used. A representative set of experimental data is shown in Fig. 1.

Interaction of the Tcf4 β-Hairpin and Its Flanking Residues (Residues 2–15)—Most of the β-hairpin formed by the xTcf3 residues 2–15 has been found to be disordered in human Tcf4 structures. The region that was well defined started with Tcf4 residue Leu$_{12}$ in the structure determined by Graham et al. (Protein Data Bank accession number 1jdh, referred to henceforth as Tcf4$_{1jdh}$) (12) and residue Gly$_{13}$ in the structure determined by Poy et al. (Protein Data Bank accession number 1jpw, referred to henceforth as Tcf4$_{1jpw}$) (13). This finding is surpris-

button on β-catenin resulted in only marginal changes of the binding constant but significantly reduced binding enthalpies. In general, mutations of polar Tcf residues in the Tcf-β-catenin interface resulted in less favorable binding enthalpies. For most mutants, this effect was largely compensated for by entropic contributions to the binding, indicating a large degree of cooperativity in this interaction. Another residue that was found to decrease binding constants and alter binding energetics when mutated to alanine was Asp$_{13}$, which has been found to be disordered in the crystal structures of the Tcf4-β-catenin complex. This finding demonstrates that even residues that are disordered in crystal structures might significantly contribute to binding affinity and alter binding energetics upon mutation. In addition, an important cluster of hydrophobic residues for the interactions of Tcf4 with β-catenin was defined by the alanine mutants of the residues Leu$_{41}$, Val$_{44}$, and Leu$_{48}$ of the C-terminal helix of the Tcf-CBD.

**MATERIALS AND METHODS**

Cloning, Expression, and Purification of Proteins—The armadillo repeat region (amino acid residues 134–671) in β-catenin was cloned and purified as described previously (11). Tcf4-CBD (residues 1–56) was cloned into the NdeI and XhoI restriction sites of pET20b. To quantitate the recombinant Tcf4 protein spectroscopically, a tryptophan residue was introduced between the CBD and the C-terminal His$_6$ tag. Site-directed mutagenesis of Tcf4 was performed using the QuikChange kit (Stratagene) and oligonucleotides that were complementary to 15 bp upstream and downstream of the mutation site on both strands. Tcf4-CBD mutants were transformed into Escherichia coli (BL21-DE3). Transformed bacteria were grown to mid-log phase at 37 °C in 2 × yeast tryptone medium, and protein expression was induced by addition of 1 mM isopropyl-1-thio-β-D-galactopyranoside. Induced cells were then grown for 3 h and harvested by centrifugation. Cell pellets were resus-
pended and lysed in PBS (Sigma) using a French press. The recombi-
nantly expressed proteins were purified using pre-charged Ni-Sepharose beads (Pharmacia) and a standard protocol (Novagen). The proteins were judged to be pure after affinity chromatography as assessed by SDS-
PAGE and matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Tcf4 wild type and mutants were quantitated using an absorbance coefficient at 280 nm of 5.69 × 10$^4$ mol/cm.

**Isothermal Titration Calorimetry**—Calorimetric measurements were carried out at constant temperatures using a VP-isothermal titration calorimetry titration calorimeter (MicroCal). Samples were extensively
ing, considering the high sequence similarity between xTcf3 and Tcf4 (Fig. 2). In xTcf3, the main side chain interactions are formed by Asp10, which forms a salt bridge with the β-catenin residue Arg274, and Glu11, which interacts with His578, Arg582, Arg612, and Tyr654 on β-catenin (Fig. 2). In the two Tcf4 structures reported, the location of the N terminus is quite different. Whereas in Tcf41jdh residues 12–16 superimpose well with the corresponding residues in xTcf3, the N terminus in Tcf41jpw does not interact with β-catenin and is oriented toward the solvent. The first 12 N-terminal residues in Tcf4 have been shown to be dispensable in solid phase competition assays (enzyme-linked immunosorbent assay) as well as in reporter gene assays (16, 15). Indeed, deletion of the first 9 (ND9) or 12 (ND12) residues of Tcf4 had only a marginal effect on binding constants (Table I), whereas binding enthalpies were found to be reduced by about 5 kcal/mol. This suggests that the Tcf4 N terminus interacts with β-catenin but that it does not contribute to binding free energy change due to enthalpy-entropy compensation mechanisms. A similar effect was observed for the mutation D10A, which bound with a $\Delta H^{\text{obs}}$ of +6.5 kcal/mol but with a binding constant similar to that of wild type Tcf4. Interestingly, the mutation D11A also reduced the $K_B^*$ of the interaction by about 5-fold. Thus, the mutation D11A has a larger effect on the binding of Tcf4 than deletions of the entire N terminus. It has been observed previously that effects of single mutations may be much stronger than the contribution of the entire site as a whole (18). Cooperative effects are usually invoked to explain such observations. However, the observed data might also be the result of local changes in protein structure, differences in the dynamics of both proteins, or changes in surface hydration. To confirm this result, we also mutated the residues on β-catenin that interact with Glu11 in xTcf3. The mutations R612A and Y654A did not significantly affect binding of wild type Tcf4 (Table II). Surprisingly, we observed an ~30-fold decrease in binding constant for wild type Tcf4 for the β-catenin mutations H578A and R582A, suggesting that wild type Tcf4 interacts with this binding site on β-catenin. The Tcf4 residue Asp12 corresponds to a glutamic acid in xTcf3. Possibly, the elongation of the side chain by one CH$_2$ group at this critical position renders this interaction more favorable. Due to the lack of structural information on the Tcf4 N terminus, we can only speculate about the molecular mechanisms that give rise to this binding behavior. However, the measured data demonstrated that under the chosen experimental conditions, the N-terminal residues of Tcf4 do interact in solution with β-catenin and that this interaction involves the β-catenin residues that are contacted by xTcf3.

**Interaction of the Tcf4 Extended Region**—This region is structurally very conserved among the three reported Tcf structures and superimposes very well with residues 671–686 of the β-catenin ligand E-cadherin as well as the Tcf antagonist ICAT (19–21). The Tcf extended region binds along a shallow groove with highly positively charged surface patches (charge buttons). Consequently, the electrostatic interactions observed in the β-catenin-Tcf complexes have been discussed as the main.

**Fig. 1.** Experimental calorimetric data of the binding of wild type Tcf4 and the Tcf4 mutant D16A to β-catenin armadillo. The experiment was carried out at 25 °C. The top panel shows raw heat data obtained over a series of injections of Tcf4 (curve a) and Tcf4 D16A (curve b) into β-catenin armadillo. PBS buffer (Sigma), 1 mM DTT was used in all experiments. Dilution heats measured by titrating Tcf4 into the corresponding buffer were in the range of the heat effects observed at the end of the titration (data not shown). The integrated heat signals of the data shown in the top panel gave the binding isotherm shown in the bottom panel. The solid lines represent a calculated curve using the best-fit parameters obtained by a non-linear least squares fit. Integrated heat signals measured using wild type Tcf4 are shown as filled squares, and those measured using the Tcf4 mutant D16A are shown as open circles.

**Fig. 2.** A, structural alignment of the β-hairpin region of xTcf3 and the two published human Tcf4 structures. Tcf residues are annotated in red, and interacting residues in β-catenin are shown in blue. The Tcf41jdh and Tcf41jpw are shown in cyan and magenta, respectively. xTcf3 carbon atoms are shown in green. Polar contacts are indicated by dotted lines, and hydrophobic contacts are shown as green dotted circles. B, structure-based alignment of Tcf4 with xTcf3, Lef1, and cadherin. Numbering corresponds to the Tcf4 sequence. Conserved residues are shown in red.
interaction sites that drive the binding of Tcf (12). Mutagenesis data on β-catenin showed that mutation of the residues Lys
106, Asn
426, His
470, Arg
469, and Lys
508 to alanine strongly reduces binding of Lef1 and Tcf4 in co-immunoprecipitation experiments (15). These residues form polar contacts with Tcf4 residues 16–19. Asp
16 has been identified previously as a critical residue for Tcf binding (11, 16). The Tcf4 residue Asp
16 forms hydrogen bonds with the β-catenin residues Lys
426 and His
470 as well as several water-mediated hydrogen bond networks (Fig. 2). We find in this study that the residue Asp
16 defines the most important interaction hot spot on Tcf4. The mutation D16A resulted in a ∆G
obs of +2.4 kcal/mol. The neighboring residue Glu
24 forms a salt bridge with Lys
508. However, upon mutation of Glu
17 to alanine, the binding constant decreased only ~3-fold (11).

The interaction with the first charge button is followed in Tcf4 by the hydrophobic residues Leu
18, Ile
19, and Phe
21, which interact with hydrophobic groups of the β-catenin residues Thr
293, Cys
295, Cys
296, Ala
367, Pro
368, and Arg
369 (Fig. 3). Single alanine mutants of Leu
18, Ile
19, and Phe
21 did not significantly affect Tcf4 binding in isothermal titration calorimetry experiments. For the related transcription factor Lef1, an important contribution of residue Phe
24 for β-catenin binding (Phe
21 in Tcf4) has been discussed using a yeast two-hybrid system. This residue has also been shown to be important for the translocation of endogenous β-catenin into the nucleus (15).

In addition, the Tcf4 double mutant I19A,F21A has been shown to reduce reporter gene activity by ~40% in transient transfection experiments (13). In light of our binding data, it is clear that mechanisms other than β-catenin binding are responsible for the observed effect of these mutants in these cell-based experiments.

The cluster of hydrophobic Tcf4 residues is followed by negatively charged residues, which form the interaction with the second charge button defined by the β-catenin residue Lys
151. The three-dimensional structure of this region differs significantly in the two Tcf4 crystal structures published. As in xTcf3, in Tcf4
1jdh the interaction with the second charge button is made by Glu
28, whereas in Tcf4
3d the similar interaction is formed by Glu
29. In this structure, Glu
24 makes no interaction with β-catenin but points to the solvent space. For the mutant E24A, we found a significant difference in binding enthalpy (∆H
obs, +7.4 kcal/mol), which is, however, largely offset by favorable entropic contributions. This mutation resulted in a ∆G
obs of only 0.9 kcal/mol and an ~4-fold decreased binding constant.

The mutant E28A resulted in only moderate reduction of ∆H
obs and in similar binding constants with respect to the wild-type protein. In addition to Glu
28, Glu
29 also makes polar contacts to the second charge button in Tcf4
3d. The observed difference in ∆H
obs of 5.8 kcal/mol for mutant E29A supports such an interaction, even though binding constants are not altered. This highly acidic region compensates for single mutations as indicated by the plasticity shown by the two structures of Tcf4-β-catenin complexes. This hypothesis is supported by co-immunoprecipitation experiments, demonstrating that the mutation of all 4 glutamate residues in that region (Glu
24, Glu
29, Glu
28, and Glu
29) abolishes binding to β-catenin, whereas single mutations had either no effect or only a small effect on binding (14).

Mutation N34A only moderately changed ∆H
obs and had a negligible effect on the binding constants. This residue is disordered in Tcf4
1jdh and forms a hydrogen bond with Arg
442 in Tcf4
3d.

Binding of the entire extended region of Tcf4 that includes residues 9–28 was probed using a synthetic peptide (Tcf
9–28). The peptide bound with an affinity of 2.4 ± 0.3 × 10
6 mol
−1, an

| β-Catenin mutant       | K_r | ∆H
obs | T∆S
obs | ∆G
obs | N°  |
|------------------------|-----|-------|-------|-------|-----|
| WT
3H16e6d                | 3.5 ± 1.5 | −24.5 ± 1.8 | −12.8 ± 0.8 | −11.7 ± 1.1 | 1.05 ± 0.2 |
| H578A                  | 0.14 ± 0.02 | −19.8 ± 1.0 | −10.1 ± 0.4 | −9.7 ± 0.3 | 0.97 ± 0.05 |
| R582A                  | 0.17 ± 0.02 | −22.8 ± 0.6 | −13.0 ± 0.4 | −9.8 ± 0.3 | 0.96 ± 0.02 |
| R612A                  | 1.5 ± 1.1 | −25.0 ± 1.0 | −17.0 ± 0.7 | −11.0 ± 0.1 | 0.97 ± 0.03 |
| V64A                   | 5.1 ± 2.1 | −27.3 ± 1.2 | −15.4 ± 1.1 | −11.9 ± 0.2 | 0.99 ± 0.05 |

* N°, stoichiometry of the interaction.
* WT
3H16e6d, wild type Tcf4 with a C-terminal tryptophane residue and His tag.

The errors that are given in the table result from differences between independent experiments, and the data represent the average calculated from all experiments "exp#." All data have been measured in PBS buffer (Sigma), 1 mm DTT at 25 °C.
enthalpy change of $-9.0 \pm 0.2$ kcal/mol, and an entropy change close to 0 (Table I). These binding data demonstrate that a large portion of the binding free energy change (about 75%) is due to interactions formed by the extended region of Tcf4.

**Interaction of the Tcf4 Helical Region**—The amphipathic $\alpha$-helix described for all three Tcf structures packs against a hydrophobic surface area on $\beta$-catenin defined by the residues Phe<sup>252</sup>, Phe<sup>293</sup>, and Ile<sup>296</sup> and the aliphatic portions of His<sup>219</sup>, Lys<sup>292</sup>, and Lys<sup>335</sup> (Fig. 4). The Tcf4 residues Leu<sup>41</sup>, Val<sup>44</sup>, and Leu<sup>48</sup> form the main hydrophobic contacts in this interface. In general, mutation of the hydrophobic residues significantly reduces binding affinities of Tcf4. We observed a 15-fold reduction for the mutations L41A and V44A as well as a 5-fold reduction for the mutant L48A (Fig. 5) (11).

The Tcf4 residues Asp<sup>40</sup> and Lys<sup>45</sup> form polar and electrostatic interactions along the edge of the $\beta$-catenin groove. Asp<sup>40</sup> is positioned between Arg<sup>376</sup> and Lys<sup>335</sup> in $\beta$-catenin, and the Tcf4 residue Lys<sup>45</sup> forms a hydrogen bond with the $\beta$-catenin residues His<sup>290</sup> and Asn<sup>291</sup>. These interactions are also preserved in the xTcf3 structure. The mutation D40A resulted in an $\sim$3-fold reduction of the Tcf4 binding constant. In contrast to that very large heat capacity change, a linear fit of binding enthalpy data measured on Tcf4<sub>9</sub>–<sub>28</sub> resulted in a $\Delta C_p$ of only $-0.18$ kcal/(mol K). Thus, part of the large negative heat capacity change observed upon binding of Tcf4-CBD to $\beta$-catenin is a consequence of the formation of the C-terminal helix observed in all Tcf-$\beta$-catenin crystal structures.

**DISCUSSION**

Binding data of a number of Tcf4 and $\beta$-catenin mutants have been reported in the literature using several diverse in vivo and in vitro assay techniques, which makes it difficult to compare the reported experimental data. We used titration calorimetry to generate a comparable set of Tcf4 mutant bind-
FIG. 7. Temperature dependence of $\Delta H_{\text{obs}}$ for Tcf4-CBD and Tcf4$^{-28}$ measured in PBS. Shown are data collected on Tcf-CBD (C) as well as Tcf9$^{-28}$ ( ). Linear least squares fits of the data shown are indicated by dotted lines and gave rise to a heat capacity increment of $-1.45$ and $-0.18$ kcal/(mol K) for Tcf4-CBD and Tcf9$^{-28}$, respectively.

Binding of Tcf does not significantly alter the overall conformation of $\beta$-catenin compared with its unbound state (12, 13). Main chain root mean square deviation between $\beta$-catenin without ligand and the two published Tcf4-3$\beta$-catenin complexes were only 0.7 Å, confirming the role of the armadillo repeat region as a rigid structural platform. However, binding to $\beta$-catenin induces structural changes in the Tcf ligand, which is mainly unstructured in solution (11). Thus, folding events in Tcf4 upon binding to the $\beta$-catenin surface additionally modulate the binding energetics of Tcf4 $\beta$-catenin complex formation. This study showed that one effect of such folding events is the determined large negative heat capacity increment of $-1.45$ kcal/(mol K). This effect can be largely assigned to folding events because the extended region of Tcf4 alone (residues 9–28) bound with a heat capacity change of only $-0.18$ kcal/(mol K).

A large number of thermodynamic studies showed that binding energetics can be calculated on the basis of polar and apolar surface area changes upon complex formation (25, 26). However, surface area changes calculated on the basis of the xTcf3-$\beta$-catenin complex predicted a heat capacity increment of $-0.067$ kcal/(mol K) (11), and the new structural data on Tcf4-$\beta$-catenin complexes did not significantly change these calculated values. Large deviations of calculated values and experimental heat capacity changes have been described for many other systems, and possible mechanisms for such discrepancies have been discussed by us and by others (11, 27–30). The comparison between experimental heat capacity increments of Tcf4-CBD and Tcf9$^{-28}$ suggests that folding events upon binding are largely responsible for the large negative heat capacity increment.

Implications for Drug Development—Because of the central role of the Tcf/Lef $\beta$-catenin interaction in Wnt signaling, the development of a low molecular weight antagonist is an interesting strategy for anti-cancer therapy. In contrast to many active sites in proteins that can be efficiently inhibited by small inhibitors, protein interaction sites usually span much larger surface areas. However, a number of studies have shown that these large interfaces often contain interaction hot spots that may be used as binding sites for much smaller antagonists (15,
31). In this study, we showed that, at least in vitro and on a single amino acid residue basis, interference with the contact site of the Tcf residue Asp16 would be the most promising strategy. A hydrophobic binding pocket on β-catenin in the vicinity of Asp16 that could harbor a small molecule has been described (15). However, such an inhibitor would also compete with the β-catenin ligand cadherin, which would lead to the retention of the cadherin in the endoplasmic reticulum and degradation of the protein (32). A recent structural study on the Tcf antagonist ICAT showed that even though ICAT, cadherin, and Tcf have partially overlapping binding sites, ICAT competed with Tcf binding but not with cadherin binding in vivo (19, 21). Possible explanations for this observation could be that cadherin binds much more strongly to ICAT/H9252 than to Tcf and ICAT or that the binding is regulated in vivo by local concentration differences and/or by post-translational modifications. Recently, it has been reported that cadherin binding is modulated by phosphorylation, which increases its binding constant to β-catenin by 3 orders of magnitude (19, 21). A large body of structural information on β-catenin complexes has been reported recently (12–14, 19, 21). This knowledge will certainly aid the structure-based design of Tcf antagonists. However, due to the large binding constants of Tcf4, the observed structural plasticity, the high degree of cooperativity, and the electrostatic nature of the interaction, the development of efficient antagonists will be challenging.

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Hot Spots in Tcf4 for the Interaction with β-Catenin
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